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Qualitative and quantitative phytochemical evaluations of *Strophanthus hispidus* stem bark

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Abstract: *The qualitative and quantitative phytochemical evaluations of aqueous and ethanol stem bark extracts of Strophanthus hispidus as well as the powdered stem bark were examined. Standard experimental procedures were used in this analysis. Aqueous, ethanol extracts and powdered stem bark tested positive for the presence of carbohydrates, flavonoids, saponins and phlobatannins. Alkaloids and tannins tested positive for both ethanolic extract and powdered sample. Glycoside tested positive for the powdered sample and aqueous extract. None of the plant preparations tested positive for the presence of anthraquinone. The quantitative analysis revealed that tannins were the major phytochemical constituent present in highest percentage (5.15%) followed by saponins (2.03%). Flavonoids and alkaloids were 0.73% and 0.38% respectively. Phenols were found to be present in the lowest percentage (0.14%). The presence of these phytochemical constituents in Strophanthus hispidus stem bark may justify its use in the treatment of many ailments by the masses.*

Key words: *phytochemical constituents, masses, Strophanthus hispidus, stem bark, Alkaloids.*

I. Introduction

Nowadays, much attention is focused on the vast botanical resources of Africa and their use as alternatives and complementary sources of medicine. This is likely to be as a result of the putative salutary effect of most Africa plants that is attributed to the presence of secondary metabolites. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Kiran *et al*., 2013). The effective substances of many plants species are isolated for direct use as drugs, lead compounds or pharmacological agents (Fabricant and Farnsworth, 2001). *Strophanthus hispidus* belongs to the species of strophanthus. A genus of 35-40 species of flowering plants in the family of Apocynaceae. It is native mainly to West Africa; the arrow- poison kombe is made by the natives from its extracts. The active principle is strophanthin, a crystallisable glycoside common to different parts of the plant but especially in the seeds which yield a large proportion. The seeds are not used in traditional medicine because of their great toxicity (Schelzer and Gurib-fakin, 2008). Decoctions of the roots or sometimes of the pulped root bark, stem bark or leaves are used externally to treat skin diseases, leprosy and ulcers and internally to treat malaria parasites, dysentery and gonorrhoea (Schelzer and Gurib-fakin, 2008). A decoction of the bark or leaf sap is taken against effects of snakebites (Burkill, 1984). In Guinea the sap from crushed leaves or young shoots is applied to kill head-lice and other parasites. In Nigeria and Ghana, a leaf and stem decoction is taken as a laxative or to treat fever and is externally applied to sores (Schelzer and Gurib-fakin, 2008). Nowadays, the glycosides extracted from the seeds are used in a number of medicines in several European Countries, the United States, Argentina and Chili as a rapid cardiac and vascular stimulant (Schelzer and Gurib-fakin, 2008). A large number of cardiac glycosides (cardenolides) isolated from *Strophanthus hispidus* collectively called strophanthins, are responsible for activity in arrow poison as well as cardiac and vascular stimulant. Ojiako and Igwe, (2009) reported the use of the plant by ethnic tribal people of Africa for the treatment of many ailment including diabetes. This study is therefore aimed at determining the qualitative and quantitative phytochemical constituents present in *Strophanthus hispidus* stem bark for the purpose of identification as well as characterization of the biomarkers responsible for its salutary effect acclaimed by ethnic tribal people of Africa.

II. Materials And Methods

The stems of *Strophanthus hispidus* were collected from Galadimawa, in Giwa Local Government, Kaduna State, Nigeria. They were identified by Mr. U. S. Gallah of the Department of Biological Science, Ahmadu Bello University, Zaria, Nigeria where a specimen with voucher number (no: 2714) was deposited.

Preparation and Extraction of Plant Material

The stems of *Strophanthus hispidus* was thoroughly washed with clean water and the barks were peeled off by incision. They were then dried under shade for two weeks and then pulverized into fine powder with the aid of a mechanical pulverizer. Measured quantities of the powdered sample were extracted separately in

aqueous and 99% ethanol for 72hrs followed by periodic stirring and they were kept in a refrigerator to avoid any microbial growth. The extracts were filtered using cheese-cloth and the filtrate re-filtered using Whatman No. 42 (125mm) filter paper. The filtrates collected were lyophilized using a freeze-dryer and stored in an airtight container for further analysis.

Qualitative Phytochemical Analysis

The crude powdered, freeze-dried aqueous and ethanolic extracts samples were used for these screening using standard procedures described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Detection of Alkaloids

A little amount (0.2g) of each sample were dissolved in dilute H_2SO_4 and thoroughly filtered. To 1ml of each of the filtrates were treated with few drops of Meyer's, Wagner's and Hager's reagents respectively. Appearance of a white or creamy precipitates, reddish-brown precipitates and yellow precipitates for Meyer's, Wagner's and Hager's reagents respectively confirmed positive result.

Detection of Saponins (Foam test)

To a little amount of each of the sample in a test tube, 2ml of distilled water were added and vigorously shaken for 15minutes. Formation of 1cm foam confirms a positive result.

Detection of Tannins (Ferric Chloride test)

To 2ml of the water extract of the crude powdered sample, and 2ml aqueous solution of each of the other extracts were added few drops of 5% ferric chloride solution. Appearance of bluish-black colour confirmed the presence of tannins (Trease and Evans, 1989).

Detection of Flavonoids (Alkaline reagent test)

2ml of aqueous extract of the crude powdered plant sample and 2ml of aqueous solutions of each of the other extracts were treated with few drops of NaOH solution. Formation of intense yellow colour which disappeared upon addition of concentrated HCl indicated the presence of flavonoids (Trease and Evans, 1989).

Detection of Glycosides (Legal's test)

2ml of aqueous extract of the crude powdered plant sample and 2ml of aqueous solutions of each of the other extracts were treated with 3ml chloroform and 10% ammonia solution. Appearance of a pink colour indicated positive result.

Detection of Phlobatannins

To 5ml of aqueous solution of the extracts and water extract of crude powdered sample were added few drops of 1% aqueous hydrochloric acid and boiled in water bath for 5minutes. Appearance of a red coloured precipitate indicated the presence of phlobatannins.

Detection of Anthraquinone

To 500mg of crude powdered sample and each of the extracts, 5ml of chloroform was added. The mixed solution was shaken and filtered after 5minutes. Equal volume of the filtrate and 10% ammonia solution were added and shaken. Appearance of a bright pink colour indicated the presence of anthraquinone.

Detection of Carbohydrates (Molisch's test)

10g of the powdered sample and 1g of each of the extracts were dissolved in 50ml and 5ml of water respectively. The solution of the powdered sample was carefully boiled in a water bath for few minutes, filtered and allowed to cool. The solutions of each of the extracts were similarly filtered. 2ml of each of the filtrates were added two drops of Molisch's reagent (α-naphthol in ethanol) and mixed thoroughly. 2ml of concentrated H_2SO_4 was carefully added to each of the solutions. Appearance of a purple ring colour at the interface indicates positive result.

Fehling's test

2ml of each of the filtrates were added 2ml of Fehling's reagents (copper sulphate/ sodium potassium tartate) and boiled in a water bath for 10minutes. Appearance of a reddish brown precipitate indicates positive result for reducing sugars.

III. Quantitative Phytochemical Screening

The amount of each phytochemical: alkaloids, flavonoids, phenols, saponins and tannins present in the crude powdered sample were evaluated using standard laboratory procedures based on the methods of Harborne (1973), Boham and Kocipai-Abyazan (1974), Obadoni and Ochuko (2001), and Van-Burden and Robinson (1981).

Estimation of alkaloids using Harborne (1973) method:

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. The solution was then filtered and the extract was concentrated on a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue if present is the alkaloid which is dried and weighed.

Estimation of flavonoids using the method of Boham and Kocipai-Abyazan (1994):

10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Estimation of total phenols by spectrophotometric method:

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 minutes. 5 ml of the extract was pipetted into a 50 ml flask, followed by the addition of 10 ml of distilled water. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The samples were made up to mark and colour developed was measured after 30 minutes at 505nm under room temperature.

Estimation of saponin by the method of Obadoni and Ochuko (2001):

The samples were ground and 20 g of each put into a conical flask followed by the addition of 100 ml of 20% aqueous ethanol. They were then heated over a hot water bath for 4 hours with continuous stirring at about 550C. The mixture was filtered and the residue re – extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 900C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether added and shaken 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. After evaporation the samples were dried in the oven to a constant weight. Saponin content was calculated as percentage.

Estimation of tannin by the method of Van-Burden and Robinson (1981):

500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipette out into a test tube and mixed with 2 ml of 0.1M FeCl3 in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes.

IV. Results

The present study has shown that *Strophanthus hispidus* stem bark contains medicinally active constituents. Flavonoids, saponins, phlobatannins and carbohydrates were present in all the plant preparation. Alkaloids, tannins were found to be present in both the ethanol extract and powdered sample while glycoside was positive in aqueous extract and powdered sample. Anthraquinone was however absent in all the plant preparation. These results are as summarized in table 1. Quantitative evaluation of the amount of individual bioactive constituents of the plant under investigation is as summarized in table 2. Tannins were observed to contain the highest percentage yield (5.15%) while phenols yielded the lowest percentage content (0.149%).

Table 1: Qualitative phytochemical evaluation of crude powdered sample, aqueous and ethanol extracts of *Strophanthus hispidus* stem bark.

Phytochemicals	Crude powdered	Aqueous Extract	Ethanol Extract
Alkaloids	+	-	+
Flavonoids	++	+	++
Saponins	+	++	++

Tannins	+	-	++
Glycosides	+	+	-
Phlobatannins	+	+	++
Anthraquinone	-	-	-
Carbohydrates	+	+	++
Reducing sugars	+	+	+

Table 2: Quantitative phytochemical evaluations of crude powdered sample of *Strophanthus hispidus* stem bark.

Phytochemicals	**%**
Flavonoids	0.73±0.12
Saponins	2.03±0.30
Alkaloids	0.38±0.12
Phenols	0.14±0.04
Tannins	5.15±0.30

Samples were analyzed in triplicate and result expressed as mean (n=3)± standard error of mean (SEM).

V. Discussion

The use of, and search for, drugs and dietary supplements derived from plants have accelerated in recent years. Researchers in the field of natural sciences are combing the Earth for *phytochemicals* and leads that could be developed for treatment of various diseases. All plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into (1) primary metabolites such as sugars and fats, which are found in all plants; and (2) secondary metabolites compounds which are found in a smaller range of plants, serving a more specific function (Meskin and Mark, 2002). It is these secondary metabolites and pigments that can have therapeutic actions in humans and which can be refined to produce drugs (Meskin and Mark, 2002).

The qualitative and quantitative phytochemical evaluation of the stem bark of *Strophanthus hispidus* showed that the plant is rich in tannins, saponins, flavonoids and alkaloids (tables 1 and 2). These bioactive constituents are known to possess medicinal activity as well as physiological activity (Sofowara, 1993). Ojiako and Igwe (2009) reported the presence of alkaloids, saponins, flavonoids, cardiac and cyanogenic glycoside in *Strophanthus hispidus* leaf, stem and root tissues in chloroform and ethanol extracts. However the percentage yield of these bioactive constituents has not been reported before (table 2). The biomarker observed in *Strophanthus hispidus* stem bark preparations belong to a large diverse groups with varied pharmacological potentials. Tannins are polyphenols that are obtained from various parts of different plants belonging to multiple species (Waterman and Mole, 1994). They are usually found in larger quantity in the barks of trees where they act as blockage to bacteria and fungi and protect the cell. Tannins are well known for their anti-oxidant and anti-microbial properties, as well as for soothing relief, skin regeneration, anti-inflammatory and diuretics (Okwu and Okwu, 2004). A lot of remedial and beneficial effects have been attributed to the consumption of tannins. The other remedial values of tannins include application on burns to heal the injury and on cuts to stop bleeding. Tannin's ability to form a strong 'leather' resistance on the exposed tissues helps in protecting the wounds from being affected further. However it has been reported that consumption of high amount of tannins could be deleterious as intake of larger quantity has been implicated in osteoporosis and anemia due to their effects on calcium and iron absorption (Brune, *et al.*, 1989). Hence tannins are known as anti-nutrients (Wheeler, 1979). The percentage yield of tannins has been observed to be highest (5.15%) in this study. This result falls within the range of tannin content obtained from plants used as Ayurvedic medicine. Edeoga *et al*., (2005) reported tannin content of 6.08%, 6.23% and 7.45% in the stems of some Nigerian medicinal plants namely: S. acuta, S. dulcis and T. procumbens respectively. Prohp and Onoagbe, (2012) also reported tannin content of 12.67% in *Triplochitin scleroxylon* stem bark extracts. Hence the value obtained from this study may be considered safe. Saponins are glycoside characterized by their ability to foam in aqueous solutions and are used as detergents (Vinken *et al*., 2007). They are basically phytochemicals which are found in most of the vegetables, beans and herbs. Like all detergents, saponins are known to be highly toxic as they are implicated in hemolysis (Francis *et al*., 2002). Recent studies have shown saponins' effects which have beneficial in the control of blood cholesterol levels, bone health, cancer and building up of the immune system (Matsuura, 2001). The percentage yield (2.03%) of saponins obtained in this study is in the range of saponin content obtained in the stem bark of *Spigellia athelmia* (2.26) reported by Edeoga *et al*., (2005). Flavonoid belongs to the family of polyphenols. They are water insoluble and are found in most plant materials. Flavonoids are well known for their anti-oxidants, anti-carcinogenic, anti-microbial and anti-tumor properties (Manikandan *et al*., 2006). Epidemiological studies have demonstrated that heart diseases are inversely related to flavonoid intake (Le, 2002). Studies have shown that flavonoids prevent the oxidation of low density lipoprotein thereby reducing the risk for the development of atherosclerosis. 0.73% percentage yield of flavonoids observed in this study may be correlated to the medicinal potentials of this plant. Similar content of flavonoid had been reported by Edeoga *et*

al., (2005) in the screening of some Nigerian medicinal plants for the presence of phytochemicals. Among all the phytochemical found in plants, alkaloids are the most powerful as well as very effective. Hence it is little surprising that the alkaloids have been researched and examined the most by the modern day scientists. The effects of alkaloids are felt in the autonomic nervous system, blood vessels, promotion of diuresis, respiratory system, gastrointestinal tract, uterus, malignant disease, infections and malaria (Trease and Evans, 1989). In addition, alkaloids also comprise of strong vegetable toxics and sedatives (Trease and Evans, 1989). Also it has been reported that medications that contains significant levels of alkaloids have a direct toxic impact. Although the alkaloid content (0.38%) obtained in this study is of lower quantity, moderate intake is advised since no specific dosage has been established for any particular ailment.

VI. Conclusion

The importance of continuing surveys of plants for biologically active substances cannot be over emphasized. However, few studies on phytochemical published usually give the idea of the amount of the various biomarkers present. Therefore this study has provided information of the medicinally important phytochemicals present in *Strophanthus hispidus* stem bark as well as the knowledge of their lower limits of detection.

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