Original Research Article **Microbiology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

ANTIMICROBIAL ACTIVITY OF CLOVE EXTRACTS AGAINST FOOD BORNE PATHOGENS *ESCHERICHIA.COLI, SALMONELLA.TYHIMURIUM, STAPHYLOCOCCUS. AUREUS AND LISTERAI .MONOCYTOGENES AND GC-MS ANALYSIS OF EXTRACTS*

BHARATH M R[1]*, M.A.AZEEM[2], KEERTHAN H V[3]

*[*1,3]Research Scholar, Research and development centre, Bharathiar University, Coimbatore, Tamil Nadu, India*
[2]Professor, Department of Pharmacognosy, Al-Ameen College of Pharmacy, Bangalore, Karnataka, India

ABSTRACT

The essential oil of flower buds (clove) of *Syzygium aromaticum* was obtained by soxhlet extraction with water, petroleum ether and ethyl acetate. The extracts were tested for the antimicrobial activity against Gram-positive and Gram-negative bacteria such as *Escherichia coli* (MTCC433), *Salmonella typhimurium* (MTCC98), *Staphylococcus aureus* (MTCC96), and *Listeria monocytogenes* (MTCC1143). Clove extracts showed antibacterial activity against all tested bacteria with zone inhibition ranged from 10mm-30mm. Antibacterial activity of clove is attributed to high eugenol, caryophyllene and farnesol content. Many components were identified by Gas chromatography-mass spectrometry, major constituents were eugenol, vanillin, humulene, ledol, adamantane derivative, caryophyllene, farnesol, alizarin and these compounds could be used as therapeutic, medicinal and preservative agents.It is concluded that this plant material can be indispensable source for secondary metabolites.

KEYWORDS: ***Antimicrobial activity, cloves, essential oils, GC-MS, soxhlet extraction, zone of inhibition.***

BHARATH M R*

Research Scholar, Research and development centre, Bharathiar University, Coimbatore, Tamil Nadu, India

Received on: 05-04-2017
Revised and Accepted on: 25-09-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.4.b321-329

INTRODUCTION

Food infection and intoxication are considered as the most common causes of food borne diseases worldwide. Food borne pathogens causing these diseases find their way in foods through cross contamination, improper handling and temperature abuse. *Escherichia coli, Staphylococcus aureus*, *Salmonella sp*. and are among the common food borne microorganisms that cause infection and intoxication. Food spoilage microorganisms such as *Pseudomonas aerugenosa*, *Bacillus subtilis*, *Lactobacillus sp*., *Saccharomyces cerevisiae*, on the other hand, cause products to lose their quality which renders them unacceptable for consumption. Short shelf-life of food products because of spoilage is one of the major problems of the food industry.[1-2] New alternatives to control these microorganisms are being explored. Recent reports indicate that *Salmonella sp*., *Escherichia coli* and *Listeria monocytogenes* are responsible for economic losses amounting to 5.9 billion dollars each year. Moreover, in last two decades, antibiotic resistance is an emerging problem worldwide. Tremendous use of antibiotics has developed multiple drug resistance (MDR) in many bacterial pathogens. At the same time, there is a growing demand among consumers for natural preservatives or additives in processed foods.[3-5] In comparison to chemical or synthetic additives herbal additives are preferred as these are safer, flavor enhancer and without any side effects. Herbal extracts are fast becoming popular as natural antimicrobial preservatives or additives.[6-8] This has led to the search for new, safe and effective antimicrobial agents from alternative natural resources like plant products. Traditionally the people of India have a long standing practice of using extensive diversity of plant products in treatment of diseases. 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.[9-10]They are used for stabilizing several food items from deterioration.[11] Spices are considered as rich sources of bio-active antimicrobial compounds.[12] Several studies have confirmed the antimicrobial activity of spices such as clove, cinnamon, mustard seed, mint, garlic, ginger, sage, thyme, rosemary, ajowan, pepper extracts against different types of microbes, including food borne pathogens are due to specific phytochemicals or essential oils.[13-16] The use of spices or herbs is gradually increasing not only in developing countries but also in developed countries. Spices offer a promising alternative for food safety.[17] However, there is limited information regarding its antimicrobial activity against food borne pathogens is available. The objective of the study is to evaluate the phytochemical properties and antimicrobial activity of the clove extract against food borne pathogens.

MATERIALS AND METHODS

Test microorganisms

Food borne pathogenic bacteria were procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India, and the cultures were sub cultured and preserved under paraffin oil. *Escherichia coli* (MTCC433), *Salmonella typhimurium* (MTCC98), *Staphylococcus aureus* (MTCC96), *Listeria monocytogenes,* (MTCC1143) were used in the study.

Preparation of Clove extract

The plant material cloves (*Syzygium aromaticum*) were collected from southern India, by observing its physical characters, like shape, smell (Kerala, India). It was dried in the laboratory for 4 to 5 days. Dried sample was then grounded by using motor and pestle. Soxhlet extraction method was followed for the essential oil extracts from the solvents[33] and water extracts prepared by shaking the sample with the water, overnight (Table 5). Cold aqueous extraction: Ten grams of the powder was soaked in 100ml cold sterile distilled water in a conical flask and left undisturbed for 24h, then filtered off using a sterile Whatman filter paper no1[34] (Ogundiya etal., 2006). The filtered extract was concentrated under vacuum below 40^0C using a rota-evaporator

Extraction of essential oils (Soxhlet extraction)

Sample of 15-20gm is weighed and packed in a cellulose thimble. Meanwhile, round bottom flask either of 500ml with solvent is prepared. Thimble with sample was placed in a soxhlet extractor and round bottom flask was filled with the ethyl acetate & petroleum ether seperately . Extraction was carried out for minimum 8 hrs to maximum requirement within limit of boiling point temperature of solvent (ethyl acetate 75-80°C and petroleum ether 40-60°C, both the extracts were used separately). Finally, solvent was evaporated in a rotavaporator at 40°C.[18] (Table 5)

Antimicrobial sensitivity testing

The antimicrobial activity of the clove (*Syzygium aromaticum*) extract was determined according to the method of Bauer.[19] Eight mm sterile discs (code DD036) were procured from the HiMedia, Laboratory Pvt.Ltd., Mumbai, India and were impregnated with 50µl of different concentration of each clove extracts before being placed on the inoculated agar plates. The inoculums of the test organisms were prepared by transferring a loopful of culture into 9 ml of sterilized nutrient broth (HiMedia) and incubated at 37^oC for 5 to 6 hrs. The bacterial culture was compared withMcFarland Standard 1 (MacFarland standard 1 is equal to $3.0x10^8$cells[20]). One hundred microlitres bacterial culture was spread on pre dried agar plates with the help of glass spreader. After the inocula dried, the impregnated discs were placed on the agar surface using forceps dipped in 70% IPA and flamed, and were gently pressed down to ensure contact. Plates were kept at 4^oC for 30 to 60 min for better absorption, during this time microorganisms will not grow, but absorption of the extracts will take place. The inoculated plates containing the impregnated discs were incubated in an upright position at 37^oC for 24 to 48 hrs. The results were expressed as the zone of inhibition around the 8mm discs. All the analyses were applied in triplicate.

GC-MS analysis

The Gas chromatography-mass spectrometry (GC-MS) analysis using Trace GC-MSD (Agilent) was performed with a gas chromatograph ultra- Trace equipped with HP-5MS capillary column (30mx0.25mm; coating

thickness 0.25µm) and mass detector.[21] Temperatures of the transfer line and the ionic source were 150^{0}C and 230^{0}C, respectively; scan range, 50-500 amu; 3.9 scans/s. Oven temperature programmed from 120^{0}C and 280^{0}C ramp of 50^{0}C /min; injector temperature was 280°C; carrier gas helium at 1 ml/min; injection of 1µl split less mode. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series to C–C alkanes, and on computer matching against commercial (NIST-MS) and laboratory-developed library mass spectra built up from pure substances and components of known oils and MS literature data, GC-MS (Graph 1-3).

RESULTS

The antibacterial activity of clove (*Syzygium aromaticum*) extracts with distilled water, ethyl acetate and petroleum ether were tested using different bacteria with zone inhibition ranged from 10mm-30mmand are indicated in Table 1. It was found that the ethyl acetate extract of clove was potentially active against all the test organisms *E.coli, S. typhimurium*, *S. aureus* and *L. monocytogenes* with zones of inhibition ranging from 14mm to 20mm. Antibacterial activity with maximum zone of inhibition 20mm was obtained against *E.coli* and *L. monocytogenes,* followed by 18mm in *S.aureus* whereas, it was least 14mm in *S. typhimurium*. However, water extracts exhibited lower antibacterial activity against all bacterial strains tested. The petroleum ether extract of clove showed widest zone of inhibition for *S. aureus* (30mm), *L. monocytogenes* (22mm). *E. coli* and *S. typhimurium* were also showed maximum zone of inhibition 18mm with petroleum ether extract of clove.

Table 1
Antibacterial activity of clove extracts measured as diameter (mm) of zone of inhibition

Extracts	Food Pathogens			
	E. coli	*S. typhimurium*	*S. aureus*	*L. monocytogenes*
Distilled Water	10	10	15	10
Ethyl acetate	20	14	18	20
Petroleum ether	18	18	30	22

Each value is the average of three independent replicates
Volume of extract/ oil in each well = 50µL

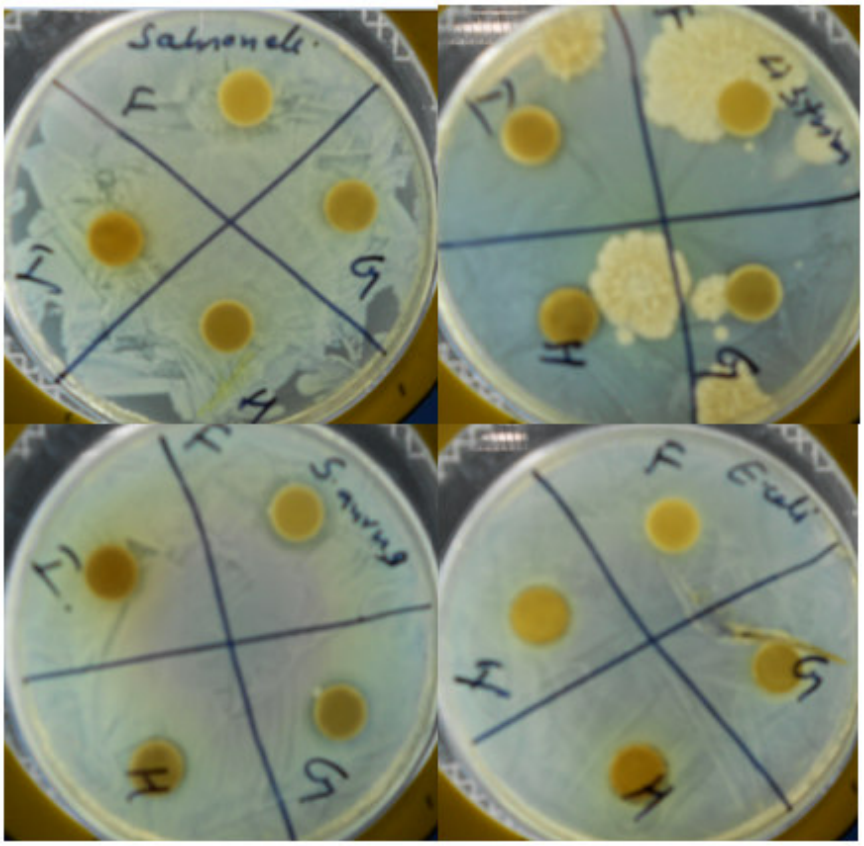

Figure 1
Antimicrobial images of water extracts, H—water extracts

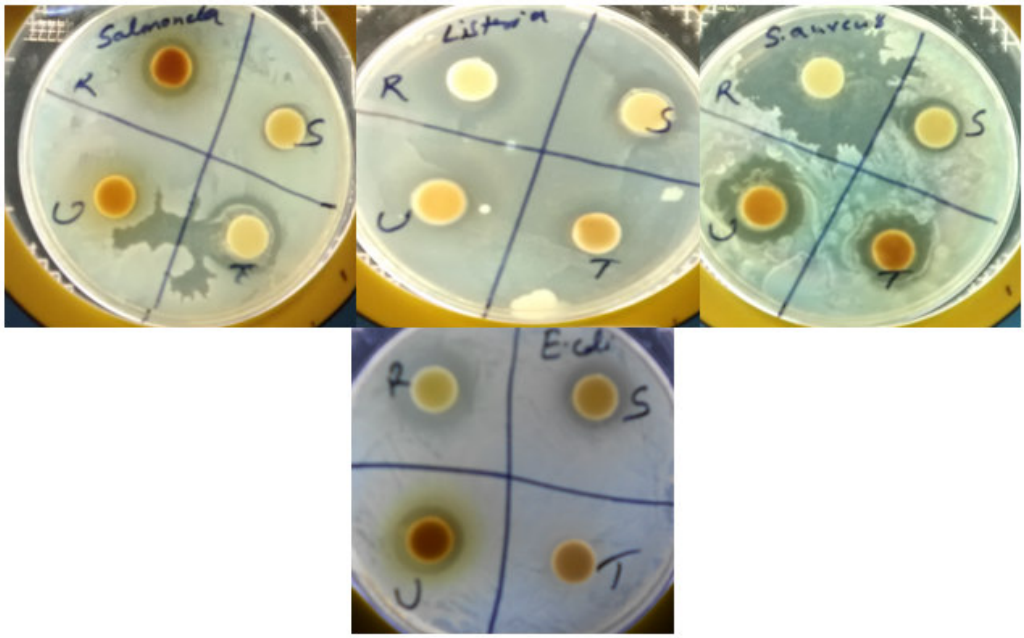

Figure 2
Antimicrobial images of water extracts, R—Petroleum ether extracts , S--Ethyl acetate extract

Cloves Extract

The GC-MS analyses (Graph 1-3) of the water, ethyl acetate and petroleum ether extracts of clove are presented in Table 2-4. Eugenol and caryophyllene are the major components are known to possess antibacterial and antifungal properties. The content of eugenol is high in ethyl acetate (34.08%) and petroleum ether (33.27%) extracts.Whereas, in water extract eugenol content is low (10.13%). In addition to eugenol in petroleum ether extract of clove, caryophyllene, farnesol and humulene significantly contributed additional antibacterial activity.

DISCUSSION

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. In the present work, ethyl acetate and petroleum ether extracts obtained from cloves showed strongest activity against all foodborne pathogens tested. Results obtained for clove extracts were found to be similar as also reported by several workers.[21-26] High eugenol content which is an antimicrobial compound having wide spectra of antimicrobial effects which may contribute to growth inhibition of enterobacteria.[27-28] Hence the antibacterial and antifungal properties demonstrated by clove and clove oil can be attributed to the compounds reported.[29-30] The components with phenolic structure such as eugenol are highly active against the test microorganisms. Members of this class are known to be either bactericidal or bacteriostatic agents, depending upon the concentration used. The modes of action by which microorganisms are inhibited by essential oil and their chemical compounds seem to involve different mechanisms. It has been hypothesized that the inhibition involves phenolic compounds, because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability, unavailability of vital intracellular constituentsand impairment of bacterial enzymes systems.[31] Identified compounds are having very good benefits to humans in many different ways, majeroly eugenol, Eugenol has been classified as 'generally recognised as safe (GRAS)' by the U.S. Food and Drug Administration. Eugenol was evaluated in vivo for the prophylaxis and treatment of experimental vaginal candidiasis on immunosuppressed rats. The results indicated that prophylactic eugenol treatment after 10 days of inoculation reduced the number of colonies of C. albicans in the vaginas of infected rats by 98.9%[35] Mequinol 2%/tretinoin 0.01% solution was found to be highly effective and well tolerated treatment for solar lentigines and related hyperpigmented lesions on the forearms and of similar efficacy for lesions on the face[36]. Inhibition of the bacterial DNA or enzymes has been proposed to be the influential mechanism for the inhibitory action of the thiazole derivatives. Inhibition of the bacterial enzyme ecKASIII (or FabH) (that is essential for the synthesis of fatty acids in Gram negative and gram positive bacteria) and the enzyme DNA-gyrase (that is needed to replicate the bacterial DNA) have been studied in some research works [37,38].Isoquinolines find many applications, including anesthetics antihypertension agents, such as quinapril, quinapirilat, and debrisoquine (all derived from 1,2,3,4-tetrahydroisoquinoline. antifungal agents, such as 2,2′-hexadecamethylenediisoquinolinium dichloride, which is also used as a topical antiseptic. This derivative, shown below, is prepared by N-alkylation of isoquinoline with the appropriate dihalide. Disinfectants, like N-laurylisoquinolinium bromide , which is prepared by simple N-alkylation of isoquinoline. Brucine has been shown to have good anti-tumor effects, on both hepatocellular carcinoma and breast cancer, its narrow therapeutic window has limited its use as a treatment for cancer.Brucine is also used in traditional Chinese medicine as an anti-inflammatory

and analgesic agent, as well as in some Ayurveda and homeopathy drugs.

STATISTICAL ANALYSIS

Mean and standard deviation of the antibacterial activity of different extracts

Mean and standard deviation[32] of the antibacterial activity of the different extracts are shown in Table 2. In the present investigation, the clove extracts showed inhibitory activity against all the four food associated bacteria in which the diameter of zone of growth inhibition varied between 5 and 18.7mm. The clove extract showed highest diameter of zone of inhibition of 18.7mm against *S,aureus* followed by *E,coli* (16mm) and *L. monocytogens* (15.7mm) and *S. typhimurium(13.3mm)*

Table 2
Mean and standard deviation

Extract	*E. coli*	*S. typhimurium*	*S. aureus*	*L. monocytogenes*
Distilled Water	10	8	8	5
Ethyl acetate	20	14	18	20
Petroleum ether	18	18	30	22
Average	16.0	13.3	18.7	15.7
Standard deviation	4.32	4.11	8.99	7.58

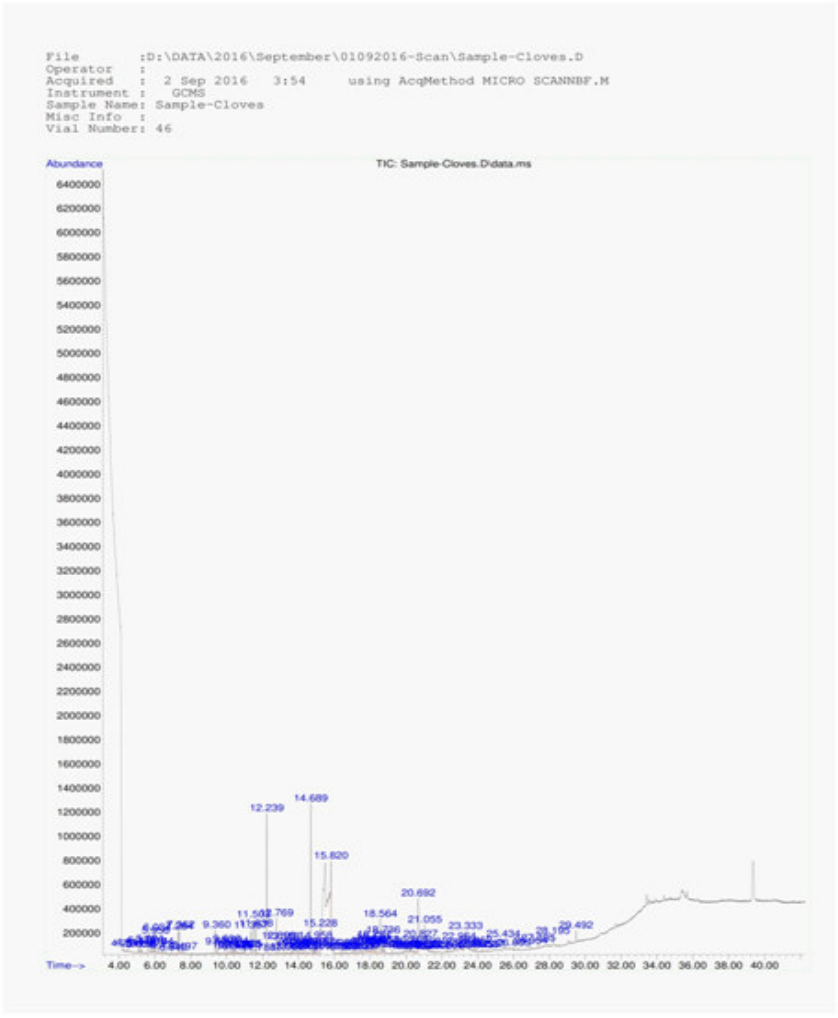

Figure 1
Chromatogram of Clove powder water extract

Table 3
Compounds found in the cloves water extract

S.No.	Compound Name	RT	Area%
1	2H-Pyran, 3,4-dihydro-	4.254	0.23
2	4-Piperidinone, 1-methyl-	5.178	0.45
3	Pyrrolidine, 1-(1-oxoheptadecyl)-	5.178	0.45
4	Propanamide,	5.266	0.23
5	Mequinol	7.497	0.21
6	Thiazole	10.958	0.36
7	Eugenol	12.239	10.13
8	Vanillin	12.898	1.25
9	Homovanillic acid	13.874	0.77
10	Pentasiloxane, dodecamethyl	14.491	0.18
11	Imidazolidinethione	15.820	7.31
12	Phytol	16.380	0.31
13	Caffeine	19.249	0.32

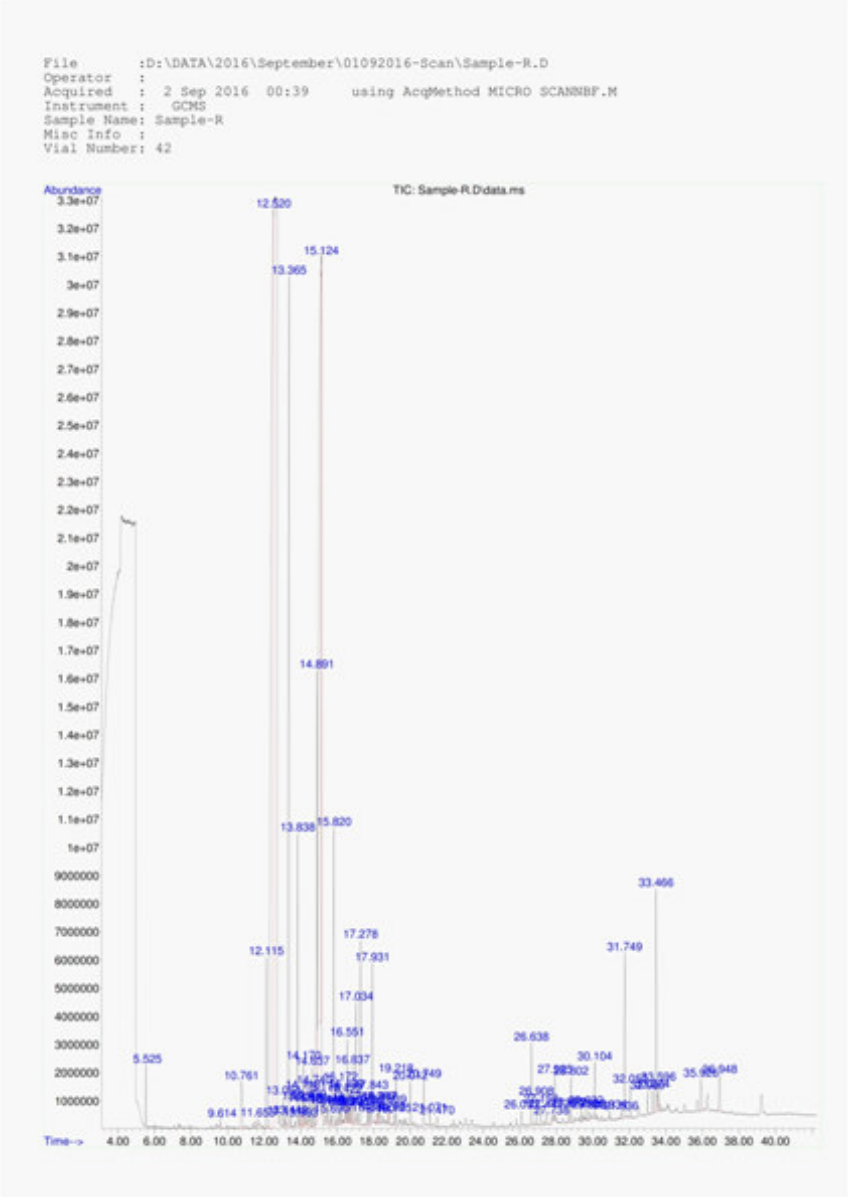

Figure 2
Chromatogram of Clove powder petroleum ether extract

Table 4
Compounds found in clove petroleum ether extract

Sl.no.	Compound Name	RT	Area%
1	Eugenol	12.520	33.27
2	Vanillin	13.064	0.18
3	Caryophyllene	13.365	11.51
4	Humulene	13.838	2.33
5	Aromandendrene	13.931	0.17
6	Alloaromadendrene	13.931	0.17
7	Homovanillyl alcohol	13.993	0.08
8	Gamma Muurolene	14.139	0.28
9	Ledol	16.089	0.15
10	Adamantane	16.551	0.55
11	tau.-Muurolol	16.795	0.10
12	Farnesol,	19.218	0.39
13	Furan	20.012	0.32
14	Alizarin	26.908	0.21
15	Isoquinoline	27.738	0.09
16	Pivalamide	27.738	0.09
17	Brucine	31.536	0.08

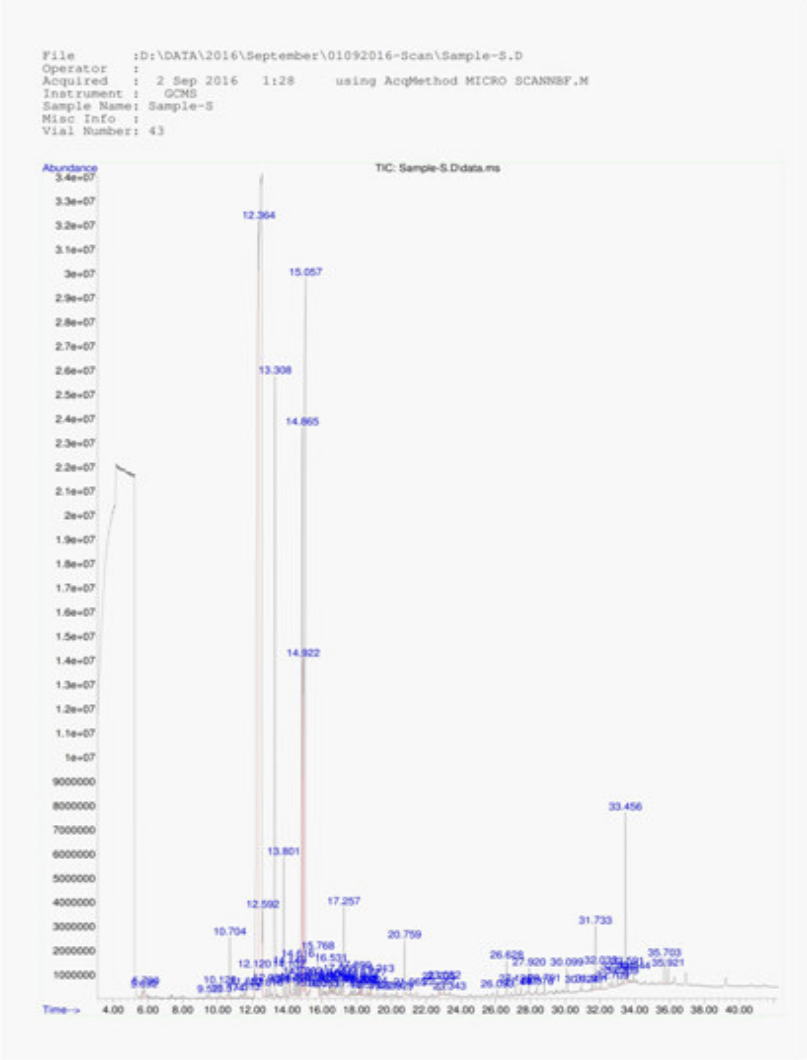

Figure 3
Chromatogram of Clove powder ethyl acetate extract.

Table 5
Compounds found in the cloves ethyl acetate extract

S. No.	Compound Name	RT	Area%
1	Aspirin methyl ester	9.521	0.09
2	Eugenol	12.364	34.08
3	6-Methylnicotinic acid	17.890	0.27
4	photocitral	18.824	0.08
5	Cycloeicosane	21.065	0.12

Table 6
Total yield obtained from the Extract this yield refers to the extracts which retainded after drying in rotavapour

Solvent	Yield in gms
petroleum ether	2.80
ethyl acetate	1.73
Water	0.50

CONCLUSION

Plants have been extensively studied in terms of pharmacological activity of its major components. In recent years, emphasis of research has been on utilizing traditional medicines that have long and proven history of treating various diseases. From the above observation and results In the current investigation, clove extract has been selected after study of such a selected plant with water extracts, petroleum ether extracts and ethyl acetate extract gave higher yield of chemical constituents like eugenol,brucine thymol, Isoquinolines, Humulene and many others ,Many studies were conducted on the medicinal uses of these compound, but availability of these compounds in the cloves were explored in this research. Our results signify the fact that natural products like spices can be seen as alternatives to chemical preservatives used in various food industries so as to minimize their side effects and simultaneously improving the shelf life of the food products. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds.

.

ACKNOWLEDGEMENTS

We are thankful to Mr. Nagabhushan H.P. for his help in GC-MS analysis. We are also grateful to MTCC, IMTECH, Chandigarh from where necessary strains were obtained.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Jay JM. Modern Food Microbiology. 6th ed. Aspen Publishers, Inc., Maryland. 2000. P 679.
2. Ray B. Fundamental Food Microbiol. 3rd ed. CRC Press, Boca Raton, Florida. 2004.
3. Walsh C. Molecular mechanisms that confer antibacterial drug resistance. Nature. 2000;406: 775-81.
4. Cohen ML. Changing pattern of infectious disease. Nature. 2002;406: 762-67.
5. Gutierrez J, Barry-Rayan C and Bourke P. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. Int. J. Food Microbiol. 2008;124: 91-7.
6. Akarpat, A, Tuthan S and Ustun NS. Effects of hot water extracts from myrtle, rosemary, nettle and lemon balm leaves on lipid oxidation and color of beef patties during frozen storage. J. Food Processing. Preservation. 2008;32(1): 117-32.
7. Pazos M, Alonso A, Sanchez I and Medina I. Hydroxytyrosol prevents oxidative deterioration in foodstuffs rich in fish lipids. J.Agri. Food Chem. 2008;56(9): 3334-40.
8. Cox S, Abu-Ghannam N and Gupta S. An assessment of the antioxidant and antimicrobial activity of six spices of edible Irish seaweeds. Int, Food Res. J. 2010;17:205-20.
9. Raman M and Gul S. Antibacterial activity of hydrodistilled essential oil of Psmmogetoncanescens N.O.Umbelliferae. Biotechnol. 2002;1: 55-60.
10. Nair R and Chanda S. Activity of some medicinal plants against certain pathogenic bacterial strains. I.J.Pharmacol. 2006;38:142-4.
11. Kizil S and Sogut T. Investigation of antibacterial effects of spices. Crop Res. 2003;3:86-90.
12. Lia PK and Roy J. Antimicrobial and Chemopreventive properties of herbs and spices. Curr. Med. Chem. 2004;47(2): 234-8.
13. Avato P, Tursil E, Vitali C, Miccollis V, Caddido V. Allyi sulfide constituents of garlic volatile oil as antimicrobial agents. Phytomed. 2000;7: 239-43.
14. Singh G, Kapoor IP, Pandey SK, Singh UK and Singh RK. Studies on essential oils: Part 10. Antibacterial activity of volatile oils of some spices. Phytotherapy Res. 2002;16(7): 680-2.
15. Sadic O and Ozean M. Antimicrobial activity of Turkish spice hydrosols. Food Control. 2003;14(3):141-3.

16. Hara-Kudo Y, A Kobayashi, Y Sugita-Konishi and K Kondo. Antibacterial activity of plants used in cooking for aroma and taste. J. Food Protect. 2004;67: 2820-4.
17. Arora DS and Kaur GJ. Antimicrobial activity of some Indian medicinal plants. J. Nat. Med. 2007;61:313-7.
18. Amit Pandey1and Parul Singh. Antibacterial activity of *Syzygium aromaticum* (clove) with metal ioneffect against food borne pathogens R&D Division, MRD Life Sciences, Lucknow, INDIA Amity University, Lucknow, INDIA Asian Journal of Plant Science and Research. 2011;1(2): 69-80.
19. Bauer AW, Kirby WMM, Sherris JC and Turck M. Antibiotic susceptibility testing ,by a stsndardized single disk method,Am J Clin pathol.1966;45: 493-6.
20. Carlos Abeyta Jr,Compendium of Methods for the Microbiological Examination of Foods,4th edition. United States of America December 17, 2013
21. Advances in Pharmacological Research of Eugenol Xiaojun KONG, Xiwang LIU, Jianyong LI, Yajun YANG Key Laboratory of Veterinary Pharmaceutical Development, Ministry of Agriculture/Key Laboratory of New Animal Drug Project, Gansu Province, Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS, Lanzhou 730050, China CurrOpin Complement Alternat Med 1:1, 8-11; January/February 2014;
22. Li Y, Xu C, Zhang Q, Liu JY and Tan RX. *In vitro* anti-*Helicobacter pylori* action of 30 Chinese herbal medicines used to treat ulcer diseases, *J. Ethnopharmacology*98 2005; 329-33.
23. Betoni JE, RP Mantovani, LN Barbosa, LC De-Stasi and FA Junior. Synergism between plant extract and antimicrobial drugs used on Staphylococcus diseases. Mem. Inst. Oswaldo Cruz. 2006;101(4): 387-90.
24. Fu Y, Zu Y, Chen L, Shi X, Wang Z, Sun S. and Efferth T. Antimicrobial activity of clove and rosemary essential oils alone and in combination. *Phytother. Res.* 2007; 21: 989-94.
25. Sonia Sethi, Aparna Dutta,,B.Lal Gupta, Saksham Gupta. Antimicrobial activity of spices against isolated food borne pathogen. International Journal of Pharmacy and Pharmaceutical Sciences Vol 5, Issue 1, 2013 2013;5(1):260-62.
26. Sharma Revathi, Chapagain Bipin, Pai (Bhat) Chitra. In Vitro antibacterial activity of seven spices against clinical isolates of enterococci. . International Journal of Pharmacy and Biological Sciences 2013;3(1): 298-304.
27. Md. MahfuzulHoque, ML Bari, Vijay K Juneja and S Kawamoto. Antimicrobial activity of cloves and cinnamon extracts against food borne pathogens and spoilage bacteria and inactivation of Listeria monocytogenes in ground chicken meat with their essential oils.Rep. Nat'l. Food Res. Inst 2008;72: 9-21.
28. Lee KG and Shibamoto T. Antioxidant property of aroma extract isolated from clove buds (*Syzygiumaromaticum*(L.) Merr. Et Perry), *Food Chemistry*. 2008; 74: 443–8.
29. Pawar VC and Thaker VS. *In vitro* efficacy of 75 essential oils against *Aspergillus niger*, Mycoses. 2006;49:316 -23.
30. Prashar A, Locke IC and Evans CS. 2006. Cytotoxicity of clove (*Syzygium aromaticum*) oil and its major components to human skin cells, Cell Proliferation. 2006; 39: 241–8.
31. Ayoola GA, Lawore FM, Adelowotan T, Aibinu IE, Adenipekun E, Coker HAB and Odugbemi . Chemical analysis and antimicrobial activity of the essential oil of *Syzigium aromaticum*(clove), African Journal.of Microbiology Research.2008; 2: 162-6.
32. Gupta C, Garg AP and Uniyal RC. Antibacterial activity of Amchur (dried pulp of unripe *Mangiferaindica*) extracts on some food borne bacteria. J. Pharm.Res.2008;1: 54-7.
33. Hussain MB, Hannan A, Akhtar N, Fayyaz GQ, Imran M, Saleem S, Qureshi IA. Evaluation of the antibacterial activity of selected Pakistani honeys against multi-drug resistant Salmonella typhi. Bio Med Central complementary and alternative medicine. 2015 Feb 26;15(1):32.
34. Y.C.Wong, M. Y. Ahmad-Mudzaqqir and W.A. Wan-Nurdiyana Extraction of Essential Oil fromCinnamon (Cinnamomum Zeylanicum) Oriental Journal of Chemisty 2014, 30(1): 37-47
35. Antimicrobial Activities of some Nigerian Chewing Sticks Ogundiya, M.O.Okunade, M.B. and Kolapo, A.L Antimicrobial Activities of some Nigerian Chewing Sticks Ethno botanical Leaflets 2006. 10: 265-271.
36. Chami.F, Chami.N, A. Evaluation of carvacrol and eugenol as prophylaxis and treatment of vaginal candidiasis in an immunosuppressed rat model. Journal of Antimicrobial. Chemotherapy. 2004 Nov, 54, 909–914.
37. Jarratt M ,Mequinol 2%/tretinoin 0.01% solution: an effective and safe alternative to hydroquinone 3% in the treatment of solar lentigines. Cutis. 2004 Nov;74(5):319-22.
38. Cheng K, Xue JY, Zhu HL. Design, Synthesis and antibacterial activity studies of thiazole derivatives as potent eckAS inhibitors. Bioorganic and Medicinal Chemistry Letters 2013 juy15 ;23(14)4235–4238.
39. Brvar M, Perdih A, Oblak M, Masic LP, Solmajer T. In silico discovery of 2-amino-4-(2,4-dihydroxyphenyl) thiazoles as novel inhibitors of DNA gyrase B. Bioorganic and Medicinal Chemistry Letters 2010 Feb 1; 20(3) 958– 962.

Reviewers of this article

Dr.P.Padmavathi M.Sc,Ph.D

Professor and Head of the department of micrbiology
center for PG studies 18/3 9th main
Jayanagar 3rd block,Jain university
Bangalore-560011

Asst.Prof.Dr. Sujata Bhattacharya

Assistant Professor, School of Biological and Environmental Sciences, Shoolini University, Solan (HP)-173212, India

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript