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Quantitative phytochemical and elemental analysis of *Guiera senegalensis* leaf extract

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Guiera senegalensis **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/diseases. In this study, some of the phytochemicals and elements present in its leaves were quantified. Alkaloids content was found to be 21.98 g/100 g, saponins 0.28 g/100 g and tannins 0.40 g/100 g. The results showed that alkaloids content is high while saponins and tannins contents are low. The results of the elemental analysis showed that the values of all the elements analyzed compares favorably with values obtained for other plants and thus indicated that *G. senegalensis* leaf contain significant amount of essential mineral elements. Their quantity is in the order Ca > K > P > Na > Mg > Fe > Zn > Cu. The results of this study has justified the widespread usage of *G. senegalensis* leaves as medicine traditionally and also showed that the plant has a lot of potentials in traditional and orthodox medicine.**

Key words: *Guiera senegalensis*, phytochemicals, elements, quantitative analysis.

INTRODUCTION

Guiera senegalensis (Family: Combretaceae) commonly known as Sabara in Hausa is a shrub of the savannah region of west and central Africa. *G. senegalensis* is widely being used in traditional medicine for the remedy of many ailments/diseases. Its leaves extract is being used against dysentery, diarrhea, gastrointestinal pain and disorder, rheumatism and fever (Sule and Mohammed, 2006). In addition, partially purified anthocyanin fraction from leaf extract of *G. senegalensis* has been shown to possess antioxidant property against ccl_4 – induced oxidative stress in rats (Sule and Mohammed, 2009). *G. senegalensis* and *Piliostigma reticulatum* commonly co-exists 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. Traditional management of these shrubs includes coppicing and burning of residues at the beginning of each cropping season, however, non-thermal management of these organic materials hold potential to add organic matter to soils and thus, be a source of nutrients such as nitrogen and phosphorus (Dossa et al., 2009).

G. senegalensis is said to provide ecological benefits to soils and also showed to dramatically increase crop productivity (in some cases 250%) particularly in the Northern Sahel region. Furthermore, it is a locally available resource 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, low input farmers (Dossa et al., 2012).

Phytochemicals are bioactive nonnutrient chemical compounds found in plants that work with nutrients and dietary fibre to protect against diseases (Johanna and Jyh-Lurn, 2007; Agbafor and Nwachukwu, 2011). They are secondary metabolites that contribute to flavor and

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color. Many phytochemicals have antioxidant activity and reduce the risk of many diseases (Agbafor and Nwachukwu, 2011). Phytochemicals are many and can be categorized into various groups that is, polyphenols, organosulfur compounds, alkaloids and nitrogen-containing compounds. The polyphenols are some of the most studied compounds and can be further divided into flavonoids (including flavonols, flavones, catechins, flavonones, anthocyanidins and isoflavones), phenolic acids, stilbenes, coumarins and tannins (Johanna and Jyh-Lurn, 2007). Their functions and mechanism of actions may include the following among others: antioxidant activity, hormonal action, stimulation of enzymes, interference with DNA replication and antibacterial properties (Sule and Mohammed, 2009).

However, *G. senegalensis* has been shown to positively contain alkaloids, tannins, flavonoids, amino acids, ascorbic acid, and anthraquinones and also displayed antimicrobial activity (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; Mohammed and Sulaiman, 2009). The determination of these elements in beverages, water, food, plants and soil is thus of utmost importance and is currently the subject of studies by various researchers (Saud and Al-Oud, 2003; Mohammed and Sulaiman, 2009)

MATERIALS AND METHODS

Sample collection and preparation

The leaves of *G. senegalensis* used for this study were collected from a bush around Sule-Tankarkar local government area of Jigawa state, Nigeria. The plant was authenticated at the Botany unit of Bayero University Kano. The leaves were allowed to dry at room temperature and then crushed into powder using laboratory mortar and pestle. The dried powdered leaves were then used for analysis.

Quantitative estimation of alkaloids

Five (5 g) of the dried powdered *G. senegalensis* leaves was extracted with 50 cm^3 of methanol. From the extract, 10 cm^3 was placed in 250 cm^3 separating funnel and 5 cm^3 of dilute H_2SO_4 and distilled water were each added. The extract was shaken twice with 10 cm^3 $CHCl_3$ (trichloromethane) containing 5 cm^3 of dilute H_2SO_4 and 10 cm^3 distilled water. The $CHCl_3$ layer was discarded after shaking and aqueous acidic layer was transferred to the content of first separating funnel. The extract was basified with ammonia solution and was shaken for 30 s. The alkaloids were completely extracted by successive portions of $CHCl_3$. The combined $CHCl_3$ extract was shaken with 5 cm^3 of water and was run through a plug of cotton wool previously moistened with $CHCl_3$. The content was covered with a little anhydrous sodium sulphate which was later washed in 5 cm^3 of $CHCl_3$. The filtrate was then placed into 25 cm^3 conical flask after which the chloroform was distilled completely, followed by the addition of 5 cm^3 neutral alcohol which was evaporated on a boiling water bath. The residue was further heated on boiling water bath for 15 min. The residue was dissolved in 2 cm^3 chloroform and 20 cm^3 N/50 H_2SO_4. The content was warmed to remove $CHCl_3$. The excess acid was titrated with N/50 NaOH using methyl red as indicator, a color change from pink to yellow was observed. The alkaloids contents of the sample were then calculated using the following formula:

Alkaloid content = Amount (cm^3) of N/50 NaOH × 0.005787 × 100 / 10 (g% w/v) according to Wasagu et al. (2005).

Quantitative determination of tannins

Five (5 g) of dried powdered *G. senegalensis* leaves were macerated in distilled water and allowed to stand overnight. The water extract (5 cm^3) was placed in a stoppered conical flask. A quantity (25 cm^3) of 0.1 N iodine and 10 cm^3 of 4% NaOH were added. The resulting mixture was kept in the dark for 15 min. Water (10 cm^3) was used to dilute the mixture and was acidified with 10 cm^3 4% H_2SO_4. The mixture was titrated with 0.1 N sodium thiosulphate solution and starch was used as indicator. The titration value corresponded to the sum of tannins and pseudo tannins-concentration A. Another 25 cm^3 of the water extract was placed in a stoppered conical flask followed by 15 cm^3 of 1% gelatin. The volume was made up with distilled water and filtered. Aliquot of 20 cm^3 was placed in a volumetric flask; 25 cm^3 of 0.1 N iodine and 10 cm^3 of 4% NaOH were added, mixed and kept in the dark for 15 min. The mixture was diluted with 10 cm^3 water and acidified with 10 cm^3 4% H_2SO_4. This was finally titrated with 0.1 N sodium thiosulphate using starch solution as indicator. The titration value obtained corresponded only to pseudo tannins - concentration B. A blank experiment was carried out simultaneously using distilled water. The tannins and pseudo tannins contents of the sample were then calculated using the following formula:

% of Pseudo tannins = Blank Expt. × 0.029 × 100 / 5

% of True tannins = A – B (g% w/v) according to Wasagu et al. (2005).

Quantification of saponins

Five (5 g) of dried powdered *G. senegalensis* leaves were macerated in methanol allowed to stand overnight. The mixture was then filtered to obtain precipitate that is, methanolic extract which was allowed to dry. The dried methanolic extract was then dissolved in water and partitioned with an equal volume of n – butanol. The n – butanol fraction was then collected and the aqueous layer was discarded. The n – butanol fraction was concentrated and dried. It was then dissolved in methanol and diethyl ether was then added drop wise to precipitate the saponins. The mixture was filtered with a pre-weighed filter paper. The precipitate corresponds to the quantity of the extracted saponins (Wasagu et al., 2005).

Elemental analysis

A 0.5 g of dried powdered *G. senegalensis* leaves were digested using 10 cm^3 of a mixture of concentrated HNO_3 and concentrated

HCl (3:1 v/v). Analytical grade reagents were used for the preparation of the standard solutions of these elements (Ca, Zn, Mg, Fe, Cu, Na, K, and P). The diluted digests were analyzed using atomic absorption spectrophotometer (AAS) for Ca, Cu, Fe, Mg and Zn, while flame photometer was used for Na, K and P.

RESULTS AND DISCUSSIONS

The results of this study are presented in Tables 1 and 2. Table 1 show the results of quantitative phytochemical analysis of *G. senegalensis* (GS) leaf extract and the result showed high content of alkaloids, low concentration of saponins and tannins. Table 2 shows the concentration of mineral elements determined for GS leaf extract. The results showed that potassium has the highest concentration while copper has the least concentration among the mineral elements analyzed.

The high content of alkaloids in GS leaf extract agrees with earlier reports that alkaloids concentration decreased in the roots with corresponding increase in the foliar parts, which suggest that alkaloids are translocated from the roots upward to the leaves and stems (Ralph and Gardner, 2003). The saponins and tannins content are relatively low when compared with values obtained from other plants; 0.386 and 0.456%, respectively (Soliz-Guerrero et al., 2002). The natural plant products that have received greatest attention with regards to possible medicinal application are the alkaloids and saponins. In addition, alkaloids and flavonoids were also reported to be responsible for antimicrobial properties in some ethno medicinal plants (Singh and Bhat, 2003). Furthermore, many tannin molecules have also been shown to reduce the mutagenic activity of a number of mutagens. Many carcinogens and/or mutagens produce oxygen free radicals for interaction with cellular macromolecules. The anticarcigenic and antimutagenic potentials of tannins may be related to their ant oxidative property, which is important in protecting cellular oxidative damage including lipid per oxidation (Chung et al., 1998).

Table 2 shows the results of mineral content determined for the GS leaf. 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. The results of calcium analysis obtained 8882.492 ppm (Table 2) were higher than those reported by Oladele and Oshodi (2007) and also higher than that reported by Mohammed and Sulaiman (2009). Copper is essential to all living organisms as a trace dietary mineral element. It is a key constituent of the respiratory enzyme complex cytochrome c oxidase which is required in aerobic respiration. Copper is also a component of the protein hemocyanin which is the oxygen carrier in most mollusks and arthropods. The results obtained for copper are lower than values reported by Aderibigbe and Brown (1993) but higher than that reported by Tokusoglu and Unal (2003) and Saud and Al-Qud (2003).

Table 1. Results of quantitative phytochemical analysis of GS leaf extract.

Phytochemical	Quantitative value (g 100^{-1} g)
Alkaloids	21.98
Tannins	0.28
Saponins	0.40

Table 2. Results of elemental analysis of GS leaf extract.

Element	Concentration (ppm)
Ca	219.03
Cu	19.80
Fe	497.36
K	5200.00
Mg	1315.57
Na	1400.00
P	1808.31
Zn	43.70

Iron (Fe) is a necessary trace element found in nearly all living organisms. It plays an important role in biology, forming complexes with molecular oxygen in hemoglobin and myoglobin which are oxygen transport proteins in vertebrates. Many enzymes vital to life also contain iron, such as catalase and lipoxygenase. The color of blood is also due to iron containing hemoglobin. The values obtained are lower than that reported by Aderibigbe and Brown (1993) for water hyacinth and water lettuce and also lower than that reported by Tokusoglu and Unal (2003) for chlorella and isochrisis algae. However, the obtained result for iron is higher than (3.39 ± 0.52 mg 100^{-1} g) reported by Oladele and Oshodi (2003) for *Jatropa cathartica* seeds and also higher than (3.19 mg dm^{-3}) reported by Mohammed and Sulaiman (2009) for tea leaf samples.

Potassium is important for reducing blood pressure and also increasing blood circulation as well as preventive aid on general health. The results in Table 2 shows the level of potassium in GS leaf which is generally lower than (987.48 ± 2.13 mg 100^{-1} g) reported by Oladele and Oshodi (2007) for *Jatropa cathartica* seeds. The result is higher than (33.1 mg dm^{-3}) reported by Mohammed and Sulaiman (2009) for tea leaf sample. Magnesium is an essential mineral element in biological systems. It is present in every cell type in every organism. ATP (adenosine triphosphate) 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 plants, magnesium is necessary for synthesis of chlorophyll and photosynthesis. The values obtained for magnesium (1315.570 ppm) are lower than that reported by Oladele and Oshodi (2007) and are higher than values obtained by Mohammed and Sulaiman (2009).

Sodium is an essential mineral element in humans that regulates blood volume, blood pressure, osmotic equilibrium and pH. Thus, it is the major cat ion in blood and extracellular fluid (ECF). In plants, sodium is a micronutrient that aids in metabolism, specifically in regeneration of phosphoenolpyruvate and synthesis of chlorophyll. The results obtained for sodium are lower than values reported by Aderibigbe and Brown (1993) for water hyacinth and water lettuce, also lower than values obtained for three microalgae by Tokusoglu and Unal (2003). However, the results are higher than that obtained for *J. cathartica* seeds by Oladele and Oshodi (2007).

The results obtained for phosphorus 1808.31 ppm (Table 2) are lower than (2125.19 ± 0.00 mg 100^{-1} g) for *J. cathartica* seed reported by Oladele and Oshodi (2007) phosphorus in GS leaf is low. However, phosphorus is also essential for all forms of life. As phosphate, it is a component of DNA, RNA, ATP and also the phospholipids that form all cell membranes. It plays a major role in DNA and RNA where it forms part of the structural framework of these molecules. Living cells uses phosphorus as phosphate to transport cellular energy in the form of ATP. ATP is important in phosphorylation, a key regulatory event in cells. Zinc is an essential mineral element of exceptional biologic and public health importance necessary for plants, animals and micro-organisms. It is important in metabolic function and for growth in man. 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. Table 2 shows the level of zinc in GS leaf (43.700 ppm). The results obtained are generally higher than (2.17 mg dm^{-3}) reported by Mohammed and Sulaiman (2009) and that reported by Tokusoglu and Unal (2003) for three microalgae. The values are lower than (47.22 ± 0.24 mg 100^{-1} g) reported by Oladele and Oshodi (2007) for *J. cathartica* seeds.

Conclusion

The results obtained have shown that GS leaf has high concentration of alkaloids and low concentration of tannins and saponins. The results of the phytochemical content have therefore justified the widespread usage of the plant as medicine traditionally. The results of mineral elements analyzed for GS leaf compares favorably with other values obtained by previous researchers and thus indicated that the leaf contain significant amount of the mineral elements. It could therefore be concluded that GS leaf is a potential source of active ingredients that could be used in both traditional and orthodox medicine and that it is also a source of essential mineral elements.

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