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Antimicrobial activity of selected spices- a bio preservative approach

Pavithra Sivakumar, Nikhishaa Sree R, Jessy Padma B, Nithyananthi MJT, Saraniya P and Smila K H*
Dept. Of Biotechnology, Vel Tech High Tech Dr.Rangarajan Dr.Sakunthala Engineering College, Avadi, Chennai.
***Corresponding author: E-Mail: smila@velhightech.com**

ABSTRACT

Antimicrobial activity of certain herbs and spices were studied. The selected spices are star anise, clove and sage. Star anise is a Chinese spice originated in Southern China. Cloves are the aromatic flower buds of the tree *Syzigium aromaticum*, a native of Maluka Islands in Indonesia. Sage is originated in Mediterranean region. These selected spices are tested for their antimicrobial activity against *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa and Staphylococcus aureus.* The antimicrobial activity of these selected spices was tested using different solvents - aqueous and ethanol extracts. The disc diffusion methodology was followed. It was observed that the antimicrobial activity was much effective on using the ethanol extract of the selected spices listed above. The potential value of these extracts as natural and biological preservatives is considered.

KEY WORDS: Bio preservative, Antimicrobial Activity, Disc diffusion, Star anise, Clove, Sage.

1. INTRODUCTION

The natural products are more effective with least side effects when compared to commercial antibiotics. Consequently they are used as a substitute medication for curing various infections. Spices are plant substances that are generally used to enhance flavor, which include leaves (coriander and mint), flower bud (clove), bulbs (garlic), fruits (black pepper), bark (cinnamon), and rhizomes (turmeric and ginger). Medicinal plants produce bioactive molecules that have both antibacterial and antifungal activities. Many medicinal plants exhibit antimicrobial and antioxidant properties which protect the host from other pathogens and cellular oxidation reactions. This highlights the significance of search for innate antimicrobial drugs. Most of the food borne bacterial pathogens is susceptible to plant extracts such as garlic, mustard, oregano and onion. Gram positive bacteria are more sensitive than Gram negative bacteria to antimicrobial compounds in spices.

Illicium verum is a medium-sized evergreen tree of northeast Vietnam and southwest China, (https://en.wikipedia.org/wiki/Illicium_verum). Star anise or Chinese star anise, is a spice that closely resembles the anise in flavor, is collected from the star-shaped pericarp of the fruit of *Illicium verum* that are harvested immediately before ripening. Star anise oil is highly aromatic and is mainly used in cooking, skin creams, perfumery, soaps, toothpastes and mouthwashes. Star anise plant is used for extraction of shikimic acid, a chemical intermediate used in the production of oseltamivir. The supercritical CO_2 and ethanol extracts of *Illicium verum* revealed considerable antibacterial property against 67 clinical drug-resistant isolates, which includes 27 *Acinetobacter baumannii*, 20 isolates each of *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus*.

Sage (*Salvia officinalis)* is a perennial, evergreen subshrub, with greyish leaves, blue to purplish flowers and woody stems. It is a member of the family Lamiaceae and is native to the Mediterranean region. However it grows naturally in several places throughout the world, it has a good history of therapeutic and culinary use. Now a day they are used as a garden plant. (https://en.wikipedia.org/wiki/Salvia_officinalis).

Clove (*Syzygium aromaticum*) is a plant cultivated extensively in Spice Islands, Pemba, Indonesia and Zanzibar. Earlier the plant was produced in China. Clove is used for seasoning food (https://en.wikipedia.org/wiki/Clove). The antimicrobial potential of clove has been confirmed when its essential oil extract eliminated many Gram negative and Gram positive organisms including a few fungi. The antimicrobial activity of clove is attributed to eugenol, oleic acids and lipids contained in its essential oils. Clove extracts were found to hinder the growth of *Salmonella choleraesuis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, Klebsiella *pneumoniae* and *Candida albicans*, (Arora and Kaur, 1999). They observed that the aqueous extracts of clove do not have antimicrobial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* or *Escherichia coli* but have some activity against *Shigella flexneri*.

The objective of this paper is to study the antimicrobial activity of spices available in our locality and to prepare an effective biopreservative. The research is progressed using the selected novel spices which have not been effectively studied in the prior research. Hence the selected spices: Star anise, Cloves and Sage extracts are used to study the antimicrobial activity of certain pathogenic bacterial strains, so as to develop an effective biopreservative.

2. MATERIAL AND METHODS

2.1. Materials: *Sage (Salvia officinalis), Clove (Syzygium aromaticum), Star anise (Illicium verum).*

2.2. Bacterial strains: Four bacterial strains used in study are: *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, and Staphylococcus aureus.*

2.3. Maintenance of bacterial culture and preparation of inoculum: Pure cultures were subcultured and maintained on nutrient agar plates regularly. The cultures were inoculated on sterile nutrient agar plates and placed

in an incubator at 37°C for 24 hours and was stored at 4°C. Bacterial cultures were subcultured after every 3 - 4 days to avoid contamination. Inoculum was prepared by inoculating the pure bacterial culture in nutrient broth and incubated overnight at 37°C.

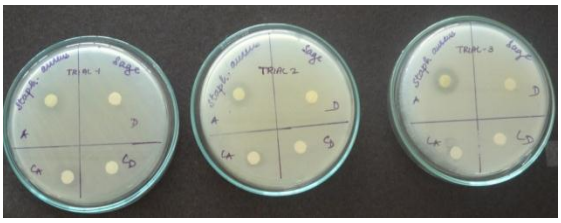
2.4. Preparation of aqueous and ethanolic extracts: Dry spices (100 gm each) Sage (*Salvia officinalis*), Clove (*Syzygium aromaticum*), Star anise (*Illecium verum*) were crushed, blended and strained through mesh cloth to obtain the fine powder. Ethanolic and aqueous extracts were prepared in the ratio 1:10 (spices in gm: distilled water/ethanol in ml) and kept at 37°C for 24hrs. 50µl of both the filtered extracts was added to the filter paper discs of Whatman no: 1 filter paper which were sterilized and the discs were impregnated using sterile inoculating loops onto the agar plates that were made in triplicate. For each dilution triplicate samples were tried. After 24hrs, the results for antibacterial activity were documented by measuring the diameter of zone of inhibition in millimetres and their mean were calculated. The zone of inhibition less than 9mm was regarded as inactive; between 9-12mm as partially active; while 13-18mm as active and more than 18mm as very active. The mean of the diameter of inhibition zones were calculated.

3. RESULTS AND DISCUSSION

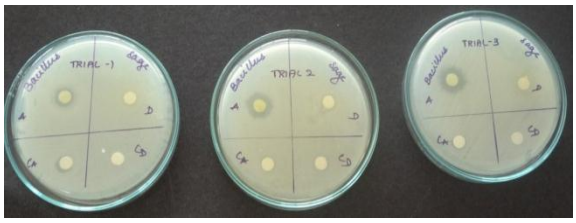
In the present study the antibacterial activity of three spices (Sage, Clove and Star anise) extracts were examined against four strains of bacteria. Amongst the three spices tested, all spices showed antibacterial activity. The results of antibacterial activity of Sage against microorganisms were given in Table 1.

Table.1.Antibacterial activity of Sage

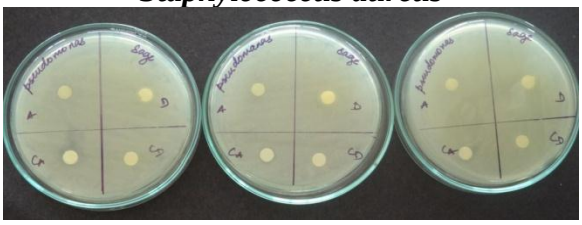
Bacterial strains	Ethanol-control	Ethanolic Extract	Distilled water-control	Aqueous extract
	Zone of inhibition in mm			
Staphylococcus aureus	8.6	12.6	-	-
Bacillus subtilis	5.6	12.3	-	-
Pseudomonas aeruginosa	-	-	-	-
Escherichia coli	8.3	12	-	-



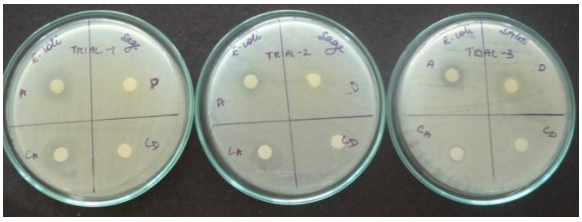
Staphylococcus aureus



Bacillus subtilis



Pseudomonas aeruginosa



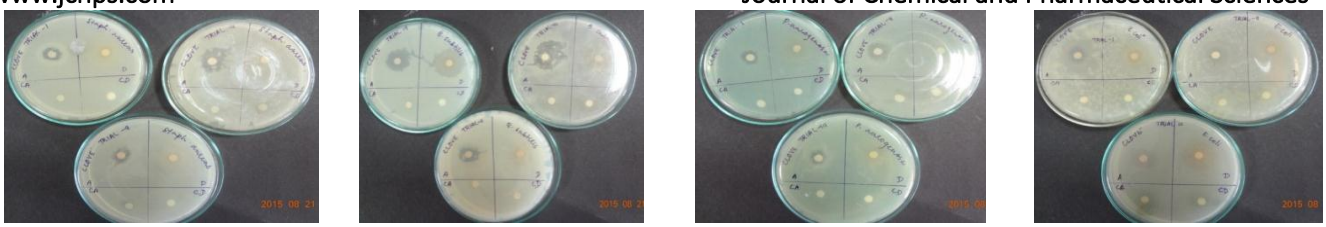
Escherichia coli

Fig.1.Antibacterial activity of Sage

Among the four bacterial species tested, all strains except *Pseudomonas aeruginosa* showed antibacterial activity in the ethanolic extract. *Staphylococcus aureus* exhibited maximum zone of inhibition (12.6 mm) trailed by *Bacillus subtilis* (12.3mm) and *Escherichia coli* (12mm). Aqueous extract did not exhibit any antibacterial activity.

Table.2.Antibacterial activity of Clove – Zone of inhibition

Bacterial strains	Ethanol - control	Ethanolic extract	Distilled water-control	Aqueous extract
	Zone of inhibition in mm			
Staphylococcus aureus	-	19.3	-	-
Bacillus subtilis	-	20.3	-	16
Pseudomonas aeruginosa	-	15	-	
Escherichia coli	-	16.3	-	14.3



Staphylococcus aureus ***Bacillus subtilis*** ***Pseudomonas aeruginosa*** ***Escherichia coli***

Fig.2.Antibacterial activity of Clove

Table 2 shows antibacterial activity of clove. The ethanolic extract of clove demonstrated antibacterial activity against all the four tested microorganisms. Among these, *Bacillus subtilis* gave highest zone of inhibition of 20.3 mm followed by *Staphylococcus aureus* (19.3 mm), *Escherichia coli* (16.3 mm) and *Pseudomonas aeruginosa* (15 mm). In aqueous extract, only *Bacillus subtilis* and *Escherichia coli* showed antibacterial activity. The zone of inhibition was prominent in size for ethanolic extracts when compared to the sage extracts. But no pigmentation was seen in *Pseudomonas aeruginosa*. *Bacillus subtilis* and *Escherichia coli* displayed clear zone of inhibition in aqueous extract while *Pseudomonas aeruginosa* produced green pigmentation in this extract. No zone of inhibition was noticed in *Staphylococcus aureus* in aqueous extract.

Table.3.Antibacterial activity of Star anise

Bacterial strains	Ethanol-control	Ethanolic extract	Distilled water – control	Aqueous Extract
	Zone of inhibition in mm			
Staphylococcusaureus	-	11.6	-	-
Bacillus subtilis	-	12.3	-	13.3
Pseudomonas aeruginosa	-	9.3	-	8
Escherichia coli	-	10.6	-	23.3

Staphylococcus aureus ***Bacillus subtilis*** ***Pseudomonas aeruginosa*** ***Escherichia coli***

Fig.3.Antibacterial activity of Star anise

Results pertaining to the antibacterial activity of star anise were presented in the Table 3. Predominant zone of inhibition was seen for all species in ethanolic extracts but no green pigmentation was observed in *Pseudomonas aeruginosa*. Among the four microorganisms, *Bacillus subtilis* showed maximum zone of inhibition (12.3mm). The acqueous extract exhibited antibacterial activity against all bacterial strains except in *Staphylococcus aureus*. The highest zone of inhibition was observed in *Escherichia coli* (23.3 mm) followed by Bacillus *subtilis* (13.3mm) and *Pseudomonas aeruginosa* (8.0mm).

The above results support the hypothesis that some common Indian spices have an inhibitory effect on the growth of certain human pathogens. The results suggest that sage, clove and star anise revealed significant antimicrobial effects.

Mosafa (2014) reported the antibacterial activity of sage against many bacterial strains of multidrug-resistant *S. aureus*, *E. coli*, *P. aeruginosa* and *K. Pneumoniae*. They reported that ethanol extract of Sage on multidrug-resistant Gram-positive *Staphylococcus aureus* is the most effective. In the present study also *Staphylococcus aureus* showed maximum antibacterial activity. Here *Bacillus subtilis* and *Escherichia coli* also have shown sensitivity to ethanol extract of the leaves of Sage.Thus, the research represents the antibacterial effects ofSage on Gram negative and Gram positive bacteria. The most sensitivity was observed in *S. aureus* and the least was seen in *E.coli*. Velickovic (2003) reported that ethanol extract of Sage possessed antibacterial activity against standard strains of Gram positive bacteria (*S. aureus* ATCC6538, *B. subtilis* ATCC6633) and Gram negative bacteria (*E. coli* ATCC25922, *P. aeroginosa* ATCC9027 and *S. enteritidis* ATCC13076).

The ethanol extracts of clove exhibited notable antibacterial activity against Gram positive and gram negative bacteria. But compared to gram negative bacteria the activity was more in gram positive bacteria. Similar results were reported by Mahfuzul (2008). This study showed that ethanol extracts of clove was more effective than that of

aqueous extract because the ethanol extract showed antibacterial activity against all the 4 bacterial strains (*Bacillus subtilis, Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa*) tested whereas aqueous extract exhibited sensitivity to *Bacillus subtilis* and *Escherichia coli* only. Amit Pandey (2011) has reported that maximum antimicrobial activity of ethanolic extract of Clove was obtained against *Pseudomonas aeruginosa* whereas least in *Staphylococcus aureus*. But in our study we got a contradictory result i.e, maximum activity was obtained in *Bacillus subtilis* (20.3mm) followed by *Staphylococcus aureus* (19.3mm) whereas least antimicrobial activity was obtained in *Pseudomonas aeruginosa* (15mm).

In the present study, the ethanolic extract of star anise have shown antibacterial activity on all the tested bacterial strains giving maximum zone of inhibition in *Bacillus subtilis* (12.3mm) followed by *Staphylococcus aureus* (11.6mm), *Escherichia coli*(10.6mm) and *Pseudomonas aeruginosa* (9.3mm). The aqueous extract of star anise displayed antibacterial activity against *Escherichia coli* (23.3mm) followed by *Bacillus subtilis (13.3mm)* and *Pseudomonas aeruginosa (8mm).*The aqueous extracts had demonstrated better activity against pathogens than ethanolic extract of star anise. These results are contrary to the reports given by Shete (2014), and Ahmad (1998).

4. CONCLUSION

In conclusion, the degree of antibacterial activity of spices tested can be put in the following order: Clove > Star anise> Sage. All the three spices were found to be excellent bactericidal agents. Owing to their antibacterial activity, these spices can be used to develop a natural biopreservative for preserving food stuffs. It is necessary to separate the active constituents, and find their pharmaco-kinetic properties, toxicity and side effects.

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