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Original Article

ANTIMICROBIAL PROFILES, ANTIDIARRHEAL AND ANTIPYRETIC CAPACITIES OF PHENOL ACID RICH-FRACTIONS FROM *XIMENIA AMERICA* L., (OLACACEAE) IN WISTAR ALBINO RATS

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ABSTRACT

Objective: The present study aim to evaluate antimicrobial and antidiarrheal activities of phenol acid-rich fractions of roots from *Ximenia americana* L., in animal models in order to confirm the ethnomedicinal use of *Ximenia Americana* L., roots.

Methods: *In vitro* antibacterial (Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) and time-kill studies) of phenol-rich fractions of roots from *Ximenia americana* L., was assessed using eight bacteria strains (Gram-negative). The anti-diarrheal activity was evaluated using castor using castor oil induced diarrhea whereas Anti-secretory activity was investigated using castor oil-induced enteropooling assay examined in animal models respectively.

Results: All test bacteria were susceptible to the phenol-rich fractions. Time-kill results showed that after 5 h exposition there was no viable microorganism in the initial inoculum. Moreover, phenol acid-rich fractions of roots (100-200 and 300 mg/kg, p. o.) produced dose-dependent and significant ($p<0.05$-0.001) protection of rats against castor oil-induced diarrhea, and delayed gastric emptying. Phenol acid-rich fractions of roots, dose-dependently and significantly ($p<0.05$-0.001) delayed the onset of castor oil induced diarrhea, decreased the frequency of defecation, and reduced the severity of diarrhea in the rats. Phenol acid-rich fractions caused dose-related inhibition of castor-oil-induced enteropooling in rats.

Conclusion: The data analysis indicates that the tested of phenol-rich fractions has significant effects when compared with the standard antibiotic. These results, therefore, justify the traditional use of *Ximenia americana* L. About antidiarrheal activity, the findings of this study indicate that phenol acid-rich fraction possesses antidiarrheal property in rats. These findings confirm the ethnomedicinal use of *Ximenia americana* L., roots valuable natural remedy for the treatment, management and/or control of diarrhea.

Keywords: *Ximenia americana* L., Phenol acid-rich fractions, Roots, Antimicrobial and antidiarrheal capacities

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INTRODUCTION

Traditional cures and plant-based remedies remain the main solution to health problems in many developing countries [1]. According [2], medicinal plants usefulness was estimated that over 80% of developing countries populations have resorted to traditional medicine. Plants have been used for medical treatments through much of human history, and such medicine is still widely practiced today. Plant parts such as leaves, seeds, bark, berries, sap, roots, or flowers are widely used for their medicinal property. Moreover, since the time immemorial, medicinal plants have played an invaluable role in the development of therapeutic agents. Currently, it is estimated that about 80% of people living in developing countries still rely on traditional medicine for their primary health care [3].

There are many medicinal plants that possess antidiarrheal activity with lesser side effects than the conventional drugs. Diarrheal disease is a leading cause of mortality and morbidity, especially in developing countries and is responsible for the death of millions of people each year. In developing countries, the majority of people almost exclusively use traditional medicines in treating all sorts of diseases, including diarrhea. WHO has encouraged studies for treatment and prevention of diarrheal diseases depending on traditional medical practices. This may reduce the mortality rate in developing countries due to diarrhea. Tannins, alkaloids, flavonoids, and terpenoids are the major constituents that are primarily responsible for the antidiarrheal activity of these medicinal herbs [4]. In Burkina Faso, a range of medicinal plants have been widely used for the management of diarrhea and related gastrointestinal disorders by traditional healers [5]. However, the safety and therapeutic potentials of some of these medicinal plants have not been validated yet. Among them, *Ximenia americana* L., is one of the popular medicinal plants being used in the traditional medicine.

Ximenia americana (Olacaceae) which is also known, is a shrub-like a plant found in abundance in the West African region. It usually flowers 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 an almond acid taste.

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 [5].

It is reported that 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 [6]. The roots are used for treating abdominal pains, dysentery, inflamed joints and mouth ulcers [7]. In the ethnobotanical claims, and particularly in Burkina Faso, this plant

and particularly the roots are used for treating abdominal pains, dysentery, diarrhea, as a poison antidote, and infectious diseases in children such as malaria, fever, pain, and also have antibacterial, anti-inflammatory, analgesic and hepatoprotective properties [5]. However, there is no scientific report available in support of the antidiarrheal and antipyretic activities of phenol acid of *Ximenia americana* L., roots. In this fact, the aim of the present work was evaluated the antidiarrheal and antipyretic activities of phenol acid of *Ximenia americana* L., roots for to provide a scientific basis for the traditional use of this ethnomedicinal plant in Burkina Faso.

MATERIALS AND METHODS

Plants material

The vegetable materials (Fresh roots) of *Ximenia america* L., (Olacaceae) were collected in August 2014 in Dedougou, 230 Km West of Ouagadougou, capital of Burkina Faso. The plants were botanically identified by Dr. Traoré Lassina from the plants Biology Department of the University of Koudougou. A voucher specimen (MR_12) was deposited at the Herbarium of the Laboratoire de Biologie et d'Ecologie Végétales, UFR/SVT of the University of Ouagadougou.

Bacterial strains and antibiotic

Microorganisms used in this study were isolated from clinical samples at Laboratory of the General Hospital of Ouagadougou in Burkina Faso. Commercially available antibiotic diffusion discs (Ciprofloxacin: 10 µg/disc) were purchased from Alkom Laboratories LTD. Clinical isolates were: *Shigella dysenteriae, Shigella boydii, Shigella flexneri, Salmonella thyphi, Klebsiella pneumonia, Klebsiella arogenes, Escherichia coli,* and *Proteus mirabilis*. The following microorganisms were all identified by the use of their biochemical profiles as recommended by the manual "Bactériologie Medical" [8].

Chemicals

Acetone, oil ether, dichloromethane were supplied by Fluka Chemie (Buchs, Switzerland) Mueller Hinton agar and broth, Mac Farland, saline solution (Nacl), dimethylsulfoxide (DMSO) were purchased from Shijiazhuang. Pharma. Group. Zhangnua (China). Metamezole sodium (Sigma), loperamide (Sigma) and castor oil (Sigma) were purchased from Sigma (St. Louis, USA).

Animals handling

Swiss NMRI mice (25–30 g) of both sexes were used for acute toxicity, and Wister albino rats (180–240 g) of both sexes were used for antidiarrhea and antipyretic activities. 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. *In vivo* studies were carried out in accordance with guidelines for the care of laboratory animals and ethical guidelines for the investigation of experimental pain in conscious animals [9].

Preparation of aqueous acetone extract for acute toxicity study

The field-grown fresh samples (roots) were washed with tap water followed by distilled water to remove the adhering dust particles. After blotting, the samples were air dried in the 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 a vacuum rotary evaporator at 40 °C under reduced pressure. Extracts were weighed and stored at 4 °C for further analysis.

Fractionation of phenol acid-rich fractions for antidiarrhea and antipyretic activities

Fifty grams (50g) of powdered plant material was extracted with 80% aqueous acetone (500 ml) in 1/10 ratio (w/v) for 24 h under mechanic agitation (SM 25 shaker, Edmund BÜHLER, Germany) at room temperature. After filtration, acetone was removed under reduced pressure in a rotary evaporator (BÜCHI, Rotavapor R-200, Switzerland) at approximately 40 °C. The aqueous extracts were subjected to sequential liquid-liquid extraction with oil either to remove chlorophyll and other low molecular weight compounds and dichloromethane. This fraction (dichloromethane fraction) was then collected and concentrated to dryness under reduced pressure to obtain phenol acids (dichloromethane fraction). The fraction was freeze-dried by Telstar Cryodos 50 freeze-dryer. The fraction residues were packed in waterproof plastic flasks and stored at 4 °C until use. For the tests, the lyophilized sample was dissolved with 10% DMSO in water at the desired concentration [5].

In vitro antimicrobial profile of phenol acid-rich fractions

Preparation of inocula

The susceptibility tests were performed by the Mueller Hinton agar-well diffusion method [10]. The bacterial strains grown on nutrient agar at 37 °C for 18 h were suspended in a saline solution (0.9%, w/v) NaCl and adjusted to a turbidity of 0.5 Mac Farland standard (108CFU/ml). To obtain the inocula, these suspensions were diluted 100 times in Muller Hinton broth to give 106colony forming units (CFU)/ml [11].

Preparation of discs

The stock solutions of phenol acid-rich fractions of roots from *Ximenia americana* L., was dissolved in 10% dimethylsulfoxide (DMSO) in water [12] at a final concentration of 100 µg/ml after a serial two-fold dilution. Each stock solution of phenol acid-rich fractions was sterilized by filtration through 0.22 µm sterilizing Millipore express filter. The sterile discs (6 mm) were impregnated with 10 µL of the sterile phenol acid-rich fractions. Negative controls were prepared using discs impregnated with 10% DMSO in water and commercially available antibiotic diffusion discs (Ciprofloxacin) from Alkom Laboratories LTD) were used as positive reference standards (10 µg/disc) for all bacterial strains.

Disc-diffusion assay

Petri plates (9 cm) were prepared with 20 ml of a base layer of molten Mueller Hinton agar (DIFCO, Becton Dickinson, USA). Each Petri plate was inoculated with 15 µl of each bacterial suspension (10^6 CFU/ml). After drying in a sterile hood, 6 mm diameter discs soaked with 10 µl of the different phenol acid-rich fractions dilutions were placed on the agar.

Discs containing Ciprofloxacin (10 µg/disc) were used as positive controls and 10% DMSO was used as a negative control. The plates were incubated for 24 h at 37 °C and at 44 °C for *Escherichia coli* because this bacterium is thermo-resistant. The diameters of the inhibition zones were evaluated in millimeters. The phenol acid-rich fractions inducing inhibition zone ≥ 3 mm around disc were considered as antibacterial. All tests were performed in triplicate, and the bacterial activity was expressed as the mean of inhibition diameters (mm) produced [13].

Micro-well dilution assay

Minimum inhibitory concentration (MIC) was determined by the microdilution method in culture broth as recommended by [14]. Eight serial two-fold dilutions of phenol acid-rich fractions were prepared as described before, to obtain a final concentration range of 400 to 3.125µg/ml. The 96-well micro-plates (NUNC, Danemark) containing 100 µL of Mueller Hinton (MH) broth were used. For each bacteria strain, three columns of eight wells to the micro-plate were used. Each well has getting: the culture medium+phenol acid-rich fractions+inoculum (10 µl of inocula) and INT (50 µl; 0.2 mg/ml). The plates were covered and incubated at 37 °C and at 44 °C for *Escherichia coli* for 24 h. All tests were performed in triplicate and the bacterial activity was expressed as the mean of inhibitions produced. Inhibition of bacterial growth was judged by rose or yellow colour. The MIC was defined as the lowest concentration of extract or fraction of extract at which no colony was observed after incubation. So, the MIC was defined as the lowest concentration at which no visible growth was observed.

Minimal bactericidal concentration (MBC)

Minimum bactericidal concentration (MBC) was recorded as a lowest phenol acid-rich fractions concentration killing 99.9% of the

bacterial inocula after 24 h incubation at 37 °C. Each experiment was repeated at least three times. MBC values were determined by removing 100 µl of bacterial suspension from subculture demonstrating no visible growth and inoculating nutrient agar plates. Plates were incubated at 37 °C for a total period of 24 h.

The MBC is determined with the wells whose the concentrations are ≥ MIC [13, 15]. The MBC was determined in Mueller Hinton (MH) agar (DIFCO, Becton Dickinson, USA) medium.

Evaluation of bactericidal and bacteriostatic capacity

The action of an antibacterial on the bacterial strains can be characterized with two parameters such as Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC). According to the ratio MBC/MIC, we appreciated antibacterial activity. If the ratio MBC/MIC = 1 or 2, the effect was considered as bactericidal but if the ratio MBC/MIC = 4 or 16, the effect was defined as bacteriostatic [16].

Time-kill assay

A bactericidal effect is defined as a 3Log decrease in the CFU/ml or a 99.9% kill over a specified time [17]. The definition of kill for this study has been used as per [18]. Kill-time can be determined at 6h [19]. A 90% kill at 6 h is equivalent to a 99.9% kill at 24 h [20]. In this study, the kill measurement was determined by the actual reduction in viable counts at 6 h for each isolate. Bacteria strains possessing the bactericidal effect were chosen to perform the time-kill assay. Thus, 0.5 Mac Farland standards suspensions of the microorganisms were diluted to have 50 ml of approximately 10^6 CFU/ml in nutriment broth, and the concentration corresponding to the best MIC, were respectively added to the corresponding culture. The cultures were incubated at 37 °C. At 0, 1, 2, 3, 4, 5 and 6 h, an aliquot of 100 µl was removed and diluted with 10 ml sterile broth. The obtained suspension was used to inoculate 9 cm diameter Petri plates with a sterile, nontoxic cotton swab on a wooden applicator as indicated before in the agar-well diffusion assay. After 24 h incubation at 37 °C, the viability of the microorganisms was evaluated by the presence of colonies on the plates. The experiment was carried out twice following [21] method with light modifications.

Biological activities

Acute toxicity study in mice of aqueous acetone extract

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 of 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 d. The number of survivors after the 14 d periods was noted. The toxicological effect was assessed on the basis of mortality for 14 d, which was expressed as the median lethal dose (LD50) (Lethal Dose 50) was estimated from the regression of log-probit mortality rate [22].

Antipyretic activity test

The method described by [23] was used for studying the antipyretic effect of phenol-acid-rich fractions of roots from *Ximenia americana* L. Thirty rats of both sex weighing 180-240 grams were divided into five groups of six rats in each. All rats were made hyperthermic by subcutaneous injection of brewer's yeast in physiological saline in a dose of 1.5 g/kg b. wt. After 17 h, the initial body temperature of each rat was measured rectally using a medical thermometer. The first group was kept as control; the second group was given metamezole sodium 50 mg/kg b. wt. as a standard antipyretic. The third, fourth and five groups were used to reveal the antipyretic effect of the tested phenol-acid-rich fractions of roots from *Ximenia americana* L., when given orally in a dose of 200, 250 and 300 mg/kg body weights, respectively. The body temperature of each rat was then recorded every hour for 3 successive hours.

Antidiarrheal activity test

Castor oil-induced diarrhea

The method of Awouters was followed for carrying out the study [24]. Rats of both sexes were fasted (without food, but water) for 18 h. The selected rats for castor oil-induced diarrheal test were divided into five groups (n=10). Animals in the positive control groups received loperamide (5 mg/kg) body weight, and the control group received 0.9% normal saline (2 ml/kg) orally while those in the test groups received phenol-rich acid fractions at the doses of 100, 200, and 300 mg/kg body weight. After 1 h, all groups received castor oil 1 ml each orally. Then they were placed in cages lined with adsorbent papers and observed for 4h for the presence of characteristic diarrheal droppings. 100% was considered as the total number of feces of the control group the activity was expressed as % inhibition of diarrhea. The percent (%) inhibition of defecation was measured.

The percentage inhibition of defecation was:

$$\% \text{ inhibition} = \frac{[A- B]}{A} \text{ x100}$$

Where *A* is mthe ean number of defecation time caused by castor oil and *B* is mean number of defecation time caused by drug or fraction.

Castor oil-induced enteropooling assay

Castor oil-induced enteropooling test helps to determine the prevention of fluid accumulation ability of fraction. The test animals were fasted (without food, but water) for 18h prior to the commencement of the experiment. They were divided into five groups (n=10). Animals in the positive control groups received loperamide (5 mg/kg) body weight, and the control group received 0.9% normal saline (2 ml/kg) orally while those in the test groups received phenol acid-rich fractions at the doses of 100, 200, and 300 mg/kg body weight. Immediately after these administrations, 1 ml of castor oil was administered orally to each rat in all the groups. After 30 min, the rats were sacrificed. At half an hour, all rats were sacrificed, and the small intestine from (the pylorus to the caecum) was isolated. The intestinal contents were collected by milking into a graduated tube, and their volume was measured [25]. The inhibition of intestinal content was computed according to the following expression:

$$\text{Mass of intestinal fluid (g)} = \frac{\text{(Control-Treatment)}}{\text{Control}} \text{ x 100}$$

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 *t*-test using Prism 4 software. The level of significance was accepted at $p \leq 0.05$.

RESULTS AND DISCUSSION

Antimicrobial profiles

In this present study, ten bacteria strain (Gram-negative and Gram-positive bacteria) were used. The antibacterial assays were performed by the agar-well diffusion and the broth microdilution methods; so that they could be qualified and quantified by inhibition zone diameters, Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) and Time-kill assays. One noticed that the susceptibility of the bacteria to the phenol acid-rich fractions on the basis of inhibition zone diameters varied according to the microorganism, the results are reported in (table 1). There is a significant variation in the diameters of inhibition zone values (DIZ) of phenol acid-rich fractions (table 1).

Table 1: Inhibition zone diameters (mm) recorder in agar well diffusion assay using phenol acid-rich fractions from *Ximenia americana* and Ciprofloxacin (10µg/disc)

Microorganisms	Ciprofloxacin (10µg)	Phenol acid-rich fractions
Proteus mirabilis	21.66±0.58	25.66±1.53
Shigella dysenteria	22.00±1.00	29.66±0.58
Shigella boydii	22.66±0.58	21.33±4.04
Shigella flexneri	nd	23.66±4.93
Salmonella thyphi	23.66±0.58	29.00±1.00
Klebsiella pneumonia	20.66±0.54	nd
Klebsiella arogenes	19.66±0.58	16.00±1.00
Escherichia coli	18.00±0.58	20.00±1.00

The results are the means of a number of the colonies±standard deviations.

nd: no detected activity

As for the micro-well dilution assay (MIC) and Minimum bactericidal concentration (MBC) of phenol acid-rich fractions, result varied according to the microorganism (table 2). The MIC values were ranged from 25 to 100 µg/ml and for the MBC values were ranged from 50 to 400 µg/ml. The bactericidal and bacteriostatic effect of phenol acid-rich fractions was determined using the ratio MBC/MIC (table 2).

Table 2: Bacteriostatic (-) and Bactericidal (+) effects of phenol acid-rich fractions of roots from *Ximenia americana* L

Microorganisms	MIC (µg/ml)	MBC (µg/ml)	Effects
Proteus mirabilis	25±0.00	50±0.00	**+**
Shigella dysenteria	50±00	100±0.00	**+**
Shigella boydii	25±0.00	50±0.00	**+**
Shigella flexneri	25±0.00	50±0.00	**+**
Salmonella thyphi	25±0.00	50±0.00	**+**
Klebsiella arogenes	100±0.00	400±0.00	**-**
Escherichia coli	100±0.00	400±0.00	**-**

The results are the means of a number of the colonies±standard deviations, **+:** bactericidal effect,**-:** bacteriostatic effect**.**

Table 3: Viability of microorganisms after 6 h exposure of phenol acid-rich fractions of

Microorganisms	Time-kill (h)						
	0h	1h	2h	3h	4h	5h	6h
Proteus mirabilis	+(UC)	+(UC)	+(UC)	+(UC)	147	12	**-**
Shigella dysenteria	+(UC)	+(UC)	+(UC)	+(UC)	102	10	**-**
Shigella boydii	+(UC)	+(UC)	+(UC)	+(UC)	37	-	**-**
Shigella flexneri	+(UC)	+(UC)	+(UC)	+(UC)	08	-	**-**
Salmonella thyphi	+(UC)	+(UC)	+(UC)	+(UC)	-	-	**-**

Roots from *Ximenia americana* L, the results are the means of a number of the colonies±standard deviations, **+:** For the presence of the colonies, **-:** for the absence of colonies

Concerning the time-kill assay of phenol acid-rich fractions (table 3), the results showed that after 5 h exposition there was no viable microorganism in the initial inoculums. The effect of phenol acid-rich fractions was faster on *Salmonella thyphi* than the other bacteria strains (table 3).

UC: uncountable

Medicinal plants have long been utilized as a source of therapeutic agents worldwide [26]. Previous studies have been carried out in different parts of the globe to extract plant products for screening antibacterial activity [27]. Plants produce highly bioactive molecules that allow them to interact with other organisms in their environment. Many investigators have evaluated the bioactivity of plant extracts and the isolated constituents against the serious infectious organisms [28]. In Africa, for the treatment of several infections, indigenous medicinal plants are often the only means [29]. Infectious due to multidrug-resistant microorganisms, pose an important clinical problem. Many of the bacterial strains are resistant to the standard antibiotic (Ciprofloxacin etc.) comparatively to the phenol acid-rich fractions. One could say that the metabolites have been shown to be responsible for the therapeutic activity of plants [30]. According to a recent study, *Ximenia americana* L. contains saponins, glycosides, flavonoids, tannins, phenolics, alkaloids, quinones, and terpenoids types [5]. The natural products were found to possess promising antimicrobial, and the metabolites have been shown to be responsible for the therapeutic activity of plants [31].

The data analysis indicates that the tested polyphenol extract showed the significant results when compared with the standard antibiotic. Indeed, the antibacterial activity profile of the isolated constituents (polyphenols) when compared with antibiotic effects shows that the activity depends on the pure form of the constituents. This may be due to the fact that the bioactive constituents such as polyphenol compounds were responsible for the antimicrobial activity. In effect, some previous studies showed that polyphenolic compounds cause inhibition of a wide range of microorganisms. Phenol is well known as a chemical antiseptic [32].

In addition, Phenolic and terpenic antimicrobial activities are well documented [13]. Polyphenols, such as tannins and flavonoids, are important for antibacterial activity [29]. The antimicrobial activity of flavonoids is due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall while that of tannins may be related to their ability to inactivate microbial adhesions, enzymes and cell envelop proteins [33]. Then, Polyphenols, such as tannins and flavonoids, are important antibacterial activity [34]. Also, polyphenols have a good antimicrobial activity against the biggest number of bacterial as such *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhimurium* [35].

Our data noticed that certain bacteria are resistant to the standard antibiotic compared to the phenol acid-rich fractions. The resistance of these bacteria towards antibacterial substances is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules and is also associated with the enzymes in periplasme space, which are capable of breaking down the molecules introduced from outside [36]. The resistances of the bacteria to the current antibiotics necessitate the further studies on the isolated constituents to find out their safety and efficacy profile. This highlights the continuous interest in laboratory screening of medicinal plants, not only to determine the scientific rationale for their usage but also to discover new active principles. Briefly, our results indicate good *in vitro* antimicrobial activities.

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. At acute toxicity level, data indicated that extract of *Ximenia americana* L., can be considered as weakly poisonous. According to Diez [21], pharmacological substances whole LD50 with less than 5 mg/kg body weight are considered not toxics; those with a LD50 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 are classified in the range of highly toxic substances. In this fact, if we refer to this classification, we could say that the extract of *Ximenia americana* L., is moderately toxic and would be regarded as being safe or of low toxicity [22]. This is an indication that the aqueous acetone extract of *Ximenia americana* L., has negligible level of toxicity when administered orally.

Antipyretic effects

The antipyretic effect of phenol-acid rich fractions of roots was studied in hyperthermic rats using brewer's yeast and data were recorded in (table 4). The subcutaneous injection of brewer's yeast suspension markedly elevated rectal temperature after 17 h of administration. Oral administration of phenol-acid rich fractions of roots induced significant antipyretic activity at a dose of 300 mg/kg b. wt., while the low dose 250 mg/kg failed to decrease the raised body temperature. The effect of the high dose of 300 mg/kg b. wt., nearly similar to that of the standard metamezole sodium (50 mg/kg b. wt.) after 3 h. Significance was indicated by lowering the body temperature were (36.66±0.14 and 36.85±0.11) after 2 h of administration of the standard drug and root fractions, respectively and at 3 h (36.62±0.1 and 36.81±0.15) when compared to the control non-treated group.

Table 4: Antipyretic effect of phenol acid-rich fractions of roots from *Ximenia americana* L., hyperthermic rats (mean±SE, N=6)

Treatment	Dose (mg/Kg b. wt.)	Rectal temperature (°C)			
		Before treatment	1 h	2 h	3 h
Control	0	37.69±0.15^{b}	37.63±0.1^{c}	37.57±0.14^{c}	37.68±0.2^{e}
Metamezole sodium (Standard)	50	37.50±0.11^{a}	36.55±0.12^{a}	36.66±0.14^{a}	36.62±0.10^{a}
phenol acid-rich fractions	200	37.76±0.1^{c}	37.72±0.11^{d}	37.76±0.11^{e}	37.62±0.11^{d}
	250	37.79±0.11^{d}	37.76±0.1^{e}	37.71±0.14^{d}	37.54±0.1^{c}
	300	37.83±0.2^{e}	37.10±0.2^{b}	36.85±0.11^{b}	36.81±0.15^{b}

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

About antipyretic potential, one could say that antipyretic activity may be attributed to the presence of phytochemical constituents such as β-sitosterol triterpenes, flavonoids, saponins, glycosides, tannins, and alkaloids [37]. The β-sitosterol reduces PG and leukotrienes synthesis and in turn shows anti-inflammatory and antipyretic activity by inhibiting the pro-inflammatory cytokines and TNF-α [38, 39]**.**

Antidiarrheal affects

Castor oil-induced diarrheal model

In case of castor-oil induced diarrheal model, phenol acid-rich fractions at the doses of 100, 200, and 300 mg/kg, exerted statistically significantly (p<0.001) decreased and dose-dependent inhibition of the total number of diarrheal feces compared with the control groups (table 5).

Castor oil-induced enteropooling assay

The administration of castor oil resulted in intestinal fluid volumes and weights of the intestinal contents of the rats (from the pylorus to the caecum) were statistically significantly (p<0.001) and dose-dependently reduced by both phenol acid-rich fractions at the doses of 200, and 300 mg/kg compared to the control group (table 6).

Table 5: The antidiarrheal effect of phenol acid-rich fractions of roots from *Ximenia americana* L., on castor oil-induced diarrhea models in rats

Treatment	Dose (mg/kg)	Total number of feces	% of inhibition	Total number of diarrheal feces	% of inhibition
Control	2 ml/kg	20.17±0.54	-	14.12±0.11	-
Loperamide	5	9.02±0.67***	55.28	6.22±0.54***	55.94
	100	12.53±0.10*	37.87	7.89±0.37*	44.12
phenol acid-rich	200	10.21±0.37***	49.38	5.80±0.11***	58.92
fractions	300	8.02±0.63***	60.23	4.78±0.23***	66.15

Each value is presented as the mean±SEM (n=10), phenol acid-rich fractions of roots from *Ximenia americana* L., ***p<0.001 compared with the control group (Dunnett's Test), **p<0.01 compared with the control group (Dunnett's Test), *p<0.05 compared with the control group (Dunnett's Test).

Table 6: The Anti-secretory effect of phenol acid-rich fractions of roots from *Ximeniaamericana* L., on castor oil-induced enteropooling assay in rats

Treatment	Dose (mg/kg)	Volume of intestinal content (mL)
Control	2 ml/kg	2.18±0.11
Loperamide	5	0.63±0.45**
phenol acid-rich fractions	100	1.12±1.63
	200	0.96±0.53*
	300	0.78±0.10**

Each value is presented as the mean±SEM (n=10), ***p<0.001 compared with the control group (Dunnett's Test), **p<0.01 compared with the control group (Dunnett's Test), *p<0.05 compared with the control group (Dunnett's Test)

Phenol acid-rich fractions of roots were evaluated for its antidiarrheal potential against castor oil induced diarrhea model in rats. In respect to demonstrate the probable mechanisms, the anti-secretory effect was also tested using castor oil induced enteropooling assay in rats. Loperamide, the standard drug, generally produces rapid and sustained inhibition of the peristaltic reflex through depression of longitudinal and circular muscle activity. It is well known to reduce the daily fecal volume and decreases intestinal fluid and electrolyte loss. The antidiarrheal activity of, phenol acid-rich fractions of roots was comparable to the standard drug, loperamide, which at present is one of the most efficacious and widely employed antidiarrheal drugs. In our investigation, loperamide proved the claims by causing effectively antagonizes diarrheal activity induced by castor oil. It also decreases the number of diarrheal feces. It is widely known that castor oil is metabolized into ricinoleic acid in the gut, reduce active Na+and K+absorption and decrease Na+/K+ATPase activity in the small intestine and colon. As with other laxatives, castor-oil changes the electrolyte permeability of the intestinal mucosal membrane [40]. In the castor-oil induced diarrhea model in rats, phenol acid-rich fractions of roots showed dose-dependent manner and significant (p<0.001) reduced in the number of feces and the number of diarrheal feces with the percentage of inhibition (table 5). In the castor oil-induced enteropooling assay, castor oil produces permeability changes in the intestinal mucosal membranes to water and electrolytes resulting in fluid and watery luminal content that flows rapidly through the small and large intestines. The Anti-secretory effect of phenol acid-rich fractions of roots significantly (p<0.001) inhibited the castor oil-induced intestinal fluid accumulation. The intestinal content was, however, more viscous in extract treated than in control rats.

CONCLUSION

The present study thus proves that phenol acid-rich fractions of roots from *Ximenia americana* L., possesses on a one hand significant antimicrobial activities to treat infectious diseases due to multi-resistant bacterial strains. On the other hand, the study also proves the anti-diarrheal activity of phenol acid-rich fractions of roots from *Ximenia americana* L., due to its inhibitory effect among castor oil induced diarrhea, and castor oil induced enteropooling assay. The study though supports the traditional claim; further studies are needed to identify the chemical constituents that are responsible for the antidiarrheal effect. In conclusion, it appears that the phenol acid-rich fractions of roots from *Ximenia americana* L., possesses antidiarrheal, anti-motility and anti-secretory activities and thus can be developed for use in the treatment of diarrhea.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

All the authors had equal contribution in the manuscript and declared no conflict of interests.

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