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Research Article

ANTIBACTERIAL ACTIVITY OF *SOLANUM PUBESCENS* - AN ETHNOMEDICINAL PLANT FROM SOUTH WESTERN REGION OF ANDHRA PRADESH

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Abstract

The present movement to find out alternative antibacterial drugs from medicinal plants and the presence bioactive phytochemicals in fruit and stem bark of *Solanum pubescens*, promoted the authors to take up the antibacterial evaluation of fruit and stem bark extracts of *Solanum pubescens.* Against clinical and plant pathogenic bacterial strains by employing the methods of National Committee for Clinical Laboratory Standards (NCCLS).

It was observed that all the extracts showed inhibition zone against one or more of the tested bacteria between 80 to 160 µg/ml concentrations with 3.17 ±0.17 to 10 ± 0.17 mm inhibition. The spectroscopic determination of MIC and MBC exhibited by extracts went between 100-300 µg/ml. In fruit extracts, fruit chloroform (FC) has inhibited all the tested organisms, similarly, fruit ethyl acetate (FEA) inhibited *Bacillus subtilis* and *Xanthomonas sp.* whereas, fruit ethanol (FET) *has* been restricted to inhibit *Bacillus subtilis* at 100 µg/ml. Furthermore, among all the active extracts of stem bark extracts bark chloroform (BC) showed potential inhibition of *Bacillus subtilis* and *Escherichia coli* with 81.44% and 82.17% respectively. Similarly, bark bottom crystals (BBC) extracts inhibited *Bacillus cereus* and *Xanthomonas sp.* with 77.38% and 77.72% respective inhibition at 100 µg/ml concentration.

The present exploration has revealed that the extracts from fruit and stem bark of *S. pubescens* revealed potential antibacterial activity against Gram (+) and Gram (-) bacteria, which are validating the ethnomedicinal claims and this the first report of investigation of above extracts for its antibacterial activity.

Key words: *Solanum pubescens;* ethnomedicinal; extracts; antibacterial; spectroscopic.

Introduction

The introduction of the sulphonamide antibiotics in the 1930s and penicillin in the 1940s revolutionised medicinal practice by dramatically decreasing the fatality rates associated with bacterial infections (Sneader 2005, Newman *et al.*, 2000, Drews2000). These discoveries led to a concerted search for new antibacterial drugs during the following 30 years and resulted in the discovery of most of the antibacterial drug classes known today, many of which were derived from natural product leads (Sneader W. 2005, Walsh CT, *et al.*, 2005, Finch RG *et al.*, 2003).

Natural products are both a fundamental source of new chemical diversity and an integral component of today's pharmaceutical compendium. However, many currently available antifungal and antibacterial agents have undesirable toxicity, and the widespread use of these drugs has led to rapid development of drug-resistant strains, which are the leading cause of failure in both clinical and agricultural applications (Muhammad Saleem *et al.*, 2009).

The prevalence of natural product-derived antibacterial drugs may be due to the evolution of secondary metabolites as biologically active chemicals that conferred selectional advantages to the producing organisms. Natural products also are likely to have evolved to penetrate cell membranes and interact with specific protein targets (Stone 1992). In addition, natural products have an element of structural complexity, which is required for the inhibition of many antibacterial protein targets (Mark, 2006).

The genus *Solanum* is the largest genera of the family Solanaceae consisting of more than 1700 species distributed all over the world. Several species of genus *Solanum* are used in the folk medicine of different countries, Brazil, India, Taiwan, Germany, South Africa and Kenya, as remedy for various ailments such as hypoglycemic (Kar *et al.*, 2006), hepatoprotective (Son *et al.*, 2003), hepatotonic (De Silva *et al.*, 2003). Laxative, appetizer, cardiotonic (Mans *et al.*, 2004), antispasmodic, renal pain, epilepsy (Perez *et al.*, 2006; Schwarz *et al.*, 2005), gastric, liver disorder (Antonio *et al.*, 2004; Mesia-Velal *et al.*, 2002), treatment of bronchitis, itches, body aches, cancer (Koduru *et al.,* 2006; Oboh *et al.,* 2005). Various chemical constituents are reported to be isolated from Solanum species, which includes alkaloids, phenolics, flavanoides, sterols saponins and their glycosides (Amir and Kumar, 2004). Alkaloides such as soladunalinidine and tomatidine were isolated from leaf and stem of Solanum species (Swapna Latha *et al.,* 2006).

Solanum pubescens is a wild plant. It is an annual erect, unarmed shrub growing upto 1.5m tall abundantly growing as weed of forest and the hills of South *Western Region* in Andhra Pradesh, India and commonly known as ushtichettu, kasivuste and pajarito in telugu and kaattu sundai kaai in tamil, flowering and fruiting is in the month of July to February.

Solanum pubescens is a traditional medicine plant used for the treatment of liver disorders, diarrhoeal diseases and cancer disorders, to treat head ache's, menstrual pain, rheumatoid arthritis, tuberculosis, ulcers, etc. (Hemamalini *et al.,* 2011, Sumalatha *et al.,* 2013). Similarly in scientific literature there are very few reports on evaluated pharmacological properties like antidiabetic (Hemamalini *et al.,* 2012), hepatoprotective (Hemamalini *et al.,* 2012), gastroprotective (Hemamalini *et al.,* 2011), anti-inflammatory (Niyogi *et al.,* 2012), anti-anxiety, anti-depressants, myorelaxant (Deepika *et al.,* 2013), and Antidiarrheal (Anurag Bhargav *et al.,* 2012). However, there are no reports on the antibacterial activity of this plant, although it has been used in the treatment of whooping cough and of certain other diseases (Reddy *et al.,* 2006). Furthermore, the quantitative analysis has revealed that the fruit and stem bark of *Solanum pubescens* is very rich in phenolics followed by flavonoid, alkaloid, saponins, carbohydrates and oils, which gives a very strong reason to select this plant for future pharmacological evaluation (Haseeb *et al.,* 2014).

According to Mathekga and Mayer (1998), *in vitro* antimicrobial screening methods could provide the needed preliminary observations necessary to select among crude extracts, those with potentially useful properties for further chemical and pharmacological investigations. Keeping this in view, the present study was designed by selecting *Solanum pubescens* wild to systematically screen the antibacterial potentials of different extracts of fruit and stem bark on clinical and plant pathogenic bacterial strains.

Materials and Methods

Plant material collection

Unripe fruits of *Solanum pubescens* and stem bark were collected from the surrounding hills of Rayadurg jurisdiction of South Western region, Anantapur Dist. Andhra Pradesh, India. The plant was confirmed by referring the Phytographia ((1794)) followed by the authentication of a taxonomist Prof. Pullaiah, Dept. of Botany, Sri Krishnadevaraya University, Anantapur, Andhra Pradesh. The specimen is deposited at Department of Biotechnology, Kuvempu University, Shankaraghatta, Karnataka.

Chemicals

Hexane, chloroform, ethyl acetate, dimethyl sulfoxide (DMSO), ethanol and all the chemicals used for antibacterial activity analysis were purchased from Merck and Himedia. All chemicals and solvents of analytical grade were used.

Soxhlet extraction

Successive extraction was done using 300 g of powdered material of fruit and stem bark in soxhlet apparatus. The solvents hexane (2L, 50ºC ˜ 15 cycles) chloroform (2 L, 45ºC ˜15 cycles), ethyl acetate (2L, 70ºC ˜ 15 cycles) and ethanol (2 L, 70ºC, ˜15–17cycles) were used. All the extracts were concentrated *in vacuo*, The yield of each dried extract was calculated.

Interestingly it was observed that the fruit rind ethanolic extract showed three different fractions. Based on that the fractions were separated as Bottom crystals (BC) and Upper liquid (UL), all the dried extracts were used for the further analysis.

Growth and maintenance of test bacterial cultures

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

Preparation of inoculum

Gram-positive *Bacillus subtilis (B. cereus)* and *Bascillus cereus (B.cereus)* and gram-negative bacteria *Escherichia coli,* (*E. coli*) and *Xanthomonas sp.* (*X. sp.*) were precultured on nutrient broth overnight in a rotary shaker at 37°C, 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 extracts except fruit hexane extract 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 80, 120 and 160 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 100,200 up to 500 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 use at 37ºC to get inoculum size of 10^8 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 80, 120 and 160 µg/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 exhibited activity are considered for further analysis.

Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) of all the nine extracts was estimated by the modified method of National Committee for Clinical Laboratory Standard (2012) 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. 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 viability.

The Investigating extracts were added to the microtiter plate in a concentration gradient 100, 200 to 500 µg/ml, to each well 150 µl of inoculum was added, negative control was inoculum without the extract and positive was with streptomycin. The plates were kept at 37ºC 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 % inhibition = $[(A_{control} - A_{test})/A_{control}] \times 100$ and MIC was considered to the concentration of the extract at which the percentage was in positive range.

Minimum Bactericidal Concentrations (MBCs)

A modified method of National Committee for Clinical Laboratory Standard (2000) and Veljic *et al.*, (2010) was employed to estimate the minimum bactericidal concentrations (MBCs) of the extracts. Serial sub cultivation of 4 µl of MIC inoculum into microtiter plates containing 100µl of broth per well and incubated for 24 hrs at 37ºC. The lowest concentration of extract with no visible growth was considered as MBC followed by spectrophotometric analysis at 600 nm. Keeping plane broth as control. The minimum bactericidal concentrations of the extracts were determined to the O.D. equal to the reference. 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 zones of inhibition with the standard reference antibiotics. The activity index of the crude plant extract was calculated by employing the method of Arya *et al.*, 2010.

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 (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).

Results

Soxhlet extraction

300 gram of the powdered material of leaves and fruit rind was refluxed separately with 1/10 (w/v) hexane, chloroform, ethyl acetate and ethanol in a soxhlet apparatus for 48 h. The percentage yield of hexane, chloroform, ethyl acetate and ethanol extracts from fruit and stem bark were calculated. Where, in fruit extracts ethanolic extract has maximum percentage of yield of (15.45%) followed by hexane (4.94%), chloroform (3.41%) and ethyl acetate extracts (1.171%). Whereas, in stem bark extract, ethanol extract showed maximum percentage of yield of (UL, 11.59% and BC 0.836 %) followed by ethyl acetate, hexane and chloroform with 3.55%, 1.24% and 1.08% respectively.

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

Antibacterial activity of fruit and stem bark extract of *S. pubescens* was examined against *B. cereus*, *B. subtilis*, *E.*

coli and *Xanthomonas sp*. The antibacterial activity of the test extracts was assayed by the agar disc diffusion method. All the tested extracts exhibited inhibition activity against one or more test organisms, and the results are tabulated in Table 1. Among the fruit extracts FC has showed favourable inhibition against all the tested strains with a zone of inhibition of (2.17 ± 0.17nm), similarly, FEA showed inhibition only against *B. subtilis,* and *Xanthomonas sp*. with ZI of (2.17 ± 0.17nm). Whereas, FET showed inhibition only against *E. coli* (2.17 ± 0.17nm) and (1.17 ± 0.17nm) respectively.

The stem bark extracts showed promising results against all the tested bacterial strains, where BH showed good inhibition against all the tested strains where high inhibition was observed against *E. coli* (4.17 ± 0.17nm) followed by *B. subtilis* (3.17 ± 0.17nm), *B. cereus* and *Xanthomonas sp*. (2.17 ± 0.17nm). Similarly, BC showed inhibition against *B. subtilis, B. cereus, E. coli* and *Xanthomonas sp*. with ZI of (2.17 ± 0.17nm). BEA also showed good inhibition against *Xanthomonas sp*. (5.17 ± 0.17nm) followed by *B. subtilis* (2.17 ± 0.17nm), *B. cereus* (2.17 ± 0.17nm), BUL has showed highest inhibition against *E. coli* and *Xanthomonas sp*. (10.17 ± 0.17mm) fallowed by *B. cereus* (9.17 ± 0.0mm) and *B. subtilis* (4.17 ± 0.17nm). Whereas, BBC also exhibited high inhibition against *B. cereus* and *Xanthomonas sp*. (9.17 ± 0.17nm) followed by *E. coli* (6.17 ± 0.17nm). The antibacterial activities of the fruit and stem bark extracts (160µg/ml) were compared favourably with that of standard antibiotics (streptomycin 10µg/ml). All the readings were statistically analyzed using ANOVA Mean ± SE

Activity index

The activity index of *Solanum pubescens* extracts are calculated by considering the zone of inhibition of extracts at different concentrations with standard antibiotics inhibition zone and the results are tabulated in the Table 2. All the extracts showed significant AI. Among the tested extracts, BUL exhibited most prominent AI followed by BBC, BEA and FC against *Xanthomonas sp.* Similarly, BUL followed by BBC, BH showed good AI against *E. coli.* Furthermore, BUL followed by BBC exhibited significant AI against *B. subtilis.* Whereas BUL has shown moderate AI against *B. cereus* when compared to standard streptomycin.

Table 1: Zone of inhibition of different extracts of *S. pubescens* against four different Bacteria.

Sl. No.	Extracts	Concentration (µg)	Zone of Inhibition (mm)			
			B. subtilis	***B. cereus***	***E. coli***	***X. sp***
1.	**FC**	80	NA	NA	NA	1.17 ± 0.17
		120	1.17 ± 0.17	1.17 ± 0.17	1.17 ± 0.17	2.17 ± 0.17
		160	2.17 ± 0.17	2.17 ± 0.172	2.17 ± 0.172	2.17 ± 0.17
2.	**FEA**	80	2.17 ± 0.172	NA	NA	2.17 ± 0.172
		120	2.17 ± 0.172	NA	NA	2.17 ± 0.172
		160	2.17 ± 0.172	NA	NA	2.17 ± 0.172
3.	**FET**	80	1.17 ± 0.17	NA	NA	NA
		120	1.17 ± 0.17	NA	NA	NA
		160	1.17 ± 0.17	NA	NA	NA
4.	**BH**	80	2.17 ± 0.17	1.17 ± 0.17	2.17 ± 0.17	2.17 ± 0.17
		120	2.17 ± 0.17	1.17 ± 0.17	3.17 ± 0.17	2.17 ± 0.17
		160	3.17 ± 0.17	2.17 ± 0.17	4.17 ± 0.17	2.17 ± 0.17
5.	**BC**	80	1.17 ± 0.17	1.17 ± 0.17	2.17 ± 0.17	2.17 ± 0.17
		120	1.17 ± 0.17	1.17 ± 0.17	2.17 ± 0.17	2.17 ± 0.17
		160	2.17 ± 0.17	2.17 ± 0.17	2.17 ± 0.17	2.17 ± 0.17
6.	**BEA**	80	2.17 ± 0.172	2.17 ± 0.172	NA	3.2 ± 0.2
		120	2.17 ± 0.172	2.17 ± 0.172	NA	5.17 ± 0.17
		160	2.17 ± 0.172	2.17 ± 0.172	NA	5.17 ± 0.17
7.	**BUL**	80	3.17 ± 0.17	9.17 ± 0.17	8.17 ± 0.17	8.17 ± 0.17
		120	4.17 ± 0.17	9.17 ± 0.17	9.17 ± 0.17	9.17 ± 0.17
		160	4.17 ± 0.17	9.17 ± 0.0	10.17 ± 0.17	10.17 ± 0.17
8.	**BBC**	80	NA	5.17 ± 0.17	6.17 ± 0.17	5.17 ± 0.17
		120	NA	6.17 ± 0.17	6.17 ± 0.17	6.17 ± 0.17
		160	NA	7.17 ± 0.17	6.17 ± 0.17	7.17 ± 0.17
9.	**Std**	10	7 ± 0	7 ± 0	7 ± 0	6 ± 0

FC: Fruit chloroform, FEA: Fruit ethyl acetate FE: Fruit ethanol, BH: Bark hexane, BC: Bark chloroform, BEA: Bark ethyl acetate, BUL: Bark upper liquid, BBC: Bark bottom crystals, Std: standard (streptomycin), NA: Not active.

Table 2: Activity index of *S. pubescens* extracts.

Sl. No.	Extracts	Conc. (µg)	Activity Index			
			B. subtilis	***B. cereus***	***E. coli***	***X. sp***
1.	**FC**	80	0	0	0	0.195
		120	0.16	0.16	0.16	0.361
		160	0.31	0.31	0.31	0.361
2.	**FEA**	80	0.31	0	0	0.361
		120	0.31	0	0	0.528
		160	0.31	0	0	0.695
3.	**FET**	80	0.167	0	0	0
		120	0.31	0	0	0
		160	0.452	0	0	0
4.	**BH**	80	0.31	0.167	0.31	0.361
		120	0.31	0.167	0.452	0.361
		160	0.452	0.31	0.595	0.361
5.	**BC**	80	0.167	0.167	0.31	0.361
		120	0.167	0.167	0.31	0.361
		160	0.31	0.31	0.31	0.361
6.	**BEA**	80	0.31	0.31	0	0.533
		120	0.31	0.31	0	0.861
		160	0.31	0.31	0	0.861
7.	**BUL**	80	0.452	1.31	1.167	1.36
		120	0.595	1.28	1.31	1.528
		160	0.595	1.31	1.452	1.695
8.	**BBC**	80	0	0.738	0.881	0.861
		120	0	0.881	0.881	1.028
		160	0	1.024	0.881	1.195

Minimum inhibitory concentration (MIC) and percentage inhibition

The determination of MIC and percentage inhibition of all extracts were tabulated in (Table 3). All the fruit and stem bark extracts showed the MIC in a range of 100 to 400 µg/ml. In fruit extracts FC showed MIC at 100 µg/ml against *B. subtilis*, *B. cereus*, *E. coli* and *Xanthomonas sp.* with 49.484%, 29.365%, 6.521% and 21.065 percentage of inhibition respectively. Similarly, FEA showed MIC at 100 µg/ml against *B. subtilis* (38.402%) and *Xanthomonas sp.* (20.581%), whereas, FET was restricted to inhibit the *B. subtilis* (16.752%) at 100 µg/ml (Fig.: 1 A-C).

The investigation of Minimum inhibitory concentration of stem bark extracts were performed (Fig. 1 D-H) where, BH showed MIC of 200 µg/ml against *B. subtilis* and 100 µg/ml against *B. cereus*, *E. coli* and *Xanthomonas sp.* with 28.09%, 25%, 59.13%, 22.518% respective percentage inhibition. Similarly, BC showed significant inhibitory concentration of 100 µg/ml against *B. subtilis* (81.443%) *B. cereus* (40.476%) and *E. coli* (73.913%), followed by *Xanthomonas sp.* (7.263%) at 200 µg/ml. Furthermore, BEA exhibited MIC at 100 µg/ml against *B. subtilis* (8.505%) *B. cereus* (30.936%) and *Xanthomonas sp.* (72.881%) with respective percentage inhibition. Whereas, BUL exhibited MIC at 100 µg/ml against *B. subtilis* and *B. cereus, E. coli and Xanthomonas sp.* with 75%, 30.952%, 20%, and 60.29% respective percentage inhibition and BBC also revealed MIC at 100 µg/ml against *B. cereus, E. coli* and *Xanthomonas sp.* with 77.38%, 68.26%, and 77.723 percentage inhibition respectively. The MIC of the fruit and stem bark extracts were compared sympathetically with that of MIC of standard antibiotics streptomycin.

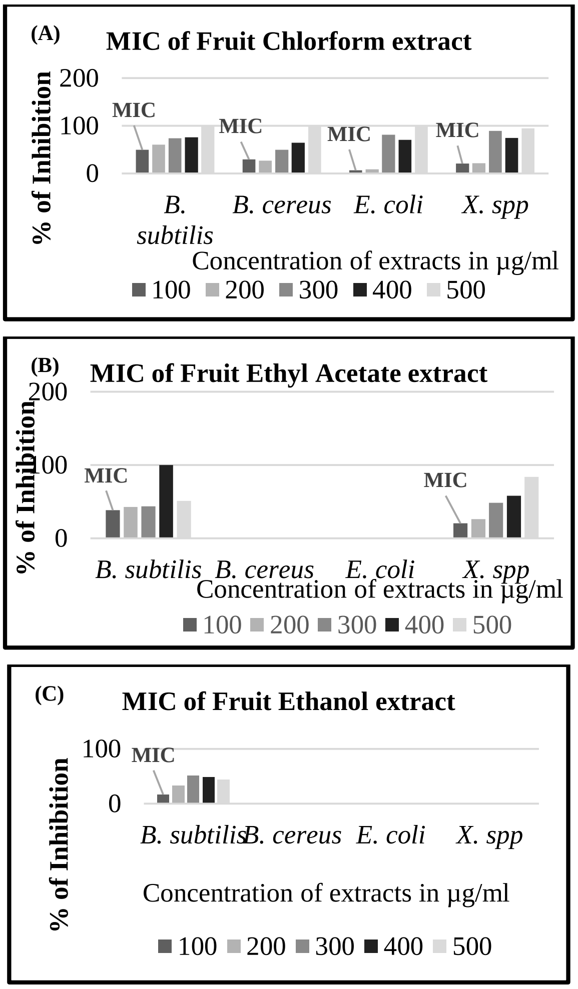
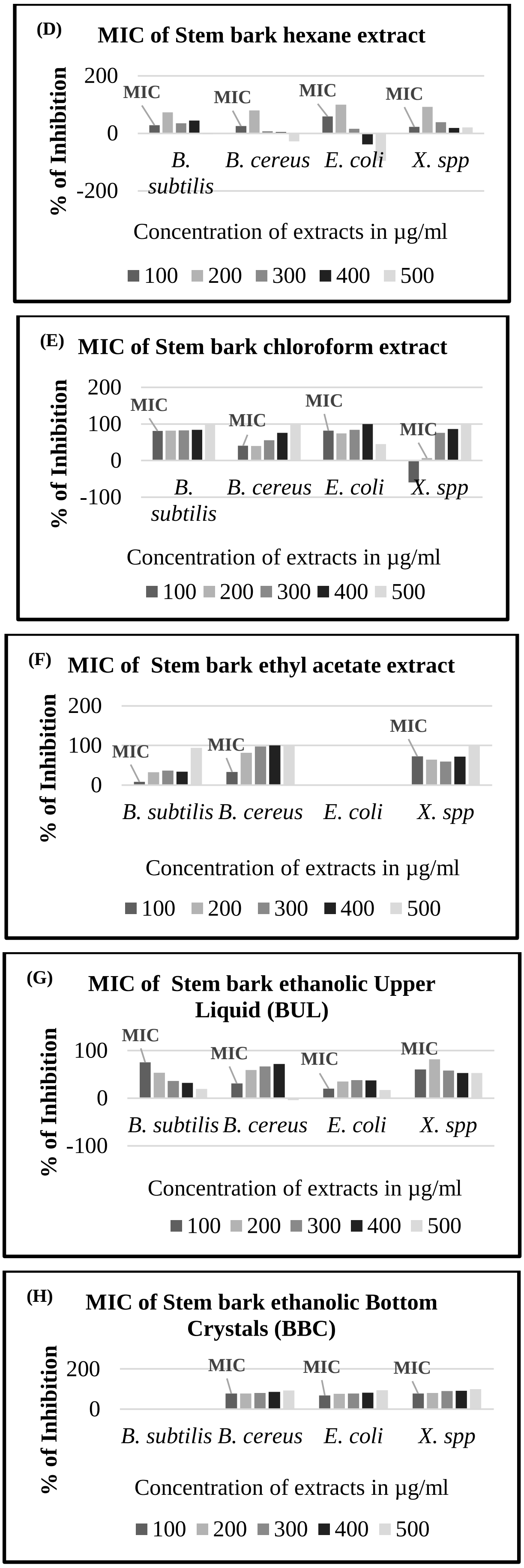


Fig. 1 (A-C): Percentage inhibition and MIC of *S. pubescens* fruit extracts



Fig, 1 (D-H): Percentage inhibition and MIC of *S. pubescens* stem bark extracts

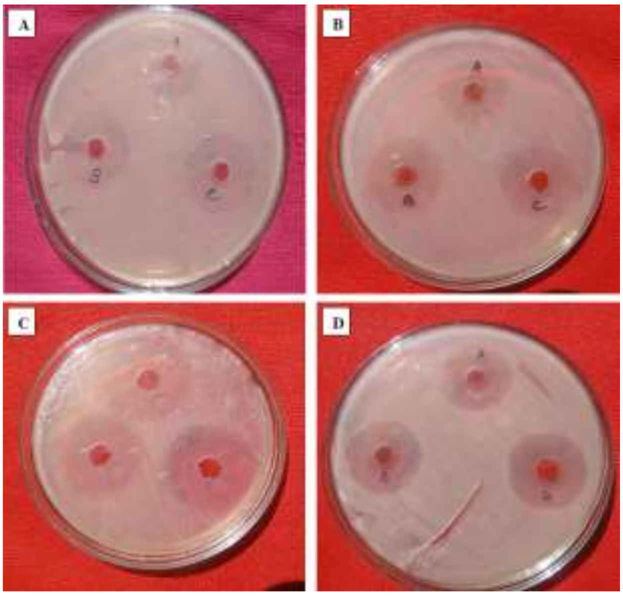


Fig. 2: A-D represents the inhibition zone of BUL extracts of *S. pubescens* against *B. subtilis, B. cereus, E. coli* and *X. sp.* respectively.

Minimum bactericidal concentrations (MBCs)

Minimum bactericidal concentrations of all the extracts showed value of ≥400 µg/ml against all the tested bacterial strains. Among the fruit extracts, FC showed MBC at 300 µg/ml against all the tested bacteria, FEA exhibited 200 µg/ml as MBC against *B. subtilis* and *Xanthomonas sp.*, whereas, FET showed MBC at 200 µg/ml against *B. subtilis.*

The stem bark extracts also exhibited significant MBCs where, BH showed 200 µg/ml concentration as MBC against all the tested bacterial strains. Similarly, *B. subtilis, B. cereus,* and *E. coli* revealed sensitivity to BC at 100 µg/ml and 200 µg/ml against *Xanthomonas sp.* as MBC. Furthermore, BEA has shown MBC at 200 µg/ml against *B. subtilis, B. cereus,* and 100 µg/ml against *Xanthomonas sp.* Whereas, BUL exhibited MBC at 100 µg/ml against *B. subtilis, Xanthomonas sp. B. cereus,* and *E. coli* are sensitive at 200 µg/ml. *B. subtilis, E. coli* and *Xanthomonas sp.* exhibited sensitivity to BBC at 100 µg/ml as MBC (Table 3).

Total antimicrobial activity

Total antimicrobial activity indicates the volume at which extract can be diluted with still having ability to kill microorganism Eloff (2004) Most of the extracts of *S. pubescens* showed high values of TAA against all the tested strains, which proves the potential of extracts to inhibit growth of the tested microorganisms even at low concentration. Among the tested extracts, it has found that FET showed highest TAA (1554 ml/g) against *B. subtilis.* Similarly, FC exhibited highest TAA against *Xanthomonas sp.* (204 ml/g) as represented in Table 3.

Table 3: Minimum inhibitory concentration, Minimum bactericidal concentration and Total antimicrobial activity of *S. pubescens* extracts.

Sl. No.	Extracts	Bacterial Type	Test organism	MIC (µg)	% inhibition	MBC	TAA (ml/g)
1.	**FC**	Gram + ve	*B. subtilis*	100	49.484	300	204
			B. cereus	100	29.365	300	204
		Gram - ve	*E. coli*	100	6.521	300	204
			Xanthomonas sp.	100	21.065	300	204
2.	**FEA**	Gram + ve	*B. subtilis*	100	38.402	200	172
			B. cereus	0	0	ND	ND
		Gram - ve	*E. coli*	0	0	ND	ND
			Xanthomonas sp.	100	20.581	200	172
3.	**FET**	Gram + ve	*B. subtilis*	100	16.752	200	1554
			B. cereus	0	0	ND	ND
		Gram - ve	*E. coli*	0	0	ND	ND
			Xanthomonas sp.	0	0	ND	ND
4.	**BH**	Gram + ve	*B. subtilis*	200	28.092	200	62.1
			B. cereus	100	25	200	124.2
		Gram - ve	*E. coli*	100	59.13	200	124.2
			Xanthomonas sp.	100	22.518	200	124.2
5.	**BC**	Gram + ve	*B. cereus*	100	81.443	100	106
			B. cereus	100	40.476	100	106
		Gram - ve	*E. coli*	100	82.17391	100	106
			Xanthomonas sp.	200	7.263	300	53
6.	**BEA**	Gram + ve	*B. subtilis*	100	8.505	200	385.3
			B. cereus	100	32.936	200	385.3
		Gram - ve	*E. coli*	0	0	ND	ND
			Xanthomonas sp.	100	72.881	100	385.3
7.	**BUL**	Gram + ve	*B. subtilis*	100	75	100	386.5
			B. cereus	100	30.952	200	3.86.5
		Gram - ve	*E. coli*	100	20	200	386.5
			Xanthomonas sp.	100	60.29	100	3.86.5
8.	**BBC**	Gram + ve	*B. subtilis*	0	0	ND	ND
			B. cereus	100	77.38	100	83
		Gram - ve	*E. coli*	100	68.26	100	83
			Xanthomonas sp.	100	77.723	100	83
9.	**Std.**	Gram + ve	*B. subtilis*	75	49.484	ND	ND
			B. cereus	85	52.380	ND	ND
		Gram - ve	*E. coli*	85	60.937	ND	ND
			Xanthomonas sp.	15	13.801	ND	ND

ND: Not determined.

Discussion

This study has evaluated the antibacterial activity of fruit and stem bark extracts of *S. pubescens* an ethnomedicinal plant used for common ailments. In our study, all extracts of *S. pubescens* showed inhibition zones against one or more tested strains at lower doses (160 µg per disc). Presumably, all the extracts contains more inhibitory principles, in our previous phytochemical examination of the taxon *Solanum pubescens,* has revealed the presence of high content of alkaloids, flavonoids and phenolics. Furthermore, the plant like *Thevetia peruviana* which has been abundantly explored for its pharmacological properties is reported for it richness in bioactive components like alkaloids, flavonoids, cardiac glycosides, phenolics etc. (Nazneen *et al.,* 2014). It was clearly stated that plants contained microbial inhibitors (i.e., flavonoids) soluble in aqueous methanol, and the flavonoid aglycones were more active than their glycosidic forms naturally present in plants (Otshudi *et al.*, 1999; Rauha *et al.*, 2000).

Among the tested extracts highest inhibition was observe in the ethanolic extracts (BUL, BBC) of stem bark with observed zone of inhibition is 10.17 ± 0.17mm against *E. coli* and *Xanthomonas sp.* However, the zone of inhibition is ranged between 6 ± 0 to 7 ± 0 mm for Streptomycin sulphate (10 mcg/disc). It is noteworthy that the fruit ethanolic extract was moderately active only against *B. subtilis* compared to other extracts.

The MIC and MBC in active extracts of the *S. pubescens* was assessed and the results indicated that in general all the tested extracts are most effective, which had MICs of 100, 200 and 400 µg/ml. The same extracts were tested for minimum bactericidal concentration, the investigations have confirmed that all the extracts exhibited MBC in a range of 100 to 400 µg/ ml which are almost equal to their minimum inhibitory concentrations. Furthermore, the analysis of percentage inhibition has revealed that among the fruit extract FC inhibited *B. cereus* 29.36% and *Xanthomonas sp.* 21.06% inhibition at MIC (100 µg/ml). Among the stem bark extracts, BC exhibited 81.44% and 82.17% inhibition of *B. subtilis* and *E. coli* at MIC of 100 µg/ml. Similarly, BBC revealed inhibition at MIC 100 µg/ml against *B. cereus* and *Xanthomonas sp.* with 77.38% and 77.72% respective inhibition. The diversity among the results of zone of inhibition and MIC and MBC determination was unknown.

It is interesting to report that the active extracts from fruit and stem bark of *S. pubescens* are more active against both Gram positive and Gram-negative bacteria. Whereas, in accordance with previous findings, Gram-negative bacteria were not susceptible to plant extracts when compared to Gram-positive bacteria (Brantner *et al.*, 1996; Nostro *et al.*, 2000; Ojala *et al.*, 2000). The inhibition of gram-positive and gram-negative bacteria by the extracts of *S. pubescens* reveals the authenticity of this plant as a vital source of natural antibacterial agents against human and plant pathogens. The findings are also 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*.

Conclusion

The present study conclusively demonstrated the antibacterial activities of *Solanum pubescens*, a ethnomedicinal plant used for common ailments. The results indicated that crude extracts of fruit and stem bark of *S. pubescens* have useful antibacterial properties. The stem bark extracts were found to be more effective at inhibiting bacterial growth than the fruit extracts. Of the four cultivars tested, BC followed by BBC and FC showed the highest level of antibacterial activity. The results of the agar well diffusion assay in the present study indicated that the ethanol extracts of stem bark of *S. pubescens* exhibited the highest level of antibacterial activity against all of the bacterial strains tested. The other results of present investigations suggests that the active extracts can be considered for further pharmacological investigations. Furthermore, *S. pubescens* could be used as a natural source for its antibacterial properties in the pharmaceutical industries.

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