

The Comparative Study of Antimicrobial Activity of Four Different Spices Formulations Using their Essential Oil and Ethanolic Extract

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Abstract:

The study was conducted to determine the antibacterial and antifungal activity of four different spices formulations each having twelve spices cinnamon (*Cinnamomum verum*), black pepper (*Piper nigrum*), ginger (*Zingiber officinale*), clove (*Syzygium aromaticum*), cumin (*Cuminum cyminum*), mustard, coriander (*Coriandrum sativum*), nutmeg (*Myristica fragrans*), mace (*Myristica fragrans*), turmeric (*Curcuma longa*), bay leaf (*Laurus nobilis*) and cardamom (*Amomum krervanh*). The essential oil and ethanolic extract of all the spices mixtures were used against the food borne pathogens including three bacteria *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis* and three fungi namely *Aspergillus niger*, *Penicillium digitatum*, *Fusarium oxysporium* using agar disc diffusion method. The observed result showed that the spices mixtures ethanolic extract has more potential against all the tested microorganism where as spices mixtures essential oil showed only activity against tested bacterial strains but no activity against the fungal strains expect spices mixture D which exhibit antifungal activity.

Keywords: Antimicrobial activity, Preservative, Spice, shelf life, inhibition, micro-organism.

1 Introduction:

Antimicrobial activity is the activity of chemical, natural compound from plants or medicine to inhibit the growth of microorganism that causes diseases or spoil the food. The chemical, natural compounds or medicines which kill the microbes are known as antimicrobial agent.

Food is spoil when its nutritious properties, texture and flavour has been changed due to microorganism or microbial growth. So the shelf life of food can be increase by controlling or inhibiting growth of microbes, some preservative (which could be chemicals or synthetic additives) used and they have toxic effect on health [1].

Today, people is more conscience about their health and well aware about their food either fresh or preserved food (processed food) [2]. Now the interest of consumer towards bio preservative or natural antimicrobial substance is increasing day by day as this food is free from harmful effect.

Biopreservatives or natural antimicrobial substance consist of natural compound from plants to reduced the microbes growth and prevent the degradation of food [3]. Among plants, spices have antioxidant, medicinal and preservative properties. Up till now the main use of spice is to enhance the flavor of food instead of increasing the shelf life [5] but several studies were done to determine the antimicrobial activity of various spices against a number of microbes and the obtained result showed these microbes possess different activity against the tested spices [6]. The different chemical compounds (sulfides, aldehydes, thiols, esters, phenols and alcohols) present in spices are responsible of their antimicrobial activity. The alcoholic compound possessed greater antimicrobial property whereas aldehyde, ether and ketones have the moderate activity and the hydrocarbon compounds showed low activity against microbes [4].

The different extracts of some spices has been studied against various [7, 8, 9] microorganism but there is currently no report is available to determine the synergistic effects of different combination of spices against food-spoiler microbes [10].

Present study was an attempt to contribute it as alternative product for food preservation. In order to determine the potential (antimicrobial activity) of four different spices mixtures A, B, C and D (each mixture contain twelve spice namely cinnamon, black pepper, ginger, clove, cumin, mustard, coriander, nutmeg, mace, turmeric, bay leaf and cardamom) against the selected food borne pathogens. The concentrations of all the spices remain same in the four mixtures except the cinnamon, black pepper, ginger and clove as they were used in high concentration in spice mixture A, B, C and D respectively.

2 Material & method:

Collection of Spices:

The fresh ginger (*Zingiber officinale*) and all the remaining spices cinnamon (*Cinnamomum verum*), black pepper (*Piper nigrum*), clove (*Syzygium aromaticum*), cumin (*Cuminum cyminum*), mustard, coriander (*Coriandrum sativum*), nutmeg (*Myristica fragrans*), mace (*Myristica fragrans*), turmeric (*Curcuma longa*), bay leaf (*Laurus nobilis*) and cardamom (*Amomum krervanh*) in dry form were collected from local market of Lahore.

Preliminary analysis:

The spices were sun- dry for 6 hour after removing the contamination by visual checking. The electrical grinder were used for making the fine power of each spices than sieved and stored in air-tight plastic jar at room temperature till required analysis. The fresh ginger outer skin was

peeled off and thin slices of ginger was made with knife after washing by tape water and dried it for 24 hr in cabinet drier at 65⁰C.

Antibacterial and antifungal activity of spices mixtures:

The agar disc diffusion method of Baydar *et al* was followed for the determination of antibacterial and antifungal activity of ethanolic extract and essential oil of spices mixtures [11] against food borne pathogens, three bacteria (*Salmonella typhi*, *Escherichia coli*, *Bacillus subtilus*) and three fungi (*Aspergillus niger*, *Penicillium digitatum*, *Fusarium oxysporium*). The selected microbial strains pure cultures were obtained from the Department of *Food and Biotechnology Research Centre of Pakistan Council of Scientific and Industrial Research Centre (PCSIR)* Lahore.

Extraction of Essential oil & Ethanolic extract of spices mixtures:

The spices mixtures oil was extracted by soxhlet following AOAC method [12] using n-hexane as an extracting solvent for 4-5 hours. The oil was stored in glass vial at 4⁰C in refrigerator until for further analysis after extraction.

The 95% ethanol was used for the preparation of spices mixtures ethanolic extract [13]. After soaking the 20g of spices mixture in ethanol at 24⁰C for 48 hours, then centrifugated & filter through filter paper (whatt man). The extract was concentrated on vacuum rotatory evaporator.

Preparation of the medium and inoculation:

The growth medium (nutrient agar for bacteria and potato dextrose agar for fungi) were prepared, autoclaved and solidify aseptically to sterilized petri plates, then test tube slants having microbial culture were separately transferred to their respective media petri plates.

The sterile and dried 4mm paper disc (Difco USA) were impregnated on microbial plates with 10µl newly extracted essential oil and ethanolic extract of spices mixtures and the four discs in each plate then they were incubated at 25 °C and 37 °C for bacteria and fungi respectively for a period 24 to 48 hrs. The presence of clear zones indicated that the oil or extract possess inhibition activity and was measured in millimeter (mm) with the help of zone reader. The result of antimicrobial activity of spice mixtures were express as high, moderate, low and no active against tested microbes.

3 Results:

3.1 Antimicrobial activity of Essential oils and Ethanolic extract of Spices Mixtures (A, B, C, D):

The result of antibacterial and antifungal activity of essential oil and ethanolic extract of spices mixtures were represented in Table 1.

Table 1 Antimicrobial activity of Essential oils of Spices Mixtures (A, B, C, D)

Test microorganism	Essential oil of spices mixtures				Ethanolic extract of spices mixtures			
	A	B	C	D	A	B	C	D
<i>Salmonella typhi</i>	+++	+	++	+	++	++	++	++

<i>Escherichia coli</i>	+++	+	++	++	+++	+++	+++	+++
<i>Bacillus subtilis</i>	+++	++	+++	+++	-	+	++	++
<i>Aspergillus niger</i>	-	-	-	+	++	++	++	+++
<i>Penicillium digitatum</i>	-	-	-	+	++	-	-	+
<i>Fusarium oxysporium</i>	-	-	-	+	+++	+	+++	+

+++ High active (14mm)

+ low active (8-6mm)

++ Moderate active (12-10mm)

- no active (5mm)

Spices mixtures A: The essential oil of spices mixture A showed high activity (14mm) against all the tested bacterial strains where as ethanolic extract was found to be highly active against only *Escherichia coli*, in case of *Salmonella typhi* its activity is moderate (12mm) while it was not active against the *Bacillus subtilis*. Among the tested fungal strains the essential oil was not active whereas ethanolic extract was found to be highly active (14mm) against *Fusarium oxysporium* and towards *Aspergillus niger* and *Penicillium digitatum* its activity is moderate (12mm).

Spices mixture B: The essential oil of spices mixture B exhibit moderate activity (11mm) against *Bacillus subtilis* and towards *Salmonella typhi* and *Escherichia coli* its activity was low where as ethanolic extract was found to be highly active, moderate active and low active against *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis* respectively. Among all the tested fungal strains the essential oil was not exhibit activity but ethanolic extract was highly active (14mm) against *Fusarium oxysporium* and moderate active (12mm) against *Aspergillus niger* while showed no activity against *Penicillium digitatum*.

Spices mixtures C: The essential oil of spices mixture C was found to be highly active against *Bacillus subtilis* and moderate active (12mm) against *Salmonella typhi* and *Escherichia coli* whereas the ethanolic extract was highly active against *Escherichia coli* and showed moderate activity against (12mm) against *Salmonella typhi* and *Bacillus subtilis*. The essential oil of spices mixture A was not active against the fungal strains *Aspergillus niger*, *Penicillium digitatum* and *Fusarium oxysporium* but the ethanolic extract was found to be highly active (14mm) against *Fusarium oxysporium* and moderate active (12mm) against *Aspergillus niger* while showed no activity against *Penicillium digitatum*.

Spices mixtures D: The essential oil of spices mixture D showed high (14mm), moderate (12mm) and low activity (8mm) against *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* respectively where as the ethanolic extract was highly active (14mm) against *Escherichia coli* and exhibit moderate activity (12mm) against *Salmonella typhi* and *Bacillus subtilis*. The essential oil of spices mixture D exhibits low activity against all the tested fungal strains while the ethanolic extract was found to be highly active (14mm) against *Aspergillus niger* and low active (8mm) against *Penicillium digitatum* and *Fusarium oxysporium*.

4 Discussion:

Antimicrobial activity of Essential oils of Spices Mixture (A, B, C, D):

The four spices mixtures essential oil showed different activity against all the tested microbes as the spices mixtures A, B, C and D had increase ratio of cinnamon, black pepper, ginger and clove respectively, antimicrobial activity exhibit by the essential oil is directly correlated with the components that it contains [14].

The oil of spices mixtures A exhibit activity against bacteria but not active against fungal strains because it contain antibacterial compound cinnamaldehyde (cinnamon). This compound may reach the internal organelles of cell by rupturing the membrane and destroyed enzyme system of bacteria, causing death [15].

The mixture B oils showed antibacterial activity due to combine effect of spices because the finding of previous studies [16] shows that black pepper oil does not exhibit antimicrobial activity.

The spice mixture C oil showed antibacterial activity as ginger contain active antibacterial compounds (gingerol, shagelol and flavonods) and they degrade the enzymes system involving the production of energy in bacterial cell [17, 18].

The essential oil of mixture D showed antimicrobial activity as clove contain eugenol antimicrobial compound, it either destroyed membrane or inhibit enzyme activities and may lost genetic material [19].

4.2 Antimicrobial activity of Ethanolic Extracts of Spices Mixture (A, B, C, D):

The ethanolic extract of all spices mixtures exhibit activity against all the tested bacterial and fungal strains as the ethanolic extract has maximum concentration of compounds tannins, terpenoids, alkaloids and flavonoids and these compounds showed activity against bacterial and fungal strains by rupturing plasma membrane of microbes or by degrading the properties of lipid fraction. The essential molecule as well as ions losted from the ruptured plasma membrane and microbes died [20].

Our results showed similarities with the previous studies [16] that the inhibitory activity of spices mixtures oil was less than that of its extracts (ethanolic).

Conculsion:

The finding of this studied revealed that the ethanolic extract of spices mixtures has more antimicrobial potential against food borne pathogens in comparation to essential oil as it was effective against only tested bacterial strains and not exhibit the fungal activity but only spices mixture D possess antifungal activity, this variation in results may depend on the active constituents of spices solubility. So spices combination could be used as alternative to preservative either chemical or synthetic additives for the processing of food in addition to taste and flavor enhancer.

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