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

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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 diarrheal. 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

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 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

ABSTRACT

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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 antipryptic activities of phenol acid of *Ximenia americana* L. roots. In this fact, the aim of the present work was evaluated the antidiarrheal and antipryptic 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 *Plants material* were purchased from 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**

Bacterial strains and antibiotic were recommended by the manual "Bactériologie Medical" [8].

**Acetone, oil ether, dichloromethane** were supplied by Fluka Chemie Swiss NMRI mice (25 – 30 g) of both sexes were used for acute toxicity. Animals handling were purchased from Sigma (St. Louis, USA).

**Preparation of inocula**

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 different 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.

**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 had: 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 inhibition diameters (mm) produced [15].

**Fractionation of phenol acid-rich fractions for antidiarrheal and antipryptic 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 mechanical 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.

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 mechanical 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 Teklar 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].

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**Minimum bactéricidal concentration (MBC)**

Minimum bactéricidal 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 10⁵ CFU/ml in nutrient 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; 2.5; 3; 4; 5 and 6g/kg. The general behavior of the mice was observed for 120 min after the method with light modifications.

**Antipyrretic activity test**

The method described by [23] was used for studying the antipyrretic effect of 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 Aowuters was followed for carrying out the study [24]. Rats of both sexes 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 absorbent papers and observed for 4 h 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:

\[
\%\text{ inhibition} = \frac{A - B}{A} \times 100
\]

Where A is the mean 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:

\[
\text{Mass of Intestinal fluid (g)} = \frac{(\text{Control-Treatment}) \times 100}{\text{Control}}
\]

**Statistical analysis**

The data were expressed as means±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≤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).
natural products were found to possess promising antimicrobial, tannins, phenolics, alkaloids, quinones, and terpenoids types [5]. The results are the means of a number of colonies±standard deviations, +: bactericidal effect,-: bacteriostatic effect.

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

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Ciprofloxacin (10µg)</th>
<th>Phenol acid-rich fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteus mirabilis</td>
<td>21.6±0.58</td>
<td>25.6±0.53</td>
</tr>
<tr>
<td>Shigella dysenteria</td>
<td>22.0±1.00</td>
<td>29.6±0.58</td>
</tr>
<tr>
<td>Shigella boydi</td>
<td>22.6±0.58</td>
<td>21.3±4.04</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>nd</td>
<td>23.6±4.93</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>23.6±0.58</td>
<td>29.0±1.00</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>20.6±0.54</td>
<td>nd</td>
</tr>
<tr>
<td>Klebsiella arogenes</td>
<td>19.6±0.58</td>
<td>16.0±1.00</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>18.0±0.58</td>
<td>20.0±1.00</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MIC (µg/ml)</th>
<th>MBC (µg/ml)</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteus mirabilis</td>
<td>25±0.00</td>
<td>50±0.00</td>
<td>+</td>
</tr>
<tr>
<td>Shigella dysenteria</td>
<td>50±0.00</td>
<td>100±0.00</td>
<td>+</td>
</tr>
<tr>
<td>Shigella boydi</td>
<td>25±0.00</td>
<td>50±0.00</td>
<td>+</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>25±0.00</td>
<td>50±0.00</td>
<td>+</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>25±0.00</td>
<td>50±0.00</td>
<td>+</td>
</tr>
<tr>
<td>Klebsiella arogenes</td>
<td>100±0.00</td>
<td>400±0.00</td>
<td>-</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>100±0.00</td>
<td>400±0.00</td>
<td>-</td>
</tr>
</tbody>
</table>

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

Table 3: Viability of microorganisms after 6 h exposure of phenol acid-rich fractions of roots from Ximenia americana L

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Time-kill (h)</th>
<th>0h</th>
<th>1h</th>
<th>2h</th>
<th>3h</th>
<th>4h</th>
<th>5h</th>
<th>6h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteus mirabilis</td>
<td>+ (UC)</td>
<td>+ (UC)</td>
<td>+ (UC)</td>
<td>+ (UC)</td>
<td>147</td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Shigella dysenteria</td>
<td>+ (UC)</td>
<td>+ (UC)</td>
<td>+ (UC)</td>
<td>+ (UC)</td>
<td>102</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Shigella boydi</td>
<td>+ (UC)</td>
<td>+ (UC)</td>
<td>+ (UC)</td>
<td>+ (UC)</td>
<td>37</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>+ (UC)</td>
<td>+ (UC)</td>
<td>+ (UC)</td>
<td>+ (UC)</td>
<td>08</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>+ (UC)</td>
<td>+ (UC)</td>
<td>+ (UC)</td>
<td>+ (UC)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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 typhi 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, LD₅₀ 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 alkaloids [37]. The *β*-sitosterol reduces PG and leukotrienes synthesis in vitro antimicrobial activities.

### Antidiarrheal effects

**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 whole LD₅₀ with less than 5 mg/kg body weight are considered not toxic; 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)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg b. wt.)</th>
<th>Rectal temperature (°C)</th>
<th>Before treatment</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>37.69±0.1*</td>
<td>37.32±0.1*</td>
<td>37.5±0.14*</td>
<td>36.6±0.2*</td>
<td></td>
</tr>
<tr>
<td>Metamizole sodium (Standard)</td>
<td>50</td>
<td>37.5±0.11*</td>
<td>37.72±0.11*</td>
<td>37.6±0.11*</td>
<td>36.6±0.10*</td>
<td></td>
</tr>
<tr>
<td>phenol acid-rich fractions</td>
<td>200</td>
<td>37.76±0.1**</td>
<td>37.76±0.1**</td>
<td>37.77±0.14*</td>
<td>36.6±0.10*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>37.79±0.1**</td>
<td>37.71±0.14*</td>
<td>37.74±0.1*</td>
<td>36.6±0.10*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>37.83±0.2</td>
<td>37.10±0.2*</td>
<td>36.85±0.11*</td>
<td>36.81±0.15*</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Total number of feces</th>
<th>% of inhibition</th>
<th>Total number of diarrheal feces</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2 ml/kg</td>
<td>20.17±0.54</td>
<td>-</td>
<td>14.12±0.11</td>
<td>-</td>
</tr>
<tr>
<td>Loperamide</td>
<td>5</td>
<td>9.02±0.7***</td>
<td>55.28</td>
<td>6.2±0.54***</td>
<td>55.94</td>
</tr>
<tr>
<td>phenol acid-rich</td>
<td>100</td>
<td>12.5±0.10*</td>
<td>78.77</td>
<td>7.89±0.11</td>
<td>44.12</td>
</tr>
<tr>
<td>fractions</td>
<td>200</td>
<td>10.21±0.37***</td>
<td>49.38</td>
<td>5.80±0.11***</td>
<td>58.92</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>8.02±0.63***</td>
<td>60.23</td>
<td>4.78±0.23***</td>
<td>66.15</td>
</tr>
</tbody>
</table>

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 (Dunnnett’s Test). **p=0.01 compared with the control group (Dunnnett’s Test), *p=0.05 compared with the control group (Dunnnett’s Test).

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Volume of intestinal content (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2 ml/kg</td>
<td>2.18±0.11</td>
</tr>
<tr>
<td>Loperamide</td>
<td>5</td>
<td>0.63±0.45**</td>
</tr>
<tr>
<td>phenol acid-rich</td>
<td>100</td>
<td>1.12±1.63</td>
</tr>
<tr>
<td>fractions</td>
<td>200</td>
<td>0.96±0.53**</td>
</tr>
</tbody>
</table>

Each value is presented as the mean±SEM (n=10), ***p=0.001 compared with the control group (Dunnnett’s Test). **p=0.01 compared with the control group (Dunnnett’s Test), *p=0.05 compared with the control group (Dunnnett’s Test).
Phenol acid-rich fractions of roots were evaluated for its anti-diarrheal 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 anti-diarrheal 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 anti-diarrheal 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 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 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 anti-diarrheal effect. In conclusion, it appears that the phenol acid-rich fractions of roots from Ximenia americana L., possesses anti-diarrheal, anti-motility and anti-secretory activities and thus can be developed for use in the treatment of diarrhea.

AUTHORS CONTRIBUTIONS

All the authors 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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