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ANTIBACTERIAL POTENTIALS OF TWO INDIAN SPICES

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ABSTRACT

Spices are indispensable components of Indian cuisines since ancient times. Spices are considered as a rich source of bio-active antimicrobial compounds. Antibacterial potentials of ethanolic extracts of two Indian spices *Syzygium aromaticum* (Lavang) and *Trigonella foenum-graecum* (Methi) were investigated. The extracts were subjected to a screening of antibacterial activity against selected test bacteria by disc diffusion assay method where tetracycline was used as standard. In present investigations, attempts were made to screen these spices as antibiotics. The results showed that the extracts possess good antibacterial activity against all the test bacteria and therefore, the results offer a scientific basis for the traditional use of *Syzygium aromaticum* and *Trigonella foenum-graecum*. Thus, the use of these spices in daily life will generate a resistance or immunity to fight against microorganisms.

NO KEYWORDS: SPICES, ANTIMICROBIAL ACTIVITIES, *Syzygium aromaticum, Trigonella foenum-graecum*

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INTRODUCTION

Nature has been a source of medicinal agents since times immemorial. Natural products still remain as one of the best reservoirs of new structural types, despite the availability of different approaches for the discovery of therapeutics. Natural products are used directly as therapeutic agents, as well as starting material for the synthesis of drugs or as models for pharmacologically active compounds (Cowan, 1999). India is well known for its strong aromatic spices. Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods (Arora and Kaur, 1999; Shelef, 1983). They have been found to reduce inflammation, protect against infection, help to detoxify the liver and cleanse the lungs and other organs and also protect from cell damage that can lead to rheumatoid arthritis, osteoporosis, heart disease and other degenerative diseases (Surh, 2002). Spices are considered as rich source of bio-active antimicrobial compounds (Lia and Roy, 2004). Fenugreek is traditionally used as a demulcent, laxative, lactation stimulant and exhibits hypocholesterolemic, hypolipidemic and hypoglycemic activity in healthy and diabetic animals and humans. The defatted seed material of fenugreek may reduce gastrointestinal absorption of glucose and cholesterol and increase bile acid secretion. Cloves have an active compound eugenol, which is used as an antiseptic and possesses local anaesthetic activity; it is therefore used for toothache (Suresh et. al., 1992). Cloves were used in folk medicine as diuretic, odontalgic, stomachic, tonicardiac, aromatic condiment properties and condiment with carminative and stimulant activity (Boulos, 1983).

In the last three decade, antibiotic resistance has become a global concern (Westh et al., 2004). By the emergence of multi drug resistant pathogens, the clinical efficacy of the existing antibiotics is being threatened (Bandow et al., 2003). Bacteria, in general, have the genetic ability to transmit and acquire resistance to drugs (Cohen, 1992). This has forced the scientists to search for new antimicrobial substances from various sources like the medicinal plants, herbs and spices. Recent work revealed that spices like garlic, ginger and turmeric have antimicrobial and antioxidant activity (Panpatil et. al., 2013). Antimicrobial activities of extracts of different plants against various microorganisms have been reported by many scientists (Sagdic and Ozcan, 2003; Nair and Chanda, 2006; Shaan et. al., 2007, Chaudhury and Tariq, 2008; Gutierrez et. al., 2008; Menghani et. al., 2011). Some traditional remedies have produced compounds that are effective against antibiotic resistant strains of bacteria (Kone et. al., 2004). The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003). In present project, attempts have been made to evaluate the antibacterial potentials of *Syzygium aromaticum* and *Trigonella foenum-graecum*. The purpose of screening is to justify, authenticate and validate the use of these spices in ethno-medicinal or folklore as traditional treasure to cure various ailments.

MATERIALS AND METHODS

Collection

The samples (*Syzygium aromaticum* and *Trigonella foenum-graecum*) were procured from Chunnilal Attar Ayurvedic Store, Ghat Gate, Jaipur in the month of February, 2010

Identification

All the samples were authenticated and were given identification number *Syzygium aromaticum* and *Trigonella foenum-graecum.* These samples were authenticated and submitted in Ethno medicinal Herbarium, Centre of Excellence funded by DST, Department of Biotechnology, MGiaS, Jaipur (Rajasthan).

Sources of test organisms

Bacteria pure culture of all test organisms, namely *Enterobacter aerogenes* (*111), *Chryseobacterium Glenn* (*1916), *Proteus vulgaris* (744), *Klebsiella pneumoniae* (109), *Shigella flexneri* (1457), *Bacillus subtilis* (Gram positive) (441) and *Staphylococcus*

aureus (Gram positive) (7443)*, the human pathogens,* were obtained through the courtesy chemical profile [TLC] of Mahatma Gandhi Institute of applied Sciences (MGiaS), Jaipur, which were maintained on Nutrient broth media.

Culture of Test Microbes

For the cultivation of bacteria, Nutrient Agar Medium (NAM) was prepared by using 20 g Agar, 5 g Peptone, 3 g beef extract and 3 g NaCl in 1L distilled water and sterilized at 15 lbs pressure and 121°C temperature for 25-30 minutes. Agar test plates were prepared by pouring approximately 15 mL of NAM into the Petri dishes (10 mm) under aseptic conditions. A saline solution was prepared (by mixing 0.8% NaCl) in distilled water, followed by autoclaving and the bacterial cultures were maintained in this medium by regular subculturing and incubation at 37°C for 24-48 h. To prepare the test plates, in bacteria, 10-15 mL of the respective medium was poured into the Petri plates and used for screening. For assessing the bactericidal efficacy, a fresh suspension of the test bacteria was prepared in saline solution from a freshly grown Agar slant.

Preparation of Test Extracts

Crushed powder (50 g) of all the species were successfully soxhlet extracted with alcohol. Later, each of the homogenates was filtered and the residue was re-extracted twice for complete exhaustion, the extracts were pooled individually. Each filtrate was concentrated to dryness in vitro and redissolved in respective solvents, out of which 80 mg/10 discs, i.e., 8 mg/disc, concentration were stored at 4°C in a refrigerator, until screened for antibacterial activity.

Bactericidal assay

For bactericidal assay, in vitro Disc diffusion method was adopted (Gould and Bowie, 1952) because of reproducibility and precision. The different test organisms were proceeded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around sterilized dried discs of Whattman No.1 paper (6 mm in diameter), which were of three different concentrations:

A1 = 1mg of test extract/disc
A2 = 5mg of test extract/disc
A3 = 10mg of test extract/disc

and tetracycline as reference drugs (standard disk) separately. Such treated discs were air-dried at room temperature to remove any residual solvent, which might interfere with the determination, sterilized and inoculated. These plates were initially placed at low temperature for 1 h so as to allow the maximum diffusion of the compounds from the test disc into the agar plate and later, incubated at 37°C for 24 h after which the zones of inhibition could be easily observed. The inhibition zone (IZ) in each case was recorded and the activity index (AI) was calculated as compared with those of their respective standard reference drugs (AI = Inhibition Zone of test sample / Inhibition zone of standard).

RESULTS

Syzygium aromaticum

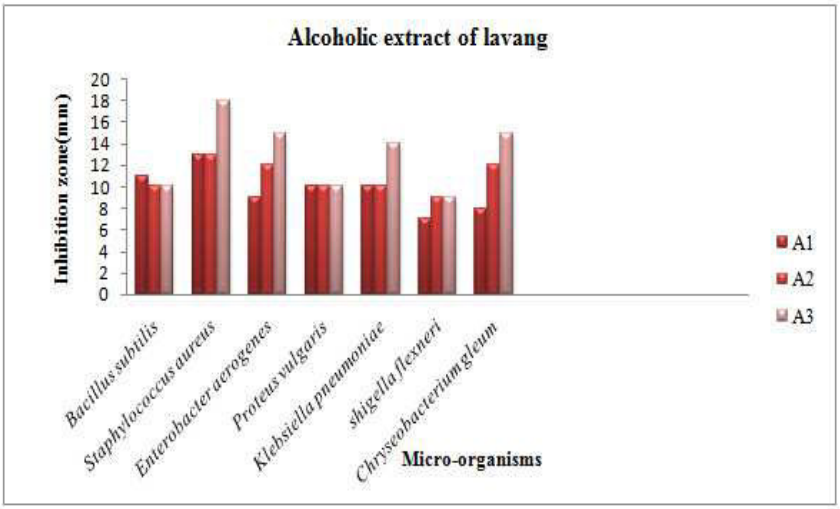
The antibacterial activity was seen in *Bacillus subtilis, Staphylococcus aureus, Enterobacter aerogenes, Chryseobacterium gleum, Proteus vulgaris, Klebsiella pneumonia* and *Shigella flexneri* (Table 1). The results showed that the given test extracts have maximum activity (A.I. = 1.25) against *Proteus vulgaris* (Fig.1) and minimum (A.I. = 0.46) against *Enterobacter aerogenes*.

Table 1
The inhibition zone and activity index of alcoholic extract of Syzygium aromaticum against selected test microorganisms.

Micro-organisms	A_1		A_2		A_3		Standard
	I.Z.(mm)	A.I.	I.Z.(mm)	A.I.	I.Z.(mm)	A.I.	I.Z.(mm)
Bacillus subtilis	11	0.47	10	0.43	10	0.43	23
Staphylococcus aureus	13	1.18	13	1.18	18	1.63	11
Enterobacter aerogenes	9	0.34	12	0.46	15	0.57	26
Chryseobacterium gleum	8	0.44	12	0.66	15	0.83	18
Proteus vulgaris	10	1.25	10	1.25	10	1.25	8
Klebsiella pneumoniae	10	0.90	10	0.90	14	1.27	11
Shigella flexneri	7	0.35	9	0.45	9	0.45	20

Trigonella foenum-graecum

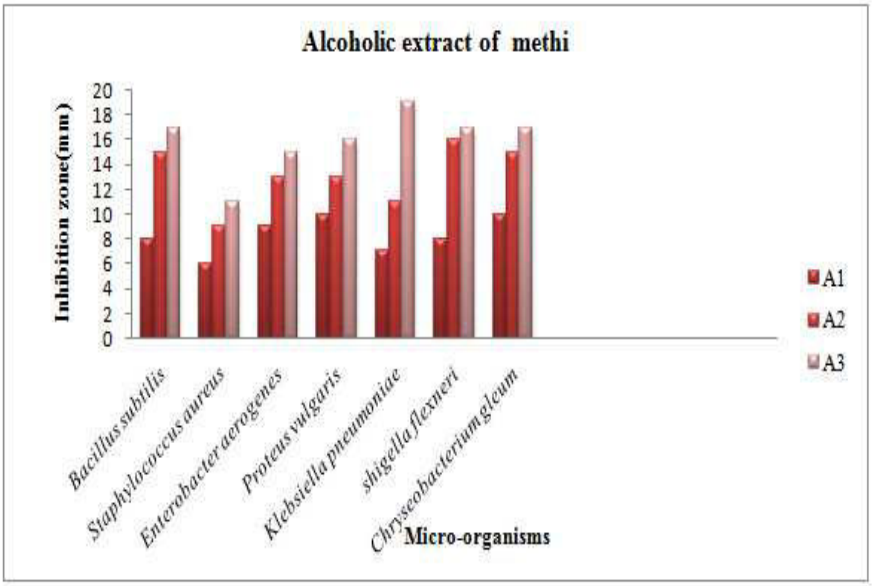
The antibacterial activity was seen in *Bacillus subtilis, Staphylococcus aureus, Enterobacter aerogenes, Chryseobacterium gleum, Proteus vulgaris, Klebsiella pneumonia* and *Shigella flexneri*



(Table 2). The results showed that the given test extracts have maximum activity (A.I. = 1.25) against *Proteus vulgaris* (Fig. 2) and minimum (A.I. = 0.47) against *Enterobacter aerogenes*.

Table 2
The inhibition zone and activity index of alcoholic extract of Trigonella foenum-graecum against selected test microorganisms.

Micro-organisms	A_1		A_2		A_3		Standard
	I.Z.(mm)	A.I.	I.Z.(mm)	A.I.	I.Z.(mm)	A.I.	I.Z.(mm)
Bacillus subtilis	8	0.34	15	0.65	17	0.73	23
Staphylococcus aureus	6	0.54	9	0.81	11	1	11
Enterobacter aerogenes	9	0.34	13	0.50	15	0.57	26
Chryseobacterium gleum	1	0.06	15	0.83	17	0.94	18
Proteus vulgaris	1	0.13	13	1.62	16	2.00	8
Klebsiella pneumoniae	7	0.63	11	1.00	19	1.72	11
Shigella flexneri	8	0.4	16	0.80	17	0.85	20



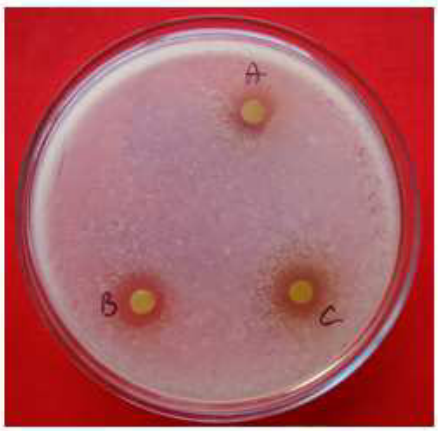


Figure 1
Activity of lavang against Proteus vulgaris

Figure 2
Activity of methi against Proteus vulgaris

DISCUSSION

In India, spices are ethnically used as active ingredients in ayurvedic medicines and reported to possess a number of pharmacological effects to treat different human ailments (Bonjar et. al., 2004). In developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is a need to search new infection fighting strategies to control microbial infections. The present results explain that the ethanolic extracts of *Syzygium aromaticum* and *Trigonella foenum*-graecum have antibacterial activity against all the test bacteria. The spices showed maximum activity against *Proteus vulgaris* and minimum against *Enterobacter aerogenes*. Thus the results offer a scientific basis for the traditional use of these spices. The results also show that the use of these spices in daily life will generate a resistance or immunity to fight against microorganisms.

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