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Pharmacognostic, acute toxicity and analgesic studies of the methanolic stem extract of *Guiera senegalensis* J. F. Gmel (Combretaceae)

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Abstract
The present study deals with the pharmacognostic, acute toxicity and analgesic studies of the methanolic stem extract of *Guiera senegalensis* J.F. Gmel, family Combretaceae which is claimed by the Hausa in the Northern Nigeria to be used traditionally for the treatment of pain. The various features of the stem were observed macroscopically using the standard description of terms. The microscopic features of the plant were also evaluated. Phytochemical screening and other physicochemical parameters of the extract were similarly determined. The extract was found to inhibit acetic acid-induced writhing in mice in a dose dependent manner. The administration of acetylsalicylic acid 30 minutes before intraperitoneal injection of acetic acid solution greatly decreased the number of writhing when compared to the control. The basis for the traditional use in the management of pain has been demonstrated.

Keywords: *Guiera senegalensis,* methanolic stem extract, analgesic, toxicity

1. Introduction
Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators such as insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total. Chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to have not only beneficial pharmacology, but also gives them the same potential as conventional pharmaceutical drugs to cause harmful side effects [1, 2]. Plants are used in medicine to maintain and augment health physically, mentally and spiritually, as well as to treat specific conditions and ailments [3].

Guiera senegalensis, very well known in its native area, generally occurs as a shrub that can grow to a height of 3 to 5 m according to habitat. Its stem presents numerous knots that send out branches. The ash-grey stem and branches have fibrous or pubescent bark and bear opposing, short petiolate 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 lend the plant an overall silver-green colour that is conspicuous in brush land [4]. Its leaves 3 to 5 cm long and 1.5 to 3.0 cm broad are opposite or sub opposite, oblong elliptic, rounded or slightly cordate at base and mucronate at the apex. They are softly tomentose on both surfaces, with scattered black glands underneath [5]. The leaves are bitter-tasting and have widespread acknowledgement in African medicine as a "cure-all" in herbal concoctions [6]. The genus *Guiera* belongs to the family Combretaceae in the order Myrtales making up a part of the 20 genera in the family. It is a shrub found in tropical and subtropical regions, mostly in Africa and India [7, 8].

It has also been reported that crude methanol extracts of G. *senegalensis* exhibit antimicrobial properties on bacteria and fungi [9]. Phytochemical studies on *G. senegalensis* showed the presence of seven active ingredients that have anti-microbial and anti-fungal activities [10]. The methanol extract of the root of *Guiera senegalensis* is used as an Antidiarrhoeal [11]. *G. senegalensis* leaf extract showed a dose dependent ulcer curative ratio in stress induced ulcers. The ulcer inhibition percentage of the extract was closer to the standard drug ranitidine [12]. The analgesic potential detected with *G. senegalensis* explains its reported application in herbal

medicine for the treatment of fevers [13]. This effect is also an added advantage to the antiplasmodial effect in the management of malaria infection [14].

2. Materials and Methods

2.1 Test material

Samples of the plant was collected from Shere-hills in Jos East L.G.A. of Plateau State and was authenticated at the Herbarium of the Federal College of Forestry, Jos and was given a voucher number; FHJ 196. The stem was then stripped of its leaves, cut into smaller parts and was dried under shade after which it was powdered using mortar and pestle. The powdered drug was sieved with a mesh of size 20 and stored in an airtight container until when required for further work.

2.2 Extraction method

The powdered stem of *Guiera senegalensis* (80 g) was extracted by maceration using 80% methanol as the extraction solvent. The extract was concentrated inside a beaker placed in a boiling water bath and produced a dark green residue. The residue was allowed to dry and was kept in a refrigerator until when required for biological tests.

2.3 Animals

Healthy Swiss albino mice (20-30 g) of both sexes at the Animal Laboratory Centre of the Department of Pharmacology University of Jos, Nigeria were used. All the animals were housed in standard cages under laboratory condition and were fed with grower mesh (poultry feed) and water *ad-libitum*. All animal experiments were conducted in compliance with NIH guidelines for care and use of laboratory animals. This study was approved by the Ethical Committee of the Faculty of Pharmaceutical Sciences, University of Jos, Nigeria.

2.4 Chemicals and Reagents

All the chemicals and reagents used in the experiments were of analytical grade.

2.5 Microscopy

Cleared samples of powdered stem of the plant was mounted in glycerol and observed under the microscope as previously described [15].

2.6 Phytochemical screening

Phytochemical screening was carried out using the standard methods [15, 16].

2.7 Quantitative Evaluation

Moisture content, ash values (total ash and acid insoluble ash values) and extractive values (alcohol soluble and water soluble values) were determined using the standard methods and procedures [15].

2.8 Acute toxicity studies

A total of ten healthy animals of equal numbers of male and female mice were used and each received a single oral-dose of 2000 mg/kg body weight of *Guiera senegalensis* extract dissolved in distilled water. After administration of drug sample, animals were observed individually once during the first 30 minutes, periodically during the first 24 hours and daily thereafter for a period of 7 days. During this period, any change in skin and fur, eye and mucus membrane (nasal), breathing and changes like salivation, lacrimation, perspiration, piloerection, urinary incontinence, ptosis, drowsiness, gait, tremors, and convulsion were noted [17].

2.9 Evaluation of Analgesic Activity

A previously described method [18] was used. Twenty five Swiss albino male and female mice (20-30 g) were used five groups of five mice each. Group I which served as the control group received 0.2 ml normal saline each. Group II, III and IV received the extract dissolved in distilled water at the dose of 1500, 2000 and 2500 mg/kg orally respectively while group V received acetylsalicylic acid 100 mg/kg dissolved in distilled water subcutaneously which were administered 30 minutes before intraperitoneal injection of 0.6% v/v acetic acid solution in normal saline at a dose of 10 ml/kg. Immediately after administering the acetic acid, mouse pairs were placed in transparent glass cages and the number of writhing or stretches were counted for 15 min. reduction in the number of writhes compared to the control groups was considered as evidence of analgesic effect. The data were computed according to the formula:

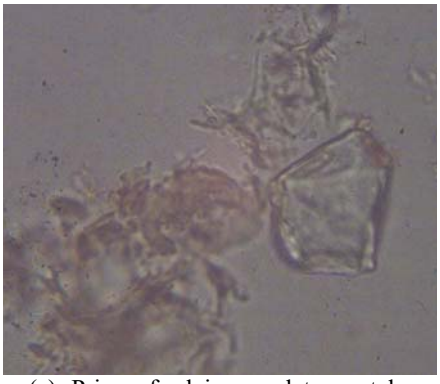
$$\text{Percentage inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

2.10 Statistical Analysis

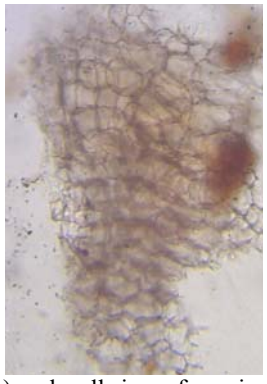
The results were expressed as mean ± Standard Deviation (SD). The mean values of control groups were compared with the mean values of treated groups using student's t-test. Results were considered significant at $p < 0.05$.

3. Results

3.1 Microscopy



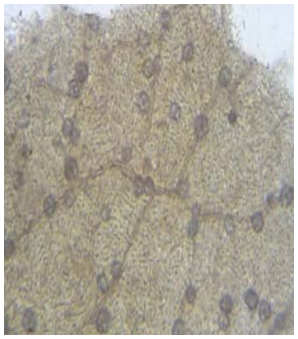
(a) Prism of calcium oxalate crystals



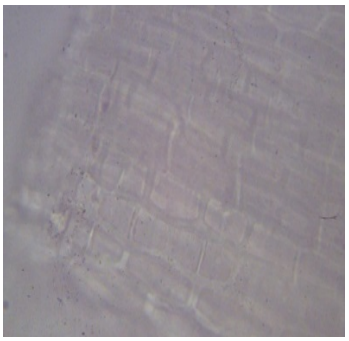
(b) cork cells in surface view

(c) single fibre

(d) Group of fibres



(e) Starch grains



(f) parenchymatous cells

Fig 1: Pharmacognostic characters of the stem of *Guiera senegalensis*

Table 1: Chemomicroscopy of the stem of *Guiera senegalensis*

Test	Observation	Inference
Cellulose	Blue- black colour observed	Cellulose present
Lignin	Red colour observed	Lignin present
Calcium oxalate crystals	Dissolution of the crystals	Calcium oxalate present
Starch grains Proteins	Blue- black Dark pink colour observed	Starch grains present Proteins present

Table 2: Quantitative values of the powdered stem of *Guiera senegalensis*

Test	Stem powder (%$^{w}/_{w}$)
Moisture content	6.75
Total ash-value	1.92
Acid insoluble ash-value	1.23
Water soluble ash-value	0.88
Alcohol extractive value	0.94
Water soluble extractive value	0.53

N=3

Table 3: Phytochemical constituents of the methanolic stem extract of *Guiera senegalensis*

Test for	Observations	Inferences
1. Tannins		
lead sub- acetate	A white colour changed was observed	Hydrolysable tannins
2. Anthraquinones	A pink rose colour in the ammonia lower phase	+
fully oxidized	A pink colour in the ammonia lower phase	+
bound		
3. Saponins		
frothing	Frothing which persisted on warming	+
Heamolytic	Complete haemolysis of red blood cells	+
4. Cardiac glycosides		
Legal	A deep red colour which faded to brownish yellow	+
Kedde	An immediate violet colour which faded gradually through reddish brown to brown yellow with a whitish crystalline solid precipitate	+
Lieberman's	A colour change from violet to blue and then green	+
Salkowski's	A reddish brown colour at the interface	+
Kella- Kiliani'	A brown ring at the interface	+
Flavonoids	A pink colour formation	+
5. Alkaloids		
Mayer's reagent	No cream precipitate	_
Dragendorff's reagent	No orange precipitate	_
Wagner's reagent	No reddish precipitate	_
Tannic acid	No black precipitate	_
Picric acid	No yellow precipitate	_

Key

+	present
-	Absent

Table 4: Effect of the methanolic stem extract of *Guiera senegalensis* on acetic acid-induced writhing in mice.

Group	Treatment	Dose (mg/Kg)	Writhing (M±SD)	% Inhibition
1	Normal saline	0.2ml	26.80±3.51	
2	Extract	1500	5.60±2.45*	79.10
3	Extract	2000	3.20±0.58*	88.06
4	Extract	2500	2.00±0.32*	92.54
5	Acetylsalicylic acid (Aspirin)	100	1.80±0.37*	93.28

Each value represents mean ± SD. *P<0.05 compared with control (student's t-test); n=6

4. Discussion

The length of various sizes of the stem ranges from about 2 to 5 cm, 3 to 4 mm in diameter and 0.5 to 1 mm in thickness were seen. The outer surface of the stem was ash-grey stem in colour while the inner surface was dark green with faint odour and slightly bitter in taste. Characteristic microscopic features of the plant seen were prism of calcium oxalate crystals, cork cells, single fibre with spindled shape and needle like ends, lignified group of fibres, spherical starch grains and phloem parenchyma which were cylindrical in shapes with thick walls were seen associated with fibres, sieve tubes which occurred in group and were not accompanied by companion cells and cork cells of thin parenchymatous cells were arranged in rows. This may serve as a diagnostic tool for its identification and differentiation. Phytochemical screening revealed the presence of tannins, saponins, glycosides, flavonoids and alkaloids. This may imply that the plant is a potential candidate for sourcing new drugs. Flavonoids, saponins and tannins might be responsible in part for the observed analgesic effect [19, 20].

The moisture content of the crude drug was found to be 6.75% $^{w}/_{w}$. The value falls within the accepted limit for moisture content of crude drugs which should not be more than 14% [21]. Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. If the water content is high, the crude drugs can easily deteriorate due to fungus and the activities of other hydrolytic micro-organisms [22].

The total ash value, water soluble ash value and acid insoluble ash value were 1.92% $^{w}/_{w}$, 0.88% $^{w}/_{w}$ and 1.23% $^{w}/_{w}$ respectively. It can be inferred based on the above data that all traces of extraneous or organic matter were removed. It also indicated the high extent of purity of the drug in the powdered plant material [22]. The result of the extractive values showed that the percentage of alcohol extractive value was 0.94% $^{w}/_{w}$ and that of water extractive value was 0.53% $^{w}/_{w}$. This implies that there is neither adulteration nor substitution with the drug. The acute toxicity study did not result in any observable symptoms or in death. No toxic effects were observed throughout the 7 days study period. No mouse showed signs of toxic effect such as changes on skins and fur, eyes and mucus membrane, behaviour patterns, tremors, salivation, diarrhea, sleep and coma or death.

In the mice writhing assay, the extract was found to inhibit the acetic acid-induced writhing in mice in dose dependent manner. It effectively reduced the pain induced by the acetic acid administered. The administration of acetylsalicylic acid 30 minutes before intraperitoneal injection of acetic acid solution greatly decreased the number of writhing when compared to the control. Administration of the extract at different doses also showed significant decrease in the number of writhes when compared to the control. The acetic acid induced writhing test is commonly used as an experimental animal model for anti- nociception. The method is very sensitive and able to detect anti-nociceptive effects of compound (s) at dose level that may appear to be inactive in other methods like tail flick test [23]. Although the test is not specific as it does not indicate whether the activity is central or peripheral [24]. The abdominal constriction response is postulated to partly involve local peritoneal receptors [25] acetic acid is known to trigger the production of noxious substances such as prostonoids like PGE_2 and PGF_2 [26] as well as Lipoxygenase products [27]. The behavioural reaction (writhing) of the animals in this model is sensitive to drugs with activity similar to aspirin, antagonists of kinin receptors and centrally/peripherally acting opioids analgesics [28]. The analgesic effect of the extracts may be either due to its action on visceral receptor sensitive to acetic acid, to the inhibition of the algogenic substances or the inhibition of transmission of painful messages at the central level [29].

5. Conclusion

The basis for the traditional use of the leaves and stem of *Guiera senegalensis* J. F. Gmel in Northern Nigeria for the treatment of pain has been justified scientifically.

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