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COMPARATIVE STUDY OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF SPICES ALONG WITH STRUCTURAL DETERMINATION USING FT-IR SPECTROSCOPY

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ABSTRACT

In today's medical world, antibiotic toxicity and multidrug resistant pathogens are the two greatest challenges which require immediate attention. In the present study, the antibacterial and antioxidant activity of spices have been investigated. The antibacterial activity of eight common Indian spices was analyzed against nine clinical pathogenic bacteria by Kirby Bauer Disc Diffusion method and maximum inhibition concentration (MIC) was determined. Maximum inhibition zones in the range of 17-25mm were seen in extracts of star anise and asafoetida. The antibacterial effect was also compared with the standard antibiotic discs. The antioxidant activity was evaluated by free radical scavenging power against DPPH free radical. The IC_{50} of the methanolic extracts ranged from 4.45 ± 0.1 to 19.6 ± 0.4 µg/ml and that of control i.e. ascorbic acid was 6.01 ± 0.1 µg/ml. Qualitative estimation was done by FT-IR analysis and major functional groups present in spices were determined.

KEYWORDS: **Spices, Pathogenic bacteria, Antibacterial activity, IC_{50} and FT-IR**

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INTRODUCTION

Since ancient times, the medicinal value of plants has encouraged mankind to search alternatives against chemicals used for preservation. Plants serve as a reservoir of chemical agents exhibiting antimicrobial properties. Since infectious diseases have been the third most threatening cause of deaths worldwide, treating them completely is a major challenge. In comparison to chemical or synthetic additives, herbal additives are preferred as they do not have any known side effects[1]. Antibacterial activities of extracts of different spices against various microorganisms have been reported by many researchers[2, 3]. Additionally, herbs and spices are considered as rich sources of bio-active antimicrobial compounds and their properties have been found to be due to the essential oils and other ingredients in those plants[4]. Antimicrobial effectiveness of spices depend on the kind of spice, its composition and concentration, type and concentrations of the target microorganism, substrate composition, and processing and food storage conditions[5]. Spices inhibit bacteria and their poisonous toxins, by producing phytochemicals as a defense mechanism Traditionally, spices have always formed an integral part of the Indian cuisine for enhancing the flavor, color and aroma of food in addition it possesses antioxidant activity also. Several biochemical reactions occurring in our body produce reactive oxygen species which are capable of damaging important bio-molecules. In case of ineffective scavenging these may lead to diseased conditions[6]. Some of the diseases caused due to oxidative damage by the free radicals are cancer, liver disease, Alzheimer's disease, premature ageing, arthritis, inflammation, diabetes, Parkinson's disease and atherosclerosis[7]. The harmful action of free radicals can be controlled by antioxidant substances, which scavenge the free radicals and detoxify the organism. Antioxidants comprise of a wide range of phenolic compounds ranging from simple phenolic acid to highly polymerized compounds such as tannins[8]. It is very important to find new sources of safe and inexpensive antioxidant substances of natural origin[9]. Thus the search for newer natural antioxidants, especially of plant origin, has been increasing ever since.

The typical Indian spices and herbs used in this study are black-pepper, nutmeg, star-anise, fenugreek, ajwain, asafoetida, coriander and mint which are usually used in curries, pickles, sauces etc. All these spices are known to possess some ethno-medicinal or antimicrobial properties[10]. In the present study, one of the objectives was to explore the antibacterial effect of spices on pathogenic bacterial strains which are a major causative agent of food-borne diseases. The antimicrobial effect of these spices was examined by Kirby-Bauer disc diffusion susceptibility method and then minimum inhibitory concentrations were determined. Structural determination of different functional groups present in spices has been carried out using FT-IR analysis. It is used for determining the presence of certain functional groups such as carbon-carbon multiple bonds, aromatic rings, carbonyl groups or hydroxyl groups in a molecule.

MATERIALS AND METHODS

(i) Collection of plant material:

Eight different spices were studied. These spices were collected from the local markets of Vellore, Tamil Nadu, India.

Leaves: Fresh leaves of coriander and mint were collected and washed thoroughly 2-3 times with running tap water and then with sterile water followed by shade drying, powdered and used for aqueous and solvent extraction.

Seed: Dried ajwain, fenugreek and black pepper seeds were grounded and powdered with the help of pestle and mortar.

Fruit: Dried star anise and nutmeg fruits were grounded and powdered with the help of pestle and mortar.

Stem: The latex extruded from the stem of asafetida was dried and powdered with the help of mortar and pestle.

(ii) Preparation of extracts

Aqueous extracts: 20 g of each dry sample was crushed and mixed with 100 ml of milli-Q water. Extracts were made either by using a rotary shaker (for 48 hours at 120 rpm; coriander, mint, nutmeg, fenugreek, asafetida, black pepper) or soxhlet apparatus (for 6 hours at 70°C; star anise, ajwain).

Solvent Extracts: 20 g of each dry sample was crushed and mixed with 100 ml of ethanol (asafetida, fenugreek, ajwain, and nutmeg), methanol (mint, coriander) and acetone (star anise, black pepper). Different solvents like methanol, ethanol and acetone were used for different spices according to their solubility, polarity and functional activity[10]. Extracts were made either by using rotary shaker (for 48 hours at 120 rpm; coriander, mint, nutmeg, fenugreek, asafetida, black pepper) or soxhlet apparatus (for 6 hours at 70°C; star anise, ajwain).

The extracts were cooled and filtered through Whatman filter paper no. 1 and evaporated using hot air oven to near dryness at 65- 70°C. Extracts were placed in dark glass bottles and stored at 4°C until further analysis.

(iii) Test microorganisms

Microorganisms used for the antimicrobial activity are as follows:

1. *Shigella dysenteriae* Type-5 NK 2440
2. *Vibrio cholerae* 0139
3. *Vibrio fluvialis* IDH 2036
4. *Vibrio parahaemolyticus* 03:K6
5. Enterotoxigenic *Escherichia coli* ETEC H 10407
6. *Salmonella typhi* strain 6
7. *Aeromonas hydrophila* IDH 1073
8. *Proteus mirabilis* ATCC 12453
9. *Escherichia coli* MTCC 1652

Bacterial cultures were maintained on nutrient agar slants.

(iv) Antimicrobial activity assay

In vitro antibacterial activity of the spices was tested against pathogenic bacteria such as *S. dysenteriae, V. cholera, V. fluvialis, V. parahaemolyticus,* Enterotoxigenic *E. coli, S. typhi, E. coli, A. hydrophila* and *P. mirabilis.* 0.2 ml of overnight culture of each bacteria was dispensed into 20 ml of sterile nutrient broth and incubated for 3- 5 hrs at 37°C to standardize the culture to 10^{-6} CFU /ml. Screening of antibacterial activity was done by Kirby-Bauer disc diffusion susceptibility method and the minimum inhibitory concentrations (MIC) were determined. The sterile discs were made by the Whatman filter paper no. 1 (5 mm in diameter). 10µl of overnight bacterial cultures were spread properly on the Mueller Hinton agar plates. After spreading, sterile discs were soaked into the extracts of spices (20µl per disc), placed on the petriplate and incubated for 18 hours at 37˚C. The zone of inhibition was measured from the centre of the disc and used for determination of MIC of the spice extracts.

(v) Antioxidant activity assay

Methanolic extract of each spice was prepared by adding 2 g of the powdered spice sample to 50 ml of 100% methanol at room temperature (25°C) in a rotary shaker for 24 hours. The filtrate was stored at 4°C until further use. 0.1 mM solution of DPPH (1,1-Diphenyl-2-picryl-hydrazyl) in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution of methanol at different concentrations (1-25 µg/ml). The absorbance was measured at 517 nm after 30 minutes. Ascorbic acid was used as the reference compound[11]. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical-scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\% \text{ inhibition} = (A_0 - A_t)/ A_0 \times 100$$

Where A_0 was the absorbance of the positive control (DPPH solution) and A_t was the absorbance in the presence of the extract. All the tests were performed in duplicate and the graph was plotted with the mean values. IC_{50} values denote the concentration of the sample, which is required to scavenge 50% of DPPH free radicals.

(vi) Functional group analysis

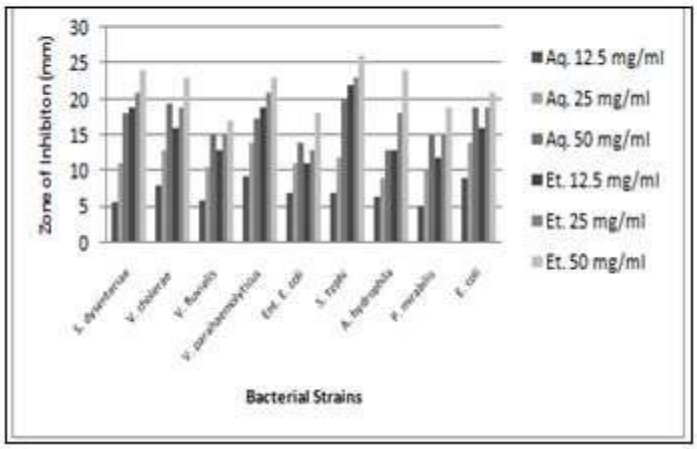
Fourier transform infrared (FT-IR) was used to identify the characteristic functional groups in the extract. 5mg of the solvent extract was dispersed in dry potassium bromide. After mixing these two well, the mixture was pressed to make a thin disc at a pressure of 6 bars for about 2 min. After placing the disc in a sample cup, the IR spectrum was obtained (Perkin Elmer 2000Infrared Spectrometer). To get better signal to noise ratio, scanning of the sample was done for 32 times from 4000 to 400 cm^{-1}.

RESULTS

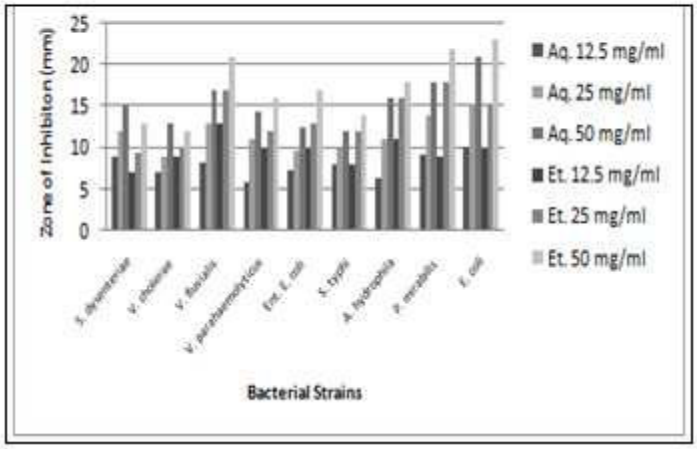
1. Antimicrobial activity assay

The antibacterial activity of eight different kitchen spices was tested against nine clinical pathogenic bacterial strains and the results were analyzed based on the zone of inhibition and the minimum inhibitory concentration (MIC) of each spice responsible for the bactericidal activity. The spices exhibited different degrees of bacterial growth inhibition, depending on the strains and concentration of the extract. Star anise exhibited strongest bactericidal activity when compared to all the other spices. The aqueous extracts of asafoetida and star-anise showed broadest antimicrobial activity with maximum activity against *S. typhi* (Graph 2 and 8). *S. dysenteriae and V. parahaemolyticus* were the most sensitive strains. Enterotoxigenic *E. coli* and *A. hydrophila* showed maximum resistance against the tested spices. In comparison to aqueous extracts, organic extracts gave better inhibitory effect. However, aqueous extracts of star-anise and asafoetida were more effective. The organic extracts of black-pepper, fenugreek and nutmeg showed greater inhibition zones (Graph 3, 5 and 7). *V. cholerae* was the most sensitive strain while *Enterotoxigenic E. coli* was the most resistant.

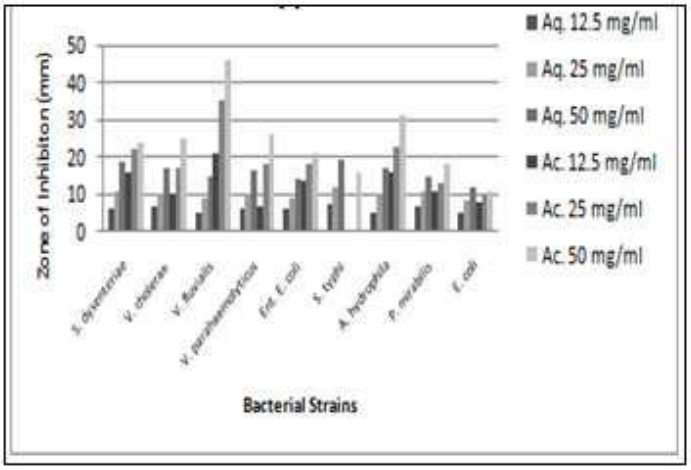
Graph 1: Antibacterial activity of ajwain extracts.



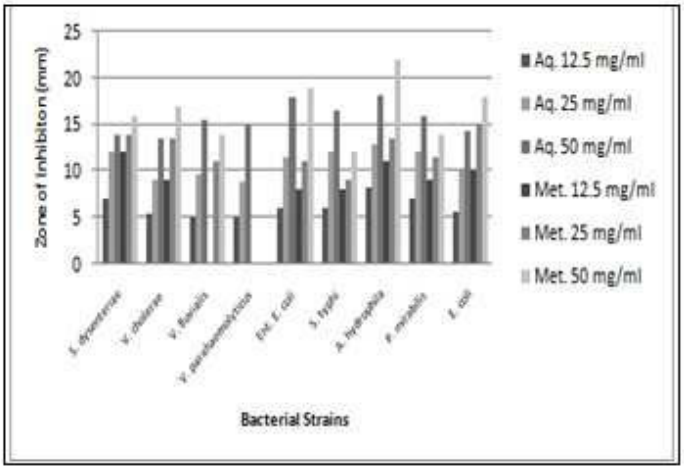
Graph 2: Antibacterial activity of asafetida extracts.



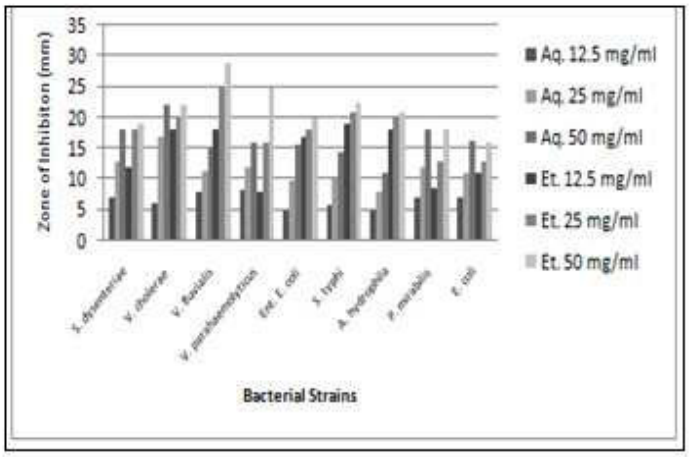
Graph 3: Antibacterial activity of black pepper extracts.



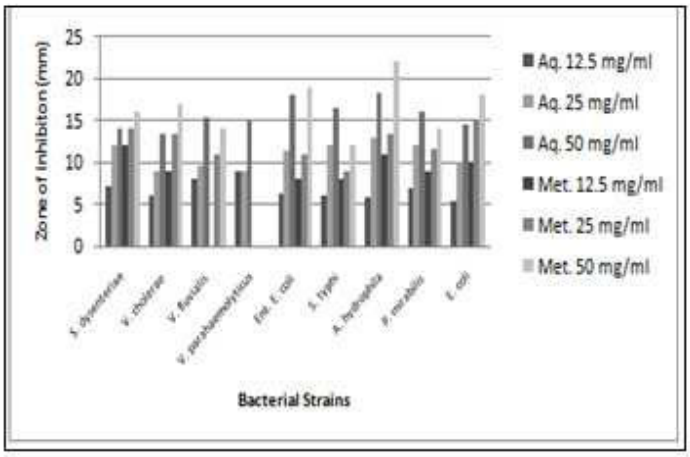
Graph 4: Antibacterial activity of coriander extracts.



Graph 5: Antibacterial activity of fenugreek extracts

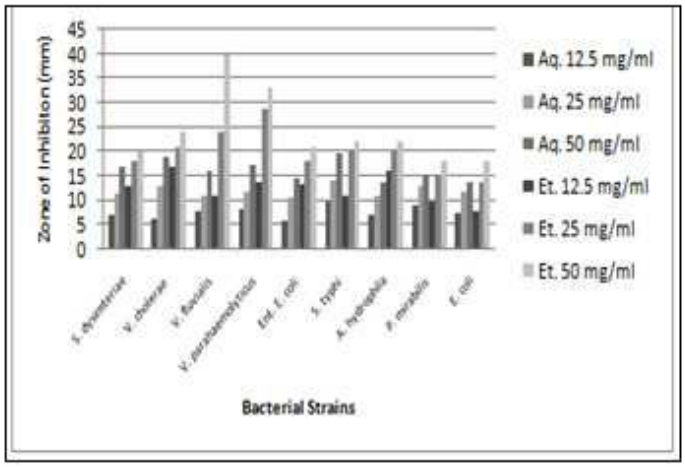


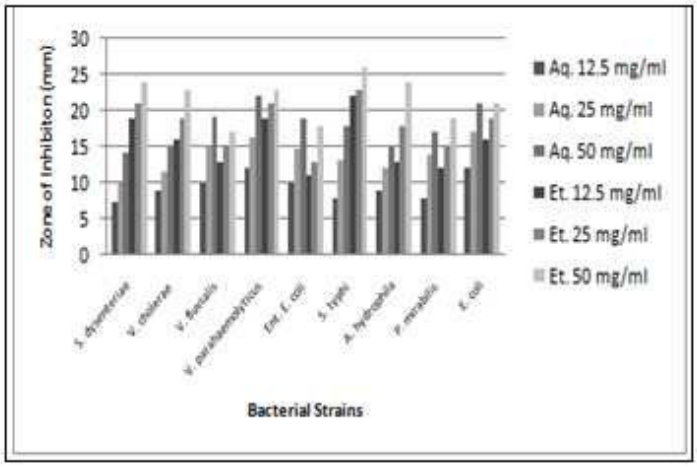
Graph 6: Antibacterial activity of mint extracts.



Graph 7: Antibacterial activity of nutmeg extracts.

Graph 8: Antibacterial activity of star anise extracts.





Extracts showed MIC of 12.5mg/ml against most of the strains. However, the MIC values of methanol extract of coriander and fenugreek was found to be 25.0mg/ml against *V. fluvialis* and *V. parahaemolyticus*, respectively (Table 1). A comparative study was done by testing standard antibiotic discs against these bacterial strains.Streptomycin (S^{10}) and kanamycin (K^{30}) showed highest inhibition of 17-25 mm against all the bacterial strains; Rifampicin (R^5) gave moderate results with smaller zone of inhibition (8-15 mm). However, Penicillin (P^{10}) and bacitracin (B^{10}) showed a negligible inhibitory effect.

Table 1
Minimum inhibitory concentration (MIC) values of different spice extracts (mg/ml)

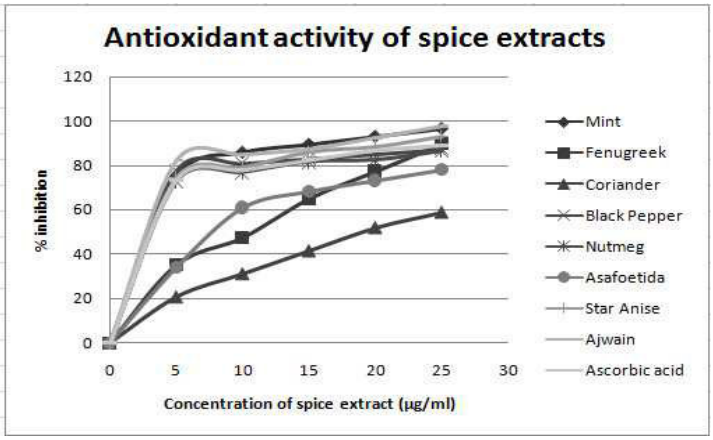
Species	Ajwain	Asafoetida	Black pepper	Coriander	Fenugreek	Mint	Nutmeg	Star anise
Shigella dysenteriae	25.0	12.5	25.0	12.5	12.5	12.5	12.5	12.5
Vibrio cholerae	12.5	12.5	12.5	25.0	25.0	25.0	25.0	12.5
Vibrio fluvialis	25.0	12.5	25.0	25.0	12.5	12.5	12.5	12.5
Vibrio parahaemolyticus	12.5	25.0	25.0	25.0	12.5	12.5	12.5	12.5
Enterotoxigenic *E. coli*	12.5	12.5	12.5	25.0	25.0	25.0	25.0	12.5
Salmonella typhi	12.5	12.5	12.5	25.0	25.0	25.0	12.5	12.5
Aeromonas hydrophila	25.0	25.0	25.0	12.5	25.0	25.0	12.5	12.5
Proteus mirabilis	25.0	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Escherichia coli	12.5	12.5	25.0	25.0	12.5	25	12.5	12.5

2. Antioxidant activity analysis

Radical scavenging activity of methanolic extracts of ajwain, asafoetida, black pepper, coriander, fenugreek, mint, nutmeg and star-anise was evaluated. In the presence of the extract capable of donating an H atom, DPPH undergoes reduction showing a color change from deep violet to yellow that was measured by the decrease in its absorbance at 517 nm. The IC_{50} value of extracts i.e. minimum quantity of spice extract required to scavenge 50% of the free radicals were also calculated. The IC_{50} value for ascorbic acid (standard) was 6.01± 0.1µg/ml (Table 2). In comparison to standard, the crude methanolic extracts of coriander and fenugreek showed lower activity, with IC_{50} value of 19.61 and 11.81µg/ml, respectively. The antioxidant activity of asafoetida extract was found to be higher than that of ascorbic acid, while that of nutmeg and black pepper was nearly similar to ascorbic acid. The radical scavenging effect of all spice extracts is depicted in the graph 9.

Table 2
IC_{50} values (µg/ml) of spices against DPPH free radical

No.	Spice	IC_{50} values
1	Asafoetida	11.67
2	Ajwain	4.45
3	Black pepper	5.29
4	Coriander	19.60
5	Fenugreek	11.81
6	Mint	4.96
7	Nutmeg	5.29
8	Star anise	5.64
9	Ascorbic acid	6.01



Graph 9
Percentage inhibition of spice extract against DPPH

3. Functional group analysis

Mint

The absorption at 3444.87cm^{-1} was due to the stretching of hydroxyl groups that were present in the extract. The bands at 2926.01 and 2854.65cm^{-1} were due to the C-H symmetric stretching of saturated (sp^3) carbon. The band at 1641.42cm^{-1} was assigned to weak intensity C=C stretch. The band at 1400.32cm^{-1} indicated the presence of NO_2 group. The band from 713.66cm^{-1} indicated the presence of benzene ring in the sample. The bands at 1261.45 and 1072.42cm^{-1} were assigned to the strong intensity C-O skeletal vibrations. The molecular structure is similar to menthol as bands of functional group –OH, C-H and isopropyl have similar band wavelength to that of mint. Thus we can say that active component responsible for activity of mint may be menthol (Figure 1).

Black Pepper

The absorption at 3446.79cm^{-1} was due to the stretching of hydroxyl groups that were present in the extract. The band at 2926.01and 2854.65cm^{-1} were due to the C-H symmetric stretching of saturated (sp^3) carbon. The bands at 1639.49 and 1631.78cm^{-1} was assigned to weak intensity N-H stretch. The bands between 1492.9- 1402.25cm^{-1} indicated the presence of NO_2 group. The band from 892.69- 713.66cm^{-1} indicated the presence of benzene ring in the sample. The bands at 1253.73 and 1031.92cm^{-1} were assigned to the strong intensity C-O skeletal vibrations. The molecular structure is similar to piperine as bands of functional group H-H, C=H, O-H and C-O aromatic have similar band wavelength to that of black pepper. Thus it could be concluded that active component responsible for activity of black pepper might be piperine (Figure 2).

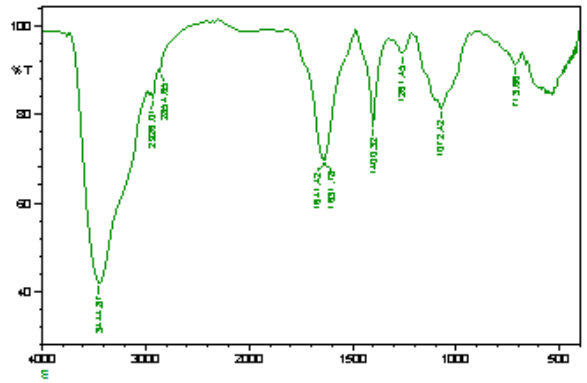


Figure 1
FTIR analysis of mint extract

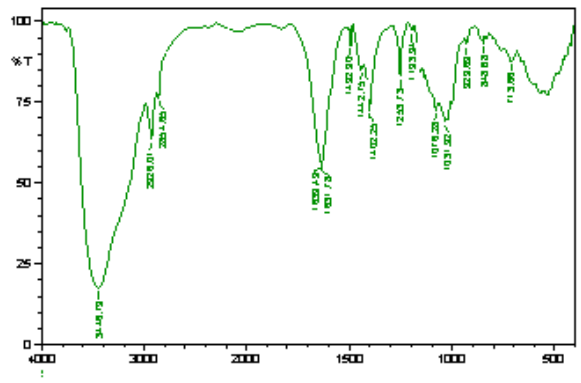


Figure 2
FTIR analysis of black pepper

Star anise

The absorption at 3383.14cm^{-1} was due to the stretching of hydroxyl groups that were present in the extract. The bands at 3132.4 and at 2924.09 and 2852.72cm^{-1} were due to the C-H asymmetric and symmetric stretching of saturated (sp^3) carbon, respectively. The band at 1631.78cm^{-1} was assigned to conjugated C=C bond. The band at 1402.25cm^{-1} indicated the presence of NO_2 group. The bands at 1247.94 and 1070.49cm^{-1} were assigned to the strong intensity C-O skeletal vibrations. The molecular structure was similar to shikimic acid as bands of functional group O-H, C-O, C=O and benzene ring have similar band wavelength to that of star anise. Hence it could be suggested that active component responsible for activity of star anise might be shikimic acid (Figure3).

Nutmeg

The absorption at 3442.94cm^{-1} was due to the stretching of hydroxyl groups that were present in the extract. The bands at 2916.37 and 2850.79cm^{-1} were due to the C-H symmetric stretching of saturated (sp^3) carbon. The band at 1737.86cm^{-1} was assigned to C=O stretch. The bands 1402.25cm^{-1} indicated the presence of NO_2 group. The band from 1631.78-1467.83cm^{-1} and from 997.2-717.52cm-1 indicated the presence of benzene ring in the sample. The bands between 1273.02-1016.49 cm^{-1} were assigned to the strong intensity C-O skeletal vibrations. The molecular structure is similar to myristicin as bands of functional group O-H, C-H, C=O, C=C and benzene ring have similar band wavelength to that of nutmeg. From these results it could be concluded that active component responsible for activity of nutmeg was probably due to myristicin (Figure 4).

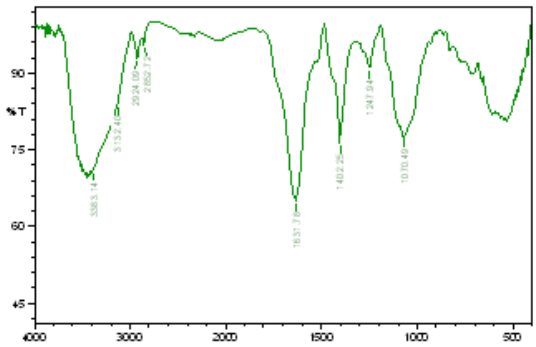


Figure 3
FTIR analysis of star anise extract

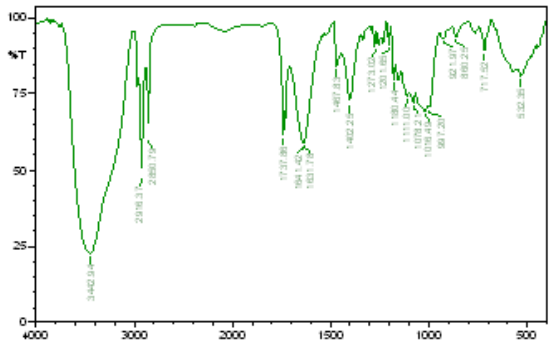


Figure 4
FTIR analysis of nutmeg extract

Asafoetida

The absorption at 3415.93cm^{-1} was due to the stretching of hydroxyl groups present in the extract. The bands at 2924.09 and 2854.65cm^{-1} were due to the C-H symmetric stretching of saturated (sp^3) carbon. The band at 1745.58cm^{-1} was assigned to C=O stretch. The bands between 1535.34– 1386.82 cm^{-1} indicated the presence of NO_2 group. The bands from 929.69- 713.66cm^{-1} indicated the presence of benzene ring in the sample. The bands at 1242.16, 1155.36 and 1078.221cm^{-1} were assigned to the strong intensity C-O skeletal vibrations. The molecular structure was similar to ferulic acid as bands of functional group O-H, C-H, C=O, C-O and benzene ring have similar band wavelength to that of asafoetida. Results suggests that the active component responsible for activity of asafoetida may be ferulic acid (Figure 5).

Coriander

The absorption at 3390.86cm^{-1} was due to the stretching of hydroxyl groups that were present in the extract. The bands at 3007.02 and at 2926.01 and 2854.65cm^{-1} were due to the C-H asymmetric and symmetric stretching of saturated (sp^3) carbon, respectively. The band at 1745.58cm^{-1} was assigned to C=O stretch. The band at 1629.85cm^{-1} indicated the presence of benzene ring in the sample. The bands at 1147.65 and 1035.77cm^{-1} were assigned to the strong intensity C-O skeletal vibrations. The molecular structure was similar to geraniol as bands of functional group O-H, C-H (sp3), C=O, and isopropyl have similar band wavelength to that of coriander. Thus it could be mentioned that active component responsible for activity of coriander may be geraniol (Figure 6).

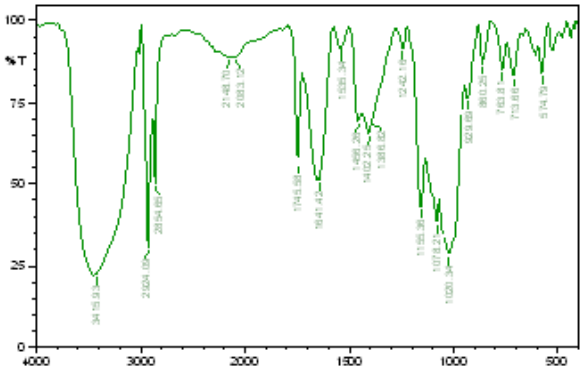


Figure 5
FTIR analysis of asafoetida extract

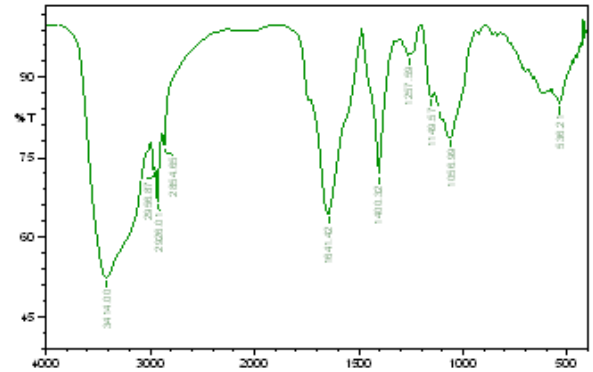


Figure 6
FTIR analysis of coriander extract

Ajwain

The absorption at 3390.86cm^{-1} was due to the stretching of hydroxyl groups that were present in the extract. The bands at 3007.02 and at 2926.01 and 2854.65 cm^{-1} were due to the C-H asymmetric and symmetric stretching of saturated (sp^3) carbon, respectively. The band at 1745.58cm^{-1} was assigned to C=O stretch. The band at 1629.85cm^{-1} confirmed the presence of benzene ring in the sample. The bands at 1147.65 and 1035.77cm^{-1} were assigned to the strong intensity C-O skeletal vibrations. The molecular structure was similar to thymol as bands of functional group O-H, C-H, C-C, isopropyl and C-O have similar band wavelength to that of ajwain. These results suggested that active component responsible for activity of ajwain may be thymol (Figure 7).

Fenugreek

The absorption at 3383.14 cm^{-1} was due to the stretching of hydroxyl groups that were present in the extract. The bands at 3010.88 and at 2956.87, 2926.01and 2854.65cm^{-1} were due to the C-H asymmetric and symmetric stretching of saturated (sp^3) carbon, respectively. The band at 1745.58cm^{-1} was assigned to C=O stretch. The bands at 1639.49, 1546.91 and 1402.25cm^{-1} confirmed the presence of benzene ring in the sample. The bands between 1240.23-1072.42cm^{-1} are assigned to the strong intensity C-O skeletal vibrations. The molecular structure was similar to trigonelline as bands of functional group O-H, C-H, C=O and C-O have similar band wavelength to that of fenugreek. From these results, it could be understood that active component responsible for activity of fenugreek may be trigonelline (Figure 8).

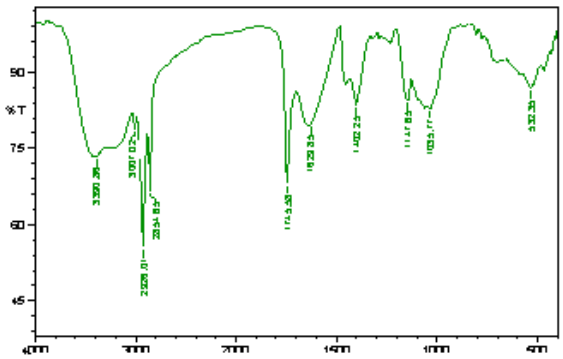


Figure 7
FTIR analysis of ajwain extract

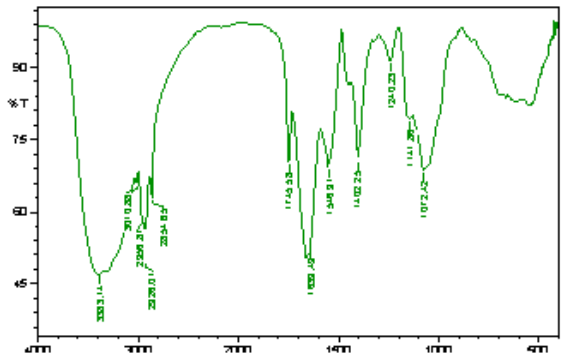


Figure 8
FTIR analysis of fenugreek extract

DISCUSSION

The main objective of this work was to analyze the active compounds such as phytochemicals present in spices for their antibacterial and antioxidant effects. Aqueous and polar solvents were used for extraction of active components. MIC values were also determined against all bacteria that were tested and were found to be between 12.5- 50 mg/ml. The antibacterial activity of spices was mainly due to the active components present in them such as pipericine (black pepper), myristicin (nutmeg), thymol (ajwain), ferulic acid (asafoetida), shikimic acid (star anise), geraniol (coriander), menthol (mint) and trigonelline (fenugreek) [3,11]. The antibacterial activity was expressed at varying degrees with the activity being both bacterial strain and dose dependent. The various crude extracts of all the spices showed significant activity against all the bacteria tested. The results of the present work showed that these spices possess potent antimicrobial activity suggesting that the spices contain effective active constituents responsible for either eliminating or inhibiting the bacterial pathogens.

Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress[12,13]. Spices and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds that act by inhibiting or preventing the deleterious consequences of oxidative stress[14,15]. The DPPH method was used to find the free radical scavenging activity of spices with ascorbic acid as reference standard. In the presence of the extract capable of donating an H atom, DPPH undergoes reduction showing a color change from deep violet to yellow that was measured by the decrease in its absorbance at 517 nm. The use of a stable DPPH radical has the advantage that it is unaffected by side reactions, such as enzyme inhibition and metal chelation. From the above results, it is clear that crude extracts of spices demonstrated good ex-vivo antioxidant activity, suggesting its therapeutic value for human health. FTIR analysis was done for structural determination of active compounds present. Some of the known active constituents were shikimic acid of star anise, volatile terpenes and thymol of ajowain, menthol of mint, ferulic ester and sulfur containing volatile oil of asafoetida, trigonelline alkaloid of fenugreek and myristicin nutmeg. The molecular structures of spices taken were similar to active compounds like myristicine, ferulic acid, shikimic acid, trigonelline etc as bands of some functional group like O-H, C-H, C=O, C-O etc have similar band wavelength to that of spices. Thus comparative analysis can be done between the spices and active constituents. Further research is required to determine the different antibacterial compounds present in these spices and their complete spectrum of efficacy. These ethno-medical spices and herbal resources or their combinations open the prospect of finding new clinically efficient antimicrobial compounds.

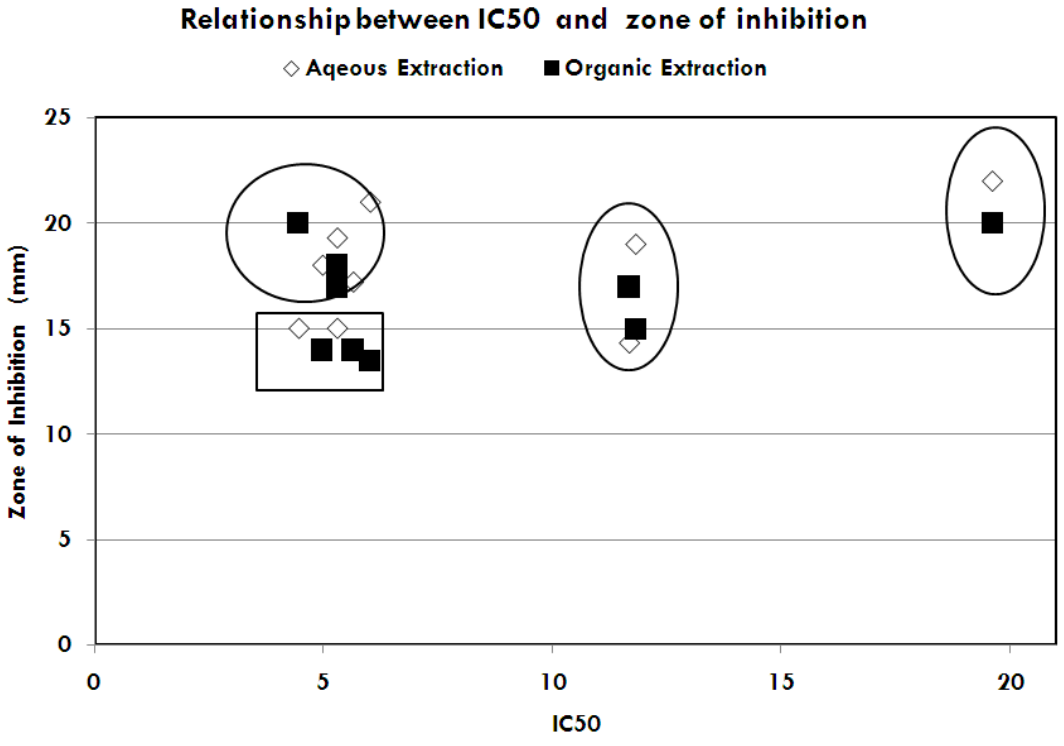


Figure 9
Relationship between inhibition concentration (IC50) and the zone of inhibition.

Results of inhibition concentration (IC50) and the zone of inhibition were presented in Figure 9. Based on these results, four different types of grouping can be identified. The coriander leaves are found to have both high IC value and inhibition zone and asafoetida and fenugreek formed a group having medium levels of IC 50 values with high levels of inhibition whereas all other spices were found to have low IC values and high inhibition zones. Further work needed to done to correlate with various other parameters to elucidate the antibacterial processes.

CONCLUSION

Nowadays, microbes are increasingly developing resistance against the drugs in use. Natural products from plants may give us a solution to combat against these drug resistant microbes. Spices, condiments and herbs, used fresh or as extracts have been reported to inhibit some microorganisms. Active compounds are chemical components present in spices which have therapeutic values. Some of the active compounds considered in this study were piperine of black pepper, shikimic acid of star anise, ferulic acid of asafoetida etc. The spice extracts used in this study were found to be effective antibacterial agents against clinical human pathogens**.** It may be concluded that the aqueous as well as organic extracts can be used as a potential source of natural antimicrobial compound which if applied to food products preservation. The antioxidant activities of methanolic extracts from 8 different spices were also determined. The results underlined that the methanolic fractions possess a free radical scavenging ability and reducing power effect against DPPH. Hence, the consumption of these spices would exert several beneficial effects by virtue of their antioxidant activity. Also**,** FT-IR analysis was done to identify the active compounds responsible for the spice's antimicrobial and antioxidant activity. Further studies should be undertaken to elucidate the exact mechanism of action by which extracts exert their antimicrobial effect. Finally, it can be concluded that the active chemical compounds present in spices should certainly find place in treatment of various bacterial infections. The results from the present study are very encouraging and indicate that further research needs to be done to explore their potential for future prospects.

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