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Antipyretic and anti-inflammatory effect of dichloromethane-methanolic extracts of *Ximenia americana* leaves and barks in rats and mice models

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Abstract. *Muthee GD, Ngugi M, Mburu D. 2018. Antipyretic and anti-inflammatory effect of dichloromethane-methanolic extracts of Ximenia americana leaves and barks in rats and mice models. Biofarmasi J Nat Prod Biochem 17: 86-96.* Extremely high body temperatures lead to the destruction of cells in the body, while excessive inflammation damages the tissues and organs of the body, requiring treatment of fever and inflammation. *Ximenia americana* L. is used in Africa as a traditional medicine to treat various ailments such as pain, helminthiasis, inflammation, fever, wounds, diarrhea, and poisoning. This study tested the antipyretic and anti-inflammatory activity of methanolic dichloromethane of *X. americana* leaf and stem bark extracts in rats and mice, respectively. The plant material was collected in Mbeere in Embu County, Kenya. The active ingredients were extracted with methanol and dichloromethane in a 1:1 ratio. Two- or three-month-old Wister rats were used to test antipyretic activities, while five- to six-week-old Swiss albino mice were used to test anti-inflammatory activities. The animals were divided into six groups of five each; negative control, reference control, normal control, and three experimental groups (50, 100, and 150 mg/kg). Pyrexia was experimentally induced with turpentine, while inflammation was induced with carrageenan. The experimental groups were treated with predetermined doses of prepared extracts, and one-way ANOVA analyzed the data. The antipyretic and anti-inflammatory effects in rats and mice were compared with aspirin (100 mg/kg) and diclofenac (15 mg/kg) as conventional standard drugs. Leaf extracts reduced rectal temperature from 0.45% to 2.11%, while stem bark extracts reduced rectal temperature from 0.71% to 2.13%. Aspirin reduced the rectal temperature by 0.74% to 1.67%. In anti-inflammatory studies, leaf extract reduced inflammation by 0.91% to 16.90%, while stem bark extract reduced inflammation by 5.84% to 29.00%. Diclofenac reduced inflammation from 1.32% to 29.60%. Qualitative phytochemical screening identified the presence of alkaloids, flavonoids, saponins, cardiac glycosides, phenols, and terpenoids. Flavonoids, alkaloids, and saponins have been associated with antipyretic and anti-inflammatory effects. The study found that *X. americana*'s DCM methanolic extract effectively treated fever and inflammation. In conclusion, taken together, *X. americana* can be explored as a potential biological source for generating a readily available herbal formulation effective in treating fever and inflammation.

Keywords: Antipyretic, anti-inflammatory, dichloromethane-methanolic extracts, *Ximenia americana*

INTRODUCTION

Fever also called pyrexia, or febrile reaction, is defined as a higher than normal body temperature due to an increase in the thermoregulatory set point (Axelrod and Diringer 2008). However, there is no agreed upper limit for the normal temperature at sources using values between 37.5 and 38.3°C (99.5 and 100.9°F) (Laupland 2009). Fever is generated from the release and conversion of arachidonic acid from cellular lipid membranes to prostaglandin E2 through the action of cyclooxygenase enzymes COX-1 and COX-2 (Purssell and While 2013).

Conversely, inflammation is part of the complex biological response of vascular tissue to noxious stimuli such as pathogens and damaged or irritating cells (Ferrero-Miliani et al. 2007). Inflammation is a protective immune-vascular response involving immune cells, blood vessels, and molecular mediators (Bairy et al. 2015). The classic signs of acute inflammation are pain, warmth, redness, swelling, and loss of function (Abbas and Lichtman 2009). Certain diseases, such as rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, retinitis, multiple sclerosis, psoriasis, and atherosclerosis, do not seem to improve. A chronic inflammatory disease may last a lifetime (Neville et al. 2004).

The main effect of antipyretics is their ability to inhibit the enzyme cyclooxygenase (COX) and disrupt the synthesis of inflammatory prostaglandins (Weissmann 1991). However, recent studies have revealed many serious side effects of conventional medications independent of COX inhibition (Biren and Avinash 2010). Non-steroidal anti-inflammatory drugs are used worldwide to treat inflammation (Narsinghani and Sharma 2014). Synthetic drug use typically causes many adverse effects (Parveen et al. 2014). Medicines of natural origin are an important source for the treatment of many diseases in the world (Pandima et al. 2003). As an alternative to chemotherapy, natural active ingredients with high efficacy and few side effects would be desirable (Rodrigo et al. 2013). Today, nearly 80% of the world's population uses herbal medicines for primary health care because of their efficacy, availability (Snyderman and Weil 2002), and minimal side effects. These plants have been used long ago as a source of antipyretic and anti-inflammatory effects, the discovery of antipyretic and anti-inflammatory chemicals has meant that they have long been ignored. Still, fortunately, many are returning to herbal remedies for various reasons. Graz and Malebo 2011).

Ximenia americana L. is used in different countries as an alternative medicine to treat various human diseases (Urso et al. 2013; Ekandjo et al.2018). In Burkina Faso, the fruit is used as a remedy for constipation and as a natural source of astringent and tonic substances (Lamien-Meda et al. 2008). Mali root treats skin diseases (Diallo et al. 2002; Grønhaug et al. 2008). In Zimbabwe, a decoction of leafy twigs is used to treat colds and coughs and as a laxative (Okigbo et al. 2009). Nigeria uses the same preparation as mouthwash to relieve toothache (Dalziel 1937). In Kenya, the communities such as Mbeere and Tharaka use the leaves, bark, and roots more diversely for human food, medicine, and animal feed (Feyssa et al. 2012).

However, in addition to the popular *X. americana*, Soro et al. (2015) data on the antipyretic and anti-inflammatory effects of aqueous plant extracts, there are no published data on the antipyretic and anti-inflammatory effects of the organic extracts of *X. americana*. In this context, the study aimed to bio-screen methanolic stem bark and leaf extracts of X DCM. Antipyretic and anti-inflammatory effects of *X. americana* in laboratory rats and mice are the first step in developing herbal antipyretic and anti-inflammatory drugs.

MATERIALS AND METHODS

Collection and preparation of plant materials

Stem bark and leaves of *X. americana* were collected from Siakago in Mbeere North Sub-district, Embu County, Kenya, with the help of local herbalists. Information collected includes common names, preparation methods, potency seasonality, and plant parts used. The materials were carefully selected, cleaned, and transported in plastic bags to Kenyatta University, Biochemistry and Biotechnology Laboratories, then dried and ground. Materials were collected using acceptable biological conservation methods. The plant material was provided to a registered taxonomist for botanical authentication, and a copy of the specimen was deposited in the Kenyatta University Herbarium, Nairobi, Kenya. The leaves and stem bark of *X. americana* were cut into small pieces and air-dried at room temperature until completely dry. They were then ground into a fine homogeneous powder using an electric grinder, followed by sieving through a mesh sieve.

Extraction

For each sample, 200 grams of leaf and stem bark powder were separately soaked in a cold 1: 1 mixture of methanol: DCM and stirred for six hours to extract the compounds. The sequential extract was filtered, and the filtrate was concentrated under reduced pressure and vacuum using a rotary evaporator (Buchi R110). The concentrate was placed in an airtight container for biological analysis studies and stored at -4°C.

Laboratory animals

Male and female Wister rats aged 2-3 months weighing 140-180 g and both sexes of Swiss albino mice aged 5-6 weeks weighing 15-35 g were used in the antipyretic test (Khan et al. 2013). Animal breeding colonies were obtained and housed in the breeding and testing facility of the Department of Biochemistry and Biotechnology, Kenya University. Animals were housed in cages and kept at room temperature. They were fed standard rodent pellets and watered ad libitum (Kirkham et al. 2002). Ethical guidelines and procedures for handling laboratory animals (Olfert et al. 1993) were followed throughout the study.

Experimental design

Determination of antipyretic activities

Thirty animals were used and divided into 6 groups of 5 rats, each treated as follows. Group, I (normal control) was normal and received DMSO. Another group was induced with fever (negative control) and administered DMSO. The third group was induced with fever (reference control) and received aspirin (reference drug). Finally, groups IV, V, and VI were experimental groups that induced inflammation and received test doses of 50, 100, and 150 mg/kg body weight, respectively (Table 1).

Turpentine solution (20%) was used as an antipyretic. Before fever induction, rats were weighed, and rectal temperature was recorded by inserting the thermistor probe of a well-lubricated digital thermometer (model YB-009). Three (3) cm in the rectum (Grover 1990). The digital thermometer is calibrated for a mercury thermometer. After baseline basal rectal temperature was measured and recorded, rats were injected with 20% turpentine at a dose of 20 mL/kg body weight to induce fever and left for 1 hour. The degree of fever induced by an intraperitoneal injection of turpentine 1 hour later was defined as a 100% febrile response. Therefore, only rats with a body temperature increase of 0.8°C were considered febrile and used in the study. All extracts are soluble in DMSO.

The temperature was recorded every hour until the fourth hour after the turpentine injection. Rectal temperatures before and after treatment were compared, and percentage changes in rectal temperature were calculated using the following formula (Hukkeri et al. 2006; Ray et al. 2006):

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

Where, B: Rectal temperature at 1 h after turpentine administration; $C_{n:}$ Rectal temperature after drug administration

Determination of anti-inflammatory activities

Those experimental animals were divided into six groups, with five mice per group, then treated as follows: Group I (normal control group) was normal and administered in DMSO. On the Group II (negative control) inflammation was induced and received DMSO. The third group (reference control) was induced with inflammation and received diclofenac (reference drug). Finally, groups IV, V, and VI are test groups that induce inflammation and receive 50 mg/kg body weight, 100 mg/kg body weight, and 150 mg/kg body weight, respectively (Table 2).

Paw inflammation was induced by injecting carrageenan (0.1 mL, 1% w/v in saline) into the subplantar tissue of the right hind paw (Winter et al. 1962). Plant extracts and DMSO were administered intraperitoneally one hour after induction of inflammation. Edema was measured immediately before carrageenan injection and compared with the volume of the same paw after carrageenan injection. Linear paw circumferences were measured at hourly intervals of 4 hours (Bamgbose and Noamesi 1981) and compared using the formula:

$$E_1 = \frac{t_1 - t_0}{t_0} X \, 100$$

Where: t_0: the initial paw circumference,t_1: the final paw circumference.

Qualitative phytochemical screening

A qualitative phytochemical screen was performed on the obtained extracts to identify the presence of selected phytochemicals using the protocols described by Harbone (1998) and Kotake (2000). In addition, the following secondary metabolites were tested: alkaloids, tannins, steroids, saponins, cardiac glycosides, phenols, and terpenoids.

Table 1. The treatment protocol to evaluate of antipyretic activities of DCM-Methanolic 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

Table 2. The treatment protocol to evaluate of anti-inflammatory activities of DCM-Methanolic leaf and stem bark extracts *Ximenia americana* in mice

Group	Status	Treatment
I	Normal control	DMSO
II	Negative control	Carrageenan 100 µg + DMSO (10%)
III	Positive control	Carrageenan 100 µg + 15 mg/kg diclofenac
IV	Experimental group A	Carrageenan 100 µg + 50 mg/kg extract
V	Experimental group B	Carrageenan 100 µg + 100 mg/kg extract
VI	Experimental group C	Carrageenan 100 µg + 150 mg/kg extract

Alkaloids

The extracts were subjected to qualitative phytochemical screening for the presence of selected phytochemicals using the protocol described by Harbone (1998) and Kotake (2000). In addition, the following secondary metabolites were examined: alkaloids, tannins, steroids, saponins, cardiac glycosides, phenols, and terpenoids.

Flavonoids (Sodium hydroxide test)

Test the extracts for flavonoids by mixing 2 mL of each extract with 2 mL of 5 M sodium hydroxide (NaOH). A strong golden yellow precipitate indicates a positive result.

Steroids

A sum of 0.5 g of each extract was dissolved in 2 mL of chloroform. Then, approximately 3 mL of 2M H_2SO_4 was gently added to the sides of the tube to form a layer. A reddish brown color on the skin indicates the presence of a steroid ring.

Saponins (Froth test)

In the saponins test, 2 mL of the plant extract was mixed with 2 mL sodium bicarbonate solution and shaken vigorously. The extract was then left to stand for 15-20 minutes. The formation of foam indicated the presence of saponins.

Cardiac glycosides (Keller-Kilian test)

To test cardiac glycosides, dissolve 0.5 g of the extract in 2 mL of glacial acetic acid containing 2 drops of 10% ferric chloride ($FeCl_3$). One (1) mL of concentrated sulfuric acid was added to it. A brown, purple, or green ring at the interface indicates the presence of a deoxysugar with a cardenolide character. A purple ring may appear below the brown ring, while a green ring may form in the acetate layer directly above the brown ring, gradually spreading through the layer.

Phenols

The extracts were tested for phenolics by adding 1 mL of ferric chloride solution to 2 mL of each extract. The formation of a blue to the green color indicated the presence of phenols.

Terpenoids (Salkowski test)

Add each 0.5 g of the extract to 1 mL of ethyl acetate/petroleum ether and mix with 2 mL of chloroform. Then carefully add 3 mL of 2M sulfuric acid (H_2SO_4) to form a layer. The interface is red-brown, indicating a positive result for terpenoids.

Data management and statistical analysis

Experimental data on changes in rectal temperature and right buttock circumference were obtained from all animals in different groups, recorded, and compiled in a spreadsheet. Descriptive statistics were performed on the results, expressed as mean ± standard error of analysis (SEM), and the hypothesis was tested at $p \leq 0.05$ to be considered significant.

Differences between groups were analyzed for statistical significance using one-way analysis of variance (ANOVA) followed by Tukey's test for pairwise separation and comparison of means. In addition, an unpaired Student's t-test was performed to compare the mean activity of leaf and root bark extracts. Analyze data using Minitab Statistical software version 17.

RESULTS AND DISCUSSION

The antipyretic activity of DCM-methanolic leaf and stem bark extracts of *Ximenia americana* in rats

The treatment with DCM-methanolic leaf extracts of *X. americana* on rats showed some antipyretic activity against turpentine fever, as evidenced by the reduction in rectal temperature (Table 3; Figure 1). In the first hour after treatment, *X. americana*'s DCM methanolic leaf extracts at doses of 50,100 and 150 mg/kg body weight elevated rectal temperatures by 0.98%, 0.93%, and 1.27%, respectively (Figure 1). In addition, the antipyretic activities of the extract at doses of 50, 100, and 150 mg/kg were statistically significant ($p<0.05$; Table 3) compared to those of the normal and negative control groups. However, the antipyretic effect of the extract was not statistically significant at all doses compared to the positive control group ($p<0.05$; Table 3).

In the second hour, the DCM-methanolic leaf extracts of *X. americana* at doses of 50, 100, and 150 mg/kg showed temperatures below 1.37%, 1.56%, and 1.76%, respectively (Figure 1). The antipyretic efficacy of the extracts was statistically significant ($p<0.05$; Table 3) compared to the normal and negative control groups but not statistically significant compared to the positive group ($p>0.05$; Table 3).

By the third hour, *X. americana* reduced the elevated rectal temperature by 0.9%, 1.45%, and 2.05% at all doses (50, 100, and 150 mg/kg body weight, respectively) (Figure 1). At this time, rats treated with doses of 100 and 150 mg/kg body weight of the extract showed antipyretic activity comparable to the positive control group ($p>0.05$; Table 3). In addition, the antipyretic activity of the extract was statistically significant ($p<0.05$; Table 3) at all doses (50, 100, and 150 mg/kg body weight) compared to normal and negative control groups.

After 4 hours of treatment, all doses of *X. americana* leaf extract (50, 100, and 150 mg/kg body weight) were found to reduce turpentine-induced fever by 0.45%, 1.19%, and 2.11%, respectively (Figure 1). The antipyretic activity of the extract at doses of 100 and 150 mg/kg body weight was comparable to the positive control with aspirin as a standard drug ($p>0.05$; Table 3) and was statistically significant compared to the normal and negative controls. ($p<0.05$) 0.05, Table 3). Moreover, the antipyretic effect of the extract at 50 mg/kg body weight was not significantly different from normal and negative control ($p>0.05$; Table 3).

Similarly, the DCM-methanol stem bark extract of B. americana had antipyretic activity against turpentine-induced fever in rats (Table 4; Figure 2). In the first hour of the study, *X. americana* DCM-methanol stem bark extract at doses of 50, 100, and 150 mg/kg body weight reduced rectal temperature in rats by 0.91%, 0.71%, and 0.88%, respectively. At the same time, aspirin reduced the rectal temperature by 0.74% (Figure 2). The activity of the extract at three dose levels (50, 100, and 150 mg/kg body weight) was comparable to that of aspirin ($p= 0.05$; Table 4). At a dose level of 100 mg/kg body weight, the activity of the extract was not significantly different from the normal control group ($p= 0.05$; Table 4). However, at 50 and 150 mg/kg body weight, the effect of the extract was significantly different from the normal and negative controls ($p<0.05$; Table 4).

In the second hour, methanolic extracts of stem bark of *X. americana* DCM at doses of 50, 100, and 150 mg/kg body weight reduced rectal temperature in rats by 1.49%, 1.54%, and 1.87%, respectively, depending on the dose. (Figure 2). The antipyretic efficacy of the extract was comparable to that of the positive control group at all three doses ($p>0.05$; Table 4; Figure 3).

In the third hour, *X. americana* stem bark extracts with methanolic DCM at 50, 100, and 150 mg/kg body weight significantly reduced the rectal temperature by 1.44%, 1.50%, and 2.13%, respectively (Figure 2). In addition, the groups treated with DCM-methanolic *X. americana* stem bark extract at doses of 50, 100, and 150 mg/kg body weight showed slightly different antipyretic activity than the positive control group ($p= 0,05$; Table 4).

During the fourth hour, stem bark extracts of DCM-methanolic *X. americana* at doses of 50, 100, and 150 mg/kg body weight reduced the rectal temperature by 1.28%, 1.28%, and 2.02%, respectively (Figure 2). Similarly, at this time, groups treated with DCM-methanolic *X. americana* stem bark extract at doses of 50, 100, and 150 mg/kg body weight showed slightly different antipyretic activity. Others, as well as the positive control group ($p>0.05$; Table 4).

Furthermore, there was no significant difference in the antipyretic effect at the three dosage levels of 50, 100, and 150 mg/kg body weight during the multi-hour test of the leaf and stem bark extract of *X. americana*. During the 4-hour experimental period, the level of significance was as follows: at a dose 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) and at 150 mg/kg body weight ($p= 0.205$, 0.608, 0.768, and 0.747, respectively).

Evaluation of the anti-inflammatory activity of DCM-Methanolic leaf andstem bark extracts in rats of *Ximenia americana*

The treatment of rats with DCM-methanolic leaf extract of *X. americana* at the 50,100 and 150 mg/kg body weight reduced carrageenan-induced inflammation in mice, which was observed by the reduction in the circumference of the hind leg.

In the first hour of the trial period, DCM-Methanol *X. americana* leaf extract at the three dose levels of 50,100 and 150 mg/kg body weight reduced the leg circumference by 0.91%, 28%, and 2.30%, respectively (Figure 4). At this time, the anti-inflammatory activity of the DCM methanolic extract of leaves of *X. americana* at the three doses was not significantly different from the positive control group (p>0.05; Table 5). However, due to the activity of the extract at the dose of 100 and 150 mg/kg body weight was significantly different compared to the normal and negative control groups (p <0.05; Table 5).

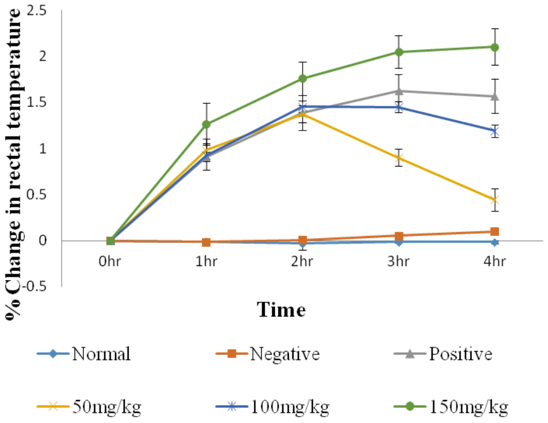

Figure 1. The percentages change in rectal temperature by DCM-Methanolic leaf extract of *X. americana* on turpentine-induced pyretic rats

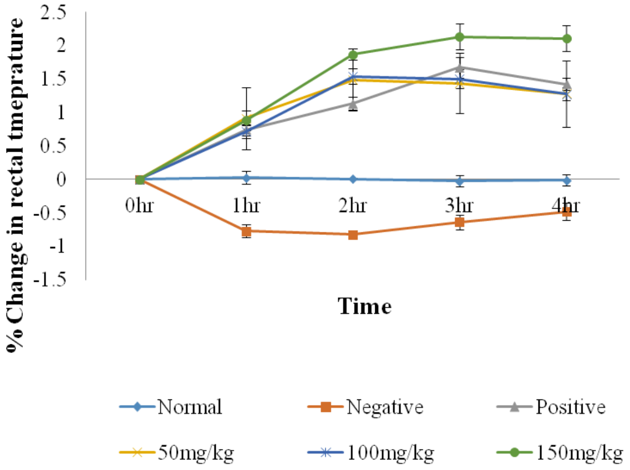

Figure 2. The percentages change in rectal temperature by DCM-Methanolic stem bark extract of *Ximenia americana* on turpentine-induced pyretic rats

Table 3. The effects of intraperitoneal administration of DCM-Methanolic leaf extracts of *X. americana* onturpentine-induced pyrexia in rats

Group	**Treatment**	**Percent change in rectal temperature (°C) after drug administration**				
		0h	**1h**	**2h**	**3h**	**4h**
Normal control	DMSO (10%)	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 (100mg/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: MethanolicLeaf extracts	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}

Note: Values are expressed as Mean ± SEM for five animals per group. Statistical comparisons were made within a column, and values with the same superscript were not significantly different by one-way ANOVA followed by Tukey‘s post hoc test ($p > 0.05$). Turpentine =20%; DMSO = 10%

Table 4. Effects of intraperitoneal administration of stem bark extracts of DCM-methanolic *X. americana* onturpentine-induced pyrexia in rats

Group	**Treatment**	**Percent change in rectal temperature (°C) after drug administration**				
		0h	**1h**	**2h**	**3h**	**4h**
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}

Note: Values are expressed as Mean ± SEM for five animals per group. According to a one-way ANOVA with Tukey’s post hoc test, values with the same superscript are not significantly different within a column (p>0.05). Turpentine =20%; DMSO = 10%; Aspirin = 100 mg/kg

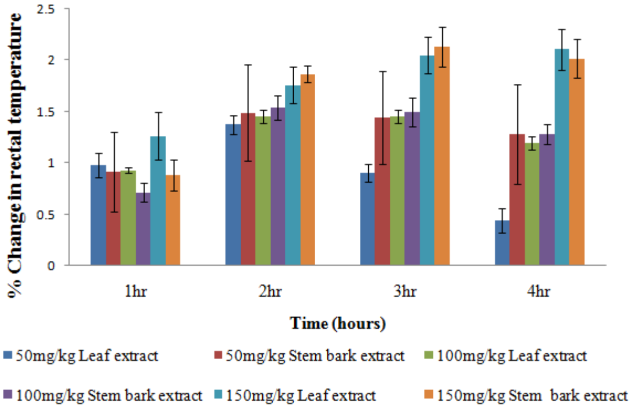

Figure 3. Comparison of change in rectal temperature of DCM-Methanolic leaf and stem bark extracts of *X. americana* on turpentine-induced pyrexia in rats

In the second hour after treatment, *X. americana* leaf extract DCM-methanol reduced paw circumference by 8.47%, 11.62%, and 7.85% at all doses (50, 100, and 150 mg/kg body weight), respectively. At this time, the anti-inflammatory activities of *X. americana* DCM methanolic leaf extract at the three dose levels were significantly different compared to the normal and negative control groups (p<0.05; Table 5). However, within this hour, the anti-inflammatory activity of the extract at doses of 50 and 150 mg/kg body weight was comparable to that of the positive control (p>0.05; Table 5). In the third hour after treatments, *X. americana*'s DCM methanolic leaf extract at doses of 50,100 and 150 mg/kg body weight reduced the paw circumference of the mice by 14.54%, 15.04%, and 16.90% (Figure 4). However, the anti-inflammatory activities of the DCM methanolic leaf extract of *X. americana* at all three dose levels differed significantly from the normal and negative control groups (p<0.05; Table 4), they were comparable to diclofenac (p>0.05, Table 5).

In the fourth hour of the trial period, *X. americana* at doses of 50, 100, and 150 mg/kg body weight reduced paw circumference in mice by 2.61%, 15.94%, and 9.01%, respectively (Figure 4). The activity of the extract at the three doses was significantly different compared to the normal and negative control groups (p<0.05; Table 4), but there was no significant difference between the positive control group and the dose of 100 mg/kg body weight (p>0.05, Table 5). Similarly, the DCM-methanolic stem bark extract of *X. americana* showed anti-inflammatory activity against carrageenan-induced inflammation in mice (Table 6; Figure 5). The reduction in leg circumference indicated this.

In the first hour after treatment, *X. americana* stem bark extract at the three dose levels of 50, 100 and 150 mg/kg body weight reduced the circumference of the inflamed hind limb by 5.848%, 9.174%, and 10.27% (Figure 5; Table 6). The anti-inflammatory activity of the extract at the three doses showed no significant difference compared to diclofenac (reference drug) (p>0.05; Table 6). However, the anti-inflammatory activity of the extract at the three doses of 50, 100, and 150 mg/kg body weight was significantly different compared to the negative control (p<0.05; Table 6).

During the second hour after treatment, *X. americana* extract at doses of 50, 100, and 150 mg/kg body weight reduced inflamed paw circumference in mice by 13.72%, 19 .17%, and 20.33%, respectively (Figure 5; Table 6). The anti-inflammatory effects of *X. americana* at the three doses were not significantly different and were comparable to diclofenac (p>0.05; Table 6). However, the anti-inflammatory activity of the extracts at the three doses (50, 100, and 150 mg/kg body weight) was significantly different compared to the normal and negative control groups (p<0.05; Table 6).

In the third hour after treatment, the extract at doses (50,100 and 150 mg/kg body weight) reduced the circumference of the inflamed hind limb in mice by 23.18%, 23.91%, and 25.97%, respectively (Figure 5). The anti-inflammatory activity of the extract at the three dose levels was not significantly different from each other nor the positive control (p>0.05; Table 6). However, the anti-inflammatory activity of the extract at the three dose levels was significantly different compared to the normal and negative control groups (p<0.05; Table 6; Figure 5).

Table 5. Effects of DCM-Methanolic leaf extract of *Ximenia americana* on carrageenan-inducedinflammation in mice

Group	**Treatment**	**Percent change in paw circumference after drug administration**				
		0h	**1h**	**2h**	**3h**	**4h**
Normal control	DMSO	100±0.00	99.997 ± 0.16^{a}	100.01 ± 0.15^{a}	99.921 ± 0.18^{a}	100.10 ± 0.10^{a}
Negative control	Carrageenan + DMSO	100±0.00	100.46 ± 0.18^{a}	103.46 ± 1.73^{a}	103.74 ± 1.73^{a}	103.79 ± 1.65^{a}
Positive control	Carrageenan + DMSO + Diclofenac	100±0.00	98.684 ± 0.40^{ab}	94.986 ± 0.67^{b}	82.34 ± 2.37^{b}	81.40 ± 1.46^{d}
DCM: Methanolic Leaf extracts	Carrageenan + 50 mg/kg	100±0.00	99.090 ± 0.22^{ab}	91.526± 0.98^{bc}	85.46 ± 1.71^{b}	97.39 ± 2.43^{ab}
	Carrageenan + 100 mg/kg	100±0.00	97.715 ± 0.58^{b}	88.38 ± 1.80^{c}	84.96 ± 1.85^{b}	84.06 ± 1.91^{cd}
	Carrageenan + 150 mg/kg	100±0.00	97.700 ± 0.87^{b}	92.149 ± 0.36^{bc}	83.10 ± 1.75^{b}	90.99 ± 2.24^{bc}

Note: Values are expressed as Mean ± SEM for five animals per group. Statistical comparisons were made within a column, and values with the same superscript were not significantly different by one-way ANOVA followed by Tukey's post hoc test (p>0.05). Carragenan =1%; DMSO = 10%;Diclofenac = 15 mg/kg

Table 6. Effects of DCM-Methanolic stem bark extract of *Ximenia americana* on carrageenan-induced inflammationin mice

Group	Treatment	Percent change in paw circumference after drug administration				
		0h	1h	2h	3h	4h
Normal control	DMSO	100±0.00	100.00 ± 0.21^{b}	99.967 ± 0.06^{b}	100.06 ± 0.07^{b}	99.939 ± 0.06^{b}
Negative control	Carrageenan + DMSO	100±0.00	130.97 ± 3.85^{a}	133.45 ± 3.32^{a}	133.97 ± 3.44^{a}	134.34 ± 3.40^{a}
Positive control	Carrageenan + DMSO + Diclofenac	100±0.00	90.020 ± 0.43^{c}	79.915 ± 0.66^{c}	73.06 ± 1.97^{c}	70.40 ± 1.70^{c}
DCM: Methanolic stem bark extracts	Carrageenan + 50 mg/kg	100±0.00	94.160 ± 1.82^{bc}	86.28± 2.29^{c}	76.82 ± 1.40^{c}	91.38 ± 2.91^{b}
	Carrageenan + 100 mg/kg	100±0.00	90.826 ± 0.90^{c}	80.83± 1.83^{c}	76.09 ± 1.19^{c}	72.27 ± 1.80^{c}
	Carrageenan + 150 mg/kg	100±0.00	89.731 ± 0.68^{c}	79.67 ±1.60^{c}	74.03 ± 1.55^{c}	71.004 ± 0.94^{c}

Note: Values are expressed as Mean ± SEM for five animals per group. Statistical comparisons were made within a column, and values with the same superscript were not significantly different by one-way ANOVA followed by Tukey's post hoc test ($p > 0.05$). Carrageenan=1%; DMSO = 10%; Diclofenac = 15 mg/kg

In the fourth hour after treatment, the extract at doses of 50 mg/kg, 100 mg/kg, and 150 mg/kg body weight, as well as diclofenac, reduced the circumference of the inflamed hind leg by 8.62%, 27.73%, 29.00%, and 29.60%, respectively. (Figure 5; Table 6). Extracts at the dose level of 100 and 150 mg/kg body weight were comparable to each other and the positive control group (p>0.05; Table 6). However, the anti-inflammatory activity of the extract at a dose of 50 mg/kg body weight was significantly different from that of the positive control group (p<0.05; Table 6). The highest anti-inflammatory activity was observed at a dose of 150 mg/kg body weight, reducing the extent of the inflamed paw by 29%. However, the anti-inflammatory activity of the extract at a dose of 50 mg/kg body weight was significantly different from 100 mg/kg and 150 mg/kg body weight (p<0.05; Table 6) but comparable to the normal control group (p>0.05; Table 6; Figure 6).

In comparison, at a dose level of 50 mg/kg, there was a significant difference in the anti-inflammatory activity of the DCM-methanolic leaf and stem bark extracts of *X. americana* during the first and third hours of the trial period (p = 0.05 and 0.006 respectively). However, there was no significant difference at this dose level during the second and fourth hours (p= 0.08 and 0.157, respectively).

At the dose level of 100 mg/kg, there was a significant difference in the anti-inflammatory activity of the DCM-methanolic extracts of leaves and stem bark of *X. americana* in the 4-hour trial period (p= 0.001.0.022, respectively. 0.007 and 0.003).

At the dose level of 150 mg/kg, there was a significant difference in the anti-inflammatory activity of the DCM-methanolic extracts of leaves and stem bark of *X. americana* in the 4-hour trial period (p= 0.000,0.002, 0.006, and 0.000 respectively).

Phytochemical screening

Qualitative phytochemical screening of DCM-methanolic leaf and stem bark extracts from *X. americana* revealed the presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins, and terpenoids (Table 7). However, alkaloids and terpenoids were absent from *X. americana* leaf extracts, and steroids were absent from leaf and stem bark extracts.

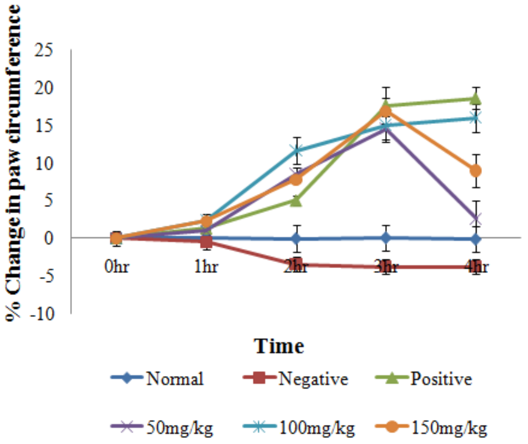

Figure 4. Anti-inflammatory effects of DCM-Methanolic leaf extract of *X. americana* on carrageenan-induced inflammation in the mice

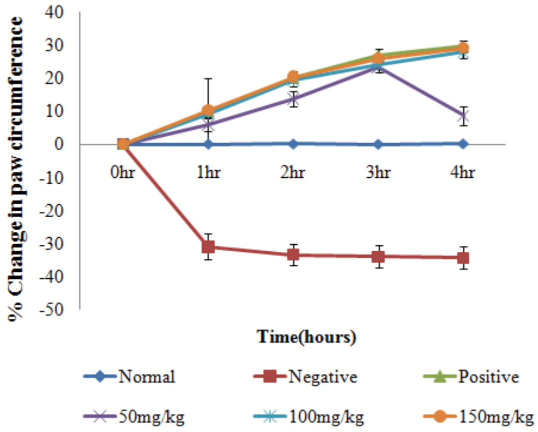

Figure 5. Anti-inflammatory effects of DCM-Methanolic stem bark extract of *Ximenia americana* on carrageenan-induced inflammation in mice

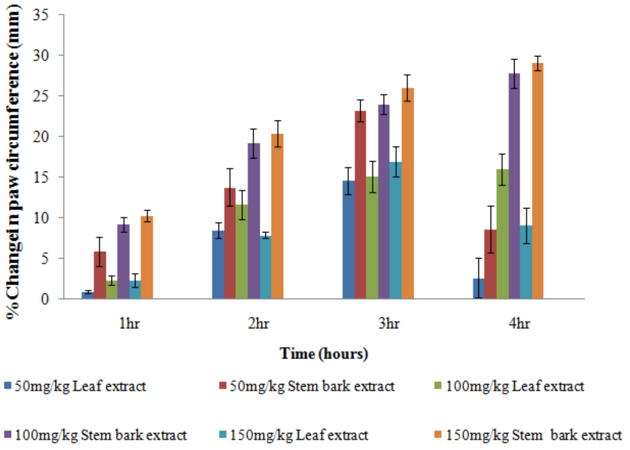

Figure 6. Comparison of Percent Reduction in Paw Circumference of DCM-Methanolic leaf and stem bark extract of *X. americana* on carrageenan-induced inflammation in mice

Table 7. Qualitative phytochemical composition of DCM-Methanolic leaf and stembark extracts of *X. americana*

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

Note: The (+) sign denotes present phytochemical; absent phytochemicals are denoted by the (-)sign, while + (trace) denotes slightly present phytochemical

Discussion

The present study aimed to evaluate the antipyretic and anti-inflammatory activities of DCM-methanolic leaf and stem bark extracts of *X. americana* in rat and mouse models. The antipyretic activity of the extracts was evaluated on turpentine-induced fever in male Wistar rats. Exogenous pyrogens such as steam distilled turpentine, lipopolysaccharides (LPS), brewer's yeast, muramyl dipeptide (MDP), and polyresin: polycytidylic acid (poly I: C) are used to induce fever in laboratory animals (Soszynski et al. 1991; Soszynski and Krajewska 2002). Turpentine is refined by distilling the resin from the pine. Intraperitoneal injection of distilled turpentine from the stem causes pyrexia as it improves prostaglandin synthesis (Zhu et al. 2011). Pyrexia caused by turpentine is rapid, and the tolerance of animals to turpentine is better than other exogenous pyrogens (Tung et al. 2006). This reason guided the choice of turpentine as an exogenous pyrogen for this study.

In this study of turpentine-induced pyrexia in rats (Tables 3 and 4), the significant antipyretic activity of the DCM-methanolic leaf and stem extracts of *X. americana* was observed. The results were consistent with other studies conducted on herbal extracts in laboratory animals. The aqueous extract of *X. americana* stem bark and its fractions showed antipyretic properties (Soro et al. 2015). In addition, Mwonjoria et al. (2011) showed that Solanum incanum root extract had an antipyretic effect on lipopolysaccharide-induced pyrexia in male Wistar rats.

Non-steroidal anti-inflammatory drugs inhibit the expression of cyclooxygenase 2 (COX-2), thereby inhibiting the biosynthesis of PGE2, leading to a reduction in elevated body temperature (Weissmann 1991). The antipyretic effect of many antipyretic drugs is achieved by inhibiting COX-2 and lowering PGE2 levels in the hypothalamus (Biren and Avinash 2010). COX-2 inhibitors are potent antipyretics and inhibit the conversion of arachidonic acid to PGE2 (Chandrasekharan et al. 2002). It was therefore believed that the DCM-Methanolic extracts of leaves and stem bark of *X. americana* reduced the rats' elevated rectal temperatures by inhibiting the COX-2 enzyme responsible for the production of PGE2 in the hypothalamus.

Dose levels of 50 mg/kg, 100 mg/kg, and 150 mg/kg were used in this study to evaluate the antipyretic activity of *X. americana* in rats with turpentine-induced pyretic. A similar assay was used by Gitahi (2015) to assess the antipyretic activity of dichloromethane: methanolic extracts of leaves and root bark of Carissa edulis in rats. Additionally, Akuodorl et al. (2013) used these dosages to evaluate the antipyretic effect of ethanolic extracts of Pseudocedrela kotschyi leaves in rats.

A dose-dependent effect was observed on the antipyretic activity of the methanolic extract of leaves and stem bark of X. American. The 150 mg/kg dose level produced the highest antipyretic activity (Figures 1 and 2; Tables 3 and 4). This dose-dependent result was similar to the work by Srivastava et al. (2013), who demonstrated the antipyretic activity of methanol extracts from Costus speciosus in experimental animals.

The antipyretic activity of the DCM-methanolic extracts of leaves and stem bark of *X. americana* were more efficient at the higher dose level of 150 mg/kg than at the dose level of 50 mg/kg and 100 mg/kg (Tables 3 and 2). Aspirin, the standard drug, showed an optimal antipyretic effect during the third hour (Figures 1 and 2; Tables 3 and 4), although its activity decreased after the third hour, which could be attributed to its metabolism and excretion. The maximum antipyretic effect of the DCM-methanolic extracts of leaves and stem bark of *X. americana* was observed in the third hour.

Passive diffusion of bioactive compounds across cell membranes into the abdominal cavity may contribute to this phenomenon (Hossain et al. 2011). The antipyretic activity of the leaf and stem bark extract of *X. americana* at a dose of 150 mg/kg body weight showed a greater effect than aspirin (Tables 3 and 4). Probably, the extracts inhibit prostaglandins better than the reference drug (aspirin) in these studies. The two parts of the plant did not differ significantly in their antipyretic activity. The presence of numerous phytochemical constituents in the DCM-methanolic extracts of leaves and stem bark of *X. americana* can be attributed to the antipyretic effects of the extracts. Phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, saponins, terpenoids, cardiac glycosides, and phenols (Table 7). Several phytochemicals show antipyretic effects in experimental animals (Okokon and Nwafor 2010). Inhibition of prostaglandin synthesis has been reported by alkaloids (Niazi et al. 2010). Several phytochemicals have been hypothesized to explain the antipyretic effect of methanolic leaf and stem bark extracts in combination with DCM.

In the second hour after treatment, DCM-methanol *X. americana* leaf extract reduced leg circumference by 8.47%, 11.62%, and 7.85% at all doses (50, 100, and 150 mg/kg body weight, respectively). At this time, the anti-inflammatory activities of the methanolic leaf extract of *X. americana* DCM at the three dose levels were significantly different compared to the normal and negative control groups ($p<0.05$; Table 5). However, by this time, the anti-inflammatory activity of the extract at doses of 50 and 150 mg/kg body weight was comparable to that of the positive control ($p>0.05$; Table 5). In the third hour after treatments, *X. americana*'s DCM methanolic leaf extract at doses of 50,100 and 150 mg/kg body weight reduced the paw circumference of the mice by 14.54%, 15.04%, and 16%, 90% (Figure 4). However, the anti-inflammatory activities of the DCM methanolic leaf extract of *X. americana* at all three dose levels differed significantly from the normal and negative control groups ($p<0.05$; Table 4), they were comparable to diclofenac ($p>0.05$, Table 5).

Methanolic extracts of DCM leaves, and stem bark from *X. americana* produced significant anti-inflammatory effects on carrageenan-induced paw edema in Swiss albino mice (Tables 5 and 6). These results relate to the effects of other medicinal plants in animal models. For example, a study by Olabissi et al. (2011) showed that the aqueous ethanolic root bark extract of *X. americana* extract (10 and 100 mg/kg body weight) significantly inhibited carrageenin-induced swelling of mouse paws, with the intensity of the anti-edema effect of the aqueous extract being proportional to the doses tested. Similar work by Zakaria et al. (2007) using aqueous extract of Bauhinia purpurea leaves in animal models showed a significant anti-inflammatory effect on carrageenan-induced inflammation. Similarly, the work of Shashank et al. (2013) showed a potent anti-inflammatory effect of ethanolic and aqueous extracts of Kalanchoe pinnata on carrageenan-induced edema in rats. Non-steroidal drugs such as diclofenac, ibuprofen, indomethacin, and others are commonly prescribed in the treatment of inflammation (Fiorucci et al. 2000). These drugs work by blocking the enzyme cyclooxygenase (COX) to inhibit; the prostaglandin biosynthesis (Blandizzi et al. 2009). Therefore, the anti-inflammatory activities of leaf and stem bark extracts of *X. americana* to control inflammation can be attributed to the inhibition of prostaglandin synthesis.

The anti-inflammatory effect of DCM methanolic extracts of *X. americana* at all doses (50, 100, and 150 mg/kg body weight) over 3 hours in carrageenan-induced inflammation was comparable to that of the positive control group. Previous studies with plants like *Maytenus obscura* (A.Rich.) Cufod., *Caesalpinia volkensii* Harms (Mwangi et al. 2015), and *Solanum trilobatum* L. (Pandurangan et al. 2010) showed a similar effect in this model. Results demonstrate that late-phase extracts act dose-dependently involving arachidonic acid metabolites, as they are associated with neutrophil mobilization-dependent edema. This dose-dependent trend can be attributed to the diffusion of bioactive principles through the cell membrane into the peritoneal cavity in a passive manner.

On the dose levels of 50,100 and 150 mg/kg body weight were used to test the anti-inflammatory activity of the extracts in mice. Similar studies conducted by Vijay and Vijayvergia (2010), Mwonjoria et al. (2011), and Mwangi et al. (2015) used similar dose ranges in their studies when evaluating the anti-inflammatory effects of medicinal plants in animal models.

The *X. americana* DCM-Methanolic leaf and stem extracts exhibited similar anti-inflammatory activities to diclofenac. American leaf extracts reduced leg edema by 0.91%-16.90%, while stem bark extracts reduced it from 5.84% to 28.99%. Diclofenac reduced paw edema from 1.32% to 29.6%. Therefore, the extracts can be considered an effective anti-inflammatory agent similar to the reference medicinal product (diclofenac). On the anti-inflammatory activity, those two parts of the plant did not differ significantly. The anti-inflammatory activity of DCM-methanolic leaf and stem bark extracts of *X. americana* can be attributed to the presence of phytochemicals. Phytochemical screening revealed the presence of flavonoids, saponins, cardiac glycosides, and phenols in the leaf extract. In contrast, the stem bark extract found alkaloids, flavonoids, saponins, cardiac glycosides, phenols, and terpenoids (Table 7). Bhaskar and Balakrishnan (2009) report that these phytochemicals exert anti-inflammatory activity in animal models.

In this study, methanolic extracts of DCM leaves and stem bark from *X. americana* were shown to be antipyretic and anti-inflammatory. *X. americana* extract has powerful antipyretic and anti-inflammatory properties, as evidenced by the significant reduction in rectal temperature and paw circumference compared to the reference drugs. Thus, DCM-methanolic extracts of the leaves and stem bark of *X. americana* can treat fever and inflammation and provide an effective alternative to synthetic drugs. Furthermore, it was found that DCM-methanolic extracts of the leaves and stem bark of *X. americana* contain phytochemicals class associated with antipyretic and anti-inflammatory effects. Accordingly, the study supports the traditional use of *X. americana* to treat fevers and inflammations, rejecting the null hypothesis.

REFERENCES

Abbas AB, Lichtman AH. 2009. Innate Immunity In Saunders (Elsevier). Basic Immunology. Functions and disorders of the immune system, 3rdedition. Saunders/Elsevier, Philadelphia.

Akuodorl GC, Essien AD, Essiet GA, Essien David-Oku, Akpan JL, Udoh FV. 2013. Evaluation of antipyretic potential of *Pseudocedrela kotschyi* Schweint. Harms (Meliaceae). Eur J Med Plants 3: 105-113. DOI: 10.9734/EJMP/2013/1097.

Axelrod YK, Diringer MN. 2008. Temperature management in acute neurologic disorders. Neurol Clin 26: 585-603. DOI: 10.1016/j.ncl.2008.02.005.

Bairy KL, Chogtu B, Nayak V. 2015. Effect of fluticasone propionate with biopolymer on skin inflammatory model in wistar rats. Res J Pharm Biol Chem Sci 6: 721-723.

Bamgbose SOA, Noamesi BK. 1981. Studies on cryptolepine inhibition of carrageenan-induced oedema. Planta Med 42: 392-396. DOI: 10.1055/s-2007-971733.

Bhaskar VH, Balakrishnan N. 2009. Analgesic, anti-inflammatory and antipyretic activities of *Pergularia daemia* and *Carissa carandas*. J Pharm Sci 17: 168-174.

Biren NS, Avinash KS. 2010. Medicinal plants as a source of anti- pyretic agents - a review. Arch Appl Sci Res 2: 188-195.

Blandizzi C, Tuccori M, Colucci R, Fornai M, Antonioli L, Ghisu N, Del Tacca M. 2009. Role of coxibs in the strategies for gastrointestinal protection in patients requiring chronic non-steroidal anti-inflammatory therapy. Pharmacol Res 59: 90-100. DOI: 10.1016/j.phrs.2008.11.004.

Chandrasekharan NV, Dai H, Roos KLT, Evanson NK, Tomsik J, Elton TS, Simmons DL. 2002. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. Proc Natl Acad Sci 99: 926-931. DOI: 10.1073/pnas.162468699.

Dalziel JM. 1937. The useful plants of west tropical Africa. DOI: 10.1093/oxfordjournals.afraf.a101471.

Diallo D, Sogn C, Samaké FB, Paulsen BS, Michaelsen TE, Keita A. 2002. Wound healing plants in Mali, the Bamako region. An ethnobotanical survey and complement fixation of water extracts from selected plants. Pharm Biol 40 (2): 117-128. DOI: 10.1076/phbi.40.2.117.5846.

Ekandjo A, Naomand E, Kahaka G. 2018. Genetic analysis of *Ximenia americana* based on DNA-DNA based on the cross-species hybridization technique. Cell Biol Dev 2: 1-7. DOI: 10.13057/cellbioldev/v020101.

Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE. 2007. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation. Clin Exp Immunol 147: 227-235. DOI: 10.1111/j.1365-2249.2006.03261.x.

Feyssa DH, Njoka JT, Asfaw Z, Nyangito MM. 2012. Uses and management of *Ximenia americana*, Olacaceae in semi-arid East Shewa, Ethiopia. Pak J Bot 44: 1177-1184.

Fiorucci S, Santucci L, Cirino G, Mencarelli A, Familiari L, Del Soldato P, Morelli A. 2000. IL-1β converting enzyme is a target for nitric oxide-releasing aspirin: New insights in the anti-inflammatory mechanism of nitric oxide-releasing non-steroidal anti-inflammatory drugs. J Immunol 165: 5245-5254. DOI: 10.4049/jimmunol.165.9.5245.

Gitahi SM. 2015. Antipyretic properties of dichloromethane: Methanolic leaf and root bark extracts of *Carissa edulis* in Rats. Asian J Biomed Pharm Sci 5: 12-20. DOI: 10.15272/ajbps.v5i43.681.

Graz B, Malebo HM. 2011. To what extent can traditional medicine contribute a complementary or alternative solution to malaria control programmes? Malaria J 10: S6. DOI: 10.1186/1475-2875-10-S1-S6.

Grønhaug TE, Glæserud S, Skogsrud M, Ballo N, Bah S, Diallo D, Paulsen BS. 2008. Ethnopharmacological survey of six medicinal plants from Mali, West-Africa. J Ethnobiol Ethnomed 4: 1. DOI: 10.1186/1746-4269-4-26.

Grover JK. 1990. Experiments in Pharmacy and Pharmacology. CBS Publisher and Distributor, Shahdara Delhi, India.

Harbone JB. 1998. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman & Hal Publishersl, London, UK.

Hossain E, Mandal SC, Gupta JK. 2011. Phytochemical screening and in-vivo antipyretic activity of the methanol leaf-extract of *Bombax malabaricum* DC (Bombacaceae). Trop J Pharm Res 10: 55-60. DOI: 10.4314/tjpr.v10i1.66541.

Hukkeri VI, Nagathan CV, Karadi RV, Patil BS. 2006. Antipyretic and wound healing activities of *Moringa oleifera* Lam. in rats. Indian J Pharm Sci 68 (1): 124-126. DOI: 10.4103/0250-474X.22985.

Khan H, Saeed M, Gilani AH, Muhammad N, Haq IU, Ashraf N. 2013. Antipyretic and anticonvulsant activity of *Polygonatum verticillatum*: Comparison of rhizomes and aerial parts. Intl J Phytother Res 27: 468-471. DOI: 10.1002/ptr.4721.

Kirkham TC, Williams CM, Fezza F, Marzo VD. 2002. Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: Stimulation of eating by 2-arachidonoyl glycerol. Br J Pharmacol 136: 550-557. DOI: 10.1038/sj.bjp.0704767.

Kotake CK. 2000. Vallabh Prakashan, New Delhi, India. Pract Pharmacogn 4: 107-111.

Lamien-Meda A, Lamien CE, Compaoré MM, Meda RN, Kiendrebeogo M, Zeba B, Nacoulma OG. 2008. Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. Molecules 13: 581-594. DOI: 10.3390/molecules13030581.

Laupland KB. 2009. Fever in the critically ill medical patient. Crit Care Med 37: S273-S278. DOI: 10.1097/CCM.0b013e3181aa6117.

Mwangi BM, Gitahi SM, Njagi JM, Mworia JK, Aliyu U, Njoroge WA. Mburu ND. 2015. Anti-inflammatory properties of dichloromethane: Methanolic leaf extract of *Caesalpinia volkensii* and *Maytenus obscura* in animal models. Intl J Curr Pharm Res 7: 83-87.

Mwonjoria JK, Kariuki HN, Waweru FN. 2011. The antinociceptive antipyretic effects of *Solanum incanum* (Linneaus) in animal models. Intl J 2: 24-26.

Narsinghani T, Sharma R. 2014. lead optimization on conventional non-steroidal anti-inflammatory drugs: An approach to reduce gastrointestinal toxicity. Chem Biol Drug Design 84: 1-23. DOI: 10.1111/cbdd.12292.

Neville AP, Cliff JW, Ian A. 2004. The journal of inflammation. BioMed Central 1 (1): 1-4.

Niazi J, Gupta V, Chakarborty P, Kumar P. 2010. Anti-inflammatory and antipyretic activity of *Aleuritis moluccana* leaves. Asian J Pharm Clin Res 3: 35-37.

Okigbo RN, Anuagasi CL, Amadi JE. 2009. Advances in selected medicinal and aromatic plants indigenous to Africa. J Med Plants Res 3: 86-95.

Okokon JE, Nwafor PA. 2010. Anti-inflammatory, analgesic and antipyretic activities of ethanolic root extract of *Croton zambesicus*. PakJ Pharm Sci 23: 383-390.

Olabissi OAF, Moussa O, Moustapha O, Edgard ZF, Marius L, Pierre GI. 2011. Acute toxicity and anti-inflammatory activity of aqueous ethanol extract of root bark of *Ximenia americana* L. (Olacaceae). Afr J Pharm Pharmacol 5: 806-811.

Olfert ED, Cross BM, McWilliam AA. 1993. Guide to the Care and Use of Experimental Animals. Canadian Councilon Animal Care, Ottawa.

Pandima DK, Sai RM, Sreepriya M, Ilavazhagan G, Devaki T. 2003. Immunomodulatory effects of Premna tomentosa extract against Cr (VI) induced toxicity in splenic lymphocytes - an in vitro study. Biomed Pharmacother 57: 105-108. DOI: 10.1016/S0753-3322(02)00345-1.

Pandurangan A, Khosa RL, Hemalatha S. 2010. Evaluation of anti-inflammatory and analgesic activity of root extract of *Solanum trilobatum* Linn. Iran J Pharm Sci 7: 217-221.

Parveen A, Shaheen K, Qamer SM, Ali SW, Hussain M, Ali SA. 2014. Prescribing patterns of antibiotics in post-operative patients in a teaching hospital. Indo Am J Pharm Res 4: 527-532.

Purssell E, While AE. 2013. Does the use of antipyretics in children who have acute infections prolong febrile illness? A systematic review and meta- analysis. J Pediatr 163 (3): 822-827. DOI: 10.1016/j.jpeds.2013.03.069.

Ray D, Sharatchandra KH, Thokchom IS. 2006. Antipyretic, antidiarrhoeal, hypoglycaemic and hepatoprotective activities of ethyl acetate extract of Acacia catechuWilld. in albino rats. Indian J Pharmacol 38: 408-413. DOI: 10.4103/0253-7613.28207.

Rodrigo B, Marcus VMN, Adryano AVC, Marize CV, José RP, Elson AC, Luiz CC. 2013. Antinociceptive and anti-inflammatory activities of the ethanolic extract from *Synadenium umbellatum* Pax (Euphorbiaceae) leaves and its fractions. Evid-Based Complement Altern Med 2013: 715650. DOI: 10.1155/2013/715650.

Shashank J, Levy I, Fishbain B, Broday D. 2013. Application of distributed sensor networks for estimating exposures to air pollution in urban areas. ISEE Conf Abstracts 2013 (1): 3445. DOI: 10.1289/isee.2013.P-1-01-08.

Snyderman R, Weil A. 2002. Integrative medicine: Bringing medicine back to its roots. Arch Intern Med 162: 395-397. DOI: 10.1001/archinte.162.4.395.

Soro TY, Zahoui OS, Néné-bi AS, Traoré F. 2015. Antipyretic activity of the fractions of the aqueous extract of *Ximenia americana* (Linnaeus) (Olacaceae). Intl J Pharmacol Toxicol 5: 104-108.

Soszynski D, Kozak W, Szewczenko M. 1991. Course of fever response to repeated administration of sublethal doses of lipopolysaccharides, polyinosinic: polycytidylic acid and muramyl dipeptide to rabbits. Experientia 47: 43-47. DOI: 10.1007/BF02041248.

Soszynski D, Krajewska M. 2002. Lack of cross tolerance between pyrogenic effects of LPS and turpentine in rats. J Thermal Biol 27: 229-237. DOI: 10.1016/S0306-4565(01)00088-2.

Srivastava S, Singh P, Jha KK, Mishra G, Srivastava S, Khosa RL. 2013. Anti-inflammatory, analgesic and antipyretic activities of aerial parts of costus speciosus koen. Indian J Pharm Sci 75: 83. DOI: 10.4103/0250-474X.113532.

Tung K, Fujita H, Yamashita Y, Takagi Y. 2006. Effect of turpentine-induced fever during the enamel formation of rat incisor. Arch Oral Biol 51: 464-470. DOI: 10.1016/j.archoralbio.2005.12.001.

Urso V, Signorini MA, Bruschi P. 2013. Survey of the ethnobotanical uses of *Ximenia americana* L. (mumpeke) among rural communities in SouthAngola. J Med Plants Res 7: 7-18.

Vijay P, Vijayvergia R. 2010. Analgesic, anti-inflammatory and antipyretic activity of *Cissus quadrangularis*. J Pharm Sci Technol 2: 64-71.

Weissmann G. 1991. Asprin. Sci Am J 264: 84-90. DOI: 10.1038/scientificamerican0191-84.

Winter CA, Risley EA, Nuss GW. 1962. Carrageenan-induced oedema in the hind paw of rat as an assay for anti-inflammatory activity. Proc Soc Exp Biol Med 111: 544- 547. DOI: 10.3181/00379727-111-27849.

Zakaria ZA, Wen LY, Abdul Rahman NI, Abdul Ayub AH, Sulaiman MR, Gopalan HK. 2007. Antinociceptive, anti- inflammatory and antipyretic properties of the aqueous extract of *Bauhinia purpurea* leaves in experimental animals. Med Principles Pract 16: 443-449. DOI: 10.1159/000107749.

Zhu ZZ, Ma KJ, Ran X, Zhang H, Zheng CJ, Han T, Qin LP. 2011. Analgesic, anti-inflammatory and antipyretic activities of the petroleum ether fraction from the ethanol extract of *Desmodium podocarpum*. J Ethnopharmacol 133: 1126-1131. DOI: 10.1016/j.jep.2010.11.042.