
Antimicrobial Activity O Indian Kitchen Spices Against Common Foodborne Pathogens

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ABSTRACT

The medicinal value of the spices used in Indian cooking has been known for centuries. The aim of this study was to evaluate the bactericidal activity of spices used in Indian kitchen on common enteropathogens endemic to our country. Aqueous extracts of mustard, clove, black pepper, cumin were used in the study. Their antimicrobial activity against diarrhoeagenic bacteria, Escherichia coli, staphylococcus were investigated by the disc diffusion and growth inhibition methods. Mustard and black pepper and clove showed antimicrobial effect against both the bacteria selected and the remaining cumin extract showed different degree of bactericidal activity. Spices, thus not only add flavor to the Indian cooking, but they may also protect us from various gastro-intestinal endemic diseases

INTRODUCTION

In Indian system traditionally the people have a longstanding practice of using extensive diversity of plant products in treatment of diseases. Spices are essential components of Indian cuisines and also in medication since ancient times⁵. Indian spices have made a significant contribution both in the health care system and the food industry. Ancient Asian literature is a treasure of information related to the problems of health care and other environmental aspects. Indian spices have been used since ages in different traditional

forms of medicine like Ayurveda, Unani and Sino-Tibetan systems⁴. The Vedic Literature (2500b.c) is the main source of the information that contributes to the development of Ayurveda. Particularly in Ayurveda, spices contributed a major amount for the treatment of key disorders of the body. Homeopathic medicine has been using spices as one of their chief ingredients in most of their preparations. In ancient India, natural herbs and spices were consumed either in food or used as medicine in order to maintain proper sanitation, health and hygiene and increase longevity of life⁴.

India on account of its wide and rich flora is considered as the source of nearly three-fourth of plant derived drugs of the world. It is estimated that even in this modern age, 80% of the medical care is provided by home care and traditional native systems of medicine and a major part of these therapies involve the use of spices, plant extracts¹.

India is one of the largest producer, consumer and exporter of spices⁴. A number of plant products are routinely used as spices and condiments in Indian kitchen which are proved to show antimicrobial activity against common food borne pathogens. Spices are also used for stabilizing several food items from deterioration of food. Spices are considered as rich source of bio-active antimicrobial compounds. Spices are used as substances that increase the taste and variation of food. The inhibitory effects of

spices are mostly due to volatile oils present in their composition⁷.

Spice:

A spice is a dried seed, fruit, root, bark, or vegetable substance primarily used for flavoring, coloring or preserving food³.

The main factors that determine the antimicrobial activity are the type and composition of the spice, amount used, type of microorganism, composition of food, pH value, temperature of the environment and proteins, lipids, salts and phenolic substances present in the food environment⁷. The Indian typical spices are cumin, fenugreek clove, black pepper, turmeric, red pepper, garlic, etc.

These have a wide variety of chemical constituents and some of them have the ability to inhibit the growth of microorganisms. . Spices are used in very minute amounts impart flavor, taste and aroma in food preparations and also to improve their palatability. A large number of plants and spices are used to combat different types of diseases and possess antimicrobial activity. Spices increase the resistance capacity of cooked food thereby preventing food spoilage. As a result food can be retained fresh for longer duration of time.

Antimicrobial activity:

Antimicrobial activity is the ability of some active chemical constituents to eliminate microorganisms by inhibiting or suppressing their growth⁶.

Apart from Indian culinary, spices also contribute to the modern Allopathic system of health care by providing large number of medicines or parent compounds. Reports indicate that spices have dual type of action. Short-term effects include pain, heat, redness, and swelling. Long-term effects include anti

inflammation, analgesic, antimicrobial, antioxidant and anti mutagenic actions⁴. An interrelationship between the health-benefiting properties of spices and their use in food needs to be scientifically re-established. Contamination of food caused by unsanitary practices compromises the health of the people at various levels. Food safety hence becomes a key concern for food-processing industry. Food safety enjoys a very low priority at all levels. There is limited awareness of its importance of hygiene practices, several food preparation techniques, such as preservation in spices have been used for thousands of years⁴.

Food borne pathogens:

Food borne pathogens are the bacteria which cause food poisoning or food illness⁹. e.g. Bacillus cereus, Campylobacter jejune, Escherichia coli ect.

Food processors, Food safety researchers, and regulatory agencies have been increasingly concerned with the growing number of food-borne illness outbreaks caused by some pathogens².

The increasing antibiotic resistance of some pathogens that are associated with food borne illness is another concern. Therefore, there has been increasing interest in the development of new types of effective and nontoxic antimicrobial compounds. Although numerous studies have published on the antimicrobial activities of a spices against many different types of microbes, including food borne pathogens. A review of the earlier literature reveals that the results reported for these different studies are difficult to compare, presumably because of the different test methods, bacterial strains, and sources of antimicrobial samples used². Three main factors

can influence the results of a test of the antimicrobial activity of a plant or spice extract.

- The composition and solubility of the extract
- The microorganism
- The method of growing and enumerating the surviving bacteria²

A unit commonly used in the measurement of antimicrobial activity is the diameter of the zone of inhibition of bacterial growth on the solid medium. For extract samples the zone of inhibition will depend on the ability of the extract to diffuse uniformly through an agar medium and the effect on bacteria by vapors released from extracts. Other variables in tests of plant antimicrobial compounds include the presence of two or more active components that may interact antagonistically, additively, or synergistically at low concentrations; changes (resulting from the partitioning of active components between the lipid and aqueous phases) in the antimicrobial activity of extracts in complex test sample (e.g. Food) compared with the activity of extract alone and substances present in complex sample reaction mixtures that may stimulate or inhibit the growth of the test microorganisms independent of the test sample. It is important to standardize test methods and evaluate factors that influence the potencies of antimicrobial agents in order to enable comparisons of results obtained in different studies².

In an era characterized by increasing consumer choice, self-medication and quest for natural therapy, herbal products are used increasingly as an alternative to drugs and supplements. In particular extracts from many kinds of oriental spice plants are known to possess antimicrobial activity besides being used for the purpose of food preservation,

appetizer promotion and medicinal purposes. Today the exploration of naturally occurring anti-microbial for food preservation receives increasing attention⁴.

Experiments are in progress to isolate the antibacterial compounds from spices. Knowledge of antimicrobial activity of spices and condiments could prove very useful in the light of emerging drug resistance to present day antibiotics. A few works on the commonly used kitchen spices have confirmed and reported the traditional therapeutic uses and also their antimicrobial activity against enteropathogens¹.

Systematic study to look for viability of different bacterial enteropathogens exposed to routinely consumed kitchen spices and condiments was required. We therefore aimed to evaluate preliminarily the bactericidal activity of four routinely used spices namely cumin, black pepper, mustard, clove against *Staphylococcus*, and *Escherichia coli* bacterial strains which are main common food borne pathogens causing illness.

The following spices were purchased from local market. The names of spices are Black Pepper, Cumin, Clove, Mustard seeds.

Black Pepper

Synonyms:

Pepper, *Piper nigrum*, Maricha.

Biological source:

Pepper is the dried unripe fruit of perennial climbing vine *Piper Nigrum* linn, family Piperaceae. It contains not less than 2.5% of Piperine on dried basis.

Chemical constituents:

Pepper contains an alkaloid piperine (5-9%), volatile oil (1-2.5%), pungent resin (6.0%), piperidine and starch.

The volatile oil contains mainly l-phellandrene and caryophyllene.

Uses;

The fruits are used as aromatic, stomachic and carminative. It stimulates taste buds, with increase in gastric juice.

The oil is used as spice and reported to enhance the bioavailability of certain drugs.¹⁵

Cumin:

Synonyms:

Jira.

Biological source

It consists of dried ripe fruits of *Cuminum Cyminum* Linn, belonging to the family Umbelliferae.

Chemical constituents:

Cumin fruits contain (2.5-4%) volatile oil, 10% fixed oil and proteins. Volatile oil contains (30-50%) cuminaldehyde, small quantities of alpha-pinene, phellandrene, cuminic alcohol, hydrated cuminaldehyde and hydro-cuminine.

Uses:

It is used as stimulant, carminative and also in diarrhea and dyspepsia.¹⁵

Mustard:

Synonyms:

Black mustard, Mohair, Avalu.

Biological source:

Mustard are the dried small ripe seeds belonging to the family *Brassica nigra*, *Brassica juncea* (Indian mustard).

Chemical constituents:

It contains not less than 92% allyl isothiocyanate.

Uses:

Mustard is used as a food flavoring, for forage, as an emetic, and diuretic, as well as a topical treatment for inflammatory conditions such as arthritis and rheumatism.

Mustard also has potential pharmacological effects in cardiovascular disease, cancer, and diabetes.¹⁵

Clove:

Synonym:

Caryophyllum, Clove flower, Clove buds.

Biological source

Clove consists of dried flower buds of *Eugenia caryophyllus*, family Myrtaceae.

Chemical constituents:

It contains about 15-20 % volatile oil, 10% tannins, resin, chrome, and eugenin. The volatile contains eugenol, eugenol acetate, caryophyllenes and small quantities of esters, ketones and alcohols.

Uses:

Clove is used as dental analgesic, carminative, flavouring agent, an aromatic and antiseptic.

The common food borne pathogens used in the experiment were *Staphylococcus* and *Escherichia coli*.¹⁵

Staphylococcus

Staphylococcus aureus (or *Staph aureus*) is a type of bacteria commonly found on the skin & hair as well as in the noses and throats of people and animals. These bacteria are present in up to 25 percent of healthy people and are even more common among those with skin, eye, nose, throat infections. *Staphylococcus* can cause food poisoning when a food handler contaminates food and then the food is not properly refrigerated. Other sources of food contamination include the equipment and surfaces on which food is prepared

These bacteria multiply quickly at room temperature to produce a toxin that causes illness. *Staphylococcus* is killed by cooking and pasteurization.

Escherichia Coli:

E. coli is the name of a type of bacteria that lives in intestines. Although most types of E. coli are harmless, some can make you sick. The worst type of E. coli, known as E. coli O157:H7 causes bloody diarrhea and can sometimes cause kidney failure and even death. E. coli O157:H7 makes a toxin called Shiga toxin and is known as a Shiga toxin-producing E. coli (STEC). One severe complication associated with E. coli infection is hemolytic uremic syndrome (HUS). The infection produces toxic substances that destroy red blood cells.

REVIEW OF LITERATURE

A perusal of literature survey shows following works were already done of these spices throughout the world.

1. Chetana vaishnavi* Sukhminderjit kaur and Manpreet kaur , they have done preliminary studies on spices and confirmed the antibacterial activity of clove and highlights the bactericidal potential of various other species and condiments used in Indian kitchen. And as the food remains in stomach for three hours the effect of bactericidal activity of spices for this period was also studied¹
2. Mendel friedman * Philp R. Henika and Roberte. Mandrell they examined the antimicrobial activities of a variety of oils and their compounds against camphylobacter jejuni, escherichia coli, listeria monocytogens and salmonella enteric strains .and found that the antimicrobial compounds identified in study are most active against four major food borne pathogens are candidates for studying synergism, compatibility and activity in foods

and mechanisms of activity for specific pathogens.²

3. Sema Agaoglu*,Nursel Dosbil and Suleyman Alemdar they have done studies on the antimicrobial activity of some food additives used in meat products such as cumin, cinnamon, cloves, crushed red pepper, fennel and anise. Against some microorganisms was investigated and found that cinnamon was most effective spice and fennel was the spice with weakest antimicrobial activity. And crushed pepper and anise were ineffective against the test strains.⁷
4. Papachan karursofia*, Rajendra Prasad, Virendra kumar, Vijay and Ashok kumar srivastav they studied the antimicrobial activity of six Indian spices extracts, namely clove cinnamon, mustard, garlic, ginger and mint. Against the common food borne pathogens Escherichia coli and staphylococcus aureus and bacillus cereus and found that clove, cinnamon, mustard had good inhibitory action at 1% concentration while garlic showed minimum activity and ginger and mint showed negligible antibacterial activity.⁴
5. Andre silverio and Maria lopes* spices condiments and herbs used fresh or as extracts have a very much reported ability to inhibit some micro-organisms and their analysis of scientific literature shown that researchers must take care when comparing results because experimental standardization has not been achieved yet. The use of natural medicines and undeniably increased in western societies. The therapeutic results and the active molecules have a variability caused by seasonal

conditions, leading to variable biological activity.⁶

6. Das.S*,Anjeza,c. and Mandal,S.they performed disc diffusion and MIC bioassays with some selected Indian spices and herbs against some enteropathogens. And widest inhibition zones were seen in cases of aqueous extracts of fenugreek, mustard and henna. Combination of spices demonstrated synergistic or additive effect. Cumin and fenugreek mustard combinations demonstrated higher synergistic antimicrobial effects.⁵
7. Arora D., S., and Kaur,j . Antimicrobial activity of spices International Journal of antimicrobial agents they have proved that certain spices have antimicrobial activity increases the resistance of food against food spoilers.⁸
8. Cox,S*,abu-ghannam,n. and gupta, .An assessment of antioxidant and Antimicrobial activity of six spices was attempted using disc diffusion method and calculated the minimum inhibitory concentration of the spices against various enteropathogens¹⁰
9. K.fisher and C.A. Phillips. They have investigated the effectiveness of oils and vapors of citrus compounds ;like lemon, sweet orange and bergamot against a number of common food borne pathogens and proved that bergamot has highest capacity to combat the growth of common causes of food poisoning.⁹
10. Shelef,L.A. Anti microbial effects of spices was done by preliminary tests.¹¹

On the basis of literature survey it has been found that all most all of Indian

kitchen spices possessed antimicrobial activity. And the medicinal value of many spices and condiments used in Indian cooking has been known for centuries. Safety and high quality of food products are some of the attributes growingly demanded by consumers worldwide. Despite of technological advances, either by chemical preservatives or mechanical equipment for inactivation or inhibition of microorganism there is still a problem concerning food spoilage of biological origin.

MATERIALS & METHODS

The following spices were purchased from local market. The names of spices are Black Pepper, Cumin, Clove, Mustard seeds.



Black pepper



Cumin

wiseGEEK



Mustard

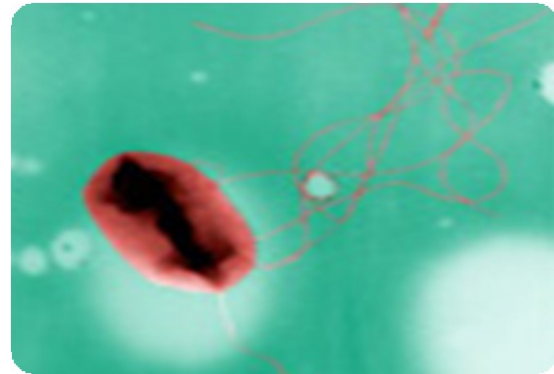


Clove

The common food borne pathogens used in the experiment were staphylococcus and Escherichia coli.



Staphylococcus Aureus



Escherichia coli

Preparation of spices extracts:

These above spices namely black pepper, clove, cumin mustard seed were bought from the local market of Nalgonda, Telangana. Care was taken to obtain clean and good quality products by visual and olfactory inspection. Then all the four spices were first cleaned using tap water in order to remove any dirt and later using sterile distilled water. After drying aqueous extracts were prepared by crushing coarsely 5gm each of the spices using mortar and pestle and boiling in adequate distilled water for 10 minutes till 25ml of extract was obtained all the extracts were then passed through Whatman filter paper No1. And then filtered through 0.45micrograms Millipore filter.¹

Nutrient agar medium:

- Agar
- Peptone
- Beef extract
- Sodium chloride
- Distilled water

Bacterial activity assay:

Two enteropathogenic, food spoilers bacterial strains were selected to assess antimicrobial activity of spices against those strains. The

bacteria used for this experimental work are the common food pathogens *Escherichia coli*, *Staphylococcus aureus*. The freeze-dried bacterial cultures were bought from Osmania University, Microbiology Department, Hyderabad.

And are then sub cultures are prepared in the laboratory in the respective nutrient broths. The bacterial activity of assays were carried out by two methods

- Disc diffusion method
- Growth inhibition method.

Paper Disc diffusion method (Kirby Bauer Technique):

Molten sterile nutrient agar (20ml) was poured over base plates in standard Petri plates and inoculated with 0.1ml of each pathogenic strain by spread plate technique. The various concentrations of spice extracts were applied in 8mm sterile filter paper disc (Whatman No 1, 5mm in diameter). The discs were placed on the surface of inoculated plates and allowed to dry in laminar flow biological safety cabinet. The sterile Petri plates were then incubated for 24 hours at 37 degrees centigrade, in an inverted position. The diameter of the zone of inhibition was recorded. Control assay discs impregnated with sterile distilled water, without spices, were used⁴.

Growth inhibition method:

Bacteria (10^8 /ml) (250 μ l) were mixed with 250 μ l extract (neat or differentially diluted in broth up to 8 times) and incubated at 37^oC. In control tubes the extract was replaced with broth. After 1 hour intervals for a period of 3 hours, a 20 μ l suspension was dispensed on

agar plate and incubated overnight at temperature and in suitable conditions. The tubes containing the lowest concentration of different extracts which showed no growth after inoculation into agar medium was regarded as minimum inhibitory concentration.¹

CONCLUSION

In conclusion the results of the present preliminary study further demonstrated that mustard, black pepper, clove, cumin possess varying degree of antimicrobial activity. These spices act through their natural inhibitory mechanisms by either inhibiting or killing of pathogens. completely and keep the intestine healthy by controlling overgrowth of commensal bacteria as food remain in the stomach for 3 hours so the study of this bactericidal activity was done for a 3 hour incubation period.

With increasing in awareness of people towards natural food and natural therapies spices might act as most obvious alternative. In India, where spices are produced and used as food additives, their use as antimicrobial agents and potential preservatives can be extremely useful.

However, in depth research is required to work up right doses of spices to be used in areas where a particular microorganism may be endemic.

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