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GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *SYZYGIUM AROMATICUM* EXTRACTS FROM TAIF, SAUDI ARABIA

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ABSTRACT

Antimicrobial activity of alcoholic and aqueous extracts from *Syzygium aromaticum* (clove) was evaluated against six Gram-negative bacteria, three Gram-positive bacteria and one fungus isolate. Clove extracts demonstrated broad spectrum antimicrobial activities against the tested microorganisms. Gram-positive bacteria were more sensitive than Gram-negative bacteria (MICs and MBCs of 62.5 to 250 µg/ml and 62.5 to 500 µg/ml) respectively. Interestingly, *Psuedomonas aeruginosa* 359 ETT which is resistant to most antibiotics was relatively more sensitive than other Gram-negative bacteria. While n-hexane extracts showed very low activity against the test microorganisms, methanol extract was recorded as the most active extract against *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC25922, *Staphylococcus aureus* 375 at MBC of 62.5 µg/ml. Clove buds essential oils were characterized by GC/MS. Benzyl Alcohol (56.2362 %) and Eugenol (32.5%) were recorded as the major components in clove oil followed by β-Caryophyllene (8.97%) and Ocimene (1.126%). Therefore, the study shed the light on the efficacy of plant extracts to combat pathogens which will help as natural antimicrobial agents of sustainable eco-friendly botanical bactericides.

KEYWORDS: ***Syzygium aromaticum , antibacterial activity,*** **GC/MS, MIC, MBC.**

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INTRODUCTION

Infectious disease account for approximately one half of all deaths in tropical countries[1] and they are considered a major threat to human health because of the unavailability of vaccines or limited chemotherapy. Ranked 5th in 1981, infectious diseases continue to be a growing public health concern[2] and they became the 3rd leading cause of death in 1992, with an increase of 58%[3]. Most of the current antibiotics have considerable limitations in terms of antimicrobial spectrum, side effects and their widespread overuse have led to increasing clinical resistance of previously sensitive microorganisms and to the occurrence of uncommon infections[4]. Plants used for traditional medicines contain a wide range of substances that can be used to treat chronic as well as acute infectious diseases [5,6], and have therefore become sources of important drugs and the pharmaceutical industries have come to consider traditional medicine as a source of bioactive agents that can be used in the preparation of synthetic medicines. Clove (*Syzygium aromaticum* L.) belong to family Myrtaceae are native to the Maluku Islands in Indonesia, and are commonly used as a spice[7]. Cloves are used in the cuisine of Asian, African, and the Near and Middle East [8]. A major component of clove taste is imparted by the chemical Eugenol which comprises 72-90% of the essential oil [9]. Cloves are used in Indian medicine, Chinese medicine, and western herbalism and dentistry where the essential oil is used as an anodyne (painkiller) for dental emergencies[10]. The oil of clove buds is known for its antimicrobial, antifungal, antiseptic, antiviral, aphrodisiac, and stimulant properties[11]. Clove Oils are used as Insect repellent, expectorant, antiseptic, perfume ingredient , flavoring agent , soap ingredient, and massage oil in soap ingradients in Southern Europe and North America[12,13]. *Syzygium aromaticum* crude extracts significantly exhibited antimicrobial activity and inhibited the growth of Gram-positive and Gram-negative bacteria[14]. *Syzygium aromaticum* essential oil is used for local anesthesia[15], eugenol present in clove oil may ameliorate effects of environmental food mutagens[16]. *Syzygium aromaticum* oil contains non-toxic disinfectant Eugenol (80-90%), Sesquiterpenoids, Caryophyllin, Eugenin, vanillin, glutamic acid and calcium oxalate[17, 18]. However, there is no detailed study concerning the antimicrobial activity of *Syzygium aromaticum* have been performed so far. Most of the studies related to *Syzygium aromaticum* have focused on the physical and chemical properties of oil. Therefore, the purpose of this study is to determine *in vitro* antimicrobial activity against Gram- negative and Gram-positive bacteria as well as the chemical compositions of *Syzygium aromaticum* extracts as well as its essential oil composition by using GC/MS.

MATERIALS AND METHODS

i. Preparation of extracts

The present study was conducted to determine the chemical composition (constituents) and antimicrobial activities of *Syzygium aromaticum* bud extracts. The clove buds were collected in April, 2013 from Taif governorate, KSA. Dried cove buds were r powdered and soaked in five volumes of 50% ethanol, 80% methanol, and 100% hexane by stirring overnight, filtered through Whatman No. 1 filter paper after 72 hours and centrifuged at room temperature at 5000 xg. The supernatants were evaporated to dryness at 45 ºC under reduced pressure. Powdered *Syzygium aromaticum* (500 g each) were extracted twice overnight with 2500 ml of distilled water at room temperature. The supernatant was collected and evaporated to dryness at 45 ºC under reduced pressure. The yield of each extract was adjusted to be less than 10% (Twenty grams of each alcoholic extract was dissolved in 100 ml distilled water containing 30% DMSO to get concentration 500 µg/ml) and stored in dark sterile bottles and become ready until use. The reconstituted alcoholic extracts were sterilized by filtering through 0.45 µm membrane filter and tested for

sterility after membrane filtration by introducing 2 ml of the extract into 10 ml of sterile nutrient broth and incubated at 37°C for 24 h. A sterile extract was indicated by the absence of turbidity in the broth after the incubation period [19].

ii. Test microorganisms

A total of 10 microbial cultures belonging to nine bacteria and one fungus species, were used in this study. These microorganisms included *Escherichia coli* (393), *Escherichiacoli* (ATCC 25922), *Psuedomonas aeruginosa* (ATCC 27853), *Psuedomonas aeruginosa* (354 ETT), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus aureus* (Nasal 375), *Streptococcus pneumoniae*(ATCC), *Salmonella enteritidis* (ATCC 13076), *Acinetobacter calcaoceuticus*(Ur) and *Aspergillus niger* (ATCC 16404). These microorganisms were obtained from the Microbiology Unit, Al-Edwani Hospital, Taif Governorate, KSA. The organisms were maintained on nutrient broth, nutrient agar (Biolab, Hungary), potato dextrose agar and potato dextrose broth. The antibacterial assays were carried out using Mueller Hinton II Agar (Oxoid, England) and broth. The antifungal assays were carried out using, potato dextrose broth and potato dextrose agar.

iii. Antimicrobial activity

All plant extracts were dissolved in sterile distilled water containing 30% DMSO to a final concentration 500 µg/ml and sterilized by filtration by 0.45µm Millipore filters. Antimicrobial tests were carried out by agar well diffusion assays. The antibacterial activity was carried out by employing 24h cultures of each microorganism. Activity of aqueous and alcoholic extracts of *Syzygium aromaticum* was tested separately using Agar well diffusion method [20, 21,22,23,24]. The medium was sterilized by autoclaving at 120 ºC (15 lb/in^2). About 30 ml of the Agar medium with the respective strains of bacteria was transferred aseptically in to each sterilized Petri plate. The plates were left at room temperature for solidification. A well of 6mm diameter was made using a sterile cork borer. Antibacterial assay plates were incubated at 37±2 ºC for 24h. The diameter of the zone of inhibition was measured. Sterile ultrapure water and DMSO were used as negative control. Samples were tested in triplicate and results are expressed as mean ± standard deviation.

iv. Determination of antifungal activity

The agar well diffusion method [25] was modified. Potato dextrose agar (PDA) was used for fungal cultures. The culture medium was inoculated with the fungal strain *Aspergillus niger* ATCC 16404 separately suspended in potato dextrose broth. A total of 6 mm diameter wells were punched into the agar and filled with plant extracts and solvent blanks (Ethanol, Methanol and hexane). Standard antibiotic (Nystatin, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 28^{o}C for 48 h. The diameters of zone of inhibition observed were measured. Antifungal activities were determined by measuring diameter of inhibition zone (DIZ) in mm. each experiment was repeated thrice and the average values of antimicrobial activity were calculated.

v. Broth micro-dilution method (BMD)

MICs and MBCs of *Syzygium aromaticum* extracts were determined by using BMD method as described by the NCCLS in flat-bottomed 96-well clear plastic tissue-cultured plates. The MIC was assayed using two-fold BMD method in MH Broth in 96-well plates. Plates contained two fold dilutions of antimicrobial agents at the concentration ranges: 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 µg/mL. 1% DMSO was used as a solvent control. Plant extracts were diluted to twice the desired initial test concentration (1 mg) with Muller Hinton broth (MHB) (Oxoid, UK). All wells, except the first were filled with MHB (50 µL). Plant extract (100 µL) was added to the first well and serial two-fold dilutions were made down to the desired minimum concentration (32 µg). An over-night culture of bacteria suspended in MHB was adjusted to turbidity equal to 0.5 McFarland standards. The plates were inoculated with bacterial suspension (50 µL/ well) and incubated at 37 °C for 24 h. Then the turbidity was observed visually by eyes. MICs and MBCs were determined for ethanol, methanol, hexane and

aqueous extracts of *Syzygium aromaticum* . MIC was determined as the lowest concentration of plant extract that inhibit the growth of each microorganism. MBC was determined as the lowest concentration of plant extract that prevent the growth of bacteria after sub-culturing on MH agar plates. To determine the MBCs, the suspensions (20 µl) were taken from each well without visible growth and inoculated in MH agar for 24 h at 37ºC. To determine the Minimal Fungicidal Concentration (MFC), 100µL of each dilution showing no growth was spread on PDA. The inoculated Petri dishes were incubated at 37°C for 48 h for fungal cultures. Tests were performed in triplicate for each test concentration.

vi. Isolation of Volatile Oil

One hundred grams of dried clove buds were grounded in a blender and then subjected to hydrodistillation for 3 h according to the standard procedure described in the European Pharmacopoeia[26]. The oil was solubilized in *n*-hexane, dried over anhydrous sodium sulphate and stored at +4 °C in the dark until tested and analyzed.

vii. Gas chromatography-mass spectrometry (GC/MS) analyses

The essential oils were analyzed on angas chromatograph (GC)–mass spectrometer (MS), Clarus 500 GC/MS (PerkinElmer, Shelton, CT). The software controller/integrator was TurboMass, version 4.5.0.007 (PerkinElmer). An Elite-1 GC capillary column (30 m, 0.25-mmID, 0.25 DF, PerkinElmer) was used. The carrier gas was helium (purity 99.9999%) at a flow rate of 0.9 mL/min (initial 7.6 p.s.i.,flow initial 36.2 cm/s, split; 50:1). Temperature conditions were: inlet line temperature, 270°C; source temperature, 210°C; trap emission, 100°C; and electron energy, 70 eV. The column temperature program was: 80°C hold for 5 min, increased to 150°C (rate, 5°C/min), and held for 5 min, increased to 270°C (rate, 20°C/min) and hold for 5 min. The injector temperature was 220°C. MS scan was from 45 to 350 *m/z*. A sample volume of 50 µL was diluted with 500 µL of chloroform, vortexed for 2 sec., and a volume of 1 µL was injected for GC-MS analysis. A volume of 50 µL from this diluted sample was transferred to total recovery vial 1-mL, mixed with 50 µL of MSTFA, capped, vortexed for 2 sec., heated at 80°C for 30 min using heating block for half insertion of vial, cooled, and a volume of 1 µL was injected for GC-MS analysis. The total ion chromatogram (TIC) was recorded from 45 – 350 *m/z*. The targeted peaks were extracted by the knowledge of major *m/z* fragments, averaged masses at peak top, and searched for matched compounds using mass spectrometry data bank NIST2008 database. The percentage composition of the essential oil was computed by the normalization method from the GC peak areas measurements.

viii. Statistical analysis

Comparison of data was performed using the one way ANOVA and is presented as mean ± standard deviation. Comparison of MIC and MBC values, tests were made in triplicate for quantification. Values of $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

In this study, *Syzygium aromaticum* alcoholic and water extracts were evaluated for their antimicrobial activities against six Gram-negative bacteria, three Gram-positive bacteria and one fungus isolate. Interestingly, *Syzygium aromaticum* ethanolic extracts (EE) showed a positive significant antibacterial activity against *Staphylococcus aureus* (ATCC 25923) and *Psuedomonas aeruginosa* (359 ETT) which were resistant to most antibiotics tested [Amoxicillin (AMC), Cefotaxime (CTX), Doxycycline (DO), Ciprofloxacin (CIP), Ceftriaxone (CRO), Cefuroxime (CXM), Chloramphenicol (CH), Erythromycin (E), and Gentamycin (CN)].The Ethanolic extracts of *Syzygium aromaticum* (EE) showed a positive significant antibacterial activity against all tested bacteria (diameter of zone of inhibition) ranged between 25 mm for *Staphylococcus aureus* (ATCC 25923) and *Psuedomonas aeruginosa* (359 ETT) (Fig 1-A9) to 21 mm for *A. niger* ATCC 16404 (Fig 1-A8). Methanolic extracts of

Syzygium aromaticum (ME) showed a highly significant antibacterial activity against *Psuedomonas aeruginosa* (359 ETT) (DIZ= 30 mm) (Fig 1-A9), *Psuedomonas aeruginosa* (ATCC 27835) (DIZ= 28mm) (Fig 1-A3), 29 mm for *Escherichia coli* 393 (Fig 1-A2), and 20 mm for *Streptococcus pneumonia* (ATCC 134) (Fig 1-A6). Hexane extracts of *Syzygium aromaticum* (HE) showed the lowest degree of inhibition against *Streptococcus pneumonia* (ATCC 134) (DIZ= zero mm) while it was 14 mm for *A. niger* ATCC 16404 and 24 mm for *Psuedomonas aeruginosa* (ATCC 27835) (Fig 1-A3). Aqueous extract of *Syzygium aromaticum* showed inhibition zones ranged between 25 mm for *Acinetobacter calcaoceuticus* (Ur) (Fig 1-A1) and 22 mm for *A. niger* ATCC 16404 while it was not detectable for *Psuedomonas aeruginosa* (359 ETT) (Fig 1-A9). Methanolic extracts of *Syzygium aromaticum* (ME) showed excellent degree of activity against most bacterial isolates tested (MIC ranged between 62.5 µg/ml for *Streptococcus pneumonia* (ATCC 134) to 125 µg/ml for *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (375) while the hexane extracts (HE) showed MICs ranged between 62.5 to 500 µg/ml. Our results of antimicrobial activity of *Syzygium aromaticum* were in agreement with that obtained by Qabaha[14] who used *Syzygium aromaticum* and other four crude extracts from commonly used medicinal plants in Jordan against four pathogenic microorganisms over a wide range of concentrations (50-5000 ppm). Ethanolic, methanolic and water extracts were found to have maximum antifungal activity in comparison to hexane extracts. Methanol extract possessed potent antifungal activity against *Aspergillus niger* showing the diameter of zone of inhibition (24 mm) while ethanolic extracts and water extracts showed diameter of zone of inhibition (21 & 22 mm), respectively. Hexane extracts of *Syzygium aromaticum* showed very low antifungal activity against *Aspergillus niger* ATTC16404 (diameter of zone of inhibition 14 mm). Water extracts of *Syzygium aromaticum* showed also a highly significant antibacterial activity against tested organisms. The diameters of zone of inhibition were ranged between 13 mm for *Streptococcus pneumonia* ATCC 134 to 25 mm for *Acinetobacter calcaoceuticus* (Ur). The results of MIC and MBC of clove extracts were promising against tested microorganisms, as the effective minimum inhibitory concentration ranged between 62.5 - 250 µg/ml. The results of MICs revealed that the *Syzygium aromaticum* alcoholic extracts exhibited the best anti-bacterial activities towards Gram-negative and Gram-positive bacteria with different degree of inhibition. MIC &MBC values of ethanolic extracts were ranged between 125 to 250 µg/ml (Table 1). Methanolic extracts of *Syzygium aromaticum* was found to be superior which has maximum antifungal activity against *Aspergillus niger* ATCC 16404(MIC= 62.5 µg/ml) while ethanolic extracts showed moderate potency against *A. niger* ATCC 16404 at highest MFC value of 125 µg /ml (Table 1). Ethanolic extracts of *Syzygium aromaticum* exhibited maximum activities against *Staphylococcus aureus* (ATCC 25923) with MIC = 62.5 µg/ml while MBC value was125 µg/ml. MIC & MBC were 62.5 to 125 µg/ml against *E. coli* 393 and 125 to 250 µg/mlagainst *E. coli* (ATCC 25922). The weakest activity was observed against *Acinetobacter calcaoceuticus* (Ur), *Salmonella enteritidis* (ATCC 13076), and *Streptococcus pneumoniae* (ATCC 134) with the highest MIC and MBC. The minimum fungicidal concentration (MFC) of water extract of *Syzygium aromaticum* found to have maximum antifungal activity (62.5µg/ml) while the MFC value of Ethanolic and Methanolic extracts was 250 & 125 µg/ml respectively. Gram-negative bacteria were more sensitive than Gram-positive bacteria. While, Gram negative bacteria were inhibited by 62.5-250 µg/ml of alcoholic and water extracts and killed by 62.5-500 µg /ml (Table 1), Gram positive, bacterium *Streptococcus pneumonia* ATCC 134 was inhibited by concentrations ranging between 62.5-250 µg /ml. The most sensitive Gram negative bacterium was *Acinetobacter calcaoceuticus* (Ur). It was inhibited and killed in a concentration ranging between 62.5 250 µg /ml (Table 1). Gas chromatography-mass spectrometry (GC-MS) analysis (Fig 2) indicated that the essential oil main compounds

of *Syzygium aromaticum* were shown in (Table 2), of which two benzoides: Benzyl Alcohol (56.2362 %) and Eugenol (32.5592%) were recorded as the major component in clove oil followed by two terpenoids: β-Caryophyllene (8.9715%), and Ocimene (1.1261%). A previous study in Turkey [27] showed that the chemical composition of *Syzygium aromaticum* oil had about 87.00% eugenol, 8.01% eugenyl acetate and 3.56% β-Caryophyllene. Another studies of GC-MS reported by Porta et al [28]; Lee and Shibamoto [29]; Tomaino et al [30] found that the clove oil contain Eugenol (82.6%), eugenol (89.2%), eugenol acetate (8.6%), eugenol acetate (77.4%), eugenol acetate (19.5%), and caryophyllene (2.01%) respectively. Our results were among the lowest and highest values reported. The inhibitory activity of clove extract can be explained due to the presence of several constituents, mainly eugenol, eugenyl acetate, beta-caryophyllene, 2-heptanone as reported by Chaieb et al[17]. On the other hand, Yang et al [31] mentioned the inhibitory activity of clove oil/extracts due to the presence of acetyl-eugenol, alpha-humulene, methyl salicylate, iso-eugenol, methyl-eugenol. While, Cai and Wu [32] reported that phenyl propanoides, dehydrodieugenol, trans-confireryl aldehyde, biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid and oleanolic acid are the main constituents of essential clove oil which are responsible for its antimicrobial activity. Our results of antifungal activity of clove extracts were agreed with several studies which have demonstrated potent antifungal [33,34,35,36,37] and antibacterial effects of clove [38,39,40,41]. 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 constituents [42] and/or impairment of bacterial enzymes systems [43]. The components with phenolic structure such as eugenol are highly active against the test microorganisms. In our study, clove oil has 32.5592% eugenol. Members of this class are known to be either bactericidal or bacteriostatic agents, depending upon the concentration used as reported by Pelczar et al [44], Dorman [45]. These compounds were strongly active despite their relatively low capacity to dissolve in water, which is in agreement with published data [46,47,48].

Table 1
MIC$_s$, MBC$_s$, and MFC$_s$ in mg/ml of Syzygium aromaticum extracts against nine bacteria and one fungal isolate

S. NO.	Name of the organism	Test sample	DIZ (mm)	MIC	MBC/ MFC	±SD
1	*Acinetobactercalcaoceuticus*(Ur)	EE	24	62.50	125.0	**±78.644**
		ME	25	62.50	62.50	
		HE	16	125.0	250.0	
		WE	25	62.50	125.0	
2	*Escherichia coli* 393	EE	17	125.0	250.0	**±157.288**
		ME	29	62.50	125.0	
		HE	10	250.0	500.0	
		WE	20	125.0	250.0	
3	*Psuedomonasaeruginosa* (ATCC 27853)	EE	**20**	125.0	250.0	**±62.500**
		ME	28	625.0	125.0	
		HE	24	62.50	250.0	
		WE	18	125.0	250.0	
4	*Salmonella enteritidis*(ATCC 13076)	EE	20	125.0	125.0	**±72.169**
		ME	24	625.0	125.0	
		HE	17	125.0	250.0	
		WE	18	125.0	250.0	
5	*Escherichia **coli*** (ATCC 25922)	EE	**16**	250.0	500.0	**±144.338**
		ME	22	125.0	250.0	
		HE	18	125.0	250.0	
		WE	16	250.0	500.0	
6	*Streptococcus pneumonia* (ATCC 134)	EE	22	625.0	250.0	**±187.500**
		ME	20	625.0	125.5	
		HE	0	250.0	500.0	
		WE	13	500.0	500.0	
7	*Staphylococcus aureus* (375)	EE	**23**	62.50	250.0	**±187.500**
		ME	22	125.0	125.0	
		HE	15	250.0	500.0	
		WE	15	250.0	500.0	
8	*Staphylococcus aureus* (ATCC 25923)	EE	25	62.50	250.0	**±187.500**
		ME	25	62.50	125.0	
		HE	17	250.0	500.0	
		WE	15	250.0	500.0	
9	*Psuedomonasaeruginosa* (359 ETT)	EE	25	125.0	250.0	**±187.500**
		ME	**30**	62.50	125.0	
		HE	12	500.0	500.0	
		WE	ND	250.0	500.0	
10	*Aspergillusniger* ATCC 16404	EE	21	125.0	250.0	**±157.288**
		ME	24	62.50	125.0	
		HE	14	250.0	500.0	
		WE	22	125.0	250.0	

EE:D.caryophyllusethanol extract	***HE:D.caryophyllus hexane extract***
ME:D.caryophyllusmethanol extract	***WE: D.caryophylluswater extract***
DIZ: diameter of inhibition zone	***±SD: standard deviation***
MIC:minimum inhibitory concentration	***MBC: minimum bactericidal concentration***
MFC: minimum fungicidal concentration	***ND: Not detectable***

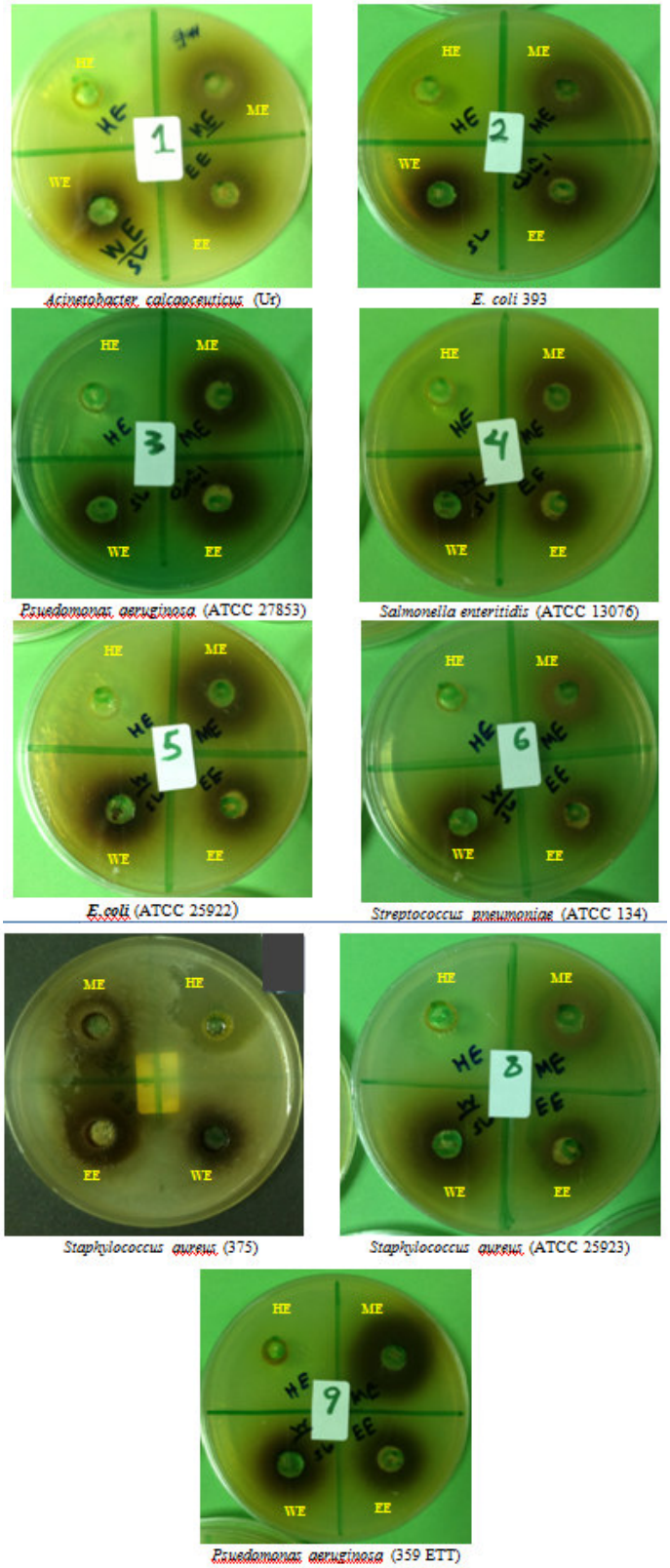

Figure 1

A1 to A9 Agar well diffusion assay showing the antibacterial activity of different extracts of Syzygium aromaticum . Each well contained 100 µg of each extract

Table 2
Compounds determined in the essential oil of Syzygium aromaticum extracted by hydro-distillation

SNo	Compound identified	Retention time (min)	Content average (%)
1	Benzaldehyde	3.30	0.0072
2	5-Hepten-2-one6-methyl	3.78	0.0065
3	β-Pinene	3.88	0.0008
4	Limonene	5.02	0.0006
5	***Benzyl Alcohol**	**5.41**	**56.236**
	Linalol	6.89	
6	Silanetrimethyl (phenylmethoxy)	8.6 to 9.1	0.0403
7	Methyl salicylate	9.359	0.0075
8	Benzoic acid trimethylsilyl ester	11.10	0.0108
	Benzaldehyde propylene glycol acetal	11.34	0.0189
9	Butanoic acid 4-[bis(trimethylsilyl)amino]]- trimethylsilyl ester	11.49	trace
10	Glycerol	13.0	0.0018
12	Cinamyl Alcohol	13.35	trace
13	α-Cubebene	14.30	trace
14	***Eugenol**	**14.62**	**32.559**
15	Copaene	14.97	0.1622
16	Benzoic acid2-[(trimethylsilyl0oxyl]-methylester	15.22	0.0062
17	Bicyclo[720] undec-4-ene 41111-trimethyl-8-methylene-[1R-(1R*4Z9S*)]	15.70	0.0017
18	***β-Caryophyllene**	**16.30**	**89.715**
19	***Ocimene**	**16.82**	**11.261**
20	α -Caryophyllene	16.84	0.0030
21	Isoeugenol	17.35	0.0450
22	Aceteugenol	17.97	0.0182
23	α -Farnesene	18.19	0.0270
24	(-)-Calamenene	18.41	0.0762
25	δ-Cadinene	18.52	0.0069
26	Naphthalene12344a7-hexahydro-16-dimethyl-4-(1-methylethyl)	18.72	0.0386
27	Caryophyllenyl alcohol	19.44	0.0211
28	Caryophyllene oxide	19.70	trace
29	12-Oxabicyclo[910]dodeca-37-diene 1558-tetramethyl- [1R-(1R*3E7E11R*)	20.34	trace
30	912-Octadecadiynoic acid trimethylsilyl ester	21.78 – 2190	trace
31	Benzyl Benzoate	24.87	0.0028

**Dominant compounds are indicated in bold*

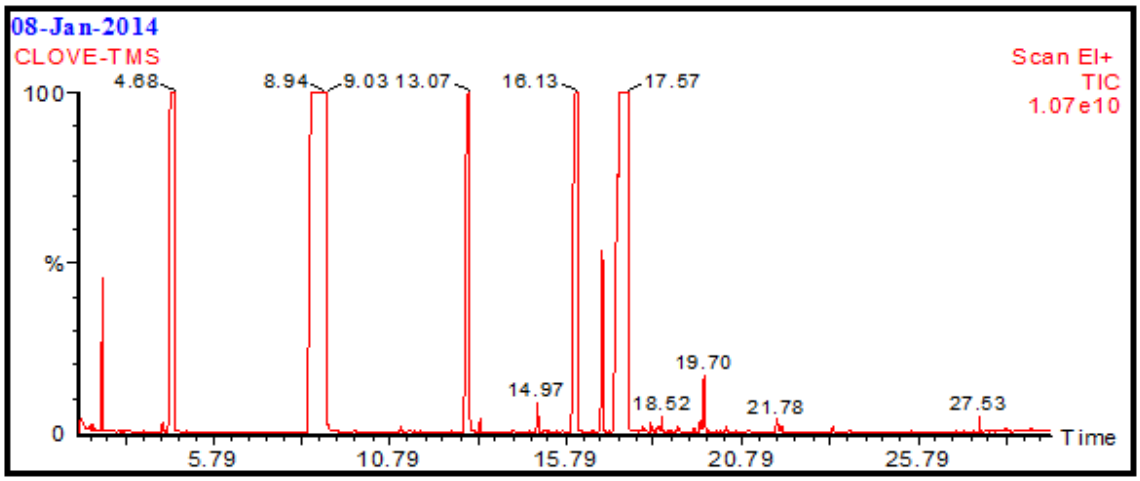

Figure 2
GC/MS chromatogram of Syzygium aromaticum essential oil

CONCLUSION

In the present study, ethanol, methanol, hexane and aqueous extracts of *Syzygium aromaticum* inhibited the growth of all clinical isolates, but their effectiveness varied. Ethanolic, methanolic and water extracts of *Syzygium aromaticum* showed considerable antimicrobial proprieties against the tested microorganisms, however, hexane extracts showed the lowest activity. Hence the methanol was discovered to be the best solvent among the three.

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