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## Phytochemical analysis and accumulation of heavy metals in some common medicinal plants

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### Abstract

The present study investigated the phytochemical constituents and levels of heavy metals in some commonly used medicinal plants; *Securidaca longipedunculata* (root bark), *Guiera senegalensis* (leaves) and *Boswellia dalzielii* (stem bark). The phytochemical analysis showed that saponins, carbohydrates, cardiac glycosides, steroids, terpenoids, alkaloids and flavonoids were present in all the three plant species. On the other hand, the heavy metals analysis showed that chromium and arsenic were not detected in all the three plant species, while lead was detected in the stem bark of *Boswellia dalzielii* ( $0.0010 \pm 0.00$  mg/kg). Also, trace amounts of cadmium were detected in the root bark of *Securidaca longipedunculata* ( $0.0002 \pm 0.00$  mg/kg) and leaves of *Guiera senegalensis* ( $0.0004 \pm 0.00$  mg/kg). The study has shown that the common toxic metals (lead, chromium and arsenic) were not detected in the root bark of *Securidaca longipedunculata* and leaves of *Guiera senegalensis*. Also, the level of lead detected in the stem bark of *Boswellia dalzielii* and level of cadmium detected in the root bark of *Securidaca longipedunculata* and leaves of *Guiera senegalensis* were below the recommended limits.

**Keywords:** Medicinal plants, heavy metals, phytochemical constituents, recommended limits

### Introduction

Medicinal plants are commonly used worldwide; they are used to cure many diseases due to their therapeutic potentials. About 80 % of the world's populations depend on traditional medicine as their primary health care. In most African countries including Nigeria, herbal medicine is recognized as an important component of health care system, especially among rural dwellers that constitute about 70 % of the population [1]. A survey conducted by the World Health Organization (WHO) in Nigeria estimated that up to 75 % of the population patronizes traditional medicine as results of the ever increasing cost of orthodox health care services [2].

*Securidaca longipedunculata* is locally called Sanya or Uwar maganguna (Hausa), Ezeogwu (Igbo) and Alali (Fulani). It is ethnomedicinally used as a remedy for numerous human and animal ailments [3]. The root is used for eye complaints such as conjunctivitis, malaria, venereal diseases, urethral discharges, stomach problems, dysentery, rheumatism, toothache, headache, sleeping sickness, cough, chest complaints, snakebite, wound dressing, aphrodisiac and expectorant [4].

*Boswellia dalzielii* is a locally known as Hanu or Ararrabi by the Hausa people of Northern Nigeria. Its stem is specifically used to treat rheumatism, septic sores, venereal diseases, diarrhea, malaria, ulcers, pain, inflammation, gastrointestinal ailments and many pediatric diseases [5].

*Guiera senegalensis* belongs to the Family Combretaceae, it is called Sabara by the Hausa of Northern Nigeria. It is traditionally used to treat malaria, dysentery and diarrhea, cough, cold, abdominal pains, leprosy, hypertension, diabetes, snakebites eczema, impotence, epilepsy, breast cancer, depressant and jaundice. Some of the biological properties reported for *G. senegalensis* include anti-tuberculosis, anti-diarrhoeal, anti-plasmodia, analgesic, antifungal, antioxidant, anti-malaria, anti-acetylcholinesterase, antilipid peroxidation [6-10].

However, medicinal plants and herbal products are not usually tested with scientific rigour required for conventional drugs, also, manufacturers of those products do not submit proof of safety and efficacy to regulatory bodies before marketing [11], thus, the safety of herbal medicines is poorly understood [12]. In view of that, the World Health Organization (WHO) recommends that medicinal plants, which form the raw materials for most herbal remedies should be checked for the presence of heavy metals. Lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg), cobalt (Co) and chromium (Cr) are the most common toxic metals that have

become a matter of concern due to the reports of their contamination in various herbal preparations and herbal ingredients [13-19].

Lead is known to cause neurological disorders, anemia, kidney damage, miscarriage, lower sperm count and hepatotoxicity in higher concentration [20]. Mercury can cause problems in neuronal cell migration and division, and can ultimately cause cell degeneration and death [21]. Arsenic is reported to cause hypertension, peripheral arteriosclerosis, skin diseases and neurotoxicity [22]. Therefore, the present study was aimed to determine the phytochemical constituents and levels of heavy metals in *S. longipedunculata* (root bark), *G. senegalensis* (leaves) and *B. dalzielii* (stem bark), which are commonly used in almost every household in Northern Nigeria.

## Material and Methods

### Collection, Identification and Preparation of plants

*B. dalzielii* and *G. senegalensis* were collected from Kumbotso Local Government Area of Kano State, while *S. longipedunculata* was collected from Gwaram Local Government Area, Jigawa State, Nigeria. All the plant species were identified in the field using taxonomic characters and the taken to Herbarium of Ethno botany Unit of Bioresource Development Centre, Kano for authentication, and reference voucher numbers (*S. longipedunculata*: BDCKN/EB/1898, *G. senegalensis*: BDCKN/EB/1616 and *B. dalzielii*: BDCKN/EB/1741) were deposited in the Herbarium. The samples were air dried and then ground into fine powder using mortar and pestle.

### Extraction of the Powdered Plant Materials

The powdered plant materials (100 g each) were separately macerated with distilled water (500 ml) for 72 hours, and each mixture was shaken occasionally. The filtrates obtained were evaporated to dryness at 40 °C using rotary evaporator and water bath.

### Preliminary Phytochemical Screening

The Preliminary Phytochemical screenings of the aqueous extracts of *S. longipedunculata* (root bark) *G. senegalensis* (leaves) and *B. dalzielii* (stem bark) were conducted using the standard laboratory procedures [23-27].

### Test for Carbohydrates

The extract (5 g) was boiled in 50 mL of distilled water for 5 minutes. The mixture was filtered while hot and allowed to cool to room temperature. The filtrate was divide into two portions and used as follows;

**Molisch test:** To the first portion, 1 mL of Molisch's reagent was added followed by 1 mL of concentrated sulphuric acid down the side of the test tube. A reddish colored interfacial ring indicates the presence of carbohydrate.

**Fehling test:** To the second portion, 5 mL of equal mixture of Fehling's solution A and B was added and the mixture boiled for minutes. A brick red colored precipitate indicates the presence of reducing sugar

### Test for Anthraquinones

Borntrager's test: The extract (200 mg) was boiled in 5 mL of 10% HCl and then filtered. The filtrate was extracted with 5 mL of benzene, and the benzene layer was shaken with 5 mL of 10% NH<sub>4</sub>OH. A rose pink or cherry red colour indicates the presence of anthraquinone derivatives.

### Test for Saponins

**Frothing test:** The extract (0.5 g) was shaken with water in a test tube. Frothing which persisted for 15 minutes indicates the presence of saponins.

### Test for Cardiac Glycosides

The extract (2 g) was boiled in 10 mL of 95 % alcohol for five minutes; it was then cooled and filtered. Lead sub acetate solution (3 mL) was added to the filtrate and then filtered again. The filtrate was divided into two portions (first filtrate). To the first portion of the filtrate, 10 mL of chloroform was added and the mixture was shaken for 5 minutes. The lower chloroform layer was run off into a beaker and divided into two portions (second filtrate).

**Keller-Killiani test:** The first portion of the second filtrate was transferred into an evaporating dish and evaporated to dryness on a water bath. The residue was dissolved in 1 mL of glacial acetic acid containing traces of FeCl<sub>3</sub> solution and then transferred into a dry test tube; 2 mL of concentrated sulphuric acid was run down the side of the test tube to form a lower layer. A purple-brown ring at the interface indicated the presence of deoxysugars, while a pale green colour in the upper acetic acid layer is due to the presence of steroid, which indicates the presence of cardiac glycosides.

**Kedde test:** To the second portion of the first filtrate, 1 mL of 2 % solution of 3, 5-dinitrobenzoic acid in 95 % alcohol was added. The solution was made alkaline by the addition of 5 % sodium hydroxide. The appearance of a purple-blue colour indicated the presence of cardenolides.

### Test for Triterpenoids/Steroids

**Liebermann-Burchard test:** Anhydrous acetic acid (1 mL) was added to 1 mL of chloroform and cooled to 0°C, and then a drop of concentrated sulphuric acid and the extract (0.5 g) were added. A blue-green ring was taken as an indication for the presence of terpenoids.

**Salkowski test:** The extract (0.5 mg) was dissolved in 2 mL of chloroform, thereafter; 1 mL of concentrated sulfuric acid was added down the test tube to form two phases. Formation of red or yellow coloration was taken as an indication for the presence of sterols.

### Test for Flavonoids

**Shinoda test:** About 0.5 g of the extract was dissolved in 2 mL of 50% methanol and filtered. Magnesium filings and 3 drops of hydrochloric acid were added to the filtrate. A pink or red color was considered as an indication for the presence of flavonoids.

**Sodium hydroxide test:** The extract (0.5 g) was dissolved in 2 mL of 10% aqueous sodium hydroxide solution and filtered to give yellow color, a change in color from yellow to colorless on addition of dilute hydrochloric acid indicated the presence of flavonoids.

### Test for Tannins

**Ferric chloride test:** The extract (200 mg) was boiled in 20 mL of distilled water and filtered. Ferric chloride (1 mL) was then added to the filtrate. The formation of a blue-black, or green precipitate indicated the presence of tannins.

### Test for Alkaloids

The extract (0.5 g) was stirred with 5 ml of 1% aqueous

hydrochloric acid on a water bath and filtered. The filtrate was divided into three portions. To the first portion, 1 mL of freshly prepared Dragendoff's reagent was added and observed for formation of orange to brownish precipitate. To the second portion, 1 mL of Mayer's reagent was added and observed for formation of white to yellowish or cream colored precipitate. To the third portion, 1 mL of Wagner's reagent was added to give a brown or reddish or reddish-brown precipitate.

### Evaluation of Heavy Metals

The methods described by [28, 29] were employed for the determination of heavy metals in all the samples. Approximately 1 g of each sample was weighed in glass beaker and 25 ml of an acid mixture of nitric acid (65 %) and perchloric acid (70 %) at molar ratio 4:1 was added. The mixture was wet digested at 130 °C in fuming hood near to dryness. The procedure was repeated until the sample digestion process was completed as indicated by appearance of white fumes and residue almost gets to dryness. The solutions were left to cool to room temperature, and each sample was filtered into a 50 ml volumetric flask and was diluted up to the mark with distilled water. The solutions were then analyzed for lead (Pb), cadmium (Cd), copper (Cu), nickel (Ni), manganese (Mn) chromium (Cr) and arsenic (As) using Atomic Absorption Spectrophotometer (AAS) available in the Department of Soil Science, Faculty of Agriculture, Bayero University Kano, Nigeria.

## Results

### Preliminary Phytochemical Screening

The preliminary phytochemical screening revealed the

presence of saponins, carbohydrates, cardiac glycosides, steroids, terpenoids, alkaloids and flavonoids in all the three plant species, in addition, anthraquinones were only detected in the stem bark of *B. dalzielii* (Table 1);

**Table 1:** Phytochemical Constituents of some Common Medicinal Plants

Phytochemicals	Medicinal Plants		
	A	B	C
Tannins	-	+	+
Anthraquinones	-	-	+
Cardiac glycosides	+	+	+
Saponins	+	+	+
Phenolic compounds	-	+	+
Flavonoids	+	+	+
Alkaloids	+	+	+
Terpenoids	+	+	+
Steroids	+	+	+
Carbohydrates	+	+	+

Key:

A = Root bark of *Securidaca longipedunculata*

B = Leaves of *Guiera senegalensis*

C = Stem bark of *Boswellia dalzielii*

+ = Present

- = Absent

### Evaluation of Heavy Metals

The heavy metals evaluation showed that Mn and Cu were detected in all the medicinal plants analyzed, while Cd was detected in the root bark of *S. longipedunculata* and leaves of *G. senegalensis*. Also, Ni was detected in the leaves of *G. senegalensis* and stem bark of *B. dalzielii*. On other hand, Cr and As were not detected in all the medicinal plants analyzed.

**Table 2:** Levels of Heavy Metals in some Common Medicinal Plants

Medicinal Plants	Heavy Metals (mg/kg)					
	Pb	Ni	Mn	Cu	Cd	Cr As
A	ND	ND	0.0454 ± 0.01	0.0103 ± 0.00	0.0002 ± 0.00	ND ND
B	ND	0.0040 ± 0.00	0.1890 ± 0.03	0.0396 ± 0.02	0.0004 ± 0.00	ND ND
C	0.0010 ± 0.00	0.0030 ± 0.00	0.0345 ± 0.01	0.0043 ± 0.00	ND	ND ND

Key:

A = Root bark of *Securidaca longipedunculata*

B = Leaves of *Guiera senegalensis*

C = Stem bark of *Boswellia dalzielii*

ND = Not detected

## Discussion

The phytochemical constituents identified in these plant species are considered as biologically active compounds of plant origin, they are very important in the discovery of new therapeutic agents, especially at this time when the scientific community is preoccupied with searching for alternative treatment to combat the increasing threat of drug resistant micro-organisms [30].

The phytochemical analysis shows that the stem bark extract of *B. dalzielii* contained all the phytoconstituents tested; however, the presence of anthraquinones didn't support the findings of [31].

Also, the absence of tannins in the aqueous root bark extract of *S. longipedunculata* did not agree with the findings of [32-34] who all reported the presence of tannins in the aqueous, ethanol and chloroform root bark extracts of the violet tree.

The present study has shown that Cd was detected in the root bark of *S. longipedunculata* (0.0002 ± 0.00 mg/kg) and leaves of *G. senegalensis* (0.0004 ± 0.00 mg/kg), but these values didn't exceed the permissible limit of Cd in medicinal plants

or herbal drugs. The permissible limit of Cd for medicinal plants was recommended to be 0.3 mg/kg [17], while the permissible limit of Cd in the European Pharmacopeia is 0.5 mg/kg [35]. Cd has been reported to be an extremely toxic metal which causes serious respiratory, liver and kidney problems. Also, accumulation of cadmium in human body causes cardiovascular, breast cancers, hemorrhagic injuries, anemia, nervous and bone diseases [18], fragile bones and osteoporosis in humans and animals [19].

The highest concentration of Mn was observed in the leaves of *G. senegalensis* (0.1890 ± 0.03 mg/kg); however, the concentrations of Mn in all three plant species tested didn't exceed the recommended limit [17].

Interestingly, Cr and As were not detected in all the plant species analyzed and only a trace amount of Pb (0.0010 ± 0.00 mg/kg) was detected stem bark of *B. dalzielii* which was far below the recommended limit [17]. The principal toxic effects of Pb are neurological effects, hematopoietic system damage and reproductive effects, while As is known to cause lung cancer and skin diseases [18].

## Conclusion

The study has shown that the most common toxic metals (Pb, Cr and As) were not detected in the root bark of *S. longipedunculata* and leaves of *G. senegalensis*. Also, the level of Pb detected in the stem bark of *Boswellia dalzielii* and level of Cd detected in the root bark of *Securidaca longipedunculata* and leaves of *Guiera senegalensis* were below the recommended limits. It is recommended that the World Health Organization and other relevant authorities should establish universally accepted values for safe levels of all heavy metals in medicinal plants and herbal products.

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