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RESEARCH ARTICLE

Bio efficacy of methanol leaf extracts of *Cissampelos pareira* Linn., *Lantana camara* Linn. and *Ocimum gratissimum* Linn. against fever in Wistar rats

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Objective: This study evaluated antipyretic potency of methanol extracts of *Cissampelos pareira*, *Lantana camara* and *Ocimum gratissimum* in Wistar rats.

Methods: The leaves samples were obtained from Embu County, Kenya. Quantitative phytochemical analysis of the extracts was conducted using LC-MS and GC-MS methods. The study was performed in eight sets each with 5 Wistar rats (n = 5): positive control, normal control, negative control as well as experimental. Group I (normal control) rats were injected intraperitoneally with a solution of Dimethyl sulfoxide (DMSO) only. Group II (negative control) constituted rats initiated with fever using turpentine alongside DMSO. Group III, (positive control) rats were induced with pyrexia and then administered with aspirin. Groups IV, V, VI, VII, and VIII composed of turpentine-induced pyretic rats that were intraperitoneally injected with various extracts dosages. The data was analysed using Statistical Package for Social Sciences (v25) software.

Results: The plant extracts possess antipyretic activities that are comparable to the standard therapy, aspirin. Quantitative phytochemistry showed that the extracts contained bioactive principles such as terpenoids, flavonoids, and alkaloids which exert antipyretic activity.

Conclusion: The studied plants are rich in secondary metabolites that have antipyretic efficacies in rats. Hence, this study validates the folkloric use of the tested plants to manage fever among the Mbeere community in Kenya.

Keywords: Pyrexia, *Cissampelos pareira, Lantana camara, Ocimum gratissimum*

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Introduction

Fever refers to a rise in body temperature occurring at the thermoregulatory set point above the normal range. The upper limits values for normal body temperature range between 37.5°C (99.5°F) and 38.3°C (100.9°F) [1]. An increase in set-point temperature initiates shivering and increased muscle contractions. This results in more heat generation as the body strives to conserve this heat. Thus, after the normal body temperature is restored, an individual becomes flushed, feels hot, and may begin to sweat [2].

Pyrogens are agents used to induce fever in animal models. They include steam-distilled turpentine, lipopolysaccharides, polyinosinic: polycytidylic acid, brewer's yeast, and muramyl dipeptide [3]. Also, endogenous pyrogenic cytokines, for example, interleukin-6, tumour necrosis factor-α, interferon-γ, and interleukin-1β**,** are applied to initiate fever in animal models [4].

Conventionally, nonsteroidal anti-Inflammatory drugs (NSAIDS), for instance, diclofenac, aspirin, and ibuprofen are used to manage fever. However, these drugs are linked with severe discomfort and side effects such as indigestion, nausea, bleeding from the stomach, bronchospasm and peptic ulcers [5], among others. Alternative approaches used to manage pyrexia include unclothing and use of cold water moisturized sponge to massage the patient. Traditionally, plant extracts and decoctions are administered to manage pyrexia [6].

Numerous medicinal plants are used widely to manage human ailments such as pain, fever, and inflammation. However, many herbal plants remain unvalidated for their therapeutic uses since, their uses are not empirically investigated. Furthermore, information on their potential side effects remains remarkably limited. Hence, it is difficult to identify the safest and effective remedies [7]. Some medicinal plants that have been scientifically validated to possess antipyretic potency include *Harrisonia abyssinica* and *Landolphia buchananii* [8], *Ximenia americana* [9] and *Senna didymobotrya* [6].

According to the information gathered from the traditional medical practitioner, in Embu County, some medicinal plants used to manage fever include extracts of *Solanun incanum, X. americana, O. gratissimum, C. pareira,* and *L. camara,* among others. Using a similar concept, this research was conceived with the aim of determining the antipyretic activities of *O. gratissimum, C. pareira,* and *L. camara* extracts to provide data on alternative sources of remedy for the management of fever.

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Materials and methods

Collection and preparation of plant samples

Fresh leaves of *C. pareira, L. camara* and *O. gratissimum* were collected from their natural habitat in Makungulu Village, Nthawa Location, Siakago Division, Mbeere North Sub-County, Embu County, Kenya. The GPS coordinates for the collection sites were 0°35'37"S, 37°38'11"E, 0°35'58"S, 37°34'45"E, and 0°35'38"S, 37°38'13"E for *C. pareira*, *L. camara,* and *O. gratissimum,* respectively. A local traditional medical practitioner assisted with folkloric information during the collection of plant samples. The plant samples were prepared by sorting and dusting before they were packed in khaki bags for transportation. The samples were transported to Kenyatta University, Department of Biochemistry, Microbiology and Biotechnology. The botanical identity of the plant samples was authenticated by an acknowledged taxonomist. In addition, voucher specimens of the plants were deposited at the Plant Science Departmental Herbarium of Kenyatta University for future reference. Specimen voucher numbers, SNK001, SNK002, and SNK003 were obtained for *C. pareira, L. camara,* and *O. gratissimum*, respectively. The plant sample materials were separately dried for three weeks at room temperature (25°C), away from direct sunlight. The dry leaves were separately ground into fine powder using an electric mill (Christy and Norris Model 8) and then stored at room temperature (25°C) in well labelled khaki bags awaiting extraction.

Extraction

Eight hundred grams of each powdered plant leaf sample (*C. pareira*, *L. camara,* and *O. gratissimum*) were separately soaked in two litres of analytical grade methanol (CAS number 6756-1; Sigma Aldrich; Germany), stirred for six hours, and then left for 48 hours in a well labelled and corked 2 litres conical flask, Borosil®. The obtained mixture was filtered using Whatman No.1 papers [10]. Each filtrate was concentrated under reduced pressure and controlled temperature of between 45 and 50°C using a rotary evaporator, Stuart RE400, Stuart, Germany [11]. The concentrates were then allowed to dry up by allowing methanol to evaporate. The plant extract concentrates were stored in pre-weighed, well labelled and airtight containers. Thereafter, they were refrigerated at 4°C awaiting use in bioassay studies. The formula as described by [12] was applied to calculate the percentage yields of the plant extracts.

Formula 1.

$$\textbf{percentaje yield} = W_2 - W_1/W_0 \times 100$$

Where:

W_2 is the weight of the extract and the container, W_1 the weight of the container alone and W_0 the weight of the initial dried sample.

Quantitative phytochemical analysis

Liquid Chromatography-Mass Spectrometry (LC-MS) analysis

Quantitative analysis of phytochemical composition of the studied plant extracts (*C. pareira*, *L. camara,* and *O. gratissimum*) was conducted at the International Centre of Insect Physiology and Ecology (ICIPE) laboratories, Kenya. The identification of phytochemicals was performed using Agilent MSD 6120-Triple Quadruple Liquid Chromatography-Mass Spectrometry (LC-MS) with electrospray source (Palo Alto, CA). LC-MS analysis system was controlled using Chemstation software (Hewlett-Packard). Reversed-phase liquid chromatography was carried out on an Agilent Technologies 1200 infinite series, Eclipse plus C_{18} column, 4.6 x 50 mm, 3.5 um (Agilent CA814112) using the following gradient program 0 min, 5 % B, 0 - 5 min, 5 - 50 % B; 5 - 10 min, 50 - 80 % B; 10 - 15 min, 80 - 100 % B; 15 - 25 min 100 % B; 25 - 30 min 5 % B; 30 - 35 % min 5 % B. The flow rate was held constant at 1 ml/min, the injection volume was 10.0 µl and data was acquired in a full-scan negative ion mode using a scan range of between 100 and 1500 *m/z*. The dwell time for each ion was 50 ms. Data acquisition and analysis were performed using Agilent LC- MS - QTOF Mass Hunter data acquisition software version B0.030.01.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the extracts was conducted at the Government Chemist, Nairobi, Kenya. This was performed by weighing 100 mg of the sample and adding 1 mL dichloromethane to it, vortexed for 10s, sonicated for 10 min, centrifuged at 14000 rpm for 5 min. The supernatant was filtered and diluted to make 100 ng/µL. The samples were analysed on a GC-MS (7890/5975 Agilent Technologies, Inc., Beijing, China) in full scan mode. The samples were prepared and analysed in 3 replicates. Specification-based gas chromatography column Agilent Technologies 5975c, a 7-inch cage GC column with a 15 m × 0.25 mm × 0.25 µm capacity, was utilized for the analyses. Flow rate (He): 1.25 ml/min, constant flow mode. Injection Mode: split mode. Oven temperature: 35°C (5 min.) to 280°C @10°C/min (10.5 min) then to 285°C @50°C/min (29.9 min); run time 70 min. A split flow rate of 60 mL/min was used to inject 1 µL into the autosampler injection volume. A 35 mL/min detector hydrogen flow, a 350 mL/min detector air flow, and a 30 mL/min detector nitrogen flow were used to operate the system. A computer-fed mass spectra data bank was cited as the source of the mass spectrum.

Undecane (standard) serial dilutions (1-100ng/L) were analysed using GC-MS in full scan mode to generate a linear calibration curve. The linear equation (y = 186096x + 1000000) was used to compute the concentration of each component in the sample. Undecane (standard) serial dilutions (1-100ng/L) were analysed using GC-MS in full

scan mode to generate a linear calibration curve. The linear equation was used to compute the concentration of each component in the sample. Compound identities were determined using NIST'11 and 14 mass spectral databases.

Laboratory animals

The present study used 240 Wistar rats of both sexes weighing 120-150 g and between 8-10 weeks of age. The rats were provided by Kenya Medical Research Institute (KEMRI) Nairobi, Kenya. The experiments only commenced after obtaining approval from the National Commission for Science, Technology, and Innovation (NACOSTI/P/20/5273). The rats were kept at Kenyatta University's Biochemistry, Microbiology, and Biotechnology Departmental animal breeding facility. The rats were allowed to acclimatize to the required laboratory conditions for one week prior to the test. Laboratory conditions were maintained at humidity (55±5%), within room temperature, and with exposure to light for 12 hours a day. The animals were acclimatized for seven days before the experiment [13].

Throughout this duration, the animals were nourished with water *ad libitum* and standard rodent pellet [14]. The animals had to fast for 12 hours before bioassays commenced. All the bioassays were done adhering to the internationally observed procedures and ethical guidelines for testing the potency as well as safety of herbal therapies in animals [15]. The study was accorded ethical approval by Egerton University Ethics Review Committee (EUREC/APP/101/2020).

Experimental design

The extracts were tested for antipyretic efficacy at doses of 50, 100, 150, 200, and 250 mg/kg body weight (bw). The doses were informed following literature review and pilot. The least effective dose in the pilot study was used as the least test dose in this study [16].

In this research, an experimental design was formulated from a completely randomized research design. Forty Wistar rats were randomly placed into eight groupings containing five rats each (n=5) for each herbal plant; 3 animals are recommended for animal studies, however, for reliability and reproducibility of the findings, five animals are recommended [17].

Group I (normal control) rats were injected intraperitoneally with a solution of 5% DMSO only. No fever was induced in this group. Group II, also referred to as the negative control group, constituted rats initiated with fever using 20% turpentine alongside 5% DMSO. Group III, also known as the positive control group, includes turpentine-induced pyretic rats that were administered with aspirin (100 mg/kg bw). Groups IV, V, VI, VII, and VIII were composed of turpentine-induced pyretic rats that were intraperitoneally injected with the extracts at dosages of 50, 100, 150, 200, and 250 mg/kg body weight individually.

The antipyretic properties bioassays of the studied methanol leaf extracts were conducted using turpentine-stimulated pyrexia in rat in reference to a methodology by Taran [18]. Before fever induction, a lubricated digital thermometer with a thermistor probe (model YB 009) was inserted approximately 3 centimetres [19] into the rats' rectum to collect the initial rectal temperature. A mercury thermometer was used to calibrate the digital thermometer. After the initial temperature was recorded, 0.01 ml of 20% turpentine was intraperitoneally injected to induce pyrexia.

The level of fever in reaction to intraperitoneal administration of turpentine was recorded as 100% pyrexia response. The rats whose temperature increased by 0.8^{o}C were categorized as pyretic and were therefore, utilized in the present study. The temperature was scored on an hourly basis. The experiment ran for four hours after administering the various treatments. Comparisons were done to the rats' rectal temperature in the 1st hour after administering turpentine as well as after the treatments. The formula described by Hukkeri et al. was used to calculate the percentage inhibition [20].

Formula 2.

$$\% \text{ fever inhibition} = \frac{B - C_n}{B} \times 100$$

Where:

B – It is the temperature of the rectum 1 hour after turpentine administration

C_n - Temperature of the rectum after treatment.

Statistical data analysis

The quantitative experimental results on rectal temperature collected from the test animals were scored and documented in MS Excel worksheets, after which they were entered in SPSS (Statistical Package for Social Sciences) Version 25 software. The Shapiro-Wilk test was employed to check the normality of the data before it was subjected to descriptive statistics. Inferential statistics were carried out with one-way analysis of variance (ANOVA) followed by Tukey's post hoc to separated and compare the means of different treatment groups. Repeated measures ANOVA was carried out, followed by Bonferroni post hoc test to analyse antipyretic activity across the four-hour treatment time for every group of treatment. Means a p values ≤0.05 were considered statistically significant. The statistics were presented in figures and tables.

Results

Percentage yield of the studied extracts

Ocimum gratissimum yielded a brown-black solid, whereas *C. pareira* and *L. camara* yielded a blue-black paste. The % yield for *O. gratissimum, C. pareira* and *L. camara* leaf extracts were 21, 23 and 24%, respectively.

Phytochemical profile of methanol extracts of *C. pareira, L. camara* and *O. gratissimum*

The GC-MS analysis of these extracts revealed the presence of phytocompounds such as cymene, limonene, α-pinene,

camphene, oleic, stearic, caprylic and lauric acid (Table 1). Besides, the LC-MS analysis revealed the presence of many phytoconstituents in the studied extracts. Examples of phytocompounds detected by LC-MS include cissamine (cyclanoline), quercetin and luteolin (Table 1).

In vivo antipyretic activity of methanol leaf extract of *C. pareira* on turpentine-induced pyrexia

The administration of turpentine substantially elevated rectal temperature of the rats in contrast to the normal control rats. The rectal temperature of rats in the turpentine control group was significantly higher than those of the extract-treated, aspirin-treated and normal control rats in the entire experiment ($p<0.05$). Notably, in the second and third hour all the extract doses exerted activities that were statistically below the effects of aspirin (the standard drug) ($p<0.05$; Table 2). The overall effectiveness of intraperitoneally administered *C. pareira* extract was found to be dose-dependent and time-dependent (Table 2).

In the first hour of the period of study, *C. pareira* extract administered intraperitoneally at the tested concentrations showed mean percentage change in rectal temperatures of 1.36, 1.31, 1.16, 0.37 and 0.16%, respectively (Table 2). The effectiveness of *C. pareira* 50, 100 and 150 mg/kg body weight test doses did not significantly vary from each other ($p>0.05$; Table 2). Further, it was noted that the mean percentage change in the rectal temperature of 200 and 250 mg/kg body weight dose levels did not significantly differ from each other ($p>0.05$; Table 2). Besides, the efficacy of 250 mg/kg bw test dose was comparable to the effects of aspirin ($p>0.05$; Table 2).

In the 2nd hour of the experiment, intraperitoneally administered *C. pareira* extract, at all the tested dosages caused mean percentage change in fever ranging from -0.21 to 1.99%, respectively (Table 2). The potencies of the five tested concentrations were less effective compared to what was exhibited by aspirin ($p<0.05$; Table 2). The 100 and 150 mg/kg bw dose extracts exhibited effects that

Table 1. Phytochemicals profiles of *C. pareira, L. camara* and *O. gratissimum*

Compound Class	Compound Name	Molecular Formula	Pareira (%)	Camara (%)	Gratissimum (%)
Terpenoids	α-pinene	$C_{10}H_{16}$	0.24	9.27	13.02
	Limonene	$C_{10}H_{16}$	0.23	2.15	1.94
	Cymene	$C_{10}H_{14}$	0.25	2.32	1.11
	Camphene	$C_{10}H_{16}$	0.26	8.61	1.22
	Carene	$C_{10}H_{16}$	0.25	3.31	-
	Longifolene	$C_{15}H_{24}$	-	1.99	1.99
Fatty Acids	Oleic acid	$C_{18}H_{36}O_2$	24.75	-	9.14
	Palmitic acid	$C_{17}H_{34}O_2$	22.72	-	3.6
	Stearic acid	$C_{18}H_{38}O_2$	15.15	-	-
	Linoleic acid	$C_{18}H_{34}O_2$	13.64	-	4.33
Alkaloids	Cissamine	$C_{20}H_{24}NO_4$	71.43	-	-
Flavonoids	Apigenin	$C_{15}H_{10}O_5$	5.74	2.1	1.9
	Quercetin	$C_{15}H_{10}O_7$	-	-	3.87
	Kaempferol	$C_{15}H_{10}O$	0.55	-	-
	Luteolin	$C_{15}H_{10}O_6$	2.83	-	7.74
	Salvigenin	$C_{18}H_{16}O_6$	-	-	1.42

Table 2. Antipyretic effect of intraperitoneally administered methanol leaf extract of *C. pareira* on turpentine-induced pyrexia in rats

Treatment	Mean percentage change in rectal temperature				
	0hr	1st hr	2nd hr	3rd hr	4th hr
5% DMSO	37.06±0.05^{bA} (0.00±0.00)^{A}	37.08±0.04^{fA} (0.05±0.05)^{cdA}	37.08±0.04^{gA} (0.06±0.16)^{dA}	37.00±0.03^{eA} (-0.16±0.11)^{dA}	37.00±0.03^{efA} (-0.16±0.16)^{cA}
Turpentine + 5% DMSO	38.06±0.05^{aA} (0.00±0.00)^{A}	38.88±0.02^{aB} (2.16±0.18)^{aB}	39.26±0.02^{aC} (3.15±0.12)^{aC}	39.50±0.00^{aD} (3.78±0.14)^{aD}	39.74±0.05^{aD} (4.41±0.13)^{aD}
Turp + Aspirin (100 mg/kg bw)	38.06±0.05^{aD} (0.00±0.00)^{D}	37.86±0.08^{eCD} (-0.53±0.12)^{dCD}	37.50±0.07^{fBC} (-1.47±0.16)^{eBC}	37.15±0.05^{eAB} (-2.31±00.05)^{fAB}	36.88±0.08^{fA} (-3.10±0.25)^{eA}
Turp + *C. pareira* (50 mg/kg bw)	38.16±0.05^{aA} (0.00±0.00)^{A}	38.68±0.02^{abB} (1.36±0.13)^{bB}	39.02±0.04^{bB} (1.99±0.14)^{bB}	38.75±0.05^{bB} (1.63±0.13)^{bB}	38.60±0.05^{bB} (1.15±0.20)^{bB}
Turp + *C. pareira* (100 mg/kg bw)	38.08±0.04^{aA} (0.00±0.00)^{A}	38.58±0.04^{bB} (1.31±0.15)^{bB}	38.56±0.02^{cB} (1.26±0.10)^{cB}	38.44±0.07^{cAB} (0.95±0.27)^{bcAB}	37.98±0.05^{cA} (-0.32±0.13)^{cA}
Turp + *C. pareira* (150 mg/kg bw)	38.02±0.02^{aB} (0.00±0.00)^{B}	38.46±0.02^{bcC} (1.16±00.06)^{bC}	38.48±0.04^{cdC} (1.21±0.14)^{cC}	38.30±0.07^{cBC} (0.74±0.23)^{cBC}	37.68±0.05^{dA} (-0.89±0.16)^{cdA}
Turp + *C. pareira* (200 mg/kg bw)	38.10±0.00^{aB} (0.00±0.00)^{B}	38.24±0.06^{cdB} (0.37±0.16)^{cB}	38.24±0.08^{deB} (0.37±0.21)^{dB}	37.98±0.05^{dAB} (-0.32±0.13)^{deAB}	37.60±0.07^{dA} (-1.31±0.19)^{dA}
Turp + *C. pareira* (250 mg/kg bw)	38.12±0.02^{aB} (0.00±0.00)^{B}	38.06±0.07^{deB} (-0.16±0.16)^{cdB}	38.04±0.08^{eB} (-0.21±0.21)^{dB}	37.76±0.07^{dAB} (-0.94±0.20)^{eAB}	37.22±0.07^{eA} (-2.31±0.24)^{eA}

Values are expressed as Mean ± SEM for n=5. Mean± SEM of percentage reduction in fever is shown in brackets. Means with different lowercase superscript letter down the column are statistically significant by one-way ANOVA accompanied by Tukey's post hoc test ($p<0.05$), while values with the different uppercase superscript letter across the row are significant statistically by repeated measures ANOVA followed by Bonferroni corrections. Turp = Turpentine.

did not vary significantly from each other (p>0.05). These effects (100 and 150 mg/kg bw) were considerably higher than those caused by 50 mg/kg bw dose extract (p<0.05) but statistically lower than what was elicited by the 200 and 250 mg/kg body weight dose extracts (p<0.05*;* Table 2).

In the third hour of the test period, the potency demonstrated by *C. pareira* dose extract was 1.63, 0.95, 0.74, -0.32 and -0.94%, in that order (Table 2). The test dose of 100 mg/kg bw produced mean percentage change in rectal temperatures that was statistically similar to the effects of the 50 and 150 mg/kg bw test doses (p>0.05*;* Table 2). However, the mean percentage change in rectal temperatures of the extract dose of 50 and 150 mg/kg bw were significantly different (p<0.05*;* Table 2). Additionally, the mean percentage change in rectal temperatures of the extract doses of 200 and 250 mg/kg bw were comparable (p>0.05*;* Table 2) and significantly higher than what was observed in the lower doses (p<0.05*;* Table 2).

In the fourth hour the effects of the intraperitoneally administered methanol leaf extract of *C. pareira*, at the five tested dosages exhibited mean percentage change in rectal temperatures of 1.15, -0.32, -0.89, -1.31, -2.31%, respectively (Table 2). It was observed that the mean percentage change in rectal temperatures of the 100 and 150 mg/kg bw test doses were comparable to each other (p>0.05*;* Table 2). Further, the effect of 250 mg/kg bw dose of extract was considerably higher than the effects evoked by the lower extract dosages (p<0.05); however, its effects were only comparable to those of aspirin (p>0.05*;* Table 2).

In vivo antipyretic activity of methanol leaf extract of *L. camara* on turpentine induced pyrexia

Overall, the potency of *L. camara* extracts administered intraperitoneally was found to be dose-dependent (Table 3). Notably, in the four hours of the test period the extract doses exerted effects that were substantially below the effects of the positive control drug aspirin (p<0.05; Table 3).

The mean percentage change in rectal temperatures of the intraperitoneally administered *L. camara* extract doses were 1.32, 0.84, 0.58, 0.47 and 0.32% in the first hour of the study period (Table 3). There was no significant variation among the tested extract dosages (p>0.05) except for the 50 mg/kg body weight dose of extract, whose effects were only statistically similar to those of 100 mg/kg bw dose of extract (p>0.05; Table 3). Further, the efficacies of 150, 200 and 250 mg/kg body weight dose extracts did not vary statistically compared to the sample control; which only compared to aspirin (p>0.05; Table 3).

In the 2nd hour of the experimental period, the mean percentage change in rectal temperature elicited by *L. camara* extract at the five dose levels were 1.52, 0.94, 0.63, 0.63 and 0.58%, respectively (Table 3). The mean percentage change in rectal temperatures of the higher test concentrations of 150, 200 and 250 mg/kg bw compared well (p>0.05; Table 3). Further, the antipyretic effects of the extract dose of 250 mg/kg bw were comparable to the effects of 5% DMSO (p>0.05). Similarly, the effects of the extract dose of 100 mg/kg bw were not significantly different from those produced by 50 mg/kg body weight dose of extract (p>0.05; Table 3).

In the third hour of the test period, the mean percentage change in rectal temperatures of methanol leaf extract of *L. camara* dose levels were 1.89, 0.84, 0.26, -0.63 and -0.68% respectively (Table 3). The mean percentage change in rectal temperatures of the extract concentrations of 100 and 150 mg/kg bw were not significantly different from each other (p>0.05); however, the effects of the extract dose of 150 mg/kg body weight were similar statistically to the normal control (p<0.05; Table 3). Additionally, potencies of the 200 and 250 mg/kg body weight dose levels were comparable (p>0.05) as well as the effects of 5% DMSO (p>0.05; Table 3).

In the 4th hour of the study period, the mean percentage change in rectal temperatures of the five *L. camara* extract dosages were 1.47, -0.37, -0.84, -1.10 and -1.31%, respec-

Table 3. Antipyretic effect of intraperitoneally administered methanol leaf extract of *L. camara* on turpentine-induce pyrexia in rats

Treatment	Mean percentage change in rectal temperature				
	0hr	1st hr	2nd hr	3rd hr	4th hr
5% DMSO	37.06±0.05^{bA} (0.00±0.00)^{A}	37.08±0.04^{eA} (0.05±00.05)^{deA}	37.08±0.04^{eA} (0.06±0.16)^{dA}	37.00±0.03^{eA} (-0.16±0.11)^{deA}	37.00±0.03^{eA} (-0.16±0.16)^{cA}
Turpentine + 5% DMSO	38.06±0.05^{aA} (0.00±0.00)^{A}	38.88±0.02^{aB} (2.16±0.18)^{aB}	39.26±0.02^{aC} (3.15±0.12)^{aC}	39.50±0.00^{aD} (3.78±0.14)^{aD}	39.74±0.05^{aD} (4.41±0.13)^{aD}
Turp + Aspirin (100 mg/kg bw)	38.06±0.05^{aD} (0.00±0.00)^{D}	37.86±0.08^{dCD} (-0.53±0.12)^{eCD}	37.50±0.07^{dBC} (-1.47±0.16)^{eBC}	37.18±0.06^{eAB} (-2.31±0.05)^{fAB}	36.88±0.08^{eA} (-3.10±0.25)^{fA}
Turp + *L. camara* (50 mg/kg bw)	38.06±0.05^{aA} (0.00±0.00)^{A}	38.64±0.04^{bB} (1.31±0.15)^{bB}	38.86±0.02^{bB} (1.52±0.18)^{bB}	38.94±0.04^{bB} (1.89±0.18)^{bB}	38.90±0.04^{bB} (1.47±0.13)^{bB}
Turp + *L. camara* (100 mg/kg bw)	38.00±0.06^{aA} (0.00±0.00)^{A}	38.54±0.02^{bB} (0.84±0.15)^{bcB}	38.70±0.03^{bC} (0.94±0.16)^{bcC}	38.76±0.04^{bcC} (0.84±0.23)^{cC}	38.64±0.07^{bBC} (-0.37±0.13)^{cdBC}
Turp + *L. camara* (150 mg/kg bw)	38.10±0.06^{aA} (0.00±0.00)^{A}	38.62±00.05^{bBC} (0.58±0.13)^{cdBC}	38.78±0.06^{bC} (0.63±0.11)^{cdC}	38.66±0.06^{cC} (0.26±0.14)^{cdC}	38.22±0.07^{cA} (-0.84±0.10)^{deA}
Turp + *L. camara* (200 mg/kg bw)	37.94±0.07^{aABC} (0.00±0.00)^{ABC}	38.32±00.06^{cC} (0.47±0.15)^{cdC}	38.14±0.05^{cBC} (0.63±0.13)^{cdBC}	38.02±0.05^{dAB} (-0.63±0.13)^{eAB}	37.76±0.05^{dA} (-1.10±0.10)^{eA}
Turp + *L. camara* (250 mg/kg bw)	38.10±0.05^{aB} (0.00±0.00)^{B}	38.48±0.02^{bcC} (0.31±00.05)^{cdC}	38.18±0.04^{cB} (0.58±0.10)^{cdB}	37.86±0.08^{dAB} (-0.68±0.13)^{eAB}	37.50±0.11^{dA} (-1.31±0.08)^{eA}

Values are expressed as Mean ± SEM for n=5. Mean± SEM of percentage reduction in fever is shown in brackets. Means with different lowercase superscript letter down the column are statistically significant by one-way ANOVA accompanied by Tukey's post hoc test (p<0.05), while values with the different uppercase superscript letter across the row are significant statistically by repeated measures ANOVA followed by Bonferroni corrections. Turp = Turpentine.

tively (Table 3). The potencies of 150, 200 and 250 mg/kg body weight dosages were comparable to each other; further, the effects of the extract dosage of 150 mg/kg bw were not significantly different from those of 100 mg/kg bw test dose (p>0.05) which was only statistically like the normal control (p>0.05; Table 3).

In vivo antipyretic activity of methanol leaf extract of *O. gratissimum* on turpentine-induced pyrexia

In general, the effectiveness of *O. gratissimum* methanol leaf extracts administered intraperitoneally was found to be dose-dependent except for the first hour of the test period (Table 4). Further, the lowest tested dose of 50 mg/kg bw elicited the least mean percentage change in fever after treatment with *O. gratissimum* extracts (Table 4).

In the first hour of the experiment, the mean percentage change in rectal temperature produced after administration of methanol leaf extract of *O. gratissimum* at all the tested dosages ranging from -0.84 to 0.63 % (Table 4). It was observed that the mean percentage change in rectal temperature of the dose extract of 150 mg/kg body weight was comparable to the activities elicited by 100 mg/kg bw test concentration (p>0.05) that was only comparable to the normal control (p>0.05; Table 4). Further, the pyrexia inhibitions of this extract dose (150 mg/kg bw) were statistically similar to those of the extract doses of 200 and 250 mg/kg bw as well as those of the standard drug (p>0.05; Table 4).

In the 2nd hour, the mean percentage change in rectal temperatures caused by *O. gratissimum* test doses were 1.21, -0.79, -0.95, -1.31 and -1.31%, respectively (Table 4). The fever inhibitions of 100, 150, 200 and 250 mg/kg body weight concentrations were statistically insignificant (p>0.05; Table 4), though significantly higher than the effects of the 50 mg/kg bw extract dose (p<0.05; Table 4). Further, the potencies of the 150, 200 and 250 mg/kg bw dose extracts did not significantly vary from the effects caused by the standard drug (p>0.05; Table 4).

In the 3rd hour of experimentation, the effectiveness of the five doses of *O. gratissimum* extract were 0.69, -1.63, -1.79, -2.20 and -2.47% respectively (Table 4). The fever inhibitions of 150 mg/kg bw dose of extract were similar statistically to the effects evoked by the extract doses of 100 and 200 mg/kg bw (p>0.05; Table 4); though the effects of the 200 mg/kg bw dose of extract were significantly different from those elicited by 100 mg/kg bw dose of extract (p<0.05; Table 4). Besides, the efficacies of the 200 and 250 mg/kg bw test doses were statistically similar (p>0.05) as well as comparable to the effects elicited by aspirin (p>0.05; Table 4).

In the fourth hour of the period of study, the potencies of *O. gratissimum* methanol extract at the five studied dose levels were -0.21, -2.21, -2.43, -2.89 and -2.89%, respectively (Table 4). It was also observed that the mean percentage change in rectal temperature 50 mg/kg bw test dose was only similar statistically to the normal control (p>0.05; Table 4). It was noted that the efficacies of the 100 and 150 mg/kg bw test doses were comparable to each other (p>0.05; Table 4).

Further, the effects of the 150 mg/kg body weight extract concentration did not vary significantly from those evoked by the 200 and 250 mg/kg body weight dose levels (p>0.05; Table 4). Notably, the extract doses (200 and 250 mg/kg body weight) elicited the greatest potencies of this extract (p<0.05); they also exerted effects that were statistically similar to those exerted by aspirin (p>0.05; Table 4).

Comparison of antipyretic activity of methanol leaf extract of *C. pareira, L. camara* and *O. gratissimum*

Interestingly, the intraperitoneally administered *O. gratissimum* methanol extracts, at the five tested concentrations showed pyrexia inhibitions that were considerably higher than those caused by *C. pareira* and *L. camara* extracts (p<0.05; Figure 1a, b, c, d and e); except, at the extract concentration of 50 mg/kg bw in the second hour along

Table 4. Antipyretic effect of intraperitoneally administered methanol leaf extract of *O. gratissimum* on turpentine-induced pyrexia in rats

Treatment	Mean percentage change in rectal temperature				
	0hr	1st hr	2nd hr	3rd hr	4th hr
5% DMSO	37.06±0.05^{bA} (0.00±0.00)^{A}	37.08±0.04^{dA} (0.05±00.05)^{cA}	37.08±0.04^{dA} (0.06±0.16)^{cA}	37.00±0.03^{eA} (-0.16±0.11)^{cA}	37.00±0.03^{cdA} (-0.16±0.16)^{bA}
Turp + 5% DMSO	38.06±0.05^{aA} (0.00±0.00)^{A}	38.88±0.02^{aB} (2.16±0.18)^{aB}	39.26±0.02^{aC} (3.15±0.12)^{aC}	39.50±0.00^{aD} (3.78±0.14)^{aD}	39.74±0.05^{aD} (4.41±0.13)^{aD}
Turp + Aspirin (100 mg/kg bw)	38.06±0.05^{aD} (0.00±0.00)^{D}	37.86±0.02^{cDC} (-0.53±0.12)^{deDC}	37.50±0.07^{cBC} (-1.47±0.16)^{eBC}	37.18±0.06^{dAB} (-2.31±00.05)^{fAB}	36.88±0.08^{dA} (-3.10±0.25)^{eA}
Turp + *O. gratissimum* (50 mg/kg bw)	37.96±0.07^{aAB} (0.00±0.00)^{AB}	38.20±0.08^{bAB} (0.63±0.11)^{bAB}	38.42±0.06^{cC} (1.21±0.07)^{bC}	38.22±0.04^{bB} (0.69±0.14)^{bB}	37.88±0.05^{bA} (-0.21±0.10)^{bA}
Turp + *O. gratissimum* (100 mg/kg bw)	38.02±0.02^{aC} (0.00±0.00)^{C}	37.92±0.04^{cBC} (-0.26±0.12)^{cdBC}	37.72±0.04^{cB} (-0.79±00.08)^{dB}	37.40±0.04^{cA} (-1.63±0.10)^{dA}	37.18±0.04^{cA} (-2.21±0.13)^{cA}
Turp + *O. gratissimum* (150 mg/kg bw)	37.94±0.02^{aC} (0.00±0.00)^{C}	37.74±0.05^{cBC} (-0.53±0.12)^{deBC}	37.58±0.04^{cB} (-0.95±0.11)^{deB}	37.26±0.02^{cdA} (-1.79±0.10)^{deA}	37.02±0.05^{cdA} (-2.43±0.10)^{cdA}
Turp + *O. gratissimum* (200 mg/kg bw)	38.12±0.02^{aD} (0.00±0.00)^{D}	37.80±0.03^{cC} (-0.84±0.10)^{eC}	37.62±0.07^{cC} (-1.31±0.19)^{deC}	37.28±0.04 ^{cdB} (-2.20±0.11)^{efB}	37.02±0.04^{cdA} (-2.89±0.08)^{deA}
Turp + *O. gratissimum* (250 mg/kg bw)	38.12±0.04^{aD} (0.00±0.00)^{D}	37.80±0.03^{cC} (-0.84±0.10)^{eC}	37.62±0.06^{cC} (-1.31±0.12)^{deC}	37.18±0.04^{dB} (-2.47±0.10)^{fB}	37.02±0.04^{cdA} (-2.89±0.14)^{deA}

Values are expressed as Mean ± SEM for n=5. Mean± SEM of percentage reduction in fever is shown in brackets. Means with different lowercase superscript letter down the column are statistically significant by one-way ANOVA accompanied by Tukey's post hoc test (p<0.05), while values with the different uppercase superscript letter across the row are significant statistically by repeated measures ANOVA followed by Bonferroni corrections. Turp = Turpentine.

with 250 mg/kg bw dose of extract in the fourth hour ($p>0.05$; Figure 1a and e).

Discussion

The present research was formulated to the determine antipyretic potential of *C. pareira*, *L. camara,* and *O. gratissimum* methanol leaf extracts in rats. The intraperitoneally administered plant extracts exhibited potent antipyretic effects, which were demonstrated by the reduction of elevated rectal temperature in rats at the third and fourth hour after treatment with higher extract doses (200 and 250 mg/kg body weight).

The rectal temperature in mice was seen to be increasing in the early hours of treatment (between zero and first hour), after administration of the studied methanol extracts. The observation could have been as a result of the effects exerted by turpentine that was used to induce fever. Turpentine is known to cause fever following successive sequences of its order, such as IL-1β, TNF-α, and IL-6 that are able to raise the temperature set point of an organism, thereby, causing fever [21]. These cytokines stimulate the production of COX-2, which initiates the hypothalamic release of prostaglandins, specifically prostaglandin E_2 (PGE_2). Prostaglandin E_2 induces the production of chemical transmitters such as cyclic adenosine monophosphate (c-AMP) and therefore, raises the body temperature [22]. This suggests that, as the effects of the crude extracts set in, the action of turpentine in the pyretic rats had not been cleared from the body system completely.

Furthermore, the crude extracts nature implied that the antipyretic phytocompounds delayed in their activity as opposed to the reference drug (aspirin). These may be ascribed to synergy among the active principles present in the studied plant extracts. According to [23] synergy and

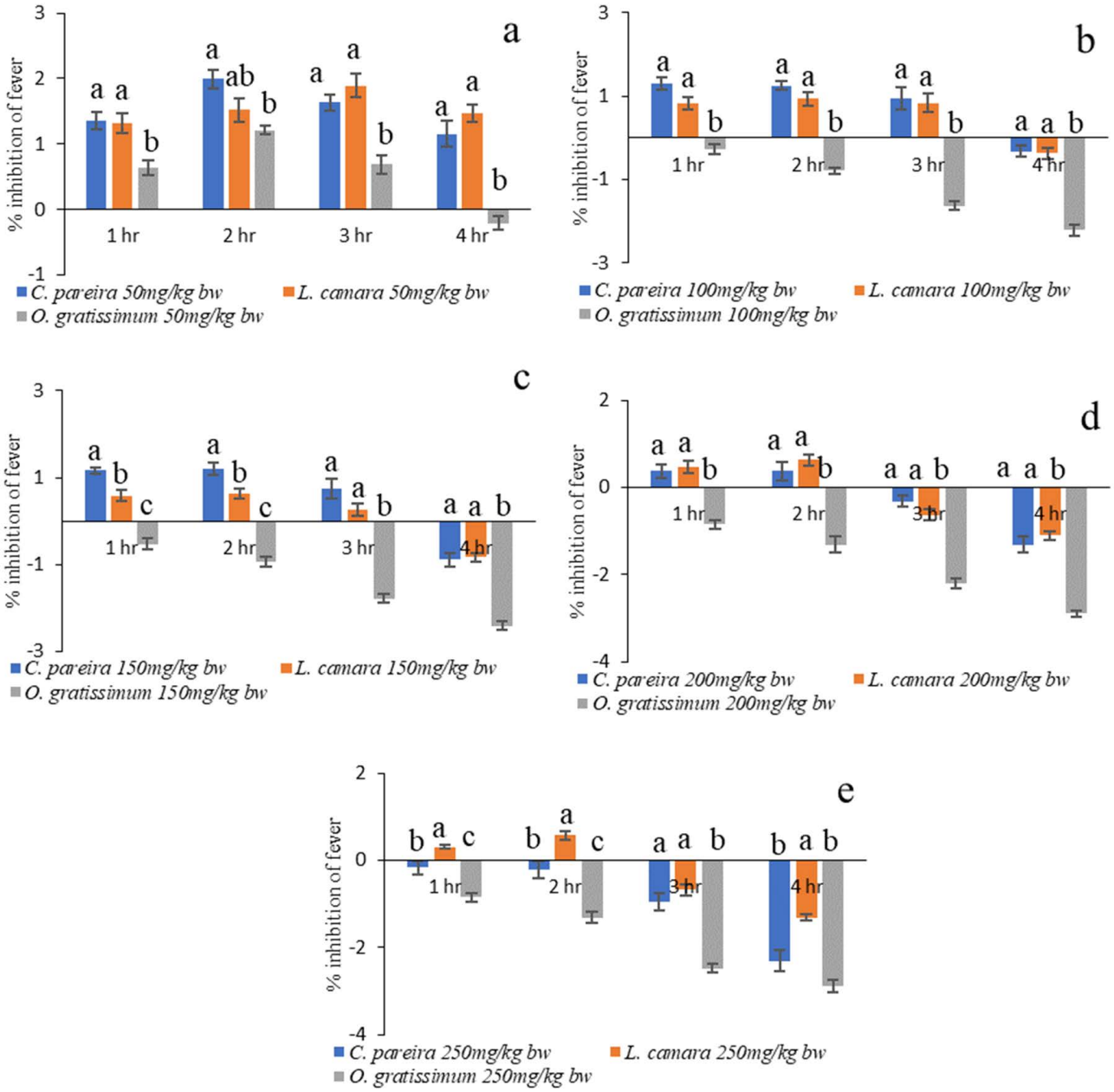

Fig. 1. Comparison of pyrexia inhibitions (%) of methanol leaf extracts of *C. pareira, L. camara* and *O. gratissimum* administered intraperitoneally. Values bearing same lowercase letter do not vary significantly ($p>0.05$).

positive interactions among various constituents of plant extracts have been reported where substances with little or no activity on the pathogen aid the major bioactive compounds to get to the target by ameliorating bioavailability, or by reducing metabolism and excretion.

In addition, the mean percentage change in rectal temperature induced by *O. gratissimum, L. camara* and *C. pareira* extracts was shown to be time-dependent. In the intraperitoneally administered extracts, the highest efficacy was achieved between the third and fourth hour of treatment. The high antipyretic effects observed in these hours (third and fourth) suggest that the bioactive molecules in the methanol extracts first underwent biotransformation before exerting a defined effect.

Biotransformation studies on terpenoids have led to the isolation of novel bioactive compounds. For instance, caryophyllene oxide showed a potent inhibitory activity against the butyryl cholinesterase enzyme and was found to be more potent than the parent caryophyllene oxide [24]. The lengthy time taken may have been essential for uptake of bioactive constituents contained in the methanol extracts. The slowed antipyretic activities may imply restrained passive diffusion of bioactive constituents past the cell membrane into the peritoneal cavity of the intraperitoneally administered extracts [25]. These results were consistent with the work performed by Mworia [6], while working on the antipyretic potential of dichloromethane leaf extracts of *Eucalyptus globulus* (Labill) and *Senna didymobotrya* (Fresenius) in rat models. Related outcomes were also reported by Subedi [26] while studying the antipyretic properties of dichloromethane: methanolic leaf and root bark extracts of *Carissa edulis* in rats.

That intraperitoneally administered *C. pareira* extract exhibited dose-dependent antipyretic effects. This implied that the highest tested dose generated the greatest antipyretic effects. The pronounced antipyretic effects at a higher test dose could have been caused by higher concentration of bioactive principles, which elicited increased antipyretic activities. Similar dose-dependent activities have been earlier reported by Subedi [26], while working on antipyretic effects of *Schoenoplectus grossus* in rat models. Furthermore, Alam [27] showed dose-dependent antipyretic activities of *Cymbopogon warancusa* ethanol extracts in rabbit models.

Inversely, *O. gratissimum* and *L. camara* extracts administered intraperitoneally demonstrated antipyretic effects that were dose-independent. These results correlated well with findings of other studies performed on plant extracts by Koech [28] while investigating the antipyretic effects of dichloromethane root extract of *Clutia abyssinica* observed antipyretic activities that were dose-independent in mice. In a related study, Veronica [29] while using mice model observed that the DCM stem bark extract of *Acacia mellifera,* at a lower dose, exerted greater effectiveness than a higher extract dose. This phenomenon can be explained in terms of certain dose limits. When certain dose limits are exceeded, the activity of the drug is reduced or is rendered inactive, probably due to saturation effects Koech [28].

The studied methanol leaf extracts at the least dosage (50 mg/kg bw) exerted the least antipyretic effects, suggesting that probably, at lower test doses, the bioactive compounds in the extracts might have been inactivated, metabolized or cleared at a faster rate. Further, the concentration of bioactive molecules at these dose levels, may have had pharmacological effects that were minimal [30].

Notably, the high antipyretic activities caused by the plant extracts in this study were comparable to those produced by the reference drug, aspirin. Aspirin has been reported to confer antipyretic effects by suppressing production of prostaglandins and thromboxanes via irreversible inactivation of the cyclooxygenase (COX) enzyme. It also acts by uncoupling oxidative phosphorylation in mitochondria and modulating signalling through NFκβ (nuclear factor kappa beta cells) pathway [31]. This means that the extracts may have similar modes of action to those of aspirin.

In the current study, the LC-MS and GC-MS profiles of *C. pareira, O. gratissimum* and *L. camara* methanol extracts showed that they contained phenols, terpenoids, fatty acids, alkaloids, and tannins among other compounds. It has been reported that numerous herbal plant species have demonstrated antipyretic activities, which have been ascribed to phytocompounds they contain [32]. It is, therefore, possible that the active principles identified in the plant extracts under this study may have mimicked mechanisms of action of aspirin to bring about the antipyretic activities observed in this study. An experimental study performed to determine the antipyretic properties of leaf extract of *Eucalyptus globulus* (DCM) in rat, showed phytochemicals comprising of borneol, **α-pinene,** and terpineol among others. The antipyretic effects observed in the *E. globulus* extract were attributed to the contained phytocompounds [6].

The antipyretic activity observed using *C. pareira*, *L. camara* and *O. gratissimum* extracts in rats could have been accorded by the phytochemical compounds revealed in these extracts. Some of the antipyretic phytocompounds identified in the studied plant extracts include limonene, cymene, eucalyptol, squalene, 3-carene, alpha and beta pinene, oleanolic acid, basilimoside, camphene, longifolene, quercetin, kaempferol, luteolin, apigenin, salvigenin, cissamine, caffeic and protocatechuic acid, as well as fatty acids, which included linoleic, oleic, palmitic, stearic, caprylic and lauric acids.

It has been reported that limonene decreases some key inflammatory mediators such as prostaglandin E_2 (PGE_2) as well as modulates pro-inflammatory cytokines. Further, it has been shown to decrease activation of nuclear factor kappa β (NFκβ) which controls downregulation of cytokines along with lowering the expression of inflammatory genes, such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). These mechanisms of action could have contributed to the antipyretic potential of limonene [33].

Studies conducted previously on cymene, have revealed that it considerably regulated interleukins (IL-6, IL-1β), and tumour necrosis factor-α (TNF-α) secretion in lipopolysaccharides (LPS) LPS-proinflammatory cytokine production (*in vitro* and *in vivo*). However, this terpenoid has been reported to increase IL-10 synthesis. Cymene regulates IL-1β, TNF-α and IL-6 production by inhibiting NFκβ signaling pathway [34]. Further, earlier experiments performed on squalene and eucalyptol reported that they also reduce pro-inflammatory cytokines by blocking the NFκβ pathway [35].

The terpenoids alpha and beta pinene have been reported to block IL-6 and TNF-α synthesis in a protocol using LPS-stimulated macrophages; further, they have been shown to considerably inhibit synthesis of PGE_2, COX-2 and iNOS. In addition, it has been reported that the activation of NFκβ is reduced by the action of alpha-pinene therapy [36]. Further, a previous study conducted on *Ocimum basilicum* revealed the presence of oleanolic acid and basilimoside as the bioactive compounds [37]. These phytocompounds were reported to possess anti-inflammatory activities by downregulating the NFκβ pathway [38]. It is possible that the three studied extracts used the NFκβ pathway to bring about the observed antipyretic activities.

Studies performed on other terpenoids like camphene, 3-carene and longifolene have also reported antipyretic activities in laboratory animals. A previous study conducted on essential oils of three *Pinus* species revealed camphene, 3-carene and longifolene as the main components in their phytochemical composition. The antipyretic effects of the *Pinus* species were alluded to inhibit IL-6 [39].

It has been shown that stearic, oleic, palmitic, linoleic, caprylic and lauric acids competitively block arachidonate metabolism through COX and 5-lipoxygenase (5-LO) pathways, thereby bringing the observed antipyretic effects in this study. In a related study performed to determine the antipyretic activities of *Linum usitatissimum* using animal models, the findings revealed presence of oleic, palmitic, stearic, linoleic, caprylic and lauric acids. The antipyretic action of *L. usitatissimum* was associated with the identified fatty acids [40].

It was reported that the mechanism of antipyretic effects was that lauric, palmitic, stearic, linoleic, caprylic and oleic acids increased their incorporation into phospholipids in place of arachidonic acid. As a result, they replaced arachidonic acid as a polymer for COX and 5- lipo-oxygenase (5-LO) that led to decreased production of leukotrienes, thromboxane and prostaglandins [41].

The antipyretic effects observed in the present study could also be associated with the presence of quercetin, kaempferol, luteolin and apigenin. A previous study performed on *Madhuca indica* reported that it has significant antipyretic effects, which were attributed to the presence of quercetin, kaempferol, luteolin and apigenin in the extract. The mechanism of action of *M. indica* was reported to be through inhibition of prostaglandin production, inhibition of nitric oxide synthase, lipo-oxygenase, phospholipase A_2 and C as well as COX-2 [42].

In addition, the phytocompound salvigenin has been reported to have inhibitory effects on cyclooxygenase, thereby preventing the formation of prostaglandins as well as inhibiting nitric oxide synthesis, thus suppressing nitric oxide (NO) production [43]. These mechanisms could have been utilized by these phytocompounds to confer the antipyretic effects observed in the current study.

Research performed by Abdoulahi [44] showed significant antipyretic effects of *X. americana* extract, which were associated with the presence of protocatechuic and caffeic acids. It was also reported that protocatechuic and caffeic acids exerted their effects by inhibiting cytokines, mainly TNF-α. It is, therefore, possible that this phytocompound used the same mechanisms to perform the antipyretic effects observed in the present study.

The antipyretic effects observed in the current study could also be associated with the presence of the alkaloid, cissamine. This alkaloid is known to be a predominant inhibitor of prostaglandin synthetase; this property is thought to confer its antipyretic effects. Further, a previous study performed to evaluate the phytochemical and biological effects of *C. pareira* identified cissamine as the bioactive compound. The antipyretic effects of *C. pareira* extracts were attributed to cissamine that was reported to act by suppressing production and expression of pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α) and inducible enzymes (iNOS and COX-2) [45].

The intraperitoneally administered extracts of *O. gratissimum* had the most pronounced antipyretic activities compared to the other two studied plant extracts. This efficacy could be as result of a higher concentration of bioactive compounds that exerted greater antipyretic activities at these extract concentrations. Coincidentally, this extract had the widest variety of flavonoids such as quercetin, apigenin, and luteolin among others, that have been shown to exert different modes of action that could have led to the observed effects.

It is, therefore, possible to attribute the antipyretic properties of *C. pareira, L. camara* and *O. gratissimum* extracts to specific compounds among the many that were revealed in this study. However, synergistic or additive effects as a result of combination and interaction between phytochemicals cannot be ignored [21]. In conclusion, the studied extracts showed appreciable antipyretic activities in rats. Therefore, the findings of this study evidently show that the methanol leaf extracts of *C. pareira, L. camara* and *O. gratissimum* can be used as bioresources for effective alternative or complementary plant-derived antipyretic agents.

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Authors' contributions

KSN, NMP and NEM conceptualized and designed the study. KSN and GSM executed the experiments and analysed the data. KSN and GSM were responsible for drafting the final manuscript. NMP and NEM supervised the study and reviewed the final manuscript. All authors read and approved the final manuscript prior to submission.

Conflict of interest

None to declare.

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