

## ANTIMICROBIAL ACTIVITY OF SPICES AGAINST ISOLATED FOOD BORNE PATHOGENS

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## ABSTRACT

Antimicrobial activity of spices with methanolic extract of *Zingiber officinale* (Ginger), *Allium sativum* (Garlic), *Syzygium aromaticum* (Clove), *Cuminum cyminum* (Cumin), *Brassica juncea* (Mustard), *Emblica officinalis* (Amla), *Aloe vera* and *Crocus sativus* (Saffron) has been evaluated against isolated food borne pathogens *E.coli*, *Bacillus subtilis*, *Pseudomonas florescens*, *Serratia marscens*, *Citrobacter freundii*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Proteus vulgaris*. They were tested by agar dilution method for determining the minimum inhibitory concentration (MIC) of spices extracts. *Syzygium aromaticum* (Clove) and *Cuminum cyminum* (Cumin) extract showed excellent antimicrobial activity against all the test organisms. *Syzygium aromaticum* showed the highest 19 mm antimicrobial zone against all except *Serratia marscens* and *Proteus vulgaris*. Clove extract was the most inhibitor followed by Cumin, whereas extracts of ginger, garlic mustard, amla aloe vera and saunf showed weak antibacterial activities against the tested strains. The most sensitive strain to spices extracts was *Citrobacter freundii* and the most resistant strain was *Proteus vulgaris*.

**Keywords:** Antimicrobial activity, Spices, Methanolic extracts, Food borne pathogens, Agar dilution method

## INTRODUCTION

Food borne disease is an increasingly serious public health problem all over the world. The main cause is determined to be microorganisms. The control of pathogens may significantly reduce the food borne disease outbreaks [1]. A considerable weight of evidence has been gathered suggesting that consumption of fruit and vegetables is beneficial for human health and may help in the prevention of chronic diseases [2], because they contain phenolic compounds [3]. Likewise, some natural substances have effective antimicrobial properties where they have been used as seasonings for centuries [4,5].

Spices and aromatic vegetable materials have long been used in food not only for their flavour and fragrance qualities and appetizing effects but also for their preservative and medicinal properties. Since the ancient times, they have been used for preventing food spoilage and deterioration and also for extending the shelf life of foods [6]. It has been extensively reported that the essential oils of spices have shown antimicrobial functions against food borne pathogens [7]. In addition, they show other beneficial biological properties, such as antimicrobial and antioxidant activities [8,9,10].

Interest in the antimicrobial properties of active compounds is strengthened by the findings that they affect the behaviour of pathogenic bacteria or fungi of agro-food or medical field. Indeed, their use as natural additives in food industry is increased in recent years [11]. Antibacterial agents, including food preservatives and organic acids, have been used to inhibit food borne bacteria and extend shelf life of processed foods. Many naturally occurring compounds found in edible and medicinal plants, herbs and spices have been shown to possess antimicrobial function and could serve as a source of antimicrobial agents against food pathogens [12].

In the present study, we have evaluated the antibacterial effect of the extracts of two widely used spices in India, such as *Zingiber officinale* (Ginger), *Allium sativum* (Garlic), *Syzygium aromaticum* (Clove), *Cuminum cyminum* (Cumin), *Brassica juncea* (Mustard), *Emblica officinalis* (Amla), *Aloe vera* and *Crocus sativus* (Saffron) against bacterial food borne pathogens such as *E.coli*, *Bacillus subtilis*, *Pseudomonas florescens*, *Serratia marscens*, *Citrobacter freundii*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Proteus vulgaris* and the results are discussed.

## MATERIALS AND METHODS

## Sample Collection

The spoiled food samples were collected from the local markets of Jaipur, Rajasthan.

## Bacteriological Analysis

Bacteriological analysis was done by selective media method by Sherman, 2005 [13].

## Morphological Characterization

The isolated microbes were characterized on the basis of simple staining and gram staining [13].

## Biochemical Characterization

The isolates were characterized by biochemical tests viz. IMViC reactions i.e. Indole test, Methyl red test, Voges proskauer test and Citrate utilization test, Nitrate reduction test, Lactose, Sucrose, Dextrose fermentation Reaction test by standard method given by Sherman, 2005 [13].

## Preparation of Spices Extracts

The fresh spices were obtained from the local market. The spices were cleaned, descaled when necessary and washed in sterile distilled water. In order to obtain spice's extracts, about 100g of washed spice were crushed with mortar and pestle. The extracts were sieved through a fine mesh cloth. This extract was considered as the 100% concentration of the extract.

## Antimicrobial Activity

The in vitro screening for antimicrobial study was carried out using selected pathogens which includes gram negative bacteria and gram positive bacteria. The antibacterial screening of the extracts were carried out by determining the zone of inhibition using well diffusion method. The strains of microorganisms obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37° C for 24 hr and were referred to as seeded broth. The extracts were prepared by reconstituting with methanol. The test microorganisms were seeded into respective medium by spread plate method 10 µl (10 cells/ml) with the 24 hr cultures of bacteria growth in nutrient broth.

One ml of this was used in flooding over nutrient agar plates in the well diffusion method of the in vitro antimicrobial sensitivity test. The plates were left for 5 mins after which they were dried at 37° C for 1 hr. Wells were bored in the plate using a sterile cork borer. One hundred microlitres of the various spices' extract were put inside the wells. Methanol were put inside the well in separate petriplates to serve as negative control while Chloramphenicol (1mg/ml) was used as positive control in the separate petriplates. The plates were left free for 1 hour after which there were incubated at 37° C for 24 hrs and were examined for zones of inhibition. The diameter of

inhibition zones were measured in mm and the results were recorded. Inhibition zones with diameter less than 12mm were considered as having no antimicrobial activity. Diameters between 12 and 16mm were considered moderately active and with >16mm were considered highly active.

#### Determination of minimal inhibitory concentration (MIC)

Agar dilution method with slight modifications was used to determine the MICs of spice extracts. Equal volumes of each bacterial strain culture containing approximately  $10^5$  CFU/ml, were applied onto nutrient broth supplemented with spice methanol extracts with concentrations ranged from 1 to 100 mg/L in tubes. Cultures were then incubated at 37 °C for 24 hr, subsequently, 100 µl of each culture was inoculated on nutrient agar and further incubated at 37 °C for 24 h. MIC was defined as the lowest concentration of spice extract that completely suppressed colony growth.

#### RESULTS AND DISCUSSION

Bacteria can cause fruits and vegetables to get mushy or slimy, or meat to develop a bad odor. There are different spoilage bacteria which grow well at room temperature. The large number of microorganisms and their waste products cause the objectionable changes in odour, taste and texture. In the present study different samples of spoiled food were collected from the market of Jaipur.

#### Morphological Characterization

The twenty isolates were recovered from the ten samples of the various categories of food. The recovered isolate were characterized on the basis of colour, shape, texture, margin, arrangement and staining characteristics.

Twenty isolates were characterized on the basis of colony morphology and the staining characteristics.

#### Biochemical Characterization

The twenty isolates were characterized on the basis of biochemical tests. The tests performed to characterize the isolates were Indole, MR, VP, citrate utilization, nitrate reduction, fructose and sucrose and dextrose fermentation [13].

In the present study, the spoiled samples of various categories of food such as fruits, vegetables, and bread were collected from the local market of Jaipur. The twenty isolates were characterized on the basis of biochemical identification [13]. The result obtained from the data shows that the bacteria found in spoiled samples was *Bacillus*,

*Klebsiella*, *Pseudomonas*, *E.coli*, *Serratia*, *Citrobacter*, *Proteus* and *Staphylococcus*. *Klebsiella pneumonia* is a potent enteroinvasive food borne pathogen and causes serious illness [14].

#### Antimicrobial Activity

In the present study, the antimicrobial activities of eight spices extracts (methanol extracts) were examined against eight food borne bacterial strains. Results obtained by agar well diffusion technique, as a qualitative method, are summarized in table (1). Most of the examined spices showed varied inhibitory activity against them tested food borne strains. The methanolic solutions of the extracts were found to have potent antimicrobial activity against all the Gram-positive and Gram-negative organisms tested. Clove extract proved to be the most inhibitor against all of tested bacteria. Results obtained for clove extract in the present study were found to be similar as also reported by several workers [15,16]. Indeed, clove oil is traditionally used in the treatment of oral candidiasis and athlete's feet. The extracts of mustard and cumin extracts exhibited notable antibacterial activities toward all examined bacteria with different potentialities. On the other hand, the spices extracts of garlic, ginger, amla, aloe vera and saffron showed weak antibacterial activities against most of the tested strains.

The minimal inhibitory concentrations for spices extracts against examined bacterial strains are presented in table (2); the obtained MICs of plant extracts toward food borne pathogens are in harmony with data obtained from table (1). The lowest MIC which could inhibit microbial growth was recorded for clove and cumin. Methanol extracts of other spices exhibited marked antimicrobial potentialities by determining their MICs against tested bacteria. From the microbial sensitivity side of view, *C.frendii* and *K. pneumoniae* was the most sensitive bacterium to the examined plant extracts, whereas *Pr. vulgaris* proved to be the most resistant among the tested food borne strains.

GC-MS analysis of the oil extract showed eugenol, eugenol acetate, caryo-phyllene as the major constituent. Eugenol and caryo-phyllene are known to possess antibacterial and antifungal properties. Hence the antibacterial and anti-fungal properties demonstrated by the clove oil extract can be attributed to the compounds identified [17]. The antibacterial activity of clove is attributed to eugenol (2-methoxy-4-allyl phenol). Clove bud oil contains high eugenol (70-90%) content [18]. This compound is an antimicrobial compound having wide spectra of antimicrobial effect [19,20] which may contribute to growth inhibition of enterobacteria. High tannin content (10-19%) in clove provides additional antimicrobial activity [21]. Similar findings have been reported by other researchers [22,23].

**Table 1: Antimicrobial Activity of the Tested Spices Extracts Against Food Borne Bacteria Measured as the Diameter of Growth Inhibition Zones (mm)\***

BACT	<i>E.Coli</i>	<i>B. subtilis</i>	<i>Ps.florescens</i>	<i>S.marscens</i>	<i>C.frendii</i>	<i>K.pneumo</i>	<i>S.aureus</i>	<i>Pr.vulgaris</i>
Ginger	11	8	11	10	10	17	8	8
Garlic	12	9	14	11	15	18	10	9
Amla	7.3	12	14	16	19	15.8	18.3	12
Musturd	14	12	14	12	19	14	14	14
Cumin	19	19	18.5	16.5	17	18	18	10
Clove	19	19	19	17	19	17	19	17
Aloe vera	3.4	2.7	1.8	3.9	1.2	2.9	1.4	0
Saffron	0.9	0.8	1.1	0.8	1.1	1.2	1	1
PC	12	14	11	14	14	12	17	12
NC	0	0	0	0	0	0	0	0

**Table 2: Minimum Inhibitory Concentration (MIC) of Spices Methanol Extracts (mg/L) Against Food Borne Strains.**

Plant used	<i>E.coli</i>	<i>B. Subtilis</i>	<i>Ps.fluorescens</i>	<i>S.marscens</i>	<i>C.frendii</i>	<i>K.pneumo</i>	<i>S.aureus</i>	<i>Pr.vulgaris</i>
Ginger	65	60	50	55	55	65	65	85
Garlic	55	35	>100	>100	85	>100	80	80
Amla	50	40	75	65	70	70	70	80
Mustard	75	70	100	90	65	75	85	60
Cumin	75	85	50	75	50	60	80	75
Cloves	95	75	80	90	80	80	65	65
Aloe vera	95	65	100	>100	80	>100	70	65
Saffron	75	60	65	100	70	>100	65	>100

## CONCLUSION

In conclusion, the degree of antibacterial property of spices tested can be put in the following order: clove > cumin > amla > mustard > ginger > garlic > aloe vera > saffron. These spices may be selected for use as potentially useful anti-microbial agents in fermented products and other foods, depending upon the desired flavor of the products. The oil fraction of these spices is recommended, with the exception of holy basil which should be used in the form of methanolic extract. However, there are some limitations in using spices, such as 1) the decreasing of antimicrobial activity when spices are added to food materials containing protein, carbohydrate, and fat, and 2) the strong flavour of some spices. The overall flavour of the products may not be acceptable if a large amount of spices need to be added in the products in order to inhibit the pathogenic bacteria. A possible way is to use spices in combination with other preservatives such as acid, salt, sugar, and other chemical preservatives, or other food preservation systems such as thermal processing, freezing, cold storage, etc [19,24].

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