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Antibacterial potential of *Thevetia peruviana* leaf extracts against food associated bacterial pathogens

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ABSTRACT

Objective: To isolate and characterize the food associated bacterial strains, and to evaluate the antibacterial activity and minimum inhibitory concentration of various solvents (acetone, chloroform, methanol and petroleum ether) leaf extracts of *Thevetia peruviana* (*T. peruviana*) against their respective isolated and standard bacterial strains and also to investigate the presence of various phytochemical constituents in the leaf extracts of test plant.

Methods: The food associated bacterial strains were isolated from students' lunch boxes in Tesfa Tewahido Primary School. The antimicrobial activity and minimum inhibitory concentrations were determined by the disc diffusion and serial dilution methods, respectively and phytochemical constituents were also detected in various solvent leaf extracts of *T. peruviana*.

Results: The result showed that all the tested solvent leaf extracts of *T. peruviana* exhibited antibacterial activity against the tested standard and isolated bacterial strains with zones of inhibition ranged from 10.0 to 17.0 mm. Amongst the tested food borne bacterial pathogens, *Salmonella typhimurium* was most sensitive towards petroleum ether leaf extracts of *T. peruviana* while, methanol leaf extracts was relatively least effective against all the tested standard and isolated bacterial strains. Minimum inhibitory concentration of various solvent leaf extracts of *T. peruviana* ranged from 16.67 to 50.00 mg/mL for all the tested standard and isolated bacterial strains. The phytochemical constituents screening on the leaf extracts of *T. peruviana* showed the presence of alkaloids, cardiac glycosides, flavonoids, polyphenols, saponins and tannins.

Conclusions: The present study suggests that *T. peruviana* could be used as prospective aspirants against the common food borne bacterial pathogens and also provide a wide array in the development of drugs against common food borne bacterial pathogens.

1. Introduction

Food-borne diseases pose serious threat to both consumers and food industries and now gradually become solemn health problems, worldwide[1]. The risks of food borne illness have been increasing prominently over the past two decades and nearly about a quarter of the total global populations are at high risk. Consequently, precluding illnesses associated with food-borne pathogenic microbes remain a foremost health challenge[2]. The common food associated infectious microbial pathogens enters into our food chains at certain levels. According to health and food safety experts more than 200 known diseases are transmitted through contaminated food such as milk, water and other food items, and masses of illness cases annually by food-borne pathogens[3]. Amongst the various infectious pathogenic microbes *Corynebacterium diphtheria*, *Escherichia coli* (*E. coli*), *Salmonella typhimurium* (*S. typhimurium*), *Staphylococcus aureus* (*S. aureus*), and *Pseudomonas aeruginosa* are of prime importance[4].

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Control of infectious pathogenic microbes could pointedly reduce the outbreak of food-borne diseases associated with food items[5]. The practices of chemical preservatives and synthetic antimicrobials raise considerable challenges to inhibit the growth pathogenic microorganisms[6]. In this regards, use of medicinal plants provides a source for the development of novel drugs for human being and play a vital role in the alteration of worse conditions produced by infectious microorganisms. It has been reported that approximately 80% of the total global population rely on use of traditional medicine for their primary health care necessities[7]. In general, the medicinal plants could serve as a potential reservoir for the development of novel chemotherapeutic agents and very few medicinal plants have been explored for their therapeutic ability till date[8].

Daljit *et al*. concluded that traditional medicine practitioners use plants because it contains a wide range of natural substances and are non-allergenic, nontoxic or selectively toxic to the host and safe without having any side effects[9]. Fatima *et al*. stated that plant extracts are comparatively inexpensive, chemically stable and easily reachable to the infected parts of the body[10]. Continuously, growing concern about the new and emerging illness by food associated infectious pathogenic microbe led to searching for the replacement of synthetic bioactive compounds with effective, nontoxic and natural compounds. The plant derived products have the stack of alkaloids, glycosides, polyphenols, steroids and terpenoids, and also possess a wide range of antibacterial activities against various Gram-negative and positive bacterial pathogens[11-13].

Ethiopia is one of the poorest country in the world and about 80% population of the country are relying on the traditional medicare system, and these medicinally important plants play a significant role in the control of various human and livestock diseases[14]. However, increase of multiple drug resistances cases among the pathogenic microorganisms has arisen owing to the indiscriminate practice of commercially available antimicrobial compounds against the food borne diseases[15]. *Thevetia peruviana* (*T. peruviana*) is a widely grown exotic plant having some medicinal properties, but this plant has not been much explored particularly in Ethiopian due to low level of awareness. The aim of the present study is to investigate the antibacterial activity and minimum inhibitory concentration (MIC) of various solvent (acetone, chloroform, methanol and petroleum ether) leaf extracts of *T. peruviana* against some selected food associated bacterial strains (*E. coli*, *S. aureus* and *S. typhimurium*) isolated from the Tesfa Tewahido Primary School student's lunch boxes and against their respective standard bacterial strains and also to investigate the various phytochemicals constituents present in the leaf extracts of *T. peruviana*.

2. Materials and methods

2.1. Isolation and characterization of food associated bacterial strains

In total, 50 food samples from the lunch boxes of Tesfa Tewahido Primary School students were collected to isolate the food borne bacterial strains. About 25 g of each collected food samples were mixed with 225 mL of buffered peptone water and homogenized for 5 min. One milliliter of resulting homogenate was further added to 9 mL of sterile buffered peptone water in a sterile test tube and diluted serially to obtain dilutions up to 10^{-5}. For the isolation of bacterial strains, about 0.1 mL of each dilution was aseptically pipetted from each test tube and spread onto pre-solidified selective and differential media (MacConkey agar, mannitol salt agar, plate count agar, violet red bile agar) in the Petri dishes and incubated for 24 h at 37 °C.

2.2. Morphological and biochemical characteration and identification

The food associated bacterial isolates were identified on the basis of morphological and biochemical characteristics as per standard protocol of Bergey's Manual of Determinative Bacteriology[16].

2.3. Collection, identification and preparation of plant materials

Fresh leaves of *T. peruviana* were collected in plastic bag from Jimma University campus, Jimma, southwest of Ethiopia and identified by the plant taxonomist at belongs to Department of Biology. After identification the collected plant materials were transported to Research and Post Graduate Laboratory, Department of Biology for further processing. After transporting the collected leaves samples were washed gently with tap water followed by sterilized distilled water to remove the adhering dust and soil particles, and dried in shaded place at room temperature for 10 d in order to prevent the decomposition of active compounds. After drying, the leaves were chopped into small pieces and grinded into fine powdery form using mechanical grinder (Hamburg 76, West Germany).

2.4. Extraction of the solvent extracts from T. peruviana

The extraction was done by the modified method of Samiduri and Saravanakumar[17]. About 100 g of powdered leaves of *T. peruviana* was extracted by Soxhlet apparatus in gradient extraction by using different polarity strength solvents (acetone, chloroform, methanol

and petroleum ether). After placing the weight amount of powdered materials into the extraction thimble, the extraction chamber was suspended with 500 mL of petroleum ether in the round bottom flask and the extraction procedure was continued for 6 h (until the extracts become colorless). The petroleum ether was then removed from each leaf extracts by Rota-vapour under reduced pressure at temperature of 40 ℃. The concentrated crude leaf extracts was then transferred to 100 mL beakers and the remaining solvent was evaporated under dissector and stored in a refrigerator at 4 ℃ till further use. Similarly, extraction was carried out for chloroform, acetone and methanol with apt temperature ranging from 59-64 ℃.

2.5. Tested standard and isolated bacterial pathogens

The tested bacterial pathogens included *E. coli* (ATCC 25722), *S. typhimurium* (ATCC 13311) and *S. aureus* (ATCC 25903). The pure cultures of all the tested bacterial strains were obtained from Ethiopian Health and Nutrition Research Laboratory, Addis Ababa, Ethiopia and their respective isolates were isolated and characterized from the food samples in lunch boxes collected from Tesfa Tewahido Primary School students in Jimma.

Active culture of the standard and isolated bacterial strains were prepared by transferring a loop-full of respective bacterial cell into test tubes containing Mueller-Hinton broth and incubated for 24 h at 37 ℃. The suspension turbidity was adjusted to a 0.5 McFarland turbidity standard (1.2×10^8 CFU/mL).

2.6. Antimicrobial activity

2.6.1. Antibacterial activity of T. peruviana

Antibacterial activity of leaf extracts of *T. peruviana* was tested by agar disc diffusion method[18]. The disk of about 6 mm diameter was prepared from Whatman No. 1 filter paper using paper puncher. About 100 mg/mL of each solvents (acetone, choloform, methanol and petroleum ether) leaf extracts of *T. peruviana* were poured onto the Whatman filter paper disc placed on the Petri plates pre-inoculated with 100 µL of bacterial strains. Dimethyl sulfoxide (10 µg/µL) was used as negative control and gentamycin (10 µg/disc) for the positive control against all the tested standard and isolated bacterial strains.

2.6.2. Determination of MIC

MIC was determined by the method of Makut *et al*.[19]. To test the MIC, the leaf extracts of the *T. peruviana* was diluted with nutrient broth in a series of six test tubes with concentration ranges from 50.00 to 1.56 mg/mL prepared from stock solution (500 mg/mL). The control test tubes were also prepared simultaneously and all the test tubes were incubated at 37 ℃ for 24 h. The lowest concentration of various solvent leaf extract of test plant that produce no visible bacterial growth compared to control tubes were considered as MIC.

2.7. Phytochemical constituents screening

Phytochemical constituents screening was performed by the methods of Ayoola *et al*[20].

2.7.1. Test for alkaloids

About 0.5 g plant extract was diluted to 10.0 mL with acid alcohol, boiled and filtered. About 2.0 mL of dilute ammonia was added to 5.0 mL of filtrate followed by addition of 5.0 mL of $CHCl_3$ and shaken gently to extract the alkaloid bases. The $CHCl_3$ layer was extracted with 10.0 mL of acetic acid. In the 5.0 mL of the resulting extracts about 3 drops of Mayer's reagent was added. The formation of a cream precipitate indicates the presence of alkaloids.

2.7.2. Test for cardiac glycosides

About 2.0 mL of filtrate plant extracts was added with 1 mL of glacial acetic acid and 2 drops of $FeCl_3$ followed by the addition of 1.0 mL of concentrated H_2SO_4. Appearance of brown ring at the interface indicated the presence of cardiac glycosides. A violet ring also appeared below the brown ring confirm positive for cardiac glycosides.

2.7.3. Test for flavonoids

To test the presence of flavanoids, first diluted ammonia (5.0 mL) was added to 5.0 mL of filtrate plant extracts followed by addition of concentrated H_2SO_4 (1.0 mL). The appearance of yellow colour, which disappear on standing, indicates the presence of flavonoids, and secondly, a few drops of 1.0% aluminum solutions were added to a portion of the filtrate. A yellow coloration indicates the presence of flavonoids.

2.7.4. Test for polyphenols

About 2.0 mL of alcoholic filtrate plant extract was added to 1.0 mL of $FeCl_3$ solution. After a minute appearance of blue or green color indicates the presence of polyphenols.

2.7.5. Test for saponins

About 0.5 g of the filtrate plant extracts was added to 5.0 mL of distilled water and the mixture was vigorously shaken to observe a stable persistent froth. The frothing was obtained by mixing with 3 drops of olive oil. It was again shaken vigorously to observe the formation of an emulsion, which indicates the presence of saponins.

2.7.6. Test for tannins

About 0.5 g of the filtrate plant extracts was dissolved in 10.0 mL

of distilled water and filtered. About 2-3 drops of 0.1% $FeCl_3$ was added to 2.0 mL of the filtrate. A blackish-blue or brownish green coloration indicates the presence of tannins.

2.8. Statistical analysis

The entire data was statistically analyzed by SPSS version 16.0 (Chicago, Inc., USA). Duncan's multiple range test was employed to denote the significance difference between the treatments at P=0.05.

3. Results

3.1. Isolation and characterization of the food borne bacterial pathogens

A total of 552 bacterial isolates were isolated form the 50 food samples (firfir, spaghetti, shiro and rice) collected from the lunch boxes of the students of Tesfa Tewahido Primary School, Jimma, southwest of Ethiopia, and characterized on the basis of the morphological and biochemical characteristics (Table 1). Rose pink colonies were aseptically picked from the MacConkey agar medium and transferred into 5.0 mL nutrient broth medium. A loop full of culture from the nutrient broth medium was streaked on nutrient agar and incubated for 24 h at 37 ºC. The Gram negative rods which grew well in aerobic conditions but failed to grow under anaerobic conditions and showed positive tests for catalase and oxidative fermentation and negative for oxidase test were identified as Enterobacteriaceae (*E. coli*) (Table 1).

Golden yellow colonies on mannitol salt agar Petri dishes were aseptically picked and transferred into 5.0 mL nutrient broth medium. A loop full of culture from the nutrient broth medium was streaked on nutrient agar and incubated for 24 h at 37 ºC. The Gram positive cocci which grew well in both aerobic and anaerobic conditions and showed positive tests for catalase and oxidative fermentation and negative for oxidase tests was dentified as staphylococci (*S. aureus*) (Table 1).

Black colonies surrounded by red color was carefully aseptically picked off from the xylose lysine desoxycholate agar and transferred to 5.0 mL of nutrient broth medium. A loop full of culture from the nutrient broth medium was streaked on nutrient agar and incubated for 24 h at 37 ºC. The Gram negative rods that grew well in aerobic conditions but fails to grown under anaerobic conditions, and showed positive tests for catalase and oxidative fermentation and negative for oxidase test were identified as Enterobacteriaceae (*S. typhimurium*) (Table 1).

Bacterial strains grew on plate count agar medium were transferred into 5.0 mL nutrient broth medium. A loop full of culture from the nutrient broth was streaked on nutrient agar and incubated for 24 h at 37 ºC. The Gram positive rods which grew well in aerobic condition and no growth under anaerobic conditions and showed positive test catalase and oxidase and negative for oxidative fermentation test were recognized as bacilli, micrococci and *Pseudomonas* isolates (Table 1). While, the bacterial isolates showed the positive catalase and negative oxidase and oxidative fermentation tests belongs to Actinobacteridae (Table 1).

3.2. Occurrence and prevalence of food associated bacterial pathogens

In total, 552 isolates, 390 (70.65%) isolated form firfir, 68 (12.32%) from spaghtei, 43 (7.79%) from shiro and 51 (9.24%) from rice. Out of 552 isolates of food associated bacterial strains isolated from the lunch boxes of the students, 269 (48.73%) were bacilli, 134 (24.28%) were staphylococci, 92 (16.66%) micrococci, 38 (13.43%) Enterobacteriaceae, 13 (2.36%) *Pseudomonas* and 6 (1.09%) Actinobacteridae (Table 2). Amongst the various types of tested

Table 1
Charateristic identification of bacterial isolates from the food samples of students' lunch boxes.

Bacterial isolates	Gram reaction	Shapes	Aerobic growth	Anaerobic growth	Endospore formation	Cell motility	Catalase test	Oxidase test	Oxidative fermentation test
Bacilli	+	Rods	+	-	+	-	+	+	-
Staphylococci	+	Cocci	+	+	-	-	+	-	+
Micrococci	+	Cocci	+	-	-	-	+	+	-
Enterobacteriaceae	-	Rods	+	-	-	+	+	-	+
Pseudomonas	-	Rods	+	-	-	-	+	+	-
Acintobacteridae	+	Rods	+	-	-	-	+	-	-

Table 2
Occurrence and prevalence of food associated bacteria isolated from the 50 food samples of student lunch boxes [*n* (%)].

Food type	No. of isolates	Bacilli	Staphylococci	Micrococci	Enterobacteriaceae	*Pseudomonas*	Acintobacteridae
Firfir	390 (70.65)	196 (72.86)	103 (76.87)	62 (67.39)	19 (50.00)	5 (38.46)	5 (83.33)
Spagheti	68 (12.32)	28 (10.41)	9 (6.71)	15 (16.30)	12 (31.58)	4 (30.76)	–
Shiro	43 (7.79)	17 (6.32)	11 (8.21)	7 (7.61)	5 (13.16)	2 (15.39)	1 (16.67)
Rice	51 (9.24)	28 (10.41)	11 (8.21)	8 (8.70)	2 (5.26)	2 (15.39)	–
Total	552 (100)	269 (48.73)	134 (24.28)	92 (16.66)	38 (6.88)	13 (2.36)	6 (1.09)

Table 3
Effect of different solvent leaf extracts of *T. peruviana* on the antibacterial activity of standard and isolated bacterial strains.

Extracts	Zone of inhibition (mm)					
	E. coli (standard)	*E. coli* (isolated)	*S. aureus* (standard)	*S. aureus* (isolated)	*S. typhimurium* (standard)	*S. typhimurium* (isolated)
TPAE	13.00±1.00^{bc}	12.70±0.58^{b}	17.00±1.00^{b}	16.50±1.50^{b}	15.50±1.80^{b}	14.50±1.32^{b}
TPCE	11.50±0.50^{cd}	11.00±1.00^{cd}	14.00±1.00^{cd}	13.50±0.50^{c}	12.50±1.80^{c}	12.00±1.00^{c}
TPME	10.50±1.50^{d}	10.00±1.00^{d}	12.50±2.18^{d}	12.00±1.00^{c}	12.30±0.58^{b}	11.50±1.50^{c}
TPPE	13.50±1.32^{b}	12.50±0.50^{bc}	16.00±2.00^{bc}	15.50±1.32^{b}	16.50±1.50^{b}	16.00±1.00^{b}
Positive control	22.00±1.00^{a}	21.50±1.32^{a}	29.00±1.00^{a}	27.50±1.32^{a}	29.50±1.50^{a}	29.00±1.73^{a}
Negative control	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}

Values are expressed as mean±SD; Values within each column followed by the same letters are not significantly different at P=0.05. TPAE: *T. peruviana* acetone extract, TPCE: *T. peruviana* chloroform extract, TPME: *T. peruviana* methanol extract, TPPE: *T. peruviana* petroleum ether extract. Positive control: Gentamycin; Negative control: Dimethylsulfoxide.

foods, firfir has encountered with high percentage of all the bacterial pathogens, while, shiro was found least (Table 2). The staphylococci (*S. aureus*) and Enterobacteriaceae (*E. coli* and *S. typhimurium*) are the common bacterial pathogens associated with the food items of the Ethiopian population.

3.3. Antibacterial activity of leaf extracts of T. peruviana

All the tested leaf extracts (acetone, chloroform, methanol and petroleum ether) of *T. peruviana* was found inhibitory against all the tested bacterial strains. Minimum inhibitory activity was found in methanol leaf extracts while, the maximum inhibitory activity was found in petroleum leaf ether extracts followed by chloroform extracts against the *E. coli* and *S. typhimurium* standard and isolated strains (Table 3). However, maximum inhibitory activity was observed in acetone leaf extracts followed by petroleum ether leaf extracts against *S. aureus* standard and isolated strains (Table 3). Acetone and petroleum ether leaf extracts showed (13.00±1.00) and (13.50±1.32) mm inhibition against *E. coli* standard strain, (12.70±0.58) and (12.50±0.50) mm inhibition against *E. coli* isolated strain, (17.00±1.00) and (16.00±2.00) mm inhibition against the *S. aureus* standard strain, (16.50±1.50) and (15.50±1.32) mm inhibition against *S. aureus* isolated strain, (15.50±1.80) and (16.50±1.50) mm inhibition against the *S. typhimurium* standard strain, (14.50±1.32) and (16.00±1.00) mm inhibition against the *S. typhimurium* isolated strain, respectively (Table 3).

The result showed that petroleum leaf extract was more effective against *E. coli* and *S. typhimurium* standard and isolated strains, while, acetone leaf extract was found to be effective against the *S. aureus* standard and isolated strains compared to other tested leaf extracts (Table 3). Gentamycin was used as positive control against all the tested standard and isolated strains with zone of inhibition (22.00±1.00) and (21.50±1.32) mm against the *E. coli* standard and isolated strains, (29.00±1.00) and (27.50±1.32) mm against the *S. aureus* standard and isolated strains, (29.50±1.50) and (29.00±1.73) mm against the *S. typhimurium* standard and isolated strains. However, dimethylsulfoxide was used as negative control against all the tested standard and isolated bacterial strains with no zone of inhibition (Table 3).

3.4. MIC of leaf extracts of T. peruviana

The MIC results showed that acetone, chloroform, and petroleum ether leaf extracts of *T. peruviana* inhibited the growth of standard strains of *E. coli* at concentration of 16.67 mg/mL, while the methanol leaf extract inhibited the growth of standard strains of *E. coli* at the concentration of 50.00 mg/mL. While, acetone, chloroform, methanol leaf extract of *T. peruviana* inhibited the growth of *S. aureus* and *S. typhimurium* at the concentration of 50.0 mg/mL (Figure 1). However, the petroleum ether leaf extract of *T. peruviana* inhibited the growth of *S. aureus* and *S. typhimurium* at the concentration of 16.67 mg/mL.

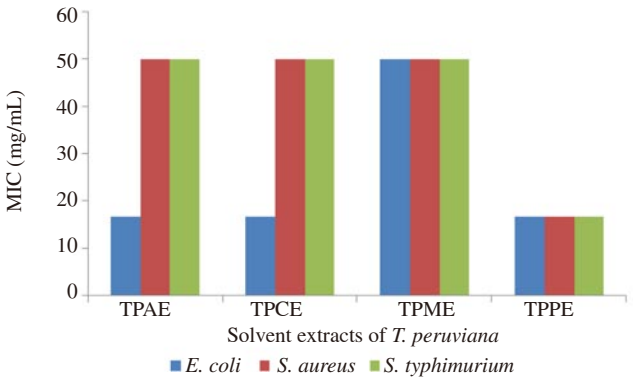

Figure 1. MIC values of different solvent extracts *T. peruviana* against the tested standard bacterial strains.
TPAE: *T. peruviana* acetone extract; TPCE: *T. peruviana* chloroform extract; TPME: *T. peruviana* methanol extract; TPPE: *T. peruviana* petroleum ether extract.

Similarly, the MIC results showed that acetone, chloroform and petroleum ether leaf extracts of *T. peruviana* inhibited the growth of *E. coli*, *S. aureus* and *S. typhimurium* at the concentration of 16.67 mg/mL. While, the MIC for the methanol leaf extracts of *T. peruviana* was recorded at the concentration of 50.0 mg/mL for all

the tested bacterial strains (Figure 2).

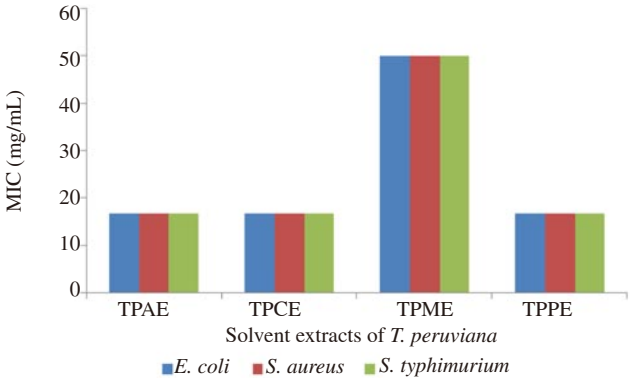

Figure 2. MIC values of different solvent extracts *T. peruviana* against the tested isolated bacterial strains.
TPAE: *T. peruviana* acetone extract; TPCE: *T. peruviana* chloroform extract; TPME: *T. peruviana* methanol extract; TPPE: *T. peruviana* petroleum ether extract.

3.5. Phytochemical screening

The phytochemicals screening of the *T. peruviana* showed the presence of alkaloids, cardiac glycosides, flavonoids, polyphenols, saponins and tannins (Table 4). Chloroform, and petroleum ether leaf extracts of *T. peruviana* showed the presence of alkaloids, cardiac glycosides, flavonoids, polyphenols, saponins and tannins, while, the acetone and methanol leaf extracts of *T. peruviana* showed the absence of flavonoids (Table 4).

Table 4
Phytochemical constituents screening of *T. peruviana*.

Extracts	Alkaloids	Cardiac glycosides	Flavonoids	Polyphenols	Saponins	Tannins
TPAE	+	+	-	+	+	+
TPCE	+	+	+	+	+	+
TPME	+	+	-	+	+	+
TPPE	+	+	+	+	+	+

+: Presence; -: Absence; TPAE: *T. peruviana* acetone extract; TPCE: *T. peruviana* chloroform extract; TPME: *T. peruviana* methanol extract; TPPE: *T. peruviana* petroleum ether extract.

4. Discussion

It was revealed from our results that 552 isolates of bacteria were isolated from food samples (firfir, spaghetti, rice and shiro) of students' lunch boxes, 269 isolates belongs to bacilli, 134 belongs to staphylococci, 92 micrococci, 38 Enterobacteriaceae, 13 *Pseudomonas* and 6 Acinetobacteridae. Okolie *et al*. isolated the pathogenic bacteria such as *Salmonella*, *Clostridium perfringes*, *S. aureus*, *Listeria monocytogens*, *C. jejuni*, *Clostridium botilinum*, *Bacillus cerus* and *E. coli* from the cooked food[21]. However, Tessi *et al*. reported that the pathogenic bacteria could also contaminate the cooked foods[22]. Similarly, Gadaga *et al*. has isolated a number of pathogenic bacteria associated with food poisoning and stated that the occurrence of these bacterial pathogens in the food was due to the unhygienic and inappropriate food handling practices[23]. The crude leaf extracts of *T. peruviana* showed antibacterial activity against both standard and isolated strains of *E. coli*, *S. aureus* and *S. typhimurium* with zone of inhibition ranges from 10.5-17.0 mm. Our finding, suggested that *T. peruviana* extracts had broad spectrum antibacterial activity against both Gram positive and negative bacteria and is in agreement with the reports of Ranpariya and Chudasama[24]. Our results showed that acetone and petroleum ether extracts of *T. peruviana* showed maximum zone of inhibition against *S. aureus*, while their inhibitory activity were similar to *E. coli* and *S. typhimurium*. Babu *et al*. stated the better expression of the active ingredients of the plant parts with acetone and petroleum ether than other solvents[25].

Reddy described that the extract of *T. peruviana* proved to be effective against *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*[26], while, Ranpariya and Chudasama reported that activity of petroleum ether extracts of leaves of *T. peruviana* against *S. aureus* with zone of inhibition 13.6 mm[24]. In our case the petroleum ether extracts of leaves of *T. peruviana* had also significant zone of inhibition 12.5-16.5 mm against all the tested standard and isolated bacterial strains followed by acetone extracts. However, Singh *et al*.[27] demonstrated that the methanolic leaf extract of *T. peruviana* showed antibacterial activity against extended spectrum beta lactamase producing bacteria like *E. coli*, *Pseudomonas, Klebsiella*, and methicillin-resistant *S. aureus* and *Salmonella*. Similarly, Hammuel *et al*.[28] reported that *T. peruviana* methanol extract showed zone of inhibition ranging from 10-26 mm against the Gram positive bacteria *S. aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Corynebacterium ulcerans*, and Gram negatives bacteria like *E. coli*, *Neisseria gonorrhoae*, *S. typhi*, and *Shigella dysenteriae*.

MIC results of crude *T. peruviana* leaf extracts showed that *S. aureus* standard and isolated strains were more sensitive. It is also revealed from our MIC results that petroleum and acetone extracts showed wide range of activity and could be potential source of antimicrobial compounds. This finding is in agreement with the reports of Gahlaut and Chhilla[29]. The methanol leaf extract have MIC value 50.0 mg/mL for the isolated and standard tested bacterial strains, however, the MIC values for other solvent leaf extracts was recorded as 16.67 mg/mL.

According to Hada *et al*. secondary metabolites are responsible for the antibacterial activities of the crude extracts of *T. peruviana* and our study identified a number of phytochemicals in the essential oil extract of the plant[30]. Joseph *et al*. observed different phytochemical constituents such as alkaloids, phenolic compounds, flavonoids, saponins, glycosides, terpenoids, steroids, coumarins, quinones, phytosterols, proteins and carbohydrates in the aqueous, acetone, petroleum ether, chloroform and ethanol extracts[31]. These

results are in line with our findings where the different solvent extracts of leaves of *T. peruviana* contained phytochemicals such as saponins, glycosides, poly phenols and alkaloids. Our finding showed that the presence of different secondary metabolites such as alkaloids, phenolic, flavonoids, saponins, and glycosides. Earlier reports indicated that the presence of flavonoids, glycosides, steroids, saponins and tannins might be the cause for the antimicrobial activity[28,32]. The secondary metabolites present in the plant extracts have the ability to crumble the cell wall of the pathogenic bacterial strains and release the lipopolysaccharides which may increase the permeability of the cytoplasmic membrane[33]. In addition, the phytochemicals also obstruct the enzymes functions in a specific pathway and inhibit the synthesis of nuclear materials and protein in pathogenic microorganism[34]. Moreover, tannins and flavonoids could inactivate the microbial adhesions, enzymes and proteins by the formation of complex compounds with the polysaccharide and amino acids[35]. However, Zibbu and Batra reported that *T. peruviana* contains high amount of phenolic compounds and exhibited maximum antioxidant activity[36]. These phenolic compounds are toxic to the pathogenic microorganisms and inhibit the enzymes synthesis[28]. Similarly, Jirovetz *et al*. also identified a number of phytochemicals from *T. peruviana* having antimicrobial activity and stated that the presence of different secondary metabolites has contributed to antimicrobial potentials[37].

It has been concluded form our study that the various solvents leaf extracts of *T. peruviana* exhibited antibacterial activities against both the tested Gram positive and negative standard and isolated bacterial strains but the acetone and petroleum ether leaf extracts have the promising results for antibacterial activity and MIC due to presence of various pharmacologically active substances in the leaf extracts of *T. peruviana*.

Conflict of interest statement

We declare that we have no conflict of interest.

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