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RESEARCH ARTICLE

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Phytochemical Screening and Thin Layer Chromatographic Studies of *Guierasenegalensis* G.F Gmel (Egyptian mimosa)

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ABSTRACT

The plant screening for phytochemical constituents seems to have the potential to act as a source of useful drugs and cures many infections as a result of the presence of various bioactive compounds that evident to have enormous activity against array human pathogens. The results of phytochemical screening indicated that G. senegalensis contains alkaloids, Flavonoids, tannins, Saponins, balsams, steroids and terpenoids. It was observed that G. senegalensis extracts differ significantly (P<0.05) in the content of glycosides, flavonoids and tannins Thin layer chromatography of the extracts was conducted and the profiles showed 15 spots. These findings suggested that G.senegalensis seeds extract could be a potential source of drugs which in future may serve for the production of synthetically improved therapeutic agents.

Keywords: *G. senegalensis*, phytochemical screening, thin layer chromatography

INTRODUCTION

Over three-quarters of the world population relies mainly on plants and plant extracts for health care and more than 30% of the entire plant species, at one time or the other was used for medicinal purposes [1]. The use of plants for medicinal purpose is probably as old as the history of mankind and their uses in the industrialized societies have led to the extraction and development of several drugs from as well as from traditionally used folk medicine. Extraction and characterization of several active phytochemicals from these green factories have given birth to some high activity profile drugs [2]. It has been estimated that in developed countries such as United States, plant-drived drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is recognized in all countries over the world and these provide two third of the plants used in modern system of medicine and the health care system of rural population depend on indigenous systems of medicine [3].

Phytochemical studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques. These techniques played a significant role in the search for additional resources of raw material for pharmaceutical industry (phytochemicals) [4]. Development of drugs based on natural products has had a long history in the US, and in 1991, almost half of the best selling drugs were natural products or derivatives of natural products [5]. Natural products are chemical compounds derived from living plants or animals. Drugs derived from natural products are usually secondary metabolites and their derivatives

G. senegalensis belongs to the family *Combretaceae* and *is* a shrub that can grow to a height of 3 to 5 m. Its stem presents numerous knots that send out branches. The ash-grey stem and branches have fibrous or pubescent bark and

bear opposing, short petiolated oval leaves, sometimes mucronate, sometimes even cordate at their base, about 2 to 4 cm long by 1 to 2 cm wide. These grey-green leaves, darker on their upper surface, display black spots on their lower surface and are slightly downy on both sides. These features give the plant an overall silver- green color that is conspicuous in brush [6]. *G. senegalensis* is widely known in traditional medicine for the remedy of many ailments. The plant leaf extract is being used against dysentery, diarrhea, gastrointestinal pain and disorder, rheumatism and fever [7] and [8].

MATERIALS AND METHODS

Collection and Preparation of Sample Materials

G. senegalensis seeds were obtained from Gidanyero area, Usmanu Danfodio University, Sokoto permanent site. The samples collected were packed separately in clean sterilized polythene bags and brought to the herbarium of the Department of Biological Sciences, Usmanu Danfodio University, Sokoto for identification and authentication. Voucher specimens of the samples were prepared and deposited in the same herbarium [9].

The fresh samples were washed with tap water and air dried under shade. Dried samples of plant material collected were milled into fine powder using high capacity grinding machine and subsequently stored separately in sterilized polythene bags until required for use [10].

Extraction Procedure

Two hundred (200) grams from powdered samples of *G. senegalensis* was extracted in water. The sample was dispensed in 1.5 liters of distilled water; the solutions were stirred, cupped with aluminum foils and kept for twenty four hours (24 hour). The resultant solutions were filtered using muslin cloth and each filtrate was separately evaporated to dryness using hot plate set at 40^0C to obtain crude extracts. Similar method was used to extract the sample in 1.5 liter of ethanol and 1.5 liter of n-hexane. The crude extract of each sample was weighed and the percentage yield of each was calculated as follows:

$$\text{Extract yield } \% = W_1 / W_2 \times 100$$

W_1= Net weight of powder in grams after extraction and W_2= Total weight of wood powder in grams taken for extraction [10]. All the crude extracts obtained were separately stored in the refrigerator until required

Phytochemical Screening

Chemical tests for the screening and identification of bioactive chemical constituents like alkaloids, carbohydrates, glycosides, saponins, flavonoids, and tannins, in extracts of E. *aromatica* under study were carried out in extracts by using standard methods. Quantitative phytochemical screening of glycocides, tannins, flavonoids, saponins and alkaloids were carried out using standard procedures.

Thin Later Chromatography

Preparation of Thin Layer Chromatography Plate

Slurry was prepared from 75 grams of silica gel, 100cm^3 of methanol 50cm^3 of chloroform and 25cm^3 of water. TLC plate was formed and activated at 110^0C using oven for one hour [11].

TLC Separation

Three grams (3g) of each ethanol, hexane, and aqueous extracts was dissolved in their respective solvent, i.e. ethanol, n-hexane and water respectively to form a sample solution. The solvent system was made from hexane and methanol 4:1 (v/v). Capillary tube was used to spot a sample solution on the silica gel TLC plate at 1cm from the edged of the plate and the drop is allowed to dry. The plate was placed in TLC (Chromo tank) and allows ascend the TLC plate by capillary action. The plate was removed and the solvent front was marked then allowed to dry. The iodine was used as the visualizing agent to detect the spot. A meter rule was used to measure the distance moved by the solvent and distance moved by spot, from which the retention factor (R_f values) of the various spots was calculated.

R_f = Distance move by spot front/ Distance move by solvent front

RESULTS

The result of the percentage yield of extract of *G. senegalensis* is presented in table 1. The results revealed that *G. senegalensis* yielded more extract when water was used as solvent followed by hexane and then ethanol with 12.80%, 12.34%, and 11.90% respectively.

Table 1: Percentage Yield of Extracts

Extracts	% extract yield (%)
G.S (aqueous)	12.80
G.S (ethanol)	11.90
GS (hexane)	12.34

Legend – GS= *Guieresenegalenss,*

Qualitative Phytochemical Contents of *G. senegalensis*

The results of qualitative phytochemical analysis of *Guierasenegalensis* extracts were presented in Table 2. The results revealed the presence of alkaloids, steroids, tannins, saponins, glycosides, flavonoids, balsams, terpenoids, proantrocyadins and anthraquins in all the three extract. There was absence of volatile oils and saponin glycosides in all the three extracts. Cardiac glycosides showed no presence in aqueous extract.

Table 2: Qualitative Phytochemical Contents of *G. senegalensis*

Constituent/component	Aqueous Extract	Ethanolic Extract	Hexane Extract
Alkaloids	+	+	+
Saponinglycocides	-	-	-
steroids	+	+	++
Tannins	+++	+++	+++
Saponins	+++	+	+++
glycosides	++	+++	+++
Flavonoids	+	+	++
balsams	+++	++	+++
Cardial glycosides	-	+	+
terpenoids	++	++	++
volatile oils	-	-	-
proantrocyadins	++	++	++
anthraquins	+	+	+

Key: - Absent, + present in a trace amount, ++ present in moderate amount, +++ present in high amount.

Quantitative Phytochemical Analysis of *G. senegalensis*

The results of quantitative phytochemical analysis of ethanol, aqueous and hexane extracts of *G. senegalensis* seeds are presented in Table 3. The results showed that, aqueous extract contained higher amount of flavonoids (1.46g %), tannins (4.41g %) and alkaloids (1.81g %). Ethanol extract contained higher amount of saponins (1.55g %), except glycosides which is presence in large quantity in hexane extract.

The result further showed that, there were no significant differences exist in flavonoid within the extracts with aqueous extract having (1.46±0.06g %) ethanol extract (1.06±0.09g %) and hexane extract (0.93±0.02g %). Tannins in aqueous extract (4.41±0.05g %) are significantly different in that of ethanol and hexane, (2.01±0.04g %) and (1.97±0.18g %) respectively. There exists significant difference in all glycosides with aqueous extract having (5.23±0.04g %) ethanol extract (3.72±0.21g %) and hexane extract (6.27±0.23g %). There exist no significant difference within all extract in saponins with aqueous extract having (1.30±0.19g %), ethanol extract (1.55±0.08g %) and hexane extract (1.47±0.07g %). Similarly there exist no significant difference within all extract in alkaloids with aqueous extract having (1.81±0.86g %), ethanol extract (1.7±0.13g %) and hexane extract (1.57±0.01g %).

Table 3: Quantitative Phytochemical Analysis of *G. senegalensis*

Plant extracts	Flavonoids	Tannins	Glycosides	Saponins	Alkaloids
aqueous Extracts	$1.46^{a}\pm 0.06$	$4.41^{a}\pm 0.05$	$5.23^{b}\pm 0.04$	$1.30^{a}\pm 0.19$	$1.81^{a}\pm 0.86$
Ethanol Extracts	$1.06^{b}\pm 0.09$	$2.01^{b}\pm 0.04$	$3.72^{c}\pm 0.21$	$1.55^{a}\pm 0.08$	$1.78^{a}\pm 0.13$
Hexane Extracts	$0.93^{c}\pm 0.02$	$1.97^{b}\pm 0.18$	$6.27^{a}\pm 0.23$	$1.47^{a}\pm 0.07$	$1.57^{a}\pm 0.01$

Values are mean ± standard error (n=3)
Means in a column with different superscripts are significantly different (P<0.05)

Thin layer chromatography of *G. senegalensis*extracts

The result ofthin layer chromatography of *G. senegalensis* extracts is presented in table 4. TheTLC studies of the ethanol extract of *G. senegalensis* Solvent system hexane and methanol 4:1 (v/v), 5 spot detected R_f values 0.51, 0.44, respectively. In hexane extract 6 spots detected $R_f$values 0.09, 0.15, 0.25, 0.33, 0.41, 0.54, 0.91, 0.65, respectively and in aqueous extract 5 spots detected $R_f$values 0.04, 0.09, 0.15, 0.43, 0.49 respectively.

Table 4: R_f values of TLC solvent system for different extract of *G. senegalensis*

Extract name	Number of spot detected	R_f values
Ethanol extract	5	0.51, 0.44
Hexane extracts	6	0.09, 0.15, 0.25, 0.33, 0.41, 0.54, 0.91, 0.65,
Aqueous extract	5	0.04, 0.09, 0.15, 0.43, 0.49

TLC fingerprints of *G. senegalensis*
a = G. senegalensis aqueous extract; b G. senegalensis ethanol extract; c = G. senegalensis hexane extract.

DISCUSSION

Puguh [12] reported that *Muntingiacalabura* has higher amount extract yield from when methanol was used as a solvent, following by water, ethanol, chloroform, ether and citric acid. The result of the present study showed that From the *G. senegalensis* yield higher amount of extract when water was used as a solvent followed by hexane and water.

The present study revealed the presence of different secondary metabolites like tannins, saponins alkaloids, glycosides, flavonoids, and others from qualitative and quantitative phytochemical analysis of *E. aromatica* which indicate that the plants are rich sources of bioactive compounds. The results showed that the concentrations of secondary metabolites differ among the extracts; this may be connected to different polarity of the solvent. Similar bioactive compounds were also earlier observed on stem back of *Detariummicrocarpum,* and *Ximeniaamericana* and leaves of *GuieraSenegalensis* [13]. The presence of bioactive compound in *Eugenia aromatica* and *Guierasenegalensis*is an indication that they have medicinal potentials due to the fact that each of the bioactive compounds identified has one or more uses therapeutically as reported by [14] and [11].

[14] reported a range of 0.24-0.52% alkaloids, 1.1-2.3% saponins and 0.32 - 0.62% flavonoids in various Malaysian herbs. This showed that plants have different quantity of constituents. Quantitative phytochemical analysis for the extract of *E. aromatica* and *G. senegalensis* extracts revealed the presence of saponin, glycosides, flavanoids, tannins compounds and alkaloids with considerable quantities. Other investigations showed the presence, distribution and importance of phytochemical compounds that are in conformity with the present study include: [13], [14] and [15]

The result of thin layer chromatographic profiles of the extracts of *E. aromatica* and *G. senegalensis* showed 15 fractions. This finding is in agreement with the work of [16] who found 42 fractions on *Centellaasiatica* using different solvent system.

CONCLUSION

Thus, the results obtained in the present study indicates *G. senegalensis* seeds extracts have the potential to act as a source of useful drugs because of presence of various phytochemical components such as carbohydrate, protein, lipids, phenols, flavonoids and tannin.

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