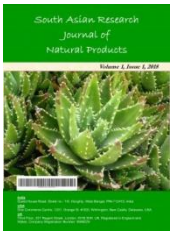

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Preliminary Phytochemical Screening, Analgesic and Anti-inflammatory Effects of the Hydroethanol and *n*-Hexane Leaf Extracts of *Maytenus senegalensis* Lam. Excell. (Celestraceae)

M. Babakura[1*], H. Usman[1], Y. A. Gaidam[1], U. A. Halima[1] and A. M. Fulata[1]

[1]Department of Chemistry, University of Maiduguri, P.M.B. 1069, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author MB designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors HU and YAG manage the statistical analysis of the study. Authors UAH and AMF managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

For long time, medicinal plants and other natural products have been used to treat varieties of diseases and as a result a number of modern drugs have been developed from them. Several families of plants have been used in ethnomedicine to treat various ailments in which Celestraceae is one. *Maytenus senegalensis* sample was collected from Takwashinge Village in Dass Local Government, Bauchi State, Nigeria. Soxhlet extraction method was adopted in this study. One thousand five hundred (1500) grams of the plant material was exhaustively extracted sequentially with *n*-hexane and 80% ethanol. The extractives were then subjected to phytochemical evaluation, analgesic and anti-inflammatory tests using Formali*n*-induced pain test and egg albumi*n*-induced paw oedema in rats.The results of the phytochemical evaluation revealed the presence of cardiac glycosides, flavonoids, saponins, tannins and terpenoids; however, anthraquinones, alkaloids and phlobatannins were absent. The results of the analgesic effect of the crude ethanol extract, and *n*-hexane extract of the leaf of *M. senegalensis*on formali*n*-induced pain in rats showed that the

**Corresponding author: Email: mohammedbabakurabama@gmail.com;*

extracts significantly inhibited the pain response at the different dosages with the most significant inhibition observed at higher doses at both phases. With a percentage inhibition of 81.31 and100, 56.82 and 46.97, for the crude ethanol extract and*n*-hexane extract respectively. The anti-inflammatory effect of the crude extract and *n*-hexane inhibited the oedema dose dependently and a maximum inhibition of 60.00% was observed at 400 mg/kg bd.wt. In the crude ethanol. Hence, the use of this part of plant in the treatment of ailments related to the analgesic and anti-inflammatory has gain scientific support.

Keywords: Analgesic; anti-inflammatory leaf extracts; Maytenus senegalensis.

1. INTRODUCTION

For thousands of years, medicinal plants and other natural products have been used to treat variety of diseases and as a result, a number of modern drugs have been developed from them [1]. The African continent has a long history with the use of plants for medicinal purposes. In some African countries, up to 90% of the population relied on medicinal plants as a source of drugs [2]. In Nigeria, World Health Organization Survey estimated that up to 75% of the population patronizes traditional medicine [3].Most ailments of the body cause pain. Pain is defined as an unpleasant, subjective, sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage [4]. Herbs such as *Maytenus senegalensis*are used in traditional medicine to treat pain.*M. senegalensis* (Lam.) Exell. locally called *'Bokaroro', 'Kunkushewa' or Namijintsada',* in Hausa; *Tultuldeor 'Yare-lesdi'* in Fulfulde; *'Afor-juru'* in Igbo and *'Sepolohun'* in Yoruba languages belongs to the family *Celestraceae*. It is a tall shrub with young branches often spiny bearing leaves and flowers. The leaves appear bluish-green in colour. The plant flowers in October and found widely distributed in Tropical and Subtropical Africa [5]. The decoction of the root bark of *M. senegalensis*is used widely in Sudan and other African countries in the traditional medicine to treat malaria [6].Traditionally, theplant is used as stimulant, pain reliever, etc. Therefore, the present study is an attempt to investigate theanalgesic and anti-inflammatory properties of the *n*-hexane and hydroethanolic extract of *M. senegalensis* leaves in experimental animals models.

2. MATERIALS AND METHODS

2.1 Plant Materials

The plant sample (leaf) was collected from Takwashinge Village in Dass Local Government, Bauchi State, Nigeria. The sample was identified by a Taxonomist in the Department of Biological Sciences, University of Maiduguri with a voucher number 12/006 deposited in Research laboratory of the Department of Chemistry, University of Maiduguri.

2.2 Extraction of Plant Materials

After shade drying in the laboratory, the plant sample (leaf) was ground into coarse- powdered form using wooden mortar and pestle. The Soxhlet extraction method was adopted in this study. One thousand five hundred (1500) grams of the plant material was exhaustively extracted sequentially with *n*-hexane and 80% ethanol. The extractives were then filtered and concentrated at low pressure to obtain a solid paste coded "HE" as *n*-hexane extract. The dried marc obtained was extracted with 80% ethanol until exhaustion. The extractive was filtered and concentrated at low pressure to obtain a solid mass coded "EE" as hydroethanol extract. The extracts were monitored while drying to ensure complete dryness; percentage yield was calculated to obtain the yield. All the extractives were concentrated by evaporation to dryness on a water bath and were then subjected to phytochemical evaluation, analgesic and anti-inflammatory tests. The extracts were kept in a desiccators pending analysis. The final marc was dried, weighed and kept for reference. Extraction with *n*-hexane followed by 80% hydroethanol yield 42.27 g (2.82%) and 312.00 g (20.80%) respectively.

2.3 Preliminary Phytochemical Evaluation

The crude hydroethanolic and *n*-hexane extracts were used to perform the phytochemical screening using standard protocol for the detection of the following:

2.4 Test for Phenolic Compounds

Exactly 0.5 g of the extract was dissolved in 5 ml distilled water and filtered. To the filtrate, few

drops of aqueous ferric chloride were added; a dark green colour indicates the presence of phenolic compounds [7].

2.4.1 General test for carbohydrates (Molisch's Test)

Distilled water was added to 0.5 g of the extract to dissolve it and then filtered, 1 mL of Molisch's reagent was added. Then along the side of the test tube, concentrated H_2SO_4 was added carefully. Formation of a brown ring at interface of the two liquids indicates presence of carbohydrates [8].

2.4.2 Test for monosaccharide (Barfoed's test)

The extract (0.5 g) was dissolved in distilled water and filtered, 1 ml of the filtrate was mixed with 1 mL of Barfoed's reagent in a test tube. This was heated on a water bath for two minutes. Appearance of red precipitates of cuprous oxide was considered as positive test [9].

2.4.3 Test for free reducing sugars (Fehling's Test)

After the extract (0.2 g) was dissolved in distilled water and filtered, the filtrate was heated with 5 mL of equal volumes of Fehling's solution A and B; Formation of a red precipitate of cuprous oxide (Cu_2O) was an indication of reducing sugar [8].

2.4.4 Test for combined reducing sugars

The extract (0.2 g) to be tested was hydrolyzed by boiling with 5 mL of dilute hydrochloric acid and the resulting solution neutralized with sodium hydroxide solution. Few drops of Fehling's solution were added and then heated on a water bath for two minutes. Formation of a reddish-brown precipitate of cuprous oxide indicated the presence of combined reducing sugars [8].

2.4.5 Test for soluble starch

For test of soluble starch, 0.2 g of the extract was boiled with 1 mL of 5% potassium hydroxide and then allowed to cooled. 1 mL of tetraoxosulphate (VI) acid was added. A yellow colouration was indicates the presence of soluble starch [10].

2.4.6 Test for tannins

To 5 mL of distilled water, 0.5 g of the extract was added, boiled and filtered. The filtrate was used for the following test: to 2 mL of the filtrate, few drops of 1% ferric chloride solution was added; occurrence of a blue or greenish-back precipitate indicates presence of tannins [9]. A solution of 10% lead ethanoate was added to 2 mL of the filtrate. Formation of a white precipitate indicates of presence of tannins. To filtrate, 3 drops of 10% HCl and 1 drop of methanol was added and boiled; a red precipitate indicates evidence for the presence of tannins [8].

2.4.7 Test for phlobatannins

To a 0.5 g of the extract was boiled with distilled water and then filtered. The filtrate was further boiled with 1% aqueous HCl. The appearance of red precipitate indicates the presence of phlobatannins [8].

2.5 Preliminary Test for Alkaloids

The extract (0.5 g) was stirred with 5 mL of 1% aqueous HCl on water bath then filtered. 3 mL of the filtrate was taken and divided equally into 3 portions in a test tube. To the first portion, few drops of Dragendorff's reagent were added. The occurrence of orange red precipitate was taken as the indication for the presence of alkaloids. To the second; 1 mL of Mayer's reagent was added and the appearance of buff-coloured precipitate was an indication for the presence of alkaloids; to the third portion, 1mL or a few drops of Wagner's reagent was added and a dark-brown precipitate was indicate the presence of alkaloids [11].

2.6 Test for Flavonoids

2.6.1 Ferric chloride test

Exactly 0.5 g of the extract was boiled in distilled water and filtered. Then few drops of 10% ferric chloride solution was added to a 2 ml of the filtrate. A gree*n*-blue or violet colouration indicates the presence of phenolic hydroxyl group [8].

2.6.2 Shinoda's test

To 0.5 g of the extract warmed in ethanol and filtered, 3 pieces of magnesium chips were added to the filtrate followed by few drops of concentrated HCl. A pink, orange or red to purple colouration indicates the presence of flavonoids [12,9].

2.6.3 Lead ethanoate test

To the extract (0.5 g) boiled in distilled water and filtered, 3 mL of lead ethanoate solution was

added. Appearance of buff coloured precipitate indicates the presence of flavonoids [13,11].

2.6.4 Sodium hydroxide Test

To 0.5 g of the extract was dissolved in distilled water and filtered, and then 2 mL of 10% aqueous sodium hydroxide was added to produce a yellow colouration. A change in the colour from yellow to colourless on addition of dilute hydrochloric acid indicates the presence of flavonoids [7,8].

2.7 Test for Glycosides

2.7.1 Liebermann*n*-Burchard's test

To the extract (0.5 g), 2 mL of acetic anhydride will be added. The mixture was cooled in ice and then conc. Tetraoxosulphate (VI) acid was added carefully. Colour development from violet to bluish-green was indicating the presence of a steroidal ring [14].

2.7.2 Salkowski's test (Test for steroidal nucleus)

To 2 mL of chloroform, 0.5 g of the extract was added. Then, tetraoxosulphate (VI) acid was carefully added by the side of the test tube to form a lower layer. Appearance of a reddish-brown colour or yellow at the intephase indicates the presence of steroidal ring [9].

2.7.3 Test for free anthraquinones (Borntrager's test)

A 10 mL of benzene was added to 0.5 g of the extract, and then shaken. The mixture was filtered. 5 mL of 10% ammonia solution was added to the filtrate. The mixture was then shaken. The appearance of a pink, red or violet colour in the lower interphase was indicating the presence of anthraquinones [8].

2.7.4 Test for combined anthraquinones (Borntrager's test)

To the extract (0.5 g), 10 mL of aqueous tetraoxosulphte (VI) acid was added and shaken and then filtered while it's still hot. The filtrate was shaken with 5 mL of benzene. The benzene layer was separated and was added half its own volume of 10% ammonia solution. The presence of a pink, red violet colouration in the ammonical (lower) phase was an indication for combined anthraquinones [8].

2.8 Test for Terpenoids

A 0.5 g of the extract was dissolved in 5 mL of ethanol. 1 mL of acetic anhydride was added, followed by the addition of conc. Tetraoxosulphate (VI) acid colour change from pink to violet indicate the presence of terpenoids [9].

2.9 Test for Saponins Glycosides

A quantity of 1 g of the extract was boiled in distilled water and filtered. The filtrate was divided into 2 portions. To the first portion; 3 mL of distilled water was added and shaken for about 5 minutes. Frothing which persisted on warming was a positive test for saponins [12]. To the second portion; 2.5 mLof a mixture of equal volume of Fehling's solution A and B was added. Appearance of brick-red precipitate indicates saponins glycosides [10].

2.10 Experimental Animals

One hundred and Ten (110) adult Wister rats of both sexes weighing between 150-300 g were obtained from the Department of Clinical Pharmacology, University of Maiduguri, they were kept under well-ventilated condition at room temperature, with light/dark cycle of approximately 12/12 hours and were allowed *ad-libitum* feeding with commercial Grower's Mash (Livestock feed®, Nigeria Ltd.) and provided fresh tap water on daily basis. All animals were allowed to acclimatized with the laboratory environment for one week before the commencement of the experiment and then handled following the international Guiding principle for Biomedical Research Involving Animals (C.I.O.M.S) as satisfied by the ethics committee of the Faculty of Veterinary Medicine, University of Maiduguri, Nigeria.

2.11 Formalin-induced Pain Test in Rats

The study was performed according to the method described by [8,11]. Thirty adult Wister rats of both sexes were grouped randomly into five groups of six rats. Rats in groups A, B and C received 100, 200 and 400 mg/kg bw, respectively, for each of the plant extract (*n*-hexaneand hydroethanol); while those in group D received standard drug (piroxicam) 10 mg/kg bd. wt., rats in group E received normal saline (0.2 mLinterperitoneally) only. All treatments were done interperitoneally. Thirty minutes later, each rat in the treatment groups was injected with

200µL of 3% formalin at plantar surface of the left paw and immediately placed in a transparent plastic chamber [15]. The rats were observed for first five minutes and then from 20-30 minutesafter formalin injection. The time spent in flinching, shaking and or licking the injected paw were recorded in seconds during these periods. The mean was determined and compared with the mean for the control group.

2.12 Egg Albumin-induced Rat Paw Oedema

Twenty five adult Wistar rats of both sexes grouped randomly into five groups of five rats each as described by [9,16]. They were deprived of food for 24 hours before the commencement of the experiment. Those in groups A, B, and C were given the plant extract 100, 200, and 400 and mg/kg bd.wt. respectively, while those in groups D (treated control) and E (no*n*- treated control) were given piroxicam 10 mg/kg bd.wt. and 0.2 mL of normal saline, respectively for each of the plant extract (*n*-hexane and hydroethanol). Hind paw oedema (in rats) test [17]. All treatment was by interperitoneal route. Thirty minutes after, oedema shall be induced by injecting 100 µL of fresh egg albumin into the plantar surface of the right hind paw of each rat. The volume was measured before and at 20 minutes interval for two hours after induction of oedema. Inflammation was assessed as the difference between the zero time volume of the treated paw (V_0) and the volume at the various times (V_t) after the administration of the extract. Egg albumi*n*-induced inflammation model is a significant predictive test for anti-inflammatory activity [18].

3. RESULTS

The results of phytoconstituents is shown on Table 1, while Table 2 shows theeffects of crude hydroethanol and *n*-hexane extracts on formalin-induced analgesic test and Table 4 shows theeffects ofcrude ethanol extract and *n*-hexane extract on egg albumin induced inflammatory test in rats and percentage inhibition.

4. DISCUSSION

The results of the phytochemical evaluation of the *M. senegalensis* showed the presence of cardiac glycosides, flavonoids, aponins, tannins and terpenoids while anthraquinones, alkaloids and phlobatannins were absent in crude hydrothanol extract and only cardiac glycosides and terpenoids were present in *n*-hexane extract as shown in Table 1. The findings of this study revealed the presence of some important bioactive metabolites of therapeutic value which is supported by different literatures. Flavonoids glycosides have been reported to be responsible for the anti-ulcerogenic effects of *M. aquifolium* and *M. llicifolia* [19].Several flavonoids, saponins, alkaloids, tannins and terpenes isolated from medicinal plant have shown to have significant antinociceptive and anti-Inflammatory activities [20]. Diaz and Dickenson [21] reported that the analgesic activity of ethanol extract of *Nigeula sativa* was due to the presence of tannins and flavonoids in the extract.

Formalin test was used in this study and the result of the analgesic effect of the hydroethanol crude extract and the *n*-hexane extract of the leaf of *M. senegalensis*on formali*n*-induced pain in rats given in Table 2 showed that the extracts significantly inhibited the pain response at the different dosages with the most significant inhibition observed at 400 mg/kg in both phases. The standard drug piroxicam had also significantly ($p<0.05$) showed a great anti-nociceptive activity by blocking both phases of the formali*n*-induced nociception. Inhibition by the extracts was higher than that observed with piroxicam (10mg/kg bw.). The ability of the extract to suppress pain responses in both phases suggests that the extract have both central and peripherial effects.Formalin test is a well-established valid model for the study of central sensitization events at the spinal level after peripheral inflammatory state [12].The two distinct phases in formalin test are due to direct effect of formalin on nociception and due to inflammation with the release of serotonin, histamine, bradykinin and prostaglandins and at least to some degrees, the sensitization of central nociceptive neurons [3]. Stimulation of opioid receptors has also been suggested as a possible mechanism of action against neurogenic pain [13].

The egg albumen-induced inflammation test in rats was used in this study. The anti-inflammatory effect and the percentage protection of the crude extract and *n*-hexane inhibited the oedema in the early phase acted dose dependently. The oedema was also significantly ($P<0.05$) reduced by the positive control (Piroxicam) in the both phases.The effect of the crude ethanol extract at all doses tested significantly ($P<0.05$) decreasedthe size ofoedemain rats, and the maximum percentage inhibition of 60.00% was observed at 400 mg/kg

bd.wt. in the first phase. The effect of the*n*-hexane portion at all doses tested significantly (P<0.05) decreased the size ofoedemain rats and the maximum percentage inhibition of 56.95% was observed at 200 mg/kg bd.wt. in the late phase.The extract induced a significant decrease in the number of inflammatory cells. This effect is probably due to higher concentrations of tannins and phenolic compounds in the extracts [22]. These results are consistent to those found by other authors. Usman et al [23]. Reported that the methanolic root bark extract of *Maytenus senegalensis* significantly (P< 0.01) reduced the number of abdominal constriction induced by acetic acid in mice. Therefore, it could be suggested that there is a dose dependant activity since 100 mg kg^{-1} extract showed higher inhibition (82.9 %) relative to other doses and even proffer more protection than does piroxicam 20 mg kg^{-1} (69.0 %). Similarly the ethanol extracts (70%) of *Maytenus heterophyla* and *M. senegalensis* were investigated in Winstar albino rats using carcigeenan-induced paw edema method and the extracts portrayed significant anti-inflammatory activity, reducing edema by 51% and 35% respectively [24,25]. The bark and leaf extracts of *M. senegalensis* were shown to inhibit cyclo-oxygenase-1, an enzyme responsible for the synthesis of inflammatory mediators, such as prostaglandins and thromboxanes]24]. The analgesic activity has been demonstrated by aqueous extracts of the leaves of *M. senegalensis* with pain inhibition of 72%. In the anti- inflammatory test, the oedema inhibition 3h after carrageenan injection was respectively 64% and 66 % for leaves and roots of *M. senegalensis* [26,27]. The chloroform extract of roots along with maytenoic acid exhibited highest anti- inflammatory by reducing the oedematous response, with potency similar to indomethacin [27]. The co-existence of analgesic and anti - inflammatory observed with the extracts is well defined for various no*n*-steroidal anti-inflammatory drugs (NSAIDS) particularly salicylates and their congeners [26]. These NSAIDS exert anti- inflammatory effects principally by inhibiting the synthesis of prostaglandin [28]. Hence, the use of this part of plant in the treatment of ailments related to the analgesic and anti-inflammatory has gain scientific support.

Table 1. Phytochemical constituents of the leaf extracts of *Maytenus senegalensis*

Phytochemical	Extracts	
	Hydroethanol	***n*-hexane**
Alkaloids	-	-
Anthraquinones	-	-
Carbohydrates	+	-
Cardiac glycosides	+	+
Flavonoids	+	-
Saponins	+	-
Tannins	+	-
Terpenoids	+	+
Phlobatannins	-	-

Key: (+) Present, (-) Absent

Table 2. Effects of hydroethanol crude extract and *n*-hexane extract on formalin induced analgesic test

Treatment/Dose (mg/kg bd.wt)	No. of Paw Licking (Mean ± SEM) and % inhibition			
	0-5 min.	**(%)**	**20-30 min.**	**(%)**
100 hydroethanol	51.20 ± 4.31^{a}	35.35	79.60 ± 5.29^{a}	34.86
100 *n*-hexane	65.80 ± 6.18^{a}	16.92	94.40 ± 4.74^{a}	22.75
200 hydroethanol	43.60 ± 4.74^{b}	44.95	64.40 ± 5.78^{b}	47.30
200 *n*-hexane	55.20 ± 4.50^{a}	30.30	82.40 ± 3.95^{a}	32.57
400 hydroethanol	14.80 ± 2.52^{c}	81.31	0.0 ± 0.0^{c}	100.00
400 *n*-hexane	34.20 ± 2.40^{b}	56.82	64.80 ± 5.18^{b}	46.97
Piroxicam 10	58.00 ± 4.14^{a}	26.77	8.80 ± 2.04^{c}	92.80
Normal saline 0.2 mL	79.20 ± 4.53^{c}	-	122.20 ± 4.21^{d}	-

Values along same column differently superscripted differ significantly (P<0.05)

Table 3. Effect of crude hydroethanol extracton egg albumin induced inflammatory test in rats and percentage inhibition

Treatment/ dose (mg/kg)	Time (min.) / Oedema level (Mean ± SEM) mm/ percentage inhibition						
	0	20	40	60	80	100	120
100	0.00±0.00	2.25±0.18[a]	2.08±0.15[a]	2.17±0.21[a]	1.83±0.17[a]	2.00±0.18[a]	2.08±0.15[a]
	0.00	23.08	44.53	34.83	33.45	38.45	8.37
200	0.00±0.00	2.75±0.28[a]	2.75±0.28[a]	2.42±0.24[a]	1.83±0.17[a]	1.75±0.17[a]	2.00±0.37[a]
	0.00	15.38	26.67	27.33	33.45	46.15	11.89
400	0.00±0.00	2.08±0.24[a]	1.50±0.26[b]	1.58±0.08[b]	1.67±0.11[a]	2.00±0.22[a]	1.67±0.28[a]
	0.00	36.00	60.00	52.55	39.27	38.45	26.43
Piroxicam 10	0.00±0.00	2.50±0.22[a]	2.08±0.22[a]	1.83±0.24[b]	1.92±0.15[a]	2.17±0.17[a]	1.67±0.21[a]
	0.00	23.08	44.53	45.05	30.18	33.23	26.43
N.Saline0.2mL	0.00±0.00	3.25±0.22[b]	3.75±0.11[c]	3.33±0.11[c]	2.75±0.25[b]	3.25±0.11[b]	2.27±0.11[b]

Values along same column differently superscripted differ significantly (P<0.05)

Table 4. Effect of *n*-hexane on egg albumin induced inflammatory test in rats and percentage inhibition

Treatment/ Dose (mg/kg)	Time (min.)/ Oedema Level (Mean ± SEM) mm/ percentage inhibition						
	0	20	40	60	80	100	120
100	0.00±0.00	2.25±0.18[a]	2.08±0.15[a]	2.17±0.21[a]	1.83±0.17[a]	2.00±0.18[a]	2.08±0.15[a]
	0.00	23.08	44.53	34.83	33.45	38.45	8.37
200	0.00±0.00	2.75±0.28[a]	2.75±0.28[a]	2.42±0.24[a]	1.83±0.17[a]	1.75±0.17[a]	2.00±0.37[a]
	0.00	15.38	26.67	27.33	33.45	46.15	11.89
400	0.00±0.00	2.08±0.24[a]	1.50±0.26[b]	1.58±0.08[b]	1.67±0.11[a]	2.00±0.22[a]	1.67±0.28[a]
	0.00	36.00	60.00	52.55	39.27	38.45	26.43
Piroxicam 10	0.00±0.00	2.50±0.22[a]	2.08±0.22[a]	1.83±0.24[b]	1.92±0.15[a]	2.17±0.17[a]	1.67±0.21[a]
	0.00	10.00	3.49	46.68	47.68	37.15	33.45
N.Saline0.2 mL	0.00±0.00	2.50±0.22[c]	2.58±0.27[b]	3.58±0.15[b]	3.67±0.17[b]	3.58±0.40[b]	2.75±0.21[a]

Values along same column differently superscripted differ significantly (P<0.05)

5. CONCLUSION

The present study revealed that the leaf of *M. senegalensis* contains pharmacologically active substances effective for management of pain. However, further chemical and pharmacological studies are required to isolate the bioactive compounds and elucidate the precise mechanisms responsible for the observed pharmacological activities of this plant.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All animals were allowed to acclimatized with the laboratory environment for one week before the commencement of the experiment and then handled following the international Guiding principle for Biomedical Research Involving Animals (C.I.O.M.S) as satisfied by the ethics committee of the Faculty of Veterinary Medicine, University of Maiduguri, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Samuelsson G. Drugs of natural origin: A textbook of pharma-cognosy. 4 Ed. Stockholm, Swedish Pharmaceutical Press; 1999.
2. Neuwinger HD. African ethnology, poison and drugs. Chapman and hall, GMBH, Germany. 1996;343.
3. Bako SP, Bakfur MJ, John I, Bala E. Ethnomedicinal and phytochemical profile of some savanna plants species in Nigeria. Int. J. Bot. 2005;1(2):147-150.
4. Merskey H, Bogduk N. Classisication of Chronic pain. 2nd Ed. Seattle: Int. Ass. Study Pain. 1994;3-4.
5. Burkill HM. The useful plants of west tropical Africa. Royal Botanical Gardens kew, United Kingdom. 1985;1:325.
6. Khalid SA, Friedrichsen G, Christensen SB, El-tahir A, Satti GM. Isolation and Characterization of Pristimerin as the AntiplasmodialAntileishmanial Agent of*M. Senegalensis* (Lam) excel. ARKIVOC. 2007;9:129-134.
7. Mir MA, Sawhney SS, MMS, Jassal MMS. Qualitative and quantitative analysis of phytochemicals of *Taraxa umofficinale*. J. Pharm. Pharmocol. 2013;2(1):1-5.
8. Trease GE, Evans WC. Textbook of Pharmacognosy, 14th Edn. Saunders WB, Company Ltd., 24-28 Oval Road, London NW17DX, UK and Printed by Harcourt Brace and Company Asia Pte Ltd. 583 Orchard Road No. 09-01 Forum Singapore. 238884. 2002;13-53, 117-139, 227,293-334, 471-511.
9. Hatil H, El-Kamali, Ahmed A. Elshikh. Premliminary phytochemical screening of 27 plants species use in ethnovetrinary in Khartoum State, Sudan. J. Advan. Life Sci. 2015;5(2):48-52.
10. Vishnoi NR. Advanced practical chemistry. Ghazaibadindia: Yikas publication house PVT ltd. 1979;447-449.
11. Brain KR, Turner TD. Wright - Scientechnica. 1st Ed. Bristol: Practical Evaluation of Phytopharmaceuticals; 1975; 144.
12. Tanko Y, Yaro AH, Isa AI, Yerima M, Saleh MIH, Mohammed A. Toxocological and Hypoglycemic Studies of the leaves of *Cissampelos mucronata* (Menispermaceae) on Blood Glucose levels of Streptozoci*n*-induced diabetic Wistar Rat. J. Med. plant Res. 2007;5:113-116.
13. Gaestel M, Kotlyarov A, Kracht M. Targeting innate immunity protection kinase signalling in inflammatory. Nat. Rev. Drug Dis. 2009;8:480-499.
14. Silver O, Duarte A, Cabrita J, Pimentel M, Diniz A, Gomes E. Antimicrobial activity of Guinea-Bissau Traditional Remedies. J. Ethnopharmacol. 1998;50:55-59.
15. Hunskar S, Hole K. Formalin test in mice: Dissociation between Inflammatory and Non-Inflammatory Pain. 1987;30:103-114.
16. Sandabe UK, Onyeyili PA, Chibuzo GA. Studies of some pharmacological activities of the aqueous extract of *Ficus sycomorous* L. (Moraceae) Stem Bark in Laboratory Animals. Nig. J. Exp. and Appl. Biol. 2004;5(2):263-268.
17. Winter CA, Risky EEA, Nuss GW. Carrageeni *n*-induced Oedema in HindPaw the rat as an assay of anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med. 1962; 111:544-547.
18. Akah PA, Nwambie AI. Evaluation of Nigerian traditional medicines: Plants used

for rheumatic (inflammatory) disorders. J. Ethnopharmacol. 1994;42:179-182.

19. Leite JPV, Rastrelli L, Romussi G, Oliveira AB, Vilegas JHW, Vilegas W, Pizza C. Isolation and HPLC quantitative analysis of flavonoid glycosides from Brazilian beverages (*Maytenus ilicifolia and Maytenus aquifolium*). J. Agric. Food Chem. 2001;49:3796-3801.
20. Suleiman MM, McGaw LJ, Naidov V, Eloff JN. Detection of antimicrobial compounds by bioautography of different extracts leaves of selected South African Tree species. Afr. J AJTCAM. 2009;7(1):64-78
21. Diaz A, Dickenson AH. Blockade of spinal *N*- and P-type, but not L-type, calcium channels inhibits the excitability of rat dorsal horn neurons produced by subcutaneous formalin inflammation. Pain. 1997;69:93-100.
22. Yeo D, Dinica R, Yapi HF, Furdui B, Praisler M, Djaman AJ, N'Guessan JD. Evaluation of the anti-inflammatory activity and phytochemical screening of *Annona senegalensis* leaves. Therapie. 2011; 66(1):73-80.
23. Usman H, Musa YM, Geidam YA, Abore DM. Phytochemical and anti-nociceptive studies of the root bark extracts of *Maytenus senegalensis* (lam) in Mice. J. of life and Environmental Sciences. 2007; 9(1):511-517.
24. Matu EN, van Staden J. Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. J. Ethnopharmacol. 2003;87:35-41.
25. da Silva G, Tanica M, Rocha J, Serrano R, Gomes ET, Sepodes B, Silva O. *In vivo* anti-inflammatory effect and toxicological screening of *Maytenus heterophylla* and *Maytenus senegalensis* extracts. Human and Experimental Toxicology. 2010;30(7): 693–700.
26. Beuoist JM, Misse JL. In: Clinical Pharmarcology: Basis of Therapeautics Ii. Giruoud JP, Mathe G, Meyniel G. (Eds.). Expansion Scientific Francaise, Paris. 1979;1049-1091.
27. Sosa S, Morelli CF, Tubaro A, Cairoli P, Speranza G, Manitto P. Anti-inflammatory activity of Maytenus senegalensis root extracts and of maytenoic acid. Phytomedicine. 2007;14: 109–114.
28. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of *action in aspirin-like Drugs Nat. New Biol. 1971;231: 232-235.

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