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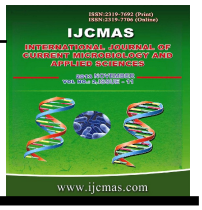

Original Research Article

Antimicrobial activity of different extracts of *Syzygiium aromaticum(Linn.)* against food borne pathogens

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A B S T R A C T

Keywords

Antimicrobial activity; Agar disc diffusion test;

The objective of the study was to characterize the antimicrobial estimation of prepared different extracts (aqueous, petroleum ether, chloroform and ethanol) of medicinal plant named as *Syzygium aromaticum(Linn.)*, commonly known as clove which acted against food borne pathogens (*E.coli, K.pneumoniae, S.aureus and S.pneumoniae)* by agar diffusion susceptibility test that revealed inhibition zone against microbes growth.

Introduction

Next, to the air we breathe and the water we drink, food has been basic to our existence. Food regulates the body process. Thus, food has many physiological functions to play (Alex.V.Ramani, 2009). Microorganisms can be detrimental to foodstuff when they cause food spoilage leading to heavy economic loss in the production phase or in the consumption phase (Vijay a ramesh., 2007). Therefore, the demand for plant based therapeutics has increased. Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens (Ahmed *et. al.*,2001) because plants are the source of energy for animal kingdom. In addition, plants can synthesize a large variety of chemical substances which have their physiological importance (Kretovich U.L.2005).

Syzygium aromaticum(Linn.) cloves the aromatic dried flower buds of a tree in the family Myrtaceae(Srivastava and Malhotra, 1991 and Chaieb *et al*., 2007a) cloves are used in Ayurveda, Chinese medicine and western herbalism. In addition, the cloves are antimutagenic (Miyazawa and Hisama, 2003), anti-inflammatory (Kim *et al*., 1998),antioxidants (Chaieb *et al*., 2007b), antiulcerogenic (Bae *et al*., 1998 and Li *et al*., 2005), antithrombotic (Srivastava and Malhotra, 1991) and antiparasitic (Yang *et al*., 2003).

Iqbal Ahmed and Arma Z Beg(2001) studied antimicrobial and phytochemical studies on 45 Indian medicinal plants against human pathogens were

demonstrated the active constituents present in ethanolic extract of *Syzygium aromaticum(Linn.)* plant inhibited the pathogens. Pundir *et al*., 2010 studied antimicrobial activity of *Syzygium aromaticum(Linn.)* against food associated bacteria where the growing concern about food safety has recently led to the development of natural activity against food borne and control spoilage microorganisms. Nazrul *et al*.,2011 demonstrated antimicrobial activity of *Syzygium aromaticum(Linn.)* extracts including petroleum ether,chloroform and ethanol tested against health hazardous microbes and reported strong inhibition for microbes.

Materials and Methods

Collection of sample

plant material of *Syzygium aromaticum (Linn.)* or Clove buds is used in this study was collected from Provision market, Usman road,T.nagar, Chennai-17,India dated on 25. October.2012 and authenticated by Mrs. Prema sambath and Vice Principal of Plant Biology and Plant Biotechnology Department from Ethiraj college for Women, Egmore, Chennai.

Extraction

The dried buds of *Syzygium aromaticum (Linn.)* were homogenised to a fine powdered and stored in airtight bottle

Preparation of aqueous extract

50g of fine powdered *Syzygium aromaticum (Linn.)* were mixed with 250ml of distilled water and boiled in a low flame for 2 hours. The extract was then filtered and used.

Preparation of petroleum ether, chloroform and ethanolic extract

20g of powder of *Syzygium aromaticum(Linn.)* were extracted with 250ml of 80% of petroleum ether, 90% of chloroform and 40% of ethanol in a flask of soxhlet apparatus for 3 hours respectively. After that the extract was concentrated in rotator vaccum evaporation with temperature ranging from 30ºC -40ºC.

Antimicrobial screening

Screening for antimicrobial activity was done by the agar disc diffusion method.

Pathogens tested for antimicrobial activity

Test strains

The strains of food borne pathogens which categorized as gram negative bacteria and gram positive. The lyophilized cultures were cultivated in the Department of Microbiology, Asan Memorial college of Arts and Science (AMCAS),Chennai-100.

Food borne pathogens

Gram negative
Escherichia coli
Klebsiella pneumonia

Gram positive
Staphylococcus aureus
Streptococcus pneumonia

Media for test organisms

33.6g of Muller Hinton Agar was added to 90ml of sterile distilled water and autoclaved at 121^{o}C for 15 minutes at 15lbs. 1.0g of dextrose was added to 10ml

of sterile distilled water and steam sterilized for 15 minutes. After cooling both the content was mixed and poured into sterile petriplates approximately 4mm and allowed to set at ambient temperature and used.

Inoculum

The microorganisms were inoculated in Nutrient broth and incubated at 37ºC for 4 hours and this was used as inoculum.

Antimicrobial activity by agar disc diffusion method

This method (Kirby Bauer *et al*., 1966) is suitable for organism that grows rapidly over night at $35^{o}C - 37^{o}C$. The antibiotic (specific concentration) impregnated disc absorbs moisture from the agar and antibiotic diffuses into the agar medium. The rate of extraction of the antibiotic from the disc is greater than the rate of diffusion. As the distance from the disc increases there is as logarithmic reduction in the antibiotic concentration. Zone of inhibition of microbial growth around each disc is measured and the susceptibility measured.

Procedure

A sterile cotton swab was inserted into the microbial suspension and then rotated and compressed against the wall of the test tube so as to squeeze out the excess fluid. The surface of the agar plate was inoculated with the swab. To ensure that the growth is uniform and confluent (or semi confluent growth) the swab is passed three times over the entire surface. Sterile disc of 5mm in diameter were impregnated with 25µl of different concentration (200mg, 400mg, 600mg, 800mg) of the each extracts were prepared using Dimethyl Sulfoxide:Methanol (1:1) solvent to dissolve the plant extract and then placed on the inoculated agar surface using sterile forceps.A standard disc containing tetracycline 10mcg/disc were used as reference controls and disc with DMSO : Methanol (1:1) was used as vehicle control. All the petriplates were sealed with sterile laboratory parafilm to avoid eventual evaporation of the test samples. The plates were left for 30 minutes at room temperature to allow the diffusion of extract and then they were incubated at 37ºc for 24 hours. After the incubation period the zone of inhibition was measured.

Result and Discussion

The aqueous extract of *Syzygium aromaticum (linn)* against *Staphylococcus aureus* showed the highest inhibition of 11mm with the concentration 200 milligram. Whereas *Streptococcus pneumoniae* ranged from 3.0 to 5.3millimeter (mm) showing the highest inhibition at 800 milligram of plant extract. Antimicrobial activity of aqueous extract of Syzygium *aromaticum (linn)* against food borne organisms *Escherichia coli* ranged from 5to 10 mm which was slightly high compared to *Klebsiella pneumoniae* the chosen other food borne microorganisms.

The effect of ethanolic extract of *Syzygium aromaticum (Linn.)* Significantly inhibited the chosen microbes (*Escherichia coli* and *Klebsiella pneumoniae)* at 200, 400, 600, 800 milligram concentration ranging from 2 mm to 10mm respectively. Against *Staphylococcus aureus* and *Streptococcus pneumoniae* showed slightly less degree of inhibition ranging from 2mm to 6 mm with the same above mentioned concentration.

Table.1 Effect of Petroleum ether extract of *Syzygium aromaticum(Linn.)* against pathogens

Name of test organisms	Zone of inhibition(mm) Mean ± standard deviation				Positive control	Vehicle control
	200mg	400mg	600mg	800mg	Tetracycline (10mcg)	
Escherichia coli	3.6± 0.94	11± 0.0	6.3± 1.41	2.3 ± 1.89	14.3 ± 6.7	-
Klebsiella pneumoniae	13.3 ± 4.7	13.6± 5.8	16.6± 0.94	13.3 ± 0.44	6.6 ± 2.3	-
Staphylococcus aureus	*21.3± 0.44	2 ± 4.24	16.3± 3.30	19 ± 0.94	25 ± 0.0	-
Streptococcus pneumoniae	8.3 ± 0.44	9.6± 2.3	9.3 ± 4.2	8.3± 0.44	6 ± 2.5	-

pneumoniae showed slightly less degree of inhibition ranging from 2mm to 6 mm with the same above mentioned concentration

Chloroform extract of buds of *Syzygium aromaticum*(*Linn*.) against food borne and respiratory organisms ranges from 4mm to 20mm whereas for *Escherichia coli* showed highest inhibition from 20mm to 21mm at 400 and 600 milligram concentrations respectively.On the other side against *Klebsiella pneumoniaee* showed 15mm and 20mm for zone of inhibition at 200 and 600 milligram concentration respectively. For *Staphylococcus aureus* have 20mm, 22mm and 23mm diameter of zone of inhibition at 200mg,600mg and 800mg and effect of chloroform extract against *Streptococcus pneumoniaee* shows 7mm, 9mm and 10mm zone of inhibition which is slightly less than other used microorganism.

Petroleum ether extract of *Syzygium aromaticum (Linn.)* against *Escherichia coli* and *Klebsiella pneumoniaee* organisms ranges from 9mm to 20mm , for *Staphylococcus aureus* ranging from 14mm to 22 mm at 200, 400, 600, 800 milligram concentrations respectively whereas against *Streptococcus pneumoniae* shows 4mm to 7mm which is lesser than other microbes at same concentration of petroleum ether extract.

The present study has been undertaken to evaluate the extracts of *Syzygium aromaticum(Linn.)* for its antimicrobial properties . In the present study petroleum ether extracts of part of the plant exhibited strong activity against the selected food borne and respiratory pathogens. The petroleum ether and chloroform extracts of leaf had strong inhibitory effect against all the chosen pathogens than aqueous and ethanol extracts. The study reported strong antimicrobial activity for all the four extracts in general petroleum ether and chloroform extracts as comparatively strong and slightly less inhibitory effects in ethanol and aqueous extracts against various pathogens.

Similarly, in another study of clove was

found active against food borne, gram positive bacteria (*Staphylococcus aureus, Bacillus cerus, Enterococcus faecalis* and *Listeria monocytogenes)* gram negative bacteria (*E.coli, Yersinia enterocolitica, Salmonella choleraesuis* and *P.aerugenosa)* (Lopez *et al*., 2005). It has also been reported that the extract of clove potentially inhibited the growth of *Helicobacter pyrlori* (Bae *et al*., 1998 and Li *et al*., 2005). In a study carried out by Betoni *et al*., (2006) clove extract showed inhibitory effect against *Staphylococcus aureus.*

In the previous study petroleum ether extract and aqueous extract shows moderate inhibition potential against bacteria suggesting that the plant extracts were bacteriostatic at lower concentration but bactericidal at higher concentration (Maji *et al*., 2010).

From the present study, petroleum ether and chloroform extract have the most potential antimicrobial activity. However ethanolic and aqueous extract was found to be inhibiting *Staphylococcus aureus* but slightly less inhibition for *Escherichia coli.* Comparison with tetracycline showed 22mm which is nearer inhibition zone of petroleum ether extract against *S.aureus.* No inhibition zone formed in solvent that could be evidence to prove the plant extract possess antimicrobial property not DMSO solvent.

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