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ORIGINAL ARTICLE

Comparative analysis of chemical composition and antibacterial activities of *Mentha spicata* and *Camellia sinensis*

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ABSTRACT

The study aim was to evaluate the antibacterial activities in the aqueous extracts of mint, tea and tea enriched with mint and to correlate the results with their mineral components and biologically active constituents. Antibacterial property of plant extracts were determined by agar gel diffusion method against microorganisms such as Staphylococcus aureus, Salmonella typhi and Pseudomonas aeruginosa. Mineral contents were determined by Spectrophotometry and the chemical constituents of the extracts were identified by HPLC and GC-MS. The plant extracts preparations demonstrated significant antibacterial property with maximum effect being observed with mint. The mineral content was high in tea extract enriched with mint. The main chemical constituents rich in antibacterial properties identified by HPLC demonstrated the presence of rosmarinic acid, luteolin and caffeic acid in mint; epigallocatechin gallate, gallocatechin and catechins in tea. GC-MS analysis showed the presence of menthone, isomenthone and hexadecanoic acid in mint; caffine, octadecenal and phytol in tea. The study shows that the plant extracts exhibits significant antibacterial effect, the bioactivity being associated with mineral content and biologically active constituents. Hence these plants extracts with the property of bioavailability and retention of certain minerals by polyphenolic compounds can be recommended for their use as an alternative anti-infective agent in natural medicine for the treatment of infectious diseases.

KEYWORDS*: Antibacterial effect; Bioactivity; Camellia sinensis; Medicinal plants; Mentha spicata; Microorganisms; Mineral analysis*

INTRODUCTION

Plants are an essential part of human society since the civilization started. Medicinal plants are the boon of nature to cure a number of ailments of human beings. In many parts of the world medicinal plants are used against bacterial, viral and fungal infections. Evaluation of plants bearing efficiency in healing various diseases is growing in recent years. Innumerable biologically active compounds of plants are found to possess antibacterial properties. Practitioners of Ayurveda and Unani system of medicine regularly employ a large number of Indian medicinal plants as antibiotic agents and over the last 40 years, intensive efforts have been made to discover clinically used herbal antibacterial and antifungal drugs.

Mint (*Mentha spicata*) and tea (*Camellia sinensis*) leaves are extensively used as herbal medicines all over the world. *Mentha spicata* commonly called as spearmint belongs to the family Lamiaceae. This herb is considered as stimulant, carminative, antispasmodic, stomachic and diuretic, and is used in the treatment of gas pain, rheumatism, toothache and muscle pain. Mint possesses antioxidant properties due to the presence of active constituents like menthone, menthol, rosmarinic acid and carvone. Tea is an infusion of flavorful leaves that has been consumed for centuries as a beverage and is valued for its medicinal properties. Tea from the leaves of plant *Camellia sinensis* has a wide range of antioxidant, anti-inflammatory and anticarcinogenic activity and presence of catechin and epigallocatechin accounts for the antioxidant property of tea extract.

Microorganisms like *Staphylococcus aureus, Salmonella typhi,* and *Pseudomonas aeruginosa* are the common pathogens of human infection. *S. aureus* is *an* opportunistic pathogen of human skin. *S. typhi* is an enteric pathogen

involved in typhoid and enteric fever pathogenicity. *P. aeruginosa* is a pathogen associated with pyogenic infection and urinary tract infection. These microorganisms are highly pathogenic and the rate of prevalence of infection caused by these microorganisms is considerably increasing in recent years. Treatment of the disease with modern medicine is often and generally associated with the development of side effects. Hence the use of plant products has been increasing world wide, to lower side effects. The present study was aimed at investigating the antibacterial activity in the extracts of mint, tea and mint tea extracts against the test microorganisms. The relationship between potent antibacterial properties and mineral contents and biologically active constituents of the extracts was also investigated.

MATREIALS AND METHODS

Plant material and extract preparation

Mint extract preparation

Mint was purchased from the local market. The leaves were separated and washed under tap water. 2.5 g of mint leaves were refluxed using 100 ml of distilled water. The filtrate was separated and further filtered using Whatman filter paper. The filtered solution was diluted (1:100) with distilled water. Measurements were accomplished using 50 L - 300 L volumes of the sample.

Tea extract preparation

2.5 g of commercially available South Indian black tea leaves were brewed and extracted in 100 ml of distilled water and it was kept below 80°C while brewing. The mixture was decanted and filtered using Whatman filter paper. The resulting filtrate was diluted (1:100) with distilled water. Measurements were accomplished using 50 L -300 L volumes of the sample.

Mint tea extract preparation

Mint tea extract was prepared by mixing the individual (mint and tea) extracts in the ratio of 1:1, mixed well for 5 minutes and then filtered. Measurements were accomplished using 50 L -300 L volumes of the sample.

Microbial strains

The strains used in this work were *Staphylococcus aureus, Salmonella typhi* and *Pseudomonas aeruginosa,* obtained from clinical isolates. The bacteria were maintained by weekly transfer in a chemically defined nutrient medium distributed in 5 ml volumes in screw-capped tubes. Cells were grown at 37° C for 48 h.

Antibacterial susceptibility test and determination of minimum inhibitory concentration (MIC)

The antibacterial tests of the mint, tea and mint tea extracts were tested on the test microorganisms using the agar-gel diffusion inhibition test. In brief, 0.2 ml of a 24 h broth culture (10^6 cfu/ml) of the bacteria was aseptically introduced and evenly spread using bent sterile glass rod on the surface of gelled sterile Muller-Hinton agar plates. Six wells of 7 mm diameter were made on the plate aseptically and the concentration of plant extract ranging from 50 L – 300 L was transferred to the wells. The plates were then incubated at 37°C for 24 h. This procedure was repeated for each organism and for each compound. The antibiotics penicillin for *S. aureus* and trimethoprim for *S. typhi and P. aeruginosa* were used as controls. The minimum inhibitory concentration (MIC) of the extracts which is regarded as the lowest concentration of the extract that did not permit the growth of the test organism was calculated by measuring the zone of inhibition around each well.

Atomic absorption studies

The plant extracts were digested with a digestion mixture of HNO_3 and H_2O_2 in the ratio of 3: 1. The resulting solution after microwave digestion was filtered through Whatmann filter paper and diluted to 50 ml with Millipore water. A sample blank containing only acid mixture was served as a control. The control and the digested samples were subjected to mineral content analysis by Perkin Elmer 2380 atomic absorption spectrophotometer. Electrode-less discharge lamp (EDL) for selenium and hollow cathode lamps for magnesium, chromium, manganese, iron, cobalt and copper are used as light sources to provide specific wavelength of the elements to be determined. Acetylene gas was used to provide constant thermal energy for atomization process. Argon gas was used as the carrier gas for purging purpose and graphite furnace was used for the analysis of Selenium.

High Performance Liquid Chromatograph studies

Fresh mint and tea leaves samples were steamed for 5 min and then placed in an oven at 80C to dry. The dried samples were ground and 0.5 g was put into a 100 ml conical flask and active components were extracted with 40 ml boiling water for 30 min in a thermostated bath set at 90C. The extract was filtered through Whatman No.1 filter paper. 1 ml of the filterate was then diluted to 4 ml with Millipore H_2O and filtered through a 0.45 m filter. The filterate (10 l) was directly injected for HPLC analysis. The active components in the plant extracts were identified by the HPLC method

using LC-10 AT vp Shimadzu liquid chromatograph (Kyoto, Japan). A reverse phase column C_{18} (250 mm x 4.6 mm, 5 m particles) was used. The mobile phase consisted of two solvents: 5%
(v/v) acetonitrite (A) and 50% (v/v) acetonitrite solvent (B) both containing 0.05% (v/v) phosphoric acid (85%). The elution program for screening the extracts was the following: 0-7 min 90% A, 10% B; 7-10 min 10-15 % B; 12-20 min 15-70% B. The flow rate was 1.0 ml min^{-1} and 10 L of the sample was injected. The column temperature was set at 40C and the monitored wavelength was 231 nm. The identify of HPLC peaks separated by HPLC was confirmed by injection of authentic standards (theobromine, catechins, caffeine, epicatechin dissolved in 5% (v/v) acetonitrite containing 0.05% (v/v) 85% phosphoric acid).

Essential oil extraction and Gas Chromatography-Mass Spectrometry (GC-MS) analysis

20 g of the powdered sample of mint and tea was taken in a beaker to which 50 ml of absolute alcohol was added and kept soaked over night. The volatiles were steam-distilled and filtered using Whatmann filter paper No. 41 along with 2 gm sodium sulfate (wetted with ethanol) to remove the sediments and traces of water in the filtrate. The filtrate was concentrated and the volume was reduced to 1 ml by bubbling nitrogen gas into the solution.

The GC-MS analysis of volatile components of mint and tea were carried out on a GC Clarus 500 Perkin Elmer, equipped with an Elite-1 (100% Dimethyl Poly Siloxane 30 m 0.25 mm ID) with 1 m film thickness. The conditions of the analysis were as follows: injected temperature was held constant at 250C during analysis; oven temperature was maintained at 110 C for 2 min, followed by a linear programmed temperature from 110 to 200 C at a rate of 10 C min^{-1} and from 200 to 280C at a rate of 5C min^{-1} for 9 min. The flow rate of the carrier gas, helium was 1 ml min^{-1}; 2 l of the sample was injected. The Turbo mass gold mass spectrometer model had electron energy of 70 eV, inlet line temperature of 200C and source temperature of 200C with a mass range (m/z) of 45-450 a.m.u. The identification of each compound was carried out by comparison of relative retention time and mass spectral data obtained with literature and a computerized MS data bank (NIST ver. 2.0- year 2005).

Statistical analysis

The results of antibacterial activities and mineral contents were expressed as means ± standard deviation. Analysis of variance was conducted and differences between variables were tested for significance by one-way ANOVA. Differences at $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The present study revealed the potent antibacterial effects of *Mentha spicata* and *Camellia sinensis* against the microorganisms *S. aureus, S. typhi* and *P. aeruginosa.* Antibacterial activity of the extracts of mint, tea and mint tea was evaluated against these three bacterial species, which are known to cause infections in humans. Mint extract possessed greater antibacterial effect against *S. typhi and P. aeruginosa* and tea extract showed significant antibacterial effect against *S. aureus.* Mint extract showed significant antibacterial effect against *S. aureus* at a concentration of 200 L during which the zone of inhibition was found to be 27.5 mm compared to 26 mm zone of inhibition produced with standard antibiotic penicillin. *S. typhi* and *P. aeruginosa* were sensitive to mint extract at 100 L concentration, where the zone of inhibition was observed around 22.6 mm and 17 mm respectively. The standard antibiotic trimethoprim was used as the control for these microorganisms which produced the zone of inhibition at 20 and 15 mm respectively (Table 1(a)). Tea extract was effective against *S. aureus* at 150 L concentration where the zone of inhibition was observed at 27 mm. 200 L and 250 L concentration of the tea extract was needed to produce a significant antibacterial effect against microorganisms like *S. typhi and P. aeruginosa* and the zone of inhibition was observed at 24.1 and 18.1 mm respectively (Table 1(b)). With regard to mint tea extract, the concentrations 200 L, 150 L and 100 L were effective against the microorganisms *S. aureus, S. typhi and P. aeruginosa* respectively and their corresponding zone of inhibitions were observed at 28.5, 23 and 18.8 mm (Table 1(c)). In accordance with our study results it is reported that mint possess excellent antimicrobial property along with antifungal, antiviral, antioxidant, antihemolytic and CNS depressant properties [1].

Table 1(a) Antibacterial activity of the aqueous extract of leave of *Mentha spicata* on *S. aureus, S. typhi* and *P. aerugenosa*

Concentration of tea (L)	Zone of inhibition (in mm) shown by test microorganism		
	Staphylococcus aureus Control (20 g)- Penicillin (26)	*Salmonella typhi* Control (20 g)- Trimethoprim (20)	*Pseudomonas aeruginosa* Control (20 g)- Trimethoprim (15)
50	14.6 2.16	14.5 1.87	10.5 1.87
100	18.16 2.86	22.6 2.8*	17 2.6*
150	22 2.37	24.16 2.31	18.5 1.87
200	27.5 1.87*	24.16 2.3	20 2.37
250	29.5 1.87	26 1.4	22 3.03
300	31.67 2.16	27.7 1.17	22 3.03

Table 1(b) Antibacterial activity of the aqueous extract of leave of *Camellia sinensis* on *S. aureus, S. typhi* and *P. aerugenosa*

Concentration of tea (l)	Zone of inhibition (in mm) shown by test microorganism		
	Staphylococcus aureus Control (20 g)- Penicillin (26)	*Salmonella typhi* Control (20 g)- Trimethoprim (20)	*Pseudomonas aeruginosa* Control (20 g)- Trimethoprim (15)
50	15.17 2.32	9.5 1.87	9.3 2.16
100	20.17 2.32	16.3 2.16	9.5 1.87
150	27 3.27*	19.5 1.87	10.5 1.87
200	28.2 2.37	24.1 2.48*	14.7 2.64
250	29 2.58	27.5 1.87	18.1 1.87*
300	30.5 2.61	28.5 2.43	23.17 1.94

*P <0.05, statistically significant when compared between the three extracts

Table 1C Antibacterial activity of the aqueous extract of tea enriched with mint leaves on *S. aureus, S. typhi* and *P. aerugenosa*

Concentration of tea (l)	Zone of inhibition (in mm) shown by test microorganism		
	Staphylococcus aureus Control (20 g)- Penicillin (26)	*Salmonella typhi* Control (20 g)- Trimethoprim (20)	*Pseudomonas aeruginosa* Control (20 g)- Trimethoprim (15)
50	12.1 3.48	10.5 1.87	9.17 2.64
100	14.67 2.16	18.5 2.43	18.8 2.64*
150	19.5 1.87	23 1.75*	20 1.79
200	28.5 2.16*	26.67 2.8	22.6 1.8
250	30.6 2.43	26.7 2.8	23.3 2.16
300	32.2 2.16	28.7 2.16	23.3 2.2

*P<0.05, statistically significant when compared between the three extracts

Tea has been reported to possess antibacterial effect against *S. aureus* and *E. coli*. Antimicrobial properties of plant extract are desirable tools in the control of undesirable microorganisms especially in the treatment of many infections. Influence of plant extracts against the test microorganism determined by minimum inhibitory concentration revealed that mint extract possessed greater antibacterial effect against the entire test microorganism except *S. aureus.* The minimum inhibitory concentration of mint extract against the microorganisms *S. typhi and P. aeruginosa* was observed at 100 L for both. Similarly, mint tea extract showed an MIC of 100 L against *P. aeruginosa*. Tea extract was effective against *S. aureus* with MIC being observed at 150 L (Table 2).

Table 2 Minimum inhibitory concentrations (MIC) of mint, tea and mint tea extracts against the microorganisms *S. aureus, S. typhi* and *P. aerugenosa*

Test microorganism	Tea (L)	Mint (L)	Mint with tea (L)	Control diameter (mm)
Staphylococcus aureus	150	200	200	26
Salmonella typhi	200	100	150	20
Pseudomonas aeruginosa	250	100	100	15

The inhibitory effects of plant extracts against test microorganism might be related to the role of mineral contents, active and effective phytochemical constituents. The presence of micronutrients and biologically active constituents in plant extract usually interfere with growth and metabolism of microorganisms to destroy them. Analysis of mineral contents of mint, tea and mint tea extracts demonstrated the prevalence of most of the essential minerals like Na, Mg, K, Ca, Cr, Fe, Co, Cu, Zn and Se in adequate amount. The mineral content was rich in the extract of tea enriched with mint followed by mint and tea extracts. Increased mineral content of the mint tea extract might be due to the cumulative effect of individual extracts, the mint and tea and a comparative study between mint and tea extracts revealed that mint is rich in mineral micronutrients compared to tea (Table 3).

Table 3 Atomic absorption spectrophotometric determination of mineral contents of mint, tea and tea extract enriched with mint

Minerals	Mint (in ppm)	Tea (in ppm)	Mint tea (in ppm)
Calcium	255.95 9.01	224.6 7.45	336.66 12.5*
Magnesium	3.90 0.14	2.68 0.10	4.97 0.15*
Sodium	147.57 4.68	138.74 4.39	157.2 5.68*
Potassium	15.56 0.51	15.56 0.51	31.06 0.96*
Iron	2.03 0.052	7.42 1.99	14.05 0.44*
Copper	0.88 0.045	0.82 0.031	1.23 0.044*
Selenium	0.26 0.008	0.02 0.004	0.34 0.005*
Chromium	0.19 0.009	0.087 0.004	0.20 0.008*
Cobalt	0.25 0.012	0.18 0.051	0.29 0.008*
Zinc	0.79 0.025	0.65 0.025	0.96 0.028*

*$P<0.05$, statistically significant when compared between the three extracts

It has been reported in many studies that the antimicrobial property of plant extracts is partly contributed by minerals. Germicidal property of Cu and Zn against the microorganism *S. typhi* and *P. aeruginosa* have been demonstrated [2]. The antibacterial effect of zinc on *Streptococci and Staphylococcus* was described as early as 1949 [3]. The effect of zinc was greater on *S. aureus* and *S. epidermidis* than *P. aeruginosa*. It has been documented that the basic mechanism that lies behind the antibacterial activity of zinc ion depends on its ability to bind to the membranes of microorganism, thereby prolonging the lag phase of growth cycle and increasing the generation time of the organisms which takes for each organism more time to complete cell division [4]. Manganese is an important element in biological system and is essential for many enzyme systems in carrying out different biochemical functions like energy production, protein metabolism, bone formation etc. It shows excellent bactericidal activity against *S. aureus* and it is also documented that this mineral increases the activity of antibiotic against bacterial strains [5]. Copper affects the ability of *Salmonella spp,* to increase the death rate of *S. typhi* [6]. Iron has been reported for its role in maintaining maternal health and reducing the risk of infection [7].

Chemical investigations by HPLC and GC-MS indicated the presence of many biologically active constituents in the extracts of both *M. spicata* and *C. sinensis*. Rosmarinic acid followed by luteolin and caffeic acid in mint (Fig. 1); epigallocatechin gallate followed by epicatechin gallate and gallocatechin in tea (Fig. 2) identified by HPLC possess antibacterial property. Similarly, among the constituents identified by GC-MS the most effective antibacterial activity was observed for menthone and isomenthone followed by hexadecanoic acid, octadecenal and phytol in mint (Fig. 3; Table 4) and 1H-purine-2, 6-dione dihydro trimethyl caffine, hexadecanoic acid followed by octadecenal and phytol with regard to tea (Fig. 4; Table 5).

The phenolic constituents of the extracts of *M. spicata* namely rosmarinic acid, luteolin and phytol are reported for their antimicrobial and antiviral activities, strong antioxidant and antitumor action [8]. Bactericidal property of menthone has been reported and caffeic acid is effective against *S. aureus, S. epidermidis* and *Bacilus subtilis* [9]. Antibacterial activity of diterpenes namely phytol has been demonstrated against *S. aureus* [10]. *In vitro* antimicrobial effect on influenza virus, *Vibrio cholerae*, *S. mutants* and *S. aureus* by tea polyphenols like epigallocatechin gallate, epicatechin gallate, gallocatechin, epigallocatechin has been reported [11]. Tiwari *et al*. [12]

has demonstrated the inhibitory activity of epicatechin gallate present in tea against *S. aureus, S. typhi, S. dysenteriae* and *E. coli.* Polyphenols are contained in black tea at an appropriate concentration of 5% and the dominant constituent of polyphenol, epigallocatechin gallate is recognized to play a major role in antimicrobial effects. A stronger inhibitory activity was observed with gallocatechin and epigallocatechin compared to catechins and epicatechin. The ability of some of the compounds to exert antibacterial effect in spite of their presence in lower concentration may be due to their involvement in some types of synergism with the other active compounds.

Antimicrobial mode of action of plant extract might be related to their phenolic compounds present. Phenolic compounds are known to be synthesized by plants in response to microbial infection. It is therefore possible that they can act as effective antimicrobial substances against a wide array of microorganisms. However, the antimicrobial activity of plant extracts depend not only on phenolic compounds, but also the property is contributed by the presence of different secondary metabolite like hydroxyl groups on the active constituents. The biologically active constituents of plant extract are considered as antimicrobial agents, because of the ability of these substances to bind to bacterial adhesions and by doing so they disturb the availability of receptors on the surface [13]. The mechanism of active compounds via which they exerts stronger bactericidal effect is attributed to their effect on cellular membranes. Some reports indicated that active constituents might attack the cell wall and cell membrane, thereby destroying their permeability barrier and causing the release of intracellular constituents like ribose and sodium glutamate. Also they interfere with electron transport, nutrient uptake, protein and nucleic acid synthesis and enzyme activity leading to the inhibition of bacterial growth [14]. Polyphenolic compounds are known to enhance the antimicrobial activity by increasing the retention of certain minerals like Cu, Mg, Zn and Fe. Grapefruit polyphenols in diets can improve bioavailability of some minerals. Polyphenols like chlorogenic acid, caffeic acid and fearulic acid as an effect of promoting digestion and absorption of minerals and is therefore useful as mineral absorption enhancers.

Table 4 the main compounds identified by GC-MS in the extracts of *Mentha spicata*

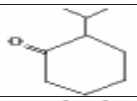
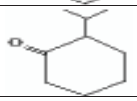
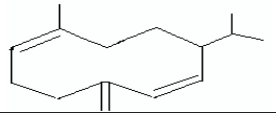
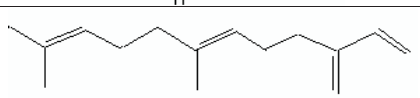
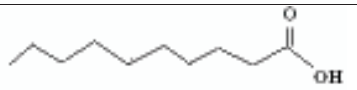
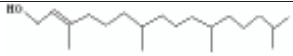
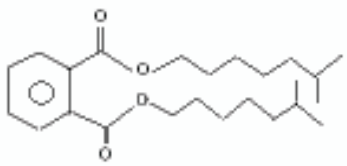
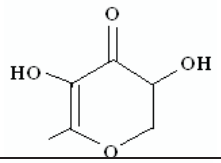
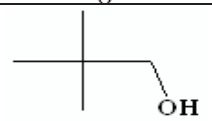
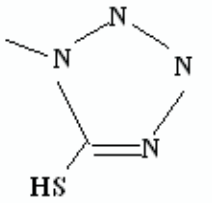
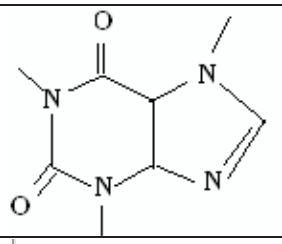
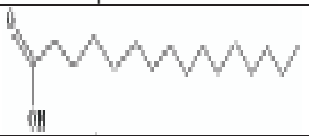
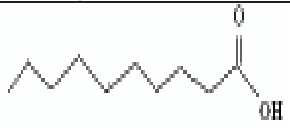
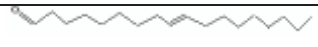
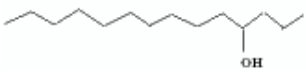
$T_{R\ (min)}$	Compound	Molecular formula and compound nature	Structure	Molecular weight	% peak area
7.54	Menthone	$C_{10}H_{14}O$		154	31.43
7.54	Isomenthone	$C_{10}H_{14}O$		154	31.43
11.02	Cyclodecadiene	$C_{15}H_{24}$ Sequiterpene		204	1.990.60
10.30	Dodecatriene	$C_{15}H_{24}$ Sequiterpene		204	0.43
14.28	n-Decanoic acid	$C_{10}H_{20}O_2$ Fatty acid		172	0.78
15.53	3,7,11,15- Tetra methyl-2-hexadecenol	$C_{20}H_{40}O$ Terpene alcohol		296	3.50
19.72	Phytol	$C_{20}H_{40}O$ Diterpene		296	2.46
20.07	Octadecanol	$C_{18}H_{34}O$ Aldehyde		266	15.03
26.14	1,2-Benzenedicarboxylic acid	$C_{24}H_{38}O_4$ Fattyacid ester		390	0.95

Table 5 The main compounds identified by GC-MS in the extracts of *Camellia sinensis*

T_R (min)	Compound	Molecular formula and compound nature	Structure	Mplecular weight	% peak area
6.22	4H-Pyran-4-One	C_6H_8O Flavonoid		144	0.12
9.97	Propanol derivative	$C_5H_{12}O$ Alcohol		88	0.44
11.15	1-methyl-5-mercaptotetrazole	$C_2H_4N_4S$ Sulfur		116	0.16
15.66	1H-purine-2,6-dione 3,7-dihydro-1,3,7-trimethyl caffeine	$C_8H_{10}N_4O_2$ Alkaloid		194	83.08
17.27	n-hexa decanoic acid	$C_{16}H_{32}O_2$ Palmitic acid		256	7.39
17.69	n-Decanoic acid	$C_{10}H_{20}O_2$ Fattyacid ester		172	0.58
19.72	Phytol	$C_{20}H_{40}O$ Diterpene		296	1.08
20.06	Octadecanol	$C_{18}H_{34}O$ Aldehyde		266	4.44
22.35	Tetradecanol	$C_{14}H_{30}O$ Aliphatic alcohol		214	0.14

The results demonstrated in this *in vitro* study provide evidence that the plant extracts are potentially a rich source of antimicrobial agent against microorganisms like *S. aureus, S. typhi* and *P. aeruginosa* with the bioactivity being attributed by their mineral contents and phytochemical constituents. Since all the three plant extracts showed significant antibacterial effects, and have other reported beneficiary effects like free radical scavenging property and antiatherogenic property [15], the current study gives credence to their ethnopharmacological use as a remedy to treat infections and diseases caused by the microorganism. Further study is recommended to determine the mechanism for

bacterial vulnerability to these plant extracts.

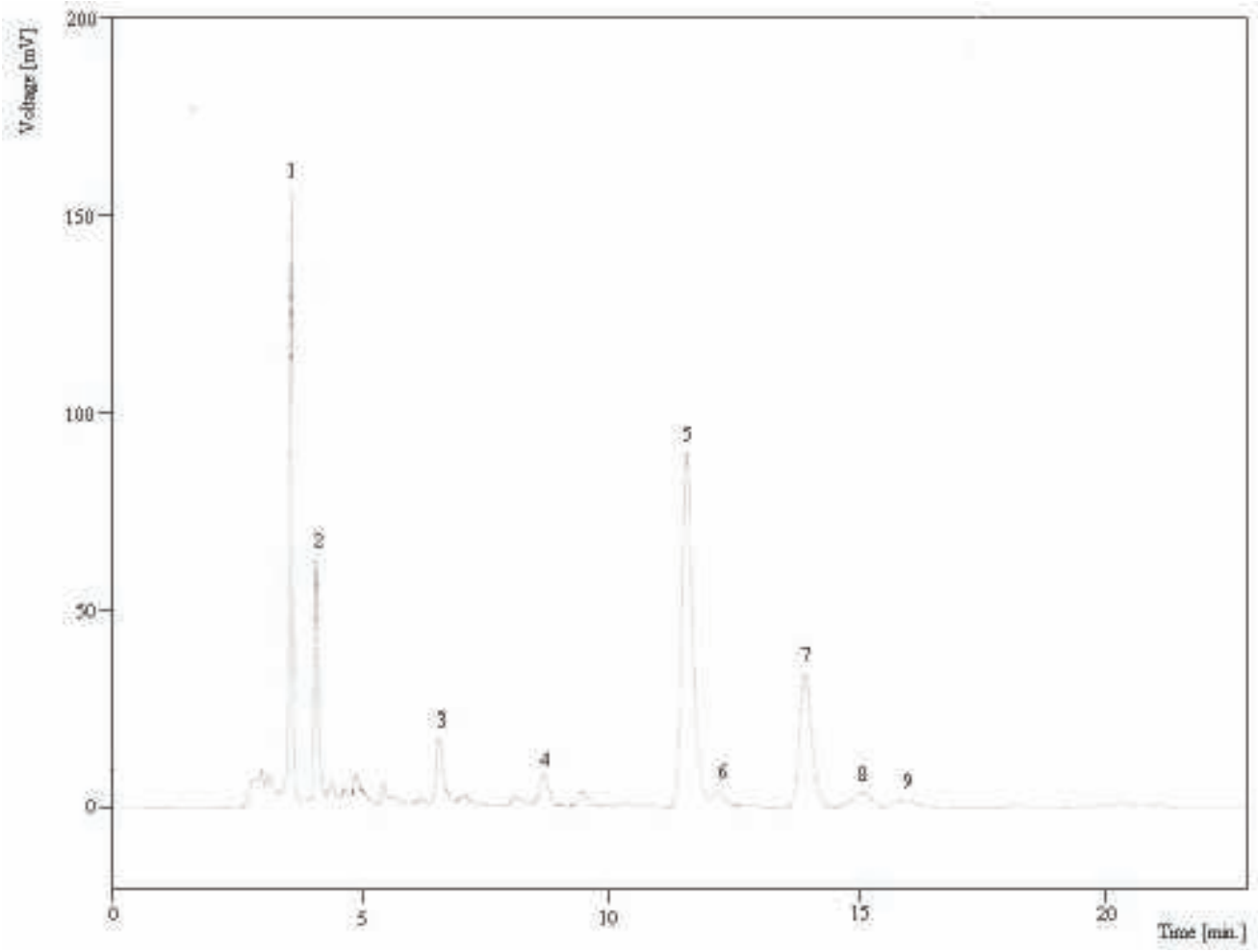


Fig.1 HPLC chromatograms of biologically active constituents in the extracts of *Mentha spicata*. The individual peaks were identified as 1 - rosmarinic acid, 2-caffeic acid, 3-myricetin, 4-eriodycitol, 5- luteolin, 6-naringenin, 7-apigenin, 8-kaempferol, 9-chrysoeriol, 10- diosmerin.

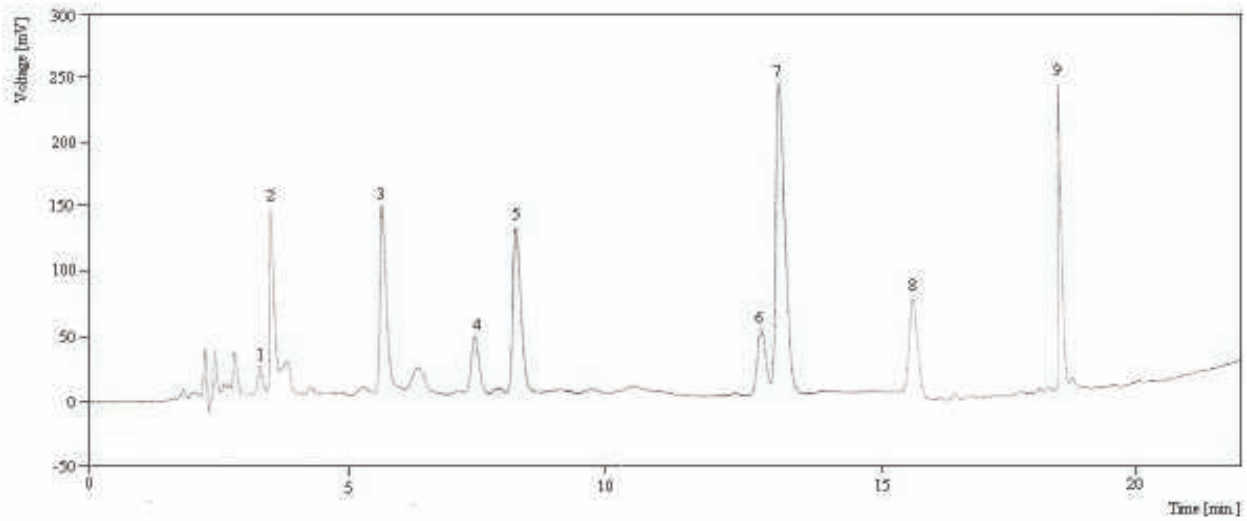


Fig.2 HPLC chromatograms of biologically active constituents in the extracts of *Camellia sinensis*. The individual peaks were identified as 1 - theobromine, 2-gallocatechin, 3-epigallocatechin, 4-catechin, 5-caffeine, 6-epicatechin, 7-epigallocatechin gallate, 8-gallocatechin gallate, 9-epicatechin gallate

Fig.3 GC-MS chromatograms of biologically active constituents in the extracts of *Mentha spicata*. The individual peaks were identified as menthone, isomenthone, T_R – 7.54; cyclobutadicyclopentene, T_R – 9.82; sucrose, T_R – 9.90; dodecatriene, T_R – 10.30; cyclodecadiene, T_R – 11.02; hexadecenol, T_R – 15.53; hexadecanoic acid, T_R – 17.28; phytol, T_R – 19.72; octadecenal, T_R – 20.07; heptadecanoic acid, T_R – 20.41.

Fig.4 GC-MS chromatograms of biologically active constituents in the extracts of *Camellia sinensis*. The individual peaks were identified as methyl mercaptotetrazole, T_R – 11.15; amyl nitrite, T_R – 12.15; epoxyhexanol, T_R – 14.28; 1H-purine, 2,6-dione, dihydro, trimethyl caffine, T_R – 15.66; hexadecanoic acid, T_R – 17.27; decanoic acid, T_R – 17.69; phytol, T_R – 19.72; octadecenal, T_R – 20.06; tetradecanol, , T_R – 22.35; vitamin E acetate, T_R – 25.21.

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