Antibacterial screening of traditional herbal plants and standard antibiotics against some human bacterial pathogens

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Abstract: Chloroformic and isoamyl alcohol extracts of *Cinnnamomum zylanicum*, *Cuminum cyminum*, *Curcuma long Linn*, *Trachyspermum ammi* and selected standard antibiotics were investigated for their *in vitro* antibacterial activity against six human bacterial pathogens. The antibacterial activity was evaluated and based on the zone of inhibition using agar disc diffusion method. The tested bacterial strains were *Streptococcus pyogenes*, *Staphylococcus epidermidis*, *Klebsiella pneumonia*, *Staphylococcus aurues*, *Serratia marcesnces*, and *Pseudomonas aeruginosa.* Ciprofloxacin showed highly significant action against *K. pneumonia* and *S. epidermidis* while Ampicillin and Amoxicillin indicated lowest antibacterial activity against tested pathogens. Among the plants chloroform and isoamyl alcohol extracts of *C. cyminum*, *S. aromaticum* and *C. long Linn* had significant effect against *P. aeruginosa*, *S. marcesnces* and *S. pyogenes*. Comparison of antibacterial activity of medicinal herbs and standard antibiotics was also recorded via activity index. Used medicinal plants have various phytochemicals which reasonably justify their use as antibacterial agent.

Keywords: Human bacterial pathogens, antibacterial activity, medicinal plants, antibiotics, agar disc diffusion assay, activity index.

INTRODUCTION

Plants have provided a good source of antibacterial agent against the infectious pathogens including bacteria (Ume-Kalsoom *et al*., 2013; Ahmed *et al*., 2012; Nasim *et al*., 2012; Bhalodia *et al*., 2011). According to Ali and Qaiser, 35,000 to 70,000 plant species has been used in folk medicine worldwide (Ali and Qaiser, 2009). Use of medicinal plants as a drug is alternative method for the management of pathogenic microbes like bacteria, fungi and viruses and is co-friendly. Number of studies had been conducted worldwide to prove the antimicrobial efficacy of traditional used medicinal plants (Thenmozhi and Rajeshwari, 2010; Sharma and Kumar, 2009). Traditional medicines are well and widely documented in developing countries. The natural products of medicinal plants may give a new source of antibacterial, antifungal and antimicrobial agents (Jahan *et al*., 2010; Runyoro *et al*., 2006).

Medicinal plants *viz*., cumin, cinnamon, turmeric and ajwain belonging to the families Apiaceae, Zingiberaceae, and Lauraceae, respectively. Various chemical constituents are reported having medicinal importance (Hawrelak *et al*., 2009; Orihara *et al*., 2008; Chih-Chun *et al*., 2007). In the recent years, the extracts of medicinal plants have been studied by a large number of researchers to extract the secondary metabolites, which have antimicrobial properties (Dahanukar *et al*., 2000). Multi drug resistant microbes can be controlled through naturally isolated therapeutic drugs (Ahmed *et al*., 2012; Bhalodia *et al*., 2011). Keeping in view the importance of medicinal plants, we screened out chloroform and isoamyl alcohol extracts of four medicinal plants like cumin, cinnamon, turmeric powder, and carum through agar disc diffusion method against human bacterial pathogens in order to detect natural source of antibacterial agents.

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MATERIALS AND METHODS

Isolation and identification of infected pathogens

Three different human infected samples such as urine, sputum, and blood etc were collected from the patients of different age and genders from microbiology section of pathology lab of Combined Military Hospital (CMH), AJK, Muzaffarabad, Pakistan. Bacterial pathogens were isolated from human infected samples. Various biochemical tests such as API- 10, urease, oxidase, catalase, indole etc and microbial growth medium were used for the identification of these bacterial pathogens (Cheesbrough, 2002; Collins *et al*., 1989). Identified bacteria were further used for antibacterial analysis in Biotechnology lab, Department of Zoology, AJK University, Muzaffarabad, Pakistan.

Preparation of extracts of medicinal plants

Four medicinal plants viz., *Cinnnamomum zylanicum* (Cinnamon), *Cuminum cyminum* (Cumin), *Curcuma long Linn* (Turmeric), *Trachiyspirum ammi* (Carom seeds) were selected based on ethanomedical importance. The medicinal herbs were purchased from the local market of

Muzaffarabad, AJ&K, Pakistan. Samples were crushed in fine powder form with pestle and mortar. Powder was soaked in chloroform and isoamyl alcohol for one week. After one week, the extract was filtered and centrifuged at 4000 rpm for 30 min at 4°C. The extract was concentrated in evaporator at 40°C and stored at 4°C for further analysis (Nasim *et al*., 2012).

Agar disc diffusion method

The antibacterial activity of different plant species was evaluated by agar disks diffusion method (Mellou *et al.*, 2005). The microorganisms were activated by inoculating a loop full of the strain in 25 ml of nutrient broth medium (NBM) and incubated at 37°C on a rotary shaker for 24 h. Next day, the old inoculated culture was mixed with freshly prepared nutrient agar medium (NAM) when the temperature reached up to 45°C and were poured the sterilized plates. All plates were placed at room temperature in laminar flow to solidify. The discs of 5 mm were prepared as follows, soaked with 200/µl of a particular extract or the corresponding solvent was applied on to a disc and then allowed to dry for the assay. Presoaked discs were placed in the Petri dishes at their labeled position. The plates were incubated for 48 h at 37°C. Discs of chloroform and isoamyl alcohol were also used as control. Microbial growth was determined by measuring the diameter of the zone of inhibition after 24 h in mm (Barry *et al.*, 1979).

Sensitivity test of standard antibiotics

Sensitivity of antibiotics against test strains was assessed by agar disc diffusion method (Baur *et al*., 1966). Sensitivity was predicted with degree of clear zone surrounding the disc after 24 h in mm (Barry *et al.*, 1979). The used concentration of standard antibiotics in µg has been shown in table 1. The results of the sensitivity tests were expressed as (0) for no sensitivity, + (below 12) for low sensitivity, ++ (12-29) for moderate sensitivity and +++ (30-45) for high sensitivity.

Table 1: Zone of inhibition and role of antibiotic discs against human bacterial pathogens

Antibiotic used	Role of antibiotic	Conc. of antibiotic	Mean values of zone of inhibition (mm)					
			K. pneumoniae	*S. pyogenes*	*S. epidermidis*	*S. aureus*	*S. marcescens*	*P. aeruginosa*
Vancomycin	Inhibits peptidoglycan synthesis	10 µg	23(++)	6(+)	26(++)	18(++)	17(++)	0
Chloramphenicol	Inhibits bacterial protein synthesis	10 µg	35(+++)	20(++)	32(+++)	29(++)	24(++)	27(++)
Streptomycin	Leaving the bacterium unable to synthesize proteins	10 µg	22(++)	25(++)	15(++)	35(+++)	29(++)	30(+++)
Tobramycin	Leaving the bacterium unable to synthesize proteins	5 µg	20(++)	5(+)	30(+++)	9(+)	10(+)	13(++)
Gentamycin	Leaving the bacterium unable to synthesize proteins	10 µg	30(+++)	13(++)	34(+++)	31(+++)	29(++)	19(++)
Ciprofloxacin	Inhibits DNA replication and transcription	10 µg	39(+++)	17(++)	36(+++)	34(+++)	26(++)	32(+++)
Oxytetracycline	Inhibits the binding of aminoacyl-tRNA to the mRNA-ribosome complex	10 µg	8(+)	0	5(+)	0	6(+)	6(+)
Nalidixic acid	Inhibits DNA replication and transcription	5 µg	24(++)	15(++)	20(++)	24(++)	21(++)	25(++)
Sulfamethoxyzol	Inhibits folate synthesis	10 µg	31(+++)	0	33(+++)	0	8(+)	6(+)
Neomycin	Leaving the bacterium unable to synthesize proteins	10 µg	25(++)	24(++)	0	22(++)	22(++)	21(++)
Tetracycline	Inhibits the binding of aminoacyl-tRNA to the mRNA-ribosome complex	10 µg	14(++)	0	10(+)	0	0	6(+)
Penicillin G	Disrupt the synthesis of the peptidoglycan layer of bacterial cell walls	10 µg	0	0	0	0	8(+)	6(+)
Trimethoprim	Inhibits folate synthesis	10 µg	31(+++)	0	33(+++)	22(++)	8(+)	21(++)
Ampicillin	Disrupt the synthesis of the peptidoglycan layer of bacterial cell walls	10 µg	6(+)	0	0	0	8(+)	0
Amoxicillin	Disrupt the synthesis of the peptidoglycan layer of bacterial cell walls	10 µg	6(+)	0	2(+)	0	6(+)	6(+)
Kanamycin	Leaving the bacterium unable to synthesize proteins	10 µg	30(+++)	11(+)	32(+++)	16(++)	16(++)	13(++)

Zone of inhibition were expressed as (0) for no sensitivity, + (below 12) for low sensitivity, ++ (12-29) for moderate sensitivity and +++ (30-45) for high sensitivity.

STATISTICAL ANALYSIS

Each experiment was repeated in triplicates and means from absolute data is mentioned. The comparison of the antibacterial activity of the medicinal extracts with standard antibiotics was evaluated by Activity Index (AI) (Shekhawat and Vijayvergia, 2010).

Phytochemical screening of extracts

Methods used for phytochemical screening of chloroform and isoamyl alcohol extracts of selected medicinal herbs as described by Trease and Evans, 2002; Siddiqui and Ali, 1997; Iyengar, 1995; Harborn, 1993 and Sofowora, 1993, are shown in table 2.

RESULTS

Identification of bacterial pathogens

Gram staining indicated that *S. aureus, S. pyogene* and *S. epidermidis* are Gram positive (+) cocci whereas *P. aeruginosa, K. pneumonia*, and *S. marcescens* are Gram negative (-), rod shaped bacteria. Besides Gram staining, API-10 was used for the differentiation of different Gram negative rods up to the species level. Some pathogens *viz.*, *S. aureus, S. pyogene, S. epidermidis, P. aeruginosa, K. pneumonia*, and *S. marcescens* were also identified through various biochemical techniques (Cheesbrough, 2002; Collins *et al*., 1989). It was observed that *K. pneumonia* and *S. marcescens* showed urease and API positive while oxidase and indole negative tests. *S. pyogene* and *S. aureus* showed catalase negative and positive test.

Inhibitory effect of selected antibiotics

Sensitivity test revealed that the *K. pneumoni*ae was highly sensitive to Nalidixic acid, Kanamycin, Vancomycin, Neomycin, Sulphamethoxyzole, Chloroamphenicol, Streptomycin, Tobramycin, Gentamicin, Trimethoprim and Ciprofloxacin (fig. 1A and table 1). The maximum zone of inhibition was measured for antibiotics namely Nalidixic acid, Ciprofloxacin, Kanamycin, Vancomycin, Neomycin, Sulphamethoxyzole, Chloramphenicol, Streptomycin, Tobramycin, Gentamicin, Trimethoprim were (24, 39, 30, 23, 25, 31, 35, 22, 20, 30, and 31 mm respectively). The results also revealed that Tetracycline had moderate effect as 14/mm (Zone of inhibition) while Oxytetracyclin, Ampicillin and Amoxicillin had lowest effect (8, 6, and 6 mm respectively). On the other hand *K. pneumoni*ae was resistant against Penicillin G (fig. 1A and table 1).

S. pyogenes was highly sensitive to Neomycin, Streptomycin and Chloramphenicol while resistance against Penicillin G, Oxytetracyclin, Tetracycline, Ampicillin, Sulphamethoxyzole, Trimethoprim and Amoxicillin. The inhibited zone was recorded as 20, 25 and 24/mm against *S. pyogenes*. Ciprofloxacin, Gentamicin and Nalidixic acid had moderate effect (13, 15 and 17/mm, respectively) whereas Kanamycin, Vancomycin and Tobramycin had low effect on the growth of *S. pyogenes* (6, 5 and 11/mm) (fig. 1A and table 1). *S. epidermidi*s was highly sensitive to Ciprofloxacin, Chloroamphenicol, Kanamycin, Trimethoprim, Gentamicin, Vancomycin, Tobramycin, Nalidixic acid and Sulphamethoxyzole. The zone of inhibition of these antibiotics was recorded as 36, 26, 32, 30, 34, 20, 33, 33, and 32/mm respectively against *S. epidermidi*s. On the other hand Streptomycin showed moderate effect on the growth of *S. epidermidi*s as 15 mm. Tetracycline, Oxytetracycline and Amoxicillin had lowest effect while Penicillin G, Ampicillin, and Neomycin had no effect on it (fig. 1A and table 1). The zone of inhibition of antibiotics was recorded against *S. aureus, S. marcescens* and *P. aeruginosa* were Streptomycin (35, 29, 30/mm), Ciprofloxacin (34, 26, 32 mm), Chloramphenicol (29, 24, 27/mm), Gentamicin (31, 29/mm), Nalidixic acid (24, 21, 25/mm) and Neomycin (22, 22, 21/mm), respectively (fig. 1.A and table 1). Medium susceptibility was measured by Kanamycin (16, 16, 13/mm), Gentamicin (19) and Vancomycin (18, 17, 16/mm).

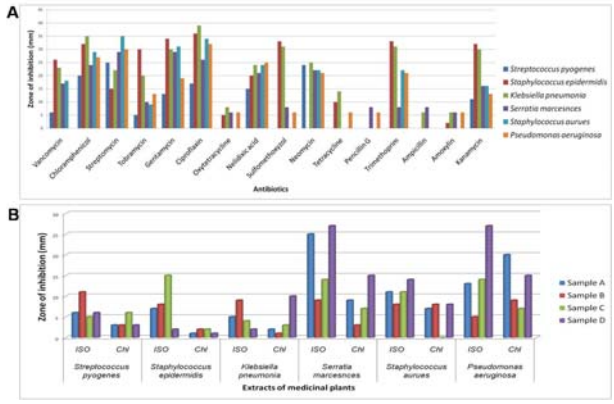

Fig. 1: Antibacterial activity of medicinal plants and antibiotics against human associated bacterial pathogens. (A) Antibacterial activity of selected antibiotics; (B) Antibacterial activity of chloroform (Chl) and isoamyl alcohol (ISO) extracts of medicinal plants. Samples A, B, C and D indicate *Cuminum cyminum*, *Cinamomum vermum*, *Trachyspermum ammi* and *Curcma longa*, respectively.

On the other hand Amoxilline, Oxytetracycline, Sulfomethoxyxol, Tetracycline, and Penicillin G had no effect on *S. aureus*, and Tetracycline had no effect on *S. marcescens*. Similarly, Ampicillin did not show such effect against all tested bacterial pathogens. It was observed that Penicillin G, Sulfomethoxyzol, Oxytetracycline, and Amoxilline had low effect on *S. marcescens* and *P. aeruginosa*. The zone of inhibition was recorded as Penicillin G (8 and 6/mm), Sulfomethoxyzol (8 and 6/mm), Oxytetracycline, (6 and 6/mm) and Amoxilline (6 and 6/mm) against *S. marcescens* and *P. aeruginosa*, respectively (fig. 1A and table 1).

Table 2: Phytochemical screening of medicinal plants

Phytochemical	Methodology used
Flavonoids	1 g powdered + 10 ml ethyl acetate, heated over a steam bath (40–50°C) for 5 min, filtrate was treated + 1 ml dilute ammonia. A yellow coloration indicated positive test.
Tannin	0.5 g powdered was boiled in 20 ml distilled water for few min, + 3 drops of 5% $FeCl_3$. Development of brownish-green or blue black coloration was taken as positive.
Saponins	1 g powdered was boiled in 10 ml distilled water for 15 min, after cooling, the extract was shaken vigorously to record froth formation.
Cardiac glycosides	2 g of extract was isolated in 10 ml methanol. Five ml of methanolic extract was treated with 2 ml glacial acetic acid + 1 drop of 5% $FeCl_3$. This solution was carefully transferred to surface of 1 ml conc. H_2SO_4. The formation of reddish brown ring at the junction of two liquids was demonstrated the presence of cardiac glycosides.
Phenols	Extract was mixed with few drops of diluted Folin Ciocalteu reagent and aqueous sodium carbonate solution. The mixture was allowed to stand for 10 min and formation of gray colour indicates the presence of Phenolic groups.
Steroids	Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample + 2 ml H_2S0_4. The color changed from violet to blue or green indicating the presence of steroids.
Alkaloids	Few drops of dilute HCL and 0.5 ml Wagner's reagent has added. A brown flocculent precipitate indicates the presence of alkaloid.

Table 3: Zone of inhibition of extracts of medicinal herbs against human bacterial pathogens

Labeling used for experiment	Conc. used gm/100ml	Name of medicinal plant	Name of solvent used for extract	Mean values of zone of inhibition (mm)					
				K. pneumoniae	*S. Pyogenes*	*S. epidermidis*	*S. aureus*	*S. marcesens*	*P. aeruginosa*
Sample A	5.73	*Cuminum cyminum* (Cumin; Zeera)	ISO	5(+)	6(+)	7(+)	11(+)	25(++)	13(++)
			Chl	2(+)	3(+)	1(+)	7(+)	9(+)	20(++)
Sample B	9.38	*Cinnnamomum zylanicum* (Cinnamon; Dalchini)	ISO	9(+)	11(+)	8(+)	8(+)	9(+)	5(+)
			Chl	1(+)	3(+)	2(+)	8(+)	3(+)	9(+)
Sample C	7.90	*Trachiyspirum ammi* (Carom seeds; Ajwain)	ISO	4(+)	5(+)	15(++)	11(+)	14(++)	14(++)
			Chl	3(+)	6(+)	2(+)	0	7(+)	7(+)
Sample D	5.25	*Curcuma long* (Turmeric powder)	ISO	2(+)	6(+)	2(+)	14(++)	27(++)	27(++)
			Chl	10(+)	3(+)	1(+)	8(+)	15(++)	15(++)

Zone of inhibition were expressed as (0) for no sensitivity, + (below 12) for low sensitivity, and ++ (12-29) for moderate sensitivity.

Inhibitory effect of medicinal plants

The antibacterial activity of solvent extracts of medicinal plants *viz*, *Cuminum cyminum* (Sample A), *Cinamomum vermum* (Sample B), *Trachyspermum ammi* (Sample C) and *Curcma longa* (Sample D) were investigated against bacterial strains through agar disc diffusion method. Antibacterial activities of solvent extracts were assessed in terms of zone of inhibition. The inhibitory effects of chloroform extract of sample (A) was measured as (3, 1, 2, 9, 7, 20/mm) while its isoamyl alcohol extract showed (6, 7, 5, 11, 25 and 13/mm) (fig. 1.B and table 3). Whilst chloroform and isoamyl alcohol extracts of sample (B) has shown lowest zone of inhibition against all bacterial pathogens (Chloroform extract: 3, 2, 1, 3, 8, and 9/mm; isoamyl alcohol extract: 11, 8, 9, 9, 8 and 5/mm). Likewise the chloroform extract of sample (C) showed resistance against *S. aureus.* The extracts of isoamyl alcohol of (C) showed moderate inhibited zone i.e. 15, 14 and 1/mm against *S. marcesnces, S. epidermidis* and *P. aeruginosa* respectively. Isoamyl alcohol extracts of sample D showed most significant zone of inhibition 27 and 27/mm against *S. marcesnces* and *P. aeruginosa* while moderate inhibition was recorded against *S. aurues* i.e. 14 mm, respectively. Moderate results of zone of inhibition of chloroform extract of (D) were measured against *S. marcesnces,* and *P. aeruginosa* as 15 and 15/mm (fig. 1B and table 3). Control solvents (chloroform and isoamylalcohol) showed lowest zone of inhibition as 1 and 2/mm, respectively. The screening results of the present study confirmed the possible use of medicinal plants as a source of antimicrobial agents.

Table 4: Activity index (AI) of extracts of medicinal plants and antibiotic discs against human bacterial pathogens

Name of medicinal plant	Activity index = Zone of inhibition of extracts / Zone of inhibition of antibiotics																Human bacterial pathogens
	Vancomycin	Chloroam-phenicol	Streptomy-cin	Tobramycin	Gentamycin	Cipro-floxacin	Oxytetra-cycline	Nalidixic acid	Sulfome-thoxyzol	Neomycin	Tetracycline	Penicillin G	Trimetho-prim	Ampicillin	Amoxicillin	Kanamycin	
Cuminum cyminum (Cumin; Zeera)	C: 0.08 I: 0.21	C: 0.05 I: 0.14	C: 0.09 I: 0.22	C: 0.1 I: 0.25	C: 0.06 I: 0.16	C: 0.05 I: 0.12	C: 0.25 I: 0.62	C: 0.08 I: 0.20	C: 0.06 I: 0.16	C: 0.08 I: 0.20	C: 0.14 I: 0.35	C: 0 I: 0	C: 0.06 I: 0.16	C: 0.33 I: 0.83	C: 0.33 I: 0.83	C: 0.06 I: 0.16	*Klebsiella pneumoniae*
	C: 0.5 I: 1	C: 0.15 I: 0.3	C: 0.12 I: 0.24	C: 0.6 I: 1.2	C: 0.23 I: 0.46	C: 0.17 I: 0.35	C: 0 I: 0	C: 0.2 I: 0.4	C: 0 I: 0	C: 0.12 I: 0.12	C: 0 I: 0	C: 0 I: 0	C: 0 I: 0	C: 0 I: 0	C: 0 I: 0	C: 0.27 I: 0.54	*Streptococcus Pyogenes*
	C: 0.03 I: 0.26	C: 0.03 I: 0.21	C: 0.06 I: 0.46	C:0.03 I: 0.23	C: 0.02 I: 0.20	C: 0.02 I: 0.19	C: 0.2 I: 1.4	C: 0.05 I: 0.35	C: 0.03 I: 0.21	C: 0 I: 0	C: 0.1 I: 0.7	C: 0 I: 0	C: 0.03 I: 0.21	C: 0 I: 0	C: 0.5 I: 3.5	C: 0.03 I: 0.21	*Staphylococcus epidermidis*
	C: 0.38 I: 0.61	C: 0.24 I: 0.37	C: 0.2 I: 0.31	C: 0.77 I: 1.22	C: 0.22 I: 0.32	C: 0.20 I: 0.32	C: 0 I: 0	C: 0.29 I: 0.45	C: 0 I: 0	C: 0.31 I: 0.5	C: 0 I: 0	C: 0 I: 0	C: 0.31 I: 0.5	C: 0 I: 0	C: 0 I: 0	C:0.43 I: 0.68	*Staphylococcus aureus*
	C: 0.52 I: 1.47	C: 0.37 I: 1.04	C: 0.31 I: 0.86	C: 0.9 I: 2.5	C: 0.31 I: 0.86	C: 0.34 I: 0.96	C: 1.5 I: 4.16	C: 0.42 I: 1.19	C: 1.12 I: 3.12	C: 0.40 I: 1.13	C: 0 I: 0	C:1.12 I: 3.12	C: 1.12 I: 3.12	C: 1.12 I:3.12	C: 1.5 I: 4.16	C: 0.56 I: 1.56	*Serratia marcescens*
	C: 0 I: 0	C: 0.74 I: 0.48	C: 0.66 I: 0.43	C: 1.53 I: 1	C: 1. 05 I: 0.68	C: 0.62 I: 0.40	C: 3.33 I:2.16	C: 0.8 I: 0.52	C: 3.33 I: 2.16	C: 0.95 I: 0.61	C: 3.33 I: 2.16	C: 3.33 I: 2.16	C: 0.95 I: 0.61	C: 0 I: 0	C: 3.33 I: 2.16	C: 1.53 I: 1	*Pseudomonas aeruginosa*
Cinnnamomum zylanicum (Cinnamon; Dalchini)	C: 0.04 I: 0.16	C: 0.02 I: 0.25	C: 0.04 I: 0.40	C: 0.05 I: 0.45	C: 0.03 I: 0.3	C: 0.02 I: 0.23	C: 0.12 I: 0.12	C: 0.04 I: 0.37	C: 0.03 I: 0.29	C: 0.04 I: 0.36	C: 0.07 I: 0.64	C: 0 I: 0	C: 0.03 I: 0.29	C: 0.16 I: 1.5	C: 0.16 I: 1.5	C: 0.03 I: 0.3	*Klebsiella pneumoniae*
	C: 0.5 I: 1.83	C: 0.15 I: 0.55	C: 0.12 I: 0.44	C: 0.6 I: 2.2	C: 0.23 I: 0.84	C: 0.17 I: 0.64	C: 0 I: 0	C: 0.2 I: 0.73	C: 0 I: 0	C: 0.12 I: 0.45	C: 0 I: 0	C: 0 I: 0	C: 0 I: 0	C: 0 I: 0	C: 0 I: 0	C: 0.27 I: 1	*Streptococcus Pyogenes*
	C: 0.07 I: 0. 30	C: 0.06 I: 0.25	C: 0.13 I: 0.53	C: 0.06 I: 0.26	C: 0.05 I: 0.23	C: 0.05 I: 0.22	C: 0.4 I: 1.6	C: 0.1 I: 0.4	C: 0.06 I: 0.24	C: 0 I: 0	C: 0.2 I: 0.8	C: 0 I: 0	C: 0.06 I: 0.24	C: 0 I: 0	C: 1 I: 4	C: 0.06 I: 0.25	*Staphylococcus epidermidis*
	C: 0.44 I: 0.44	C: 0.27 I: 0.27	C: 0.22 I: 0.22	C: 0.88 I: 0.88	C: 0.25 I: 0.25	C: 0.23 I: 0.23	C: 0 I: 0	C: 0.33 I: 0.33	C: 0 I: 0	C: 0.36 I: 0.36	C: 0 I: 0	C: 0 I: 0	C: 0.36 I: 0.36	C: 0 I: 0	C: 0 I: 0	C: 0.5 I: 0.5	*Staphylococcus aureus*
	C: 0.17 I: 0.52	C: 0.12 I: 0.31	C: 0.10 I: 0.31	C: 0.3 I: 0.9	C: 0.10 I: 0.31	C: 0.11 I:0.34	C: 0.5 I: 1.5	C: 0.14 I: 0.42	C: 0.37 I: 1.12	C: 0.13 I: 0.40	C: 0 I: 0	C: 0.37 I: 1.12	C: 0.37 I: 1.12	C: 0.37 I: 1.12	C: 0.5 I: 1.5	C: 0.18 I:0.56	*Serratia marcescens*
	C: 0 I: 0	C: 0.25 I: 0.51	C: 0.23 I: 0.46	C: 0.53 I: 1.07	C: 0.36 I: 0.73	C: 0.21 I: 0.43	C: 1.16 I: 2.33	C: 0.33 I: 0.56	C: 1.16 I: 2.33	C: 0.33 I: 0.66	C: 1.16 I: 2.33	C: 1.16 I: 2.33	C: 0.33 I: 0.66	C: 0 I: 0	C: 1.16 I: 2.33	C: 0.53 I: 1.07	*Pseudomonas aeruginosa*
Trachiyspirum ammi (Carom seeds; Ajwain	C:0.13 I: 0.17	C: 0.08 I: 0.11	C: 0.13 I: 0.08	C: 0.15 I: 0.2	C: 0.1 I: 0.16	C: 0.07 I: 0.10	C: 0.37 I: 0.5	C: 0.12 I: 0.16	C: 0.09 I: 0.12	C: 0.12 I: 0.16	C: 0.21 I: 0.28	C: 0 I: 0	C: 0.09 I: 0.12	C: 0.5 I: 0.16	C: 0.5 I: 0.16	C: 0.1 I: 0.13	*Klebsiella pneumoniae*
	C: 1 I: 0.83	C: 0.3 I: 0.25	C: 0.24 I: 0.2	C: 1.2 I: 1	C: 0.46 I: 0.38	C: 0.35 I: 0.29	C: 0 I: 0	C: 0.4 I: 0.33	C: 0 I: 0	C: 0.25 I: 0.20	C: 0 I: 0	C: 0 I: 0	C: 0 I: 0	C: 0 I: 0	C: 0 I: 0	C: 0.54 I: 0.45	*Streptococcus Pyogenes*
	C:0.07 I: 0.57	C: 0.06 I: 0.46	C: 0.13 I: 1	C: 0.06 I: 0.5	C: 0.05 I: 0.44	C: 0.05 I: 0.41	C: 0.4 I: 3	C: 0.1 I: 0.75	C: 0.06 I: 0.45	C: 0 I: 0	C: 0.2 I: 1.5	C: 0 I: 0	C: 0.06 I: 0.45	C: 0 I: 0	C: 1 I: 7.5	C: 0.06 I: 0.46	*Staphylococcus epidermidis*
	C: 0 I: 0.61	C: 0 I: 0.37	C: 0 I: 0.31	C: 0 I: 1.22	C: 0 I: 0.35	C: 0 I: 0.32	C: 0 I: 0	C: 0 I: 0.45	C: 0 I: 0	C: 0 I: 0.5	C: 0 I: 0	C: 0 I: 0	C: 0 I: 0.5	C: 0 I: 0	C: 0 I: 0	C: 0 I: 0.68	*Staphylococcus aureus*
	C: 0.41 I: 0.82	C: 0.29 I: 0.58	C: 0.24 I: 0.48	C: 0.7 I: 1.4	C: 0.24 I: 0.48	C: 0.26 I: 0.53	C: 1.16 I: 2.33	C: 0.33 I: 0.66	C: 0.87 I: 1.75	C: 0.31 I: 0.63	C: 0 I: 0	C: 0.87 I: 1.75	C: 0.87 I: 1.75	C: 0.87 I: 1.75	C: 1.16 I: 2.33	C: 0.43 I: 0.87	*Serratia marcescens*
	C: 0 I: 0	C: 0.25 I: 0.51	C: 0.23 I: 0.46	C: 0.53 I: 1.07	C: 0.36 I: 0.73	C: 0.21 I: 0.43	C: 1.16 I: 2.33	C: 0. 33 I: 0.56	C: 1.16 I: 2.33	C: 0.33 I: 0.66	C: 1.16 I: 2.33	C: 1.16 I: 2.33	C: 0.33 I: 0.66	C: 0 I: 0	C: 1.16 I:2.33	C: 0.53 I: 1.07	*Pseudomonas aeruginosa*
Curcuma long (Turmeric powder)	C: 0.43 I: 0.08	C: 0.28 I: 0.05	C: 0.45 I: 0.09	C: 0.5 I: 0.1	C: 0.33 I: 0.06	C: 0.25 I: 0.05	C: 1.25 I: 0.25	C: 0.41 I: 0.08	C: 0.32 I: 0.06	C: 0.4 I: 0.08	C: 0.71 I: 0.14	C: 0 I: 0	C: 0.32 I: 0.16	C: 1.66 I: 0.33	C: 1.66 I: 0.33	C: 0.33 I: 0.06	*Klebsiella pneumoniae*
	C: 0.5 I: 1	C: 0.15 I: 0.3	C: 0.12 I: 0.24	C: 0.6 I: 1.2	C: 0.23 I: 0.46	C: 0.17 I: 0.35	C: 0 I: 0	C: 0.2 I: 0.4	C: 0 I: 0	C: 0.12 I: 0.25	C: 0 I: 0	C: 0 I: 0	C: 0 I: 0	C: 0 I: 0	C: 0 I: 0	C: 0.27 I: 0.54	*Streptococcus Pyogenes*
	C: 0.03 I: 0.07	C: 0.03 I: 0.06	C: 0.06 I: 0.13	C: 0.03 I: 0.06	C: 0.02 I: 0.05	C: 0.02 I: 0.05	C: 0.2 I: 0.4	C: 0.5 I: 0.1	C: 0.03 I: 0.06	C: 0 I: 0	C: 0.1 I: 0.2	C: 0 I: 0	C: 0.03 I: 0.06	C: 0 I: 0	C: 0.5 I: 1	C: 0.03 I: 0.06	*Staphylococcus epidermidis*
	C: 0.44 I: 0.77	C: 0.27 I: 0.48	C: 0.22 I: 0.4	C: 0.88 I: 1.55	C: 0.25 I: 0.45	C: 0.23 I: 0.41	C: 0 I: 0	C: 0.33 I: 0.58	C: 0 I: 0	C: 0.36 I: 0.63	C: 0 I: 0	C: 0 I: 0	C: 0.36 I: 0.63	C: 0 I: 0	C: 0 I: 0	C: 0.5 I: 0.87	*Staphylococcus aureus*
	C: 0.88 I: 1.58	C: 0.62 I: 1.12	C: 0.51 I: 0.93	C: 1.5 I: 2.7	C: 0.51 I: 0.93	C: 0.57 I: 1.03	C: 2.5 I: 4.5	C: 0.71 I: 1.28	C: 1.87 I: 3.37	C: 0.68 I: 1.22	C: 0 I: 0	C: 1. 87 I: 3.37	C: 1.87 I: 3.37	C: 1.87 I: 3.33	C: 2.5 I: 4.5	C: 0.93 I: 1.68	*Serratia marcescens*
	C: 0 I: 0	C: 0.55 I: 1	C: 0.5 I: 0.9	C: 1.15 I: 2.07	C: 0.78 I: 1.42	C: 0.46 I: 0.84	C: 2.5 I: 4.5	C: 0.6 I: 1.08	C: 2.5 I: 4.5	C: 0.71 I: 1.28	C: 2.5 I: 4.5	C: 2.5 I: 4.5	C: 0.71 I: 1.28	C: 0 I: 0	C: 2.5 I: 4.5	C: 1.15 I: 2.07	*Pseudomonas aeruginosa*

* Activity index was calculated by using mean values of both zone of inhibitions of extracts and medicinal herbs; C used for chloroform; I used for isoamylalcohol.

Table 5: Phytochemical screening of medicinal plants

Phytochemicals	*Cinnnamomum zylanicum* (Cinnamon)	*Cuminum cyminum* (Cumin)	*Curcuma long Linn* (Turmeric)	*Trachiyspirum ammi* (Carom seeds)
Flavonoids	++	++	++	++
Tannin	++	++	++	++
Saponins	--	--	--	--
Cardiac glycosides	++	++	++	++
Phenols	++	++	++	++
Steroids	++	++	++	++
Alkaloids	++	++	++	++

++ presence of phytochemicals; -- absence of phytochemicals

Analysis through Activity Index (AI)

The significant use of chloroformic and isoamyl alcohol extracts of medicinal plants with standard antibiotics was calculated through activity index (table 4). More than 1 activity index (AI) value indicated the considerable role of herbal extracts while below zero showed the strong effect of antibiotics against tested pathogens. More AI values evaluated the more significant results (table 4).

Phytochemical screening of medicinal plants

Qualitative phytochemical analysis showed the presence of alkaloids, flavonoids, tannins, saponins and cardiac glycosides in selected medicinal plants (table 5). The detected phytoconstituents in the plant materials could be responsible for their antimicrobial activity.

DISCUSSION

The antimicrobial activity of antibiotics can be administered through various ways to treat both human and veterinary diseases likewise these antibiotics can also be used to promote growth in food animals like fish and poultry. The sensitivity of antibiotics were consistent with the reported literature (White and Hancock, 2007; Nelson *et al*., 2007; William and Cromie, 2000). The overuse of antibiotics leads to produce multidrug resistant microorganism. In the current scenario herbal products are considered as safe alternatives of synthetic drugs. Results from the present investigation showed that the growth of bacterial pathogens was inhibited with the crude extracts of medicinal plants. The inhibition of growth of *S. marcesnces* and *P. aeruginosa* was more pronounced with both chloroform (Chl) and isoamyl alcohol (ISO) extracts as compared to antibiotics used. Obtained results through AI were consistent with Shekhawat and Vijayvergia, (2010) study.

T. ammi is used frequently for the treatment of abdominal discomfort, cough, diarrhea, and stomach troubles (Anilkumar *et al*., 2009). Paul *et al*. (2011) reported remarkable antibacterial activity of *T. ammi* against food borne bacteria. Phytochemical analysis indicated that Ajwain (*Trachiyspirum ammi*) contains two phenolic compounds which are responsible for antiseptic, antibacterial, and antifungal properties (Treas and Evans, 2002). Several reports also indicated the pharmacological activities of turmeric such as antioxidant, anti-protozoal, anti-microbial, antivenom, anti-tumor, anti-inflammatory, hepatoprotective, anti-allergic, anti-ulcer, antidyspeptic and antidepressant (EL-Ansary *et al.,* 2006; Masuda *et al.*, 2002; Das and Das, 2002; Deitelhoftt *et al.,* 2002; Yu *et al.,* 2002; Surh *et al.,* 2001; Ozaki *et al.,* 2000; Yano *et al.,* 2000). The all tested medicinal plants contain phytochemicals like saponin, steriods, glycosides and flavonoids, which indicated that plants rich in tannin and phenolic compounds, and may possesses antimicrobial activities against a number of microorganisms (Riaz *et al*., 2010; Parekh and Chanda, 2007).

CONCLUSION

In conclusion, these plants possessed good inhibitory activity against the *S. marcesnces* and *P. aeruginosa*. Activity index (AI) showed almost comparable antibacterial activity, which support the traditional use of medicinal herbs against infectious pathogens. Unfortunately, resistance to available antibiotics is on the rise and there are a limited number of antibacterial agents with reliable activity. The extracted phytochemicals are responsible for their therapeutic effects. It further reveals a hope for the development of novel chemotherapeutic drugs from such plants which may serve for the production of synthetically improved therapeutic agents.

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