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Comparative Study on Selected Phytochemicals and Antioxidant Activities of the Methanol Extracts of *Guiera senegalensis* J. F. Gmel. (Combretaceae)

[1]Awwalu, S. [2]Kassoum, D.B. and [3]Nasir, I.

[1]Department of Pharmaceutical and Medicinal Chemistry,
Ahmadu Bello University,
Zaria, Nigeria

[2]Department de Pharmacie Sciences,
Faculte de Science de la Sante/
Universite Dan Dicko Dankoulodo de Maradi,
Republique du Niger

[3]Department of Pharmaceutical and Medicinal Chemistry,
Faculty of Pharmaceutical Sciences,
Usmanu Danfodiyo University,
Sokoto, Nigeria

Email: salislauwali@gmail.com, awwalus@abu.edu.ng

Abstract
The shrub Guiera senegalensis is rich in medicinal properties which are linked to its phytochemical constituents especially phenolic and flavonoid compounds. The aim of this research is to compare the antioxidant activities viz-a-viz the phenolics and alkaloids in the root, stem bark and leaf methanol extracts of this shrub. Total phenolic, total flavonoid and antioxidant activities were determined by Folin-Ciocalteu, aluminum chloride and DPPH free radical methods respectively. Results were presented as mean ± SD and were further analysed by ANOVA at 95 % confidence interval. Significant ($p<0.05$) differences were observed in total phenolic, total flavonoid and antioxidant activity between the extracts. In all the activities tested, the root was found to be the highest followed by the stem bark and then the leaf. It can be concluded that the root of this plant should be used for antioxidant activity in preference to its stem bark and leaf.

Keywords: *G. senegalensis*, total phenolic, total alkaloid and antioxidant activity

INTRODUCTION
Guiera senegalensis, commonly known as Guiera, is a perennial shrub that grows up to a height of 3 to 5 m (Silva *et al*., 2008). It is known as Sabara and Kishishi in Hausa and Kanuri respectively (Fiot *et al*., 2006). It is widely distributed in savannah region of west and central Africa, Nigeria, Senegal, Gambia, Mali, Niger, Burkina Faso and Ghana (Shettima *et al*., 2012).

Guiera is reported to be rich in alkaloids, tannins, terpenoids menthol, coumarins, saponins, flavonoids (quercetin), cardiotonics and cynogenic hetrosides (Ficarra *et al*., 1997; Bucar *et al*.,

**Author for Correspondence*

1998; Bouchet *et al*., 2000; Fiot *et al*., 2006). This shrub has been reported to have numerous medicinal uses (Fiot *et al*., 2004; Faye *et al*., 1980; Sanogo *et al*., 1998; Diatta *et al*., 2007; Benoit *et al*., 1996; Ancolio *et al*., 2002; Azas *et al*., 2002; Aniagu *et al*., 2005). The flavonoids in *G. senegalensis* are responsible for its use in the treatment of aches, pains and for its venolymphatic and antiviral effects (Narayana *et al*., 2001).

Comparative antioxidant study of the different morphological parts of this plant is essential as it will suggest the best part of the plant to be used for this purpose. The aim of this research is to compare the total phenolic, total alkaloid and antioxidant properties of the leaf, stem back and root methanol extracts of this plant.

METHODOLOGY

Collection, identification and processing

The leaf, stem bark and root of *G. senegalensis* was collected from forest in Ahmadu Bello University, Zaria, Kaduna State, Nigeria in the month of March, 2021 and identified at the herbarium in Department of Botany, Ahmadu Bello University Zaria where a voucher number, 1823, was assigned. The samples were washed under running tap, shade-dried, size-reduced and then stored in labeled polyethylene bags. Quantities (30 g) of the processed samples were extracted with 200 mL methanol for 2 days using cold maceration with occasional shaking. The solutions were filtered and the filtrates were evaporated to dryness at room temperature. The percentage yields were then calculated.

Preparation of stock solutions

Ten grams of the methanol extracts were individually dissolved in labeled conical flasks containing 10 mL methanol to obtain 1 mg/ mL stock solutions.

Determination of total flavonoids

Construction of quercetin calibration curve

Five points quercetin calibration curve was constructed by preparing quercetin working solutions (20, 40, 60, 80 and 100 µg/ mL) from quercetin stock solution using standard addition method. Each working concentration was transferred into labeled test tubes containing 0.3 mL of 5 % sodium nitrate solution. The solutions were allowed to stand for 5 minutes after which 0.3 mL of 10 % aluminum chloride solution was added and allowed to stand for 5 minutes. To these solutions, 2 mL of 1.0 M sodium hydroxide solution, 4 mL of distilled water were added. Absorbance of the resultant mixtures were then taken at 430 nm (Hossain and Shah, 2015). Quercetin calibration curve was then constructed using Microsoft Office Excel 2016.

Sample preparation

A quantity (1 mL of 1 mg/mL) of each extract was transferred into labeled test tubes containing 0.3 mL of 5 % sodium nitrate solution. The solutions were allowed to stand for 5 minutes after which 0.3 mL of 10 % aluminum chloride solution was added and allowed to stand for 5 minutes. To these solutions, 2 mL of 1.0 M sodium hydroxide solution, 4 mL of distilled water were added. Absorbance of the resultant mixture was then taken at 430 nm (Hossain and Shah, 2015). Total flavonoids of the various extract were then extrapolated from the quercetin calibration curves.

Determination of total phenolics

Construction of garlic acid calibration curve

Garlic acid working solutions (20, 40, 60, 80 and 100 µg/ mL) from 1 mg/mL garlic acid stock solution. The working solutions were neutralized using 2 mL of 15 % sodium bicarbonate, and diluted with 4 mL of distilled water. Two drops of 1 % aqueous ferrous ammonium sulphate (FAS) were added to each test tube, shaken and placed on a water bath (40 - 50 ^{o}C) for 15 min. The solutions were cooled and absorbance was measured at 575 nm (Hossain and Shah, 2015). Garlic acid calibration curve was then constructed using Microsoft Office Excel 2016.

Sample preparation

An aliquot (1 mL of 1 mg/mL) of the extracts were neutralized using 2 mL of 15 % sodium bicarbonate, and diluted with 4 mL of distilled water. Two drops of 1 % aqueous ferrous ammonium sulphate (FAS) were added to each test tube, shaken and placed on a water bath (40 - 50 ^{o}C) for 15 min. The solutions were cooled and absorbance was measured at 575 nm (Hossain and Shah, 2015). Total phenolics of the various extract were then extrapolated from the garlic acid calibration curves.

Determination of antioxidant activity

Working concentrations (20-100 µg/mL) of the various extracts were prepared from the stock solutions (1 mg/mL). To each 2 mL quantity of the working solutions, 2 mL DPPH solution was added, shaken and incubated for 30 minutes at room temperature. The absorbance was measured at 517 nm using CARY-100 UV-Vis. spectrophotometer (Chen *et al*., 1999). Percentage DPPH scavenging activity (% inhibition) was then calculated at each concentration of the extract.

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{Absorbance\ of\ blank - Absorbance\ of\ sample}{Absorbance\ of\ blank} x\ 100$$

Thereafter, graphs of % inhibition against working concentrations were constructed using Microsoft Office Excel 2016. Half maximal inhibitory concentrations (IC_{50}) of the various extract were then extrapolated from the graphs.

Statistical analysis

Results were expressed as mean ± standard deviation. Differences between extracts were determined using one-way Analysis of variance (ANOVA) followed by Tukey post hoc test at 95 % confidence interval using IBM SPSS statistics 20.

RESULTS

Percentage yield

The percentage yields were found to be 20.67, 19.30 and 16 % for root, leaf and stem bark methanol extracts respectively.

Total Phenolic and Flavonoid Contents

Garlic acid and quercetin calibration curves are shown in Figure 1 and 2 respectively while total phenolic and total flavonoid contents are shown in Table 1.

Table 1: Total Phenolic and Total Flavonoid Contents

Samples	Conc. (µg/ mg)	
	Total Phenolics	Total Flavonoids
Stem bark	221.67 ± 0.14	38.71 ± 0.02
Leaf	398.00 ± 0.5	40.14 ± 0.02
Root	449.67 ± 0.19	91.86 ± 0.02

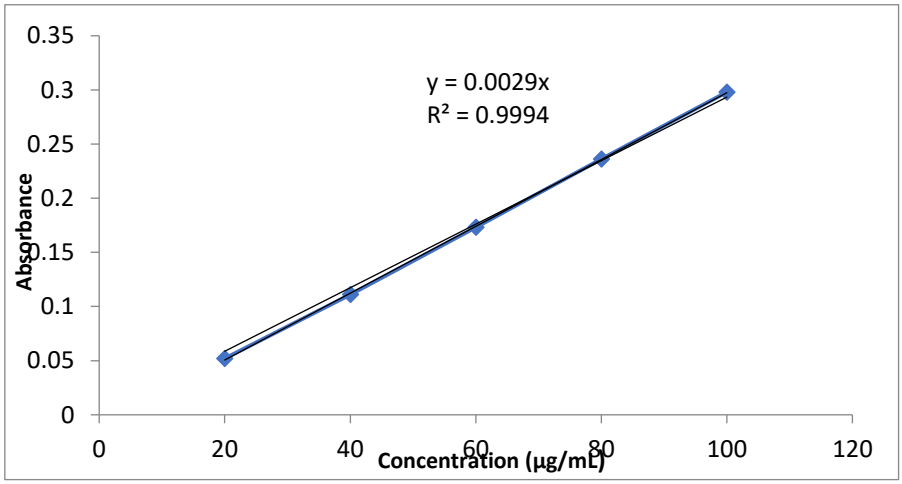

Fig. 1: Calibration Curve of Garlic Acid Standard Powder

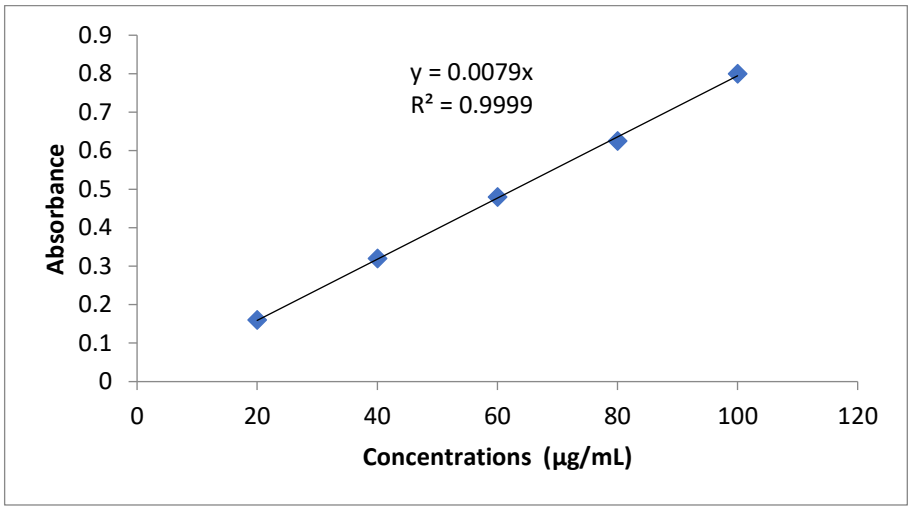

Fig. 2: Calibration Curve of Quercetin Standard Powder

Antioxidant Activity

The results of the antioxidant activity are shown in Figures 3 and 4 below.

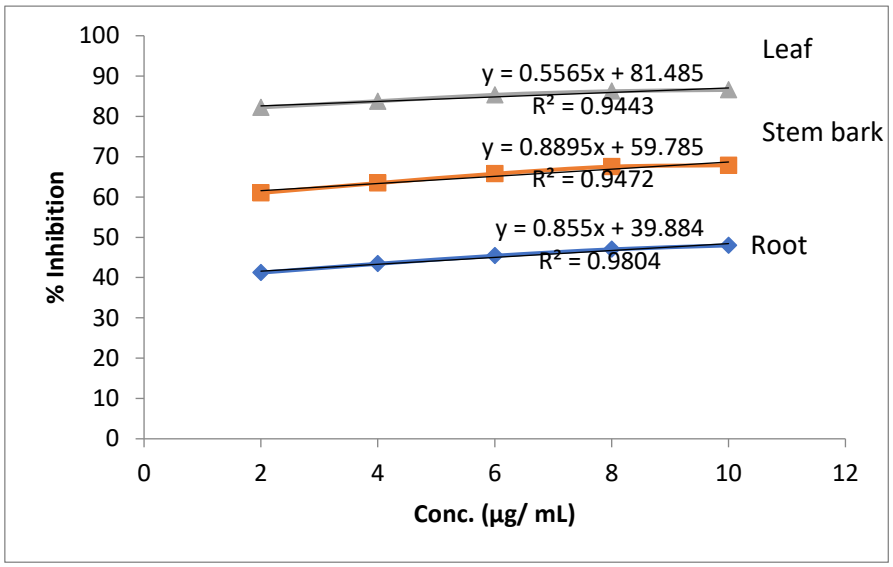

Fig. 3: Antioxidant Activity of the Morphological parts of *G. senegalensis* Methanol Extract

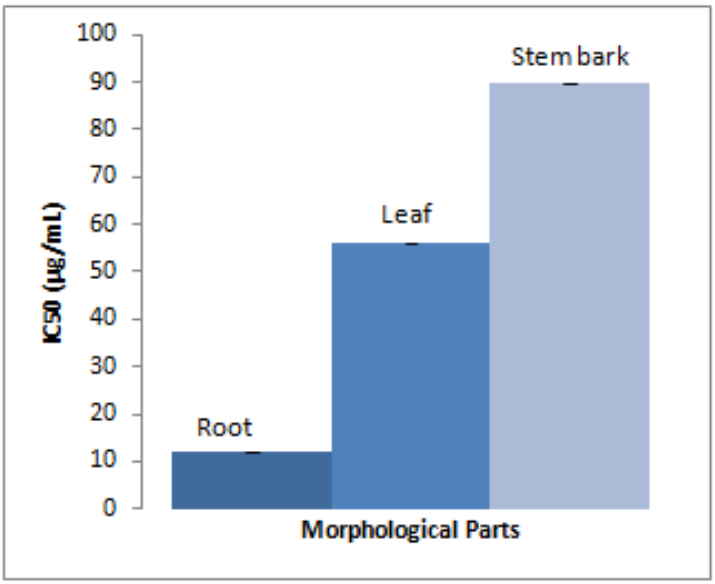

Fig. 4: IC50 of Different Morphological Parts G. *Senegalensis* Methanol Extract

Discussion

G. senegalensis is said to possess strong antioxidant activity attributed to its phytochemical constituents especially flavonoid and phenolic (Ancolio *et al.*, 2002; Silva and Gomes, 2003). Significant ($p<0.05$) differences in phytochemical constituents between the root, stem bark and leaf methanol extracts were observed as indicated by their significant ($p<0.05$) differences in percentage yields. The root extract was found to be the richest in phytochemical constituents when compared with the leaf and stem bark methanol extracts.

The garlic acid and quercetin calibration curves were found to be suitable for the quantification of the total flavonoid and total phenolic in the samples as their coefficients of determinations (r^2) were close to unity (Figure 1 and 2) signifying strong relationship between absorbance and concentrations. The root extract was found to contain the highest amount of phenolics and flavonoids while the stem bark had the least (Table 1). Hence, there is a strong correlation between percentage yield, total phenolics and total flavonoids.

Free radicals play a vital role in the manifestation of diseases (Bouchet *et al*., 2000). Antioxidants fight these free radicals and protect humans from various diseases by either scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms (Bouchet *et al*., 2000). In all the extracts (root, stem bark and leaf), the percent inhibition was found to be dose dependent as indicated by the r^2 (Figure 3).

The lower the IC_{50} value, the higher is the antioxidant activity (Brand-Williams *et al*., 1995). Hence, the methanol root extract was found to be the most potent while the stem bark was found to be the least as evident by their IC_{50} values (Figure 4).

Conclusion

Significant ($p<0.05$) difference in total phenolic, total flavonoid and antioxidant activity was observed in the order root> leaf> stem bark methanol extracts. The root of *G. senegalensis* should be used for antioxidant activity in preference to the stem bark and leaf.

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