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Phytochemical screening and antipyretic activities of dichloromethane-methanolic leaf and stem bark extracts of *Ximenia americana* in rat models

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ABSTRACT

Introduction: In most parts of Africa, *Ximenia americana* is used in folklore to treat various disorders such as oedema, pain, fever, helminthiasis, diarrhoea, burns among other diseases. This study tested the antipyretic activities of dichloromethane-methanolic (DCM-MeOH) stem bark and leaf extracts of *X. americana* in rats. Qualitative phytochemical screening was also done to evaluate the presence of alkaloids, flavonoids, steroids, saponins, cardiac glycosides, phenolics and terpenoids in the extract.

Methods: The plant materials were collected from Mbeere North sub-county, Embu county, Kenya. Methanol and dichloromethane in the ratio of 1:1 was used to extract the active compounds. Two to three months old male Wister rats were employed for the antipyretic studies. Animals were divided into six groups of five rats each: normal, negative, reference and three experimental groups (50, 100 and 150 mg/kg body weight). Pyrexia was induced experimentally using turpentine. The experimental groups were treated with predetermined dose quantities of prepared extracts. Aspirin was used as the reference drug. Data were analyzed using one-way analysis of variance (ANOVA).

Results: The extracts from the leaves lowered rectal temperature by 0.45% to 2.11% while the stem bark extracts lowered rectal temperature in the range of 0.71% to 2.13%. Aspirin lowered the rectal temperature in the range of 0.74% and 1.67%. Qualitative phytochemical screening showed presence of alkaloids, flavonoids, saponins, cardiac glycosides, phenolics and terpenoids in the extract.

Conclusion: DCM-MeOH leaf and stem bark extracts of *X. americana* is effective in management of fever and therefore it can be explored as a possible bio-resource in the development of herbal antipyretic medicines.

Implication for health policy/practice/research/medical education:
This study confirms the antipyretic potential of the leaf and stem bark of DCM-MeOH extracts of *X. americana* in experimental animals. Therefore, the DCM-MeOH leaf and stem bark extracts of *X. americana* might prove useful in managing pyrexia and thus serve as an alternative treatment bioresource.

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Introduction

Conventional treatment of fever by synthetic drugs may cause side-effects and may not be universally affordable. Therefore, use of herbal medicines is more attractive in treatment because it is generally affordable, closely conforms to patient's ideology, arguably more tolerable than synthetic drugs and satisfies desire for more personalized healthcare (1).

Aqueous extracts of *Ximenia americana* have been traditionally used in fever management. However, there is no documented evidence on activities of organic extracts of *X. americana*. This study scientifically evaluated and provided preliminary information on organic leaf and stem bark extracts of *X. americana* as an alternative, arguably affordable and with less side effects in fever management. The study also tries to reveal the gaps for further research.

Fever is defined as elevation of the body temperature

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above the normal range due to an increase in temperature regulatory set-point (2). It is caused by prostaglandin E_2 which is synthesized from arachidonic acid by the action of cyclooxygenase (COX) enzymes (3). Fever is part of acute-phase response to inflammatory response or an infection (4). it is induced by a class of inflammatory mediators known as cytokines. Examples of such cytokines are interleukin-1, interleukin-6 and tumor necrosis factor-α (TNFα) (5).

Turpentine induces fever in laboratory animals by increasing prostaglandins synthesis (6). This fever is better tolerated by animals than that induced by other exogenous pyrogens. Hence, turpentine was selected to be used in current study as a pyrogen (7).

Since pyrexia is a product of prostaglandins of the E series activities (8), blockade of prostaglandin E_2 synthesis helps alleviate fever. The synthesis of prostaglandin E_2 occurs in three steps: synthesis of arachidonic acid from cell membrane phospholipids, a process mediated by phospholipase A_2; synthesis of prostaglandin H_2 from arachidonic acid, a step catalyzed by COX and the last step is isomerization of prostaglandin H_2 into prostaglandin E_2 by the action of terminal prostaglandin E synthase (9). COX enzymes exist in two isoforms. The first is constitutive isoform, referred to as COX-1. COX-1 has clear physiologic functions. The second is inducible isoform, referred to as COX-2 (10). The non-steroidal anti-inflammatory drugs exert anti-inflammatory activities through COX-2 inhibition. The inhibition of COX-1 causes side-effects, such as erosion of gastric mucosa (10). The side effects associated with such synthetic drugs can be avoided by use of reliable naturally occurring agents which are effective with minimal side effects (11).

Medicinal plants are either directly or indirectly used. They are directly used as home remedies or indirectly in development of conventional drugs (12). Traditionally, *X. americana* is used in the treatment of a variety of disorders such as: pain, fever and inflammation (13,14), treatment of helminthiasis (15), diarrhea, wounds (16), headaches, skin ulcers, kidney and heart problems (17). Mbeere community (a community living upper eastern Kenya) use pounded stem bark and raw leaves of *X. americana* to treat stomach discomforts, fever, oedema, and pain. Despite the broad folklore use of *X. americana*, there is no scientific evaluation of its organic extracts. The current study was designed against this background to specifically bioscreen dichloromethane-methanolic (DCM-MeOH) stem bark and leaf extracts of *X. americana* for antipyretic potential.

Materials and Methods

Collection and preparation of plant samples

Leaves and stem barks of *X. americana* were collected with the help of local traditional herbalists from Mbeere-North in Embu county, Kenya. The plant materials were properly sorted, cleaned and packed in polythene bags and transported to Biochemistry and Biotechnology laboratories of Kenyatta University for further processing. The botanical authentication of the materials was done by a qualified taxonomist and the voucher specimen deposited in Kenyatta University Herbarium. The plant materials chopped, completely air dried at room temperature followed by grinding into fine homogenous powder with an electric mill and then sieved through mesh sieve.

Extraction

For each sample, 200 g of the powder was soaked in cold 1:1 mixture of methanol and DCM and stirred for six hours to extract the active compounds. This was followed by successive filtering of the extracts and the filtrate concentrated under reduced pressure and vacuum using rotary evaporator (Buchii R110). The concentrate was stored in airtight containers at -4°C before use in the bioassay studies (18).

Laboratory animals

Male Wister rats, *Rattus norvegicus,* aged 2-3 months and weighing between 140-180 g were used (19). The animals were acquired and bred at the animal breeding and experimentation laboratory in Kenyatta University, Department of Biochemistry and Biotechnology. The animals were kept in standard cages, under the conditions of a standard laboratory (ambient temperature of 25°C and 12-hour light followed by 12 hour dark cycle) throughout the experiments. The feeding was on standard pellets for rodents and water was supplied *ad libitum* (20). Throughout the study, all ethical guidelines and procedures on animal handling were followed (21).

Determination of antipyretic activities

The experimental animals (30 male Wistar albino rats) were divided into 6 groups of five rats each and treated as shown in the table (Table 1).

The digital thermometer was calibrated against mercury thermometer. The rectal temperature was recorded by inserting about 3 cm of the thermistor probe of the thermometer (model YB-009) into the rectum. The probe was well lubricated (22). For fever induction, turpentine 20% at a dose of 20 mL/kg body weight was injected intraperitoneally after recording of the initial basal rectal temperature. The animals were left for one hour. The fever magnitude response after one hour of intraperitoneal turpentine injection was defined as 100% fever response. The animals that recorded 0.8°C raise in rectal temperature were con-

Table 1. Treatment protocol for evaluation of antipyretic activities of DCM-MeOH leaf and stem bark extracts *Ximenia americana* in rats

Group	Status	Treatment
I	Normal control	DMSO
II	Negative control	Turpentine (20%) + DMSO (10%)
III	Positive control	Turpentine (20%) +100 mg/kg aspirin
IV	Experimental group A	Turpentine (20%) +50 mg/kg extract
V	Experimental group B	Turpentine (20%) +100 mg/kg extract
VI	Experimental group C	Turpentine (20%) +150 mg/kg extract

DMSO was used as a vehicle.

sidered pyretic and were employed in the study.
The rectal temperature was recorded at an hourly interval for four hours. The calculation of rectal temperature percentage change was done by comparing temperature before and after treatment as per the formulae below (23,24):

$$\frac{B-Cn}{B}\times 100$$

B: Temperature of the rectum, 1 hour after turpentine administration
C_n: Rectal temperature after drug administration.

Qualitative phytochemical screening

Qualitative phytochemical screening was done on the extracts by established methods to find out whether selected phytochemicals were present (25,26). The secondary metabolites tested were: alkaloids, tannins, steroids, saponins, cardiac glycosides, phenolics and terpenoids.

Management and statistical analysis of the data

The data on rectal temperature changes were recorded and tabulated in a spread sheet. The data was then imported to Minitab statistical software version 17 for descriptive statistical analysis. The results expressed as mean ± standard error of mean for analysis. One-way analysis of variance (ANOVA) was performed to compare the group means followed by Tukey's post hoc test for pair-wise mean separations and comparisons to obtain the specific significant differences among the different groups. Un-paired student *t* test was used to compare mean antipyretic activities between leaf and stem bark extracts of *X. americana*.
The statistical significance was considered at $P \leq 0.05$. The data on the percentage change in rectal temperature was presented using graphs.

Results

Antipyretic activity of DCM-MeOH leaf extract of *Ximenia americana* in rats

The DCM-MeOH leaf extracts of *X. americana* reduced rectal temperatures of turpentine-induced pyrexia in rats (Table 2; Figure 1).
The leaf extract of *X. americana* at three dose levels (50, 100 and 150 mg/kg body weight), reduced the elevated rectal temperatures by 0.98%, 0.93% and 1.27%, respectively, after one hour of the treatment (Figure 1). At all dose levels, antipyretic activities of the extract were significant in comparison to the normal and negative control groups ($P < 0.05$; Table 2) but there was no significant difference when compared to the positive group ($P > 0.05$, Table 2).
The DCM-MeOH leaf extract of *X. americana* at three dose levels (50, 100 and 150 mg/kg body weight) caused a reduction in elevated rectal temperature by 1.37%, 1.56% and 1.76%, respectively after the second hour of the treatment (Figure 1). The antipyretic effectiveness of the extracts was statistically significant compared to the normal and negative control groups ($P < 0.05$; Table 2), although there was no statistical significance when compared to positive group ($P > 0.05$, Table 2).
The leaf extract of *X. americana* at the three dose levels (50, 100 and 150 mg/kg body weight) was found to lower the elevated temperature by 0.9%, 1.45% and 2.05%, respectively in the third hour (Figure 1). After three hours, the antipyretic activities of the rats treated with 100 and 150 mg/kg body weight of the extract was comparable to the reference group ($P > 0.05$; Table 2.). At all dose levels

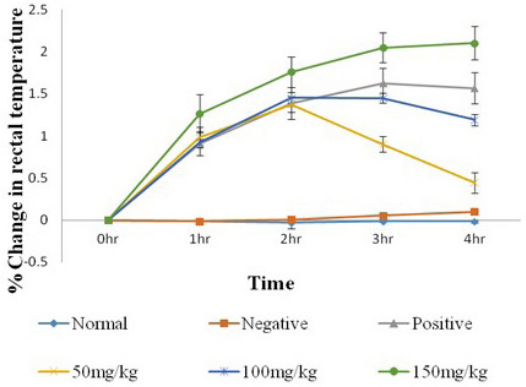

Figure 1. The change in percentage of rectal temperature caused by DCM-MeOH leaf extract of *Ximenia americana* on turpentine-induced pyretic rats

Table 2. Effects of intraperitoneal administration of DCM-MeOH leaf extract of *Ximenia americana* on turpentine induced pyrexia in rats

Group	Treatment	Percent change in rectal temperature (°C) after drug administration				
		0 h	1 h	2 h	3 h	4 h
Normal control	DMSO	100±0.00	100.01 ± 0.02^{a}	100.03 ± 0.07^{a}	100.02 ± 0.01^{a}	100.02 ± 0.01^{a}
Negative control	Turpentine	100±0.00	100.02 ± 0.02^{a}	99.995 ± 0.02^{a}	99.947 ± 0.01^{a}	99.900 ± 0.02^{a}
Positive control	Turpentine + aspirin (100 mg/kg b.w)	100±0.00	99.089 ± 0.14^{b}	98.610 ± 0.19^{b}	98.373 ± 0.18^{cd}	98.431 ± 0.19^{bc}
DCM: methanolic Leaf extract	Turpentine + 50 mg/kg	100±0.00	99.018 ± 0.12^{b}	98.626 ± 0.09^{b}	99.098 ± 0.09^{b}	99.554 ± 0.12^{a}
	Turpentine + 100 mg/kg	100±0.00	99.071 ± 0.04^{b}	98.544 ± 0.06^{b}	98.549 ± 0.06^{c}	98.808 ± 0.07^{b}
	Turpentine + 150 mg/kg	100±0.00	98.734 ± 0.23^{b}	98.239 ± 0.18^{b}	97.950 ± 0.18^{d}	97.892 ± 0.20^{c}

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 one-way ANOVA followed by Tukey's post hoc test ($P > 0.05$). Turpentine = 20%; DMSO = 10%;

of the extract, the antipyretic activities were significant in comparison to the normal group and the negative group ($P < 0.05$; Table 2).

The three dose levels of the leaf extract (50, 100 and 150 mg/kg body weight) were found to reduce pyrexia by 0.45%, 1.19% and 2.11%, respectively after the fourth hour (Figure 1). With the aspirin as standard drug, the antipyretic activity of the extract was comparable to the positive control at the dosages of 100 and 150 mg/kg body weight ($P > 0.05$; Table 2). There was significance in comparison to the negative group and normal group ($P < 0.05$; Table 2.). The dose level of 50 mg/kg body showed no significant difference from the negative group and the normal group ($P > 0.05$; Table 2).

Antipyretic activity of DCM-MeOH stem bark extract of *Ximenia americana* in rats

The DCM-MeOH stem bark extract of *X. americana* showed similar antipyretic activities (Table 3; Figure 2). The dichloromethane-MeOH stem bark extract of *X. americana* at three dose levels (50, 100 and 150 mg/kg body weight) decreased the elevated rectal temperature by 0.91%, 0.71% and 0.88%, respectively, after one hour of the treatment while aspirin reduced rectal temperature by 0.74% (Figure 2). At the three dose levels, the activity of the extracts was similar to that of aspirin ($P > 0.05$; Table 3). At the dose of 100 mg/kg body weight, the activity was not significantly different from the normal control group ($P > 0.05$; Table 3). This was as opposed to the dose levels of 50 and 150 mg/kg body weight of the extract that was significantly different from negative group and the normal group ($P < 0.05$; Table 3).

The dichloromethane-MeOH stem bark extract of *X. americana* at the dose levels of 50, 100 and 150 mg/kg body weight decreased the rats' elevated rectal temperature by 1.49%, 1.54% and 1.87%, respectively after the second hour of the treatment in a dose dependent manner (Figure 2). These effects were similar to that of aspirin ($P > 0.05$; Table 3).

Ximenia americana stem bark extract at the three dosages (50, 100 and 150) in mg/kg body weight, was found to reduce the elevated rectal temperature by 1.44%, 1.50% and 2.13% respectively in the third hour (Figure 2). The antipyretic activities at the three dose levels were not significant in comparison to positive group ($P > 0.05$; Table 3).

All the three dose levels (50, 100 and 150 mg/kg body weight), were found to reduce fever by 1.28%, 1.28% and 2.02%, respectively after the fourth hour (Figure 2). The antipyretic activities had no statistically significant difference from the positive group ($P > 0.05$; Table 3).

Comparison between the antipyretic activities of leaf and stem bark extract of *Ximenia americana*

In comparison, there was no significant difference in the antipyretic activities at the three dose levels (50, 100 and 150 mg/kg body weight), at the various test hour period of leaf and stem bark extract of *X. americana* (Figure 3). During the 4 hours of the test period, the level of significance was as follows: at the dosage of 50 mg/kg body weight ($P = 0.872$, 0.821, 0.307 and 0.176 respectively), at 100 mg/kg body weight ($P = 0.084$, 0.549, 0.769 and 0.501 respectively) and at 150 mg/kg body weight ($P = 0.205$, 0.608, 0.768 and 0.747 respectively).

Qualitative phytochemical screening

The qualitative phytochemical screening of DCM-MeOH leaf and stem bark extracts of *X. americana* showed the presence of alkaloids, cardiac glycosides, flavonoids, phenolics, saponins and terpenoids. However, alkaloids

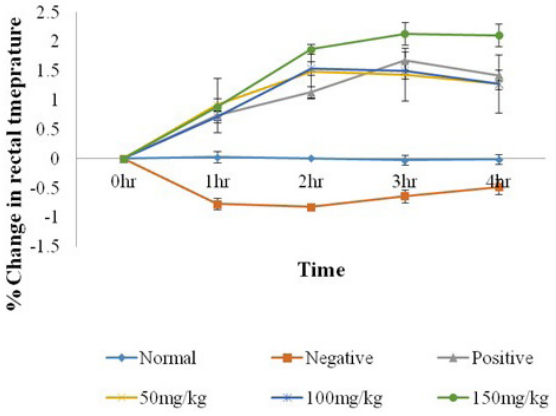

Figure 2. The change in percentage of rectal temperature caused by DCM-MeOH stem bark extract of *Ximenia* americana on turpentine-induced pyretic rats.

Table 3. Effects of intraperitoneal administration of DCM-MeOH stem bark extracts of *Ximenia americana* on turpentine-induced pyrexia in rats

Group	Treatment	Percent change in rectal temperature (°C) after drug administration				
		0 h	1 h	2 h	3 h	4 h
Normal control	DMSO	100±0.00	99.973 ± 0.09^{ab}	99.995 ± 0.01^{a}	100.02 ± 0.09^{a}	100.01 ± 0.09^{a}
Negative control	Turpentine + DMSO	100±0.00	100.77 ± 0.09^{a}	100.82 ± 0.06^{a}	100.64 ± 0.11^{a}	100.48 ± 0.13^{a}
Positive control	Turpentine + DMSO + aspirin	100±0.00	99.261 ± 0.08^{bc}	98.865 ± 0.09^{b}	98.329 ± 0.16^{b}	98.575 ± 0.09^{b}
DCM: Methanolic stem bark extracts	Turpentine + 50 mg/kg	100±0.00	99.088 ± 0.39^{c}	98.510± 0.47^{b}	98.562 ± 0.45^{b}	98.721 ± 0.49^{b}
	Turpentine + 100 mg/kg	100±0.00	99.288 ± 0.09^{bc}	98.459 ± 0.12^{b}	98.502 ± 0.14^{b}	98.723 ± 0.09^{b}
	Turpentine + 150 mg/kg	100±0.00	99.118 ± 0.15^{c}	98.131 ± 0.08^{b}	97.869 ± 0.19^{b}	97.984 ± 0.19^{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 one-way ANOVA followed by Tukey's post hoc test ($P > 0.05$). Turpentine = 20%; DMSO = 10%; Aspirin = 100 mg/kg.

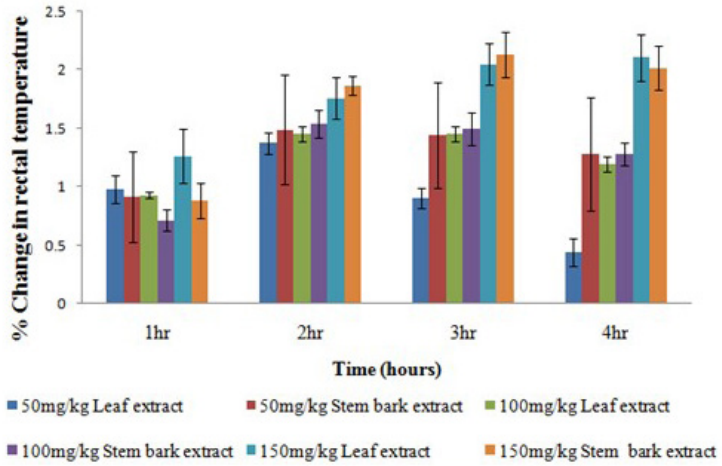

Figure 3. The percentage change comparison of rectal temperature caused by DCM-MeOH leaf and stem bark extracts of *Ximenia americana* on turpentine-induced pyrexia in rats.

and terpenoids were absent in the leaf extract of the *X. americana* while the steroids were absent in both leaf and stem bark extracts (Table 4).

Discussion

The present study was designed to evaluate the antipyretic activities of DCM-MeOH leaf and stem bark extracts of *X. americana* in rat models.

Pyrexia in rats was used to evaluate the antipyretic activity of steam distilled turpentine. Examples of other exogenous pyrogens which are used in fever induction in laboratory animals are: LPS, brewer's yeast, polyinosinic polycytidylic acid (poly I: C) and muramyl dipeptide (27,28).

Turpentine is injected intraperitoneally into the laboratory animals to induce fever. The turpentine-induced fever is through increase in prostaglandins synthesis (6). Different phytochemicals found in *X. americana* reduce fever by inhibition of these prostaglandins. Turpentine-induced fever is tolerated by the animals better than that induced by other exogenous pyrogens (7) and it is for this reason it was chosen for this study.

As shown in Tables 2 and 3, there was significant antipyretic activity in pyretic rats that was associated with administration of DCM-MeOH leaf and stem bark extracts of *X. americana*. The findings were consistent with other studies performed on some other herbal extracts in experimental animals. The aqueous stem bark extract of *X. americana* and its fractions showed the antipyretic properties (29). The study performed on *Solanum incanum* roots extract confirmed that there was antipyretic effect associated with the extract on pyrexia induced with lipopolysaccharide in male Wistar rats (30). Nonsteroidal anti-inflammatory drugs inhibit COX-2 expression and thus inhibiting PGE_2 biosynthesis leading to the reduction in elevated body temperature (31). The antipyretic activity of many antipyretic drugs is achieved through inhibition of COX-2 and the reduction of PGE_2 levels within the hypothalamus (32). Inhibitors of COX-2 are potent antipyretics and inhibit the transformation of arachidonic acid to PGE_2 (33). It is against this background that it was believed the extracts from *X. americana* used in this study conferred the antipyretic activities by inhibiting COX-2 that lead to reduced PGE_2 concentration.

The dose levels of 50, 100 and 150 mg/kg body weight were used in this study for the evaluation of antipyretic activity of *X. americana* in turpentine-induced pyrexia in rats. Similar dose levels were used to evaluate antipyretic activity of organic extracts of *Carissa edulis* in rats (34). In addition, the same dose levels were used in evaluating the antipyretic effect of *Pseudocedrela kotschyi* ethanolic leaf extract in rats (35).

Dose dependent antipyretic activities were observed in the DCM-MeOH leaf and stem bark extracts of *X. americana*. The dose of 150 mg/kg produced the highest antipyretic activity (Tables 2 and 3). These dose dependent results were similar to the work performed on methanol extracts of *Costus speciosus* which had dose dependent antipyretic activities in the laboratory animals (36).

The antipyretic activities of DCM-MeOH leaf and stem bark extracts of *X. americana* were most efficient at 150 mg/kg dose level and least at 50mg/kg dose level (Tables 2 and 3).

Aspirin, the standard drug, exhibited optimum antipyretic effect during the third hour (Tables 2 and 3). Its activity

Table 4. Qualitative phytochemical composition of DCM-MeOH leaf and stem bark extracts of *Ximenia* americana

Phytochemicals	*X. americana* leaf extract	*X. americana* stem bark extract
Alkaloids	-	+
Flavanoids	(+)	(+)
Steroids	-	-
Saponins	(+)	(+)
Cardiac glycosides	+	+
Phenolics	(+)	(+)
Terpernoids	-	+

Present phytochemical is denoted by (+) sign, absent phytochemical are denoted by (-) sign while + (trace) denotes slightly present phytochemical.

declined after the third hour which might be attributed to its metabolism and excretion. The maximum antipyretic effect of the *X. americana* DCM-MeOH leaf and stem bark extracts was observed in the third hour which may be attributed to the transport of the bioactive molecules across the plasma membrane by passive diffusion from peritoneal cavity (37). There was better antipyretic activity at the dose level of 150 mg/kg body weight for both extracts of *X. americana* than that of aspirin (Tables 2 and 3). These findings indicated that probably there is a better inhibition of prostaglandin synthesis by the extracts than by the reference drug (aspirin).

The presence of several phytochemical constituents in the DCM-MeOH leaf and stem bark extracts of *X. americana* might be attributed to the extracts' antipyretic activities. Qualitative phytochemical screening confirmed the presence of various phytochemicals such as: alkaloids, flavonoids, saponins, terpenoids, cardiac glycosides and phenolics, in the extracts. Several of these phytochemicals have been shown to exhibit antipyretic effects in the experimental animals (38). Alkaloids have been reported to inhibit prostaglandin synthesis (39). The antipyretic effect observed by the DCM-MeOH leaf and stem bark extracts was believed to be due to the presence of the several phytochemicals.

Conclusion

In conclusion, the present study confirms the antipyretic potential of the leaf and stem bark DCM-MeOH extracts of *X. americana* in experimental animals. The significant reduction in rectal temperature which was comparable to the reference drug, showed that these extracts are endowed with significant antipyretic activities. Therefore, the DCM-MeOH leaf and stem bark extracts of *X. americana* might prove useful in managing fever and thus serve as an alternative treatment bioresource, which is more effective than the conventional synthetic drugs.

The present study showed that DCM-MeOH leaf and stem bark extracts of *X. americana* contain a class of phytochemicals attributable to antipyretic properties. Therefore, the study scientifically confirms and supports the use of *X. americana* in traditional management of fever.

Authors' contributions

GDM prepared the manuscript and was involved in execution of the work. MMA took care of the experimental animals and followed ethical issues. GGM collection and processing of plant materials. NMP performed experimental design development and literature review. MND performed data analysis and discussion. All read and confirmed publication of the article.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

We confirm that our work is consistent with this Journal's guidelines and position on ethical publication issues.

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