Plant Archives Volume 20 No. 2, 2020 pp. 6077-6083 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

FUNCTIONAL CHARACTERIZATION, PHYTOCONSTITUENTS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF LEAF EXTRACT OF *SYZYGIUM AROMATICUM*

V.S. Murali and V.N. Meena Devi*

Department of physics, Noorul Islam Centre for Higher Education, Kumaracoil, Thuckalay (Tamilnadu), India.

Abstract

Syzygium aromaticum (*S. aromaticum*) commonly known as clove. The work is designed to study and evaluate the antibacterial potency of phytochemicals from *Syzygium aromaticum* leaves and characterized by FTIR. However, the present study analysis the qualitative phytochemical contents of aqueous, diethyl ether and ethanol extract of *Syzygium aromaticum* (clove) leaves. Phytochemicals are a powerful group of chemicals that are derived from natural resource, especially with plants origin. Phytochemical are important for its chemoprevention and chemotherapeutic effects. Phytochemicals are useful for the metabolization of food and drugs. Antioxidants are biomolecules which are cable to fight against the free radicals in the body. Free radicals are harmful if it is present in higher level. They are linked up with multiple illnesses, including diabetes, heart disease, cancer etc. Results revealed the phytochemicals namely alkaloid, flavonoids, steroids, phenol, terpenoids, saponins and tannins are present in the leaf extract. The antioxidant activity of the aqueous and ethanol extract was evaluated by ferrous ion chelating assay, nitric oxide radical scavenging assayand DPPH assay. The ethanol extract showed better radical scavenging activity than aqueous extract except the nitric oxide radical scavenging activity. FTIR results showed more than ten peaks for represent the main fine composition of the extract. The bands were Probable assign with the components present in various extracts. By this analysis, functional groups such as amines, amides and alkyl halides are present in all the two extracts. Antibacterial potential of solvents extracts of clove leaves was assessed against three Gram positive and three Gram negative bacteria. All the tested pathogens were sensitive to aqueous and ethanol extract of clove leaves. Ethanol extract of leaves shows maximum zone of inhibition against *Staphylococcus aureus* and *Bacillus subtilis* whereas aqueous extracts exhibited good activity against *Staphylococcus aureus*, *Bacillus subtilis*and *Streptococcus mutans*.This study illustrates that compounds present in *S. aromaticum* could be a potent against the microorganism tested and it can could be used for the advancement in developing different drugs in pharmaceutical industries.

Key words: *Syzygium aromaticum,* phytochemicals, antioxidants, antibacterial activity

Introduction

Syzygium aromaticum (*S. aromaticum*) commonly known as clove, from the Mirtaceae family is a median size tree grow upto 8-12 m and abundant in Maluku islands in East Indonesia. Among the spices, clove is a potent antioxidant and antimicrobial agent (Shan *et al*., 2005). The major components present in clove are phenolic compounds as flavonoids, hydroxybenzoic acid, hydroxycinnamic acid acids and hydroxyphenyl propane (Neveu *et al.,* 2010). In recent years, many reports confirm the spice plants have antibacterial, antifungal, antiviral and anticarcinogenic properties.In Ayurveda, cloves are mostly used as medicines in china and as an herb in western countries. It is also known to be much effective against symptoms of diarrhoea, gastric, vomiting and dental problem (Phyllis and James, 2000; Prashar *et al.,* 2006). Cloves have high content of flavonoids and antioxidants and very effective in mutagens, inflammation, ulcer and damages caused by free radicals (Kim *et al.,* 1998; Jin and Cho, 2011).

Clove bud oil traditionally as flavoring and antimicrobial agents in food products (Jasna *et al.*, 2013; Nazrul *et al.*, 2010). It possesses biological activities such as antibacterial, antifungal, insecticidal, antioxidant properties. Mahmoud *et al.* (2011) reported clove oil has therapeutic effects including anti-phlogistic, anti-vomiting, analgesic, antispasmodic, anti-carminative and antiseptic

***Author for correspondence :** E-mail : vndevi@gmail.com

and its Strong antibacterial activity is mainly due to the presence of eugenol in high levels. Living system produced free radicals or reactive oxygen species (ROS) which can able to damage the biomolecules and initiate the diseases (Gulcin *et al.,* 2012; Edziri *et al.,* 2011). Antioxidants can inhibit or slow down the oxidizing reactions occurring in the cells and protect cell damage causing by free radicals (Halliwell and Gutteridge, 2007). Plant based natural antioxidants gain much interest in recent days and it is employed as alternate antioxidant substance (Skerget *et al*., 2005). Plants have several antioxidative chemicals such as vitamins, carotenes, tannins, phenolics, etc. (Govindhan-Singh *et al*., 2013; Gian *et al*., 2012).

Based on literature search, it was found that there was no report on antioxidant activity of the leaves of *Syzygium aromaticum.*The aim of the study is to evaluate the functional characters of the phytochemical constituents, antioxidant and antibacterial effect of crude and different extracts of *Syzygium aromaticum* leaves. Hence the study focused on analysis of the chemical composition and functional group of the extract of *Syzygium aromaticum* leaves by phytochemical analysis and Fourier Transform Infrared Spectroscopy (FTIR) analysis.

Materials and Methods

Sample collection and processing

The matured leaves of *Syzygium aromaticum* was collected from Pathanamthitta, Kerala. The plant was taxonomically identified by Professor Dr. P. Nagendra Prasad, Head, Department of Biotechnology, Sri Paramakalyani College, Alwarkurichi, Tirunelveli. Freshly collected *S. aromaticum* leaves were washed in distilled water thoroughly to remove residues. Excess moisture was removed from the sterilized leaves.Then they were subjected to solvent and crude extraction.

Preparation of crude and solvent Extracts

About 10 g fresh leaves of *S. aromaticum* was macerated in mortar and pestle at room temperature and then filtered using muslin cloth under strict aseptic conditions and the filtrate crude sample was collected in fresh sterilized glass tubes and stored at 4°C until use (Goyal *et al.,* 2008). Fresh leaves were cut into small pieces and to 5 g of leaves 10 ml of solvent like ethanol, diethylether and aqueous were added separately and grounded with motor and pestle. The extracts were boiled at 60°C for 3 hours, kept overnight at 37°C and then filtered with Whatman No. 1 filter paper. The extracts were stored at 4°C.

Qualitative Phytochemical analysis

Phytochemical screening of leaf extracts of *S. aromaticum* subjected to qualitative phytochemical test for the presence of various active chemical constitutes such as carbohydrate, protein, amino acid, steroids, saponins, tannin, terpenoid, glycosides, alkaloid, flavonoid and phenol using standard procedure.

Determination of antioxidant activity

Ferrous ions chelating ability

The ferrous ion chelating potential of the extracts was evaluated by Dinis *et al*., (1994) method. The reaction mixture contained 1.0 ml of various concentrations of the extracts (2-10 mg/ml) and 0.05 ml of 2 mM $FeCl_3$. The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine. The reaction mixture was shaken vigorously and left standing at room temperature for 10 minutes and the absorbance of the reaction mixture was measured at 562 nm against a reagent blank. A lower absorbance of the reaction mixture indicated a higher ferrous ion chelating ability. The control contained all the reagents except sample. Ascorbic acid was used as standard for comparison.

Percentage of inhibition = [(Control- Test)/ control]×100

Nitric oxide radical scavenging assay

Nitric oxide was generated when sodium nitroprusside interacts with oxygen to produce nitrite ions which was measured by the Griess reaction. This assay was done by the procedure described by Green *et al*., (1982). The reaction mixture contained 3.0 ml of 10 mM sodium nitroprusside in phosphate buffered saline (pH 7.4) and various concentration of (2-10 mg/ml) extracts. The resulting solution was then incubated at 25°C for 60 minutes. To the incubated sample 5.0 ml of Griess reagent (1% sulphanilamide, 0.1% NEDD in 2% H_3PO_4) was added and the absorbance of the chromophore formed was measured at 546 nm against a reagent blank. Percentage inhibition of the nitrite ions generated is observed. The standard ascorbic acid and BHT was used for comparison. The free radical scavenging activity was determined by evaluating % inhibition as above.

DPPH radical scavenging activity

The free radical scavenging activity of the fractions was measured *in vitro* by $2, 2^2$ - diphenyl-1-picrylhydrazyl (DPPH) assay according to the standard method (Williams *et al.,* 1995). The stock solution was prepared by dissolving 24 mg DPPH with 100 ml of ethanol stored at 20°C until required. The working solution was obtained by diluting DPPH solution with ethanol and 3 ml aliquot

of this solution was mixed with 1 ml of methanol extracts at various concentrations (50, 100, 150, 200 and 250 µg/ml). The reaction mixture was shaken well and incubated in the dark for 15 min at room temperature. Then the absorbance was taken at 517 nm. The control was prepared without any sample and scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation.

Percentage of inhibition = [(control OD"sample OD)/(control OD)] × 100

FTIR Analysis

Fourier transform infrared (FTIR) spectroscopy is a technique used to obtain an infrared spectrum of absorption of solid, liquid or gas sample. For measurements the samples were air dried and mixed with potassium bromide in the ratio of 1: 100. FTIR spectrum of samples was recorded on Shimazdu IR Prestige-21 FTIR instrument with a diffuse reflectance mode (DRS-8000) attachment. All measurements were carried out in the range of 400-4000 cm-1 at a resolution of 4 cm-1 at room temperature using a double beam. This range was used to study the fundamental vibrations and associated rotational vibrational structure (Anjana and Anitha, 2012).

Antibacterial activity

Antibacterial activity of leaf extracts of *S. aromaticum* was determined by using agar disc diffusion method on Muller Hinton agar (MHA) medium (Salah-Alhashimi *et al.,* 2013). The bacterial strains were first cultured in a nutrient broth for 18 hours prior to use. Test organisms used are three Gram positive and three Gram negative bacteria. 25 µl of sample with 100 µg concentration is used as test sample. Streptomycin 25 µg was used as positive control and sterile disc (Hi-media) was used as negative control.

Result and discussion

Phytochemical screening

The result reveals that some of the phytochemicals were present in the extracts of *S. aromaticum*. The presence of active phytochemical constituents such as alkaloids, flavonoids, phenol, tannins, saponins, terpenoids and others are the basic reasons for a plant to exhibit medicinal activity. Carbohydrate, protein, alkaloid, flavonoids, steroids, phenol and terpenoids were present in ethanolic extract; protein, steroid, phenol and terpenoids were present in diethyl ether extract; whereas carbohydrate, protein, alkaloid, flavonoids, steroids, saponins, tannin and terpenoids present in aqueous extract of *S. aromaticum* table 1. Reports reveals presence of flavonoids helps as antioxidants Nahak and Sahu, (2011),

Table 1: Phytochemical analysis of *S. aromaticum* leaf extracts.

Test name	Ethanol	Diethyl ether	Aqueous
Carbohydrate	+	-	+
Protein	+	+	+
Alkaloid	+	-	+
Flavonoid	+	-	+
Glycoside	-	-	-
Steroid	+	+	+
Saponins	-	-	+
Phenol	+	+	-
Tannin	-	-	+
Terpenoids	+	+	+

saponins as anti-feedants in plants (Jain, 1987), tannins protect the plants from phytophagous insects and herbivores, terpenoids helps in phytoalexin property (Waqas-Ahmed, 2016). Similarly essential oil from *Syzygium caryophyllatum* revealed it contains flavanoids, steroids, tannins, terpenoids, alkaloids, saponins, and cardiac glycosides (Soni and Dahiya, 2014; Bhuiyan *et al.,* 2010).

Antioxidant activity

Antioxidant helps to prevent cell damage caused by free radicals, unstable molecules that are produced by our body.Naturally obtained antioxidants are very important due to their use in food as well as in the pharmaceutical industry as they have the ability to scavenge free radicals. As compared to synthetic drugs the antioxidant potential of medicinal plants and herbs are most important.Nitric oxide scavenging assay of all extracts were determined at five different concentrations (50, 100, 150, 200 and 250 µg/ml) and results of scavenging efficiency of extracts are depicted in table 2, 3, 4. The results revealed that scavenging is maximum (82.5%) at higher concentration of ethanol extract likewise the ferrous iron chelating assay was measured with five concentrations of all extracts. The ferrous iron chelating activity also increased with increasing concentration. The chelating power was 82.5% by the aqueous extract. Antioxidant activity of the extracts were also estimated through the DPPH radical scavenging activity. It was

Table 2: Nitric oxide scavenging activity of *S. aromaticum* leaf extracts.

Concentration	Ethanol	Aqueous	Standard (Ascorbic Acid)
50 µg/ ml	20.2±0.25	16.4±0.28	26.5±0.35
100 µg/ ml	44.5±0.32	27.1±0.26	34.9±0.60
150 µg/ ml	52.5±0.32	51.3±0.24	56.8±0.52
200 µg/ ml	71.4±0.25	63.4±0.29	77.0 ±0.78
250 µg/ ml	82.5±0.81	76.3±0.29	91.5±0.36

Table 3: Ferrous ion chelating activity of *S. aromaticum* leaf extracts.

Concentration	Ethanol	Aqueous	Standard (GallicAcid)
50 µg/ ml	14.8±0.29	24.9±0.38	32.1±0.54
100 µg/ ml	33.3±0.28	37.8±0.28	53.6±0.38
150 µg/ ml	53.2±0.21	54.2±0.29	68.0 ±0.70
200 µg/ ml	65.8±0.28	68.1±0.18	77.5±0.35
250 µg/ ml	81.4±0.24	82.5±0.31	88.4±0.36

Table 4: DPPH radical scavenging activity.

Concentration	Ethanol	Aqueous	Standard (GallicAcid)
50 µg/ ml	80.5±0.31	82.4±0.28	87.6±0.10
100 µg/ ml	83.4±0.21	83.5±0.32	90.5±0.17
150 µg/ ml	88.3±0.28	85.3±0.24	92.4±0.22
200 µg/ ml	88.4±0.21	88.5±0.32	95.2±0.17
250 µg/ ml	91.3±0.22	90.2±0.17	97.5±0.14

reported the ethanolic extract of *S. aromaticum* showed antioxidant capacity of91.3% whereas the antioxidant activity exhibited by aqueous extract was 90.2%.

FTIR analysis of*S. aromaticum* leaf extract

Fourier transform infrared spectrophotometry was used to study functional biomolecules present in the aqueous and ethanol extracts of *S. aromaticum*.

Table 5: FTIR analysis of aqueous leaf extract of *S. aromaticum.*

Peak	Class	Structure	Assignments
591.60	Alkyl halides	R-Br	C-Br stretch
1635.05	Amides	RCONH2	NH out of Plane
2114.48	Misc	Si-H silane	Si-H silane
3310.33	Amines	R2NH	NH stretch

Table 6: FTIR analysis of ethanol leaf extract of *S. aromaticum.*

Peak	Class	Structure	Assignments
572.59	Alkyl halides	R-Br	C-Br stretch
623.69	Alkyl halides	R-Br	C-Br stretch
802.65	Alkanes	R2C=CHR	=CH out of plane
879.52	Aromatic	1,2,4 –Trisub	CH out of plane
1044.45	Amines	RNH2	C-N stretch
1086.95	Amines	RNH2	C-N stretch
1273.77	Misc	P=O Phosphoramide	P=O Phosphoramide
1325.44	Alkyl halides	R-F	C-F stretch
1379.45	Alkanes	RCH2CH3	CH2 and CH3
1659.11	Misc	C=N	C=N
2880.01	Alkanes	RCH2CH3	CH stretch
2927.76	Alkanes	-CH2-	-CH2-
2973.19	Alkanes	RCH2CH3	CH stretch
3325.18	Carboxylic acid	RCO-OH	Dimer OH

Absorbtion values and their functional moieties were determined with the aid of introduction to spectroscopy written by Donald *et al*., (2001). The spectrum showed that the aqueous showed the high broad peak at 3310 cm^{-1}, high sharp peak at 591 cm^{-1}, medium sharp peak at 1635 cm^{-1} and low peak at 2114 cm^{-1}in the absorption spectra of amines, alkyl halides, amides and misc with NH stretch, C-Br stretch, NH out of plane and Si-H silane bonds respectively. Where the spectrum of ethanol extract shows the presence of various bioactive compounds with different range of peaks. The high sharp peak at 1044cm^{-1} with amines showing C-N stretch, medium sharp peak at 1087 cm^{-1} and 879 cm^{-1} with amines and aromatic with C-N stretch and CH out of plane, moderate sharp peak at 2973 cm^{-1}, 623 cm^{-1} and moderate broad peak at 3325 cm^{-1} suggest alkanes, alkyl halides and carboxylic acid with CH stretch, C-Br stretch and dimer OH respectively table 5, 6; Fig. 1, 2 & 3. The wave number (cm^{-1}) retrieved from the clove sample was noted such as 591, 623, 879, 1044, 1087, 1635, 2114, 2973 and 3310

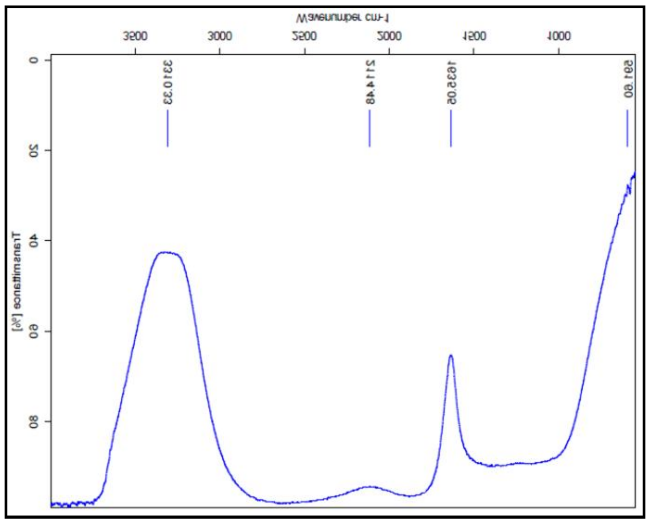

Fig. 1: FTIR analysis of aqueous leaf extract of *S. aromaticum.*

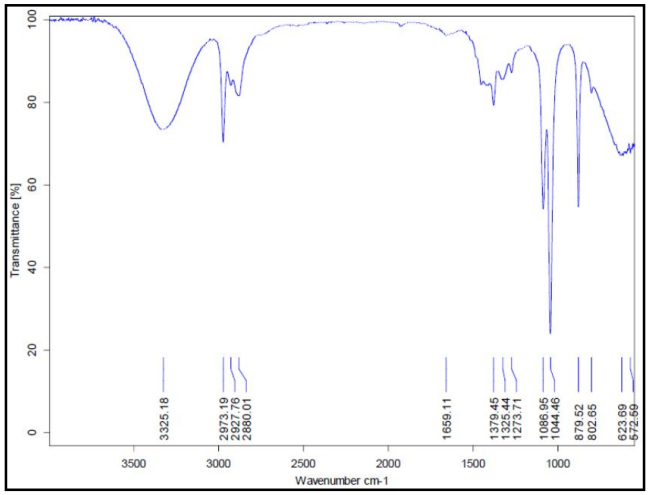

Fig. 2: FTIR analysis of ethanol leaf extract of *S. aromaticum.*

Table: Antibacterial activity of *S. aromaticum* leaf extracts.

Bacteria	Zone of inhibition (mm)			
	SA-Aq	SA-E	PC	NC
Staphylococcus aureus	10	17	16	-
Bacillus subtilis	12	14	19	-
Streptococcus mutans	10	13	19	-
Klebsiella pneumonia	7	9	20	-
Escherichia coli	9	12	18	-
Proteus vulgaris	8	11	28	-

SA-Aq -*S. aromaticum* leaves aqueous; SA-E -*S. aromaticum* leaves ethanol; PC - positive control; NC - negative control.

which confirms the existence of various groups such as carboxylic acids (O-H), anhydrides, alkanes (C-H), amide (C=O) and alkenes (C=C) (Meena *et al*., 2010 a, b).

Antimicrobial activity

Antibacterial activity of aqueous and methanol extracts of clove leaves was screened against three Gram positive bacteria and three Gram negative bacteria. All the bacteria tested were sensitive to aqueous and ethanol extract of clove leaves. Ethanol extract of leaves shows maximum zone of inhibition against *Staphylococcus aureus* (17 mm) and *Bacillus subtilis* (14 mm). This result agrees the findings of earlier researcher Pandey *et al*., (2014), Kuang *et al*., (2011) against *S.aureus* by ethanolic extract of clove leaves.

Aqueous extracts exhibited good activity against *Staphylococcus aureus* (10 mm), *Bacillus subtilis* (12 mm) and *Streptococcus mutans* (10 mm). *In-vitro* and *in-vivo* study of aqueous extract clove against pathogenic microorganisms *S. aureus* and *E. coli* also revealed the same result (Nassan *et al*., 2015, Liu *et al*., 2017).

Conclusion

From the present studywe confirmthe presence of essential phytochemical components, functional groups, antioxidant activityand antimicrobial effectsof various extracts from *S. aromaticum* leaves. Hence it could be concluded that the*S. aromaticum* leaves possess good medicinal properties which will be useful to treat various ailments and diseases. Further studies has to be carried out to determine the efficacy of each phytochemical

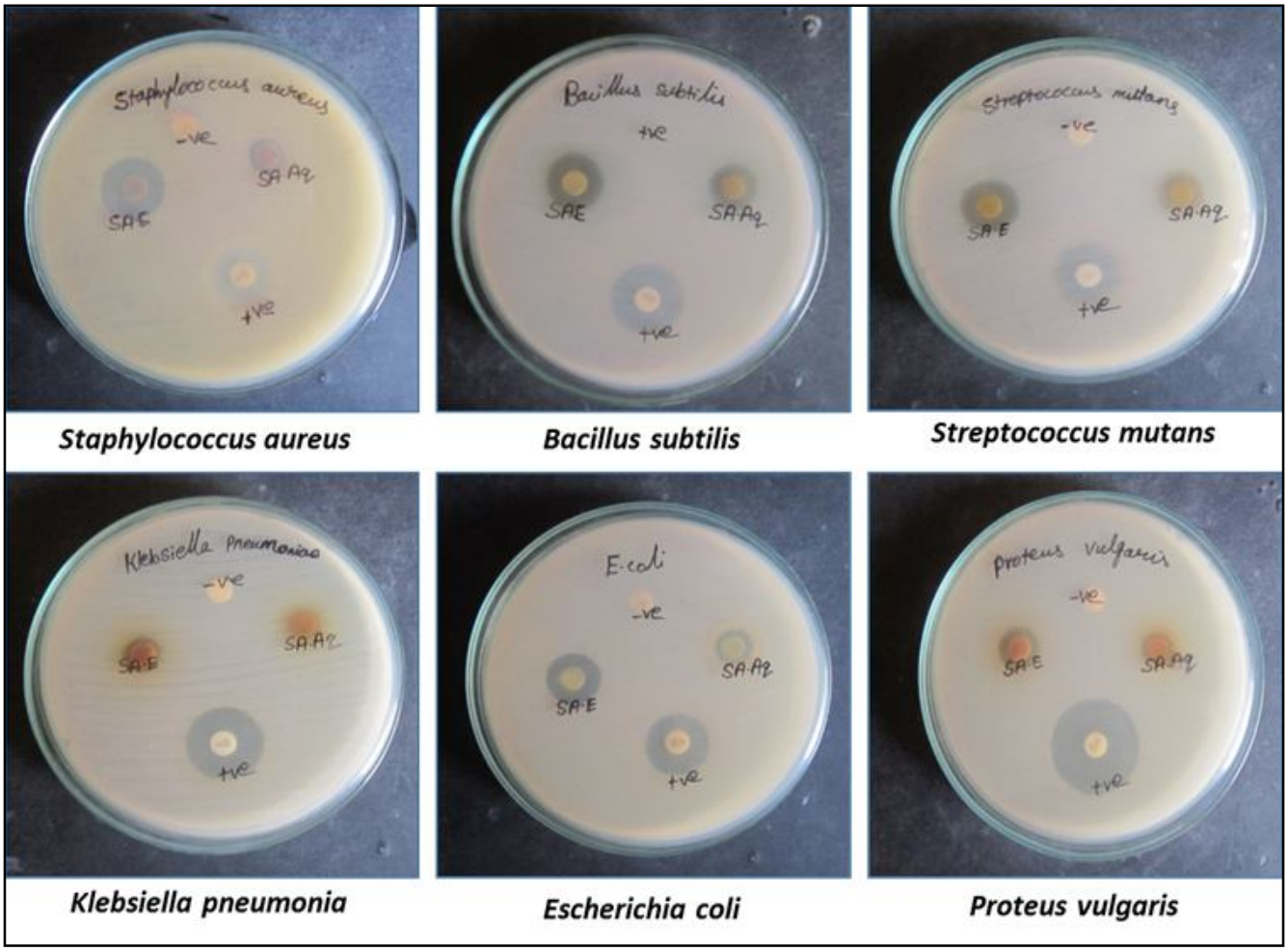

Fig. 3: Antibacterial activity of *S. aromaticum* leaf extracts.

components against human therapeutic usage.

References

Anjana, E. and R. Anitha (2012). Efficacy of lectin-silver nanoparticle conjugate in the control of rice pathogen, *Curvularia lunata* (Wakker) Boedijn. *International journal of current science*. 17-23.

Bhuiyan, N.I., J. Begum, N.C. Nandi and F. Akter (2010). Constituents of the essential oil from leaves and buds of clove (*Syzigium caryophyllatum* L. Alston). *African journal of Plant Science*, **11:** 451-54.

Brand-Williams, W., M.E. Cuvelier and C. Berset (1995). Use of a free-radical method to evaluate antioxidant activity. *Food science and technology*, **28(1):** 25-30.

Dinis, T.C.P. and V.M. Maderia and L.M. Almeida (1994). Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers. *Archives of Biochemistry and Biophysics,* **315:** 161-169.

Donald, L.P., M.L. Gary and S.K. (2001). George Introduction to Spectroscopy. 3rd ed. Brooks/Cole (US): Thomson Learning.

Edziri, H.L, M.A. Smach, S. Ammar, M.A. Mahjoub, Z. Mighri, M. Aouni and M. mastouri (2011). Antioxidant, antibacterial and antiviral effects of *lactuca sativa* extracts. *Industrial Crops and Products,* **34:** 1182-1185.

Gian, C.T., N. Ettore and B. Adriana (2012). Nutraceutical potential and antioxidant benefits of red pitaya (*Hylocereus polyrhizus*) extracts. *Journal of Functional Food*, **4:** 129-136.

Govardhan-Singha, R., S. Pradeep, S. Negi and C. Radha (2013). Phenolic composition, antioxidant and antimicrobial activities of free and bound phenolic extracts of *moringaoleifera* seed flour. *Journal of Functional Food*, **5:** 1883-1891.

Goyal, P., A. Khanna, A.G. Chauhan, G. Chauhan and P. Kaushik (2008). In vitro evaluation of crude extracts of *Catharanthus roseus* for potential antibacterial activity. *International Journal of Green Pharmacy*, **2(3):** 176-181.

Green, L.C., D.A. Wagner, J. Glogowski, P.L. Skipper, J.S. Wishnok and S.R. Tannenbaum (1982). Analysis of nitrate, nitrite and 15N in biological fluids. *Analytical Biochemistry,* **126:** 131-6.

Gulcin, I., M. Elmastas and H.Y. Aboul-Enein (2012). Antioxidant activity of clove oil - a powerful antioxidant source. *Arabian Journal of Chemistry*, **5:** 489-499.

Halliwell, B. and J.M.C. Gutteridge (2007). Free radicals in biology and medicine. 4th. Oxford: Oxford University Press.

Jain, D.C. (1987). Antifeedant active saponins from *Balanites roxburghii* stem bark. *Phytochemistry*, **26(8):** 2223- 2225.

Jasna, I., D.B. Suzana, M. Dusan, R. Mihailo and Z. Irena (2013). Evaluation and improvement of antioxidant and antibacterial activities of supercritical extracts from clove buds. *Journal of Functional Food*, **5:** 416-423.

Jin, S. and K.H. Cho (2011). Water extracts of cinnamon and clove exhibits potent inhibition of protein glycation and anti-atherosclerotic activity in vitro and in vivo hypolipidemic activity in zebra fish. *Journal of Food Chemistry and Toxicology*, **49:** 1521-1529.

Kim, H.M., E.H. Lee, S.H. Hong, H.J. Song, M.K. Shin, S.H. Kim and T.Y. Shin (998). Effect of *Syzygium aromaticum* extract on immediate hypersensitivity in rats. *Journal of Ethnopharmacology*, **60:** 125-131.

Kuang, X., B. Li, R. Kuang, X.D. Zheng, B. Zhu, B.L. Xu and M.H. Ma (2011). Granularity and antibacterial activities of ultra-ûne cinnamon and clove powders. *Journal of Food Safety*, **31:** 291–296.

Liu, Q., X. Meng, Y. Li, C. Zhao, G. Tang and H. Li (2017). Antibacterial and Antifungal Activities of Spices. *International Journal of Molecular Sciences*, **18:** 1283.

Mahmoud, H., K.A. Mina and R. Hassan (2011). Analgesic effect of clove essential oil in mice. *Avicenna Journal of Phytomedicine*, **1:** 1-6.

Meena-Devi, V.N., P. Nagendra-Prasad and K. Kalirajan (2010). Infrared Spectral studies on siddha drug pavalaparpam. *International journal of Pharma and Bioscience*, **4(1):** 474 - 483.

Meena-Devi, V.N., P. Nagendra-Prasad and K. Kalirajan (2010). Standardization of Siddha Drug 'SanguParpam' using Infrared Spectrum. *International journal of Pharma and Technology*, **3(2):** 640-647.

Nahak, G. and R.K. Sahu (2011). Phytochemical Evaluation and Antioxidant activity of *Piper cubeba* and *Piper nigrum*. *Journal of Applied Pharmaceutical Science*, **01(08):** 153-157.

Nassan, M.A., E.H.S. Mohamed, S. Abdelhafez and T.A. Ismail (2015). Effect of clove and cinnamon extracts on experimental model of acute hematogenous pyelonephritis in albino rats: Immunopathological and antimicrobial study. *International Journal of Immunopathology and Pharmacology*, **28:** 60-68.

Nazrul, I.B., M.D. Jaripa, C.N. Nemai and A. Farhana (2010). Constituents of the essential oil from leaves and buds of clove (*syzigium caryophyllatum* L. Alston) *African Journal of Plant Science*, **4:** 451-454.

Neveu, V., J. Perez-Jimenez, F. Vos, V. Crespy, L. du Chaffaut, L. Mennen, C. Knox, R. Eisner, J. Cruz, D. Wishart and A. Scalbert (2010). Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *The journal of biological databases and curation*. doi:10.1093/database/bap024.

Pandey, B., S. Khan and S. Singh (2014). A Study of antimicrobial activity of some spices. *International journal of current microbiology and applied sciences*, **3(3):** 643-650.

Phyllis, B. and B. James (2000). Prescription for Nutritional Healing, 3rd ed., Avery Publishing, pp.111.

Prashar, A., I.C. Locke and C.S. Evans (2006). Cytotoxicity of clove (*Syzygium aromaticum*) oil and its major components to human skin cells, *Cell Proliferation*, **39:** 241-248.

Salah-Alhashimi, K., I. Khaleel-Rashid, S. Ghoson-Saleh, M. Alea-Abdulhadi and A. Tara-Taher (2013). The antimicrobial activity of leaves and callus extracts of *Thevetia peruviana* In vitro, *Journal of Biotechnology Research Center*, **7:** 74-80.

Shan, B., Y.Z. Cai, M. Sun and H. Corke (2005) Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *Journal of Agricultural and Food Chemistry*, **53(20):** 7749-7759.

Skerget, M., P. Kotnik, M. Hadolin, A.R. Hras, M. Simonic and Z. Knez (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chemistry*, **89:** 191-198.

Soni, A. and P. Dahiya (2014). Phytochemical analysis, antioxidant and antimicrobial activity of *syzygium caryophyllatum* essential oil. *Asian Journal of Pharmaceutical and Clinical Research*, **7(2):** 1-9.

Waqas-Ahmed (2016). Monitoring antioxidant and antityrosinase activity of clove aromatic flower buds. *Journal of Medicinal Plants Studies*, **4(2):** 163-169.