Invitro Antibacterial Activity of four Indian Spices against Some Pathogenic Organisms

B.Umamaheswari & S.Rajalakshmi
Department of Microbiology, Idhaya College for women Kumbakonam. Guided by: Miss. B.Umamaheswari, M.Sc., M.Phil,-lecturer in microbiology, Idhaya College for women

ABSTRACT

In the present study the Indian spices extracts of *Piper nigrum, Foeniculum vulgare, Syzyigum aromaticum and Elettaria cardamomum* was used for antibacterial activity assay. The test organism such as *Escherichia coli, klebsiella pneumonia, Staphylococcus aureus and Pseudomonas aeruginosa* were selected. This Indian spices have been reported to have antiseptic and disinfectant influences on the microbial word and are considered as antimicrobial agents. Spices extracts of *Piper nigrum*, Foeniculum vulgare, Syzyigum aromaticum, Elettaria cardamomum maximum antibacterial activity against microorganisms Escherichia coli, Pseudomonas aeruginosa, staphylococcus aureus. All other bacterial species showed moderate antibacterial activities.

INTRODUCTION

A Spice is a dried seed, fruit, root, bark or vegetative substance primarily used to flavouring, colouring or for preserving food. It have other uses, including medicinal, religious ritual, cosmetics or perfume production or as a vegetable. Turmeric roots are consumed as a vegetables and garlic as an antibiotic.

NUTRITION

Herbs and spices have substantial antioxidant activity. Owing primarily to phenolic compounds especially flavonoids which influence nutrition through many pathways. These antioxidants also can act as natural preservatives, preventing or slowing the spoilage of food.

THE USES AND BENEFITS OF SPICES

Spices are used in India and other countries make better use of spices by discovering for its benefits provided by the spices. Among like many spices Black pepper, Fennel, Cardamomum and cloves are taken for the investigation of antimicrobial activity for this study.

SCIENTIFIC CLASSIFICATION OF BLACK PEPPER

Kingdom	:	Plantae
Unranked	:	Angiosperms
Unranked	:	Magnolids
Order	:	Piperales
Family	:	Piperaceae
Genus	:	Piper
Species	:	P. nigrum
Binomial name	:	***Piper nigrum***

Black pepper (*Piper nigrum*) is a flowering vine in the family *Piperaceae*, cultivated for its fruit, which is usually dried and used as a spice and seasoning. Black pepper is native to south India. It is used for flavour and as a medicine. The spiciness of black pepper is due to the chemical piperine. It is added in food as flavouring agent.

SCIENTIFIC CLASSIFICATION OF FENNEL

Kingdom	:	Plantae
Unranked	:	Angiosperms
Unranked	:	Eudicats
Unranked	:	Asterids
Order	:	Apiales
Family	:	Apiaceae
Genus	:	Foeniculum
Species	:	F. vulgare
Binomial name	:	***Foeniculum vulgare***

It is a hardy, perennial, umbelliferous, herb, with yellow flowers and feathery leaves. It is grown in many parts of the world, especially on dry soils near the sea coast and on river banks. It is a highly aromatic and flavourful herb with culinary and medicinal uses.

SCIENTIFIC CLASSIFICATION OF CARDAMOMUM

Kingdom	:	Plantae
Unranked	:	Angiosperms
Unranked	:	Monocots
Unranked	:	Commelinids
Order	:	Zingiberales
Family	:	Zingiberaceae
Genus	:	Elettaria
Species	:	E. cardamomum
Binomial name	:	***Elettaria cardamomum***

Cardamomum of commerce is the dried ripe fruit often referred as the ‘Queen of Spices’ because of its very pleasant aroma and taste cardamomum is a perennial, herbaceous, rhizomatous plant. Based on

the nature of panicles, three varieties are recognized viz. Malabar with prostrate panicle, Mysore with erect panicle and vazhukka with semi erect panicle.

SCIENTIFIC CLASSIFICATION OF CLOVE

Kingdom	:	Plantae
Unranked	:	Angiosperms
Unranked	:	Eudicats
Unranked	:	Rosids
Order	:	Myrtales
Family	:	Myrtaceae
Genus	:	Syzyigum
Species	:	S. aromaticum
Binomial name	:	***Syzyigum aromaticum***

Cloves are the aromatic dried flower buds of a tree in the family Myrtaceae, syzugium aromaticum, cloves are native to the melaku islands in Indonesia and used as spice in all cuisines all over the world. Cloves are harvested primarily in Indonesia. India, Madagascar, Zanzibar, Pakistan and srilanka. They have a numbing effect on mouth tissues.

Staphylococcus aureus

Staphylococcus aureus is a major pathogen for human. Almost every person will have some type of staphylococcus aureus infection during a life time ranging in infections staphylococcus aureus cause impetigo superficial skin infection that is common in sepsis with suppuration in any organ. Staphylococci of low invasiveness are involved in many skin infections (e.g. acne, pyoderma or impetigo)

Pseudomonas aeruginosa

Pseudomonas aeruginosa, on abuitous inhabitant of soil and fresh, water causes several diseases. It causes 4% urinary tract infections, *Pseudomonas aeruginosa*, a gram negative bacteria is a clinically important pathogen because of therapeutic problem because it produces a high rate morbidity and mortality and because of the possibility of drug resistance developing during therapy. The main reason for this bacterial resistance is thought to be organism low outer membrane permeability to antibacterial agents. It affects invictims of severe burns in cancer patients who have been treated with immuno suppressive drugs.

Klebsiella pneumoniae

Klebsiella pneumonia is among the most common gram negative bacterial encountered by physicians worldwide. It is common hospital acquired pathogen causing urinary tract infections nasocomical *K. pneumoniae* is also a potential community acquired pathogen. In this interaction collaborative study we evaluated geographic differences and trends in three prominent presentations of community acquired Klebsiella infection chest radiography abnormalities such as bulging interlobar tissure and cavitary abscsses are prominent. However, the incidence of community acquired Klebsiella pneumoniae has apparently declined in the United States.

Escherichia coli

A strain of *Escherichia coli* a sub group within the species that has unique characteristics that distinguish it from other *Escherichia coli* strains. These differences are often detectable only at the molecular level however they may result in changes to the physiology or life cycle of the bacterium. For example ability to take upon a particular ecological niche or the ability to resist antimicrobial agents. Different strains of *Escherichia coli* are often host specific, making it possible to determine the source of faecal contamination in environmental samples and knowing which *Escherichia coli* strains are often host-specific. For example knowing which *Escherichia coli* strains are present in a water sample allows to make assumptions about a human another mammal or a bird.

DISC DIFFUSION METHOD

Antibiotic assay is performed by using disc diffusion technique two great. Scientist William Kirby and A.W. Bauer developed it during the year 1996. In this antibiotics present in the disc diffuse in to the remote area success fullness of disc diffusion is depends on amount of inoculums, nature of disc, moisture content of media and incubation condition. This test is done to determine which antibiotic is effectiveness against the particular pathogen. In this method both test and control organisms were used in two separate plate's width of the zone of inhibition is usually measured.

MATERIALS AND METHODS

SPICES COLLECTION

The spices like black pepper (*Piper nigrum*), Fennel seed (*Foeniculum vulgare)*, Cloves (*Syzyigum aromaticum)*, cardamom *(Elettaria cardamomum*) was collected from shop Thiruvannamalai and in Kumbakonam, Thanjavur district, Tamil nadu.

BACTERIAL CULTURE COLLECTION

Antibacterial activity of spices extract and synthetic compounds were investigated on four bacterial spices. The bacteria organisms were used in this study includes one gram positive bacteria, ***Staphylococcus aureus*** and three gram negative bacterial strains that is ***Escherichia coli, Klebsiella***

pneumoniae and ***Pseudomonas aeruginosa***. The microorganisms were obtained from diagnostic centres of Trichy, Thanjavur District and Tamil nadu.

GENERAL MICROBIOLOGICAL TECHNIQUES

General laboratory techniques for the preparation of media inoculation maintenance of culture were followed.

CLEANING OF GLASS WARE

All glass wares were kept in chromic acid cleaning solution (10%) potassium dichromate in 25% sulphuric acid for a few hours. The glass wares were washed thoroughly in tap water followed by detergent solution and finally rinsed with distilled water.

STERILIZATION

Media were sterilized in autoclave at 121^{o}c (151os) pressure for 15 minutes. The glass wares were sterilized in a hot air oven at 110^{o}c for 3 hours.

MEDIA USED

MULLER-HINTON AGAR

Beef infusion	-	300g
Casein acid hydrolysate	-	17.5g
Starch	-	15.0g
Agar	-	17.0g
Distilled water	-	1000ml
$_{p}$H	-	7.3 ± 0.2

NUTRIENT BROTH

Beef infusion	-	3.0gm
Peptone	-	5.0gm
Sodium chloride	-	5.0gm
Distilled water	-	1000ml
$_{p}$H	-	7.0

PREPARATION OF MEDIA

MULLER HINTON AGAR

10gm of Muller-Hinton agar was suspended in 1000ml of distilled water and the $_{p}$H was adjusted to 7.3 ± 0.2 and the agar was boiled to dissolve the medium completely. The medium was sterilised by autoclaving at (121^{o}c) (15lbs pressure) for 15 minutes and mixed well before pouring.

EXTRACTS PREPARATION

ETHANOL EXTRACT

The fine powder of 4 spices, like black pepper, fennel seeds, cloves and cardamom was taken. 2.5gm of each spice were mixed with 7.5ml of ethanol separately. 25% was kept for 12hrs for proper mixing of bioactive compounds. From this µl of extract was used for antibacterial assay.

INVITRO SUSCEPTIBILITY TESTING FOR BACTERIAL SPECIES

Gram positive bacteria Staphylococcus aureus and gram negative bacterial strain such as *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were used for the study.

INOCULUM PREPARATION

The test organism of each strain was suspended in 5ml of nutrient broth and incubates overnight culture were diluted 1/10 with nutrient broth before use.

DISC-DIFFUSION METHOD

PREPARATION OF DISC

What Mann No.1.6mm filtered paper disc were prepared and sterilized by autoclaving. These discs were plated and each disc was impregnated with appropriate quantity of stock solution and dried overnight at 37^{o}c. This was carried out under sterile condition inside a laminar air flow.

INOCULATION AND TESTING

Antimicrobial activity at the extracts was tested using the disc diffusion methods 10µl of each extract was impregnated into empty sterilized antibiotic disc. Each Muller Hinton agar plate was inoculated with the standard inoculums suspension by soaking a swab and rotating it over the agar plate. The paper disc was placed over the inoculated agar. After 24 hour of incubation at 37^{o}c zone of an inhibition was measured and recorded.

THERAPEUTIC USES OF CERTAIN MEDICINAL INDIAN SPICE

SCIENTIFIC NAME THE PLANTS	LOCAL NAME	PARTS USED	MEDICINAL USES
Piper nigrum	Melaku	Fruit	Digestive disorder, Vomiting, diarrhoe Flatulence, blood Purification, urinary Problems and skin Diseases.
Foeniculum vulgare	Sombu	Seed	Reduces blood cholesterol
Elettaria cardamomum	Elakkai	Fruits	Help to control bad breath And digestive disorder. A Whole cardamom chewed is good for diabetes
Syzugium aromaticum	Kirambu	Flower	Reduce blood sugar dental pain, oral u vomiting, and diarrhoea and spleen sto coldness.

RESULT

The present study investigated the antibacterial activity of ethanol extract is presented in the table the four spices. The extracts tested exhibited different degree of antibacterial activity against tested microorganisms, such as Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa.

Ethanolic extract of Black pepper (*Piper nigrum*) (25%) showed the zone of inhibition ranging from *Escherichia coli* (36mm), *Klebsiella pneumoniae* (10mm), *Staphylococcus aureus* (30mm), *Pseudomonas aeruginosa* (28mm). Among these organisms *Escherichia coli* showed the higher zone of inhibition (Table 1).

Ethanolic extract of Fennel seed (*Foeniculum vulgare*) (25%) showed the zone of inhibition ranging from *Escherichia coli* (33mm), *Klebsiella pneumoniae* (10mm), *Staphylococcus aureus* (34mm) and *Pseudomonas aeruginosa* (30mm). Among these organisms *Staphylococcus aureus* showed the higher zone of inhibition (Table 2).

Ethanolic extract of Cloves (Syzygium aromaticum) (25%) showed the zone of inhibition ranging from *Escherichia coli* (25mm), *Klebsiella pneumoniae* (10mm), *Staphylococcus aureus* (27mm), *Pseudomonas aeruginosa* (28mm). Among these organisms Pseudomonas aeruginosa showed the higher zone of inhibition (Table 3).

Ethanolic extract of cardamom (*Elettaria cardamomum*) (25%) showed the zone of inhibition ranging from *Escherichia coli* (31mm), *Klebsiella pneumoniae* (12mm), *Staphylococcus aureus* (30mm), *Pseudomonas aeruginosa* (28mm). Among these organisms *Escherichia coli* showed the higher zone of inhibition (Table 4).

ANTIBIOTIC SENSITIVITY

Antibiotic sensitivity of pencilium, Gentamycin against *Escherichia coli* (30mm), *Staphylococcus aureus* (28mm), *Klebsiella pneumoniae* (6mm), *Pseudomonas aeruginosa* (25mm). Among the tested organism *Escherichia coli* showed maximum zone of inhibition.

TABLE 1: ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF Piper nigrum

S.NO	BACTERIAL SPECIES	DIAMETER OF THE ZONE OF INHIBITION IN (mm) (M±SD)
		Ethanol
1	***Escherichia coli***	36 ± 0.7
2	***Staphylococcus aureus***	30.3 ± 0.7
3	***Klebsiella pneumoniae***	10 ± 0.1
4	***Pseudomonas aeruginosa***	28 ± 0.4

TABLE 2: ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF Foeniculum vulgare

S.NO	BACTERIAL SPECIES	DIAMETER OF THE ZONE OF INHIBITION IN (mm) (M±SD)
		Ethanol
1	***Escherichia coli***	33 ± 0.8
2	***Staphylococcus aureus***	34.3 ± 0.8
3	***Klebsiella pneumoniae***	10 ± 0.1
4	***Pseudomonas aeruginosa***	30.8 ± 0.6

TABLE3: ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF Syzugium aromaticum

S.NO	BACTERIAL SPECIES	DIAMETER OF THE ZONE OF INHIBITION IN (mm) (M±SD)
1	***Escherichia coli***	25.1 ± 0.5
2	***Staphylococcus aureus***	27 ± 0.7
3	***Klebsiella pneumoniae***	10 ± 0.1
4	***Pseudomonas aeruginosa***	29 ± 0.8

TABLE 4: ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF Elettaria cardamomum

S.NO	BACTERIAL SPECIES	DIAMETER OF THE ZONE OF INHIBITION IN (mm) (M±SD)
1	***Escherichia coli***	31 ± 0.8
2	***Staphylococcus aureus***	30 ± 1.2
3	***Klebsiella pneumoniae***	12 ± 0.77
4	***Pseudomonas aeruginosa***	28 ± 0.8

DISCUSSION

The present study investigated the antibacterial activity of ethanol extracts. The four spices the extract tested exhibited different degree of antibacterial activity against tested microorganism. Such as Escherichia coli, Klebsiella pneumoniae, staphylococcus aureus and Pseudomonas aeruginosa.

Antibacterial activity of black pepper from the Ethanolic extract by agar disc diffusion method. The zone of inhibition range from *Salmonella (25mm), Bacillus (20mm), Escherichia coli (30mm) and Staphylococcus aureus (27mm).* Among this black pepper showed higher zone of antibacterial activity against *Escherichia coli*. (ELGAYYAR M ET el., 2001).

The present study investigated the antibacterial activity of black pepper (*Piper nigrum).* Ethanolic extract of pepper were evaluated for antibacterial activity by Muller Hinton agar disc diffusion method. Among these organisms *Escherichia coli* showed the higher zone of inhibition.

The Ethanolic extract of Fennel seeds were evaluated method. The zone of inhibition against various bacteria was measured. The result indicate excellent inhibition of Fennel Ethanolic extract was ranged from *Staphylococcus aureus 30.4* ± 0.6, *Bacillus cereus 12* ± 0.8, *Pseudomonas aeruginosa 30* ± 0.18 and *Escherichia coli 25* ± 0.77. Showed extract higher zone of inhibition for *Pseudomonas aeruginosa* **(Singh et al., 2008)**

The present study investigated the antibacterial activity of fennel seeds the mode of action of a bacteria were done. The fennel extract showed higher zone of inhibition for *Staphylococcus aureus.*

The antibacterial properties of *Syzygium aromaticum* commonly known as clove tested food borne pathogens. Agar diffusion susceptibility test revealed inhibition zone of clove sample compare to ethanolic extract was showing best results against gram positive culture *Staphylococcus aureus* and two gram negative culture *Pseudomonas aeruginosa and* Escherichia coli. The MIC value was determined by using broth dilution methods. (**Amit pandey et al., 2011)**

The present study investigated the antibacterial activity of cloves extract was found against five disease causing bacteria. Among these bacteria cloves showed higher antibacterial activity against *Pseudomonas aeruginosa*.

Antibacterial activity of cardamom ethanolic extract was evaluated by agar disc diffusion method. The diameter of the zone of inhibition against various gram positive and gram negative bacteria *Salmonella typhi*. The zone of inhibition large from *Salmonella typhi* (**konning TAMIL et al., 2004).**

The present study of antibacterial activity of ethanolic extract of cardamom was evaluated by disc diffusion method. Among these organisms *Escherichia coli* showed the higher zone of inhibition.

Among the spices Black pepper showed higher zone of inhibition for *Escherichia coli*, Fennel showed higher zone of inhibition for staphylococcus aureus, cloves showed higher zone of inhibition for *Pseudomonas aeruginosa and* cardamom showed higher zone of inhibition for *Escherichia coli*.

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