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Antibacterial Evaluation of Some Plant Extracts Against Some Human Pathogenic Bacteria

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Abstract: The aqueous, different solvent extracts and isolated constituents of eight higher medicinal plants viz., *Argemone mexicana*, *Caesalpinia coriaria*, *Decalepis hamiltonii*, *Euphorbia tirucalli*, *Leucas aspera*, *Phyllanthus amarus*, *Phyllanthus niruri*, *Tinospora cordifolia* and *Tribulus terrestris* were screened *in vitro* for antibacterial activity by cup diffusion method against eleven human pathogenic bacteria. *Caesalpinia coriaria* (Jacq.) Willd. (Caesalpinaceae) recorded significant antibacterial activity against all the test bacteria. The Minimal Inhibitory concentration (MIC) value of the aqueous extract of leaves and pod of *C. coriaria* for the test bacteria ranged between 2 to 10 mg/ml and 0.5 to 4 mg/ml, respectively. Similarly the MIC value of the methanol extracts of leaves and pod of *C. coriaria* for the test bacteria varied between 0.5to 4 mg/ml and 0.5 to 4 mg/ml, respectively depending on the bacterial species. Phytochemical analysis of leaf and pod materials revealed that the antibacterial activity is due to presence of acidic and phenolic fractions. Further separation of active fraction resulted in the loss of antibacterial activity, indicating a synergistic effect of the isolated active fraction. Comparison of the inhibitory activity with the antibiotics Gentamicin and Streptomycin revealed that methanol extract of both leaf and pod and aqueous pod extracts were significantly higher than that of the antibiotics tested. The results suggest that *C. coriaria* is a potential candidate plant for further exploitation in medical microbiology.

Key words: Plant extracts · Antibacterial activity · Human pathogenic bacteria

INTRODUCTION

The emergence of multiple drug resistant bacteria (MDR) has become a major cause of failure of the treatment of infectious diseases [1, 2]. As a result, society is facing one of the most serious public health dilemmas over the emergence of infectious bacteria displaying resistance to many and in some cases all, effective antibiotics [3]. Much like the situation in human medicine, the use of antibiotics in agriculture, livestock and poultry has accelerated the development of antibiotic resistant strains of microbial pathogens, potentially complicating treatment for plants, animals and humans [4, 5]. Furthermore changing patterns of susceptibility and the availability of new antimicrobial agents require continuous updating of knowledge concerning treatment of disease caused by such pathogens. Thus there is a need to look for alternative strategies for the management of disease resistant bacteria. One of the possible strategies towards this objective involves the rational localization of bioactive phytochemicals which have antibacterial activity, may be one of the important approaches for the containment of antibiotic resistance [6, 7]. Even today plants are the almost exclusive source of drugs for the majority of the world population. People in developing countries utilize traditional medicine for their primary health care needs [8, 9]. The potential of higher plants as a source for new drugs is thus still largely unexplored [10]. This is also true in India and only a small percentage of plants of this region have been evaluated for antibacterial activity against human pathogens [11,12]. Thus considering the vast potentiality of plant as a source of new therapeutic agents, hence detail investigations were conducted to test the efficacy of some plant extracts against important human pathogenic bacteria.

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MATERIALS AND METHODS

Plant Materials: Fresh plant materials viz., *Argemone mexicana* L. (Papaveraceae) (Leaf), *Caesalpinia coriaria* (Jacq.) Willd. (Caesalpinaceae) (Leaf and pod), *Decalepis hamiltonii* Wight & Arn.(Asclepiadaceae) (Rhizome), *Euphorbia tirucalli* L. (Euphorbiaceae) (Leaf), *Leucas aspera* Spr. (Lamiaceae)(Leaf), *Phyllanthus niruri* L. (Euphorbiaceae) (Leaf), *Tinospora cordifolia* Miers. (Menispermaceae)(Leaf), *Tribulus terrestris* L. (Zygophyllaceae) (Leaf), free from disease were collected from Mysore region of Karnataka, washed thoroughly 2-3 times with running tap water and once with sterile water, shade-dried, powdered and used for extraction.

Preparation of Aqueous Extracts: Samples (50 gm) of each, plant materials were macerated with 100 ml sterile distilled water in a warring blender (Waring International, New Hartford, CT, USA) for 10 min. The macerate was first filtered through double-layered muslin cloth and then centrifuged at 4000 g for 30 min. The supernatant was filtered through Whatman No. 1 filter paper and sterilized at 120°C for 30 min. The extract was preserved aseptically in a brown battle at 5°C until further use [13]. Only *C. coriaria,* which recorded highest antibacterial activity, the minimal inhibitory concentration (MIC) was determined and subjected to successive solvent extraction.

Preparation of Solvent Extract: Sample (50 gm) of the shade-dried powder of *C. coriaria* was extracted in a Soxlet extractor successively with 200 ml Petroleum ether, Benzene, Chloroform, Methanol and Ethanol until colourless extract was obtained on the top of the extractor. Each of the solvent extract was concentrated separately under reduced pressure [14]. After complete solvent evaporation, each of these solvent extracts was weighed and subjected to antibacterial activity assay. For only methanol extract, which recorded highest antibacterial activity, the minimal inhibitory concentration (MIC) was determined.

Separation of the Active Fractions from Methanol Extract of *C. Coriaria*: 26.8 g of methanol extract of leaf and 31 g of methanol extract of pod obtained from 50 g of plant material were subjected to active fraction separation following the procedures of Roberts *et al*. [15]. All the four fractions viz., Acidic (fraction 1), Basic (fraction 2), Phenolic (fraction 3) and Neutral (fraction 4) were dried under reduced pressure and weighed to determine the yield. All the fractions were subjected to antibacterial activity (5mg/ml concentration). The fraction which showed activity was selected for further isolation of the active principle.

Isolation of Antibacterial Active Compound from Acidic and Phenolic Fraction of C. Coriaria by TLC System: The acidic and phenolic fraction which showed antibacterial activity were subjected to compounds separation by TLC with chloroform:acetone (1:1.5(v/v)) as an eluting solvent [14]. After the solvent front moved up to 17 cm, the plate was taken out from chromatographic tank and allowed for air-drying. The separated bands were identified under iodine vapor and retardation factor (R_f) values of the spots were determined. The respective bands were scraped out separately along with silica and dissolved in methanol and filtered through Whatman No. 1 filter paper and the filtrate was collected in chromic acid washed glass vials and allowed to dry. After complete evaporation of methanol, all the bands were subjected to antibacterial activity assay.

Human Pathogenic Bacterial Cultures: *Escherichia coli* (Migula) Castellani and Chalmers (MTCC 443), *Klebsiella pneumoniae* (Schoreter) Trevisan (MTCC109), *Proteus mirabilis* Hauser (MTCC1429), *Pseudomonas aeruginosa* (Schroeter) Migula (MTCC1688), *Salmonella paratyphi* A (Brion and Kayser) Castellani and Chalmers (MTCC735), *Shigella flexneri* Castellani and Chalmers (MTCC1457), *Salmonella typhi* (Schroter) Warren and Scott (MTCC733), *Salmonella typhimurium* (Loeffler) Castellani and Chalmers(MTCC98), *Shigella sonnei* (Levine) Weldin (MTCC2957), *Staphylococcus aureus* Rosenbach (MTCC 737), *Streptococcus faecalis* (Andrewes and Harder) Schleifer and Klipper-Balz (MTCC459) were obtained from MTCC Chandigrah, India. All the test strains were subculture on Muller Hinton Agar (MHA) medium. These bacteria served as test pathogens for the assay.

Antibacterial Activity Assay: Antibacterial activity was determined by cup diffusion method on MHA medium. The sterile medium (20ml) was poured into a 9 cm petriplates. The medium was allowed to cool in a sterile condition and plates were then inculcated with $1x10^5$ cfu cultures of test bacteria. The concentration of bacterial cells in the suspension was adjusted to minimum of $1x10^5$ cfu/ml in Muller Hinton broth solution. Agar cup of 5 mm diameter were made in the plates. The desired

different concentrations of the extracts, fractions and pure compounds were prepared by first reconstituting in methanol then diluting in sterile distilled water. A 50µl volume of each dilution was introduced in triplicate wells into MHA plates already seeded with the standardized inoculums (5×10^5) of the test bacterial cells. All test plates were incubated at 37°C for 24h. The least concentration of each extract showing a clear zone of inhibition was taken as the MIC.

Negative controls were prepared using the same solvent employed to dissolve the extracts. Gentamicin and Streptomycin were used as positive reference to determine the sensitivity of each bacterial species tested.

RESULTS

Antibacterial Activity

Aqueous extracts: Antibacterial activity of aqueous extract of eight plants against eleven human pathogenic bacteria is presented in Table 1. Only one plant viz., *Caesalpinia coriaria* (Jacq.) Wild. (Leaf and pod) showed significant antibacterial activity. *Argemone mexicana* L., *Decalepis hamiltonii* Wight and Arn., *Euphorbia tirucalli* L., *Leucas aspera* L., *Phyllanthus niruri* L., *Tinospora cordifolia* Miers. and *Tribulus terrestris* L. showed least antibacterial activity. Antibacterial activity of different concentration of aqueous extract of leaf and pod of *C. coriaria* against tested pathogenic bacteria is presented in Table 2 and 3. Tukey HSD analysis of the data revealed that among the eleven pathogenic bacteria *Staph. aureus* was highly susceptible, followed by *Strep. faecalis*, where as *E. coli* was least susceptible. Aqueous extract of pod showed higher antibacterial activity than leaf extract. The lowest MIC value was observed in *Staph. Aureus* (2mg/ml and 0.5mg/ml) and *Strep. Faecalis* (4mg/ml and 0.5mg/ml), where as highest MIC value was observed in *E. coli* (8mg/ml and 2mg/ml) from leaf and pod respectively.

Table 1: Antibacterial activity of aqueous extract of different medicinal plants against some human pathogenic bacteria at 10mg/ml concentration

	Zone of inhibition [mm]								
	Argemone mexicana.	*Caesalpinia coriaria*		*Decalepis hamiltonii*	*Euphorbia tirucalli*	*Leucas aspera*	*Phyllanthus niruri*	*Tinospora cordifolia*	*Tribulus terrestris*
Human pathogenic bacteria	L	L	P	R	L	L	L	L	L
Escherichia coli MTCC443	0.00	08.8±0.3	12.8±0.3	0.00	0.0	0.00	0.00	0.00	0.00
Klebsiella pneumoniae MTCC109	0.00	09.8±0.3	15.5±0.4	0.00	7.8±0.3	0.00	0.00	0.00	0.00
Proteus mirabilis MTCC1429	0.00	08.8±0.3	17.5±0.4	7.3±0.3	8.0±0.3	0.00	0.00	0.00	0.00
Pseudomonas aeruginosa MTCC1688	0.00	07.8±0.3	17.1±0.3	0.00	0.0	0.00	0.00	0.00	0.00
Salmonella paratyphi A MTCC735	0.00	09.1±0.3	18.1±0.3	7.1±0.3	7.1±0.3	0.00	0.00	0.00	0.00
Salmonella typhi MTCC733	0.00	07.8±0.3	17.1±0.4	0.00	6.8±0.3	0.00	0.00	0.00	0.00
Salmonella typhimurium MTCC98	0.00	07.8±0.3	16.1±0.3	0.0	9.1±0.3	0.00	0.00	0.00	0.00
Shigella flexneri MTCC1457	0.00	07.8±0.3	18.1±0.3	7.1±0.3	8.8±0.3	0.00	0.00	0.00	0.00
Shigella sonnei MTCC 2957	0.00	09.1±0.3	18.8±0.3	0.00	7.1±0.3	0.00	0.00	0.00	0.00
Staphylococcus aureus MTCC 737	8.5±0.3	11.8±0.3	19.8±0.3	9.8±0.3	11.8±0.3	0.00	0.00	0.00	0.00
Streptococcus faecalis MTCC459	8.4	10.1±0.3	24.8±0.3	8.2±0.3	9.1±0.3	0.00	0.00	0.00	0.00

The values are mean of six replicates ± standard error.
Note: Zone of inhibition was 0.00 in aqueous control against all the test bacteria, L: Leaf; P: Pod; R: Rhizome

Table 2: Antibacterial activity of aqueous extract of different concentrations of *C. coriaria* (leaf) against some human pathogenic bacteria

	Zone of inhibition [mm]								
	Concentration of aqueous extract of *C. coriaria* (leaf)								
Human pathogenic bacteria	0.25mg/ml	0.5mg/ml	1.0mg/ml	1.5mg/ml	2mg/ml	4mg/ml	6mg/ml	8mg/ml	10mg/ml
Escherichia coli MTCC443	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	08.8±0.3
Klebsiella pneumoniae MTCC109	0.0	0.0	0.0	0.0	0.0	0.0	6.8±0.3	8.1±0.3	09.8±0.3
Proteus mirabilis MTCC1429	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.0±0.3	08.8±0.3
Pseudomonas aeruginosa MTCC1688	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	07.8±0.3
Salmonella paratyphi A MTCC735	0.0	0.0	0.0	0.0	0.0	0.0	6.5±0.2	7.1±0.3	09.1±0.3
Salmonella typhi MTCC733	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.8±0.3	07.8±0.3
Salmonella typhimurium MTCC98	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.8±0.3	07.8±0.3
Shigella flexneri MTCC1457	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.8±0.3	07.8±0.3
Shigella sonnei MTCC 2957	0.0	0.0	0.0	0.0	0.0	0.0	7.1±0.3	8.1±0.3	09.1±0.3
Staphylococcus aureus MTCC 737	0.0	0.0	0.0	0.0	6.1±0.3	7.1±0.3	8.1±0.3	10.8±0.3	11.8±0.3
Streptococcus faecalis MTCC459	0.0	0.0	0.0	0.0	0.0	6.1±0.3	7.8±0.3	8.8±0.3	10.1±0.3

The values are mean of six replicates ± standard error.
Note: Zone of inhibition was 0.00 in aqueous control in all the concentrations against all the test bacteria

Table 3: Antibacterial activity of aqueous extract of different concentrations of *C. coriaria* (pod) against some human pathogenic bacteria

	Zone of inhibition [mm]								
	Concentration of aqueous extract(pod)								
Human pathogenic bacteria	0.25mg/ml	0.5mg/ml	1.0mg/ml	1.5mg/ml	2mg/ml	4mg/ml	6mg/ml	8mg/ml	10mg/ml
*Escherichia coli*MTCC443	0.0	0.0	0.0	0.0	0.0	07.8±0.3	08.8±0.3	10.5±0.4	12.8±0.3
*Klebsiella pneumoniae*MTCC109	0.0	0.0	0.0	0.0	0.0	08.3±0.3	10.8±0.3	11.8±0.3	15.5±0.4
*Proteus mirabilis*MTCC1429	0.0	0.0	0.0	6.4±0.4	08.8±0.3	09.8±0.3	12.8±0.3	14.1±0.3	17.5±0.4
*Pseudomonas aeruginosa*MTCC1688	0.0	0.0	0.0	0.0	08.5±0.4	10.5±0.4	13.8±0.3	15.1±0.3	17.1±0.3
Salmonella paratyphi AMTCC735	0.0	0.0		8.6±0.4	10.6±0.3	12.8±0.3	14.6±0.4[c]	16.0±0.3	18.1±0.3
*Salmonella typhi*MTCC733	0.0	0.0	0.0	0.0	9.16±0.3	10.8±0.3	13.8±0.3	15.0±0.4	17.1±0.4
*Salmonella typhimurium*MTCC98	0.0	0.0	0.0	7.6±0.3	08.8±0.3	11.1±0.3	13.5±0.4	14.8±0.3	16.1±0.3
*Shigella flexneri*MTCC1457	0.0	0.0	7.2±0.3	9.2±0.3	10.8±0.3	12.1±0.3	13.5±0.4	16.1±0.3	18.1±0.3
*Shigella sonnei*MTCC 2957	0.0	0.0	0.0	6.2±0.5	08.1±0.3	12.1±0.6	13.8±0.4	14.8±0.3	18.8±0.3
*Staphylococcus aureus*MTCC 737	0.0	0.0	6.4±0.3	7.6±0.3	09.8±0.4	10.8±0.3	13.5±0.4	16.1±0.3	19.8±0.3
*Streptococcus faecalis*MTCC459	0.0	6.2±0.4	8.4±0.3	10.6±0.3	12.1±0.3	14.8±0.3	19.1±0.8	22.1±0.3	24.8±0.3

The values are mean of six replicates ± standard error.

Note: Zone of inhibition was 0.00 in aqueous control in all the concentrations against all the test bacteria

Table 4: Antibacterial activity of methanol extract of *C. coriaria* (leaf) against some human pathogenic bacteria

	Zone of inhibition [mm]								
	Concentration of methanol extract(leaf)								
Human pathogenic bacteria	0.25mg/ml	0.5mg/ml	1.0mg/ml	1.5mg/ml	2mg/ml	4mg/ml	6mg/ml	8mg/ml	10mg/ml
Escherichia coli MTCC443	0.0	0.0	0.0	0.0	0.0	07.1±0.3	08.1±0.3	09.8±0.3	11.1±0.3
Klebsiella pneumoniae MTCC109	0.0	0.0	0.0	0.0	10.1±0.3	13.8±0.3	15.8±0.3	17.5±0.2	19.8±0.3
Proteus mirabilis MTCC1429	0.0	0.0	5.8±0.3	8.6±0.3	11.0±0.3	16.0±0.3	18.8±0.3	20.1±0.3	20.0±0.2
Pseudomonas aeruginosa MTCC1688	0.0	0.0	0.0	7.3±0.3	09.1±0.3	10.1±0.3	12.8±0.3	15.1±0.3	17.1±0.3
Salmonella paratyphi A MTCC735	0.0	0.0	0.0	6.5±0.3	08.8±0.3	11.8±0.3	12.8±0.3	14.5±0.4	17.1±0.4
Salmonella typhi MTCC733	0.0	0.0	0.0	0.0	0.0	09.8±0.3	13.5±0.4	14.5±0.4	16.5±0.4
Salmonella typhimurium MTCC98	0.0	0.0	0.0	0.0	0.0	09.1±0.3	10.1±0.3	11.5±0.4	11.8±0.3
Shigella flexneri MTCC1457	0.0	0.0	6.9±0.3	8.4±0.3	10.8±0.3	12.1±0.6	15.8±0.3	18.1±0.3	20.1±0.3
Shigella sonnei MTCC 2957	0.0	0.0	0.0	6.6±0.3	08.1±0.3	12.8±0.3	15.1±0.3	18.8±0.3	20.8±0.3
Staphylococcus aureus MTCC 737	0.0	5.9±0.3	7.8±0.3	10.7±0.3	14.8±0.3	16.8±0.3	18.8±0.3	21.1±0.3	22.8±0.2
Streptococcus faecalis MTCC459	0.0	5.7±0.3	7.3±0.3	9.6±0.3	14.1±0.3	16.1±0.3	18.1±0.3	21.1±0.3	24.7±0.3

The values are mean of six replicates ± standard error.

Note: Zone of inhibition was 0.00 in methanol control in all the concentrations against all the test bacteria

Table 5: Antibacterial activity of methanol extract of *C. coriaria* (pod) against some human pathogenic bacteria

	Zone of inhibition [mm]								
	Concentration of methanol extract (pod)								
Human pathogenic bacteria	0.25mg/ml	0.5mg/ml	1.0mg/ml	1.5mg/ml	2mg/ml	4mg/ml	6mg/ml	8mg/ml	10mg/ml
Escherichia coli MTCC443	0.0	0.0	0.0	0.0	0.0	7.8±0.3	08.3±0.3	10.5±0.4	12.8±0.3
Klebsiella pneumoniae MTCC109	0.0	0.0	0.0	0.0	0.0	8.8±0.3	10.8±0.3	11.8±0.4	15.5±0.4
Proteus mirabilis MTCC1429	0.0	0.0	0.0	7.1±0.3	8.8±0.3	9.8±0.3	12.8±0.3	14.1±0.3	17.5±0.4
Pseudomonas aeruginosa MTCC1688	0.0	0.0	0.0	0.0	8.5±0.4	10.5±0.4	13.8±0.3	15.1±0.3	17.1±0.3
Salmonella paratyphi A MTCC735	0.0	0.0	0.0	6.4±0.3	8.5±0.2	9.0±0.2	10.1±0.3	11.1±0.3	13.1±0.3
Salmonella typhi MTCC733	0.0	0.0	0.0	7.3±0.3	9.1±0.3	10.8±0.3	13.5±0.4	15.5±0.4	17.1±0.3
Salmonella typhimurium MTCC98	0.0	0.0	0.0	6.9±0.3	8.8±0.3	11.1±0.3	13.5±0.4	14.8±0.3	16.6±0.3
Shigella flexneri MTCC1457	0.0	0.0	6.4±0.3	8.2±0.3	9.8±0.3	12.1±0.3	14.5±0.4	16.1±0.3	19.1±0.4
Shigella sonnei MTCC 2957	0.0	0.0	0.0	7.1±0.3	8.1±0.3	12.1±0.6	13.8±0.4	14.8±0.3	18.8±0.3
Staphylococcus aureus MTCC 737	0.0	5.1±0.3	7.3±0.3	8.8±0.3	9.8±0.4	10.8±0.3	13.5±0.4	16.1±0.3	19.8±0.3
Streptococcus faecalis MTCC459	0.0	5.4±0.3	7.5±0.3	9.4±0.3	12.1±0.3	14.8±0.3	19.1±0.8	24.1±0.3	29.8±0.3

The values are mean of six replicates ± standard error.

Note: Zone of inhibition was 0.00 in methanol control in all the concentrations against all the test bacteria

Table 6: Antibacterial activity of different fractions of *C. coriaria* against some human pathogenic bacteria at 10mg/ml concentration

	Zone of inhibition [mm]									
	Acidic fraction		Basic fraction		Neutral fraction		Phenolic fraction			
Human pathogenic bacteria	L	P	L	P	L	P	L	P	Gentamicin (10mcg/disc)	Streptomycin (10mcg/disc)
Escherichia coli MTCC443	13.6±0.4	14.5±0.3	0.0	0.0	0.0	0.0	11.6±0.3	12.1±0.4	14.8±0.2	10.5±0.1
Klebsiella pneumoniae MTCC109	15.8±0.2	17.5±0.3	0.0	0.0	6.0±0.3	7.8±0.3	5.2±0.3	7.8±0.3	15.9±0.2	11.8±0.2
Proteus mirabilis MTCC1429	18.0±0.3	18.2±0.4	7.4±0.3	0.0	7.8±0.3	7.8±0.2	15.4±0.3	16.5±0.4	10.6±0.2	9.3±0.3
Pseudomonas aeruginosa MTCC1688	14.3±0.3	16.1±0.3	0.0	0.0	0.0	0.0	13.7±0.3	15.1±0.3	14.8±0.3	8.9±0.2
Salmonella paratyphi A MTCC735	13.5±0.4	15.2±0.4	0.0	6.1±0.3	5.5±0.3	7.8±0.3	.2±0.3	7.8±0.3	18.7±0.2	16.8±0.2
Salmonella typhi MTCC733	13.0±0.4	14.5±0.3	0.0	0.0	7.2±0.3	7.8±0.3	5.2±0.3	7.8±0.3	20.4±0.3	11.9±0.2
Salmonella typhimurium MTCC98	12.5±0.4	12.6±0.3	0.0	0.0	6.2±0.4	0.0	13.5±0.3	15.3±0.3	16.7±0.2	8.4±0.2
Shigella flexneri MTCC1457	16.3±0.3	17.6±0.3	7.1±0.3	5.3±0.3	7.2±0.3	6.7±0.3	15.1±0.3	15.1±0.3	14.7±0.2	10.1±0.2
Shigella sonnei MTCC 2957	16.1±0.3	16.8±0.3	0.0	0.0	0.0	0.0	12.7±0.3	13.8±0.3	18.8±0.2	9.3±0.3
Staphylococcus aureus MTCC 737	19.0±0.4	19.8±0.4	6.2±0.3	7.8±0.3	8.2±0.3	7.8±0.3	15.1±0.3	17.2±0.3	21.6±0.3	20.1±0.3
Streptococcus faecalis MTCC459	18.1±0.3	20.0±0.3	7.2±0.3	8.2±0.4	7.0±0.4	8.0±0.2	16.2±0.3	18.6±0.3	19.5±0.2	17.0±0.5

The values are mean of six replicates ± standard error.

Note: Zone of inhibition was 0.00 in methanol control in all the concentrations against all the test bacteria

Solvent Extracts: The average yield of extracts of leaf and pod respectively in different solvents were 1.8 & 1.1g in petroleum ether, 0.5 & 0.4g in benzene, 1.2 & 0.8 g in chloroform, 26.8 & 31.0g in methanol and 3.2 & 2.5g in ethanol. Among the five solvents tested, all the test pathogens were highly sensitive to methanol extract. The antibacterial activity of different concentration of methanol extract of leaf and pod is presented in Table 4 and 5. Among the test bacteria *Staph. aureus* and *Strep. faecalis* were highly susceptible. With increasing concentration there was increased activity in all the species. In methanol extract, the lowest MIC value was observed in *Staph. aureus*(0.5mg/ml) and *Strep. Faecalis* (0.5mg/ml), where as highest MIC value was observed in *E. coli* (4mg/ml) from both leaf and pod.

Separation of the Active Fraction from Methanol Extract of *C. Coriaria*: The yield of different fractions of methanol extract of leaf and pod of *C. coriaria* is 14.6 and 18.2gm in acidic fraction, 2.2 and 2.7gm in basic fraction, 6.4 and 6.6gm in neutral fraction and 4.7 and 5.2gm in phenolic fraction. The yield of the pod was generally more than the leaf and among the fraction highest yield of both leaf and pod extract was obtained in acidic fraction and lowest yield were obtained in basic fraction. The inhibitory effect of different fractions of methanol extract of leaf and pod of *C. coriaria* is presented in Table 6. The inhibitory activity was observed in acidic and phenolic fraction, where as basic and neutral fraction did not show any antibacterial activity against all the test bacteria.

Isolation of Antibacterial Active Compound from Acidic and Phenolic Fraction of C. Coriaria by TLC System: Acidic fraction showed five bands (R_f values 0.036, 0.079, 0.434, 0.565 and 0.876) and phenolic fraction showed four bands (R_f values 0.087, 0,333, 0.701 and 0.964). All the compounds separated by TLC of both acidic and phenolic fractions did not show any antibacterial activity against tested human pathogenic bacteria.

The overall analysis revealed that methanol extract of leaf and pod and aqueous extract of pod were found to be highly significant in the activity when compared with the synthetic antibiotics tested.

DISCUSSION

Plants have formed the basis of sophisticated traditional medicine system and natural products make excellent leads for new drug development [7]. Approximately 80% of the world inhabitants rely on traditional medicine for their primary health care and plants also play an important role on the health care system of the remaining 20% of the population [16]. The rediscovery of the connection between plants and health is responsible for launching new generation of botanical therapeuticals, multicomponent botanical drugs, dietary supplements, functional foods and plant produced recombinant proteins [17]. Species of higher plants are much less surveyed for antibacterial activity [18].

The World Health Organization (WHO) is encouraging, promoting and facilitating the effective use of herbal medicine in developing countries for health

programs. The potential of higher plants as a source of new drugs is still largely unexplored. Hence, last decade witnessed an increase in the investigations on plants as a source of new biomolecules for human disease management [19].

In vitro evaluation of plants for antimicrobial property is the first step towards achieving the goal for developing eco-friendly management of infectious diseases of humans by search for new bio-molecules of plant origin. Considering these, eight plants were screened *in vitro* for antibacterial activity against eleven human pathogenic bacteria known to cause diseases in humans. These plants were selected based on traditional medicine knowledge. On the basis of zone of inhibition, the result of the present investigation revealed that among the eight plants screened, aqueous extract of *C. coriaria* (leaf and pod) showed significant antibacterial activity. Further investigation on isolate the active principle for the activity by successive solvent extraction revealed that methanol is the most suitable solvent for the extraction of antibacterial principle from *C. coriaria*. The present investigation has also clearly demonstrated for the first time the antibacterial activity of acidic and phenolic fraction of methanol extract of leaf and pod of *C. coriaria*. It is also interesting to note that the activity was high in pod extract compared with that of leaf extract. Further separation of methanol extract to isolate and identify the active principle responsible for antibacterial activity revealed presence of five bands in acidic fraction and four bands in phenolic fraction. Antibacterial activity assay of all fraction of different R_f value was conducted. It is surprising to note that even though significant antibacterial activity was observed in the phenolic fraction, none of the four bands separated by TLC revealed for antibacterial activity did not show any significant activity. Thus the result of the present investigation suggests that these compounds present in the acidic and phenolic fraction are not antibacterial activity individually but are highly antibacterial active synergistically. Thus the finding of the present investigation suggests the need for further work on identification of the active principle synergistically responsible for antibacterial activity assay.

Gentamicin and streptomycin are known for their broad spectrum activity against Gram-positive and Gram-negative bacteria [20]. The results of the present investigation demonstrate that *Pr. mirabilis*, *Salm. typhimurium* and *Sh. sonnei* were least susceptible to streptomycin. However these bacteria were effectively inhibited by aqueous extract of pod and methanol extract of both leaf and pod of *C. coriaria*. *Proteus mirabilis* which was least susceptible to gentamicin, showed highly significant activity in the methanol extract of leaf of *C. coriaria*. Thus the result of the present investigation point to the fact that methanol extract of *C. coriaria* showed broad spectrum of activity.

Caesalpinia coriaria (Jacq.) Willd is a wild plant distributed in tropical and subtropical region belonging to the family Caesalpinaceae. It grows to approximately 30 feet tall and the leaves are bipinnate. The individual leaflets are 7mm long and 2mm broad. The fruits are twisted pod 5cm long. The pods are a rich source of tannin. Earlier report reveal that pods are used in the treatment of bleeding piles and also good for emollient properties useful in treating freckles and alleviates acute colic pain [21]. A scientific and systematic investigation with regard to the various biological activities of this plant is lacking. Considering these antibacterial activity guided assay of the different solvent extracts of this plant in the present investigation clearly revealed highly significant antibacterial activity against all the tested human pathogenic bacteria.

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