

Root bark of *Cordia millenii* essential oil: anti-inflammatory and anti-nociceptive activities

ABSTRACT

SAMPLE ABSTRACT:

Aims: Considering the lack of scientific studies focused on the pharmacological activity of *Cordia millenii* essential oil, this work was designed to evaluate the anti-inflammatory and anti-nociceptive activities of essential oil from the root bark.

Study design: The design of the study include collection of root bark of *Cordia millenii*, hydrodisitllation? of essential oil from the plant and evaluation of its anti-inflammatory and anti-nociceptive potentials.

Place and Duration of Study: Department of Chemistry, Lagos State University, Nigeria between May 2017 and April 2018.

Methodology: The root bark of *C. millenii* were collected from Ayetoro, Ilesha (7°37'0N 4°43'0E), Osun State, Nigeria in June 2017. Essential oils were obtained from the air-dry sample by hydrodistillation procedure in an all glass Clevenger-apparatus. The anti-inflammatory activity was evaluated by carrageenan-induced rat paw edema. The anti-nociceptive action was established from the hot-plate analysis. Statistical analysis was performed using GraphPad Prism (version 7.02).

Results: The anti-inflammatory activity of the essential oil was statistically significant ($p < 0.001$) at 1st and 3rd h for the 100 mg/kg p.o., at 1st-3rd h for the 200/kg mg p.o. and at 3rd and 4th h for the 400 mg/kg p.o. In addition, the 100 mg/kg p.o. showed significant activity ($p < 0.01$) at 2nd h. Also, the anti-inflammatory activity was significant ($p < 0.05$) for 100 mg/kg p.o. (4th h), 200 mg/kg p.o. (4th h) and 400 mg/kg p.o. (1st h). The essential oil of *C. millenii* displayed high activity ($p < 0.001$) for all doses in the hot plate anti-nociceptive assay which was time and dose independent.

Conclusion: Results demonstrate that the essential oil of *C. millenii* was effective in the treatment of inflammatory conditions, thereby supporting the traditional use of this herb.

Keywords: *Cordia millenii*, essential oil, anti-inflammatory activity, anti-nociceptive activity

1. INTRODUCTION

Cordia millenii (Bak.) is a medicinal plant belonging to Boraginaceae family. It is widely distributed in tropical Africa. The plant can grows to a height of 60 to 100 ft, bole cylindrical, but rarely straight, 30 to 40 ft. in length; trunks about 3 ft in diameter above buttresses [1]. The plant has been used in ethnomedicine for the treatment of fever, cough, stomachache, mild tonic, astringent, toothache and inflammation related disorders. Extracts from *C. millenii* have shown the antifertility [1], antimicrobial [2] and antioxidants [2] effects. In addition, the extracts have prevented lipopolysaccharide-induced neuroinflammation [3]. The phytochemical compounds previously isolated from the plant include cordiachromes A–F [4]. Previously, the main constituents of essential oil from the leaf of *C. millenii* [5] were identified as limonene (19.9%), diallyl disulfide (18.4%), β -caryophyllene (16.6%), linalool (13.4%) and nonanal (10.6%). In addition, the leaf essential oil did not possess any significant anti-

26 nociceptive property [5]. However, the essential oil only displayed anti-inflammatory activity
27 at the 1st h ($P < .01$) for the 200-mg p.o. Till moment, no information is available on biological
28 activity of essential oils from other parts of *C. millenii*.

29 Generally, the inflammatory process involves a series of events that can be elicited
30 by numerous stimuli such as infectious agents, ischemia, antigen-antibody interaction, and
31 thermal or physical injury. Inflammation is usually associated with pain as a secondary
32 process resulting from the release of analgesic mediators: nonsteroidal anti-inflammatory
33 drugs (NSAIDs), steroidal drugs, and immunosuppressant drugs, which have been used
34 usually in the relief of inflammatory diseases by people around the world for a long time [6].
35 However, these drugs were often associated with severe adverse side effects, such as
36 gastrointestinal bleeding and peptic ulcers [6]. Recently, many natural medicines derived
37 from medicinal plants were considered as effective and safer for the treatment of various
38 diseases including inflammation and pain [7].

39 This paper describes the observed anti-inflammatory actions of *C. millenii* essential
40 oil. Recently, the chemical constituents, anti-inflammatory and anti-nociceptive activities of
41 essential oils from Nigerian plants were reported [5,8-10].

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44 2. MATERIAL AND METHODS

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46 2.1 Drug and chemicals

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48 Carrageenan drug (Batch Number: SLBR0530V) of analytical grade was obtained from
49 Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Acetylsalicylic? salicylate injection (RX,
50 Nigeria Ltd; Batch Number: MT2056) and Diclofenac Injection (FITZKING LINK LIMITED,
51 Nigeria Ltd; Batch Number: 180606) were purchased from Lagos State University Pharmacy.

52 2.2 Animals

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54 Wistar rats (150-200 g) of both sexes were accommodated in the Biochemistry Department
55 animal facility of Lagos state University, Ojo-Lagos. The animals were kept in metal steel
56 cages, where they had unrestricted supply to water and standard pellet food. They were
57 acclimatized for two weeks before commencement of experiment. The animals were
58 assigned at random to a group of 5 consisting of 6 animals per group:

59 Group 1- Control group (Saline solution); Group 2- Diclofenac treated group 100 mg/kg
60 (Standard Group); Group 3- 100 mg/kg of essential oil of *C. millenii*; Group 4- 200 mg/kg of
61 essential oil; and Group 5- 400 mg/kg of *C. millenii*.

62 The rationale for selecting the studied doses was that animals of similar weight were
63 grouped together to obtained average weight. The weight recorded was similar across the
64 groups of animals. The dose was therefore determined from the weight of animals in the
65 assigned group. The essential oil of *C. millenii* was dissolved in a saline vehicle and
66 administered to the animal in the order of 100, 200 and 400 mg/kg.

67 All experimental procedures were approved under the Lagos State University
68 Research Ethical Clearance Committee (RECC) of the University (Approval no:
69 012/2017/LASU/BCH).

70 2.3 Plant sample

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72 The root barks of *C. millenii* were collected from Ayetoro, Ilesha (7°37'0N 4°43'0E), Osun
73 State in June 2017. Botanical identification was achieved by Mr. Dotanus E. of Herbarium,
74 Department of Botany, University of Ibadan, Nigeria. A voucher specimen (UIH-22607) was
75 deposited at the herbarium. Samples were air-dried under laboratory shade (27°C) for two
76 weeks.

77 **2.3.1 Hydrodistillation of essential oil**

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79 In this experiment, 260 g of air-dried and pulverized roots of *C. millenii* was used. The
80 pulverized sample was carefully introduced into a 5 L flask and distilled water was added
81 until it covered the sample completely. Essential oils were obtained by hydrodistillation which
82 was carried out in distillation unit designed according to the specification as described
83 previously [5, 8-10]. The distillation time was 3 h and conducted at normal pressure. The
84 volatile oils which distilled over water were collected by running through the tap in the
85 receiver arm of the apparatus into clean and weighed sample bottles. The oils after drying
86 were kept under refrigeration (4°C) until the moment of analyses.

88 **2.4 Anti-inflammatory and anti-nociceptive tests**

89 **2.4.1 Toxicity test**

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91 The essential oil was tested for acute toxicity study. Wistar rats were administered 500,
92 1000, 1500 and 2000 mg/kg of *C. millenii* per oral route. One group received normal saline
93 that served as a negative control. The animals were observed for 12 h continuously for
94 changes in their behavior. Mortality for the next 14 days was also noted.

96 **2.4.2 Carrageenan-induced paw edema in rats**

97 Carrageenan induced rat paw edema experiment was carried out according to a modification
98 form of an established procedure as described previously [5,8-10]. Thirty Wistar rats (both
99 sexes, 150-200 g each) divided into 6 animals in each groups used for study. The animals
100 were induced by subcutaneous injection of 0.1 mL of 1% freshly prepared carrageenan in
101 saline in the right hind paw. In addition, 1mL of all other solutions was administered for all
102 doses. Paw volume of the injected rats was measured every hour for four hours using a
103 plethysmometer (Ugo Basile, Italy). All treatments were administered orally using the canula
104 syringe.

105 **2.4.3 Hot plate anti-nociceptive test**

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107 The experiment was carried out according to the method described previously [5,8-10].
108 Twenty-five (25) mature Wistar rats of both sexes were randomly divided into 5 groups of
109 equal rats. The animals were fasted for 12 h with provision of clean water *ad libitum*. Doses
110 were administered as follows: Group 1- 10 mL/kg of saline solution (control); Group 2- 10
111 mg/kg (sodium salicylate, ASA, standard control; Group 3- 100 mg/kg of *C. millenii* oil p.o.;
112 Group 4- 200 mg/kg of *C. millenii* oil p.o.; Group 5- 400 mg/kg of *C. millenii* oil p.o.

113 Each mouse was placed upon the heated metal plate (Hot plate) maintained at the
114 temperature of about 50-55 °C within the restraining glass cylinder. Animal response to the
115 heat varies and such changes includes kicking of hind foot and jumping about, licking of foot,
116 raising the foot, holding the foot tightly to its body or shaking of the foot. The reaction time
117 was recorded 30, 60, 90 and 120 min after the administration of the treatments. The
118 maximum reaction time was fixed at 30 s to prevent any injury to the tissues of the paws. If
119 the reading exceeds 30 s, it would be considered as maximum analgesia.

120 **2.4.4 Statistical analysis**

121 Repeated Measures Two way ANOVA Analysis using Bonferotti multiple comparisons post
122 hoc test was performed using GraphPad Prism (version 7.02), San Diego CA, USA,
123 www.graphPad.com) to compare activity between the control groups and rat treated with the
124 test compounds and values were considered significant at $P < .05$ and above. Results were
125 expressed as mean \pm SEM [5,8-10].

126 **3. RESULTS AND DISCUSSION**

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128 **3.1. Acute toxicity**

129 Test doses of 500, 1000, 1500 and 2000 mg/kg body weight of WIEO showed no adverse
130 effects on the behavioural and physical responses in the tested rats following an observation
131 for 14 days. There was no mortality, flesh or skin peeling, swollen limb or neck, and no
132 weight loss was observed. Therefore, a higher dose of 400 mg/kg given to rats in this study
133 was considered to be safe.

134 **3.2. Anti-inflammatory activity**

135 The evaluation of the anti-inflammatory activity in vivo was conducted using the model of
136 carrageenan-induced paw edema. Edema formation in the paw is the result of a synergism
137 between various inflammatory mediators that increase vascular permeability and/or the
138 mediators that increase blood flow [11]. This is a well-defined model of acute inflammation
139 and has been applied in the study of anti-edematous effect of extracts due to the production
140 of different inflammatory mediator in the Wistar rat. This development is time dependent
141 characterised by biphasic release of mediators. The initial phase involves the release of
142 mediators such as histamine, serotonin and bradykin last within the first 1 h, while the latter
143 phase is characterized by infiltration of leukocytes and prostaglandins biosynthesis [12].



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145 Figure 1: Effect of the essential oils of *C. millenii* roots bark on Carrageenan-induced inflammation.
146 Control, standard and *C. millenii* represent 1mL saline solution, 100 mg/kg of diclofenac injection and
147 1mL of 100, 200 and 400 mg/kg of *C. millenii* leaves essential oil respectively. * $P < .05$, ** $P < .01$, ***
148 $P < .001$ statistically compare to the control.

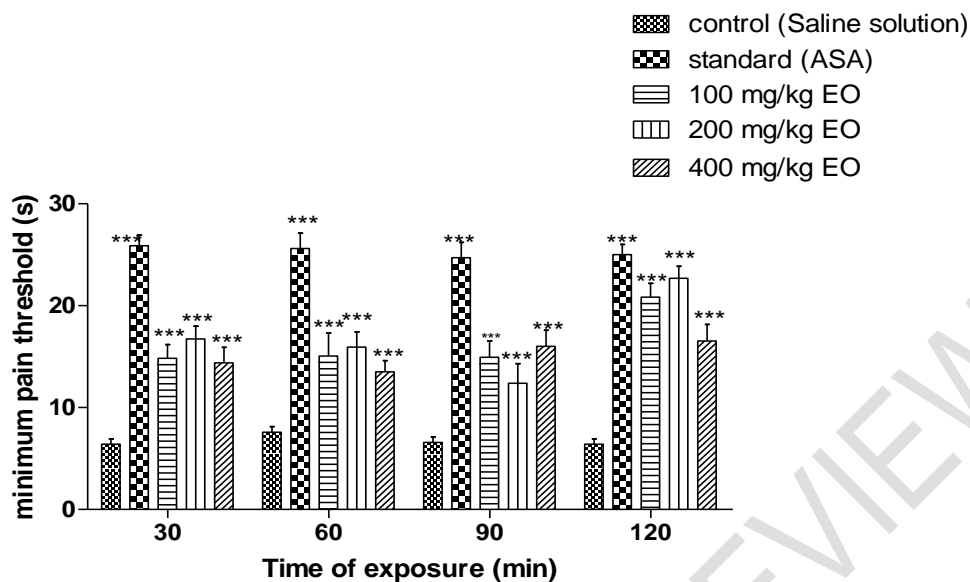
149 The anti-inflammatory activity of *C. millenii* root essential oil was statistically ($P < .001$) for the
150 100 and 200 mg/kg p.o., at 1st and 3rd h, while the 200 mg/kg p.o., also displayed significant
151 activity ($P < .001$) at 2nd h. The 400 mg/kg p.o. displayed anti-inflammatory actions ($P < .001$)
152 at 3rd and 4th h. The anti-inflammatory activity was also statistically ($P < .01$) for the 100
153 mg/kg p.o at 2nd h, while activity was significant ($P < .05$) for 100 and 200 mg p.o. (4th h) and
154 400 mg/kg p.o (1st h).

155 The anti-inflammatory inhibitory activity of *C. millenii* root oil was highly significant. As
156 shown in **Fig. 1**, mediators released in all phases were significantly inhibited. The oil activity
157 at these doses was also equivalent to that of the standard drug used (Ibuprofen). However,
158 at the 4th h, there was significant reduction in the inhibitory activities of the 100 and 200
159 mg/kg doses. The carrageenan-induced paw edema in rats is believed to be biphasic. The
160 former phase is due to the release of histamine or serotonin (0-1 h post treatment), and the
161 latter phase is characterised by the release of bradykinin, protease, prostaglandin, and
162 lysosome (2nd to 4th h post treatment) [13]. In the present study, oral treatment with *C.*
163 *millenii* root oil markedly inhibited carrageenan-induced paw oedema in rats in a dose and
164 time dependent manner. This treatment steadily attenuated the paw oedema induced by
165 carrageenan, as well as by numerous inflammatory mediators participating in the
166 carrageenan-induced inflammation such as bradykinin, histamine, substance P and platelet-
167 activating factor [14,15]. This evidence suggests that the anti-inflammatory action of the
168 essential oil of *C. millenii* are related to the inhibition of one or more inflammation mediator
169 pathways involved in the effects of these mediators.

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171 **3.2. Anti-nociceptive activity**

172 The anti-nociceptive activity of the essential oils was investigated using the hot plate model.
173 The hot plate test was carried out to ascertain either the peripheral or the central acting
174 effect of the essential oils [16]. The test is widely used to clarify the analgesic and most
175 especially the effect of opioid drugs on the spinal cord. In our study, we found that the
176 essential oil of *C. millenii* showed a very prominent activity at all doses. The ability of the
177 essential oil to inhibit the expressions of the nociceptive neurons was highly significant at all
178 doses ($p < 0.001$). The activity of the oil showed a similar activity as the standard drug (ASA),
179 due to statistical significance as shown in **Fig. 2**.



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Figure 2 : Effect of the essential oils of *C. millenii* roots bark on hot plate-induced anti-nociceptive. Control, standard and *C. millenii* represent 1mL saline solution, 100 mg/kg of aspirin injection and 1mL of 100, 200 and 400 mg/kg of *C. millenii* leaves essential oil respectively. * $P < .05$, ** $P < .01$, *** $P < .001$ statistically compared to the control.

Essential oil has been reported as a good source of anti-inflammatory agent due to their quick penetration after dermal, oral, or pulmonary administration. Their metabolism and elimination occurs in the kidney in the form of phase-II conjugates [17]. Recent information indicated that essential oils and their composition have significantly ameliorated inflammation related ailments. The anti-inflammatory activity of essential oil of *C. millenii* root competes favourably with data from essential oils from other plants studied under the same experimental model. The root barks oil of *C. millenii* posse's considerable anti-inflamamtory activity when compared with the leaf oil [5]. The essential oil of *Phyllanthus muellerianus* [8] and *Waltherica indica* [10] displayed anti-nociceptive effect ($P < .001$) and suppression of inflammatory mediators ($P < .001$) at a rate independent of reaction time and dose. The anti-nociceptive property of the essential oil of *Bounganvillea glabra* [9] was statistically significant ($P < .001$), while for the 1st and 2nd h, at doses of 100 and 200 mg/kg, the anti-inflammatory activity was statistically very significant ($P < .001$). The essential oil of *Melissa officinalis* showed pronounced reduction and inhibition of edema induced by carrageenan at 6 h at 200 and 400 mg/kg [18]. The essential oils of *Senecio flammeus* [19] and *Pycnocycla bashagardiana* [20] significantly reduced inflammation mediators ($P < .05$) 4 h after of carrageenan injection. The anti-inflammatory activity of essential oil of *Cinnamomum longepaniculatum* [21] and *Artemisia aucheri* [22] occurred both in early and late phase and peaked at 4 h after carrageenan injection.

4. CONCLUSION

For the first time, the anti-inflammatory and anti-nociceptive activities of essential oil from the root barks of *C. millenii* were evaluated against carrageenan-induced paw edema and hot plate test, respectively, in rats. Results in this study demonstrated that the essential oils of *C. millenii* were statistically significant and effective in the treatment of both pains and inflammatory conditions, thereby supporting the traditional use of this herb.

214 **COMPETING INTERESTS**

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216 “Authors have declared that no competing interests exist.”

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220 **CONSENT**

221

222 Not applicable

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224 **ETHICAL APPROVAL**

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226 “All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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