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Research Article

PHARMACOGNOSTIC, ACUTE TOXICITY, AND ANALGESIC STUDIES OF THE STEM OF *GUIERA SENEGALENSIS* J.F GMEL (COMBRETACEAE)

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ABSTRACT

Objectives: This study was undertaken to report the various features of the whole stem of *Guiera senegalensis* J.F. Gmel macroscopically and microscopically using the standard description of terms and to also determine the acute toxicity and the analgesic activity of the powdered extract in mice.

Methods: The methods employed in this study are as reported below.

Results: The powdered stem revealed lignified fibers, prism calcium oxalate crystals, medullary rays on the fibers, parenchyma cells, and cork cells. The transverse section of the stem shows cork cells of thin parenchymatous cells arranged in rows, thick-walled and lignified sclereids, sieve tubes, and medullary rays. The moisture content of the crude drug was found to be 6.75% w/w. 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. The percentage of alcohol extractive value was found to be 0.94% w/w, and the water extractive value was found to be 0.53% w/w. In the mice writhing assay, the extract was found to inhibit the acetic acid-induced writhing in mice in a dose-dependent manner. The acute toxicity study did not result in any observable symptoms or death. No toxic effects were observed throughout the 7-day study period. No mouse showed signs of toxic effect such as changes on skin, fur, eyes, mucus membrane, and behavioral patterns. There were no tremors, salivation, diarrhea, sleep/coma, or death of any mouse.

Conclusion: The acute toxicity and the analgesic study of the plant extract were successfully determined and should be further explored for further studies.

Keywords: Mice, Acute toxicity, Analgesic, *Guiera senegalensis* J.F. Gmel, Ash value.

INTRODUCTION

Medicinal plants have been identified and used throughout human history. They are used in medicine to maintain and augment health physically, mentally, and spiritually, as well as to treat specific conditions and ailments [1]. Early 19th century was a turning point in the knowledge and use of medicinal plants. The discovery, substantiation, and isolation of alkaloids from poppy (1806), ipecacuanha (1817), strychnos (1817), quinine (1820), pomgranaze (1878), and other plants, then the isolation of glycosides, marked the beginning of scientific pharmacy [2]. The Combretaceae family consists of as many as 600 species of trees, shrubs, and lianas in about 20 genera. Plants belonging to this family are found in tropical and subtropical regions, mostly in Africa and India [3]. It is usually rich in tannin. *Guiera senegalensis,* (Fig. 1) very well known in its native area, generally occurs as a shrub that can grow to a height of 3-5 m according to habitat.

The galls of *G. senegalensis* possess effective antiacetylcholinesterase, anti-lipid peroxidation in rat brain homogenates, and erythrocytes hemolysis inhibitory activities [4]. Crude methanolic extracts of *G. senegalensis* exhibit antimicrobial properties on bacteria and fungi [5]. Phytochemical studies showed the presence of seven active ingredients that have antimicrobial and antifungal activities [6]. The analgesic potential detected explains its reported application in herbal medicine for the treatment of fevers [7]. The leaves and stem of *G. senegalensis* J. F. Gmel are locally used in Northern Nigeria for the treatment of pain, but so far, there is no scientific work carried out on the stem to justify the claim [8]. Therefore, this research work shall determine the pharmacognostic, acute toxicity, and analgesic studies of the stem of *G. senegalensis* J. F. Gmel.

EXPERIMENTAL

The microscope used is the light microscope rating: 85-265 v, 50'/60 HZ (Fisher Scientific UK), the extract was dried in a hot air oven, and the powdered extract was weighed using the Fischer Scientific UK Weighing balance. All reagents used are products of Sigma-Aldrich.

METHODS

Collection, identification, and preparation of the sample

Samples of the plant were collected from Shere Hills in Jos East L.G.A. of Plateau State, Nigeria and were authenticated at the Herbarium of the Federal College of Forestry, Jos Nigeria and were given a voucher number; FHJ 196. The stem was stripped of its leaves, cut into smaller parts and dried under shade, and powdered using mortar and pestle. The powdered drug was sieved with a mesh of size 20 and stored in an airtight container.

Preparation of alkaloidal reagents

The various preparations for alkaloidal test reagents include: Dragendorff's reagent, Hager's reagent, Mayer's reagent, and Wagner's reagent. They were all prepared through standard methods.

Macroscopical examination

The features such as color, taste, and odor of the powder were observed for proper identification [9].

Microscopical examination

A small quantity of the powdered sample was placed on a clean slide; few drops of chloral hydrate solution was added and covered with a clean cover slip. The slide was heated over spirit lamp for 20-30 seconds, then

a few drops of dilute glycerol were added, and the slide observed under the compound microscope.

Chemomicroscopical examination

Examination of the powder for starch grains, lignin, calcium oxalate crystals, oils, proteins, and tannins was carried out using standard techniques [10].

Quantitative evaluation

Determination of ash values: Total ash value

A nickel crucible was heated at 105°C to a constant weight 2 g of the powdered stem was accurately weighed into the crucible, and both were gently heated in an electric furnace until "moisture free" and completely charred, and most of the carbon volatilized leaving the organic ash. The heating and cooling exercise were repeatedly done until a constant weight of ash was obtained. The weight of the ash was calculated by subtracting the weight of the crucible from the final weight of the crucible and the ash, and the total ash value was calculated as the percentage of the weight of the crude [11].

Determination of ash values: Acid-insoluble ash value

The crucible and the ash obtained above was transferred into a beaker, and 25 ml of dilute hydrochloric acid was added and boiled for about 5 minutes and filtered through ash less filter paper. The insoluble ash value was then determined adequately using the formula:

$$\% \text{ Acid-insoluble ash value} = {}^{y}\!/\!_{x} \times 100$$

Where, weight of powdered sample used = x, Mean acid-insoluble ash = y.

Determination of extractives: Alcohol-soluble extractive value

The powdered stem (5 g) was weighed into 250 ml stopper conical flask and 100 ml of 90 % ethanol was added, and the flask was shaken on a mechanical shaker for 6 hrs and was allowed to stand for 18 hrs after which it was filtered using a suction pump. The weight of a flat-bottom evaporating dish was heated (105°C), cooled, and weighed. The % alcohol extractive value was then calculated with reference to the initial weight of the powdered sample. The mean of three determinations was used [12].

$$\% \text{ Alcohol extractive value} = \frac{\text{Weight of residue in 100 ml}}{\text{Weight of powder}} \times 100$$

Determination of extractives: Water-soluble extractive value

The above experiment was repeated but with chloroform water instead of ethanol as the extractive solvent. The water-soluble extractive value was calculated. The mean of three determinations was used.

$$\text{Percentage water-soluble extractive value} = \frac{W_4 \times 100}{W_0}$$

W_4 - Weight of residue from 100 ml extracts, W_0 - Weight of powdered sample.

Determination of moisture content

About 2 g of the powdered drug was added into an evaporating dish (heated at 105°C) and weighed then the content dried in an oven to a constant weight. The total loss in weight (weight of the moisture) was determined by subtracting the weight of the dish and powdered sample after heating from the weight of the dish and its content before heating.

Fig. 1: *Guiera senegalensis* J.F. Gmel

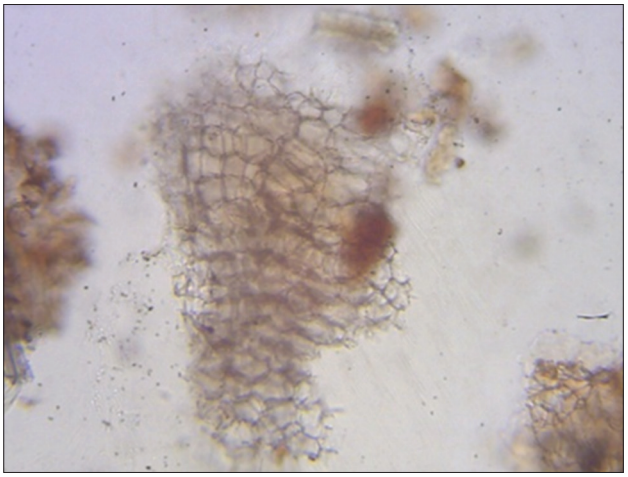

Fig. 3: Cork cells in surface view (Magnification, ×40)

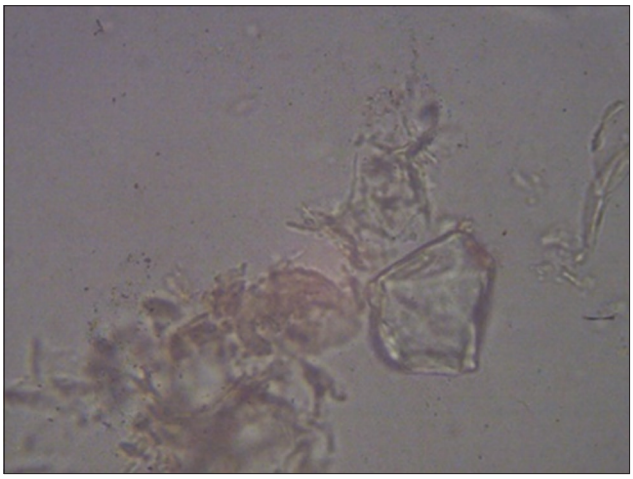

Fig. 2: Prisms of calcium oxalate crystals (Magnification, ×40)

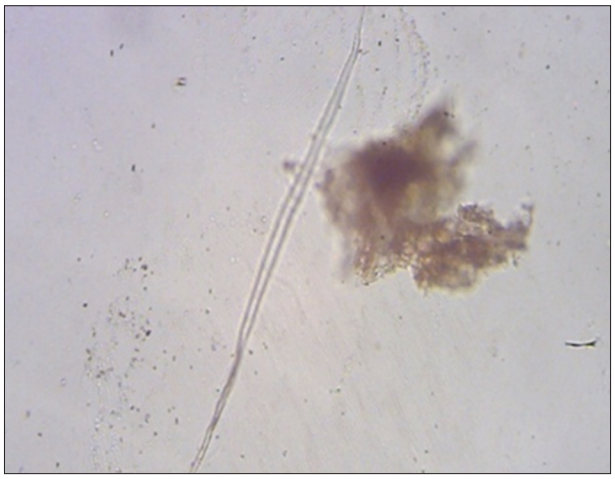

Fig. 4: Single fiber with spindle shape (Magnification, ×40)

$$\% \text{ Moisture content} = \frac{\text{Average yield of moisture content}}{\text{Weight of drug taken}} \times 100$$

Average yield = Weight of crucible + (Sample after heating − Weight of crucible) + Sample before heating.

Phytochemical screening of the powdered stem of *G. senegalensis*

The phytochemical components of *G. senegalensis* stem were screened using standard methods described by Harborne [13]. These screening include the following:

- Tests for tannins: Lead sub-acetate test as described by Trease and Evans [14]
- Tests for anthraquinones as described by Sofowora [15]
- Tests for saponins: Frothing test as described by Sofowora [16]
- Tests for cardiac glycocsides: Keller-Kiliani test as described by Sofowora [17]
- Tests for flavonoids as described by Brain and Turner [18]
- Tests for alkaloids as described by Brain and Turner [19]. This involves the use of Mayer's reagent test and Dragendorff's reagent test
- Tests for carbohydrates: Molisch test as described by Evans [20].

Extraction of the powdered stem of *G. senegalensis*

The powdered stem of *G. senegalensis* (80 g) was extracted by maceration using 80% methanol. The extract was concentrated inside a beaker on a boiling water bath and the dark green residue produced was air dried and refrigerated.

Experimental animals

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. This study was approved by the Ethical Committee of the Faculty of Pharmaceutical Sciences, University of Jos, Nigeria [21].

Acute toxicity test

A total of 10 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 *G. senegalensis* extract dissolved in distilled water. The animals were observed individually once during the first 30 minutes then periodically during the first 24 hrs and daily thereafter for 7 days. Changes in skin and fur, eye, mucus membrane (nasal), and breathing and changes such as salivation, lacrimation, perspiration, piloerection, urinary incontinence, ptosis, drowsiness, gait, tremors, and convulsion were all noted [22].

Evaluation of analgesic activity

Acetic acid-induced abdominal writhing in mice

The method described by Onansawo *et al.* [23] was used. 25 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. Groups II, III, and IV received the extract dissolved in distilled water at the dose of 1500, 2000, and 2500 mg/kg orally, respectively, whereas 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 10 ml/kg. Subsequently, mouse pairs were placed in transparent glass cages, and the number of writhing/stretches were counted for 15 minutes, 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:

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

RESULTS

Plant collection land identification

The identity of the plant was further confirmed by the Department of Forestry Technology, Federal College of Forestry, Jos, Nigeria and the Herbarium Specimen Number, FHJ 196 was given.

Macroscopical examination of the stem of *G. senegalensis*

The stem presents numerous knots that send out branches. The ash gray stem and branches have fibrous or pubescent bark, having a characteristic odor and a slightly bitter taste. The length of the stem ranges from 1 to 3 m and 5 to 10 mm in diameter.

Microscopical features of the stem of *G. senegalensis*

Characteristic features include: Lignified fibers (Fig. 4 and 5), prism type of calcium oxalate crystals (Fig. 2), and medullary rays seen mostly on the fibers, parenchyma cells (Fig. 6), and cork cells (Fig. 3).

Transverse section of the stem of *G. senegalensis*

Characteristic features: Cork cells of thin parenchymatous cells arranged in rows, thick-walled and lignified sclereids, sieve tubes, and medullary rays.

Chemomicroscopical examination of the stem of *G. senegalensis*

This includes test for cellulose, lignin, crystals of calcium oxalate (Fig. 2), starch grains (Fig. 7), and proteins as shown in Table 1.

Quantitative values of the stem of *G. senegalensis*

The quantitative values obtained are shown in Table 2.

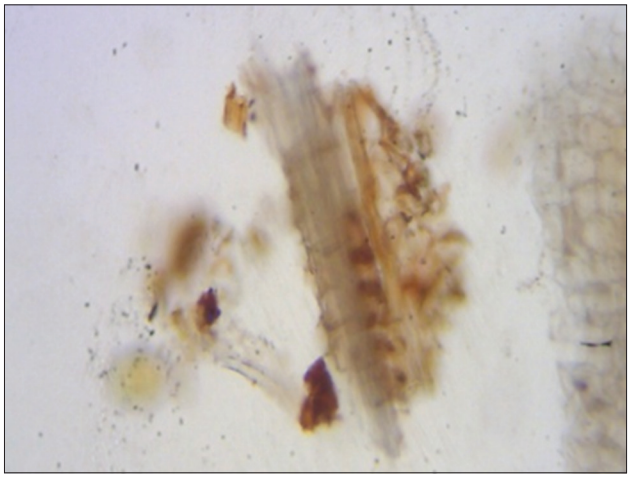

Fig. 5: Group of fibers (Magnification, ×40)

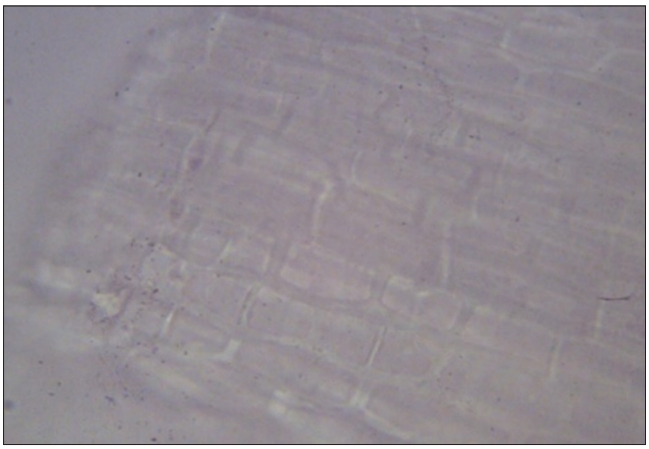

Fig. 6: Parenchyma cells (Magnification, ×40)

Phytochemical screening of the stem of *G. senegalensis*
The results obtained were shown in Table 3.

Evaluation of biological activity

Results of acute toxicity studies of the stem extract

No mice showed signs of toxic effect such as changes in skin and fur, eyes and mucus membrane, behavior pattern, tremors, salivation, diarrhea, sleep, and coma or death.

Evaluation of analgesic activities
The extract was found to inhibit the acetic acid-induced abdominal writhing in mice in the dose-dependent manner. Administration of the extract at doses also showed significant decreases in the number of writhing when compared to the control.

DISCUSSION AND CONCLUSION

Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. If the moisture content is high, the crude drugs can easily deteriorate due to fungus and the activities of other hydrolytic micro-organisms [24]. The moisture content of the crude drug was found to be 6.75% w/w which is within the acceptable limit of 14% w/w according to the African Pharmacopoeia for moisture content of crude drugs. 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. These low values also indicate the extent of purity of the powdered plant material [25]. The percentage of alcohol extractive value (0.94% w/w) was higher than that of the water extractive value (0.53% w/w), this indicates that more constituents are better extractable using alcohol than water and if adulteration is to be checked then the use of alcohol instead of water should be considered. In the preliminary phytochemical screening, the presence of flavonoids, saponins, lignins, and cardiac glycosides were observed. This makes *G. senegalensis* J.F. Gmel a good, promising, and a highly potential medicinal plant that should be under intense research. In the absence of alkaloids, we yet expect activity such as analgesic due to the presence of flavonoids, saponins, and tannins as reported by Calixto *et al*. [26]. The safety of the plant extract was ascertained when nil acute toxicity was observed neither was there any death of the animals recorded as observed in the acute toxicity studies. Moreover, the extract's ability to manage pain was demonstrated when it was able to reduce the pain induced by the administration of acetic acid (Tables 4 and 5).

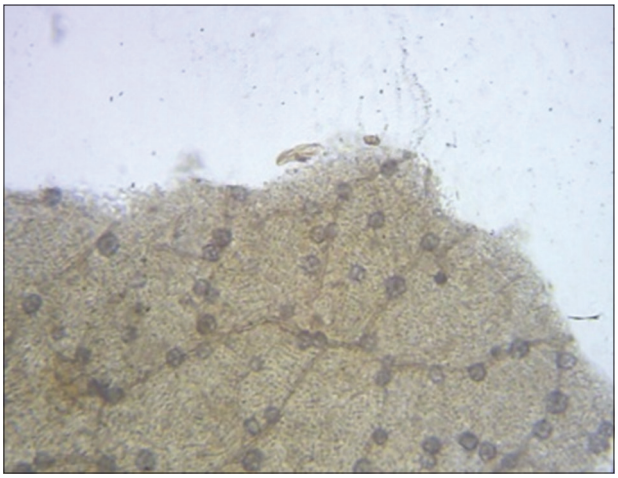

Fig. 7: Starch grains (Magnification, ×40)

Table 1: Summary of the results of chemomicroscopy

Test	Observation	Inference
Cellulose	Blue-black color observed	Cellulose present
Lignin	Red color observed	Lignin present
Calcium oxalate crystals	Dissolution of the crystals	Calcium oxalate present
Starch grains	Blue-black (Figure 7)	Starch grains present
Proteins	Dark pink color observed	Proteins present

Table 2: Results for the quantitative values of the powdered stem of *G. 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. *G. senegalensis*: *Guiera senegalensis*

Table 3: Summary of the phytochemical constituents of *Guiera senegalensis*

Test for	Observations	Inferences
Tannins		
Lead sub-acetate	A white color changed was observed	Hydrolysable tannins
Anthraquinones		
Fully oxidized	A pink rose color in the ammonia lower phase	+
Bound	A pink color in the ammonia lower phase	+
Saponins		
Frothing	Frothing which persisted on warming	+
Hemolytic	Complete hemolysis of red blood cells	+
Cardiac glycosides		
Legal	A deep red color which faded to brownish yellow	+
Kedde	An immediate violet color which faded gradually through reddish brown to brown yellow with a whitish crystalline solid precipitate	+
Lieberman	A color change from violet to blue and then green	+
Salkowski	A reddish brown color at the interface	+
Kella-Kiliani	A brown ring at the interface	+
Flavonoids	A pink color formation	+
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	-

+: Present, -: Absent

Table 4: Mean writhing number across group compared to normal group

Group	Treatment	Dose (mg/kg)	Writhing (Mean±SEM)	p value
1	Normal saline	0.2 ml	26.80±3.51	
2	Extract	1500	5.60±2.45	**0.004**
3	Extract	2000	3.20±0.58	**0.002**
4	Extract	2500	2.00±0.32	**0.002**
5	Acetylsalicylic acid (aspirin)	100	1.80±0.37	**0.002**

N=5. Bold p value indicates a significant difference in mean writhing number of group compared to normal control group ($p<0.05$). SEM: Standard error of mean

Table 5: Mean writhing number across group compared to reference group

Group	Treatment	Dose (mg/kg)	Writhing (Mean±SEM)	p value
	Normal saline	0.2 ml	26.80±3.51	
2	Extract	1500	5.60±2.45	**0.000**
3	Extract	2000	3.20±0.58	**0.078**
4	Extract	2500	2.00±0.32	**0.694**
5	Acetylsalicylic acid (aspirin)	100	1.80±0.37	

N=5. Bold p value indicates a significant difference in mean writhing number of group compared to reference group ($p<0.05$). SEM: Standard error of mean

CONCLUSION

The pharmacognostic, acute toxicity, and the analgesic studies of *G. sennegalensis* J.F Gmel were successfully determined as reported.

REFERENCES

1. Aboelsoud NH. Herbal medicine in ancient Egypt. J Med Res 2010;4(2):82-6.
2. Dervendzi V. Contemporary Treatment with Medicinal Plants. Skopie: Tabernakui; 1992. p. 5-43.
3. Hutching A, Scott AH, Lewis G, Cunningham AB. Zulu Medicinal Plants - An Inventory. Pietermaritzburg, South Africa: University of Natal Press; 1996. pp. 27-41.
4. Sombié PA, Hilou A, Coulibaly AY, Tibiri A, Kiendrebeogo M, Nacoulma OG. Brain protective and erythrocytes haemolysis inhibition potentials from galls of *Guiera senegalensis* J.F. Gmel (*Combretaceae*). J Pharmacol Toxicol 2011;6(4):361-70.
5. Bassene E, Mahamat B, Lo M, Boye CS, Faye B. Comparaison de l'activité antibactérienne de trois *Combretaceae*: *Combretum micranthum, Guiera senegalensis, Terminalia avicennioides*. Fitoterapia 1995;66:86-7.
6. Elrahaman OF, Abuelgais AI, Galal M. Toxicopathological effects of *Guiera senegalensis* extracts in Wister strain albino rats. J Med Plants Res 2009;3(10):699-702.
7. Zeljan M, Marica M, Franz B. Flavonoida of *Guiera senegalensis* - thin layer chromatography and numerical methods. Croatica Chemica Acta 1998;71(1):69-79.
8. Berhaut J. Flore du Sénégal. 2nd éd. Paris: Claire Afrique; 1967. p. 484.
9. Wallis TE. Textbook of Pharmacognosy. London: Published By SK Jain; 1985.
10. Evans WC. Trease and Evans Pharmacognosy. 15th ed. Edinburg, UK: W.B. Saunders Company Ltd.; 2002. p. 30, 518.
11. Evans WC. Trease and Evans Pharmacognosy. 14th ed. London: W.B. Saunders Company Ltd.; 1996a. p. 119-59.
12. Brain KR, Turner TD. The Practical Evaluation of Phytopharmacenticals. Brustal, France: Wright Science Technical; 1975a. p. 45, 81-2, 153.
13. Harbone JB. A Guide to Modern Techniques of Plant Analysis. New York, NY, USA: Chapman and Hall; 1973.
14. Evans WC. Trease and Evans Pharmacognosy. 14th ed. London: W.B. Saunders Company Ltd.; 1996b. p. 119-59.
15. Sofowora A. Medical Plants and Traditional Medicine in Africa. 3rd ed. Ibadan, Nigeria: Spectrum Book Ltd.; 2008a. p. 200-2.
16. Sofowora A. Medical Plants and Traditional Medicine in Africa. 3rd ed. Ibadan, Nigeria: Spectrum Book Ltd.; 2008b. p. 200-2.
17. Sofowora A. Medical Plants and Traditional Medicine in Africa. 3rd ed. Ibadan, Nigeria: Spectrum Book Ltd.; 2008c. p. 200-2.
18. Brain KR, Turner TD. The Practical Evaluation of Phytopharmacenticals. Brustal, France: Wright Science Technical; 1975b. p. 45, 81-2, 153.
19. Brain KR, Turner TD. The Practical Evaluation of Phytopharmacenticals. Brustal, France: Wright Science Technical; 1975c. p. 45, 81-2, 153.
20. Evans WC. Trease and Evans Pharmacognosy. 15th ed. London: W.B. Saunders Company Ltd.:2009. p. 334-5, 340-4, 542-78.
21. Rollin BE, Kesel ML. The experimental animal in biomedical research. Survey of Scientific and Ethical Issues for Investigators. Vol. 1. London, Boca Raton, FL: CRC Press; 2007. p. 22-35.
22. OECD. Guidelines for the Testing of Chemicals. Section 4: Health Effects Test No. 423: Acute Oral Toxicity-Acute Toxic Class Method. Paris: Organization for Economic Cooperation and Development; 2002.
23. Onasanwo SA, Pal A, George B, Olaleye SB. Pharmacological and toxicity studies of the crude extracts and fractions of *Hedranthera barteri* leaf in rats and mice. Afr J Biomed Res 2008;11:311-21.
24. Kadam PV, Yadav KN, Navasare VS, Bhiwade SK, Patil MJ. *Eclipta alba*: A Phytopharmacognostic study. Int J Pharm Phytopharmacol Res 2012a;1(6):350-3.
25. Kadam PV, Yadav KN, Navasare VS, Bhiwade SK, Patil MJ. *Eclipta alba*: A Phytopharmacognostic study. Int J Pharm Phytopharmacol Res 2012b;1(6):350-3.
26. Calixto JB, Beirith A, Ferreira J, Santos AR, Cechinel FV, Yunes RA. Naturally occurring antinociceptive substances from plant. Phytother Res 2000;14(6):401-18.