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Quantitative Phytochemical Evaluation of Stem Bark Extracts of *Harungana madagascariensis*

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Authors' contributions

This work was carried out in collaboration between all authors FNE and OJA. Author FNE designed and wrote the protocol, conducted the study, performed the statistical analysis and wrote the first draft. Author OJA helped with plant collection, processing, literature searches and correction of the draft. Both authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Aims: The study was conducted to quantify some phytochemicals present in hydroethanol (absolute ethanol: water 1:1 v/v) and methanol extracts of *Harungana madagascariensis* stem bark traditionally used in the management of diabetes mellitus.
Methodology: Hydroethanol and methanol extracts of *H. madagascariensis* were separately prepared from the stem bark powder. The quantitative phytochemical analysis of the hydroethanol and methanol crude extracts were carried out by employing standard conventional protocols for total phenols, tannin, saponin, alkaloids, and anthraquinone

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from plants.
Results: Both samples showed the presence of all phytochemicals investigated. The study revealed that hydroethanol extract of *H. madagascariensis* stem bark contained higher amounts of bioactive compounds (65.72%±3.36, 7.22%±0.20, 2.45%±0.42 and 0.54%±0.04) in comparison with the methanol extract (52.54%±2.35, 3.50%±0.17, 0.60%±0.05 and 0.42%±0.02) for total phenols, tannin, alkaloids, and anthraquinone respectively. Amongst these, only total phenols and tannins were significantly (P<0.05) higher. The exception to this trend was in saponin which was higher in the methanol extract (0.59%±0.06) as opposed to 0.38%±0.11 in the hydroethanol extract.
Conclusion: Thus in all phytochemicals studied, hydroethanol extract of *H. madagascariensis* stem bark was found to be richer than the methanol extract. Saponin was the only exception. It can be concluded that bioactive compounds are more abundant in hydroethanol extract, and this potential could further be exploited in drug development.

Keywords: Phytochemicals; Harungana madagascariensis; hydroethanol extract; stem bark.

1. INTRODUCTION

Plants have always been an essential part of man's existence. Since ancient times humans have explored their environment for plants that could be used to cover all their basic needs: food, shelter, fuel and health. This has resulted in the use of a large number of plants. The number of medicinal plants has been estimated to be on the order of 40,000 to 70,000 [1], which means that almost 25% of all plant species have some sort of medicinal use somewhere in the world [2]. Plants have formed the foundations of sophisticated traditional medicine systems among which are Ayurveda, Unani, Chinese Traditional Medicine, and Traditional African Medicine amongst others. These systems of medicine have been in existence for thousands of years and continue to provide man with new remedies [2]. At the moment there is increasing acceptance of herbal medicine worldwide for the treatment of various maladies. These herbal medical products generally comes in the form of herbal drugs, nutraceuticals, functional foods, food supplements, etc. [3]. This increase in acceptance and popularity has been due to the drawbacks associated with synthetic drugs such as unwanted side effects, toxicity, inefficiency, among other problems [3]. Though ancient medicinal treaties have documented a large number of medicinal plants, most have remained undocumented and uncharacterized, the knowledge of their use being passed down from generations to generations through oral traditions. Although some of the therapeutic properties attributed to plants have been erroneous, many plants have given rise to some drugs still in use today [2].

Harungana madagascariensis is a tropical shrub abundant to the tropical rainforest margins and stream banks. The genus is monotypic, the single species being found in Africa, Madagascar, Mauritius and Mascareign Island. *H. madagascariensis* is one of the most popular trees in African traditional medicine system. It is a well established fact that every part of the plant has an application in curing human diseases [4]. Among Yoruba herbalists (Southwest Nigeria) the plant is known as "amuje" and the aqueous root decoction of the plant is employed in the treatment of suspected liver or kidney diseases [5]. The plant exudates is used to cure acute enteritis, scabies and jaundies [6]. Decoction of the plant root and extract is also used as remedy for dysentery, bleeding piles, trypanosomiasis, fever, cold and cough [6] The efficacy of medicinal plants is majorly due to the presence of phytochemicals and the successful extraction of these compounds from the plant material is largely dependent on the type of solvent used in the extraction procedure [7].

The aim of this study was to quantify some phytochemicals present in hydroethanol and methanol extracts from *H. madagascariensis* stem bark traditionally used in the management of diabetes mellitus.

2. MATERIALS AND METHODS

2.1 Chemicals

All chemicals used were of analytical grade and were products of BDH Chemicals Ltd, Poole, England unless otherwise stated.

2.2 Plant Collection and Authentication

Harungana madagascariensis stem bark used for this work was collected around University of Benin, Ugbowu campus, Benin City. The plant was identified and authenticated by Mr. Sunny Nweke of the Department of Pharmacognosy, University of Benin, Benin City and was deposited at the University of Benin herbarium with the Voucher Number: UBHh0265.

2.3 Processing Plant Materials

The stem bark was washed in running water and cut into small bits to facilitate drying. The pieces of plant material was air-dried under a shade at room temperature for a period of three weeks. The dried plant materials (stem bark) was ground using an electric blender to obtain a fine powder. The powder was further passed through a 2mm sieve to obtain finer particles. 250g portions of powdered plant materials were each separately dispersed in 2500ml of each methanol and hydroethanol (absolute ethanol: water 1:1v/v). The solution was left to stand at room temperature for 48hrs and was filtered with Whatman No. 1 filter paper. The filtrates were concentrated using a rotary evaporator at 37ºC-40ºC. These were concentrated to complete dryness by leaving the extracts under a rotating ceiling fan at room temperature. The extracts were stored in a refrigerator from where aliquots were taken for phytochemical analyses. The quantitative phytochemical analysis of the methanol and hydroethanol extracts of *H. madagascariensis* stem bark were carried out in order to ascertain the amount of some active constituents employing standard conventional protocols [8]. These active compounds include alkaloids, total phenols, tannins, anthraquinones, and saponins

2.4 Determination of Alkaloids

This was done by the alkaline precipitation gravimetric method described by Mboso et al. [9]. A measured weight of the sample was dispersed in 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4h at 28ºC. It was later filtered via Whatman No. 42 grade of filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of conc. aqueous NH_4OH until the alkaloid was precipitated. The precipitate was collected on a pre-weighed filter paper, washed with 1% ammonia solution. It was then oven-dried at 80ºC to a constant weight. The percentage yield of alkaloid was calculated from the weights of precipitate and that of the original sample.

2.5 Determination of Total Phenols

Determination of total phenols was done by spectrophotometric method. The fat free sample was boiled with 50ml of ether for the extraction of the phenolic component for 15min. 5ml of the extract was taken into a 50ml flask, then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development [10]. This was measured at 505nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250mg/l solutions of gallic acid in methanol:water (50:50,v/v).

2.6 Determination of Tannin

Tannin content was determined as described by Mboso et al. [9]. 20ml of 50% methanol was added to 0.2g of the sample in a 50ml beaker. This was covered with paraffin and introduced to a water bath set at temperature of 77 to 80ºC. Continuous stirring of the sample ensured no formation of lumps. The extract was filtered into a 100ml volumetric flask using a doubled-layer Whatman filter number 1 paper. The filtrate was rinsed with 50% methanol and the content of the volumetric flask made up to mark with distilled water. The content was mixed thoroughly. 1ml of the content was extracted with a pipette into a 50ml volumetric flask. Unto the extract was added 20ml of distilled water, 2.5ml of Folin-Denis reagent and 10ml of 17% Na_2CO_3. The content of the volumetric flask was thoroughly mixed and made up to mark with distilled water. It was then allowed to stand for the development of a bluish-green colouration. Tannic acid standard solutions ranging from 0 to 10ppm were also treated as the 1ml sample above. After colour development, the absorbance of the tannic acid standard as well as that of the 1ml sample were measured at 750nm with a spectrophotometer. The total tannin content was then measured and reported in percentage (%).

2.7 Determination of Anthraquinone Contents

50mg of the fine powder sample was soaked in 50ml of distilled water for 16 hours. This suspension was heated in water bath at 70ºC for one hour. After the suspension was cooled, 50ml of 50% methanol was added to it and then filtered. The clear solution was measured by spectrophotometer at a wavelength of 450nm and compared with a standard solution containing 1mg/100ml alizarin and 1mg/100ml purpurin with the absorption-maximum 450nm.

2.8 Determination of Saponin

The method used was as described by Gracelin et al. [11]. 20g of the sample was put into a conical flask followed by the addition of 100ml of 20% aqueous ethanol. It was then heated over a hot water bath for 4 hours with continuous stirring at about 55ºC. The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The extracts were combined and concentrated over a water bath at a temperature of 90ºC to give a 40ml concentrate. The concentrate was transferred to a 250ml separating funnel. Unto the extract was added 20ml diethyl ether and vigorously shaken. Two layers were formed. The ether layer was discarded while the aqueous layer was retained. This process was repeated. 60ml of n-butanol was then added to the extracts. The n-butanol extracts were combined, washed twice with 10ml of 5% aqueous sodium chloride. The solution left was concentrated in a

water bath and the concentrate was evaporated to a constant weight in an oven. Saponin content was calculated as percentage.

2.9 Statistical Analysis

All the values of phytochemicals were expressed as mean±Standard Deviation (S.D.) and analyzed using ANOVA. Inter-sample comparison was achieved by Duncan Multiple Range Test using SPSS 20.0. Differences between samples were considered significant at $P<0.05$ levels.

3. RESULTS AND DISCUSSION

Plants synthesize a wide range of organic compounds which do not take part in primary metabolism, hence are often referred to as secondary metabolites. The biological activities of plants are attributed to the presence of secondary metabolites. Also, secondary metabolites are of interest because of their use as dyes, fibres, glues, oils, waxes, flavouring agents, drugs, perfumes, and they are viewed as potential sources of new natural drugs, antibiotics, insecticides, and herbicides [12]. Literature search revealed that phytochemicals present in the stem-bark of this plant have not been quantified hitherto. Also, it is common knowledge that the type of solvent used for extraction is important for both quantification and classification of compounds occurring in plants [7]. The present quantitative phytochemical screening clearly validates the rationale for using different solvents when quantifying compounds in plant materials.

For instance, when the hydroethanol and methanol crude extracts were compared, data from (Table 1) clearly revealed that with the exception of saponins, the hydroethanol extract of the plant was richer in phytochemicals than the methanol This comparison demonstrated that hydroethanol is a better extractant for this plant. Also, it showed that *H. madagascariensis* stem bark is a relatively good source for plant phenolic compounds, tannins, alkaloids, anthraquinone, and to a lesser extent, saponins. These phytochemicals and phenolic compounds have been shown to contribute immensely to the medicinal and nutritional quality of plant and plant products [13]. It has been reported that intake of these bioactive constituents to an extent has protective and therapeutic effects essential to preventing diseases and maintaining a state of wellbeing [14].

Table 1. Quantitative phytochemical evaluation of hydroethanol and methanol extracts of *H. madagascariensis* stem bark

Extracts	Phytochemical constituents (%)				
	Total phenols	**Tannin**	**Saponin**	**Alkaloids**	**Anthraquinone**
Hydroethanol	65.72±3.36	7.22±0.20	0.38±0.11	2.45±0.42	0.54±0.04
Methanol	52.54±2.35*	3.50±0.17*	0.59±0.06	0.60±0.05	0.42±0.02

Values represent mean of triplicate readings±standard deviation. Significantly different when compared to hydroethanol extract at the level P<0.05*

Phenolic compounds of plant origin exhibit anticarcinogenic effect which is attributed to their antioxidant properties, as well as their capability to modulate the activity of enzymes, block hormone receptors, and lower the activity of mutagens. Polyphenols also protect blood vessels, reduce the aggregation of blood platelets and lower the LDL-cholesterol level in the blood [15]. Tannins are phenolic compounds and their derivatives are also considered as

primary antioxidants or free radical scavengers important in protecting cellular oxidative damage including lipid peroxidation [16]. Alkaloids are one of the most investigated phytochemicals. They play some metabolic role and control development in living systems. They are also involved in protective function in animals, and are used as medicine especially the steroidal alkaloids [17]. Saponins are another type of bioactive chemical constituents which are involved in plant disease resistance because of their antimicrobial activity [18]. Saponins help reduce congestive heart failure by inhibiting sodium efflux via the blocking of the entrance of the sodium ions into the cell [19], hence activating sodium-calcium antiporter producing elevated cytosolic calcium which strengthens the contraction of heart muscle. The plant aromatic compound known as anthraquinone content is not on the high side in this plant (Table 1). Several health benefits have been derived from anthraquinones such as antimalarial, antineoplastic and laxatives [20]. The presence various amounts of phytochemicals in the hydroethanol and methanol extracts part explains the therapeutic claims attributed to this plant in folk medicine.

4. CONCLUSION

Medicinal plants are a major source for chemical ingredients, antimicrobial and antioxidant agents for the treatment and management of different diseases. A comparison of the hydroethanol and methanol extracts of *Harungana madagascariensis* stem bark showed that the former contains higher amounts of bioactive compounds. These phytochemicals could be exploited as new leads for potential drug development.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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