

# Antitoxic, Antifungal and Phytochemical Analysis of Medicinal Compounds of *Guiera senegalensis* Leaves in Sudan

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## Abstract

*Guiera senegalensis* (Gs) is a well-known traditional medicinal plant in Africa, whose leaves extract and roots powder are used for treatment of diseases and wounds in Western Kordufan, Sudan. The aim of this study was to investigate the phytochemical analysis, toxicity, and the antifungal activity of Gs leaves extract. Extract of the leaves of Gs was tested for its antimicrobial activity against *Stemphylium solani*, *Aspergillus flavus*, *Trichoderma viride*, *Penicillium* Spp., *Fusarium verticillatum*, *Cladosporium cladosporioides*, and *Fusarium solani*. This present study showed that Gs leaves extract has no inhibition activity against all of the tested fungal strains. On the other hand, the toxicity test, which was conducted by using brine shrimp, suggests that Gs leaves extract is apparently not toxic. The phytochemical screening revealed that Gs leave extract contains alkaloids, flavonoids, terpenoids, tannin, carbohydrates, proteins, steroids, and saponins. The results of this preliminary investigation suggests that the medicinal plant extract may be safe to use as a drink for treatment of various diseases as has been practiced for years in the villages of Western Sudan. More research is needed to investigate if there is any side effect when the extract is taken orally. Further, the medicinal properties of the phytochemical compounds of Gs need to be further investigated.

**Keywords:** *Guiera senegalensis*; Antifungal activity; Brine shrimp toxicity; Phytochemical analysis; Sudan

## Introduction

Several traditional medicinal plants, including *Guiera senegalensis* (Gs), a shrub that grows well in sub-Saharan Africa and Sudan [1], have been candidates for research because of their perceived medicinal properties. Evaluation of compounds such as, tannins, alkaloids, flavonoids saponins, terpenoids and phenols have been used as a method of screening of medicinal plants [2]. Gs has been used in Western Kordofan of Sudan and elsewhere in traditional medicine as a cure for infections and wounds [3,4]. In the Sudan, Gs is locally known as Ghubaysh of which the leaves extract and the roots powder are used for treatment of a variety and diseases and wounds, respectively. In a companion paper [4], we found that Gs leaves' extract has been used for treating jaundice which represents more than 51.5% of conditions treated; and 48.5% of the other diseases that include diabetes mellitus, hypertension, cough, arthritis, enteritis, diarrhea and malaria. In addition, the majority of people surveyed have used roots' powder of Gs for treatment of wounds, including diabetics wounds, and inflammation of skin, and injuries. Gs extract was also used in the folk medicine in other countries and found to be effective against leprosy, fever, and was helpful against increased blood pressure and high blood sugar levels [5]. In addition to its usefulness as traditional medicinal plant, Gs has been shown to increase soil fertility and crop production without use of fertilizers in in the Northern Sahel region, especially in low-yields-low input farms [6]. The importance of Gs in traditional medicine became more apparent with the recent increase in fungal infections in Africa, and elsewhere. Extracts of Leaves, shoots and galls of Gs were found to be useful against bacteria and fungi infections in Burkinabe folk medicine [7]. These antimicrobial properties were credited to the crude methanolic extracts of Gs [8]. Studies of medicinal plants usually concentrate on the part of the plant that has been shown to have value in traditional medicine. Although Gs leave extract has been used to treat jaundice and many diseases in Western part of Sudan, the antifungal activity of leave extract has not been documented. The plant extract is prepared by boiling the leaves or soaking them

in water [4]. Since there are thousands of species of fungi present in the environment and at least a hundred of them are pathogenic [9], antifungal activity of medicinal plants needs to be addressed. It worth mentioning that the antifungal activity of many medicinal plant species in Africa has not been satisfactorily explored. The aim of study was to examine the phytochemical properties, antimicrobial efficacy and toxicity of Gs leaves' extract in order to find out their biological activity.

## Materials and Methods

### Plant materials

The leaves of Gs used in this study were randomly collected from Gs bushes around Ghubaysh village area of Western Kordufan State, West of the Sudan. The plant leaves were dried in the shade. After drying, the leaves were ground well into fine powder using mechanical blender, and the powder was transferred into airtight containers with proper labeling for future use.

### Water extraction

Five grams of the plant powder was placed in a beaker containing 100 ml. distilled water; soaked and shaken well. The solution was filtered with the help of filter paper and the filtrate was kept and used for further phytochemical analysis. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use.

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### Toxicity test

Brine shrimp (*Artemia*) lethality bioassay, was carried out to investigate the cytotoxicity of plant extract. Fifty mg of *Artemia salina* (Leach) eggs were added to a hatching chamber containing sea salt water (30 gr/L). The hatching chamber was kept under an inflorescent bulb for 48 h for the eggs to hatch into shrimp larvae. Different concentration of the extract (250, 500 and 1000) µg in 40 ml distilled water were prepared in triplicate vials. Ten brine shrimp larvae were then placed in each of the three duplicate vials. Others were placed in distilled water to serve as a negative control and incubated for 24 h. After 24 h the nauplii were examined against a lighted background, with a magnifying glass and the average number of survived larvae was determined [10].

### Fungal mycoflora

One mg of plant powder under investigation was cultured in each of the seven petri dishes containing Potato Dextrose agar (PDA) and incubated at room temperature for seven days.

### Antifungal test

Antimicrobial activity testing was carried out by using agar spread method. One ml of the *Gs* extract (5%) was spread to each one of seven (PDA) that were cultured with *Stemphylium solani*, *Aspergillus flavus*, *Trichoderma viride*, *Penicillium* spp, *Fusarium ver ticillatum*, *Cladosporium cladosporioides*, *Fusarium solani*. The petri dishes were incubated for seven days at room temperature. At the end of incubation, zone of inhibition that developed were measured with a ruler. Distilled water was used as a control.

### Test for flavonoids

For the confirmation of flavonoids in the *Gs* leave powder, 0.5 g of plant extract was placed in a test tube and 10 ml of distill water was added, 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of the plant extract followed by addition of 1 ml concentrated H<sub>2</sub>SO<sub>4</sub>. Indication of a yellow color shows the presence of flavonoids in each extract [11].

### Test for alkaloids

The procedure used for determination of alkaloids in *Gs* sample is similar to that described by [11]. Briefly, 0.2 g of *Gs* leave powder was added to a glass test tube that has 3 ml of hexane. The powder and hexane were thoroughly mixed, shaken well, and filtered. To the hexane and *Gs* leaves extract mixture, 5 ml of 2% HCl were added, and the mixture heated until boiling. The mixture was then filtered, and 1-3 drops of picric acid were added to the hexane, HCl and *Gs* leave extract filtrate. The presence of alkaloids in the *Gs* sample was confirmed by the yellow- colored precipitate that was formed.

### Test for terpenoids

An amount of 0.8 gram of the *Gs* leave powder was placed in a test tube, then 10 ml. of methanol was poured in it, shaken well and filtered. Five ml of the plant extract of plant sample was taken. Then 2 ml of chloroform were mixed with the extract and 3 ml of sulphuric acid were added. Formation of reddish brown color indicates the presence of terpenoids in the selected plants [11].

### Test for tannins

Crude extract of *Gs* powder was mixed with 2 ml of 2% solution of FeCl<sub>3</sub>. A blue-green or black coloration indicated the presence of tannins [12].

### Test for proteins, ninhydrin test

The Crude extract of *GS* leaves powder was boiled with 2 ml of 0.2% solution of Ninhydrin. Violet color appearance will suggest the presence of amino acids and proteins [12].

### Test for carbohydrates, Fehling's test

Two ml. of equal volumes of Fehling A and Fehling B reagents mixed together were added to the crude extract and gently boiled. A brick red precipitate appearing at the bottom of the test tube indicates the presence of reducing sugars [12].

### Test for steroids

*Gs* leaves powder was mixed with 2 ml of chloroform and concentrated H<sub>2</sub>SO<sub>4</sub> was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2 ml of chloroform. Then 2 ml of each of concentrated H<sub>2</sub>SO<sub>4</sub> and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids [12].

### Test for saponins

The crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins [12].

## Results and Discussion

### Toxicity test

In the present study, brine shrimp (*artemia*) toxicity test was used to find out if the leaves extract is safe to consume. The *artemia* toxicity test is used routinely because it convenient to use in addition to being inexpensive. As a routine test, it is employed as an initial step in the assessment and evaluation of the toxic characteristics of a substance [13], for screening drugs, and for determination of lethal doses, and for assessing health hazards that may arise from short term exposure to drugs [14]. On these grounds, we decided to use the brine shrimp (*artemia*) toxicity test to evaluate if the traditional medicinal plants *Gs* leave extract has potential toxicity. In the present study, the leave extracts from *Gs* showed no toxicity to brine shrimps at concentrations of 250,500, and 1000 µg in 40 ml of distilled water, and that was confirmed by the number of dead brine shrimp larvae, which was zero. From this result, we can conclude that the *Gs* leaves extract is apparently not toxic. Our result are in general agreement with that obtained by Ref. [14] who observed that water extract of *Gs* given at the rate of 2 g/kg to Wister male and female rats showed no toxicity as confirmed by the evaluation of pathological changes in different organs, hematological parameters, and urinalysis. The results of the present study are also in agreement with Ref. [15] who concluded that *Gs* aqueous leave extract is not toxic and may be safe, especially at the dose used for treatment, which is greatly lower than the doses used in their experiments. However, the findings of the present study are not in agreement with that found by Ref. [16] who reported signs of toxicity when experimental animals were drenched with the leaves powder of *Gs*. At the dosage of 1 or 2 g/k/day. This disagreement could be explained by the fact that the present study used the aqueous extract of the leaves while Ref. [16] used the actual leaves powder. Interestingly, Ref. [16] observed that the treated animals became drowsy suggesting an effect of *Gs* powder on the cardiovascular system function and blood pressure. This may explain the folk use of the plant for hypertension control. Further, Ref. [17] reported that spontaneous motor activity

was decreased in mice by administration of the aqueous extract of *Gs*, and that the sleeping time induced by pentobarbital was prolonged in treated mice.

### Fungal test

Physical and microscopic examination of the cultured *Gs* powder showed growth of *Aureobasidium pullulans*, *Penicillium* spp. and *Rhizopus* (Figure 1).

### Antifungal activity

In developing countries, medicinal plants are gaining popularity because of their perceived effects against microbes, and cost or invariability of health care. The World Health Organization (WHO) reported that approximately 80% of the world population use traditional medicinal plants the extracts or the active ingredients in medicinal plants [18]. On the other hand, more than 60% of the successful drugs developed against infections during the last twenty years utilized natural products [19]. Therefore, the interest in traditional medicinal plants will continue to grow as emerging infectious diseases continue to expand, and the numbers of drug resistant microbes continue to escalate. Further, plant extract are often consumed without paying attention to safety issues. In the present work, the antimicrobial activity of the extracts of *Gs* were studied against seven fungal strains. For the antifungal susceptibility test, the leaves extracts obtained from *Gs* showed no activity (No inhibition) against all of the tested fungal strain. Ref. [20] indicated that since solvents used for extraction are expected to have different range of solubility for the phytochemical component of the medicinal plant, ethanolic extract would be more effective in treatment than an aqueous extract. In Western Sudan, people use the aqueous extract of the leaves as a medicine. However, from drug discovery point of view, it would be interesting to quantify antimicrobial activity of *Gs* ingredients extracted using ethanolic solvent in which plant material is expected to dissolve better.

### Phytochemical analysis

This study also showed the presence of different phytochemicals whose biological activity can be of valuable therapeutic index. The result of the presence of the phytochemicals in *Gs* extract, shown in Table 1, is indicated by the colour intensity in a scale of 4 (+, ++, +++, ++++).

Since medicinal plants, such as *Gs*, are expected to be a valuable source for a variety of drugs, no wonder that 80% of individuals from developed countries use traditional medicines extracted from plants according to WHO [18]. It, therefore, is important to investigate traditional medicinal plants to assess, following scientific

methodologies, their properties, safety, and efficiency of treatment, and the optimum dose [21]. Table 1 shows that plant under investigation contains flavonoids, alkaloids, terpenoids, tannin, proteins, carbohydrates, steroids and saponins. Our results are in the same line with the findings obtained by Ref. [22] who indicated that *Gs* leave extract had been found to contain alkaloids, tannins, flavonoids, amino acids, in addition to its antimicrobial activity. The results of the present study also agrees with that reported by Ref. [23] who also confirmed the presence in *Gs* leave extract of flavonoids, saponins, tannins, terpenes, and carbohydrates. One of ingredients that we found in *Gs* leave extract are terpenoids, which are found to have a variety of pharmacological activities such anti-bacterial, anti-inflammatory and anti-malarial activities [24,25]. The presence of flavonoids in *Gs* leaves extract documented in this study supports the findings of Ref. [26] who stated that the antimicrobial properties in some traditional medicinal plants can be due to the presence of flavonoids. Furthermore, several biological effects such as antioxidants abilities, and anti-inflammatory effects are attributed to flavonoids and phenolic compounds in medicinal plants [27]. These observations and the findings of the present study suggest there is an urgent need for investigating why *Gs* extract is credited for curing several diseases and infections in Africa. It would important to find out which phytochemical compound in *Gs* is responsible for what activity.

The content of alkaloids, tannin and saponins found in the present study agree with that reported by Ref. [28] who reported high levels of alkaloids and low tannins and saponins content in *Gs*. Alkaloids found in medicinal plants used as anesthetic agents [29], a premise that was not investigated in the present study. In addition, tannin have been found by Ref. [30] to have a wide range of antimicrobial and anti-inflammatory effects. The anticarcinogenic potentials of tannins could be attributed to their antioxidative properties that protect living cells from oxidative damage [31]. It is documented in this study that steroids were present in *Gs* leave extract. These steroids have antibacterial effects [32], and are important because of its relationship with steroid hormones such as sex hormones estrogen and testosterone [33].

The observed antibacterial effects of the medicinal plants extracts have been related to the presence of tannins, flavonoids and saponins [34]. This is in agreement with Ref. [35] who investigated the effect *Eucalyptus Camaldulensis* extract against pathogenic bacteria (*Salmonella typhi* and *Escherichia coli*) and attributed the effect of the plant extract to the active phytochemical compounds it has, namely tannins, flavonoids and saponins. *Gs* has been shown to contain compounds such as alkaloids, tannins, flavonoids that demonstrated their antimicrobial activity [36].

### Conclusion

In conclusion, medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs for treatment of various diseases. The results obtained in the present study have shown that *Gs* leaves have high concentration of alkaloids and low concentration of tannins and saponins and have no toxic effect and show no antimicrobial activities in case of fungi. These observations and the findings of the present study suggest an urgent need for investigating why *Gs* extract is credited for curing several disease and infections in Africa. It would important to find out which phytochemical compound in *Gs* is responsible for what activity. Thus, we hope that the important phytochemical compounds identified by our study in the *Gs*, will be helpful in treating different diseases of this particular region of Africa, and may be in other regions. The plant extract may be safe, especially at the therapeutic dose which



Figure 1: Growth of fungi in Potato Dextrose agar.

Plant species	Flavonoids	Alkaloids	Terpenoids	Tannin	Proteins	Carbohydrates	Steroids	Saponin
<i>Guiera senegalensis</i>	+++	++++	++	+	++	++	++	+

+indicates presence of phytochemicals; ++=indicates small presence; +++=indicates moderate presence; ++++=indicates High presence

**Table 1:** Phytochemical component of *Guiera senegalensis* leaf extract.

is far lower than the tested doses. The results could serve for further pharmacological and phytochemical research. Also more research needed to evaluate the potential effectiveness of the crude extracts as the antimicrobial agents.

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