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Antidiarrheal and antimicrobial profiles extracts of the leaves from *Trichilia emetica* Vahl. (Meliaceae)

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PEER REVIEW

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Comments

It is a good study in which the authors justify the traditional uses of *Trichilia emetica* and good antidiarrhoeal and antimicrobial activities of EAF from *Trichilia emetica*. The manuscript was well done. The authors described their study thoroughly. The writing style was easy to comprehend.
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ABSTRACT

Objective: To evaluate anti-diarrhoeal and antimicrobial activities of the bioactive fraction of *Trichilia emetica* in order to provide a scientific basis for the management of gastroenteritis in Burkina Faso.

Methods: To do this, polyphenols content of extract and fractions were investigated. Folin-Ciocalteu, $AlCl_3$ methods and tannic acid respectively were used for polyphenols content. The *in vivo* antidiarrhoeal activity was done using Swiss albino mice of both sexes. *In vitro* antimicrobial activity (disc-diffusion assay, minimum inhibitory concentration and minimum microbicidal concentration or minimal bactericidal concentration) was assessed using seven bacteria strains (Gram-negative and Gram-positive).

Results: About our study, it was found that ethyl acetate fraction effective attenuation factior (EAF) elicits the higher total phenolics and total flavonoids contents compared to the extracts of leaves of *Trichilia emetica*. EAF of *Trichilia emetica* Vahl., has positive effects in a dose dependent manner against diarrhoea induced by castor oil in experimental mice. The bioactive fraction also showed good antimicrobial activity against all tested Gram-negative and Gram-positive bacteria strains. It was shown that experimental bacteria strains were more sensitive to the EAF effect compared to the ciprofloxacin.

Conclusions: The obtained results allow justifying the traditional uses of *Trichilia emetica* and possess good antidiarrhoeal and antimicrobial activities of EAF from *Trichilia emetica*. Results of the present study have clearly supported the utilization of *Trichilia emetica* in Burkina Faso traditional medicine.

KEYWORDS
Trichilia emetica, Bioactive fraction, Antidiarrhoeal and antimicrobial profiles

1. Introduction

Diarrhoea is one of the main water-borne diseases endemic in many regions of the world and considered to be the major health threats to the world populations, both in tropical and subtropical poor countries[1]. The disease is responsible for morbidity and

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mortality in developing countries leading to the death of millions of people each year[2]. Diarrhoea may be defined as a situation in which an adult daily stool exceeds 200 g and contains 60%-95% water[3].

The major causative agents of diarrhea in human beings include various enteropathogens like *Shigella flexneri, Escherichia coli, Staphylococcus aureus, Salmonella typhi* and *Candida albicans*[4-6]. For the treatment and management of diarrhea in developing countries, the world health organization has constituted a diarrhoea disease control programme. This programme includes studies of traditional medicine practices together with the evaluation of health education and prevention approaches (Syder medicine), which is predominantly based on herbal products[7]. The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics have led researchers to investigate the antimicrobial activity of herbal extracts. Plants containing flavonoids, terpenoids, steroids, phenolic compounds and alkaloids have been reported to have antimicrobial activity[8].

In developing countries, majority of people use traditional medicines in the treatment of various diseases, including diarrhoea. Therefore, a search for plants with antidiarrhoeal and antimicrobial activities that could be used against any type of diarrhoeal disease is of high interest. A range of medicinal plants with antidiarrhoeal and antimicrobial properties have been widely used by traditional healers. Among these plants, *Trichilia emetica* which is widely distributed throughout tropical Africa enjoys a number of ethnomedical uses in Burkina Faso[9]. In traditional medicine, *Trichilia emetica* (Meliaceae) is used to treat various diseases like diarrhea, abdominal tumours, asthma, epilepsy, eye infections, fever, inflammation, leprosy, oedema, paralysis, rheumatic pain, skin diseases, urinary disorders, ulceration and vomiting, anthelmintic, antihypertensive, aphrodisiac, diuretic, remedy against poisons and tonic to the brain, liver and spleen[9,10]. Some pharmacological properties such as antioxidant properties and bioavailability of free and bound phenolic acids as well as antibacterial activity of this plant have been investigated before[10,11].

Regardless of the wide use of this plant species as a popular medicine for its antidiarrheal properties, amazingly no research has been carried out to evaluate the *in vivo* antidiarrheal activity of the leaves extract from *Trichilia emetica*. The present study was thus carried out to investigate the polyphenolic constituents, antioxidant and *in vitro* anti-inflammatory activities of aqueous acetone extract and fractions of *Trichilia emetica* and then evaluate antidiarrheal and antimicrobial profiles of bioactive fraction of the leaves from *Trichilia emetica* evaluating the traditional folklore medicinal use of the plant.

2. Materials and methods

2.1. Chemicals

To carry out different bio-activities, we used solvents and various classic reagents. All reagents were of analytical grade. Folin-Ciocalteu reagent, carbonate de sodium (Na_2CO_3), gallic acid, quercetin and trichlorure d'ammonium ($AlCl_3$) were purchased from Sigma-Aldrich Chemie (Steinheim, Germany); acetone, methanol and ethanol were supplied by Fluka Chemie (Buchs, Switzerland). *p*-Iodonitrotetrazolium chloride was purchased from Sigma-Aldrich Chemie (Steinheim, Germany).

2.2. 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. Ciprofloxacin (25 μg) was purchased from Alkom Laboratories LTD. Clinical isolates were *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Shigella boydii, Shigella flexneri* and *Shigella dysenteriae.* All the test microorganisms were identified by the use of their biochemical profiles as recommended by the manual "Bactériologie Medical"[12].

2.3. Identification of plants material

Trichilia emetica was collected in August 2010 in Gampela, 25 km east of Ouagadougou, Burkina Faso. The plants were identified by a botanist, Prof. Millogo-Rasolodimby, Department of Botany, University of Ouagadougou and voucher specimen was deposited at the Herbarium of the "Laboratoire de Biologie et d'Ecologie Végétale, UFR/SVT of University of Ouagadougou".

2.4. Extraction and fractionation

Fifty grams 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, Rotavopor R-200, Switzeland) at approximately 40 ˚C. The aqueous extracts were subjected to sequential liquid-liquid extraction with *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol. Each fraction was then collected and concentrated to dryness under reduced pressure to obtain *n*-hexane fraction (*n*-HF), dichloromethane fraction (DCMF), ethyl acetate fraction (EAF) and *n*-butanol fraction. The different fractions were freeze-dried by Telstar Cryodos 50 freeze-dryer. The fraction residues were packed in waterproof plastic flasks and stored at 4 ˚C until use.

2.5. Phytochemical analysis of extract and fractions

2.5.1. Total phenolics content

Total polyphenols were determined by using Folin-Ciocalteu reagent[13]. Aliquots (125 μL) of solution of extracts (extract and fractions) in methanol (10 mg/mL) were mixed with 625 μL Folin-Ciocalteu reagent (0.2 mol/L). After 5 min, 500 μL of aqueous Na_2CO_3 (75 g/L) were added and the mixture was vortexed. After 2 h of incubation in the dark at room temperature, the absorbencies were measured at 760 nm against a blank (0.5 mL Folin-Ciocalteu reagent + 1 mL Na_2CO_3) on a UV/visible light spectrophotometer (CECIL CE 2041, Cecil Instruments, England). The experiments were carried out in triplicate. A standard calibration curve was plotted using gallic acid (y=0.0289x-0.0036; R^2=0.9998). The results were expressed as mg of gallic acid equivalents (GAE)/100 mg of extracts.

2.5.2. Total flavonoids content

The total flavonoids were estimated according to Lamien-Meda *et al*[13]. A total of 0.5 mL of methanolic $AlCl_3$ (2%, w/v) were mixed with 0.5 mL of methanolic extract solution (0.1 mg/mL). After 10 min, the absorbencies were measured at 415 nm against a blank (mixture of 0.5 mL methanolic extract solution and 0.5 mL methanol) on a UV/visible light spectrophotometer (CECIL CE 2041, Cecil Instruments, England) and compared to quercetin calibration curve (y=0.0289x-0.0036; R^2=0.9998). The data obtained were the means of three determinations. The amounts of flavonoids in plant extracts were expressed as mg of quercetin equivalents (QE)/100 mg of extracts.

2.6. Antidiarrheal profile of bioactive fraction (EAF)

2.6.1. 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/12-h dark cycle and 25 ℃. They received pellets food enriched with protein 20% 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 animals were performed in accordance with the ethical guidelines of the Ethical Committee of laboratory animals for biomedical research approved by the Medical Ethical Committee of "University of Ouagadougou, Burkina Faso"[14].

2.6.2. Acute toxicity study in mice

Healthy male and female Swiss mice (25-30 g) 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 *Trichilia emetica* L. suspended in 10% DMSO were administered intraperitoneally at doses of 1, 2, 2.5, 3, 4, 5 and 6 g/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 days' period was noted. The toxicological effect was assessed on the basis of mortality for 14 d, which was expressed as the median lethal dose (LD_{50}) estimated from the regression of log-probit mortality rate[14].

2.6.3. Antidiarrhoeal activity: castor oil induced diarrhea

The method described by Shoba and Thomas[15], was followed for this study with slight modification. The animals were all screened initially by giving 0.5 mL of castor oil one week before the actual experiment. Only those showing diarrhoea were selected for the final experiment. Twenty five mice fasted for 24 h were randomly allocated to five groups of five animals each. Group I (received 1% tween 80 at a dose of 10 mL/kg) served as control group, Group II received the standard drug loperamide 3 mg/kg *p.o.*, Group III, IV and V received EAF of *Trichilia emetica* at the doses of 100, 200 and 300 mg/kg *p.o.*, respectively. One hour after administration, all animals received 0.5 mL of castor oil and then they were individually placed in cages, the floor of which was lined with transparent paper. During an observation period of 4 h, the time of onset of diarrhoea, the total number of faecal output (frequency of defecation) and weight of faeces excreted by the animals were recorded.

2.7. In vitro antimicrobial profile of EAF

2.7.1. Preparation of inocula

The susceptibility tests were performed by Mueller Hinton agar-well diffusion method. The bacterial strains grown on nutrient agar at 37 ℃ 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 (10^8 CFU/mL). To obtain the inocula, these suspensions were diluted 100 times in Muller Hinton broth to give 10^6 CFU/mL[16].

2.7.2. Preparation of discs

The stock solution of EAF was dissolved in 10% DMSO in water at a final concentration of 10 mg/mL[16]. The stock solution of extract and each fraction 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 extract and each fraction solution from *Trichilia emetica*. Negative controls were maintained by using discs impregnated with 10% DMSO in water and commercially available antibiotic diffusion discs ciprofloxacin (25 μg) from Alkom Laboratories LTD were used as positive reference standards for all bacterial strains.

2.7.3. 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 EAF (20 mg/mL) of *Trichilia emetica* were placed on the agar. Discs containing ciprofloxacin (25 µg) were used as positive control and 10% DMSO was used as a negative control. The plates were incubated for 24 h at 37 ˚C. The diameters of the inhibition zones were evaluated in millimeters. The 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[16].

2.8. Minimum inhibitory concentration (MIC) and minimum microbicidal concentrations (MMC)

MIC was determined by the microdilution method in culture broth as recommended by Konaté *et al.* with modifications[16]. Seven serial two-fold dilutions of fraction solutions or conventional antibiotic were prepared as described before, to obtain final concentration range of 10 mg/mL to 125 µg/mL and 25-0.391 µg/mL for ciprofloxacin. The last wells (n°8) served as sterility controls (contained broth only) or negative control (broth + inoculums). The 96-well micro-plates (NUNC, Denmark) containing 100 µL of Mueller Hinton broth were used. For each bacteria strain, three columns of seven wells to the micro-plate were used. In each well, the culture medium + fraction solution or ciprofloxacin + inoculum (10 µL of inocula) was filled. The plates were sealed with parafilm, then agitated with a plate shaker to mix their contents and incubated at 37 ˚C and at 44 ˚C for *Escherichia coli* for 24 h and an hour before the end of incubation, 40 µL of 0.2 mg/mL p-iodonitrotetrazolium salt solution was added to each well. All tests were performed in triplicate and the bacterial activity was expressed as the mean of inhibitions produced. Viable microorganisms reduced the yellow dye to a pink colour. The MIC was defined as the lowest concentration of fraction substance at which no colony was observed after incubation. So, the MIC was defined as the lowest concentration where no change was observed, indicating no growth of microorganism.

The MMC or minimum bactericidal concentration (MBC) was determined by transferring 50 µL aliquots of the clear wells into 100 µL of freshly prepared broth medium and incubating at 37 ˚C for 24 h (bacteria). The MMC was regarded as the lowest concentration of test sample which did not produce a colour/turbidity change as above, indicating no microbial growth. All tests were performed in triplicates. Ciprofloxacin for bacteria was used as positive controls.

2.9. Evaluation of bactericidal and bacteriostatic capacity

The action of an antibacterial on the bacterial strains can be characterized at two parameters as MIC and MBC. According to the ratio MBC/MIC, we can apperceive antibacterial activity. If the ratio MBC/MIC=1 or 2, effect is bactericidal but if the ratio MBC/MIC=4 or 16, effect is bacteriostatic[16].

3. Results

3.1. Phytochemical screening

Phytochemical screening of extract and fractions of *Trichilia emetica* revealed the presence of phenolics and flavonoids. The total phenolics content per 100 mg of extract and fractions from *Trichilia emetica* ranged from (61.23±0.12) mg GAE to (23.60±0.03) mg GAE. The highest content of phenolics in *Trichilia emetica* was detected in EAF with (61.23±0.12) mg GAE followed by DCMF with (35.16±0.04) mg GAE. The lowest total phenolics were obtained in *n*-HF with (23.60±0.03) mg GAE. The total flavonoids content per 100 mg of extract and fractions from *Trichilia emetica* ranged from (10.83±0.02) mg QE to (3.83±0.07) mg QE. The highest content of total flavonoids was detected in EAF with (10.83±0.02) mg QE. The lowest total flavonoids content were obtained in *n*-HF with (3.83±0.07) mg QE (Table 1).

Table 1

Polyphenols contents of extracts (aqueous acetone extract and fractions) from *Trichilia emetica* L.

Extracts	Total phenolics (mg GAE/100 mg extract or fraction)	Total flavonoids (mg QE/100 mg extract or fraction)
AA extract	35.19±0.00^{d}	10.74±0.07^{c}
n-HF	28.55±0.01^{e}	7.78±0.03^{e}
DCMF	62.23±0.09^{b}	13.51±0.01^{b}
EAF	66.18±0.01^{a}	15.79±0.07^{a}
n-BF	40.00±0.04^{c}	10.03±0.01^{d}

AA extract: aqueous acetone extract; *n*-BF: *n*-butanol fraction. Values are mean±SD (*n*=3). $P<0.05$ (^{a} compared to ^{b}); $P<0.01$ (^{a} and ^{b} compared to ^{c}); $P<0.001$ (^{a} and ^{b} comparaed to ^{d} and ^{e}).

3.2. Acute toxicity study of the plant extract

The acute toxicity of extract was evaluated in mice. The effect of intraperitoneal treatment of aqueous acetone extract from *Trichilia emetica* on mortality and LD_{50} value were determined. The value of LD_{50} is 568.5 mg/kg for intraperitoneal administration and the various observations showed normal behavior of the treated mice.

3.3. Antidiarrhoeal effects of EAF

EAF from *Trichilia emetica* L., was found to be effective in a dose dependent manner against castor oil induced diarrhoea in Swiss NMRI mice. At the dose of 300 mg/kg body weight, the fraction produced a significant decrease in the severity of diarrhoea in terms of the rate of defecation and consistency of faeces in Swiss NMRI mice. At the same dose, the EAF (300 mg/mL *p.o.*) showed significant antidiarrhoeal activity by 79.75% reduction in diarrhoea

comparable to that of the standard drug loperamide which showed 86.07% reduction in diarrhoea (Table 2).

Table 2

Effect of EAF on castor oil induced diarrhea in mice.

Treatment	Dose (mg/kg, *p.o.*)	Time of onset of diarrhea (min)	Total number of faeces in 4 h (frequency of defecation)	% Inhibition of defecation	Weight of stool (g)
Group I		90.0±1.5	7.9±1.0	-	0.69±0.05
Group II	3	231.8±1.0***	1.1±0.2***	86.07	0.05±0.02***
Group III	100	147.2±10.8*	4.2±0.9*	46.83	0.30±0.05***
Group IV	200	195.0±0.4***	3.4±0.4**	56.96	0.20±0.02***
Group V	300	210.2±0.2***	1.6±0.2***	79.75	0.01±0.02***

Results are mean±SEM and significantly different when compared with that of the control at $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

3.4. Antimicrobial profile of EAF

In this study, seven bacteria strains (Gram-negative and Gram-positive bacteria) were used. The antibacterial assays were performed by the agar-well diffusion and the broth micro dilution methods so that they could be qualified and quantified by inhibition zone diameters, MIC, MMC or MBC. We noticed that the susceptibility of the bacteria to the EAF on the basis of inhibition zone diameters varied according to the microorganism and bacteria strains were more sensitive to the EAF than reference compound (ciprofloxacin); the results are reported in Table 3. There is a significant variation in the diameters of inhibition zone values of EAF (Table 3). As for the micro-well dilution assay (MIC) and MBC of EAF, result varied according to microorganism (Table 4). The MIC values were ranged from 156 to 1250 μg/mL and the MBC values were ranged from 313 to >10000 μg/mL. The bactericidal and bacteriostatic effect was determined using the ratio MBC/MIC (Table 4).

Table 3

Inhibition zone diameters (mm) recorder in agar-well diffusion assay using EAF from *Trichilia emetica* L. and ciprofloxacin.

Microorganims	Ciprofloxacin	EAF
Staphylococcus aureus	13.00±1.20	24.33±1.10
Echerichia coli	nd	14.00±1.21
Pseudomonas aeruginosa	23.66±1.90	21.33±1.00
Salmonella typhi	nd	22.00±2.10
Shigella boydii	28.66±1.10	24.66±1.52
Shigella flexneri	29.00±1.40	22.33±0.10
Shigella dysenteriae	29.66±1.10	24.00±1.10

The results are the means of number of the colonies ± standard deviations. nd: no detected activity.

Table 4

MIC and MBC or MMC determination, bactericidal (+) and bacteriostatic (-) effects of EAF from *Trichilia emetica* L.

Microorganisms	MIC (μg/mL)	MBC (μg/mL)	MBC/MIC	Effect
Staphylococcus aureus	156	313	2	+
Echerichia coli	1250	>10000	>4	nd
Pseudomonas aeruginosa	313	625	2	+
Salmonella typhi	313	1250	4	-
Shigella boydii	313	625	2	+
Shigella flexneri	625	1250	2	+
Shigella dysenteriae	313	625	2	+

The results are the means of number of the colonies ± standard deviations. nd: no detected activity.

4. Discussion

Diarrhoea may be characterized as the abnormally frequent defecation of faeces of low consistency which may be due to a disturbance in the transport of water and electrolytes in the intestines. Diarrhoea is one of the major causes of child morbidity and mortality in the developing countries[17]. Worldwide distribution of diarrhea accounts for more than 5-8 million deaths each year in infants and small children less than 5 years. Some plants have been evaluated for their antidiarrhoeal properties[18,19]. Many medicinal plants used traditionally for antidiarrhoeal treatment have been in use by man without any scientific basis to explain the action of such plants. The aim of this study was experimentally to evaluate the acclaimed use of *Trichilia emetica* leaves, which are regarded to confer protection in diarrhoea in Burkina Faso traditional medicine. Several studies have validated the use of antidiarrheal medicinal plants by investigating the biological activity of extracts of such plants, which have antispasmodic effects, delay intestinal transit, suppress gut motility, stimulate water adsorption, or reduce the intraluminal fluid accumulation[20]. This experimental model was therefore employed to validate antidiarrhoeal efficacy of the bioactive fraction from *Trichilia emetica* in the current study.

At acute toxicity level, data indicated that the aqueous acetone extract of *Trichilia emetica* can be considered as weakly poisonous. According to Diez[21], pharmacological substances whole LD_{50} with less than 5 mg/kg body weight are considered not toxics; those with a 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 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 *Trichilia emetica* 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 *Trichilia emetica* has negligible level of toxicity when administered orally.

The EAF of leaves from *Trichilia emetica* exhibited broad spectrum of antibacterial activity. It was observed in the present study that EAF of *Trichilia emetica* inhibited the growth of all pathogenic bacteria tested. Preliminary phytochemical screening of *Trichilia emetica* showed the presence of a number of bioactive constituents such as total phenolics, total tannins, total flavonoids, total flavonols and sterols/terpenes[9]. The antimicrobial activity could be due to the presence of these phytoconstituents. Moreover, data of the present study also revealed that the plant extracts have *in vitro* broad spectrum antibacterial activity[23]. In effect, various publications have documented the antimicrobial activity of plant extracts[24-26]. The results obtained in this study indicate a considerable difference in antibacterial activity with EAF. The bacteriostatic and bactericidal activity could be ascribed to the presence of polyphenol compounds. In effect, some previous studies showed

that polyphenolic compounds caused inhibition of a wide range of microorganisms. Phenol is well known as a chemical antiseptic[27]. In addition, phenolic and terpenic antimicrobial activities are well documented[28]. Polyphenols, such as tannins and flavonoids, are of important antibacterial activity[29,30]. Tannins and flavonoids in general have been reported to have antidiarrhoeal activity through inhibition of intestinal motility, antimicrobial action and antisecretory effects[31]. 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[32]. The results indicated that fraction inhibited *Staphylooccus aueus* (Gram-positive) as compared to Gram-negative bacteria. This is further confirmed by the previous studies that describe the high sensibility of Gram-positive bacteria towards plant extracts and their component[33,34]. Certain authors reported that Gram-negative bacteria were more resistant to the plant-based organic extracts because the hydrophilic cell wall structure of Gram-negative is constituted essentially of a lipopolysaccaharide that blocks the penetration of hydrophobic oil and avoids the accumulation of organic extracts in target cell membrane[35-38]. This is the reason why Gram positive bacteria were found to be more sensitive to various extracts. One notice that extracts are more sensible on certain bacteria strains compared to the standard drug (ciprofloxacin). According to a study[39], a probable degree of lipophilicity might be responsible for the extracts being higher in activity than standard drugs used lipophilicity toxicity due to the interactions with the membrane constituents and their arrangement. Considering the above, Gram-positive bacteria should be more susceptible since they have only an outer peptidoclycans layer which is not an effective permeability barrier as reported by Nostro *et al*[40].

The present study validates the use of *Trichilia emetica* leaves as anti-diarrhoeal agent in traditional medicine in Burkina Faso. We suppose that anti-inflammatory, anti-diarrhoeal and antimicrobial capacities of fractions of *Trichilia emetica* are connected with polyphenolic compositions which induce high antioxidant capacities. Therefore, further investigation is required to isolate and identify other active constituents responsible for anti-diarrhoeal potential.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The background of this research sounds sufficient. In particular, as long as diarrhea is a disease causing the child morbidity and mortality in the developing countries, *Trichilia emetica* accepted to use as an alternative medicine against diarrhea.

Research frontiers

The animal model induced by castor oil -utilized for this current investigation by the researchers- is still a valid model. Moreover, the researchers employed the clinical isolates for assessing the antimicrobial profiles, making this study more interesting.

Related reports

Currently, there are at least 231 published articles in PubMed based on the terms "plant and extract and antidiarrheal" for searching. The field of herbal medicine with antidiarrheal activity is still of great interest.

Innovations and breakthroughs

This current work provides the scientific data to support the use of *Trichilia emetica* extract. The novel finding that EAF exhibited the higher total phenolics and total flavonoids contents compared to other fractions of leaves of *Trichilia emetica* was very interesting. So, the researchers subsequently tested the antidiarrheal and antimicrobial activities of EAF.

Applications

If their data on acute toxicity are validated, the applications are potentially advantageous and encouraged for patients with diarrhea.

Peer review

It is a good study in which the authors justify the traditional uses of *Trichilia emetica* and good antidiarrhoeal and antimicrobial activities of EAF from *Trichilia emetica*. The manuscript was well done. The authors described their study thoroughly. The writing style was easy to comprehend.

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