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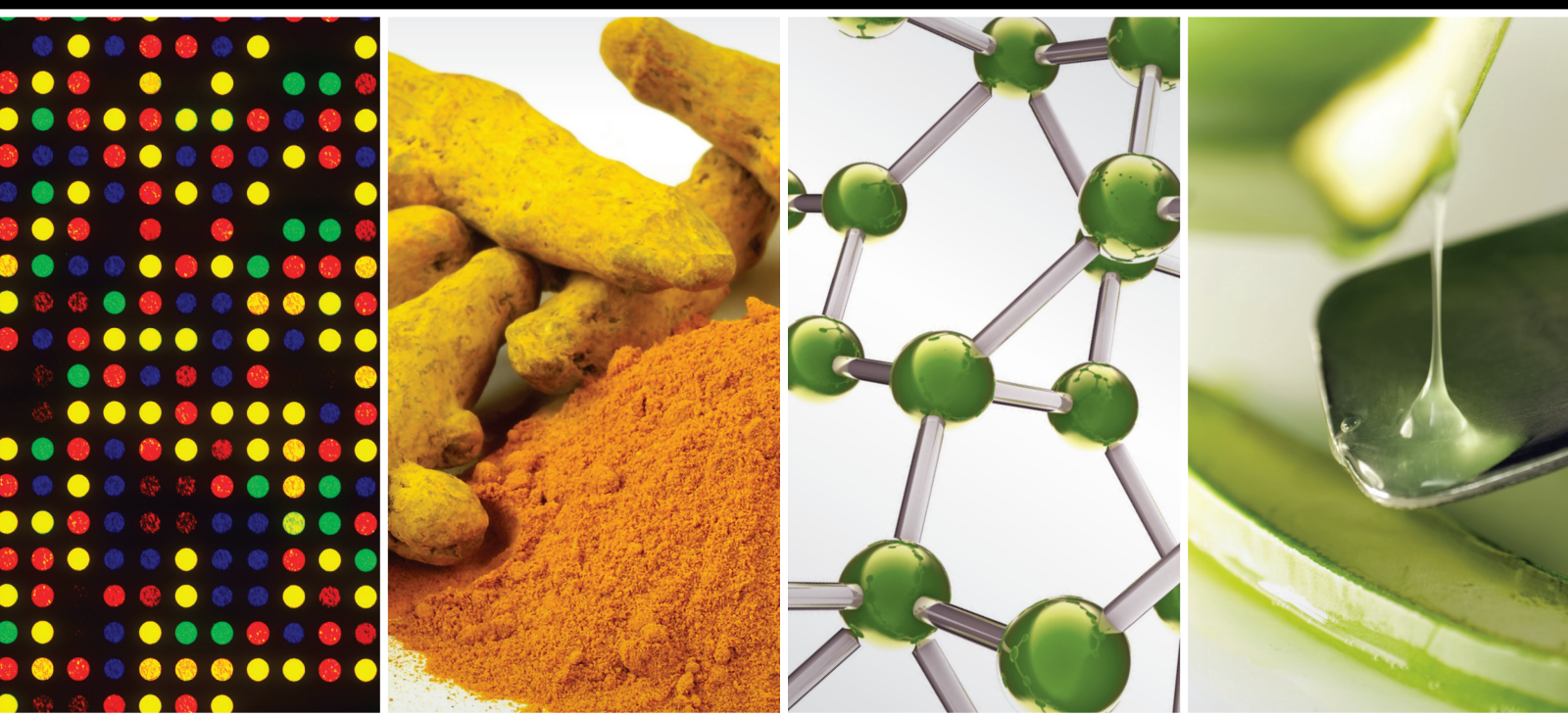
Natural Products and/or Isolated Compounds on Wound Healing

Lead Guest Editor: Christian Agyare
Guest Editors: Abidemi J. Akindele and Vanessa Steenkamp



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Editorial Board

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Editorial

Natural Products and/or Isolated Compounds on Wound Healing

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The effective treatment of wounds remains a major global health challenge. Failure to heal or elongation of the wound healing process results in increased financial and social stress being placed on health institutions, care-givers, patients, and their families. The occurrence of various forms of wounds such as chronic and acute wounds, pressure ulcers, venous stasis ulcers, and diabetic ulcers has increased over the years in most countries, especially developed countries where life expectancy has been increasing over time and is accompanied by geriatric diseases. Acute and chronic, nonhealing wounds impose heavy financial and quality of life burdens on patients [1]. Chronic wounds are normally characterized by intense pain, infection, loss of function, and loss of mobility and may lead to amputations and in some cases even death. With an increase in the prevalence of wounds and the high cost of orthodox medicines, most patients, especially those in developing countries, resort to herbal preparations or remedies which are believed to be readily available and cheap for the treatment thereof. The urgent need for the identification of effective, safe, and cost efficient wound healing promoters which can be introduced into clinical practice is unequivocal [2, 3]. This has driven an increase in the search for potent, cost effective wound healing agents from natural products including medicinal plants.

Medicinal plants have been used in the management of various diseases since ancient time. It is estimated that about 70 to 80% of the world's population depends on medicines of plant origin for treatment of diseases. The use of medicinal plants in the management of acute and chronic wounds is common in most traditional medicine practices in the world. Based on this, many plants in the tropical and subtropical regions of the world have been screened for their wound healing activity. Yet there are still many medicinal plants that need to be screened in the search for newer, efficacious, and cost effective wound healing agents. This special issue provides the platform for bringing to the limelight recent efforts in this regards.

This issue contains eight articles which focus on studies on the current trends for managing wounds and associated complications, newer natural products including animal products, and/or isolated compounds possessing wound healing activity.

R. Komakech et al. presented a review on the wound healing potential of *Aspilia africana* (Asteraceae), a plant used in African traditional medicine to treat wounds. The authors reported that *in vitro* and *in vivo* investigations provided evidence of the wound healing properties of the plant's derived extracts and phytochemicals, including alkaloids, saponins, tannins, flavonoids, phenols, terpenoids, β-caryophyllene, germacrene D, α-pinene, carene, phytol, and linolenic acid. These phytoconstituents were linked with strong anti-inflammatory, antimicrobial, and antioxidant activity, all essential for wound healing. Specific activities of the extracts of *A. africana* and its constituents beneficial to wound healing were reported to include inhibitory effects on bleeding, enhancement of wound contraction, increases in the levels of basic fibroblast growth factor (BFGF) and platelet derived growth factor, and stimulation

of haematological parameters like white and red blood cells.

M. Gulumian et al. reported on African herbal remedies with antioxidant activity as potential resource base for wound treatment in a form of a review article. This is based on the premises that excess free radicals have been linked with wound chronicity and antioxidant therapy facilitates wound healing. The review highlighted tests that have been used to assess antioxidant activities of African medicinal plant extracts; compounds isolated from African medicinal plant extracts with confirmed antioxidant activities; and crude extracts of African medicinal plants with confirmed antioxidant activities. The authors reported that either single assays or *in vitro* analysis were used to determine the antioxidant activity of listed extracts and compounds, warranting the use of alternative testing methods, including *in vivo* assays, for confirmation of observed effects. They also clamoured for identification of compounds responsible for the antioxidant activities and evaluation of wound healing properties of isolated compounds in applicable cases.

J. E. T. do Nascimento identified and isolated the main chemical compounds present in extracts of *Ouratea fieldingiana* (Ochnaceae) and investigated their possible antifungal, antioxidant, and anticholinesterase activities. The plant is popularly used in Brazilian folk medicine for wound healing and treatment of inflammation and infectious diseases. Amentoflavone and kaempferol 3-O-rutinoside were isolated, respectively, from the ethanol seeds and leaves extracts of the plant. The extracts and compounds were shown to possess antifungal activity against several *Candida* strains via inhibition of ergosterol biosynthesis, antioxidant, and anticholinesterase activities.

K. Xu et al. in a review article on plant-derived products for treatment of vascular intima hyperplasia (IH) described the different originating cells involved in vascular IH and highlighted the effect of different natural products on inhibiting abnormal cellular functions, such as vascular smooth muscle cells (VSMC) proliferation and migration. The review article covered diverse cells involved in vascular IH; antiproliferation, migration, and cellular functions of abnormal VSMCS as a target to decrease intimal hyperplasia; typical signal pathways involved in the growth and physiology of VSMCs in IH disease; different natural compounds being used for preventing neointimal formation; and selective inhibition of VSMCs versus vascular endothelial cells (VECs).

In another review article, L. Tan et al. evaluated the clinical effective rate, safety, and financial cost of traditional Chinese medicine injections (TCMIs) in treating diabetic foot and ulcer wound healing. The findings from the study suggest that TCMIs are beneficial to patients with diabetic foot ulcers, increasing the clinical effective rate of conventional therapies, and eliciting better performance in safety and financial burden. The authors however suggested more rigorous designed randomized control trials (RCTs) with large sample size to provide more high-quality evidence to support the benefits of TCMIs in the treatment of various types of wounds.

K. Jenwitheesuk et al. evaluated the efficacy of *Centella asiatica* (Apiaceae) extract in cream for the prevention of scar development of the split-thickness skin graft (STSG) donor site. A prospective randomized, controlled, double-blind trial was conducted on the *Centella* cream for scar improvement. The amelioration of hypertrophic scar by the extract cream was attributed to better pigmentation, improvement of objective measurements, and longer follow-up times.

M. Çalışır et al. investigated the effect of humic acid on the healing of excisional wounds in the palate of rats as a follow-up to previous demonstration of enhancement of cutaneous wound healing and antibacterial properties. The findings from the study showed that humic acid treatment enhanced the rate of wound closure and recovery. This provides a basis for the use of humic acid as an alternative in the treatment of oral wounds.

In the last publication in this special issue, J. Kim et al. in a research article investigated the effect of tracheloside, a plant lignin, on keratinocyte proliferation using scratch wound healing and cell proliferation assays and western blot analysis based on the fact that cell migration and proliferation are important for proper wound healing after skin injury. In the study, it was demonstrated that tracheloside positively affects the proliferation of HaCaT keratinocyte cell line through the regulation of ERK1/2 phosphorylation. The authors concluded that tracheloside is a potential therapeutic candidate for promotion of wound healing.

The high quality articles constitute authors located at affiliations and institutions from nine different countries and parts of the world. This special issue has extended the frontiers of knowledge in exploring the therapeutic application of natural products and/or isolated compounds in wound healing which accounts for significant morbidity and mortality worldwide. It has also provided the desired basis for future research in complementing and/or replacing existing therapeutic agents used in the treatment of wounds.

Conflicts of Interest

The editors declare that they have no conflicts of interest regarding the publication of this special issue.

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Christian Agyare
Abidemi J. Akindele
Vanessa Steenkamp

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Review Article

The Wound Healing Potential of *Aspilia africana* (Pers.) C. D. Adams (Asteraceae)

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Guest Editor: Abidemi J. Akindele

Wounds remain one of the major causes of death worldwide. Over the years medicinal plants and natural compounds have played an integral role in wound treatment. *Aspilia africana* (Pers.) C. D. Adams which is classified among substances with low toxicity has been used for generations in African traditional medicine to treat wounds, including stopping bleeding even from severed arteries. This review examined the potential of the extracts and phytochemicals from *A. africana*, a common herbaceous flowering plant which is native to Africa in wound healing. *In vitro* and *in vivo* studies have provided strong pharmacological evidences for wound healing effects of *A. africana*-derived extracts and phytochemicals. Singly or in synergy, the different bioactive phytochemicals including alkaloids, saponins, tannins, flavonoids, phenols, terpenoids, β-caryophyllene, germacrene D, α-pinene, carene, phytol, and linolenic acid in *A. africana* have been observed to exhibit a very strong anti-inflammatory, antimicrobial, and antioxidant activities which are important processes in wound healing. Indeed, *A. africana* wound healing ability is furthermore due to the fact that it can effectively reduce wound bleeding, hasten wound contraction, increase the concentration of basic fibroblast growth factor (BFGF) and platelet derived growth factor, and stimulate the haematological parameters, including white and red blood cells, all of which are vital components for the wound healing process. Therefore, these facts may justify why *A. africana* is used to treat wounds in ethnomedicine.

1. Introduction

A wound can be defined as the disruption of living tissue integrity associated with loss of function [1]. The wound healing process is a complex dynamic process which represents an attempt to restore a normal anatomical structure and function [2, 3]. Wounds can be broadly categorized as acute wounds which are caused by external injury to the skin and include surgical wounds, bites, burns, minor cuts and abrasions, and more severe traumatic wounds such as lacerations and those caused by crush or gunshot injuries or chronic etiology wounds which includes vascular, diabetic, and pressure ulcers [1, 4]. In fact, wounds impose significant health, social, and economic burdens to the individuals, the healthcare system, and the community as a whole [5, 6]. Recent statistics showed that approximately 3% of the healthcare budget is spent on treating wound-related complications in developed countries [6]. The aim of treating a wound is to prevent pain discomfort to the patient and promote wound healing which occurs mainly in four phases: hemostasis, inflammation, proliferation, and remodeling [1, 7, 8]. Plants have immense potential that can be explored for the treatment and management of wounds [2, 9]. Indeed, several medicinal plants have been used in traditional medicine for the treatment and management of all kinds of wounds across the globe since time immemorial

[3, 10, 11]. *Aspilia africana* (Pers.) C. D. Adams (Asteraceae), commonly referred to as wild sunflower, is one of the highly valued wound healing plants throughout its distribution range and beyond [12–14]. This unique wound healing plant species is commonly referred to as "haemorrhage plant" due to its distinguished ability to stop bleeding, even of severed artery [15, 16]. Apart from its enormous potential in wound healing, *A. africana* is reported to be vital in the treatment and management of myriad of other diseases and disorders in African traditional medicine, including headache, corneal opacities, stomach disorders, cough, gonorrhea, rheumatic pains, and tuberculosis; the leaf infusion is taken as a tonic for women immediately after delivery [17, 18]. *A. africana* plant is known to possess great anti-inflammatory, antimalarial, and antimicrobial activities [12, 16]. Several scientific studies have attributed the numerous medicinal properties of *A. africana* to the abundant bioactive secondary metabolites in it such as alkaloids, saponins, tannins, glycosides, flavonoids, and terpenoids [18, 19].

The use of *A. africana* in wound treatment and management has been assessed and discussed in a number of peer reviewed journal articles over the years. This review therefore sought to examine the wound healing potential of *A. africana* both *in vitro* and *in vivo* with the goal of finding new drugs for treatment and management of wounds.

2. Methods

In this review, we obtained information from original peer reviewed articles published in scientific journals, with a focus on the botany, distribution, and potential of *A. africana* for treatment and management of wounds. We critically searched electronic literature databases including but not limited to Google Scholar, PubMed, and Scopus for all available peer reviewed data. The following key search terms were used ("*A. africana*" OR "Wild sunflower" AND "wounds" OR "wound healing" OR "Phytochemicals") OR ("Phytochemicals in *A. africana*" OR "Wild sunflower" AND "wound" OR "wound healing"), OR ("Phytochemicals in *A. africana*" OR "Wild sunflower" AND "Anti-inflammation" OR "Antimicrobial"), OR ("Plants" OR "Natural products" AND "wound" OR "wound healing") OR ("*A. africana*" OR "Wild sunflower" AND "Botany" OR "Distribution"). The data obtained were verified independently for their accuracy and any inconsistencies were settled through discussions between the authors. The final data obtained through discussions among the authors were then summarized, analyzed, and compared, and conclusions were made accordingly.

3. Botany and Distribution of *Aspilia africana*

The genus *Aspilia* is a genus of common herbaceous flowering plants which are native to Africa and comprised of approximately 140 species [18, 64]. Morphologically, *A. africana* is a herb measuring about 1-2 m in height covered with bristles; stem is stiff at the base, with many branches and rough to touch (Figures 1(a) and 1(b)); leaves are rough, opposite, ovate-lanceolate, creased accordion-style covered with trichomes, average 10 cm long and 5 cm wide, and rounded at the base with petioles about 1 cm long with 3 prominent veins (Figure 1(c)); inflorescence consists of capitula which is terminal, solitary, or in lax racemes with hairy stalk of about 7 cm long on average; flowers have numerous showy-yellow florets; fruits are 4-angled achenes (Figure 1(d)) [12, 64, 65].

A. africana is native to Africa occurring in a number of countries throughout the tropical African region on waste land of the savanna and forested zones between altitude of 800 and 1800 m (Figure 2), and its rapid growth characteristics make it a difficult weed in cultivated land and fallows [65].

4. Toxicological Effects of *Aspilia africana*

Generally, this unique wound healing plant can be classified among agents with low toxicity [66]. In an *in vivo* study by Okokon et al. [67] using Swiss albino mice, the acute toxicity of the ethanolic extract of the plant showed that doses of 2000 $mgkg^{-1}$ and above were lethal to the animals and the determined LD_{50} of the extract was 1414.2 $mgkg^{-1}$. Further, *in vivo* study by Oko et al. [68] on Swiss albino mice concluded that oral administration of up to 10,000 $mgkg^{-1}$ body weight of aqueous and ethanolic extracts of the plant was safe for animal and human use. However, a recent study showed that the aqueous leaf extract of *A. africana* may be teratogenic to the developing placenta of Wistar rats in a dose-dependent manner; more severe outcomes were observed in female rats that received up to 1250 mg/kg body weight of the aqueous extract [69]. Similarly, other previous studies also showed that intraperitoneal administration of the extracts of *A. africana* leaf caused significant delay in estrus cycle and in addition did not only distort the histology of ovaries and reduce its weight, but also damaged the uterine tissues and fallopian tubes in Wistar rats [17, 67, 70, 71]. Furthermore, methanolic extracts of *A. africana* have also been found to significantly decrease the weight of testis, epididymis, seminal vesicle, and prostate gland of experimental male Wistar rats [72]. Therefore, despite the safety associated with *A. africana*, caution must be taken during its long term oral consumption as it may have adverse effects on reproductive organs.

5. Effects of Leaf Extracts of *Aspilia africana* on Wound Healing

A. africana is one of the many medicinal plants containing large quantities of bioactive compounds making it such a potent plant in wound sepsis treatment and management and other microbe induced disease conditions [19, 20]. Over the years, several *in vitro* and *in vivo* scientific studies have been conducted to validate the wound healing ability of this plant. In an *in vivo* study by Eweka and Eweka [73]; they examined the effects of aqueous extract of *A. africana* administered orally for fourteen days on the duodenum of adult Wistar rats exposed to varied concentrations of hydrochloric acid. The histological findings indicated sections of the small intestine of treated rats showed varying degrees of cellular proliferation and epithelia regeneration. This showed that *A. africana* consumption may have antiulcer effects on duodenal ulcer by its healing effects on the Brunnals gland and epithelia cells of the small intestine of adult Wistar rats. Similarly,

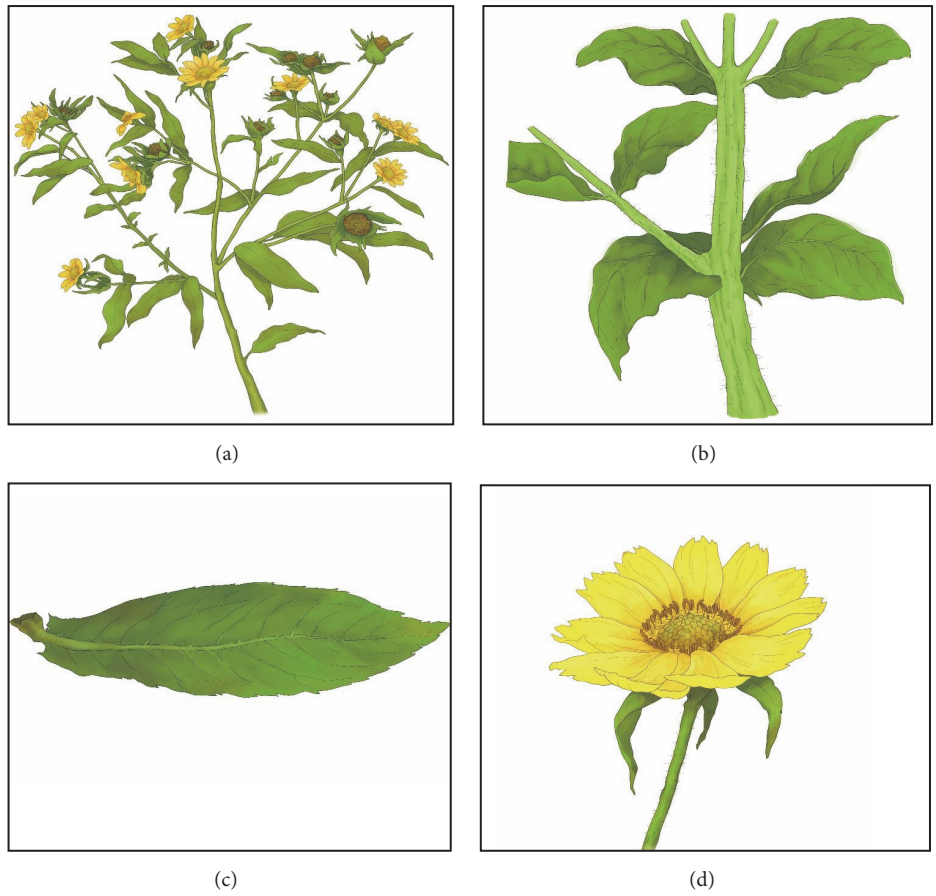


Figure 1: The morphological illustration of the main features of *A. africana*. (a) *A. africana* plant with numerous branches. (b) *A. africana* stem with numerous bristles. (c) Simple leaf of *A. africana,* oppositely arranged on the plant. (d) Inflorescence of *A. africana* consisting of outer ray and inner disc florets.

earlier study by Nguelefack et al. [74] also showed that the methanolic extract of fresh leaves of *A. africana* at the dose of 1 g/kg reduced gastric lesion in the pylorus ligated rats by 52%, a further proof of the potential of *A. africana* in wound healing. In a study by Attama et al., 2011 [75] where they examined the methanol leaf extract of *A. africana* formulated as gels for its potency on experimentally induced wound in rats, 100% wound closure was observed by the 17th day of treatment in both gel formulations of the plant methanolic extract and the standard gel, an indication of effectiveness of *A. africana* in wound healing. Similarly, a study by Osunwoke et al. [76] on the wound healing effects of the leaves extract of *A. africana* on Wistar rats showed that the rate of contraction of the excised wounds in the experimental group on days 6 and 9 was significant ($P<0.05$) with a mean wound closure of 12.6±1.17 cm compared to those in the control group which was 15.0±1.86 cm. Furthermore, they observed that the concentration of neutrophils and macrophages was intense in the experimental group relative to than the control group in the excised tissue samples. The total wound closure and increased inflammatory response suggests that the aqueous extract of the leaves of *A. africana* promotes wound healing activity through increased inflammatory response and neovascularization. In another *in vivo* experimental evaluation by Okoli et al. [12] using Wistar rats, they observed that the methanolic and hexane extracts and methanolic fractions of *A. africana* significantly ($P<0.05$) reduced bleeding (clotting time) in the rats and caused varying degrees of inhibition of the growth of microbial organisms known to cause wound infections such as *Pseudomonas fluorescens* and *Staphylococcus aureus*. The study showed that the extracts reduced epithelialization period of wounds that were experimentally excised in the rats, hence validating the fact that *A. africana* possesses constituents capable of accelerating wound healing. At different concentrations, *A. africana* also showed varied stimulating effects on haematological parameters including white and red blood cells due to the enormous micronutrients found in the plant [77]. Indeed, increased haematological changes especially in the red blood cells count are known to result in increased level of oxygen supply to the wounds resulting in faster wound healing [78]. Additionally, the wound healing ability of *A. africana* has also heavily been attributed to its anti-inflammatory activity resulting in inhibition of prostaglandins synthesis, decreased vascular permeability, inhibition of neutrophil migration into inflamed tissues, and stimulation of lymphocyte accumulation, thus enhancing tissue repair and healing [12]. Indeed, anti-inflammatory activity is essential for wound healing,

Figure 2: Modified map on distribution of *A. africana* [65].

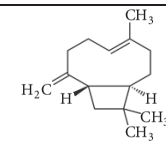
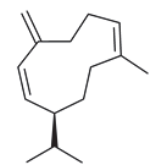
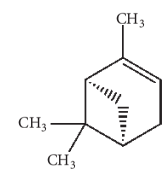
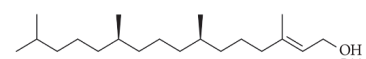
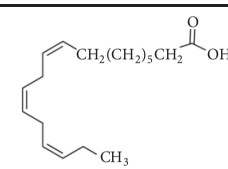
since a long duration of the inflammatory phase causes delay in the wound healing process [79]. Additionally, the strong antimicrobial activities of *A. africana* play a vital role in the ability of this plant to heal wound sepsis [80–84]. In fact, a study by Anibijuwon et al. [85] showed that *A. africana* has strong antimicrobial activities. These findings further showed that the anti-inflammatory and antimicrobial agent play vital roles in wound healing process.

6. The Potential of the Phytochemicals from *Aspilia africana* in Wound Healing

As discussed above, *in vivo* studies have provided strong pharmacological evidence for wound healing potential of the extracts obtained from *A. africana*. The plant is endowed with myriad of classes of bioactive secondary metabolites including alkaloids, saponins, tannins, flavonoids, and phenols (Figure 3) [12, 18, 20, 86, 87] and terpenoids [19, 20]. *A. africana* also contains a number of other compounds (Table 1) such as sesquiterpenes including β-caryophyllene and germacrene D, and linolenic acid [20]. The presence of these phytochemicals suggests that *A. africana* might be of medicinal importance and supports the basis for its use in ethnomedicine as a wound healing plant.

The high content of alkaloids in *A. africana* may be one of the major contributing factors to the wound healing activity of this plant [64, 68]. A number of alkaloids have been known to have great wound healing activities [18]. In an *in vivo* study, topical application of an alkaloid enriched-ointment exhibited higher dermal healing activity of the wounds on rats [45]. Similarly, alkaloids have been observed to promote early phases of wound healing in a dose-dependent manner

Table 1: Constituent compounds in *A. africana* extract and associated activities that enhance wound healing.

S/No	Class of compound	Phytochemical compounds	Compound structure	Activities that enhances wound healing	Reference
a	Monoterpenes	carene		(i) Anti-inflammatory (ii) Antimicrobial	[19–26]
b	Phytocannabinoids	Caryophyllene		(i) Antimicrobial (ii) Anti-inflammatory	[19, 27–35]
c	Sesquiterpenes	Germacrene D		(i) Anti-inflammatory (ii) Anti-microbial and (iii) Anti-oxidant	[36–44]
d	Terpene	α-pinene		(i) Anti-microbial (ii) Anti-inflammatory (iii) Increases basic fibroblast growth factor (BFGF) (iv) Increases platelet derived growth factor	[45–53]
e	Acyclic diterpene alcohol	Phytol		(i) Induces oxidative stress on microbial organisms (ii) Reduces interleukin-1β and tumor necrosis factor-α levels	[4, 20, 54–60]
f	Fatty *acid*	Linolenic acid		(i) Anti-microbial (ii) Down regulate inflammatory inducible nitric oxide synthase (iNOS).	[20, 61–63]

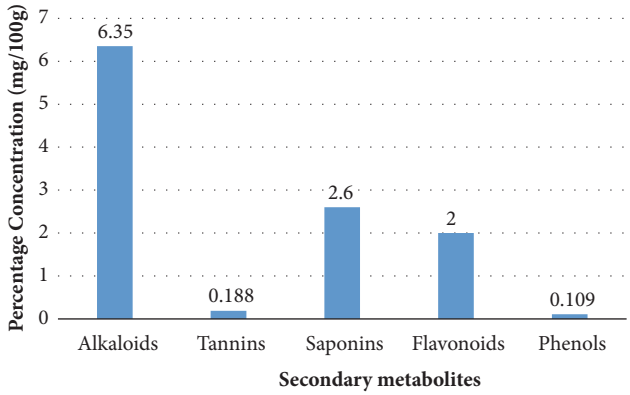


Figure 3: The percentage composition of phytochemical analysis of some of the nonvolatile secondary metabolites in the leaf extract of *A. africana* [88].

with the ability to stimulate chemotaxis for fibroblasts *in vitro* [89]. Alkaloids have also been observed to enhance significant wound healing activity ($P<0.05$) as evidenced by the increased rate of wound contraction and reduction in the period of epithelialization [90]. Sahib et al. [21] reported that the wound healing potential of *Ruta graveolens* L. plant may be due to the presence of alkaloids. These findings therefore suggest that the wound healing potential of *A. africana* may be due to the presence of large quantity of alkaloids.

Flavonoids are antioxidants with free radical scavenging ability and are therefore able to prevent oxidative damage in cells and have great anti-inflammatory activities [22], a basis for wound healing. Furthermore, flavonoids are also known to promote the wound healing process mainly due to their astringent and antimicrobial properties which are responsible for wound contraction and increased rate of epithelialization [23, 24]. Consequently, the wound healing ability of flavonoids has been observed to be even greater than that of silver sulfadiazine [25]. Flavonoids have also been observed to increase collagen synthesis, promote the cross-linking of collagen, shorten the inflammation period, and provide resistance against infections, important factors in enhancing the wound healing process [26]. These findings in part may be the reason behind the use of *A. africana* in the treatment of wounds, ulcers, and burns in traditional medicine.

Saponins' antioxidant and haemolytic properties make them one of the most important secondary metabolites in the treatment and management of a number of diseases, including wound healing [28, 29]. Indeed, saponins' ability to treat wounds and stop bleeding is due to the fact these phytochemicals precipitate and aggregate red blood cells [18]. Saponins are also known to enhance wound healing by causing wound contraction and bringing about high collagen deposition [29, 30]. In fact, saponins are also known to promote angiogenesis during skin wound repair [31]. Therefore, the high quantity of saponins in *A. africana* could explain why the plant has got such a potent ability to treat wounds in traditional medicine.

The presence of phenols in the plant leaf extract of *A. africana* is an indication that the extract may have antimicrobial properties [18] which greatly offers a basis for wound healing.

Tannins have been reported as having astringent activities which helps to quicken wound healing and treat inflammations [18]. Owing to its antibacterial activity and NIH3T3 cell proproliferative effect, tannins have been observed to promote wound contraction, improve healing rate, and promote healing of infectious wounds [32]. Specifically, tannins have been observed to reduce colonization of wounds by *S. aureus* resulting in a hasten wound healing [33]. Therefore, the presence of tannins may be one of the reasons why *A. africana* is renowned for wound healing in traditional medicine.

Terpenoids isolated from the leaves of *A. africana* include 3β-O-[α-rhamnopyranosyl-(1⟶6)-β-glucopyransyl-(1⟶3)-ursan-12-ene, 3β-Hydroxyolean-12-ene, and 3β-acetoxyolean-12-ene (Figure 4) [27]. Other terpenes present include α-pinene [34], carene, and phytol [19, 20] (Table 1).

Terpenoids are known to promote the wound healing process, mainly due to their astringent and antimicrobial properties, which seem to be responsible for wound contraction and an increased rate of epithelialization [35]. Carene (monoterpene) (Table 1) wound healing ability may be due to its antimicrobial activity in which it can inhibit the growth of *S. aureus* and *P. aeruginosa* in wounds [36–40]. Carene as an example of monoterpenes exhibited strong anti-inflammatory activity [41]. Therefore, the anti-inflammatory and antimicrobial activities of carene and other monoterpenes contained in *A. africana* somewhat validate the use of this plant in wound healing.

Alpha-pinene (Table 1) is an organic compound of the terpene class contained in *A. africana* [34, 42]. This vital compound was found to have potent anti-inflammatory activity [43]. The anti-inflammatory activity is due to its ability to suppress mitogen-activated protein kinases (MAPKs) and the nuclear factor-kappa B (NF-κB) pathway which makes it a vital compound in the treatment of inflammatory diseases [44]. Beside its anti-inflammatory activity, singly or in synergy with other compounds, α-pinene has been observed to have interesting antimicrobial properties [46–48]. An *in vivo* study on *Pistacia atlantica* resin extract with 46.57% α-pinene as the main content had a concentration-dependent effect on the healing of burn wounds after 14 days of treatment by increasing the concentration of basic fibroblast growth factor (BFGF), platelet derived growth factor, and improving angiogenesis [49]. Indeed, increased concentration of basic fibroblast growth factor is known to greatly enhance wound healing [49, 50]. Therefore, the antimicrobial, anti-inflammatory, and ability to increase BFGF level may explain why *A. africana* with α-pinene as one of the major compounds has been used in wound healing for generations.

Phytol (Table 1) is an acyclic diterpene alcohol with a percentage abundance of about 13% in the chloroform extract of *A. africana* [20]. This phytochemical has been shown to have wound healing activity. In an *in vivo* study, topical application of *Stachytarpheta jamaicensis* plant extract cream containing phytol on diabetic excision wound significantly improved ($P<0.05$) the percentage of wound contraction

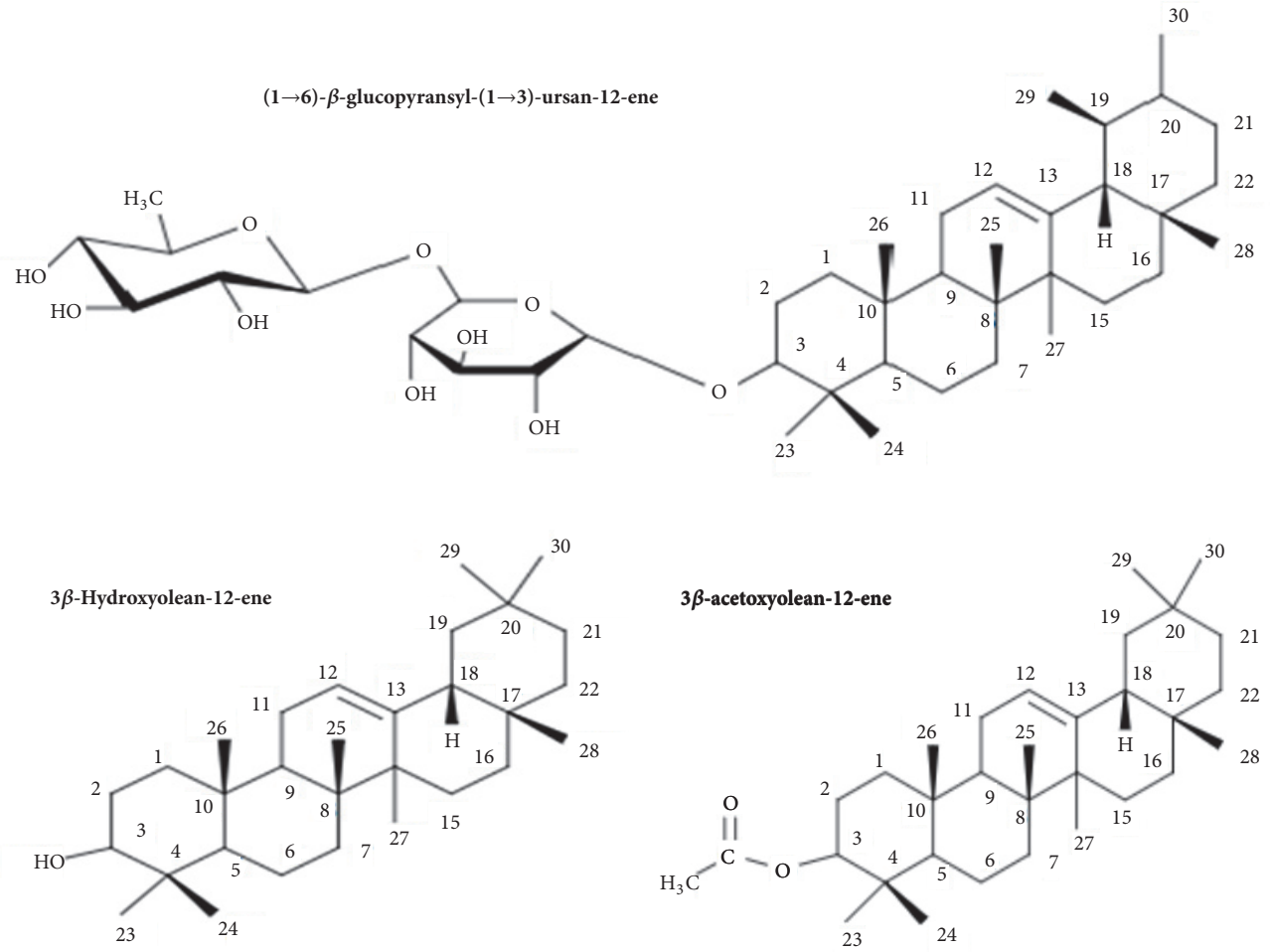


Figure 4: Isolated terpenoids from the leaves of *Aspilia africana* [27].

(88%) when compared to untreated diabetic rats in a period of 20 days [51]. It is important to note that wound healing can be greatly delayed due to infection by microorganisms [4]. *Pseudomonas aeruginosa* is one of the most common bacteria isolated from chronic wounds and can express virulence factors on the surface proteins affecting wound healing [52]. Phytol is known to exert antibacterial property on *P. aeruginosa* via inducing oxidative stress [53]. Indeed, this compound is known to have high antimicrobial activity, high stability, and low toxicity [54]. In addition to the antimicrobial potential, phytol is also known to be one of the compounds with highly potent anti-inflammatory property [55, 56]. An *in vivo* study showed that phytol attenuated the inflammatory response by inhibiting neutrophil migration that is partly caused by reduction in interleukin-1β and tumor necrosis factor-α levels and oxidative stress [57]. The presence of phytol in *A. africana* therefore may explain why this plant has great antimicrobial and anti-inflammatory activities and hence its potent wound healing ability.

Caryophyllene (Table 1) is a natural bicyclic sesquiterpene that is a constituent of many essential oils belonging to a class of phytocannabinoids, one of the many compounds found in the extract of *A. africana* [19]. This compound has been shown to have potent antimicrobial property [58, 59]. Indeed, β-caryophyllene has demonstrated selective antibacterial activity against *S. aureus* (minimum inhibitory concentration (MIC) 3±1.0 μM) and more pronounced antifungal activity [60]. Similarly, β-caryophyllene presented rapid bacterial killing for *S. aureus* (MIC <1.0 mg/Ml) in 4 h [61]; *S. aureus* is one of the main microbial organisms that enhances wound sepsis [62]. β-caryophyllene has also been shown to exhibit great anti-inflammatory activity [63, 91, 92]. In a study by Dahham et al. [93], it was observed that β-caryophyllene elicited significant (P<0.01) reduction in paw volumes and low intensity of fluorescent signal in experimental animals when compared with negative control. Furthermore, the result indicated that the compound has a low toxicity, with high ability of skin penetration, greatly enhancing anti-inflammatory and analgesic activities making it useful for prevention and management of inflammation-related diseases, including wounds. Therefore, the antimicrobial and anti-inflammatory activities exhibited by β-caryophyllene contained in the extracts of *A. africana* could explain why this plant is so effective in wound healing.

Germacrene D (Table 1) is a volatile organic hydrocarbon compound belonging to the class sesquiterpenes contained

in *A. africana* plant [27, 94, 95]. The compound possesses potent antimicrobial, anti-inflammatory, and antioxidant potentials activities [96–99]. Indeed germacrene D showed broad spectrum antibacterial activity against important human pathogenic Gram-positive and Gram-negative bacteria including *S. aureus* [100–102]. Therefore, the antimicrobial and anti-inflammatory activities exhibited by germacrene D contained in the extracts of *A. africana* could explain why this plant is so effective in wound treatment and management. However, more studies on isolated germacrene D needs to be conducted to validate further its wound healing potential.

Linolenic acid (Table 1) has been reported to have very strong antimicrobial activity against a number of microbes including those known to infect wounds and delay its healing such as *S. aureus* [103]. In addition, it is also an important anti-inflammatory agent [104]. Linolenic acid has been observed to down regulate inflammatory inducible nitric oxide synthase (iNOS), cyclooxygenase-2, and tumor necrosis factor-alpha gene expressions through the blocking of nuclear factor-kappaB and mitogen-activated protein kinases activation in lipopolysaccharide-stimulated murine macrophages cell line (RAW 264.7 cells), which may be the mechanistic basis for the anti-inflammatory effect of linolenic acid [105]. The presence of linolenic acid in *A. africana* therefore may explain why this plant has great antimicrobial and anti-inflammatory activities and hence its potent wound healing ability.

Through synergistic interactions of the different phytochemicals in *A. africana,* the plant has exhibited very strong antimicrobial, anti-inflammatory, and antioxidant activities which are vital components of the wound healing processes.

7. Conclusion

Throughout the world, wounds impose significant health burdens on millions of people. Consequently, all possible measures have to be taken to tackle it. Natural products have been used over the years for treatment and management of wounds. *A. africana* is one of the plants with immense attributes to enhance wound healing. The synergistic effects of the major phytochemicals in *A. africana* including alkaloids, saponins, tannins, flavonoids, β-caryophyllene, germacrene D, α-pinene, carene, phytol, and linolenic acid confer potent anti-inflammatory, antimicrobial, and antioxidant activities on the plant. This probably explains why this plant has such a potent wound healing ability. However, due to the reported adverse effects on the reproductive organs of the experimental animal models when administered orally, we recommend that future clinical studies focus on its topical application for wounds. Furthermore, although several studies have been carried out regarding chemical screening in *A. africana*, our review did not find any study on major nonvolatile chemical isolation and structure determination except for a limited study on terpenoids. Therefore, further studies on *A. africana* need to be done in this regard. Future studies also need to focus on the wound healing potential of the individual isolated compounds in *A. africana*. Furthermore, more preclinical and subsequently clinical studies need to be done to validate and understand the mechanism(s) of action of these phytochemicals in *A. africana* either in isolation or in combination for possible future wound healing drug development.

Disclosure

Richard Komakech is first author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Richard Komakech carried out the data search and was the major contributor in writing the manuscript. Motlalepula Gilbert Matsabisa and Youngmin Kang technically designed and helped in writing the manuscript. All the authors read and approved the final manuscript.

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Review Article

African Herbal Remedies with Antioxidant Activity: A Potential Resource Base for Wound Treatment

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The use of traditional herbal remedies as alternative medicine plays an important role in Africa since it forms part of primary health care for treatment of various medical conditions, including wounds. Although physiological levels of free radicals are essential to the healing process, they are known to partly contribute to wound chronicity when in excess. Consequently, antioxidant therapy has been shown to facilitate healing of such wounds. Also, a growing body of evidence suggests that, at least, part of the therapeutic value of herbals may be explained by their antioxidant activity. This paper reviews African herbal remedies with antioxidant activity with the aim of indicating potential resources for wound treatment. Firstly, herbals with identified antioxidant compounds and, secondly, herbals with proven antioxidant activity, but where the compound(s) responsible for the activity has not yet been identified, are listed. In the latter case it has been attempted to ascribe the activity to a compound known to be present in the plant family and/or species, where related activity has previously been documented for another genus of the species. Also, the tests employed to assess antioxidant activity and the potential caveats thereof during assessment are briefly commented on.

1. Introduction

Human cells are continuously exposed to exogenous oxidants as well as to those produced endogenously during normal physiological processes. Antioxidants form part of protective mechanisms that exist in human cells to scavenge and neutralize these oxidants. Oxidants such as the reactive oxygen species (ROS) and reactive nitrogen species (RNS) are involved in several diseases [1, 2]. Antioxidant defenses are defective in these diseases and therefore it is possible to limit oxidative damage and ameliorate disease progression with antioxidant supplementation [3].

With reference to wounds, antioxidants play pivotal roles that consequently restore normalcy to injured skin. Basal levels of ROS and other free radicals are essential in almost all phases of the wound healing process (Figure 1) [4]. During haemostasis, ROS regulates the constriction of blood vessels to limit loss of blood. Furthermore, ROS facilitates the migration of neutrophils and monocytes from surrounding blood vessels towards the injury site. The presence of ROS and other free radicals in the wound vicinity during the inflammatory phase of the healing process is also required for infection control and general maintenance of sterility. Finally, ROS promotes the proliferation of keratinocytes, endothelial cells, and fibroblasts, thereby enhancing angiogenesis and collagen deposition. However, uncontrolled release of ROS could cause oxidative stress, resulting in cellular and tissue damage, thereby causing delayed healing [1].

To keep ROS within physiological levels, antioxidants serve as electron donors, thereby preventing them from capturing electrons from other molecules which ultimately leads to their destruction [4]. Both nonenzymatic antioxidants such as glutathione, ascorbic acid, and α-tocopherol, as well as enzymatic antioxidants like catalase and peroxiredoxin, have shown potential to normalize high ROS levels and thus stimulate healing [4]. By normalizing ROS, antioxidants can enhance their physiological roles and thereby accelerate the wound healing process. Naturally occurring antioxidants are

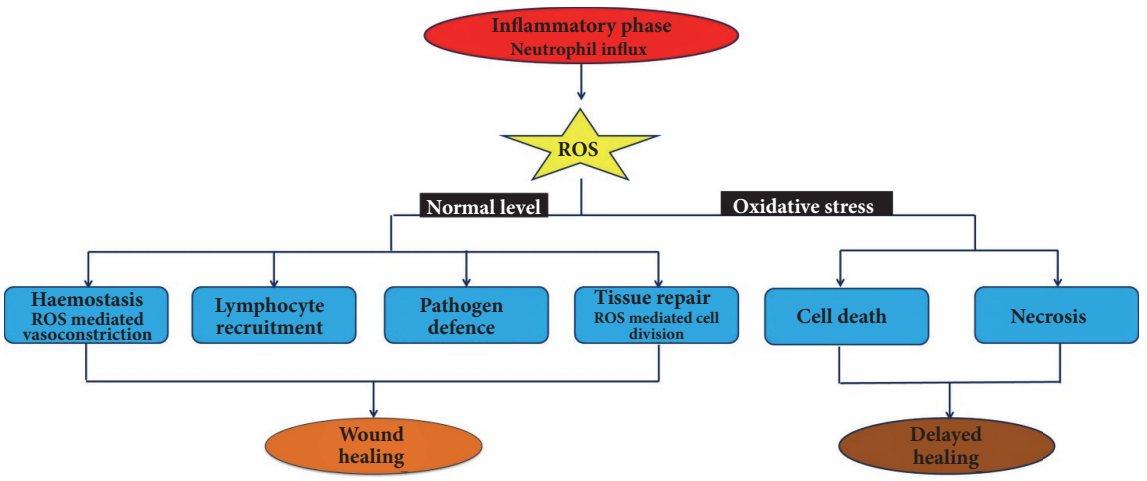

Figure 1: Role of reactive oxygen species (ROS) in the wound healing process.

generally favoured over their synthetic counterparts, as the latter are suspected to cause or promote negative health effects [5]. This has resulted in the restricted use of synthetic antioxidants in several countries [6].

This review provides a comprehensive list of African medicinal plants and isolated compounds with antioxidant activities, with the aim of highlighting the continent's rich herbal resource base for possible management of wounds and allied conditions. Previous reviews have listed a number of these African medicinal plants with antioxidant properties [7–9]. The present work has therefore aimed to expand the list to include medicinal plant species with antioxidant properties that are used in different African countries including those from Madagascar and Mauritius. For the sake of inclusivity, plants that have been shown to contain compounds that hold the potential of being novel antioxidants are also considered. In addition, those with anti-inflammatory properties were also included due to an earlier observation that the anti-inflammatory activities of the same extracts could be explained, at least in part, by their antioxidant properties [10]. Additional efforts were also made to include information, where available, on their vernacular names, their regional distribution, and medicinal use and plant parts used for these preparations or for the isolation of the antioxidant ingredient(s). Table 1 lists medicinal plants that have been investigated and have confirmed antioxidant and/or anti-inflammatory activity and that contain compounds which are known to have such activities. Table 2 on the other hand lists medicinal plants that have confirmed antioxidant activity but the compounds responsible for their antioxidant property have not yet been identified.

Many edible and culinary herbs and condiments were also included in these two tables as they were used in certain instances as medicinal herbs to treat diseases. These included fruits and seeds of *Balanites aegyptiaca*, leaves of *Boscia senegalensis*, leaves of *Entada africana* and seeds of *Parkia biglobosa*, from Niger [11], also leaves, seeds, and stem-bark of *Mangifera indica* from Benin and Burkina Faso [12, 13], leaves of *Cynara scolymus* from Ethiopia [14, 15], leaves of *Aspalathus linearis* from South Africa [16–21], leaves of *Cinnamomum zeylanicum* from Madagascar and Ethiopia [22–24], essential oils from the bark and leaves of *Ravensara aromatica* from Madagascar [23, 25], buds of *Syzygium aromaticum* from Madagascar [23], seeds of *Trigonella foenumgraecum* from Ethiopia and Morocco [26–28], and oils in seeds of *Nigella sativa* from African countries of the Mediterranean region [29–31].

2. Tests Used to Assess Antioxidant Activities of African Medicinal Plant Extracts

A variety of test systems were employed to assess the antioxidant properties of the medicinal plant extracts and compounds listed in Tables 1 and 2. A comprehensive list of the methods used in antioxidant activity determination, as well as their merits and demerits, has already been published [343–346]. The methods used in the determination of antioxidant activity of natural products and isolated compounds result in varied outcomes when the same samples are tested in different laboratories and by other researchers [347]. Furthermore, results of different methods cannot be correlated, as contradictory results are usually obtained. Hence, although several assays are available, none of them is capable of accurately and completely determining the antioxidant activity of a test substance because of the complex nature of the redox-antioxidant system *in vivo* (Figure 2). Based on this complexity, antioxidants are broadly classified as (i) inhibitors of free radical formation, (ii) free radical scavengers, (iii) cellular and tissue damage repairers, and (iv) signalling messengers [347].

The inhibition of free radical formation could protect against oxidative damage by suppressing the formation of active ROS/RNS. This typically involves reduction or inhibition of substrates required for free radical formation such as metal ions like iron (Fe) and copper (Cu). The sequestration of these metal ions by antioxidant compounds like ellagic acid and glutathione is known to suppress formation of

Table 1: Medicinal plants with confirmed antioxidant activity, shown to contain compounds that are known to have such activity.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Aloaceae						
Aloe barbadensis Mill.	Burn plant, siber, sbar/essouktouri /mar, sbar	Leaf exudate	Algeria, Morocco, Tunisia	Antioxidant activity. Used as laxative, purgative, diuretic, asthma, baldness, cuts, bounds, skin rash.	Flavonoids, two dihydrocoumarin derivatives and two flavone glycosides	[32–34]
Aloe claviflora Burch.	Kraal aloe	Leaf exudate	South Africa	Radical scavenging activity and moderate activity in the lipid peroxidation assay	Chromone glycoside	[35, 36]
A. saponaria (Ait.) Haw.	Mpelu Mnemvu Soap aloe, African aloe	Leaf exudate	South Africa	Radical scavenging activity and moderate activity in the lipid peroxidation assay	Chromone glycoside	[35, 37]
A. thraskii Baker	Dune aloe, ikhala, umhlaba	Leaf exudate	South Africa	Radical scavenging activity and moderate activity in the lipid peroxidation assay	Chromone glycoside	[35, 36]
Amaranthaceae						
Amaranthus caudatus L.	Tassel flower	Seed; Young shoots	Ethiopia	Antioxidant properties	Tocopherols, phenolic acids	[38–40]
Anacardiaceae						
Anacardium occidentale L.	Not signalized	Stem-bark	Nigeria	Anti-inflammatory properties.	Agathisflavone, quercetin 3-*O*-rutinoside, quercetin 3-*O*-rhamnoside	[41, 42]
Lannea edulis Engl.	Wild Grape	Root-bark	Zimbabwe	Semipolar extracts high activity both as radical scavengers and lipoxygenase inhibitors. Lipophilic extracts inhibitor of 15-lipoxygenase. Used for painful menstruation, urogenital infection, sexually transmitted diseases.	Two alkylphenols (cardonol 7 and cordonol 13) and three dihydroalkylhexenones	[43–45]
Lannea velutina A. Rich	Bemmbeyi Raisinier velu, Lannéa velouté	Leaves, bark, root	Mali	Antioxidant properties	Proanthocyanidins	[46, 47]
Mangifera indica L.	Mango Mangoro	Leaves, seeds, stem-bark	Benin Burkina Faso	Anti-inflammatory, analgesic, and hypoglycemic effects. Used to treat urogenital infection, tonic, diarrhoea, tooth ache, gingivitis, liver disease, diabetes.	Polyphenolics, flavonoids	[12, 13, 46, 47]

Table 1: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Apiaceae						
Centella asiatica (L.) Urb.	Gotu kola	Leaves	South Africa	Antioxidant and anti-inflammatory activities. Used for wound healing. Protection against radiation-induced injury. Cardio protective effect. Oral treatment increased antioxidant enzymes.	Quercetin and tetrandrine	[48–55]
Apocynaceae						
Alstonia boonei De Wild.	Awun, Egbu	Stem-bark Root-bark	Nigeria Ghana	Anti-inflammatory activity. Used for its analgesic and anti-inflammatory properties.	Rutin, Quercetin robinobioside, Kaempferol-3-O-rutinoside, Kaempferol-3-O-robinobioside	[56–59]
Catharanthus roseus (L.) G. Don	Madagascar periwinkle kaka poul, karaktè dezosm blan, zèb sosyé	Whole plant	Madagascar	Antioxidant activity and ability to increase antioxidant enzymes. Used for conjunctivitis.	Phenols	[60]
Arecaceae						
Elaeis guineensis Jacq.	Ori	Nuts	*Ghana* Nigeria	Anti-inflammatory activity. Used to treat rheumatoid arthritis.	3,4 hydroxybenzaldehyde, p-hydroxybenzoic acid, vanillic acid, syringic acid, ferulic acid, carotenoids, α-tocopherol	[12, 61]
Asclepiadaceae						
Secamone afzelii Rhoem.	Ahaban Kroratima	Stem	Central Africa	Antioxidant and anti-inflammatory properties. Used for wound healing.	Flavonoids, caffeic acid derivatives and α-tocopherol.	[62–64]
Asphodelaceae						
Bulbine capitata Poelln.	Scented grass bulbine	Roots Aerial parts	South Africa	Anti-inflammatory and weak antioxidant and free radical scavenging and lipid peroxidation inhibition activities. Knipholone as a selective inhibitor of leukotriene metabolism. Used as a mild purgative and to cure gonorrhoeal infections.	Anthraquinone Knipholone	[65–73]
Bulbine frutescens Willd.	Snake flower, cat's tail, burn jelly plant	Leaf juice Roots	South Africa	Anti-inflammatory and weak antioxidant and free radical scavenging and lipid peroxidation inhibition activities. Knipholone is a selective inhibitor of leukotriene metabolism. Used to treat burns, rashes, blisters, insect bites, cracked lips, acne, cold sores, mouth ulcers and areas of cracked skin.	Phenylanthraquinones, Isofuranonaphthoquinones, Gaboroquinones A and B and 4′-O-demethylknipholone-4′-O-beta-D-glucopyranoside, and Knipholone (anthroquinone)	[65, 67, 70, 74, 75]

Table 1: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Kniphofia foliosa Hochst.	Red-not-peker		Kenya	Anti-inflammatory and weak antioxidant and free radical scavenging and lipid peroxidation inhibition activities. Knipholone as a selective inhibitor of leukotriene metabolism. Used for abdominal cramps, wound healing	Anthraquinone: Knipholone	[65, 76–78]
Asteraceae						
Artemisia abyssinica Sch.Bip.	Chikugn (Amharic) *Arrtta bera (Or)*	Whole plant	Ethiopia	Radical scavenging and antioxidant activities. Used for stomach pain and wound healing.	Essential oils and flavonoids	[79–82]
A. afra Jacq. ex Willd.	African wormwood Wild wormwood	Roots, stems and leaves	Ethiopia South Africa	Radical scavenging and antioxidant activities. Used for stomach pain, coughs, colds, fever, loss of appetite, colic, headache, earache, intestinal worms to malaria.	Essential oils and flavonoids	[79, 82–84]
A. arvensis L.	Mugwort Wormwood	Whole plant	Algeria	Radical scavenging and antioxidant activities.	Phenolic compounds and flavonoids.	[85]
A. campestris L.	Field sagewort Field wormwood	Whole plant	Algeria	Radical scavenging and antioxidant activities. Used to treat insomnia	Phenolic compounds and flavonoids.	[85–87]
Bidens pilosa L.	Black jack	Leaves Roots	South Africa	Antioxidant and anti-inflammatory, antibacterial, antihypertensive activities. Used to treat diabetes and backache.	Phenolic compounds: quercetin 3-O-rabinobioside, quercetin 3-O-rutinoside. Two novel methoxylated flavone glycosides: quercetin 3,3′-dimethyl ether 7-O-c¢-L-rhamnopyranosyl-(1 ~ 6)-fl-D-glucopyranoside and the known quercetin 3,3′-dimethyl ether 7-O-fl-D-glucopyranoside	[19, 88–91]

Table 1: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Cynara scolymus L.	Globe artichoke	Leaves	Ethiopia	Antioxidative and lipid-lowering properties and eNOS up-regulating ability. Used to treat chronic liver and gall bladder diseases, jaundice, hepatitis and atherosclerosis.	Polyphenolic flavonoid compounds	[14, 15, 92, 93]
Helichrysum dasyanthum Sweet	Afrikaans common name of kooigoed (bedding material)	Leaves	South Africa	Antioxidant, radical scavenging and anti-inflammatory activities. Used to treat wounds, infections, respiratory conditions.	Essential oils	[94–96]
H. petiolare Hilliard & B.L. Burtt.	Everlasting, Imphepho	Leaves	South Africa	Antioxidant, radical scavenging and anti-inflammatory activities. Used to treat wounds, infections, respiratory conditions, asthma, chest problems and high blood pressure	Essential oils	[94–96]
Tagetes minuta L.	Khaki bush stinking roger muster John Henry, wild marigold	Leaves	Madagascar	Antimicrobial and antioxidant activity. Used as anthelmintic, antispasmodic, purgative and for the treatment of gastritis, indigestion and internal worms.	Essential oils.	[23, 97]
Balanophoraceae						
Thonningia sanguinea Vahl.	Nkomango	Roots	Ghana	Antioxidative and radical scavenging activities and lipid peroxidation inhibitory activity. Used for bronchial asthma, rheumatoid arthritis, atherosclerosis and diabetes.	Ellagitannins: Thonningianin A and B	[98–103]
Balanitaceae						
Balanites aegyptiaca (L.) Delile	Hausa: aduwa Desert date	Bark and roots	East Africa	Antioxidant properties *in vitro* confirmed. The bark and roots are used as laxatives, and for colic. The bark is used for sore throats, and as a remedy for sterility, mental diseases, epilepsy, yellow fever, syphilis, and tooth aches.	Coumarins, flavonoids, saponins (Balanin 1 (3β,12β,14β,16β) cholest-5-ene-3,16-diyl bis (β-d -glucopyranoside)- 12-sulphate, a new sterol sulfonated and Balanin 2 (3β,*20S*,*22R*,*25R*)-26-hydroxy-22-acetoxyfurost-5-en-3-yl-rhamnopyranosyl-(1⟶2)-glucopyranoside, a novel furostanol saponin)	[11, 104–106]
Bignoniaceae						
Jacaranda mimosaefolia D.Don.	Sharpleaf Jacaranda	Leaves Stem-bark	Nigeria	Shown to have antimicrobial activity and used to treat infections	Phenylethanoid glucoside, jacaranone	[107–109]
Spathodea campanulata P.Beauv.	African tulip	Stem-bark	Nigeria, Ghana, Cameroon (Yaounde region)	Anti-inflammatory, antioxidant, hypoglycemic, anticomplement and anti-HIV activities. Used to treat itching, arthritis, and diabetes.	Flavonoids and caffeic acid derivatives	[63, 110]

Table 1: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Tecoma stans (L.) H.B. & K.	Yellow trumpet bush	Leaves Stem-bark	Nigeria	Anti-diabetic activity is shown.	4-O-E-caffeoyl-alpha-L-rhamnopyranosyl-(1′ ⟶ 3)-alpha/beta-D-glucopyranose, E/Z-acetoside, isoacetoside	[107, 111]
Capparaceae						
Cleome arabica L.	Cleome efeina	Leaves	Egypt	Antioxidant activity, inhibited lipoxygenase activity and calcium ionophore-stimulated LTB4 synthesis in human neutrophils. Used to treat wounds and prevent inflammation	Rutin and quercetin.	[112, 113]
Clusiaceae						
Garcinia kola Heckel	Bitter cola/aku ilu, agbu ilu. Nigeria Hausa: Góórò *pl.* gwârráá *or* gòòràrràkáí	Seeds	Nigeria	Inhibit lipid peroxidation and protective against H_2O_2-induced DNA strand breaks and oxidized bases. Used for laryngitis, coughs, liver disease, bronchitis and throat infections. Inhibits Aflatoxin B1 induced genotoxicity.	Biflavonoid: kolaviron	[114–120]
Harungana madagascariensis Poir.	Otori	Stem-bark	Eastern Nigeria	Significant antioxidant activity. Used to treat skin diseases.	Prenylated Anthronoids: harunmadagascarin A [8,9-dihydroxy-4,4-bis-(3,3-dimethylallyl)-6-methyl-2,3-(2,2-dimethylpyrano)anthrone], harunganol B	[121–123]
Hypericum carinatum Griseb.	Not signalized	Leaves	Egypt	Antioxidant and radical scavenging activities.	Benzophenones: cariphenone A (6-benzoyl-5,7-dihydroxy-2,2,8-trimethyl-2H-chromene) and cariphenone B (8-benzoyl-5,7-dihydroxy-2,2,6-trimethyl-2H-chromene).	[124, 125]
H. perforatum L.	Common St.-Johns' wort	Whole plant	Egypt	Anti-inflammatory and anti-oxidant activities. Free radical scavenging, metal-chelation, and reactive oxygen quenching activities. Protective against scopolamine-induced altered brain oxidative stress status and amnesia in rats. Ability to suppress the activities of 5-lipoxygenase (5-LO) and cyclooxygenase-2 (COX-2), key enzymes in the formation of proinflammatory eicosanoids from arachidonic acid (AA). Analgesic, antiseptic, antispasmodic, digestive, diuretic and sedative.	Flavonoids: Rutin, hyperoside, isoquercitrin, avicularin, quercitrin, and quercetin.	[124, 126–131]

Table 1: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Cochlospermaceae						
Cochlospermum tinctorium A.Rich.	N'tiribara	Roots	Sudan, Uganda West Africa	Antioxidant activity. Used for malaria, jaundice.	Polyphenols: gallotannins and ferulic acids	[35]
Combretaceae						
Combretum woodii Drum.	Large-leaved forest bushwillow	Leaf	South Africa	Antioxidant and antibacterial activities. Also tannins showed inhibitory effect on Fe^{2+}-induced lipid peroxidation and radical scavenger activity. Used for pneumonia, syphilis, abdominal pain and conjunctivitis.	Polyphenols: Combretastatin B5 (2′,3′4-trihydroxyl,3,5,4′-trimethoxybibenzyl). Tannins.	[132–137]
Combretum imberbe	Not signified		South Africa	Combretum species are widely used for treating abdominal disorders (e.g. abdominal pains, diarrhea) backache, bilharziasis, chest coughs, colds, conjunctivitis, dysmenorrhoea, earache, fattening babies, fever, headache	1α,3β-dihydroxy-12-oleanen-29-oic, 1-hydroxy-12-olean-30-oic acid, 3,30-dihydroxyl-12-oleanen-22-one, and 1,3,24-trihydroxyl-12-olean-29-oic acid, a new pentacyclic triterpenoid (1α,23-dihydroxy-12-oleanen-29-oic acid-3β-O-2,4-di-acetyl-l-rhamnopyranoside)	[138]
Guiera senegalensis J.F.Gmel.	N'kundjè	Leaf	Western Africa	Antioxidant and radical scavenging activities. Used to treat dysentery, diarrhoea, gastro-intestinal pains and disorders, rheumatism, diabetes and fever.	Flavonol aglycones, flavonol glycosides and flavonoids (catechin, myricitrin, rutin and quercetin) as well as tannins (galloylquinic acids (hydrolysable tannins).	[139–143]
Terminalia sericea Burch. ex DC.	Silver cluster-leaf	Bark	South Africa	Radical scavenging and antioxidant activities. Used to treat diabetes and pneumonia and to relieve colic	Pentacyclic triterpenoids Anolignan B	[21, 136, 144]
Commelinaceae						
Commelina diffusa Burm.f.	Wandering Jew Climbing day flower	Leaves	Ghanna	Anti-inflammatory and antioxidant properties. Used to treat fever and is diuretic	Flavonoids	[63, 145]
Palisota hirsuta K.Schum.,	Not signified	Aqueous leaf extracts	Nigeria	Anti-inflammatory effects against carrageenan induced hind paw oedema	Not identified	[146, 147]

Table 1: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Crassulaceae						
Bryophyllum pinnatum (Lam.) Oken Synonym: *Kalanchoe pinnata* (Lam.) Pers.	Ufu ivo	Leaves	Nigeria, South Africa	Anti-inflammatory properties. Used for earache.	Flavonoids, polyphenols, triterpenoids	[12, 148, 149]
Cupressaceae						
Juniperus procera Hochst ex. Endl.	African Juniper	Young twigs and buds	Ethiopia	Antioxidant and free radical scavenging activities. Used to relieve stomach pain.	Essental oils	[79, 150, 151]
Dioscoreaceae						
Dioscorea dumetorum (Kunth) pax	Yam	Tubers	Nigeria	Antioxidant activity to modify serum lipid and anti-inflammatory activity. Used to treat diabetes.	Dioscorea and Dioscoretine	[152–154]
Drosera madagascariensis (DC.) *D. ramentacea* Burchell	Sundew	Roots and flowers	Madagascar	Anti-inflammatory effects. Used to treat coughs and asthma	Flavonoids: hyperoside, quercetin and isoquercitrin	[155, 156]
Drosera rotundifolia L.	Round-leaf Sundew	Roots and flowers	Madagascar	Anti-inflammatory effects. Used to treat coughs and asthma	Flavonoids: hyperoside, quercetin and isoquercitrin	[155, 157]
Euphorbiaceae						
Alchornea laxiflora (Benth) Pax & K. Hoffm.	Wild banana	Leaf and root	Nigeria	Antioxidant and anti-microbial activity. Used to treat jaundice and liver disorders. Also used in food preservation.	Quercetin-7,4′-disulphate, quercetin, quercetin-3′,4′-disulphate, quercetin-3,4′-diacetate, rutin and quercetrin	[158–161]
Bridelia ferruginea Benth.	Ora	Leaves, stem and bark	West Africa Democratic republic of Congo, Nigeria	Anti-inflammatory. Used to treat diarrhea, dysentery, gastro-intestinal disorders, gynecological disorders (including sterility), and rheumatic pains.	A bioflavonoid: Gallocatechin-(4′ ⟶O ⟶7)-Epigallocatechin.	[12, 57, 162–166]
Mallotus oppositifolius (Geiseler) Muell. Arg.	Jororo Káfàr mútúwàà Senampendi Mvundza jembe	Leaves, roots	West Africa Nigeria	Antioxidant, anti-inflammatory and antimicrobial activities. Used for abortion.	Flavonoids: quercetin and quercitrin.	[167–172]

Table 1: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Fabaceae						
Aspalathus linearis (Brum. F.) R. Dahlgr.	Rooibos	Leaves	South Africa	Radical Scavenging Capacity Used to treat stomach cramps, insomnia, and to reduce stress.	Phenolic Fractions, Tannins and monomeric flavonoids aspalathin, nothofagin, quercetin, rutin, isoquercitrin, orientin, isoorientin, luteolin, vitexin, isovitexin, and chrysoeriol.	[16–21, 173, 174]
Burkea africana Hook	Wild Syringa	Bark	Mali and Sub-Saharan Africa	Antioxidant and radical scavenging activity. Used to treat coughs, colds, stomach obstruction, infusions against gonorrhoea and syphilis.	Proanthocyanidins; fisetinidol-(4alpha->8)-catechin 3-gallate and bis-fisetinidol-(4alpha->6, 4alpha- >8)-catechin 3-gallate, with smaller amounts of flavan-3-ols (catechin, epicatechin and fisetinidol)	[175, 176]
Crotalaria podocarpa DC.	Crotalaria	Roots	South Africa	Anti-inflammatory activity. Used for the treatment of sore-eyes and boils. Expectorant.	Flavonoids	[67, 177]
Cyclopia intermedia E. Mey. and *C. subternata* Vog.	Honeybush	Leaves and stem	South Africa	Antioxidant activity. Used as tonic for colds, catarrh and tuberculosis.	Pinitol, shikimic acid, p-coumaric acid, 4-glucosyltyrosol, epigallocatechin gallate, the isoflavone orobol, the flavanones hesperedin, narirutin and eriocitrin, a glycosylated flavan, the flavones luteolin, 5-deoxyluteolin and scolymoside, the xanthone mangiferin and the flavonol C-6-glucosylkaempferol. Phenolic content: tyrosol and a methoxy analogue, 2-[4-[O-alpha-apiofuranosyl-(1″ ⟶6′)-beta-d-glucopyranosyloxy] phenyl]ethanol, 4-[O-alpha-apiofuranosyl-(1″ ⟶2′)-beta-d-glucopyranosyloxy]benzaldehyde, five glycosylated flavonols, two isoflavones, four flavanones, two isoflavones, and two flavones	[19, 21, 178–181]

Table 1: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Eriosema robustum		Twigs	Burundi, Ethiopia, Kenya, Rwanda, Tanzania, Uganda, Democratic Republic of Congo and Cameroon	Used traditionally for the treatment of coughs in East Africa and skin diseases in Central Africa	2′,3′,5′,5,7-pentahydroxy-3,40-dimethoxyflavone, 2′,3,5′,5,7-pentahydroxy-4′-methoxyflavone	[182, 183]
Erythrina latissima E. Mey.	Broad-leaved coral tree	Stem Wood Root wood Seeds	South Africa Botswana	Antimicrobial activity and weak radical scavenging properties. Purgative.	Flavonoids and isoflavonoids. Isoflavones: erylatissin A and B. Flavanone: erylatissin C and flavonoids and Isoflavone glycosides: 4′-hydroxyisoflavone-7-O-beta-D-glucopyranoside (compound 1); 4′-hydroxyisoflavone-7-O-alpha-L-rhamnosyl (1⟶6)-beta-D-glucopyranoside (compound 2); and a new compound 4′, 8-dimethoxy isoflavone-7-O-alpha-L-rhamnosyl (1⟶6) glucopyranoside (8-O-methylretusin-7-O-alpha-L-rhamnosyl (1-6)-beta-D-glucopyranoside) (compound 3) Isoflavonoids: 5,7-dihydroxy-2′,4′,5′-trimethoxyisoflavanone.	[67, 184–186]
E. lysistemon Hutch.	Common coral tree; lucky bean tree	Bark	South Africa	Mild antioxidant activity. Used to treat sores, wounds, abscesses and arthritis.	Three prenylated flavonoid derivatives; 5,7,4′-trihydroxy-8-(3‴-methylbut-2‴-enyl)-6-(2″-hydroxy-3″-methylbut-3″ enyl) isoflavone (isoerysenegalensein E), 5,7,2′-trihydroxy-4′-methoxy-5′-(3″-methylbut-2″-enyl) isoflavanone (lysisteisoflavanone), 5, 4′-dihydroxy-6-(3‴-methylbut-2‴-enyl)-2″-hydroxyisopropyl dihydrofurano [4″,5″:8,7] isoflavone (isosenegalensin), together with the four known flavonoids abyssinone V-4′-methylether, alpinumisoflavone, wighteone and burttinone	[187–190]

Table 1: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Melilotus elegans Salzm. ex Ser. (syn. *M. abyssinica* Baker)	Egug, Gugi, Yemen berri Elegant sweet clover	Leaves	Ethiopia	Anti-inflammatory properties. Used for asthma, haemorrhoid, wounds, excavated sore, piles, ulcers mouth infection, lacerated wounds, haemorrhoids, bronchial asthma (personal communication)	Flavonoids: kaempferol	[191–194]
Millettia griffoniana Baill.	Not signalized	Root-bark and seeds	Cameroon	Anti-inflammatory activity. Used as an antimalarial.	Coumarin: 4-hydroxy-3-(3′,4′-methylenedioxyphenyl)-5,6,7-trimethoxycoumarin, durmillone, odorantin, 7-methoxyebenosin, calopogonium isoflavone B and 7,2′-dimethoxy-4′,5′-methylenedioxy isoflavone maximaisoflavone G (5) and 7-hydroxy-6-methoxy-3′,4′-methylenedioxyisoflavone and new prenylated isoflavonoids griffonianones A, B, C, D and E.Griffonianone D ((7E)-(6″,7″-dihydroxy-3″,7″-dimethyloct-2″-enyl)oxy-4′-methoxyisoflavone), an isoflavone.	[195–202]
Parkia biglobosa (Jacq.) Benth	African Locust Bean Nèrè Ojinyi	Bark Seeds	Mali Sudan Ivory Coast	Anti-inflammatory activity. Used as antiseptic and to treat coughs, chest pain, and wound healing	Tocopherol, ascorbic acid (Seeds)	[12, 33, 34, 36–39, 43–53, 55, 64, 66–72, 118, 119, 121, 138, 159, 182, 195, 203–235]
Peltophorum africanum Sond.	Weeping wttle	Root and bark	South Africa	Antioxidant and antibacterial activities Used to treat diarrhoea, dysentery, sore throat, wounds, back and joint pains, HIV-AIDS, venereal diseases and infertility.	Flavonol glycosides and flavonol glucoside gallates	[236–238]
Piliostigma thonningii (Schum.) Milne- Redh	Camel's foot tree, Monkey Bread Niama (Mali). Abefe Kalgo Okpoatu Omepa	Root, bark, pods, leaves	Nigeria, Ethiopia Botswana, Kenya, Namibia, Senegal, South Africa, Sudan, Tanzania, Uganda, Zambia	Anti-oxidant and anti-inflammatory properties. Used to treat wounds, chronic ulcers, cough, respiratory disorders and toothache, gum inflammation, arthritis, headache, backache, and antioxidant supplement.	Proanthocyanidins epicatechin, catechin trimers and oligomers, flavonoids, polyphenolics, C-methylflavonols (in the leaf extract)	[12, 58, 239–245]
Sutherlandia frutescens R.Br.	Cancerbush Phetola	Leaves	South Africa	Superoxide and hydrogen peroxide scavenging activities. Used as tonic to boost the immune system.	Canavanine, pinitol	[246–248]
Trigonella foenumgraecum L.	Fenugreek	Seeds	Ethiopia, Morocco	Protective effect against Oxidative stress during ischemia-reperfusion. It is hypolipidemic, and is also used to treat boils and to improve appetite.	Free phenolics and Vit C.	[26–28, 249, 250]

Table 1: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Humiriaceae						
Sacoglottis gabonensis Urb.	Cherry tree, ozouga	Stem-bark	West Africa	Antioxidant activity.	Bergenin	[251–254]
Hypoxidaceae						
Hypoxis hemerocallidea Fisch. & C.A. Mey.	African potato	Corms	South Africa	Antioxidant activity. Used to treat tuberculosis, cancer, bladder disorders, benign prostatic hyperplasia.	Rooperol	[188, 255–257]
Lamiaceae						
Ocimum basilicum L.	Mükandu Basil	Leaves	Burkina Faso Ethiopia	Intermediate antioxidant activity and high antibacterial activity. Used in Ethiopia to treat Conjunctivitis and in Kenya to treat colds and stomacheache.	Linalool basil oil Methyl chavicol, eugenol, (E)-methyl cinnamate, thymol, linalool	[23, 258]
Ocimum gratissimum L.	Tea bush, Scent leaf/Nchuanwu. Ujuju okpevu Basil	Leaves	Popular republic of Congo (ex Brazaville Congo) Eastern Nigeria	Antioxidant activity Popular republic of Congo it is used as a laxative, purgative, and to treat snakebite, diabetes, tooth ache, gingivitis.	Xanthomicrol, cirsimaritin, rutin, kaempferol 3-*O*-rutinoside and vicenin-2 were identified as the major flavonoids, whereas luteolin 5-*O*-glucoside, luteolin 7-*O*-glucoside, apigenin 7-*O*-glucoside, vitexin, isovitexin, quercetin 3-*O*-glucoside and isothymusin were detected as minor constituents.	[12, 58, 258–262]
Lauraceae						
Cinnamomum zeylanicum Breyne	Cinnamon leaf	Leaves	Madagascar Ethiopia	Very high antioxidant and high antimicrobial activities. Used to treat diarrhoea, rheumatism, colds and hypertension	Cinnamaldehyde, eugenol and eugenyl acetate to be the main constituents of cinnamon oil.	[22–24, 263]
Ocotea bullata (Burch.) Baill.	Black stinkwood Unukane (Zulu)	Bark	South Africa	Anti-inflammatory, cyclooxygenase inhibitory activity. Urinary disorders, headaches.	Monoterpenoids	[188, 264]
Ravensara aromatica Sonn.	Nutmeg havozo	Bark Leaf	Madagascar	Low antioxidant and antimicrobial activity. Useful for chronic respiratory conditions, and sometimes helpful in cases of asthma.	Essential oils, principally composed of the monoterpene hydrocarbons a-pinene, sabinene, myrcene, limonene, & the azulene: iso-ledene. In barks, estragole (methyl chavicol) but leaves contain b-myrcene, 1,8-cineole, linalool, and carotol.	[23, 25, 265]

Table 1: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Malvaceae						
Hibiscus sabdariffa L.	Red tea, sorelle Rosella	Flowers	Nigeria South Africa	Antimutagenic activity and free radical scavenging effects on active oxygen species Used against insomnia, colic.	Flavonol glucoside hibiscitrin Anthocyanins. Such as cyanidin 3-*O*-*β*-D-glucopyranoside, cyanidin 3-*O*-(2-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranoside, delphinidin 3-*O*-*β*-D-glucopyranoside and delphinidin 3-*O*-(2-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranoside.	[19, 21, 266–269]
Meliaceae						
Trichilia roka Chiov.	Soulafinzan	Root	Tropical Africa Mali	Significantly protective against CCl_4-induced liver damage and prevented perisinusoidal fibrosis. Used to treat malaria, abdominal pain and dermatitis.	Polyphenols	[270, 271]
Menispermaceae						
Sphenocentrum jollyanum Pierre	Akerejupon ajo	Fruit Root	West Africa	Anti-inflammatory activity. Used to treat inflammatory-based diseases	Furanoditerpenes: columbin, isocolumbin. Flavonoids-rich fraction.	[272–274]
Tinospora bakis		Whole plant	Sudan	Anti-inflammatory activity. To treat headache and rheumatism	A diterpenoid furanolactone, columbin	[275]
Moraceae						
Dorstenia barteri var. *subtriangularis* (Engler) M.E.E.Hijman & C.C.Berg	Contrayerva	Twigs/leaves	Cameroon	Antioxidant properties account for the anti-inflammatory action of these extracts Used to treat arthritis, rheumatism, gout, headache and other forms of body pains.	Prenylated flavonoids: Three diprenylated chalcones: bartericins A (-)-3-(3,3-dimethylallyl)-5′-(2-hydroxy-3-methylbut-3-enyl)-4,2′,4′-trihydroxychalcone, bartericins B (+)-3-(3,3-dimethylallyl)-4′,5′-[2‴-(1-hydroxy-1-methylethyl)-dihydrofurano]-4,2′-dihydroxychalcone and bartericins C 3,4-(6″,6″-dimethyldihydropyrano)-4′,5′-[2‴,-(1-hydroxy-1-methylethyl)-dihydrofurano]-2′-hydroxychalcone and also two novel diprenylated chalcones: 3,5′-di-(2-hydroxy-3-methylbut-3-enyl)-4,2′,4′-trihydroxychalcone, 3, 4-(2,2-dimethylpyrano)-3′-(2-hydroxy-3-methylbut-3-enyl)-2′,4′-dihydroxychalcone, 4,2′, 4′-trihydoxy-3′-prenylchalcone and 4,2′,4′-trihydoxy-3, 3′-diprenylchalcone; and 5,7,4′-trihydoxy-8-prenylflavone. Other known compounds such as stipulin, 4-hydroxylonchocarpin, kanzonol B, 3′-(2-Hydroxy-3-methylbut-3-enyl)-5′-(3,3-dimethylallyl)-4,2′,4′-trihydroxychalcone, and dorstenone.	[67, 276–281]

Table 1: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
D. ciliata Engl.	Contrayerva	Aerial parts	Cameroon Central Africa	Antiradical and antioxidant activities. Used as food additive.	phenolic compound (6-prenylapigenin) Flavones: (ciliatin A) 5,4′-Dihydroxy-5″-isopropenyldihydrfuranol[2″,3″:7,6]flavone (ciliatin B) 7,4′-Dihydroxy-3′-methoxy-6″,6″-dimethyldihydropyranol[2″,3″:5,6].	[282–284]
D. convexa De Wild.	Contrayerva	Twigs and leaves	Democratic Republic of the Congo	Antioxidant properties account for the anti-inflammatory action of these extracts. Used to treat arthritis, rheumatism, gout, headache and other forms of body pains.	Prenylated flavonoids	[67, 276, 280]
D. mannii Hook.f.	Contrayerva	Twigs/leaves Aerial parts	Central Africa	Antioxidant action against copper-induced LDL oxidation, this activity is like the non-prenylated flavonoid quercetin. Also, inhibition of platelet aggregation and influence of cyclooxygenase and lipoxygenase activity. Used to treat rheumatism, stomach disorders. Anti-trichomonal activity.	Grenylated and prenylated flavonoids and flavonones: Flavonones: 6,8-diprenyl-5,7,3′4′-tetrahydroxyflavanone, 4-hydroxylonchocarpin, 4-methoxylonchocarpin, 6-prenylchrysoeriol, 6,8-diprenyleriodictyol, gancaonin P and Prenylated flavonoids: 6,8-diprenyleriodictyol, dorsmanin C 7,8-(2,2-Dimethylchromeno)-6-geranyl-3,5,3′,4′-tetrahydroxyflavone and dorsmanin D 6,8-Diprenyl-3,5,7,4′-tetrahydroxy-3′-methoxyflavone, dorsmanins 1, J and 2″-epimers of dorsmanins F (6,7-(2,2-dimethylpyrano)-8-prenyl-5,3′,4′-trihydroxyflavanone, G (6,7-(2,2-dimethyldihydro-pyrano)-8-prenyl-5,3′,4′-trihydroxflavanone). Also, dorsmanins F and G. Four new prenylated flavanones, named dorsamine F (7,8-[2″-(1-hydroxy-1-methylethyl)-dihydrofurano]-6-prenyl-5,3′,4′-trihydroxyflavanone), dorsmaine G (6,7-[2″-(1-hydroxy-1-methylethyl)dihydrofurano]-8-prenyl-5,3′,4′-trihydroxyflavanone) and dorsamine H (6-prenyl-8-(2-hydroxy-3-methylbut-3-enyl)-5,7,3′,4′-tetrahydroxyflavanone).	[67, 187, 207, 285–287]

Table 1: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
D. poinsettifolia var. *angusta* Engl.	Dingetenga	Whole plant	Cameroon	Antiradical and antioxidant activities. Used to treat infected wounds.	Grenylated and prenylated flavonoids. The unusual 4-phenyl-substituted dihydrocoumarin and the rare geranyl-and prenyl-substituted Chalcone.	[207, 288, 289]
D. psilurus Welw.	Dingetenga	Roots	Cameroon Central Africa	Antiradical and antioxidant activities. Used against snakebite and to treat rheumatism, headache and stomach disorders.	Grenylated and prenylated flavonoids. Three phenolic compounds: 6,8-diprenyl-3′ [O],4′-(2,2-dimethylpyrano)-3,5,7-trihydroxyflavone, 3,6-diprenyl-8-(2-hydroxy-3-methylbut-3-enyl)-5,7,2′,4′-tetrahydroxyflavone and an unusualB/C ring modified flavonoid derivative for which the names dorsilurins C, D and E, respectively, are proposed. Two new flavones, dorsilurins A and B, and a new benzofuran derivative have been isolated from Dorstenia psilurus, together with three known phenylpropanoid derivatives, stearyl-p-coumarate [octadecanyl 3-(4-hydroxyphenyl)prop-2-enoate], stearyl ferulate [octadecanyl 3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate] and psoralen.	[206, 282, 290–292]
Myrtaceae						
Eugenia elliptica Sm. *Syzygium smithii* (Poir.) Nied.	Lilly Pilly	Leaves	Mauritius	Modulate the expression of the antioxidant enzyme genes.	Quercetin-3-O-galactoside (hyperoside), kaempferol-3-glucoside (astragalin), quercetin-3-O-glucoside (isoquercitrin), (+)-catech	[293, 294]
E. fasciculata Wall.	Not signalized	Leaves	Mauritius	Modulate the expression of the antioxidant enzyme genes.	Quercetin-3-O-galactoside (hyperoside), kaempferol-3-glucoside (astragalin), quercetin-3-O-glucoside (isoquercitrin), (+)-catech. procyanidin B2 dimer and (-)-epicatechin	[293]
E. orbiculata Lam.	Not signalized	Leaves	Mauritius	Modulate the expression of the antioxidant enzyme genes.	Quercetin-3-O-galactoside (hyperoside), kaempferol-3-glucoside (astragalin), quercetin-3-O-glucoside (isoquercitrin), (+)-catech. quercetin-3-O-rutinoside (rutin),	[293, 295]

Table 1: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
E. pollicina J.Gueho & A.J.Scott	Not signalized	Leaves	Mauritius	Modulate the expression of the antioxidant enzyme genes.	Quercetin-3-O-galactoside (hyperoside), kaempferol-3-glucoside (astragalin), quercetin-3-O-glucoside (isoquercitrin), (+)-catech. (-)-epicatechin gallate	[293, 296]
Monimiastrum acutisepalum J. Gueho & A.J. Scott.	Not signalized	Leaves	Mauritius	Modulate the expression of the antioxidant enzyme genes.	Quercetin-3-O-galactoside (hyperoside), kaempferol-3-glucoside (astragalin), and quercetin-3-O-glucoside (isoquercitrin). (+)-catechin	[293–295]
M. globosum J.Gueho & A.J.Scott	Not signalized	Leaves	Mauritius	Modulate the expression of the antioxidant enzyme genes.	Quercetin-3-O-galactoside (hyperoside), kaempferol-3-glucoside (astragalin), and quercetin-3-O-glucoside (isoquercitrin). (-)-epicatechin gallate	[293]
Syzygium aromaticum (L.) Merr. & L.M.Perry	Clove bud	Dried flowers Buds	Madagascar Sudan	Antioxidant and antimicrobial activities. Used to treat tooth ache and throat inflammation.	Eugenol Methyleugenol	[23, 297, 298]
S. coriaceum J.Bosser & J.Guého	Bois de pomme		Mauritius	Abilities to modulate the expression of the antioxidant enzyme genes.	Phenols and flavonoids: Quercetin-3-O-rutinoside, kaempferol-3-glucoside (astragalin) and quercetin-3-O-glucoside (isoquercitrin), (+)-catechin, procyanidin B1 dimer, (-)-epicatechin gallate	[293]
S. glomeratum DC.	Bois de pomme	Leaves	Mauritius	Abilities to modulate the expression of the antioxidant enzyme genes. Used to treat boils, abscesses, fever and wounds and as expectorant.	Phenols and flavonoids: kaempferol-3-glucoside (astragalin) and quercetin-3-O-glucoside (isoquercitrin), procyanidin B1 dimer, (-)-epicatechin gallate, chlorogenic acid, (-)-epicatechin	[293]
S. guehoii	Not signalized		Mauritius	Abilities to modulate the expression of the antioxidant enzyme genes.	Phenols and flavonoids: quercetin-3-O-rutinoside (rutin), kaempferol-3-glucoside (astragalin) and quercetin-3-O-glucoside (isoquercitrin), (+)-catechin, chlorogenic acid, procyanidin B2 dimer	[293]
S. mauritianum J.Gueho & A.J.Scott	Not signalized	Leaves	Mauritius	Abilities to modulate the expression of the antioxidant enzyme genes.	Phenols and flavonoids: quercetin-3-O-rutinoside (rutin), kaempferol-3-glucoside (astragalin) and quercetin-3-O-glucoside (isoquercitrin), (+)-catechin, chlorogenic acid	[293]
S. petrinense J.Bosser & J.Guého	Not signalized		Mauritius	Abilities to modulate the expression of the antioxidant enzyme genes.	Phenols and flavonoids: quercetin-3-O-rutinoside (rutin), kaempferol-3-glucoside (astragalin) and quercetin-3-O-glucoside (isoquercitrin), procyanidin B1 dimer, chlorogenic acid	[293]

Table 1: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
S. venosum (Lam.) J.Gueho & A.J.Scott	Not signalized		Mauritius	Abilities to modulate the expression of the antioxidant enzyme genes.	Phenols and flavonoids: quercetin-3-O-rutinoside (rutin), kaempferol-3-glucoside (astragalin) and quercetin-3-O-glucoside (isoquercitrin), (+)-catechin, procyanidin B2 dimer	[293, 295, 299]
Oleaceae						
Olea europaea subsp *africana* (Mill.)P.S. Green	African wild olive	Leaves	South Africa	Potent antioxidant activity. Used as eye lotions and tonics, lower blood pressure, improve kidney function and deal with sore throats. The early Cape settlers used the fruits to treat diarrhoea	Oleuafricein (mixture of oleanolic acid and ursolic acids), Triterpenoids and oleoropein.	[84, 300, 301]
Pedaliaceae						
Harpagophytum procumbens DC. ex Meissner	Devil's claw	Root	South Africa Native to the Kalahari Desert of southern Africa, Namibia and Botswana.	Anti-inflammatory and ability to inhibit the expression of cyclooxygenase-2 and inducible nitric oxide by suppression of NF-kappaB activation. Used for pain, muscular tension, osteoarthritis, degenerative rheumatism or painful arthrosis and tendonitis as well as tonic for loss of appetite and dyspeptic complaints.	Roots contain iridoid glycosides mainly harpagoside. Other constituents are flavones and flavonols kaempferol, and luteolin.	[302–312]
Piperaceae						
Piper guineense Schum. & Thonn.	West African black pepper Bush pepper Ikom, Amana kakwale iyeyeh ashoesie taquale Meshoro	Fruit, seed and leaf	Ghana, West Africa Nigeria Cameroon	Antioxidant activity.	Volatile oil components-monoterpenes, sesquiterpenes, terpenoids, lignans and sterols.	[313–316]
Podocarpaceae						
Podocarpus species *Podocarpus elongates Podocarpus falcatus, Podocarpus henkelii and Podocarpus latifolius*		Leaves and young stems	Eastern and Southern Africa	These species are used to treat fevers, asthma, coughs, cholera, chest complaints, arthritis, rheumatism, painful joints and venereal diseases	Diterpenoids, bioflavonoids and Totarol	[317]

Table 1: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Ranunculaceae						
Nigella sativa L.	Black cumin	Seed	African countries in the Mediterranean region	Antioxidant potentials through scavenging ability of different free radicals including the superoxide anion radical, inhibition of lipid peroxidation, and protection of liver against carbon tetrachloride (CCl4)-induced liver fibrosis in rabbits Used to treat diarrhoea, asthma, and as gastroprotective agent.	Oil: Thymoquinone	[29–31, 318, 319]
Rosaceae						
Crataegus monogyna Jacq.	Hawthorn, May Blossom, May Day Flower, White Thorn.	Fresh vegetative and reproductive organs	Mauritius, Northern Africa	Antioxidant activities. Used for its neuro- and cardiosedative actions.	Polyphenols: (proanthocyanidin, flavonoid, anthocyanin, (-)-epicatechin, procyanidin B2, chlorogenic acid). Flavonoids:quercetin and quercetrin, glycosides, proanthocyanidins, anthocynaidins, saponins, tannins, and cratetegin Also, Vitamin C.	[320–323]
Leucosidea sericea		Leaf, bark and roots	Southern Africa	Antimicrobial and anti-inflammatory properties	Phenolics, alkaloids and saponins	[210]
Pygeum africanum Hook. f.	African plum tree Red Stinkwood	Bark	South Africa	Anti-inflammatory. Used to treat against benign prostatic hyperplasia, prostatitis	14% triterpenes (urolic acids, oleanolic acid, crataegolic acid), 0.5% n-docosanol Phytosterol (β-sitosterol, β-sitosterone, Campesterol	[188, 324–327]
Rubiaceae						
Crossopteryx febrifuga Benth.	Roger Blench "rima jogoo-hi/je"	Seeds Leaf and roots	Mali Nigeria	Radical scavenging and lipoxygenase inhibition activities. Used to treat fever and various respiratory diseases	Flavonoids	[328–330]
Rutaceae						
Agathosma betulina (Berg.) Pillans.	Round-leaf buchu	Leaves, stems	South Africa	Hydroxyl radical ion scavenging ability. Used for stomach problems, kidney and urinary track diseases.	Essential oils and flavonoids	[188, 331, 332]
A. crenulata (L.) Pillans	Oval-leaf buchu	Leaves, stems	South Africa	Anti-inflammatory activity. Used to treat benign prostatic hyperplasia, prostatitis, diabetes, inflammation of the colon, gums, and mucous membranes. Leaves chewed to relieve stomach complaints.	Essential oils and flavonoids	[84, 188, 331, 332]

Table 1: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Fagara zanthoxyloides Lam.	xeti, xe Wô	Roots, root-bark	Cameroon, Uganda	Antioxidant activity. Used to treat gingivitis, toothache, urinary and venereal diseases, rheumatism and lumbago, malaria and other infections.	Phenylethanoid derivative, lignans and fagaronine	[333–336]
Sapindaceae						
Dodonaea viscosa Jacq. Synonyms: *Dodonaea angustifolia* L. f.; *Ptelea viscosa* L.	Umusasa	Leaves	Rwanda	Anti-inflammatory activity by inhibiting the synthesis of prostaglandin (PG) E(2). Used to treat rheumatism, skin infections, diarrhea, stomachaches, pains of hepatic and splenic origin, uterine colic. It is also used as an antipruritic in skin rashes and for the treatment of some throat, dermatitis and hemorrhoids.	Quercetin, isorhamnetin	[337–341]
Xanthorrhoeaceae						
Aloe ferox Mill.	Bitter aloe or Cape aloe	Leaves	South Africa, Lesotho	*A. ferox* gel contains at least 130 medicinal agents with anti-inflammatory, analgesic, calming, antiseptic, antiviral, antiparasitic and anticancer effects	Chromones, anthraquinones, anthrone, anthrone-C-glycosides, and other phenolic compounds Barbaloin	[9]
Zingiberaceae						
Siphonochilus aethiopicus (Schweinf.) B.L. Burtt.	Wild ginger Natal ginger African Ginger	Rhizome	South Africa	Anti-inflammatory activity through cyclooxygenase inhibitory (prostaglandin-synthetase inhibition), activity. Used to treat Coughs, colds, asthma.	Sesquiterpenoid	[188, 264, 342]

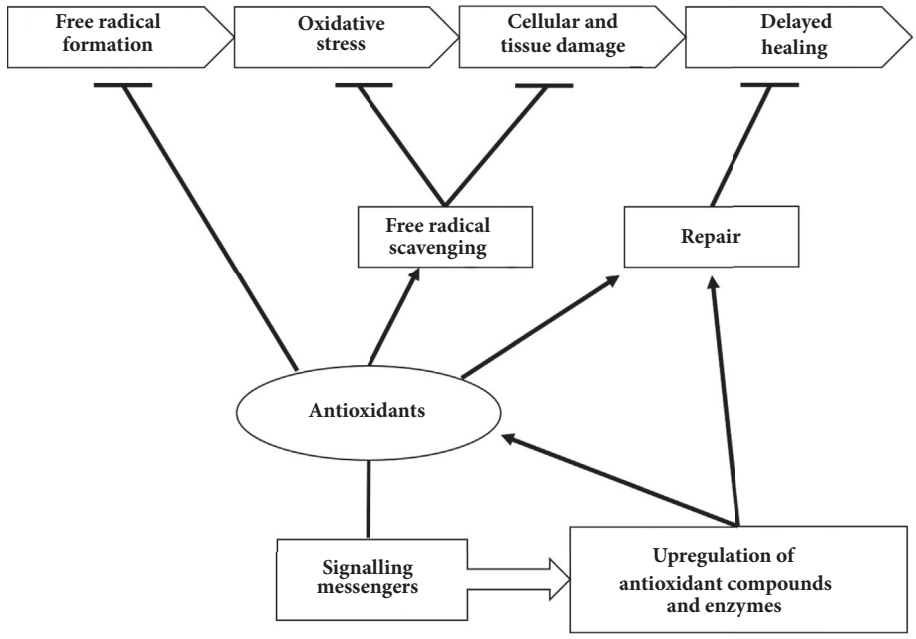

Figure 2: Mechanism of antioxidant action in wounds.

hydrogen peroxide (H_2O_2) and other free radicals [348, 349]. Furthermore, increasing evidence suggests a relationship between metal overload and several chronic diseases through the induction of oxidative stress [350]. Therefore, inhibition of free radical formation using metal ions as targets could be useful therapeutically. Antioxidant assays designed for this purpose include the cupric and ferric reducing antioxidant power (CUPRAC/FRAP). These methods measure the ability of antioxidants to reduce cupric (Cu^{2+}) and ferric (Fe^{3+}) ions, respectively.

Another mechanism by which antioxidants act is through the suppression of oxidative stress by directly scavenging active free radicals. Most commonly reported antioxidant assays such as 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 2,2′-diphenyl-p-picrylhydrazyl radical (DPPH), oxygen radical absorbance capacity (ORAC), Trolox equivalent antioxidant capacity (TEAC), total oxyradical scavenging capacity (TOSC), and total radical antioxidant parameter (TRAP) are focused on testing the ability to scavenge free radicals. Furthermore, there are diverse cellular antioxidant assays that assess the ability of antioxidant compounds and substances to protect cells against excessive free radical generation. Such assays involve the use of a fluorescent compound such as 2,7-dichlorofluoroscein to determine the ability of test samples to quench intracellularly generated free radicals and inhibit radical formation and lipid peroxidation [345].

There are also numerous reports of the ability of antioxidants to repair damaged tissues and improve healing. Topical application of kojic acid and deferiprone, two compounds with the ability to scavenge free radicals, enhanced healing of wounds in rats [351]. Also, the mitochondria-targeted antioxidant, 10-(6′-plastoquinonyl) decyltriphenylphosphonium, accelerated wound closure, stimulated epithelialization, granulation tissue formation, and vascularization, and lowered lipid peroxidation in mice [352]. Moreover, an antioxidant peptide (cathelicidin-OA1) promoted wound healing in a mouse model with full-thickness skin wounds, accelerated reepithelialization and granulation tissue formation by enhancing the recruitment of macrophages to the wound site, and induced cell proliferation and migration [353]. Some antioxidants have also been reported to contribute to healing by enhancing the activity of endogenous antioxidant compounds and enzymes. The induction of the nuclear factor E2-related factor 2-(Nrf2) mediated antioxidative pathway by a rhomboid family protein (RHBDF2) promoted healing of injured tissues, suggesting a relationship between antioxidant gene induction and healing [354]. Niconyl-peptide enhanced wound healing and protected against hydrogen peroxide-induced cell death by increasing the expression of Nrf2 expression in human keratinocytes [355].

The most common tests used to determine the antioxidant activity of samples included the assessment of the ability to scavenge free radicals such as DPPH, $ABTS^+$ [16, 19, 35, 62, 85, 94, 98, 99, 139, 158, 175, 184, 187, 266, 282, 302, 356–364], or the hydroxyl radicals [79, 188, 267, 365, 366], as well as the hydroperoxyl radicals by the Briggs-Rauscher reaction [104]. The ability of the extracts to chelate metal ions was also determined as further indication of their ability to contribute in the reduction of free radicals such as the hydroxyl radical [114]. In addition, assessment of the ability of these medicinal plant extracts to protect against lipid peroxidation was also included, which in turn was measured by the malondialdehyde-thiobarbituric acid (MDA) test [320, 367], the modified thiobarbituric acid reactive species (TBARS) assay [18, 22], or conjugated diene

formation [367]. Moreover, lipid peroxidation was assessed using the fluorescent probe, diphenyl-1-pyrenylphosphine (DPPP) [188], or using the inhibition of Cu(2+)-mediated oxidation of human low-density lipoprotein (LDL) [187, 367]. The ability of extracts to protect against damage to DNA using the Comet assay was also employed [114, 188].

The antioxidant capacity of the medicinal plant extracts was determined using either the TEAC or FRAP assays [11, 85, 302, 313, 321, 368]. The ability of extracts to modulate the gene expression of the antioxidant enzymes, such as Cu, Zn-superoxide dismutase (Cu, Zn-SOD), Mn-superoxide dismutase (Mn-SOD), catalase, and glutathione peroxidase (GPx), was also used as a measure of their antioxidant properties [293]. The photochemilumiescence (PLC) assay is a more recent antioxidant capacity assessment method and was employed for the evaluation of antioxidant capacity of baobab fruit pulp extracts [369].

Anti-inflammatory properties of these extracts were assessed by their ability to inhibit 5-lipoxygenases [94, 370, 371] or cyclooxygenase (COX-1 and COX-2) activities [65, 275, 317, 372, 373]. Using the former [374] and the latter [264, 331] methodologies, respectively, a great number of South African medicinal plant extracts were screened for their anti-inflammatory properties. The effect of medicinal extracts on the biosynthesis of different prostaglandins was assessed as a measure of their anti-inflammatory effect [239, 337, 375]. Extracts of *Podocarpus* species were shown to inhibit the activities of the COX enzymes [317]. Once again, using this test, the anti-inflammatory properties of the aqueous and ethanolic extracts of 39 plants used in traditional Zulu medicine were screened [376]. The Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) assay which utilizes the CAM's capillary system in bred hen eggs was also used to assess the anti-inflammatory activity through antiangiogenic effects of the ethanol and aqueous extracts of *Drosera rotundifolia* and *D. madagascariensis* [155].

The antioxidant and anti-inflammatory abilities of the herbal extracts were further assessed by evaluating their ability to control the production of ROS produced by oxidative burst in neutrophils stimulated with L-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP) [21, 246]. The inhibition of neutrophils elastase was used as a measure of anti-inflammatory property and it was proposed that the presence of flavonoids such as hyperoside, quercetin, and isoquercitrin in *D. rotundifolia* [377] and five flavonoid compounds in two *Polypodium* species (*P. decumanum* and *P. triseriale*) [378] were thought to contribute to this anti-inflammatory activity. These and other *in vitro* tests were used to assess the antioxidant properties of three Ghanaian species: *Spathodea campanulata*, *Commelina diffusa*, and *Secamone afzelii* [63].

Inflammation is a complex mechanism with many pathways. Several extracts derived from medicinal plants have been shown to modulate or inhibit the activities of mediators of inflammation. For instance, kolaviron, a bioflavonoid compound isolated from the seeds of *Garcinia kola*, has been reported to possess anti-inflammatory and antioxidant activities via its effects on COX-2 and inducible nitric oxide synthase (iNOS) by inhibiting the expression of nuclear factor kappa B (NF-κB) [115]. Quercetin is a flavonoid molecule ubiquitous in nature and functions as an antioxidant and anti-inflammatory agent. Dose- and time-dependent effects of quercetin have been investigated on proinflammatory cytokine expression and iNOS, focusing on its effects on NF-κB signal transduction pathways in lipopolysaccharide-stimulated RAW 264.7 cells by using real time polymerase chain reaction (RT-PCR) and immunoblotting. Curcumin, a yellow pigment of turmeric, has been shown to exhibit anti-inflammatory activity. Curcumin has been found effective in the treatment or control of chronic inflammatory conditions such as rheumatism, atherosclerosis, type II diabetes, and cancer [203]. Calixto et al. reported that the anti-inflammatory action of active spice-derived components results from the disruption of the production of various inflammatory proteins (e.g., cytokines such as tumour necrosis factor-alpha (TNF-α), iNOS, and COX-2) [379].

Animal studies were also conducted to assess the antioxidant properties of several medicinal extracts. The antioxidant potential of *Hypericum perforatum,* containing many polyphenolic compounds, was evaluated on splanchnic artery occlusion (SAO) shock-mediated injury [477] and also against elevated brain oxidative status induced by amnestic dose of scopolamine in rats [126]. Some medicinal plant extracts were tested for their ability to protect against carbon tetrachloride-, 2-acetylaminofluorene- (2-AAF-), and galactosamine-induced liver as well as aflatoxin B1-(AFB1-)induced genotoxicity. Using this test, it was found that an extract of *Garcinia kola* seeds [116, 478, 479], a decoction of *Trichilia roka* root [270], extracts of *Entada africana* [442], and *Thonningia sanguinea* [98, 480] possessed protective abilities. The antioxidant properties of plant extracts against potassium bromate (KBrO(3))-induced kidney damage showed the ability of *G. kola* seed extract to protect the kidneys [481].

Animal studies were also used to assess the anti-inflammatory ability of a great number of medicinal plant extracts using the carrageenan-induced rat paw oedema model. Plants investigated include seed extracts of *Picralima nitida* [399], crude methanol extract of the root of *Moringa oleifera* [469], powdered leaves and root of *Mallotus oppositifolium* [167], methanolic extract of *Picralima nitida* fruit [400], hot water extract of *Alstonia boonei* root-bark, *Rauvolfia vomitoria* root-bark, and *Elaeis guineensis* nuts [56], secondary root aqueous extract of *Harpagophytum procumbens* [303], crude extracts of *Sphenocentrum jollyanum* [272], aqueous and methanolic extracts of *Hypoxis hemerocallidea* corm [482], aqueous and methanolic extracts of *Sclerocarya birrea* stem-bark [483], aqueous extract of *Mangifera indica* stem-bark [13], aqueous extracts of *Leonotis leonurus* leaves [484], leaf extracts of *Bryophyllum pinnatum* [148], methanol extracts of the stem-bark of *Alstonia boonei* [485], aerial parts of *Amaranthus caudatus* [486], methanolic extracts of *Kigelia pinnata* flower [415], and leaf and twig extracts of *Dorstenia barteri* [276]. In all of these studies, the anti-inflammatory effect against carrageenan-induced rat paw oedema was attributed to flavonoids and other polyphenolic compounds. Animal tests also employed to assess the anti-inflammatory effects of the medicinal plant extracts included inflammatory cell response such as neutrophil chemotaxis

Table 2: Medicinal plants with confirmed antioxidant activity or medicinal plants that contain compounds that are not known to have antioxidant activity.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Acanthaceae						
Barleria species B. albostellata, B. greenii, B. prionitis		Leaves, twigs and roots	South Africa	Anti-inflammatory and antioxidant activities	Not identified	[212, 213]
Hypoestes rosea Decne.	Not signalized	Leaf extract	Nigeria	Anti-inflammatory activity due in part to its ability to inhibit NF-kappaB activation through direct inhibition of IkappaB kinase (IKK).	Diterpene: Hypoestoxide (a bicyclo [9,3,1] pentadecane)	[380, 381]
Aizoaceae						
Glinus lotoides L.	"Mettere" Hairy carpet -weed	Seeds	Cameroon Ethiopia, Sudan, Uganda, Egypt.	Used to treat cardiovascular and gastrointestinal system.	Three flavonoids: apigenin-7-O-glucoside, isovitexin, and luteolin-7-O-glucoside Three isoflavonoids: 5,7,2′,4′-tetrahydroxy-6-(3,3-dimethylallyl)isoflavone, 5,7,4′-trihydroxy -6,3′-di-(3,3-dimethylallyl)isoflavone, and 5,7,2′,4′-tetrahydroxy-6,3′-di-(3,3-dimethylallyl) isoflavone.	[290, 382–386]
G. oppositifolius (L.) Aug. DC.	Balasa	Whole plant	Mali	Antioxidant and radical scavenging abilities.	kaempferol 3-O-galactopyranoside	[387, 388]

Table 2: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Aloaceae						
Aloe claviflora Burch.	Kraal aloe		South Africa	Free radical scavenging and moderate inhibition in lipid peroxidation. Used as a purgative.	Not identified	[35]
A. maculata Forssk. (=*A. saponaria*)	"Yellow Form" Tiger Aloe, Soap Aloe		South Africa	Free radical scavenging and moderate inhibition in lipid peroxidation. Used as a purgative.	Not identified	[35]
A. thraskii Baker	Dune aloe		South Africa	Free radical scavenging and moderate inhibition in lipid peroxidation. Used as a purgative.	Not identified	[35]
Anacardiaceae						
Sclerocarya birrea (A.Rich.) Hochst	Marula	Stem-bark		Anti-inflammatory activity. Used to treat diabetes, tonsillitis, snake bite and also diarrhoea.	Not identified	[389]
Annonaceae						
Enantia chlorantha Oliver	Erenbavbogo, Mföl Muamba	Root, stem-bark	Nigeria	Anti-inflammatory activity. Used to treat ulcers and leprous spots wounds. Bark sap is taken as decoction against diarrhoea.	Not identified	[390–393]
Uvaria afzelii Sc. Elliot	Pareho-houon, Bahie oulin	Leaves, roots and stem-bark	Ivory Coast	Used as for its antiparasitic activity	Anthocyanins and other flavonoids	[394–396]
U. chamae P.Beauv.	Okandii Anweda tsoGa	Stem, bark Leaves, root	Ivory Coast Nigeria	Used for its antiplasmodial activity.	Polyphenols	[12, 397, 398]

Table 2: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Apocynaceae						
Picralima nitida Th. & H. Dur.	Ghana: Kpetepetetso, Kanwini, Kanwinu Cameroon: *motoko-toko*	Seeds Stem-bark	Ghana	Anti-inflammatory activity. Used for its analgesic and anti-inflammatory properties.	Not identified	[168, 399–402]
Rauvolfia vomitoria Afzel.	Asofeyeje, adapopo Mwanje	Root-bark	Ghana	Anti-inflammatory activity. Used for its analgesic, antipyretic and anti-inflammatory activities. Also to treat scabies, high blood pressure, fever and snakebites.	Not identified	[56]
Araliaceae						
Cussonia barteri Seem.	Cabbage tree	Leaves Roots	Nigeria, Mali	Antioxidant and radical scavenging abilities. Inhibitory activity on 5-lipoxygenase and cyclooxygenase-1.	Not identified	[357, 403]
Arecaceae						
Hyphaene thebaica Mart.	Not signalized	Shell	Niger	Antioxidant activity	Not identified	[11]
Asclepiadaceae						
Calotropis procera (Aiton) W.T.Aiton	African milk weed Sodom apple/Giant milkweed/	Latex	Ethiopia	Anti-inflammatory and antioxidant activities.	Not identified	[404]
	Swallow-wort/Auricula tree.		Sudan	Used to control dermal fungal infections and for pain relief. Latex used against scorpion stings and roots for jaundice.		
Gongronema latifolium Benth.	Not signalized	Leaves	Nigeria	Antioxidant activity	Not identified	[405–407]
Leptadenia hastata Decne.	Not signalized	Leaves	Niger	Antioxidant activity	Not identified	[11]
Pachycarpus rigidus E. Mey.	Not signalized	Bark	South Africa	Antioxidant activity. Used to treat pain in the joints	Not identified	[188]

Table 2: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Asparagaceae						
Asparagus virgatus Baker Refug. Bot. (Saunders)	Broom asparagus	Bark	South Africa	Antioxidant activity. Used to treat syphilis, anthelmintic	Not identified	[35]
Asteraceae						
Ageratum conyzoides L.	Inkuruba Herbe à bouc	Whole plant	Central Africa, Rwanda Ethiopia	Antioxidant and anti-inflammatory properties. Used to treat mastitis and urogenital infections and to dress wounds. Also as a gastroprotective.	Not identified	[12, 408, 409]
Artemisia herba-alba	Desert wormwood, shih	Aerial parts	Algeria, Tunisia, Israel, Morocco	Herbal tea from *A. herba-alba* has been used as analgesic, antibacterial, antispasmodic, and hemostatic agents in folk medicines	Camphor (17–33%), α-thujone (7–28%), and chrysanthenone (4–19%)	[9]
Artemisia judaica L.	Wormwood	Leaves	Egypt	Used for gastrointestinal disorders	Flavonoids with antioxidant activities.	[410]
Callilepis laureola DC.	Ox-eye daisy, Impila	Tuber	South Africa	Antioxidant and radical scavenging activities. Used to induce fertility, impotence, tapeworm infestations but induces hepatic and renal tubular necrosis.	Not identified	[188, 411, 412]
Psiadia punctulata (DC) Vatke	Mwendathigo	Leaf exudate	Kenya, East Africa	Used to treat colds, fevers and abdominals pains.	Flavones: 5,7-dihydroxy-2′,3′,4′,5′-tetramethoxyflavone, 5,4′-dihydroxy-7,2′,3′,5′-tetramethoxyflavone, 5,7,4′-trihydroxy-2′,3′,5′-trimethoxyflavone, 5-hydroxy-7,2′,3′,4′,5′-pentamethoxyflavone and 5,7,3′-trihydroxy-2′,4′,5′-trimethoxyflavone.	[359, 413]
Vernonia kotschyana Sch. Bip. ex Walp.	Buaye	Leaves, roots	Mali	Anti-inflammatory activity. Used to treat gastritis, gastro duodenal ulcers, as an aid to ameliorate digestion and as a wound healing remedy. Immunomodulating activities.	Not identified	[187, 414]

Table 2: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Bignoniaceae						
Kigelia pinnata DC.	Suasage tree, Cucumber tree	Root fruit	Egypt	Used as dressing for ulcers and used to treat rheumatism Anti-inflammatory activity	Naphthoquinones: kigelinone, isopinnatal, dehydro-alpha-lapachone, and lapachol and the phenylpropanoids: p-coumaric acid, ferulic acid (root), kigelinone and caffeic acid (fruits).	[415, 416]
Tabebuia rosea (Bertol.) DC.	Pink tecoma Pink trumpet tree	Leaves Stem-bark	Nigeria	Used to treat arthritis.	Tannins, flavonoids, alkaloids, quinones and traces of saponins	[107]
Crescentia cujete L.	Calabash Gourd tree	Leaves Stem-bark	Nigeria	Used as purgative and to treat coughs.	Tannins, flavonoids, alkaloids, quinones and traces of saponins	[107]
Bombacaceae						
Bombax costatum Pellegrin & Vuillet	Not signalized	Fruit	Niger	Antioxidant activity	Not identified	[11]
Boraginaceae						
Heliotropium indicum L.	Nonsikou	Leaves	Mali	Moderate antioxidant activity. Used for wound healing and for ocular infection.	Not identified	[417–419]
Buddlejaceae						
Buddleja madagascariensis Lam.	Butterfly-bush	Leaves	Egypt	Used to treat coughs, asthma, and bronchitis.	Flavonoids triglycosides: hesperetin and diosmetin 7-O (2″,6″ - di-O-alpha-L-rhamnopyranosyl)-beta-D-glucopyranosides	[420]
Caesalpiniaceae						
Cassia fistula L.	Golden shower tree	Fruit	Mauritius	Laxative.	Phenolics and flavonoids	[368]

Table 2: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Canellaceae						
Warburgia salutaris (Bertol F.) Chiov.	Pepper-bark tree Isibaha	Bark	South Africa	Antioxidant and radical scavenging activities. Used to treat coughs, stomach ulcers, malaria, rheumatism, liver and venereal diseases	Not identified	[188]
W. ugandensis Sprague	Fever tree	Stem-bark Leaves	Kenya Ethiopia	Used to treat stomach ache, chest pains, malaria, toothache and coughs.	Flavonol glycoside Kaempferol, kaempferol 3-rhamnoside, kaempferol 3-Rhamnoside-7,4′-digalactoside and Quercetin: 3-Rhamnosyl(1⟶6[glucosyl(1⟶2)glucoside]-7-rhamnoside, kaempferide 3-O-beta-xylosyl (1⟶2)-beta-glucoside, kaempferol 3-O-alpha-rhamnoside-7,4′-di-O-beta-galactoside, kaempferol 3,7,4′-tri-O-beta-glucoside, kaempferol 3-rutinoside, myricetin, quercetin 3-rhamnoside, kaempferol 3-arabinoside, quercetin 3-glucoside, quercetin, kaempferol 3-rhamnoside-4′-galactoside, myricetin 3-galactoside and kaempferol 3-glucoside.	[421–424]
Capparaceae						
Boscia senegalensis (Pers.) Lam. ex Poiret	Senegal Boscia	Fruit hull Roots and leaf	Mali Niger	Antioxidant activity. Used to treat diarrhoea, cholera, tachycardia, pectoral pain.	Not identified	[12]
Gynandropsis gynandra Merr.	Not signalized	Leaves	Niger	Antioxidant activity	Not identified	[11]
Celastraceae						
Salacia leptoclada Tul.	Lemon rope	Root	South Africa	Antioxidant activity. Used as an aphrodisiac.	Not identified	[188]
Chenopodiaceae						
Salsola somalensis N.E.Br.	Dingetegna	Roots	Ethiopia	Used as taenicide.	Nine new isoflavones, 5,3′-dihydroxy-6,7,2′-trimethoxy isoflavone, 5,8,3′-trihydroxy-7,2′-dimethoxyisoflavone, 8,3′-dihydroxy-5,7,2′-trimethoxyisoflavone, 5,6,3′-trihydroxy-7,2′-dimethoxyisoflavone, 6,7,3′-trihydroxy-5,2′-dimethoxyisoflavone, 5,8,3′-trihydroxy -2′-methoxy-6,7-methylenedioxyisoflavone, or 5,6,3′-trihydroxy-2′-methoxy-7,8-methylenedioxyisoflavone, 3′-hydroxy-5,6,7,2′-tetramethoxyisoflavone, 7,3′-dihydroxy -5,6,2′-trimethoxyisoflavone and 6,3′-dihydroxy-5,7,2′-trimethoxyisoflavone.	[425]

Table 2: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Clusiaceae						
Psorospermum guineense Hochr.	Karidjakouma	Leaves	Mali	Antioxidant activity. Used as diuretic and febrifuge.	Not identified	
Combretaceae						
Pteleopsis suberosa Engl. & Diels.	Girga	Stem-bark	Mali	Antioxidant properties. Used to treat gastric and duodenal ulcers.	Not identified	[329, 426]
Dioscoreaceae						
Dioscorea dumetorum Th.Dur.et Schinz	Cluster yam African bitter yam Trifoliate yam	Tubers	Nigeria Tropical West Africa	Antioxidant and hypolipidemic activities. Used to treat diabetes.	Not identified	[152, 153, 427]
Ebenaceae						
Diospyros abyssinica (Hiern) F. White	Giant diospyros	Leaves, roots Root-bark	Mali	Radical scavengers and lipoxygenase inhibitors.	Not identified	[357]
Euclea divinorum Hiern	Diamond-leaved euclea Magic guarri	Roots	Ethiopia	Used to treat venereal diseases, chest pains, pneumonia, internal body pains, stomach-ache and diarrhea. Chewed roots ease toothache.	Flavonoids	[428]
Euphorbiaceae						
Acalypha hispida Burm. f.	Chenille plant Red-hot cattail	Leaves Flowers	Nigeria	Used as anti-bacterial agent.	Gallic acid and Quercetin 3-O-rutinoside and kaempferol 3-O-rutinoside The main anthocyanin is the known cyanidind 3-*O*-(2-*O*-galloylgalactose, but a minor pigment (5%) is the new cyanidin Cy 3-*O*-(2-*O*-galloyl-6-*O*-rhamnosylgalactoside	[228, 429]
A. wilkesiana Müll. Arg.	Copper leaf	Leaves	Nigeria	Used to treat ailments of microbial origin	Gallic acid and Quercetin 3-O-rutinoside and kaempferol 3-O-rutinoside	[430]
Croton gratissimus Burch.	Lavender fever-berry	Bark	South Africa	Used as purgative for abdominal disorders, fever. The charred and powdered bark is used to treat bleeding gums	Flavonoids.	[188]
Euphorbia hirta L.	Kasandasanda Ufu idire	Whole plant Leaves	Ethiopia	Used to treat diarrhoea and asthma.	**Flavonoid**: quercitrin Flavonol: Euphorbianin (3-(6‴-Acetylglucosyl) (1⟶3)galactoside)	[12, 431–433]
Fabaceae						
Acacia caffra (Thunb.) Wild.	Hook-thorn Cat-thorn	Bark	South Africa	Used to treat diarrhoea and as emetics.	Proanthocyanidins: oritin-(4alpha⟶5)-epioritin-4beta-ol, ent-epioritin-(4alpha⟶5)-epioritin-4beta-ol and epioritin-(4beta⟶5)-epioritin-4alpha-ol and ent-oritin-(4beta⟶5)-epioritin-4alpha-ol.	[434–436]

Table 2: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
A. galpinii Burtt Davy.	Monkey-thorn	Bark	South Africa	Used to treat diarrhoea.	Proanthocyanidins: oritin-(4alpha⟶5)-epioritin-4beta-ol, ent-epioritin-(4alpha⟶5)-epioritin-4beta-ol and epioritin-(4beta⟶5)-epioritin-4alpha-ol and ent-oritin-(4beta⟶5)-epioritin-4alpha-ol.	[434, 435]
Afzelia bella Harms	Pretty Afzelia	Stem-bark	Ivory Coast	Used to treat skin diseases and cough.	An acylated dihydroflavonol glycoside identified as 2R,3R-trans-aromadendrin-7-O-beta-D-glucopyranoside-6″-(4″-hydroxy-2″-m ethylene flavonoids:butanoate), along with five known flavonoids and the lignan glycoside (+)-isolariciresinol 9-O-xyloside.	[437]
Bolusanthus speciosus Harms	Tree Wisteria	Root Stem-bark	South Africa, Botswana, Mozambique, Zimbabwe, Zambia.	Used to treat abdominal pains, emetism and tuberculosis.	Three new flavonoids from the root: 5,7,4′-trihydroxy-6-[1-hydroxy-2-methylbuten-2-yl]isoflavone (isogancaonin C), 7,2′-dihydroxy-4′-methoxyisoflav-3-ene (bolusanthin III), 6,6′-dihydroxy-4′-methoxy-2-arylbenzofuran (bolusanthin IV) in addition to eight known derrone, medicarpan, genistein, wighteone, lupiwighteone, gancaonin C, 7-hydroxy-4′-methoxyisoflavone and 7,3′-dihydroxy-4′-methoxyisoflavone flavonoids 2*R*,3*R*-Aromadendrin 7-(6-[4-hydroxy-2-methylenebutanoyl]glucoside). Two new **i**soflavonoids from the combined ethyl acetate/methanolic extracts of the stem bark of Bolusanthus speciosus have been established as 4,7,2′-trihydroxy-4′methoxyisoflavanol (**1**) and 5,7,3′,4′-tetrahydroxy-5′-(2-epoxy-3-methylbutyl)isoflavanone (**2**). Five other known isoflavonoids, 5,7,3′-trihydroxy-4′-methoxy-5′-γ, γ-dimethylallyisoflavanone, 5,7,2′trihydroxy-4′-methoxy-6,5′-di(γ, γ-dimethyla)isoflavanone, 5,7,2′,4′-tetrahydroxy-8,5′-di(γ, γ-dimethylallyl)isoflavanone, 5,7,2′,4′-tetrahydroxy-8,3′-di(γ, γ-dimethylallyl)-isoflavanone, and derrone.	[67, 358, 438]

Table 2: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Crotalaria lanceolata E. Mey.	Lanceleaf rattlebox	Root	South Africa	Antioxidant activity. Used to treat coughs.	Not identified	[188]
Derris trifoliata Lour.	Common derris	Root-bark. Stem-bark. Seeds.	Kenya	Used for prevention of cancer. Entire plant is used as stimulant, antispasmodic. Bark is used as an alternative in rheumatism.	An isoflavonoid derivative, named 7a-O-methyldeguelol, a modified rotenoid with an open ring-C, representing a new sub-class of isoflavonoids (the sub-class is here named as rotenoloid). In addition, the known rotenoids, rotenone, deguelin and alpha-toxicarol. In addition, two unusual rotenoid derivatives, a rotenoloid (named 7a-O-methyl-12a-hydroxydeguelol) and a spirohomooxarotenoid (named spiro-13-homo-13-oxaelliptone). In addition a rare natural chromanone (6,7-dimethoxy-4-chromanone) and the known rotenoids rotenone, tephrosin and dehydrodeguelin were identified. Also one new rotenoid, 6-alpha,12-alpha-12a-hydroxyelliptone.	[438–441]
Entada africana Guill. & Perr.	Samanere	Leaves	Mali Niger	Antioxidant properties. Protective against carbon tetrachloride-induced liver damage. Used to treat fever and various respiratory diseases.	Not identified	[329, 357, 442, 443]
Erythrina abyssinica Lam.	Red hot poker tree	Stem bark Root bark	Kenya	Used to treat malaria.	New isoflav-3-ene [7,4′-dihydroxy-2′,5′-dimethoxyisoflav-3-ene] in addition to the known compounds erycristagallin, licoagrochalcone A, octacosyl ferulate and triacontyl 4-hydroxycinnamate were identified. A new chalcone, 2′,3,4,4′-tetrahydroxy-5-prenylchalcone (trivial name 5-prenylbutein) and a new flavanone, 4′,7-dihydroxy-3′-methoxy-5′-prenylflavanone (trivial name, 5-deoxyabyssinin II) along with known flavonoids	[444, 445]

Table 2: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
E. burttii Baker f.	Not signalized	Stem-bark Root-bark	Kenya	Used as antifungal and antibacterial agent.	Two new flavanones: 5,7-dihydroxy-4′-methoxy-3′,5′-di-(3-methylbut-2-enyl)flavanone (trivial name, abyssinone V-4′-methyl ether) and 5,7-dihydroxy-4′-methoxy-3′-(3-hydroxy-3-methylbut-1-enyl)-5′-(3-methylbut-2-enyl)favanone (trivial name, burttinone). A new isoflavone, 5,2′,4′-trihydroxy-7-methoxy-6-(3-methylbut-2-enyl)isoflavone (trivial name, 7-O-methylluteone) and a new flavanone, 5,7-dihydroxy-4′-methoxy-3′-(3-methylbutadienyl)-5′-(3-methylbut-2-enyl)flavanone, 3 isoflavonoids (8-prenylluteone, 3-O-methylcalopocarpin and genistein) Three isoflav-3-enes, 7,4′-dihydroxy-2′-methoxy-6-(1″,1″-dimethylallyl)isoflav-3-ene (trivial name, burttinol-A), 4′-hydroxy-2′-methoxy-2″,2″-dimethylpyrano[5″,6″:8,7]isoflav-3-ene (trivial name, burttinol-B), 7,4′-dihydroxy-2′-methoxy-8-(3″,3″-dimethylallyl)isoflav-3-ene (trivial name, burttinol-C), and 2-arylbenzofuran, 6,4′-dihydroxy-2′-methoxy-5-(1″,1″-dimethylallyl)-2-arylbenzofuran (trivial name, burttinol-D).	[446–449]
E. eriotricha Harms.	Not signalized	Root-bark	Cameroon	Anti-microbial activity	A novel isoflavanone, named eriotrichin B, one new prenylated flavanone, named sigmoidin L, one flavanone (sigmoidin A), four isoflavones (scandenone, 6,8-diprenylgenistein), flemiphilippinin B and 8-prenyldaidzein	[450, 451]
E. sacleuxii Hua	Kinyarwanda	Bark	Kenya	Used to treat fever, malaria and leprosy.	Two new isoflavanones, (R)-5,7-dihydroxy-2′,4′,5′-trimethoxyisoflavanone (trivial name, (R)-2,3-dihydro-7-demethylrobustigenin) and (R)-5-hydroxy-2′,4′,5′-trimethoxy-2″,2″-dimethylpyrano[5″,6″:6,7]isoflavan one (trivial name, (R)-saclenone)	[452, 453]
Millettia ferruginea (Hochst.) Baker	Birbira Sotallo Sari	Bark	Ethiopia	Used for skin disorders.	O-Geranylated and O-prenylated flavonoids, C-prenylated isoflavones Geranylated and prenylated flavonoids	[199]
M. dura Dunn.	Runyankore Uumuyogoro	Stem-bark	Rwanda Uganda	Used for blood parasitism	Flavonoids: A new isoflavone (7,3′-dimethoxy-4′,5′-methylenedioxyisoflavone) and three known isoflavones [isoerythrinin A 4′-(3-methylbut-2-enyl) ether, isojamaicin and nordurlettone].	[454, 455]
Ostryoderris stuhlmannii (Taub.) Dunn ex Harms	Mnyinga	Leaves	Mali	Antioxidant activity. Used to treat painful menstruation, peritonitis, gastritis, colitis and gingivitis.	Not identified	[357]

Table 2: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Piliostigma reticulatum (DC.) Hochst	Kalga	Leaves Bark	Nigeria	High antioxidant activity. Used to treat wounds, bronchitis, malaria, sterility (leaves) and diarrhoea and dysentery (bark).	Not identified	[240]
Sesbania pachycarpa DC.	Not signalized	Leaves	Niger	Antioxidant activity	Not identified	[11]
Tephrosia polyphylla (Chiov.) J.B. Gillett	Hoary pea	Aerial part	Kenya		Flavonoids	[456]
T. deflexa Baker	Hoary pea	Aerial part	Senegal		Flavonoids: Rutin 1 – quercetine 3-O-a-L-rhamnopyrannosyl (1-6) glucopyrannose – and morin 2 – 3,5,7,2′,4′-pentahydroxyflavone.	[457]
T. albifoliolis A.Nongonierma & T.Sarr	Hoary pea	Aerial part	Senegal		Flavonoids: Rutin 1 – quercetine 3-O-a-L-rhamnopyrannosyl (1-6) glucopyrannose – and morin 2 – 3,5,7,2′,4′-pentahydroxyflavone.	[457]
Taverniera abyssinica A. Rich.	Dingetegna	Root	Ethiopia	Used to treat fever, discomfort and pain, stomach ache.	Four isoflavonoids	[290, 458, 459]
Flacourtiaceae						
Flacourtia flavescens Willd.	Not signalized	Leaves	Mali	Antioxidant activity.	Not identified	[357]
Geraniaceae						
Pelargonium reniforme Spreng.	Xhosa (Umckaloabo)	Root	Southern Africa	Used to treat liver disorders, laxative, purgative, cancer, and pulmonary disorders	Polyphenols: catechol (3′4′-dihydroxy) element in the B-ring, which possesses higher antioxidant activity than ascorbic acid.	[362, 460, 461]
Gunneraceae						
Gunnera perpensa L.	River pumpkin Ugobho	Root Leaves and stem.	South Africa	Decreased lucigenin enhanced chemiluminescence. Used to treat wounds and psoriasis.	Not identified	[21, 462]
Irvingiaceae						
Irvingia gabonensis (Aubry-Lecomte ex O'Rorke) Baill.	Bush mango Ono	Seeds	Nigeria Cameroon	Antioxidant activity. Used as laxative and for stomach and kidney pain. Shown to lower total cholesterol.	Not identified	[12, 313, 463]

Table 2: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Lamiaceae						
Leonotis leonurus (L.)R.Br.	Wild dagga	Leaves	South Africa	Anti-inflammatory properties. Used to treat headaches, dysentery, coughs and colds.	Not identified	[13]
Salvia stenophylla Burch. ex Benth.	Sage	Leaves	South Africa	Solvent extracts: antioxidant activity but poor anti-inflammatory properties. Essential oils: anti-inflammatory activity but poor anti-oxidant activity. Used against fever and digestive disorders.	Not identified	[360]
S. repens Burch. ex Benth.	Not signalized	Leaves	South Africa	Solvent extracts: antioxidant activity but poor anti-inflammatory properties. Essential oils: anti-inflammatory activity but poor anti-oxidant activity. Used for fevers and digestive disorders.	Not identified	[360]
S. runcinata L.f.	Not signalized	Leaves	South Africa	Solvent extracts: antioxidant activity but poor anti-inflammatory properties. Essential oils: anti-inflammatory activity but poor anti-oxidant activity. Used against fever and digestive disorders.	Not identified	[360]
Loranthaceae						
Tapinanthus globiferus Tiegh.	Not signalized	Leaves	Niger	Antioxidant activity	Not identified	[11]

Table 2: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Malvacea						
Adansonia digitata (L.)	English: baobab, Afrikaans: kremetart, Hausa: kuka, Sotho: seboi, Tswana: mowana, Tsonga: shimuwu, Venda: muvhuyu, Arabic: tabladi	Leaves, root, bark and fruits	All over Africa, but limited trees in Central Africa	Antioxidant, analgesic and anti-inflammatory properties of extracts	L-ascorbic acid	[36, 464]
Mimosaceae						
Albizia lebbeck (L.) Benth.	East Indian walnut, frywood, koko, lebbek, lebbek tree, rain tree, raom tree, silver raintree, siris rain tree, siris tree, soros-tree, woman's tongue.	Leaves and bark	Egypt	Used to treat asthma and skin disorders (bark) and eye diseases and dysentery (leaves)	Two new tri-O-glycoside flavonols: kaempferol and quercetin 3-O-alpha-rhamnopyranosyl(1⟶6)-beta-glucopyranosyl(1⟶6)-beta-galactopyranosides	[465]
Moraceae						
Dorstenia angusticornis Engl.	Not signalized	Twigs	Cameroon	Used for snakebite and to treat infection, rheumatism, headache, cough and stomach pain.	Two novel **diprenylated chalcones**: 3,5′-di-(2-hydroxy-3-methylbut-3-enyl)-4,2′,4′-trihydroxychalcone, 3, 4-(2,2-dimethylpyrano)-3′-(2-hydroxy-3-methylbut-3-enyl)-2′,4′-dihydroxych alcone and the known stipulin. 3-(2-Hydroxy-3-methylbut-3-enyl)-5′-(3,3-dimethylallyl)-4,2′,4′-trihydroxy chalcone and the known compounds: gancaonin Q, paratocarpins C, F, and lupeol.	[67, 278]

Table 2: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
D. dinklagei Engl.	Not signalized	Twigs	Cameroon	Used for snakebite and to treat infection, rheumatism, headache, cough and stomach pain.	Three prenylated flavonoids, dinklagins A, B and C identified, respectively, as (dinklagin B): (+)-5,4′,5″ξ-Trihydroxy-6″,6″-dimethyldihydropyranol[2″,3″:7,6]flavone. (dinklagin C): (+)-6-(2ξ-Hydroxy-3-methyl-3-butenyl)-5,7,4′-trihydroxyflavone (-)-6-(3,3-dimethylallyl)-7-hydroxy-6‴, 6‴-dimethylchromeno-(4′,3′,2‴,3‴)-flavanone, (+)-5,4′,5″ξ-trihydroxy-6″,6″-dimethylchromano-(7,6,2″,3″)-flavone and (+)6-(2ξ-hydroxy-3-methyl-3-butenyl)-5,7,4′-trihydroxyflavone. 6-prenylapigenin, 4-hydroxylonchocarpin, stipulin and 5,4′-dihydroxy-6″,6″-dimethylchromano-(7,6,2″,3″)-flavone.	[67, 226]
D. elliptica Bur.	Not signalized	Twigs	Botswana	Used to treat eye infection.	Monoprenylated flavan	[466]
D. Kameruniana. Engl.	Not signalized	Leaves	Botswana	Used for snakebite and to treat infection, rheumatism, headache, cough and stomach pain.	Two novel favonoids: 6,7-(2,2-dimethylchromano)-5,4′-dihydroxyfavone and 3,4-,4′,5′-*bis*-(2,2-dimethylchromano)-2′-hydroxychalcone together with the known 6-(3-methylbut-2-enyl)apigenin and two chalcones (*E*)-1-[2,4-dihydroxy-3-[3-methylbut-2-enyl]phenyl]-3-[4-hydroxyphenyl]-prop-2-en-1-one and (*E*)1-[2,4-dihydroxy-5-[3-methylbut-2-enyl]phenyl]-3-[4-hydroxy-3-[3-methylbut-2-enyl]phenyl]-prop-2-en-1-one.	[467]

Table 2: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
D. prorepens Engl.	Not signalized	Twigs	Botswana	Used for snakebite and to treat infection, rheumatism, headache, cough and stomach pain.	Digeranylated chalcone, 5,3′-(3,7-dimethyl-2,6-octadienyl)-3,4, 2′,4′-tetrahydroxychalcone. 4-Hydroxylonchocarpin Chalcone: 3,4,2′,4′-Tetrahydroxy-5,3′-digeranylchalcone	[67, 468]
D. poinsettiifolia Engl.	Not signalized	Twigs	Botswana	Used for snakebite and to treat infection, rheumatism, headache, cough and stomach pain.	Grenylated and prenylated flavonoids. In addition, the flavone 5,7,4-trihydroxy-8-prenylflavone (licoflavone C), the chalcones 4,2′,4′-trihydroxy-3′-prenylchalcone (isobavachalcone) and isobavachromene, the triterpene butyrospermol, and the carotenoid lutein.	[67, 206, 289]
D. zenkeri Engl.	Not signalized	Twigs	Botswana	Used for snakebite and to treat infection, rheumatism, headache, cough and stomach pain.	3′,4′-(3-hydroxy-2,2-dimethyldihydropyrano)-4,2′-dihydroxychalcone and a bichalcone. 4-Hydroxylonchocarpin. p-hydroxybenzaldehyde, dorsmanin A, 4,2′,4′-trihydroxychalcone and 4,2′,4′-trihydroxy-3′-prenylchalcone Chalcones: 4,2′,5″-Trihydroxy-6″,6″-dimethyldihydropyranol[2″,3″:4′,3′]chalcone	[67, 468]
Moringaceae						
Moringa oleifera Lam.	Horse-radish tree Drumstick Moringo Zakalanda	Root	West Africa Zimbabwe	Anti-inflammatory activity. Used as aphrodisiac and to treat asthma, gout and rheumatism.	Not identified	[469]

Table 2: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Myrtaceae						
Eucalyptus camaldulensis Dehnh.	Not signalized	Leaves	Egypt	Antioxidant activity	Not identified	[470]
Polygonaceae						
Polygonum senegalense Meisn.	Fotsimbarin'akoholahy	Leaves	Madagascar		Flavonoids: quercetin, kaempferol and luteolin and their glycosides such as dihydrochalcone glucoside and quercetin glycosides.	[413, 471]
Rumex abyssinicus Jacq.	Mekmeko	Leaves	N. Africa - Ethiopia	Anti-inflammatory properties Used to treat itching, skin eczema and leprosy.	Flavonoids.	[337, 472]
R. nervosus Vahl.,	Alcgango Dengogo	Leaves	Ethiopia	Anti-inflammatory properties. Used to treat acne, wounds, eczema, typhus and as an ophthalmic antiseptic.	Not identified	[337]
Rubiaceae						
Nauclea latifolia Smith	Pin Cushion Tree Ìgíyàà	Leaves and root	Nigeria	Used as anthelmintic and to treat malaria, fever, stomachache and liver diseases.	Proanthocyanidins.	[12, 58, 473–475]
Solanaceae						
Datura stramonium L.	Thorn-apple rwiziringa	Seeds	South Africa	Antioxidant activity. Used to treat asthma, headaches and wounds.	Not identified	[188]
Tiliaceae						
Grewia occidentalis L.	Cross-berry Four-corner	Bark	South Africa	Antioxidant activity. Used to treat bladder ailments, wounds, impotence and sterility, and to help in childbirth.	Not identified	[188]

Table 2: Continued.

Family and plant name	Vernacular name	Plant part	Country/area	Medicinal use and/or experimental validation	Compounds isolated	Reference
Vahliaceae						
Vahlia capensis (L.f) Thunb.	Vahlia of the Cape	Zimbabwe		Used to treat bacterial infections.	Kaempferol, quercetin, afzelin, astragalin, quercitrin, isoquercitrin, rutin, gallic acid, chiro-inositol, dulcitol, and a novel biflavonoid, VC-15B (vahlia biflavone)	[475]
Vitaceae						
Cyphostemma natalitium (Szyszl.) J.v. d. Merwe	Tick-berry bush	Root	South Africa	Anti-inflammatory and anti-microbial agents with significant inhibition of COX-1	Not identified	[374]
Rhoicissus digitata Gilg. & Brandt	Wilde patatat	Roots, stems and leaves	South Africa	At high concentrations possessed some prooxidative properties. Anti-inflammatory and anti-microbial agents with significant inhibition of COX-1. Used to facilitate delivery.	Not identified	[364, 374]
R. rhomboidea (E. Meyer ex Harvey) Planchon	Glossy forest grape	Roots, stems and leaves	South Africa Mozambique	Radical scavenging activity, inhibitory effect on xanthine oxidase activity, prevention of lipid peroxidation and damage to DNA and ability to chelate iron. Anti-inflammatory through inhibition of COX-1.	Not identified	[364, 374]
R. tomentosa (Lam.) Wild & R.B.Drum.	Wild grape Forest Grape, Monkey rope,	Roots, stems and leaves	South Africa	Antioxidant and anti-inflammatory activities. Anti-inflammatory through inhibition of COX-1. Used to facilitate delivery.	Not identified	[364, 374]
R. tridentata (L.f.) Wild & Drum.	Bitter grape Bushman's grape Isinwazi	Roots, stems and leaves	South Africa : Venda	Radical scavenging activity, inhibitory effect on xanthine oxidase activity, prevention of lipid peroxidation and damage to DNA and ability to chelate iron. Anti-inflammatory through inhibition of COX-1. Used to treat colds, infertility and stomach ailments.	Not identified	[364, 374, 476]

and degranulation [112, 487], antiatherosclerosis effects [486], and pain assessment in experimental animals [117].

The effect of the medicinal plants on the induction or inhibition of drug metabolizing enzymes was also studied in animals. The effect of the aqueous extract of *Thonningia sanguinea* on 7-ethoxyresorufin O-deethylase (EROD, CYP1A1), 7-pentoxyresorufin O-dealkylase (PROD, CYP2B1/2), 7-methoxyresorufin O-demethylase (MROD, CYP1A2), aniline hydroxylase (aniline, CYP2E1), *p*-nitrophenol hydroxylase (PNPH, CYP2E1), and erythromycin N-demethylase (ERDM, CYP3A1) in rat liver was found to selectively modulate CYP isoenzymes [100] and suppress CYP3A2 and CYP1A2 gene expression [101].

3. Compounds Isolated from African Medicinal Plant Extracts with Confirmed Antioxidant Activities

Several medicinal plant extracts were studied at research centres in African countries for their antioxidant properties. The major findings of these investigations have indicated that, in addition to known antioxidant compounds such as ascorbic acid in the seeds of *Parkia biglobosa* [204] and fruits pulp of *Adansonia digitata* [369], alpha-tocopherol in methanol extracts of the stems of *Secamone afzelii* [62] or from the seeds [38] and methanol extracts of leaves of *Amaranthus caudatus* [39], and apigenin and luteolin in aerial parts of *Bulbine capitata* [66], several other antioxidant compounds were identified. Although known antioxidant compounds such as ascorbic acid have been confirmed to promote wound healing, not all the newly identified compounds have been tested for such activity [488–491].

The identified compounds included mainly flavonoids such as flavones and flavonols, flavone and flavonol glycosides, chalcones and dihydrochalcones, and flavonones, although some anthocyanins, proanthocyanidins, and anthrones were also isolated with antioxidant properties. A wide range of plant extracts investigated have been shown to contain flavonoids. *Dorstenia* species are rich in flavonoids some of which are unique to this genus [67, 205], namely, prenylated flavonoids as found in *Dorstenia kameruniana* and twigs of *D. mannii* [206, 207]. Earlier studies have shown that prenylated flavonoids had antioxidant properties, which protected human LDL from oxidation [208]. Those isolated from African medicinal plant extracts were also tested and their antioxidant properties confirmed. The antioxidant activities of three prenylated flavonoids from *D. mannii* (6,8-diprenyleriodictyol, dorsmanin C, 7,8-(2,2-dimethylchromeno)-6-geranyl-3,5,3′,4′-tetrahydroxyflavonol and dorsmanin F, (+)-7,8-[2″-(1-hydroxy-1-methylethyl)-dihydrofurano]-6-prenyl-5,3′,4′-trihydroxyflavanone) against LDL oxidation and also their free radical scavenging activity have been indicated [187]. Similarly, a diprenylated chalcone, Bartericin A, present in *D. barteri* leaf and twig extracts was shown to have potent antioxidant properties. It was found that this and other prenylated and geranylated chalcones were as active as the prenylated flavones and may account for the anti-inflammatory action of these extracts [276]. Free radical scavenging activity was also confirmed for prenylated anthronoids isolated from the stem-bark of *Harungana madagascariensis* [121] and for proanthocyanidins isolated from the bark of *Burkea africana* [175]. The anti-inflammatory and antioxidant activities of kolaviron, a biflavonoid isolated from a *Garcinia kola* seed extract to scavenge free radicals, which protect against lipid peroxidation and H_2O_2-induced DNA strand breaks and oxidized bases, were also reported [114, 116–119, 209]. In addition, the ability of free radical scavenging activity and ability to inhibit lipid peroxidation of Thonningianin A and Thonningianin B, ellagitannins, isolated from *Thonningia sanguinea* have been shown [99, 366]. The anti-inflammatory ability of Griffonianone D ((7E)-(6″,7″-dihydroxy-3″,7″-dimethyloct-2″-enyl)oxy-4′-methoxyisoflavone), an isoflavone present in *Millettia griffoniana*, has been established [195]. Prenylated anthronoids, harunmadagascarins A (8,9-dihydroxy-4,4-bis-(3,3-dimethylallyl)-6-methyl-2,3-(2,2-dimethylpyrano)anthrone and B (8,9-dihydroxy-4,4,5-tris-(3,3-dimethylallyl)-6-methyl-2,3-(2,2-dimethylpyrano)anthrone), harunganol B, and harungin anthrone from the stem-bark of *Harungana madagascariensis* have exhibited significant antioxidant activity [121]. Saponins and isofuranonaphthoquinones isolated from different medicinal plant extracts showed antioxidant properties and include the saponin, Balanin 1 (3β,12β,14β,16β) cholest-5-ene-3,16-diyl bis (β-d-glucopyranoside)-12-sulphate, sterol sulfonated, Balanin 2 (3β,20*S*,22*R*,25*R*)-26-hydroxy-22-acetoxyfurost-5-en-3-yl-rhamnopyranosyl-(1⟶2)-glucopyranoside, and a furostanol saponin isolated from *Balanites aegyptiaca* [104]. Isofuranonaphthoquinones isolated from the roots of *Bulbine capitata*, 5,8-dihydroxy-1-tigloylmethylnaphtho[2,3-c]furan-4,9-dione, 1-acetoxymethyl-8-hydroxynaphtho [2,3-c]furan-4,9-dione, and 1-acetoxymethyl-5,8-dihydroxynaphtho[2,3-c]furan-4,9-dione possess antioxidant activities [68]. Though none of these antioxidant compounds has been directly assessed for wound healing potential, the enhanced wound closure observed with treatment of prenylated flavonoids such as genistein [492] and the demonstrated effect of chalcones on the inflammation process [493] attest to the potential of isolated antioxidants in wound management.

4. Crude Extracts of African Medicinal Plants with Confirmed Antioxidant Activities

The antioxidant properties of a larger proportion of African medicinal plants listed in Tables 1 and 2 were tested using either aqueous or organic plant extracts. After confirming antioxidant properties, a correlation was proposed between this property and the general groups of antioxidant compounds that are present in these extracts. No further attempts were made to isolate the specific compounds that may have contributed towards this property. Flavonoids in *Aloe barbadensis* [32], chromone glycosides in *A. claviflora* [35], essential oils in *Artemisia abyssinica*, and *Juniperus procera* [79] as well as *Helichrysum dasyanthum*, *H. felinum*, *H.*

excisum, and *H. petiolare* [94], proanthocyanidins in *Burkea africana* bark [175], polyphenols in extracts of *Crataegus monogyna* [321], saponins, and alkaloids in extracts of *Leucosidea sericea* [210, 211] are all considered as major compounds that have contributed to the antioxidant properties of these plants. Reports on a number of *Barleria* species, which includes *B. albostellata, B. greenii*, and *B. prionitis*, have indicated their anti-inflammatory [212] and antioxidant capacities [213]. Unlike the isolated compounds, most of the plants listed for possessing antioxidant activity, including extracts of *Agerantum conyzoides*, *Euphorbia hirta, Kigelia africana, and Nauclea latifolia,* have been shown to possess wound healing ability [494–496].

Furthermore, studies have focused on screening a vast number of plants, used in a specific region, so as to determine their antioxidant properties, Mali [357], South Africa [19, 188, 267, 364], Cameroon [182, 313], Algeria [85], Ghana [98], Burkina Faso [266], Madagascar [23], and Mauritius [293], and anti-inflammatory properties, South Africa [168, 264, 374, 376] and West Africa [400].

5. Discussion and Conclusion

The use of traditional herbal remedies as alternative medicine plays a significant role in Africa since it features extensively in primary health care. The search for natural antioxidants, especially from plant sources, as a potential intervention for treatment of free radical mediated diseases is an important research field, especially for those in developing countries. Many polyphenols, including phenolic acids, flavonoids (anthocyanins and anthoxanthins), tannins, and lignans, are known to act as antioxidants and protect against various pathological conditions such as coronary artery disease and wounds, in addition to their anti-inflammatory, antimicrobial, and anticancer activities [214–216].

Flavonoids are a large group of compounds containing several hydroxyl groups on their ring structures and include isoflavonoids and isoflavonoid glycosides, flavones, and flavone glycosides, flavonols and flavonol glycosides, anthocyanins, chalcones and dihydrochalcones, aurones, flavonones and dihydroflavonols, and flavans and biflavonyls. To date, approximately 9000 different flavonoids have been identified from plant sources [217]. Great interest has been dedicated to the antioxidant properties of flavonoids that may function as potent free radical scavengers, reducing agents, and protectors against peroxidation of lipids [208, 218]. Reviews have been published documenting numerous studies on antioxidant efficacy of flavonoids and phenolic compounds as well as on the relationship between their antioxidant activities, as hydrogen donating free radical scavengers, in relation to their chemical structures. The importance of the unsaturation in the C ring of quercetin compared to catechin in the increased antioxidant activity of the former has been presented [216, 219–223]. Also, the importance of the position and number of hydroxyl groups on the phenolic rings in increasing or decreasing the antioxidant properties of these compounds has been emphasized [216, 219–223].

Although many flavonoids have been isolated from different African medicinal plant extracts, the structure-activity relationship of these compounds has not yet been investigated. Recent studies have also shown that some flavonoids are modulators of proinflammatory gene expression, thus leading to the attenuation of the inflammatory response [224]. Examples of these include the lipophilic flavones and flavonols 5,7-dihydroxy-2′,3′,4′,5′-tetramethoxyflavone, 5,4′-dihydroxy-7,2′,3′,5′-tetramethoxyflavone, and 5,7,4′-trihydroxy-2′,3′,5′-trimethoxyflavone isolated from *Psiadia punctulata* [225] and Dinklagin B and C isolated from *Dorstenia dinklagei* [226]. Isolated flavone and flavonol glycosides include kaempferide 3-O-beta-xylosyl (1⟶2)-beta-glucoside, kaempferol 3-O-alpha-rhamnoside-7,4′-di-O-beta-galactoside, kaempferol 3,7,4′-tri-O-beta-glucoside and quercetin 3-O-[alpha-rhamnosyl (1⟶6)] [beta-glucosyl (1⟶2)]-beta-glucoside-7-O-alpha-rhamnoside from *Warburgia ugandensis*, and quercetin-7,4′-disulphate from *Alchornea laxiflora* [159]. Flavanones and dihydroflavonols include dorsmanin I and J and epidorsmanin F and G isolated from *Dorstenia mannii* [227] and Dinklagins A, isolated from the twigs of *Dorstenia dinklagei* [226] and two flavones isolated from the twigs of *Eriosema robustum* [182] and 1α,3β-dihydroxy-12-oleanen-29-oic (1), 1-hydroxy-12-olean-30-oic acid (2), 3,30-dihydroxyl-12-oleanen-22-one (3), and 1,3,24-trihydroxyl-12-olean-29-oic acid (4), a new pentacyclic triterpenoid (1α, 23-dihydroxy-12-oleanen-29-oic acid-3β-O-2,4-di-acetyl-l-rhamnopyranoside) (5) from *Combretum imberbe* [138]. Anthocyanins isolated include the cyanidins 3-*O*-(2″-galloyl-β-galactopyranoside) and 3-*O*-(2″-galloyl-6″-*O*-α-rhamnopyranosyl-β-galactopyranoside) from *Acalypha hispida* [228] and cyanidin 3-*O*-β-D-glucopyranoside and cyanidin 3-*O*-(2-*O*-β-D-xylopyranosyl)-β-D-glucopyranoside from *Hibiscus sabdariffa* [266]. When revising the literature, it became apparent that even though most of these medicinal plants and compounds have confirmed antioxidant activity, not many of them have been screened for wound healing potential. As there is an association between antioxidative therapy and wound healing, research in this direction is as imminent as it is important. Furthermore, structure-activity studies on the isolated compounds from African medicinal extracts will be of great interest.

Antioxidants may exert their protective effects via different mechanisms at different stages of the oxidation process. There are those that are able to inhibit the production of free radicals via their ability to chelate transition metal ions and those that are able to quench and stabilise free radicals [229, 230]. Additionally, they are further subdivided into categories according to their functions [230]. Such classification of the newly isolated antioxidant compounds from African medicinal plant extracts is warranted to better understand their antioxidant properties.

It should be noted that the antioxidant activity of the extracts and compounds listed in this review was mostly determined using either single assays or *in vitro* analysis. It is therefore possible that some of these extracts and compounds may not show antioxidant activity when alternative testing methods are used. Furthermore, although *in vivo* studies are encouraged, most studies cited used *in vitro* assays. As

antioxidant activity *in vitro* does not necessarily translate to activity *in vivo*, due to pharmacokinetic and pharmacodynamic processes that occurs *in vivo*, it is possible that samples may not be active when tested in animals. Activity of such samples should therefore be confirmed using animal models.

Additionally, attempts should be made to identify the compounds responsible for the proven antioxidant properties where not yet done, and in cases where they have been isolated, their wound healing properties should be investigated. If the activity of the compounds and plants identified in this review is confirmed *in vivo*, they could serve as viable sources for the treatment of wounds in future.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Chemical Composition and Antifungal *In Vitro* and *In Silico*, Antioxidant, and Anticholinesterase Activities of Extracts and Constituents of *Ouratea fieldingiana* (DC.) Baill

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Ouratea fieldingiana (Gardner) Engl is popularly used for wound healing. This study describes the main chemical compounds present in extracts of *O. fieldingiana* and evaluates their biological potential by investigating antifungal, antioxidant, and anticholinesterase activities. The action mechanism of main antifungal compound was investigated by molecular docking using the enzyme sterol 14-α demethylase, CYP51, required for ergosterol biosynthesis. The seeds and leaves were extracted with ethanol in a Soxhlet apparatus and by maceration, respectively. Both extracts were subjected to silica gel column chromatography for isolation of main constituents, followed by purification in sephadex. The structures of compounds were established by ^{1}H and ^{13}C-NMR spectroscopy and identified by comparison with literature data as amentoflavone and kaempferol 3-*O*-rutinoside, respectively. The antioxidant activities of the extracts were determined by the DPPH and ABTS free radical inhibition methods. In general, the extracts with the highest antioxidant activity corresponded to those with higher content of phenolic compounds and flavonoids. The ethanol extracts and two isolated compounds presented relevant antifungal activity against several *Candida* strains. The *in silico* findings revealed that the compound amentoflavone coupled with the CYP450 protein due to the low energy stabilization (-9.39 kcal/mol), indicating a possible mechanism of action by inhibition of the ergosterol biosynthesis of *Candida* fungi.

1. Introduction

This work investigates the therapeutic potential of *Ouratea fieldingiana*, a shrub of the Ochnaceae family, found mainly in Ceará and Rio Grande do Norte states (Brazil). This family presents tropical and subtropical characteristics, with arboreal, shrubby, and rare herbaceous representatives, including about 40 genera and 600 species.

The oil of *O. fieldingiana* seeds, extracted by decoction, is popularly used for healing skin wounds [1]. The oil obtained by extraction with hexane from *O. fieldingiana* seeds presented antibacterial and antifungal activities [2]. The results of another study showed that the anti-inflammatory activity *O. fieldingiana* seed oil was also associated with the presence of phenolic compounds and fatty acids [3]. The treatment with *Ouratea* sp. seed oil showed a collagen effect, which may be associated with the high levels of omega-6 and omega-9. Finally, [4] concluded that *Ouratea* sp. oil has good therapeutic potential in a model of cutaneous wound healing.

Plants of the *Ouratea* genus contain several types of flavonoids, like flavones, flavonols, isoflavones, chalcones, and anthocianins in the form of monomers, glycosides, bi- or bisflavonoids, like hexaspermone, amentoflavone, agatisflavone, robustaflavone, and lanaraflavone. The biflavonoid 7″-O-methylagatisflavone (from *O. hexasperma*), amentoflavone (from *O. semiserrata*), and the acetylated derivative of amentoflavone presented DNA inhibitory activity to poisomerase type I, and potent inhibition of the growth of Ehrlich carcinoma cells [5].

Alzheimer's disease is neurodegenerative and has a strong socioeconomic impact, being associated with neurotransmitter deficits in the brain [6]. A known treatment for this disease is restoration of the cholinergic function, for which compounds that inhibit the acetylcholinesterase enzyme (AChE) improve the content of the neurotransmitter acetylcholine [7]. Then the oil and extracts of *O. fieldingiana* were also tested for inhibitory activity of acetylcholinesterase (AChE), aiming for new compounds to fight against Alzheimer's disease.

The wound healing action is closely associated with the antimicrobial [8] and antioxidant [9] activities of medicinal plants, so in this study several extracts and constituents of *O. fieldingiana* were evaluated as antioxidant and antifungal activities against *Candida* strains.

It is estimated that *Candida* infections (*Candida albicans*, *Candida parapsilosis*, *Candida glabrata*, *Candida tropicalis*, and *Candida krusei*) [10] correspond to 80% of the fungal infections detected in the urinary and blood flow of patients, as well as in the surgical environment [11]. Polyenes, flucytosine, and azole drugs are the most recommended for the treatment of *Candida* infections [12]. Some drugs with antifungal properties have inhibited the enzyme sterol 14-α demethylase, CYP51 [13], which is required for the biosynthesis of ergosterol, essential for the fungal membrane maintenance [14]. Thus, recently *in silico* studies examining the drug-protein interaction by the molecular docking method [15] have become a powerful tool to characterize possible compound activity in a given protein [16].

2. Materials and Methods

2.1. General Experimental Procedures. The isolation of compound was performed in an open column chromatography (60 cm length and 3 cm diameter) using silica gel 60 (60–120 mesh size, Merck) as stationary phase, being eluted with solvents hexane, chloroform, ethyl acetate, and methanol in mixtures of increasing polarity. The compounds were visualized by UV detection and/or sprayed with a solution of vanillin/sulphuric acid/EtOH. The 1D and 2D NMR data were acquired with a Bruker Avance DPX-500 spectrometer. Chemical shifts, given on the δ scale, were referenced to the residual undeuterated portion of the deuterated solvent $CDCl_3$.

2.2. Plant Material. Leaves, branches, and seeds of *O. fieldingiana* collected in the city of Itapipoca, Ceará, Brazil, were used. An exsiccate with plant parts was prepared and deposited in the Prisco Bezerra Herbarium of the Federal University of Ceará, under number 57817.

2.3. Preparation of Ethanol Extracts with Leaves and Branches of O. fieldingiana. The plant material was collected and dried in the sun. After drying, 1 kg of leaves and 590 g of branches were obtained, which were shredded in a domestic multiprocessor. The leaves were macerated with 10 L of ethanol PA 96° GL, for seven days at room temperature; then the solution was evaporated under reduced pressure (40 rpm at 60°C) leaving the leaf ethanol extract, LEE (50.82 g). Also, 590 g of branch was placed in 5 L of 96% ethyl alcohol to obtain the branch ethanol extract, BEE (43.43 g), by the same procedure. The LEE and BEE were passed through a filtration column and eluted with the solvents hexane, chloroform, ethyl acetate, and methanol to obtain the respective fractions, LEE (HF: hexane fraction), LEE (CF: chloroform fraction), LEE (EAF: ethyl acetate fraction), LEE (MF: methanol fraction); BEE (HF: hexane fraction), BEE (CF: chloroform fraction), BEE (EAF: ethyl acetate fraction), and BEE (MF: methanol fraction).

2.4. Preparation of O. fieldingiana Seed Extracts. The crushed seeds were submitted to extraction with hexane and then ethanol in a Soxhlet apparatus, obtaining the respective hexane (SHE) an ethanol extracts (SEE).

2.5. Determination of the Total Phenol Content of the Leaf, Branch, and Seed Extracts of O. fieldingiana. Extracts samples (7.5 mg) were dissolved in 25 mL of methanol and 100 μL aliquots were taken for analysis by the Folin-Ciocalteu method, which is based on the oxyreduction reactions between the phenolic compounds and metal ions, causing the formation of a blue complex, which is absorbed at 750 nm [17]. A standard curve was prepared with gallic acid. Indicate formula used in the calculation of total phenol content and total flavonoid content. The equation for the calibration curve of gallic acid was Y = 0.0013X - 0.018, where X is the concentration of gallic acid, Y is the absorbance at 750 nm, and the correlation coefficient R = 0.998. All analyses for calculations of total phenol content were performed in triplicate. The results were analyzed by Microsoft Excel 2010 [18].

2.6. Determination of the Flavonoid Content of Leaf, Branch, and Seed Extracts O. fieldingiana. Quantification of the flavonoid content of extracts (at the concentration of 2 mg/mL) was performed using 2% aluminum chloride in methanol in a spectrophotometer, with readings at 425 nm. A standard curve was prepared with quercetin. The quercetin

calibration curve equation was Y = 0.04215X - 0.0118, where X is the quercetin concentration, Y is the absorbance at 425 nm, and the correlation coefficient R = 0.996. All analyses for calculations of flavonoid content were performed in triplicate. This test was performed in triplicate and followed the method proposed by [19].

2.7. Determination of Antioxidant Activity of Leaf, Stem, and Seed Extracts of O. fieldingiana

2.7.1. By the DPPH Method. Several solutions of the extracts of *O. fieldingiana* were prepared in the following concentrations: 250, 125, 25, 12.5, 1.25, 0.25, 0.125, and 0.025 μg/mL. The negative control was a DPPH methanol solution and the positive control was prepared by mixing a standard (quercetin) and DPPH. Methanol solutions of the extracts (100 μL) were mixed with 3.9 mL of a DPPH solution; then the solutions were stored in the dark for 60 minutes and the reading was performed by spectrophotometer at the wavelength of 515 nm [18, 20]. The DPPH free radical inhibition was calculated by the scavenging index (SI_{50}) = ($Abs_{DPPH} - Abs_{Sample}$) x 100 / Abs_{Sample}.

2.7.2. By ABTS Method (2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic Acid). The $ABTS^{+\bullet}$ solution (7 mM, 5 ml) was mixed with 88 μl of potassium persulfate (140 mM). The mixture was shaken and kept in the dark at room temperature for 16 h. Then, 1 ml of this solution was added to 99 ml of ethanol. The absorbance was read at 734 nm (0.715). Several solutions of decreasing concentrations of plant extracts (10000 to 5 μg/mL) were prepared and 3.0 ml of an $ABTS^{+\bullet}$ solution was added to 30 μl of these solutions, and after 6 min readings were taken at 734 nm [21]. In order to evaluate the radical scavenging activity, the percentage of inhibition was obtained according to the following equation: IP% inhibition = ($Abs_{ABTS} - Abs_{Sample}$) x 100 / Abs_{ABTS}

The effective concentration of the antioxidant required to decrease the initial ABTS concentration by 50% (EC_{50}) was calculated through a linear regression curve plotted with Excel. To plot the points, the values of the means obtained from triplicates were used for each of the tests.

2.8. Isolation and Characterization of the Constituents of the Leaf and Seed Ethanol Extracts of O. fieldingiana.

The extracts from the leaves and seeds were passed through a vacuum-filter chromatographic column and eluted with solvents of increasing polarities: hexane, chloroform, ethyl acetate, and methanol. Fractions were obtained and compared by thin layer chromatography (CCD) for the isolation of the constituents. The isolated compounds were subjected to spectroscopic analyses, mainly ^{1}H and ^{13}C-NMR nuclear magnetic resonance and mass spectrometry, to determine the chemical structures

2.9. Qualitative Determination of Acetylcholinesterase Inhibitory Activity.

The bioassay consisted of application of the samples in TLC plates, which were prepared by mixing of 5,5-dithiobis-2-nitrobenzoic acid (DTNB or Ellman's reagent) and a buffer solution of acetylthiocholine iodide (ATCI). Subsequently, the AChE enzyme was sprayed and after 3 min the presence of spots (halos) was observed and measured (in mm) on the yellow plate [22, 23]. Physostigmine was used as a positive control because it is the best chemical substance to inhibit acetylcholinesterase.

2.10. Quantitative Determination of Acetylcholinesterase Inhibitory Activity.

The anticholinesterase activity was quantitatively measured using a Biotek ELISA microplate reader (model ELX 800 with Gen5 V2.04.11 software), based on the method described by [16], as modified by [24].

2.11. Antifungal Susceptibility Tests.

The extracts SHE, SEE, LEE, LEE (FM), BEE, and BEE (AF) were tested against two fungal strains from the culture collection, *Candida parapsilosis* (ATCC® 22019™) and *Candida krusei* (ATCC® 6258™), and two other fluconazole-resistant strains, from the Laboratory of Bioprospection and Experimentation of Yeasts of Federal University of Ceará (LABEL). These were seeded in Sabouraud dextrose agar and incubated at 35°C for 24 h. In the antifungal tests with amentoflavone and kaempferol 3-*O*-rutinoside, three strains from the collection were used: *Candida albicans* (ATCC® 14053™), *Candida parapsilosis* (ATCC® 22019™), and *Candida krusei* (ATCC® 6258™), as well as one clinical fluconazole-resistant strain. These were also seeded in Sabouraud dextrose agar and incubated at 35°C for 24 h.

The microdilution method was used in accordance to the document M27-A3 [25], using the culture medium RPMI 1640 (pH 7.0 ± 0.1) buffered with 0.165 M of morpholinepropanesulfonic acid (MOPS) (Sigma, USA). All samples were prepared in dimethyl sulfoxide (DMSO) (Sigma, USA) in a maximum 2% proportion to avoid interference in the microorganism structure. The extracts SHE, SEE, LEE, LEE (FM), BEE, and BEE (AF) were tested in concentrations ranging from 1000 to 1 μg/mL and the compounds amentoflavone and kaempferol 3-*O*-rutenoside in the range from 500 to 0.97 μg/mL.

Compounds were tested together to ascertain synergism at a concentration range from 2.5 to 0.0049 mg/mL (1:1). An initial inoculum suspension was prepared from 24 h culture of the yeasts to be tested, adjusted to 0.5 on the McFarland scale using Sabouraud dextrose agar. Serial dilutions were then prepared in RPMI 1640 medium to obtain final inocula containing 0.5 to 2.5 x 10^3 CFU / mL. The microplates were incubated for 24 h at 35°C (± 2°C). The readings were performed visually as recommended by the Clinical and Laboratory Standards Institute (CLSI).

The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the drug capable of inhibiting 50% and 100% growth of the microorganism, for the tests of the substances SHE, SEE, LEE, LEE (MF), BEE, and BEE (MF) compared to the control also containing only the culture medium and the standardized inoculum [25]. The tests were performed in triplicate.

For the tests of the isolated compounds, the minimum inhibitory concentration (MIC) was determined as the lowest concentration of the drug capable of inhibiting 50% growth of the microorganism, compared to that in the

control also containing only the culture medium and the standardized inocula [25]. The tests were performed in triplicate.

2.12. In Silico Analysis of the Properties of Amentoflavone. The geometric arrangement of a given molecule can present structural similarity with other compounds already identified, establishing a correlation in biological activity [26]. Therefore, the structural data of the amentoflavone molecule were obtained from the PubChem database [27] for analysis of the structural similarity by comparison with the DrugBank database, with similarity limit, ST: 0.7 [28]. Then a three-dimensional optimization of the conformation of the amentoflavone was prepared using the MarvinSk program [29], with valence checking and geometric analysis of the molecule, to minimize the steric hindrance, and the energy minimization was performed by the MMFF94 force field [30]. Finally, the theoretical LD_{50} of the amentoflavone was calculated by the ProTox server [31], and the toxicity of the compound was predicted using the Toxin-Toxin target database (T3DB), which presents 3,673 toxins [32].

2.13. Obtaining the Candida CYP51 Protein Molecule and Optimization of Its Spatial Structure. The protein *Candida albicans*, CYP51 (id: 5v5z), used as a molecular target in the computational simulations, was obtained from the Protein Database, PDB [33], and was edited to remove the water molecules around the surface of the protein and to add polar hydrogen atoms by the PyMOL 2.0 program [34].

2.13.1. Molecular Redocking and Docking of Itraconazole Coupled to C. albicans Protein. The itraconazole linked to the catalytic site of the *C. albicans* CYP51 (PDB: 5v5z) protein was removed by the PyMOL 2.0 program and then redocked in the autodock Vina, serving as reference for the docking. The molecular docking of amentoflavone was carried by addition Kollman charges [35], using AutoDockTools 1.5.6 [36]. Also, the chosen program used the protein as input, whereas the amentoflavone molecule was in the flexible form, having greater freedom regarding torsion angles in the search for a favorable conformation [37]. In this way, a grid box was created respecting the protein group HEME (C_{34} H_{32} Fe N_4 O_4), around the active site of the protein with coordinates x: -43.454; y: -13.913; z: 23.332, in AutoDockTools 1.5.6 [36]. After the execution of the program, the molecules with the best position within the active site of the protein were chosen, with lower binding energy found in kcal/mol (score = -ΔG) [38], and the root mean square deviation (RMSD) was calculated [39], using the VMD program [40].

2.14. Statistical Analysis. For each parameter evaluated, the mean ± standard deviation (SD) was calculated. In the case of multiple comparisons between groups, the homogeneity of the variables involved was tested through the Bartlett test. When homogeneity was observed between the variables, analysis of variance (ANOVA) was applied, followed by the Tukey test. All conclusions were taken at the significance level of 5% ($p < 0.05$). The tests were performed in triplicate and the values expressed as mean ± standard deviation. The experiment was completely randomized, where the extracts were the treatments.

In the antifungal test, experiments measuring the susceptibility of compounds and the expression of synergism profiles *in vitro* were performed in triplicate. The geometric mean of the three trials was used to compare the MIC_{50} results statistically.

3. Results

3.1. Structural Characterization of Chemical Constituents. The chromatographic treatment of the seed ethanol extract led to the isolation of a compound identified by spectral data in comparison to literature data [41, 42] as kaempferol 3-*O*-rutinoside, not previously reported in this plant. The ^{1}H-NMR spectra of this compound showed several peaks in the aromatic region from 6.21 to 8.06 ppm and hydrogens of two sugar units with a signal at 5.12 ppm and another at 4.51 ppm, characteristic of glucose and rhamnose anomeric hydrogen, respectively.

The methanol fraction of the leaf ethanol extract was chromatographed in a silica gel column and a compound was isolated, whose chemical structure was established by ^{1}H and ^{13}C-NMR analyses. The ^{1}H-NMR of this compound showed several peaks in the aromatic region from 6.29 to 8.41 ppm and the ^{13}C-NMR spectrum displayed 30 signals in the Csp^2 region, revealing the dimeric flavonoid characteristic. By comparison with literature data [41, 42] it was characterized as amentoflavone, a biflavonoid present in other *Ouratea* species [5]. The chemical structures of two isolated compounds are displayed in Figure 1.

The complete spectral assignments of hydrogens and carbons are shown below.

Amentoflavone Assignments. ^{1}H NMR (MeOD, 300 MHz): δ 6.60 (s, H-3), 6.16 (d, 2.0, H-6), 6.22 (d, 2.0, H-8), 8.14 (d, 2.2, H-2'), 7.09 (d, 8.6, H-5'), 7.87 (dd, 8.6, 2.2, H-6'), 6.29 (s, H-6"), 7.59 (d, 8.7, H-2"'), 6.64 (d, 8.7, H-3"'), 6.64 (d, 8.7, H-5"'), 7.59 (d, 8.7, H-6"').

^{13}C NMR (MeOD, 75 MHz): δ 165.11 (C-2), 102.23 (C-3), 182.36 (C-4), 161.67 (C-5), 98.90 (C-6), 164.95 (C-7), 93.80 (C-8), 155.18 (C-9), 103.78 (C-10), 120.61 (C-1'), 131.40 (C-2'), 122.31 (C-3'), 161.06 (C-4'), 118.25 (C-5'), 126.68 (C-6'), 164.21 (C-2"), 101.80 (C-3"), 182.58 (C-4"), 157.95 (C-5"), 100.96 (C-6"), 167.71 (C-7"), 106.16 (C-8"), 161.54, (C-9"), 103.04 (C-10"), 121.89 (C-1"'), 127.90 (C-2"'), 115.38 (C-3"'), 160.91 (C-4"'), 115.38 (C-5"'), 127.90 (C-6"') [42].

Kaempferol-3-O-Rutinoside NMR Assignments. ^{1}H NMR (MeOD, 300 MHz): δ 6.21 (1H, d, J = 1.9, H-6), 6.41 (1H, d, J = 1.9, H-8), 8.06 (2H, d, J = 8.8, H-2′,6′), 6.88 (2H, d, J = 8.8, H-3′,5′), 5.12 (1H, d, J = 7.2, Glc H-1), 4.51 (1H, s, Rha H-1), 0.88 (3H, s, Rha-CH_3), 3.25–3.82 (other H). ^{13}C NMR ($CDCl_3$, 75 MHz) [41].

^{13}C NMR (MeOD, 75 MHz): δ 159.58 (C-2), 135.68 (C-3), 179.57 (C-4), 163.15 (C-5), 100.15 (C-6), 166.16 (C-7), 95.09 (C-8), 158.71 (C-9), 105.85 (C-10), 121.00 (C-1'), 132.51 (C-2'), 116.30 (C-3'), 161.62 (C-4'), 116.30 (C-5'), 132.51 (C-6'), 104.77

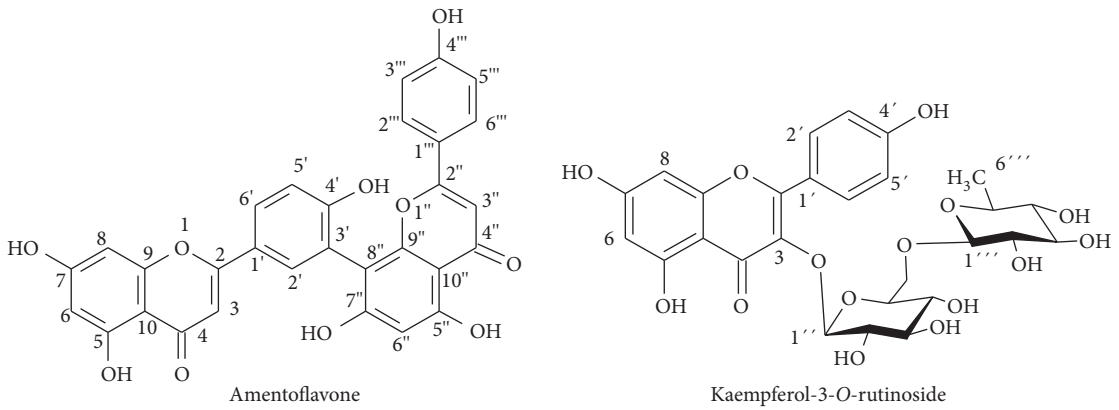


Figure 1: Chemical representation of compounds present in *Ouratea fieldingiana*.

Table 1: Phenol and flavonoids content and antioxidant activity of *O. fieldingiana* extracts.

Extracts and constituents	**Total Phenols (mg GAE/g)***	**Flavonoids (mg QE/g)****	**DPPH IC_{50} (μg/mL)**	**ABTS (μg/mL)**
LEE	35.33 ± 22.15[b]	8.978 ± 0.267[a]	4.953 ± 0.884[a]	5.117±2.605[a]
LEE (HF)	16.03 ± 14.29	3.798 ± 0.284	64.345 ± 0.227	63.210±1.577[d]
LEE (CF)	12.70 ± 8.38	1.261 ± 0.929	72.436 ± 0.359	59.613±1.916[d]
LEE (EAF)	23.30 ± 16.93[b]	5.216 ± 0.523	57.147 ± 2.085	9.654±0.161[b]
LEE (MF)	26.89 ± 9.46[a]	8.657 ± 0.195[a]	5.394 ± 0.03[a]	5.235±0.195[a]
BEE	61.81 ± 7.15[a]	7.225 ± 0.779[a]	5.898 ± 0.291[a]	4.195±0.0283[a]
BEE (HF)	9.60 ± 3.39	1.517 ± 0.241	310.486 ± 34.743	194.213±3.418[d]
BEE (CF)	8.013 ± 7.403	1.259 ± 0.094	108.386 ± 4.653	31.753±2.386[d]
BEE (EAF)	32.07 ± 1.29[b]	4.283 ± 0.123	7.577 ± 0.216[a]	6.166±0.164[a]
BEE (MF)	13.69 ± 6.99	0.979 ± 0.198	63.433 ± 3.456	62.455±0.018[d]
SEF	7.344±1.1482	0.229±0.0721	31.324±6.289	23.898±1.036[c]
SEE	6.007±3.007	0.688±0.095	71.092±1.116	27.676±0.467[c]
SAE	46.274±19.6[a]	5.849±0.296	16.096±0.636	64.228±0.298[d]
Amentoflavone	-	-	35.612±2.440	83.306±2.6354[d]
Kaempferol 3-*O*-rutinoside	-	-	30.962±1.5317	75.752±6.082[d]
Quercetin	-	-	4.779 ± 0.507[a]	1.738±0.089[a]

*Total phenols are quantified in milligrams per gallic acid equivalent. *Flavonoids are quantified in milligrams per quercetin equivalent. *Confidence interval: 95%; LEE: leaf ethanol extract (LEE), BEE: branch ethanol extract, SEF: seed ethanol fraction, SEE: seed ethanol extract, and SAE: seed aqueous extract. HE: hexane fraction, CF: chloroform fraction, EAF: ethyl acetate fraction, and MF: methanol fraction.

(C-1"), 74.07 (C-2"), 78.32 (H-3"), 71.62 (H-4"), 78.05 (H-5"), 68.74 (H-6"), 102.58 (H-1'''), 72.25 (H-2'''), 72.49 (H-3'''), 73.62 (H-4'''), 69.89 (H-5'''), 18.05 (H-6''', CH_3) [42].

3.2. Determination of the Total Phenol and Flavonoids Content and Antioxidant Activity of Extracts of Leaves, Branches, and Seeds of O. fieldingiana, Table 1. Regarding phenolic content, LEE, LEE (MF), BEE, and BEE (EAF) showed the best results. In relation to flavonoid content, the extracts LEE and LEE (MF) were better. In general, the more polar extracts had higher yields of phenolic compounds.

To evaluate the antioxidant activity of the samples, two different methods, using $DPPH^{\cdot}$ and $ABTS^{+\cdot}$ radicals, were performed. In the $DPPH^{\cdot}$ test, the IC_{50} (concentration capable of inhibiting the radical by 50%) was evaluated and better results were shown by LEE, LEE (MF), BEE, and BEE (FEA).

In relation to the $ABTS^{+\cdot}$ method, the extracts BEE, BEE (MF), BEE (EAF), LEE, and LEE (MF) presented IC_{50} values similar to the standard quercetin. Like the more polar extracts, which presented higher yield of phenolic compounds, these also presented higher antioxidant activities according to both methods.

3.3. Acetylcholinesterase Enzyme Inhibition Test by ELISA. All fractions were submitted to the quantitative anti-acetylcholinesterase test to find the IC_{50} and the results of *O.*

Table 2: Evaluation of acetylcholinesterase inhibition action of extracts and constituents of *Ouratea fieldingiana*.

Extract	IC_{50} in ELISA (μg/mL)
Leaf ethanol extract	0.816 ± 0.004^{a}
Branch ethanol extract	11.89 ± 0.048^{b}
Leaf ethanol extract (MF)	36.81 ± 0.024^{d}
Branch ethanol extract (EAF)	57.58 ± 0.088^{e}
Seed hexane extract	20.51 ± 0.387^{c}
Seed ethanol extract	12.15 ± 0.003^{b}
Seed aqueous extract	9.19 ± 0.030^{b}
Kaempferol-3-*O*-rutinoside	17.70 ± 0.030^{c}
Amentoflavone	11.92 ± 0.046^{b}
Physostigmine (standard)	1.15 ± 0.046^{a}
Eserine (standard; Penido et al., 2016)	19.53 ± 0.08^{c}

Data presented are mean ± standard deviation, according to ANOVA followed by the Tukey test. Values with different small letters differ statistically ($p<0.05$); EAF: ethyl acetate fraction; MF: methanol fraction.

fieldingiana fractions are shown in Table 2. The leaf ethanol extract showed the best anticholinesterase action, similar to physostigmine, the alkaloid standard. The biflavonoid amentoflavone is present in the leaf ethanol extract, but the extract showed superior results probably due to the synergism among the main constituents, Table 2.

3.4. Antifungal Tests. In general, the extracts demonstrated antifungal activity against all isolates except for EHS, and especially against ATCC strains, because resistant strains were less active, Table 3.

Among the isolated compounds, amentoflavone was more active against fungal isolates than kaempferol-3-O-rutinoside, but the mixture of the two constituents revealed synergism since MIC 50 values were lower against all strains tested, Table 4.

3.4.1. In Silico Analysis of the Properties of Amentoflavone. The atomic coordinates of the amentoflavone molecule, obtained from PubChem (CID 5281600), had no valence error. In the geometric analysis, the molecule was optimized by MMFF94 energy = 163.74 kcal/ mol, minimal projection area = 74.63 $Å^2$, maximal projection area = 126.50 $Å^2$, minimal projection radius = 6.79 Å, maximal projection radius = 9.39 Å, length perpendicular to max area = 10.03 Å, length perpendicular to min area = 18.40 Å, van der Waals volume = 432.58 $Å^3$, for improved geometry: MMFF94 energy = 76,24 kcal/ mol, minimal projection area = 62.78 $Å^2$, maximal projection area = 133.27 $Å^2$, minimal projection radius = 6.16 Å, maximal projection radius = 8.65 Å, length perpendicular to max area = 9.33 Å, length perpendicular to min area = 15.58 Å, and van der Waals volume = 441.42 $Å^3$, Figure 2.

In the analysis of the structural similarity by the DrugBank server, amentoflavone ($C_{30}H_{18}O_{10}$) resembled the following molecules: (DrugBank-DB02375) - myricetin ($C_{15}H_{10}O_8$); (DrugBank-DB04216) - quercetin ($C_{15}H_{10}O_7$); (DrugBank-DB07352) – apigenin ($C_{15}H_{10}O_5$); (DrugBank-DB07795) - 3,7,3′,4′ tetrahydroxyflavone ($C_{15}H_{10}O_6$); (DrugBank-DB08230) - 5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-4H-chromen-4-one ($C_{15}H_{10}O_7$); (DrugBank-DB11259)-diosmetin ($C_{16}H_{12}O_6$); (DrugBank-DB12672) -icaritin ($C_{21}H_{20}O_6$). In the toxicity study, amentoflavone obtained an LD_{50} of 3919mg/kg, being classified in category 5 according to the globally harmonized system of classification of labeling of chemicals (GHS).

3.4.2. Molecular Redocking and Docking. A valuable alternative for the study of drug-protein interaction is the molecular docking technique, which evaluates the behavior of a given molecule within the active site of the protein [43]. Therefore, in the simulation of the molecule redocking, the itraconazole pose was obtained at the active site of the CYP51 protein with the lowest binding free energy of -12.73 kcal/mol, with the approximation of the itraconazole pose in the native conformation of the protein CYP51, being observed in the overlap of the links, lower root mean square deviations, RMSD > 2.0 Å [44], Figure 3.

The residues involved in the interaction were Gly65(A); Pro230(A); Phe233(A); Ala61(A); Phe380(A); Met508(A); Ser378(A); Leu376(A); Tyr188(A); Gly303(A); Gly307(A); and Thr311(A), along with the Hem601(A) group, as shown in Figure 4.

The amentoflavone molecule coupling with the CYP51 protein, and the spatial conformation with the lowest free energy of -9.39 kcal/mol, showed 15 binding residues (Ser378(A); Leu376(A); Val509(A); Met508(A); His310(A); Gly307(A); Phe228(A); Ile304(A); Phe126(A); Leu300(A); Leu139(A); Ile131(A); Tyr132(A); Tyr118(A); Ile379(A)), with the group HEME (Hem601(A)), performed hydrophobic interactions, and also two hydrogen bonds with residues: Gln142(A), with a distance of 2,65 Å, and Phe380(A), with a distance of 2.75 Å, with a root mean square deviation (RMSD) >2.0 Å [44], as shown in Figure 5.

4. Discussion

The wound healing action found for the *Ouratea* sp. seed oil displays a collagen effect, attributed to the presence of w-6 and w-9 fatty acids [4]. The main fatty acid in *O. fieldingiana* was oleic (43.06%), a w-9 fatty acid, which can contribute to the healing action. In another work, the effect of the anti-inflammatory activity *O. fieldingiana* seed oil was also associated with the presence of phenolic compounds besides fatty acids [3]. The activity of antioxidant compounds in a mixture depends on several physicochemical factors, like the interactions among other antioxidant compounds and with other constituents, such as fatty acids linked to phospholipids or triacylglycerides. Therefore, an individual antioxidant constituent could give limited results when associated with other constituents in a plant extract [45]. The seed oil of *O. fieldingiana* is also popularly used for wound healing on the skin. This action can be related to the presence of phenolic compounds with antioxidant and antifungal properties, besides the presence of unsaturated fatty acids.

Table 3: Evaluation of the antifungal effect of *Ouratea fieldingina* extracts against *Candida* spp.

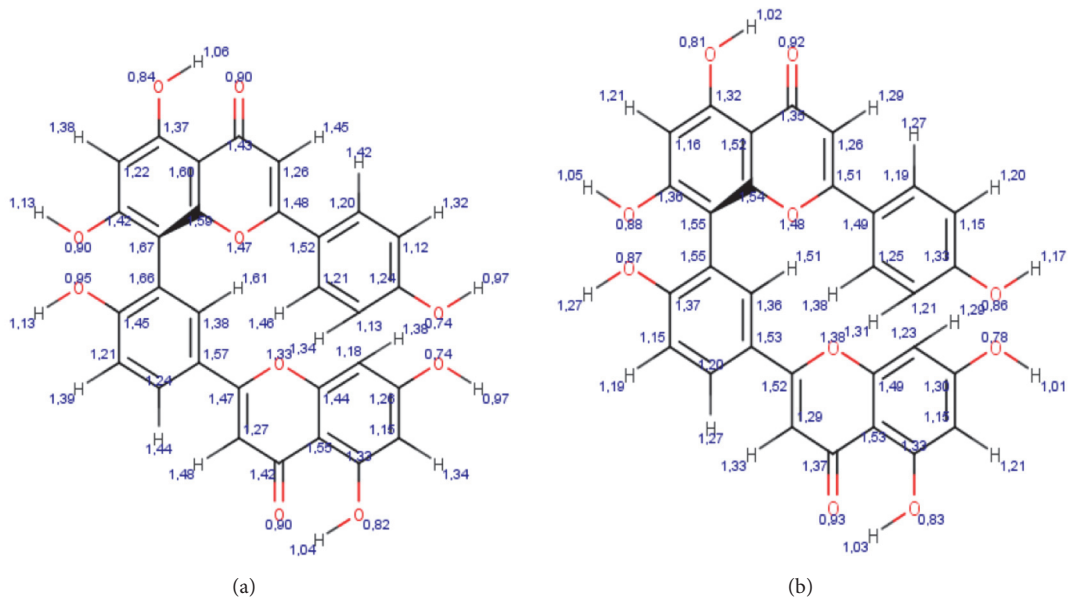
	MIC 50/100 [b] (μg/mL)			
	Strains[a]			
Extracts	ATCC *C. parapsilosis* 22019	ATCC *C. krusei* 6258	*C. tropicalis* (R)	*C. parapsilosis* (R)
SHE	>1000 / >1000	>1000/>1000	>1000/>1000	>1000/ >1000
LEE	500 / > 1000	7.8 / 31.25	500 / > 1000	500 / > 1000
LEE (MF)	500 / > 1000	3.9 / 31.25	1000 / > 1000	125 / > 1000
BEE (EAF)	125 / 500	62.5 / 250	500 / > 1000	500 / > 1000
BEE	15.62 / > 1000	1.95 / 15.6	250 / > 1000	62.5 / > 1000
SEE	250 / 1000	62.5 / 250	1000 / > 1000	1000 / >1000

[a]Yeast strains isolated from collection. [b]MIC was defined as the lowest concentration which produced 50% and 100% reduction of fungal cell growth after 24 h incubation. [c]The procedure was performed according to protocol M27-A3 of CLSI 2008. The range of compounds tested varied from 1000 to 1.95 μg/mL. SHE: seed hexane extract; LEE: leaf ethanol extract; LEE (MF): leaf ethanol extract (methanol fraction): BEE: branch ethanol extract, BEE (EAF): branch ethanol extract (ethyl acetate fraction), and SEE: seed ethanol extract.

Table 4: Evaluation of antifungal effect of amentoflavone and kaempferol against *Candida* spp. Isolates.

	MIC_{50} [b] (μg/mL)			
	Strains[a]			
Compounds	ATCC *C. albicans* 14053	ATCC *C. parapsilosis* 22019	ATCC *C. krusei* 6258	*Candida parapsilosis* (R)
Amentoflavone	125	15.62	15.62	250
Kaempferol 3-*O*-rutinoside	> 500	250	125	> 500
Amentoflavone + kaempferol 3-*O*-rutinoside	1.25	1.25	0.625	2.5

[a]Yeast cells isolated from collection. [b]MIC was defined as the lowest concentration which produced 50% reduction of fungal cell growth after 24 h incubation. The procedure was performed according to protocol M27-A3 of CLSI 2008. The concentrations of compounds tested ranged from 500 to 0.97 μg/mL.



(a) (b)

Figure 2: Optimization of the geometry of the amentoflavone molecule. (a) Before energy minimization by the MMFF94 force field. (b) After energy minimization by the application of the force field MMFF94.

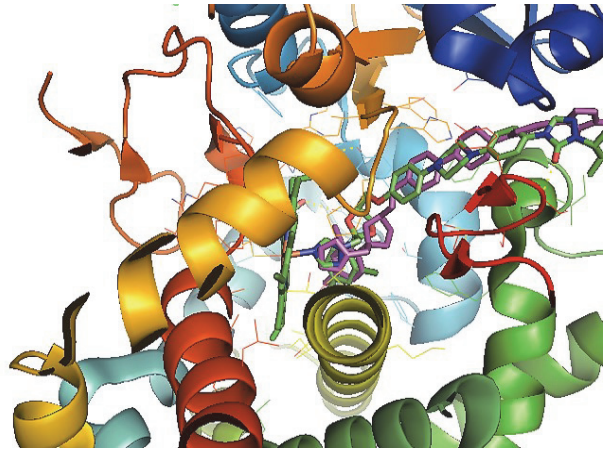


Figure 3: Overlap of the itraconazole binders to the catalytic site of the protein CYP51.

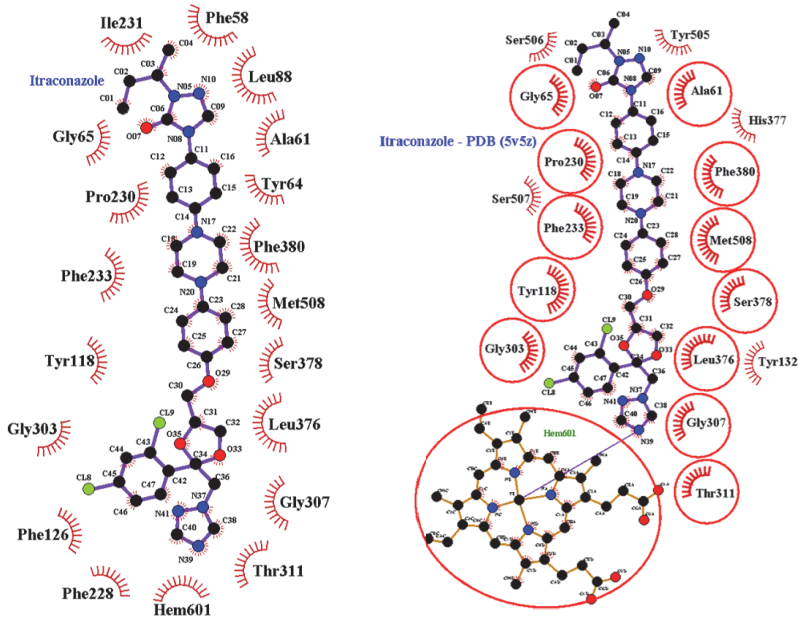


Figure 4: Conformation of compound submitted to the computational test. (Itraconazole) Pose obtained from re-docking molecule. (PDB-5v5z) Itraconazole complexed the CYP51 protein in its native conformation.

Plant derived products have been used to treat many diseases worldwide and are still playing a major role in healthcare. It is known that the wound-healing process can be aided by the presence of antioxidants. The presence of effective antioxidants in various plant extracts is well known and many plant extracts or plant-derived compounds possessing high antioxidant properties also show wound-healing activities [3, 9].

The glycosylated flavonoid nicotiflorin (kaempferol-3-*O*-rutinoside), extracted from the seeds, and amentoflavone, a biflavonoid obtained from the ethanol extract of the leaves of *O. fieldingiana*, displayed antioxidant activity, as previously reported [46]. In that study, the authors showed the antioxidant activity of several flavonoids and their rutinoside derivatives, O-glycosides, and dimers, deducing that the higher the number of hydroxyls, the greater the antioxidant activity. However, the 3′,4′-dihydroxy system, present in quercetin, used as standard in the study of *O. fieldingiana*, probably confers greater antioxidant activity on the molecule.

Infected wounds heal less rapidly, so there is a need to stimulate the healing process and restore the normal functions of the affected part of the body, preventing infection and activating tissue repair processes. Antibacterial and healing compounds in a traditional remedy can induce this occurrence and may be beneficial in treating wounds [8].

The oil from the seeds of *O. fieldingiana* fruits presented antibacterial and antifungal activities [2]. Our study corroborated this action of the oil and extends it to the leaf and branch extracts and the constituents amentoflavone and kaempferol 3-*O*-glucoside, isolated from the leaves and the

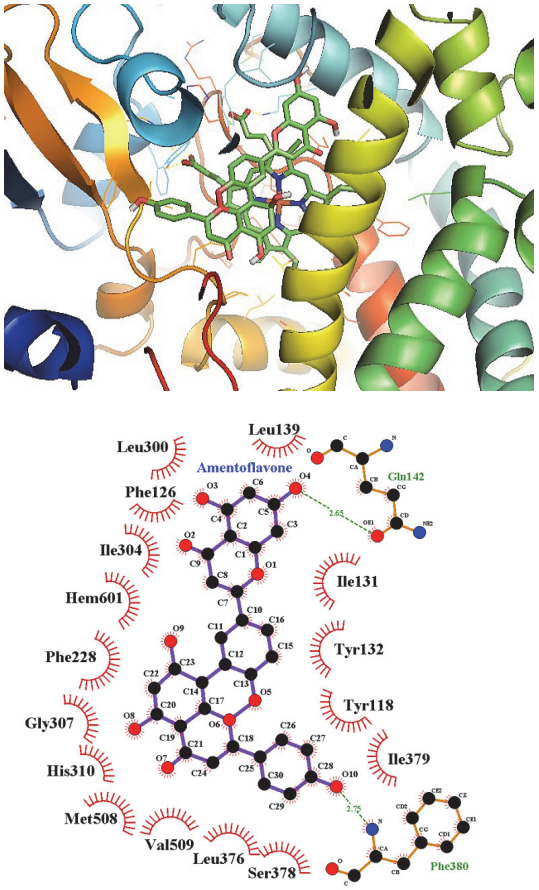


Figure 5: Molecular docking of amentoflavone with the CYP51 protein, characterizing the residues involved in the hydrophobic interaction and hydrogen bond.

seeds, respectively, which also display antimicrobial activities, a useful characteristic in the wound healing process. All the extracts and compounds tested showed antifungal activity within the tested range (1000 to 1.95 μg/mL) against all isolates, except for the hexane seed extract (SHE). However, the active extracts showed selectivity for the *C. krusei* isolate. The substances amentoflavone and kaempferol 3-O-rutinoside used in combination showed superior antifungal activity than the extracts alone.

On the other hand, in the study of drug-protein interaction with the molecular coupling method, it was observed in the simulation of the molecular redocking of the ligand (Itraconazole) with the CYP51 protein of *Candida albicans* that coupling was established at the catalytic site of the protein (-12.73 kcal/mol), with a high level of similarity to the CYP51 native structure site, demonstrating reliability in the coupling simulations [47] (Figure 2). Furthermore, in the coupling simulation of amentoflavone with the CYP51 protein, it was observed that this compound showed favorable interactions with several residues of the active site of the protein and the group (Hem601). This result demonstrates that amentoflavone is a strong candidate as an antifungal drug, when compared to the Irfan and Abid results [48], where the molecular fit with 18 synthetic antifungal triazole derivatives in the CYP51 protein of *Candida albicans* showed a free energy range of -9.8 to -7.4 kcal/mol, including the antifungal fluconazole (-8.6 kcal/mol). Moreover, in the present study, low binding energy (-9.39 kcal/mol) was obtained for amentoflavone, CYP51, near to that of fluconazole, which is considered by ANVISA as a drug of choice for the treatment of fungal infections (especially vaginal candidiasis caused by *Candida* species).

Reactive oxygen species (ROS) are directly involved in the pathogenesis of many diseases and the antioxidants from *O. fieldingiana* act by attenuating the cellular oxidative damage. In this respect, free-radical scavenging was observed in experimentally induced liver injuries [49].

The search for plants that have antimicrobial and antioxidant properties can lead to relatively nontoxic and cost-effective antifungal products [50]. So, using substances with antioxidant properties is a useful way to achieve this goal, since in general good antioxidant plant extracts contain phenolic compounds like flavonoids and organic acids that also display antimicrobial activity. Seeds, leaves, and branches of *O. fieldingiana* were submitted to maceration with several solvents to prepare the respective extracts. These extracts were treated in silica gel chromatographic columns and two flavonoids were isolated. The structure was elucidated by NMR analysis and amentoflavone and kaempferol 3-*O*-rutinoside were identified.

Biflavones, including amentoflavone, have been reported mainly in leaves of various *Ouratea* species [51]. Kaempferol 3-*O*-rutinoside is an antioxidant found in fruits and vegetables that fights free radicals, which promote the development of cancer, besides being a potent promoter of apoptosis [52]. This substance is much less toxic to normal cells than standard chemotherapy drugs [53].

The extracts of *O. fieldingiana* showed excellent antioxidant activity. The standard used was quercetin, a compound that belongs to the class of the most common flavonoids and stands out for its great antioxidant potential. The extracts with the best antioxidant activity were BEE (EAF) and LEE (MF), those containing the largest amount of phenolic compounds. The good antioxidant activity presented by the extracts can be correlated to the presence of flavonoids and other phenols, present in all samples analyzed.

The results found in this study indicate that this species has antioxidant chemicals capable of capturing free radicals. These substances are promising for studies aimed at preventing diseases due to oxidative stress. However, the antioxidant tests performed do not allow a precise definition of the antioxidant effects because they are *in vitro*. *In vivo* study is required to determine whether this medicinal plant can be used effectively for this purpose [45].

Oxidative damage is considered to be one of the most important mechanisms involved in the pathogenesis of Alzheimer's disease, which results in the chemical modification of the biological molecules, leading to neuronal death. It has been reported that plants containing vitamins (C, E, carotenoids, etc.), flavonoids (flavones, isoflavones, flavonones, anthocyanins, and catechins), and polyphenols (ellagic acid, gallic acid, and tannins) possess remarkable antioxidant activities [54].

Other studies have shown that the antioxidant capacity of vegetable oils can be influenced by the concentration of some tocopherols, such as γ- and δ-tocopherols, or phenolic compounds [3, 55]. In a study carried out by [4], the results of the chemical evaluation (by gas chromatography together with mass spectrometry) of the fixed oil of *Ouratea* sp. detected the major components as being the unsaturated fatty acids linoleic acid (40.88%) and oleic acid (28.29%), along with palmitic acid as the saturated fatty acid (20.65%).

The anticholinesterase action of plant extracts in combination with antioxidant properties can also contribute to complementary therapy for Alzheimer's disease.

The inhibition of acetylcholinesterase by the ethanol extract of the *O. fieldingiana* leaves was excellent, with IC_{50} of 0.81μg/mL ± 0.004, being statistically equal to the physostigmine standard, followed by the ethanol extracts of the seeds and branches and isolated compounds. Compared to eserine, another standard used as AChE inhibitor, almost all samples presented activity. Amentoflavone, isolated from the root extract of *Cnestis rustina*, demonstrated antidepressant and anxiolytic effects [56, 57], so our findings confirm the potential of this substance for nervous system problems.

5. Conclusion

O. fieldingiana is a good source of flavonoids, biflavonoids, and fixed oils and is used in folk medicine, mainly for wound healing and relief of inflammation and infectious diseases. It was observed that *O. fieldingiana* extracts are important source of antioxidants. It was possible to isolate a biflavonoid among the more polar constituents of the leaf extracts, while from the seeds a flavonoid glycoside, kaempferol 3-*O*-rutinoside, was identified, which when tested in association with known antimicrobial agent demonstrated superior antifungal action in relation to all other tested materials.

The *in silico* findings revealed that the compound amentoflavone coupled with the CYP450 protein due to the low energy stabilization, indicating a possible mechanism of action by inhibition of the ergosterol biosynthesis of *Candida* fungi.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Additional Points

Highlights. (i) This study corroborates the traditional use for wound healing of *O. fieldingiana*. (ii) Amentoflavone was the main constituent of the plant leaf extract. (iii) The plant extracts displayed antioxidant and anticholinesterase activities. (iv) The antimicrobial activity was demonstrated in *in vitro* and *in silico* tests.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

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Supplementary Materials

Graphical abstract figure shows the chemical compounds and biological actions of *Ouratea fieldingiana* (DC.) Baill. *(Supplementary Materials)*

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Review Article

Plant-Derived Products for Treatment of Vascular Intima Hyperplasia Selectively Inhibit Vascular Smooth Muscle Cell Functions

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Natural products are used widely for preventing intimal hyperplasia (IH), a common cardiovascular disease. Four different cells initiate and progress IH, namely, vascular smooth muscle, adventitial and endothelial cells, and circulation or bone marrow-derived cells. Vascular smooth muscle cells (VSMCs) play a critical role in initiation and development of intimal thickening and formation of neointimal hyperplasia. In this review, we describe the different originating cells involved in vascular IH and emphasize the effect of different natural products on inhibiting abnormal cellular functions, such as VSMC proliferation and migration. We further present a classification for the different natural products like phenols, flavonoids, terpenes, and alkaloids that suppress VSMC growth. Abnormal VSMC physiology involves disturbance in MAPKs, PI3K/AKT, JAK-STAT, FAK, and NF-κB signal pathways. Most of the natural isolate studies have revealed G1/S phase of cell cycle arrest, decreased ROS production, induced cell apoptosis, restrained migration, and downregulated collagen deposition. It is necessary to screen optimal drugs from natural sources that preferentially inhibit VSMC rather than vascular endothelial cell growth to prevent early IH, restenosis following graft implantation, and atherosclerotic diseases.

1. Introduction

Intimal hyperplasia (IH) is a fibroproliferative disorder observed in vascular pathogenesis particularly in vessel anastomotic stenosis, atherosclerosis, blockage of vessel grafts, angioplasty, and in-stent restenosis [1]. IH is characterized by enhanced cell migration, proliferation, and differentiation that cause narrowing of the tunica intima. Several cells are associated with initiation and progression of IH, namely, vascular smooth muscle cells (VSMCs) [2], vascular adventitial cells [3], vascular endothelial cells (VEC) [4], and circulating bone marrow-derived cells [5]. These cells have different origins but may contribute to IH formation. For example, endothelial cells may undergo endothelial-to-mesenchymal transition (EndMT) acquiring a fibroproliferative mesenchymal phenotype whereas adventitia-derived stem cells may migrate to the intimal lesion site and differentiate into fibroblasts. VSMCs play a critical role in the initiation and development of intimal thickening and formation of neointimal hyperplasia [6, 7].

Many herbal medicines sourced from plants or foods have been used to prevent cardiovascular disease over the millennia. For example, green tea contains various flavanols that have antioxidative [8, 9], anti-inflammatory

[10], antimicrobial [11], and hypolipidemic [9] effects. This pharmacological profile helps prevent atherosclerotic plaque formation caused by inflammation and oxidative stress. Red wine, another commonly enjoyed beverage, has long been believed to be rich in polyphenols [12], which act as powerful antioxidants. These assist in preventing lower density lipoprotein oxidation in heart disease and attenuating development of atherosclerotic disease in the hamster model [13, 14]. Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene), a typical polyphenol extracted from red wine, has been proven to inhibit proliferation of VSMCs *in vitro* [15]. Many natural compounds have been reported to be active and to have potential utility as clinical medicines. Tanshinone is an isolate from *Salvia miltiorrhiza* that has been used against cardiovascular disease in China [16]. Therefore, many active compounds with cosmopolitan distribution are being used as herbal medicines or foods, giving hope for screening for potential therapeutic agents against IH (Figure 1).

Recent clinical studies have shown that rapamycin A, an VSMC inhibitor, prevents development of IH-induced vascular endothelial dysfunction [17]. This nonspecific cytotoxicity leads to stenosis and eventually to failure of vascular reconstruction after injury. Therefore, the ideal drug to prevent restenosis or IH is one that inhibits VSMC proliferation selectively while having minimal inhibitory effect on VEC proliferation.

2. Diverse Cells Involved in Vascular IH

As stated earlier, four different cell types are involved in the initiation and progression of IH. These are VSMCs, vascular adventitial cells, VECs, and circulating bone marrow-derived cells (Figure 2). VSMCs play a critical role in the initiation of intimal thickening and the formation neointimal hyperplasia. Physiologically VSMCs exist in two phenotypes, i.e., differentiated cells and proliferating cells, which are responsible for maintaining the homeostasis and function of vascular vessels [2, 6]. Stimulation by certain growth and inflammatory factors, such as platelet-derived growth factor, tumor necrosis factor-α (TNF-α), and thrombin, results in dedifferentiation into mature VSMCs [18, 19]. Mature and differentiated VSMCs exhibit loss of contractility and increased proliferation and expression of ECM protein and various cytokines. This phenomenon is responsible for intimal thickening leading to the neointimal hyperplasia formation that is observed in early-phase atherosclerosis.

Endothelial-to-mesenchymal transition is a phenomenon where endothelial cells acquire a fibroproliferative mesenchymal phenotype through differential stimulation [20]. These transitioned endothelial cells mimic fibroblasts and have increased ECM production and migration capabilities. In vascular diseases, these transitioned endothelial cells can quickly migrate and differentiate into smooth muscle-like cells serving as a potential contributor to IH [21, 22]. EMT is reported to be modulated by shear stress in an ERK5-dependent manner, to contribute to neointimal hyperplasia, and to induce atherogenic differentiation [23]. In addition to adventitia-derived stem cells, circulating smooth muscle progenitor cells have also been implicated in the pathogenesis of neointimal hyperplasia [24] and in the recruitment of endothelial precursor cells after vascular trauma. The presence of bone marrow-derived cells in solid neointima or allograft lesions suggests their crucial involvement in lesion formation following vascular injury [5, 25]. Although various cells contribute to IH pathogenesis, smooth muscle cells are the main culprits in lesion formation. Therefore, therapeutic strategies that maintain VSMCs in a terminally differentiated state and inhibit their proliferation and migration can be useful in preventing neointimal hyperplasia.

3. Antiproliferation, Migration, and Cellular Functions of Abnormal VSMCs as a Target to Decrease Intimal Hyperplasia

VSMCs in the normal vascular tunica media express a range of smooth muscle cell markers including smooth muscle cell myosin heavy chain (MYH11), 22-kDa SMC lineage-restricted protein (SM22α/tagln), alpha smooth muscle actin (ACTA2), and smoothelin. VSMCs *in vitro* and in atherosclerosis undergo phenotypic switching with reduced expression of these markers, while increasing capacity for cell proliferation, migration, and secretion of various ECM proteins and cytokines. These phenotypic switches have long been considered of fundamental significance in IH progression.

Most studies investigating inhibition of VSMCs adopt drugs like rapamycin, sirolimus, or tacrolimus to induce VSMC apoptosis and cell cycle arrest at G1/S phase, suppress ROS production, inhibit VSMC migration, and downregulate collagen deposition. These approaches do not recover the mature VSMC immunophenotypes, but they do decrease neointimal formation and prevent stenosis following vascular injury. To investigate the anticellular function of drugs on VSMCs many models have been established *in vitro* and *in vivo*. For the *in vitro* experiments, inflammatory cytokines like TNF-α or some growth factors such as platelet-derived growth factor (PDGF) are used for inducing abnormal proliferation and migration of VSMCs. For the *in vivo* experiments, IH is usually induced using the vascular endothelial denudation model or carotid artery ligation injury.

Dietary supplements and traditional herbal medicines are complementary medication approaches used in every society and are widely used for preventing IH in Asia and in other developed countries [26]. Many herbal drugs and foods have been verified as suppressing abnormal VSMC growth and inhibiting intima formation. The positive effects of the herbal medicines and plants depend on their active natural compounds including phenols, flavonoids, terpenes, and alkaloids. These natural products are involved in different signaling pathways that regulate abnormal VMSCs to attenuate IH.

4. Typical Signal Pathways Involved in Growth and Physiology of VSMCs in IH Disease

The six signaling pathways involved in most drug inhibitory VSMC studies (Figure 3) are mitogen-activated protein

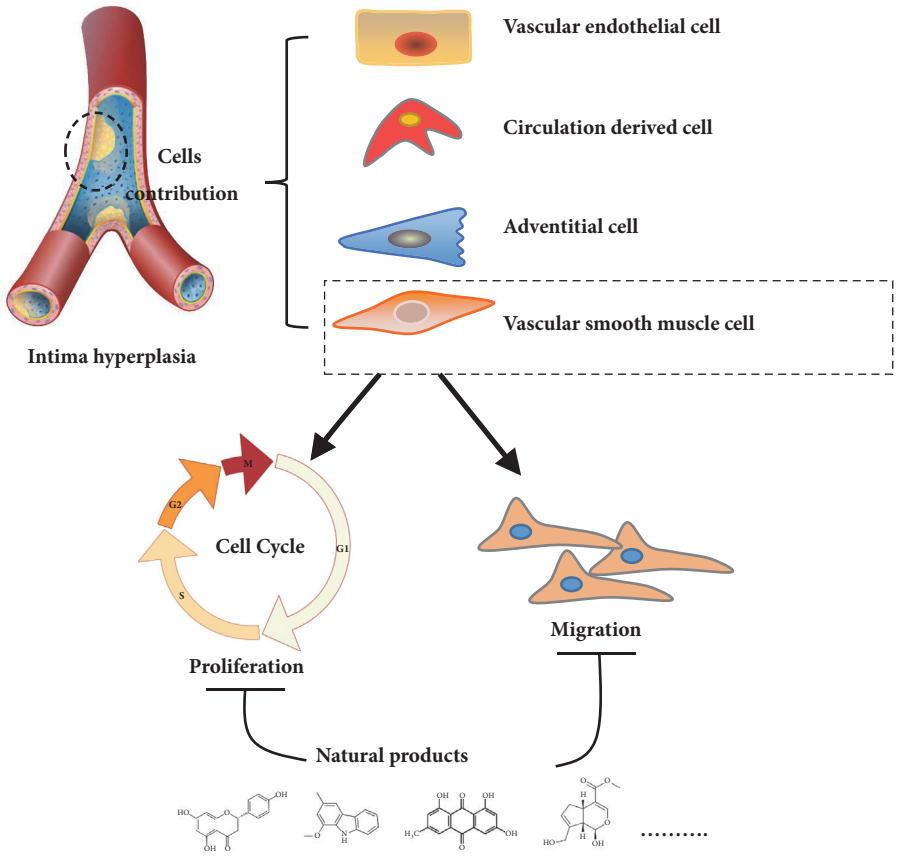


Figure 1: Graphic abstract for different natural compounds for inhibiting vascular smooth muscle cells proliferation and migration.

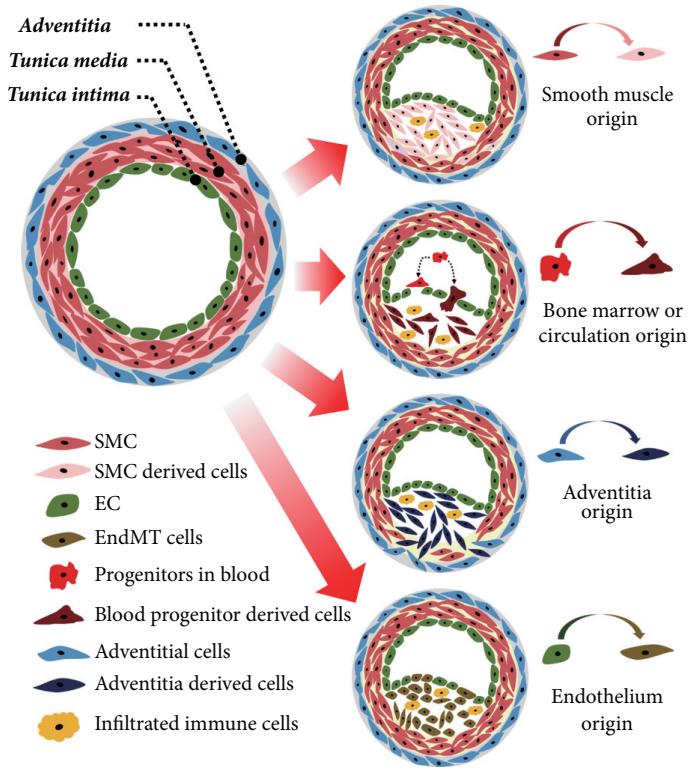


Figure 2: Four different cell origins contribute to blood vessel stenosis.

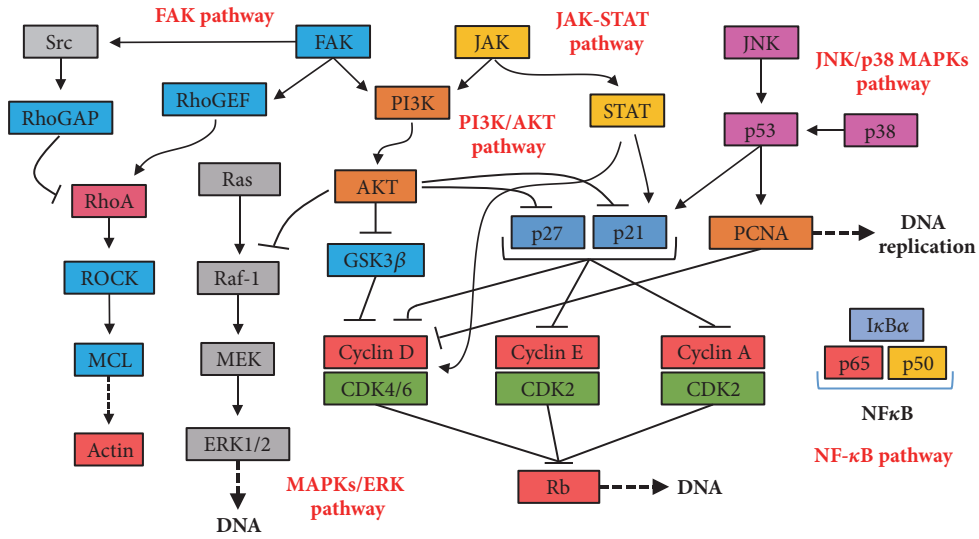


Figure 3: Key genes and pathways involved in restraining cell cycle and movements of VSMCs with natural products.

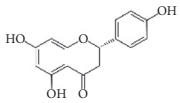
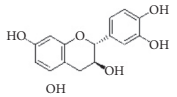
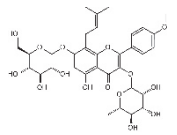
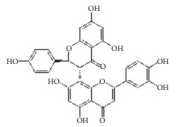
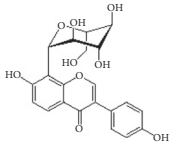
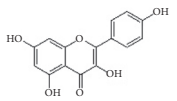
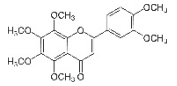
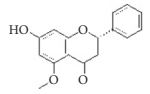
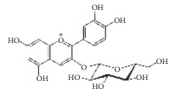
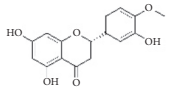
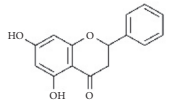
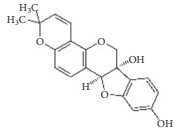
kinases/extracellular signal-regulated kinase (MAPKs/ERK), phosphatidylinositol 3-kinases/Akt (PI3K/Akt), Janus kinase-signal transducer and activator of transcription (JAK-STAT), focal adhesion kinase (FAK), and nuclear factor kappa-light-chain-enhancer of activated B (NF-κB). MAPKs are involved in cell proliferation, differentiation, mitosis, cell survival, and apoptosis [27]. Three major families of MAPKs are extracellular signal-regulated kinase (ERK) [28], p38 kinase, and c-Jun N terminal kinase (JNK). These contribute to the two important signaling pathways, Ras/ERK-MAPK and JNK/p38-MAPK, which are involved in regulating VSMCs [29]. In antiproliferation studies of VSMCs, PI3K/Akt signaling pathway includes many key factors such as GSK3β, p21, and p27, which all inhibit cyclins and CDKs thereby interfering with cell cycle processes. GSK3β is one of the critical downstream molecules of the Akt signaling pathway involved in cell proliferation, metabolism, growth, and survival. It is reported that cyclin D is regulated by GSK3β [30] and that activation of GSK3β leads to exportation into cytoplasm for proteolysis, thus downregulating cyclin D1 expression [31]. The JAK-STAT signaling pathway transmits information from extracellular chemical signals to the nucleus resulting in DNA transcription and expression of genes involved in immunity, proliferation, differentiation, and apoptosis [32]. The downstream proteins in this pathway include cyclin D, p21, Bcl-2, and c-Myc, which are all directly involved in growth, apoptosis, and cell cycle progression in VSMC studies [33]. FAK is involved in cellular adhesion and migration [34]. FAK is typically located at structures known as focal adhesions, which are multiprotein structures including actin, filamin, and vinculin which link the ECM to the cytoplasmic cytoskeleton [35–37]. In addition, FAK interacts with PI3K and p53 [38, 39] and with the PI3K/Akt and MAPKs signaling pathways that are involved in cell cycle regulation. NF-κB controls many genes involved with inflammation which are crucial to progression of diseases including arthritis, asthma, and atherosclerosis [40, 41]. Inflammation also mediates abnormal movement and growth of VSMCs, while suppressing inflammation could attenuate neointimal hyperplasia significantly [42–45]. Therefore, different signaling pathways are involved in VSMC inhibition, which provides preferential protein targets for future drug screening.

5. Different Natural Compounds Being Used for Preventing Neointimal Formation and Focus on VSMCs

5.1. Flavonoids Regulate Cell Cycle and Functions Inhibiting VSMCs Proliferation and Migration. Flavonoids are distributed throughout the plant kingdom and fulfill a diverse range of biological and pharmacological effects such as anti-inflammatory [46], antioxidant [47], antibacterial [48], antitumor [49], and antidiarrheal activities [50]. For treatment of cardiovascular disease, flavonoid studies have focused on reducing hypertension, risk of atherosclerosis, oxidative stress, and related signaling pathways in blood vessel cells, as well as modifying vascular inflammatory mechanisms [51, 52]. In this review, we described the chemical structure, category, source, and mechanism of action of some typical flavonoids that suppress VSMC function and inhibit IH (Table 1).

Nobiletin is widely distributed in citrus fruits and has been reported to inhibit VSMC proliferation and migration *in vitro* [44]. In addition, carotid balloon injured rats given nobiletin 25 mg/kg/day by gavage had significantly decreased neointimal hyperplasia via regulation of the ROS derived NF-κB pathway and decreased serum TNF-α and IL-6 concentrations [44]. Cyanidin-3-O-glucoside, an anthocyanin flavonoid, inhibited TNF-α-induced NoxA1 (a type of NADPH oxidase) and downregulated expression of both TNF-α and NoxA1 at transcriptional and translational levels [53]. (2S)-Naringenin, a typical flavonoid isolated from *Typha angustata*, inhibited PDGF-BB-induced proliferation

Table 1: The structure, cells, category, source, and mechanism of typical flavonoid compounds on inhibiting VSMCs proliferation and migration.

Compound name	Structure	Cells and animals	Category	Sources	Mechanism
(2S)-naringenin		rASMCs	Flavonoid	*Typha angustata*	G0/G1 ↓; cyclins D1 ↓; cyclins E ↓; CDK2/4 ↓; PCNA ↓; pho of rb protein ↓
Catechins		rASMCs and rat balloon injury	Flavonoid (Flavanols)	Green tea	TIMP-2 ↑, in vivo: TIMP-2 ↑
Icariin		hASMCs	Flavonoid (Prenylated flavonol glycoside)	*Epimedium brevicornum*	pERK1/2 ↓; G1/S ↓; PCNA ↓
Morelloflavone		mVSMCs and mouse artery injury	Biflavonoid	*Garcinia dulcis*	FAK ↓; Src ↓; ERK ↓; RhoA ↓
Puerarin		rASMCs and rat balloon injury	Isoflavone	*Radix puerariae*	ROS ↓; Nox ↓; PKC;PKCβ2 ↓; Rac1 ↓; p47phox ↓; p67phox ↓
Kaempferol		hpASMCs	Flavonoid	Widely (grapefruit, Ginkgo biloba)	miR-21 ↑; ROCK4/5/7 ↓
Nobiletin		rASMCs and rat balloon injury	Flavonoid	Widely (citrus fruits)	ROS ↓; pERK1/2 ↓; NF-κB p65 ↓, in vivo: TNF-α ↓; IL-6 ↓
Alpinetin		rASMCs	Flavonoid	Widely *(Alpinia katsumadai, Amomum subulatum, and etc.)*	LDH ↓; NO ↓
Cyanidin-3-O-glucoside		mASMCs	Flavonoid	*Hibiscus sabdariffa*	ROS ↓;NoxA1 ↓; pSTAT3 ↓
Hesperetin		rpASMCs	Flavonoid	Widely (lemons and sweet oranges)	Block G1/S; cyclin D1 ↓; cyclin E ↓; CDK2/4 ↓; p38 ↓; p27 ↑; regulate AKT/GSK3β signaling pathway
Pinocembrin		rAMSCs and rat aortic rings injury	Flavonoid	Propolis	ERK1/2 ↓; MLC2 ↓; AT1R ↓
Glyceollins		hASMCs	Isoflavone	Soybean	Arrest G1/S phase; CDK2 ↓; cyclin D1 ↓; p27kip1 ↑; p53 ↑; ROS ↓; pPDGFr-β ↓; phospholipase Cγ1↓; Akt ↓; ERK1/2 ↓

of VSMCs via a G0/G1 arrest by suppressing cyclin D1/E and CDK 2/4 [54]. Hu and colleagues found that icariin reduced the amount of ox-LDL-induced proliferation of VSMCs through suppression of PCNA expression and inactivation of ERK1/2 [55]. Puerarin, isolated from *Radix puerariae*, exerted inhibitory effects on high glucose-induced VSMC proliferation via interfering with PKCβ2/Rac1-dependent ROS pathways, thus resulting in attenuation of neointimal formation [56]. Alpinetin is a well-known flavonoid isolated from a variety of plants such as *Alpinia katsumadai*, *Amomum subulatum*, and *Scutellaria rivularis*. It may have some protective effects on VSMCs as it decreases LDH leakage and inhibits production of NO in TNF-α-induced VSMC [57]. Hesperetin, a flavonoid, inhibits PDGFa-BB-induced pASMC proliferation via the AKT/GSK3β signaling pathway through upregulating p27 expression while suppressing cyclin D1/E, CDK2/4 and p38 [58]. Pinocembrin reduces the increased ERK1/2 phosphorylation that occurs in response to angiotensin II in both rat aortic rings *ex vivo* and VSMCs *in vitro* [59]. Glyceollins, which are isoflavonoids, inhibit PDGF-BB-induced hVSMC proliferation and migration by downregulating CDK2, cyclin D1, pPDGFr-β, phospholipase Cγ1, Akt, and ERK1/2 and interfering with ROS generation, while upregulating $p27^{kip1}$ and p53 expression levels [60]. Morelloflavone is a biflavonoid, which has been found to block injury-induced neointimal hyperplasia via inhibition of VSMC migration and downregulation of FAK, Src, ERK and RhoA expression [61]. Some studies have demonstrated that a natural flavonoid, kaempferol, may induce miR-21. This results in downregulation of ROCK4, 5, and 7, which are critical for cytoskeletal organization and cell motility, leading to decreased cell migration [62]. Finally, green tea is beneficial for health due to its antioxidant, anticarcinogenic, anti-inflammatory, and antiradiation effects [63–65]. A large number of flavonoids, especially flavan-3-ols ("catechins"), inhibit IH in a rat balloon injury model through upregulation of TIMP-2 expression to modulate MMP activity [66]. From the above review, flavonoids are an important candidate compound type for screening natural drugs capable of inhibiting VSMC growth.

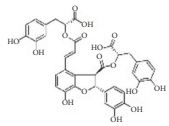
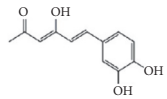
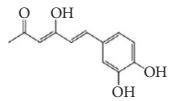
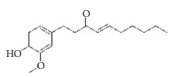
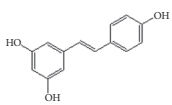
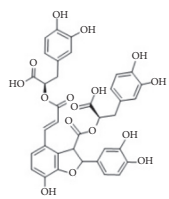
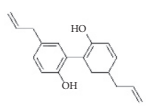
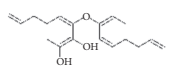
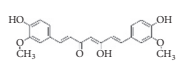
5.2. Polyphenols as an Antioxidants Restrain VSMC Proliferation and Migration to Attenuate IH. Polyphenols are distributed widely in vegetables and plants, green tea, black tea, and red wine. Recent studies have shown that they possess antioxidant, anti-inflammatory, and cardioprotective effects [67–69]. Some typical polyphenols prevent IH by restraining VSMC function including proliferation, migration, and fibrosis (Table 2). Salvianolic acid B is a typical polyphenol that is usually isolated from *Salvia miltiorrhiza*. It markedly reduces neointimal thickness by inducing neointimal cell apoptosis through upregulating p53 expression levels [70]. In another study, salvianolic acid B protected hAECs and neointimal formation through inhibition of LDL oxidation by reducing ROS generation [71]. Magnesium lithospermate B, a derivative of salvianolic acid B, prevented diabetic atherosclerosis via the Nrf2-ARE-NQO1 transcriptional pathway [72]. Magnolol (a phenol) is a powerful antioxidant that inhibited balloon injury-induced rabbit IH by downregulating MCP-1 expression [73]. In another work, magnolol inhibited VSMC migration via the cytoskeletal remodeling pathway through inhibition of β1-integrin expression, phosphorylation of FAK and MLC20, and activation of RhoA and Cdc42 [74]. Lithospermic acid, a polyphenol, arrested cell cycle progression at the G1 phase via downregulating expression of cyclin D1 and inhibiting ROS generation and ERK1/2 phosphorylation [75]. Moreover, lithospermic acid attenuated LPS-induced VSMC migration by inhibiting MMP-9 expression in a dose-dependent manner (25-100 μmol/L). Hispolon blocked balloon injury-induced neointimal hyperplasia via inhibition of VSMC proliferation. It also inhibited VSMC migration by lowering MMP-2/9 expression and increasing TIMP-1/2 expression through suppression of the FAK signaling pathway [76]. Lim and colleagues were of the view that obovatol blocked the cell cycle in G1 phase by downregulating expression of cyclins and CDKs, while selectively upregulating expression of $p21^{Cip1}$, a well-known CDK inhibitor, both *in vitro* and *in vivo* [77].

Some studies have shown that curcumin (diarylheptanoid phenol) has potent antioxidant properties, which can be used for attenuating neointimal hyperplasia [78]. Curcumin has also been shown to inhibit PDGF-induced VSMC migration, proliferation, and collagen synthesis in a concentration-dependent manner [79], with a concentration range of 0.01 to 10 μmol/L inhibiting VSMC proliferation and migration. Curcumin-coated stents inhibited neointimal formation in the rabbit iliac artery stent model. Moreover, curcumin inhibited LPS-induced MMP-2 activity in rat VSMCs through Ras/MEK1/2 and NF-κB signaling [80].

Curcumin shows the ideal biological effects of inhibiting abnormal VSMC proliferation and migration without compromising VEC proliferation or delaying reendothelialization after blood vessel injury. Curcumin inhibited platelet adhesion to brain microvascular endothelial cells by decreasing expression of P-selectin, E-selectin, and GPIIb/GPIIIa in a concentration-dependent manner (30-240 μmol/L). Curcumin antagonized the detrimental effect of rapamycin on aortic endothelial cells *in vitro,* through upregulation of eNOS [81]. Hence, curcumin very selectively inhibited abnormal VSMC functions, such as PDGF-induced proliferation or migration, without impairing VECs. As a result, curcumin has been regarded as an ideal drug for attenuating atherosclerosis and restenosis. In summary, polyphenols exhibit beneficial and wide ranging biological effects relevant to prevention of IH. Polyphenols are worthy candidate compounds to be screened as natural drugs for inhibiting VSMCs.

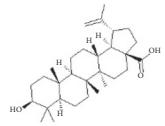
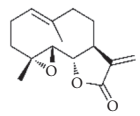
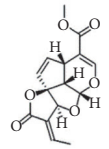
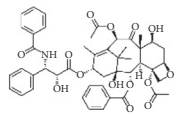
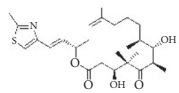
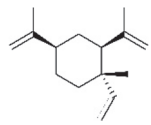
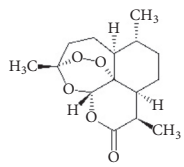
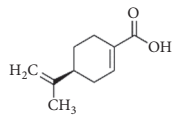
5.3. Terpenes Suppress Abnormal VSMC Function against Neointimal Formation. Terpenes are proven cell cycle inhibitors for various cell types, especially tumor cells [82, 83]. Like similar compounds with active sites for regulating VSMC mitosis and DNA synthesis, terpenes lead cell proliferation and function arrest via cell cycle blockade or apoptosis induction (Table 3). Betulinic acid, a typical terpene, has been reported to inhibit growth and proliferation of VSMCs via arresting G1/S cell cycle in a dose-dependent manner [84]. A monoterpene, (S)-(-)-perillic

Table 2: The structure, cells, category, source, and mechanism of typical polyphenols compounds on inhibiting VSMCs proliferation and migration.

Compound name	Structure	Cells and animals	Category	Sources	Mechanism
Salvianolic acid B		NeCs; HAECs and cholesterol-fed rabbits; rTASMCs and rats balloon injury	Polyphenol	*Salvia miltiorrhiza*	(1) p53 ↑; NeCs apoptosis, (2) ROS ↓; LDL oxidation ↓; lipid deposition ↓, (3) PCNA ↓; NQO1 ↓; via Nrf2-ARE-NQO1 pathway
Caffeic acid phenethyl ester (CAPE)		rASMCs	Polyphenol	*Honeybee propolis*	Blocking G0/1 to S phase; pp38 ↑;HiF1α ↑;HO-1 ↑
Hispolon		rTA-A10-VSMCs	Polyphenol	*Phellinus linteus*	MMP2 ↓;MMP9 ↓; TIMP-1 ↑;TIMP-2 ↑; pFAK ↓; pERK1/2 ↓;PI3K/AKT ↓
[6]-shogaol		rASMCs	Phenols	*Zingiber officinale*	Inhibit DNA synthesis; activation of (Nrf2)/HO-1 pathway
Resveratrol		ncTASMCs; mASMCs	Polyphenol	Widely *(grapes, blueberries, raspberries, and etc.)*	c-Src ↓, Rac1 ↓, cdc42 ↓, IRS-1 ↓, MEKK1 ↓, MEKK4 ↓; p-Src; pFAK ↓; pAKT ↓; pERK1/2 ↓
Lithospermic acid		rTASMCs	Polyphenol	*Salvia miltiorrhiza*	ROS ↓; pERK1/2 ↓; cyclin D1 ↓; arresting cell cycle progression at the G1 phase; MMP9 ↓
Magnolol		Cholesterol-fed rabbits; rVSMCs; rats balloon injury	Polyphenol	*Magnolia officinalis*	(1) MCP-1 ↓, (2) Reduce collagen type I deposition; β1-integrin ↓;pFAK ↓;pMLC20 ↓; RhoA ↓;Cdc42 ↓
Obovatol		rASMCs; rats balloon injury	Biphenol	*Magnolia obovata*	Blocks the cell cycle in G1 phase; CDKs ↓;p21cip1 ↓
Curcumin		rTASMCs; rabbit artery injury; VECs; RAECs	Phenols	*Curcuma longa*	(1) Inhibits PDGF Receptor Binding; PDGFr ↓; pERK1/2 ↓; pAkt ↓, (2) P-selectin ↓; E-selectin ↓; GPIIb/GPIIIa ↓, (3) MMP2 ↓; pRas ↓; MEK1/2 ↓; NF-κB p65 ↓, (4) Curcumin protects aortic endothelial cells; eNOS ↑; caveolin-1 ↓;

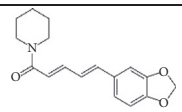
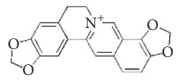
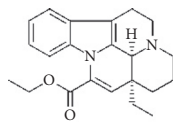
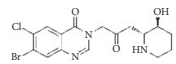
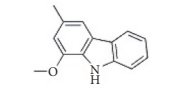
acid, has been reported to decrease protein prenylation leading to DNA synthesis and inhibition of VSMCs [85]. A sesquiterpene lactone, parthenolide, arrested VSMC G0/G1 cell cycle via upregulating p21 and p27. It also increased IκBα expression and reduced Cox-2 expression in a time-dependent manner [86]. A special terpene, plumericin, arrested VSMCs in the G1/G0 phase of the cell cycle along with causing abrogated cyclin D1 expression, hindered $Ser^{807/811}$-pRb protein [87], and blockade of STAT3 signaling via S-glutathionylation. Paclitaxel, a diterpenoid, has been used as an anticancer drug for decades and has been shown to prevent neointimal formation in oral administration studies [88]. Moreover, paclitaxel arrested VSMC G1/S phase by upregulating p21 and p53 *in vitro* [89]. Epothilone D is a paclitaxel-like microtubule-stabilizing agent that was isolated originally from the myxobacterium *Sorangium cellulosum*. It inhibits neointimal hyperplasia through blockade of VSMC CDK2 and pRb [90]. β-Elemene protected VECs from injury induced by H_2O_2 *in vitro* via downregulating MDA while upregulating T-AOC, SOD, GSH-Px, and CAT [91]. Meanwhile, β-elemene selectively inhibited VSMC proliferation/migration and inhibited neointimal formation *in vivo* following vascular injuries [91]. Recent studies have indicated that artemisinin effectively inhibited VSMC proliferation induced by TNF-α through apoptotic induction of the caspase pathway and cell cycle arrest [92, 93]. It also significantly inhibited neointimal formation in rat balloon injured carotid arteries. Therefore, terpenes are also notable candidate compounds for screening natural drugs capable of inhibiting VSMCs.

Table 3: The structure, cells, category, source, and mechanism of terpenes on inhibiting VSMCs abnormal proliferation, migration, and functions.

Compound name	Structure	Cells and animals	Category	Sources	Mechanism
Betulinic Acid		VSMCs	Terpene	Various plant sources widespread throughout the tropics	Inducing G1 Arrest and Apoptosis
Parthenolide		rVSMCs	Sesquiterpene lactone	*Tanacetum parthenium*	G0/G1 cell cycle arrest; p21 ↑; p27 ↑; IκBα ↑;Cox-2 ↓
Plumericin		rAVSMCs	Iridoid (Terpene)	*Himatanthus sucuuba*	Block STAT3 signaling; arrest VSMCs in the G1/G0-phase; cyclin D1 ↓; pRb ↓
Paclitaxel		Rat balloon injury; hCASMCs (CC-2583) and VSMCs (CC-2571); rTASMCs and VECs	Diterpenoid	*Taxus cuspidata*	(1) prevent neointimal formation via oral administration, (2) arrest G1/S phase; p21 ↑; p53 ↑
Epothilone D		rTASMCs; carotid artery injury	Diterpenoid	*Sorangium cellulosum*	CDK2 ↓; pRb ↓
β-elemene		hUVECs and VSMCs (A7r5); rat balloon injury	Terpene	*Curcuma wenyujin*	Antioxidant; Casp 3/7/9 ↑; Migration ↓
Artemisinin		rVSMCs and rat balloon injury; rTASMCs	Sesquiterpene lactone	*Artemisia annua*	(1) arrest G0/G1 phase; cyclin D1/E ↓; CDK2/4 ↓; caspase 3/9 ↑; Bax ↑; Bcl-2 ↓, (2) PCNA ↓; caspase 3↑; Bax ↑; Bax/Bcl-2 ratio ↑
(S)-(-)-Perillic acid		rASMCs	Monoterpene	Widely	Protein prenylation ↓

5.4. Alkaloids Exhibit Antiproliferation Biological Effect on VSMCs. Alkaloids are a group of naturally occurring chemical compounds that mostly contain basic nitrogen atoms. Alkaloids have diverse biological effects including those against tumors, hypertension, and pain. For vascular IH, some studies indicate that alkaloids hinder cell cycle progress, decrease ROS production, and inhibit VSMC migration (Table 4). A classic alkaloid, piperine, selectively inhibits VSMC proliferation with an IC50 of 11.8 μmol/L without influencing VEC growth [94]. Coptisine was isolated from *Coptis chinensis* and suppresses VSMC proliferation selectively at lower concentrations with a GI_{50} of 3.3 μmol/L (1.2 μg/mL) [95]. Vinpocetine, a potential derivative of vincamine, inhibits high glucose-induced proliferation of VSMCs by preventing ROS generation and affecting MAPK, PI3K/Akt, and NF-κB signaling, Wang, Wen, Peng, Li, Zhuang, Lu, Liu, Li, Li, and Xu [96]. Vinpocetine arrested G1/S phase of the cell cycle by downregulating cyclin D1 and pERK1/2. Alongside these effects, vinpocetine also inhibited VSMC migration and ROS production [97]. A quinazolinone alkaloid, halofuginone, selectively inhibited cell proliferation, ECM deposition, and type I collagen synthesis in VSMCs versus VECs, which attenuated injury-induced IH [98]. Carbazole or murrayafoline A inhibited PDGF-BB induced abnormal proliferation of VSMCs by downregulating cyclin D1/E, CDK2/4, and PCNA and phosphorylation of Rb [99].

Table 4: The structure, cells, category, source, and mechanism of alkaloids on inhibiting VSMCs abnormal proliferation, migration, and functions.

Compound name	Structure	Cells and animals	Category	Sources	Mechanism
Piperine		rASMCs	Alkaloid	*Piper nigrum*	Selectively inhibit VSMCs
Coptisine		rVSMCs	Alkaloid	*Coptis chinensis*	Arrest G1/S phase
Vinpocetine		rVSMCs and rat balloon injury; rASMCs and mice carotid artery ligation injury	Alkaloid vincamine	Lesser periwinkle plants	(1) ROS ↓; apoptosis ↓; pAkt ↓; pJNK1/2 ↓; IκBα ↓; PCNA ↓; cyclin D ↓; Bcl-2 ↓, (2) Arrest G1/S phase; cyclin D1 ↓; $p27^{Kip1}$ ↑; inhibit migration; pERK1/2 ↓; ROS ↓
Halofuginone		bASMCs	Quinazolinone alkaloid	*Dichroa febrifuga*	ECM synthesis and deposition ↓; Col I ↓
Murrayafoline A		rASMCs	Carbazole alkaloid	*Glycosmis stenocarpa Guillaumin*	Arrest G1/S phase; cyclin D1/E ↓; CDK2/4 ↓; PCNA ↓; pRb ↓

Review of these recent studies on the effects of alkaloids provides hope for identification of useful drugs capable of inhibiting VSMC growth and preventing IH.

5.5. Other Promising Natural Compounds for Preventing Intima Hyperplasia. As shown in Table 5, emodin is a typical anthraquinone compound beneficial for prevention of atherosclerosis due to its effects against inflammation, proliferation, and migration and its ability to induce apoptosis in VSMCs [100]. Moreover, emodin arrested growth and induced apoptosis and autophagy via enhanced ROS production and upregulation of p53 expression [101]. Emodin inhibited VSMC proliferation induced by angiotensin II through downregulation of PCNA and c-myc expression [102]. Moreover, emodin showed anti-inflammatory effects by inhibiting Hcy-induced CRP generation, a key inflammatory molecule in atherogenesis in VSMCs [103]. Emodin has also been shown to inhibit TNF-α-induced hASMC proliferation via caspase signaling and a mitochondrial-dependent apoptotic pathway by downregulating Bcl-2 and upregulating Bax expression [104]. Additionally, emodin reduced TNF-α induced migration of VSMCs by suppressing NF-κB activation and MMP2/9 expression levels [105]. Our recent study demonstrated that emodin efficiently and concentration-dependently (0.05 to 5 μmol/L) inhibited hVSMC proliferation more than hVEC proliferation *in vitro,* with less influence on reendothelialization of VECs in rat carotid artery balloon injury [106].

Methyl-protodioscin is a steroidal saponin that has been reported to inhibit neointimal formation by restraining VSMC proliferation and migration through downregulation of ADAM15, FAK, ERK, PI3K, Akt, and MMP-2/9 expression levels [107]. *Salvia miltiorrhiza* has been used to prevent cardiovascular diseases in traditional Chinese medicine over the millennia. Tanshinone-IIA is a principal active component of *Salvia miltiorrhiza* that suppresses abnormal VSMC proliferation by cell cycle arrest at G0/G1 phase and inhibits phosphorylation of ERK1/2 and c-fos expression [108]. It has been reported that ajoene (1-50 μnol/L) interfered with progression of the G1 phase in the cell cycle and restrained rat VSMC proliferation via inhibiting protein prenylation [109]. Gastrodin influenced the S phase entry of VSMCs and stabilized $p27^{KIP1}$ expression. It also inhibited VSMC proliferation and attenuated neointimal hyperplasia by suppressing phosphorylation of ERK1/2, p38 MAPK, Akt, and GSK3β [110]. Genipin has been reported to inhibit TNF-α induced VSMC proliferation and migration in a dose-dependent manner by upregulating HO-1 expression, preventing ERK/MAPK and Akt phosphorylation, and additionally blocking generation of ROS [111]. Ginsenoside Rg1 is one of the main active components of *Panax ginseng* and is said to arrest G1/S phase in VSMCs by interfering with GRKs, PKC, and N-ras while upregulating p21 expression [112]. Vascular IH is significantly decreased when carotid artery balloon injured rats are intraperitoneally injected with ginsenoside Rg1 for 14 days [113]. Moreover, ginsenoside Rg1 significantly inhibited TNF-α-induced hASMC proliferation dose-dependently through downregulating cyclin D1, inactivating ERK1/2 and PKB, and upregulating expression of p53, $p21^{WAF/CIP1}$, and $p27^{KIP1}$ [114]. A coumarin called ostruthin is a major bioactive constituent of *Peucedanum ostruthium* and inhibited serum (10%)-induced VSMC proliferation in a dose-dependent manner [115].

Most foods contain various biologically active constituents that act to prevent and cure neointimal hyperplasia by inhibiting abnormal VSMC proliferation and migration. A well-known carotenoid, lycopene, is abundant in tomatoes and its products and has been reported to inhibit neointimal hyperplasia in a rabbit restenosis model. It does this by

Table 5: The structure, cells, category, source, and mechanism of promising compounds on suppressing VSMCs.

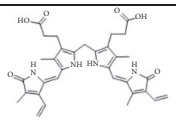
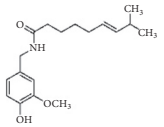
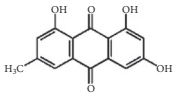
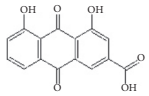
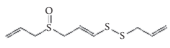
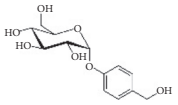
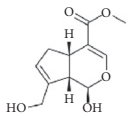
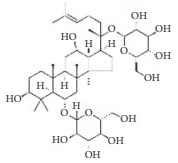
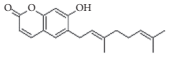
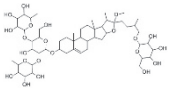
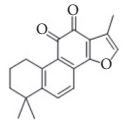
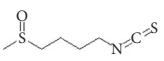
Compound name	Structure	Cells and animals	Category	Sources	Mechanism
Bilirubin		rVSMCs and mVSMCs; rat balloon injury	Ferric porphyrins	Heme	Inhibit MAPK signaling pathway; CDK2 ↓; Cyclin A/D1/E ↓; pRb ↓; YY1 ↓; p38 ↓
capsaicin		rASMCs	Capsaicinoids	Chili peppers	Inhibit DNA synthesis
Emodin		hUVSMCs; rTASMCs; hASMCs; rat balloon injury	Anthraquinone	*Rheum officinale*	(1) Arrest cell cycle, induce apoptosis and autophagy; ROS ↑; p53 ↑, (2) PCNA ↓; c-myc ↓, (3) CRP ↓;ROS ↓; pERK1/2 ↓; p38 ↓; PPARγ ↑, (4) Induce apoptosis; Bcl-2 ↓; Bax ↑, (5) MMP2/9 ↓; NF-κB activation ↓
Rhein		hASMCs	Anthraquinone	*Rheum palmatum*	Col I/III ↓; Wnt4/Dvl-1/β-catenin ↓; miR-126 ↑
Ajoene		rASMCs	Organosulphur compound	*Allium sativum*	Inhibit protein prenylation and cholesterol biosynthesis
Gastrodin		rASMCs, mice artery injury	Glucoside	*Gastrodia elata B1*	Block S-phase; stabilised p27Kip1; PCNA ↓; pERK1/2 ↓; pp38 ↓; pAkt ↓; pGSK3β ↓
Genipin		rTASMCs	Aglycon	*Gardenia jasminoides*	HO-1 ↑; pERK/MAPK ↓; pAkt ↓; ROS ↓
Ginsenoside Rg1		hASMCs; rat balloon injury	Steroid glycosides	*Panax ginseng*	(1) PCNA ↓; pERK2 ↓; c-fos ↓; MKP-1 ↑; (2) Arrest G1/S phase; GRKs ↓; PKC ↓; N-ras ↓; p21 ↑, (3) Cyclin D1 ↓; p53 ↑; p21WAF/CIP1 ↑; p27KIP1 ↑; inactivate PKB and ERK1/2
Ostruthin		rTASMCs	Coumarins	*Peucedanum ostruthium*	Inhibit DNA synthesis
Lycopene		Rabbit artery injury	Carotenoid	Widely (tomatoes, red carrots,)	TG ↓; TC ↓; LDL-C ↓; HDL-C ↑; SOD ↑; T-AOC ↑; MDA ↓; PCNA ↓; pERK1/2 ↓; Nox1 ↓; $p22^{phox}$ ↓; HMG-CoA ↓; ABCA1 ↑
Methyl Protodioscin		A7r5 VSMCs; rat balloon injury	Steroidal saponin	*Dioscorea collettii*	Arrest G1/S phase; ADAM15 ↓; MMP2/9 ↓; FAK ↓; ERK ↓; PI3K ↓; Akt ↓
Tanshinone IIA		rASMCs; rat balloon injury	Phenolic acids	*Salvia miltiorrhiza*	Block cell cycle in G0/G1 phase; pERK1/2 ↓; c-fos ↓
Sulforaphane		rASMCs; rat balloon injury	Organosulfur compounds	*Widely (cruciferous vegetables such as broccoli, Brussels sprouts, and cabbages)*	p21 ↑; p53 ↑; CDK2 ↓; Cyclin E ↓; PCNA ↓

Table 6: The selected potential targets of the compounds.

Seq	Idarubicin	Halofuginone	Piperine	β-elemene	Curcumin	Coptisine
	Predicted target names (most related top 15)					
1	MAPT	BCHE	MAOA	MAPT	MAPT	CHRM4
2	MBNL1	ACHE	MAOB	TDP1	TLR9	CHRM1
3	MBNL2	MAPK8	SIGMAR1	CXCR3	TDP1	CHRM2
4	MBNL3	MAPK9	MBNL1	SLC6A2	Unknown	CHRM5
5	MMP2	MAPK10	MBNL2	SLC6A3	MBNL1	CHRM3
6	MMP9	MAPK11	MBNL3	LDLR	MBNL2	BCHE
7	APP	MAPK14	MAPT	VLDLR	MBNL3	ADRA2A
8	SNCA	HTR1A	DRD2	LRP8	GLO1	CYP2D6
9	APLP2	HTR1B	DRD3	HSD11B1	AKT1	ADRA2B
10	SNCG	MAPT	HDAC3	BACE1	AKT2	ADRA2C
11	SNCB	HTR2A	HDAC1	HSD11B1L	AKT3	ACHE
12	TDP1	DRD2	HDAC2	BACE2	HSD17B3	HTR2A
13	EGFR	DRD1	DYRK1A	HTR1A	HSD17B12	HTR2C
14	ERBB2	OPRM1	HDAC6	HTR1D	CRYZ	HTR2B
15	ERBB3	OPRD1	CTSL1	HTR1B	APP	SIGMAR1

regulation of blood lipid concentrations and suppression of oxidative stress [116]. Sulforaphane, an organosulfur compound, mostly found in cruciferous vegetables significantly inhibited PDGF-BB-induced VSMC proliferation by upregulating p21 and p53 expression, while CDK2, cyclin E, and PCNA expression was suppressed [117].

6. Selective Inhibition of VSMCs versus VECs Shows Significant

Although many natural products inhibit VSMC function, most anti-smooth muscle proliferation drugs such as rapamycin (in-stent coating) also inhibit VEC proliferation and delay reendothelialization. This nonspecific cytotoxicity leads to restenosis and final graft or stent implantation failure. When screening for selective natural drugs that inhibit smooth muscle cell proliferation and migration, it is necessary to combine computer-aided design, bioinformatics, and a high-throughput screening platform. In this review, we selected certain drugs including chemosynthetic (idarubicin) and some natural (β-elemene, coptisine, halofuginone, piperine, and curcumin) compounds that possess specificity for suppressing proliferation of VSMCs over VECs. The chemical structure of the natural compounds has no typical similarity and cannot be analyzed using structural-activity relationships of molecular-protein binding sites. However, an online tool "Swiss Target Prediction" was used to predict potential targets of these compounds [118]. Most of the predicted targets of these drugs were membrane receptors, enzymes, kinases, proteases, or transporter proteins (Table 6). The analyses showed that microtubule-associated protein TAU (MAPT) is the most frequent protein target among them (Figure 4). This stabilizes microtubules and influences transportation of cellular secretory proteins. Moreover, MAPT has been reported to accelerate cancer cell growth [119], while its inactivation through gene knockdown suppressed cell proliferation [120]. Therefore, it is speculated that the diverse affinity of a natural drug to different functional protein targets may be one of the key factors for different selectivity profiles on VSMCs or VECs. Common targets like MAPT could be used as one of the important indicators in screening selective inhibitory drugs in future studies.

7. Conclusion

This review highlighted the originating four cells that may contribute to IH and then focused on VSMCs due to their involvement in intima formation as a consequence of abnormal proliferation, migration, and physiology. It further summarized typical signaling pathways such as MAPKs, PI3K/Akt, JAK-STAT, FAK, and NF-κB and their involvement in the abnormal activities of VSMCs. Based on these the above cell origins and pathways, we organized and classified different natural isolates including phenols, flavonoids, terpenes, and alkaloids that have suppressing effects on VSMCs. In addition, many natural drugs not only induce apoptosis and arrest cell cycle in VSMCs, but also impair VECs leading to vascular restenosis and failure of blood vessel remodeling. Thus, it is crucial to screen desirable drugs from natural sources that preferentially inhibit VSMCs versus VECs to prevent IH in the early stages, restenosis following graft implantation, and even atherosclerotic diseases.

Abbreviations

IH: Intimal hyperplasia
EndMT: Endothelial-to-mesenchymal transition
rASMCs: Rat aortic smooth muscle cells
rTASMCs: Rat thoracic aortic smooth muscle cells
VSMCs: Vascular smooth muscle cells
CA: Carotid artery
RAECs: Rat aortic endothelial cells

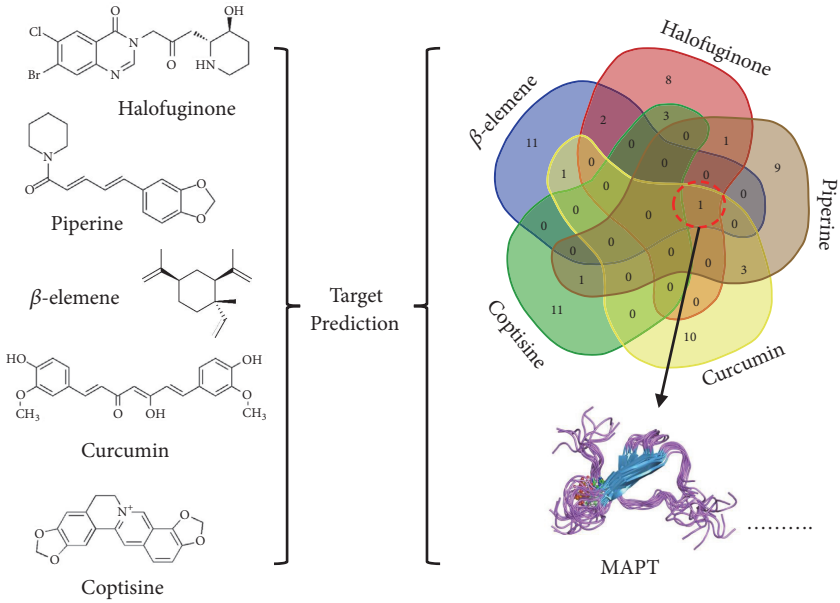


Figure 4: The compounds potential target: MAPT which is a common target.

HAECs: Human aortic endothelial cells
VECs: Vascular endothelial cells
hUVECs: Human umbilical vein endothelial cells
hUVSMCs: Human umbilical vein smooth muscle cells
NeCs: Neointimal cells
rTA-A10-VSMCs: Rat thoracic aorta A10 vascular smooth muscle cells
ncTASMCs: Newborn calf thoracic aorta smooth muscle cells
mASMCs: Mice aortic smooth muscle cells
hPASMCs: Human pulmonary artery smooth muscle cells
rPASMCs: Rat pulmonary artery smooth muscle cells
hCASMCs: Human coronary artery smooth muscle cells
bASMCs: Bovine aortic smooth muscle cells
MYH11: Smooth muscle cell myosin heavy chain
SM22α/tagln: SMC lineage-restricted protein
ACTA2: Alpha smooth muscle actin
ECM: Extracellular matrix
TNF-α: Tumor necrosis factor-α
PDGF: Platelet-derived growth factor
ERK: Extracellular signal-regulated kinase
MMP: Matrix metalloproteinase
MAPK: Mitogen-activated protein kinase
JNK: c-Jun N terminal kinase
PCNA: Proliferating cell nuclear antigen
PI3K: Phosphatidylinositol-4,5-bisphosphate 3-kinase
AKT: Serine/threonine kinase 1
CDK: Cyclin-dependent kinase
JAK: Janus kinase
STAT: Signal transducer and activator of transcription protein
FAK: Focal adhesion kinase
NF-κB: Nuclear factor kappa B
LDL: Low-density lipoprotein
ROS: Reactive oxygen specie
IL-1β: Interleukin 1-β
LPS: Lipopolysaccharide
Nox: NADPH oxidase
TIMP: Tissue inhibitors of metalloproteinase
NOS: Nitric oxide synthase
IC50: Half maximal inhibitory concentration
miR-21: MicroRNA-21
NO: Nitric oxide
LDH: Lactate dehydrogenase
eNOS: Nitric oxide synthase
pPDGFr-β: β-type platelet-derived growth factor receptor
ROCK: Rho-associated protein kinase
Rb: Retinoblastoma tumor suppressor protein family.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Kang Xu, Mohanad Kh Al-ani, and Xin Pan designed the project, performed the experiments, collected the data, and wrote the manuscript. Qingjia Chi analyzed the data and wrote and revised the manuscript. Nianguo Dong and Xue-feng Qiu designed the project, gave financial support, and wrote and revised the manuscript. All authors read and approved the final manuscript.

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Review Article

Traditional Chinese Medicine Injections in the Treatment of Diabetic Foot: A Systematic Review and Meta-Analysis

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Context. The role of traditional Chinese medicine injections (TCMIs) in diabetic foot (DF) has not been well estimated. *Objective*. To evaluate the clinical effective rate, safety, and the financial cost of TCMIs in treating DF and ulcer wound healing. *Methods*. We searched PubMed, Embase, CENTRAL, China National Knowledge Infrastructure (CNKI), VIP database, and Wanfang database from inception to May 2018 to find all randomized control trials (RCTs) related to TCMIs in DF treatment. The search items were "Traditional Chinese Medicine Injection" AND "Diabetic foot or Diabetic foot ulcer" AND "random". *Study Selection and Synthesis*. Only RCTs of TCMIs combined conventional therapies versus conventional therapies and that can be quantitatively synthesized were included. Finally, 17 studies and 1294 participants were included after extraction. Two investigators independently extracted and analyzed the data using RevMan5.3 software. *Results*. The overall clinical effective rate of TCMI groups is higher than that of control groups [RR=1.27, 95CI % (1.20, 1.34), P<0.00001] based on fixed effect model analysis. Regarding motor nerve conduction velocity of median nerve and peroneal nerve, TCMI group showed a significant improvement (MD=3.84[2.28, 5.41], P<0.00001; MD=2.89[0.63, 5.15], P=0.01). Regarding plasma viscosity TCMI group showed a statistically difference (MD=0.27[0.04, 0.49], P=0.02). In terms of blood viscosity at high shear rate, there was an improvement of TCMI group (MD=0.36[0.05, 0.67], P=0.02). However, sensory nerve conduction velocity of peroneal nerve and median nerve showed a contradiction to motor nerve conduction velocity, respectively (MD=2.59[-1.69, 6.87], p=0.24; MD=2.73[-0.96, 6.43], P=0.15). *Conclusion*. The data of this study shows that TCMIs can bring benefits to patients with diabetic foot. However, due to low methodological quality of included RCTs, more rigorous designed RCTs with large sample size are recommended to provide more high-quality evidence.

1. Introduction

Diabetic foot (DF) is the infection, ulceration, or destruction of tissues of the foot associated with neuropathy and/or peripheral vascular disease (PVD) in the lower extremity of people with diabetes [1]. Diabetic peripheral neuropathy (DPN) and microangiopathy are the most significant risk factors for DF [2]. It is one of the most severe and costly chronic complications of diabetes mellitus (DM) [3]. People with diabetes with foot ulcers experience health expenditures five times higher than those without foot ulcers [4]. It always develops from mild or moderate neural symptoms into diabetic foot ulcers (DFU) on lower extremities even leading to amputations. The amputation rate population with DM is ten to twenty times more than the nondiabetic population [5]. And there is also a twofold risk of mortality for DM population with a history of DFUs compared to those without DM [6].

The prevalence of foot ulcers of people having diabetes mellitus is 4% to 10%, and the annual population-based incidence is 1.0% to 4.1% [7, 8]. The lifetime incidence of DM people having DFU could be as high as 25% [9]. Given the rapid growth of DM population which will increase by 48% in 2045 compared to the number of 425 million [4], we are facing a rapid growing of DF patients in the following 30 years.

However, the DFU is preventable and a timely treatment for ulcers can help in the reduction of severe outcomes. A comprehensive intervention including DFU risk assessments,

foot care based on prevention, education for patients and their healthcare attendants, and a multidisciplinary treating approach will lower foot complications and amputations by 85% at most [4].

Being a widely practiced and long-time-used healthcare method, traditional Chinese Medicine plays a significant role in treating DM and glycaemic control [10–15]. According to IDF DIABETES ATLAS 8th edition, intensive glycaemic control is the primary preventive method of DFU and associated with a lower risk of amputation and sensory numbness [4, 16]. Traditional Chinese medicine injection (TCMI) is sterile liquid of active ingredients that extracted from the natural drugs. The TCMIs with the clinical efficacy of promoting blood circulation to remove blood stasis are now being widely used in China on preventing and treating of DPN and they are subsequent [17, 18]. However, there was no sufficient evidence-based medicine (EBM) support of that for clinicians and specialists. We performed this systematic review and meta-analysis to investigate the clinical efficacy and safety for TCMIs on DFU.

2. Methods

We strictly followed the instruction of Preferred Reporting Items for systematic reviews and meta-analyses: the PRISMA statement during the process of this review [19].

2.1. Inclusion and Exclusion Criteria. We included all the randomized controlled trials (RCTs) applying TCMIs in the treatment of DFU in patients with DM. Participants are diagnosed as diabetic foot and there are no restrictions of age, gender, and course of disease. TCMIs are the injections extracted from herbs, single or mixture herbal formulas. Interventions in trial group are one kind of TCMI with basic care or this TCMI combined conventional therapies with basic care. The basic care and conventional therapies should remain the same in the control group in the same RCT.

Exclusion criteria were as follows: ① duplicates; ② systematic reviews and/or meta-analyses; ③ catalogue, indexes, and conferences; ④ irrelevant topics; ⑤ RCTs using more than one traditional Chinese medicine injection; and ⑥ studies that cannot be quantitative synthesized.

There were no limits on publication status or language.

2.2. Search Strategy. PubMed, Embase, CENTRAL, China National Knowledge Infrastructure (CNKI), VIP Database for Chinese Technical Periodicals (VIP), and Wanfang databases were searched from inception to May 2018. The search items were "Traditional Chinese Medicine Injection or Zhong Yao Zhu She Ye or Zhu She Ye" AND "Diabetic foot or Diabetic foot ulcer or Tang Niao Bing Zu or Tang Niao Bing Zu Kui Yang" AND "random".

2.3. Data Extraction. Two reviewers screened and extracted the basic information independently by using a standardized data extraction form of our own and a cross check had been made after the extraction. Disagreements were resolved by discussion and we attempted to contact the authors for the missing data. We used Zotero5.0 software to manage the bibliographies. The information we filled into the form included the following:

(i) General information: title, authors' names, journal, publish date, etc.

(ii) Characteristics of the RCTs: sample size, age, gender, course of disease, interventions, etc.

(iii) Outcomes

(iv) Adverse reactions

2.4. Types of Outcome Measures

Primary Outcomes. Clinical effective rates are as follows.

Clinical efficacy was defined as one or more Wagner score reductions after treatment.

Secondary Outcomes. Nerve conduction velocity includes motor nerve conduction velocity (MCV) and sensory nerve conduction velocity (SCV).

Hemorheology includes blood viscosity and plasma viscosity.

2.5. Risk of Bias. Two reviewers made the assessment following the Cochrane Handbook for Systematic Reviews of Interventions 5.1.0 and the systematic review of the methodological quality assessment tools [20, 21].

(i) Random sequence generation (selection bias)

(ii) Allocation concealment (selection bias)

(iii) Blinding of participants and personnel (performance bias)

(iv) Blinding of outcome assessment (detection bias)

(v) Incomplete outcome data (attrition bias)

(vi) Selective reporting (reporting bias)

(vii) Other bias

2.6. Data Synthesis. We conducted this meta-analysis through Revman5.3 software [22]. The categorical variables were analyzed by risk ratio (RR) and the continuous variables take the mean difference (MD) as the effect index, and they are all with 95% confidence interval (95% CI).

The heterogeneity among the included studies was analyzed using the chi-square test (the test level was α=0.1), and the heterogeneity was quantitatively determined using I^2. If there is no heterogeneity or heterogeneity test result is P>0.1 or I^2 <50%, the fixed effect model was applied for meta-analysis. Otherwise, we will further identify the sources of heterogeneity and then reanalyze after reducing the heterogeneity. If there still exists heterogeneity, we will run the analysis with random effects model. Subgroup analyses were conducted based on types of traditional Chinese medicine injections and we illustrated the publication bias of primary outcomes in funnel plot.

3. Results

3.1. Study Selection. We finally included 17 studies from 595 studies. The process is demonstrated in Figure 1.

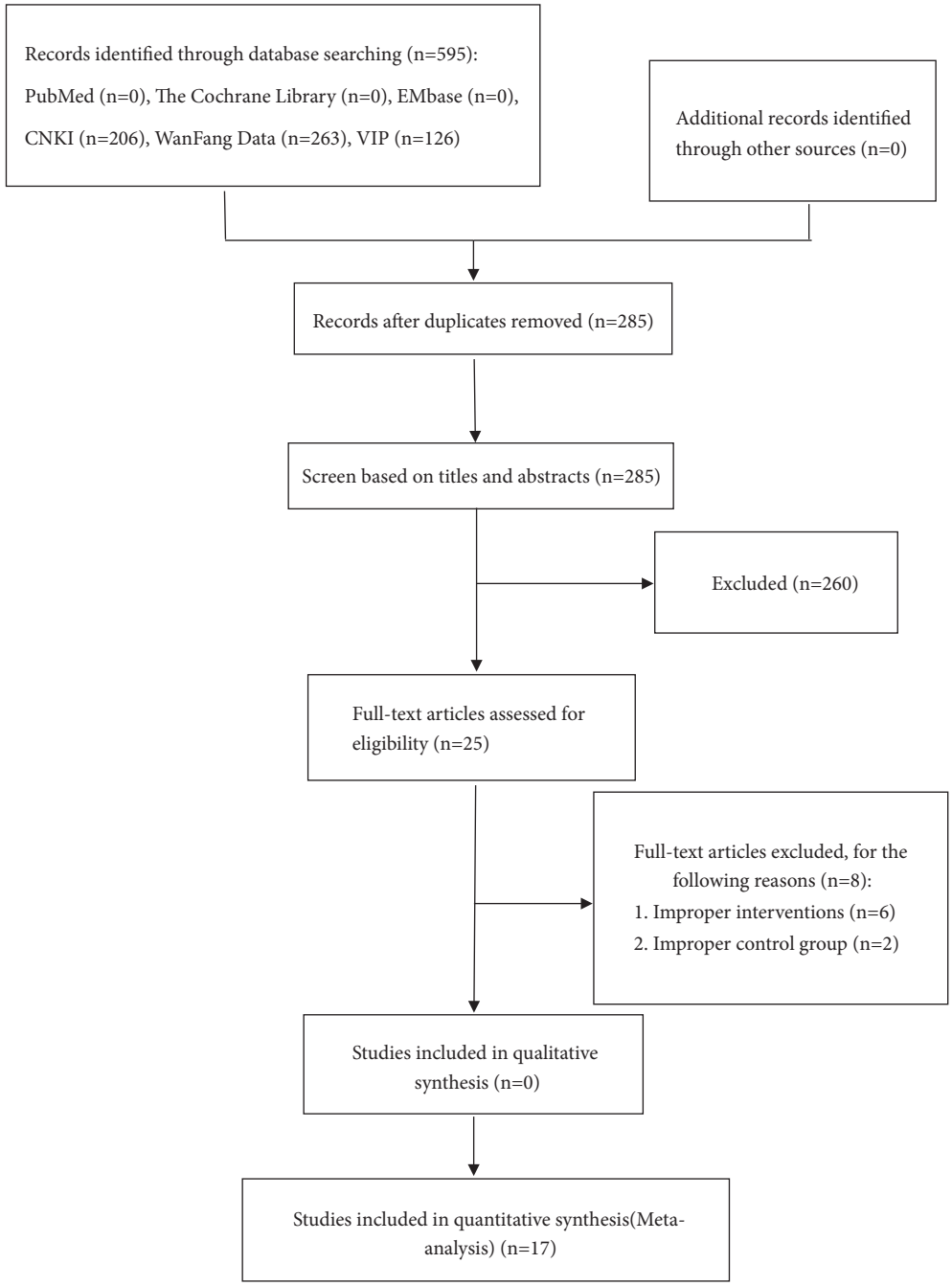

Figure 1: Flow diagram of study selection and identification.

3.2. Study Characteristics. A total of 1294 participants were included from the 17 studies [23–39]. All the data were illustrated in Tables 1 and 2 including study size, interventions, and basic information of studies.

All studies utilized Wagner scale for the classification of DFU patients when initially enrolled [40]. Among them, 13 participants were grade 0 (1%), 232 were grade 1 (17.93%), 358 were grade 2 (27.67%), 140 were grade 3 (10.82%), and 41 were grade 4 (3.17%). And 510 (39.41%) were without specific grade classification information.

3.3. Risk of Bias. We used Revman5.3 software to explicitly report the methodological features for each study (Figure 2). Regarding random sequence generation, 14 studies reported "random" without specific method, 2 studies are quasi-randomized for their obvious selection bias [27, 36], and only 1 study reported using random number table [34]. Regarding blinding for patients and personnel, 2 studies had high risk and 15 studies had unclear risk. As for blinding for outcome assessment, 13 studies had low risk and 4 studies had unclear risk. Regarding incomplete outcome data, all studies had low

Table 1: Basic characteristics of the included studies.

Study IDs	Sample size (T/C)	Age (T/C)	Intervention T	Intervention C	Duration (weeks)	Outcomes
Chen 2018	30/30	66.06 ± 0.47(62~82)/ 67.36 ± 0.55(64~84)	Ginkgo biloba extract injection (20ml/d); Basic care	Basic care	4	(1) Total effective rates; (2) Hemorheology
Chi 2012	41/41	NA	Erigeron Breviscapus extract injection (30ml/d); Basic care	Basic care	2	(1) Total effective rates; (2) Nerve conduction velocity; (3) Hemorheology
Guo et al. 2017	100/100	43.1±3.4/44.3±3.7	Ginkgo biloba extract injection (20ml/d); Alprostadil (10μg/d); Basic care	Alprostadil (10μg/d); Basic care	4	(1) Total effective rates;
Nuerzada et al. 2018	31/31	72.5±9.5(63~ 82)/72.5±10.5(61~83)	Ginkgo biloba extract injection (10ml/d); Basic care	Basic care	NA	(1) Total effective rates; (2) Hemorheology
He et al. 2010	40/38	68±14.5/69±15.2	Erigeron Breviscapus extract injection (30ml/d); Basic care	Basic care	2	(1) Total effective rates; (2) Nerve conduction velocity; (3) Hemorheology
Jin et al. 2017	70/70	62.79±5.43(53~78)/ 61.97±6.25(52~79)	Compound Salvia Miltiorrhiza injection (20ml/d); Alprostadil (10μg/d); Basic care	Alprostadil (10μg/d); Basic care	4	(1) Total effective rates; (2) Blood lipid levels; (3) Renal function and urine protein
Lin 2008	36/36	61.36(51~75)/ 60.35±5.3(49~72)	Erigeron Breviscapus extract injection (400mg/d); Basic care	Basic care	2	(1) Total effective rates; (2) Hemorheology; (3) Ulcer size
Liu 2008	37/33	67(55~81)/ 65(56~79)	Danhong injection (NA); Anisodamine (20mg/d); Basic care	Anisodamine (20mg/d); Basic care	4	(1) Total effective rates
Shi 2010	42/42	(43~74)/ (38~66)	Compound Salvia Miltiorrhiza injection (12ml/d); Ozagrel (160mg/d); Basic care	Ozagrel (160mg/d); Basic care	4	(1) Total effective rates; (2) Nerve conduction velocity; (3) Hemorheology

Table 1: Continued.

Study IDs	Sample size (T/C)	Age (T/C)	Intervention T	Intervention C	Duration (weeks)	Outcomes
Wang 2010	22/18	57.6(38~73)/ 56.2(41~71)	Danhong injection (20ml/d); Basic care	Basic care	4	(1) Total effective rates; (2) Hemorheology
Wen 2014	20/20	61.8(38~76)/ 63.8(36~75)	Panax notoginsenosides injection (250mg/d); Basic care	Basic care	NA	(1) Total effective rates; (2) Lower limbs blood
Wu et al. 2003	36/36	63(48~72)/ 62(46~68)	Compound Salvia Miltiorrhiza injection (10~30g/d); Pancreatic kininogenase (480~720IU/d); Mecobalamin (500~1000μg/d); Basic care	Pancreatic kininogenase (480~720IU/d); Mecobalamin (500~1000μg/d); Basic care	4	(1) Total effective rates; (2) Nerve conduction velocity; (3) Hemorheology
Xiang 2017	50/50	58.91±8.54/ 59.42±7.89	Danhong injection (40ml/d); Basic care	Basic care	4	(1) Total effective rates; (2) Ulcer area (3) Arterial diameter and Blood velocity
Yu et al. 2008	25/25	57.6(38~73)/ 56.2(41~71)	Danhong injection (40ml/d); Basic care	Basic care	3	(1) Total effective rates
Zhang et al. 2008	16/16	52.3±2.5/ 51.8±2.7	Danhong injection (20ml/d); Basic care	Basic care	4	(1) Total effective rates; (2) Nerve conduction velocity
Zheng et al. 2014	33/33	58.2±5.3/ 56.7±6.6	Danhong injection (30ml/d); Basic care	Basic care	4	(1) Total effective rates; (2) Oxidative stress status
Zhu 2010	30/16	NA	Panax notoginsenosides injection (450mg/d); Basic care	Basic care	1.7(10d)	(1) Total effective rates

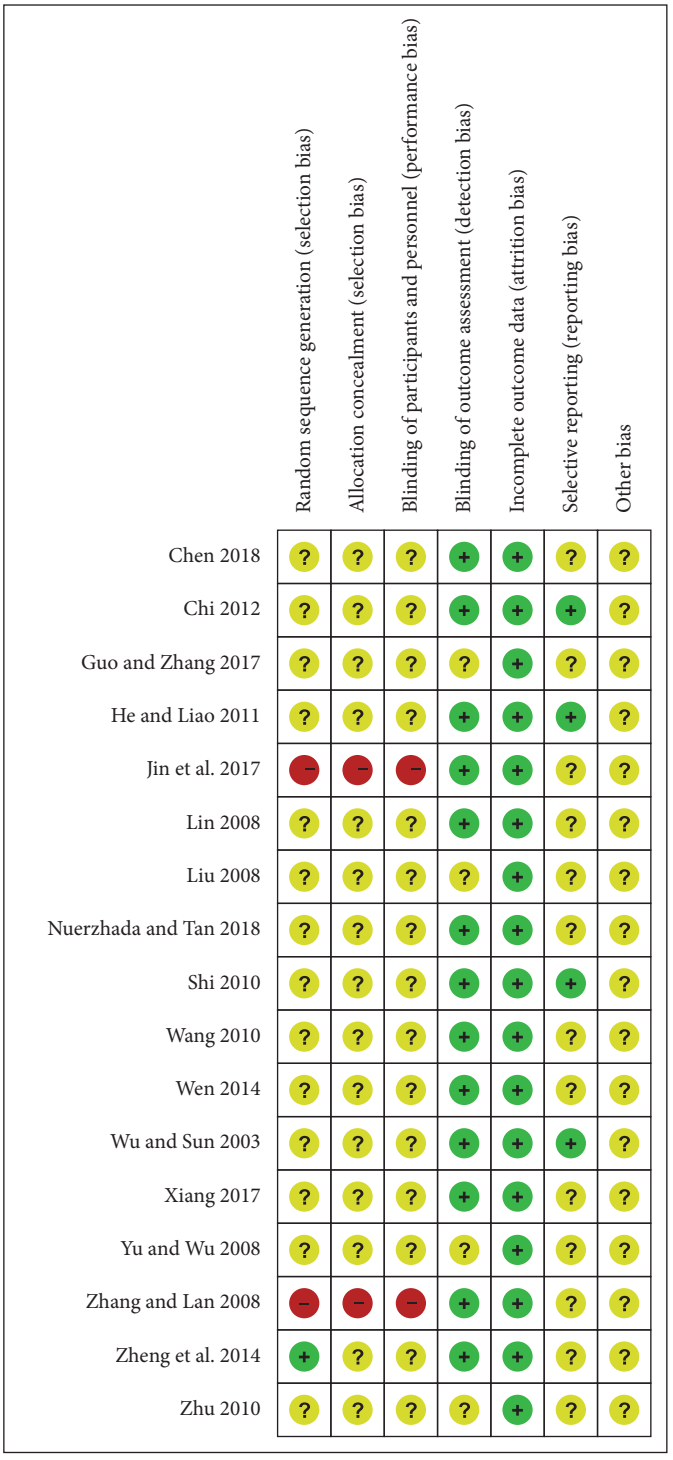

Figure 2: Risk of bias summary.

risk of bias. Concerning selective reporting, 4 studies had low risk of bias and 13 studies had unclear risk of bias.

3.4. Meta-Analysis Results

3.4.1. Primary Outcome: Clinical Effective Rate. All the 17 studies and 1294 patients receiving treating were included. The overall clinical effective rate of TCMI groups is higher than that of control groups (RR=1.27, 95CI% [1.20, 1.34], P<0.00001). Analysis results of different subgroups of conventional therapies based on fixed effect model showed that all TCMI groups outperformed the conventional therapies groups (Danhong injection RR=1.24[1.10, 1.41], P=0.0005; Erigeron Breviscapus extract injection

Table 2: Course of disease.

Study IDs	Women (%)	Course of Diabetes Mellitus (yrs.)		Course of Diabetic Foot	
		Treatment group	Control group	Treatment group	Control group
Chen 2018	45%	9.19±0.28 (5~15)	9.21±0.33 (6~15)	38.24±1.09 (32~78) days	37.94±0.98 (30~76) days
Chi 2012	NA	NA	NA	NA	NA
Guo 2017	41%	9.6±2.9	9.2±3.5	NA	NA
Nuerzada 2018	47%	(5~14)	(5~15)	NA	NA
He 2010	46%	15±4.2	16±4.5	5.7±1.5 yrs.	6.8±1.8 yrs.
Jin 2017	46%	15.92±4.37 (8~20)	16.35±4.42 (8~21)	1.29±0.34 (0.08~3) yrs.	1.31±0.26 (0.08~3) yrs.
Lin 2008	46%	11.2 (5~20)	10.56 (6~18)	15.5 (6~66) mos.	18 (4~72) mos.
Liu 2008	46%	11.2 (5~32)		NA	NA
Shi 2010	50%	(5~20)	(2~25)	NA	NA
Wang 2010	50%	11.7 (4~19)	12.4 (3~21)	13 (3~31) mos.	13.8 (2.5~30) mos.
Wen 2014	22.5%	12.4 (2~20)	13.4 (3~21)	NA	NA
Wu 2003	50%	7.6 (2~20)	7.8 (1.5~22)	NA	NA
Xiang 2017	42%	NA	NA	7.8 ± 2.3 mos.	6.9 ±2.4 mos.
Yu 2008	40%	11.7 (4~19)	12.4 (3~21)	13 (3~31) mos.	13.8 (2.5~30) mos.
Zhang 2008	40%	5.4±0.3	5.39±0.27	10.1±0.8 mos.	9.5±1.0 mos.
Zheng 2014	42.4%	NA	NA	27.0 ±3. 9 mos.	29. 2 ±4.0 mos.
Zhu 2010	65%	(5~25)		NA	NA

RR=1.39[1.19, 1.62], P<0.0001; Compound Salvia Miltiorrhiza injection RR=1.25[1.12, 1.38] P<0.0001; Ginkgo Biloba extract injection RR=1.17[1.08, 1.27] P=0.0003; Panax Notoginsenosides injection RR=1.69[1.23, 2.33] P=0.001) (Figure 3).

3.4.2. Secondary Outcomes. All the data were analyzed on random effect model due to the heterogeneity.

(i) Nerve conduction velocity of median nerve (Figure 4)

MCV: 4 studies and 262 participants were included [23, 27, 28, 31]. TCMI group showed a significant improvement (MD=3.84[2.28, 5.41], P<0.00001).

SCV: 4 studies and 263 participants were included [23, 27, 28, 31]. There was no statistical difference between two groups (MD=2.59[-1.69, 6.87], p=0.24).

(ii) Nerve conduction velocity of peroneal nerve (Figure 5)

MCV: 4 studies and 264 patients were included [23, 27, 28, 32]. TCMI group showed a statistical difference (MD=2.89[0.63, 5.15], P=0.01).

SCV: 4 studies and 265 patients were included [23, 27, 28, 32]. There was no statistical difference (MD=2.73[-0.96, 6.43], P=0.15).

(iii) Hemorheology of plasma viscosity (Figure 6)

A total of 5 studies and 256 participants were included [23, 28, 29, 31, 32]. It showed a statistical difference (MD=0.27[0.04, 0.49], P=0.02).

(iv) Hemorheology of blood viscosity (Figure 7)

High shear rate: 6 studies and 428 participants were included [23, 24, 28, 29, 31, 32]. There was an improvement of TCMI groups (MD=0.36[0.05, 0.67], P=0.02).

Median shear rate: 4 studies and 268 participants were included [23, 24, 28, 29]. No statistical difference existed (MD=-0.02[-0.15, 0.12], P=0.81).

Low shear rate: 4 studies and 268 participants were included [23, 24, 28, 29]. TCMI groups showed an improvement (MD=1.05[0.14, 1.96], P=0.02).

3.5. Adverse Events. Only 3 studies reported adverse events. Four patients had facial redness and headache in study of Chi 2012 [32]. Two patients were with rash and pruritus in study of Jin 2017 [36] and two with the same symptom in study of Xiang 2017 [37]. The adverse events were mild and disappeared afterwards, so there was no sample loss. All the other studies reported no adverse events happening.

3.6. Publication Bias. We evaluated the possibility of publication bias by funnel plot of the clinical effective rate (Figure 8). As shown, it was generally symmetrical representing a low risk of publication bias.

4. Discussions

4.1. Summary of Main Results. We finally included 17 studies after extraction. The TCMIs they chose to use as the trail interventions concentrating on 5 different kinds are Danhong injection [25–27, 29, 34, 37], Erigeron Breviscapus extract injection (Dengzhanxixin injection) [24, 31, 32], Compound Salvia Miltiorrhiza injection (Fufang Danshen injection) [23, 28, 36], Ginkgo Biloba extract injection (Shuxuening injection) [35, 38, 39], and Panax Notoginsenosides injection (Xueshuangtong injection) [30, 33]. And we run the subgroup meta-analysis based on that.

Regarding clinical effective rate, all the five TCMI groups showed an improvement compared to conventional therapies groups no matter if it is the overall rate or subgroup rate,

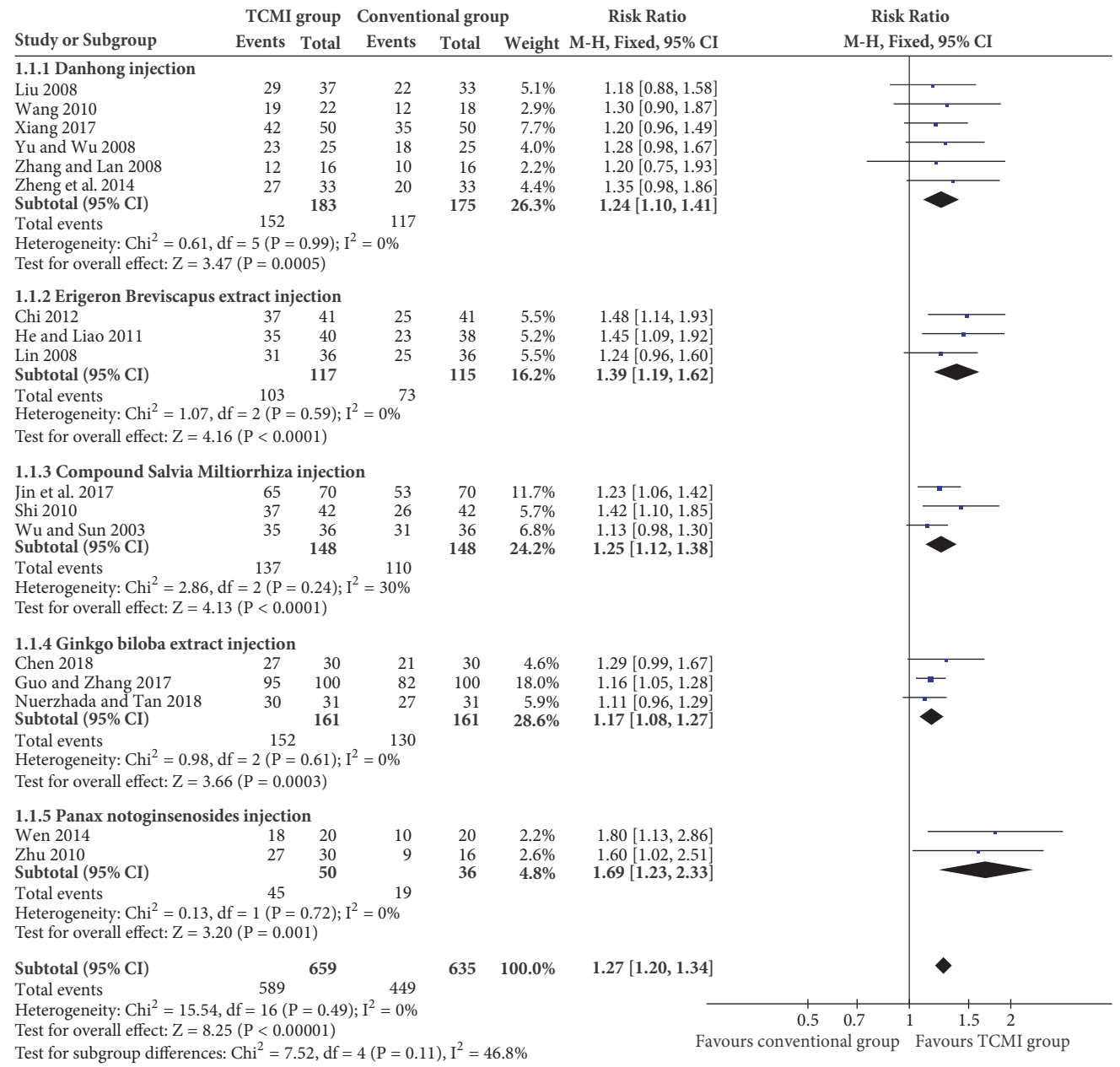

Figure 3: Effective rates of TCMI.

respectively.Using TCMIs can significantly raise the rate by 27% (P<0.00001) generally. Danhong injection was most widely used in clinic; however, evidence showed that it is not the most effective type to improve the clinical effective rate (RR=1.24[1.10, 1.41], P=0.0005). Meanwhile, the most effective type, Panax Notoginsenosides injection (RR=1.69[1.23, 2.33] P=0.001), is being used the least. Therefore, more qualified clinical trials and further researches need to be done.

Regarding the secondary outcomes, evidence suggested an improvement of TCMI groups in reducing the plasma viscosity and blood viscosity of high shear rate and low shear rate. And our evidence also supported an improvement of MCV of median and peroneal nerve, whereas no evidence supported the improvement of blood viscosity of median shear rate and SCV of both nerves. Given this contradiction, we consider a further analysis based on more qualified RCTs would help.

4.2. Strength and Limitations. We included 17 studies and 1294 participants totally. No sample loss happened, and all the outcomes were integrally reported at last. Regarding blinding for outcome assessors, 13 studies were evaluated with low risk of publication bias for they measured objective laboratory indexes. Also, test for subgroup difference showed no statistical differences (P=0.11, I^2=46.8%). With no heterogeneity (I^2=0%, P=0.49) in the analysis of overall clinical effective rate and a low heterogeneity (the largest I^2=30%, P=0.24) in subgroups, we considered the internal validity moderate.

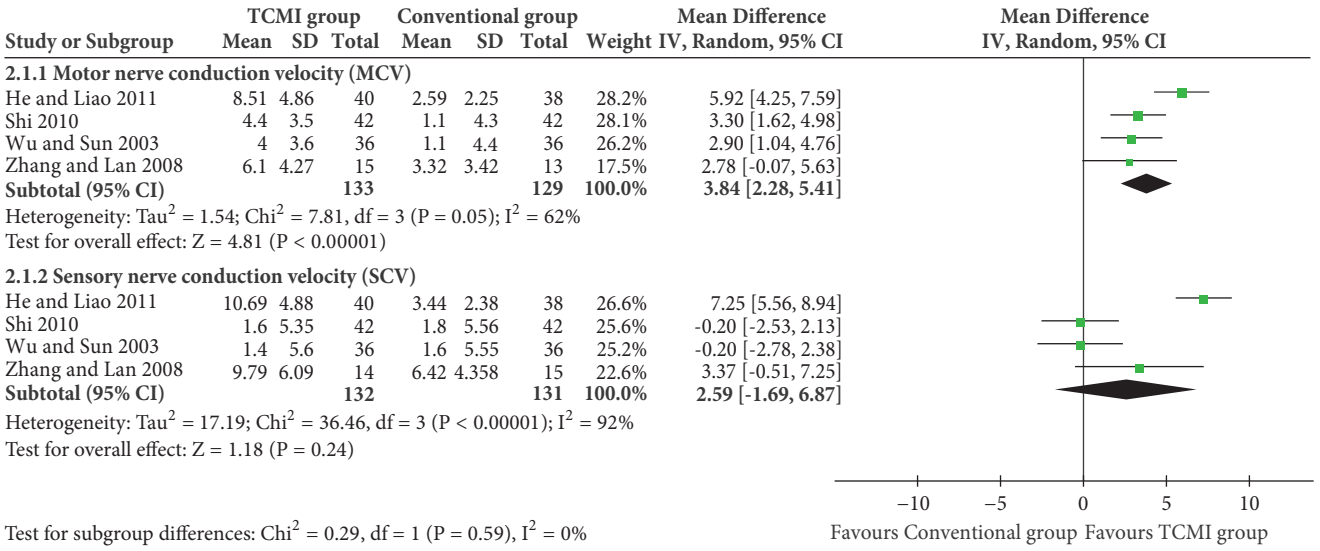

Figure 4: Nerve conduction velocity of median nerve.

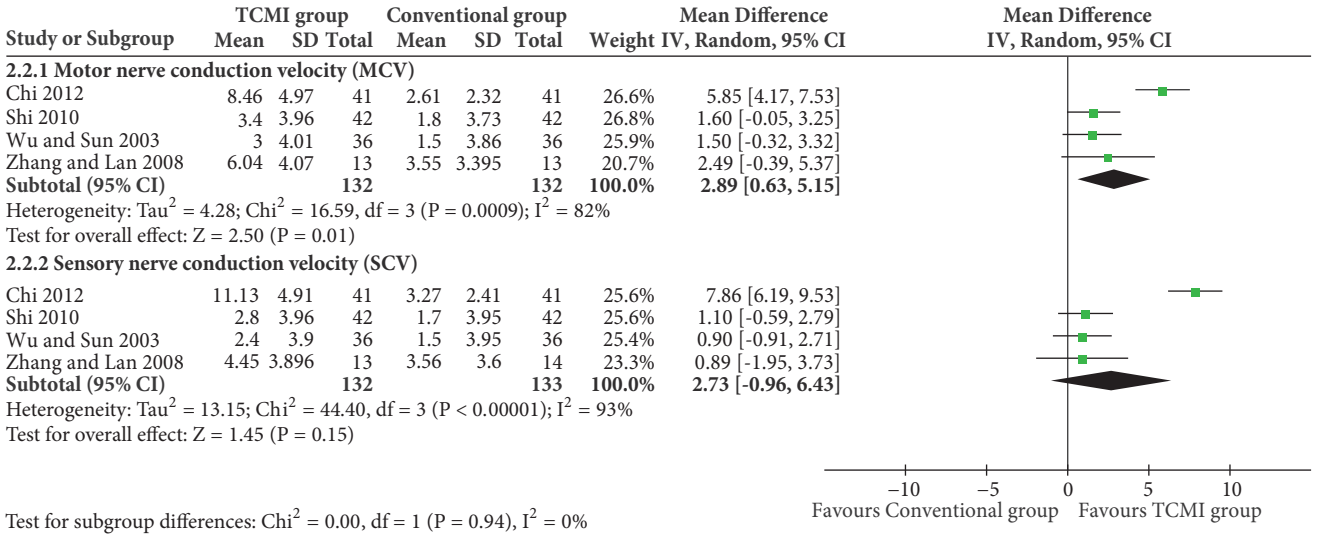

Figure 5: Nerve conduction velocity of peroneal nerve.

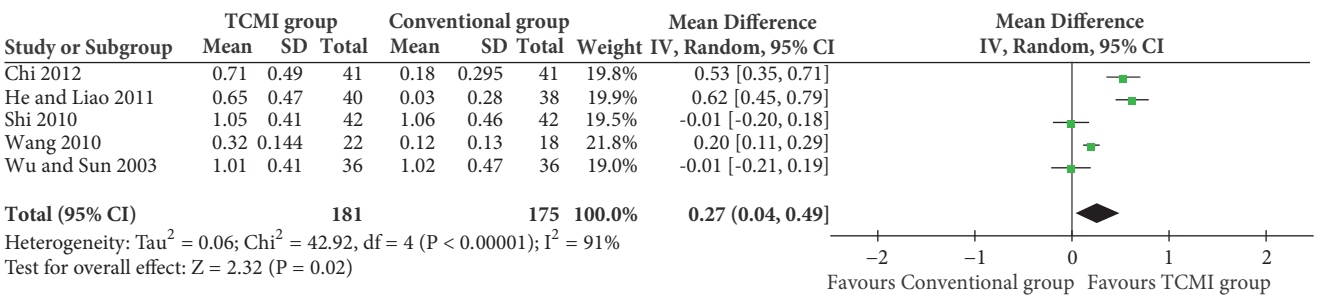

Figure 6: Hemorheology of plasma viscosity.

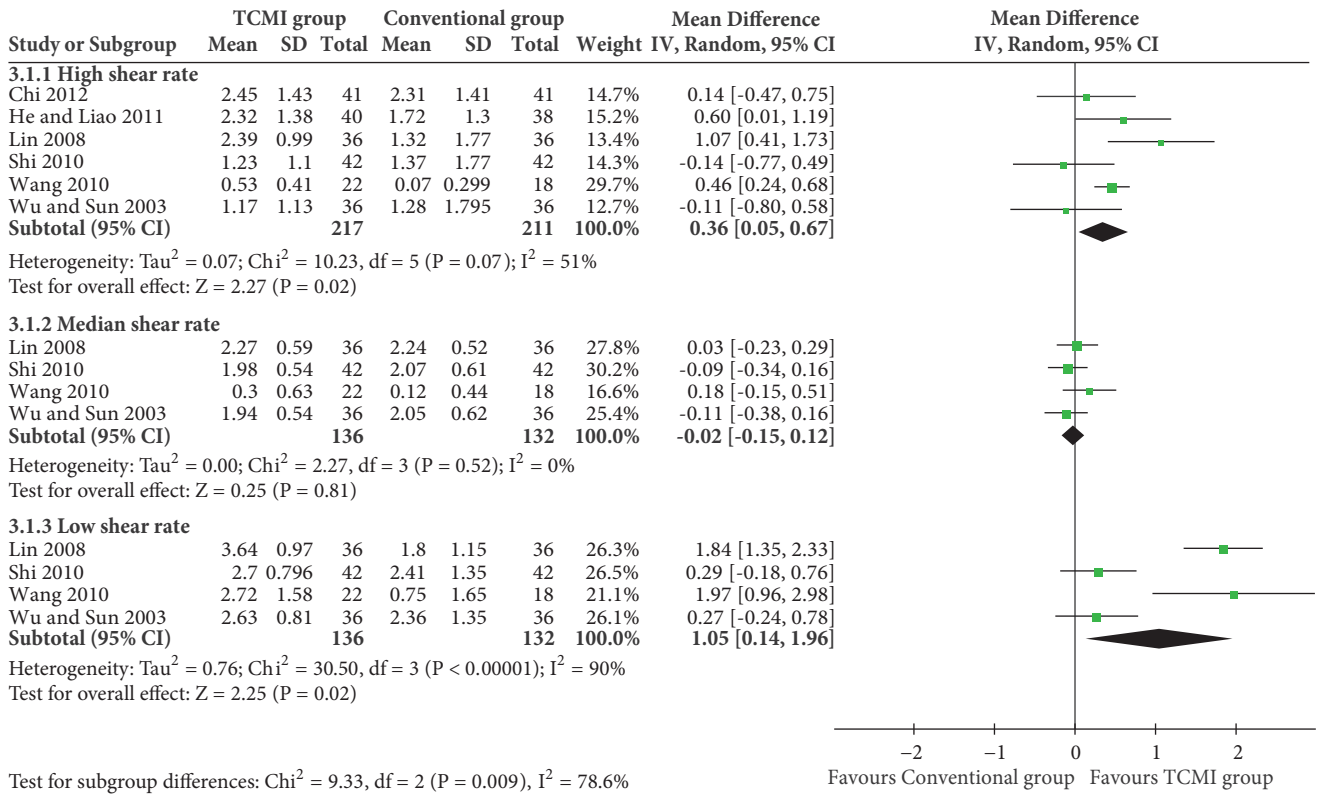

Figure 7: Hemorheology of blood viscosity.

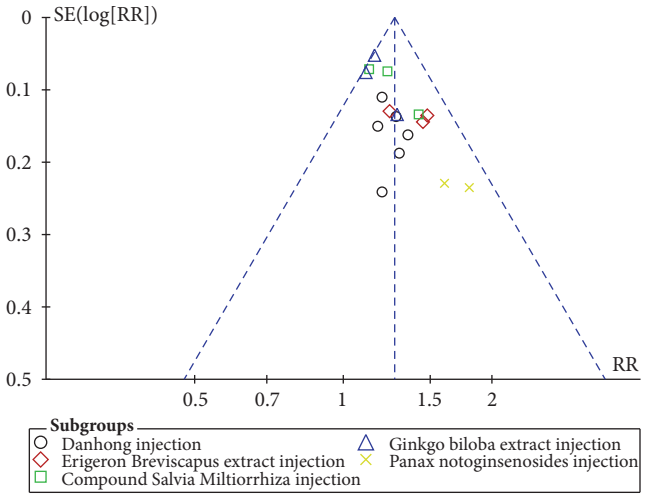

Figure 8: Funnel plot of the clinical effective rate.

All participants are enrolled from different regions of mainland China with a balance gender ratio and most of them are middle-aged and elderly people. Within the 17 studies, only 1 reported the random number table and 14 mentioned "random" without the specific approach. Furthermore, 2 are quasi-randomized with an obvious selection bias. None of them mentioned the allocation concealment and the two quasi-randomized trial cannot conceal its allocation. That indicates a high risk of allocation bias. Besides, no participants included are classified into grade 5 in Wagner scale. Thus, we only recommend the TCMI interventions to clinicians in treating the middle-aged and elderly patients with a mild to moderate DFU classification (with a Wagner scale lower than grade 5).

Few adverse events happened in all the studies and the events happened are mild to moderate degree which will disappear after some resting. And the cost of TCMI is cheap, because most of them are in the Chinese national medical insurance list (Danhong injection, Erigeron Breviscapus extract injection, Ginkgo Biloba extract injection, Panax Notoginsenosides injection) which means 80% of the expense is covered [41]. According to the course of treatment reported, mostly 28 days, the total cost will be no more than 436.24 CNY. Compared to the significant improvement of clinical effective rate as 27%, TCMIs will only increase the average cost for an ulcer episode by 1.5% [42]. It can be considered as a cost-effective and safe strategy with a low treatment expense increase.

Although the heterogeneity of primary outcome is low, there may exist potential bias. The courses of disease were inconsistent (Table 2) and the conduction of basic care may differ from practitioners such as debridement and dressing change.

More qualified RCTs need to be included to explain the high heterogeneity in the meta-analysis of secondary outcomes. And due to a contradictory result of secondary outcomes, we hereby recommend more qualified RCTs with

a report of objective laboratory indexes in treating DFU with TCMIs such as nerve conduction velocity and hemorheology indexes.

5. Conclusion

In management of DF, TCMIs can increase the clinical effective rate of conventional therapies by 27%. Along with a better performance in safety and financial burden, the management of DF can be improved by TCMIs. However, the overall methodological and reporting quality of the included studies was limited. Moreover, there are some contradictions in secondary indexes. Therefore, more high-quality large sample-size RCTs are needed to prove and explain it.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this article.

Acknowledgments

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Research Article

A Prospective Randomized, Controlled, Double-Blind Trial of the Efficacy Using Centella Cream for Scar Improvement

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Objective. This study was performed to evaluate the efficacy of *Centella asiatica* extract in cream, a preparation for the prevention of scar development of the split-thickness skin graft (STSG) donor site. *Methods*. A prospective randomized, double-blind control study was performed to evaluate the efficacy of Centella cream in 30 patients who underwent a STSG operation. Both Centella cream and placebo were applied equally to the donor site at least 2 weeks after epithelialization was completed. A scar assessment using the Vancouver Scar Scale (VSS) was taken at 4, 8, and 12 weeks. *Results*. Of the original 30 patients, 23 patients completed evaluation. There were significant differences in pigmentation parameter of VSS and comparative total VSS scores between 4 and 12 weeks in Centella cream group. *Conclusion*. The effect of Centella cream on scar development of a STSG operation may be attainable in terms of better pigmentation. By means of objective measurements and longer follow-up times, Centella cream may prove to be an alternative product for hypertrophic scar amelioration.

1. Introduction

Wound healing is a process that takes place with almost all medical treatments. The history of the wound care dates back to the days when natural substances, such as honey and other various remedies, were the norm for treatment since 2600-2200 BCE [1]. Later studies found that natural substances contained in herbs and plants have many positive properties that assist in and enhance the wound healing process, substances such as antioxidants, anti-inflammatories, and antibacterial agents [2]. Many species of plants support the theory that herbs have served a major role in assisting the process of recovery and healing. Studies from different parts of the world have produced concrete findings that support such theories in the era of modern medicine. Evidence stems from research regarding plants and herb usage for treating wounds in different geographical locations, countries such as Africa [3, 4], India [5], China [6] Thai [7], and even the United States, Canada, or Europe [8].

When wounds occur, the chances of a scar forming are greatly increased; scars usually have negative impact on patient. There are many methodologies such as occlusive dressings, compression therapy, intralesional corticosteroid injections, radiation therapy, laser therapy, interferon therapy, and topical silicone gel application, and herbal extracts have been prescribed as the norm. The high cost of these treatments has been the greatest obstacle for patients to continue with treatment, especially in developing countries. "Ugly scars" is a term referring to scar contracture, stretching or hypertrophic scarring resulting from abnormal response by fibroblasts during the proliferative stage and imbalance between collagen synthesis and degradation during the remodeling stage [9]. Nowadays, herbs have an important role in restoring the healing wound process and ameliorating scar. Current studies on *C. asiatica* have been conducted to improve the outcome of the wound healing process in small wound types and hypertrophic scar as well as burns, psoriasis, and scleroderma [10–14].

Centella asiatica, also commonly known as Gotu kola, is a small plant that depends on the soil with water trapped, as a herbaceous that originates from Asia. It is an annual plant of the family Apiaceae. This plant has been used in folk medicine

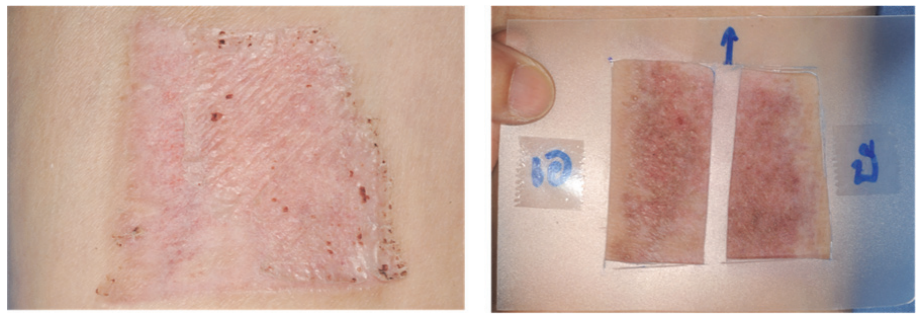

Figure 1: Two-hole template using which patients have to apply the Centella and placebo cream in the frame.

as well as in western medicine [15]. This study was performed to evaluate the efficacy of *Centella asiatica* extract in a cream preparation for the prevention of scar development on the STSG donor site.

2. Methods

2.1. Design Overview. All patients who participated consented to join the study on a voluntary basis as the donor site of STSG. Subjects were divided randomly into 2 groups, with various cream applied to each part of the donor's scar site. Cream A (7% w/w *Centella asiatica* extract in cream preparation) or Cream B (placebo) was randomly applied on the subjects. The gels were applied for a total treatment period of 12 weeks. The assessment was conducted by one experienced nurse who was blinded to the subject grouping and was trained to administer all the assessments in standardized manner.

2.2. Setting and Participants. From January 2014 to February 2015, 36 patients who underwent split-thickness skin graft harvesting were enrolled in this study, but six patients were excluded because they declined to participate at the Outpatient Unit, Department of Surgery, Faculty of Medicine Srinagarind Hospital. All 30 patients in this study were aged over 20 years and met the inclusion criteria. This study was approved by Khon Kaen University Ethics Committees for Human Research.

Inclusion Criteria

(1) The donor site of the patients who underwent STSG operation completed more than 14 days of epithelialization.

(2) All of the participants were 20 years or older.

Exclusion Criteria

(1) Patients with critical illnesses such as those with systemic infection or hemodynamic instability.

(2) Patients with a major acute or chronic medical illness that could have impact on the wound healing process.

(3) Patients who were pregnant.

(4) Patients who could not read the Thai language.

(5) Patients who declined to participate.

2.3. Sample Size. 30 voluntary patients were included in this study. The donor site of skin graft in each subject was divided into 2 parts, with different treatments randomly applied to each part. For sample analysis, with 23 subjects per method, the statistical power to remark a mean difference of 1 unit on the rating scale, assuming an SD of 1.71, was 80%. A 2-tailed test with type 1 error rate 5% was supposed. In statistical method, 1.00 corresponded to Mu (M) and 0.02 to the Beta (β). Concerning defense against the withdrawal of the patients, it was necessary to raise the number of patients of each group to be 30.

2.4. Randomization and Interventions. The *Centella asiatica* was prepared by being extracted with 70% alcohol in cream preparation (Chao Phya Abhaibhubejhr Hospital, Prachin Buri, Thailand). It was formulated from 7% w/w Centella extract, 100 gram, combined with Centella extract 7 g., cetyl alcohol 15 g., stearyl alcohol 12 g., mineral oil 5 g., cetomacrogol-1,000 3 g., propylene glycol 1 g., paraben concentrate 1.5g, and water refill for total of 100 g. for the whole combination. The Centella extract comprised asiaticoside 5.12% and madecassoside 5.1%. The placebo cream was similar in color and consistency to the *Centella asiatica* extract cream and was packed in the same sealed packages. For the composition, it was the same as *Centella asiatica* extract cream except 7 g. of paraben concentrate. Centella and placebo cream were marked A or B. The patients were advised to clean their hands before applying each cream and to use the template (Figure 1). After placing the template on the wound, the patient had to gently apply approximately 1 gram of each cream and wait until they were completely absorbed. This application was done twice daily. Each patient was scheduled for follow-up at 4, 8, and 12 weeks (Figure 2).

2.5. Outcomes and Measurements. Patient demographic data were recorded. The scars were examined and rated using the Vancouver Scar Scale (Table 1) to determine pigmentation, vascularity, pliability, and height. The digital photos of each scar were also recorded by using a digital camera each time the assessment was performed, and the photos taken were standardized with fixed distance and lighting. A digital camera was used to ensure that the images taken were clear and comparable.

The Vancouver Scar Scale measured vascularity, pliability, and height, each on a 3- to 6-point ordinal scale; pigmentation

Table 1: Vancouver Scar Scale (VSS).

Score	0	1	2	3	4	5
Vascularity	Normal	Pink	Red	Purple		
Pigmentation	Normal	Hypopigmentation	Mixed	Hyperpigmentation		
Pliability	Normal	Supple	Yielding	Firm	Ropes	Contractures
Height	Flat	<2 mm	2-5 mm	>5 mm		

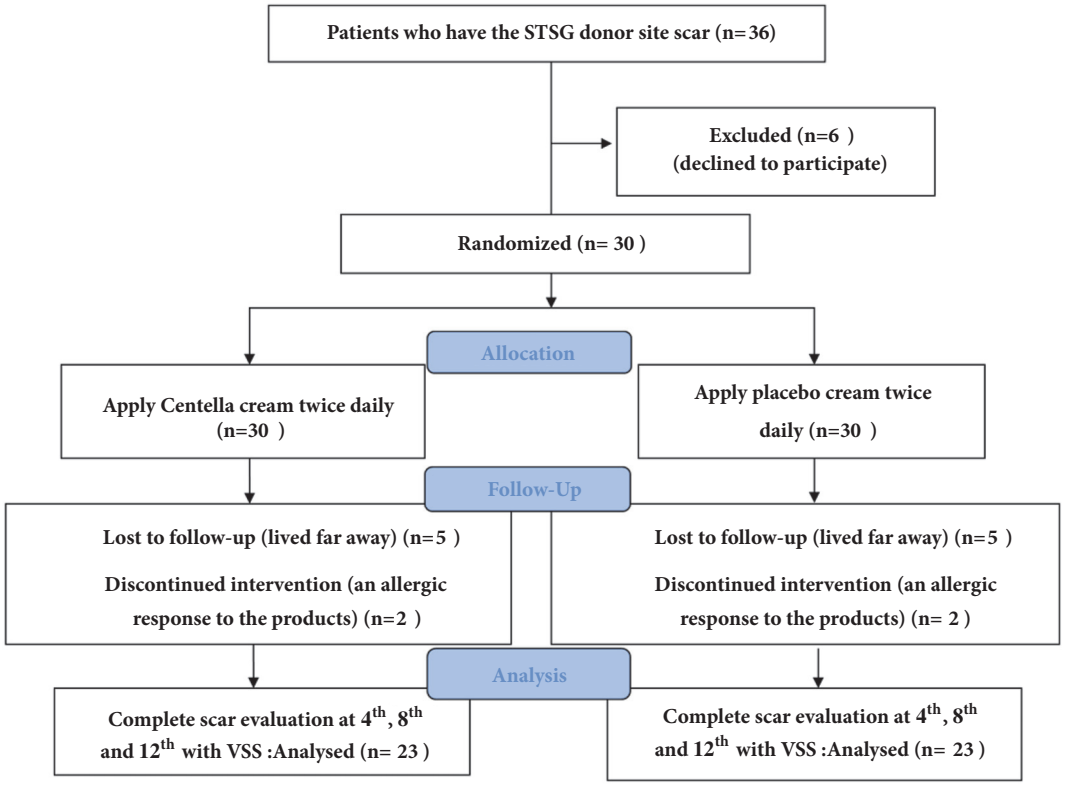

Figure 2: Diagram of study protocol that was initiated.

was measured on a 3-point categorical scale. The results were recorded and patients identified as to which of the 2 groups they belonged after the protocol was finished. If the treatment decreased in the score of each parameter, the result concluded with improvements. If the score was the same or higher, no response was reported.

2.6. Statistical Analysis. Greenhouse-Geisser correction (p value) and one-way repeated measures ANOVA were used to assess two more groups who were participants in the same group. All statistical analyses were operated using SPSS 16.0 software (SPSS, Chicago, IL.). A p value equal to or below 0.05 was regarded as statistically significant

3. Results

Only 23 of 30 patients completed the study protocol. 2 patients were excluded due to rash at the scar, which may have been caused by an allergic response to the products. 5 patients were lost to follow-up because they lived far away. Of 23 patients, there were 13 males and 10 females. The average age was 54 years old (range: 20-65 years old). As shown in Table 2 and Figures 3–7, differences within groups are as follows: For the Centella cream group, there were differences from baseline including the pigmentation score at 8 and 12 weeks and between 4 and 12 weeks (-0.443, p value 0.019; -0.707, p value 0.001; -0.557, p value 0.001) and the overall Vancouver Scar Scale scores between 4 and 12 weeks (-1.279, p value 0.041). However, for height, it was worse at 4 weeks (0.300, p value 0.043). For the placebo group, there were 2 differences from the baseline including the pigmentation score between 4 and 12 weeks (-0.399, p value 0.020) and after 12 weeks (-0.549, p value 0.002), while the pliability and the height scores of both groups were compared before and after treatment and still were not different.

4. Discussion

The split-thickness skin graft donor site is a superficial partially thickness wound in which losing the epidermis and part of the dermis exists. Epithelialization is the natural act of healing dermal tissue resulting in minimal or no scarring

Table 2: Comparison within groups after 12 weeks of both groups.

Vancouver Scar Scale	Centella cream	Contrast	95% CI	P-value
After	week 4th	0.400	-0.799 - 1.599	0.510
After	week 8th	-0.089	-1.312 - 1.133	0.885
After	week 12th	-0.879	-2.102 - 0.344	0.157
Between	8&4 weeks	-0.489	-1.712 - 0.733	0.429
Between	12&4 weeks	-1.279	-2.502 - -0.056	0.041*
Between	12&8 weeks	-0.789	-2.020 - 0.441	0.206
Vancouver Scar Scale	**Placebo**			
After	week 4th	0.150	-1.049 - 1.349	0.805
After	week 8th	0.312	-0.911 - 1.535	0.614
After	week 12th	-0.793	-2.016 - 0.429	0.201
Between	8&4 weeks	0.162	-1.061 - 1.385	0.794
Between	12&4 weeks	-0.943	-2.166 - 0.279	0.129
Between	12&8 weeks	-1.105	-2.336 - 0.125	0.078
Vascularity	**Centella cream**			
After	week 4th	0.100	-0.426 - 0.626	0.707
After	week 8th	-0.055	-0.591 - 0.481	0.838
After	week 12th	-0.213	-0.749 - 0.323	0.432
Between	8&4 weeks	-0.155	-0.691 - 0.381	0.567
Between	12&4 weeks	-0.313	-0.849 - 0.223	0.249
Between	12&8 weeks	-0.158	-0.697 - 0.381	0.563
Vascularity	**Placebo**			
After	week 4th	-0.050	-0.576 - 0.476	0.851
After	week 8th	-0.051	-0.587 - 0.485	0.850
After	week 12th	-0.367	-0.903 - 0.169	0.177
Between	8&4 weeks	-0.001	-0.537 - 0.535	0.996
Between	12&4 weeks	-0.317	-0.853 - 0.219	0.244
Between	12&8 weeks	-0.316	-0.855 - 0.224	0.248
Pigmentation	**Centella cream**			
After	week 4th	-0.150	-0.478 - 0.178	0.366
After	week 8th	-0.443	-0.777 - -0.109	0.010*
After	week 12th	-0.707	-1.041 - -0.373	0.001*
Between	8&4 weeks	-0.293	-0.627 - 0.041	0.084
Between	12&4 weeks	-0.557	-0.891 - -0.223	0.001*
Between	12&8 weeks	-0.263	-0.599 - 0.073	0.124
Pigmentation	**Placebo**			
After	week 4th	-0.150	-0.478 - 0.178	0.366
After	week 8th	-0.233	-0.567 - 0.101	0.170
After	week 12th	-0.549	-0.883 - -0.215	0.002*
Between	8&4 weeks	-0.083	-0.417 - 0.251	0.624
Between	12&4 weeks	-0.399	-0.733 - -0.065	0.020*
Between	12&8 weeks	-0.316	-0.652 - 0.020	0.065
Pliability	**Centella cream**			
After	week 4th	0.200	-0.202 - 0.602	0.326
After	week 8th	0.179	-0.231 - 0.589	0.389
After	week 12th	-0.137	-0.547 - 0.273	0.509
Between	8&4 weeks	-0.021	-0.431 - 0.389	0.919
Between	12&4 weeks	-0.337	-0.747 - 0.073	0.106
Between	12&8 weeks	-0.316	-0.728 - 0.097	0.132
Pliability	**Placebo**			
After	week 4th	0.150	-0.252 - 0.552	0.461
After	week 8th	0.312	-0.098 - 0.722	0.134
After	week 12th	-0.004	-0.414 - 0.406	0.985

Table 2: Continued.

Vancouver Scar Scale	Centella cream	Contrast	95% CI	P-value
Between	8&4 weeks	0.162	-0.248 - 0.572	0.435
Between	12&4 weeks	-0.154	-0.564 - 0.256	0.458
Between	12&8 weeks	-0.316	-0.728 - 0.097	0.132
Height	**Centella cream**			
After	week 4th	0.300	0.009 - 0.591	0.043*
After	week 8th	0.229	-0.067 - 0.525	0.129
After	week 12th	0.176	-0.120 - 0.473	0.241
Between	8&4 weeks	-0.071	-0.367 - 0.225	0.636
Between	12&4 weeks	-0.124	-0.420 - 0.173	0.410
Between	12&8 weeks	-0.053	-0.351 - 0.246	0.727
Height	**Placebo**			
After	week 4th	0.200	-0.091 - 0.491	0.176
After	week 8th	0.284	-0.012 - 0.581	0.060
After	week 12th	0.126	-0.170 - 0.423	0.400
Between	8&4 weeks	0.084	-0.212 - 0.381	0.575
Between	12&4 weeks	-0.074	-0.370 - 0.223	0.623
Between	12&8 weeks	-0.158	-0.456 - 0.140	0.296

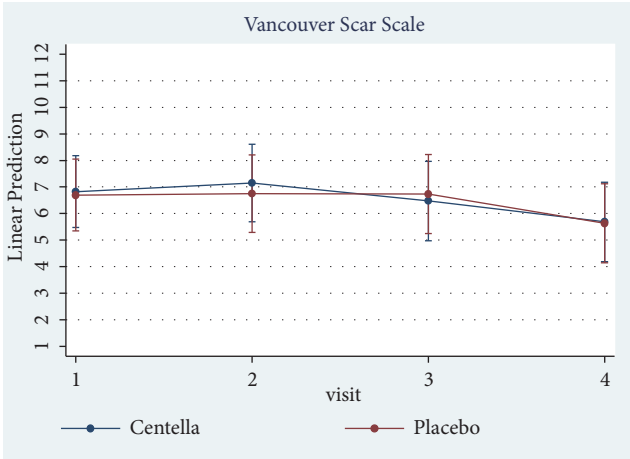

Figure 3: Comparison of Vancouver scar scores within groups after 12 weeks.

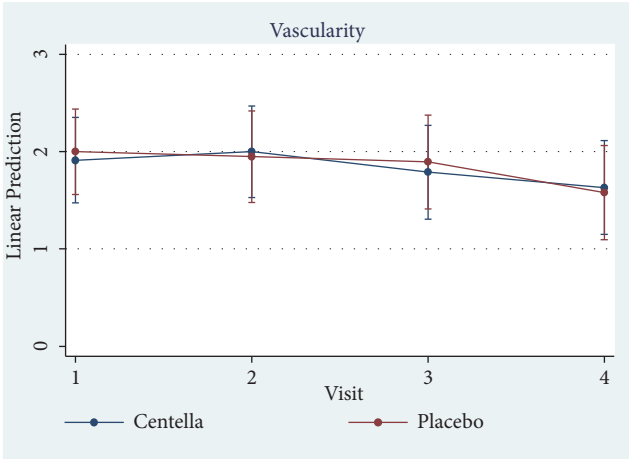

Figure 4: Comparison of vascularity scores within groups after 12 weeks.

[16]. In most cases, scars occur if the depth reaches the dermis layer; the exposed area of the scar can be more problematic. Treatment for scars can be difficult, and in some cases the skin cannot return to its normal condition causing additional suffering to the patient. Some patients complained about discoloration, both hyperpigmentation and hypopigmentation. When hypertrophic scar or keloid developed, it may induce itching, pain, and uninviting scar damage, sometimes in the form of scar contracture which may cause organ dysfunction. In some cases, patients were unhappy and expressed a great deal of regret, anger, rejection, and isolation despair [17]. The knowledge of scar protection/prevention and causes of the scar is an important factor and helps reduce its severity. However, some Asians displayed a condition referred to as "Fitzpatrick Skin" (type III or type IV): commonly known as hypervascular, hyperpigmented, hypopigmented, or hypertrophic scars when other related characteristics are the anatomic region, patient's skin type or genetic factors, nature of injury, skin tension, and prolonged inflammatory process [18]. Numerous attempts have been made to introduce natural substances to reduce scars such as onion extract [19, 20], resveratrol in grape's skin [21], curcumin [22], and *Centella* [23].

Scar protection applications can be made by starting from coagulation, inflammation, and proliferation phase, which tried to make each phase without any complications and was time consuming. In the remodeling phase, collagen was rearranged and was broken by enzyme matrix metalloproteinase (MMPs), which is controlled by tissue inhibitors of MMP (TIMPs) [24]. The balance between regeneration and

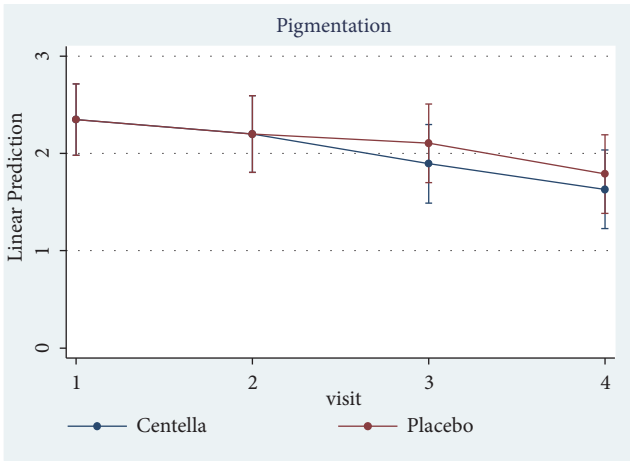

Figure 5: Comparison of pigmentation scores within groups after 12 weeks.

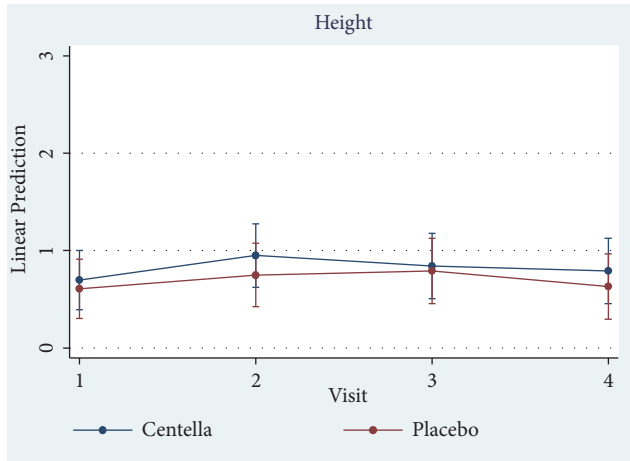

Figure 7: Comparison of height scores within groups after 12 weeks.

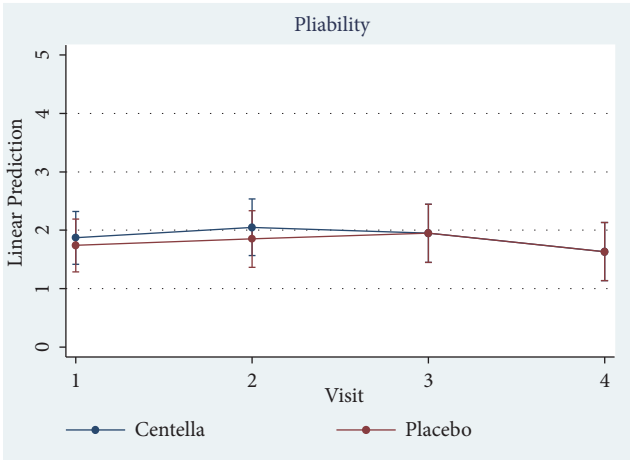

Figure 6: Comparison of pliability scores within groups after 12 weeks.

degradation of collagen was initiated during the time periods of 6 months to 1 year. The scar was entirely abnormal in appearance, the architectural arrangement of collagen, and ECM. The strategy for minimized scar needed many factors like efficiently control inflammation [25] and low levels of cytokines such as TGF βI, TGF βII, and platelet-derived growth factor [26, 27] but high levels of TGF β III [26–28]. TGF β I and TGF β II played a role in activating proliferation of fibroblasts, whereas TGF β III was antagonist and that is why it could prevent scar formation [29].

Prior studies have shown the effectiveness of *C. asiatica* extract in promotion of wound healing and prevention of hypertrophic scars [19, 30, 31]. The active compounds of *C. asiatica* responsible for those activities are pentacyclic triterpenes, including asiaticoside, and madecassoside. In vitro study demonstrated that asiaticoside decreased fibroblast proliferation in a dose-related manner and reduced the expression of both TGF- β I and TGF- β II at the transcriptional and translational level [32]. Asiaticoside also slows down scar formation possibility by increasing the activity process of Smad7 which is a negative regulator of TGF-β signaling [33]. The other active composition, madecassoside, is related to inhibiting the migration of fibroblasts from keloids [34]. Both active chemical substances promote *C. asiatica* to induce fibroblast proliferation and collagen synthesis. It involves the improvement of the tensile strength of newly formed skin and stimulation of maturation of the scar by the production of type I collagen. Inversely, the expression of TGF-βI in hypertrophic scars and keloid is reduced [32, 34, 35]. Both asiaticoside and madecassoside affected the healing process mechanism in which inflammatory, proliferation, and remodeling phases initiate the improvement of wound healing and scar prevention [10]. In the case of animal research they showed effective result of diminished hypertrophic scar [30]. Numerous data confirmed that *C. asiatica* extract in a combination form with other herbs could prevent abnormal scars from median sternotomy or split-thickness skin graft donor sites; however, no exact demonstration revealed that one was stronger for preventing scar [19, 31]. The aim of this study was to evaluate the efficacy of Centella cream with only one chemical substance, not combined with other herbal substances, on the prevention of scar development. Our study revealed that Centella cream improved scar outcomes against placebo. The Centella cream significantly improved the overall Vancouver scores between 4 and 12 weeks and pigmentation from the baseline since 8th week, in comparison with the improved pigmentation from the baseline since the 12th week in the placebo group. This evidence concluded that the nature of scar development of the donor site of the split- thickness skin grafts got better over time, ameliorating by Centella effect. Figures 8 and 9 demonstrated the better outcome in skin pigmentation after applying the Centella cream. In this study, an allergic dermatitis was observed in 2 patients. Gomes reported in the reported case that the patient had contact dermatitis due to *Centella asiatica* extract [36]. Since the allergic reaction occurred with both Centella cream and placebo, the ingredients of the placebo may be responsible for the allergic

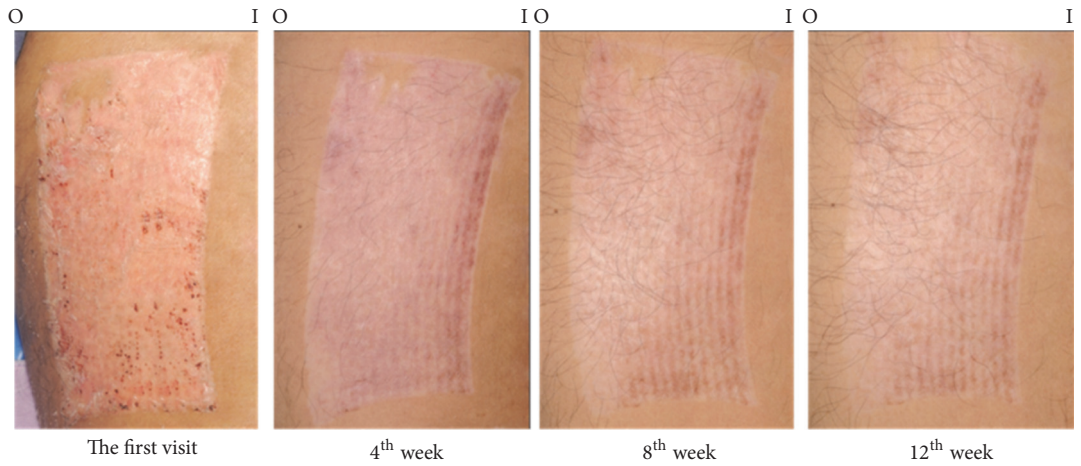

Figure 8: A 58-year-old male patient with a split-thickness skin graft harvested from right thigh. Centella cream was applied at the outer part with placebo at the inner part of thigh (I: inner part, O: outer part).

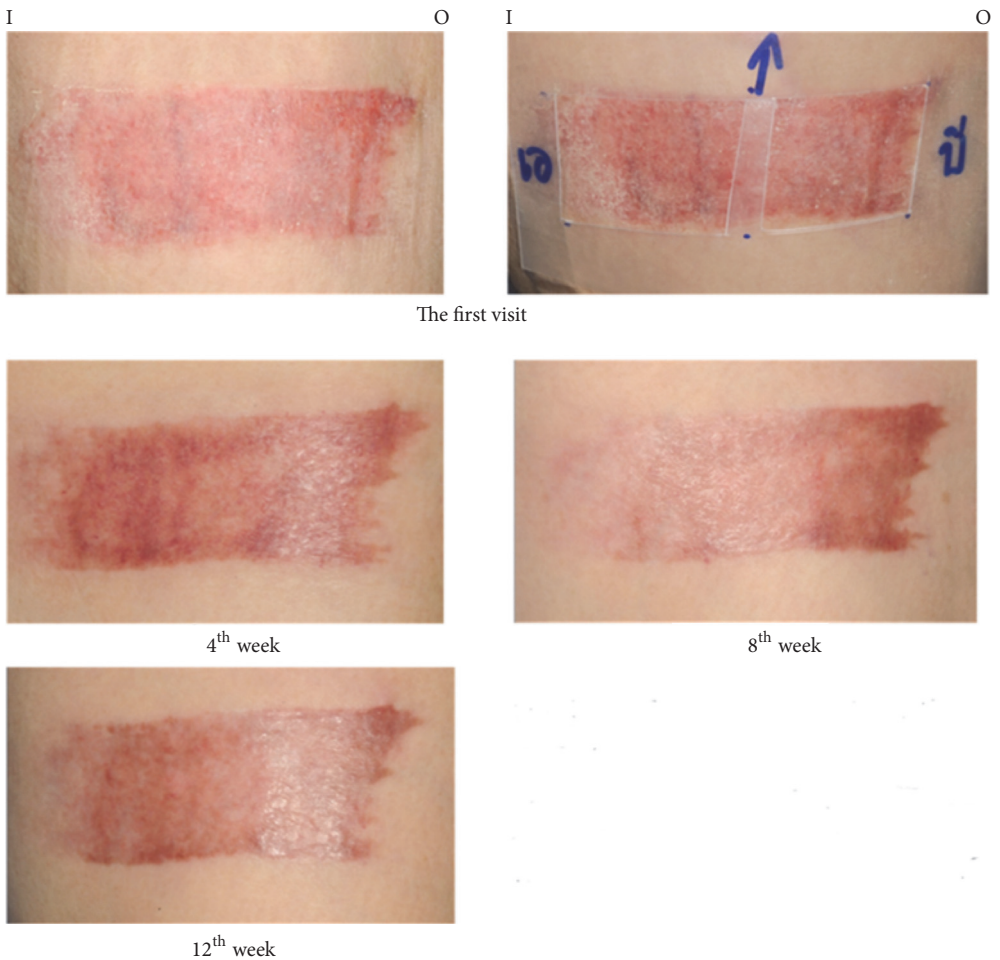

Figure 9: A 66-year-old female patient with a split-thickness skin graft harvested from left thigh. Centella cream was applied at the inner part with placebo at the outer part of thigh (I: inner part, O: outer part).

dermatitis. Previous reports showed that contact dermatitis developed when paraben-containing products were applied [37].

The efficacy of *C. asiatica* formulation may be affected by the release of active compounds from the formulation. The animal (rat) model demonstrated the promoted cellular proliferation and collagen synthesis effects of the aqueous extract *C. asiatica*. The gel formulation provided significantly better healing outcome than the ointment and cream formulations [38].

The VSS is more subjective measurement. Studies with longer follow-ups may be required to confirm the benefits of this product. In the future, the dosage and the manner evaluation of efficacy of *Centella asiatica* need standardized experimentation in the healing process and scar prevention.

5. Conclusion

The effect of Centella cream on scar development of the donor site of the split- thickness skin grafts may be attainable in terms of better pigmentation. By means of objective measurements and longer follow-up times, Centella cream may prove to be an alternative product for hypertrophic scar amelioration.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors have no financial interest or commercial association with any information mentioned in this article.

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Research Article

Humic Acid Enhances Wound Healing in the Rat Palate

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Introduction. Humic acid was previously shown to enhance cutaneous wound healing and show antibacterial properties; however, it has not been used for wound healing in the oral cavity. Thus, the goal of this study was the evaluation of the effect of the humic acid on the healing of excisional wounds in an experimental rat study. *Materials and Methods*. A circular wound on mid-palatal surfaces was made on a total of 77 Wistar rats by using a 3-mm biopsy punch under anesthesia. The animals were divided into 4 groups as baseline, saline control (0.09%), chlorhexidine gluconate (0.05%), and humic acid (80 mg/kg) and were treated with these materials for 7 days. *Results*. The rats were observed for 3 weeks in order to track the wound closure rates. Both humic acid treatment and chlorhexidine gluconate treatment resulted in statistically significant enhanced rate of wound closure compared to the saline control on both the 1st and 2nd weeks of treatment. Humic acid treatment for the wounds in the palate resulted in enhanced recovery compared to not only saline control but also chlorhexidine gluconate treatment. *Conclusion*. In this study, humic acid was shown to enhance healing of oral wounds for the first time in the literature. These findings indicate that humic acid can be used as an alternative to current treatment methods for oral wounds.

1. Introduction

Wounds in the oral cavity constitute an important health concern for many people due to the warm oral microenvironment that is home to many bacteria and the constant wear and tear that occurs due to physical activity caused by eating and drinking [1, 2]. Wound healing is a complex process that is characterized by three stages as inflammation, proliferation, and remodeling [3]. During the inflammation phase, the wound area is protected against pathogens and the dead cells are removed, whereas, during proliferation phase, the cells that secrete extracellular matrix materials proliferate and secrete high amounts of fibrous extracellular matrix proteins in order to rapidly block and protect the wound area against pathogens [4]. During remodeling phase, these fibrous blocking components are removed and more functional tissue is generated.

Since the highly humid and warm environment of the oral cavity is a supportive environment for microbial growth, it is customary to use antibacterial products to aid in rapid and infection-free wound healing for wounds in the palate [5, 6]. Other than the antibacterial products that are already commercially used, few factors were previously shown to aid palatal wound healing [5–7].

Humic substances, which are present mostly in lignite, peat, soil, and water, have antiviral, antibacterial, antitoxic, antiulcerogenic, antiarthritic, antiallergic, immunomodulatory, and anti-inflammatory properties [8–15]. Microorganisms convert plant and animal tissue into peat [16], and humic substances such as humus, peat, sapropel, and mumie have been used in medicine for different applications against different illnesses as far as 3000 years ago [11]. The toxicity of humic acid is very low [17]. Use of humate results in reduction in paw volume of the carrageenan-induced edema in rats [18]

Table 1: Composition of the humic acid sample.

Analysis Parameters	Unit	Methods	Analysis Results W/W'
Total humic acid	%	TS 5869 ISO 5073	6.5
Total P	%	ICP-AES	0.015
Total Si	%	ICP-AES	1.36
Total Se	mg/kg	ICP-AES	0.15
Total Ca	%	ICP-AES	1.26
Total Mg	%	ICP-AES	0.014
Total Fe	%	ICP-AES	0.056
Total Mo	mg/kg	ICP-AES	89.50
Total Zn	mg/kg	ICP-AES	31.13
Total Na	%	ICP-AES	1.79
Total Cl	%	Titrimetric	-

and sodium humate was shown to enhance wound healing in rats [19].

In the oral cavity, we have previously shown that humic acid prevents alveolar bone loss and reduce inflammation in rats [20]. In addition, carbohydrate-derived fulvic acid, which is a major constituent of humic acids, has been shown to have a broad-spectrum antimicrobial activity against orally active microorganisms [21]. Although these results suggest the possibility that these specific properties of humates may be useful in wound healing in the palate, currently, there is no evidence showing the effects of humates on wound healing in the oral cavity. Thus, the aim of this study was the evaluation of the effect of the humic acid on the healing of excisional wounds in the palate of rats.

2. Materials and Methods

2.1. Animals and Study Groups. The study protocol and experimental design were approved by the Animal Ethics Committee of Cumhuriyet University School of Medicine (approval number: B.30.2.CUM.0.01.00.00-50/59, 312). In total, 77 three-month-old male Wistar rats were used in the experiment. Their body weight ranged from 280 to 320 g at the beginning of the experiment. Rats in each group were fed in different cages under the same conditions in a well-lit and well-ventilated room. All rats were fed *ad libitum* and kept at 12 h/12 h light/dark cycle and at 21 ± 1°C temperature and 40–60% humidity. Rats were acclimated to their living environment for 10 days prior to the study to alleviate stress related interference with experimental set-up. The experimental stages of this study were performed in the Animal Laboratory of Cumhuriyet University's Faculty of Medicine. The animals were randomly divided into four groups as follows:

(i) Control (C) group (n = 5)

(ii) Saline (0.9%) (S) group (n = 24)

(iii) Chlorhexidine gluconate (0.05%) (CHG) group (n = 24)

(iv) Humic acid (HA) group (n = 24)

Each main group was divided to three subgroups containing 8 rats in each to observe changes after 1st, 2nd, and 3rd weeks.

2.2. Formation of Experimental Palatal Wound Surface. After an adaptation period of 10 days, animals were anesthetized with xylazine hydrochloride (Rompun; 10 mg/kg, Bayer Animal Health GmbH, Leverkusen, Germany) and ketamine hydrochloride (Ketalar; 40 mg/kg, Eczacibasi Ilac Sanayi, Istanbul, Turkey) intraperitoneally. A punch biopsy tool with 3 mm diameter was used to create a standardized circular wound outline on the anterior palate in the mucoperiosteum of midline of the hard palate. The soft tissue was removed by sharp dissection to expose the underlying bone. Cotton gauze was placed over the wound until hemostasis was achieved. No medication was used throughout the experiment.

2.3. Preparation of Humic Acid. Humic acid was obtained from peat coming from the western Black Sea region and was diluted in sterile saline solution to reach the study concentrations (80 mg/kg) [20]. The concentration of the trace elements in the humic acid solution such as Si, Se, Ca, Mg, Fe, and Zn is provided in Table 1. Titrimetric method was used for the assessment of Cl, whereas inductively coupled plasma-atomic emission spectrometry (ICP-AES) was used for all the other elements.

2.4. Study Process. Five animals were sacrificed immediately to get the baseline values (C group). The remaining 72 animals were randomly divided into three experimental groups. 0.5 mL of 0.09% saline solution, 0.05% chlorhexidine gluconate (Irrisept, Irrimax Corporation, Innovation Technologies, Inc., Lawrenceville, GA), or 80 mg/kg humic acid preparation was applied to the respective wound site once daily for 1 min each day by using cotton pellets. Eight animals from each group were sacrificed at 7, 14, and 21 days postoperatively. The maxillae were dissected out and the samples were photographically assessed and compared with the histological findings.

2.5. Photographic Assessment. Photographs of the specimens were taken (25X magnification) using a stereomicroscope (Stemi DV4, Carl Zeiss, Jena, Germany). The surface area

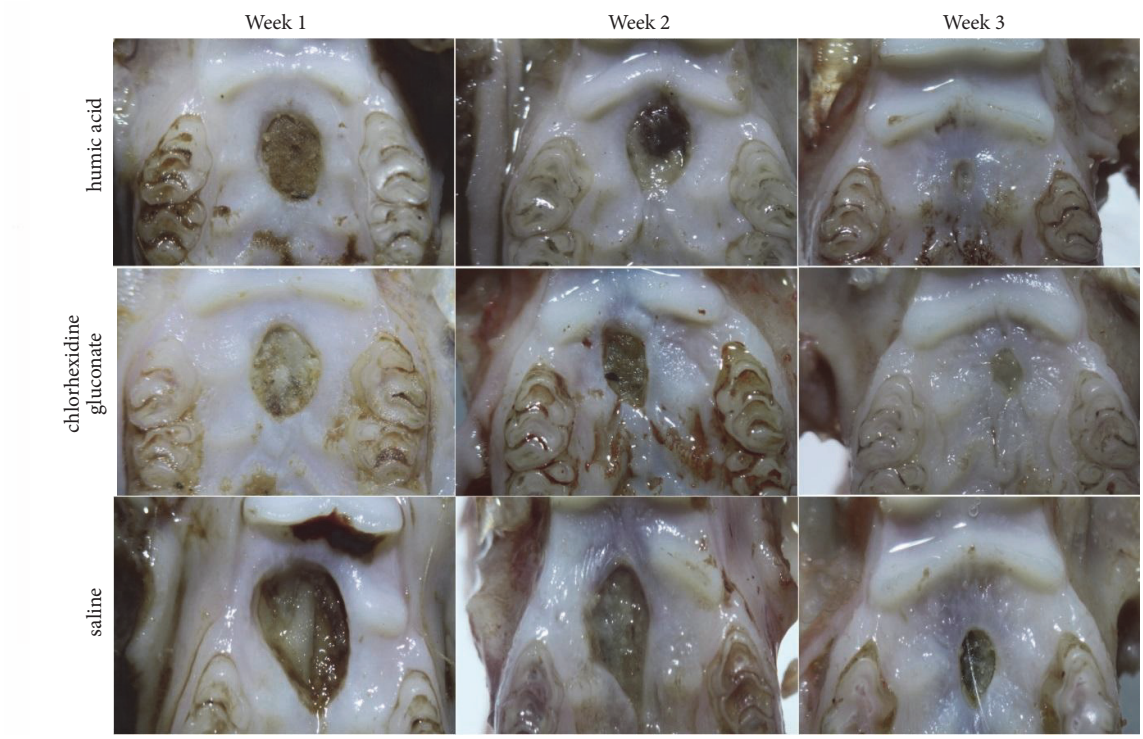

Figure 1: The representative light microscopic photographs of the wound areas on weeks 1, 2, and 3.

of the wound was morphometrically measured using the "Biowizard - D Winter, Version 3" software. The photographic assessment was performed by a single examiner (Dr. Talmac) who was unaware of the identity of samples.

2.6. Histopathological Assessment. Histological analysis was performed by a single examiner (Dr. Goze) who was also blinded to the identity of samples. Specimens were fixed in 10% neutral formalin for 48 h. The samples were then decalcified in formic acid (10%) and nitric acid (10%) for 72 h. Then, the samples were embedded in paraffin. 5 μm serial sections were prepared perpendicular to the palatal midline at the greatest diameter of the wound by using a microtome. The sections were stained with eosin and hematoxylin. Slides were evaluated for histological changes under light microscopy (Nikon Eclipse, E 600, Tokyo, Japan).

2.7. Statistical Analysis. The control and experimental group data were compared with each other and to the baseline values. Statistical analysis was done using the SPSS software and the GraphPad Prism program. Two-way ANOVA or one-way ANOVA with Tukey's multiple comparisons analysis and Student's t-test were applied.

3. Results

The humic acid was obtained as a paste and was further diluted at a concentration of 80 mg/kg in saline solution. After surgical operation, photographs of the wound areas were obtained with a light microscope and the images were measured by an observer who was blind to the study groups. The negative control "saline solution", the positive control "chlorhexidine gluconate solution", and the humic acid solution were applied to the wound area daily for one minute. The representative pictures of the wound areas that were taken with a light microscope are shown inFigure 1.

After the first week of treatment, there was a statistically significant difference between groups by one-way ANOVA analysis with a p value of 1.89×10^{-17} (Table 2). When the groups were individually compared with each other through Tukey's multiple comparisons test, there was a statistically significant difference between the saline control and the humic acid group as well as saline control and chlorhexidine gluconate group, with p values 2.06×10^{-13} and 5.01×10^{-13}, respectively. On the other hand, there was no statistically significant difference between the humic acid group and the chlorhexidine gluconate group after one week of treatment.

At the end of the second week of treatment, the wound areas were measured again, and the groups were statistically analyzed with one-way ANOVA. There was a statistically significant difference between groups with a p value of 4.61×10^{-10} (Table 2). Similar to the first week of treatment, there was a statistically significant difference between the saline control and the humic acid group as well as the saline control and the chlorhexidine gluconate group, with p values 9.21×10^{-8} and 3.05×10^{-8}, respectively. However, at the end of the two weeks of treatment, there was still no statistically significant difference between the humic acid group and the chlorhexidine gluconate group.

Table 2: Analyses of wound areas during 21 days of observation.

	1st week of treatment	2nd week of treatment	3rd week of treatment
Control (mean ± sem)	8.22	5.131	2.194
Chlorhexidine gluconate (mean ± sem)	5.276	4.02	1.215
Humic acid (mean ± sem)	5.193	3.97	0.7788
F-value	400.6	70.85	201.9
R^2-value	0.9745	0.8709	0.9506
95% CI between control and humic acid	2.637 - 3.251	0.8832 - 1.439	1.233 - 1.597
95% CI between control and chlorhexidine gluconate	2.720 - 3.335	0.8332 - 1.389	0.7970 - 1.161
95% CI between chlorhexidine gluconate and humic acid	-0.2234 - 0.3909	-0.2280 - 0.3280	0.2545 - 0.6180

sem = standard error of mean and CI = confidence interval.

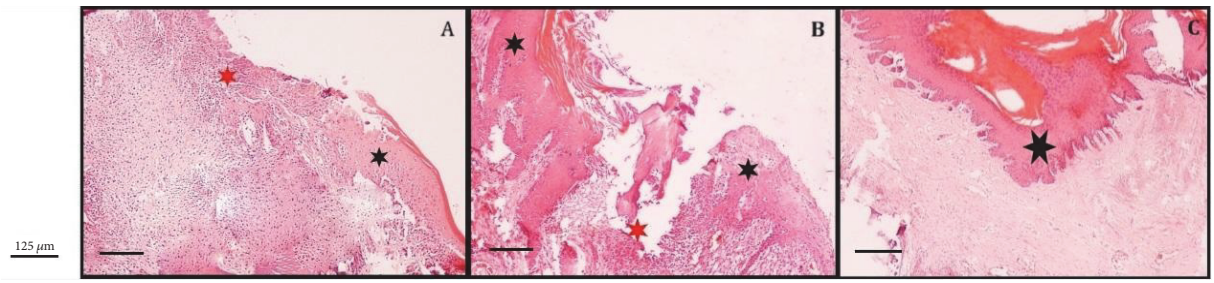

Figure 2: The representative histological image of the wound areas. (A) Representative histological image of the humic acid treated wounds at 1st week. The red star shows the wide necrotic and severely inflamed area, while the black star points to the mucosal epithelium. (B) Representative histological image of the humic acid treated wounds at 3rd week. The red star shows the mildly inflammatory area and granulation tissue, while the black stars show the constricted mucosal epithelial layer. (C) A representative section of humic acid treated tissues at the end of the study. Black star shows complete mucosal epithelial repair and healing.

The study was performed for three weeks, and at the end of the study, the wound area measurements were again compared with one-way ANOVA and multiple comparisons between groups were done with Tukey's test. Overall, there was a statistically significant difference between groups with a p value of 1.94×10^{-14} (Table 2). Similar to first and second week measurements, there was a statistically significant difference between the saline control and the humic acid group as well as saline control and chlorhexidine gluconate group. The p values of these comparisons were 3.83×10^{-11} and 3.78×10^{-10}, respectively. Strikingly, at the end of three weeks, there was also a statistically significant difference between the humic acid group and the chlorhexidine gluconate group with a p value of 0.0001 (3.95×10^{-5}) as well.

When all three weeks' measurements were compared by using two-way ANOVA with repeated measures, there was a statistically significant difference between not only weeks but also treatment groups, with p values of 1.55×10^{-41} and 1.68×10^{-64}, respectively.

When the tissue sections were analyzed after being processed with hematoxylin and eosin staining, the sections from the one-week treatment group showed necrotic and inflamed areas. After three weeks of treatment with humic acid, however, the inflammation areas were much reduced and granulation tissue with constricted mucosal epithelial layer was observed. At the end of the study, sections from the humic acid treated animals showed complete mucosal epithelial repair and healing (Figure 2).

4. Discussion

In this study, humic acid was shown to enhance healing of oral wounds for the first time in the literature. The experiments were performed in rats and humic acid treatment was compared to saline treatment and traditional chlorhexidine gluconate treatment.

Bacterial microflora in the oral cavity is very diverse and these bacteria colonize the wounds [22]. Wounds in palate are usually treated with antibacterial treatments to prevent infections. In this study, we also used an antibacterial material, chlorhexidine gluconate, as a positive control. Humic acid was also previously reported to have antibacterial properties [9, 12]; therefore, this control enabled us to compare the effectiveness of the antibacterial treatment for wound healing. In the first two weeks of treatment, we observed the positive effects of the antibacterial on oral wound healing for both humic acid treatment and chlorhexidine gluconate treatment, which were much more effective in enhancing wound healing than the saline control. On the other hand, within the first two weeks, there was almost no difference between the humic acid treatment group and chlorhexidine gluconate treatment group, which shows the importance of antibacterial properties for the initial stages of wound healing. The histological analysis which showed granulation tissue with constricted mucosal epithelial layer and complete mucosal epithelial repair and healing after humic acid treatment also support the critical effect of humic acid in wound healing in palatal wounds.

Humic acid was also shown to have anti-inflammatory properties, which can aid in healing of the wounds on different tissues [10, 15, 18, 23]. This property might be more influential at later stages of wound healing, since we observed that the humic acid treated wound healed statistically significantly faster than the chlorhexidine gluconate treated wounds on the third week of the treatment. In addition, at the end of three weeks of treatment, the humic acid treated wounds showed reduced inflammation areas compared to both the saline control and the chlorhexidine treated group when the samples were analyzed through histological staining.

Humic acids can have a positive effect on wound healing and cancer therapy, as suggested by Jurcsik [24]. The healing process requires extra oxygen, and this demand appears in the first minute after wounding due to phagocytosis, the main event in wound healing process, which is very oxygen-consumptive [24].

The composition of humic acid is complex and the samples from different areas are different from each other. Although drug substances that are prepared by using natural materials as starting materials are routinely used and are allowed to differ to a certain extent as batch-to-batch variations, it might be beneficial to use synthetic humic acid preparations for future wound healing experiments as alternatives to these natural samples to achieve more chemically defined drug products.

5. Conclusion

Overall, the results of this study showed that humic acid, which has previously been shown to have antibacterial and anti-inflammatory properties, enhances wound healing in the oral cavity. The humic acid treatment was even superior to chlorhexidine gluconate, which is widely used for the treatment of oral wounds. To the best of our knowledge, this is the first study to show that humic acid treatment can be used for the treatment of wounds in the oral cavity.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors have no conflicts of interest to disclose.

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Research Article

Promotion of Keratinocyte Proliferation by Tracheloside through ERK1/2 Stimulation

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Cell migration and proliferation are important for proper wound healing after skin injury. Recent studies have shown that compounds from plants could promote cell migration and proliferation. Tracheloside, which is a plant lignan, has been found to promote the growth of HaCaT cells over 40% compared to other compounds tested based on a cell proliferation assay. An *in vitro* scratch assay confirmed the healing activity of tracheloside (more than 2-fold increased healing activity after 24 hours of treatment compared with the control) and revealed that this activity is better than that of allantoin (1.2-fold increased after 24 hours of treatment compared with the control), a positive control. With western blot results, wound healing with tracheloside occurred through the phosphorylation of ERK1/2. Therefore, tracheloside is a good candidate to promote wound healing and could be developed as a therapeutic agent for wound treatment or used as a leading compound with higher activity.

1. Introduction

Skin wound healing is a complex process involving the reepithelialization of missing cellular structures and tissue layers through three phases: inflammation, proliferation, and remodeling. Of the many cell types required during the wound healing process, keratinocytes are important for epithelialization in the proliferative phase as they are the predominant cell of the outermost layer of the skin. In addition, complex interactions and cross communication between keratinocytes and other cell types during all three phases of wound healing are critical for successful wound closure and repair [1, 2].

As keratinocytes proliferate and migrate toward the upper layers of the epidermis, they are differentiated and transformed through different cell layers to reach their final maturation stage [3]. Cell proliferation is activated by growth factors and cytokines that are released into the injury site. Combined key events such as signaling, cytoskeletal reorganization, and adhesion processes are required [4].

Mitogen-activated protein (MAP) kinase family members such as ERK1/2, JNK, and p38 are well known for their importance in wound healing, cell survival, differentiation, and proliferation [5, 6]. The major mechanism in these processes is the regulation of cell cycle entry and progression. For example, ERK1/2 regulates cyclin D1, which controls cell proliferation [7, 8].

Tracheloside, which belongs to plant lignans, is a component of *Trachelospermum jasminoides* used as herbal medicines in Japan, China, and Korea. It is already known that plant lignans have various effects related to growth factor actions, steroid biosynthesis, cell differentiation, cell transformation, and proliferation [9–11].

In the present experiment, we showed the effect of tracheloside on keratinocyte proliferation. To confirm this effect, we used a scratch wound healing assay, cell proliferation assay, and western blot analysis for signaling related to cell proliferation.

2. Materials and Methods

2.1. Chemicals. All plant extracts were purchased from ChemFaces (Wuhan, China), and the tracheloside chemical structure is shown in Figure 1. Allantoin as a positive control

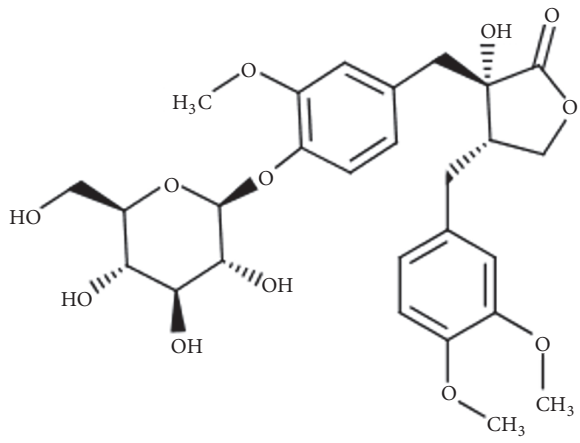

Figure 1: Structure of tracheloside.

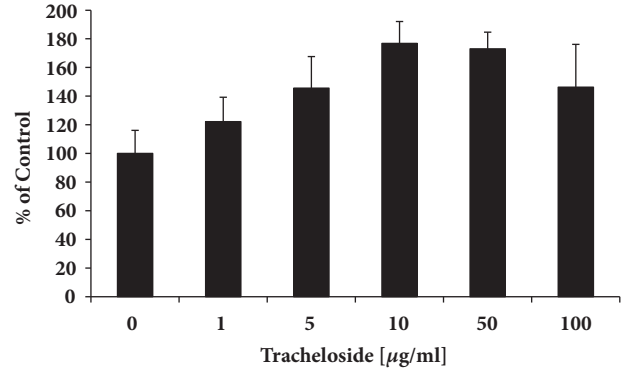

Figure 2: Tracheloside induced the cell proliferation rate. Various concentrations of tracheloside were applied to HaCaT cells cultured for 24 hours in serum-free medium and checked the cell proliferation rate using MTT.

was obtained from Sigma-Aldrich (St. Louis, MO, USA) [12, 13]. The chemicals were dissolved in dimethyl sulfoxide (DMSO), and the stock solutions were stored at -20°C.

2.2. Animal Cells and Culture. Human keratinocyte cell line HaCaT were maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin at 37°C in a 5% CO_2 atmosphere [14].

2.3. Cell Proliferation Assay. The proliferation of HaCaT cells by test compounds was tested using a slightly modified cell-based MTT assay [14]. HaCaT cells in DMEM were added to the wells of a 96-well plate at a density of 10^3 cells per well. Serum-free medium including various concentrations of tracheloside (0, 1, 5, 10, 50, and 100μg/ml) were added and further incubated for 48 hours. MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma) in PBS was added into each well at a final concentration of 0.5 mg/ml, followed by incubation for 3 hours at 37°C. The medium was then removed, and cells were suspended in 100 μl DMSO for 10 minutes. Cell proliferation was calculated from optical density (OD_{540}) values measured using a microplate reader (BioTek Instruments, Korea) and were reported as a percentage of the vehicle control [14, 15].

2.4. In Vitro Wound Healing. HaCaT cells were seeded into 6-well plate and cultured to nearly confluent cell monolayers. A linear vertical and horizontal wound was then generated in the monolayer with a sterile 20-200 μl plastic pipette tip. Any cellular debris was removed by washing with phosphate-buffered saline (PBS). Serum-free medium with various concentrations of tracheloside (1, 5, and 10μg/ml) was added in triplicate and incubated for 24 hours at 37°C with 5% CO_2 atmosphere. Images of the scratched areas were photographed to estimate the relative proliferation of cells at 0 and 24 hours posttreatment. The data were analyzed using an EVOS XL imaging system (Fisher Scientific, USA) by calculating the percentage of scratch closure at each dose point relative to the control. The experiments were repeated three independent times [15].

2.5. Western Blot Analysis. Protein was extracted with RIPA buffer and quantified with the Bradford reagent (Sigma). Protein samples with equal amounts (25 μg) were separated by 8-10% SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF, Bio-Rad, USA) membranes. The membranes were blocked with 5% bovine serum albumin (BSA, GenDEPOT, Korea) and then incubated with a 1:2000 dilution of primary antibodies (p38α, p-p38, ERK1/2, JNK, p-JNK, and GAPDH from Santa Cruz Biotechnology, CA, USA; p-ERK1/2 from Cell Signaling Technology, MA, USA) overnight at 4°C. The membranes were washed with TBST and incubated with a secondary horseradish-peroxidase-conjugated antibody for 1 hour at room temperature. The membranes were developed using enhanced ECL (Bio-Rad, USA) on a UVITEC imaging system (UVITEC Cambridge, UK). Each experiment was repeated at least twice for consistency of the results [7].

2.6. Statistical Analysis. Results are expressed as means ± SD. Statistically significant differences were analyzed with one-way ANOVA with Tukey's post hoc test.

3. Results

3.1. Enhanced Cell Growth Effect of Tracheloside to Keratinocytes. Several available extracts from plants were used to test and compare their effects of proliferation to keratinocyte HaCaT cells (Table 1). It was found that tracheloside increased cell proliferation against human cells (Figure 2).

In 10 μg/ml concentration, HaCaT cells grew over 45.58% more compared to the control.

3.2. Tracheloside Increased Wound-Healing. To determine the effect of tracheloside on keratinocyte proliferation, various concentrations of tracheloside were used to treat HaCaT cells. Tracheloside increased the proliferation of HaCaT cells in a dose-dependent manner (Table 2 and Figure 3) compared to

Table 1: Cell proliferation effect of several plant extracts (% of control).

Compounds	Proliferation rate (100 μg/ml)	Compounds	Proliferation rate (100μg/ml)
Bisdemethoxy-curcumin	2.60 ± 0.70	Falcarindiol	27.71 ± 0.79
Sophoraflavanone G	0.97 ± 0.13	Pimaric acid	81.11 ± 5.35
Acetylshikonin	30.42 ± 5.36	Anwulignan	103.59 ± 1.36
Lobatoside C	2.40 ± 1.25	6,8-Diprenylorobol	3.69 ± 0.05
Alpinumisoflavone	73.56 ± 4.09	Galangin	113.66 ± 3.01
Eupatilin	78.30 ± 1.73	Corosolic acid	72.59 ± 3.27
Kurarinone	10.49 ± 0.49	Tracheloside	145.58 ± 22.1

Table 2: Tracheloside increased wound healing after 24 hours of treatment through an i*n vitro* scratch assay.

Concentration of treatment	% of wound healing from 0 hours		
	Control	Tracheloside	Allantoin
1 μg/ml	10.12 ± 1.29	13.98 ± 3.21	13.55 ± 2.38
5 μg/ml	9.13 ± 1.94	18.82 ± 5.95	12.33 ± 2.26
10 μg/ml	10.38 ± 0.19	17.94 ± 2.03	12.50 ± 2.42

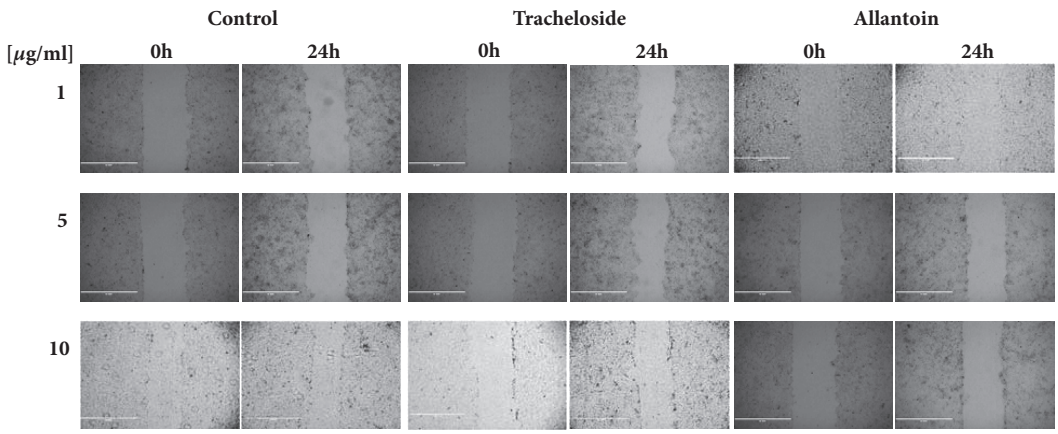

Figure 3: Effect of tracheloside on the proliferation of HaCaT cells through the wound healing assay. HaCaT cells were cultured in a 6-well plate, scratched, and treated with different concentrations of DMSO only, allantoin, or tracheloside. The results were photographed and demonstrated healing of the scratched wound with various concentrations of compounds.

the control or allantoin as a positive control. Tracheloside increased the cell proliferation rate by 13.98%, 18.82%, and 17.94% at 1, 5, and 10 μg/ml, respectively after 24-hour treatment. As a result of those findings, 38.14%, 106.13%, and 72.83% increased healing activity was observed, respectively, compared with the control.

3.3. Tracheloside Induced ERK1/2 Phosphorylation. Tracheloside treatment promoted the proliferation of HaCaT cells. To investigate whether signaling kinases including p38, JNK, and ERK1/2 participated in proliferation, western blot analysis was performed after treatment with tracheloside (Figures 4(a) and 4(b)). Phosphorylated ERK1/2 increased dose-dependently 1.3-, 1.67-, and 2.73-fold by 1, 50, and 10 μg/ml, but phosphorylated JNK was slightly decreased and phosphorylated p38 did not show any change after treatment with tracheloside.

4. Discussion

Tracheloside is a type of plant lignan and an analogue of another plant lignan, arctiin. Arctiin has exhibited some clinical effects including anti-inflammatory, improved immune response to influenza, and antidiabetic activities [16–18]. However, arctiin has also shown antiproliferative effects [19, 20]. Tracheloside is a known antiestrogenic lignan [21]. Other effects of tracheloside have yet to be found.

In the present study, we showed that tracheloside positively affects the proliferation of the keratinocyte, HaCaT, which is comparable with allantoin as the positive control, which exhibited less effect on cell proliferation than tracheloside [12].

ERK1/2, one of the MAP kinase family members, are phosphorylated and activated by MEK, a tyrosine/threonine

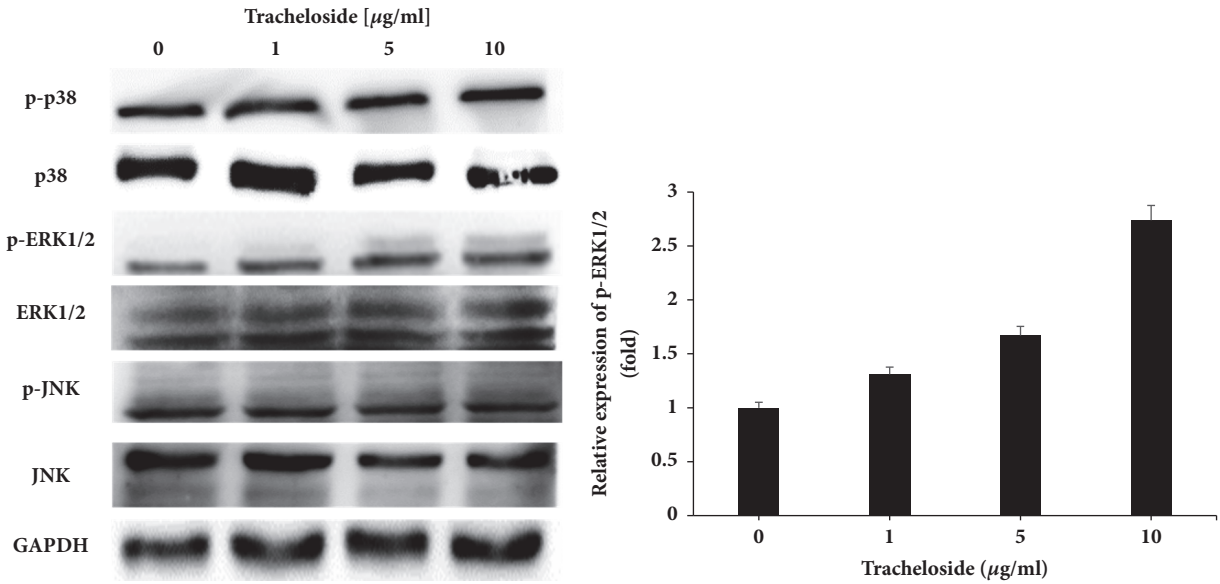

Figure 4: Tracheloside dose-dependently induced ERK1/2 phosphorylation on the HaCaT cells. HaCaT cells were treated with various concentration of tracheloside and the protein was used in western blot analysis. (a) GAPDH was used as a control, and p38, ERK1/2, and JNK, which are related with cell proliferation in their phosphorylated forms, were detected. (b) The p-ERK1/2 was quantified with densitometric analysis and normalized with GAPDH.

kinase [22]. Activated ERK1/2 (p-ERK1/2) can change extracellular stimulus to intracellular signal that control gene expression, which contributes to the regulation of cell proliferation [23]. Western blot results show that phosphorylation of ERK1/2 was increased as tracheloside was treated. Therefore, tracheloside affects proliferation through the regulation of ERK1/2 phosphorylation [6, 7].

In vivo testing and experiments with epidermal tissue were not performed in this study. These additional data will show a clearer effect of tracheloside in cell proliferation. Based on our research results, tracheloside could be recommended as a lead compound related to wound healing and skin proliferation. Western blot analysis under a pathway of ERK1/2 and RT-PCR results about interleukins will aid in the understanding of how tracheloside stimulates keratinocytes [24–26].

In conclusion, tracheloside can be used as a good candidate to promote wound healing. Furthermore, it could be utilized as therapeutic uses for wound treatment.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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