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ABSTRACT

The present study was to investigate preliminary toxicity; antioxidant and some pharmacological activities include analgesic and anti-inflammatory activity of polyphenol-rich fractions of *Ximenia americana* L. The toxicological pattern was studied by the determination of LD_{50} in mice. The antioxidant potential of the sample was evaluated using three separate methods, hydroxyl radical scavenging assay; hydrogen peroxide scavenging assay and phosphomolybdate assay for the total antioxidant capacity. The analgesic activity used two separate methods such as the test of writhing induced by acetic acid and hot pot test. The anti-inflammatory profile was evaluated using induction in the right-hand paw by injection of formalin. Polyphenol-rich fractions from *Ximenia americana* L., exhibit maximum radical scavenging activity. Concerning preliminary toxicity study; LD_{50} is 3270.8 mg/Kg body weight for intraperitoneal administration. It could say that extracts of roots are no toxic.Fraction exhibited significant analgesic and anti-inflammatory activity in doses of 200, 250 and 300 mg/kg b. wt. and particularly higher dosage level (300 mg/kg b. wt) induced significant anti-inflammatory and analgesic activities. The results indicated that the polyphenol-rich fractions of *Ximenia americana* L., possessed antioxidant, anti-inflammatory and analgesic properties, and may give credence to some of its ethnopharmacological uses. We undertook this study of roots from this plant in order to provide a scientific basis for the traditional use of *Ximenia americana* L., in traditional medicine. Our results of this study appeared to show the safety of acute toxicity of extract from of *Ximenia americana* L., which can, therefore, be continuously used with safety in traditional medicine.

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INTRODUCTION

Pain is always a subjective an unpleasant sensory and emotional experience associated with actual or potential tissue damage and described in terms of such damage [1]. There may be a strong emotional component contributing to the pain experience, but that does not mean that the suffering is less important [2]. It is the most common reason a patient sees a physician. For most patients, it is of short duration and quickly forgotten [3]. When chronic, it markedly decreases individuals' health status and quality of life and can detrimentally affect the families of patients. It often interferes with everyday work activities [5]. Unrelieved acute pain can cause chronic pain, and long-standing pain can cause anatomical and even genetic changes in the nervous system [2].

Inflammation, on the other hand, is a physiological response of living tissues to injury [5]. Although the inflammatory response is essential for host defense, it is very much a double-edged sword, which can lead to an organ failure and/or death [6]. To relieve the damage they cause and to reduce their effect on quality of life, it might be necessary to take pharmacological agents against pain and inflammation. Non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and opiates have been used classically in these conditions [7, 8]. However, due to extensive use of analgesic and anti-inflammatory agents, the toxicity and untoward effects occur many times, especially when therapy of pain and inflammation involves use of higher doses for longer periods [9]. Gastrointestinal disturbances, respiratory depression, possible dependence[7], constipation [10], renal dysfunction [11], peptic ulcer and bleeding [12] are some of the commonly encountered untoward effects of analgesic and anti-inflammatory agents.

Natural products derived from medicinal plants are becoming preferred alternative remedies. By screening medicinal plants with acclaimed analgesic and anti-inflammatory use, safe and effective analgesic and anti-inflammatory drugs might be discovered [13]. It is therefore essential that efforts should be made to introduce new compounds derived from medicinal plants to the drug arsenal against pain and inflammation [14].

Indigenous medicinal plants were and are still one of the sources of modern medicines [15]. Moreover, the trend of using phytotherapy as alternative medicine has increased the interest for the tropical plants' pharmacognosy [16].

In Africa, especially in Burkina Faso, medicinal plants still play an important role in healthcare of an important portion of the population. This is because they are cheap, are locally available and efficient. Generally, the effects of medicinal plants are attributed to their content in active chemicals [17]. In developing countries, there is a general belief among the consumers that the use of medicinal plants is always safe because they are "natural". However, evidences suggest otherwise and some studies suggest that some of the plants can be associated with health hazards. Medicinal plants can contain many active chemical compounds and other substances of great complexity like mucilage's, polyphenols, polysaccharides, etc [17]. That may modulate and modify the effects of any "active principles". Thus, some plant remedies can be toxic or can act as either agonists or antagonists of the active principles. Therefore, the study of toxicity is an essential prerequisite for the efficiency assessment of plant extracts.

Ximenia americana (Olacaceae) which is also known is a shrub-like plant found in abundance in the West African region. It flowers usually in the second part of the dry season, producing cream – white to greenish yellow flowers. The fruits are green but turn golden-yellow or red. The fruit when eaten is very refreshing and has the almond acid taste. In Northern Nigeria, the plant is found in the Savannah areas. The plant is used in traditional medicine for treatment of malaria, fever, leprotic ulcers and skin infections of mixed origin in Northern parts of Nigeria [18].

An ethnobotanical investigation in the central region of Burkina Faso has shown that many species are traditionally used to treat various kinds of pain diseases. Among such plants, *Ximenia americana* L. (Olacaceae) is the most frequently and widely used. This plant and particularly the roots are used for treating abdominal pains, dysentery, diarrhea, as the poison antidote, and infectious diseases in children such as malaria, fever, pain, and have antibacterial, anti-inflammatory, analgesic and hepatoprotective properties [17]. In most cases, the drug is administrated over a long period of time and without any proper monitoring of the dosage.

Phytochemical screening of the leaves and stem bark revealed the presence of saponins, glycosides, flavonoids, tannins, phenolics, alkaloids, quinones and terpenoids types. In addition, the plant is potentially rich in fatty acids and glycerides and the seeds contain derivatives cyanide [17].

Some recent studies have been reported on analgesic and antipyretic of stem barks and leaves of this in certain countries in Africa such as Tanzania, Senegal, Zimbabwe and Nigeria [19; 20]. Still no scientific and methodical investigation has so far been reported in literature regarding antioxidant, anti-inflammatory and analgesic properties of polyphenol-rich fractions of roots from *Ximenia americana* L. Therefore, the choice of our investigated plant is based on two criteria: First, in this domain there is no study about bioactive fraction of roots in Burkina Faso that deals this plants and second criterion is that this plant has ethnopharmacological data indicating their traditional utilization in treatment of inflammatory diseases and including analgesic properties of these roots. Despite the extensive use of this plant in traditional health care, the literature provides little information regarding the toxicity of roots of *Ximenia americana* L. So that the toxicities effect of those, very used are unknown. Therefore, the objective of the present study was to assess the toxicological study of acetone aqueous extract and then to evaluate the anti-inflammatory and analgesic properties of bioactive fraction of roots from *Ximenia americana* L.

MATERIALS AND METHODS

Plants material

The vegetable materials (Fresh roots) of *Ximenia americana* L.,(Olacaceae) were collected in August 2014 in Dedougou, 230 Km West of Ouagadougou, capital of Burkina Faso. Dr. Traoré Lassina botanically identified this plant from the plants Biology Department of the University of Koudougou.

Animals Handling

Swiss NMRI mice (25–30 g) of both sexes were used for this study. All animals were housed in cages under controlled conditions of 12 h light/and 12 h without light and 25°C.They received pellets of food enriched with 20% protein and water ad libitum. They were deprived of food for 15 h (but with access to drinking water) and weighed before the experiments. Experiments on the animals were performed according to the protocols already approved by the Institute of Health Sciences Research/University of Ouagadougou (Burkina Faso) and met the international standards for animal study [21].

Preparation of aqueous acetone extract for Acute Toxicity Study

The field-has grown fresh samples (roots) were washed with tap water followed by distilled water to remove the adhering dust particles. After blotting, samples were air dried in shade. The dried plant materials (roots) were ground to fine powder and stored in clean airtight containers. A sample of 50 g of stem barks was placed in the soxhlet and run by using 80% aqueous acetone (500 ml) in 1/10 ratio (w/v) for 24 h under mechanic agitation at room temperature. After filtration, all the extracts were dried in vacuum rotary evaporator at 40 °C under reduced pressure. Extracts were weighed and stored at 4 °C for further analysis.

Polyphenol-rich fractions extraction

The harvested plant materials fresh (roots) were dried in the laboratory at room temperature

(20-25°C), afterward, samples were ground to pass a sieve of 0.3 mm. Polyphenol-rich fractions were extracted with aqueous acetone (80%, v/v). The extract was then washed with hexane to remove chlorophyll and other low molecular weight compounds. Acetone was evaporated and the extract was lyophilized and stored at 22°C prior to biological tests. For the tests, the lyophilized sample was dissolved with 10% DMSO in water at the desired concentration [22].

***In vitro* Antioxidant profiles of Polyphenol-rich fractions of roots**

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was measured by the ability of the polyphenol-rich fractions(roots**)** to scavenge the hydroxyl radicals generated by the Fe^{3+}- ascorbate - EDTA - hydrogen peroxide system [23; 24]. The reaction mixture (1 mL) contained 100 µL of 2-deoxy -2 -ribose (28 mM in 20 mM KH_2PO_4 buffer, pH 7.4), 500 µL of the fraction (roots) at various concentrations (100 - 500 μgmL^{-1}) in buffer, 200 µL of 1.04 mM EDTA and 200 µM ferric chloride (1:1 v/v), 100 µL of 1.0 mM hydrogen peroxide (H_2O_2) and 100 µL of 1 mM ascorbic acid. Test samples were kept at 37 °C for 1 h. The free radical damage imposed on the substrate deoxyribose was measured using the thiobarbituric acid test. About 1 mL of 1 % thiobarbituric acid (TBA) and 1 mL 2.8 % trichloroacetic acid (TCA) were added to the test tubes and were incubated at 100 °C for 20 min. After cooling, the absorbance was measured

at 532 nm against a blank containing deoxyribose and buffer. Quercetin (100-500 μgmL^{-1}) was used as a positive control.

Hydrogen peroxide scavenging assay

Hydrogen peroxide solution (2 mML^{-1}) was prepared with standard phosphate buffer (pH 7.4). Various concentrations of the polyphenol-rich fractions roots (100-500 μgmL^{-1}) in distilled water were added to 0.6 mL of hydrogen peroxide solution respectively. Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging activity at different concentrations of the polyphenol-rich fractions was determined and the IC_{50} values were compared with the standard, α-tocopherol [25].

Phosphomolybdate assay

The total antioxidant capacity of the polyphenol-rich fractions (roots) was determined by phosphomolybdate method using α-tocopherol as the standard [26]. An aliquot of 0.1 mL of the polyphenol-rich fractions of roots (100 µg) solution respectively was combined with 1 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated in boiling water bath at 95°C for 90 min. The samples were cooled to room temperature and the absorbance was measured at 695 nm against the blank. The total antioxidant capacity was expressed as gallic acid equivalent per gram.

Biological activities

Acute Toxicity study in mice

Healthy male and female Swiss mice (25-30g) were randomly divided into 7 groups (1 control group and 6 treated assay groups) of 6 animals (3 male and 3 female). The control group received water containing 10% dimethylsulfoxide (DMSO) administered intraperitoneally. The water/acetone extract of roots from *Ximenia americana* L., suspended in 10% DMSO was administered respectively intra-peritoneally at doses of 1; 2; 2.5; 3; 4; 5 and 6g/kg. The general behavior of the mice was observed for 120 min after the treatment. The animals were observed for morbidity and mortality once a day for 14 days. The number of survivors after the 14 days period was noted. The toxicological effect was assessed on the

basis of mortality for 14 days, which was expressed as the median lethal dose (LD_{50}) (Lethal Dose 50) was estimated from the regression of log-probit mortality rate [27].

Analgesic properties of polyphenol-rich fractions of roots

Test of writhing induced by acetic acid

This experiment was carried out as described by [28] with minor modifications in the process. Thirty mice of both sexes weighing 25-30 g body weight each which previously showed positive writhing (stretch torsion to one side drawing up of hind limb, retraction of abdomen and opisthotonus, so that the belly of the mouse touch the floor) with glacial acetic acid were selected and divided into five groups with six mice in each group. Mice of the first group were kept as control no treated, animals received vehicle (10% DMSO in water, 10ml/kg body weight), and those of the second group was orally administrated aspirin 100 mg/kg body weight as a standard group. Mice of the third, fourth and five groups were orally administered respectively polyphenol-rich fractions of roots dissolved in 10% DMSO, in a dose of 200; 250 and 300 mg/Kg body weight. After 30 minutes, each mouse was intraperitoneally injected with 0.25 ml of 0.7% glacial acetic acid in distilled water, and the mice were then placed in transparent boxes for observation. The numbers of writhes were observed after 1, 2, 3, 4 and 5 hours post administration. The number of writhes for each animal in all groups was recorded and the analgesic potency of the tested extract was determined as protection % against writhing according to the following formula below:

$$\textbf{(\%) Inhibition} = \frac{\text{(Control mean- treated mean)}}{\text{Control means}} \text{ x100}$$

Hotplate test

The experiment was carried out as described by [29] and modified by [30] using hot plate apparatus, thermostatically controlled at 56 ± 0.5˚c.Thirty mice of both sexes weighing 25-30 g body weight were divided into 5 groups, 6 animals each. Reaction time was measured prior to extract (min 0) and after the drug treatment. Group I was kept under normal control. The flavonoid-rich fractions were administrated orally to mice of groups II, III and IV in doses of 200; 250 and 300 mg/kg, respectively. Mice of group V (standard) were treated orally with

acetylsalicylic acid at a dose rate of 100 mg/kg b. wt. The reaction time was measured at 15 min and repeated at 30, 60, and 90 min post-administration.

Anti-inflammatory effect polyphenol-rich fractions

According to the method described by [31]' thirty mice of both sexes weighing 25-30 grams body weight were used. Inflammation was induced in the right-hand paw of all mice by subcutaneous injection of 0.1 ml formalin 6% solution in normal saline. After four hours, the thickness of each rat paw was measured in mm using vernier caliber to detect the inflammatory process achieved by the formalin solution. Mice were then divided into five equal groups of six mice each. Mice of the first group were left in control with induced inflammation only. Those of the second group were orally administered diclofenac sodium (Voltarin®) in a dose of 30 mg /kg body weight as a standard. Mice of the third, fourth and five groups were orally administrated polyphenol-rich fractions of *Ximenia americana* L., in a dose 200, 250 and 300 mg/kg body weight. Thirty minutes after drug or test compound administration, 0.1ml of 6% formalin solution in normal saline was injected subcutaneously in the right-hand paw of all animals for induction of edema the thickness of each mice paw was measured in mm by vernier caliber after 1, 2, 3, and 4 hours post administration.

Statistical analysis: The data were expressed as Mean±Standard deviation (SD) of six determinations (n=6). Results were analyzed by one-way ANOVA followed by Dunnett's*t*-test using Prism 4 software. The level of significance was accepted at $p \leq 0.05$.

RESULTS

***In vitro* Antioxidant activity**

The measures of antioxidant activity were obtained using three described methods. Hydroxyl radical scavenging activity was quantified by measuring the inhibition of the degradation of deoxyribose by the free radicals generated by the Fenton reaction. The Hydroxyl radical scavenging activity of the polyphenol-rich fractions of roots is very similar to the (quercetin, 0.31 $mgmL^{-1}$). The results are reported in (Table 1).

For scavenged hydrogen peroxide, polyphenol-rich fractions of roots scavenged hydrogen peroxide in a concentration-dependent manner. The polyphenol-rich fraction of roots

exhibited hydrogen scavenging activity (IC_{50}= 0.13 $mgmL^{-1}$) whereas the standard, α-tocopherol had potent scavenging activity with 0.07 $mgmL^{-1}$(Table 1).

The phosphomolybdate method is quantitative since the total antioxidant capacity is expressed as gallic acid equivalents. Polyphenol-rich fractions of root contained 55.23 mg GAE/g (Table 1).

Acute toxicity study in mice

The effect of intraperitoneal treatment of the aqueous acetone extract from *Ximenia americana* L., on mortality, LD_{50} is 3270.8 mg/Kg body weight for intraperitoneal administration. No significant difference in body weight gain of the treated assay groups over the period of observation. No statistical difference was observed between the organ weights in the control and the intraperitoneal route groups.

Analgesic properties

Acetic-acid writhing test

The polyphenol-rich fractions have effectively reduced the number of abdominal muscle contractions induced by 0.7% acetic acid solution. Oral administration of the fraction in a dose of (200, 250 and 300 mg/kg b. wt.) exhibited significant analgesic activity with 51.2%; 60.4% and 73.14 % protection percentage for 5 hours. Thus, fraction produced significant inhibition of writhing induced by acetic acid. The inhibition was dose-dependent. Standard group orally administrated acetylsalicylic acid (100 mg/kg b. wt.) showed 76% protection against writhing induced glacial acetic acid for 5 hours. The results are presented in Table 2.

Hotplate test

Results of central anti-nociceptive activity of the polyphenol-rich fractions in mice are recorded in table 3. Data showed that polyphenol-rich fractions in a dose of 200, 250 and 300 mg/kg b. wt., induced significant analgesic activity in a dose-dependent manner since the tested dose relieved the pain in mice exposed to the hot plate at different time intervals compared to the control (non treated group). Oral administration of the standard at a dose of 100 mg/kg b. wt. significantly increased the reaction time (Table 3).

Anti-inflammatory properties of polyphenol-rich fractions

The anti-inflammatory effect of polyphenol-rich fractions of *Ximenia americana* L. was studied using formalin-induced edema in mice paws and data were compared with that of control in table 4. Oral administration of polyphenol-rich fractions of *Ximenia americana* L., in a dose of 200, 250 and 300 mg/kg b.wt induced a significant decrease in inflamed rat paw thickness in a dose-dependent manner when compared with control non treated group for 4 hours. Therefore, results demonstrated a concentration dependent anti-inflammatory activity at all test doses (100, 200 and 300 mg/kg) (Table 4).

DISCUSSION

Nowadays, it is noteworthy that traditional medicine is gaining popularity in developing countries. Medicinal plants are often believed to be harmless because they are natural and are commonly used for self-medication without supervision. This increase in popularity and the scarcity of scientific studies on their safety and efficacy have raised concerns regarding toxicity and adverse effects of these remedies [32]. These products of plants contain bioactive principles with the potential to cause adverse effects [33]. About the acute toxicity of our study plant, data indicated that the root extracts of *Ximenia americana* L., are low poisonous. During the 14 day period of acute toxicity evaluation, some signs of toxicity were observed, but they were all quickly reversible. According to [34], pharmacological substances whole LD_{50} is less than 5 mg/kg body weight are classified in the range of highly toxic substances, those with an LD_{50} between 5 mg/kg body weight and 5000 mg/kg body weight are classified in the range of moderately toxic substances and those with the lethal dose is more than 5000 mg/kg body weight not toxic. In this fact, if we refer to this classification we could say that the extracts of *Ximenia americana* L., are moderately toxic and would be regarded as being safe or of low toxicity [35].

Medicinal plants are an important source of antioxidants [36] and natural antioxidants increase the antioxidant capacity of the plasma, reduce the risk of certain diseases such as cancer, heart diseases, and stroke [37]. The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots and bark [38]. Concerning antioxidant profiles of polyphenol-rich fractions of roots of *Ximenia americana* L., data showed the three methods used in our study, showed a good antioxidant potential. In fact,

certain secondary metabolites such as saponins, tannins, and flavonoids, so polyphenols, in general, have been shown to be responsible for the therapeutic activity of plants [39]. In effect, according to [40], plant phenols are a major group of compounds acting as primary antioxidants or free radical scavengers due to their hydroxyl groups [41] which contribute directly to the antioxidative action. Phenolic compounds are effective hydrogen donors, making them good antioxidants [42]. Then, our results showed effectively that total phenolics are responsible for the antioxidant capacity and there is a correlation between total phenolics and the antioxidant capacity.

About analgesic properties, general it could say that nociception is a sensory signal indicating potential harm which most of the time perceived as pain. The sensation of pain develops with the activation of nociceptor mediated by mechanical, thermal or chemical stimuli [43]. Our present study is the first time to demonstrate the antinociceptive activity using flavonoid-rich fractions of roots of *Ximenia americana* L., in classical pharmacological models of pain. Although roots of *Ximenia americana* L., is widely used in the folk medicine in all over Burkina Faso the antinociceptive activity of this plant root has not been reported yet. The study of plant species that are traditionally used for the relief of the pain should still be seen as a logical research strategy to investigate new analgesic drugs.

The acetic acid-induced writhing response was the firstest to evaluate the antinociceptive activity of the roots of *Ximenia americana* L., is a well-recommended protocol in evaluating medicinal agents for their peripherally acting analgesic property. The intraperitoneal administration of acetic acid causes abdominal contractions, whole body movements and twisting of the dorsal-abdominal muscles. In this model, pain is generated indirectly via proinflammatory agent capsaicin and, endogenous mediators like bradykinin and serotonin, which stimulate peripheral nociceptive neurons that are sensitive to NSAIDs and narcotics [44].

Preliminary phytochemical screening of *Ximenia americana* L. qualitatively identified the presence of flavonoids, tannins, alkaloids, saponins, phenolics, steroids, and glycosides. Therefore, polyphenols such as flavonoids of the fraction of roots might be responsible for the antinociceptive activity. The results of our extracts in acetic acid-induced abdominal contraction exhibited prominent inhibition of writhing response. These findings imply deep insights regarding the strong peripheral analgesic activity of polyphenol-rich fractions, and

their mechanisms of action may be mediated by inhibition of cyclooxygenase activity or prostaglandin synthesis.

Hotplate test, thermal nociception model, was used to evaluate central analgesic activity. Flavonoid-rich fractions in different concentrations displayed analgesic effect in the hot plate test. As the hot plate test is a specific central antinociceptive test, it is possible that root fractions exert an analgesic effect at least in part through central mechanisms.Non-prescription use of medicinal plants is reported today as an important health problem, particularly in nephrotoxicity [45]. In this regards, brine shrimps cytotoxicity assay has been considered as a pre-screening assay and suggested to be a convenient probe for the pharmacological activities of plant extracts [46].The central analgesic activity may be attributed to the presence of tannins and flavonoids, which inhibit prostaglandin synthesis, which plays the significant role in different phases of inflammatory reactions.

At last, for anti-inflammatory profiles, induction of edema in mice paw by formalin is a biphasic response, in which the first phase is mediated by histamine, serotonin, and kinins whereas the second phase is mediated by prostaglandins (cyclooxygenase product of arachidonic acid metabolism) and production of reactive oxygen species [47]. The anti-inflammatory effect of polyphenol-rich fractions of roots may be attributed to the inhibition of various chemical mediators of inflammation like histamine and 5-HT during the initial phase [48] or inhibition of pro-inflammatory cytokines and Cox-2 synthesis and subsequent reduction in prostaglandin synthesis [49] and may be attributed to inhibition of neutrophils infiltration and stabilizing lysosomal enzymes which play key role in the development of inflammation [50]**.** The link between both anti-nociceptive activity and moderate anti-inflammatory effect observed with the extract has been indicated in non-steroidal anti-inflammatory drugs (NSAIDs). It is a well-established fact that NSAIDs exert their analgesic and anti-inflammatory activity by the inhibition of cyclooxygenase activity [51]. Based on the pharmacological tests results, flavonoid-rich fractions of roots of *Ximenia americana* L. showed both anti-nociceptive and anti-inflammatory activities.

CONCLUSION

Ximenia americana L., roots extract is considered to be moderate safe as its LD_{50} is 3270.8 mg/Kg body weight with antioxidant profile and many pharmacological activities include analgesic and anti-inflammatory actions that may be a result of its active biochemical

ingredients alkaloids, flavonoids, glycosides, saponin, and tannin. This study on this Olacaceae confirms that *Ximenia americana* L. is a good candidate for inflammatory diseases and possess antioxidant properties. Thus, which may explain the traditional basis of using this plant in the treatment of various diseases such as infectious, microbial infections and cardiovascular diseases in Burkina Faso. Further investigations are required to identify the active constituents of the plant extracts responsible for the antioxidant and pharmacological properties.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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Table 1.Antioxidant Properties of polyphenol-rich fractions of the root of *Ximenia americana* L.

flavonoid-rich fractions	Hydroxyl radical scavenging (IC_{50} mg/mL)	Hydrogen peroxide scavenging (IC_{50} mg/mL)	Total antioxidant assay (mg GAE/g)
Root fraction	0.31 ± 0.01^{b}	0.13 ± 0.01^{b}	55, 23±0.01
Quercetin	0.30 ± 0.01^{a}	----------	----------
A-tocopherol	----------	0.07 ± 0.62^{a}	----------

Values are Mean±SD (n=3), values in each column with different superscript letters (a,b) are significantly different at $P < 0.05$.

mgGAE/g= mg equivalent Gallic acid for 1g dried extracts;

Table 2: Analgesic activity of polyphenol-rich fractions of *Ximenia americana* L., in mice, using writhing test (n=6)

Treatment	Dose (mg/Kg b.wt.)	Protection percentage against writhing after 5 hours				
		1 hour	2 hour	3 hour	4 hour	5 hour
Control	0	0	0	0	0	0
aspirin (standard)	100	76	76	76	76	76
Polyphenol-rich fractions	200	51.2	51.2	51.2	51.2	51.2
	250	60.4	60.4	60.4	60.4	60.4
	300	73.14	73.14	73.14	73.14	73.14

Table 3: Effect of Polyphenol-rich fractions of *Ximenia americana* L., on hot plate reaction time in mice (n=6)

Treatment	Dose (mg/Kg b.wt)	1 hour	2 hours	3 hours
Control	0	9.2 ± 0.3^{e}	8.8 ± 0.5^{e}	7.6 ± 0.1^{e}
Acetyl Salicylic Acid (Standard)	100	21.7 ± 0.4^{a}	23.1 ± 0.7^{a}	23.8 ± 0.2^{a}
Polyphenol-rich fractions	200	15.7 ± 0.2^{d}	16.9 ± 0.5^{d}	18.7 ± 0.4^{d}
	250	17.1 ± 0.3^{c}	20.2 ± 0.7^{c}	20.8 ± 0.5^{c}
	300	19.4 ± 0.5^{b}	21.1 ± 0.3^{b}	21.9 ± 0.3^{b}

Values represent the mean ± S.E. of six animals for each group, values in each column with different superscript letters (a,b,c,d,e) are significantly different at P < 0.05.

Table 4: Anti-inflammatory effect of polyphenol-rich fractions in formalin-induced edema in the paw of rats (n=6).

Treatment	Dose (mg/Kg b. wt.)	Mean of right paw thickness in mm				
		Pretreatment	1 hour	2 hours	3 hours	4 hours
Control	0	0.64± 0.03^{b}	0.64± 0.1^{e}	0.64± 0.01^{e}	0.62±0.02^{e}	0.62± 0.02^{e}
Acetyl Salicylic Acid (Standard)	100	0.64± 0.01^{b}	0.49±0.03^{a}	0.50± 0.1^{a}	0.48± 0.02^{a}	0.47± 0.01^{a}
Polyphenol-rich fractions	200	0.63±0.01^{a}	0.55±0.03^{d}	0.56±0.01^{d}	0.53±0.1^{c}	0.54±0.01^{d}
	250	0.64±0.02^{b}	0.54±0.1^{c}	0.55±0.01^{c}	0.53±0.02^{d}	0.53±0.02^{c}
	300	0.63±0.002^{a}	0.52±0.02^{b}	0.52±0.01^{b}	0.51±0.1^{b}	0.51±0.01^{b}

Values represent the mean ± S.E. of six animals for each group, values in each column with different superscript letters (a,b,c,d,e) are significantly different at P < 0.05.