Journal of Experimental Sciences Vol. 1, Issue 7, Pages 33-36 [2010]
www.jexpsciences.com

Regular Article

Screening and Comparision of Antibacterial Activity of Indian Spices

Madhumita Rakshit and C. Ramalingam*

School of BioSciences and Technology, V. I. T. University, Vellore -632014, TN, India

ABSTRACT: Spices have been shown to possess medicinal values, particularly antimicrobial property. This study compares the sensitivity of some human and food borne pathogenic bacteria to various spice extracts. Of the different spices tested only Garlic, Turmeric, Fenugreek, Cardamom, Star anise, Red chilly, Coriander and Clove were found to possess broad spectrum antimicrobial activity. When exposed to higher temperature, turmeric looses the antimicrobial property. But at higher temperature, the antimicrobial activity of Clove and Ajowain increases. Thus spices might have a great potential to be used as antimicrobial agents at different food products processed at different temperature.

Key words: Spices, Microorganism, Antimicrobial activity, Zone of inhibition

Introduction

India is well known for its strong aromatic spices. A huge amount of spices are exported to all over world. In 2009-2010, the export of spices from India stood at 502,750 tonnes. In ancient time spices was the most attractive trade item which led to colonisation of India. Among 80 types of spices in the world, India alone produces 50 types.India produces a variety of spices. From ancient time people are going on using spices as food additive, either to add color, flavor or aroma to the food. Spices and herbs are also being well known for its medicinal value. The most essential spices in India are Pepper, Garlic, Cumin, Coriander, Mustard, Curry leaves. Cinnamon, Cardamom, Turmeric, Ginger, Chilly.

In India spices are not only used to add flavor, aroma and color to food, but also it is used for many medicinal values. It is used to heal skin disease, cold and cough, indigestion, diabetes, heart disease and many others. (DeSouza et al., 2005; Saeed & Tariq, 2006).

Many spices and vegetables are also known for their antioxidant activity and are usefull in preventing oxidation of fat and lipid tissues in food and living tissues.

With the advancement of science and people consciousness to health, people are getting concerned to the food they are eating .There is a great demand of free synthetic preservative added to food because many of them have been proven to be toxic. But spices itself are also used as preservative by inhibiting the food spoiling bacteria growing on the food. A large number of plants are used to combat disease (Gibbons A, 1992; Chopra et al., 1956). Garlic with its antibacterial properties is widely used for a number of infectious diseases. Eugenol, an active principle of clove is used as an antiseptic and possesses local anaesthetic activity; it is therefore used for toothache (Suresh P et al., 1992). Some spices are used as a preservative in pickles and chutneys. Little information is available on the preservative and antimicrobial role of spices and their role in prevention of spoilage in food .The present study was made on the antimicrobial activity against food borne and common pathogens. A comparison of the antimicrobial activity of spices to that of different temperature 180°C and 360 °C was been carried out which can yield significant information as to whether spice extracts can be employed as preservative to well established synthetic preservative in raw, cooked, deep fried and grilled food products.

Materials and Method

Microorganism

The bacterial culture was maintained at 1% Nutrient Agar(NA) slants and was stored at 4°.It was subcultured every month. Five microbial strains i.e. four gram positive *Bacillus cereus, Staphylococcus aureus*, *Enterococcus faecalis* ,*Proteus mirabil*is and one gram negative bacteria *Escherichia coli* were used for the antimicrobial assay. For the purpose of antimicrobial evaluation the microorganism was cultured in Nutrient Broth (NB) at 37°C overnight.

Spices

Fourteen different spices were brought from the local market in and around Vellore.

Preparation of aqueous infusion

Aqueous infusion of the spices was prepared by steeping 15 g in 50 mL sterile distilled water respectively in separate conical flasks. The flasks were kept for two days with occasional shaking. The contents of flasks were kept for two days with occasional shaking. The contents of the flask were filtered.

Preparation of ethanol extract

Spices were washed with sterile water, dried overnight at 40°C. It was grinded by a mixer. 15 g of each spice was added in 50 mL of absolute ethanol and kept at 40°C for 24 hours with occasional shaking. The filtrate was kept at room temperature for 24 hours for evaporation of the solvent.

Preparation o spice extract at different temperature

Spices were heated at 180 °C and 360 °C for 10 minutes in microwave oven and then grounded in mixer. Then the same procedure as that of ethanol extraction was carried on.

Screening of antimicrobial activity

Media

Nutrient Agar (NA) (Hi-Media) was used as base medium for screening of antibacterial activity and Nutrient Broth (NB) (Hi-Media) for preparation of inoculums.

Preparation of Nephelometer standard

McFarland tube number 0.5 was prepared by mixing 9.95 ml 1% Sulfuric acid in NB and 0.05 ml 1% Barium chloride in distilled water in order to estimate bacterial density (Baron et al., 1994). The tube was sealed and used for comparison of bacterial suspension with standard whenever required.

Preparation of inoculums

Four to five colonies from pure growth of each test organism were transferred to 5 ml of NB. The broth was incubated at 35-37°C for 18-24 hours. The turbidity of the culture was compared to 0.5 McFarland Nephelometer standards to get 150 x 106 CFU/ml.

Well diffusion technique

Screening of antibacterial activity was performed by well diffusion technique (Kivanc & Kunduhoglu, 1997). The NA plates were seeded with 0.1 ml of the standardized inoculum of each test organism. The inoculum was spread evenly over plate with loop or sterile glass spreader. The seeded plates were allowed to dry in the incubator at 37°C for 20 minutes. A standard cork borer of 6 mm diameter was used to cut uniform wells on the surface of the NA and 100 µl of each aqueous and ethanol extract was introduced in the well respectively.

Incubation

The inoculated plates were incubated at 35-37°C for 24 hours and zone of inhibition was measured to the nearest centimeter (cm).

** Corresponding Author, Email: cramalingam@vit.ac.in, Mob:9487044822*

Statistical analysis
All tests were done in triplicates. Mean zone of inhibition and standard deviations were calculated.

Results and Discussion

Spices which are the different parts of plant (flowers, buds, leaves, stem, skin, pulp) have been used for thousands of years to enhance the flavor and aroma of food. In addition, plants are rich in a wide variety of secondary metabolites such as tannins, terpenoides, alkaloids and flavonoides which have been found in vitro to have antimicrobial properties (Cowan, 1999). In this connection, the present study was conducted to evaluate the antibacterial activity of the fourteen spices that are daily used in our food.

Table 1. Antimicrobial sensitivity test with aqueous infusion of Spices against test organism

Microorganism	Curry leaves	Tomato	Onion	Garlic	Ginger	Sesame
Enterococcus faecalis	+	+	_	+	+	_
Bacillus cereus	_	_	_	+	_	_
Staphylococcus aureus	_	_	_	+	++	__
Escherichia coli	_	_	-	++	+	_
Proteus mirabilis	_	_	_	+	_	_

\+ ⟶ Zone of inhibition within 1.0 ± 0.057 to 1.3± 0.57 cm
_ ⟶ No zone of inhibition

Among all these spices which are used to add flavour and taste to the food, only garlic was found to be the broad spectrum antibacterial spice. Bioactive components are allicin, diallyl disulfide, allyl isothiocyanate which may be responsible for the antibacterial activity of garlic. (Fareed G et al., 2007)

Table 2. Antimicrobial sensitivity test with aqueous infusion of Spices against test organism

Microorganism	Cloves	Turmeric	Ajowain	Mustard	Cumin	Cardamom	Coriander	Red chilly	Star Anise	Jayathri	Bay leaf	Marathi magu	Fenugreek
A	++	++	_	_	+	++	+	++	++	_	+	_	++
B	++	++	_	_	+	++	+	++	++	_	+	_	++
C	++	++	_	_	+	++	+	++	++	_	+	_	++
D	++	++	+	_	+	++	+	++	++	_	+	_	++
E	++	++	+	+	+	+	+	++	++	_	+	_	++

A: *Proteus mirabilis*
B: *Escherichia coli*
C: *Entererococcus faecalis*
D: *Bacillus cereus*
E: *Staphylococcus aureus*
++ ⟶ Zone of inhibition greater than 1.4 ± 0.057 cm
\+ ⟶ Zone of inhibition within 1.0 ± 0.057 to 1.3± 0.57 cm
_ ⟶ No zone of inhibition

Table 3. Antimicrobial sensitivity test with ethanolic extract of Spices against test organisms

Microorganism	Cloves	Turmeric	Onion	Garlic	Cardamom	Coriander	Red chilly	Star Anise	Jayathri	Bay leaf	Marathi magu	Fenugreek
A	++	+	_	++	++	+	+	++	_	+	_	+
B	++	+	_	++	++	+	+	++	_	+	_	+
C	++	+	_	++	++	+	+	++	_	+	_	+
D	++	+	-	++	++	+	+	++	_	+	_	+
E	++	+	_	++	+	+	+	++	_	+	_	+

A : Proteus mirabilis
B: Escherichia coli
C: Entererococcus faecalis
D: Bacillus cereus
E: *Staphylococcus aureus*
++ → zone of inhibition greater than 1.4 ± 0.057 cm
+ → Zone of inhibition within 1.0 ± 0.057 to 1.3± 0.57 cm
_ → No zone of inhibition

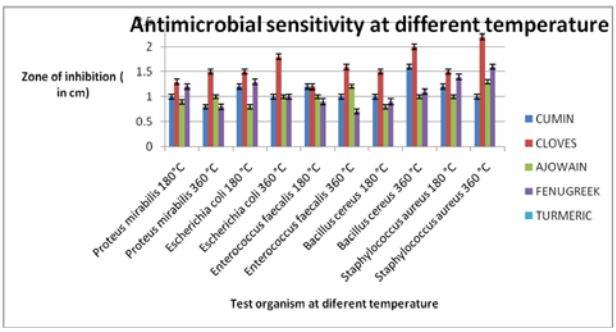
The aqueous infusion of Turmeric, Fenugreek, Red chilly shows more antibacterial activity than that of the ethanol extract, whereas Cloves, Cardamom, Star Anise showed the same result for the both extracts. Clove, Cardamom and Garlic showed considerable bactericidal property against the tested organisms.

Table 4. Effect of two temperatures on the antimicrobial activity of spices

Test organism	Diameter of zone of inhibition (in cm)				
	Cumin	Clove	Ajowain	Fenugreek	Turmeric
Proteus mirabilis 180°C	1	1.3	0.9	1.2	0
Proteus mirabilis 360 °C	0.8	1.5	1	0.8	0
Escherichia coli 180 °C	1.2	1.5	0.8	1.3	0
Escherichia coli 360 °C	1	1.8	1	1	0
Enterococcus faecalis 180 °C	1.2	1.2	1	0.9	0
Enterococcus faecalis 360 °C	1	1.6	1.2	0.7	0
Bacillus cereus 180 °C	1	1.5	0.8	0.9	0
Bacillus cereus 360 °C	1.6	2	1	1.1	0
Staphylococcus aureus 180 °C	1.2	1.5	1	1.4	0
Staphylococcus aureus 360 °C	1	2.2	1.3	1.6	0

The antimicrobial activity of Cumin and Fenugreek decreases with high temperature, but interestingly the antibacterial proerty of Clove and Ajowain increase with higher temperature. Whereas Turmeric did not show any antibacterial activity against the test orgnasim either at 180°C or at 360°C.

Fig: Antimicrobial sensitivity at different temperature



Conclusion

The success story of natural preservative lies in the continuous search for new ones to counter the challenge posed by chemical preservatives. The investigation of certain indigenous plant material like spices for their antimicrobial properties may yield useful results**.** Clove, Cumin, Fenugreek, Turmeric, Cardamom, Garlic and Star anise can act as a good natural antimicrobial agent, which can be used both for chemotherapy and as food preservative. The antimicrobial compound of Cloves and Ajowain remains active even at 360 °C, and thus it has an application as food preservative for specific food products including the grilled and deeep fried food products.

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