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Research Article

ANTIBACTERIAL ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF GARLIC, CINNAMON AND TURMERIC AGAINST *ESCHERICHIA COLI* ATCC 25922 *AND BACILLUS SUBTILIS* DSM 3256.

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ABSTRACT: Many of the spices used daily have been documented to be antimicrobial and have medicinal value as well. Most bacteria are sensitive to the extracts from plants such as clove, garlic, mustard, onion, oregano, turmeric etc. spices such as garlic turmeric and cinnamon has been used as antimicrobial agents in their raw form for the treatment of wounds and injuries and joint pains etc. The present study was conducted to investigate the antibacterial activity of garlic, cinnamon and turmeric. Different concentrations of extracts were prepared by using two solvents water and ethanol. The antibacterial activity was tested against *Bacillus subtilus* (DSM 3256) and *E.coli* (ATCC 25922) at different concentration of extracts of spices by using disc diffusion method. According to the results among the selected spices garlic had the best inhibitory activity showing maximum zone of 26mm against *Bacillus subtilis* DSM and a zone of 22mm against *E.coli* ATCC 25922. The aqueous extracts of garlic were more effective than ethanolic extract. In the case of cinnamon and turmeric, the ethanolic extracts were more effective exhibiting zones of 16mm against *B.subtilis* DSM 3256 and 17mm against *E.coli* , which showed that the cinnamon ethanolic extracts are equally effective against both Gram negative and Gram positive bacteria. The widest zones formed by ethanolic extract of turmeric against *B.subtilis* was measured as 14mm and it was 11mm for *E.coli* ATCC 25922. The results showed that *B.subtilus* is more susceptible to test spices as compared to *E.coli.*

Key words: Garlic, Turmeric, Cinnamon, antibacterial activity, *Escherichia. coli and Bacillus subtilis*, disc diffusion, ethanolic extracts.

INTRODUCTION

The natural products are found to be more effective with least side effects as compared to commercial antibiotics so that reason they are used an alternated remedy for treatment of various infections. (Tepe et al., 2004). Spices are defined as plant substances used to enhance flavor, they include leaves(mint and coriander), flower (clover), bulbs (garlic, turmeric), fruits(black pepper), stem (cinnamon), rhizomes (ginger and turmeric) (Shelef ,1983). Medicinal plants produce certain bioactive molecules which show both antibacterial and antifungal activities. (Chopra et al. (1992, Bruneton, 1995). Many medicinal plants produce antioxidant and antimicrobial properties which protect the host from cellular oxidation reactions and other pathogens highlighting the importance of search for natural antimicrobial drugs. (Mothana and Lindequist, 2004, Bajpai et al., 2005; Wojdylo et al., 2007). Most of the foods borne bacterial pathogens are sensitive to extracts from plants such as garlic, mustard, onion and oregano (Chopra et al., 1956). Gram positive bacteria are more sensitive to antimicrobial compounds in spices than G-negative bacteria (Lawson, 1996).

Garlic is therapeutically effective because of its oil and water soluble organosulfur compounds , thiosulfinates is mainly responsible for its antibiotic activity as Hughes and Lawson, (1991) reported that if extract is free from thiosulfinates the antimicrobial capacity will be lost. Garlic has antibacterial and antifungal activity and contains powerful sulfur and numerous phenolic compounds. (Benkeblia, 2004).

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Previously conducted researches confirmed that garlic is not only effective against many Gram positive and Gram negative bacteria but also possess antiviral and antifungal activity. (Sivam et al., 1997, Ankri and Mirelman., 1999; Whitemore and Naidu; 2000, Rosss et al., 2001; Tsao and Yin., 2001, Martin and Ernst., 2003). Turmeric contains phenolic compounds called curcuminoids that possess all the bio-protective properties of turmeric. Crude turmeric extracts have both antioxidant and antimicrobial capacities so that turmeric could be a potent alternative to common antibiotics. (Goel, 2009). Turmeric extracts are found to show antibacterial activity against methicillin resistant *Staphylococcus aureus*. (Kim et al., 2005, Smith, 1991). Antimicrobial activity of cinnamon bark and oil was reported against many bacterial and fungal strains .Muthuswami et al., (2008) (Gupta, et al., 2008). Antibacterial activity may vary between different strains of same species and moreover depends on the form that is used such as dried, fresh or extracted and also on the harvesting seasons and geographical area as well. (Nanasombat et al., 2005, Arras et al., 1992, Mcgimpsey and Douglas, 1994, Cervenka et al., 2006). The present study was aimed to investigate the antibacterial activity of garlic, cinnamon and turmeric.

MATERIAL AND METHODS

Materials

Garlic, *Allium sativum*, turmeric (*Curcuma longa*), cinnamon (*Cinnamomum zeylanicum*)

Bacterial strains:

Two bacterial strains *Escherichia coli* (ATCC 25922) and *Bacillus subtilis* (DSM 3256) were used in study. Pure cultures of these were obtained from Microbiology Research Laboratory of Quaid-I-Azam University, Islamabad, Pakistan.

Maintenance of bacterial culture and inoculum preparation

Pure cultures were refreshed and maintained on nutrient agar slants and plates on regular basis. The cultures were streaked on sterile nutrient agar plates and kept in incubator for 24 hours at 37°C and stored at 4 °C. Bacterial cultures were refreshed after every 3 to 4 days to avoid contamination. Inoculum was prepared by growing the pure bacterial culture in nutrient broth over night at 37°C.

Preparation of aqueous and ethanolic extracts

The spices including garlic (*Allium sativum*) , turmeric (*Curcuma longa*), cinnamon (*Cinnamon zeylanicum*) were purchased from local market. The three spices were washed with distilled water thoroughly. Garlic(100gm) was washed first by distilled water and then by 95% ethanol. Garlic was homogenized using sterile mortar and pestle. And then sieved through double layer of sterile fine mesh cloth to make 100% extract. Dry spices (100 gm each) were crushed and sieved through mesh cloth to get the fine powder. Powdered spices were soaked in 200ml of distilled water and were kept at room temperature for 24 hours, then were filtered using Whatman no. 1 filter paper. The filtrate was heated at 40-50°C using water bath, until thick paste is formed. The thick paste was considered as 100% concentration of extract. These extracts were stored at 4°C in refrigerator. Extracts of these spices were further diluted to make different concentrations such as 80%,60%,40%, 20% and 10% by mixing with appropriate volumes of distilled water. The ethanolic extract was prepared following same procedure with the exception of solvent which was 95% ethanolic instead of sterilized distilled water.

Antibacterial sensitivity testing using disc diffusion method

Filter paper disc of 5mm diameter using Whatman no. 1 filter paper was prepared and sterilized. The test microorganisms were transferred from nutrient broth to sterile Muller Hinton agar plates with the help of sterile cotton swabs. Using an ethanol dipped and flamed forceps the discs were aseptically placed over the Muller Hinton agar plates seeded with the test microorganisms .10-µL of the various spice extracts i.e. pure extract, ethanol extract and aqueous extract were septically transferred to each disc at all dilutions that were made in triplicate.

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Plates were incubated in an upright position at 37°C for 24hours. 10 µL of 95% ethanol was added in sterile filter paper disc as negative control. Triplicate sample of each dilution was tested. After 24hrs the diameter of zone of inhibition were measured in mm and results were recorded. The antibacterial activity results were expressed in term of the diameter of zone of inhibition and <9mm zone was considered as inactive; 9-12mm as partially active; while13-18mm as active and >18mm as very active (Junior and Zanil, 2000). The mean and standard deviation of the diameter of inhibition zones were calculated.

Antimicrobial sensitivity testing

The test microorganisms i.e. *Escherichia coli* and *Bacillus subtilis* were also tested for their sensitivity against the antibiotics nalidixic acid (30 µg), imepenem (10 µg) by disc diffusion method.

RESULTS

These spices namely garlic (*Allium sativum*), turmeric (*Curcuma longa*) and cinnamon (*Cinnamomum zeylanicum*) were tested against *Bacillus subtilis* DSM3256 and *Escherichia coli* ATCC25922. All the spices used in research study were effective against the test bacterial strains but the best activity was shown by garlic forming a maximum zone of 26mm against *Bacillus subtilus* DSM 3256 and 22mm against *E.coli* ATCC 25922. The aqueous extracts of garlic made wider zones as compared to ethanolic extracts. The ethanol extracts of turmeric and cinnamon showed better results as compared to the aqueous, cinnamon ethanolic extract showed maximum zone of 17mm against *E.coli* (ATCC 25922 and 16mm against *Bacillus subtilus* DSM 3256, while aqueous extracts of garlic have shown better results as compared to the ethanol extracts. Maximum zone of inhibition given by ethanolic extract of turmeric was 14mm against *Bacillus subtilis* DSM 3256 and 11mm against *E.coli* ATCC 25922 .The diameter of zone of inhibition obtained against the three spices at 100% concentration by disk diffusion method was also compared to those obtained against two standard antibiotics imipenem and nalidixic acid as shown in table #3garlic extract produced wider zone of inhibition of 26mm as compared to nalidixic acid for *Bacillus subtilis* DSM 3256 while *E.coli* ATCC 25922 was resistant against nalidixic acid but the growth of *E.coli* was inhibited by garlic, turmeric and cinnamon. Imipenem was effective against both *E.coli* and *Bacillus subtilis* forming zones of 35mm and 23mm, respectively.

Table1 . Antibacterial activity of different concentrations of garlic, cinnamon and turmeric against *B.subtilis* (DSM 3256) by disk diffusion method.

Mean diameter of inhibition zone (mm) of garlic, cinnamon and turmeric						
Concentration	Garlic		Cinnamon		Turmeric	
	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol
10%	11±0.5	9.7±0.57	9±0.34	9±0.38	9±0.35	11±0.38
20%	12±0.42	10.3±0.45	10.3±0.45	10±0.55	10±0.42	12±0.35
40%	16±0.34	11±0.35	11±0.38	12±0.44	10±0.45	12.3±0.48
60%	20±0.52	14±0.35	12±0.36	14±0.52	10.6±0.45	13±0.52
80%	25±0.48	20±0.52	13±0.53	15±0.54	11.3±0.55	13.5±0.62
100%	26±0.45	24±0.52	14±0.42	16±0.45	12.5±0.52	14±0.47

Table 2. Antibacterial activity of different concentrations of garlic, cinnamon and turmeric against *E.coli* (ATCC 25922) by disk diffusion method.

Mean diameter of inhibition zone (mm) of Garlic, Cinnamon and Turmeric						
Concentration	Garlic		Cinnamon		Turmeric	
	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic
10%	11±0.37	10±0.58	0	8±0.32	8±0.38	9±0.38
20%	13±0.35	11±0.40	0	11±0.28	9±0.54	9±0.32
40%	15±0.39	12±0.38	0	12±0.50	9.3±0.56	9.6±0.41
60%	18±0.51	14±0.44	9.3±0.38	14±0.52	9.6±0.35	10.3±0.42
80%	21±0.50	17.6±0.35	10±0.40	16±0.52	10±0.37	10.6±0.52
100%	22±0.38	18±0.36	10.3±0.41	17±0.52	10±0.38	11±0.42

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Table: 3 Comparison of the antibacterial activity of spice extracts and standard antibiotics

Mean diameter of inhibition zone (mm)					
Bacterial strains	Antibiotics		Spices extracts		
	imepenem	Nalidixic acid	Garlic	Cinnamon	Turmeric
			100%	100%	100%
B.subtilis	35±0.35	19±0.42	Aq26±0.42/eth. 24±0.52	Aq.14±0.42, eth.16±0.52	Aq.12.5±0.34,eth. 14±0.36
E.coli	23±0.45	-	Aq.22±0.25/eth .18±0.42	Aq.10.3±0.34/et h.17±0.35	Aq.10±0.42/eth.11 ±0.44

DISCUSSION

Among all the tested spices garlic has shown the best activity at all concentrations both in aqueous and ethanol solvents. Garlic has shown better activity against *B.subtilis* as compared to *E.coli*. Aqueous extract of garlic was more effective as compared to ethanolic extract. The activity of 100% garlic extract was comparatively more than of nalidixic acid but less than imipenem. Cinnamon extract was also effective against *B.subtilis*. The ethanolic extract of cinnamon was more efficient in its antibacterial activity as compared to the aqueous extract. The reason is that the antimicrobial component of the cinnamon bark is more soluble in ethanol as compared to water but its activity was reported less as compared to the garlic. Turmeric was least effective against *B.subtilis* among the three test spices. The ethanol extract of turmeric showed better results as compared to the aqueous ones. Zones of inhibition formed by garlic was less in diameter against *E.coli* as compared to the *B.subtilis*. Nalidixic acid was ineffective against *E.coli* while garlic showed and inhibition zone approximately equal to that of imipenem .The ethanol extract of cinnamon showed better zones at all concentrations against *E.coli* as compared to the aqueous extracts which was effective at only higher concentration and when compared with impenem it produce smaller zone. Both the aqueous and ethanolic extracts were effective against *E.coli* but ethanolic extract showed comparatively better results. Zone formed by turmeric was smaller as compared to imipenem. The findings agree with the work of Srinivasan *etal.*(2009). In that study the antibacterial activity of garlic was checked against Gram positive and Gram negative bacteria. Among the two bacterial strains tested maximum activity was shown against *B.subtilis.* The Gram negative *E.coli* was comparatively resistant to Gram positive *Bacillus subtilis*, this may be due to the structural differences in the cell membrane and cell wall structure, Gram negative has outer membrane as well which further block the penetration of antibiotics including the extracts of spices making them resistant. Indu et al.(2006) suggested that the garlic extract is effective against different serotypes of *E.coli.* The study also showed that the antibiotic Nalidixic acid is effective against both the *Bacillus subtilis* and *E.coli*. Indu et al. (2006), reported that the cinnamon extract posses effective antibacterial properties against *B.subtilis* and *E.coli* . Gur et al.(2006) suggested that the turmeric extract is effective against both the test bacteria *B.subtilis* and *E.coli.* This agrees with the results of the present study. Chandara et al. (2005) reported that turmeric is effective against *E.coli, B.subtilus and S.aureus* due to the presence of a phenolic compound, curcuminoid,. The presence of essential oil, an alkaloid, curcumins and turmerol and veleric acid are responsible for antibacterial activity of turmeric. Odhav et al. (2002), suggested that the mechanism of antibacterial action of spices involve the hydrophobic and hydrogen bonding of phenolic compounds to membrane proteins, membrane disruption and destruction of electron transport systems and cell wall disruption. The antimicrobial activity of aqueous extracts could be due to anionic components such as thiocyanate, nitrate, chlorides and sulphates in addition to many other compounds naturally present in plants. (Darout , 2000). The ethanolic extracts showed better results as compared to aqueous as being organic dissolves more organic compounds resulting in the release of greater amount of active antimicrobial components (Cowan, 1999).

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The thick structural constituents of Gram-positive microbes in this instance can be held responsible for the increase in the interaction between the active compound, curcumin and the structural lipoproteins. This increased interaction may results in the inhibition of the Gram-positive microbes .The antibacterial activity of cinnamon might be due to the presence of cinnamaldehyde compound which inhibits the amino acid decarboxylation activity in the cell which leads to energy deprivation and microbial cell death (Wendakoon and Sakaguchi, 1995). Previously stated, these are phenolic compounds that are capable of further cellular destruction and inhibition by establishing the hydrophobic and hydrogen bonding of these degradative phenolic compounds to membrane proteins resulting in portioning of the lipid bilayer (Juven et al., 1994).

It is concluded from the present study that these spices can be used to produce new therapeutics. Among three spices used garlic was most effective among all so it can be used to develop new antimicrobials. Further research is required to investigate the bioactive molecules of garlic, turmeric and cinnamon.

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