Summary

Loranthus europaeus is an important medicinal plant, which contains a lot of bioactive compounds. The dried plant fruits were extracted in 80% methanol by maceration. Chemical detection of crud plant extracts was performed. The total flavonoids were isolated, subjected to thin layer chromatography (TLC) using different mobile systems. The purified material was augmented by using high performance liquid chromatography (HPLC).

Inflammation was induced in experimental animals (rabbits) by subcutaneous injection of 2.5% formalin. The anti-inflammatory effect of the extract was evaluated in healing skin wounds in comparison with the synthetic pharmaceutical medication Piroxicalm Gel. Simple preparation was applied by mixing the crude compound with Vaseline and Glycerin. After healing of the wound, histopathological study was necessary to support the results.

Results indicated that, *L. europaeus* fruits were rich with flavonoids of the total flavonoids were estimated, the major components were rutin and lueteolin and trace of quareciten and kaempferol.

The healing of the skin was clear by disappearance of odema and reduction in scar size, enhancement of fibroblast proliferation, angiogenesis, keratinization and epithelialization as compared with the control groups.

L. europaeus extracts could be considered as one of the promising plants for the treated of skin wound. Flavonoids of the plant have the ability of suppression of acute inflammation induced by chemical substance and seemed to be the most active component for healing the wound.

1. Introduction and Literature Review

1.1 Introduction

Chemical components of the plant medicinal are the most important for pharmaceutical companies. People are interested in medicines prepared from plants due to their little side effects, cheap and almost available compared with synthetic drugs. This may be because of the low concentrations of the active compounds found in plants which the human body would need (Mackin, 1993).

Arokiyaraj *et al*. (2007) mentioned that a large number of these plants, plant extracts, plant derivatives and/or their isolated constituents have shown beneficial biological effects: including immunomodulatory, anti-oxidant, anti-inflammatory, anti-mutagenic, anti-carcinogenic and anti-microbial effects. Modulation of immune response by using medicinal plant products has become a subject of scientific investigations; several plants have important secondary metabolites that can interfere with different immunological functions; for instance, activation of humoral and cell-mediated immune responses, which are crucial for the integrity of immune system against invading pathogens or a carcinogenic transformation.

These secondary metabolites have different structures that correlate with different chemical terminologies (flavonoids, alkaloids, tannins, glycosides and others), and therefore they may have different functions with regard to their biological potentials (XD *et al*., 2008).

Wounds are the result of injuries to the skin that disrupt the other soft tissue. Healing of wound is a complex and protracted process of tissue repair and remodeling in response to injury. Inflammation is the response of living tissue to mechanical injuries, burns, microbial infections, and other noxious stimuli that involve changes in blood flow, increased vascular permeability, activation and migration of leucocytes and the synthesis of local inflammatory mediators (Wiart, 2006).

Various plant products have been used in treatment of wounds over the years. Wound healing herbal extracts promote blood clotting, fight infection, and accelerate the healing of wounds. A list of medicinal plants that have been investigated for the skin and skin structure protection are used for healing wounds: such as Coconut Palm; Oak Gall; Eucalyptus; Fenugreek; Onion; Black Nightshade, Cinnamon, Coffee, Digitalis (Fleming, 2002).

Most earlier systematic treatments contain all mistletoe species with bisexual flowers (though some species have reversed to unisexual flowers), while most modern systematisms treatment as a monotypic genus with the only species *Loranthus europaeus* Jacq. The summer mistletoe or European yellow mistletoe in contrast to the well-known European or Christmas mistletoe (*Viscum album* L., Santalaceae or Viscaceae) is deciduous. The systematic situation of *Loranthus* is not entirely clear, and some showy mistletoe in Asia may be true parts of this genus (Waly *et al.,* 2012).

Aims of the Study

1) Detection the classes of active compounds present in *Loranthus europaeus* methanolic extract.
2) Quantitative and qualitative estimation of the total flavonoids in dried fruit *Loranthus europaeus* plant extract.
3) Identification of the purified flavonoids using (HPLC).
4) Histopathological study of the extract affected in healing skin wounds and inflammation in rabbits in comparison with the synthetic pharmaceutical medication.

1.2 Literatures Review

1.2.1 *Loranthus europaeus*

The family Loranthaceae, is a large family that belong to the order Santalales, it includes about 75 genus belong to 1000 species, some of these species are parasites on the roots while the remaining parasites on branches and stems trees and known as Mistletoe (Waly *et al.*, 2012*)*.

Loranthus europaeus Jacq. (Loranthaceae) is hemiparasitic mistletoe of South-Eastern Europe, Anatolia and South Russia (Balle *et al*., 1960). *L. europaeus* has a similar branching pattern to the evergreen mistletoe *Viscum album* L., but it is deciduous, yellow-berried mistletoe, with dull brown twigs, with flowers located in stipulate inflorescences and respectively berries (Briggs, 2009).

L. europaeus grows mostly on branches of *Quercus* species and occasionally of chestnuts as host trees (Harvala *et al*., 1984).

Barnhart and Steinmetz in 2008 found the alliance of oaks and mistletoes became a symbol of knowledge and strength, and it was aptly rendered in the word “Druid” (i.e. the oak-knower), which is derived from the Greek word for oak.

Mistletoes on oaks have a symbolism and a healing status that is very interesting, because both species were highly prized by ancient people, all chemists and herbalists (De Cleene and Lejeune, 2003).

1.2.2 **Common Names and Taxonomy** (Watsonand Dallwitz, 1991)

- Kingdom : *Plantae*
- Subkingdom : *Tracheobionta*
- Superdivision : *Spermatophyta*
- Division : *Magnoliophyta*
- Class : *Magnoliopsida*

- Subclass : *Rosidae*
- Order : *Santalales*
- Family : *Loranthaceae*
- Genus : *Loranthus*
- Scientific Name: *Loranthus europaeus*
- Common Name: Mistletoe

Figure (1-1) Mistletoes on oaks (Watsonand Dallwitz, 1991)

1.2.3 Plant distribution

European Mistletoe is found mostly in Europe and as far as Iran, not found in America or Australia. It is grow in central Europe and China. The showy mistletoes can be found throughout the world (Watsonand Dallwitz, 1991).

1.2.4 Plant description

The flowers of *L. europaeus* are small, green, usually have four to six parts and may be either unisexual or bisexual. Other species of a broader *Loranthus* have very large, showy flowers, with blooms in lively colors. Fruits are berries, usually containing a single seed, that are dispersed by birds (Watsonand Dallwitz, 1991).


Figure (1-2): Fruits of *Loranthus europaeus* (Watsonand Dallwitz, 1991)

1.2.5 Active constituents of *L. europaeus*

Generally the studies on some species of the Loranthaceae family have indicated the presence of several chemical compounds, including flavonoids and alkaloids (Fernandez *et al.*, 1998). Lectins and viscotoxins (Park *et al.*, 1999), arginine and polysaccharides (Sinha *et al.*, 1999), *Loranthus* species are known to produce variety of bioactive compounds, like sesquiterpene lactones from *Loranthus parasiticus* (Okuda *et al*., 1987).

In a study by Egbuonu and Nwankwo (2011) and Orji *et al*. (2012) occurrence of many other chemical components was known such as tannin, terpenoids, phenols, flavonoids, glycosides, triterpenoids and resins in *L. micranthus* Linn.

On *L. bengwensis* species screening process, alkaloids, flavonoids, tannins, cardiac glycoside, terpenes and steroids were present in both aqueous and ethanolic extract. The quantitative phytochemical screening of the aqueous extract revealed that the plant contains 0.30% flavonoids (Olatunde and Dikwa, 2014).

Preliminary chemical analysis for *L. europaeus* oil extract showed the presence of glycosides, carbohydrates, aldehydes and ketone, triterpenoids groups, protein and polysaccharides (Jawad *et al*., 2007).

Qualitative analysis and thin layer chromatography (TLC) for extract of *L. europaeus* seed, showed the presence of: Alkaloids, Flavonoids, Saponins, Glycosides and Terpenoids (Al-Fartosy and Al-Rikaby, 2007).

In the study by Hamad *et al.* (2013) many flavonoids were detected included: Kaempferol, Quercetin and Rutin in methanol and ethyl acetate extract.

1.2.6 Biological potentials of *L. europaeus*

Steiner (1989) indicated historically, that the intentions of mistletoe uses were manifold and conflicting in several cases (i.e., swellings or tumors, epilepsy, hysteria, delirium, vertigo, antispasmodic, tonic and narcotic, labour-pains, weakness of the heart and edema, eczema, ulcers of the feet, burns, and granulating wounds).

1.2.6.1. Antimicrobial Activity

The species *Loranthus micranthus* was studied for its antimicrobial activities at various concentrations (15, 30 and 60µg/disc) using agar diffusion method, and was tested on *Staphylococcus aureus*, *Escherichia coli*, *Salmonella paratyhi-1*, *Klebbsiella pneumoniae*, and *Proteus mirabilis*, the results of inhibition were increase in concentration results in wider zone of inhibition (Egbuonu and Nwankwo, 2011).

Several workers reported that other species of *Loranthus capitellatus* were active against yeast Candida species with MIC at the concentration of 10 to 40 mg /ml (Parekh *et al.*, 2005; Buwa and van Staden, 2006).

1.2.6.2. Antioxidant Activity

The antioxidant activity of extracts of aerial tissues (i.e. flowers, fruits, leaves, stems, twigs and berries) of the mistletoe, in the mainland of Greece was study the total phenolic content and antioxidant potential of aerial issues of *L. europaeus* have reducing antioxidant power assay. Extracts of twigs and stems of *L. europaeus* exhibited higher antioxidant activity in comparison to that of fruits, leaves and flowers. (Katsarou *et al*., 2012).

1.2.6.3. Antidiabetic Activity

Loranthus micranthus possesses optimum antidiabetic activity, Uzochukwu and Osadeb (2007) have studied a comparative evaluation of antidiabetic activities of crude methanolic extract and flavonoids extract of *L. micranthus* harvested from *Kilifia acuminate* in alloxan-induced diabetic rats. This flavonoids extract (400 mg/kg) showed significant anti-diabetic effect within one hour of administration, while the methanolic extract (200 mg/kg) showed the significant antidiabetic effect within three hours of administration.

1.2.6.4. Anticancer activity

Mistletoe extracts were introduced for the first time in a cancer treatment by Steiner (1989), founder of anthroposophy. He recommended a drug extract produced in a complicated manufacturing process combining sap from mistletoe harvested in winter and summer. Based on his recommendations, several anthroposophic doctors have treated their cancer patients with these extracts within the last century. Clinical evaluations of mistletoe as an adjuvant cancer treatment have expanded.

During the 1965, Vester and Nienhaus isolated carcinostatic protein fractions which were recognized later as the cytotoxic viscotoxins and mistletoe lectins. The scientific researches has confirmed the evidence that mistletoe extracts (1)induce apoptosis, (2)stimulate immunocompetent cells which slow the

growth of cancer cells, and (3)protect the DNA of mononuclear cells. The immuno-modulating effects were ascribed to the mistletoe lectins, poly-oligosaccharides, viscotoxins and several other components like alkaloids.

1.2.7. Pharmacological Activities of Flavonoids

Flavonoids are polyphenol compounds that are ubiquitous in nature. Flavonoids are natural products widely distributed in plant kingdom and currently consumed in large amounts in the daily diet (Hanneken *et al.,* 2006).

Tsuchiya (2010) suggested that flavonoids differ in their arrangement of hydroxyl, methoxy and glycosidic side groups and in the conjunction between A and B rings. A variation in C ring provides division of subclasses figure (1-3). According to their molecular structure, they are divided into eight classes:-

(1) Flavone
(2) Flavonones
(3)Flavonol
(4) Isoflavone
(5) Anthocyanidin
(6) Catechin
(7) Dihydroflavonol
(8)Chalcone

Flavonoids have been reported to exert wide range of biological activities, these includes: anti-inflammatory, antibacterial, antiviral, antiallergic, cytotoxic antitumour, treatment of neurodegenerative diseases, vasodilatory action.

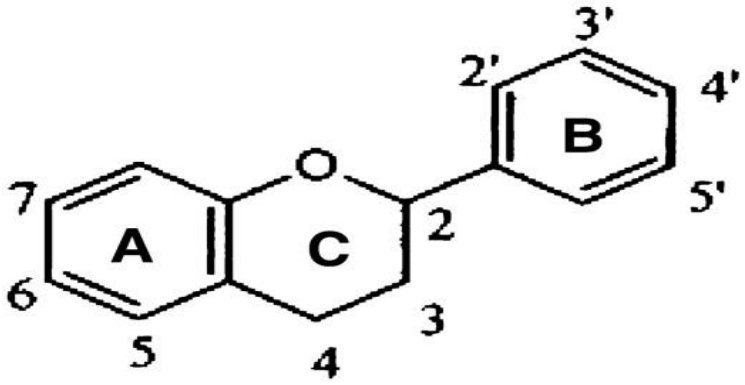


Figure (1-3): Basic structure of flavonoids (Tsuchiya, 2010)

In addition flavonoids are known to inhibit lipid-peroxidation, platelet aggregation, capillary permeability and fragility, cyclooxygenase and lipoxygenase enzyme activities, flavonoids exert these effects as:-

1. Antioxidants, free radical scavengers, chelators of divalent cation. (Narayana *et al., 2001*).
2. In treatment of hepatotoxicity: Flavonoids bind to subunit of DNA-dependent RNA polymerase I, thus activating the enzyme. As a result, protein synthesis gets increased leading to regeneration and production of hepatocytes (Murray, 1998).
3. In treatment of allergy: Flavonoids inhibit cyclic AMP phosphodiesterase and calcium-dependent ATPase which are responsible for histamine release from mast cells and basophils (Murray, 1998).
4. In treatment of inflammation: Flavonoids have been found to be prominent inhibitors of COX or LOX (Pal *et al.*, 2009).

1.2.8. Mechanism of Action of Flavonoids as Anti-inflammatory Agents

A number of flavonoids are reported to possess anti-inflammatory activity *in vitro* and *in vivo*. Although not fully understood, several mechanisms of action are proposed to explain *in vivo* anti-inflammatory action. The important mechanism for anti-inflammatory activity is inhibition of eicosanoid generating enzymes including phospholipase A2, cyclooxygenases and lipoxygenases, thereby reducing the concentrations of prostanoids and leukotrienes. Other mechanisms include inhibition of histamine release, phosphodiesterase, protein kinases and activation of transcriptase (Kim *et al.,* 2004).

The anti-inflammatory effect of flavonoids extract of *Aegle marmelos* seeds was evaluated using carrageenan induced paw edema and cotton pellets induced granuloma in rats. (Sharma *et al*., 2011).

1.2.9. Skin Histopathological Anatomy

The skin is a continuously self-renewing organ that covers the surface of the body and separates it from the outside world with which it connects in a dynamic way. It provides protection against external agents such as mechanical and chemical insults; heat, infections, water, and electromagnetic radiation (Breitkreutz *et al*., 2009).

There are three structural layers considered in the skin, table (1-1): the epidermis, the dermis and hypodermis (subcutis). Hair, nails, sebaceous, sweats and apocrine glands are regarded as derivatives of skin, as cells of the outer layers are continuously shed and replaced by inner cells moving up to the surface. (Ro and Dawson, 2005).

Table (1-1): Layers of the Skin

Skin layer	Description
Epidermis	The external layer mainly composed of layers of keratinocytes but also containing melanocytes, Langerhans cells and Merkel cells.
Basement membrane	The multilayered structure forming the dermoepidermal junction.
Dermis	The area of supportive connective tissue between the epidermis and the underlying subcutis: contains sweat glands, hair roots, nervous cells and fibers, blood and lymph vessels.
Subcutis	The layer of loose connective tissue and fat beneath the dermis.

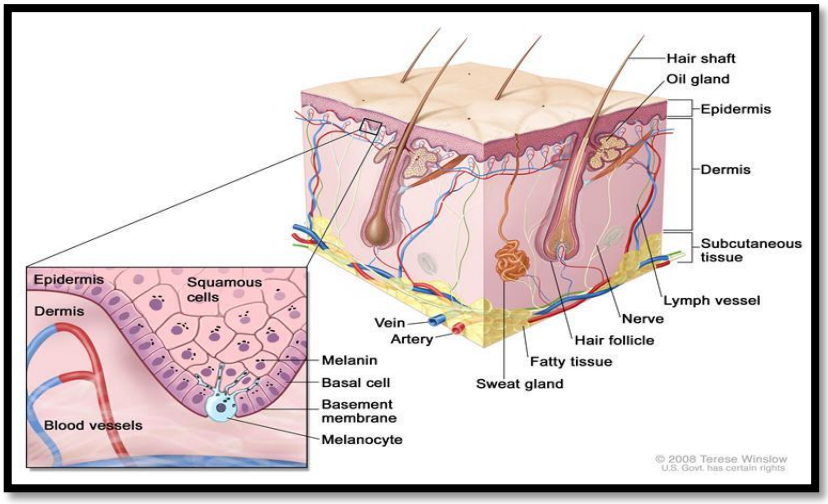


Figure (1-4): Cross-Section of the Skin, Showed the Layers of the Skin

(Ro and Dawson, 2005)

1.2.10. Wound Healing

Wound in a simple word, is a loss of cellular and functional continuity of living tissue. Although the process of wound healing is natural, an infection can delay healing (Subramoniam *et al.*, 2001). Traditional use of plants for wound healing was because of their fibroblast growth stimulation and antioxidant effects (Houghton *et al*., 2005).

Wound healing process holds several steps which involve coagulation, inflammation, formation of granulation tissue, matrix formation, remodeling of connective tissue, collagenization and acquisition of wound strength (Suresh *et al.*, 2002). According to Puratchikody *et al.*, (2006) the four phases of wound healing requires collaborative efforts of different tissues. This involves blood clotting and platelet aggregation, inflammatory response to injury, fibrin formation, altered ground substances, re-epithelialization and angiogenesis. Healing will be complete only after the disrupted surface was firmly knit by collagen.

1.2.11. Medicinal Plants with Wound Healing Activity

Historically all medicinal preparations were derived from plants, whether in the simple form of plant parts or in the more complex form of crude extracts, mixtures, etc. Today a substantial number of drugs are developed from plants. Which are active against a number of diseases? The majority of these involve the isolation of the active ingredient (chemical compound) triterpenes, alkaloids, β-sitosterol, flavonoids and biomolecules found in a particular medicinal plant and its subsequent modification. In the developed countries 25 percent of the medical drugs are based on plants and their derivatives and the use of medicinal plants is well known among the indigenous people in rural areas of these developing countries (Principe, 2005).

A list of medicinal plants with wound healing activity as *Aloe* spp., Glycoproteins from its gel showed significant wound healing activity *in vivo* with enhancement of granulation and epithelialization, as well as wound healing activity *in vitro* via cell proliferation activity, and enhancement of epidermal tissue, proliferation markers (fibronectin and keratin), epidermal growth factor receptor, and fibronectin receptor (Choi *et al.,* 2001), while *Arnebia euchroma*. ,the roots possess significant wound healing activity on bilateral round skin wounds with enhancement of fibroblasts' cell, collagen, and capillary tissue production, and also basic fibroblast growth factor (b-FGF) expression (Pei *et al.,* 2006).

Blechnum spp.: *B. orientale* leaves showed wound healing activity on an excision model with enhancement of epithelization, collagen synthesis, tissue regeneration, fibroblasts cell production, and angiogenesis (Lai *et al.*, 2011).

B. occidentale leaves showed anti-inflammatory activity on rat paw edema and inhibit carrageenan-induced neutrophil migration to the peritoneal cavity (Nonato *et al.,* 2009). *B. orientale* leaves demonstrated antioxidant and antibacterial activity *in vitro* (Lai *et al.*, 2010).

Mathew *et al.*, (2004) showed that the rhizomes of *Gentiana lutea* L. has significant wound healing activity on excision, incision, and dead space wound

models with enhancement of wound contraction, tensile strength, tissue granulation, fibroblast proliferation, collagen content, and hydroxyproline content. The root of this plant also manifested wound healing activity *in vitro* via enhancement of collagen production and mitotic activity.

1.2.12. Piroxicam, Drug Class and Mechanism

Piroxicam is a non-steroidal anti-inflammatory compound with analgesic and antipyretic effects, used for the treatment of rheumatoid arthrits, osteoarthrits , traumatic contusions and inflammation in the body (Swetman, 2009). Dermal delivery route requires a formulation which ensures deep skin penetration, allowing therapeutic effect at localized site. Although piroxicam is not easily absorbed after topical application, some studies have been carried out to predict the percutaneous absorption of piroxicam using different substances as permeation enhancers (Marks and Dykes, 1994).

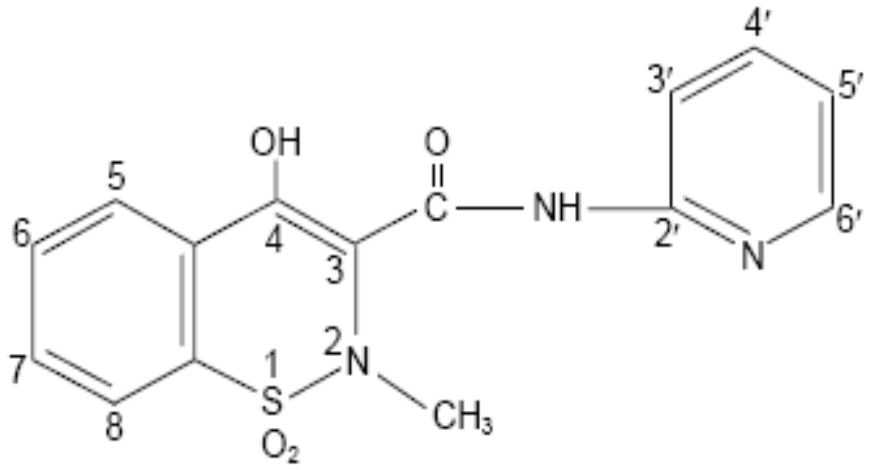


Figure (1-5): Structural Formula of Piroxicam

(Marks and Dykes, 1994)

Within the major group of semisolid preparations, the use of transparent gels has increased both in cosmetics and in pharmaceutical preparations (Okuyama *et al*., 1999).

A gel is colloid that is typically 99% by weight liquid, which is immobilized by surface tension between it and a macromolecular network of fibers built from a small amount of a gelating substance present. When gels and emulsions are used in combined form the dosage forms are referred as emulgels. Emulgels for dermatological use have several favorable properties (Khular *et al*., 2011).

Piroxicam works by preventing the production of a certain type of body chemical called prostaglandins (Brayfield, 2014).

In recent years the relative importance of inducible cyclooxygenase (COX-2), in mediating inflammation via prostaglandin production has been highlighted. Other postulated mechanisms by which NSAIDs suppress inflammation include inhibition of leucocyte adherence and function, reduction of platelet aggregation and inhibition of free radical formation (Vane, 1994).

The FDA approved piroxicam in 1982.Generic name: piroxicam and the brand name: feldene.

2.1. Materials

2.1.1 Chemicals and Reagents

The specific chemicals, reagents and drugs which were used in the present study with their origin are listed in table (2-1) and table (2-2).

Table (2-1): Chemical, materials, drugs and their suppliers.

Chemical	Company	origin
Absolute Methanol	BDH	England
Acetic anhydride	BDH	England
$AlCl_3$	Fluka	Switzerland
Basic Bismuth Nitrate	BDH	England
Canada balsam	Sigma	USA
Chloroform ($ChCl_3$)	BDH	England
Copper II sulphate pentahydrate	Sigma	USA
Eosins stain	BDH	England
Ethanol	BDH	England
Ethyl acetate	BDH	England
Ferric Chloride ($FeCl_2$)	Fluka	Switzerland
Formalin	Analar	England
Formic acid	BDH	England

Glacial acetic acid	BDH	England
Glycerin	Media	Syria
Hydrochloric acid (HCl)	BDH	England
Kaempferol	Xian	China
Lead acetate	Leo	France
Mercuric chloride ($HgCl_2$)	Fluka	Switzerland
n-hexane	BDH	England
Petroleum ether	Fluka	Switzerland
Piroxicalm Gel	Pharmaline s.a.l.	Lebanon
Potassium chloride(KI)	Fluka	Switzerland
Potassium hydroxide(KOH)	BDH	England
Potassium iodide	Fluka	Switzerland
Quercetin	Xian	China
Rutin	Xian	China
Sodium bicarbonates ($NaHCO_3$)	BDH	England
Sodium chloride	Fluka	Switzerland
Sodium citrate	Fluka	Switzerland

Sodium hydroxide NaOH	BDH	England
Sodium nitrite	Fluka	Switzerland
Sulphoric acid (H_2SO_4)	BDH	England
Vaseline	Laleh Ekatan	I.R.I
Xylene	BDH	England

2.1.2. Equipment and Instruments

The following specific equipment and Instruments which were used in the present study with their origin.

Table (2-2): Instruments and their suppliers

Apparatus	Company	origin
Autoclave	Webeco Gmph	Germany
Centrifuge	Beckman	England
Digital Camera	Mercury	China
Disposable blade	Dorco	China
Disposable Syringes	Abu Dhabi Medical	U.S.E
Electric Balance	Sartorius	Germany

Electric blender	Sartorius	Germany
Electrical Oven	Memmert	Germany
Filter Paper	Fushun Civil Administration	China
HPLC Apparatus	waters/487	USA
Incubator	Memmert	Germany
Laminar Flow Hood	Heraeus	Germany
Lyophilizer	Martin Christ	Germany
Microscope	Meiji	Japan
Reflex Apparatus	Electrothermal	England
Refrigerator	Ariston	Japan
Rotary Evaporator	Heidolph	Germany
Rotary Microtome	Gallenkamp	England
Spectrophotometer	LABOMED.INc.	Japan
Surgical blade	carbon	spain
TLC Paper(silica gel Gf254 aluminum plates)	barcelona	spain
U.V. Light	shighi	England
Water Bath	Gallenkamp	England
Water Distiller	GLF	Germany

2.1.3 Solution Preparation.

2.1.3.1 Mayer's reagent:

Two solutions were firstly prepared; the first one was prepared by dissolving 1.58 grams of mercuric chloride ($HgCl_2$) in 60 ml of distilled water, while the second solution was prepared by dissolving 5 gm of potassium iodide (KI) in 10 ml of distilled water. Both solutions were mixed and the volume was made-up to 100 ml with distilled water (Smolensk *et al*., 1972).

2.1.3.2 Benedict reagent:

A general Benedict's test was done as primary qualitative determination for polysaccharide (Harborne, 1998). Briefly the test employed preparation of the following reagents

-Solution A:

Sodium carbonate anhydrous----------10 g

Sodium citrate ----------17.3 g

Distilled water ----------80 ml

-Solution B:

Copper (II) sulfate pentahydrate ---------- 17.3 g

Distilled water ---------- 100ml

20 ml from solution B was added immediately to solution A.

2.1.3.3 Ferric chloride solution (1%):

The solution was prepared by dissolving 1gm of ferric chloride in 100 ml of distilled water (Stahl, 1969).

2.1.3.4 Haematoxylin stain and Eosins stain (1%):

The stain solutions were ready supplied by the Histopathology Department at Educational Laboratories of Medical Baghdad City.

2.1.3.5 Potassium hydroxide solution:

Prepared by dissolving (50g) of potassium hydroxide in (100ml) D.W (Jaffer *et al*., 1983).

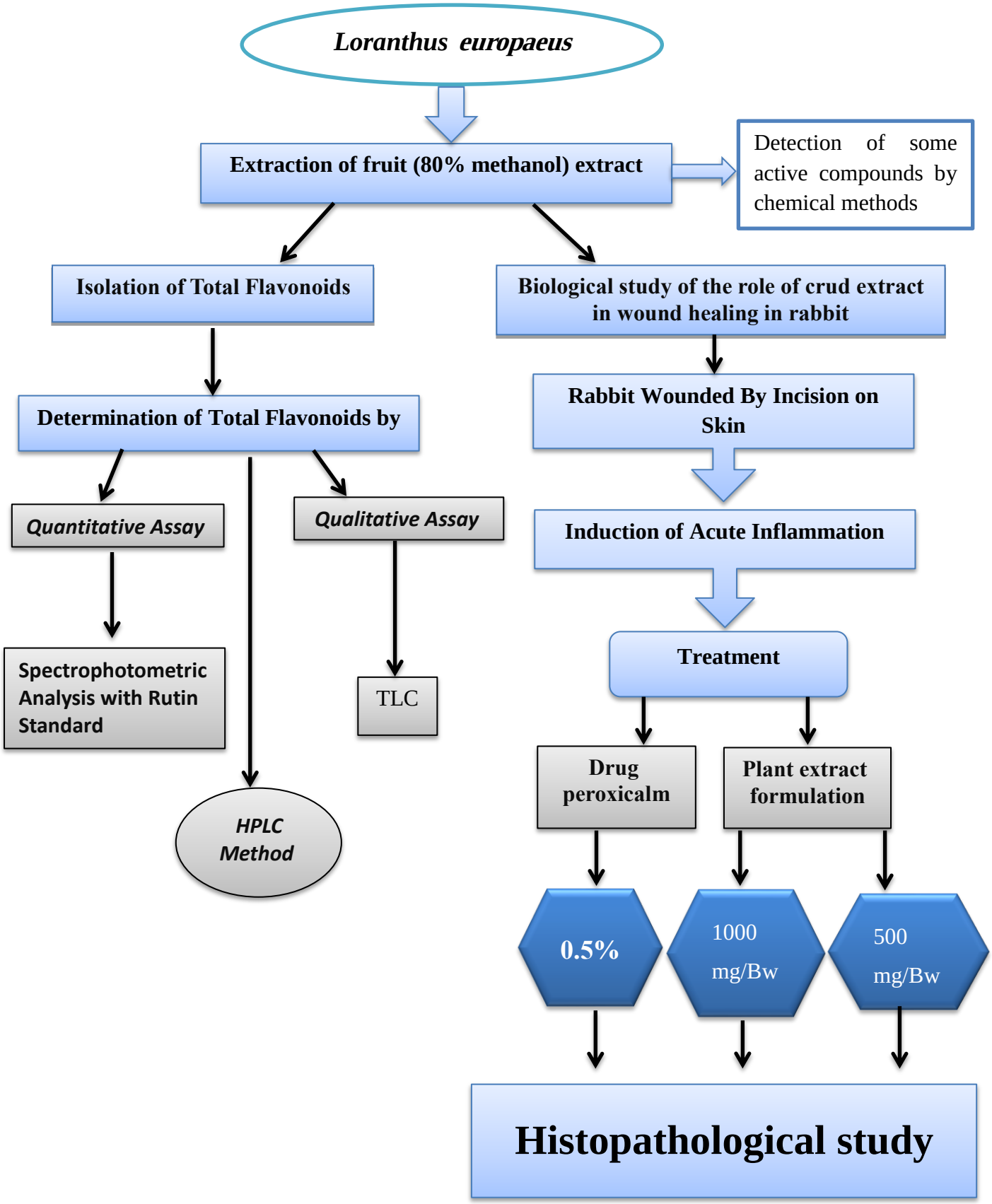


Figure (2-1): Main steps of the research plan

2.2. Methods.

2.2.1. Plant Collection:

The plant was bought from Iraqi market and authenticated by prof. Dr. Ali AL-Mossawy, Biology Department, College of Science, Baghdad University. The plant fruits were at room temperature crashed by blunder to be extracted.

2.2.2. Plant Extraction

Fifty grams of dried plant fruits were extracted overnight in 250ml of methanol by maceration, using shaker incubator 25°c. The extract solution was filtered by Buchner funnel. Then concentrated at 40c° by rotary evaporator, finally evaporated by lyophilizer, and the resultant crude powder e was kept at -20^{0}C until use**.** (Arokiyaraj *et al*., 2007)**.**

2.2.3. Chemical Detection of Plant Extracts.

i. Alkaloids: One ml of the plant extract was added to a glass watch containing 2 ml of Mayer′s reagent. The appearance of white precipitate was an indicator for the presence of alkaloids (Smolensk *et al*., 1972).

ii. Flavonoids: The detecting solution was prepared by mixing 10 ml of ethanol (50%) with 10 ml of potassium hydroxide (50%), and then 5 ml of this solution was added to 5 ml of the plant extract. The appearance of yellow color was an indicator of the presence of flavonoids (Jaffer *et al*., 1983).

iii. Glycosides: About 3ml from the extract was hydrolyzed with HCl for 1-2 hours on water bath. Then the hydrolysate transferred to glass tube and heated with 7ml Benedict's reagent. The reagent contained blue copper(II) ions(Cu^{+2}) which were reduced to copper(I)ions(Cu^{+1}) in the presence of reducing sugar and heating, which precipitated as insoluble red copper(I) oxide (Harborne, 1998).

iv. Detection of Terpenes and Steroids: One gram of the plant methanolic extract was participated in a few drops of chloroform, then a drop of acetic anhydride and drop of concentrated sulphuric acid were added, brown precipitate appeared which representing the presence of terpene, which the appearance of dark blue color after few minutes would represent the presence of steroids (Harborne, 1984).

v. Saponins: Two methods were employed in the detection of saponins from Stahl (1969) methods:

- The first one by adding 1-3 ml of ferric chloride solution to 5 ml of the extract (methanol), and then the formation of white precipitate was an indicator of the presence of saponins.
- In the second method, the tube containing the extract was shaken, and the formation of thick foam that remained for few minutes was an indicator of the saponins.

2.2.4. Extraction of Flavonoids from *L. europaeus*

As presented by Harborne (1984) six gram, from dried methanol extract was reflected for 8hr using 200 ml of 2M HCl solution. Filtered and the filtrate was cooled and transferred to a separator funnel. The aglycon moiety was extracted by 50 ml X3 ethyl acetate. The collected ethyl acetate layers were washed with distilled water to get rid of the excess acid then evaporated to dryness by rotary evaporator at 40ºC. The dried residue then re-dissolved in 30 ml 50% ethanol.

2.3 Determination of Total Flavonoids.

A. Quantitative Assay (Wang *et al.*, 2009)

Rutin standard stock solution was prepared (1mg/ml in 50%ethanol), from which serial dilutions were made to get Rutin standard solutions with concentration of (0.2, 0.5, 1, 2.5 and5) mg/ml in 50% ethanol. Amount of 1ml was transferred from each standard solution and from the extracted flavonoid

into a glass tubes, then 0.75 ml of 5% sodium nitrite solution was added and mixed well to be left to stand at room temperature for 5 minutes. To all tubes 1.5 ml of 10% $AlCl_3$ in 50% ethanol was added, shacked well and left to stand at room temperature for another 5 minutes. At last 5ml of 1N NaOH solution was added to all tubes.

The absorbance was read at 510nm, and a standard curve was plotted between the concentration and the absorbance, then the amount of total flavonoid was calculated as rutin from the equation of straight line that obtained from the plotted curve.

B. Qualitative Assay (Marcica *et al*., 2009***)***

Thin-layer chromatography (TLC) is a very commonly used technique because it is simple. For determining of the extracted flavonoids the following solvent systems were used as a mobile phase to choose the most proper one that separate the extracted *L. europaeus* flavonoids efficiently, as shown in table (2-3)**.**

Table (2-3): Different TLC Solvent System and their Ratios

Solvent System	Ratio	Symbol
n-Hexane: Ethyl acetate: Glacial acetic acid	31:14:5	A
Chloroform: Glacial acetic acid: Formic acid	44:3.5:2.5	B
n-Butanol: Glacial acetic acid: Distilled water	20:5:25	C

Standard solutions was prepared 0.1mg/ml in 50%ethanol from rutin, kaempferol, quercetin, luteolin, and mixed of standard solutions then by using one spot of the extracted plant flavonoid and from each standard solutions, a thin layer chromatography was performed on silica gel Gf254 aluminum plates activated at 100°C for 30 minutes in an oven and cooled at room temperature before use.

c. HPLC Method *(*Hamad *et al*., 2013)

HPLC application for flavonoids standards rutin, kaempferol, quercetin and luteolin, and the extracted plant flavonoid was used to measure the concentration of the flavonoid extract. The condition for this assay as follow:

Mobile phase : Methanol: Water (70:30)

Column : C18 (25cm)

Flow rate : 0.5ml/min.

Injected volume : 10µl.

Wave length : 280nm.

Instrument : waters/487 USA.

2.4 Laboratory Animals and Experimental Design.

Healthy mature locally rabbit 4-6 month old and 1.300-1.500 kg weight were supplied by the Biotechnology Research Centre (Al-Nahrain University), the animals were maintained at a temperature of 20- 25°C, and had free excess to food (standard pellets) and water throughout the experimental work. The rabbits were kept two weeks for adaptation. As the experiment was designed to assess the histopathological effects therefore, the animals were divided into seven groups:

- **Group 1:** Normal.
- **Group 2:** Animal skin wound **without** inflammation **(no** treatment).
- **Group 3**: Animal skin wound **with** inflammation **(no** treatment**).**
- **Groups 4**: Animal skin wound **with** inflammation **(**treatment **with carrier** Vaseline and glycerin).

- **Groups 5:** Animal skin wound **with** inflammation (treatment **with** Piroxicalm Gel 0.5%).

- **Group 6**: Animal skin wound **with** inflammation **(**treatment **with** dose 500mg of extract**).**

- **Groups 7:** Animal skin wound **with** inflammation **(**treatment **with** dose 1000 mg of extract**).**

The tested preparation of plant crude extract as a single dose (500 or 1000mg\BW) was mixed with Vaseline and Glycerin in ratio (3:2:1) applied topically daily and for 10 days. In the case of Piroxicalm Gel 0.5%, it was applied topically with a single dose then the rabbits were sacrificed at day 11 of experiment (Nielsen, 1992).

2.5 Animal's treatment.

- Both sides of the animal were cleaned with sterile distilled water and then shaved with sharp blade.
- After removal of hair from both sides, all animals were wounded by making an incision at the dorsal surface (1cm) below the scapula, which was 3 cm long and 1cm deep (from the skin and panniculus carnosus muscle). (Radenahmad *et al.*, 2012).
- All animals were weighted before and through the time of experiment.

2.6 Induction of Acute inflammation

As described by Graham *et al.* (1995), the induction of acute inflammation was done by a subcutaneous injection of 0.1 ml of 2.5% formalin on the second day of wound incision.

Figure(2-2): Rabbit wound and inflamation befor treatment

2.7 Measurement wound contraction

The periphery of the incision wound was traced on a transparent paper on every 5th day, till the wound gets healed. Wound area was measured by retracing the wound on a millimeter scale graph paper. The evaluated surface area was used to calculate the percentage of wound contraction (Sadaf *et al.*, 2006).

$$\%\ \text{of wound contraction} = \frac{\text{Initial would size - Specific day wound size}}{\text{Initial wound size}} x\,100$$

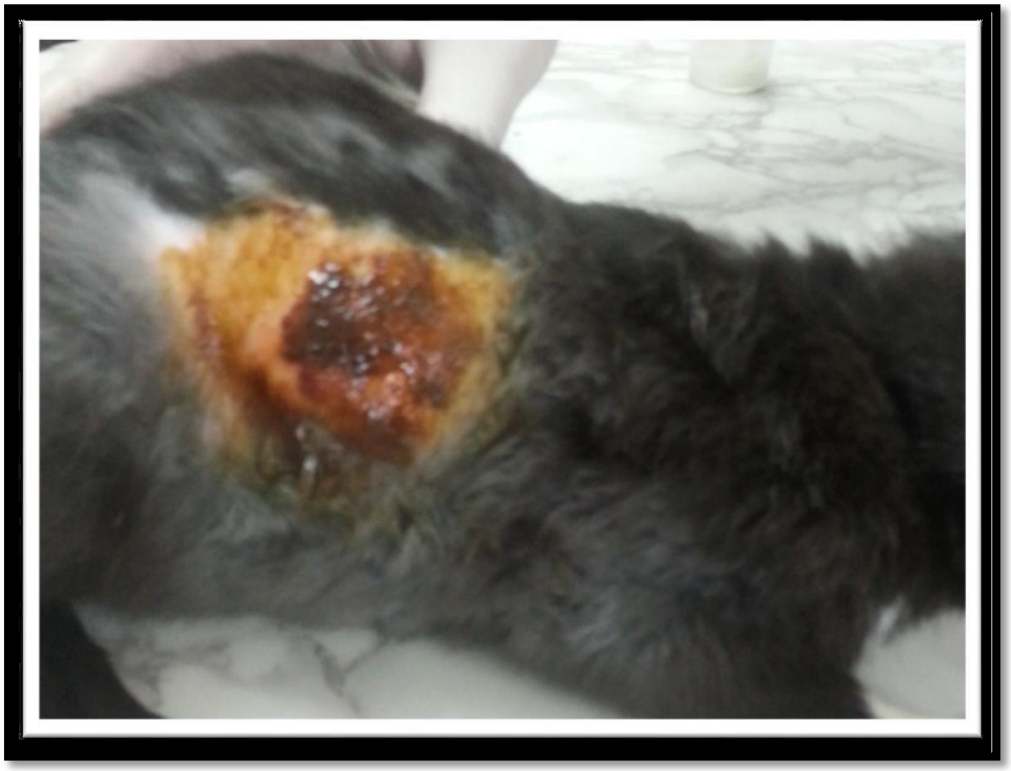


Figure (2-3): Rabbit wound at first day of treatment

2.8 Histopathological Study

All rabbits were scarified by heart puncture, and then dissected to obtain the wounded skin, the whole initial wound area was biopsied and processed and the procedure of Bancroft and Stevens (1982) was followed to prepare sections for histopathological examinations after the obtained sections were fixed in 10% formalin for 48 hours.

- **Sample preparation**

i. Dehydration: dehydration of sample was made with ascending concentrations (50, 70, 90 and 99%) of ethanol, one hour for each concentration.

ii. Clearing: xylene was used in clearing of the sample by dipping for half to one hour.

iii. Infiltration: The sample was first placed in paraffin-xylene (1:1) for 30 minutes at 57-58ºC, and then in paraffin alone for half to one hour at 60-65ºC in the oven.

iv. Embedding: The sample was embedded in pure paraffin wax (melting temperature: 60-70ºC) in L-shape copper block and left to solidify at room temperature.

v. Sectioning: The paraffin block was sectioned (rotary microtome) at a thickness of 5 microns, and then the sections were transferred to a slide covered with Mayer's albumin. The section of tissue was placed in a water bath (35-40ºC) for few seconds.

vi. Staining: The slide was placed in xylene for 15-20 minutes, then put in descending concentrations (70, 80 and90%) of ethanol (two minutes for each concentration) and finally distilled water 5 minutes. After that, the slide was stained with haematoxylin for 5-10 minutes and then washed with tape water for

5 minutes. Then, the slide was placed in acidic alcohol by dipping few second and washed with distilled water. After washing, the slide was placed in eosin stain for 15-30 seconds, and washing in tape water then in ascending concentrations (70, 80, 90 and 99%) of ethanol (two minutes for each concentration). Finally, dry and the slide was cleared with xylene for 10 minute at least.

vii. Mounting: The slide was mounted with a Canada balsam and covered with a cover slip, then examined microscopically to inspect the histopathological changes.

3.1. Plant Active Components Extraction

3.1.1. Methanol extract

Fifty gram of *L. europaeus* powdered fruits yielded a residue weighted 6g which represents 12% of the original fruits sample. The appearance of the residue was brown in color.

3.1.2. Detection of some active compounds in the plant extract

Results of chemical detections of secondary metabolites in the fruits of *L. europaeus* crude methanolic extract were shown in table (3-1).

Table 3-1: Chemical detections of secondary metabolites in the fruits of *L. europaeus* methanolic extract[1]

Secondary Metabolites	Reagents	indication	Result of detection
Alkaloids	Mayer's reagent	white precipitate.	+
Flavonoids	Ethanol with KOH	yellow color	+
Glycosides	Benedict reagent	Red precipitate	+
Saponins	Shaking Extract ferric chloride	foam white Precipitate	+
Terpenes and Steroids	chloroform, acetic anhydride, sulphuric acid	brown precipitate	+

[1]: : Positive reaction indicates the presence of this active compound.

In this study chemical detections of secondary metabolites in the fruits of *L. europaeus* methanolic extract showed presence of flavonoids, alkaloids, glycosides, saponins and terpenes and steroids by using different method used routinely in lab.

Previous pharmacological and chemical studies on some species of the Loranthaceae have indicated the presence of several chemical compounds, including flavonoids, alkaloids (Fernandez *et al.*, 1998) and polysaccharides-glycosides-(Sinha *et al.*, 1999). Many chemical components such as tannin, terpenoids, phenols, flavonoids, glycosides, triterpenoids and resins present in *Loranthus micranthus* Linn in methanolic extract (Egbuonu and Nwankwo, 2011).

Also *Loranthus bengwensis* was proved by Olatunde and Dikwa (2014) indicated presence of alkaloids, flavonoids, tannins, cardiac glycoside, terpenes and steroids.

Chemical analysis of *Loranthus ferrugineus* showed the presence of significant amounts of polyphenolic and flavonoid constituents. (Ameer *et al.*, 2009).

3.1.3. Extraction of Flavonoids and Determination of Total Flavonoids

The dried powdered fruits yielded about (6g) residue which was reflected with acidic solvent (HCl) to break down the glyosidic linkage. The non-aqueous aglycon residue was dissolved in 30 ml of 50%v/v ethanol for following investigations.

A. Quantitative Assay.

The absorbance of the spectrophotometric was recorded by *L. europaeus* extract total flavonoids and rutin standard solutions at 510nm were shown in table (3-2).

Table(3-2): Spectrophotometric analysis of *L. europaeus* total flavonoids and rutin standard solutions at 510nm.

Concentration (mg/ml)	Absorbance (at 510nm)
0.01	0.006
0.1	0.062
0.2	0.172
0.25	0.266
0.5	0.55
1	1.263
The extracted solution	1.843

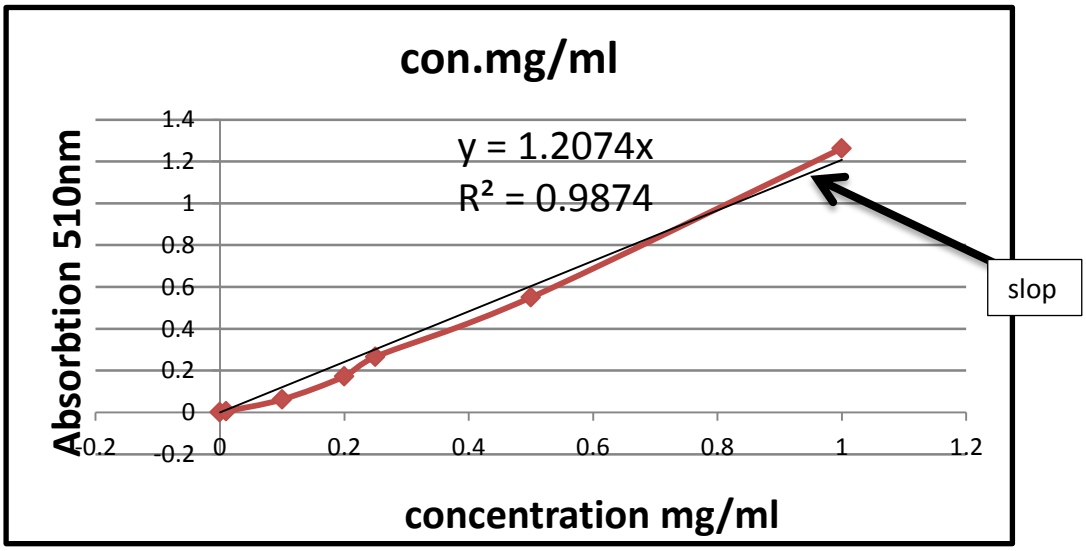


Figure (3-1): Standard curve for rutin as determined spectrophotometrically at 510 nm.R^2=0.9874.

The plotting process for the standard rutin solutions and the equation of a straight line was shown in figure (3-1).

Results indicated that total flavonoid in (1g) *L. europaeus* dried fruits was: 2.545 mg determined as rutin according to straight line equation.

In a study by Bamane *et al.*(2012) showed the determination of total flavonoids content in selected plants belonging to family Loranthaceae, in the three plants *Plicoseplus acacia*, *Plicoseplus curviflorus* and *Plicoseplus austro* Arabica, were found to be 5.39, 5.82, 6.2 g /100 g of dry plant weight respectively.

Total flavonoid content was found to be 22.5 mg/g in methanolic extracts of *Macrosolen parasiticus* (L.) which belongs to the family Loranthaceae (Lobo *et al.*, 2011).

The quantitative phytochemical screening of *Loranthus bengwensis* revealed that the plant contains 0.20% saponins, 0.30% flavonoids, 0.10% alkaloids and 0.21% anthraquinones (Olatunde and Dikwa, 2014).

B. Qualitative Assay.

During comparison of different mobile phases, it was found that the solvent system (B) was the best one as long as it gave good separation of the components, as shown in figure (3-2) for B system.

Previous work by Wagner and Bladt (1996) is in agreement with this study that Quareciten was identified as one of the flavonoid in this plant. Result depends on TLC technique.

Harvala *et al.* (1984) showed that spots of methanol extract of *L. europaeus* on TLC paper indicated that Quareciten and Kaempferol.

Rutin present in *L. micranthus* (Osadebe *et al.*, 2010) and Lueteolin present in this extract proved by many workers.

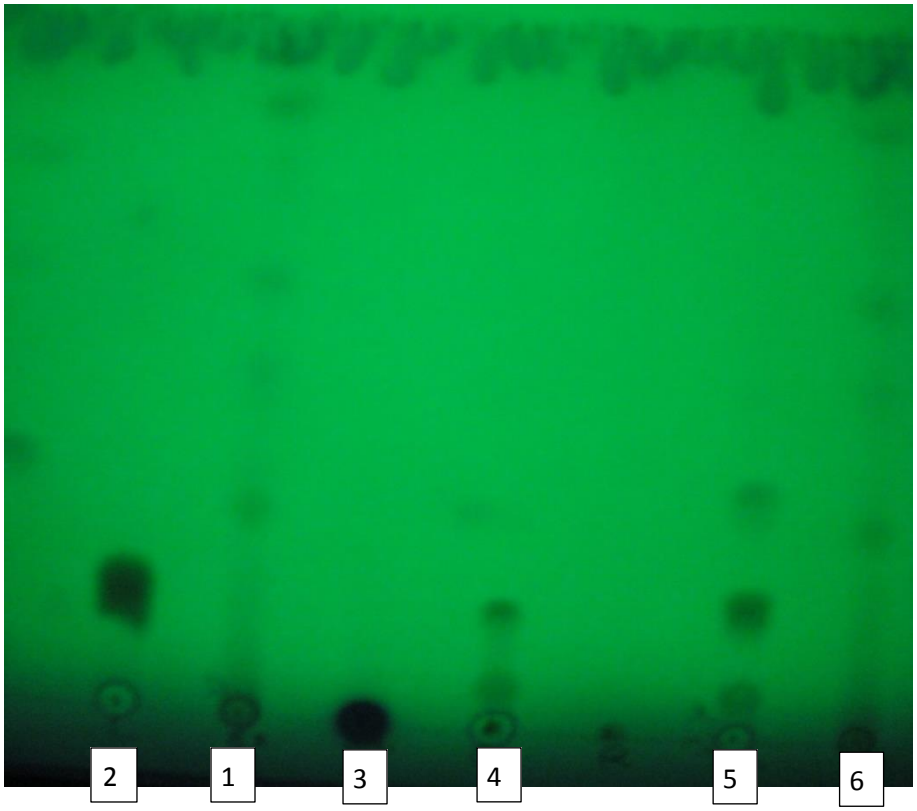


Figure (3-2): TLC chromatogram for the mobile phase [B].
***L. europaeus* flavonoids extract (1), Kaempferol (2), Quareciten (3), Luteoline (4), Rutin (5), mixed standers (6).**

C. HPLC Analysis

HPLC analysis of the methanolic extract for *L. europaeus* obtained from dried fruits flavonoid extract indicated the presence of**:**

A. Rutin, with retantion time (1.894)minutes, figure(3-3) in comparsion with Rutin standard (1.866) as figure (3-4A).

B. Lueteolin, with retantion time (2.470)minutes, figure(3-3) in comparsion with Lueteolin standard (2.458) as figure (3-4B).

Retantion time of two flavonoid standards: Quareciten and Kaempferol are (2.317 and 2.798) minutes respectily, figure (3-5 A and B), in comparsion with retantion time of tested flavonoid (2.470) minutes.

When applied the data for peak area under the curve and retention time of the standard and extracted flavonoids, the concentration for total flavonoids were calculated as follow:

Total flavonoid(mg) in 1g dried fruit powder

$$= \frac{Peak\ area\ of extracts}{Peak\ area\ of standard} \times Standard\ solution\ concentration \times total\ volume\ of\ extract$$

That is:

- Rutin

$$\frac{16419597}{23273538} \times 1\frac{mg}{ml} \times 100ml = 70.5\ mg$$

- Lueteolin

$$\frac{9670273}{26534042} \times 0.5\frac{mg}{ml} \times 100ml = 18.2\ mg$$

- One gram of dried fruit contains:-

Rutin 70.5 %of total flavonoid $\Longrightarrow$ Rutin =1.8mg

Lueteolin 18.2% of total flavonoid $\Longrightarrow$ Lueteolin =0.46mg

The rest quantities 0.28mg may be suggested as Quareciten and Kaempferol.

The present study focuses on estimation of total flavonoid in *L. europaeus* fruits. It became clear that *L. europaeus* plant is rich with flavonoid [2.545 mg\1g dried powder fruit] that might give an emphasis for the plant pharmacological action.

According to the phytochemical evaluation of the extract in our study *L. europaeus* (fruits flavonoids extract) flavonoids are found to be the major constituents of the extract. According to HPLC results, in previous study,

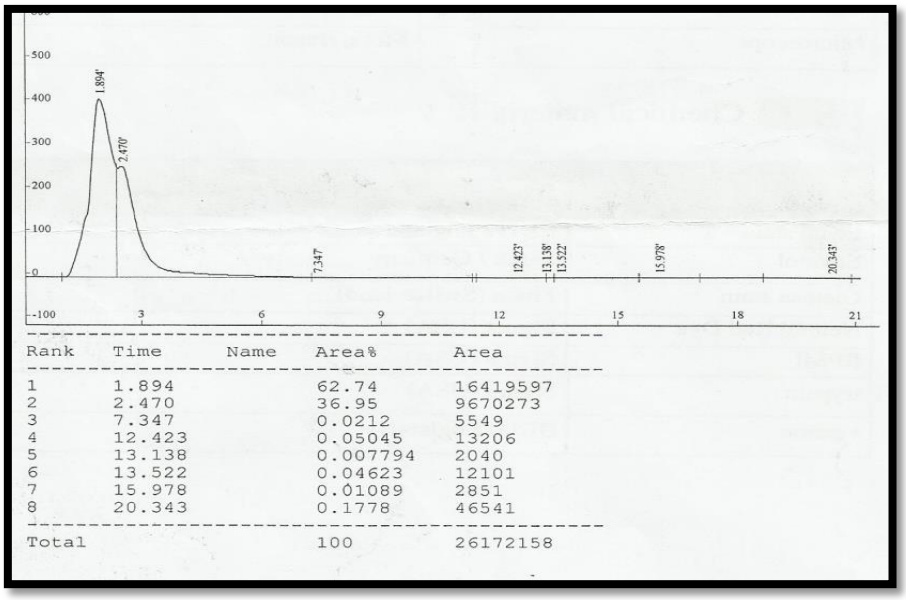
flavonoids were identified by Carroll *et al*. (1998) as (Kaempferol, quercetin and rutin).

Fraction of *L. europaeus* using HPLC technique, previous study by Bamane *et al.*(2012) gave different concentration of quercetin varied in selected plants belonging to family Loranthaceae, with 0.157 (*P. austro arabica*) to 0.062 g% (*P. acacia*) and *P. curviflorus* contained 0.115 g% w/w quercetin.

While it was known that only Rutin which match the preliminary phytochemical investigations was present as flavonoids in methanol fractions and HPLC (Hamad, 2013).

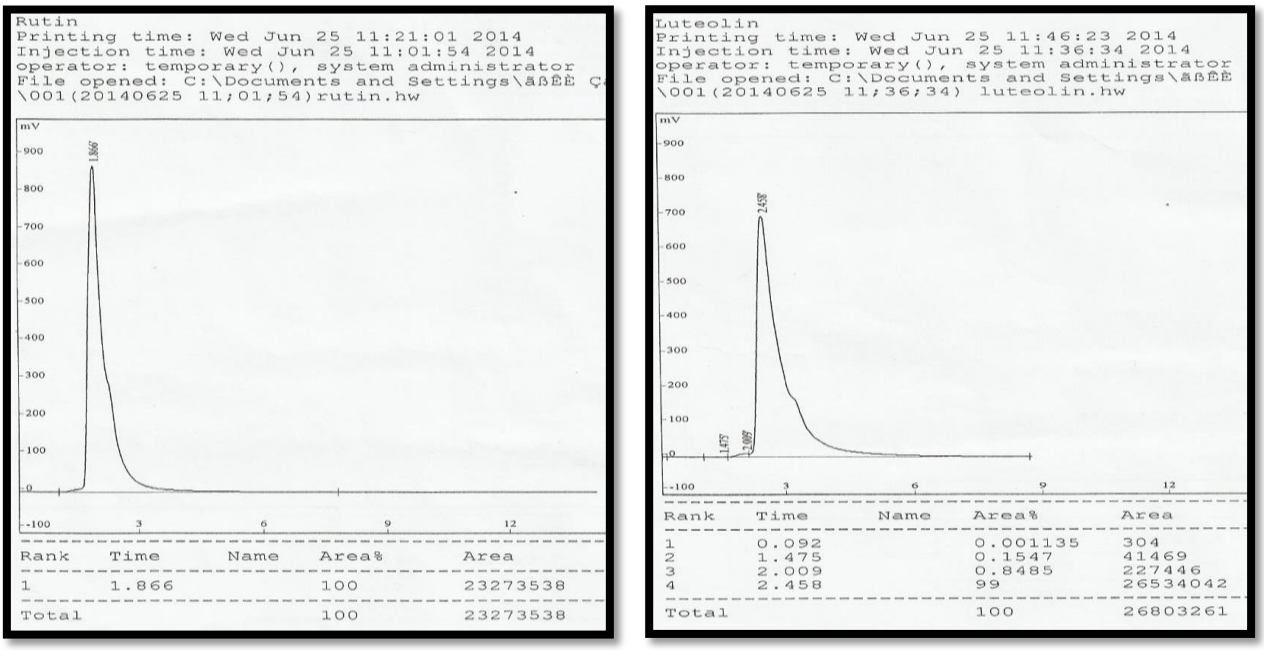
The results showed that the flavonoids found in the extract, were in variation in their conclusion starting with the major flavonoid present Rutin=1.8 mg. And Lueteolin is the second major component in this plant with the quantity = 0.46mg.

Our study did not point the presence of Quareciten and Kaempferol in spite of their appearance by TLC technique; this may need other different mobile phase in further work.



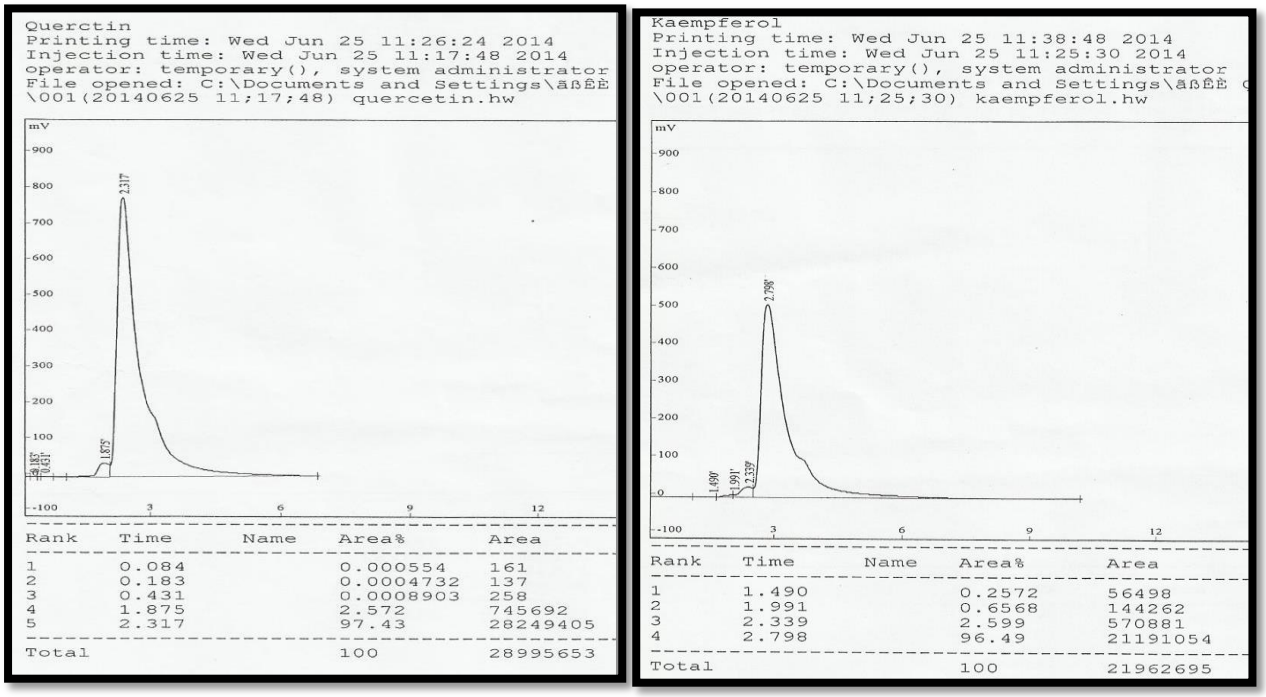
Rank	Time	Name	Area%	Area
1	1.894		62.74	16419597
2	2.470		36.95	9670273
3	7.347		0.0212	5549
4	12.423		0.05045	13206
5	13.138		0.007794	2040
6	13.522		0.04623	12101
7	15.978		0.01089	2851
8	20.343		0.1778	46541
Total			100	26172158

Figure [3-3]: HPLC analysis of the *L. europaeus* dried fruits flavonoid extract.



[A] **[B]**

Figure (3-4): HPLC analysis for [A] Rutin and [B] Lueteolin as stander



[A] **[B]**

Figure (3-5): HPLC analysis for [A] Quareciten and [B] Kaempferol as standers.

3.2. Effect of *L. europaeus* dried fruits extract on wounds healing

3.2.1. General observation

The following figures show obviously results of treatment and healing of wounds with plant extract:

Treatment with extract -ve control[1] +ve control[2]

At 5th days of treatment

Figure(3-6): show morphological changes and repair of skin by the treatment of wounds with plant crude extract at 5th day of experiment as compared with negative control.

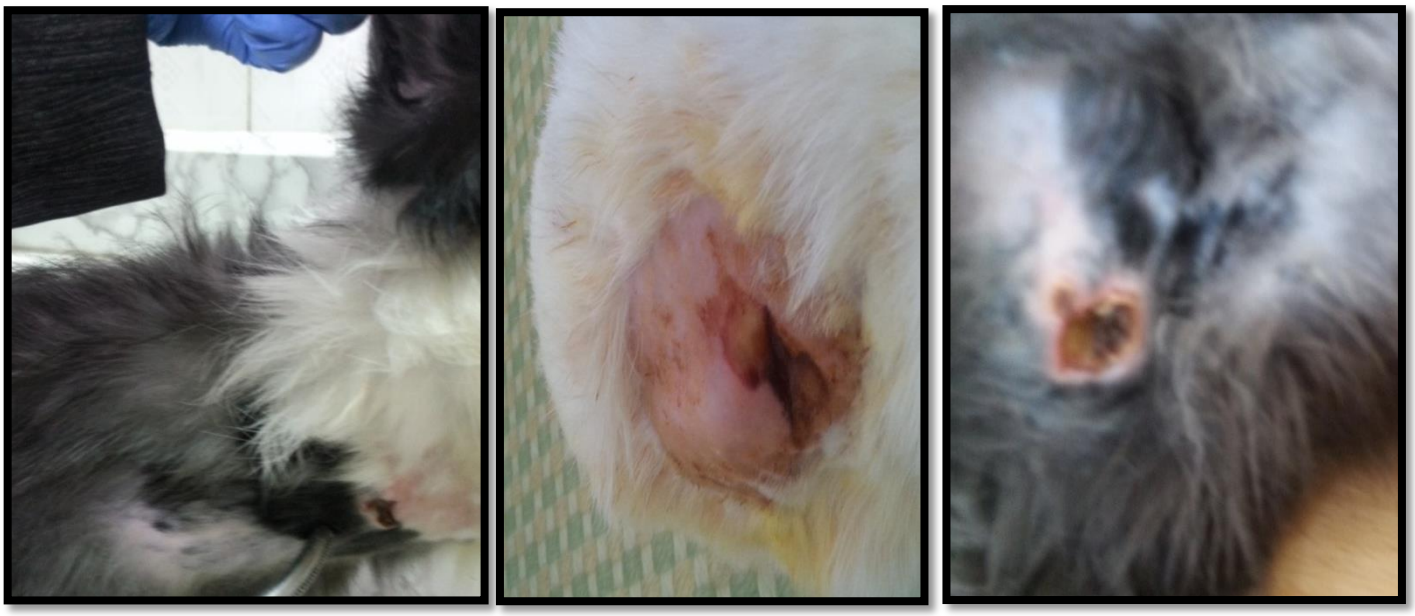
According to the results, improved wound healing activity has been observed at fifth day of treatment compared to control skin, treatment wound showed in figure (3-6) a rate of wound closer, length of incision became smaller which means has skin breaking strength. Inflammation edema size became smaller; with begin of hair growth at site of barber.

According to The above results demonstrated that the treatment with the extract for five days was not enough to produce significant healing changes in

[1] : -ve control: animals not treated

[2] : +ve control: animals treated with Piroxicalm Gel 0.5%.

the wound area, as compared to controls. However, such changes were prominent when the treatment continued for more than five days.



Treatment with extract -ve control[3] +ve control[4]

At 10th days of treatment

Figure(3-7): Show morphological changes and repair of skin after the treatment of wounds with plant crude extract at 10th day of experiment as compared with negative control.

While improved wound healing activity has been observed at tenth day of treatment compared to control skin, in figure (3-7) treated wound showed a rate of wound closer with small scar, length of incision became smaller which means has skin breaking strength. Inflammation edema size disappeared, with complete hair growth at site of barber.

[3] : -ve control: animals not treated
[4] : +ve control: animals treated with Piroxicalm Gel 0.5%.

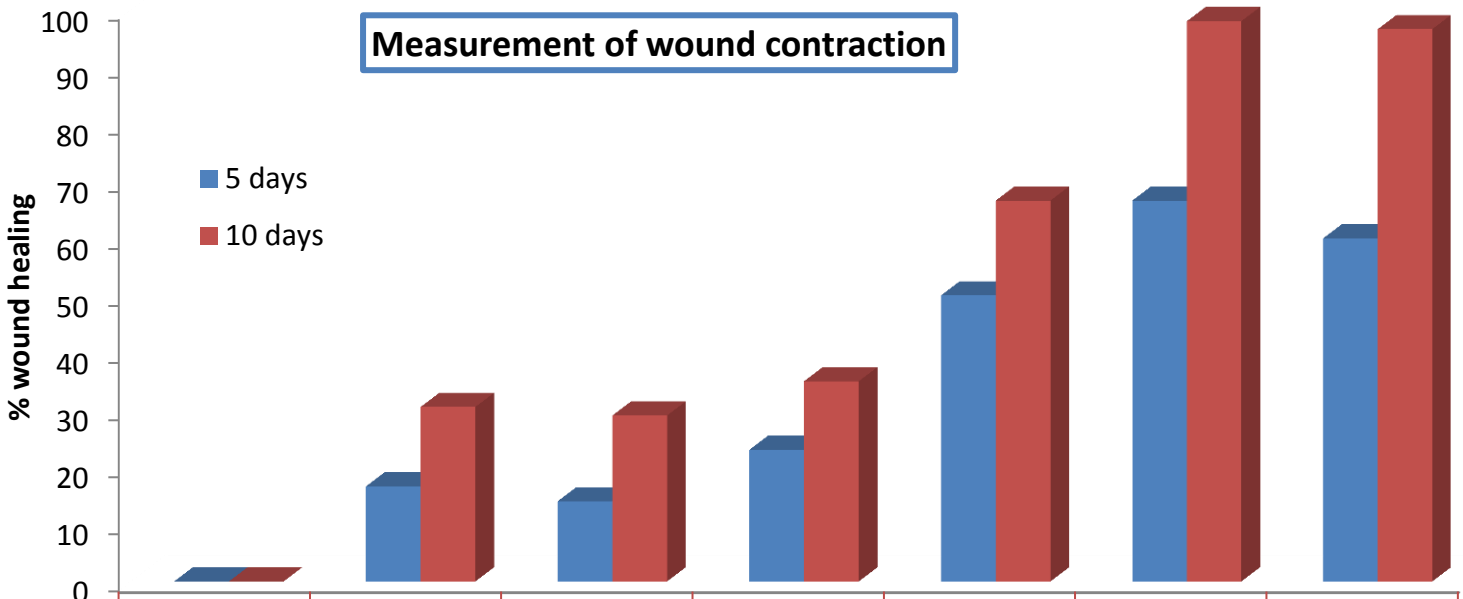
The effect of plant extract was clear and rapid after tens day of treatment, through reduced scar formation, exhibits enhanced fibroblast proliferation, angiogenesis, keratinization and epithelialization as compared to reference standard and control groups.

Gross examination of the skin clearly showed that all treated wounds groups with extract has the fastest healing rate, which was associated with less redness, swelling, and exudates, as compared to the other groups.

3.2.2. Wound contraction studies

Reduction of wound area of different groups on 5th and 10th day for incision wound model figure (3-8) was recorded. Control groups that not treated and that treated with gel showed least rate of wound healing. Faster rate of wound healing was seen in groups treated with 500 and 1000 plant extract formulation.

Wound contraction as Levinson and coworkers (2004) a shrinkage of open skin wounds, leaves a remarkably small scar as the surrounding normal skin moves centripetally to close the wound. Wound contraction progresses through fibroblasts generating thicker collagen fibers, using traction forces; rather than by myofibroblasts utilizing cell contraction forces.



	normal	wound	wound\ inflamation	with carrier	with peroxicalm	extract 50%	extract100%
5 days	0	16.6	14	23	50	66.6	60
10 days	0	30.5	29	35	66.6	98	96.6

Figure (3-8): Measurement of wound contraction

3.2.3. Histopathological evaluation by light microscopic results

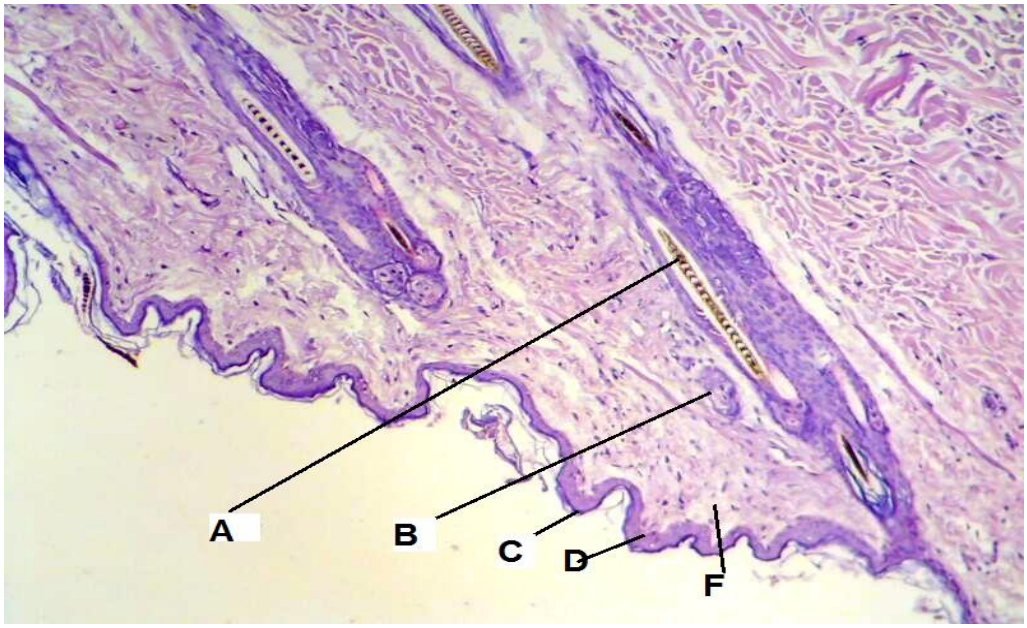


Figure (3-9): Longitude section shows the normal skin without any incision or inflammation (x200 H and E). A: hair follicle. B: sebaceous gland. C: keratin. D: epidermis. F: dermis.

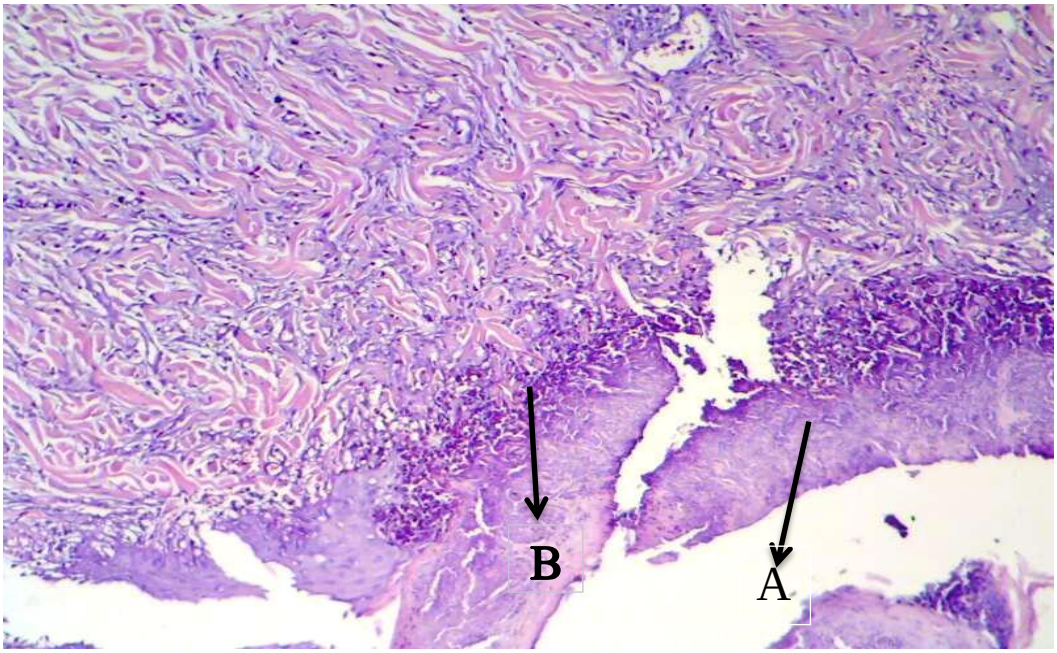


Figure (3-10): Incision animal skin without injection with formalin (non treated). Section showing sever inflammatory reaction, debris, scar and remnant of epithelial cell (X200 H and E).

A: the clear scarp. B: numerous inflammatory cells.

Histological features of incision animals skin are illustrated in figure (3-10), (Group 2). After 10 day of incision, the untreated wound showed prominent fibroid necrosis in the sub-epidermal region, this was characterized by permeation of collagen with fibroid and additional degenerative changes. This was very clear when compared with control animals (Group I) showed in figure section (3-10), which have normal structure of the epidermis and dermis layers of the skin with the appearance of hair follicle and sebaceous glands.

While in figure (3-11), which represent the inflammation (group 3) induced by formaldehyde, there will be empty spaces in the dermal region, indicating evidence of edema[5].

Large edemas under the skin surface are present, as a result of increased fluid from the blood vessels allowing more infection-fighting white blood cells to enter the affected area. Re-epithelialization was incomplete with little skin appendages. The wound surface was covered with scar in both group 2and 3.

Sebum and sebaceous glands prevent invasion and infection by pathogens and toxic substances. Additionally, the sebaceous glands control water loss from the skin and maintain moisture in the horny cell layers as suggested by Ro and Dawson (2005).

[5]: [Contact with formaldehyde solutions, vapor or resins can cause eczema (dry, flaking and itching skin) and in extreme cases can lead to allergic dermatitis or hives]. (World Health Organization, 1989).

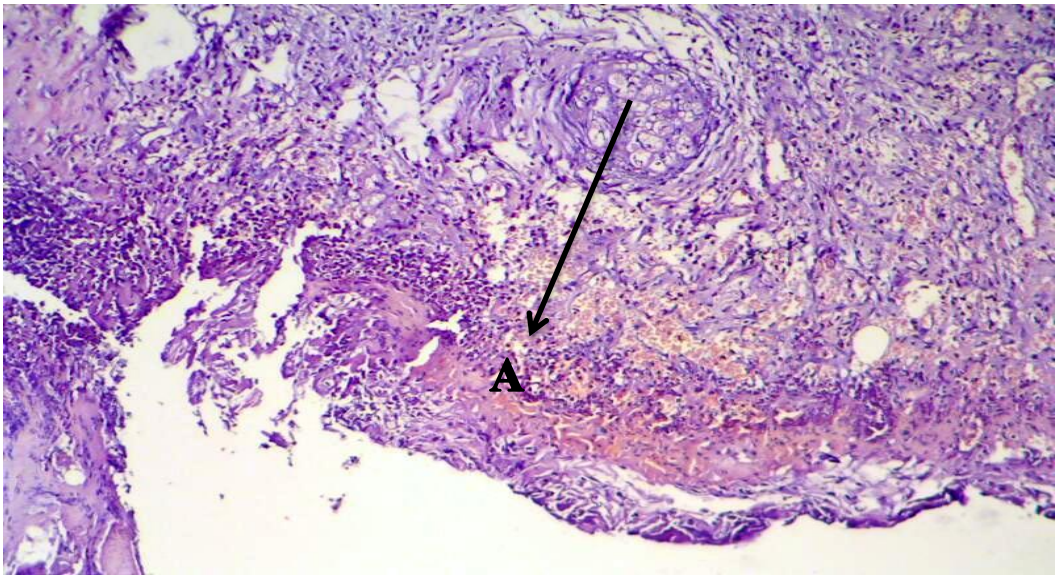


Figure (3-11): Section of incision animal skin with inflammation; (non treated). Showing sever inflammatory reaction–abundant inflammatory cell presence (H and E; X200). A: sebaceous gland.

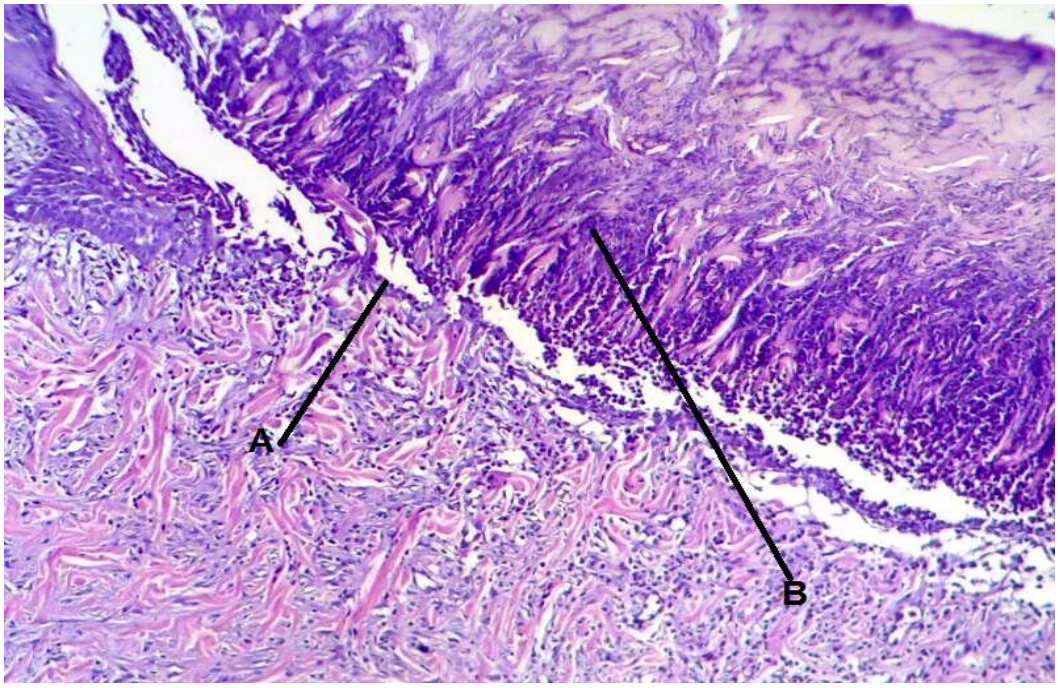


Figure (3-12): Section of incision animal skin with inflammation has treated with carrier Vaseline and Glycerin. (H and E; X100).
A: Re-epithelialization. B: Granulation tissue.

Examination of figure section (3-12) from the recovery vehicle-treated group (Group 4) showed the formation of the epidermal cells and the epithelialization was observed after 5^{th} day as Vaseline and glycerin moisture the area and allowed the wound to form a scar. Vaseline (petroleum jelly) not only keeps wounds clean and moist but also provides an occlusive layer, thus keeps the wound covered. It keeps germs out decreasing the risk of infection. Apart from that it hydrates the wound stimulating the healing process (Mark, 2006).

The effect of glycerin on skin is a healthier, more natural-looking appearance. Glycerin, or glycerol, can fight the effects of skin diseases such as psoriasis. When applied to the skin, signals the cells to mature in normal fashion. The application of glycerin can interrupt this abnormal process, and allows the cells to reach full maturation before shedding. The compound can also help wounds heal more quickly in some cases. Glycerin helps maintain the skin's water balance on an intercellular level. Glycerin in lotions or other skin care products can help prevent or combat dry skin. The fact that glycerin can have a therapeutic effect on wounds and other skin diseases also contributes to skin looking healthier and smoother. Glycerin is an emollient, making skin not only moist, but soft and supple to the touch. (Zheng and Bollinger Bollag, 2003).

Whereas the group 5 in figure section (3-15), showed little cured in the used - Piroxicalm Gel- synthetic drugs, all the steroidal and non-steroidal anti-inflammatory drugs (NSAIDs), despite their great number, cause undesired and serious side effects, therefore, development of new and more powerful drugs is still needed. Medicinal plants have long been used worldwide in folk medicine as an alternative treatment of inflammatory processes of diverse origins (Farnsworth, 1989).

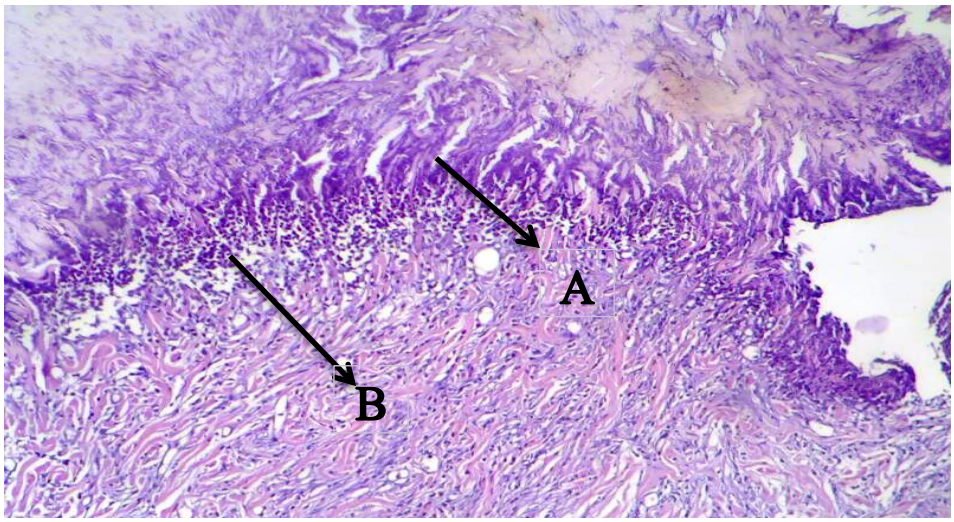


Figure (3-13): Section of incision animal skin with inflammation after treatment with Piroxicalm Gel 0.5%. Sever inflammatory cell, abundant of epithelium tissue, scar formation and re-epithelium(x100).
A: Re-epithelialization. B: numerous inflammatory cells.

The last two figures (3-14) and (3-15) showed that all extract-treated wounds appeared to heal better than the controls based on gross examination, degree of wound healing, and histopathological evaluation. The wounds in all extract-treated groups have doses 500 and 1000mg\Bw, showed developed epithelialization and keratinization, skin appendages were almost normal, there was no noticeable necrosis or inflammation.

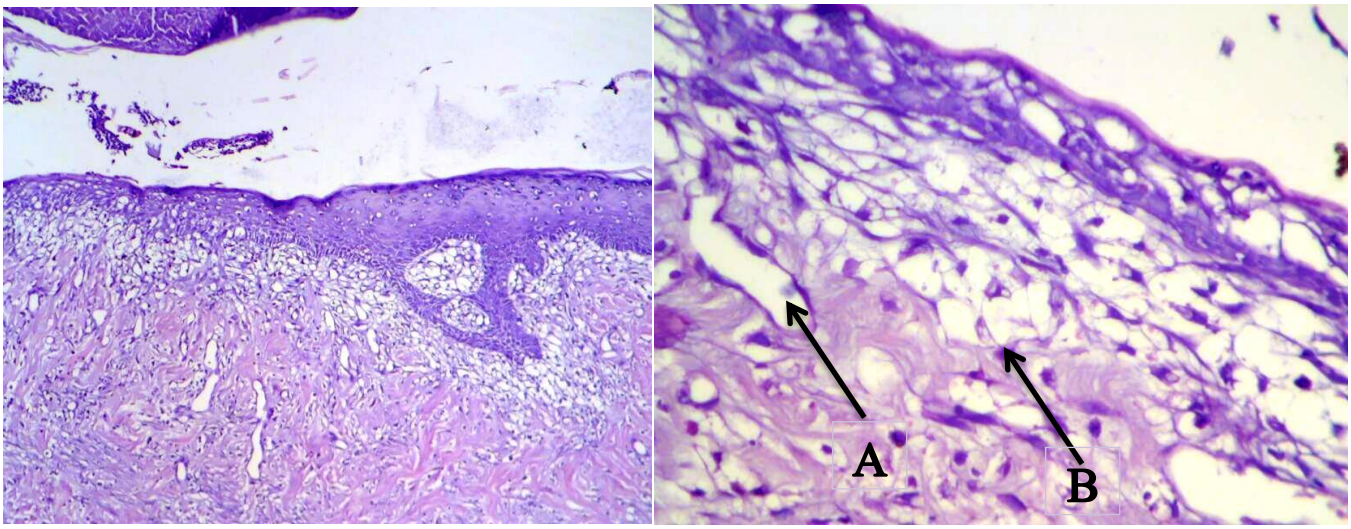
From this preliminary study showed that the rabbits treated with 500mg of plant extract had significantly better wound healing, including less scarring, brighter skin, and softer hair, as compared to that treated with double the dose of plant extract and the controls. However, these observations were only based on microscopic level the changes taking place inside the wound following the treatment of rabbit on a ten days period. Morphological evaluation on the effect of plant extract was evident in the accelerated wound healing that was characterized by reduced wound depth and width, increased thickness of the epidermis and dermis, thicker and more abundant collagen fibers and hair follicles.

Wound closure intentions by primary or secondary intention. In secondary intention, the wound is allowed to granulate, surgeon may pack the wound with a gauze or use a drainage system, granulation results in a broader scar, healing process can be slow due to presence of drainage from infection and wound care must be performed daily to encourage wound debris removal to allow for granulation tissue formation (Kuhl and Steinmann).

According to figure (3-13) presence of hemorrhage after treatment of high dose of plant medication could be disused as followed:

The sugar binding B chain of lectins is able to bind galactoside residues on the cell membrane preferring certain confirmations. While using low doses will not cytotoxic and enhancing the lectin in the proinflammatory activity of natural immune system. (Hostanska *et al*.,1995).

The used of plant extract with Vaseline and glycerin for the effect that aforementioned group in No. 4.



X100 x200

Figure (3-14): Section of incision animal skin with inflammation treated with 500 of plant extract [x100 and x200]; A: formation a new capillary, B: youth fibroblast.

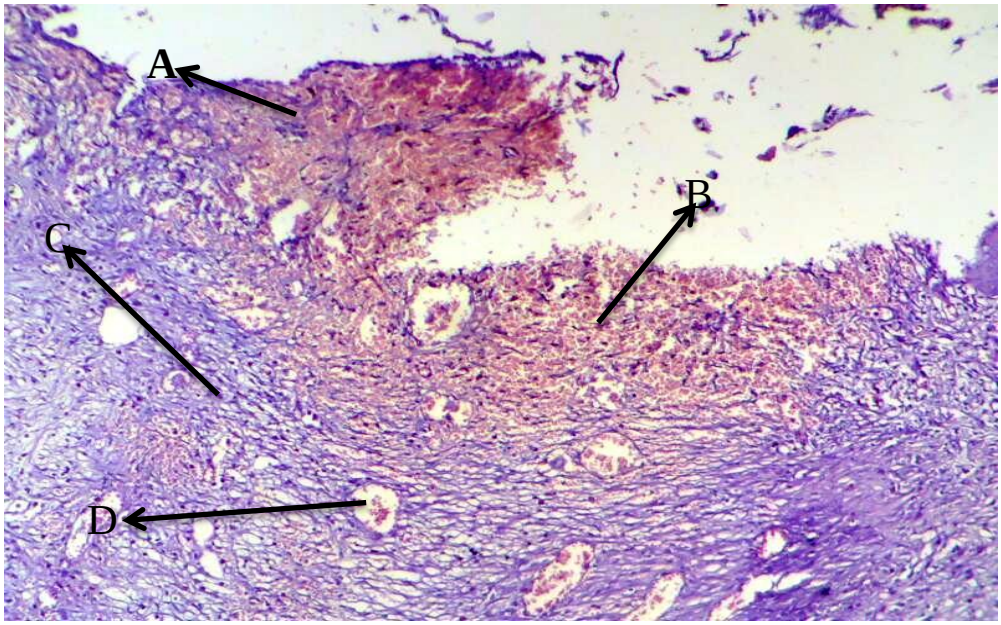


Figure (3-15): Section of incision animal skin with inflammation treated by 100% of plant extract (x100 H and E).
A: re-epithelialization, B: hemorrhage, C: fibroblast,
D: new capillary.

3.2.4. Role of *L. europaeus* fruits Plant Compounds in Wound Healing

Wound healing restores cellular structures and tissue layers in damaged tissue near to its normal state. Wound healing process is divided into sequential, yet overlapping phases, these are: hemostasis; inflammation; proliferation (also known as granulation and contraction); and remodeling (also known as maturation). (Kerstein,1997).

L. europaeus dried fruit has been used for its various therapeutic effects. The preliminary phytochemical analysis of the plant extract showed the presence of Flavonoids, Glycosides, Saponins, Steroids, Triterpenoids and Alkaloids. All these phytochemical constituents present in *L. europaeus* can be contributing to the wound healing activity.

Tissue repair after injury depends on the synthesis of a fibrous extracellular matrix to replace damaged tissue, which is then re-modelled over time to emulate normal tissue. Acute inflammation, re-epithelialization, and contraction all depend on cell extracellular matrix interactions this minimize infection and promote rapid wound closure. Vogel *et al.* (1980) showed granulation tissue formed in the proliferative phase is primarily composed of fibroblasts, collagen and newly formed blood vessels. The importance of collagen in wound healing is highly appreciated because of the simple reason that the ultimate outcome of tissue repair in the higher vertebrate is the formation of scar tissue composed of collagenous fibers (Omale and Emmanuel, 2010).

Phytochemical constituents like flavonoids of *L. europaeus*, produced many biological activities when used both in pure form and within extracts. Methanolic extracts was chosen to separate out total flavonoids, represent by the rutin, luteoline, kaempferol and quercetin.(Lee *et al.*,2009).

The healing effect of the skin wound may be attributed to flavonoid. The healing results agree with Bharti and his co-worker in 2011 presented that, the flavonoid extract of *Euphorbia hirta* Linn (with total flavonoids on methanolic extracts 212.6 mg/gm) showed decrease in the epithelization period, as

evidenced by the shorter period for the fall of eschar tissue in turn facilitating the rate of wound contraction. Wound contracture occurs throughout the healing process resulting in shrinkage of wound area. Wound contraction accelerates by flavonoids of *E. hirta* may be due to increased number of myofibroblast recruited into the wound area enhancing the contractile property.

Midwood and his coworker (2004) showed that, the flavonoid increase skin tensile strength along with increase in granulation tissue weight, protein and hydroxyproline content in incision and dead space wounded animals. Wound healing starts with an acute inflammatory phase followed by epithelization, angiogenesis and collagen deposition in the proliferative phase.

It has been reported that flavonoids and phenolic acids are the sources of antioxidants in plants (Cook and Samman, 1996).

Flavonoids are reported to possess potent antioxidant and free radical scavenging effect, which is believed to be one of the most important determinants of wound healing. Bioflavonoids are thought to benefit connective tissue turnover by biding to elastin, preventing its degradation by elastases. The flavonoids and saponins present in the *E. hirta* herb can speed up wound healing by strengthening the connective tissues (Basha and Sudarshanam, 2011). Extensive research has been done on the role of flavonoid as antioxidants and also its relation with wound healing many flavonoids are reported to reduce lipid peroxidation by preventing or slowing onset of necrosis in turn improving vascularity. The increased production of reactive oxygen species during injury results in consumption and depletion of the endogenous scavenging compounds such as superoxide dismutase, catalase, and glutathione peroxidase. Flavonoids may have an additive effect to the endogenous scavenging compounds. The delay in wound healing is mostly due to insufficient fibroblast activity. (Wang *et al.*, 2000).

Flavonoids prevent synthesis of proteoglycans that suppress T-cells and by inhibiting cycloxygenase-2 (COX2) and inducible nitric oxide synthase.

Luteoline and quercetin are known to possess anti-inflammatory activity, Kaempferol, quercetin, are reported to possess COX and LOX inhibitory activities (Tapas *et al.*, 2008).

Saponins were detected in *L. europaeus*, because of the antioxidant and antimicrobial activity of saponins; they may be responsible for wound contraction and elevated rate of epithelialization. (Shenoy *et al.*, 2009)

One of *L. europaeus* active compounds is triterpenoids which are supposed to enhance the wound healing process. (Scortichini and Roosi, 1991)

Triterpenoids induces type I collagen synthesis in human dermal fibroblast cells via activation of the TGF-beta receptor I kinase-independent S mad signaling pathway (Lee *et al.*, 2006) and also elevates antioxidant levels in a punch wound model, thereby promoting wound healing (Shukla *et al.*, 1999). Somboonwong *et al.* (2012) on extracts of *Centella asiatica* in incision wound found that triterpenoids was the most active extract for wound healing. Treated wound showed complete epithelization on 12th day post-operative day with increase in tensile strength as compared with control groups. Under the effect of cumin terpenoids responsible for wound healing activity(Bairy and Rao, 2001).

Another active component in phytochemical analysis of the plant extract, are glycosides and polysaccharides which can activate the macrophages in turn stimulate the fibroblast proliferation with subsequent proliferation of myofibroblast at periphery of the wound, the contraction of the contractile protein of myofibroblast play important role in wound concentration. This was proved on *C. asiatica*, it is extract facilitate treat burn wound healing. (Won *et al*., 2010).

Depending on the results, alkaloids with their analgesic and bactericidal effects stimulate chemotaxis for fibroblasts and promotes early phase of wound healing. (Stary, 1998; Okwu and Okwu, 2004).

4. Conclusions and Recommendations

4.1. Conclusions

1. The fruits of *L. europaeus* were rich with flavonoid, total estimated flavonoid =2.545 mg\1gm and the major components were Rutin with concentration=1.8 followed by Lueteolin with concentration =0.46 and trace of Quareciten and Kaempferol=0.28, proved by TLC and HPLC technique.
2. Methanolic extract of *L. europaeus* has therapeutic effect on wound during ten days.
3. Histopathological study proved that *L. europaeus* has anti-inflammatory effects as a result of wound healing.
4. *L. europaeus* may be a promising plant that could be applied in pharmaceutical preparation.

4.2. Recommendations

1) Further studies with purified phyto-constituents of *L. europaeus* extract are needed to comprehend the complete mechanism of wound healing activity.
2) Further isolation and evaluation of major active principles present in the plant material and test their efficiency against various multidrug resistant microbial strains**.**
3) Extended the period of treatment with the extract until complete keratin skin appeared and measure the exact time needed for healing.

References

❖ **Al-Fartosy**, A. J. and Al-Rikaby, A. K. (2007). The Antioxidative Action of Monoterpene from *Loranthus europaeus L. seeds*. Basra Journal of Agricultural Sciences, 20(1):23.

❖ **Ameer**, O. Z.**;** Salman, I. M.**;** Yam, M. F.**;** Abd Allah, H. H.**;** Abdulla, M. H.**;** Shah, A. M.**;** Sadikun, A. and Asmawi, M. Z. (2009). Vasorelaxant properties of *Loranthus ferrugineus* Roxb. methanolic extract. International Journal of Pharmacology. 5(1):44-50.

❖ **Arokiyaraj**, S.; Perinbam, K.; Agastian, P. and Balaraju, K. (2007). Immunosuppressive effect of medicinal plants of kolli hills on mitogen-stimulated proliferation of the human peripheral blood mononuclear. Indian.J.Pharmacol., 39:180-183.

❖ **Bairy**, K. L. and Rao, C. M. (2001). Wound healing profile of gimko biloba. J. Nat. Rem. 1:25-27.

❖ **Balle,** S.; Dandy, J. E.; Gilmour, J. S. ; Holltum, R. E. ; Stearn, W. T. and Thoday, D. (1960). *Loranthus, Taxon,* 9:208-210.

❖ **Bamane**, F. H.; Badr, J. M. and Amin, O.A. (2012). Antioxidant activities and flavonoid contents of selected plants belonging to family Loranthaceae. [Plicosepalus acacia, P. curviflorus and Phragmanthera austroarabica showed comparable antioxidant activity, apparently associated with their quercetin content.].African Journal of Biotechnology 11(78): 14380-14385.

❖ **Bancroft**, J. D. and Stevens, A. (1982). Theory and Practice of Histological Technique.2 ed. Churchill living stone. Edinburgh, London, Pp: 662.

❖ **Barnhart**, R. K. and Steinmetz, S. (2008). Chambers Dictionary Of Etymology. Chambers Harrap Publishers Ltd., Edinburgh.

❖

❖**Basha**, S. K. and Sudarshanam, G. (2011). Multiple herbal therapy-Antimicrobial activity of wound healing paste (Pasuru) used by Sugali tribes of Yerramalais of Kurnool district, Andhra Pradesh, India. Int. J. Pharm Tech Res., 3(3):1238-1241.

❖**Bharti**, M.; Saxena, R.C.; Arya, N.; Saxena, G.; Saxena, R. and Apte, K.G. (2011). Wound healing activity of alcoholic extract of *Nyctanthes arbortristis* L. in Wistar rats, Int. J. Pharmacy Pharm Sci. 3(5):253-255.

❖**Brayfield**, A, (2014). "Piroxicam". *Martindale: The Complete Drug Reference*. London, UK: Pharmaceutical Press. Retrieved.

❖ **Breitkreutz**, D.; Mirancea, N. and Nischt, R. (2009). Basement membranes in skin: unique matrix structures with diverse functions? Histochem Cell Biol. 132:1-10.

❖ **Briggs**, J. (2009). *Loranthus europaeus*, new to Britain, and notes on *Viscum album*, both new to Kew Gardens, *Haustorium.* 56:2-3.

❖ **Buwa**, L. V. and van Staden, J. (2006). Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South African. J. Ethnopharmacol. 103:139-142.

❖**Carroll**, K.K.; Guthrie, N.; So, F.V. and Chambers, A.F., (1998). Anticancer properties of flavonoids, with emphasis on citrus flavonoids, in Flavonoids in Health and Disease (Rice-Evans CA and Packer L (eds), Marcel Dekker Inc., New York, 437–446.

❖ **Choi**, S. W.; Son, B. W.; Son, Y. S., Park, Y. I., Lee, S. K. and Chung, M. H. (2001). The wound-healing effect of a glycoprotein fraction isolated from aloe vera. Br. J. Dermatol., 145(4):535-545.

❖ **Cook**, N. C. and Samman**,** S. (1996). Flavonoids-Chemistry, metabolism, cardioprotective effects, and dietary sources. Br. J. Dermatol. 7(2):66-76.

- **De Cleene**, M. and Lejeune, C. (2003). In: Compendium of symbolic land ritual plants in Europe, De Cleene, M. and C. Lejeune (*ed*s.), Man and Culture Publishers, Ghent, Pp: 411-425.
- **Egbuonu**, A. and Nwankwo, N. (2011). Phytochemical properties of some solvent fractions of petroleum ether extract of the African mistletoe (*Loranthus micranthus* Linn) leaves and their antimicrobial activity, Afr. J. Biotechnol. , 11(62): 12595-12599.
- **Farnsworth,** N. R. (1989). Screening plants for new medicines*:* in "Biodiversit: Part II". Wilson, E.O. (ed.), National Academy Press, Washington, 83-97.
- **FDA**: Brief of Food and Drug Administration, is a federal agency of the United States Department of Health and Human Services**,** http://www.fda.gov/Food**.**
- **Fernandez,** T., Wagner, M., Varela, B., Ricco, R., Hajos, S. and Gurni, A. (1998). Study of an argentine mistletoe: The hemiparasite Ligaria cuneifolia (Loranthaceae). J. Ethnopharmacol, 62:25-34.
- **Fleming,** T. (2002). Physicians' Desk Reference (PDR) for Herbal Medicines. Medical Economics Company, New Jersey**.**
- **Graham**, M., Pitcher Parent, A. and Terence, J. (1995). Noxious Thermal and Chemical Stimulation Induce Increases in 3H-Phorbol 12,13=Dibutyrate Binding in Spinal Cord Dorsal Horn as Well as Persistent Pain and Hyperalgesia, the Journal of Neuroscience, 5(5): 3263-3272.
- **Hamad**, M.; Hassan, A. and Numan, I. (2013). Evaluation of anti-inflammatory effect of ethyl acetate and methanol extracts of *Loranthus europaeus* in experimental models of acute inflammation in rats. Int. J. Pharm. Sci. Rev. Res., 23(2): 170-174.

- **Hanneken**, A.; Fen Lin, F.; Johnson, J. and Maher, P. (2006). Flavonoids Protect Human Retinal Pigment Epithelial Cells from Oxidative-Stress–Induced Death. 47:7.
- **Harborne**, J. B. (1984). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, second edition, Chapman and Hall, London.
- **Harborne**, J. B. (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, **3rd** edition, Chapman and Hall Int. Ed., New York.
- **Harvala**, E.; Exner, J. and Becker, H. (1984). Flavonoids of *Loranthus europaeus, J. Nat. Prod.* 47: 1054- 1055.
- **Hostanska**, K., Hajto, T.; Spagnoli, G. C.; Fischer, J.; Lentzen, H. and Hermann, R. (1995). A plant lectin derived from *Viscum album* induces cytokine gene expression and protein production in cultures of human peripheral blood mononuclear cells. *Nat Immun*. 14:295-304.
- **Houghton**, P.; Hylands, P.; Mensah, A.; Hensel, A. and Deters, A. (2005). *In vitro* tests and ethnopharmacological investigations; wound healing as an example. *J Ethnopharmacol.* 100: 100-107.
- **Jaffer**, H.T.; Mahmoud, M.; Jawad, A.; Nagi, A. and Alnailb, A. (1983). Phytochemical and biological Screening of some Iraqi plant. Fitoterapialix, Pp:299.
- **Jawad**, A.; Al-Rubaee; M. and Al-Diab, J. (2007).Oil Extraction of *Loranthus europaeus* Seeds Promotes Wound Healing. Bas. J. Vet. Res. 6(1):63-74.
- **Katsarou,** A.; Sophia, R. and Panagiotis K. (2012). Antioxidant potential of the aerial tissues of the mistletoe *Loranthus europaeus* Jacq. *Rec.* Nat. Prod. 6(4): 394-397.

References

- **Kerstein**, M.D. (1997). The scientific basis of healing. Adv. Wound Care. 10:30-36.
- **Khular**, R.; Saini, S.; Seth, N. and Rana, A. (2011). Emulgels: A surogate aproach for topicaly used hydrophobic drugs. International Journal of Pharmacy and Biological Sciences. 1:17-28.
- **Kim**, H. P.; Son, K. H.; Chang, H. W. and Kang, S. S. (2004). Anti-inflammatory plant flavonoids and cellular action mechanisms. J. Pharmacol. Sci. 96:229-245.
- **Kuhl**, E. and Steinmann, P. (2004). "Computational modeling of healing: An application of the material force method". Biomechanics and Modeling in Mechanobiology. 2 (4): 187–203.
- **Lai**, H.Y.; Lim, Y.Y. and Kim, K.H. (2010). *Blechnum Orientale* Linn - a fern with potential as antioxidant, anticancer and antibacterial agent. BMC Complement Altern Med.10:15.
- **Lai**, H.Y.; Lim, Y.Y. and Kim, K.H. (2011). Potential dermal wound healing agent in *Blechnum orientale* Linn. BMC Complement Altern Med., 11:62.
- **Lee**, J.; Jung, E.; Kim, Y.; Park, J.; Hong, S.; Kim, J.; Hyun, C.; Kim, Y.S. and Park, D. (2006). Asiaticoside induces human collagen I synthesis through TGFbeta receptor I kinase (TbetaRI kinase)-independent Smad signaling. Planta Med. 72:324–328.
- **Lee,** T.; Chen, C. L. and Shieh, Z. H. (2009). Study on antioxidant activity of *Echinacea purpurea* L. extracts and its impact on cell viability. Afr. J. Biotechnol; 8:5097-5105.

- **Levinson**, H.; Moyer, K.E.; Saggers, G. C. and Ehrlich, H.P. (2004). Calmodulin-myosin light chain kinase inhibition changes fibroblast-populated collagen lattice contraction, cell migration, focal adhesion formation, and wound contraction. Wound Repair Regen, 12:505.
- **Lobo**, R.; Sodde, V.; Dashora, N.; Gupta, N. and Prabhu, K. (2011). Quantification of flavonoid and phenol content from *Macrosolen parasiticus* (L.) Danser. *J. Nat. Prod. Plant Resour*. 1(4): 96-99.
- **Mackin,** J. (1993). Cornell Book of Herbs and Edible Flowers. Cornell Cooperative Extension Ihaca, New York, U.S.A., Pp: 78- 99.
- **Marcica**, M. S.; Vesna, R.; Mirza, B. and Zeljan, M. (2009). From functional food to medicinal product: Systematic approach in analysis of polyphenolics from populist and wine. Nutrition Journal. 8:33.
- **Mark,** D. P. (2006). The Claim: Wounds Heal Better When Exposed to Air. Website of the New York Times.
- **Marks**, R. and Dykes, P. (1994). Plasma and cutaneous drug levels after topical aplication of piroxicam gel: a study in healthy volunters. Skin Pharmacol. 7:340.
- **Mathew**, A.; Taranalli, A.D. and Torgal, S.S. (2004). Evaluation of anti-inflammatory and wound healing activity of *Gentiana lutea* rhizome extracts in animals. *Pharm Biol*. 42(1):8-12.
- **Midwood**, K.S.; Williams, L.V. and Schwarzbauer, J.E. (2004). Tissue repair and the dynamics of the extracellular matrix, Int. J. Biochem. Cell Biol. 36(6): 1031-1037.
- **Murray**, M. (1998). Quercetin: Nature's antihistamine. Better Nutrition.
- **Narayana**, K.; Reddy, S.; Chaluvadi, M. and Krishna, D. R. (2001). Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Indian Journal of Pharmacology. 33: 2-16.

- **Nielsen**, I. C. (1992). *Flora Malesiana: Mimosaceae.* 1: (45). OECD 1987. Guidelines for the Testing of Chemicals/Section 4: Health Effects: *Acute Dermal Toxicity* Test No. 402.
- **Nonato**, F. R.; Barros, T. A. and Lucchese, A. M. (2009). Antiinflammatory and antinociceptive activities of Blechnum occidentale L. extract. *J Ethnopharmacol*. 125(1):102-107.
- **Okuda**, T.; Yoshida, T.; Chen, X. M.; Xie, J. X. and Fukushima, M. (1987). Corianin from Coriaria japonica A. Gray and sesquiterpene lactones from Loranthus parasiticus Merr. used for treatment of schizophrenia. *Chem. Pharm. Bull*., 35:182-187.
- **Okuyama**, H.; Ikeda, Y. and Kasai, S. (1999). Influence of nonionic surfactants, pH and propylene glycol on percutaneous absorption of piroxicam from cataplasm. International Journal of Pharmaceutics. 186:141-148.
- **Okwu,** D.E. and Okwu, M.E. (2004). Chemical composition of *Spondias mombin* linn plant parts. J. Sustain Agric. Environ. 6(2): 140-147.
- **Olatunde**, A. and Dikwa, M. A. (2014). Qualitative and Quantitative Analysis of Phytochemicals of *Loranthus bengwensis* Leaf. Int. R. J. Pharm Sci. 5(1): 10.
- **Omale**, J. and Emmanuel, T.F. (2010). Phytochemical composition, bioactivity and wound healing potential of *Euphorbia heterophylla* (Euphorbiaceae) leaf extract, Int. J. Pharm Biomed Res. 1(1): 54-63.
- **Orji**, F.; Nwachukwu, N.; Onyia, A. and Nkwocha, M. (2012). Phytochemical and antimicrobial properties of leaves of African mistletoes (*Lorathus micranthus*) on some selected microbial pathogens in Abia State, Nigeria. Glob. Adv. Res. Microbiol., 2(1):011-016.

❖ **Osadebe**, P.; Omeje, E.; Kawamura, A. and Okoye, F. (2010). Lupeol derivatives from the Eastern Nigeria mistletoe, Loranthus micranthus Linn. (Loranthaceae) with enhanced cell proliferative potentials. Planta Medica. 76:091.

❖ **Pal**, R.; Ariharasivakumar, G.; Girhepunjhe, K. and Upadhay, A. (2009). In-vitro antioxidative activity of phenolic and flavonoids compounds extracted from seeds of *Abrus precatorius*. International Journal of Pharmacy and Pharmaceutical Sciences. 1:136-140.

❖ **Parekh,** J.; Jadeja, S. and Chanda, S. (2005). Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity. Turkish J. Bio., 29:203-210.

❖ **Park**, J. H.; Hyun, C. K. and Shin, H. K. (1999). Cytotoxic effects of the components in heat-treated mistletoe (*Viscum album*). Cancer Lett. 139:207-213.

❖ **Pei,** X.W.; Wang, K.Z.; Dang, X.Q. Song, J.H.; Shi, Z.B. and Gao, D.F. (2006). Arnebia root oil promotes wound healing and expression of basic fibroblast growth factor on the wound surface in rabbits. [in Chinese] Zhong Xi Yi Jie He Xue Bao. 4(1):52-55.

❖ **Principe**, P. (2005). Monetising the Pharmacological Benefits of Plants. US Environmental protection Agency, Washington, D.C. Pp: 1991.

❖ **Puratchikody**, A., Nithya, C. and Nagalakshmi, G. (2006). Wound healing activity of *Cyperus rotundus* Linn. *Indian J Pharm Sci., 68*:97-101.

- **Radenahmad**, N.; Saleh, F.; Sayoh, I.; Sawangjaroen, K.; Subhadhirasakul, P.; Boonyoung, P.; Rundorn, W. and Mitranun, W. (2012). Young coconut juice can accelerate the healing process of cutaneous wounds. BMC Complementary and Alternative Medicine. 12:252.
- **Ro**, B. I. and Dawson, T. L. (2005). The role of sebaceous gland activity and scalp micro-oral metabolism in the etiology of seborrheic dermatitis and dandrug. J. Investigative Dermatol SP., 10(3): 194.
- **Sadaf**, F.; Saleem, R.; Ahmed, M. and Ahmed, S.I. (2006). Healing potential of cream containing extract of Sphaeranthus indicus on dermal wounds in guinea pigs. J Ethnopharmacol 107: 161-163.
- **Scortichini**, M. and Roosi, M.J. (1991). Preliminary in vitro evaluation of the antimicrobial activity of terpenes and terpenoids towards *Erwinia amylovora*. Appl Bacterioly. *71*: 109-112.
- **Sharma**, N. G.; Dubey, S. K.; Sati, N. and Sanadya, J. (2011). Anti-inflammatory Activity and Total Flavonoid Content of *Aegle marmelos* Seeds. International Journal of Pharmaceutical Sciences and Drug Research. 3(3): 214-218.
- **Shenoy**, C.; Patil, M. B.; Kumar, R. and Patil, S. (2009). Preliminary phytochemical investigation and wound healing activity of *Allium cepa* Linn (Liliaceae), International Journal of Pharmacy and Pharmaceutical Sciences. 2(2):167–175.
- **Shukla**, A.; Rasik, A. M. and Dhawan, B. N. (1999). Asiaticoside induced elevation of antioxidant levels in healing wound. Phytother. Res. 13:50–54.
- **Sinha**, A.; Taylor, W.; Khan, I.; McDaniel, S. and Essko, J. (1999). Glycoside primers of *Psittacanthus cucullaris*. J. Nat. Prod, 62:1036-1038.

- **Smolensk**, S. J.; Silinis, H. and Fransworth, N. R. (1972). Alkaloid screening. Annals of Biochem. 35:31-34.
- **Somboonwong,** J.**;** Kankaisre**,** M.**;** Tantisira**,** B. and Tantisira, M. (2012). Wound healing activities of different extracts of *Centella asiatica* in incision and burn wound models: an experimental animal study. BMC Complementary and Alternative Medicine. 12:103.
- **Stahl, E. (1969).** Thin-Layer-Chromatography, a Laboratory Hand book, 2nded. Translated by Ashworth, M. R. F. Spring. Verag, Berlin,Heidelberg, New York.
- **Stary,** F. (1998). The Natural Guide to Medicinal Herbs and Plants. Tiger Books International, London. p. 12-16.
- **Steiner,** R. (1989). Discussions with Practicing Physicians: In Physiologically-therapeutic based on spiritual science, Dornach and Steiner, R. (eds.), Verlag, 285-301.
- **Subramoniam**, A.; Evans, D.; Rajasekharan, S. and Nair, G.S. (2001). Effect of *Hemigraphis colorata* (Blume) leaf on wound healing and inflammation in mice. Hallier H. G. (*ed*.). Indian J Pharmacol., 33:283-285.
- **Suresh**, J., Rao, P. and Reddy, M. (2002). Wound healing effects of *Heliotropium indicum*, *Plumbago zeylanicum* and *Acalypha indica* in rats. J. Ethnopharmacol., 79:249–251.
- **Swetman**, S. (2009). Martindale the Complete Drug Reference: the Pharmaceutical Pres. 17-8.
- **Tapas,** A. R.; Sakarkar, D. M. and Kakde, R. B. (2008). Flavonoids as nutraceuticals: A Review. Tropical Journal of Pharmaceutical Research. 7:1089-1099.

- **Tsuchiya**, H. (2010). Structure-dependent membrane interaction of flavonoids associated with their bioactivity. Food Chemistry. 120:1089-1096.
- **Uzochukwu,** I. and Osadeb, P. (2007). Comparative evaluation of antidiabetic activities of flavonoids extract and crude methanol extract of Loranthus micranthus parasitic on Kola acuminate. Journal of Pharmaceutical and Allied Sciences, 4(1):2–7.
- **Vane**, J. (1994). Towards a better aspirin. Nature. 367: 215.
- **Vester**, F. and Nienhaus, J. (1965). Cancerostatic protein components from Viscum album. *Experientia*. 21:197-199.
- **Vogel**, H.G. and De Souza, N.J. (1980). Effect of terpenoids isolated from *Centella asiatica* on granuloma tissue, Acta Therapeutica, 16(4): 285-298.
- **Wagner**, H. and Bladt, S. (1996). Drugs Containing Lignans in Plant Drug Analysis, Second Edition, Springer, Pp: 265.
- **Waly**, N., Ali, A. and Jrais, R. (2012). Botanical and biological studies of six parasitic species of family Loranthaceae growing in Kingdom of Saudi Arabia. International Journal of Environmental Sciences, 4(1):196-205.
- **Wang**, C.; Chang, S.; Stephen, B. and Chen, B. (2009). Isolation of carotenoids, flavonoids and polysaccharides from *Lycium barbarum* and evaluation of antioxidant activity. Food chemistry.120:184-192.
- **Wang**, W.; Heideman, L.; Chung, C. S.; Pelling, J. C.; Koehler, K. J. and Birt, D. F. (2000). Cell-cycle arrest at G2/M and growth inhibition by apigenin in human colon carcinoma cell lines, Mol Carcinog. 28(2):102-110.
- **Watson**, L. and Dallwitz, M. J. (1991). The families of angiosperms: automated descriptions, with interactive identification and information retrieval. *Aust. Syst. Bot.*; 4: 681–95.

References

- **Wiart**, C. (2006). Ethnopharmacology of Medicinal Plants. In Asia and the Pacific, Humana Press Inc., New Jersey.
- **Won**, J. H.; Shin, J. S. and Park H. J. (2010). Anti-inflammatory effects of madecassic acid via the suppression of NF-κB pathway in LPS-induced RAW 264.7 macrophage cells, Planta Medica. 76(3): 251–257.
- **World Health Organization** (1989). *Formaldehyde*: Environmental Health Criteria, Geneva: 89.
- **XD**, X.; XR, H. and JS, Y. (2008). Review of studies on active components of antitumor medicinal plants. Zhongguo Zhong Yao Za Zhi. 33: 2073-2081.
- **Zheng**, X. and Bollinger Bollag, W. (2003). Aquaporin 3 Colocates with Phospholipase D_2 in Caveolin-Rich Membrane Microdomains and Is Down regulated Upon Keratinocyte Differentiation. the Journal of Investigative Dermatology. 121, 1487–1495.

List of content

List of Figures

List of tables

الملخص

يُعد حب نبات الدبج (بلوط الهدال) من النباتات الطبية المهمة و يحتوي على مجموعة من المركبات ذات الفعالية الحيوية. اجري استخلاص الثمار الجافة باستخدام الميثانول بتركيز 80% بالتنقيع وباستخدام الحاضنة الهزاز في25 م° ; أعقبه الكشف الكيمائي لمكونات المستخلص الخام. وتم استخلاص الفلافونويدات من ثمار نبات الهدال باستخدام ال Reflex وتقدير المحتوى الكلي لها ، ثم الكشف عن الفلافينويدات للمستخلص المنقى باستخدام تقنية كروماتوغرافيا الطبقة الرقيقة و باستخدام مذيبات مختلفة كوسيط ناقل.ثم استخدام تقنية ال HPLC.

تم عمل الالتهاب في حيوانات التجارب (الأرانب) عن طريق الحقن تحت الجلد ب2.5٪ من الفورمالين. و تقييم التأثير المضاد للالتهابات للمستخلص في شفاء الجروح الجلدية بالمقارنة مع الأدوية الصيدلانية الاصطناعية Piroxicalm جل. و تحضير المستحضر بطريقة خلط المادة الخام مع الفازلين والجلسرين. و بعد شفاء الجرح كانت الدراسة النسيجية المرضية اللازمة.

وأشارت النتائج إلى أن مستخلص *L. europaeus* غني بالفلافونيدات المكونة من الروتين والليتيولين ونسبة من كورايسيتين وكايمبفيرول.

اوضحت الدراسة النسيجية بان المركبات الفعالة ساعدت في الشفاء من خلال اختفاء odema وانخفاض في حجم ندبة الجرح ، وتعزيز انتشار الخلايا الليفية، والأوعية الدموية، والتقرن والاندمال بتشكل النسيج الظهاري بالمقارنة مع مجموعات المراقبة.

L. europaeus يمكن ان يعد احد النباتات الواعدة لعلاج الالتهابات الجلدية.

قال الله تعالى :

وَيَسْـَٔلُونَكَ عَنِ ٱلرُّوحِ ۖ قُلِ
ٱلرُّوحُ مِنْ أَمْرِ رَبِّى وَمَآ أُوتِيتُم
مِّنَ ٱلْعِلْمِ إِلَّا قَلِيلًا ﴿٨٥﴾

سورة الإسراء

السيرة الذاتية

- الاسم: إصلاح شاكر رجب.
- خريجة جامعة النهرين كلية العلوم قسم التقانة الاحيائية عام 2010-2011 بترتيب الثانية على دفعتها والرابعة على كلية العلوم.
- تقدمت على الماجستير في 2012-2013.
- خلال المراحل الدراسية شاركت بعدة ندوات ومؤتمرات منها:
  - ❖ المشاركة في ورشة العمل (مسارات في النشر البحثي) عام 2013.
  - ❖ المشاركة في ورشة العمل الموسومة (تقديم البحث للنشر العملي)التي اقيمت في كلية العلوم(2013).
  - ❖ شاركت في دورة اقيمت في مركز البحوث للتقنيات الاحيائية الخاص بالتدريب العملي للتقطيع وتصبيغ الانسجة والفحص المجري لها بعنوان (tissue processing).
  - ❖ شاركت في مؤتمر النهرين للدراسات العليا التي اقامتها كلية العلوم في جامعة النهرين 2014\12\17.
  - ❖ حصلت على قبول نشر في المؤتمر الخامس عشر لل Ethnopharmacology المقام في جامعة البلقاء في الاردنية في 2015\5\5 بعنوان البحث:

"Anti-inflammatory Effect of *Loranthus europaeus* Extract on Wound Skin in Rabbit"

- ❖ حصلت على قبول نشر في مجلة مركز البحوث للتقنيات الاحيائية في 2015\4\19 بعنوان البحث الموسوم:

Purification and Identification of Flavonoids Extracted from *Loranthus Eurpaeus* Fruits.

- ❖ هناك بحثين قيد النشر.

Republic of Iraq
Ministry of higher education
And scientific research
Al- Nahrain University
College of science
Department of biotechnology

Study the Effect of *Loranthus europaeus* Extract on Wound Healing Process

A thesis

Submitted to College of Science/ Al-Nahrain University as a partial fulfillment of the requirement for the Degree of Master of Science in Biotechnology.

By

Eslah Shaker Rajab
B.Sc. Biotechnology/ College of Science /Al-Nahrain University (2011)

Supervised by
Dr. Khulood W. Al-sammarrae
(Professor)

April 2015 Jumada al-thani 1436

Supervisor Certification

We, certify that this thesis entitled "**Study the Effect of *Loranthus europaeus* Extract on Wound Healing Process**" was prepared by "**Eslah Shaker Rajab**" under our supervision at the College of Science\ Al-Nahrain University as a partial requirement for the degree of Master of Science in Biotechnology.

Signature:

Name: Dr. Khulood W. Al-sammarrae.

Scientific Degree: Professor

Date: \ \ 2015

In view of the available recommendations, I forward this thesis for debate by the examining committee

Signature:

Name: Dr. Hameed M. Jasim

Scientific Degree: Professor

Title: Head of Biotechnology Department

Date: \ \ 2015

Committee Certification

We, the examining committee, certify that we have read this thesis and examined the student in its contents and that, according to our opinion, is accepted as a thesis for the degree of Master of Science in Biotechnology.

Signature:

Name: salim al-obadi

Scientific Degree: prof.

Date:

(Chairman)

Signature:

Name: Rehab S. Ramadhan

Scientific Degree: Prof.

Date:

(Member)

Signature:

Name: abedaaljasim M. Aljibouri

Scientific Degree: Prof.

Date:

(Member)

Signature:

Name: Khulood W. Al-sammarrae

Scientific Degree: Prof.

Date:

(Member)

I hereby certify upon the decision of the examining committee

Signature:

Name: Dr. Hadi M. A. Abood

Scientific Degree: Assistant Professor

Title: Dean of College of Science

Date:

Acknowledgments

At the beginning thanks to great "Allah" who gave me the reality and strength to accomplish this work.

I would like to extend my thanks, respect and appreciation to my supervisor prof. ***Dr. Khulood W.* Al-sammarrae**, for her great efforts to explain things clearly and simply, and throughout my thesis writing period. She provided encouragement, sound advice, good teaching, good accompany, and lots of good ideas.

Also, I would like to express my great thanks to ***Dr. Zainab Yaseen*** and *Mrs. Raghad Kadhim* for their valuable guidance, advice, support, beside the continuous encouragement through my study.

My deep appreciation to the Head of Biotechnology Department College of Science of Al-Nahrain University: prof. *Dr. Hameed M. Jasim* for his support. Very much thanks to all staff of Biotechnology Department, *Dr. Rehab, Mrs. Hind, Mrs. Raya, Mrs. Madeha, Mrs*. zyna and all my College.

My special thanks go to faculties in *Biotechnology Research Center/Al-Nahrain University*, who helped to raise my morale during the difficult times, and kept me in a positive frame of mind, among them:*, Dr. Jasim Mohamed, Dr. Hazim I. Al-Ahmed, Mr. Ahmad Abdl-Almonem, and Bashar sabry*.

Special thanks go to *Dr. saleem alobadi* for their helped.

My special thanks go to my friends: *Yasamien Thaer, Weham Saad, Albab Fawaz, Mena Majid, Nuha Basheer, Sara Kahtan, Noor Ali, Marwa Adle and all my colleagues who stood with me.*

To all these and other forgotten names who assist me in accomplishing this study, my great thankful and gratitude.

Eslah shaker

الإهداء

إلى رمز العزة.. و الشموخ. و منقذ البشرية.. من الجهل وباني مدينه العلم

الرسول الأعظم محمد (صلى الله عليه وسلم)

إلى قبس النور الذي أضاء طريقي. و هيأ لي أسباب النجاح...إلى سندي في الحياة ....و فخري.. و اعتزازي. **والدي حفظه**

إلى البساطة و الطيب ...إلى بحر الحنان و الحب...إلى جنتي في الأرض...نور عيني

أُمي حفظها الله

إلى أحبائي.. و قرة عيني..و سندي في مسيرتي... **أخوتي وأخواتي**

الى زوجي الهبة التي وهبها الله لي... **عمر**

إلى رفقة در ب لن أنسى لهم ما قدموه لي ما حييت....

إصلاح

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم

دراسة تأثير مستخلص نبات حب الدبج في عملية إلتئام الجروح

رسالة

مقدمة الى كلية العلوم - جامعة النهرين وهي جزء من متطلبات نيل

درجة الماجستير في علوم التقانة الاحيائية

من قبل

إصلاح شاكر رجب

بكالوريوس تقانة احيائية (2011)

إشراف

أ.د. خلود وهيب السامرائي

جمادى الثاني 1436 نيسان2015

CHAPTER ONE

Introduction and Literature Review

CHAPTER TWO

Materials and Methods

Chapter Three

Results and Discussion

Chapter Four

Conclusions and Recommendations

References