RESEARCH ARTICLE

Antibacterial activity of spice essential oils against uropathogenic bacteria

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ABSTRACT

The medicinal values of many spices and condiments used in Indian cooking have been known from centuries. The aim of this study is to evaluate the antibacterial activity of spice essential oils which use in kitchen on uropathogenic bacteria. Urinary tract infection is second most common infection next to Respiratory tract in our human body. The indiscriminate use of antimicrobial drugs has led to the resistance in uropathogens globally. In present scenario, alternative and safe therapy is urgently needed. Essential oils of ajwain (*Trachyspermum ammi*), cardamom (*Amomum subulactum*), clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum zeylanicum*), black pepper (*Pipera nigrum*) and turmeric (*Curcuma longa*) were used in this study. Their antibacterial activity against various uropathogenic bacteria viz. *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* was determined by disc diffusion method. The minimal inhibitory concentrations (MICs) of oils were determined by broth macrodilution method. Among all oils ajwain showed highest inhibitory activity with zone of inhibition ranging from 40 to 35 mm diameter against all uropathogenic bacteria. Turmeric was found to be ineffective against all uropathogenic bacteria. Cardamom and clove also showed strong activity for all uropthogens. Activity of black pepper and cinnamon oil was found to be moderate against uropathogens.

Keywords: Spice, Spice essential oils, Uropathogens, Antibacterial activity.

INTRODUCTION

Urinary Tract Infections (UTI) is the second most common infection in humans (Tabiban *et al*., 2008). Different sex and age group of people are affected by urinary tract infection. Sometimes, the UTI is symptomatic or asymptomatic and complicated or uncomplicated. Usually UTI infection is confirmed by significant bacteriuria. i.e., 10^5 organisms/ml (Anandraj *et al.* 2015). UTI refers to the presence of clinical signs and symptoms arising from the genitourinary tract associated with the presence of one or more microorganisms. UTIs are usually localized to the bladder, kidneys or prostate. *Escherichia coli* is the predominant uropathogen

responsible for roughly 80% of all UTI cases, followed by *Staphylococcus*, *Klebsiella*, *Enterobacter*, *Proteus* and *Enterococci* species (Ronald, 2003). Isolation, characterization, early detection and antibiotic therapy are also very important for Urinary tract infection. Current management of UTI's are usually empirical, without the use of urine culture or susceptibility testing to guide therapy (Manjunath *et al*., 2011). Today many broad-spectrum antibiotics are used to treat many urinary tract diseases pose serious threat of drug resistance and hypersensitivity reactions. The success of chemotherapy lies in the continuous search for new drugs to couter the challenge posed by resistant strains (Gibbons, 1992). The present investigation evaluates the antibacterial effects of essential oils of various spices against urinary tract pathogens.

MATERIALS AND METHODS

The following standard cultures were procured from microbial Type culture collection (MTCC), & American Type Culture Collection (ATCC)
Escherichia coli ATCC 14948
Klebsiella pneumoniae MTCC 4030
Pseudomonas aeruginosa MTCC 4676
Staphylococcus aureus ATCC 33591
The standard cultures were maintained on the culture media as recommended by MTCC and ATCC

Essential Oils

In the present study, seven genuine essential oils of, clove, ginger, turmeric, ajwain, black pepper, cardamom & cinnamon were obtained from different commercial sources. The oils were stored in glass bottles at 4^{o} C under refrigeration.

Antibacterial assay of spice essential oils

In the present work, essential oils of seven different spices were used. The test organisms were grown in nutrient broth. The fresh bacterial cultures were compared with 0.5 Mc Farland turbidity standard, which is equivalent to approximately $1x10^{6}$ CFU/ml were used. Then the test organisms were inoculated on the Muller-Hinton agar plates. Sterile swabs were used for inoculation purpose. The sterile discs were placed on agar surface. 20 µl essential oil of each spice was added on the sterile discs. These plates were incubated at 37^{o} C for 24 hours. The zone of inhibition was observed and recorded.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations of six essential oils namely ajwain, cardamom, clove, cinnamon, black Pepper and ginger oil were determined by Broth Macrodilution Sensitivity testing method. This test was performed in round bottom sterile glass tubes (12x75 mm) using Muller Hinton Broth supplemented with 0.15% agar. Serial two fold dilutions of stock solution of each spice essential oils were prepared in tubes over the range of 0.02-10µl/ml with a final oil concentration range 0.01-5µl/ml.

A working inoculum suspension of $1x10^{4}$ CFU/ml was prepared by diluting the stock inoculums. The inoculums were added in each tube & one tube kept as a control i.e. inoculum was not added in this tube. The tubes were then incubated at 37^{o} C for 24 hrs and observed for the presence or absence of visible growth. (Kamble 2006)

RESULTS AND DISCUSSION

The antibacterial activities of seven spice essential oils were screened against four organisms by disc diffusion method. Essential oils showed antibacterial activity in varying magnitudes. Ajwain oil, cardamom oil, cinnamon oil, black pepper, clove oil, & ginger oil showed inhibitory activity against all uropathogens (Table 1).

Out of these seven spice essential oils, ajwain oil was found to be most effective with > 30mm diameter of inhibition zone against all uropathogenic bacteria. This result is in agreement with the report of Hassanshahian et al. (2014) reported that essential oil of ajwain was found to be very effective against *E. coli, K. pneumoniae & S. aureus*.

Cardamom oil showed strong inhibitory activity with 33mm, 30 mm, 27mm & 23 mm inhibition zone diameter for *E. coli, K. pneumoniae*, *P. aeruginosa* & *S. aureus,* respectively. Kumar et al. (2010) reported that the antimicrobial property of cardamom is due to having wide verity of secondary metabolized such as tannins, alkaloids & flavonoids. Ritender et al . (2013) concluded that, the essential oil of cardamom (*Ammomum subulatum*) is useful for treatment of infection caused by *S. aureus P. aeruginosa & E.coli.*

Table 1. Antibacterial activity of undiluted essential oils of spices against uropathogens.
Zone of Inhibition in mm

Organisms	Essential Oils						
	Ajwain	Cardamom	Clove	Cinnamon	Black Pepper	Ginger	Turmeric
E.coli	40	33	25	26	16	12	No Zone
K. pneumoniae	42	30	22	8	20	13	No Zone
P. aeruginosa	41	27	22	18	15	12	No Zone
S. aureus	35	23	23	24	18	13	No Zone

Table 2. Minimum inhibitory concentrations (MICs) of essential oils against uropthogens

Organisms	MICs of essential oils (µl /ml)					
	Ajwain	Cardamom	Clove	Cinnamon	Black pepper	Ginger
E. coli	0.62	1.25	1.25	1.25	2.5	5.0
K. pneumoniae	0.62	1.25	1.25	2.5	2.5	5.0
P.aeruginosa	0.62	2.5	1.25	2.5	2.5	5.0
S. aureus	1.25	2.5	1.25	1.5	2.5	5.0

Aneja & Joshi (2010) studied in importance of clove & clove oil & indicated that clove oil can be used as an antimicrobial agent. Present investigation supports the findings of Aneja & Joshi. In the present work clove oil showed inhibitory activity for all uropathogens. The essential oil of cinnamon was found to be inhibitory for *E.coli , S. aureus & P. aeruginosa* & showed little inhibition for *K. pneumoniae.* Poppachan et al .(2007) evaluated the antimicrobial activity of six indian spices extracts namely clove, cinnamon & mustard, garlic, ginger & mint against *E .coil S .aureus* and *B. cereus* & reported that the extract of clove, cinnamon & mustard had good inhibitory activity. Ginger oil showed inhibition ranging from 12 to 13 mm diameter zone of inhibition. This result is in agreement with Nader *et al.* (2009) who reported ginger essential oil to be moderately effective against bacteria. In present studies turmeric oil was found to be ineffective against all uropathogens. Jelena et al. (2016) also reported the ineffectivity of turmeric oil against *S. aureus, E. coli* and *L. monocitogen*.

In the present investigation MICs of six spice essential oils was determined by broth macro dilution method. *E. coli, K. pneumoniae*, & *P. aeruginosa* were inhibited by Ajwain oil at concentration 0.62 µl/ml. Ajwain oil inhibited *S. aureus* at concentration of 1.25 µl/ml. Omidpanah et al. also (2016) reported that, essential oil of ajwain inhibited the growth of *E. coli* at concentration of 0.5 µl/ml and *S. aureus* at concentration 1 µl/ml The cardamom oil inhibited *E. coli*, *K. pneuononiae*, *P. aeruginosa* & *S. aureus* at concentration 1.25, 1.25, 2.5 & 2.5 µl/ml respectively. Cinnamon oil inhibited *E. coli* at concentration of 1.25 µl/ml, *K. pneumoniae* & *P. aeruginosa* at 2.5 µl/ml & *S. aureus* at 1.5 µl/ml. All the bacteria were inhibited by the black pepper and ginger oil at 2.5 µl/ml & 5.0 µl/ml concentration respectively. MICs for all bacterial test cutures are shown in Table 2.

CONCLUSION

Present study concluds that the spice essential oils have antimicrobial property against uropathogens. By the view of therapeutic management spice and herbs are of low cost and biodegradable. Therefore spice essential oils may be the natural and safe way rather than use of expensive and nonbiodegradable antibiotics for treatment of UTI.

Conflicts of interest: The authors stated that no conflicts of interest.

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