Chemical Composition and Polyphenolic Antioxidant Properties of active fractions of Guiera Senegalensis (J.F.Gmel) Leaves

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Abstract

Guiera senegalensis J.F. Gmel. (Combataceae) is one of the most used plants in Sudan folkloric medicine. The powdered leaves of G. senegalensis were extracted using 70% methanol; the methanolic extract was then fractionated by Petroleum ether, Chloroform, Ethyl acetate and n-butanol. Phytochemical profiling was carried out for all the four fractions of Guiera senegalensis leaves by using Thin Layer Chromatography (TLC). The TLC chromatogram revealed the presence of Flavonoids, Phenolic acids, Coumarins, Terpenoids and Alkaloids. However, the ethyl acetate fraction was the richest fraction with polyphenols. The radical scavenging activity of G. senegalensis leaves fractions was screened by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH), the antioxidant results were expressed as concentration of inhibition (IC₅₀) of the free radical DPPH, the ethyl acetate fraction showed the highest scavenging activity with IC₅₀ = 0.954334 µg/ml, compared to the control standard Gallic acid which yield IC₅₀ = 4.424024 µg/ml, followed by petroleum ether IC₅₀ = 9.048009 µg/ml, n-butanol IC₅₀ = 10.46394 µg/ml and Chloroform IC₅₀ = 24.53962 µg/ml. The scavenging activity of G. senegalensis leaves fractions increases correspondingly with the increasing polyphenolic content. Judging from the high antioxidant activity of the ethyl acetate fraction of G. senegalensis leaves, it can be said that G. senegalensis leaves have a promising efficacy in treating oxidative stress-induced diseases.

Keywords: Guiera senegalensis; antioxidant; phytochemical profiling; leaves fractions

Introduction

Medicinal plants continue to be a major source of medicine as they have always been from the beginning of human civilization, (Ampofo 1977; Feansworth, et al., 1985). Management of many diseases by using traditional remedies is very common in Africa in rural as well as urban communities. The increasing number of patients who are using traditional herbs is driven by combination of factors including financial constraints or inadequacy of the healthcare systems as well as ease of accessibility to traditional medicines. Medicinal properties of plants are normally dependent on the presence of certain phytochemical principles such as alkaloids, anthraquinones, cardiac glycosides, saponins, tannins and polyphenols (Mann, et al., 2008).

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G. senegalensis is a shrub of 3 m height. The leaves are 3-5 cm long and 1.5-3.0 cm broad arranged opposite or sub-opposite on the stem (Hutchinson et al. 2012). It is widely distributed in the savannah region of west and central Africa Nigeria, Senegal, Gambia, Mali, Niger, Burkina Faso, Ghana and Sudan. In north Nigeria it is used extensively for wide range of medicinal purposes, (Fiot, et al., 2004) such as its activity against cough, respiratory congestion and fever, (Adedapo, et al., 2009; Ali et al., 2011). It is also used against malaria fever, (Ancolio et al., 2002). A tea made from its leaves is prescribed through oral route to treat eczema, (Somboro et al., 2012).

Phytochemical screening of Guiera senegalensis leaves showed positive results for alkaloids, flavonoids, tannins, saponins, carbohydrates, sterol and triterpenes (Houacine et al., 2012).
Polyphenols are well known antioxidants (Tiwari and Rao, 2002) their Oxidant properties may be both antioxidant and/or pro-oxidant based upon the structure of the particular polyphenol and the cellular redox context that may include increased levels of oxidant scavenging proteins or decreased levels of oxidized proteins and lipids (Chen et al., 2013).

Free radicals were reported to be the main reason behind various type of diseases such as; cancer, neuropathy, diabetes and cardiovascular problems. Special attention is focused on the isolation of natural phenolic antioxidants from inexpensive sources. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity and toxicity. Therefore, the search for preparations of useful natural antioxidants is highly desirable.

The objective of this study is to investigate, the phytochemical profile of *Guiera senegalensis* (J.F. Gmel) leaves and antioxidant activity of its polyphenolic constituents.

**Material and Methods**

**Plant material preparation and extraction**

*Guiera senegalensis* leaves (Fig.1) were collected from North Kordofan State, taxonomical identification was made at the Medicinal and Aromatic Plant Research Institute (MAPRI), the fresh leaves were rinsed with tap water then they were shade dried and grounded. Hundred grams of dry powdered leaves were macerated in 70% methanol for four successive days, and in each day the extract is filtered and additional volume of 70% methanol is added. The four portions of methanolic extract was then collected together and fractionated (liquid/liquid) in sequence using solvents with increasing polarities petroleum-ether (PE), chloroform (CHCl₃), ethyl acetate (EtoAc) and normal Butanol (n. butanol).

**Thin Layer Chromatography (TLC)**

Aluminum silica gel plates 60 F254 (Merck 5554) and pre-coated TLC plates SII, RP -18W /UV 254 (Macherey-Nagel) were used as stationary phases in carrying out TLC of the different extracts of *Guiera senegalensis* leaves. Standard chromatograms were prepared by applying 20 µl of the dissolved extract to a silica gel plate and developing it in different solvents systems depending on the type of extract. Chromatograms were detected under UV light (254 nm and 366 m) and sprayed with diagnostic reagents which include: Vanillin -H₂SO₄ reagent, Dragendorff , KOH and Natural Product Reagent (NPR).

**Free radical scavenging procedure**

This method was carried out according to that described by (Shyur et al., 2005) with some modifications. Stock solution was prepared by dissolving 1mg of the sample in 1ml of absolute ethanol (98%). Stock solution was diluted to final concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 µg /ml in ethanol. 0.9ml Tris-HCL and 1ml of 0.1 mM DPPH in methanol solution were added to each concentration and incubated at room temperature in the dark for 30 minutes. The absorbance of the resulting mixture was measured at 517 nm and converted to percentage antioxidant activity using the formula below:-

\[
\text{Scavenging activity(%) = } \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100
\]

solution of 0.9 ml Tris-HCL+ 0.1ml absolute ethanol+ 1ml absolute ethanol was used as blank, while solution of 0.9 ml tris-HCL+0.1 ml absolute ethanol+1ml DPPH was used as a control.

A freshly prepared DPPH solution exhibits a deep purple colour with a maximum absorbance at 517 nm. The purple colour disappears when an antioxidant is present in the medium. Thus, the change in the absorbance of the reduced DPPH was used to evaluate the ability of test compound to act as free radical scavenger. Furthermore, the "Inhibitory concentration" or IC₅₀ value (the concentration of antioxidant that causes 50% loss of the DPPH activity) was also used to assess the antioxidant activity of the plant extract compared to the standard drug. The higher the antioxidant activity, the lower is the value of IC₅₀ (Molyneux, 2004).
Data analysis
- TLC chromatogram will be analyzed for secondary metabolite using UV light at 254 and 366 wave length.
- Anti-oxidant activity of the different fractions with different concentrations will be recorded as IC_{50} which will be calculated using Microsoft Office Excel 2007.

Results
Yield percent of Guiera senegalensis leaves
From the 100 g powdered Guiera senegalensis leaves, 23.76% methanolic extract was obtained, the methanolic extract was fractionated into four fractions, which gives, n-butanol 8.2%, ethyl acetate 6.9%, Petroleum ether 1.77% and Chloroform 1.09%.

Thin Layer Chromatography (TLC) of Guiera senegalensis leaves
TLC profiles of the different fractions of the methanolic extract of Guiera senegalensis leaves are presented in Figures (2-5). (Wagner et al., 1984) is used as a reference in color detection of the different phytochemical compounds.

Thin layer chromatography (TLC) of petroleum ether fraction
Typical blue to purple colors were developed upon spraying with vanillin H_2SO_4 (heat 110°C), by six compounds (Rf: 0.32, 0.37, 0.45, 0.52, 0.62, 0.95) (Fig 2-2). Vanillin H_2SO_4 is a universal reagent that detects components of essential oils, terpenoids, phenols.

The presence of flavonoids was confirmed by their colour change from quenching fluorescence (366 nm) to yellow or orange colour (Rf: 0.05, 0.097, 0.39, 0.50, 0.55) and prominent blue or green colour in case of flavonoidal acids or other phenolic acids (Rf: 0.16, 0.49, 0.64) after spraying with natural product reagent (NPR). Eight compounds with (Rf: 0.05, 0.097, 0.16, 0.33, 0.39, 0.49, 0.64, 0.77) reacted positively with vanillin H_2SO_4 (heat 110°C) producing blue to purple color, which according to (Wagner, et al., 1996) they are might be terpenoids. (Fig 4).

Alkaloids were detected in the ethyl acetate fraction of the methanolic extracts of the leaves after spraying with Dragendorff which developed brown or orange visual day light zones immediately upon spraying reported in (Rf: 0.037, 0.087, 0.43, 0.68) (Fig 4).

Upon spraying with KOH the fluorescence of coumarins became more prominent, giving us green to blue fluorescence. Only one spot was detected in the ethyl acetate fraction with (Rf: 0.50), (Fig 4).

Thin layer chromatography (TLC) of n-butanol fraction
Typical pink to purple colors were developed after spraying with vanillin H_2SO_4 and heating the plate at 110°C indicating the presence of terpenoids. Only one compound with (Rf: 0.05) was observed in n-butanol fraction of G. senegalensis leaves. (Fig 5).

Flavonoids were detected by spraying of natural product reagent NPR on the chromatogram, developing yellow or orange color (Rf: 0.06, 0.09) and blue or green colors in case of phenolic acids (Rf: 0.19, 0.47, 0.63). (Fig 5).

Upon spraying with KOH the fluorescence of coumarins became more prominent, giving us green to blue fluorescence, this reaction was observed in n-butanol fraction by two compounds (Rf: 0.06, 0.19), (Fig 5).
Figure 2: Petroleum ether fraction NP-TLC chromatograms of G. senegalensis leave using different diagnostic reagents and petroleum ether: acetone 7:3 solvent system.

Figure 3: Chloroform fraction NP-TLC chromatograms of G. senegalensis leaves using different diagnostic reagents and Toluene: EtOAc: Formic acid 5: 4: 1 solvent system

TLC of n-butanol fraction revealed two compounds (Rf: 0.05, 0.41) which reacted positively with Dragen-dorff reagent suggesting the presence of alkaloids. Alkaloid develops brown or orange visual day light zones immediately on spraying, (Fig 5).

Antioxidant activity of Guiera senegalensis leaves fractions

The results of in vitro investigation of the antioxidant activities of G. senegalensis leaves extracts using
DPPH radical scavenging techniques are presented in Table 1 and Fig (6-10).

All fractions of *G. senegalensis* leaves produced high to moderate radical scavenging activity. The antioxidant activity are been expressed as IC$_{50}$. On the level of *G. senegalensis* leaves fractions the ethyl acetate fraction produced the lowest IC$_{50}$ 0.954334 followed by the Petroleum ether fraction 9.048009, n-butanol fraction 10.46394, last of all was the Chloroform fraction 24.53962. This study compared the in
vitro antioxidant potential of different fractions of *G. senegalensis* leaves. The ethyl acetate fraction showed the highest scavenging capacity followed by petroleum ether, n-butanol and chloroform as evidenced from their IC\textsubscript{50} values 0.954334, 9.048009, 10.46394 and 24.53962 µg/ml respectively. The results were compared to the control standard Gallic acid which yield IC\textsubscript{50} = 4.424024 µg/ml which suggest that the ethyl acetate fraction of *G. senegalensis* leaves possess a stronger antioxidant activity than Gallic acid.

The results found justify this strong antioxidant activity of the ethyl acetate fraction, that the accumulation of polyphenols in this fraction could be the main reason behind this activity, this come in consistent with (Mariod et al., 2006) who stated that *G. senegalensis* leaves shows a very remarkable antioxidant activity, only 0.08 mg were used to reach 50% reduction in DPPH, he related his finding to the total phenolic content of the root and leaves 275.6±0.1 mg/g and 240.1±0.05 mg/g respectively.

The literature revealed that Flavonoids are important antioxidant as reported by (Rice-Evans 2001). The major structural characteristics which contribute to their reducing properties are in the B-ring, an unsaturated 2, 3 double bond and a 3-hydroxyl group in ring C. flavonoids, including rhamnetin, showed significant antioxidant and radical scavenging activities in vitro, according to (Bucar et al., 1989) rhamnetin from *Guiera senegalensis* strongly inhibited peroxidation of phospholipids liposomes.

**Discussion**

**Chemical Composition of *Guiera senegalensis* leaves fractions**

The TLC profiling of different fractions of *G. senegalensis* leaves revealed the presence of different phytochemicals, including: Flavonoids, Phenolic acid, Coumarins, Terpenoids and Alkaloids. These findings are in consistency with who reported the presence of Alkaloids, Flavonoids, Coumarins and terpenoids in a methanolic extraction of *G. senegalensis* leaves.

Quercetin, rutin, myricitrin, catechin, (Ficarra et al., 1997; Maleš et al., 1998) quercitrin, kaempferol, rhamnetin and apigenin (Charles et al., 2005) are flavonoids that were reported in leaves of *G. senegalensis*. While (Bouchet et al., 1996) reported the presence of Gallic acid and its derivatives in the galls of *G. senegalensis*. All these studies are in agreement with our findings that Flavonoids and Phenolic acids were detected in *G. senegalensis* leaves fractions, and mainly they are accumulated in the ethyl acetate fraction, followed by the petroleum ether fraction, n-butanol fraction and lastly the chloroform fraction.

Regarding coumarins the ethyl acetate also was found to be the richest fraction, while the rest of fractions were contain equal amounts of coumarins. (Williams et al., 2009) reported the presence of coumarins in the Leaves of *G. senegalensis*. In contrast the coumarins were not detected by (Charles et al., 2005).

Terpenoids also were accumulated in the Ethyl acetate fraction, followed by the Chloroform fraction, Petroleum ether and n-butanol. Similarly the presence of tepenoides in *G. senegalensis* leaves extract was also reported by (Tijjani et al., 2012).

Alkaloids were mainly accumulated in the ethyl acetate fraction, then the Chloroform fraction followed by n-butanol and lastly the Petroleum ether fraction. Hyoscyamine and solanine, are alkaloidal compounds that were found in *G. senegalensis* leaves by (Somboro et al., 2011). According to (Koumaré et al., 1968; Silva and Gomes, 2003) the roots of *G. senegalensis* contain tetrahydroharman (eleagnine) which consider as the main alkaloid in the roots, and harman and harmalan (dihydroharman) as minor compounds in the root. Where, the leaves contain the alkaloids tetrahydroharman and Harman. From these finding we observed that the ethyl acetate is the richest fraction that contain the highest amount of Polyphenols, Terpenoids and Alkaloids. We also observed that the total polyphenols are the dominant phytochemicals in this plant leaves, this was also reported by (Charles et al., 2005) who found that the total Phenolic compounds in acetone extract of *G. senegalensis* leaves made up half of the contents (50.6±0.7 g /100 g extract) while the total flavonoid content was 1.61±0.01 g /100 g extract. The total phenolic content in the aqueous decoction was 34.6±0.04 g /100 g extract and the flavonoid content was 0.79±0.00 g /100 g extract.

**Conclusion and Recommendations**

- The current study showed that *G. senegalensis* leaves extract fractions contain Polyphenols, Terpenoids and Alkaloids.
- The total phenolic content represents the highest amount of all phytochemicals followed by Terpenoids and Alkaloids.
- The polyphenols that were detected are Flavonoids, Phenolic acids and coumarins.
- The ethyl acetate Fraction was the richest fraction with polyphenols in contrast to the petroleum ether, n-butanol and Chloroform fractions.
- This study also revealed the antioxidant potential of *G. senegalensis* leaves, all fractions gave positive results and scavenge the free radical DPPH. While Ethyl acetate fraction present the most reactive fraction due to its high polyphenolic content.
Table 1: DPPH radical scavenging activity of *G.senegalensis* leaves fractions

<table>
<thead>
<tr>
<th>Concentration µg/ml</th>
<th>Ethyl acetate</th>
<th>Petroleum ether</th>
<th>n-butanol</th>
<th>Chloroform</th>
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</thead>
<tbody>
<tr>
<td>100</td>
<td>85</td>
<td>86.38</td>
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<td>IC50</td>
<td>0.954334</td>
<td>9.048009</td>
<td>10.46394</td>
<td>24.53962</td>
</tr>
</tbody>
</table>

Figure 6: DPPH radical scavenging activity of ethyl acetate fraction of *G. senegalensis* leaves

- The high antioxidant activity of the ethyl acetate fraction makes *G. senegalensis* leaves a significant source of natural antioxidants.
- Employ other techniques for purification and identification of compounds such as HPLC.
- Verification of the antioxidant activity of each purified compound from *G. senegalensis*.
- Study the antioxidant activity of different parts of *G. senegalensis* mainly roots and bark.
- Proof the potentiality of *G. senegalensis* to work as antidiabetic, antihypertensive and antimicrobial.
Figure 7: DPPH radical scavenging activity of Petroleum ether fraction of *G. senegalensis* leaves.

Figure 8: DPPH radical scavenging activity of n-butanol fraction of *G. senegalensis* leaves.
Figure 9: DPPH radical scavenging activity of Chloroform fraction of *G. senegalensis* leaves.

Figure 10: Concentration of inhibition (IC$_{50}$) of all the four fractions of *G. senegalensis* leaves compared to the control stander Gallic acid.
References


