Nigerian Research Journal of Chemical Sciences
(ISSN: 2682-6054)
Vol. 7, Issue 2, 2019

Phytochemical Screening of Some Selected Medicinal Plants Marketed in Three Major Selling-outlets within Sokoto Metropolis, Nigeria

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ABSTRACT

Secondary plant metabolites are the bioactive, non-nutritive chemical compounds produced by plants during metabolic processes. These bioactive compounds in plants have provided a source of drugs for pharmaceutical industries. These compounds also serve as starting material for drug development. The aim of the study was to qualitatively analyse for phytochemicals in the methanolic extract of ten different medicinal plants marketed at three major selling-outlets in Sokoto town, Nigeria. The plant materials were extracted by maceration using methanol. The extracts obtained were oven dried at low temperature and subsequently subjected to phytochemical screening using standard methods. The results of the phytochemical screening showed the presence of saponins, flavonoids, tannins, alkaloids, steroids/triterpenes and cardiac glycosides in all the ten samples except for anthraquinone which was detected in *Prosopis Africana* and *Detarium microcarpum*. The presence of these phytochemicals in the extractsof these medicinal plants has demonstrated that they could be a source of useful compounds for drug design and development.

Keywords: Medicinal plants, secondary metabolites, methanolic extract, phytochemicals

INTRODUCTION

For over thousands of years mankind uses plants in healing ailments and diseases, and are still widely used in many cultures around the world. For decades, traditional remedies (using medicinal plants) were empirically practiced in Nigeria, and indeed Africa to treat various diseases. These plants had been indispensably used by many cultures and traditions in folklore medicines for thousands of years. There has been an increasing awareness about the importance of medicinal plants in the recent years. Drugs from the plants are easily available, less expensive, safe, efficient and rarely have side effects. 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 [1].

The use of plants and plant products as medicines could be traced as far as the beginning of human civilization. The World Health Organization (WHO) estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs, for their primary health care needs [2]. Medicinal plants are a source of great economic value all over the world. Around 80% of products were of plant origin and their sales exceeded US $65 billion in 2003 [3].

Phytochemicals are the bioactive non-nutritive, chemical compounds occurring naturally in plants during metabolic processes. The word 'phyto' is derived from the Greek word phyto which means plant. These bioactive phytochemicals can be grouped under two catagories; primary and secondary metabolites. Primary metabolites include proteins, carbohydrates, amino acids and chlorophyll while polyphenols, alkaloids, terpenoids are some examples of secondary metabolites. Most of these active plants phytochemicals can be classified into sub-groups according to their chemical structure, which include terpenoids (e.g. carotenoids), phytosterols, polyphenols (e.g. tannins, flavonoids, phenolic acids) and glucosinolates [4]. Secondary metabolites are the chemicals that are not required for the immediate survival of the plant but synthesized to increase the survival of the plant by allowing it to interact with pathogens, herbivores insects and environment [5]. Secondary metabolites such as alkaloids, glycosides, flavonoids, steroids, saponins and terpenoids play an important role in the protection of the plant from environmental

stress, attacks of pathogens and insect pests [6]. Generally, these compounds enable the plants to interact with the environment and may act as a defence system against physiological and environmental stress as well as predators and pathogens. The presence of these bioactive phytochemicals are said to confer them with resistance against bacterial, fungal and pesticidal pathogens. Several of these secondary plant metabolites have beneficial effect in food product and metabolism [7].

Due to the presence of these bioactive phytochemicals, plants provide a source of medicine since historic times and now these are an important part of all the world's pharmaceuticals and serve as starting material for drug development [8]. The beneficial effects of phytochemicals may arise from activation of feed intake and secretion of digestive secretions, immune stimulation, anti-bacterial, coccidiostatic, anthelmintic, antiviral, anti-inflammatory activity and inhibition and antioxidant [9]. Phytochemical studies also suggest that these natural phytochemicals modulate various molecular signal transduction pathways, involved in the phenomenon of inflammation, thereby preventing the onset of various chronic diseases like cancer, atherosclerosis, neurodegradation, obesity, articular rheumatism, skin aging and diabetes [10]. Most phytochemicals function as antioxidants in vitro and they can reduce oxidative stress and inflammation which are involved in the progression of type II diabetes mellitus [11]. These specific organic compounds of plant origin have shown antidiabetic activity and represent a source for the discovery and development of new type of antidiabetic molecules [12]. Polyphenolic compounds, immensely distributed throughout the plant kingdom, serve as antioxidants and neutralize deleterious free radicals, quenching singlet or triplet oxygen, or decomposing peroxides, and regulate carbohydrate metabolism [13]. Besides combating various afflictions, and possessing antidiabetic and antioxidative properties, phytochemicals could provide health benefits as, substrate for biological reactions, Co-factors for enzymetic reactions, inhibitors for enzymatic reactions, absorbants/sequestrants that bind to and eliminate undesirable constituents in the intestine, ligands that agonize or antagonize cell surface/ intracellular receptors, compounds that enhance the absorption or stability of essential nutrients, selective growth factors for beneficial gastrointestinal bacteria and selective inhibitors of deleterious

intestinal bacteria [14]. Therefore, the main purpose of the present study was to preliminary screen for the presence of phytochemicals in the methanolic extracts of ten different medicinal plants marketed at three major selling-outlets in Sokoto town, Nigeria.

MATERIALS AND METHODS

Sample Procurement

The sampling was carried out in May, 2015. The samples were collected from three different sale outlets (*Kara* Market, Old Market and *Marina* Market). The plants collected include: *Cassia singueana* (leaves), *Guiera senegalensis* (leaves), *Combretum micranthum* (leaves) and *Senna italic* (leaves) were collected at *Kara* Market. *Anogeissus leiocarpus* (leaves), *Boswellia dalzielli* (bark) and *Cassia arereh* (bark) were collected atOld Market. *Prosopis africana* (bark), *Anogeissus leiocarpus* (bark) and *Detarium microcarpum* (bark) were collected at *Marina* Market. The samples were collected in polyethylene bags. The plant parts (leaves, flowers and fruits) were collected for identification purpose. The plants were identified by a consultant taxonomist in the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. Voucher specimens of the plant samples were prepared and voucher numbers were assigned and deposited at the Herbarium of the Department for reference. The plant scientific and local names, parts of the plant employed in the research and their identification codes as well as the voucher number are shown in Table 1.

Table 1: List and Name of Medicinal plants

S/N	Hausa Name	Code	Scientific Name	Plant Part used	Voucher No.
01	Runhu	RH	*Cassia singueana*	Leaves	PCG/UDUS/Legu/0001
02	Sabara	SB	*Guiera senegalensis*	Leaves	PCG/UDUS/Comb/0002
03	Geza	GZ	*Combretum micranthum*	Leaves	PCG/UDUS/Legu/0002
04	Fulasko	FK	*Senna italic*	Leaves	PCG/UDUS/Caes/0002
05	Marke	MK-L	*Anogeissus leiocarpus*	Leaves	PCG/UDUS/Comb/0001
06	Hanu	HN	*Boswellia dalzielli*	Bark	PCG/UDUS/Burs/0001
07	Malga	MG	*Cassia arereh*	Bark	PCG/UDUS/Caes/0001
08	Ƙirya	ƘY	*Prosopis africana*	Bark	PCG/UDUS/Legu/0003
09	Marke	MK-B	*Anogeissus leiocarpus*	Bark	PCG/UDUS/Comb/0001
10	Taura	TR	*Detarium microcarpum*	Bark	PCG/UDUS/Legu/0004

Preparation of plants extract

The plant samples were ground to coarse powder using wooden pestle and mortar. The powdered samples were sieved separately to very fine particles. A hundred gram (100 g) of each of the sample was macerated in 100cm^3 of 70% methanol for 72 hours. The extracts were filtered through a Whatman No. 1 filter paper and the filtrates were concentrated to dryness using rotary evaporator under reduced pressure.

Qualitative phytochemical analysis

Preliminary qualitative phytochemical screening was carried using standard methods.

Tests for Saponins

Crude extract was mixed with 5cm^3 of distilled water in a test tube and it was shaken and allowed to stand for 10 minutes. The formation of stable foam was taken as an indication for the presence of saponins [15].

Tests for Flavonoids

a) Shinoda's test

The methanolic extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. The appearance of pink scarlet colour after few minutes was used as an indication of the presence of flavonoids [16].

b) Ferric Chloride Test

Methanolic extract (2cm^3) was diluted into a ratio of 1:4 with distilled water and few drops of ferric chloride solution were added. A green or blue colour indicates the presence of phenolic nucleus [17].

Tests for Tannins

a) Ferric chloride test.

Methanolic extract (1cm^3) was diluted with distilled water. Few drops of ferric chloride solution were added. The appearance of blue-black colour indicates the presence of tannins [18].

b) Lead Sub acetate test.

To 1cm^3 of the extract, 2 drops of lead sub acetate solution was added. A whitish or milky precipitate indicates the presence of tannins [19].

Tests for Alkaloids

a) Mayer's Test

The methanolic filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a white precipitate indicates the presence of alkaloids [20].

b) Wagner's Test

The methanolic filtrate of the extract was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids [20].

Tests for Steroids/Triterpenes

a) Liebermann-Burchard's Test

To the methanolicfiltrate, 1cm^3 acetic anhydride was added, followed by the addition of 1cm^3 of concentrated sulphuric acid down the side of the test-tube. Formation of blue-green colour in the upper layer indicates the presence of steroids while pink or purple colour indicates the presence of triterpenoids [15, 21].

b) Salkowski's Test

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes [15,21].

Tests for Anthraquinones

Borntrager's Test

To the 2cm^3 of the extract filtrates were treated each with 5cm^3 of benzene. This formed two layers; in each case the clear colourless upper layer was pipette and treated with 3cm^{3}of 10% ammonia. The lower layer with the ammonia becomes rose pink red. This indicates the presence of anthraquinones [20].

Tests for Cardiac Glycosides

Keller-Killiani's Test

In a clean test-tube, 2 cm^3 of the filtrate was added 3cm^3 of 3.5% ferric chloride and then 3cm^3 of acetic acid. This gave a green precipitate and a dark brown coloured solution respectively. Finally, concentrated sulphuric acid was carefully poured down the side of the test-tube which resulted in the formation of brownish-red layer. This appeared at the interface due to aglycone and the upper layer which is acetic acid layer becomes greenish-blue due to the deoxy-sugar present [15].

RESULTS AND DISCUSSION

Table 2: Phytochemical constituents of ten samples selected from the three locations in Sokoto State

S/N	Compounds (Test)	*Cassia singueana* (leaves)	*Cassia singueana* (leaves)	*Combretum micranthum* (leaves)	*Senna italic* (leaves)	*Anogeissus leiocarpus* (leaves)	*Boswellia dalzielli* (bark)	*Cassia arereh* (bark)	*Prosopis africana* (bark)	*Anogeissus leiocarpus* (bark)
01	Saponins (frothing)	+	+	+	++	+	+	+	++	+
02	Flavonoids:									
	i)Ferric chloride test	+	+++	+++	-	+	++	++	-	+
	ii) Shinoda's Test	++	-	++	-	+	+	-	-	+
03	Tannins:									
	i)Ferric chloride test	+	+++	+++	+	+	++	++	+	+
	ii)Lead acetate test	+	+++	++	+	+	++	+	+	++
04	Alkaloids:									
	i)Mayer's Test	++	+	-	+	+	+	+	+	+
	ii)Wagner's Test	+	+	-	+	+	+	+	+	+
05	Steroids/triterpens:									
	i)Salkowski's Test	+	+	+	+	+	+	+	+++	+
	ii)Liberman									
	Buchard's Test	+	+	+	++	++	+	+	+	+
06	Anthraquinones:									
	Bontrager's	-	-	-	-	-	-	-	+	-
07	Cardiac glycosides	+	++	+	++	+	+	+	+	+

Key: + = Low amount; ++ = Moderate amount; +++ = High amount

Table 2 shows the results of the phytochemical constituents of 10 samples; *Cassia singueana* (leaves), *Guiera senegalensis* (leaves), *Combretum micranthum* (leaves) and

Senna italic (leaves) were collected from Kasuwar Kara, *Anogeissus leiocarpus* (leaves), *Boswellia dalzielli* (bark) and *Cassia arereh* (bark) were collected from Tsohuwar Kasuwa, while *Prosopis Africana* (bark), *Anogeissus leiocarpus* (bark) and *Detarium microcarpum* (bark) were collected from 'Yan Marina. In this table, saponins, flavonoids, tannins, alkaloids, steroids/triterpens, anthraquinones and cardiac glycosides were all detected. This shows medicinal potentials of these samples.

Saponins were detected in almost all the samples, saponins which are highly toxic when injected into the blood stream, cause haemolysis of the red blood cells and destroy them. They are also used to reduce body cholesterol by preventing its re-absorption and suppressing rumen protozoa by reacting with cholesterol in the protozoan cell membrane thereby causing it to lyse. Saponins also find applications in foaming fire extinguishers, emulsifiers and insecticides [22].

Flavonoids were detected in all the samples except *Senna italic* (leaves) and *Prosopis africana* (bark). Flavonoids are other plant constituents with antibacterial and antifungal properties. They are used in treating stomach ulcer and inhibit HIV – 1 integrase and HIV – 1 protease enzymes which are responsible for the HIV replication [23].

Tannins using both ferric chloride and lead acetate tests were detected in all the samples. Tannins are active detoxifying agents by precipitating the protein component and hence inhibiting their growth. This effect agrees with the mode of action of aminoglycoside antibiotic (e.g. streptomycin in which they first latch onto surface receptor on the bacteria before exerting their action). Other uses of tannins are to stop haemorrhage, to treat diarrhoea, as well as local burns treatment where they precipitate proteins in the burned area, thus forming a protective layer [24].

Alkaloids were also found in almost all the samples except *Combretum micranthum* (leaves), using Mayer's and Wagner's tests. Alkaloids have many pharmacological activities including antihypertensive effects (many indole alkaloids), antiarrhythmic effect (quinidine), antimalarial activity (quinine), and anticancer actions (dimeric indoles, vincristine, vinblastine). These are just a few examples illustrating the great economic importance of this group of plant constituents [23]. Some alkaloids have stimulant

property as caffeine and nicotine, morphine are used as the analgesic and quinine as the antimalarial drug [26].

Salkowski's and Liberman Buchard's tests confirmed the presence of steroids/triterpenes in all the samples under study. Steroids are also another important class of plant constituents. Due to their relationship with sex hormone [23] vegetable containing steroids are given to expecting or breast feeding mothers to enhance hormonal balance [23].

Anthraquinones were detected in only two of the samples *Prosopis africana* (bark) and *Detarium microcarpum* (bark) by Bontrager's test. Anthraquinones are used as anticeptics in certain skin diseases (eczema) and represent the basic structure of a number of important laxatives and dyestuffs [27].

The cardiac glycosides were identified in all the samples. The glycosides are reported to possess strong antibacterial activities. They exist as antibiotics like streptomycin, neomycin, kanamycin, paromomycin, gentimycin and tobramycin [28].

CONCLUSION

The phytochemicals such as saponins, flavonoids, tannins, alkaloids, steroids/triterpens and cardiac glycosides were all detected in the samples, except for anthraquinones which were detected only in *Prosopis africana* and *Detarium microcarpum* (barks).

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