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Phytochemical Screening and Quantitative Determination of Phytochemicals in Leaf Extracts of *Hannoa undulata*

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Abstract: *Preliminary phytochemical screening on the dried leaf extracts of Hannoaundulata using cold maceration by serial exhaustive extraction method with solvents of increasing polarity (Hexane, chloroform, ethylacetate, acetone and ethanol) was investigated. The phytochemical screening of the crude leaf extracts revealed the presence of alkaloids, flavonoids, phenols, saponins and glycosides in all extracts. Quantitative analysis on some detected phytochemicals reveals high content of saponins (1.033%/g), followed by flavonoids (0.77%/g) and alkaloids (0.72%/g).*

Keywords: *Phytochemical screening, Hannoaundulata, Polarity, cold maceration, leaf extracts*

1. Introduction

Medicinal plants form an important component of flora and are widely distributed in the world. The pharmacological evaluation of substances from plants is an established method for the identification of lead compounds which can result to the development of novel and safe medicinal agents. Medicinal plants are composed of some certain organic compounds called phytochemicals which produce definite physiological actions in the human body and these bioactive substances include but are not limited to tannins, alkaloids, terpenoids, steroids and flavonoids (Edoega *et al*., 2005). The development of pharmaceutical products necessitates an exhaustive investigation of medicinal plants to improve our knowledge about their biological activities and the phytoconstituents responsible for them (Jothy *et al.*, 2012). Furthermore, the need for comprehensive investigations in this area is more evident owing to the fact that only a limited number of medicinal plant species have received complete scientific inspection (Mazumder *et al.*, 2008).

Hannoaundulata belongs to the family simaroubaceae. *Hannoaundulata* is locally known as "Gbur" in the Tiv dialect of Benue State (Shomkegh *et al.*, 2016). It is used as a cure for various ailments in this area. Air dried roots bark are used for treating stomach ache and the bark and leaves for jaundice and malaria. The leaves are very coriaceous (leathery), obovate and are narrowed or cuneate at the base. It is a fast growing plant and usually grows up to 6-8 meters tall. It has a woody bark that is longitudinally fissured. Prot and Kornprobst reported in 1985 that quassinoid fractions extracted from the seeds of *H. undulata*prevented the penetration of juveniles of *meloidogynejavanica*into tomato roots. According to the report, full inhibition of penetration occurred during three days of continuous exposure to a 5 ppm quassinoid solution in the soil water. Quassinoids are highly oxygenated triterpenes which have attracted much attention because of their wide spectrum of biological activities.

2. Materials and Methods

2.1. Sample Collection and Preparation

The leaves of *Hannoa undulata* were collected from its natural habitat in Wukari local Government area of Taraba state, Nigeria. The samples were air dried and pulverized into a coarse powder. This was to increase the surface area to facilitate extraction.

2.2. Extraction

Cold maceration was used in the extraction by serial exhaustive extraction method which involves successive extraction with solvents of increasing polarity (n-hexane, chloroform, ethylacetate, acetone

and ethanol).The extracts of the leaves were prepared by soaking 200g of the coarsely powdered leaves in n-hexane in a stoppered container for a defined period (4 days) with frequent agitation. The resulting mixture was filtered and concentrated using rotary evaporator. The procedure was repeated on the residues using chloroform, ethyl acetate, acetone and ethanol sequentially in order of polarity. The extracts were kept in the refrigerator until required for analysis.

2.3. Phytochemical Screening

Preliminary phytochemical screening was carried out on all the crude leaf extracts (hexane, chloroform, ethyl acetate, acetone and ethanol) of *Hannoaundulata* using standard procedures as described by Trease and Evans (1989), Sofowora (1993), Ushie *et al.*,2016) and Santhi *et al.*,2016).

2.4. Detection of Alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

2.5. Mayer's Test

Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

2.6. Wagner's Test

Filtrates were treated with Wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

2.7. Detection of Flavonoids

2.7.1. Lead Acetate Test

Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

2.7.2. Alkaline Test

Extracts were treated with few drops of dilute sodium hydroxide solution. Formation of a yellow color, which turned colorless on addition of dilute acid, indicated the presence of flavonoids.

2.7.3. Detection of Steroids

2 mL of acetic anhydride was added to 0.5 mL of each extract in a test tube, followed by the addition of 2 mL of sulfuric acid. A color change from violet to blue or green indicated the presence of steroids.

2.8. Detection of Phlobatannins

2 mL of each extract of the plant sample was boiled with 1% aqueous hydrochloric acid. Disposition of red precipitate indicated the presence of phlobatannins.

2.9. Detection of Anthraquinones

2.9.1. Borntrager s Test

0.5 mL of each extract was boiled with 2 mL of 10% HCl for few minutes in a water bath. The resultant solution was filtered and allowed to cool. Equal volume of chloroform was added to the filtrate. Few drops of 10% NH_3solution was added to the mixture and heated. Formation of rose pink color indicated the presence of anthraquinones in the extracts.

2.10. Detection of Terpenoids

2.10.1. Salkowski s Test

0.5 mL of each extract was mixed with 2 mL of chloroform, and 3mL of concentrated H_2SO_4 was carefully added to form a layer. An appearance of a reddish brown color interface indicated the presence of terpenoids.

2.11. Detection of Phenols

2.11.1. Ferric Chloride Test

10 mL of each extract was treated with few drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenol.

2.12. Detection of Glycosides

Glycoside Test

0.5 mL of each extract was dissolved in 1 mL of water and then aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides.

2.13. Detection of Tannins

1 mL of each of the extracts was mixed with water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. Formation of a dark green color indicated the presence of tannins.

2.14. Detection of Saponins

Foam Test

1 mL of each of the extracts was shaken with 5 mL of distilled water. Formation of stable persistent foam indicated the presence of saponins.

Quantitative Phytochemical Analysis

The phytochemicals detected in the extracts of *Hannoaundulata* were quantified using standard procedures as described by Harborne (1973), Obdoni and Ochuko (2001), Krishnaiah *et al.*, 2009) and Sathya *et al*., 2013).

Estimation of Alkaloids

Determination of alkaloids was done by using Harborne (1973) method. To 5 g of the sample, 200 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. It was filtered and the filtrate was concentrated on a water bath to one quarter of the original volume. Concentrated NH_4OH was added drop wise to the filtrate until precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH and then filtered. The residue is the alkaloid, which was dried and weighed.

Estimation of Flavonoids

10 g of plant sample was repeatedly extracted with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through a Whatman No.42 filter paper into a pre weighed 250 mL beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed (Krishnaiah*et al.*, 2009*).*

Estimation of Total Saponins

The method used was that of Obdoni and Ochuko (2001). To 20 g of the ground sample, 100 mL of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90°C. The concentrate was transferred into a 250 mL separation funnel and 20 mL of diethyl ether was added and shaked vigorously. The aqueous layer was recovered while the ether layer was discarded.

The purification process was repeated. 60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath for evaporation and were further dried in the oven to a constant weight; the saponin content was calculated.

3. RESULTS

3.1. Preliminary Phytochemical Screening of Leaves Extracts of *Hannoaundulata*

The extracts (hexane, chloroform, ethyl acetate, acetone and ethanol) of the leaves of *Hannoaundulata* were screened for the presence of some phytochemicals. Table 1 presents the results of phytochemical screening of crude leaf extracts of *Hannoaundulata*.

Table1. *Preliminary Phytochemical Screening of the Various crude Leaf Extracts of Hannoaundulata.*

S/N	Name of test	Test applied/Reagent used	HE	CE	EAE	AE	EE
1	**Alkaloids**	Mayer's reagent Wagner's reagent	- +	- +	+ +	+ +	+ +
2	**Flavonoids**	Lead acetate test Alkaline reagent test	+ +	+ +	+ +	+ +	+ +
3	**Terpenoids**	Salkowski test	-	-	-	-	+
4	**Phenols**	Ferric chloride test	+	+	+	+	+
5	**Steroids**	Extract + acetic anhydride + H_2SO_4	+	+	+	+	-
6	**Saponins**	Foam test	+	+	+	+	+
7	**Tannins**	Ferric chloride test	-	-	+	+	+
8	**Anthraquinones**	Borntrager's test	-	+	-	-	-
9	**Glycosides**	Extract + H_2O +NaOH	+	+	+	+	+
10	**Phlobatannins**	Extract + 1%HCl + heat	-	-	-	-	+

Key: HE=hexane extract, CE=chloroform extract, EAE=ethylacetate extract, AE=acetone extract, EE= ethanol extract, (+) = Present, (-) =Absent

3.2. Quantitative Analysis of Some Detected Phytochemicals

The result of quantitative Phytochemical analysis is presented in Table 2 below.

Table2. *Quantitative Phytochemical Analysis of Hannoaundulata*

S/N	Test Phytochemical	Weight of sample (g)	Weight of dried filtrate (g)	% Crude calculated
1	Flavonoids	10.00	0.77	7.70
2	Alkaloids	5.00	0.18	3.60
3	Saponins	20.00	4.13	20.65

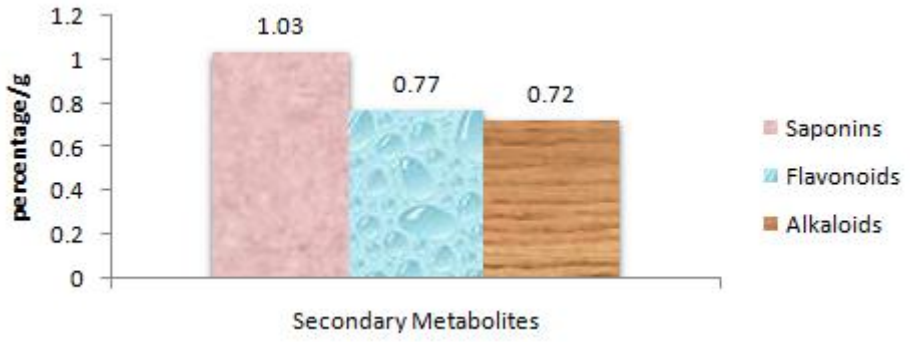

Figure1. *Histogram for Quantification of Secondary Metabolites of Hannoaundulata*

4. DISCUSSION

*Hannoaundulata*is used as traditional medicine in different parts of Nigeria. The present study was conducted on preliminary phytochemical and quantitative analysis of the leaf extracts of the plant.

4.1. Preliminary Phytochemical Analysis

The phytochemical analysis of the hexane, chloroform, ethyl acetate, acetone and ethanol extract of the leaves of *Hannoaundulata* revealed the presence of anthraquinones, steroids, terpenoids and terpenes, phlobatannins, phenols, saponins, tannins, flavonoids, glycosides and alkaloids.

The results obtained revealed the absence of anthraquinones in hexane, ethyl acetate, ethanol and acetone extracts. However, this substance was found to be present in chloroform extract. Tannins were present in all extracts except hexane and chloroform extracts. Phlobatannins were absent in all the extracts except ethanol extract. Steroids were present in all extracts except ethanol extract. Terpenoid was absent in all extracts exceptin ethanol extract. Phenols, alkaloids, saponins, flavonoids and glycosides were found to be present in all extracts as shown in Table 1. Quantitative analysis carried out on three detected phytochemicals; alkaloids, flavonoids and saponinsreveals that these secondary metabolites are present in different amount in the leaves of the plant (Table 2). Amongst the quantified phytochemicals, Saponin content of the leaves of *H. undulata* was found to be 1.033 %

/g followed by flavonoid 0.77 % / g and then alkaloid which was found to be 0.72 % / g. Therapeutically, Saponins are very important as they are shown to have hypolipidemic and anti-cancer activity. The natural anti-cancer agent saponin reacts with cholesterol rich plasma membrane of various cancer cells and arrests their proliferation (Rao*et al.,* 1995). This high level of saponin present in the leaves of *Hannoaundulata* indicates that the plant can be used to produce anti-cancer drugs. Alkaloids and flavonoids contribute various medicinal properties such as analgesic, anti-oxidant and astringent activity (Chung *et al.*, 1998).

These classes of phytochemical compounds are known to show therapeutic activity against several bacteria and it is not surprising that these plant extracts are used traditionally by herbalist to cure bacteria related ill-health (Njoku and Obi, 2009)**.**

*Hannoaundulata*can be used as anti-inflammatory, antispasmodic, anti-cancer and analgesic agent. This is attributed to the presence of flavonoids, alkaloids, steroids, glycosides and saponins in their compositions (Savithramma*et al.*, 2011). Saponins are precursors of important therapeutic drugs such as cortisone and contraceptive estrogens (Karеru*et al.,* 2008). There is tremendous, commercially driven promotion of saponins as dietary supplements and nutriceuticals (Ushie*et al.,* 2016). Saponins are a mild detergent used in intracellular histochemistry staining to allow antibody access to intracellular proteins. The saponins are used in hypercholestrolaemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory activity and weight loss (Manickam*et al.*, 2014). Saponin are also known to cause complexation with cholesterol to form pores in cell membrane bilayers, e.g., in red cell (erythrocyte) membranes, where complexation leads to red cell lysis (hemolysis) on intravenous injection (Francis *et al.;* 2002).*Hannoaundulata* can be used as an analgesic, anaesthetic and as social drugs in view of the fact that it contains alkaloids. The alkaloids contained in plants are used in medicine as anaesthetic agents (Herourat*et al.,* 1988). Harborne (1988) also reported on the analgesic properties of alkaloids. Alkaloids has contributed to the majority of the poisons, neurotoxins and traditional psychedelics and social drugs [e.g. nicotine, caffeine, methamphetamine (ephedrine) co-caine, and opiates] consumed by humans (Zenk and Juenger, 2007). Flavoniods were detected in all the extract. According to Okoli and Okere (2010), flavonoids are potent water soluble super antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and inhibit tumor growth. The beneficial effects of fruit, vegetables, and tea or even red wine have been attributed to flavonoid compounds rather than to known nutrients and vitamins (Félicien, 2008).*Hannoaundulata*is important in pharmacy because it contains steroidal compounds. Okwu (2001) reported that steroidal compounds are of importance and interest in pharmacy due to their relationship with sex hormones. They are known to effect the development and control of the reproductive tract in humans and molt insects. Other function includes inducing sexual reproduction in aquatic fungi (Ushie *et al., 2016)*. *Hannoaundulata* can be used as an anti-inflammatory, antiseptic, antioxidant agent due to the presence of tannins. De Bruyne *et al.,* (1999) reported that in medicine, especially in Asian (Japanese and Chinese) natural healing, tannin-containing plant extracts are used as astringents, against diarrhoea, as diuretics, against stomach and duodenal tumours and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals (Dolara*et al.,* 2005). Saxena*et al.,* (2013) pointed out that recently, tannins have attracted scientific interest, especially due to the increased incidence of deadly illnesses such as acquired immune deficiency syndrome (AIDS) and various cancers (Blytt*et al*., 1988). Pharmacologically**,** glycosides have been found to be useful in treatment of several illnesses for instance cardiac glycoside have long been employed as important ingredient for arrow poisons and drugs (Trease and Evans 1989). The presence of terpenoids that have carboxylic acid groups could also be responsible for the activity of the organic extracts of *Hannoaundulata* (Njoku and Obi 2009). Langenheim, 1994 and Dudareva, 2004 also reported that terpenoids have medicinal properties such as anti-carcinogenic (e.g. perilla alcohol), anti-malarial (e.g. artemisinin), anti-ulcer, hepaticidal, antimicrobial or diuretic (e.g. glycyrrhizin) activity. Anthraquinones are extensively applied in medicine. In the pharmaceutical industry, the natural and synthetic derivatives of 9, 10 anthraquinone are beneficial to mammals and humans as they can display antibacterial, antitrypanosomal and antineoplastic activities (Heyman *et al.,* 2009; Tarus*et al.,* 2002; Velez-Cruz and Osheroff, 2004). Phlobatannins have been reported for its wound healing properties. They also have anti-inflammatory and analgesic (Ayinde*et al.,* 2007) and anti-oxidant properties (Okwu *et al*., 2004)

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