Comparative Determination of Minerals and Phytochemical Constituents in the Leaves of *Guierasenegalensis* from Selected Areas

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Abstract
Guierasenegalensis is a shrub found in the savannah region of West and Central Africa that is widely used in traditional medicine for the remedy of many ailments and diseases. In this study, some of the phytochemical and mineral elements present in its leaf sample were qualitatively and quantitatively analyzed. The phytochemical screening carried out on G. senegalensis leaf samples of some selected state in Northern part of Nigeria (Kano, Bauchi and Jos) gave a positive result for alkaloid, tannins, saponins and flavonoids. The quantitative analysis revealed that the alkaloid and tannin content (17.0mg/kg and 8.60mg/kg) of the Jos' G. senegalesis is higher than that of Bauchi (15.8mg/kg and 5.50mg/kg) and that of Kano (15.8mg/kg and 5.20mg/kg). In terms of flavonoids content that of Kano (24.0mg/kg) has the highest, followed by that of Jos (23.2mg/kg) andthen that of Bauchi (20.6mg/kg). The result of the elemental analysis for the three states indicate that G. senegalensis leaf contain significant amount of essential mineral elements. Their quantities are in the decreasing oder: K > Mg >Na >Fe >Ca >Mn >Zn > Cu and this oder is the for all the three states . The result of this study has justified the widespread usage of G. senegalensis leaves as traditional medicine and that the plant has a promising potential in both traditional and orthodox medicine.

Introduction

Guierasenegalensis (family *combretaceae*) commonly known as *Sabara* in Hausa, *Kashishi* in Tawun, *ShatiPitu* in Margi and *Tadar* in Tangale languages (Habila, 1999), is a shrub of the savannah region of west and central Africa; it is widely being used in traditional medicine to cure many ailments and diseases. Its leaves extract is being used to cure diseases such as *dysentery, diarrhoea, gastrointestinal pain* and *disorder, rheumatism and fever* (Sule and Mohammad, 2006). Partially purified anthocyanin fraction from leaf extract of *G. senegalensis* has been shown to possess antioxidant property against tetrachloromethane induced oxidative stress in rats.(Sule and Muhammed, 2009). *Guierasenegalensis* and pillostigmareticulalum commonly co-exist with crops in the farmer's field throughout the Sahel and their presence can potentially provide more organic inputs to cropped fields than any other source by the traditional burning of residues at the beginning of each cropping season (Sule and Muhammed, 2009). However, non thermal management of these organic material hold potential to add organic matter to soils and thus, be a source of nutrients such as nitrogen and phosphorous (Dossa *et al.*, 2009). *Guierasenegalesis* is said to provide ecological benefits to soils and also showed to dramatically increase crop productivities (in some cases 50%) particularly in the Northern sahel region

(Dossa*et al.,* 2009). Furthermore is locally available resources that can provide crop yield responses even with low or no fertilizer applications, thus making its co-existence with crops in the farm well suited for subsistence and low inputs farmers (Dossa*et al.* 2012). The term crude drug of natural origin is used by pharmacist and pharmacologist to describe whole plant or plant part, which have medicinal properties (Sofowara *et al.,* 1984). There are hundred for chemical substance that have been derived for use as drugs and medicine. Medicinal plant are therefore defined as plant or plant part used for the treatment of ailment through methods like decoction, infusion or concoction (Sofowara, 1984) Phytochemicals are bioactive non-nutrient chemical compounds found in plant that work with nutrients and dietary fibre to protect against diseases (Johanna and Jyl-lurn, 2007: Agbafor and Nwachukwu, 2011). They are secondary metabolites that contribute to flavor and color.(Agbafor and Nwachukwu, 2011).Many phytochemical have antioxidant activity and reduse the risk of many disease (Agbofor&Nwachukwu, 2011). Phytochmicals are many and can be categorized into various groups that is polyphenols, organo sulfur compounds, alkaloid and nitrogen containing compounds. The polyphenols are some of the most studied compound and can be further divided into flavonoids (including flovonols, flavones, catechins, flavonones, anthocyanidins and isoflavones), Phenolic acids, stilbenes, coumarins and tannins, (Johanna and Jyh- Lurn, 2007). *Guierasenegalensis* has been shown to positively contain alkaloid, tannins, flavonoids, amino acids, ascorbic acid, and anthraquinones and also displayed anti microbial activities (Sule *et al.,* 2002). Human body requires both metallic and non-metallic elements for healthy growth, development and the proper functioning of the body. Many elements present in the food at major, minor and trace levels are reported to be essential to man's well being. However, their ingestion in excess or limited amount can cause severe health problems (Kumar *et al.,* 2005; Mohammad and Sulaiman, 2009). The determination of these elements in beverages, water, food and soil is thus of utmost importance and is currently the subject of studies by various researchers (Saud Al-Qud, 2003; Mohammed and Sulaiman, 2009).

Justification

As a result of frequent uses of this medicinal plant (*Guierasenegalensis*) especially in northern part of Nigeria, studies by many researchers has shown that the plant leaves contains some phytochemical and mineral elementswhich are responsible for its medicinal properties. The content of these phytochemical and minerals varies with the vaiations of soil and climatic conditions, hence there is need to determine and compare the different contents of their medicinal attributes. This work is planned to determine the concentration of phytochemical (Alkaloids, Tannins and flavonoids) and some of the mineral constituents in the leaves' sample of *Guierasenegalensis* from some selected states in Northern Nigerian. The aim of the research is to determine the qualitative and quantitative phytochemical and mineral elements composition of *Guierasenealensis* leaf samples.

Material And Methods

Sample Collection and Identification

The leaves of the *Guierasenegalensis* used for the study was collected from Gwarzo local government area of Kano state, Kafin-Tafawa in Bauchi state and Narakuta in Jos local government area of Plateau state. The plant was identified in the Department of Biological Sciences Bayero University Kano (B.U.K.).

Sample Preparation

The leaves of the plant were allowed to dry at room temperature under shade (away from the sun) for 2 weeks and then the dried leaves were crushed into powdered form using ceramic pestle and mortar. The dried powder was then used for the analysis as samples A (Kano), B (Bauchi) and C (Jos).

Phytochemical Screening

Qualitative Analysis

Test for Alkaloids

About 0.2g of the powered sample was warmed with 40ml of 2% H_2SO_4 for 2 minutes, it was filtered and a few drops of dragendroffs reagent were added, orange red precipitates indicates the presence of alkaloids (Harbone, 1973).

Test for Tannins

About 0.5g of the dried powdered sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration to indicate the presence of tannins (Harborne,1973).

Test for Flavonoid

A portion of the powdered leaf sample was heated with 10ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colorations was observed indicating a positive test for flavonoids (Sofowara, 1995).

Quantitative Determination

Alkaloid Determination

Precisely 5.0g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol added and allowed to stand for 4 hours. This was filtered and the extract was concentrated in a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with 20ml of dilute ammonium hydroxide and then filleted. The residue is the alkaloid which was dried and weighed (Harbone, 1973) Method

Flavonoid Determination

About 10.0g of the powdered sample was extracted rapidly with 100ml of 90% aqueous methanol at room temperature. The whole solution was filtered through Whatman Filter Paper No. 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight (Bohm and KocipalAbyazan, 1994).

Tannin Determination

About 0.5g of the sample was weighed into 50ml plastic bottle. 50ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into 50ml volumetric flask and made up to the mark. Then 50ml of the filtrate was pipette out into a test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120nm within 10minutes(Van-Burden and Robson, 1981)**.**

Elemental Analysis

Determination of some mineral element concentration was conducted using Atomic absorption spectrophotometer (AAS) and flame photometer (for Na^+, K^+ and Ca^{2+}).

Dry Ashing Procedure

Dry ashing is suitable for the determination of Ca, Fe, Li, Mg, Mn, Na, K, and Zn in plant tissue and may be applicable to the determination of other elements as well except the volatile element like As and Se.

Procedure for Digestion of the Sample

Precisely 1.0g of the grounded powdered sample was put into crucible and ashed in a muffle furnace at 500^{o}C for 4 hours and then it was allowed to cool and the ash is then dissolve in 5ml of 20% (2M) Hydrochloric acid. The solution was filtered and washed into a 50ml volumetric flask and then dilute to volume with distilled water and was allowed to cool before the elemental analysis using the AAS and flame photometer.

RESULT

The following results were obtained during the experimental analysis.

Table 1: Qualitative photochemical analysis of *Guierasenegalansis* .

Sample	Alkaloids	Tannin	Flavonoids
A	+	+	+
B	+	+	+
C	+	+	+

Key: + = Present (Positive) - = Absent (Negative), A = Kano B = Bauchi, C = Jos

Table 2: Quantitative phytochemnical analysis of *Guierasenegalensis*.

Sample	Alkaloids (Mg/kg)	Tannin (mg/kg)	Flavonoids (mg/kg)
A	8.40	5.20	24.0
B	15.8	5.50	20.6
C	17.0	5.60	23.2

Key: A = Kano B = Bauchi C = Jos

Table 3: The mean concentrations (mg/kg) of mineral element in the *Guierasenegalensis* leaf samples from some selected state in northern Nigeria

Samples	Zn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg)
A	21.52	13.23	276.04	24.00	212.50	900.00	822.58	2012.50
B	16.66	11.76	247.91	23.00	237.50	800.00	874.193	1857.50
C	18.75	10.29	266.67	22.00	225.00	850.00	645.16	1675.00

Key: A = Kano B=Bauchi C=Jos

Discussions

The results of this work were presented in table 1, 2, and 3. Table 1 show the result for the qualitative phytochemical analysis of *G. senegalensis* leaf samples A (kano), B (Bauchi) and C (Jos) . The result of the analysis indicates the presence of alkaloid, tannin, and flavonoid which agrees with the earlier reports that these plants contain such phytochemicals. (Sule *et al*, 2002). These classes of phytochemicals are known to have curative activity against several diseases and therefore could suggest their use traditionally for the treatment of various ailments. (Hassan *et al*, 2004, Usman and Osuji, 2007). The quantitative phytochemical analysis of the *G. senegalensis* leaf sample presented in table 2, showed that the alkaloid and tannins content of the *G. senegalensis* of Jos is the highest, followed by that of Bauchi and then Kano. The high content of alkaloid and tannins in *G. senegalensis* leaf sample also agrees with earlier reports that alkaloid concentration decreases in the roots with corresponding increase in the leaves parts, which suggest that mostly alkaloid are translocated from the root upward to the leaves and stem (Ralph and Gadner, 2003). *Alkaloids* are present in all parts of the plant. Three *alkaloids: harman, tetrahydroharman* and *harmalan* with antimalarial properties and a low *cytoxicity* had already been isolated and identified from leaves and roots of the plant. This might account for the prescription of decoctions of leaves to treat malaria (Somboro *et al*. 2011). Total *alkaloids* extracted from leaves were proven to have an *antitussive* activity. In terms of flavonoids content, the *G. senegalensis* of Kano, contains the highest content of flavonoid followed by that of Jos and then that of Bauchi. High concentration of flavonoid in plant is very important, because flavonoid and related polyphenols compound protect plant against microbial invasion (Harbome, 1994). The natural products that have received the greatest attention with regards to possible medicinal applications are the alkaloid and saponin. In addition, alkaloids and flavonoids were also reported to be responsible for antimicrobial properties in some ethno medicinal plants (Singh and Bhat, 2003). *Flavonoids* occurr in different forms. These forms are known to have *vascular* protective and *venotonic* effects, although (Somboro *et al*. 2011). Even so, this diverse occurrence of *flavonoids* in the plant parts might account for its use in the treatment of aches and pains and its *venolymphatic* effects. In addition, the *antiviral* effects of *flavonoids* extracted from *G. senegalensis* against several *DNA* and *RNA viruses* have also been described (Somboro *et al*. 2011). Several studies of *gallic* and *catechin tannins* compounds have been published. The presence of *tannins*, in particular *gallic tannins*, might explain the use of *G. senegalensis* to treat *respiratory tract* disorders and cough (Somboro *et al*. 2011). Certain *gallic tannins* possess an *antidiarrhoeal* activity, which might account for the use of *G. senegalensis* to treat severe *diarrhoea* and *dysentery*. It has been shown that *3,4,5-tri-Ogalloylquinic acid* isolated from *G. senegalensis* displays an *anti-HIV* activity (Somboro *et al*. 2011).

Table 3 shows the results of some mineral constituents determined in the leaves samples of *G.* senegalensis. The quantities in all the three states is in decreasing order ; K > Mg > Na > Fe >Ca>Mn> Zn > Cu., The variation in the concentration of the mineral elements from the different states is due to variation in the soil and climatic conditions in this areas. The results in table 3 also shows that potassium ion concentration in the (*G. senegalensis*) leaf samples from the three States (Sample A: 2012.50mg/kg), (Sample B: 1675.00mg/kg) and (Sample C: 1857. 50mg/kg) is higher than that of the other elements and Sample C has the highest among them. Potassium is important for reducing blood pressure and also increasing blood circulation as well as a preventive aid on general health. (Sulaiman and Muhammad, 2009). Potassium is one of the most important elements in the human diet, and it is the third most abundant element in the human body

after calcium and phosphorus (Weiner and Goss, 1986). The value of magnesium ion concentration obtained from the analysis of the Samples A, B and C' are (900, 850 and 800) mg/kg respectively. This relatively high Mg^{2+} content is expected from a leaf sample because of its presence in chlorophyll. Magnesium is an essential mineral element in biological system. It is present in every cell type in every organism (Muhammad and Suleiman, 2009). Adenosine triphosphate (ATP), the main source of energy in cells must be bound to a magnesium ion in order to be biologically active. Over 300 enzymes require the presence of Mg^{2+} for their catalytic action including all enzymes utilizing or synthesizing ATP or those that use other nucleotides to synthesize DNA and RNA. In plant, magnesium is necessary for synthesis of chlorophyll and photosynthesis. The Kano *G. senegalensis* has the highest Mg^{2+} concentration among them. The values obtained for magnesium in three different States are lower than that reported by Oladele and Oshodi (2007) and are higher than values obtained by Sulaiman and Mohammad (2009). Sodium is an essential mineral element in human that regulates blood volumes, blood pressure, osmotic equilibrium and pH. In plant, sodium is a micronutrient that aids in metabolism, especially in regeneration of phosphoenolpyruvates (PEP) and synthesis of chlorophyll (Sulaiman and Muhammad, 2009). The sodium ion content from the Samples A, B and C are (822.58, 874.19 and 645.16) mg/kg respectively. This shows that Sample B contains the highest content of sodium followed by Sample A and then Sample C. The results obtained for sodium are lower than that reported by Aderibigbe and Brown (1993) for water hyacinth and water lettuce. Iron, is a necessary trace element found in nearly all living organisms. It plays an important role in biological systems forming complexes with molecular oxygen in hemoglobin and myoglobin which are oxygen transport proteins in vertebrates (Muhammad and Sulaiman, 2009). The result obtained from iron content in the three samples are: Sample A (276.04) mg/kg which is the highest, followed by Sample C (266.67) mg/kg and then sample B (247.91) mg/kg having the lowest content.Iron is also an important constituent of cytochrome and certain non-hemeproteins useful in phagocytosis and for killing of bacteria by neutrophilis. Iron is associated with effective immune competence of the body.

Calcium which is the most common mineral element in the body helps in the transport of long chain fatty acids which aid in prevention of diseases, high blood pressure and other cardiovascular diseases (Muhammad and Sulaiman, 2009). The calcium content obtained are: sample A (212.50mg/kg) which is the lowest, sample B (237.50mg/kg) the highest and sample C (225.00mg/kg). The function of calcium is to acts in cooperation with phosphorous to build and maintain bone and teeth. The deficiency disease of calcium includes arthritis, high blood pressure, osteoporosis, constipation, lack of appetite etc. About 99% of calcium in the body is deposited in the bone and teeth and the remaining 1% is in the soft tissues, The value of calcium ion content obtained (Table 3) is higher than that reported by Oladele and Oshodi (2007) and also higher than that reported by Mohammad and Sulaiman, (2009).

The results obtained for manganese content in decreasing order are (table 3): Sample A (24.00mg/kg), Sample B (23mg/kg) and Sample C (22.00mg/kg). Manganese; the biochemical function of manganese are: it serve as a co-factor for several enzymes, this include arginase, pyruvate carboxylase, isocitrate dehydrogenase, superoxide dismutase. (Safyanarayana and Chakrapani, 2011). Manganese is required for formation of bone, proper reproduction and normal function of nervous system (Safyanarayana and Chakrapani, 2011). The concentrations of zinc obtained (in deacreasing order) are: Sample A (21.52mg/kg) ,Sample C (18.75mg/kg) and then Sample B (16.66mg/kg). The values obtained from the three samples are generally higher than (2.17mg/kg) reported by

Mohammad and Sulaiman (2009). Zinc is an essential mineral element of exceptional biological and public health importance necessary for plant, animals and microorganisms. Zinc is also found in nearly 100 specific enzymes. Enzymes with zinc atom in the reactive center are widespread in biochemistry such as alcohol dehydrogenase in humans. The concentration of zinc in plants varies based on levels of the element in soil (Mohammad and Sulaiman, 2007). Copper is essential to all living organism as a trace dietary mineral element. It is a key constituent of the respiratory enzymes complex; cytochrome C oxidase which is required in aerobic respiration (Muhammad and Sulaiman, 2009).
The copper ion concentration is lower than that of other element for each of the three samples. Sample A the highest has (13.23mg/kg), followed by sample B (11.76mg/kg) and then sample C (10.29mg/kg). The result obtained for the three samples are lower than the values reported by Aderigbe and Brown (1993) but higher than that reported by Tokusoglu and Unal (2003) and Saud and Al-Qut (2003).

Conclusion

Based on the result obtained from the *G. senegalensis* leaf samples; from Kano, (Sample A), Bauchi; Sample B and Jos; Sample C, it can be deduced that *G. senegalensis* has high concentration of alkaloids, flavonoids and low concentration of tannins. The result of phytochemical content determination (qualitative and quantitative) has therefore justified the reasons for the widespread usage of the plant in traditional medicine. The result of mineral element analysis for *G. senegalensis* leaf sample compares favourably with values obtained by previous researchers and thus indicated that the leaf contains significant amount of useful minerals element. It is therefore reasonable to accept that *G. senegalensis* is potential sources of active ingredient and antioxidant that could be useful in both traditional and orthodox medicine and that it is also a source of essential mineral elements that are helpful in maintaining good health.

Recommendations

The research work carried out showed that the *G. senegalensis* leaf samples collected from Kano, Bauchi and Jos are good sources of phytochemical and mineral elements. It is therefore reasonable to encourage the continous consumption of the plant by both human and animals for herbal medicine purposes. It is also recommended that further research on antidiabetic properties, proximate analysis etc. should be carried out on this medicinal plant.

References

Aghafor, K.W. and Nwachukwu, N. (2011), **Phytochemical Analysis and Antioxidant Property of Extract of *Vitexdoniana* and M*ucunapruriens***.Biochem. Res. Intem. 2011:1-4.

Aderibigbe, A.O. and Brown, A.A. (1993). Nutritive Characteristics of Two Tropical Aquatic Weeds for Ruminants. The *J. Agric.* 16817: 42 – 45.

Boham, B. A. and Kocipai-Abyazan, R. (1974). Flavonoids and Condensed Tannins from Leaves of Hawaiian *Vacciniumvaticulatum* and *V. calycinum*. *Pacific sci*. 48:458-463.

Dossa*et al.,* (2009).Carbon, Nitrogen and Phosphorus Mineralization Potential of Semiarid Sahelian Soil Amendent with Native Shrub Residues.*Geoderma* 148:251-160

Dossa*et al.,* (2012). Crop Productivity and Nutrient Dynamics in a Shrub (*Guierasenegalensis*) Based Farming System of the Sahel. *agron. J. 104: 1285 –*

1264.

Harbone, J. B. (1973). **Phytochemical Methods.** London, Chapman and Hall, Ltd. Pp. 49-188.

Hussain, I., Khattak, M. and Khan, K. (2011).Analysis of Heavy Metal in Selected Medicinal Plant from Air, Sweat and Peshawar. *Journal of Chemistry Soc. Paker.*33: 495 – 498.

Johanna, W. L, and Jyh-Lurn, C. (2007).Inter-individual Differences in Phytochemical Metabolism and Disposition. *Sem. Cancer Biol*. 17:347-353.

Saud, G. and Al-Qut (2003). Heavy Metal Contents in Tea and Herb Leaves.*Park. J. Biol. Sci. 6: 208 – 212.*

Satyararayana, U. and Chakrapani, U. (2011).**Biochemistry,** Published by Arunabha Sen. Books and Allied (P) Ltd.

Singh, B. and Bhat, T.K. (2003).Heavy Metal Contents in Tea and Herb Leaves. Pak. *J. Biol. Sci.* 6: 208 – 212.

Somboro A.A., Patel k., Diallo d., Sidibe L., Chalchart J.C., Figueredo G., Duckie s., Torin B., and Chalard P. (2011). An Ethnobotanical and Phytochemical study of the African Medicinal Plant *Guera senegalensis J.F. Gmel. Journal of medicinal plants research.* 5(9); 1639-1651.

Sule, M.S, Bichi, L.A, Atiku, M.K (2002). Antimicrobial are Preliminary phytochemical Screening of *Guierasenegalensis*, EuphoiaLaterflora and mitracapusscaber. *W. Afr.J. pharmacol. Drug Res.* 18:12-13.

Sule, M. C, and Mohammed, S. Y. (2006). Toxicological Studies on the Leaves of *Guierasenegalensis* and *Psidiumguajava* in Rats. Biol. Environ. *Sci.J. Trop.* 3(1):81-83.

Sule, M.S. Mohammad, S.Y. (2009). Potency of Partially Purified Anthocyamin from Leaf Extract of *Genegalensis*Agent Carbon Tetrachloride-Induced Lipoperoxidation in Rats.Bayero*J. Pure Applied Sci.* 2(2): 155 – 158.

Tokusoglu, O., Una, M.K. (2007). Biomass Nutrient Profiles of Three Microalgae: *Sirulinaplatensis, Chrorella vulgaris and Isochrisisgalbana. J. Food Sci.* 68 (4): 1144 – 1148.

Trease, G. E and Evans, W.C (1978).**A Textbook on Clarendon Press** Oxford W.K, Vol. 11, P.372.

Trease, G.E and Evans, W.C (1989).**Pharmacognsy.**11th ed. BrailliarTiridel Can. Macmillan Publisher.

Usman, H. and Osuji, J.C. (2007). Phytochemical and In Vitro Anti Microbial Assay of the Leaf Extract of New BoultiaLeavis. *African Journal of Traditional Medicine* (AM 4(4): 476 – 480.

Van-Burden, T. F, and Robinson, W.C (1981). Formation of Complexes Between Protein and Tannin Acid. *J Agric. Food chem.* 1:77.

Weiner, M.H. and Gossa, T. (1986). Mineral Nutrition for Immunity in; Maximum Immunity, Library of Congress Catalogue Publication, Prinket in USA. Pp. 110 – 116.