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Research Article

SPECTROPHOTOMETRIC SCREENING OF POTENT BACTERICIDAL PROPERTY OF *THEVETIA PERUVIANA* SCHUM. LEAF AND FRUIT RIND EXTRACTS ON CLINICAL AND PLANT PATHOGENS

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Abstract

The development of multi drug resistance in human and plant pathogens, reconsidering the traditional medicines as antibacterial source and presence of promising phytochemicals in leaf and fruit rind of *T. peruviana* (S) (Nazneen *et al.,* 2014) prompted the authors to take up the antibacterial evaluation of different extracts. Antibacterial activity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of various extracts of *T. peruviana* (S) were measured using methods of National Committee for Clinical Laboratory Standards (NCCLS).

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) spectrophotometric determination of active extracts has found that the MICs of all the active extracts lies between the range of 250 µg to 1250 µg and bactericidal concentration in a range of 500 to 1250 µg. The percentage of inhibition was analysed, where in among all the extracts tested against *B. subtilis* and *E. coli*, fruit upper liquid (FUL) exhibited highest inhibition percentage of 33.75% and 30.31 % at 500 µg/ml respectively. Similarly, fruit hexane (FH) extract has the highest inhibition of 15.60% against *B. cereus* at 750 µg/ml. The *Xanthomonas sp.* was susceptible to leaf chloroform (LC) with 32.29% of inhibition at 1000 µg/ ml. The activity index and total antimicrobial activity indicates the antibacterial action of extracts.

The present investigations have revealed that among the extracts, the fruit rind extracts have most prominent inhibition abilities against tested bacteria, which are validating the use of this plant in traditional system of medicine and this is the first report of exploration of above extracts for their antibacterial activities against *B. subtilis, B. cereus, E. coli* and *Xanthomonas sp*.

Key words: *Thevetia peruviana* (S)**;** Yellow oleander; traditional medicine; extracts; Antibacterial property.

Introduction

Antibiotics are the first preference for the treatment of infections. The prolong use of the antibiotic like penicillin, chloramphenicol or neomycin in dermatological as well as cosmetic products have turned into the sensitisation (Manten, 1981). Nephrotoxicity is the selective binding of antibiotic to the kidney tissue leading to the impairment of renal functions. Ototoxicity, neurotoxicity, mutagenesis and horizontal gene transfer (Manten, 1981 and Cogliani *et al.*, 2011) are some of the side effects of antibiotics. European Union has banned the Antibiotic growth promoters, as they are the main reason for the development of antibiotic resistance in microbes. The severity of this situation can be understood by the estimation of the non typhoidal *Salmonella* cause between two hundred million and 1.3 billion cases of intestinal diseases including 3 million of death each year worldwide (Goburn *et al.*, 2007).

The development of resistance against antibiotics is another dark face of the antibiotic treatment against infections. The bacterial strains have resistance to the available drugs where *Bacillus subtilis* has resistance to ampicillin, *Escherichia coli* resistant to amikacin, ampicillin, cephalothin, cefpirome, chloramphenicol, carbenicillin, gentamicin, netilmicin, piperacillin, sulfamethoxazole, tobramycin and tetracycline (Nascimento *et al.*, 2000). *B. cereus* strains have developed high levels of resistant (up to 1 mg/ml) to ampicillin, colistin, and polymyxin. These properties are therefore, most likely chromosomally determined in *B. cereus*. In contrast, resistance to kanamycin, tetracycline, bacitracin, or cephaloridin was observed in only a few strains.

Research during the last decade has convincingly shown that natural products play an important role in pharmacological industry. The abundant presence of secondary metabolites like alkaloids, phenols, flavonoids coumarins and tannins etc. such as present in *Solanum pubescens* (Haseeb *et al.*, 2014) are mainly for the plants defence mechanism, from which humans can also get benefitted as many of them stands as the potent and powerful, pharmaceuticals having antimicrobial, anti-

cancer and anti-arthritic drugs. These drastic side effects of antibiotics and the development of resistance in microbes are may be responsible for the great paradigm shift of infectious disease treatment in future from antibiotics to the phytochemicals or plant derivatives of traditional medicines.

Thevetia peruviana (S) is widespread on the American, Asian and African continents, its seeds, leaves, fruits and roots being used in traditional medicine as a purgative, as an emetic and for intermittent fever treatment (Correa *et al.*, 1931). In Nigeria and Ghana the bark is used as antipyretic. Recent reports from The National Cancer Institute's (NCI) have revealed that in folklore system of medicine *Thevetia peruviana* (S) has been extensively used for antitumor therapy (Richard *et al.*, 2005).

T. peruviana (S) has been claimed that all parts of these plants are toxic, and contain a variety of cardiac glycosides that have a relatively high therapeutic index (Mantu *et al.*, 1980). Phytochemical analysis of its seed oil revealed that it is rich in bioactive compounds (Hammuel *et al.*, 2011). Similarly, the flowers of this plant were reported to possess good medicinal value. Phytochemical screening has shown the presence of alkaloids, glycosides, tannins phenolic compounds, proteins, essential oils, gums, mucilage and fixed oils (Kumar *et al.*, 2012).

Our earlier research on systematic phytochemical analysis of leaf and fruit rind extracts of *T. peruviana* (S) using different solvents revealed the presence of bioactive compounds in the form of alkaloids, flavonoids, saponins, cardiac glycosides, anthraquinone glycosides, coumarins, phenols, tannins, steroids, oils and fats etc. Which confirmed that *T. peruviana* (S) is very rich in alkaloid and saponins followed by flavonoid as well as phenolics (Nazneen *et al.*, 2014), the abundant presence of phytochemicals in plant part promoted the authors to screen the antibacterial potential of leaves and fruit rind part of *T. peruviana* (S).

Materials and Methods

Plant material collection

Unripe fruits and leaves of *Thevetia peruviana* (S) were collected from the surroundings of Kuvempu University, Shankaraghatta, Dist. Shimoga, Karnataka, India. The plant was authenticated by Prof. V. Krishna, Taxonomist, Dept. of Biotechnology, Kuvempu University. The specimen has been deposited at the Department of Biotechnology. The fruits were processed by separating the epicarp (fruit rind) and the material was shade dried and powdered.

Chemicals

Hexane, chloroform, ethanol etc. were purchased from Merck and Himedia. All the chemicals and solvents used were of analytical grade.

Soxhlet Extraction

Successive extraction was done using 500 g of powdered material of leaf and fruit rind in soxhlet apparatus. The solvents hexane (2L, 500C ˜ 15 cycles) chloroform (2 L, 450C ˜15 cycles) and ethanol (2 L, 700C, ˜15–17cycles) were used. All the extracts were concentrated *in vacuo*, followed by the cold extraction of fruit rind material using 9:1 ratio of water and ethanol respectively for 24 h with frequent agitation. Water extract was filtered and concentrated *in-vacuo*. The yield of each dried extract was calculated.

Growth and maintenance of test bacterial cultures

Clinical samples of *Bacillus subtilis* (*B. subtilis*), *Bacillus cereus (B. cereus) Escherichia coli* (*E. coli*), were obtained from the Department of Microbiology, Shivamogga Institute of Medical Sciences, Karnataka, India, and *Xanthomonas Sp. (X. sp.)* was isolated from the citrus canker. All the four bacterial species were used as test organisms and maintained on nutrient broth (NB) at 37°C.

Preparation of inoculum

Gram positive *B. subtilis, B. cereus* and gram negative bacteria *E. coli, Xanthomonas sp,* were precultured on nutrient broth overnight in a rotary shaker at 37°C then centrifuged at 10,000 rpm for 5 min. Pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{600} nm) to $1X10^8$ cfu (colony forming units).

Preparation of the extracts

All nine extracts were collected and stock solution of 20 mg /5ml of each extract was dissolved in respective vehicle solvent, among which water and DMSO was preferred the most. A concentration gradient of 1000, 1500 and 2000 µg/ml extract was selected for the analysis of agar-well diffusion test of antibacterial activity. To determine the MIC of all extracts concentration gradient was adjusted in a range of 250, 500 and up to 2000 µg/ml.

Agar-well diffusion test

The antibacterial activity of the crude extracts was determined by the agar-well diffusion method. The inoculum was prepared by inoculating a loopful of the strain in the nutrient broth (25 ml) and incubated at room temperature on a rotary shaker for 18 hours before the use at 37ºC to get inoculum size of 108 cfu/ml as per McFarland standard. Around 20 ml of nutrient agar was added onto the petri plate, after solidification 200 µl of the standardized cell suspensions were spread using sterilized non-absorbent cotton swab. Wells were then bored into the agar using a sterile 6 mm diameter cork borer. 50 µl of the crude extract containing 1, 1.5 and 2 mg/ml were loaded into wells, plates were then incubated for 1 hour to allow the diffusion of solution in to the medium.

The plates were incubated at 37°C overnight. Negative and positive controls were set up in parallel, the solvents that

were used to dissolve the extract were set as negative control and streptomycin as positive control (10 µg/ml). The plates were observed for zones of inhibition after 24 hours. The effects were compared with those of standard. The zone of inhibition was measured from the edge of the well. The extracts which exhibited the inhibition were considered for further analysis.

Minimum inhibitory concentration (MIC)

Minimum Inhibitory Concentration (MIC) of all the nine extracts was estimated by following the method of National Committee for Clinical Laboratory Standard (2012 and 2000) with some modifications by using micro-dilution Technique in 96-well microtiter plates, followed by spectrophotometric analysis to obtain more appropriate results and quantitative data. Bacterial species were cultured overnight at 37ºC in nutrient broth. The inoculum suspension was adjusted to a concentration of approximately 1.0 X 10^8 cfu. The inocula were stored at ±4°C for further use. Dilutions of the inocula were cultured on solid nutrient agar for bacteria to verify the absence of contamination and to check the validity.

The investigating extracts (100 µl) were added to the microtiter plate in a concentration gradient of 250, 500 to 2000 µg/ml and 150 µl of inoculum was added to each well, negative control was inoculum with sterile distilled water and streptomycin was added to the positive control. The plates were kept at 37 ºC for 24 hrs. All the tests were conducted in triplicates and the effects were compared with those of standard. After incubation the plates were examined for the presence or absence of visible growth using inverted microscope (Nikon, Japan) followed by the spectrophotometric analysis at 600 nm. Further, the percentage of inhibition was calculated by means of formula and MIC was considered to the concentration of the extract at which the percentage was in positive range.

% inhibition = [(Acontrol - Atest)/Acontrol] × 100 *A control: O. D. of control.

A test: O. D. of tested sample.

Minimum bactericidal concentrations (MBCs)

The minimum bactericidal concentrations (MBCs) of the extracts were estimated by a adopting the method of National Committee for Clinical Laboratory Standard (2000) (Veljic *et al.,* 2010) with some modifications modified. Serial sub cultivation of 4 µl of MIC inoculum into microtiter plates containing 100µl of broth per well and incubated for 24 hrs at 37 ºC. The lowest concentration of extract with no visible growth was considered as MBC followed by spectrophotometric analysis at 600 nm. Plane broth has been used as control. The minimum bactericidal concentrations of the extracts were determined to the O.D. equal to the reference control, which indicates 99.5% killing of the original inoculum. Further, the results were confirmed by sub-culturing the samples of MBC on the plane NA medium.

Activity index (AI)

Activity Index is a comparison between the extract's zone of inhibition with the standard reference antibiotics (Arya *et al*, 2010). The activity index of the crude plant extract was calculated as

Activity index (A.I.) = Mean of zone of inhibition of the extract / Zone of inhibition obtained for standard antibiotic drug

Total antimicrobial activity (TAA) determination

Total antimicrobial activity is the volume at which the test extract can be diluted with the ability to kill microorganisms. It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract or compound isolated and is expressed in ml/g. TAA has been calculated by adopting the method of (Eloff *et al.,* 2004).

Total Activity = Extract per gram dried plant part / MIC of extract

Statistical analysis

Data are expressed as Mean ± S.E. All the assays were analysed by one-way analysis of variance (ANOVA) Using EZANOVA software.

Results

Soxhlet Extraction

The percentage yield of hexane, chloroform and ethanol and water extract from leaf and fruit rind were calculated. Ethanolic extract from leaves exhibited a maximum yield of 10.47% whereas, fruit rind water extract showed a maximum yield of 25.36% followed by ethanolic fruit rind extract with 16.66%. Chloroform showed least yield in both samples with 1.44% in leaves and 1.67% in fruit rind. The hexane extract had moderate yield of 3.81% and 2.32%in both the plant materials. Interestingly it was observed that the fruit rind ethanolic extract showed three different fractions. Based on that the fractions were separated as Bottom residue (BR), Middle Crystals (MC) and Upper Liquid (UL) and all the dried extracts were used for the further analysis.

Antibacterial activity

The activity was performed by agar-well diffusion method based on the observations only the active extracts were further selected for MIC and MBC determination.

Agar-well diffusion method

The result of the antimicrobial activity is tabulated in Table -1. The results clearly indicates that the leaf extracts LC have shown considerably high inhibition against E. coli (7.33 ± 0.33mm), followed by Xanthomonas sp. (4.67± 0.33mm), B. cereus (4.33± 0.33mm) and B. subtilis (2 ± 0 mm). Similarly, LE extract has inhibited E.coli (5.33 ± 0.33mm), *Xanthomonas* sp. (4.67 ± 0.33mm), B. cereus

(2.67 ± 0.33mm) and *B. subtilis* (2.33 ± 0.33mm) respectively at a concentration of 2mg/ml. Interestingly LE extract has shown highest zone of inhibition against *B. subtilis* (3.67mm) at 1.5mg/ml, whereas, LH extract did not exhibit any activity against tested bacterial species.

Table 1: Zone of inhibition of all the extracts of *T. peruviana* (S) against four different Bacteria.

Sl. No.	Extracts	Conc. (µg)	Zone of inhibition			
			B. subtilis	***B. cereus***	***E. coli***	***X. sps.***
1.	**LH**	1000	0	0	0	0
		1500	0	0	0	0
		2000	0	0	0	0
2.	**LC**	1000	2.33 ± 0.33	2.33 ± 0.33	4 ± 0.58	2.33 ± 0.33
		1500	4.33 ± 0.33	2 ± 0	4.67 ± 0.33	3.33 ± 0.33
		2000	2 ± 0	4.33 ±0.33	7.33 ± 0.33	4.67 ± 0.33
3.	**LE**	1000	2 ± 0	1 ± 0.33	3.33 ± 0.33	2.67 ± 0.33
		1500	3.67 ± 0.3	1.67 ± 0.33	4.33 ± 0.33	2.67 ± 0.33
		2000	2.33 ± 0.33	2.67 ±0.33	5.33 ± 0.33	4.67 ± 0.33
4.	**FH**	1000	2.67 ± 0.33	2.33 ± 0.33	1.83 ± 0.17	2.67 ± 0.33
		1500	4.33 ± 0.3	3.33 ± 0.33	4 ± 0.01	2.67 ± 0.33
		2000	5 ± 0.33	6 ±0.58	5.2 ± 0.2	5.33 ± 0.33
5.	**FC**	1000	2 ± 0.33	2 ± 0.33	2.33 ± 0.17	0
		1500	3.67 ± 0.3	3.33 ± 0.33	4.33 ± 0.01	0
		2000	5.33 ± 0.33	4.67 ±0.58	6.33 ± 0.2	0
6.	**FUL**	1000	2.67 ± 0.33	3.33 ± 0.33	1.93 ± 0.07	2.33 ± 0.33
		1500	3.33 ± 0.3	4.67 ± 0.33	2.33 ± 0.01	6 ± 0
		2000	4.33 ± 0.33	7.33 ±0.58	3.6 ± 0.2	7.33 ±0.03
7.	**FMC**	1000	2 ± 0	2.33 ± 0.33	0	0
		1500	3.67 ± 0.3	3.33 ± 0.33	2.4 ± 0.01	0
		2000	4.67 ± 0.33	4.67 ±0.58	3.3 ± 0.2	0
8.	**FBR**	1000	2.33 ± 0.33	1 ± 0	1.77 ± 0.15	0
		1500	4.33 ± 0.33	2 ± 0	3.33 ± 0.33	0
		2000	4.67 ± 0.33	2.33 ± 0.33	4.67 ± 0.31	0
9.	**FEW**	1000	0	0	0	0
		1500	0	0	0	0
		2000	0	0	0	0
10.	**Std.**	10	7	7	7	6

LH: leaf hexane, LC: leaf chloroform, LE: leaf ethanol, FUL: Fruit rind upper liquid, FBR: Fruit rind bottom residue, FMC: Fruit rind Middle Crystals FW: Fruit rind water extracts

The fruit rind extracts shown promising results, where FH extract had highest inhibition against *B. cereus* (6 ±0.58mm) followed by *Xanthomonas sp.* (5.33 ± 0.33mm), *E. coli* (5.2 ± 0.2mm) and *B. subtilis* (5 ± 0.33mm). Similarly, FC extract exhibited considerable inhibition against *E. coli* (6.33 ± 0.2mm), *B. subtilis* (5.33 ± 0.33mm) and *B. cereus* (4.67 ±0.58mm), whereas *Xanthomonas sp.* was resistant to FC extract. All the fractions of ethanolic extracts have shown good inhibitory effects except FBR, where FUL showed highest inhibition against *B. cereus* (7.33 ±0.58mm), *Xanthomonas sp.* (7.33 ± 0.03mm), *B. subtilis* (4.33 ± 0.33mm) and *E. coli* (3.6 ± 0.2mm). In the same way FMC showed inhibition against *B. cereus* (4.67 ±0.58mm), *B. subtilis* (4.67 ± 0.33mm) and *E. coli* (3.3 ± 0.2mm) but *Xanthomonas sp.* had complete resistance against FMC upto 2000 µg/ml. FBR showed poor inhibition towards *E. Coli* (2.33 ± 0.33), *B. subtilis* (1.83 ± 0.17) *B. Cereus* (2.33 ± 0.33) whereas *Xanthomonas sp.* showed total resistance. The FEW extract did not exert any inhibitory activity against tested bacterial strains up to 2000 µg/ml. All the readings were statistically analyzed using ANOVA Mean ± SE.

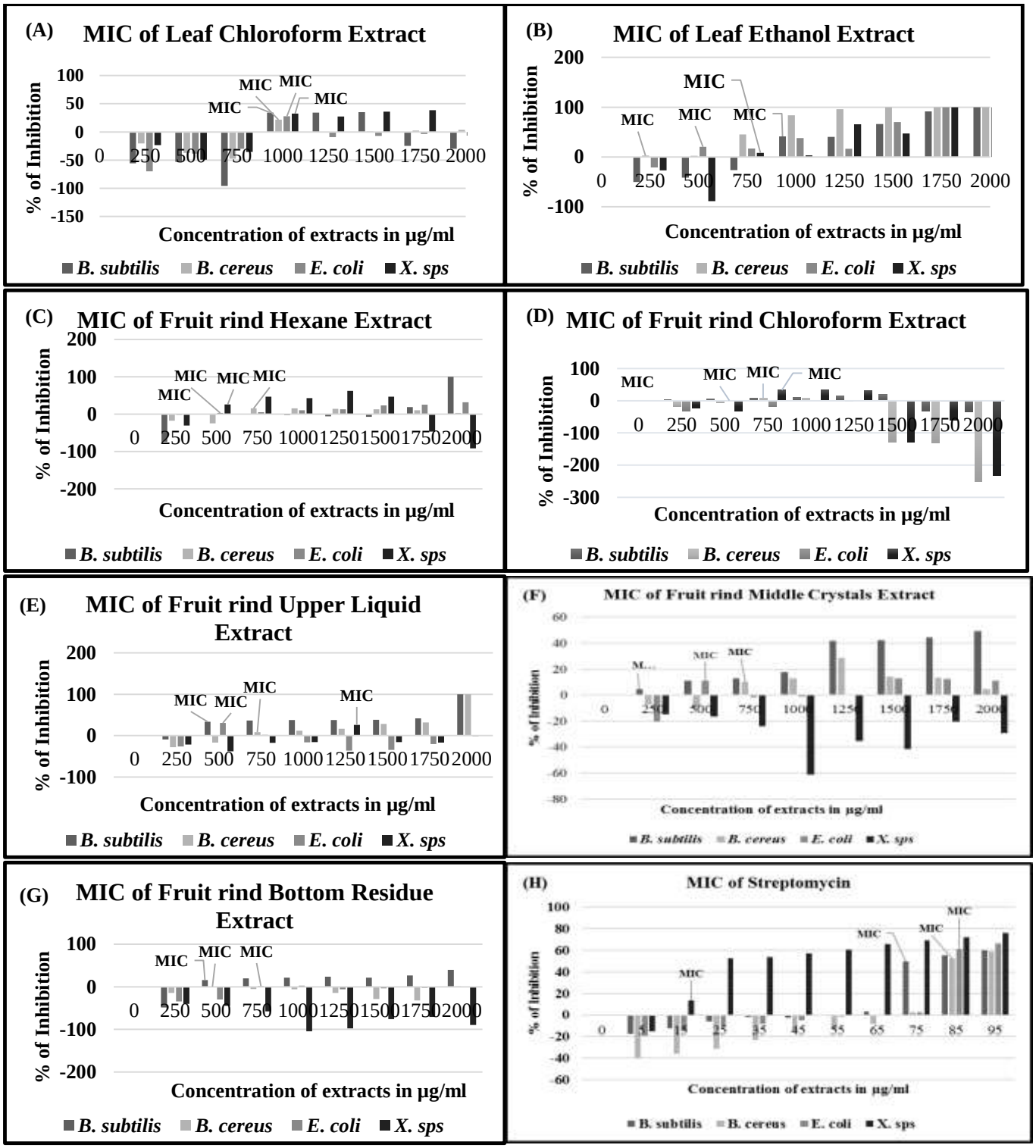

Fig. 1 (A-H): Represents MIC of different extracts of leaves and fruit rind of *T. peruviana* (S).

Minimum inhibitory concentration (MIC)

Among the tested extracts, only active extracts are considered for further analysis and the results are represented in Fig. 1 and Table-2. The percentage of inhibition of all the extracts were calculated and MIC was determined. All the extracts showed the MIC in a range of 250 to 2000µg/ml. Starting from LC extract which showed inhibition concentration of 1000 µg/ml for all four bacterial strains where *E. coli* (27.812%), *B. subtilis* (34.1666%), *B. cereus* (21.631%) and *Xanthomonas sp.* (32.298%) with their respective percentage of inhibition. Similarly, LE extract showed MIC in a range of 250 to 1000 µg/ml with lowest MIC at 250 µg/ml against *B. cereus* (2.836%) followed by *E. coli* (20.312%) and *Xanthomonas sp.* (8.0745%) at 500 µg/ml and 750 µg/ml. The MIC against *B. subtilis* (41.25%) shown by LE extract was 1000 µg/ml.

Table 2: Minimum inhibitory concentration, Minimum bactericidal concentration and Total antimicrobial activity of *T. peruviana* (S) extracts.

Sl. No.	Extracts	Bacterial Type	Test strain	MIC µg/ml	% inhibition	MBC µg/ml	TAA ml/g
			B. s	1000	34.166	1000	14.3
		Gram + ve	B. c	1000	21.631	1000	14.3
1.	**LC**		E. c	1000	27.812	1000	14.3
		Gram - ve	X. s.	1000	32.298	1000	14.3
			B. s	1000	41.25	1000	104.7
		Gram + ve	B. c	250	2.836	1000	418.8
2.	**LE**		E. c	500	20.312	500	209.4
		Gram - ve	X. s.	750	8.0745	750	139.6
			B. s	500	0.833	500	46.2
		Gram + ve	B. c	750	15.602	750	30.8
3.	**FH**		E. c	500	3.437	500	46.2
		Gram - ve	X. s.	500	26.086	750	46.2
			B. s	250	2.5	500	66.4
		Gram + ve	B. c	750	8.865	750	22.133
4.	**FC**		E. c	500	0.3125	500	33.2
		Gram - ve	X. s.	750	32.919	750	22.133
			B. s	500	33.75	500	157.6
		Gram + ve	B. c	750	8.1560	750	105.066
5.	**FUL**		E. c	500	30.312	500	157.6
		Gram - ve	X. s.	1250	26.086	1250	66.04
			B. s	250	4.583	1250	145.2
		Gram + ve	B. c	750	10.283	750	48.4
6.	**FMC**		E. c	500	10.937	500	72.6
		Gram - ve	X. s.	ND	ND	ND	ND
			B. s	500	15.833	500	103
		Gram + ve	B. c	500	0.354	500	103
7.	**FBR**		E. c	750	1.5625	750	68.66
		Gram - ve	X. s.	ND	ND	ND	ND
			B. s	75	35.051	ND	ND
		Gram + ve	B. c	85	10.317	ND	ND
8.	**Standard**	Gram - ve	E. c	85	8.75	ND	ND
			X. s.	25	22.76	ND	ND

LH: leaf hexane, LC: leaf chloroform, LE: leaf ethanol, FUL: Fruit rind upper liquid, FBR: Fruit rind bottom residue, FMC: Fruit rind Middle Crystals FW: Fruit rind water extracts, ND: Not determined.

Table 3: Activity Index of *T. peruviana* (S) extracts.

Sl No.	Extracts	Conc. (µg)	Activity Index			
			B. subtilis	***B. cereus***	***E. coli***	***X. sps.***
1.	**LC**	1000	0.285	0.285	0.57142	0.333
		1500	0.571	0.285	0.7142	0.5
		2000	0.285	0.571	1	0.66
2.	**LE**	1000	0.285	0.142	0.428	0.33
		1500	0.428	0.285	0.571	0.33
		2000	0.285	0.285	0.714	0.66
3.	**FH**	1000	0.285	0.285	0.285	0.33
		1500	0.571	0.428	0.571	0.66
		2000	0.714	0.857	0.714	0.833
4.	**FC**	1000	0.285	0.285	0.285	0
		1500	0.428	0.428	0.571	0
		2000	0.714	0.571	0.857	0
5.	**FUL**	1000	0.285	0.428	0.285	0.333
		1500	0.428	0.571	0.285	1
		2000	0.571	1	0.428	1.166
6.	**FMC**	1000	0.285	0.285	0	0
		1500	0.571	0.428	0.285	0
		2000	0.571	0.571	0.428	0
7.	**FBR**	1000	0.285	0.142	0.285	0
		1500	0.571	0.285	0.428	0
		2000	0.571	0.285	0.571	0

LH: leaf hexane, LC: leaf chloroform, LE: leaf ethanol, FUL: Fruit rind upper liquid, FBR: Fruit rind bottom residue, FMC: Fruit rind Middle Crystals

Fig. 2(1-4) shows inhibition zone observed in FC, FUL, LC and FUL against *B. subtilis*, *B. cereus*, *E. coli* and *X. sp*. respectively. The Minimum inhibitory concentration of FH extract against *B. subtilis* (0.833%), *E. coli* (3.437%) and *Xanthomonas sp* (26.086%) was observed at 500 µg/ml followed by *B. Cereus* (15.602%) at 750 µg/ml. In the same way FC extract showed significant inhibitory concentration of 500 µg/ml against *B. subtilis* (2.5%) and *E. coli* (0.31%), followed *B. cereus* **(**8.865%) and *Xanthomonas sp.* (32.92%) at 750 µg/ml. Among the ethanolic extract fractions, FUL has shown its MIC at 1250 µg/ml against *Xanthomonas sp.* (26.09%) and *B. cereus* (8.16%), followed by *B. subtilis* (33.75%) and *E. coli* (30.31%) at 500 µg/ml. The minimum inhibitory concentration of FMC against *B. subtilis* (4.58%) at 1250 µg/ml, followed by 750 µg/ml against *B. cereus* (10.28%), and *E. coli* (10.94%) was inhibited at a concentration of 500 µg/ml. Similarly, MIC of FBR against *E. coli* **(**1.56%) was at the concentration of 750 µg/ml, followed by 500 µg/ml against *B. subtilis* **(**15.83%) and *B. cereus* **(**0.35%) respectively. *Xanthomonas sp.* seems to be resistant against both the FMC and FBR fractions.

Minimum bactericidal concentrations (MBCs)

Almost all the extracts showed MBC value of ≥1250 µg/ml against all the tested bacterial strains. The LC extract against all four bacteria and LE against *B. subtilis* and *B. cereus*, showed the same MBC value of 1000 µg/ml. The

leaf ethanol extract showed a MBC value of 500 µg/ml and 750 µg/ml against *E. coli* and *Xanthomonas sp*. respectively. Moreover, FH extract showed MBC value of 500 µg/ml for *E. coli* and *B. subtilis*, and 750 µg/ml for *B. cereus* and *Xanthomonas sp*. FC extract followed the same pattern and showed 500 µg/ml of MBC value for *E. coli* and *B. subtilis*, and 750 µg/ml for *B. cereus* and *Xanthomonas sp*.

Similarly, among ethanolic extracts fractions FUL showed 500 µg/ml of MBC value for *E. coli* and *B. subtilis*, and 750 µg/ml for *B. cereus*, whereas, for *Xanthomonas sp*. the MBC value was increased to 1250 µg/ml. The FMC bactericidal concentration for *B. subtilis* was 1250 µg/ml, followed by *B. cereus* 750 µg/ml and *E. coli* with 500 µg/ml of MBC value. Lastly, the MBC values of 500 µg/ml of FBR was observed to be the same against both *B. subtilis* and *B. cereus*. Followed by *E. coli* with MBC value of 750 µg/ml. It has been observed that both the ethanolic fractions FBR and FMC are found to have zero activity even at 2000 µg/ml against *Xanthomonas sp*.

Activity index

The inhibition zone of extracts of *Thevetia peruviana* (S) at different concentrations were compared with standard antibiotics inhibition zone to calculate the activity index and the results are depicted in Table-3. All the extracts showed significant AI. Among the tested extracts, most prominent AI was observed in FUL followed by FH, LE and LC extracts against *Xanthomonas sp.* Similarly, LC followed by FC, LE, FH extracts showed good AI against *E. coli*. Furthermore, FUL followed by FH, LC, FC and FMC extract have also exhibited significant AI against *B. cereus*, whereas FH and FC extracts followed by FUL, FMC and FBR had shown moderate AI against *B. subtilis* when compared to standard streptomycin.

Total antimicrobial activity

Total antimicrobial activity indicates the volume at which extract can be diluted with still having ability to kill microorganism (Table 2). Most of the extracts of *T. peruviana* (S) showed great values of TAA against all the tested strains, which proves the potential of extracts to inhibit growth of the experimental microorganisms even at low concentrations. Among the tested extracts, it has been found that LE extract showed highest TAA (418.8 ml/g) against *B. cereus*, followed by *E. coli* (209.4 ml/g) and *Xanthomonas sp.* (139.6 ml/g). Similarly, FUL fraction exhibited highest TAA against *B. subtilis* (157.6 ml/g).

The activity index and total antimicrobial activity investigations supports the antibacterial effect of extracts. It has been observed that LE, FUL and FC are found to be the most prominent extracts as per as the AI is concerned, whereas, TAA of LE exerted highest activity (418.8 ml/g) against *B. cereus*, *E. coli* (209.4 ml/g) and *Xanthomonas sp.* (139.6 ml/g) and FUL showed highest TAA against *B. subtilis* (157.6 ml/g).

The previous reports of phytochemical investigations of *Thevetia peruviana* (S) has revealed the presence of high content of alkaloids, flavonoids and phenolics among them flavonoids and tannins have been reported to possess antimicrobial activity (Nazneen et al., 2014) (Yebpella *et al.*, 2011). It may be due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall, whereas tannins may be related to their ability to inactivate microbial adhesion enzymes and cell envelop proteins (Dahot *et al.*, 1999). Furthermore, present investigations have revealed that among the extracts, the fruit rind extracts have most prominent inhibition abilities against tested bacteria, which are validating the use of this plant in traditional system of medicine and this is the first report of exploration of above extracts for their antibacterial activities against *B. subtilis*, *B. cereus*, *E. coli* and *Xanthomonas sp*.

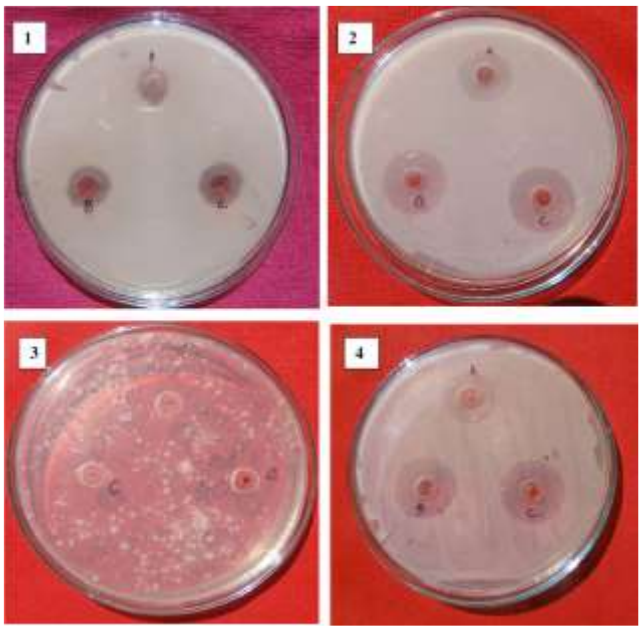

Fig. 2: 1-4 represents the inhibition zone of FC, FUL, LC and FUL against B. subtilis, B. cereus, E. coli and X. sp. respectively.

Conclusion

The results of present investigation clearly indicate that both the leaf and fruit rind extracts have showed significant antibacterial activity when compared to the standard in different bacterial strains. The results clearly revealed the importance of extracts of *Thevetia peruviana* (S) when compared with antibiotics, to control resistant bacteria, which are becoming a threat to human health. Furthermore, fruit rind extracts have shown promising results, which can be considered for further pharmacological investigations.

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