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Effects on Anti-Microbial Activity of Vathal Kulambu Dry Spice Mix on Food Borne Pathogens

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A B S T R A C T

Spices are rich source of phytochemicals having specific health benefits. Spices are used individually or in combination as food adjuncts to impart flavor, colour and aroma. Traditional knowledge existing in countries like India has shown the medicinal properties of many spices for treating wounds, cough, cold and fever, hyperglycemic and hyperlipidemic conditions. Some of the important constituents of spices which are shown to possess medicinal value include curcumin from turmeric, capsaicin from red pepper, piperine from black pepper, eugenol from cloves, allyl sulfides from garlic and onion. These compounds are shown to possess antioxidant, anti-inflammatory, antimicrobial, hypolipidemic and anti-lithogenic activities and anti-cancer properties. In this study was under taken to standardize the dry spice mix of Chettinadu ethnic cuisine *Vathalkulambu* dry spice mix were used in different packaging materials such as P1 – Low density poly ethylene, P2 – High density poly ethylene, P3 – Poly propylene and P4 – Aluminium foil are used.

Introduction

Dinesh *et al.,* (2011) studied that 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. 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.

Antibiotic toxicity and multi drug resistant pathogens are the two greatest challenges being faced by today's medical world. The antimicrobial activity of spices has been investigated as an alternative to antibioticsin order to tackle these dangers. In search of bioactive compound, methanol and acetone extract of 5 Indian spices were screened for antibacterial property. The choice of spice as an alternative is based on two basic reasons: firstly, plants have been the model source of medicine since ancient times and secondly, the increasing acceptance of herbal medicines by general population methanolic and acetone extracts were used to determine antibacterial properties of the spices. The antibacterial activity of five common Indian spices namely clove, ajwain, turmeric, dalchini and black pepper against two bacteria *Klebsiella pneumoniae* and *Staphylococcus aureus.*

The results revealed that the methanol extracts of spices (MIC values of 20- 100 µl/ml) have high antimicrobial activities on all test organisms (range of inhibition, 6- 16 mm) as compare to acetone extracts of spices in same concentration. These spices contain high amount of secondary metabolites due to these metabolites they have high antimicrobial activity and it can be used as good bio-preservater and it can also use for medicinal purpose (Bhawana, Shabina, Sheetal, 2014).

Kumar *et al.*, (2007) studied the antimicrobial activity of black pepper (*Piper nigrum*) and Turmeric (*Curcuma longa*) extracts. A total of six extracts of these two spices in three solvents were evaluated for their antibacterial and antifungal activity. The antibacterial activity was measured by Agarwell Diffusion Method and antifungal activity by poisoned food technique.

Materials and Methods

Bay leaf, coriander seed, dry chilli, turmeric powder, cardamom, garlic, cinnamon, clove, fenugreek, fennel, star aniseed, salt, sundaikaivathal, mustard, cumin, asafoetida and tamarind were purchased from the local market. These items were purchased in bulk properly cleaned and kept in an air tight container at ambient temperature till the day of use.

Test microorganism

Four pathogenic bacteria *Listeria monocytogenes* MTCC1143, *Staphylococcus aureus* MTCC 1144, *Bacillus cereus* MTCC 1272 and *Escherichia coli* MTCC 2622 were obtained from Microbial Type Culture Collection, Chandigarh.

The test bacteria were cultured on nutrient agar at 28^{o}C for 24 h. The cultures were subcultured regularly (every 30 days) and stored at 4^{o}C.

Inoculums preparation

Ten millilitre of distilled water was taken into the screw cap tube and pure colony of freshly cultured pathogenic bacteria was added into the tube and mixed. The OD (optical density) was measured with the colorimeter and microbial population was confirmed to be within 10^{7} ml^{-1} to 10^{8} ml^{-1}. This suspension is used as inoculum.

Preparation of spice extract

The four spice mix were cleaned with deionized water and dried in oven at 70^{o}C for about 24 h. Twenty gram of each spice mix was weighed and transferred into separate 100 ml conical flasks. Then 40 ml of ethanol and 40 ml of acetone was added. The conical flasks were closed with aluminium foil paper and put on orbital shaker for four days (120 rpm room temp). The crude ethanol and acetone extracts were filtered by passing the extracts through Whatman no. 1 filter paper and then concentrated. The residual extracts were stored in refrigerator at 4^{o}C for further studies.

Agar well diffusion method

The antibacterial activity of all the four spice mix extracts against the four pathogenic bacteria *Listeria monocytogenes* MTCC1143, *Staphylococcus aureus* MTCC 1144, *Bacillus cereus* MTCC 1272 and *Escherichia coli* MTCC 2622 were evaluated by using agar well diffusion method (Ahmed and Beg, 2001). Nutrient agar plates were inoculated by spread plate method with 1 ml of pathogenic bacteria (10^{7} to 10^{8} ml^{-1}). Well of 8 mm diameter were made with sterile borer into agar plates containing the test bacterial inoculum. 50 µl of the spice extract was poured into the well of the each inoculated plate. Sterile distilled water or solvent (ethanol) was used as control which was introduced into a well instead of spice extract. The plates were incubated at 28^{o}C for 2 days and observed for the zone of inhibition around the well. The zone of inhibition was measured in mm and expressed '+' for presence of inhibition '-' for no inhibition. The antimicrobial activity of the dry spice mixes were analysed at the initial and final day of storage by agar well diffusion method described by Ahmed and Beg, (2011).

Results and Discussion

Antimicrobial activity of Vathalkulambu mix

The antimicrobial activity of traditional spice mixes against four important food borne pathogens, such as *Listeria monocytogenes* MTCC1143, *Staphylococcus aureus* MTCC 1144, *Bacillus cereus* MTCC 1272 and *Escherichia coli* MTCC 2622 were evaluated. The antimicrobial activity of *vathalkulambu mix* extract before and after storage was presented in Table1.

Plate.1 Preparation of spice mix extract for well diffusion method

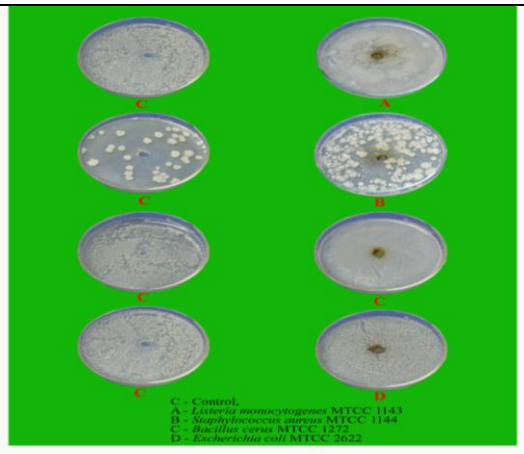

Plate.2 Antimicrobial activity of Vathalkulambu mix against human pathogens

Table.1 Antimicrobial activity of vathalkulambu mix on food borne pathogens

S. No	Culture name	Vathalkulambu mix							
		Initial				Final			
		P_1	P_2	P_3	P_4	P_1	P_2	P_3	P_4
1	*Listeria monocytogenes* MTCC 1143	+	+	+	+	-	-	-	-
2	*Staphylococcus aureus* MTCC 1144	-	-	-	-	-	-	-	-
3	*Bacillus cereus* MTCC 1272	++	++	++	++	-	-	-	-
4	*Escherichia coli* MTCC 2622	++	++	++	++	-	-	-	-
Medium: Nutrient agar **Method : Well diffusion method**						++ : zone of inhibition > 12 mm + : zone of inhibition ≤ 12 mm - : no inhibition			

P_1 : Low density Poly Ethylene 200 gauage
P_2 :High density Poly Ethylene 400 gauage
P_3: Poly Propylene 200 gauage
P_4 : Aluminium foil 200 gauage

Vathalkulambu extract before storage inhibited the growth of all the pathogens except *Staphylococcus aureus* MTCC 1144. The inhibition zone was above 12 mm in case of *Bacillus cereus* MTCC 1272 and *Escherichia coli* MTCC2622, as where the spice extract resulted in less inhibition against *Listeria monocytogenes* MTCC1143. Usha *et al.,* (2002) observed similar inhibition results when crude ethanol extract of cinnamon was tested for antimicrobial activity.

Mukhtar and Ghori (2012) resulted show that similar the antibacterial activity of garlic, cinnamon, turmeric 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 17mmagainst *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.*

Vathalkulambu extract inhibited the growth of all the pathogens except *Staphylococcus aureus* MTCC 1144 only at the in initial day of storage.

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