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ORIGINAL RESEARCH ARTICLE

Antibacterial Potential of Selected Herbs and Spices against Human Pathogenic Bacteria

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ABSTRACT

Objective of the study was to evaluate antibacterial potential of aqueous and methanolic extracts roots of ginger, valerian, shallot bulb, flower of borage and whole fruit of lime against *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus cereus* and *Staphylococcus aureus* as test bacteria, by well diffusion method. Aqueous and methanolic extracts of shallot and lime exhibited higher antibacterial potential against all test bacteria. Minimum Inhibitory Concentration (MIC) of these two extracts was ranging between 0.5mg/well to 1mg/well against all test bacteria. In conclusion, the shallot and lime extracts have potential to kill human pathogenic bacteria *in vitro*, which could be analyzed for isolation and characterization of antibacterial active compound/ fraction(s) and further to conduct pharmacological investigations.

Key words: Borage, ginger, lime, shallot, valerian, antibacterial activity.

INTRODUCTION

The potential of plant extracts for curative and preventive properties is increasing being realized by the scientific community and the focus of present day research is mostly on the use of such herbs and spices and other materials for medicinal treatment. The realization that the effective life span of antibiotic is limited and indiscriminate usage of antibiotics is causing bacterial resistance is another reason for undertaking such research investigations (Alam *et al.*, 2011). Nearly 30% or more of the modern pharmacological drugs derived directly or indirectly from plants and their extracts dominate in alternative therapies such as Homeopathy and Ayurveda (Banso, 2009; Jabeen *et al.*, 2009; Ahameethunisa and Hopper, 2010; Kyung 2012; Murugesan, 2011). Treating disease with natural foods may be the future choice of people due to various side effects of synthetic drug formulations. Many plant extracts have been shown to possess antibacterial potential against microorganisms *in vitro*, for example, garlic, clove, ginger, allium species (Indu *et al.*, 2006; Pundir *et al.*, 2010; Silva and Fernandes Junior, 2010; Kyung, 2012). Bioactive components derived from plant sources such as flavones, flavonoids and flavones are found to be active against microorganisms (Ekwenye and Elegalam, 2005; Nazaruk and Jakoniuk, 2005). In our previous work, we had observed the presence of high polyphenol and flavonoid content in selected herbs and spices (ginger root, valerian root, whole lime, shallot bulb and petals of borage) (Adel Pilerood and Prakash, 2010; Adel Pilerood and Prakash, 2011); also some of these samples are traditionally used for treating infections. Hence, the study was planned to evaluate the antibacterial potential of aqueous and methanolic extracts of selected samples against different human pathogenic bacteria.

MATERIALS AND METHODS

Samples

Fresh petals of borage (*Echium amoenum*; Family: Boraginaceae), valerian root (*Valerian officinalis*; Family: Valerianaceae), whole lime (*Citrus aurantifolia*; Family: Rutaceae) and shallot (*Allium ascalonicum*; Family: Amaryllidaceae) were collected from a farm in Iran and ginger (*Zingiber officinale*; Family: Zingiberaceae) was procured from Indian market. All samples were initially washed with running tap water followed by sterile distilled water and kept under shade for

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drying. Dried samples were powdered and stored in air tight container under refrigeration at 4 °C.

Bacterial cultures

Bacillus cereus (*B. cereus*) (MTCC 1272), *Bacillus subtilis* (*B. subtilis*) (MTCC 121), *Staphylococcus aureus* (*Staph. aureus*) (MTCC 7443), *Escherichia coli* (*E.coli*) (MTCC 7410), *Klebsiella pneumoniae* (*Kleb. pneumoniae*) (MTCC 7407), *Pseudomonas aeruginosa* (*P. aeurginosa*) (MTCC 424) and *Salmonella typhi* (*Salm. typhi*) (MTCC 733) were obtained from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India- were served as test bacteria. The test bacteria were maintained on nutrient agar (Hi-Media, Mumbai) slants and were sub-cultured once every two-week.

Antibacterial activity

Antibacterial activity of aqueous and methanolic extracts was determined by Well diffusion method on nutrient agar (Srinivasan, 2001). Wells were made in nutrient agar plate using sterile cork borer (5 mm) and inoculums containing 10^{-6}CFU/ml of bacteria swabbed on the solidified media plates. 50 µL of the aqueous extract and methanolic extracts were placed in the well, along with 50µL of sterilized distilled water and methanol as negative control and gentamicin as positive control. All the plates were incubated for 24 hrs at 37 °C and zone of inhibition, if, any was measured in mm and experiments were carried out in triplicates.

Determination of Minimal Inhibitory Concentration (MIC)

It was determined by well diffusion dilution methods. Wells were made in nutrient agar plate using sterile cork borer (5 mm) and inoculums containing 10^{-6}CFU/ml of bacteria swabbed on the solidified media plates. Different concentration (ranging from 5mg to 0.5mg) of extracts were prepared and to each well 50µL test extracts were placed in the well, along with 50µL of sterilized distilled water & methanol as negative control and gentamicin as positive control. All the plates were incubated for 24 hrs at 37 °C and zone of inhibition at the lowest concentration was considered, if any, measured in mm and experiments were carried out in triplicates.

RESULTS AND DISCUSSION

Antibacterial activity and MIC of aqueous extract

The results of the study are compiled in Tables 1 and 2. As presented in Table 1, shallot and lime showed highly significant antibacterial activity against all bacteria. Against *E. coli*, shallot showed more activity than lime with diameter of zone of inhibition at 26.5 mm against a zone of inhibition on 19.0mm. Minimum inhibitory concentration (MIC) was determined using different concentration ranging from 0.5mg, 1.0mg, 2.5mg and 5.0mg/well. Inhibition was observed in the range of 27 to 14mm in shallot and 19 to 12mm in lime. Borage, valerian and ginger did not show any activity against *E.coli*. Observation for ginger was in agreement with other studies (Azu *et al*., 2007; Ekwenye and Elegalam, 2005; Gupta and Ravishankar, 2005; Valdeira *et al*., 2003).

Onyeagba *et al*., (2004) evaluated aqueous extract of dried ginger. They extracted 20 g dried ginger with 100 ml water and added the sample to the disk with 5mm diameter and observed that it did not inhibit *E.coli*. They also reported that at same concentration fresh lime juice inhibited the growth of *E.coli* with diameter zone of 11 mm. Indu *et al*., (2006) determined the antibacterial properties of 100% fresh ginger juice, and 75, 50 and 25% diluted ginger juice against 20 serogroups of *E.coli* (both pathogenic and non-pathogenic). The pathogenic serogroups included enterotoxigenic *E.coli* and enterohemorrhagic *E.coli*. They observed that 100% ginger showed activity only against 2 pathogenic strain of *E.coli* and no activity was seen in remaining 18 strains. Inhibition was reported as 10 and 18 mm in enterotoxigenic *E.coli* and enterohemorrhagic *E.coli* respectively. Diluted juice did not show activity in any strain and any concentrations except 75% ginger which showed activity of 13mm against enterohemorrhagic *E.coli*. In another study Ekwenye & Elegalam, (2005) reported the antibacterial potential of aqueous extract of ginger against *E.coli*. They extracted 5g of ginger in 200 ml of distilled water. The extract was dried and reconstituted at the ratio of 1:2, sterilized and evaluated for inhibition. The aqueous extract of ginger did not show activity against *E.coli* at concentration of 1g of sample.

Fresh ginger and commercial ginger paste were inoculated with a three strain cocktail of overnight cultures of *E.coli* O157: H7 and stored at 4°C and 8°C for 2 weeks. Each paste exhibited different antimicrobial effects alone and in ground beef or buffered peptone water at 4°C and 8°C for 2 weeks. Commercial ginger paste showed strong antimicrobial properties with complete inactivation of *E.coli* O157:H7 in the paste at 3 days at 4°C and 8°C. However, fresh ginger paste showed antimicrobial activity only at 8°C. Only

commercial ginger paste had antimicrobial activity in buffered peptone water at 4°C for 2 weeks. However, commercial ginger paste showed antimicrobial activity in ground beef for 3 days (about 1–2 log CFU/g) compared to control samples at 8°C for 2 weeks (Gupta and Ravishankar, 2005).

Aqueous extract of both shallot and limes exhibited a remarkable inhibition in growth of *Staph. aureus* and shallot showed more inhibition (33.5 mm) than lime (23 mm) (Table 2). Other test plant aqueous extracts did not exhibit antibacterial properties against *Staph. aureus*. When MIC was examined, it was observed that shallot and lime showed activity in the range of 33-12 and 22-0 mm respectively. In similar conditions inhibition was observed with gentamicin antibiotic which interestingly was 1mm lesser than shallot at the same concentration. Other samples did not show inhibition. Onyeagba et al., (2004) reported that aqueous extract of dried ginger did not exhibit antibacterial activity against *S. aureus*, whereas fresh lime juice could inhibit with zone of 17 mm.

B.subtilis was inhibited only with shallot and lime and zone of inhibition of shallot was 29 and lime 28.8 mm. MIC was in the range of 29-18mm and 28-20mm for shallot and lime respectively.

Typhoid fever causes an estimated 16.6 million cases and 600,000 deaths worldwide each year (Perilla, 2003), hence it may be useful to find a medicinal plant which has property to inhibit this bacteria. Shallot showed an inhibitory zone of 32.5 and lime showed 30.5 mm. As illustrated in Table 2, MIC of aqueous extracts of shallot and lime were in the range of 32-15mm and 29-12 mm respectively.

Ekwenye and Elegalam, (2005) studied the antibacterial potential of aqueous extract of ginger against *Salm. typhi* and reported that *Salm. typhi* is sensitive to aqueous extract of fresh ginger and showed an inhibitory zone of 8 mm at 1000 mg/ml of sample. But since we used dry ginger in present study, activity was not seen which could be due to sensitivity of antibacterial active compound to storage and drying. This finding is supported by the study conducted by Onyeagba et al., (2004). They found that dried ginger did not exhibit antibacterial potential against *Salmonella* spp, however, lime juice showed inhibition of zone diameter by 13mm.

Shallot aqueous extract showed a remarkable activity against *Kleb. pneumoniae* (29.5mm), which was 3.5 mm more than gentamicin (26.5mm). With lime, zone of inhibition was measured as 18.5 mm. Activity was observed in 4 different concentration and found to be 29-19mm and 21-0 mm in shallot and lime respectively.

Lime had slightly more inhibition than shallot, against *P. aeruginosa*. At concentration of 5mg/well, lime showed zone of 23.5mm and shallot had 21.5mm inhibition. MIC was in the range of 20-13 and 21-11 mm in shallot and lime respectively. Gentamicin showed inhibition of 27mm.

Both shallot and lime showed antibacterial activity against *B.cereus*. Shallot showed slightly more activity than lime. As presented in (**Table 1**), zone of inhibition at 23.5 and 18.5 mm was observed in shallot and lime respectively.

Antibacterial activity and MIC of methanolic extract

Antibacterial activity of all samples in methanolic extract was studied and similar to aqueous extract, ginger, valerian and borage did not show any inhibition of test bacteria (**Table 3**).

Diameter of inhibition zone (25 mm) was shown by shallot and lime against *Escherichia coli*. MIC was found to be in the range of 25-18mm and 26-12mm in shallot and lime respectively. But borage, ginger and valerian did not show activity. Results are in agreement with other studies. Mansouri *et al*., (2005) also examined antibacterial properties of methanolic extract of *Echium amoenum* petals against *E.coli* and results were negative. Onyeagba *et al*., (2004) reported that the ethanolic extract of dried ginger did not have antibacterial potential against *E.coli*.

Lime and shallot showed similar activity against *Staph. aureus*. Diameter of inhibition zone was 25mm for both lime and shallot. Mansouri (1999) tested ethanolic extracts of *E. amoenum* against *S. aureus* (489 samples) and observed that it did not inhibit bacterial growth. *P. aeruginosa* was inhibited with methanolic extract of shallot and lime. Zone of inhibition were found to be 22.5 and 23.5 mm in shallot and lime respectively. At different concentration zone of inhibition were in the range of 22-15mm and 23-15mm respectively. Inhibition was found to be similar to gentamicin. Diameter of inhibition zone was 32 mm in shallot extract against *B.subtilis* and lime also showed a similar inhibition zone (31 mm).

The MIC of methanolic extracts of samples, against gram negative and gram positive test

bacteria are presented in (**Table 4**). MIC of shallot was found to be in the range of 31-11 mm and that of lime was 30-21 mm. In this experiment gentamicin antibiotic showed a zone of 38 mm. Shallot and lime showed 24 and 25 mm zone respectively. The MIC of shallot and lime were in the range of 24-18 and 25-17 mm respectively. Gentamicin was found to inhibit the growth with the diameter of 24mm.

Shahidi, (2004) examined the antibacterial properties of ethanolic extract of *E. amoenum* and ginger against *Kleb.pneumoniae* and observed that both ginger and borage did not show antibacterial properties in ethanolic extract against *Kleb.pneumoniae*. Against *B.cereus*, lime exhibited higher diameter of inhibition zone (27 mm) than shallot. The activity of lime was found to be closer to the inhibition by gentamicin (28 mm). When activity was evaluated at different concentration, a range of 25-18 mm and 26-16 mm were observed in shallot and lime respectively.

Shallot and lime had 25 and 24 mm inhibition zone diameter against *Salm. typhi*. Inhibition was considerably lesser than gentamicin (30 mm). Inhibition at different concentration was in the range of 24-10mm in shallot and 24-16mm in lime. Onyeagba *et al*., (2004) reported that *Salmonella* spp was not inhibited with ethanolic extract of dried ginger. Azu *et al*., (2007) studied the antimicrobial properties of various extracts of fresh *Allium cepa* (onions) and fresh *Zingiber officinale* (ginger) against *E. coli, Salm. typhi* and *B. subtilis.* Sensitivity pattern of *Staph. aureus* and *P.aeruginosa* to cold-water extract of ginger was reported to be in the range of 19-13mm against *Salm. typhi and* 17-12 mm against *P.aeruginosa* in the concentration of 0.1-0.8 gml^{-1}. Inhibition was not seen in the hot water extract against *Salm. typhi*. It can be explained by sensitivity of antibacterial component of ginger to thermal treatment. However, activity was seen in hot water extract against *P.aeruginosa*. When the inhibition was compared with cold water extract, inhibition was found to be lesser in hot water extract.

In contrast, in this study, with aqueous and methanolic extract of ginger, inhibition was not seen for any of the bacteria. It could be due to the variety and also the type of ginger as in the present study we used dried ginger whereas Azu *et al*., (2007) used fresh ginger for the study. It was evident that extraction media affected the degree of antibacterial activity of the extracts.

In aqueous extract of *C. aurantifolia* and *Allium scallion,* difference in activity was not significant between gram positive and gram negative tested bacteria. But between shallot and lime, aqueous extract of shallot showed more activity in both gram positive and gram negative bacteria. In methanol extract, lime showed slightly more inhibition compared to shallot. Diameter zone of inhibition in shallot and lime did not show significant difference between gram positive and gram negative tested bacteria.

It can be concluded that dry whole lime and shallot have antibacterial potential which could be effective as synthetic antibiotics against some pathogenic bacteria. However, other samples tested viz., valerian, borage and dry ginger, did not exhibit any antibacterial activity against the human pathogenic bacteria investigated.

Table 1: Antibacterial Activity of Aqueous Extracts of Herbs and Spices against Pathogenic Bacteria (Zone of inhibition in mm)

Plant extracts	Test bacteria						
	Gram positive			Gram negative			
	B. cereus	***B. subtilis***	***Staph. aureus***	***E. coli***	***Kleb. pneumoniae***	***P. aeruginosa***	***Salm. typhi***
Shallot	23.5 ±0.71	29.0 ±1.41	33.5 ±0.71	26.5 ±0.71	29.5 ±0.71	21.5 ±0.71	32.5 ±0.71
Borage	-	-	-	-	-	-	-
Valerian	-	-	-	-	-	-	-
Lime	18.5 ±0.71	28.5 ±0.71	23 ±0.71	19.0 ±1.41	18.5 ±0.71	23.5 ±1.41	30.5 ±2.12
Ginger	-	-	-	-	-	-	-

Concentration, 5 mg/well.

Table 2: Minimum Inhibitory Concentration Of Aqueous Extracts Of Herbs And Spices Against Pathogenic Bacteria

Test bacteria	Test extracts															
	Shallot				Lime				Gentamicin				Water			
Concentration* (µl)	50	25	10	05	50	25	10	05	50	25	10	05	50	25	10	05
B. cereus	24	22	20	18	18	16	15	14	31	-	-	-	-	-	-	-
Staph. aureus	33	31	29	21	22	20	15	-	32	-	-	-	-	-	-	-
B. subtilis	29	24	20	18	28	25	21	20	34	-	-	-	-	-	-	-
Salm. typhi	32	27	24	15	29	25	23	12	29	-	-	-	-	-	-	-
P. aeruginosa	20	18	15	13	21	17	15	11	-	-	-	-	-	-	-	-
Kleb. pneumoniae	29	26	23	19	21	18	12	-	26	-	-	-	-	-	-	-
E. coli	27	25	23	14	19	17	15	12	25	-	-	-	-	-	-	-

* Concentration starts with 5mg/well and ends with 0.5mg/well.

Table 3: Antibacterial Activity of Methanolic Extracts of Herbs and Spices against Pathogenic Bacteria (Zone of inhibition in mm)

Plant extracts	Test bacteria						
	Gram positive			Gram negative			
	B. cereus	***B. subtilis***	***Staph. aureus***	***E. coli***	***Kleb. pneumoniae***	***P. aeruginosa***	***Salm. typhi***
Shallot	25.0 ±1.41	32.0 ±1.13	25.0 ±1.41	25.5 ±0.71	24.0 ±0.71	22.5 ±1.28	25.0 ±1.41
Borage	-	-	-	-	-	-	-
Valerian	-	-	-	-	-	-	-
Lime	27.0 ±0.71	31 ±2.12	25.0 ±0.71	25.0 ±1.41	25.0 ±0.71	23.5 ±0.71	24.0 ±0.00
Ginger	-	-	-	-	-	-	-

Concentration, 5 mg/well

Table 4: Minimum Inhibitory Concentration of Methanolic Extracts of Herbs and Spices against Pathogenic Bacteria

Test bacteria	Test extracts															
	Shallot				Lime				Gentamicin				Water			
Concentration* (µl)	50	25	10	05	50	25	10	05	50	25	10	05	50	25	10	05
B. cereus	25	24	21	18	26	24	21	16	30	-	-	-	-	-	-	-
Staph. aureus	24	19	16	14	25	22	20	13	34	-	-	-	-	-	-	-
B. subtilis	31	29	25	11	30	26	24	21	38	-	-	-	-	-	-	-
Salm. typhi	24	20	14	10	24	19	18	16	30	-	-	-	-	-	-	-
P. aeruginosa	22	19	18	15	23	20	17	15	24	-	-	-	-	-	-	-
Kleb. pneumoniae	24	22	20	18	25	22	19	17	24	-	-	-	-	-	-	-
E. coli	25	23	21	18	26	19	17	12	29	-	-	-	-	-	-	-

* Concentration starts with 5mg/well and ends with 0.5mg/well

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