Antipyretic Properties of Dichloromethane: Methanolic Leaf and Root Bark Extracts of Carissa edulis in Rats

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ABSTRACT :
Although pyrexia benefits the nonspecific immune response to invading microorganisms and tissue damage, it is also viewed as a source of discomfort and hence is commonly suppressed with some medication. However, conventional medications are expensive and arguably associated with various severe adverse side effects hence the need to develop herbal agents that are effective, cheaply available as alternative and complementary to conventional therapeutic drugs. Today herbal remedies are gaining popularity because the efficacy of conventional medicine is on the wane. Through ages *Carissa edulis* has been used as folklore remedy for various common ailments afflicting people in various parts of the world. However, although C. edulis (Forssk.)Vahl is widely used for pyrexia in the traditional medicine, an extensive search of the literature reveals no scientifically investigated report of its described effects. Thus, this study was to scientifically evaluate the ethnomedicinal importance of C. edulis (Forssk.)Vahl as an alternative and complementally intervention for the treatment of fever. The experimental groups were treated with leaf and root bark extracts at concentration of 50mg/kg, 100mg/kg and 150mg/kg. Antipyretic activities in rats were compared with aspirin (100mg/kg) as the standard conventional drug. The leaf extracts reduced the rectal temperature by between 0.02% - 2.98% while the root bark extracts reduced it by between 0.15% - 2.55%. Aspirin reduced the elevated rectal temperature by between 1.08% - 2.53%. Results indicate that both leaf and root bark extracts of C. edulis have profound antipyretic activities comparable to the conventional antipyretic drug, aspirin.

Keywords: Pyrexia, *Carissa edulis*, Rectal temperature, Leaf extracts, Root bark extracts.

INTRODUCTION:

Fever is a complex physiologic response triggered by infectious or aseptic stimuli, normally caused as a secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive1. The infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediators (cytokines like interleukin 1β, α, β and TNF-α), which increase the synthesis of prostaglandin E2 (PGE2) near preoptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature1.

Fever is a common medical sign reflected by an elevation of temperature. In human it means a temperature above the normal range of 36.5-37.5°C2. Fever occurs with the increase in the concentrations of prostaglandin E2 (PGE2) in the hypothalamus thus altering the firing rate of neurons that control thermoregulation in the hypothalamus3. Fever leads to the disturbance of metabolism including: increase in blood pressure, pulse rate, cardiac output, respiration rate among others hence the need to eliminate the fever using the antipyretic agents4.

As5 state, physicians have, for long, used various physical means to manage pyrexia. Conventionally, non-steroidal anti-inflammatory analgesics are used

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for the treatment of pyrexia. Further, opioids and anti-depressants are also used for the same purpose[6,7]. The principal action of antipyretics rests in their ability to inhibit the enzyme cyclooxygenase (COX) and interrupt the synthesis of inflammatory prostaglandins[7]. Recent studies on the mechanism of antipyretic action of these drugs, however, have revealed many severe side effects of the conventional drugs independent of COX inhibition as well[3].

The conventional drugs may have various and severe adverse 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 an alternative and complementally intervention to the chemical therapeutic agents[8]. 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. Search for herbal remedies with potent antipyretic activity received momentum recently as the available antipyretics, such as paracetamol, nimusulide among others, have toxic effect to the various organs of the body[3].

Carissa edulis is the best known member of the genus Carissa as it has been used as a traditional medicinal plant over thousands of years in the ayurvedic system of medicine as it is practiced on the Indian sub continent[9].

Like other terrestrial plants, *Carissa edulis* has ethno-pharmacological relevance and has also been exploited by the local people in the search for remedies for various ailments[10]. The plant was much more used in traditional as well as in modern era. The plant parts are used in ethnomedicine for wide variety of illnesses, such as epilepsy[11], headache, chest complaints, gonorrhea, syphilis, rheumatism, rabies and as well as a diuretic[12]. Other folkloric uses of *Carissa edulis* include fever, sickle cell anaemia and hernia[13].

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

Collection and preparation of plant materials

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 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 drying and crushing.

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, 200grams of powder was soaked 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 rotary evaporator. The concentrate was put in an airtight container and stored at -40C before use in bioassay studies.

Experimental design

Laboratory animals

This study tested for antipyretic activities using adult wister albino rats, *Rattus norvegicus*. The rats were of either sex, between 2-3 months old and weighing

Group	Status	Treatment
I	Normal control	None
II	Negative control	Turpentine (20%) + DMSO (10%)
III	Positive control	Turpentine (20%) +100 mg/kg Aspirin + DMSO (10%)
IV	Experimental group A	Turpentine (20%) +50 mg/kg extract + DMSO (10%)
V	Experimental group B	Turpentine (20%) +100 mg/kg extract + DMSO (10%)
VI	Experimental group C	Turpentine (20%) +150 mg/kg extract + DMSO (10%)

Table 1: Treatment protocol for evaluation of antipyretic activities

between 140-180gm14. The animals' breeding colony was acquired and bred in the animal breeding and experimentation facility of the Department of Biochemistry and Biotechnology, Kenyatta University. The animals were allowed to acclimatize for 48 hours before beginning the experiment. The animals were kept in the standard cages and maintained under the standard laboratory conditions of ambient temperature (250C) and with 12 hour day light. The experimental animals were fed on a standard rodent pellets and supplied with water ad libitum15. Ethical guidelines and procedures for handling experimental animals were followed.

Evaluation of antipyretic activities

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

A 20% turpentine solution was acquired from the local market and was used as the fever inducing agent. Before fever induction, the rats were weighed and their rectal temperature was taken. After the initial basal rectal temperatures were measured and recorded, the rats were injected with 20 ml/kg turpentine for pyrexia induction according to body weight and left for 1hour. Induction of fever took about 1 hour16,17. Only those whose temperature rose by 0.8°C were noted as pyretic and, therefore, were used for the studies. One hour later, the rats were treated with the vehicle, standard drug and various extract doses. The vehicle solvent (10% DMSO- 3ml) was given on the negative control group, turpentine only was given on the baseline group, the known antipyretic agent (aspirin solution- 3ml of 100mg/kg) was given on the positive control group and each sample solution (1ml) was given to the corresponding experimental group (Table 1). Plant extracts were prepared on the day of the experiment. All treatments were dissolved in DMSO solvent. Intraperitoneal route was used to turpentine, aspirin solution, solvent, and sample solutions.

The rectal temperatures of the rats were recorded by inserting a well lubricated thermistor probe of a digital thermometer about 3 cm17 in to the rectum. The digital thermometer was calibrated against a mercury thermometer. The mean body temperature measured at 15 minutes intervals over the 1 hour before injection of turpentine was calculated as the baseline/initial temperature. The mean magnitude of the fever which developed in response to intraperitoneal injection of 20mg/ kg body weight turpentine (20%) after one hour was defined as 100% fever response.

Temperature was recorded at hourly intervals up to fourth hour after turpentine injection. Rectal temperature before and after treatment was compared and the percentage change in rectal temperature was calculated by the following formula[18,19:]

$$(B-Cn/B)\times100$$

Where,

B - Rectal temperature at 1 hour after turpentine administration

Cn - Rectal temperature after drug administration

Qualitative phytochemical screening

The extracts obtained were subjected to qualitative phytochemical screening to identify presence or absence of selected chemical constituents using methods of analysis as described by20,21. Standard screening tests for detecting the presence of different chemical constituents were employed. Secondary metabolites tested for include were flavonoids, phenolics, saponins, alkaloids, cardiac glycosides, sterols and terpenoids.

Data management and statistical analysis

Experimental data on the rectal temperatures was obtained from all the animals in different groups, recorded and tabulated on a broad sheet using Ms Excel program. Analysis of the data was done using Minitab statistical software. The results were expressed as mean ± standard error of mean (SEM) for analysis. Statistical significance of difference among groups were 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.

RESULTS

Treatment of rats with DCM: methanolic leaf extracts of *C. edulis* showed some antipyretic activity against turpentine-induced pyrexia, which was indicated by reduction in rectal temperature (Table 2; Figure 1). In the first hour after treatment, the groups of rat treated with aspirin and DCM: methanolic leaf extracts of *C. edulis* at the dose levels of 50 and 100 mg/kg body weight lowered the elevated rectal temperature by 99.7% and 99.98%, respectively (Figure 1). However, the group treated with 150 mg/kg of extract showed no antipyretic activity (Table 2; Figure 1). Actually this high dose was pyretic and it increased the temperature by 100.41%. The anti- and pyretic activities recorded by the herbal extracts were statistically insignificant (p > 0.05; Table 2; Figure 1) when compared to baseline and negative control group, and this was indication that the values were similar.

In the second hour, all rats treated with the leaf extracts of *C. edulis* at doses of 50, 100 and 150 mg/kg recorded lower temperatures of 99.69%, 99.13% and 99.51%, respectively (Table 2). Although, the anti-

pyretic effectiveness of DCM: methanolic of *C. edulis* at the three dose levels was not significantly different from negative control groups (p > 0.05; Table 2), the extracts at dose levels of 100 and 150 mg/kg body weight was comparable to reference drug, aspirin (p > 0.05; Table 4.1).

In the third hour, *C. edulis* at all dose level (50, 100 and 150 mg/kg body weight) was found to lower the elevated rectal temperature by 1.02%, 1.19% and 2.12%, respectively (Table 2). At this hour, the rats treated with 150 mg/kg of the herbal extract exhibited the highest antipyretic effect (Figure 1; Table 2). The antipyretic properties of DCM: methanolic leaf extracts of *C. edulis* at the three dosages was comparable to aspirin (p > 0.05; Table 2), the effectiveness of leaf extracts at dose levels of 50 and 100 mg/kg body weight was not significantly different compared to the other control groups (p > 0.05; Table 2).

Four hours after treatment, *C. edulis* at all dose levels (50, 100 and 150 mg/kg body weight) was found to lower the turpentine-induced fever to 98.69%, 98.72% and 97.02%, respectively (Table 2). The antipyretic effectiveness of DCM: methanolic leaf extract of *C. edulis* at the three dose levels was comparable to reference drug (p > 0.05; Table 2). However, the group of rats treated with 150 mg/kg was even better than the other dose levels as well as aspirin for it lowered the activity by 2.98% (Figure 1).

Similarly, the DCM: methanolic root bark extracts of C. edulis showed antipyretic activity against turpentine-induced fever in rats (Table 3; Figure 2).

In the first hour of the test period, the DCM: methanolic root bark extracts of *C. edulis* at dose levels of 50, 100, and 150 mg/kg body weight reduced rectal temperature in rats to 99.66%, 99.88% and 99.53% respectively, while aspirin reduced rectal temperature

Group	Treatment	Percent change in rectal temperature (oC) after drug administration				
		0h	1h	2h	3h	4h
Baseline	None	100±0.00	100.02 ± 0.02[a]	100.02 ± 0.02[a]	100.02 ± 0.02[a]	100.02 ± 0.02[a]
Negative control	Turpentine + DMSO	100±0.00	100.03 ± 0.12[a]	99.88 ± 0.41[a]	99.910 ± 0.22[a]	100.12 ± 0.15[a]
Positive control	Turpentine + DMSO + Aspirin	100±0.00	98.920 ± 0.63[b]	98.38 ± 0.47[b]	98.250 ± 0.42[b]	97.920 ± 0.31[b]
Leaf extract	Turpentine + 50 mg/kg	100±0.00	99.700 ± 0.51[ab]	99.69 ± 0.78[a]	98.980 ± 1.40[ab]	98.690 ± 1.38[ab]
	Turpentine + 100 mg/kg	100±0.00	99.980 ± 0.23[ab]	99.13 ± 0.24[ab]	98.810 ± 0.31[ab]	98.720 ± 0.24[ab]
	Turpentine + 150 mg/kg	100±0.00	100.41 ± 1.25[a]	99.51 ± 1.38[ab]	97.880 ± 1.34[b]	97.020 ± 1.36[b]

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). Turpentine =20%; DMSO = 10%; Aspirin = 100 mg/kg.

Table 2: Effects of intraperitoneal administration of DCM: methanolic leaf extracts of *Carissa edulis* (Forssk.)Vahl on turpentine induced pyrexia in rats.
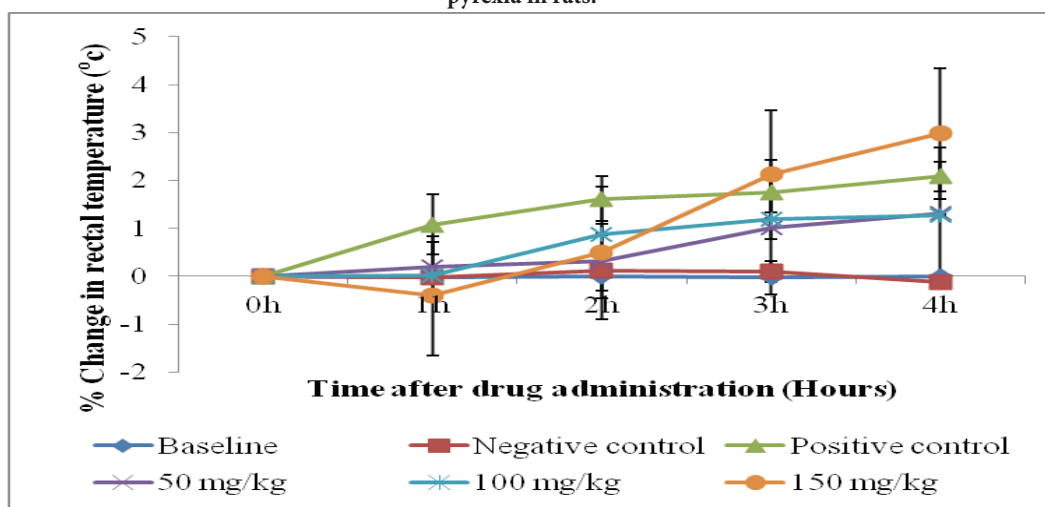
Figure 1: The percent change in rectal temperature by DCM: methanolic leaf extracts of *Carissa edulis* (Forssk.)Vahl in turpentine induced pyretic rats

to 98.33% (Table 3). However, the antipyretic activity of the DCM: methanolic root extracts of *C. edulis* at all the three dose levels (50, 100 and 150 mg/kg body weight) was not significantly different from each other and baseline (p > 0.05; Table 3). Further, the antipyretic effectiveness of the DCM: methanolic root extracts of C. edulis at all the three dose levels (50, 100 and 150 mg/kg body weight) was not comparable to reference drug (aspirin) (p < 0.05; Table 3).

In the second hour, pyrexia was reduced in a dose dependent manner and the trend was maintained up to the fourth hour of the test period (Table 3; Figure 2). The rectal temperature reduction by DCM: methanolic root bark extracts of C. edulis at the three dose levels (50, 100 and 150 mg/kg body weight) was by 0.53%, 0.59% and 0.64% respectively (Figure 2). However, although all the three dose levels of DCM: methanolic root bark extracts of C. edulis lowered elevated rectal temperature, their effectiveness was not comparable to the reference drug (aspirin) (p < 0.05; Table 3).

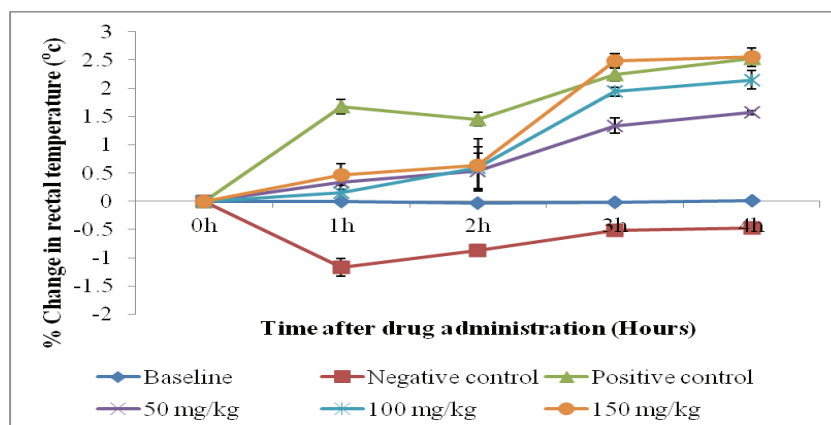In the third hour, DCM: methanolic root bark extracts of *C. edulis* at dose levels of 50, 100 and 150 mg/kg body weight lowered rectal temperature appreciably to 98.66%, 98.06% and 97.52% respectively (Table 3). The groups treated with DCM: methanolic root bark extracts of *C. edulis* at dose levels of 50, 100 and 150 mg/kg body weight demonstrated antipyretic activity significantly different from each other as well as baseline and negative controls (p < 0.05; Table 3). However, the DCM: methanolic root bark extracts of *C. edulis* at dose levels of 100 and 150 mg/kg body weight lowered the elevated rectal temperature as effective as reference drug (aspirin) (p > 0.05; Table 3). Further, the group of rats treated with DCM: methanolic leaf extracts of C. edulis at the dose level of 150 mg/kg body weight exhibited pyrexia lowering activity by 2.48%, which was even better than the other dose levels as well as aspirin (Figure 2).

In the fourth hour, pyrexia in rats was reduced in a dose dependent manner. DCM: methanolic root bark extracts of C. edulis at dose levels of 50, 100 and 150 mg/kg body weight lowered rectal temperature by 1.57%, 2.15% and 2.55% respectively (Figure 2). At this hour, the group treated with DCM: methanolic root bark extracts of *C. edulis* at the dose level of 150

Group	Treatment	Percent change in rectal temperature (ºC) after drug administration				
		0h	1h	2h	3h	4h
Baseline	None	100±0.00	100.02 ± 0.02[a]	100.03 ± 0.02[a]	100.03 ± 0.02[a]	100.02 ± 0.02[a]
Negative control	Turpentine + DMSO	100±0.00	101.17 ± 0.16[c]	100.87 ± 0.06[Ac]	100.52 ± 0.07[C]	100.48 ± 0.05[C]
Positive control	Turpentine + DMSO + Aspirin	100±0.00	98.330 ± 0.13[b]	98.050 ± 0.12[B]	97.750 ± 0.12[B]	97.470 ± 0.06[B]
DCM: Methanolic Extract	Turpentine + 50 mg/kg	100±0.00	99.660 ± 0.13[a]	99.470 ± 0.32[A]	98.660 ± 0.13[F]	98.430 ± 0.04[D]
	Turpentine + 100 mg/kg	100±0.00	99.880 ± 0.16[a]	99.410 ± 0.37[A]	98.060 ± 0.08[Bd]	97.850 ± 0.16[B]
	Turpentine + 150 mg/kg	100±0.00	99.530 ± 0.19[a]	99.360 ± 0.46[A]	97.520 ± 0.13[Be]	97.450 ± 0.16[B]

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). Turpentine =20%; DMSO = 10%; Aspirin = 100 mg/kg.

Table 3: Effects of intraperitoneal administration of DCM: methanolic root bark extracts of *Carissa edulis* (Forssk.) Vahl on turpentine induced pyrexia in rats.

Figure 2: The percent change in rectal temperature by DCM: methanolic root bark extracts of *Carissa edulis* (Forssk.) Vahl in turpentine induced pyretic rats.

mg/kg body weight exhibited the highest antipyretic effect (Figure 2; Table 3). Both the DCM: methanolic root bark extracts of *C. edulis* and reference drug (aspirin) lowered the turpentine-induced pyrexia significantly different compared to the baseline and negative control groups ($p < 0.05$; Table 3). However, the groups treated with DCM: methanolic root bark extracts of *C. edulis* at dose levels of 100 and 150 mg/kg body weight lowered the elevated rectal temperature not significantly different compared with reference drug (aspirin) ($p > 0.05$; Table 3).

In comparison, the DCM: methanolic root bark extracts of *C. edulis* exhibited more antipyretic effect than the leaf extracts at all dose levels in the first and third hours of the test period. However, in the second and fourth hours of the test period, the DCM: methanolic leaf extracts of *C. edulis* had the most efficacious antipyretic effect than the root bark extracts though only at the dose levels of 100 and 150 mg/kg body weight respectively (Figure 3).
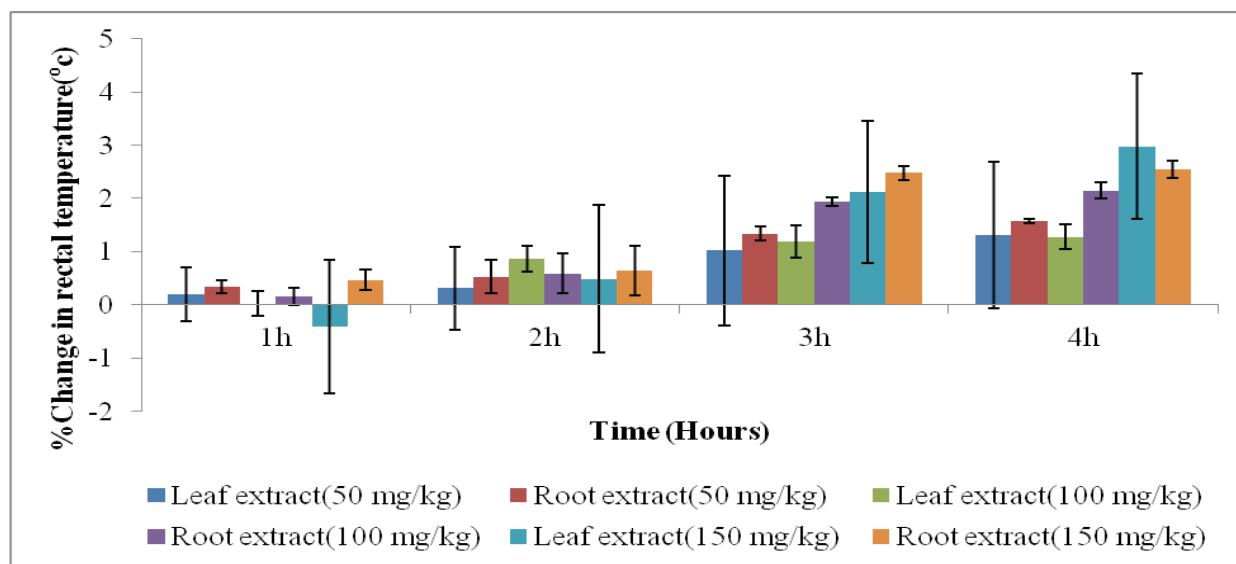

Figure 3: Comparison of percent change in rectal temperature by DCM: methanolic leaf and root bark extracts of Carissa edulis (Forssk.) Vahl at various hours of the test period.

Phytochemical screening

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

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

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

DISCUSSION:

The evaluation of antipyretic properties of the extracts was done using turpentine induced pyrexia tests in rats. Turpentine is a pyrogen which is widely used in the experimental studies of fever since the mechanism of turpentine-induced fever is well understood. Turpentine is refined from pinus palustris mill and is an agent that causes local inflammation with a robust acute phase fever, when administrated into experimental animals 22. In this study injection with turpentine (20 ml/kg body weight) induced pyrexia with a rise by 0.8ºC after 1hour [16,17].

Exogenous pyrogens like turpentine, lipopolysaccharides (LPS), amphetamines and sulphur induce fever by their ability to act on macrophages, monocytes and other immune cells to release proinflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor a (TNF-a), which act as endogenous pyrogens [23.] Turpentine induces fever through a serial pathway of its regulation such as IL-1 interacts with the IL-1 type I receptor 1 (IL-1r1) resulting in the production of IL-6 and induction of fever (Leon, 2002). Moreover, as [23] showed in their research report, it is less easy for experimental animals to acquire the tolerance to turpentine than to other pyrogens.

After four hours of the test period, the DCM: methanolic leaf and root bark extracts of *C. eduli*s produced appreciable antipyretic activity against turpentine induced fever in rats. The DCM: methanolic leaf and root bark extracts of C. edulis at dose level 150 mg/kg body weight demonstrated the greatest rectal temperature lowering activity by 2.98% and 2.55%, respectively, which compare well with aspirin that caused a reduction of between 1.08% to 2.53%. These findings were in agreement with the effects of other medicinal plants in laboratory animals. Similar work carried out by 24 demonstrated effective antipyretic activity of methanolic leaf extract of *P. kotschyi* on brewer's yeast- and amphetamine-induced pyrexia in rats. In addition, related results were also observed by [25] who demonstrated antipyretic activity against brewer's yeast-evoked hyperthermic mice intraperitoneally injected with crude methanolic extracts and various organic fractions of *Diaspyros lotus*.

The NSAIDs like aspirin are the drug of choice for the control of febrile conditions in routine practice. Over a period of time and after thorough investigations, it is believed that NSAIDs inhibit prostaglandins synthesis via blockage of enzyme cyclooxygenase pathway [26]. In general, non steroidal anti inflammatory drugs produce their antipyretic action, through inhibition of prostaglandin biosynthesis within the hypothalamus [27.] Therefore, it is possible that the antipyretic action of DCM: methanolic extracts of *C. edulis* (Forssk.)Vahl was related to the inhibition of prostaglandin synthesis in hypothalamus. However, other alternative mechanisms for blocking fever cannot be ruled out.

The DCM: methanolic root bark extracts of *C. edulis* demontrated a dose dependent response on rectal temperature lowering effect in turpentine-induced pyretic rats that was consistent with 28 who observed dose dependent antipyretic effects of *H. zeylanica* alkaloid extracts in yeast-induced hyperthermic rats. In another related study, 29 demonstrated a dose dependent antipyretic effect of the methanolic extract of *Capparis zeylanica* Linn in brewer's yeast- induced elevation of body temperature in experimental rats. This dose dependant antipyretic effect of DCM: methanolic leaf and root bark extracts of *C. edulis* could be explained by passive diffusion of the active principles across the cell membrane in the peritoneal cavity.

The dose ranges used in this study were within the dose ranges used by [24,25.] while examining the antipyretic effect ethanolic leaf extracts of Pseudocedrela kotschyi Schweint. Harms (*Meliaceae*), used dose levels of 50, 100 and 150 mg/kg body weights in rats. 25 used doses of 50 and 100 mg/kg body weight while evaluating the antipyretic activity of methanolic extracts and various solvent fractions of Diospyros lotus. It was observed that the leaf and root bark extracts of *C. edulis* at lower dose levels of 50 and 100 mg/kg body weight were not as effective as the higher dose of 150 mg/kg body weight, and thus may be explained by the fast metabolism, clearance and inactivation of the lower concentration of the active principles. The DCM: methanolic leaf and root bark extracts of *C. edulis* at all the dose levels, did not lower rectal temperature in the first and second hours as effectively as in the third and fourth hours. The highest antipyretic effect of the leaf extracts was by 1.30% and 0.87% in the first and second hours, respectively while 2.12% and 2.98% was observed in the third and fourth hours, respectively. Similarly, the highest antipyretic effect by root bark extracts was by 0.47% and 0.64% in the first and second hours, respectively while 2.48% and 2.55% was observed in the third and fourth hours, respectively. These findings could have been due to the fact that the active principles in the extracts required biotransformation so as to become antipyretic.

That the dose level of 150 mg/kg body weight of the DCM: methanolic root bark extracts of *C. edulis* was marginally effective than aspirin suggests a possibly better blockage of prostaglandins biosynthesis or mimicry of aspirin action by the active principles in the extracts. It is also possible that the herbal extracts

were efficiently inhibiting alternative mechanisms for blocking fever. The decline in rectal temperature in case of treatment with *C. edulis* extracts was not as sudden as that of aspirin administration. Therefore, the extracts offer some advantage over the standard drug (aspirin).

The antipyretic activity of the DCM: methanolic extracts of *C. edulis* (Forssk.)Vahl could be due to one or more groups of active principle(s) present in the extracts. Qualitative phytochemical screening in this study revealed that the DCM: methanolic extracts of *C. edulis* (Forssk.)Vahl contains steroids, saponins, phenolics, alkaloids, flavonoids and terpenoids. A number of these phytochemicals have been shown to exhibit inhibitory action on cyclooxygenase enzyme and, as a result, produce antipyretic activity by preventing the formation of prostaglandins or by increasing the concentration of body's own antipyretic components [30].

Flavonoids like baicalin have been shown to exert antipyretic effect by suppressing TNF- α [31] and its related compounds also exhibit inhibition of arachidonic acid peroxidation, which results in reduction of prostaglandin levels therefore reducing fever 32.Therefore, the presence of flavonoids in the DCM: methanolic extracts of *C. edulis* (Forssk.)Vahl may be contributory to its antipyretic activity. The presence of alkaloids in these extracts could also be responsible for the antipyretic activity. For instance, according to [28,] while evaluating on antipyretic effects of alkaloids extracted from the stem bark of *Hunteria zeylanica*, reported that alkaloids also possesses antipyretic effects.

The antipyretic activity of the DCM: methanolic extracts of *C. edulis* (Forssk.)Vahl may also be attributed to the presence of saponins, which are involved in inhibition of prostaglandin synthesis. According to the study of [33] saponins are suggested to act synergistically to exert antipyretic activity. In a related study, the antipyretic effect of ethanolic root extracts of Asparagus racemosus on yeast-induced hyperthermia in rats was attributed to the saponins in the extracts [34].

CONCLUSION

In conclusion, this study demonstrated the antipyretic potential of DCM: methanolic leaf and root bark extracts of *Carissa edulis* (Forssk.)Vahl in animal models. The significant reduction in pyrexia in rats when treated with standard drugs as well as different doses of extracts, reflect that *Carissa edulis* (Forssk.)Vahl is endowed with potent antipyretic properties. It is also evident from the study that the antipyretic activity of DCM: methanolic leaf and root bark extracts of *Carissa edulis* (Forssk.)Vahl at 150 mg/kg body weight is almost similar to the standard aspirin group. 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 antipyretic secondary metabolites in the extracts. This will further inform the efforts to elucidate more efficacious plant-derived antipyretics.

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