Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 12, Issue 4, 2023, 176
https://doi.org/10.33263/LIANBS124.176

An Assessment of the Antinociceptive Effect of Methanolic Root Extract of *Chasmanthera dependens*

Valentine Nwanelo [1], Linus Agwu [2], Sabastine Ugwuoke [1], Dionysius Osuji [1,*], Chigozie Odo [1], Rita Aguchem [1], Kenneth Nwangene [1], Linus Ngwu [1], Chigbo Ekemezie [1], Sylvia Chikelue [1], Charles Okoroafor [1], Chioma Anosike [1]

[1] Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria Nsukka, Enugu State
[2] Department of Biochemistry, Faculty of Biological Sciences, Federal University of Technology Owerri, Imo State, Nigeria
* Correspondence: obinna.osuji.pg90178@unn.edu.ng;

Scopus Author ID 57219936267
Received: 13.10.2022; Accepted: 24.11.2022; Published: 25.02.2023

Abstract: The antinociceptive property of the methanolic root extract of *C. dependens* (MRECD), based on the folkloric treatment of pain, was evaluated in this study. The LD50 of the root extract appeared safe at 5000 mg/kg b.w. Acetic acid-induced writhing, tail clip, and formalin-induced pain models were all utilized in the antinociceptive study in mice. Forty-five albino mice were randomly separated into three experimental models (n=15), each further distributed into five groups (n=3). In the three models, a group I was administered 1% Tween 80, groups II-IV with 100, 200, and 400 mg/kg b.w of the MRECD respectively, whereas group V, 100 mg/kg b.w of Aspirin. The acetic acid writhing test yielded significant ($p < 0.05$) and dose-dependent reduction in pain - 47.37%, 50.87%, and 60.53%, respectively, for the extract-treated groups. In the formalin-induced pain model, the extract, administered at varying doses, had significantly ($p < 0.05$) higher pain latency time compared to those of the normal control and Aspirin-treated groups after 30 minutes. Additionally, in the tail clip test, the mice demonstrated a dose-dependent reduction in pain for the extract-treated groups. These results provide pharmacological bases for the ethnomedicinal use of *C. dependens* plant in managing pain-related disorders.

Keywords: pain; *Chasmanthera dependens*; inflammation; non-steroidal anti-inflammatory drugs.

1. Introduction

In recent times, medicinal plants have assumed greater relevance in primary health care delivery in many countries, especially least-developed countries such as Africa and some parts of Asia. This is because products from these plants have been found to be safe, affordable, and readily accessible, unlike orthodox medicine. The traditional medicine system consists of herbal medicine as a major component, and currently, considerable inputs have been channeled toward the development of natural products from different plant sources [1-3]. Today, a reasonable number of medications, which are potent and active against some infections and/or ailments, have been developed from plants by simple isolation and modification of their active ingredients. Over 25% of the medications prescribed worldwide are derivatives of plants [4,5].

Chasmanthera dependens is one of the tropical plants extensively used by many herbalists and bone-setters to treat various health conditions, especially pain-related ones. The leaf sap is usually applied as emergency treatment to halt blood loss in injuries [6,7]. This plant is a climbing shrub belonging to the family of Menispermaceae. Its common name is

Chasmanthera, and it is known locally in Nigeria as Ato (Yoruba) and Onochie (Igbo) [8,9]. It is a popular medicinal plant usually reaped from the wild for use locally. Additionally, it is frequently grown in backyard gardens and sold in nearby markets due to its accessibility. They are widely distributed throughout tropical regions of Africa, including Nigeria, Sierra Leone, Ghana, Somalia, Ethiopia, Angola, Zimbabwe, and Zambia [10]. *C. dependens* grows to a height of 5.0 meters in cocoa plantations, where it is occasionally thought to lower yields [11,12]. Locally, the fibrous stalk is trodden and utilized as a sponge. The plant has traditionally acquired eminence in the treatment of a number of disease conditions. For example, treating injuries such as sprains, bruises, and stiffness locally entails administering the sap from the leaf and stem [13,14]. This plant's methanol and chloroform root extracts have been reported for their antimicrobial activities [15,16]. The stem maceration of *C. dependens* has also been used in combination with the root extracts of other plants as a remedy for convulsions. The roasted stem was found to be efficacious against febrile convulsions [17]. The bark is consumed as a treatment for venereal discharge or as a general tonic for physical/mental fatigue and inflammatory conditions [18,19]. This plant has reportedly been used to treat malaria, cough, snakebites, and dementia. Several reports have shown that the chloroform and methanol extracts of *C. dependens* contain several bioactive compounds [13,20-22]. However, many other pharmacological uses have been reported about this plant, all of which point to the need to explore further its other medicinal relevance and potential.

Several injuries and diseases are most accompanied by pain and fever. [23,24] stated that non-steroidal anti-inflammatory drugs (NSAIDs), such as diclofenac, indomethacin, and Aspirin, are usually prescribed drugs for their management, but gastrointestinal complications like peptic ulcers, perforation, bleeding, obstructions, as well as hepatotoxicity have all led to minimal use of these drugs in clinical settings. These drugs work by inhibiting cyclooxygenase (COX) and interrupting inflammatory mediators' synthesis, prostaglandins (PGs) [25,26]. Moreover, current analgesics are not absolute in subsiding pain but only offer partial relief to patients in some cases [27,28]. The above limitations trigger the search for novel drugs. *C. dependens* has successfully received scientific attention for its remedial usefulness in various disease conditions, yet, no study has been carried out on its antinociceptive potential. It exhibits therapeutic potential for diverse inflammation-relevant disease conditions and boasts traditional usage over the years in various societies. As a result, we are tasked with proceeding to our present study on *C. dependens* with great curiosity regarding studies that promise to offer a scientific core for the potential antinociceptive effects.

2. Materials and Methods

2.1. Materials

2.1.1. Plant materials.

Fresh roots of *Chasmanthera dependens* were gathered from Orba Nsukka in Enugu State, Nigeria. Identification and assignment of voucher specimens were done and placed in the botanical garden of the Department of Botany, University of Nigeria, Nsukka.

2.1.2. Experimental animals.

Sixty-three (63) albino Wistar mice weighing between 22 and 38 g were used for this study, eighteen of which were used for the acute lethality study. In contrast, forty-five (45)

males were used for the *in vitro* antinociceptive studies. The mice were obtained from the animal section of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, after which they underwent adaptation for seven (7) days under typical laboratory circumstances before the commencement of the experiment. Experimental protocols were carried out in harmony with the National Institute of Health Guidelines for Use and Welfare of Research Animals (Publication No. 85 – 23, revised 1985) and the authorization of the Institutional Ethical Committee regarding the usage of lab animals.

2.1.3. Chemical and reagents.

Analytical grade reagents and chemicals were employed after being procured from the Springboard Research Laboratory, Awka, Anambra State, Nigeria.

2.2. Methods

2.2.1.Extraction of plant material.

Fresh roots of *Chasmanthera dependens*, gathered and desiccated under atmospheric temperature (25 ˚C to 45 ˚C) for fourteen (14) days, were pulverized by means of grinding. The sample got immersed in 50% methanol, shaken vigorously, and made to stand for 48 h. After that, there was occasional stirring and filtration. The filtrate was concentrated using a rotary evaporator at 45 ˚C and thenceforth kept in a refrigerator at 4˚C until required.

2.2.2. Acute toxicity and lethality (LD_{50}) study

The acute toxicity of the methanol root extract of *C. dependens* in mice (n=18) was ascertained using [18] described method of determining acute toxicity. Tests were performed in phases I and II- where phase I mice (n=3) received 10, 100, and 1000 mg/kg b. w of the extract orally, while Phase II mice (n=3) received oral administration of higher amounts of the extract- 1600, 2900, and 5000 mg/kg b.w. Both phases were monitored for 24 h for alterations in behavior or death.

$$LD_{50}= \sqrt{Minimum\ dose\ at\ which\ death\ occured\ \times\ Maximal\ dose\ at\ which\ a\ number\ of\ death\ was\ recorded}$$

2.2.3. Experimental protocol.

Forty-five (45) male albino rats (25-38 g) were acclimatized for 7 days and separated into 3 experimental segments of 15 rats. The 15 animals in each experimental segment were further distributed randomly into five groups of three rats per group.

2.2.3.1. Experiment I: acetic acid-induced writhing test.

The writhing induction using acetic acid was carried out using a modification of [29] as was reported by [30]. This experimental segment involved fifteen (15) mice randomly separated into 5 groups of 3 mice each. They were fasted overnight, followed by treatment with tween 1% 80 (p.o), *C. dependens* methanol extract (100, 200,400 mg/kg b.w, p.o), and Aspirin (100 mg/kg b. w). Acetic acid (0.6% v/v, 10 ml/kg, i.p) was administered 60 minutes after oral treatment and 30 minutes post-cutaneous administration. The number of writhes, portrayed by abdominal musculature contraction and limb extension, was taken note of during a 5 minutes

intervals for 30 minutes. The percentage inhibition was deduced from the obtained data according to the method of [31]. The protocol for experiment I is summarized below;

Group I: Control (1% tween 80)
Group II: 100 mg/kg of MRECD + 0.6% acetic acid
Group III: 200 mg/kg b.w of MRECD + 0.6% acetic acid
Group IV: 400 mg/kg b.w of MRECD + 0.6% acetic acid
Group V: 100 mg/kg b.w of Aspirin + 0.6% acetic acid

2.2.3.2. Experiment II: tail clip test.

Initially, the mice were screened by applying a metallic artery clip to the tail root to cause distress. Animals that were unsuccessful in removing the clip in 10 sec were removed. The method employed in this test was as reported by [32]. Mice that qualified for the test were randomly separated into five groups of three mice each. Afterward, treatment ensued, as summarized below:

Group I: Control (1% tween 80) (p.o)
Group II: 100 mg/kg b.w of MRECD (p.o)
Group III: 200 mg/kg b.w of MRECD (p.o)
Group IV: 400 mg/kg b.w of MRECD (p.o)
Group V: 100 mg/kg b.w of Aspirin

Reaction time assessment was done 60 minutes after oral administration and 30 s after cut-off treatment time, according to methods of [33].

2.2.3.3. Experiment III: formalin-induced pain test.

Pain induction by formalin was performed as proposed by [34] and [35] with slight adjustments. Fifteen (15) male mice were starved overnight and partitioned into five groups of three mice each. The control (group 1) received plain vehicle (1% tween 80, p.o), test groups (groups 2-4) received varied measures of the extract (p.o) respectively, and standard control (group 5) received the standard drug, Aspirin (100 mg/kg, p.o); followed by injecting 1% formalin (20µL) subcutaneously into the mice right hind paw after 60 minutes' post-oral treatment as summarized below:

Group I: Control (1% tween 80) (p.o) + 1% formalin (20µL)
Group II: 100 mg/kg b.w of MRECD (p.o) + 1% formalin (20µL)
Group III: 200 mg/kg b.w of MRECD (p.o) + 1% formalin (20µL)
Group IV: 400 mg/kg b.w of MRECD (p.o) + 1% formalin (20µL)
Group V: 100 mg/kg b.w of Aspirin + 1% formalin (20µL)

Responses such as biting and licking following the injection of the paw indicated the onset of pain, or pain latency, and were recorded (in a sec) for each mouse.

2.3. Data analysis.

Data were evaluated using one-way analysis of variance (ANOVA) with repeated measures using Statistical Product and Service Solution (SPSS) version 21. GraphPad Prism 5.0 for windows (GraphPad Software, San Diego, Califonia, USA) was employed for statistical analysis. Results were displayed as mean ± SD, and p-value < 0.05 were considered significant.

3. Results and Discussion

3.1. Results.

3.1.1. Acute toxicity study.

No lethality was recorded in the methanol root extract of *C. dependens* up to 5000 mg/kg b.w (Table 1).

Table 1. Phases I and II of the acute toxicity test of the methanol root extract of *C. dependens*

S/N	Phases/groups	Dosage of extract (mg/kg b.w)	Mortality rate
1	Group 1	10	0%
2	Group 2	100	0%
3	Group 3	1000	0%
	Phase II		
4	Group 1	1600	0%
5	Group 2	2900	0%
6	Group 3	5000	0/3

N=3 for each group.

3.1.2. Effect of methanol root extract of *C. dependens* on acetic acid-induced writhing in mice.

A significant and dose-dependent decline in the number of writhes was noticed between the groups treated with the extract and the control group (Table 2). Extracts at 100, 200, and 400 mg/kg b.w generated writhing percentage inhibitions of 7.37%, 50.87%, and 60.53%, respectively. The inhibition (%) offered by 400 mg/kg b.w was non-significantly ($p > 0.05$) higher in comparison to the standard control (57.89%).

Table 2. Effect of MRECD on acetic acid-induced writhing in mice.

Groups	Number of Writhes	% Inhibition
I	38.00 ± 2.01^{d}	-
II	20.00 ± 1.10^{c}	47.37 ± 2.12^{a}
III	18.67 ± 1.25^{b}	50.87 ± 1.90^{b}
IV	15.00 ± 1.00^{a}	60.53 ± 4.14^{c}
XV	16.00 ± 1.05^{a}	57.89 ± 3.10^{c}

Results are demonstrated as mean ± S.D (n =3), and values with varied superscripts selfsame column are significantly different ($p < 0.05$).

3.1.3. Effect of MRECD on tail clip-induced pain in mice.

A significant ($p < 0.05$) decrease in the number of times reacted by the extract-treated groups was observed compared to the control. In addition, the test groups exhibited a significant ($p < 0.05$) dose-dependent percentage rise in pain inhibition compared to the control. Also, a non-significant ($p > 0.05$) difference was detected between groups IV and V.

Table 3. Effect of MRECD on tail clip-induced pain in mice.

Groups	Number of Writhes	% Inhibition
I	13.67 ± 2.01^{a}	-
II	9.33 ± 1.10^{b}	31.7 ± 7.23^{a}
III	6.67 ± 1.25^{c}	51.21 ± 9.06^{b}
IV	2.67 ± 1.00^{d}	80.47 ± 14.40^{c}
V	3.67 ± 1.05^{d}	73.15 ± 5.21^{c}

Results are demonstrated as mean ± S.D (n =3), and values with varied superscripts selfsame column are significantly different ($p < 0.05$).

3.1.4. Effect of MRECD on formalin-induced pain in mice.

Table 4 shows the post-treatment latency time on pain induced by formalin in mice. Results indicate that the control group becomes significantly different from the treated groups after 30 minutes. The extract administered at varying doses (100 and 200 mg/kg b.w) significantly increased the pain latency time of the mice compared to the Aspirin-treated group. Nevertheless, the difference in the latency time observed between group IV and group V was non-significant.

Table 4. Effect of MRECD on formalin-induced pain in mice.

Groups	Post-Treatment Latency Time (s)
I	194.00 ±7.50[a]
II	258.67 ±13.28[b]
III	314.25 ±19.16[c]
IV	373.33 ±11.33[d]
V	381.00 ±8.00[d]

Results are demonstrated as mean ± S.D (n =3), and values with varied superscripts on the selfsame column are significantly different ($p < 0.05$).

3.1.5. Effect of MRECD on percentage pain latency time of formalin-induced pain in mice.

Table 5 shows the pain latency time (percentage) of pain induced by formalin in mice. A significant dose-dependent increased latency time (percentage) was noticed among the extract-treated groups. However, a non-significant difference in the percentage latency time was recorded in group IV mice when compared to group V mice

Table 5. Effect of MRECD on percentage pain latency time of formalin-induced pain in mice.

Groups	Percentage Latency Time (%)
I	-
II	33.33±3.52[a]
III	61.19±19.16[b]
IV	92.24±11.74[c]
V	96.39±15.41[c]

Results are demonstrated as mean ± S.D (n =3), and values with varied superscripts on the selfsame column are significantly different ($p < 0.05$).

3.2. Discussion.

C. dependens plant has been well-researched and found to possess plenty of bioactive compounds responsible for its diversified therapeutic potential. This research has shown that the methanolic root extract of *C. dependens* exhibits antinociceptive activities. The antinociceptive activity was investigated using different induction models viz: Acetic acid, tail clip, and formalin induction.

MRECD did not show any form of behavioral alterations, morbidity, and mortality when administered to the mice up to the dose of 5000 mg/kg b.w, implying its safety and being devoid of toxicity at this dose. The above outcome corresponds with that of [15] on the safety of methanolic root extract of *C. dependens.*

The writhing model is associated with the inducement of outermost receptors, particularly localized peritoneal receptors found at the exterior of the cell bordering the peritoneal cavity [36]. However, evaluation of the antinociceptive potency of MRECD following writhing induction with acetic acid shows that oral dispensation produced a significant dose-dependent inhibition of writhes compared to the control. This indicates the peripherally mediated antinociceptive activity of the MRECD since agents that reduce the

number of writhes demonstrate analgesic potentials by inhibiting the synthesis of prostaglandins (PGs), a common pain inhibition mechanism [37]. There could also be an antipyretic potency of MRECD, possibly attributed to its ability to cause a reduction in the PGE_2 brain level, particularly in the hypothalamus, via the action of cyclooxygenase-2 (COX-2), or by amplifying the body's production of antipyretic substances such as arginine and vasopressin by metabolites such alkaloids (bolidine), sterols (β-sitosterol) and flavonoids, which are all pharmacologically active (baicalin) [18,38]. These antipyretic substances are well-known for lowering pro-inflammatory mediators, which address the anterior hypothalamus inhibiting COX-2 from releasing PGE2, thereby reducing the body temperature [39].

To confirm the participation of central mechanism(s) in the antinociceptive activity of *C. dependens*, the tail clip test was employed. The tail clip test reveals a remarkable decline in the number of times reacted by the test groups compared to the control. In addition, the test groups exhibited a significant dose-dependent rise in the pain inhibition percentage compared to the control. This suggests that a high amount of the methanolic extract of *C. dependens* offered an excellent percentage inhibition and proves that its antinociceptive potency remarkably involves a central mechanism of action.

Adoption of the formalin test was performed to buttress further a possible mechanism for the antinociceptive activity of the extract. Production of clear-cut biphasic nociceptive response has been associated with this test. The initial phase is marked by association with the influence inflicted on nociceptors by formalin, whereas the latter phase (15-30 min) is believed to be concerned with injurious actions [41]. The early phase (first 5 min) involves intensive licking and biting activities and results predominantly from C-fibre stimulation due to formalin-induced peripheral stimulus [42]. Drugs acting peripherally, like Aspirin, inhibit solely nociceptive response at the late phase, unlike centrally acting drugs, e.g., morphine [43]. The results of this test revealed after 30 minutes that the pain latency time for the test groups (II, III, and IV) was significantly higher in contrast to the normal control, while the extract at 100 and 200 mg/kg b.w significantly increased the pain latency time of the mice when compared to the group treated with Aspirin. More so, a significant dose-dependent increase in the percentage latency time among the group treated with *C. dependens* methanolic root extract was observed. The result proves that the extract demonstratively blocked formalin response and was more noticeable in the late phase of nociceptive response, which occurred after 15 to 30 minutes.

4. Conclusions

These results have demonstrated that the methanolic root extract of *C. dependens* exhibits antinociceptive activities, possibly through a central mechanism augmented by peripheral actions. The extract was relatively potent in writhing induced by acetic acid and pain induced by tail clip and formalin tests, thus, indicating peripheral anti-nociception. However, the results obtained provide the pharmacological bases for the ethnomedicinal use of *C. dependens* in managing pain-related disorders.

Funding

This research had no funding.

Acknowledgments

The authors wish to acknowledge the Pharmacological Biochemistry Department of the University of Nigeria for providing laboratory equipment used in this research.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Fokunang, C.N.; Ndikum, V.; Tabi, O.Y.; Jiofack, R.B.; Ngameni, B.; Guedje, N.M; Tembe-Fokunang, E.A.; Tomkins, P.; Barkwan, S.; Kechia, F.; Asongalem, E.; Ngoupayou, V.; Torimiro, N.J.; Gonsu, K.H.; Sielinou, V.; Kom, K. Traditional Medicine: Past, Present and Future Research and Development Prospects and Integration in the National Health System of Cameroon. *African J Tradit Complement Altern Med*. **2011**, *8*, 295, https://doi.org/10.4314/AJTCAM.V8I3.65276.
2. Ozioma, E-O.J.; Okaka, A.N. Herbal Medicines in African Traditional Medicine. In: *Herbal Medicine*. IntechOpen; **2019**, https://doi.org/10.5772/INTECHOPEN.80348
3. Alshammari, K.F.; Alradaddi, F.M.; Alshammari, K.F.; Almutairi, M.Q.; Almakhalfi, N.S.; Almeshari, R.A.; Alaezaimee, S.M. Assessment of Knowledge about Traditional Medicine Reveals Overuse as a Potential Risk for Aggravating COVID-19 and Underlying Diseases in Geriatrics and Women's Health in the Saudi Population. *Clin Pract.* **2022,** *12*, 363–73, https://doi.org/10.3390/clinpract12030041.
4. Noronha, M.; Pawar, V.; Prajapati, A.; Subramanian, R.B. A literature review on traditional herbal medicines for malaria. *South African J Bot*. **2020,** *128*, 292-303, https://doi.org/10.1016/J.SAJB.2019.11.017
5. Yuan, H.; Ma, Q.; Ye, L.; Piao, G. The Traditional Medicine and Modern Medicine from Natural Products. *Mol* **2016**, *21*, 559, https://doi.org/10.3390/MOLECULES21050559
6. Ogbozor, C.S.; Anosike, C.A. The Effect Of Chloroform Extract Of Chasmenthera Dependens On Carbon Tetrachloride (Ccl 4) Induced Hepatotoxicity. *Glob Sci Journals* **2020**, *8*, 339-344, www.globalscientificjournal.com.
7. Adepiti, A.O.; Reuben, T.T.; Odeyinka, I.D.; Akinwunmi, K.F. Assessment of the Activities of Chasmanthera Dependens Hochst. Combined with Other Plants on Chloroquine-Sensitive and Chloroquine-Resistant Plasmodium Berghei. *Sci. Afr.* **2022**, *15*, e01103, https://doi.org/10.1016/J.SCIAF.2022.E01103.
8. Aworinde, D.O.; Erinoso, S.M.; Ogundele, A.A.; Teniola, O.A.; Ojo, S.O. The Doctrine of Signatures in herbal prescriptions in Ikale and Ilaje communities of Ondo State, Southwestern Nigeria. *J Med Plants Res*. **2018**, *12*, 222-227, https://doi.org/10.5897/JMPR2018.6576
9. Enenebeaku, C.K.; Ogukwe, C.E.; Nweke, C.O.; Anyado-Nwadike, S.O.; Obi, M.; Duru, I.A.; Enenebeaku, U.E.; Ogidi, O.I. Antiplasmodial and in Vitro Antioxidant Potentials of Crude Aqueous and Methanol Extracts of Chasmanthera Dependens (Hochst). *Bull Natl Res Cent*. **2022,** *46*, 1–12, https://doi.org/10.1186/S42269-022-00721-3.
10. Iloh, A.C.; Ogundipe, O.T. Using ecological niche models to plan conservation in a changing environment: A case for the plant Chasmanthera dependens Hochst (Menispermaceae) in West Africa. *J Ecol Nat Environ*. **2016**, *8*, 1-8, https://doi.org/10.5897/JENE2015.0525
11. Iloh, A.C.; Schmidt, M.; Muellner-Riehl, A.N.; Ogundipe, O.T.; Paule, J. Pleistocene refugia and genetic diversity patterns in West Africa: Insights from the liana Chasmanthera dependens (Menispermaceae). *PLoS One*. **2017,** *12*, e0170511, https://doi.org/10.1371/JOURNAL.PONE.0170511
12. Enenebeaku, U.E.; Okotcha, E.N.; Oguoma, L.M.O.; Mgbemena, I.C.; Enenebeaku, C.K.; Onyeka, C.A. Biochemical and Haematological Enhancement Activities of Aqueous and Methanol Leaves, Stem and Roots Extracts of Chasmanthera Dependens (Hochst) and Dictyandra Arborescens (Welw.). *Bull Natl Res Cent*. **2021,** *45*, 1–15, https://doi.org/10.1186/S42269-021-00642-7.
13. Watuma, B.M.; Kipkoech, S.; Melly, D.K.; Ngumbau, V.M.; Rono, P.C.; Mutie, F.M.; Mkala, E.M.; Nzei, J.M.; Mwachala, G.; Gituru, R.W.; Hu, G.W.; Wang, Q.F. An Annotated Checklist of the Vascular Plants of Taita Hills, Eastern Arc Mountain. *PhytoKeys*, **2022**, *191*, 1–158, https://doi.org/10.3897/PHYTOKEYS.191.73714.
14. Chasmanthera dependens - Useful Tropical Plants. Accessed October 8, **2022**, https://tropical.theferns.info/viewtropical.php?id=Chasmanthera+dependens

15. Anosike, C.; Nwachukwu, J.; Ogbozor, C.; Madueke, A. Phytochemicals and antimicrobial studies on methanol and chloroform extracts of Chasmanthera dependens roots. *J Pharmacogn Phyther*. **2018**.
16. Quadri, A.L.; Yakubu, M.T. "Semen Quality and Gonadometric Assessment of the Male Wistar Rats Treated with Aqueous Leaf Extract of Chasmathera Dependens (Hochst)." *Afr. J. Biomed. Res*, **2022**, *25*, 95–99, https://doi.org/10.1111/AND.12775.
17. Wahab, O.M. Ethnomedicinal Antiepileptic Plants Used in Parts of Oyo and Osun States, Nigeria. *Bot Res Int*. **2015**, *8*, 77-81, https://www.idosi.org/bri/8(4)15/3.pdf.
18. Kola-Mustapha, A.T.; Yohanna, K.A.; Ghazali, Y.O.; Ayotunde, H.T. Design, formulation and evaluation of Chasmanthera dependens Hochst and Chenopodium ambrosioides Linn based gel for its analgesic and anti-inflammatory activities. *Heliyon* **2020**, *6*, e04894, https://doi.org/10.1016/J.HELIYON.2020.E04894
19. Tijani, A.S.; Farombi, E.O.; Olaleye, S.B. Mechanisms Underlying the Healing Potentials of the Methanol Extract of Chasmanthera Dependens Stem on Indomethacin-Induced Gastric Ulcer. *Taylor & Francis*, **2021**, *8*, 17–31, https://doi.org/10.1080/2314808X.2021.1881746.
20. Abiola, T.S.; Susan, A.O.; Olusegun, B.O.; Abiola, T.S.; Susan, A.O.; Olusegun, B.O. Tannin-Rich Extract of Chasmanthera dependens Stem Potential in Piroxicam-Induced Nephrotoxicity in Adult Male Wistar Rats. *Am J Mol Biol*. **2019**, *10*, 29-43, https://doi.org/10.4236/AJMB.2020.101003
21. Madueke, A.; Nwanelo, V.; Tabansi, E.; Onoh, A.; Anichebe, R.; Okosisi, A.; Anosike, C. Evaluation of Antioxidant Properties of Choloroform Extract of Chasmanthera dependens Roots. *Med Sci Forum* **2021**, *2*, 21, https://doi.org/10.3390/CAHD2020-08606
22. Miranda, G.M.; Ramos, E.; Santos, V.O.; Bessa, J.R.; Teles, Y.; Yahouédéhou, S.; Goncalves, M.S.; Ribeiro-Filho, J. Inclusion Complexes of Non-Steroidal Anti-Inflammatory Drugs with Cyclodextrins: A Systematic Review. *Biomol* **2021**, *11*, 361, https://doi.org/10.3390/BIOM11030361
23. Osafo, N.; Agyare, C.; Obiri, D.D.; Antwi, A.O. Mechanism of Action of Non-steroidal Anti-Inflammatory Drugs. In: *J. Rheumatol*. **2017**, *2017*, 5-14, https://doi.org/10.5772/68090
24. Kushner, P.; McCarberg, B.H.; Grange, L.; Kolosov, A.; Haveric, A.L.; Zucal, V.; Petruschke, R.; Bissonnette, S. The Use of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) in COVID-19. *NPJ Prim. Care Respir. Med.* **2022,** *32*, 1–10, https://doi.org/10.1038/s41533-022-00300-z.
25. Bannister, K.; Sachau, J.; Baron, R.; Dickenson, A.H. Neuropathic Pain: Mechanism-Based Therapeutics. *Annu Rev Pharmacol Toxicol* **2020,** *60*, 257-274, https://www.annualreviews.org/doi/abs/10.1146/annurev-pharmtox-010818-021524.
26. Monteiro, C.; Silvestre, S.; Duarte, A.P.; Alves, G. Safety of Non-Steroidal Anti-Inflammatory Drugs in the Elderly: An Analysis of Published Literature and Reports Sent to the Portuguese Pharmacovigilance System. *Int. J. Environ. Res.* **2022,** *19*, 1–18, https://doi.org/10.3390/ijerph19063541.
27. Zhou, Q.; Zhao, S.; Gan, L.; Wang, Z.; Peng, S.; Li, Q.; Liu, H.; Liu, X.; Wang, Z.; Shi, Q.; Estill, J.; Luo, Z.; Wang, X.; Liu, E.; Chen, Y. Use of Non-Steroidal Anti-Inflammatory Drugs and Adverse Outcomes during the COVID-19 Pandemic: A Systematic Review and Meta-Analysis. *EClinicalMedicine*, **2022,** *46*, 101373, https://doi.org/10.1016/j.eclinm.2022.101373.
28. Ribeiro, H.; Rodrigues, I.; Napoleão, L.; Lira, L.; Marques, D.; Veríssimo, M.; Andrade, J.P.; Dourado, M. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), Pain and Aging: Adjusting Prescription to Patient Features. *Biomed. Pharmacother*, **2022,** *150*, 112958, https://doi.org/10.1016/J.BIOPHA.2022.112958.
29. Lorke, D. A new approach to practical acute toxicity testing. *Arch Toxicol*. **1983**, *54*, 275-287, https://doi.org/10.1007/BF01234480
30. Koster, R.; Anderson, W.; Beer, E. Acetic Acid For Analgesic Screening. *Fed Proc*. **1959**, *18*, 412-417.
31. Singh S, Majumdar, D.K. Analgesic Activity of Ocimum sanctum and its Possible Mechanism of Action. **2008,** *33*, 188-192, http://dx.doi.org/103109/13880209509065361.
32. Mbagwu, H.O.; Anene, R.A.; Adeyemi, O.O. Analgesic, antipyretic and anti-inflammatory properties of Mezoneuron benthamianum Baill (Caesalpiniaceae). *Nig Q J Hosp Med*. **2007,** *17*, 35-41, https://doi.org/10.4314/NQJHM.V17I1.12540
33. Ishola, I.O.; Olayemi, S.O.; Oreagba, I.A.; Ifeanyi, C.I.; Popoola, T.D. Antinociceptive and anti-inflammatory properties of Tetracera alnifolia Willd. (Dilleniaceae) hydroethanolic leaf extract. *J Basic Clin Physiol Pharmacol*. **2015**, *30*, 39-49, https://doi.org/10.1515/jbcpp-2016-0190
34. Adeyemi, O.O.; Okpo, S.O.; Okpaka, O. The antinociceptive effect of the methanolic extract of Acanthus montanus. *J. Ethnopharmacol* **2004**, *90*, 45-48, https://doi.org/10.1016/J.JEP.2003.09.021
35. Rosland, J.H.; Hunskaar, S.; Hole, K. The Effect of Diazepam on Nociception in Mice. *Pharmacol Toxicol*. **1987,** *61*, 111-115, https://doi.org/10.1111/J.1600-0773.1987.TB01786.X

36. Young, H.Y.; Luo, Y.L.; Cheng, H.Y.; Hsieh, W.C.; Liao, J.C.; Peng, W.H. Analgesic and anti-inflammatory activities of [6]-gingerol. *J Ethnopharmacol*. **2005**, *96*, 207-210, https://doi.org/10.1016/J.JEP.2004.09.009
37. Mielcarska, M.B.; Bossowska-Nowicka, M.; Toka, F.N. Cell Surface Expression of Endosomal Toll-Like Receptors-A Necessity or a Superfluous Duplication? *Front Immunol*. **2021,** *11*, 620972, https://doi.org/10.3389/FIMMU.2020.620972
38. Forkuo, A.D.; Mensah, K.B.; Ameyaw, E.O.; Antwi, A.O.; Kusi-Boadum, N.K.; Ansah, C. Antiplasmodial and Antipyretic Activity and Safety Evaluation of the Methanolic Leaf Extract of Murraya exotica (L.). *J Parasitol Res*. **2020,** *2020*, 1-8, https://doi.org/10.1155/2020/1308541
39. Venkateswarlu, G.; Ganapathy, S. Antipyretic effect of methanolic extracts of Limnophila repens and Argyreia cymosa whole plant on albino rats. *MOJ Bioequivalence Bioavailab*. **2018**, *5*, 303-306, https://doi.org/10.15406/MOJBB.2018.05.00119
40. Khan, A.S. Antipyretic and Analgesic Activities of Some Economically Important Woody Plants. In: *Medicinally Important Trees*. Springer, Cham, Germany; **2017**; 159-185, https://doi.org/10.1007/978-3-319-56777-8_7
41. Fan, S.H.; Ali, N.A.; Basri, D.F. Evaluation of Analgesic Activity of the Methanol Extract from the Galls of Quercus infectoria (Olivier) in Rats. *Evid Based Complement Alternat Med*. **2014** *2014*, 1-6, https://doi.org/10.1155/2014/976764
42. Rezaee-Asl, M.; Sabour, M.; Nikoui, V.; Ostadhadi, S.; Bakhtiarian, A. The Study of Analgesic Effects of Leonurus cardiaca L. in Mice by Formalin, Tail Flick and Hot Plate Tests. *Int Sch Res Not*. **2014**, *2014*, 1-5, https://doi.org/10.1155/2014/687697
43. Yam, M.F.; Loh, Y.C.; Oo, C.W.; Basir, R. Overview of Neurological Mechanism of Pain Profile Used for Animal "Pain-Like" Behavioral Study with Proposed Analgesic Pathways. *Int J Mol Sci*. **2020**, *21*, 1-26, https://doi.org/10.3390/IJMS21124355
44. Sofidiya, M.O.; Oloruntola, O.M.; Sofola, I.; Fageyinbo, M.S. Antinociceptive activity of Euadenia trifoliolata (Schum. & Thonn.) Oliv. leaves and roots in mice. *J Tradit Complement Med*. **2015,** *6*, 289-293, https://doi.org/10.1016/J.JTCME.2015.07.006