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Spices as Potent Antibacterial Agents against Staphylococcus Aureus

[1] Sanchali Padhye*, [2] Sapna Rai, [3] Nidhi Narula Harleen K Lamba, [4] Manisha Upadhyay
Department of Microbiology, MGMM, Jabalpur

[1] padhyesanchali@yahoo.com

ABSTRACT

Antibacterial activity of aqueous decoctions of Clove (Syzygium aromaticum), Star anise (Illicium vernum), Asafoetida (Ferula asafoetida), Holy basil (Ocmium sanctum), Ginger (Zingiber officinale), Nutmeg (Myristica fragrans), Garlic (Allium sativa), Bay leaf (Laurus nobilis), Turmeric (Curcuma longa), were investigated against Staphylococcus aureus isolated from college ground soil of apparently. Screening of antibacterial activity was performed using disc diffusion method (Kirby- Bauer method) and cup-plate method (Heatley`s original cylinder-plate method). The highest antibacterial potential was observed from the aqueous decoction of Star anise which inhibited the tested microorganisms than aqueous decoctions of Clove> Asafoetida > Holy basil > Ginger> Nutmeg > Garlic > Bay leaf = Turmeric.

Keywords: *Clove (Syzygium aromaticum), Star anise (Illicium vernum), Asafoetida (Ferula asafoetida), Holy basil (Ocmium sanctum), Ginger (Zingiber officinale), Nutmeg (Myristica fragrans), Garlic (Allium sativa), Bay leaf (Laurus nobilis), Turmeric (Curcuma longa) and aqueous decoction*

1. INTRODUCTION

The spices have unique aroma and flavor which are derived from compounds known as phytochemicals or secondary metabolites **(Avato et al.; 2002).** the phytochemicals are antimicrobial substances in the spices which are capable of attracting benefits of repel harmful organisms, also serve as photo protestants and responds to environmental changes. Numerous classes of phytochemicals including the isoflavonoids, anthocyanin and flavonoids are found associated with the spices **(Chang; 1988).** Spices are an important part of the human diet. They have been used for thousands of years to enhance the flavor, colour, aroma of foods. In addition to boosting flavor, herbs and spices are also known for their preservative and medicinal value **(De Souza; 2005).**Which forms one of the oldest sciences.

The spread of multi –drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. **Parekh et al.; 2005** reported that down the ages, spices have evoked interest as sources of natural product for their potential uses as alternative remedies to heal many infectious diseases. Spices are the common dietary adjuncts that contribute to the taste and flavor of food as well as are recognized to stabilize the foods from the microbial deterioration **(Kizil & So gut; 2003). Arora and Kaur; 1999** have described the inhibitory effect of spices on a variety of microorganisms, although considerable variation for resistance of different microorganisms to a given spice and of the same microorganisms to different spices has been observed. Spices are rich sources of biologically active antimicrobial compounds .The gram positive bacterial strains are more sensitive to the antimicrobial compound of spices than gram negative **(Lia and Ray; 2004; Russell ;1991)**.The extent of antimicrobial activity of spices depend on several factors which includes-

a. Kind of spice.
b. Composition and concentration of spice.
c. Microbial spice and its occurrence level
d. Processing conditions and storage **(Shelf; 1993)**

Although as natural substances spices are easily absorbed by our bodies and generally do not have any adverse effects, spices as medicine should be used judiciously. This is because substances' being derived from a plant does not mean it is always harmless. The chemical present in spices can be allergens, carcinogens and mutagens **(Chand Arana et al.; 2005).** Keeping this view the present study was conducted to determine the antibacterial potential of aqueous decoction of bay leaf, turmeric, garlic, ginger, clove, star anise, holy basil, , asafoetida, nutmeg, against Staphylococcus aureus.

Spices are essential components of Indian cuisines since ancient times. These are used in minute amounts to impart flavor, taste and aroma in food preparation to improve their palatability **(Rahman and Gul, 2002; Nair and Chanda, 2006).** Spices are also used for stabilizing several food items from deterioration **(Kizil and Sogut, 2003).** Spices are considered as rich source of bio-active antimicrobial compounds **(Lia and Roy, 2004).** The typical Indian spices and herbs like cumin, black cumin, mustard, fenugreek, ajowain, curry-leaf, nutmeg and henna are usually used in curries, pickles, sauces etc. These spices are also known to have some ethno-medicinal or anti-microbial properties **(Singh et al., 2002).** Plants traditionally used for medicinal purpose in different parts of the world have been screened for possible antimicrobial action by several workers **(Bonjar, 2004).** Antibacterial activities of extracts of different plants against various microorganisms have been

reported by many scientists **(Sagdic and Ozcan, 2003; Nair and Chanda, 2006; Shan et al., 2007; Chaudhury and Tariq, 2008; Gutierrez et al., 2008).** Some medicinal herbs have also been assessed **(Ahmad and Beg, 2001).** Some spices were specifically tested for anti-microbial activities **(Shelef, 1983; Sagdic et al., 2003).** But there are little reports on some of the Indian spices and herbs **(Singh et al., 2002; Arora and Kaur, 1999; Romson et al., 2011).**

2. MATERIAL & METHODS

2.1 Isolation of Bacteria from Soil

Ground soil was procured from the college campus. From the above soil, serial dilutions ranging from 10^{-2} to 10^{-10} were prepared and the soil micro flora was isolated by pour plate method.

2.1.1 Pour Plate Method

0.5ml of 10^{-2} dilution was poured in a sterile Petri plate. Then sterile nutrient agar was poured in the Petri plate. The contents were mixed properly and the media was allowed to solidify. All the 5 plates were incubated at 37ºC 24-48 hours to solidify.

2.2 Identification of Isolated Bacteria

After 48hrs, different types of bacteria were observed which were identified on the basis of morphological, microscopic characteristics by performing the Gram staining and biochemical tests.

2.2.1 Biochemical Test

a. Sugar Fermentation Test

Three different sugar media i.e. Dextrose, Sucrose and Lactose broth were prepared and sterilized by autoclaving. These sugar media were inoculated with the bacterial isolates. The test tubes were then incubated at 37ºC for 24-48 hrs. Then the test tubes were observed for acid and gas production. Change in colour of broth from green to yellow is an indicative of acid production. Gas production was observed in Durham`s tube.

b. Indole Production Test

The ability of some bacteria of some bacteria to decompose the amino acids tryptophan to indole was demonstrated in peptone water. The peptone water was first sterilized by autoclaving. The broth was then inoculated with bacterial isolates and one tube was kept as control. The test tubes were then incubated 37 ºC for 48 hrs. After 48hrs incubation, 1ml of Kovac`s reagent was added drop wise in each test tube including control. Formation of cherry red colored ring indicates positive indole test.

c. Methyl Red and Voges - Proskauer Test

MR-VP broth was prepared and sterilized by autoclaving. The broth was then inoculated with bacterial isolates and one test tube was kept as control. The test tubes were then incubated at 37ºC for 48hrs.

i. Methyl Red Test

This test is used to demonstrate the production of sufficient amount of acid by fermentation of glucose. After 48hrs, 5 drops of methyl red indicator was added to each tube. Development of red colour indicates a positive test.

ii. Voges-Proskauer Test

In this test 10 drops of α-naphthol and 4 drops of 40% KOH was added to each inoculated tube. The contents were mixed properly and the caps of the tubes were removed to provide oxidation. The reaction was allowed to occur for 15-20 min. Then the tubes were observed for colour change.

d. Citrate Utilization

This test is performed to check the ability, of bacteria to utilize citrate as sole source of carbon. In this test, Simmon's citrate agar was prepared. The test organism was then streaked in the slants were incubated at 37ºC for 24-48hrs .Development of blue colour indicates positive test.

e. **Triple Sugar Iron Agar test**.

The test is performed to differentiate bacteria on the basis of carbohydrate utilization pattern (dextrose, lactose and sucrose) .triple sugar iron agar was prepared and autoclaved. Then slants were prepared and test organism was streaked on the slant as well as stabbed in the butt of the same tube .The slants were then incubated at 37ºC for 24-48hrs and observed for change in colour of slant and butt as well as production of H_2S.

2.3 Test Organism-

Gram positive Staphylococcus aureus from soil suspension .The isolate was maintained on Tryptone Soya agar medium and Mannitol Salt medium.

2.4 Collection of spices

All the sample of spices viz. turmeric, garlic, ginger, clove, star anise, holy basil, asafoetida, nutmeg, bay leaf were purchased from the local market.

2.5 Preparation of Aqueous Decoction

Aqueous decoction of turmeric, garlic, ginger, clove, star anise, holy basil, asafoetida, nutmeg, bay leaf were prepared by boiling 10gm in 100ml sterile distilled water over low flame for 15minutes. The flasks were then plugged and removed from heat and allowed to cool. After cooling the contents of flasks were filtered.

2.6 Determination of antibacterial activity

Screening of antibacterial activity was performed using disc diffusion method (Kirby- Bauer

method) and cup-plate method (Heatley`s original cylinder-plate method).

The cup plate method, as used in the bio assay, a modification of Heatley`s original cylinder-plate method. In assaying spices including coriander, cumin, nigella seeds, poppy seeds, chilli, turmeric, garlic, ginger, clove, star anise, holy basil, brown mustard seeds, black cardamom, fenugreek, asafoetida, nutmeg, mace, bay leaf by this technique the test organism is grown on a suitable complete agar medium in petridishes. Cups cut out of the agar are filled with appropriate dilutions of the spices after incubation the cups are found to be surrounded by circular zone of inhibition, in which the organism has failed to grow.

The antibacterial action of the spices which diffuses into the medium the transparent zones are sufficiently clearly demarcated from the rest of the medium, opalescent or opaque from the growth of the test organism, for the measurement of these diameter are found to bear, over a range of spices concentrations wide enough for practical assay purposes, a linear relationship to the amount of spices in the cup.

2.7 Base Medium

Tryptone Soya Broth (TSB) and Tryptone soya Agar(TSA); were used for the determination of antibacterial activities of turmeric, garlic, ginger, clove, star anise, holy basil, asafoetida, nutmeg, bay leaf.

2.8 Preparation of Standardization of Inoculum

A sterile inoculating loop was touched to 4-5 isolated colonies of the bacterial spices grown on agar and then used to inoculate a tube of Tryptophan soya broth. The inoculated tryptophan soya broth tube was incubated for 24hrs35-37ºC and was matched to 0.5 turbidity standard.

2.9 Disc Diffusion Assay

100 sterilized filter paper disc of 2mm. diameter were soaked in 1ml of aqueous infusion or aqueous decoctions of, garlic, ginger, clove, star anise, holy basil, asafoetida, nutmeg, bay leaf for 1-2minutes.Thus, potency of each disc was 10µl. The soaked discs were used for screening.

2.10 Statistical Analysis

The results were calculated as mean diameter of zone of inhibition in cm.

3. RESULTS & DISCUSSION

Table 1: Macro morphological characteristics of the bacteria identified from the soil

CHARACTERSTICS	10^{-2}a	10^{-2}b	10^{-2}c	10^{-2}d	10^{-4}a	10^{-6}a	10^{-6}b	10^{-8}a
COLOUR PIGMENT	Milky white	Creamy white with yellow centre	Creamish	White	Creamish	Creamish	Creamish	Creamish
COLONY FORM	Circular/ Spherical	Circular	Circular	Circular	Shapeless	Circular	Irregular	Irregular
MARGIN	Entire	Entire	Entire	Entire	Absent	Entire	Absent	Absent
ELEVATION	Convex	Centrally raised	Raised convex	Flat	Flat	Raised	Flat	Flat
TEXTURE	Smooth, Shiny, Glistening opaque	Smooth, Shiny	Smooth shiny	Glistening	Glistening	Smooth & Shiny	Rough	Rough opaque
GRAM STAINING	Gram positive bacilli	Gram negative bacilli	Gram negative bacilli	Gram negative bacilli	Gram positive bacilli	**Gram positive mono cocci**	Gram negative bacilli	Gram positive mono cocci

Isolation of Staphylococcus aureus from college ground soil.

In total, 8 bacterial strains were isolated from college ground soil.

3.1 Identification of Isolated Bacteria

The bacterium isolated from the soil was identified on the basis of cultural, Morphological and Biochemical characteristics.

Table 2: Carbohydrate Fermentation

2: (a) Dextrose Fermentation

S.No	Colonies/Tube No. (Dextrose)	24 Hours Acid	24 Hours Gas	48 Hours Acid	48 Hours Gas
1.	10^{-2} a	+ + +	_	+ + +	+
2.	10^{-2} b	+ + +	_	+ + +	_
3.	10^{-2} c	+ + +	+	+ + +	+
4.	10^{-4} a	+ + +	+	+ + +	++
5.	**10^{-6} b**	**+ + +**	**_**	**+ + +**	**_**
6.	10^{-6} c	+ + +	+	+ + +	+
7.	10^{-8} a	+ + +	+	+ + +	++

2: **(b)** Sucrose Fermentation

S.No	Colonies/Tube No. (Sucrose)	24 Hours Acid	24 Hours Gas	48 Hours Acid	48 Hours Gas
1.	10^{-2} a	+ +	_	+ +	+
2.	10^{-2} b	+ +	_	+ + +	+
3.	10^{-2} c	+	++	+ + +	+++
4.	10^{-4} a	+ + +	_	+ + +	_
5.	**10^{-6} b**	**+ + +**	**_**	**+ + +**	**_**
6.	10^{-6} c	+ +	_	+ +	_
7.	10^{-8}	+ +	++	+ + +	++

2: (c) Lactose Fermentation

S.No	Colonies/Tube No. (Lactose)	24 Hours Acid	24 Hours Gas	48 Hours Acid	48 Hours Gas
1.	10^{-2} a	_	_	_	
2.	10^{-2} b	_	_	_	
3.	10^{-2} c	_	+	_	
4.	10^{-4} a	_	+	_	
5.	10^{-6} b	_	_	_	
6.	10^{-6} c	_	+	_	
7.	10^{-8} a	_	+	_	

Table 3: Triple sugar iron test

S.No.	Tube No.	Acidic Butt	Acidic Slant	Alkaline Butt	Alkaline Slant	H_2S
	Control	-	-	-	-	-
1.	10^{-2} a	+	_	_	+++	_
2.	10^{-2} b	+++	+	_	+	_
3.	10^{-2} c	++	++	_	+	_
4.	10^{-4} a	+++	_	_	+++	_
5.	**10^{-6} a**	**+++**	**++**	**_**	**+**	**_**
6.	10^{-6} b	_	_	+++	+++	_
7.	10^{-8} a	+++	_	_	+++	_

Table 4: IMVIC TEST

ISOLATES	10^{-2} a	10^{-2}b	10^{-2}c	10^{-4}a	10^{-6}a	10^{-6} b	10^{-8}a
Indole Production Test	_	+	+	_	_	_	_
Methyl Red Test	_	+	+	+	+	_	+
Voges Proskauer Test	_	_	_	_	_	_	_
Citrate Utilization Test	_	_	_	_	_	+	+

Table 5: Antibacterial activities of aqueous decoctions

SPICES	Aqueous Decoction
Turmeric	1.0cm.
Ginger	1.1-1.4cm.
Garlic	0.9-1.2cm.
Nutmeg	1.0-1.3cm.
Asafoetida	1.2-1.6cm.
Holy basil	1.1-1.5cm.
Clove	1.1-1.8cm.
Star anise	1.2-2.0cm.
Bay leaf	1.0cm

Spices are frequently used as an active ingredient in certain medicines and reported to possess a number of pharmacological effects to treat different human ailments **(Bonjar et al., 2004).** Several investigations have been directed towards their antibacterial properties **(Voravuthikunchai et al., 2005).** The present study gives an account on the antibacterial activities of aqueous decoctions of turmeric, garlic, ginger, clove, star anise, holy basil, asafoetida, nutmeg, bay leaf.

The results pertaining to the antibacterial potential of the tested spices are given in Table 5. Among the spices screened, the aqueous decoction of Star anise showed highest antibacterial potential against Staphylococcus aureus. Aqueous decoction of Star anise exhibited promising antibacterial activity against Staphylococcus aureus than aqueous decoctions garlic, ginger, clove, holy basil, asafoetida, nutmeg, bay leaf.

In the present study, the antibacterial activity of aqueous decoction of Clove was found next to Star anise.

4. CONCLUSION

The degree of antibacterial activity of the spices tested can be represented in the following order: Star anise > Clove> Asafoetida > Holy basil > Ginger> Nutmeg > Garlic > Bay leaf = Turmeric; all the spices were found to be sensitive against Staphylococcus aureus. Turmeric and Bay leaf indicates similar effect on Staphylococcus aureus.

Star anise, Asafoetida, Clove, Holy basil, Ginger, Nutmeg, Garlic, Bay leaf and Turmeric were sensitive against Staphylococcus aureus, indicating that these spices can be used as a substrate which stopped the growth of Staphylococcus aureus and also can be used as potential medicine against the wound infections. These spices are beneficial for-

- Star anise oil is beneficial for rheumatism. It is helpful for digestion and avoiding bad breath.
- Clove oil is beneficial for coping with tooth ache and sore gums. It is also beneficial remedy for chest pains, fever, digestive problems, cough and cold.
- Bay leaf oil possesses antifungal and anti-bacterial.
- Basil (Tulsi) - Due to its antispasmodic properties, you can use basil to ease an upset stomach. Basil can also stimulate the cilia in the nose to help clear the nasal passages.
- Garlic is thought to prevent heart disease, stroke and hypertension.
- Ginger helps to stimulate the heart and circulatory system its ability to reduce inflammation.
- Nutmeg stimulates the cardiovascular system. It also reduces stomach problems such as nausea and diarrhea.
- Turmeric can be used to reduce the risk of gallstones and is also an anti-inflammatory.
- Asafoetida used for whooping cough and stomach ache caused due to gas.

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