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Proximate and Phytochemical Analysis and Levels of Heavy Metal (Pb, Cd, Cr, Ni and Cu) of Some Medicinal Plants Collected from Gombe State, Nigeria

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Abstract: *Medicinal plants are by far the crucial source of life saving drugs for nearly 80% of world's population. Proximate analysis is carried out with a view to determining macronutrients contents in a plant, compound or feed. Studies available on ethnomedicinal plants have indicated that potent phytochemicals capable of mitigating many health problems can be developed. Medicinal plants are thought to contain reasonable amounts of heavy metals that may constitute health hazards. This study aims at evaluating the proximate and phytochemical compositions as well as levels of Cadmium (Cd), Lead (Pb), Chromium (Cr), Nickel (Ni) and Copper (Cu) of medicinal plants including Senna singuena, Nymphaea lotus, Cochlospermum planchoni and Acacia nilotica. The plants extracts were prepared using cold aqueous maceration method. The proximate analysis for each plant was conducted using standard procedure; preliminary phytochemical screening was carried out on each extract using method of Trease and Evans; and assay of heavy metal was quantitatively measured using atomic absorption spectrophotometer. S. singuena was investigated to have the lowest moisture content (5%), highest fat and crude protein (3.9 and 39.5%), S. singuena and N. lotus have same carbohydrate content (41%), N. lotus has the highest ash content (20%) while C. planchoni was found to have the highest fiber content (42%). Preliminary screening of phytochemicals of the plant extracts revealed different classes of secondary metabolites including saponons, phenolics, phlobatanins, quinines, alkaloids, tannins, anthroquinones, steroids, flavonoids, terpenoids and glycosides. Cd level in S. singuena and N. lotus was found to be above the permissible limit recommended by WHO. Ni and Cu levels in the four plants were found to be below the WHO recommended permissible limit in the plants except the Ni level of A. nilotica that resulted to be above WHO permissible limit. Conversely, Pb and Cr were absent in all the plants assayed. All together, S. singuena has the lowest moisture content and highest crude protein and fat; C. planchoni has the highest fiber content and Cd was found to be totally absent; S. singuena and N. lotus contain toxic level of Cd; the four plants contain different secondary metabolites which may be responsible for their ethnomedicinal usage.*

Keywords: *Heavy Metals, Medicinal Plants, Phytochemicals, Proximate.*

INTRODUCTION

Nature has been reported for thousands years to be a source of ethnomedicinal agents and an impressive number of drugs used today were derived from natural sources (Owolabi *et al.* 2007). A significant proportion of individuals and communities in developing countries depend on traditional medicines for their primary health care needs (WHO, 2002). For example, the vinca alkaloids (vincristine, vinblastine and vindesine), derived from *Catharanthus roseu,* have been employed for their anticancer properties (Builders *et al.* 2012). The medicinal plants recruited in this study are *S. singuena, N. lotus, C. planchoni and A. nilotica. Senna singuena* (Delile) Lock belongs to the family fabaceae. It is a widespread plant in semi-arid parts of tropical Africa. It is usually found in savanna and abundantly available where shrubs used to grow; its common name is Winter Cassia and is called Rumfu and Lotorki in Hausa and Fulfulde respectively. *Nymphaea lotus* belongs to the family of Nymphaeaceae, it is an aquatic flowering plant, native to Egypt, central and west Africa, and Madagascar. It is called water lily or white Egyptian lotus; Bado and Badoje in Hausa and Fulfulde respectively. Cochlospermum planchoni is a perennial plant with a woody subterranean found in savanna and forest savanna. It belongs to the family Bixaceae. It is called False cotton; Rawaya and Feru in Hausa and Yoroba respectively. Acacia nilotica belongs to the family fabaceae, it is usually found in regularly flooded areas. Its common name is gum Arabic tree and Bagaruwa in Hausa (Usman *et al.*, 2018).

Infusion of *S. senguena* is used in treating malaria, powdered plant is used in treating wounds caused by leprosy and syphilis (World Agroforestry Centre, 2006). The root is used in treating Bilhazia and impotence caused by diabetes mellitus (Sepasal, 2006). Nymphaea lotus is used as an aphrodisiac, anodyne, astringent, sedative, demulcent, pain killer and anti-inflammatory (Madhusudhanan *et al.*, 2011). The plant was reported to have calming and sedative effects and therefore used for the treatment of insomnia, anxiety and other related disorders. Rhizomes and leaves of Cochlospermum planchoni are used in the treatment of jaundice, diarrhea, stomach disorder and urinary tract infections (Yakubu *et al.*, 2010). The root extract of the plants possesses CNS depressing activity (Anaga & Opara, 2009), and is used to treat Schistosomiasis, intestinal worms and typhoid fever (Togotla *et al.*, 2008).

S. singuena was reported to have anti-oxidant, anti-inflammatory, anti-cancer, anti-pyretic, anti-syphilis, anti-ulcer and antibacterial activity against both gram positive and negative bacteria (Sepasal, 2006). Many biological activities, including anticancer and antiviral activities of N. lotus have been attributed to gallic acid and ellagic acid isolated from the plant (Adebayo *et al.,* 2009). *C. planchoni* was reported to have antibacterial, antimalarial, antityphoid, antidiabetic, anti-inflammatory, analgesic, and anti-hyperglycemic activities (Anaga and Opara, 2009). The published reports of the various biological activities of A. nilotica include anti-inflammatory, hypoglycemic and anti-tumor activities (Lam & Ng, 2010); anti-fungal (Beltramini *et al.*, 2009); antiplatelets aggregation, antihypertentive and antihepatitis activities (Lee *et al.*, 2011); anti-oxidant activity; wounds healing and anti-nociceptive activities (Kumar *et al.*, 2016). Hepatoprotective activities of *S. singuena, N. lotus, C. planchoni and A. nilotica* were formally reported by Usman *et al.* (2018).

Although a lot of studies on *S. singuena, N. lotus, C. planchoni and A. nilotica* have been conducted as extensively cited above, to the best of our knowledge, their nutritive and phytochemical contents as well as levels of Pb, Cd, Cr, Ni and Cu have not been documented nor reported in the study area. Hence, this study aims at evaluating these gaps by taking into cognizance the analysis of heavy metals, phytochemicals and proximate composition of the abovementioned plants.

Materials and Methods

Plants collection, identification and authentication

Fresh leaves of *S. singuena* and *N. lotus* were collected from the western part of Kashere town, Akko local Government and Wuro ibba Dam, Kwami Local Government, Gombe State, Nigeria respectively in December and January, 2017. Root of *C. planchoni* and the pod of *A. nilotica* were collected from Kashere and Bajoga towns respectively, all in Gombe State in December and January, 2017. The plants were identified and authenticated by a taxonomist, Dr. Kolawale Opeyemi Saheed of the Department of Biological Sciences, Federal University, Kashere, Gombe State, where voucher specimen No. was prepared and deposited at the Herbarium of Federal University, Kashere (FUKH) for future reference. Batch/code number for all the plant samples were issued by Mr. Umar Galadima, (Head of Biological laboratory, FUK) as follows: *Senna singueana* leaf; FUKH077, *Nymphaea lotus* leaf; FUKH078, *Cochlospernum planchonmi* root; FUKH076 and *Acacia nilotica* seed; FUKH079.

Preparation of Plants Extract

The fresh leaves of *Senna singuena* and *Nymphaea lotus,* root of *Cochlospermum planchoni* and the pods of *Acacia nilotica* were washed and shade-dried until constant weights were obtained. The plants samples were grinded into fine powdered forms. The powdered forms were poured into Bama bottle for each sample and labeled. Three hundred and fifty grams (350 g) each of *S. singuena, N. lotus, C. planchoni* and *A. nilotica* powdered form was soaked in 1 L of distilled water, shaken for three minutes and then allowed to stay for three days. The mixtures were filtered with Whattman No. 1 filter paper on the third day. The filtrates were evaporated to dryness for two weeks using water bath evaporator at 40 ^{o}C.

Proximate Analysis

- **Sample Preparation for Proximate Analysis**

The plant samples collected were air dried at room temperature for one week. The dried sample of each plant was chopped into smaller pieces and ground into fine powder using pestle and mortar and this was stored in an airtight container with proper labelling prior to use. They were then subjected to proximate analysis to determine the proximate compositions and mineral contents of each plant.

- **Method of Proximate Analysis**

Proximate parameters: carbohydrate, fats, protein, fiber and ash of the plants under study were obtained using the Association of Official Analytical Chemists (AOAC, 2005) method. The percentage nitrogen of the plant extracts was determined by micro-Kjeldhal method. The value of nitrogen obtained was multiplied by 6.25 leading to its conversion to crude protein thus:

$$\%N = 14 \text{ X } VA \text{ X } N \text{ X } W/100$$

Then,

$$\% \text{ Crude Protein} = 6.25 \times \%N$$

Where

14 = atomic weight of Nitrogen

VA = volume of acid used

N = Normality of HCl

W= Weight of the sample (2 g)

V = Volume taken for distillation

The weight difference methods were used to determine moisture and ash content levels of plants under study as follows:

The percentage moisture was calculated by the following formula:

$$\%\text{Moisture} = (W2 - W3)/ (W2 - W1) \times 100$$

Where:

W 1 = Initial weight of empty dish

W2 = Weight of dish + sample before drying

W 2 = Final weight of dish + Sample after drying

% Total solid (dry matter) = 100 – % moisture

The formula for the calculation of ash content is given below:

$$\% \text{ Ash} = (W3 - W1)/ (W2 - W1) \times 100$$

Where:
W1= weight of empty crucible
W2= weight of crucible + sample before ashing
W3= weight of crucible+ ash
The crude fat and fiber contents of the plants were determined using the AOAC (2003) procedure with petroleum ether as solvent thus:
The percent crude fat was determined by using the formula below:

$$\%\text{CrudeFat} = \frac{\text{Wt. of ether extract } \times 100}{\text{Wt. of sample}}$$

The percent crude fiber was calculated using the formula below:

$$\%\text{Crude fiber} = \frac{(W_1 - W_2)\ \times 100}{W_0}$$

The carbohydrate content was determined by calculation using the method belows:

$$\text{NFE} = 100 - (\text{crude protein} + \text{moisture} + \text{Ash} + \text{crude fat})$$

- **Procedure of Phytochemical Screening**

Preliminary phytochemical screening of the plants under study was conducted in accordance with the method of Trease and Evans (2002). The plant extracts were screened for the presence and/or absence of alkaloids, phenolics, phlobatanins, terpenoids, glycosides, saponins, quinines, anthroquinones, steroids, flavonoids and tannins.

Determination of Heavy Metals

Determination of heavy metal was done using Atomic Absorption Spectrophotometer (AAS) after digesting the plant samples using Hydrochloric acid (HCl) and Sodium hydroxide (NaOH) using the method described by Ibrahim *et al.* (2016). The procedure includes: 2 g of the plant sample was placed into the crucible and ashed for 2 hours using furnace maintained at 400 ^{0}C. 5 mL of 1 M of Nitric acid (HNO_3) solution was added to the ash contained in the crucible, followed by evaporation to dryness using hot plate at a low heat under ventilation. The sample was then returned to furnace and heated at 400 ^{0}C for ten minutes and perfectly white ash was obtained. The sample was again cooled using desiccator before adding 10 mL of 1 M of HCl and then the solution was filtered in to 50 mL volumetric flask. The crucible and the filter paper were washed with additional 10 mL of 1 M HCl three times and the volume was made up to 100 mark with distilled water. The filtrate was then transferred into sample bottle, ready for analysis. The filtered was send to Biochemistry Laboratory of Gombe State University for AAS test. Usually, the sample solution was nebulized (to convert liquid in to fine spray of aerosols, by means of oxygen, compressed air or ultrasonic vibration) using nebulizer. Elemental ions are then atomized and the atoms formed absorbed radiation of the characteristic wavelength from the hollow-cathode lamp. The absorbance measured, were proportional to the concentration of the analyte in the sample solution (Ibrahim *et al.*, 2016).

Statistical Analysis

Descriptive and explanatory statistical data analysis was carried out.

Results

Table 1 shows the results of proximate compositions of the plants under study. Proximate analysis revealed that *S. singuena* has the lowest moisture content (5.0%) when compared with *N. lotus* (8.00%), *C. planchoni* (9.0%) and *A. nilotica* (9.0%), in contrast, *N. lotus* (20.0%) has the highest ash content when compared with *S. singuena* (6.0%), *A. nilotica* (5.0%) and *C. planchoni* (4.0%). Crude protein and lipid contents of *S. singuena*, *N. lotus*, *C. planchoni* and *A. nilotica* were virtually the same except *S. singuena* (3.9% and 39.5%) which was found to be slightly higher for crude protein and lipid respectively. *C. planchoni* was found to have the highest fiber content of 42.0% while its carbohydrate content (9.98%) was the lowest when compared with *S. singuena*, *N. lotus* and *A. nilotica*. Based on our findings, it can be deduced that *S. singuena* has the highest nutritive value as it has the highest crude protein, lipid and carbohydrate as well as lowest moisture content compared to other plants.

Table 1: Proximate composition (%) of *Senna singuena, Nymphaea lotus, Cochlospermum planchoni and Acacia nilotica.*

Plant sample	Moisture (%)	Ash (%)	Crude protein (%)	Crude lipid (%)	Crude fiber (%)	Carbohydrate (%)
S. singuena	5.0	6.0	3.9	39.5	4.0	41.5
N. lotus	8.0	20.0	2.3	24.5	4.0	41.2
C. planchoni	9.0	4.0	2.0	33.0	42.0	9.9
A. nilotica	9.0	5.0	2.1	35.5	9.0	39.4

Table 2 shows the results of preliminary phytochemical screening of the plants under study. Saponins, phenolics, tannins, glycosides, alkaloids, terpenoids, anthraquinones, steroids, phlobatanins, flavonoids and quinines were screened and out of the eleven (11) phytochemicals screened, *C. planchoni* contained ten (10) followed by *S. singuena* (9)*, N. lotus* (8) and *A. nilotica* (7), even though one or more phytochemicals were found to be absent. This shows that *C. planchoni* has the highest number of phytochemicals and may have more pharmacological activities compared to other plants.

Table 2: Phytochemical Constituents of *Senna singuena, Nymphaea lotus, Cochlospernum planchoni and Acacia nilotica.*

Phytochemicals	S. singuena	N. lotus	C. planchoni	A. nilotica
Saponins	-	-	+	+
Phenolics	+	+	+	-
Phlobatanins	-	-	+	-
Quinines	+	+	-	-
Tannins	+	+	+	+
Terpenoids	+	+	+	+
Glycosides	+	+	+	+
Alkaloids	+	+	+	+
Anthroquinones	+	-	+	+
Flavonoids	+	+	+	-
Steroids	+	+	+	+

KEY: – = Absent; **+** = Present

Table 3 presents the levels of heavy metals (Pb, Cd, Cr, Ni and Cu) contained in the plants under study. The four plants used in the study were found to be non-toxic in relation to the levels of Cu, Ni, and Cd; though Cd level in *S. singuena* (1.50mg/Kg), *N. lotus* (1.00 mg/Kg) and *A. nilotica* (0.50 mg/Kg) were found to be slightly above the maximum permissible limit recommended by WHO. Equally, the Ni level in *A. nilotica* (15 mg/Kg) was resulted to be above the WHO maximum permissible limit. Importantly however, none of the plant samples contained Pb, and Cr. This shows that long-term ingestion *S. singuena*, *N. lotus* and *A. nilotica* of may result in cadmium-induced toxicity. This finding will be substantiated by doing preclinical sub-chronic and chronic toxicity studies.

Table 3: Heavy Metals Composition of *Senna singuena, Nymphaea lotus, Cochlospernum planchoni and Acacia nilotica.*

Plant sample	Lead (mg/kg)	Cadmium (mg/kg)	Chromium (mg/kg)	Copper (mg/kg)	Nickel (mg/kg)
S. singuena	ND	1.50	ND	1.05	7.00
N. lotus	ND	1.00	ND	3.65	7.50
C. planchoni	ND	ND	ND	1.25	8.00
A. nilotica	ND	0.50	ND	1.35	15.00

ND = Not detected

Discussion

Proximate analysis refers to the determination of major constituents/compositions of a feed and is valuable in assessing the nutritive status of a feed or plant materials. This analysis involves determination of percentage ash, moisture, crude protein, fat, crude fiber and carbohydrate. The percentage moisture of the root of *C. planchoni* (9%) and the pod of *A. nilotica* (9%) was found to be higher than that of the leaf of *S. singuena (5%).* The moisture content of the leaf of *N. lotus* is slightly lower (8%) than that of the root of *C. planchoni* and the pod of *A. nilotica.* From these findings, *S. singuena* has the lowest moisture content and this indicates that its shelf life will be longer than the other plants, and also implies a great economic importance since high moisture content is associated with increase in microbial activities during storage (Abdullahi *et al.,* 2016), thus, the leaf of *S. singuena* would be least susceptible to microbial attack, unlike the root of *C. planchoni* and the pod of *A. nilotica*, which have the highest moisture content; and as such, are more prone to microbial growth particularly fungi as noted during drying of the root of *C. planchoni*. High moisture content also promotes susceptibility to enzymes activity in a given plant sample (Aliyu *et al.,* 2009). This may cause rapid decomposition of the important phytochemicals. Moreover, moisture content levels over 8% favour the presence of insects, and over 14% there is risk of contamination by fungi and bacteria (Cockerell *et al.,* 1971), henceforth, reducing the economic importance of the index plant. With regard to ash content, the leaf of *N. lotus* was found to have highest ash content (20%), followed by the leaf of *S. singuena* (6%) and the pods of *A. nilotica* (5%). The root of *C. planchoni* was found to have the lowest ash content (4%) which is thought to result in high moisture content of the plant (Abdullahi *et al.,* 2016). This finding was corroborated with the proximate values obtained by Oyeleke (1984) and Abdullahi *et al.* (2016). Looking at the percentage composition of crude protein of the plants used in this study, the leaf of *S. singuena* was found to have the highest crude protein (3.99%) followed by the leaf of *N. lotus* (2.26%) and the pods of *A. nilotica* (2.09). The root of *C. planchoni* was found to have the lowest protein content (2.02%). High crude protein content may be a probable indicator of nutritional superiority in general and relatively, in this research, it is an indication of the nutritional superiority of the leaf over the root (Wasagu *et al*., 2014).

With regard to the crude lipid/fat content, the leaves of *S. singuena* have the highest fat (39.50%) followed by the pods of *A. nilotica* (35.50%) and the root of *C. planchoni* (33.00%). The leaves of *N. lotus* were found to have the lowest fat content (24.50%). Three of the plant samples used (*S. singuena, A. nilotica and C. planchoni*) have high fat content when compared with the reported values (8.3 – 27 %) in some vegetables consumed in Nigeria and Republic of Niger (Senna *et al.,* 1998), proving that they can also add weight/help in building the body apart from their ethnomedicinal uses. The crude fiber content of the root of *C. planchoni* was found to be highest (42%) of all other three plants, followed by that of the pods of *A. nilotica* (9 %). The crude fiber of the leaves of both *S. singuena* and *N. lotus* was found to be the same (4.00%) which are far lower than that of the *C. planchoni* (42%) and slightly lower than that of *A. nilotica* (9%). From the findings of this research, *C. planchoni* has the highest crude fiber and this can be clearly observed from the root of the plant which is highly fibrous in nature. High fiber content in any plant/food will support regular bowel movement and help maintain normal cholesterol and blood sugar levels as well as protect against heart disease and certain types of cancer (WHO, 2008; Wasagu *et al.,* 2014). *C. planchoni* can also facilitate effective digestion of food and defecation process smoothly. Carbohydrate content estimated as nitrogen free extract was found to be higher in the leaves of *S. singuena* and *N. lotus* (both 41.0%), followed by the pods of *A. nilotica* (39.4%) and root of *C. planchoni* (9.98%). Growing plants store very little carbohydrate (Osagie, 1992). This shows that the leaves of *S. singuena* and that of *N. lotus* are good sources of carbohydrate, in effect, they can provide energy to the body in addition to their numerous ethnomedicinal uses.

Medicinal plants are useful for treatment as well as for prevention of human diseases because of the presence of phytochemical constituents (Nostro *et al.*, 2000). Naturally occurring compounds derived from plants such as roots, stem, leaves and vegetables that have defense mechanism and capable of protecting humans from various diseases are termed as phytochemicals and are by far divided into primary and secondary compounds. Chlorophyll, proteins and carbohydrates are primary constituents, in contrast to secondary ones which involve terpenoid, alkaloids, flavonoids, tannins, phenolics, among others, (Krishnaiah, *et al*., 2007).

Preliminary screening of phytochemicals of the plants under study revealed that *C. planchoni* contained saponins, phenolics, tannins, glycosides, alkaloids, terpenoids, anthroquinones, steroids, phlobatanins and flavonoids while quinines was found to be absent. *S. singuena* contains alkaloids Phenolics, quinines, terpenoids, steroids, tannins, glycosides and flavonoids while phlobatanins and saponins were resulted to be absent. *N. lotus* contains glycosides, tannins, terpenoid, alkaloid, steroid, phenol, quinines, flavonoid, while saponins, phlobatanins and anthroquinones were absent. *A. nilotica* contains tannins, glycosides, saponins, alkaloids, anthroquinones, terpenoids and steroids while phenolics, phlobatanins, quinines and flavonoids were absent. The phytochemicals contained in these plants may be attributed to their ethnomedicinal values.

The concentration of Cadmium in *S. singuena* and *N. lotus* was found to be above the permissible limit recommended by WHO which is 0.02 mg/kg (1996). Likewise, the concentration in *A. nilotica* is slightly above the permissible limit. Long-term ingestion of these plants may result in accumulation of cadmium to toxic level. Cadmium was reported to be highly toxic metal that could be detrimental to biological systems at doses that are much lower than most heavy metals (Nazir *et al.,* 2015). Cadmium accumulates irreversibly in the human body most notably in the kidneys and other vital organs such as the lungs and the liver (Jarup *et al.,* 1998; Bernard, 2004). *C. planchon*i does not contain Cadmium. The copper concentration in all the plants samples (1.05 – 3.65 mg/kg) was below the permissible limit recommended by the WHO, which is 10mg/kg (WHO, 1996). This shows that the plants recruited in this study are free of copper toxicity. The Nickel concentration in all the plants ranges between 7.5 mg/kg and 15 mg/kg. The permissible limit of Nickel according to WHO standard is 10 mg/kg (Nazir *et al.,* 2015). Lead and Chromium were absent in all the four plants used in this study.

Conclusion

The proximate and phytochemical compositions of the plants used in this study were determined. *S. singuena* was found to have the lowest moisture content and highest crude protein and fat; *S. singuena* and *N. lotus* contained toxic level of Cadmium; the four plants contained different secondary metabolites which may be responsible for their ethnomedicinal usage.

Conflict of interest

The authors declare that they have no conflict of interest.

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