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Study on Antibacterial Activity of Common Spices

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Abstract

Spices have been shown to possess medicinal value, in particular, antimicrobial activity. They are used as household medicines as well as preservatives of food materials. This study compares the sensitivity of some human pathogenic bacteria to various spice extracts viz. essential oils, acetone and methanol extracts by agar well diffusion method. Of the different spices tested clove, ajowan and cinnamon were found to possess relatively higher antimicrobial activities. Essential oil of cinnamon showed broad spectrum of inhibition against all tested bacteria while essential oil of ajowan and clove inhibited 90% and 70% bacteria respectively. Acetone and methanol extracts of clove showed better antibacterial activity among the spices. The MBC value ranged from 0.39 to 25mg/ml. The lowest MBC (minimal bactericidal concentration) value was given by essential oil of cinnamon against *E.coli*, *S. aureus* and S. Typhi. Gram positive bacteria were found to be more sensitive to spices than Gram negative bacteria. Spices might have a great potential to be used as antimicrobial agents.

Key words: spices, essential oil, acetone extract, methanol extract, antibacterial activity, MBC

Introduction

Herbs and spices are the most important parts of human diet. In addition to boosting flavor, herbs and spices are also known for their preservative and medicinal value (DeSouza *et al.* 2005, Saeed & Tariq 2006). Antimicrobial activity of spices and herbs has been known and described and used for medicinal purposes for several centuries (Bagamboula *et. al*. 2003, Arora & Kaur, 1999).

Since the introduction of antibiotics there has been tremendous increase in the resistance of diverse bacterial pathogens (Gold & Moellering 1996), and the side effects of conventional treatment in healing and treatment of various diseases has been rise in the last few decades (Saeed & Tariq 2007), the world has been diverting towards the traditional medicine or herbal medicine. At present, it is estimated that about 80% of the world population rely on botanical preparations as medicines to meet their health needs (WHO 2002). Herbs and spices are generally considered safe and proved to be effective against certain ailments. It is only in recent years that modern science has started paying attention to the properties of spices (Chaudhry & Tariq 2006). They are also extensively used, particularly, in many Asian, African and other countries. In recent years, because of their beneficial effects, use of spices and herbs has been gradually increasing in developed countries also.

Food-borne illness caused by eating food or drinking beverage contaminated with bacteria, parasites or viruses, can cause symptoms that range from an upset stomach to more serious symptoms such as diarrhea, fever, vomiting, abdominal cramps and dehydration. Natural products and naturally derived components from the plants have applications in controlling pathogens in foods. Due to antimicrobial properties, Spices can be the alternative to chemical food additives (Chandarana *et. al.* 2005). But, its application has been minimal. The antioxidant and antimicrobial property of spices are very important to preserve the quality of food material and at the same time provide safety to consumer (Singh *et al*. 2007). Medicinal value of spices is due to antimicrobial activity exhibited by different bioactive compound like alkaloids, flavonoids, isoflavonoids, tannins, cumarins, glycosides, terpens and phenolic compounds. Spices are also of great economic value as they are incorporated in perfumes, cosmetic and some dietary products and also in medicine due to their sweet scent (El-Gammal 1993; Loewenfeld and Back 1979).

Therefore, the investigation of the antimicrobial properties of spices used as food additives to control the growth of food-borne pathogen bacteria may gives useful results.

This study mainly focused on the antibacterial properties of the some common spices used in Nepal like clove, coriander, turmeric, black pepper, ajowan and cinnamon.

Methodology

This study was conducted from October, 2007 to July, 2008, partly in Department of Plant Resource (DPR), Thapathali and partly in Central Department of Microbiology, TU. The extraction part of the study was carried out in DPR and antibacterial activity of the spices was carried out in laboratory of Central Department of Microbiology.

Plants Materials

The spices listed in Table 1 were brought from the market in packaged form. These spices were identified and confirmed in the Department of Plants Resources (DPR), Thapathali.

Sample processing

The samples were grinded in a grinder. The powdered spices were used for extraction of essential oils and other extracts successively.

Extraction of spices material

Extraction of essential oils

Essential oils from spice were extracted by hydro-distillation method using Clevenger apparatus for 6 hrs or more. The water was drained off and the oil was collected on a small well capped bottle. The oils were dried over anhydrous sodium sulphateand stored in refrigerator in dark at 4ºC until use.

Soxhlet extraction with acetone and methanol

After extraction of essential oils, spices powered were dried at 45ºC. Dried spices powder were loaded in soxhlet apparatus and subjected to continuous extraction with solvent like acetone and methanol to obtain crude extracts, acetone extract and methanol extract respectively. At first the extraction was done using acetone. The process was allowed to run for 8-15 hrs or till the color solvent appeared in the siphon. Then, the acetone solvent was removed and the extraction was continued using methanol. After complete extraction, the solvent was removed with the help of rotary vacuum evaporator.

The crude extract of spices was transferred to a bottle. It was labeled and store in a refrigerator at 4ºC until use.

Preparation of stock/working solution

The stock solution of essential oil was made in 2% Tween80 in physiological saline (0.85g of sodium chloride dissolved in 100 ml water, sterilized by autoclaving). 1g of an essential oil was dissolved in 10ml of 2% Tween80.

The crude extract was dissolve in dimethyl sulphoxide (DMSO) (10-40%) by dissolving 1gm extract in 10ml of it. The concentration of the stock solution becomes 100mg/ml. The solutions were stored in refrigerator.

Standard bacterial culture

Ten different human pathogenic bacteria were selected for the study. The cultures were collected from Department of Microbiology, TUTH, and Central Public Health Laboratory, Teku. The bacterial cultures were maintained on nutrient agar slant. The organisms were sub- cultured every two weeks.

Three or four colonies of bacteria were transferred to the test tube containing 5ml of sterile nutrient broth. It

was incubated at 37ºC for 3 or 4 hr. The tubes were compared with McFarland Nephelometer Standard 0.5 (turbidity standard) recommended by WHO (1991). A blank nutrient broth was used as a control.

Evaluation of antibacterial activities of extracts

Screening and evaluation of antibacterial activity: The crude extracts of plants were screened for its antimicrobial activity against the organisms by agar well diffusion method given by Dingle *et al.* (1953).

Sterile cotton swab was dipped in to the prepared inoculums and seeded all over the MHA plate by rotating through an angle of 60° after each swabbing finally the swab was passed round the edges of the agar surface and left to dry for few minutes at room temperature with lid closed. Then with the help of sterile cork borer (no. 9 and no. 6), wells were made in the inoculated plate and labeled properly. 100µl and 50µl of the working suspensions of the spices extract were dispensed in the respective wells with the help of the micropipette. The solvent itself was tested for its activity as a control as the same time. The plates were then left for half an hour with the lid closed. Then the plates were incubated at 37°C for 24 hr then observed for the zone of inhibition which is suggested by the clear area around the well (WHO, 1991).

Determination of minimal bactericidal concentration (MBC)

MBC was determined for those extract which showed the antibacterial activities by two fold dilution method.

Table 1. Lists of spices used in the evaluation of antimicrobial activities

Common name	Botanical name	Family	Part(s) used	*Major components
Clove (Lwang)	***Syzygium aromaticum* L.**	Myrtaceae	Bud	Eugenol
Black pepper (Marich)	***Piper nigrum* L.**	Piperaceae	Fruit	Piperine
Turmeric (Beshar)	***Curcuma longa* Linn.**	Zingiberaceae	Rhizome	curcumin
Ajowan (Jwano)	***Trachyspermum ammi* L.**	Umbelliferae	Seed	Thymol
Coriander (Dhaniyaa)	***Coriandrum sativum* L.**	Umbelliferae	Fruit	Linalool
Cinnamon (Daalchini)	***Cinnamomum zeylanicum* Bl.**	Lauraceae	Bark	trans-cinnamaldehyde and eugenol

*source Wealth of India

Resuts and Discussion

Table 2. summarises the antimicrobial activities of the essential oils, acetone extracts and methonal extracts of spices. Among the six spices, clove, cinnamon and ajowan were found to be effective against tested bacteria.

Essential oil of cinnamon shown to be broad spectrum inhibiting all the bacteria tested with the highest inhibitory effects producing inhibition zones of 37mm (100ml) of diameter against *S. dysenteriae*. Essential oil of ajowan inhibited all the bacterial except *P. aeruginosa*. Essential oil of clove inhibited *E. coli, S. aureus, K. pneumoniae, K. oxytoca. S.* Typhi and *P. aeruginosa*. Essential oil of black pepper inhibited only *S. aureus*, that of turmeric inhibited *E. coli* and *S. aureus* and that of coriander inhibited *E.coli, S. aureus and S. dysenteriae*.

Acetone fraction of clove showed higher antibacterial activity inhibiting all test bacteria except *K. oxytoca* S. Typhi *P. vulgaris* , that of cinnamon inhibited all except *P. aeroginosa,* that of black pepper inhibited *E.coli* and *S.aureus* and that of turmeric and coriander inhibeted only *S. aureus*. Methanol fraction of clove showed higher antibacterial activity inhibiting *S. aureus* S. Paratyphi *P. aeroginosa S. dysenteriae P. mirabilis* and *P. vulgaris,* whereas that of cinnamon and black pepper inhibited only *S. aureus*. In case of clove the bacterial which were not inhibited by essential oil were inhibited by acetone and methanol fraction.

Table 3 showed the MBC of different effective fraction. The MBC ranged from 0.39mg/ml to 25mg/ml in case of essential oils, 6.25mg/ml to 25mg/ml in case of

acetone and methanol fractions. The essential oils showed better antibacterial activity against test bacteria at tested concentration (100mg/ml). The minimum MBC value was given by cinnamon essential oil against *E. coli, S. aureus* and *S*. Typhi (0.39mg/ml). Gram positive bacteria are more susceptible to the spices than Gram negative bacteria.

The oils and extracts though less effective at lower volume, gave rise to high inhibition zone when their volume increased. The result of clove is supports the results obtained by Agaoglu (2006) and Agnihotri and Vaidya (1995). According to Singh *et al.* (2005) and Pradhan *et al.* (1999), black pepper was effective against to *S. aureus* which supports with the result of study. Singh *et al.* (2007) reported that essential oil of turmeric was effective against *E. coli, S. aureus*, and *Bacillus* species but acetone fraction didn't show any antibacterial activity. Singh *at al.* (2007) reported that essential oil of Ajowan was also active against *P. aeruginosa* that contradicted with the result and acetone fraction was didn't showed any antibacterial activity that supports the result. The fractions of coriander showed weak inhibitory activity. The result supports the finding of the study done by Singh *et al.* (2007). The result obtained for the cinnamon oil is supported research done by Rajendhran *et al.* (1998), Sofia *et al*. (2007), Fabio *et al*. (2007) and Agaoglu *et al*. (2007). However, the contradiction of the result may be due to concentration variation, various test environments and methods. The volatilility and poor solubility of most essential oils are problematic especially with diffusion and dilution of the test substance in a microbiological medium. Antimicrobial activity of spices depend on several factors, including kinds of spices, composition and concentration of spices, microbial species and its occurrence level, substrate composition and processing conditions and storage (Shelef, 1983).

Table 2: Antibacterial activities of essential oils of the spices

spices	Zone of Inhibition (mm)																			
	E. coli (ATTC 25922)		*S. aureus* (ATTC 25923)		*K. pneumoniae*(ATTC 25922)		*K. oxytoca*		S. Typhi		S. Paratyphi		*P. aeroginosa* (ATTC 25922)		*S. dysenteriae*		*P. mirabilis* (ATTC 25922)		*P. vulgaris*	
Volatile oil	50µl	100µl	50µl	100µl	50µl	100µl	50µl	100µl	50µl	100µl	50µl	100µl	50µl	100µl	50µl	100µl	50µl	100µl	50µl	100µl
Cinnamon	20	26	29	35	17	24	21	27	22	32	22	30	18	25	25	37	20	28	21	28
Clove	14	17	13	16	13	17	13	18	14	18	-	-	12	17	-	-	-	-	-	-
Black pepper	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turmeric	10	15		14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ajowan	15	22	14	22	-	15	15	22	15	23	-	15	-	-	11	19	14	20	15	21
Coriander	11	15	20	28	-	-	-	-	-	-	-	-	-	-	-	15	-	-	-	-
Acetone extracts																				
Cinnamon	10	15	13	19	-	13	-	14	12	17	11	16	-	-	12	19	10	16	12	15
Clove	12	17	13	16	13	17	-	-	-	-	12	18	-	15	-	13	-	14	-	-
Black papper	12	17	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turmeric	-	-	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ajowan	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
coriander	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methanol extract																				
Cinnamon	-	-	10	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clove	-	-	13	17	-	-	-	-	-	-	12	18	12	17	15	22	11	15	11	16
Black papper	-	-	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turmeric	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ajowan	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
coriander	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table. 3 MBC of the spices

ices	Test Bacteria									
olatile oil	*E. coli*	*S. aureus*	*K. pneumoniae*	*K. oxytoca*	S. Typhi	S. Paratyphi	*P. aeroginosa*	*S. dysenteriae*	*P. mirabilis*	*P. vulgaris*
innamon	0.39	0.39	0.78	0.78	0.39	0.78	0.78	0.78	0.78	0.78
love	6.25	3.13	3.13	1.56	1.56	-	12.50	-	-	-
lack pepper	-	25.00	-	-	-	-	-	-	-	-
ırmeric	25.00	25.00	-	-	-	-	-	-	-	-
jowan	1.56	1.56	3.13	1.56	1.56	6.25	-	1.56	1.56	1.56
oriander	3.13	3.13	-	-	-	-	-	3.13	-	-
cetone extracts										
innamon	6.25	12.50	12.50	12.50	3.13	12.50	-	12.50	12.50	12.50
love	6.25	25.00	25.00	-	-	25.00	25.00	25.00	25.00	25.00
lack papper	-	25.00	-	-	-	-	-	-	-	-
ırmeric	-	25.00	-	-	-	-	-	-	-	-
jowan	-	25.00	-	-	-	-	-	-	-	-
oriander	-	-	-	-	-	-	-	-	-	-
[ethanol :tract										
innamon	-	25	-	-	-	-	-	-	-	-
love	-	12.5	-	-	-	6.25	25	3.13	25	3.13
lack papper	-	-	-	-	-	-	-	-	-	-
ırmeric	-	-	-	-	-	-	-	-	-	-
jowan	-	-	-	-	-	-	-	-	-	-
oriander	-	-	-	-	-	-	-	-	-	-

Based on the above result, it can be concluded that the essential oil of cinnamon, clove and ajowan possess excellent antibacterial properties following the acetone extract of clove and cinnamon. Lowest MBC values were given by essential oil of cinnamon against *E. coli, S. aureus* and *S.* Typhi. So spices could be remedies for certain ailments and may be alternative to chemical additives.

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