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Screenning of extracts of *Hibiscus sabdariffa* and *Azardirachta indica* for bioactive compounds

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Abstract

It is important to screen secondary metabolites in plants as a major step towards the elucidation of their therapeutic mechanisms. This study focused on screening secondary metabolites of *Azardirachta indica* (neem) plant parts and *Hibiscus sabdariffa* calyx extracts as a major step toward the elucidation of therapeutic potential. Standard biochemical procedures were used to analyze aqueous (hot and cold) and ethanol extracts of the plant parts for bioactive components. Tannin and alkaloids in neem were high in both bark (5.23-6.54/ mg/g; 12.6-19.9%) and leaf (5.19-17.31 mg/g; 7.3-53.2%); the highest value was recorded in the ethanol leaf extract (17.31mg/g; 53.2%). The highest yield of all the bioactive components in *H. sabdariffa* was found in the hot water extract. Oxalate yield was highest in the hot water extracts of all the plant parts. Extracts of *Azardirachta indica* (leaf, root and bark) and *Hibiscus sabdariffa* (calyx) contain useful bioactive components which may be responsible for the folklores and scientifically investigated beneficial medicinal effects. However, there may be needed to be cautious in excessive consumption of hot water extracts of the plants because of the presence of oxalate.

Keywords: *Azardirachta indica*, Hibiscus *sabdariffa*, bioactive extracts, malaria beverage.

Introduction

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al*., 1999) this is because some of plants contain multitudes of naturally occurring chemical compounds (Amrit, 2006).

Medicinal plants contain secondary metabolites, which are organic compounds that are not directly involved in the normal growth, development, or reproduction of organisms. However, they often play an important role in plant defences (Harbone and Baxter, 1993) and are also capable of destroying or inhibiting the growth of microorganisms (Houghton, 2002).

The use of plants and plant products as medicines could be traced back to the beginning of human civilization. Herbal medicine is still the mainstay of about 75-80% of the whole population, and the major part of traditional therapy involves the use of plant extracts and their active constituents (Ahmad *et al*., 1998).

A significant proportion of plants branded medicinal in the western world originate from the rich biodiversity in developing countries, especially Africa and Asia. The plants are screened, analyzed and used in drug preparations and marketed to developing countries as high priced medicines to developing countries. As a result, many people in developing countries cannot afford such drugs. In Nigeria for example, the high cost of basic and essential drugs force over 60% of the rural population to depend on traditional medicine (Ghani *et al*., 1989: Edema and Alaga, 2012). Such traditional medicines originate from the wide variety of untapped biodiversity in Nigeria.

Azadirachta indica (Neem) belong to the mahogany family Meliacea (Thomson, 1992), and known with other names such as Nim (Bengali), Margosa, Neeb (Arabic), Azad Dirakht (Persian), Paraiso (Spanish), Indian Lilac (English) and Dogonyaro in some parts of Nigeria languages (Rahman and Jairajpuir, 1996).

The plant is drought resistant and grows well even in the arid parts of Nigeria up to 25 m high mostly as medium sized trees (Oliver, 1959). Various parts of this plant are employed in Nigerian traditional medicine for the treatment of variety of ailments. Dogonyaro is used extensively in Nigeria for the treatment of malaria using aqueous infusion, decoction or alcoholic extracts of the leaves and stem bark (Katsayal *et al.,* 2008).

All plant parts have been used medicinally for centuries. It has been used in Ayurvedic medicine for more than 4000 years due to its medicinal properties. The earliest Sanskrit medical writings refer to the benefits of Neem's fruits, seeds, oil, leaves, roots and bark. Each has been used in the Indian Ayurvedic and Unani medicine, and is now being used in pharmaceutical and cosmetics industries (Brotiti and Kaplay, 2011).

The leaves of *A. indica* (medium to dark green in colour) are known for use medically in the treatment of vatic disorders (Caldecott, 2006), acting also as an antitoxin (CDC, 2011), while the seeds are bitter and have anti-helminthic properties, antibacterial and insecticidal (Caldecott, 2006). Neem bark acts as an astringent and refrigerant herb (Atwal and Pajni, 1964), while other parts of this all-important plant Neem (including the fruits, flowers and roots) serve other purposes ranging from purgative, anti-helminthic and insecticidal respectively (Ganguli, 2002).

The effect of *Azadirachta* extract on methaemoglobin generation and the conversion of glutathione to its oxidized counterpart have also been investigated (Iwu *et al*., 1986). The neem plant and its derived products have shown a variety of insecticidal properties on a broad range of insect species (Isman, 2006). Neem products have shown to exhibit a wide range of effects that are potentially useful for malaria control and include antifeedancy, ovicidal activity, fecundity suppression, insect growth regulation and repellency. These activities are frequently attributed to the azadirachtin contents and other constituents of the plant or its products (Modue and Blackwell, 1993).

Hibiscus sabdariffa (roselle) is a member of the Malvaceae family. It has been used in different countries around the world as a culinary and therapeutic resource. It is considered to be one of the most important and popular medicinal plant and it has several properties such as antiseptic, aphrodisiac, cholagogue, digestive and stomachic (Akindahunsi and Olaleye, 2003). It is as well used for preparing candies, jelly, and hot and cold beverages. According to different ethnobotanical studies, some traditional medicines use the aqueous extract of this plant as diuretic, for treating gastrointestinal disorders, and hypercholesterolemia, and as diaphoretic, and antihypertensive. The dry calyx from this plant possesses great commercial value not because of its use as a plant colorant, but principally due to its use as beverage and, recently, for its antihypertensive properties (Rovesti, 1936; Bhaaskara and Seshadri, 1945; Sharaf, 1962; Haji-Faraji and Haji-Tarkhani, 1999).

This study sought to carry out preliminary phytochemical screening of *Azardirachta indica* (neem) leaves, bark and root extracts and *Hibiscus sabdariffa* calyx extracts as likely scientific justification for their use in herbal medicine. Furthermore, it is hoped this study will reveal the most effective method of extraction which will be useful for future studies in the verification of claimed medicinal uses.

Materials and Methods

Sample collection, identification and preparation

The fresh parts of *Azadirachta indica* (leaf, bark and root) and *Hibiscus sabdariffa* (calyx) used in this study were sourced locally from South Western part of Nigeria. Sample identification and deposit was done at the herbarium section of the University of Ado – Ekiti, Nigeria, 2005.

Fifty gram (50 g) of fresh plant parts were separately chopped into pieces and subsequently blended. Aqueous extracts of the pulverized parts were obtained under two conditions. First, by soaking in 200ml of ordinary distilled water for 24 hours at room temperature (29-31^0 C) and second, by soaking in 200ml of previously boiled distilled water for 24 hours.

Alcoholic extracts of another set of plant parts were also obtained by soaking 50g of chopped and milled parts separately in 200ml of 95% (v/v) alcohol for 24 hours. All the extracts were thereafter filtered using Whatman filter paper No. 1. The filtrate so obtained was concentrated using rotary evaporator at 40^{o}C and refrigerated until used.

Quantitative phytochemical analysis of extracts

Standard procedure as described by Harbone (1992), Sofowora (1996), Trease and Evans (1996) and Edeoga *et al*., (2005) were used to carry out chemical assay of the concentrated extracts in triplicates. Values are expressed as mean ± standard error of mean.

Results

LEGEND: CWE- Hot water extract, HWE- Cold water extract, EOE- Ethanol extract

The data of Phytochemistry of cold water, hot water and ethanol root, bark and leaf extractions of *Azardirachta indica* are shown in Table 1. Tannin was high in both bark (5.23-6.54 mg/g) and leaf (5.19-17.31 mg/g); the highest value was recorded in the ethanol leaf extract (17.31mg/g). The pattern of alkaloids content was similar to that of tannin with the ethanol leaf extract giving the highest (53.20%) yield. Saponin was best extracted with hot water; the highest yield was from the root closely followed by the bark and leaf. The value of extracted polyphenol was generally low in all the plant parts (0.03-0.25 mg/g). The highest yield was in the ethanol leaf extract, 0.25 mg/g. Oxalate yield was highest in the hot water extracts of all the plant parts (3.5-7.5 %). Interestingly, the lowest (1.00%) and highest (7.50%) oxalate was from the ethanol and hot water leaf extracts, respectively.

Table 2 shows the results of phytochemical assay of yield of *Hibiscuss sabdariffa* (calyx) extracted differently with cold water, hot water and ethanol. The highest yield of all the bioactive components was found in the hot water extract.

Table 1: Composition of Tannin, Alkaloid, Saponin, Total polyphenol and Oxalate in *Azardirachta indica* (neem)

COMPONENT/ SAMPLE	Neem root	Neem bark	Neem leaf
TANNIN(mg/g)			
CWE	3.00±0.2	5.23±0.3	5.19±0.4
HWE	6.31±0.4	6.54±0.3	6.31±0.3
EOE	2.00±0.1	5.54±0.2	17.31±0.4
TOTAL POLYPHENOL(mg/g)			
CWE	0.03±0.1	0.03±0.1	0.05±0.1
HWE	0.07±0.1	0.05±0.1	0.13±0.1
EOE	0.07±0.1	0.02±0.1	0.25±0.1
ALKALOIDS (%)			
CWE	10.64±0.5	12.64±0.3	7.32±0.4
HWE	24.94±0.7	19.95±0.4	18.25±0.9
EOE	3.99±0.6	16.96±0.8	53.20±0.5
SAPONIN (%)			
CWE	1.68±0.4	4.62±0.6	1.47±0.2
HWE	6.05±06	5.88±0.7	3.15±0.6
EOE	2.35±0.1	3.78±0.2	4.20±0.1
OXALATE (%)			
CWE	1.00±0.2	2.75±0.1	3.50±0.2
HWE	3.60±0.3	3.50±0.2	7.50±0.2
EOE	1.40±0.4	2.25±0.3	1.00±0.2

Table 2: Composition of Tannin, Alkaloid, Saponin, Total polyphenol and Oxalate in *Hibiscus sabdariffa* calyx extract

COMPONENT/SAMPLE	Calyx
TANNIN(mg/g)	
CWE	12.82±0.5
HWE	15.58±0.4
EOE	10.90±0.6
TOTAL POLYPHENOL(mg/g)	
CWE	0.14±0.1
HWE	0.20±0.2
EOE	0.12±0.1
ALKALOIDS (%)	
CWE	53.20±0.4
HWE	59.85±0.3
EOE	46.55±0.8
SAPONIN (%)	
CWE	1.89±0.1
HWE	2.33±0.1
EOE	1.59±0.1
OXALATE (%)	
CWE	6.66±0.4
HWE	8.33±0.5
EOE	5.66±0.4

Discussion

In the light of the data from the present study a plausible case can be made for the use of local gin in extracting the combined plant parts of neem as antimalarial. Other folklore uses of neem include its use in ameliorating jaundice, skin infections such as eczema and ringworm; as a laxative, antiemetic and for the treatment of sore throat (Van Der Nat *et al*. 1987). Different types of alkaloids serve specific functions in the body system and are biologically active in low doses. The principal among them are tropane alkaloids, sanguinarine (possesses antimicrobial properties), quinine alkaloid (used occasionally for the prevention of nocturnal leg cramps caused by vascular spasms and malaria), berberine (used topically and good for gastrointestinal complaints) (Mahata *et al.* 2011). Berberine could have side effects and allergic reactions when taken and could be poorly absorbed by oral administration. It can be inferred from this study that the bark and leaf extracts (especially ethanol extract) of neem has a high concentration of sanguinarine, berberine and quinine alkaloids. Isolation of one form of alkaloid from the leaf of neem extract has been used in suppressing egg-hatch of root nematodes (Amba, 1993). Further isolation of the different forms of alkaloids will be a laudable step in drug discovery using a plant, neem that is employed for various uses by the natives in Nigeria. Aside alkaloids, tannins have been shown to have antimicrobial activity (Abalaka, 2011). The antimicrobial effects of alkaloids and tannin seems to result from the combined bark and leaves used commonly in traditional medicine and would be expected to act synergistically. Insight into the antimicrobial mechanism of action of alkaloids and tannin was offered by Abalaka *et al.* (2011). It was postulated that they form irreversible complexes with praline-rich proteins which would lead to inhibition of cell-wall-protein synthesis. This inevitably would lead to cell wall disintegration and bacterial cell death; a mechanism similar to that of β lactam group of antimicrobials.

The observed highest extract yield of *Hibiscus sabdariffa* using hot water justifies the mode of preparation of the calyx as a local beverage by boiling. Other uses include diuretics, cough reliever and antimicrobial. Studies (Ekor *et al.,* 2010) have demonstrated the hypolipidaemic and antioxidant activities of extracts of the calyx in experimental animals. These beneficial effects could be as a result of the bioactive components. Alkaloids are known to be effective as antimicrobials while polyphenols act as antioxidants.

Saponin, a bioactive compound, has a broad spectrum of biological and pharmacological activities such as anti-inflammatory, hepatoprotective, hypoglycaemic, antimicrobial and antiviral activities (Shagal *et al.* 2012). These are potential that are waiting to be exploited for medicinal purposes in these plants. Triterpenoid and

steroidal saponins are the two known forms. It can be inferred that triterpenoid saponin, a strong expectorant, is present in *Hibiscus sabdariffa* since it is used as cough reliever.

The observed high oxalate yield in the present study calls for caution in excess consumption of the hot water extracts as oxalate is noted for toxicity. Reported toxic effects of excess consumption of oxalate have been shown to involve among others gastrointestinal irritation, precipitation of blood calcium and production hypocalcaermia syndrome of muscular weakness and paralysis (Littledoke *et al*., 1976; Yuen, 2001).

In conclusion, extracts of *Azardirachta indica* (leaf, root and bark) and *Hibiscus sabdariffa* (calyx) contain useful bioactive components which may be responsible for the folklores and scientifically investigated beneficial medicinal effects. However, there may be need to be cautious of excessive consumption of hot water extracts of the plants because of the presence of oxalate. Further studies will be necessary to isolate the different forms of the bioactive compounds and also investigate other possible/ likely beneficial effects of these plants arising from the high presence of the active components noted for the effects.

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