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RESEARCH ARTICLE

Comparative Phytochemical Analysis of selected Medicinal Plants in Nigeria

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Phytochemical screening of twenty-two medicinal plants distributed in fifteen different famsilies were carried out in order to evaluate the secondary metabolites in them, while their ethnomedicinal significance was gathered through secondary data mining. It was discovered that alkaloids, tannins and saponins were present in all. Flavonoids was absent only in *Peperomia pellucida*; five out of these plants lack terpenes, ten of them have steroids; eleven of them have cardiac glycosides while only four of these twenty-two have phlobatannins. The ethnomedicinal uses of these selected plants was widely ranged, from arthritis, inflammations, eye problems, respiratory problems, piles, fever, urinary problems, diabetes, sexually transmitted diseases, stomach problems, to jaundice and many other human disease conditions. Cissus aralioides was found to test positive to all the 8 phytochemicals evaluated, while majority of the rest possess between 5 and 7 phytochemicals. The significance of phytochemical screening for bioprospecting was discussed

Key words: Phytochemical, ethnomedicinal, medicinal plants, secondary metabolites.

INTRODUCTION

Phytochemicals are chemical compounds formed during the plants' normal metabolical processes (Okigbo et al., 2009). These chemicals are often referred to as secondary metabolites of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids (Okwu, 2004). In contrast to synthetic pharmaceuticals based upon single chemicals, many medicinal plants exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process (Okigbo et al., 2009). In modern medicine, plants are used as sources of direct therapeutic agents, as models for new synthetic compounds, and as a taxonomic marker for discovery of new compounds. They serve as a raw material base for the elaboration of more complex semisynthetic chemical compounds (Akerele, 1992). The synthesis of bioactive compounds is chemically difficult, because of their complex structure and high cost (Shimomura et al., 1997). Wide variations in medicinal quality and content in phytopharmaceutical preparations have been observed. These are influenced mainly by cultivation period, season of collection, plant-to-plant variability in the medicinal content, adulteration of medicinal preparations with misidentified plant species, a lack of adequate methods for the production and standardization of the crop, a lack of understanding of the unique plant physiology or efficacy with human consumption and consumer fraud (Nalawade & Tsay, 2004). According to Heldt (2005), most of these phytochemicals are produced through biosynthesis in the metabolic pathways. The primary metabolites are of major importance to plants (Trease & Evans, 1989). The secondary metabolites are of medicinal value to man and these can equally be obtained from various anatomical structures of plants (Fahn, 1974). Plants are so diverse in West Africa to the extent that hardly could there be any disease that cannot be tackled by these plants (Hutchinson *et al*, 1963). Therefore, plants, especially the higher ones have been described as the sleeping giants of drug and development and these medicinal plants have been screened for their chemicals that are potentially potent (Fahnsworth, 1988). Many of the medicinal plants, especially in Nigeria have been documented (Gill, 1992). The importance of medicinal plants, and the contribution of phytomedicine to the well-being of a significant number of the world's population, has attracted interest from a variety of disciplines (Biapa et al., 2007). Nigeria is rich in varieties of medicinal plants. Among the 50,000 species of medicinal plants recognized all over the world (Schippman et al., 2002), and 7,895 plants identified in Nigeria (FGN, 2006), more than 900 types of precious medicinal plants are estimated to be found in Nigeria. Unfortunately, only few of them are used for their medicinal value. Our approach involved the collection, identification, extraction and phytochemical of selected medicinal plants found in Nigeria.

MATERIALS AND METHODS

Collection of Information and Plant Samples

The ethnomedicinal significance of the plants was obtained from secondary data mining, mainly from previously published research papers. A modified Whitetaker Nested-Quadrant method (Stohgren *et al*, 1994) and normal field techniques for plant collections and herbarium development were used for vegetation sampling and plant collections for this study.

The selected plants used for the study are Spondias mombin, Cissus aralioides, Combretum racemosum, Combretum sp., Cucurbita pepo, Momordica foetida, Ipomoea batatas, Merremia aegyptia, Ficus exasperata, Ficus thonningii, Myrianthus arboreus, Boerhavia diffusa, Tetracera potatoria, Harungana madagascariensis, Rauvolfia vomitoria, Passiflora foetida, Parquetina nigrescens, Piperomia pellucida (syn. Piper pellucidum), Spermacoce verticillatus (syn. Borreria verticillata), Spermacoce ocymoides (syn. Borreria ocymoides), Lapportea aestuans (syn. Fleurya aestuans), and Poulzolzia guineensis. The fresh leaves of the plant materials used in the work were simultaneously collected from forest, abandoned farms, cultivated farms and the open field in Southern part of Nigeria. The plants were identified in their fresh state, and authenticated by one of the authors, who also is the Herbarium Curate of Department of Plant Science Herbarium (UHAE) in University of Ado-Ekiti, Ekiti State. The leaves were then cut into bits, and air dried for two weeks, after which they were pulverized, using a warring mechanical blender before being subjected to phytochemical screening. Duplicate samples of these plants were prepared and registered in the Herbarium of the Department of Plant Science Herbarium (UHAE) in University of Ado-Ekiti, Ekiti State.

Phytochemical Screening

The extracts were examined for the presence of the following phytochemicals: alkaloids, tannins, saponin, steroid, terpenes, flavonoids, phlobatannin and cardiac glycosides, as follows:

Alkaloids

Drangendoff's reagent was used and the method described by Harborne (1973) was adopted. Leaves powdered (0.2g) were extracted with 95% ethanol and 30mL boiling water respectively in a Sohlet extractor for six hours and the extract evaporated to dryness using a vacuum evaporator. The residue was redissolved in 5mL of 1% HCl and 5 drops of Drangendoff's reagent were added. The formation of orange precipitate indicates the presence of alkaloids.

Saponins

The persistent frothing test for saponin described by Odebiyi and Sofowora (1978) was used. To 1g of the extract, 30mL of tap water was added. The mixture was vigorously shaken and heated to boil. Frothing that persisted for 30 minutes shows the presence of saponin.

Phlobatannins

The extract (0.2g) was dissolved in 10mL of distilled water and filtered. The filterate was boiled with 2% HCl solution. Red precipitate shows the presence of phlobatannins.

Tannins

The method of Trease and Evans (1989) was adopted. 0.5g powdered crude drug was dissolved in 5mL of distilled water, then boiled gently and cooled. 1mL of this solution was put in a test tube and 3 drops of Ferric Chloride solution was added. A deep greenish-black colouration indicates a positive test for tannins.

Terpenes/Terpenoids

The Salkowski test was used. 5mL of each extract was mixed in 2mL of Chloroform, and 3mL concentrated sulphuric acid was carefully added to form a layer. A reddish brown colouration of the inter-face was formed to show positive result for the presence of terpenes or terpenoids.

Steroids

2mL of acetic anhydride was added to 0.5g ethanolic extract of each sample with 2mL of H_2SO_4. The colour changed from violet to blue, indicating the presence of steroids.

Cardiac glycosides

The Keller-Killani test was used. 5mL of each extracts was treated with 2mL of glacial acetic acid, containing one drop of ferric chloride solution. This was underlayed with 1mL of concentrated sulphuric acid. A browning of the interface indicates a deoxysugar characteristic of 'cardiac glycosides' (cardenolides). Below the 'brown', a violet ring was observed, while in the acetic acid layer, a greenish ring was observed.

Flavonoids

Three methods were used to determine or confirm the presence of flavonoids in the plants' powdered samples (Trease & Evans, 1989). The method adopted for this study is the one in which 5mL of diluted ammonia solution was added to a portion of the aqueous filtrate of each plant extract, followed by addition of concentrated sulphuric acid. A yellow colouration was observed in each extract, indicating the presence of flavonoids.

RESULTS AND DISCUSSIONS

Table 1 reveals the ethnomedicinal values of the plants used for this particular study. The diseases treated with part of the plants used for this study, as revealed on table 1 includes arthritis, inflammations, eye problems, respiratory problems, piles, fever, urinary problems, diabetes, sexually transmitted diseases, stomach problems, jaundice and many other human disease conditions. Table 2 shows the results of the phytochemical screening for the selected plants. It could be observed that alklaloids, tannins and saponins are present in all the 22 selected plants used for this study, while flavonoids were present in all but one of the plant samples. Steroids was present in only 10 samples, cardiac glycosides were present in 11 samples, terpenes in 17 samples and phlobatanins in only 4 samples. It is important to note that *Cissus aralioides* is the only plant that tested positive for all the 8 phytochemicals. Five of the remaining plant samples tested positive for 7 of the phytochemicals, while majority tested positive for only 5 of the phytochemicals, indicating a wide distribution of the phytochemicals among the selected plant samples. The use of phytochemical screening has significance in plant taxonomy, and this was further demonstrated in the two species of *Combretum* representing the family *Combretaceae*. All the two species contain alkaloid, tannin, saponin and flavonoid. *Combretum racemosum* contains both terpene and cardiac glycoside in addition, while the other representative lacks them. However, both of them do not have phlobatannin. Similar results of different phytochemical content among members of the same plant family was observed between

Table 1: Ethnomedicinal Significance Of The Selected Plant Samples

S/N	Plant Name	Family	Ethnomedicinal uses
1	*Spondias mombin* L.	Anacardiaceae	Leaves used to treat eye problems, cough, fever, yaws and as diuretic; for gastroenteritis. The fruits decoction is drunk as a diuretic and febrifuge, the decoction of the bark and the leaves is used as an emetic, anti-diarrhoea, dysentery recipe and for haemorrhoids as well as for gonorrhoea and leucorrhoea. A tea of the flowers and the leaves is taken to relieve stomachache, biliousness, urethritis, cystitis and eye and throat inflammations (Ayoka et al., 2008). A decoction of mashed leaves is used to wash swollen face.
2	*Cissus aralioides* (Welw ex Bak) Planch	Ampellidaceae	For treatment of wounds, cuts, internal and external microbial infections. It is also used for treating arthritis, rheumatism, dropsy, swellings, oedema, gout; fabrifuges; pain-killers; pulmonary troubles while the sap is used for eye treatments; venereal diseases (Aluka, 2010).
3	*Combretum racemosum* P. Beauv.	Combretaceae	Leaves used for treatment of wounds, stings, cuts, for cough and fever. Decoction of the roots and leaves used for abortion (Ibe & Nwufo, 2005). The plant is also. used for the treatment of haemorrhoids, convulsive coughing, tuberculosis, toothache and male sterility (BurkilI, 1985;Oliver-Bever, 1986)
4	*Combretum sp.* Loeft	Combretaceae	For treatment of fever and piles
5	*Cucurbita pepo* L.	Cucurbitaceae	The seed has been used in traditional medicine as an antihelmintic agent and for supportive treatment in functional disorders of the bladder and for difficulties in urination (Srivastava & Singh, 1967); childhood enuresis nocturnal and irritable bladder had been treated successfully with pumpkin seed (Weiss, 1988).
6	*Momordica foetida* Schum & Thorn	Cucurbitaceae	For treatment of diabetes, piles/haemorrhoid, gastroenteritis, snake bites, pregnancy, small pox, stomach ache, dropsy, fever, ear ache; and as anthelmintic, for tumours. Used as antidiabetic agent (Olaniyi, 1980).
7.	*Ipomea batatas* (L.) Lam	Convolvulaceae	Leaves as blood tonic. The leaves are grounded with salt to treat witlow (Oke et al 1999).
8	*Merremia aegyptia* (L.)	Convolvulaceae	For treatment of diabetes, wounds, infections and tumors
9	*Ficus exasperata* (Vahl.)	Moraceae	For treatment of microbial infections, sexually transmitted diseases and gastroenteritis. The leaf extract from *F. exasperata* reported to have diverse uses such as treating hypertensive patients (Buniyamin et al., 2007), heamostative opthalmia, coughs and heamorrhoid (Odunbaku et al., 2008). In Nigeria, young leaves of *F. exasperata* are prescribed as a common anti-ulcer remedy. The viscid non-milky sap is used for treating sores eye trouble and stomach pains (Burkill, 1997).
10	*Ficus thonningii* Blume	Moraceae	It is commonly used for the treatment of diarrhea, cold, sore throat, wounds and for stimulation of lactation in women. The young leaf of *Ficus thonningii* has also been used for the treatment of ulcer. It is used in treating colds, sore throat, dysentery, wounds, constipation, and nose bleed. Latex is used for wound fever, while an infusion of the root is taken orally to help prevent abortion. Powdered root is taken in porridge to stop nosebleed; the milky latex is dropped into the eye to treat cataracts (Orwa et al, 2009).

11	*Myrianthus arboreus* P. Beauv.	Moraceae	Seeds are used for boils. The bark decoction is administered for diabetes. The Leaves are used in preparations to treat dysentery, diarrhoea and vomiting. In the Igala area of Nigeria the leaves are an ingredient of a febrifuge given to young children. In eastern Nigeria a plaster made of beaten leaf-petioles is applied to boils (Okafor, 2004). The leaves serve as an analgesic given to young children against fever, applied as an enema to treat pain in the back and loins, chopped leaves are eaten raw with salt for heart troubles, pregnancy complications, dysmenorrheal, incipient hernia and a plaster made of beaten leaf applied to boils. Sap from the leaves is applied topically for toothache, to the chest for bronchitis or as throat paint for sore throat (Agwa, 2011)
12	*Boerhaavia diffusa* L.	Nyctaginaceae	Useful for treating rheumatism, skin infections and microbial problems. The roots have been widely used for the treatment of dyspepsia, jaundice, enlargement of spleen, and abdominal pain (Kirtikar and Basu, 1956). The powdered leaves are made into a paste and are applied to the chest to relieve asthma. The leaves are applied to the forehead to treat violent headache and around the ears against earache. Root sap as a lotion for friction is used to treat kidney troubles, rheumatism, generalised pain and sprains (Muzila, 2006).
13	*Tetracera potatoria* Afzel ex G. Don	Dilleniaceae	For the treatment of back ache and diabetes. The leaves of the plant boiled in its own sap are used for the treatment of gastrointestinal sores (Burkil, 1985).
14	*Harungana madagascariensis* Lam ex. Poir	Clusiaceae	For treatment of microbial skin infections, tapeworm and gastroenteritis. The leaves and stem bark are used for the treatment of anaemia, the stem bark is also used for nephrosis, malaria, gastro-intestinal disorders and fever (Erah et al., 2003). Traditionally, the leaves and stem bark are used for the treatment of anaemia, while the stem bark is indicated for nephrosis, malaria, gastro-intestinal disorders and fever.
15	*Rauvolfia vomitoria* Afzel	Apocynaceae	Leaves for treating yellow fever, internal pains, gastroenteritis, constipation and mental disorder. It is useful in the lowering of blood pressure (Amole, 2003), as an antimalarial (Amole et al., 1993). R. vomitoria is used by Nigerian traditional healers to treat psychiatric patients. The bark has purgative and emetic properties. The root extracts have arbotifacient properties.
16	*Passiflora foetida* L.	Passifloraceae	For the treatment of skin infections, hypertension, fever and asthma. The decoction of leaves and fruits to treat asthma and biliousness
17	*Parquetina nigrescens* (Afzel) Bullock	Periplocaceae	Leaves are used an anti-sickling agent and for treating gastroenteritis. The leaves and whole plant are usually used for the treatment of gonorrhoea, jaundice, rickets and asthma (Schlage 2002). The leaf juice is generally known to taken orally as blood tonic.
18	*Peperomia pellucida* L.	Piperaceae	The aerial parts are applied against abdominal pain, abscesses, acne, rheumatic pain, gout, headache, kidney-problems, cardiac arrhythmia and fatigue. It is used as an ingredient of an infusion for treating convulsions. The warmed leaves are applied to sores and boils. The leaves are applied as a poultice to treat breast cancer (Mosango, 2008).
19	*Spermacoce verticillata* L.	Piperaceae	Leaves are used to treat abdominal pains and Gonorrhea (Kone, 2004)
20	*Spermacoce ocymoides* Burm. F.	Piperaceae	Leaf of this plant, leaf of *Garcinia pictoria* and stem bark of *Syzygium cumini* are mixed, ground into a paste and heated with gingelly oil. The mixture thus obtained is applied topically on affected places to heal wounds (Ayyanar & Ignacimuthu, 2009). The leaves are used to treat dysentery and diarrhea.
21	*Laportea aestuans* (L.) Chew.	Urticaceae	The cooked leaves of L. aestuans are eaten as a remedy for stomach-ache and cooked with peanuts, which they are given to pregnant women (Bosch, 2004), According to Lans (2007), an infusion prepared of L. aestuans by soaking leaves in water is taken to deliver the placenta after child birth. Extract leaves of L. aestuans can be used to treat arthritis, enemia, hay fever, kidney problems and pain.
22	*Poulzolzia guineensis* Benth.	Urticaceae	Leaves for wound healing and treating stomach-ache. Asthma is treated with a mixture of leaves kneaded with kaolin, leaf sap is taken to treat diarrhoea and dysentery, and a leaf decoction is given by draught against vomiting during pregnancy. A decoction of the whole plant is taken as an aphrodisiac (Bosch, 2004)

Table 2: Phytochemical Screening of the 22 Plant Species Selected For The Study

S/N	Plant Name & Family	Alkaloids	Tannins	Saponins	Steroids	Phlobatannins	Terpenes	Flavonoids	Cardiac glycosides
1	*Spondias mombin*	+	+	+	+	-	-	+	-
2	*Cissus aralioides*	+	+	+	+	+	+	+	+
3	*Combretum racemosum*	+	+	+	-	-	+	+	+
4	*Combretum sp.*	+	+	+	-	-	-	+	-
5	*Ipomoea batatas*	+	+	+	-	-	+	+	+
6	*Merremia aegyptiaca*	+	+	+	-	-	+	+	-
7	*Cucurbita pepo*	+	+	+	-	-	-	+	+
8	*Momordica foetida*	+	+	+	+	-	+	+	-
9	*Ficus exasperata*	+	+	+	+	-	-	+	+
10	*Ficus thonningii*	+	+	+	+	-	+	+	-
11	*Myrianthus arboreus*	+	+	+	-	-	+	+	-
12	*Boerhavia diffusa*	+	+	+	-	-	+	+	+
13	*Tetracera potatoria*	+	+	+	-	+	+	+	-
14	*Harungana madagascariensis*	+	+	+	+	-	+	+	+
15	*Rauvolfia vomitoria*	+	+	+	-	-	-	+	+
16	*Passiflora foetida*	+	+	+	+	+	+	+	-
17	*Parquetina nigrescens*	+	+	+	-	-	+	+	-
18	*Piperomia pellucida*	+	+	+	-	-	+	-	+
19	*Spermacoce ocymoides*	+	+	+	+	-	+	+	-
20	*Spermacoce verticillatus*	+	+	+	-	+	+	+	-
21	*Lapportea aestuans*	+	+	+	+	-	+	+	+
22	*Poulzozia guineensis*	+	+	+	+	-	+	+	+

Ipomea batatas and *Merremia aegyptia* both members of the family *Convolvulaceae;* and *Cucurbita pepo* and *Momordica foetida*, both members of *Cucurbitaceae*; *Ficus exasperata, Ficus thonningii, Myrianthus arboreus* all from Moraceae; as well as *Spermacoce verticillatus* and *Spermacoce ocymoides* from Rubiaceae. Previous studies revealed that Arjunolic acid and a new triterpene acid, myriantic acid, have also been isolated from the root, wood of *Myrianthus arboreus* (Ojinnaka et al., 1984). Similarly, earlier phytochemical studies showed that *F. thonningii* contains tannins, saponins, flavonoids, and anthraquinone glycosides as the active agents (Hutchinson & Dalziel, 1956). *Boerhavia diffusa* contains all the phytochemicals except steroids and phlobatannins. Investigations on the chemical constituents of *Boerhavia diffusa* have indicated the occurrence of two novel alkaloids, Punarnavine-1 and Punarnavine- 2, belonging to the group quinolizidine (Nandi & Chatterjee, 1974). *Tetracera potatoria* contains all the phytochemicals, except steroids and cardiac glycosides. *Harungana madagascariensis* contains all the phytochemicals, except phlobatannins. *Rauvolfia vomitoria* contains 5 phytochemicals, but lacks steroids, phlobatannins and terpenoids. Two new indole alkaloids, 3-epi-rescinnamine and 3,4-dimethoxybenzoyl-reserpic acid methyl ester, are isolated from the root bark of *R. vomitoria* (Orwa et al, 2009).

Passiflora foetida was observed to possess seven of the eight phytochemicals, with the exception of cardiac glycosides. This agrees with earlier findings of Dhawan et al. (2004) who reported that *Momordica foetida* contain alkaloids, phenols, glycoside flavonoids and cyanogenic compounds while Echeverri et al. (2001) further identified passifloricins, polyketides and alpha-pyrones in *P. foetida*. *Parquetina nigrescens* contains five of the phytochemicals, while steroids, cardiac glycosides and phlobatannins were absent. *Piperomia pellucida* contains five phytochemicals, except steroids, phlobatannins and flavonoids. *Spermacoce verticillatus* and *Spermacoce ocymoides.* These two plants possess alkaloid, tannin, saponins, flavonoids and terpenoids. In addition, *Spermacoce verticillata* has phlobatannins, but it does not have steroids, while *Spermacoce ocymoides* has steroids, but lacks phlobatannins. However, both of them lack cardiac glycosides. Both *Lapportea aestuans* and *Poulzolzia guineensis* possess seven of the phytochemicals, except phlobatannins. Despite the fact that *Laportea aestuans* and *Poulzolzia guineensis* were found to be rich in phytochemical compounds, yet little is known or applied about their usefulness in Southwestern Nigeria. Therefore, it is recommended that more intensive ethnobotanical surveys be carried out about the plants within wider scope of human settlements. Phytochemicals, generally have a wide range of pharmacological activities or actions (Trease and Evans, 1989). Most of these phytochemical constituents are potent bioactive compounds found in medicinal plant parts which are precursors for the synthesis of useful drugs (Sofowora, 1993). The synthesis of bioactive compounds is chemically difficult, because of their complex structure and high cost (Shimomura *et al*, 1997). All plant parts synthesize some chemicals in themselves which metabolize their physiological activities. These phytochemicals are used to cure the disease in herbal and homeopathic medicine. Alkaloids, the most revered of all the phytochemicals, are said to be pharmacologically active and their actions are felt in the autonomic nervous system, blood vessels, promotion of diuresis, respiratory system, gastrointestinal tract, uterus, malignant diseases, infections and malaria (Trease and Evans, 1989). In addition, alkaloids are antispasmodiac, analgesic and also have bactericidal effects (Okwu & Okwu, 2004). Tannins are well known for their anti-oxidant and anti-microbial properties, as well as for soothing relief, skin regeneration, as anti-inflammatory and diuretics (Okwu & Okwu, 2004). Saponins lower the cholesterol level; have anti-diabetic and anti-carcinogenic properties (Trease and Evans, 1989). In addition, Saponins are expectorants, cough suppressants and for haemolytic activities (Sofowora, 1993; Okwu, 2005). Flavonoids are significantly recognized for their anti-oxidant, anticarcinogenic, antimicrobial and antitumor properties (Manikandan et al., 2006), while cardiac glycoside acts on the heart muscles and increase renal flow (diuresis). Terpenes or terpenoids have anti-hepatoxic properties, thus helping to prevent liver damage (cirrhosis), they equally have anti-microbial or anti-septic properties. Steroids regulate carbohydrate and protein metabolism, and possess anti-inflammatory properties. Phlobatannins on the other hand, have astringent or styptic properties. These plants have proved to be very important in the medicinal plants' research and because of the phytochemicals that they possess, these plants are useful in drug research and development. Therefore, they are recommended for further studies on the pharmacological significance of the phytochemicals they possess in the body. Further studies are therefore, needed for the isolation and characterization of the specific phytochemical compounds responsible for a particular disease treatment narrated in table 1. From this, it will be easy to synthesize an effective drug for the treatment of the disease on a large scale. This study is another confirmation of the earlier stated facts that these plants are good sources of income for individuals, revenue generation and bioprospecting.

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