

Identification of an Active Component in *Guiera senegalensis* Plant Used for Healing Diabetes Wounds

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Abstract: The African species Ghobash (*Guiera senegalensis* J. F. Gmel), is a small herb found mainly in West Africa. It had wide spread used in traditional medicine. The aim of this study is to identify the bioactive ingredient responsible of healing diabetes wound. The aim was obtained using different process; first the plant crude oil was extracted using the soxhlet apparatus and two organic solvents (Chloroform and Methanol). Then the natural products present in dry plant leave were investigated by phytochemical screening analysis tests which confirm the main classes of secondary metabolites, namely terpenoids, alkaloids, flavonoids and steroides. The anti-bacterial activity test was carried out to show the bioactivities properties of the plant. The tests were positive in two types of bacteria: staphylococcus aureus and pseudomonas aeruginosa. The (GC/MS) analysis was carried out to separate and identify the components of the plant and their structures. The obtained results showed the presence of different active ingredients such as: flavonoids, alkaloids and steroids in high percentage. The expected compound responsible of healing diabetes wound was suggested to be stigmasterol.

Keywords: *Guiera Senegalensis*, J. F Gmel, Active Component, Healing Diabetes Wounds, Stigmasterol

1. Introduction

Guierasenegalensis is one of the most popular West African medicinal plants, and is used to treat a wide variety of diseases. Its uses are comparable to those of *Combretummicranthum* G. Don, commonly called 'kinkeliba', and the plants are often used combined, especially, to treat common colds, fever and respiratory problems. The bitter leaves are most frequently used in Sudan aleaf is taken to treat diabetes. The wood is used for the framework of wells, bed posts and roof lattice work and it is also, commonly, used to fence farms. It is also an important source of fuel. The roots are split and used as chew sticks and tooth picks. The fruit yields a black dye. The leaves enter into various medico-magical preparations, e.g. to free people from evil spirits and to bring good luck. Leaves and roots contain β -carboline alkaloids (0.15–0.2%), and the

methoxylatednaphthyl butanone guieranone A. In the roots tetrahydroharman (eleagnine) was the main alkaloid, with harman and harmalan (dihydroharman) as minor compounds. The leaves contain the alkaloids tetrahydroharman and harman, as well as guieranone A.

Tannins were present in large amounts in all plant parts, including the galls. The different plant parts showed quantitative and qualitative differences with respect to the chemical composition of the tannins. Nine gallotannins with a quinic acid core and two condensed tannins (epicatechin and epigallocatechin gallate) have been isolated so far. The major tannin in all plant parts is 3,4,5-tri-O-galloylquinic acid. From the leaves, roots and galls a range of flavonoids was isolated, including catechin, myricitrin, several myricetin derivatives, rutin, rhamnetin, quercetin,

quercetrin, kaempferol, tiliroside, apigenin and gallic acid. From the leaves the naphthopyrones 5-methylhydroflavasperone and 5-methylflavasperone and the amino acid ascorbic acid have been isolated. Mucilage was also present in all plant parts, but mostly in the fruits. *Guiera senegalensis* is acclaimed as a common herbal antipyretic and antimalarial among some tribal groups in northern Nigeria. Leaf extracts of the plant were thus tested for antiplasmodial, analgesic and anti-inflammatory effects in vivo. Results indicated the safe dose of extracts as 600 mg/kg body weight of mice with LD₅₀ of 1100 mg/kg bw. Only the methanolic fraction had antiplasmodial effect while ethylacetate and hexane fractions were ineffective. [3] Furthermore the methanolic extract produced a significant ($p < 0.05$) suppression of up to 67.52% levels. The extracts had no prophylactic effect and high parasitaemia including mortality of sub-inoculated mice were obtained on day 14 post treatment. [3] It gave 44.83% analgesic effect but was devoid of anti-inflammatory activity. Phytochemical screening indicated the presence of alkaloids, glycosides, tannins and flavonoids [3]. The antimicrobial activities of aqueous and methanol extracts of *Guiera senegalensis* were studied against some enteropathogens by agar-well diffusion method. Both extracts inhibited the growth of *Streptococcus Pneumonia*, *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter cloacae*, *Salmonella* spp and *Pseudomonas aeruginosa*. [3] The methanol extract was more effective on *S. Pneumonia*, *E. coli*, *S. aureus*, *E. cloacae*, *Salmonella* spp, while the aqueous extract was more active on *Salmonella* spp. and *P. aeruginosa*. [3] The methanol extract showed quicker curative action in, experimentally, induced diarrhoea in adult male mice when administered once or twice at a dose of 556 mg/kg daily. [4] From this work, *G. senegalensis* root appears to be an effective measure for treating diarrhoea. Where food poisoning or metal poisoning are the causative agents. The phytochemical screening results of the aqueous extract, showed the presence of tannins, saponins, alkaloids, cardiac glycosides, coumarin and anthraquinones.

However [4] anthraquinones were not detected in the methanol extract and cardiac glycosides were not detected in the aqueous extract. Ethno medicinal use of this plant in the treatment of diarrhoea is justified by this work and is encouraged [4]. Recent study the claimed antimicrobial activity of Sudanese medical plant, *Guiera senegalensis* leaves studies. This involved preparation and use of methanolic extract of *Guiera senegalensis* leaves. The study conducted on Guinea pig and rat with induced inhibition and slight contraction. The extract showed dose dependant inhibition on force and rate of contraction of isolated Guinea pig atrium. In isolated rat uterus preparation the extract showed slight contraction which blocked by (2 mg/mL-1) cyproheptidine [5]. The chemical composition of total alkaloids from leaves and roots of *Guiera senegalensis* was investigated [6]. Three beta-carboline alkaloids were purified: in addition to harman and tetrahydroharman, known in roots and leaves, harmalan (dihydroharman) was

isolated for the first time from roots of *Guiera senegalensis*. Guieranone A, a naphthylbutenone, was also purified from leaves and roots. The in vitro antiplasmodial activity and the cytotoxicity of extracts and pure compounds were evaluated. Each total alkaloid extract and beta-carboline alkaloids presented an interesting antiplasmodial activity associated with a low cytotoxicity. Harmalan was less active than harman and tetrahydroharman. Guieranone A showed a strong antiplasmodial activity associated with a high cytotoxicity toward human monocytes. Its cytotoxicity was performed against two cancer cell lines and normal skin fibroblasts in order to study its anticancer potential: guieranone A presented a strong cytotoxicity against each cell strains. Finally, evaluated the potent synergistic antimalarial interaction of *Guiera senegalensis* and two plants, commonly, used in traditional remedies: *Mitragyninermis* and *Pavetta crassipes*. Three associations evaluated were additive. A synergistic effect was shown between total alkaloids extracted from leaves of *Guiera senegalensis* and those of *Mitragyninermis*. This result justified the traditional use of the plants in combination to treat malaria.

GC-MS analysis and antifungal activity from galls of *Guiera senegalensis* J. F Gmel (Combretaceae) have been investigated [7] The *G. senegalensis* exhibited an interesting antifungal activity against all strains tested. Quantitative phytochemical and elemental analysis of *Guiera senegalensis* leaf extract was carried out [8] 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 $\text{Ca} > \text{K} > \text{P} > \text{Na} > \text{Mg} > \text{Fe} > \text{Zn} > \text{Cu}$. General Phytochemical Screening and Antioxidant Activity of Some Sudanese Medicinal Plants including *Guiera senegalensis* were reported [9] and Acaricidal properties of two extracts from *Guiera senegalensis* J. F. Gmel. (Combretaceae) against *Hyalomma anatolicum* (Acari: Ixodidae) was studied [10]. Antitoxic, Antifungal and Phytochemical Analysis of Medicinal Compounds of *Guiera senegalensis* had been extensively studied by many authors [11-13]. Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts was also investigated [14], recently. Phytochemical Analysis, Cytotoxicity and Antifungal Activities of *Guiera Senegalensis* Leaves Extract Review was carried out [15] The aim of the review was to investigate the phytochemical analysis, toxicity, and the antifungal activity of *Guiera senegalensis* leaves extract and compare methods and they concluded that 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 *Guiera senegalensis* need to be further investigation. The aim of this study is to Identify of an Active Component in *Guiera senegalensis* Plant Used for Healing Diabetes Wounds.

2. Materials and Methods

2.1. Materials

- i Samples of *Guierasenegalensis* leaves were collected from West Sudan, Northern Kordfan (700Km west khartoum)
- ii All chemicals used were of analytical grade type and includes:

Chloroform (99%), Methanol (99%), Wagner's reagent, Hager's reagent, Alkaline reagent, Copper acetate, Hydrochloric acid, Lead acetate, deionized Water.

- iii Micro Organism (*Staphylococcus aureus* ATCC6538 Passage No 4, *Pseudomonas aeruginosa* ATCC9027 Passage No 4).

- iv Glass ware and Apparatus:

Soxhlet. Apparatus, Beakers, Flasks, Petri dishes, pipette

- v Instrument:

Gas Chromatography Mass Spectrometry (GC/MS).

Instrument information:

Detector: Mass spectrometer

Model: GC/MS-QP2010Ultra

Company: Shimadzu

Country: Japan

Column: Rtx-5MS... Length (30)... Diameter (0.25)...

Thickness (0.25μI)

Carrier gas: Helium

2.2. Methods

Sample preparation for photochemical screening:

Guiera senegalensis leaves were collected from West Sudan, Northern Kordfan (700Km west khartoum) and dried in shadow. 10 g of the dried leave were ground by mortar and extracted by soxhlet using chloroform, Extraction was repeated using methanol.

Phytochemical screen:

Alkaloid test:

- a) In dry and clean test tube 1 ml of the extract were added and 3 drops of dilute Hydrochloric acid then it were treated with Wagner's reagent (Iodine in Potassium Iodide). the formation of brown /reddish precipitate indicates the presence of alkaloids.
- b) 1 ml of the extract were added to dry and clean test tube and 3 drops of dilute Hydrochloric acid were added then it were treated with Hager's reagent (saturated Picric acid solution). the presence of alkaloids confirmed by the formation of yellow colored precipitate.

Phytosterols, libermann test, 2 ml of the extract few drops of chloroform, 3 drops of acetic anhydride, and 1 drop of

concentrated sulphuric acid were added. The appearance of purple color shows the presence of steroid.

Terpens test, Copper acetate test, The extract were dissolved in water and treated with 3 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpens.

Flavonoids:

- a) Alkaline Reagent test: In clean and dry test tube 1 ml, of extract, was treated with few drops of sodium hydroxide solution. Formation of intense yellow color which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

- b) Lead Acetate test: In dry and clean test tube 1 ml, of extract was treated with, few, drops of lead acetate solution. Formation of, ayellow, precipitate indicates the presence of flavonoids.

Anti-Bacterial test:

Preparation of Chloroform extract (Reagent 1):

0.5 g of chloroform extracted sample were dissolved in 2.5 ml of Methanol (Absolute) analytical grade, well, mixed and sonicated for 15 min, the volume was completed to 20 ml with water for injections, well mixed and used.

Preparation of Methanol extract (Reagent 2):

0.396 g of methanol extracted sample were dissolved in 2.5 ml of absolute Methanol analytical grade, mixed well then sonicated for 15 min, the volume completed to 20 ml with water, for injections, well mixed, and used.

2.3. Methodology of Antimicrobial Tests

In a rack of 20 test tubes 1 ml of tryptone soya broth placed in each tube, tilted in screw capped bottle. At 121°C for 15 min the media were sterilized using an autoclave, at 121°C and 15bar pressure, and left Then to cool at room temperature.

Suspensions of organisms were prepared in normal saline and compared with Macfarland turbidity. Reagents (1&2) were diluted in four, separate, set, of ten test tubes, with previously prepared tryptone soya broth (each reagent in 2 set of tubes), suspensions of 1 ml of organisms were added in four set of tubes (each bacterium suspension in two set of different diluted Reagent). After mixing the four set were incubated at 32°C for 24 hour.

3. Results

Tables 1 and 2 showed the results of Phytochemical screening of the Chloroform and Methanol extract respectively whereas table 3 showed the Anti-bacterial activity of the extracts.

Table 1. Resultsof phytochemical screeing of the Chloroform extract.

Natural Product Test	Name of Reagent	Results
Alkaloids	Wagner, s	+
	Hagers	+
Flavonoids	Alkaline	+
	Lead Acetate	+
Terpens	Copper Acetate	+
Phytostrols	LibermannBurehards	+

Table 2. Phytochemical screening of the Methanol extract.

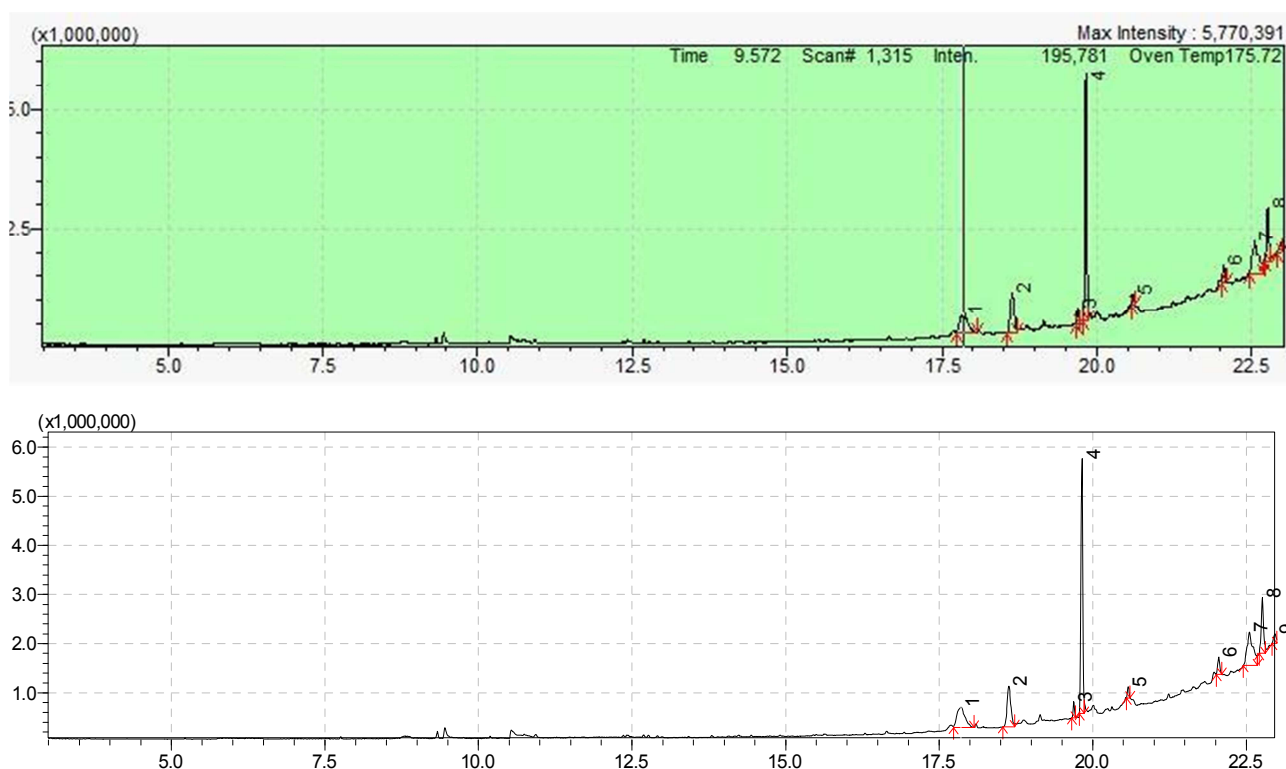
Natural Product Test	Name of Reagent	Results
Alkaloids	Wagner, s	+
	Hagers	+
Flavonoids	Alkaline	+
	Lead Acetate	+
Terpens	Copper Acetate	+
Phytostrols	LiebermannBurehards	+

Table 3. Anti-bacterial activity of the extracts.

Bacteria Organism	Type of extract	%Minimum Inhibitory concentration (MIC)
Staphylococcus aureus ATCC6538 Passage No 4	Chloroform	1.25%
	Methanol	2%
Pseudomonas aeruginosa ATCC9027 Passage No 4	Chloroform	2.5%
	Methanol	2%

Table 4. Peak report of TIC.

Peak	R. Time	Area	Area %	Name
6	22.049	803094	2.87	Stigmasterol

**Figure 1.** Gas chromatography mass spectrometry chromatogram of the mixture extract of the GuieraSenegalsis dry leaves.

4. Discussion

The phytochemical screening shows the presence of natural products as the results show in Tables 1 and 2. Identical quantities of alkaloids, terpenes, flavonoids and steroids were detected in the two solvents extracts which explain the high value of the use of this plant in the traditional medicine.

Antibacterial test, show significant activity against two types of bacteria, gram-positive cocci bacterium and gram-negative, rod-shaped bacterium which classify this plant as

one of strong antibiotics that work in wide spectrum as shown in Table 3.

GC/MS analysis shows many bioactive components as shown in Tables 4 and Figure 1 which lead to identification of the component used for healing diabetes wound, the main objective of this study. This component is Stigmasterol which industrial company uses as the starting raw material for the synthesis in the medical field which reduces inflammations, swellings and attendant pain and at the site of injury.

One of the components of the (GC/MS) analysis is Quinacridone which is a quinoline derivative used to treat malaria. This explains the antiparasitic activity of plant extract.

These components have different activities which explain the many uses of this plant in traditional medicine.

5. Conclusion

In the present study the *Guiera senegalensis* J. F. Gmel species extract were investigated, the phytochemical screening showed the presence of alkaloid, terpenes, flavonoids and steroids, the GC/MS analysis show different bioactive components which confirmed by the activity against some microorganism.

The bioactive component responsible for healing diabetes wound was identified as Stigmasterol.

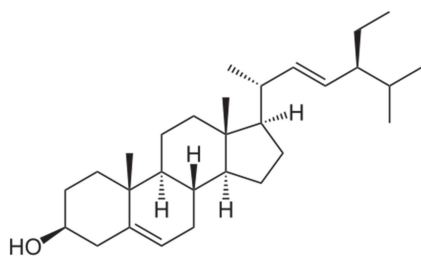


Figure 2. Stigmasterol.

6. Recommendations

The use of traditional, herbal, medicine is recommended for the following reasons:

1. More available.
2. Less expensive
3. limited risk of contamination or expiry.
4. No side effects as general.

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