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Antinociceptive properties of dichloromethane: methanolic leaf and root bark extracts of *Carissa edulis* in rats

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Abstract

Conventional medications are expensive and arguably associated with various severe adverse effects, hence the need to develop herbal agents that are effective as alternative. *Carissa edulis* (Forssk.) Vahl is the herb that has previously made thousands of people flock to a remote Loliondo village in Northern Tanzania, for its cure said to apply to all diseases such as cancer, HIV/AIDS, ulcers, hypertension, and diabetes. Although *C. edulis* (Forssk.)Vahl is widely used for pain in the traditional system of medicine; review of the literature shows no scientifically investigated report of its described effects. This study was, therefore, designed to bioscreen the DCM: methanolic extract of the leaf and root bark of *C. edulis* on anti-nociceptive potential. The plant parts were collected from Siakago-Mbeere north sub-county, Embu County, Kenya. Pain was induced into the rats experimentally using formalin. Anti-nociceptive activities in rats were compared with diclofenac (15 mg/kg) as the standard conventional drug. The leaf extract reduced pain by between 47.04% - 47.19% (in the early phase) and 38.96% - 89.26% (in the late phase) while the root bark extracts reduced it by between 21.5% - 41.89% (in the early phase) and between 21.4% - 90.62% (in the later phase). Diclofenac reduced pain by between 27.37% - 34.9% (in the early phase) and 88.24% - 90.28% (in the late phase). Further, the phytochemical screening results showed that the extract had alkaloids, flavonoids, steroids, saponins, phenolics and terpenoids which have been associated with anti-nociceptive activities. Therefore, the study has established that the DCM: methanolic extracts of *C. edulis* (Forssk.)Vahl are effective in the management of pain.

Keywords: Nociception, *Carissa edulis*, Licking time, Leaf extracts, Root bark extracts.

Introduction

Pain is an unpleasant sensory affliction and emotional experience usually associated with actual or potential tissue damage, or described in terms of such damage.[1] It is not a disease, but it is manifested in certain disease or pathological conditions in the organism body. It acts as a warning signal against disturbances in the internal or in the external environment of an individual, which is essential for the organism's survival and wellbeing.[2]

The ability to detect noxious stimuli is essential to an organism's survival and wellbeing. This is dramatically illustrated by examination of individuals who suffer from congenital abnormalities that render them incapable of detecting painful stimuli. These people cannot feel piercing pain from a sharp object, heat of an open flame, or even discomfort associated with internal injuries, such as a broken bone. As a result, they do not engage appropriate protective behaviors against these conditions, many of which can be life threatening.[2]

When body tissues and cells receive any harmful stimulation or disturbance, protons (H^+), prostaglandin E_2 (PGE_2), serotonin (5-HT), among others, are released and consequently cause pain[1], while some pain may be minor or acute, other diseases, for example, rheumatoid arthritis can cause chronic pain. This persistent pain associated with injury or diseases (such as diabetes, arthritis, or tumor growth) can result from alterations in the properties of peripheral nerves and occur as a consequence of damage to nerve fibers, leading to increased spontaneous firing or alterations in their conduction or neurotransmitter properties.[2]

Although pain benefits the nonspecific immune response to invading microorganisms, they are also viewed as sources of discomfort and are commonly suppressed with analgesic medications respectively.[3] Pain is often treatable. Since antiquity, physicians have even used various physical means to reduce pain.[4] Conventionally, over the counter non-steroidal anti-inflammatory analgesics are used for the symptomatic treatment of pain.[5] In addition, opioids and antidepressants are also used for the same

same purpose. The conventional drugs may have various and severe side effects, such as gastric disorders, kidney, liver, and heart failure, prolonged bleeding after injury or surgery, adrenal suppression, insomnia, redness, increased appetite, Cushing's syndrome, and diabetes.

Naturally occurring agents, with high effectiveness and very few side effects are, therefore, desirable as alternative and complementary interventions to the chemical therapeutic agents.[6] According to[7], nature is a good source of salvation for man's health because it provides numerous remedies from its plants, animals and other sources cure most ailments of mankind. Herbal agents are favoured over the synthetic ones for their compatibility with the human physiological system, easy availability, little or no side effects and the rich knowledge about the traditional healing system.[3]

Carissa edulis is commonly known as the Magic herb from the Apocynaceae family.[8] The herb has been used for the treatment of gonorrhea, breast cancer, headache, chest pains and malaria among some communities in Kenya.[8-10] The root is used to treat glandular inflammation, lumbago and other pains.[11] Various parts of the plant are used in traditional medicine for the treatment of fever, oedema, toothache, cough, ulcer, sickle cell anaemia and hernia.[12,13] Preparations of *Carissa edulis* have been used in the Nigerian traditional medicine for the management of fever, sickle cell disease, epilepsy, pain and inflammation for many years and their efficacy is widely acclaimed among the Hausa communities of northern Nigeria.[14]

This study was aimed at bioscreening the dichloromethane:methanolic root bark and leaf extracts of *Carissa edulis* (Forssk.)Vahl, for antipyretic potential in rats models, as a preliminary step towards development of a more efficacious plant-derived antipyretic agent.

Materials and Methods

The plants were collected from Siakago division, Mbeere North sub-county, Embu County, Kenya. The fresh leaves and root barks were identified with the help of local herbalists. The information gathered included vernacular names, plant parts used and the ailment treated. The plant sample was provided to an acknowledged taxonomist for botanical authentication and a voucher specimen (Specimen no. SGM 01/2014) deposited at the Kenyatta University Herbarium. Samples were properly sorted out, cleaned, and transported in polythene bags to Kenyatta University, Biochemistry and Biotechnology laboratories for use in the study.

Sample processing and extraction

The leaves and root barks of *C. edulis* were chopped into small pieces and air dried at room temperature for two weeks until properly dried. They were then ground into fine homogenous powder using an electric mill followed by sieving through mesh sieve. For each sample, 200 grams of powder were oaked separately in a cold 1:1 mixture of DCM and methanol and stirred for six hours to extract the active compounds. The successive extract was filtered using Whatman's filter papers and the filtrate concentrated under reduced pressure and vacuum using a rotary evaporator. The concentrate was put in an airtight container and stored at -4^{0}C before use in bioassays.

Experimental design

Laboratory animals

This study tested for antinociceptive activities using adult Wister albino rats, *Rattus norvegicus*. The rats were of either sex, between 2-3 months old and weighing between 140-180 gm.[15] The animals' breeding colony was acquired and bred in the animal breeding and experimentation facility at the Department of Biochemistry and Biotechnology, Kenyatta University. The animals were allowed to acclimatize for 48 hrs before beginning the experiment. The animals were kept in the standard cages and maintained under the standard laboratory conditions of ambient temperature (25^{0}C) and with 12 hrs day light. The experimental animals were fed on a standard rodent pellets diet and supplied with water *ad libitum*.[16] Ethical guidelines and procedures for handling experimental animals were followed.

Evaluation of antinociceptive activity

The rats were divided into six groups (n = 5) and then treated as shown in Table 1.

Table 1: Treatment protocol for evaluation of antinociceptive activities of DCM: methanolic leaf and root bark extracts of *Carissa edulis* (Forssk.)Vahl in rats

Group	Status	Treatment
I	Normal control	2.5% Formalin only
II	Negative control	2.5% Formalin + 10% DMSO
III	Positive control	2.5% Formalin + 15 mg/kg Diclofenac + 10% DMSO
IV	Experimental group A	2.5% Formalin + 50 mg/kg extract + 10% DMSO
V	Experimental group B	2.5% Formalin + 100 mg/kg extract + 10% DMSO
VI	Experimental group C	2.5% Formalin + 150 mg/kg extract + 10% DMSO

Thirty minutes after the administration of the treatments, the formalin test was carried out as described by[17], where all the animals were injected intraperitoneally with 0.1 ml of 2.50% formalin in the sub-plantar region of the left hind paw to induce nociceptive behavior of lifting, licking and biting.[18,19] The application of the irritant compound into the hind paw makes the nociceptive response more specific, since during grooming the animals most frequently use their forelegs.[19]

The time that the rats spent lifting, licking or biting the injected paw was hence recorded according to the described response pattern described by[19]. Two distinct periods of intensive licking/biting activity were identified and scored separately. The first period (Early Phase - direct chemical stimulation of nociceptors) was recorded 1-5 minutes after formalin injection and the second period (Late Phase-release of inflammatory mediators) was recorded 15-30 minutes after formalin injection. The percentage inhibition of the licking was then calculated using the following formula;

$$\frac{C-T}{C} X100$$

Where;
C- The vehicle treated control group value for the each phase
T - The treated group value for each phase

Qualitative phytochemical screening

The extracts obtained were subjected to a qualitative phytochemical screening to identify the presence or absence of selected chemical constituents using methods of analysis as described by[20,21]. Standard screening tests for detecting the presence of different chemical constituents were employed. Secondary metabolites tested for were flavonoids, phenolics, saponins, alkaloids, cardiac glycosides, sterols and terpenoids.

Data management and statistical analysis

Experimental data on the licking time in seconds was obtained from all the animals in different groups, recorded and tabulated on a broad

sheet using Ms Excel program. The results were expressed as mean ± standard error of mean (SEM) for analysis. Statistical significance of differences among groups was analysed using one-way analysis of variance (ANOVA) followed by Tukey's tests to separate the means and obtain the specific significant differences among the different groups. Un-paired student t-test was done to compare between the mean activities of leaf and root bark extracts. The values of $p \leq 0.05$ were considered to be significant. Analysis of the data was done using Minitab statistical software.

Results

Formalin induces pain in two phases; early phase, which takes one to five minutes and the late phase, which takes fifteen to thirty minutes after formalin injection. Generally, the administration of DCM: methanolic leaf extracts successfully reduced the formalin-induced pain in both phases which was indicated by reduction in paw licking time (Table 2).

In the early phase, treatment of rats with DCM: methanolic leaf extracts of *C. edulis* at dose levels of 100 and 150 mg/kg body weight, significantly reduced paw licking time compared to the negative control ($p < 0.05$; Table 2). The percent licking inhibition of these two dose levels (100 and 150 mg/kg body weight) were 47.04% and 47.19% respectively, which was not significantly different from each other ($p < 0.05$; Table 2). In this phase, DCM: methanolic leaf extracts of *C. edulis* at dose levels of 100 and 150 mg/kg body weight exhibited a significant decrease in pain compared to diclofenac, which had percent paw licking inhibition of 27.37% ($p < 0.05$; Table 2). However, in this phase, the group treated with DCM: methanolic leaf extracts of *C. edulis* at the dose level of 50 mg/kg body weight did not lower the paw licking time significantly (Table 2).

In the late phase, DCM: methanolic leaf extracts of *C. edulis* reduced formalin induced pain in rats but not in a dose dependent manner (Table 2). At the dose level of 100 mg/kg and 150 mg/kg body weight, the DCM: methanolic leaf extracts exhibited significant antinociceptive effect compared with negative control and baseline groups ($p < 0.05$; Table 2). The two dose levels inhibited the late phase pain in rats by 89.26% and 84.93% respectively (Table 2). The antinociceptive effectiveness of the two extracts dose levels was not significantly different from each other ($p > 0.05$; Table 2). Diclofenac (reference drug), significantly reduced the paw licking time by 88.24% in the late phase of the formalin induced pain test compared to baseline and negative control groups. In this phase, the extracts at dose levels of 100 and 150 mg/kg body weight were as effective as diclofenac. However, the group treated with DCM: methanolic leaf extracts of *C. edulis* at the dose level of 50 mg/kg body weight showed a slight though the significant antinociceptive effect compared to the baseline and negative control groups ($p < 0.05$; Table 2).

Table 2: Antinociceptive effects of DCM: methanolic leaf extracts of *Carissa edulis* (Forssk.)Vahl in rats

Group	Treatment	Phase I	Phase II
Baseline	Formalin only	281.60 ± 8.94[a] (-4.14)	843.6 ± 12.0[a] (02.62)
Negative control	Formalin + DMSO	270.40 ± 6.50[a] (00.37)	840.0 ± 23.2[a] (00.12)
Positive control	Formalin + DMSO + Diclofenac	196.40 ± 11.7[b] (27.37)	98.8 ± 16.30[c] (88.24)
DCM: Methanolic Leaf Extracts	50 mg/kg + Formalin + DMSO	274.80 ± 4.84[a] (-1.75)	515.0 ± 68.8[b] (38.96)
	100 mg/kg + Formalin + DMSO	142.80 ± 12.7[c] (47.04)	89.0 ± 13.80[c] (89.26)
	150 mg/kg + Formalin + DMSO	142.60 ± 16.6[c] (47.19)	123.6 ± 23.3[c] (84.93)

Values are expressed as Mean ± SEM for five animals per group. Statistical comparison were made within a column and values with the same superscript are not significantly different by ANOVA followed by Tukey's post hoc test ($p > 0.05$). Figures in paranthesis indicate percent paw licking inhibition. Formalin = 2.5%; DMSO = 10%; Diclofenac = 15 mg/kg.

On the other hand, the administration of DCM: methanolic root bark extracts of *C. edulis* also generally reduced the formalin-induced pain in both phases, although not in a dose dependent fashion (Table 3). In the early phase, DCM: methanolic root bark extracts of *C. edulis*, at the three dose levels (50, 100 and 150 mg/kg body weight), reduced formalin induced pain by 42%, 30% and 22% respectively (Table 3). Treatment with root bark extracts at dose levels of 50 and 100 mg/kg body weight significantly inhibited the paw licking response time in rats compared to the control groups, although their antinociceptive effectiveness was not significantly different from each other ($p > 0.05$; Table 3). In this phase, the DCM: methanolic root bark extracts of *C. edulis* at dose levels 50 and 100 mg/kg body weight exhibited a decrease in pain as effectively as diclofenac (reference drug) which had percent licking inhibition of 35% ($p > 0,05$; Table 3). However, the group treated with the DCM: methanolic root bark extracts of *C. edulis* at the dose level of 150 mg/kg body weight did not exhibit a significant reduction in paw licking time compared to baseline and negative control groups ($p > 0.05$; Table 3).

In the late phase, DCM: methanolic root bark extracts of *C. edulis* at the three dose levels (50, 100 and 150 mg/kg body weight) also reduced formalin induced pain in rats (Table 3). The percent paw licking inhibitions of the root bark extracts at the three dose levels were 90.62%, 81.12% and 21.4% respectively (Table 3). Just like in the early phase, antinociceptive effect of the root bark extracts at the dose levels of 50 and 100 mg/kg body weight were not significantly different from each other ($p > 0.05$; Table 3). The extract dose levels of 50 and 100 mg/kg body weight were as effective as diclofenac, which had percent paw licking inhibition of 90.28%. The group treated with DCM: methanolic root bark extracts of *C. edulis* at the dose level of 150 mg/kg body weight produced slight but significant antinociceptive effect compared to the baseline, positive and negative control groups ($p < 0.05$; Table 3).

In comparison, the DCM: methanolic root bark extracts of *C. edulis* exhibited more effective antinociceptive effect, than the leaf extracts at the dose of 50 mg/kg body weight in both early and late phases. However, at the dose levels of 100 and 150 mg/kg body weight, the DCM: methanolic leaf extracts of *C. edulis* exhibited more effective antinociceptive activity than the root bark extracts (Figures 1 and 2).

Phytochemical screening

Qualitative phytochemical screening of the DCM: methanolic leaf extracts of *C. edulis* revealed the presence of alkaloids, flavonoids, phenolics, terpenoids and traces of steroids. Phytochemical constituents of the root barks extract contained alkaloids, flavonoids, steroids, saponins, phenolics and terpenoids. However, saponins were absent in the leaf extracts while cardiac glycosides were absent in both leaf and root bark extracts (Table 4).

Table 3: Antinociceptive effects of DCM: methanolic root bark extracts of *Carissa edulis* (Forssk.) Vahl in rats

Group	Treatment	Phase I	Phase II
Baseline	Formalin only	266.80 ± 13.1[a] (03.40)	839.4 ± 16.2[a] (02.62)
Negative control	Formalin + DMSO	276.20 ± 6.17[a] (00.36)	862.0 ± 5.65[a] (00.12)
Positive control	Formalin + Diclofenac + DMSO	179.80 ± 20.0[b] (34.90)	83.80 ± 14.6[b] (90.28)
DCM: Methanolic Root bark Extracts	50 mg/kg + Formalin	159.40 ± 18.6[b] (41.89)	80.40 ± 18.8[b] (90.62)
	100 mg/kg + Formalin	194.40 ± 13.8[b] (29.60)	162.6 ± 15.8[b] (81.12)
	150 mg/kg + Formalin	216.00 ± 15.5[a] (21.50)	678.8 ± 60.0[c] (21.40)

Values are expressed as Mean ± SEM for five animals per group. Statistical comparison were made within a column and values with the same superscript are not significantly different by ANOVA followed by Tukey's post hoc test ($p > 0.05$). Figures in paranthesis indicate percent paw licking inhibition. Formalin = 2.5%; DMSO = 10%; Diclofenac = 15 mg/kg.

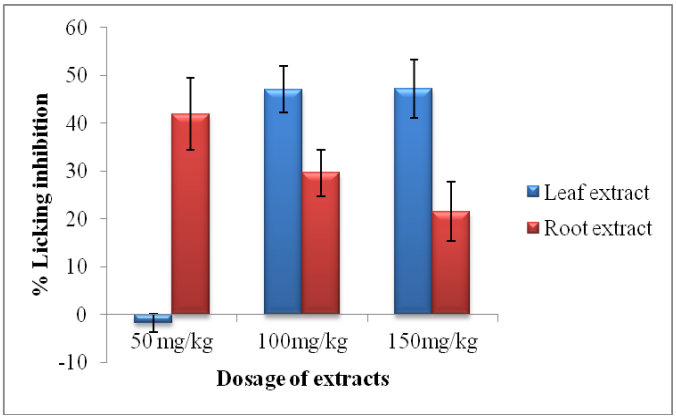

Figure 1: Comparison of percent licking inhibition by DCM: methanolic leaf and root bark extracts of *Carissa edulis* (Forssk.)Vahl in phase I of formalin test.

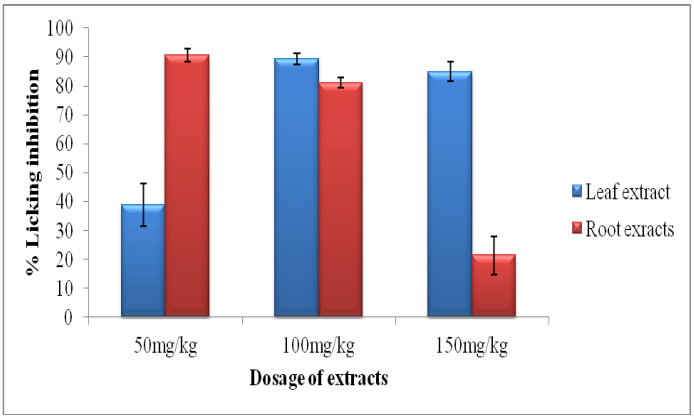

Figure 2: Comparison of percent paw licking inhibition by DCM: methanolic leaf and root bark extracts of Carissa edulis (Forssk.)Vahl in phase II of formalin test.

Table 4: Phytochemical composition of DCM: methanolic leaf and root bark extract of *Carissa edulis* (Forssk.)Vahl

Phytochemicals	Leaf extracts	Root bark extracts
Alkaloids	+	+
Flavonoids	+	+
Steroids	+ (trace)	+
Saponins	-	+
Cardiac glycosides	-	-
Phenolics	+	+
Terpenoids	+	+

Present phytochemicals are denoted by (+) sign, absent phytochemicals are denoted by (-) sign while + (trace) denote slightly present phytochemicals

Discussion

To evaluate antinociceptive properties of the extracts, formalin-induced paw licking test was used for its applicablility, reliable and high specificity for antinociceptive responses.[19,22] The model is considered ideal for clinical pain and it is suggested as a suitable test for chronic pain compared to others.[23] The intraperitoneally administered formalin-induced paw licking test demonstrate intense nociception in two distinct phases of intensive nociceptive behavior, which involve different mediators.[17, 24-28]

The first phase of nociception, commonly known as neurogenic phase, starts immediately after formalin injection, extending for the following five minutes and is believed to occur due to direct chemical stimulation of nociceptors[28], predominantly afferent C fibers and partly A$\delta\delta$ fibers.[22] This phase is inhibited by opioid agonists such as morphine and fentanyl, bradykinin B1 and B2 receptor antagonists, N-methyl-D-aspartic-acid (NMDA) receptors, as well as vanilloid receptor antagonists.[22,26,28-30] The second phase of this model takes place fifteen to thirty minutes after formalin injection. Its response corresponds to inflammatory pain and is most likely secondary to the development of an inflammatory response and relates to the release of several proinfammatory analgesic mediators like serotonin, histamine, bradykinin and prostaglandins.[24,31,32] Analgesics that act centrally such as narcotics inhibit both phases equally while peripherally-acting drugs such as steroids (dexamethazone, hydrocortisone) and non-steroidal anti-inflammatory drugs like aspirin suppress primarily only the late phase pain.[33,34]

In this study, DCM: methanolic leaf and root bark extracts of *C. edulis* (Forssk.)Vahl showed a significant antinociceptive effect by reducing the formalin-induced licking time in both phases. The highest analgesic effect of leaf extracts was by 47.19% and 84.93% in the early and late phases, respectively, while by root bark extracts was by 41.89% and 90.62% in the early and late phases, respectively. These findings suggest both direct analgesic effects on the nociceptor blockage and an inhibition of the synthesis and/or release of inflammatory pain mediators such as prostagradin. The extracts, however, did not inhibit both phases equally. For instance, the highest analgesic effects of leaf extracts was by 47.19% and 84.93% showing different pain inhibitory activity in the early and late phases, respectively. Therefore, it is possible to suggest that DCM: methanolic leaf and root bark extracts of *C. edulis* (Forssk.)Vahl contain centrally and/or peripherally acting antinociceptive phytochemicals.

These results are similar to other previous studies on evaluation of antinociceptive activities of medicinal plant extracts. That the DCM: methanolic leaf and root bark extracts of *C. edulis* (Forssk.)Vahl demonstrated a reduction in the formalin-induced paw licking time in both phases is consistent with[35] who observed antinociceptive activity of aqueous extracts of Radix *Aconiti Carmichaeli* against formalin-induced pain in laboratory animals. Similarly, the crude n-hexane fraction of *Dudleya variegata* have been shown to demonstrate related antinociceptive effect in acetic acid and tail immersion models.[34]

That the DCM: methanolic leaf and root bark extracts of *C. edulis* (Forssk.)Vahl, produced non-dose dependent analgesic activity is related to studies by [36] who observed the antinociceptive activities of *Melissa officinalis* leaf extracts in laboratory animals. The dose ranges used in this study were within the dose ranges used by[37-39]. [37]used dose levels of 50, 100, and 200 mg/kg in evaluating analgesic activity of the root extracts of *Alafia barteri* Oliver (Apocynaceae), *Combretum mucronatum* Schumach (Combretaceae) and *Capparis thonningii* Schum (Capparaceae) on acetic acid, formalin and hot plate induced pain tests[39] used dose levels of 50 and 100 mg/kg while evaluating antinociceptive activity of fieldgrowth plants and tissue culture of *Cleome spinosa* (Jacq.) in mice. However, in the evaluation of antinociceptive activities of extract and fractions of *Eugenia jambolana* root bark, [38] used a dose range of 100 to 400 mg/kg body weight.

The DCM: methanolic leaf extracts of *C. edulis* (Forssk.)Vahl, at the lower dose level of 50 mg/kg body weight was not as effective as the two higher doses (100 and 150 mg/kg body weight) in both phases. The percent licking inhibition by the leaf extracts at the dose level of 50 mg/kg body weight was -1.75% and 38.96% in early and late phases, respectively, while, at both dose levels of 100 and 150 mg/kg body weight, percent licking inhibition was 47.19% and 84.93% in early and late phases, respectively. These findings may be explained by the fast metabolism, clearance and inactivation of the lower concentration of the active principle.

The DCM: methanolic root bark extracts of *C. edulis* (Forssk.)Vahl, at the higher dose of 150 mg/kg body weight reduced the formalin-induced paw licking time not as effective as at the lower dose level of 50 mg/kg body weight. This may be due to the fact that the high dose takes longer to be absorbed across the peritoneum cavity.

The antinociceptive effect of DCM: methanolic leaf and root bark extracts of *C. edulis* (Forssk.)Vahl can be attributed to one or more groups of the phytoconstituents contained in the extracts. Several studies have shown the antinociceptive activity of such compounds. A study by[40] showed that saponins of *Carissa edulis* roots have potential analgesic properties[41] demonstrated that saponins in the methanolic stem bark extracts of *Ficus platyphylla* are involved in analgesic effects. Furthermore, saponins isolated from different plants such as *Bupleurum falcatum, Phytolacca americana* and M*adhuca longifolia* have been shown to have significant antinociceptive activities.[42,43]

Terpenoids present in the DCM: methanolic leaf and root bark extracts of *C. edulis* (Forssk.)Vahl could also be responsible for the antinociceptive activity. For instance, terpenoids have been attributed for the antinociceptive activities of *M. officinalis* extracts in laboratory animals.[44]

According to a study carried out by[45], evaluation of the antinociceptive profile of five cyclopeptide alkaloids isolated from *Ziziphus oxyphylla*, showed that alkaloids significantly attenuate painful sensation in both phases. In a similar study[46], reported that alkaloid extracts of *K. macrophylla* were shown to possess analgesic actions[35] also reported that the aqueous extracts of Radix *Aconiti Carmichaeli* exhibits antinociceptive activity effect probably due to the presence of high content of mesaconitine alkaloids. Therefore, alkaloids present in the DCM: methanolic leaf and root bark extracts of *C. edulis* (Forssk.)Vahl could also be responsible for the observed antinociceptive activity in rats.

The presence of flavonoids in the extracts could also be responsible for the antinociceptive activity. For example, according to[44], the anti-nociceptive effects of *M. officinalis* are attributed to the flavonoids present in the extracts. In addition, according to several studies, flavonoids are widely shown to target prostaglandins which are involved in the pain perception through moderating opioidergic mechanism.[29,47,30]

Conclusion

The present study has demonstrated the antipyretic and antinociceptive potential of DCM: methanolic leaf and root bark extracts of *Carissa edulis* (Forssk.)Vahl in animal models. The extracts were able to inhibit the pain sensation of both phases. It is, therefore, possible to find opioid analgesics as well as analgesics in *Carissa edulis* that act by inhibition of inflammatory pathways responsible for pain. Furthermore, the classes of phytochemicals in, DCM: methanolic leaf and root bark extracts of *Carissa edulis* (Forssk.)Vahl leaf and root bark extracts have previously been observed to contribute to antipyretic and antinociceptive activities. Therefore, the DCM: methanolic leaf and root bark extracts of *Carissa edulis* (Forssk.)Vahl can be studied further, in an effort to isolate the specific analgesic secondary metabolites in the extracts. This will further inform the efforts to elucidate more efficacious plant-derived analgesics.

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Conflict of interest

No conflict of interest among authors

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