ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 8, Issue 3, 2015

ISSN - 0974-2441

Research Article

GAS CHROMATOGRAPHY-MASS SPECTROSCOPY EVALUATION OF BIOACTIVE PHYTOCHEMICALS OF COMMERCIAL GREEN TEAS (*CAMELLIA SINENSIS*) OF INDIA

SENTHILKUMAR SR[1]*, SIVAKUMAR T[1], ARULMOZHI KT[2], MYTHILI N[3]

[1]Department of Botany, Annamalai University, Chidambaram - 608 002, Tamil Nadu, India. [2]Physics Wing (DDE), Annamalai University, Annamalai Nagar - 608 002, Tamil Nadu, India. [3]Department of Physics, Annamalai University, Annamalai Nagar - 608 002, Tamil Nadu, India. Email: s.rsenthilkumar1980@gmail.com

Received: 09 March 2015, Revised and Accepted: 30 March 2015

ABSTRACT

Objective: An attempt has been made to identify the presence and composition of bioactive phytochemcials present in six commercial green tea (*Camellia Sinensis*) samples acquired from diverse locations of India. **Methods:** Gas chromatography-mass spectroscopy technique was used for the qualitative and quantitative analysis of the phytochemicals present in the green tea samples. **Results:** The results indicated the presence of various secondary metabolites like pyrogallol, quinic acid, caffeine, xanthine alkaloid palmitic acid, diterpene, linolenic acid in the studied samples. **Conclusion:** The total peak area percentages of health beneficial phytochemicals in the green tea samples were in the range of 83 to 96.

Keywords: Gas chromatography-mass spectroscopy analysis, Phytochemical screening, Green tea, Bioactivity.

INTRODUCTION

Plants are the natural sources of many biochemical used in the different fields of science. Higher plants are rich in secondary metabolites possessing important and interesting bioactivities. The bioactive components derived from medicinal plants have been traditionally used to prevent and cure many diseases. Medicinal plant-based drugs exhibit several advantages like being simple, cost effective, broad spectrum activity, preventive and curative actions, and above all shows fewer side effects [1-3].

In any plant species of interest, it is essential to discover the composition of its chemical constituents so as to obtain new biomedical sources for the remedy of illness. Gas chromatography coupled with mass spectroscopy (GC-MS) is normally used for direct qualitative and quantitative analysis of compounds present at molecular levels with very high precision. GC is used to separate the individual chemical components and the MS ionizes and identifies them by their structure and molecular weight. Further, the main advantage of GC-MS is that it can quantitative determining materials present even at very low concentrations. This feature of GC-MS has been widely utilized in forensic science, trace element analysis, pollution studies, quality control, etc., [4]. Recently GC-MS studies have been increasingly used for the analysis of non-polar components, volatile substances, alkaloids, phenols long chain and branched chain hydrocarbons, alcohols, acids, esters, and other bioactive components [5-11].

Camellia sinensis is a flowering plant in the family Theaceae, whose leaves and buds are used to produce the most widely and popularly used beverage tea. Green tea (GT) is a "non-fermented" tea derived from this plant and so contains higher amounts of phenolic compounds. Earlier phytochemical analysis and preliminary screening methods have revealed the presence of alkaloids (caffeic), saponins, amino acids, proteins, phenolic acids, carbohydrates, aluminum, fluoride, vitamins (A, C, E), flavonoids (quercetin, kaempferol, myricetin), and trace elements (Zn, Mg) [12-15].

However, the complex chemical composition of tea clones varies with the position of leaf in the shoot, climate, season, location of cultivation, horticultural practices, processing methods storage conditions, etc., [16]. The present study was aimed at the GC-MS analysis of the bioactive phytochemicals present in the six different commercial GT samples collected from diverse locations in India.

METHODS

About 10 g of GT samples in each variety were finely ground separately in an electric blender and sieved with muslin cloth. The fine powder was dissolved in 100 ml of methanol and the extracts were obtained by maceration (48 hrs). The solvent was concentrated at temperature 40°C and the resulting dry extracts, in fine powder form were used for further studies. The percentage yield was about 5-6% of the GT material taken initially. The names of the source location/(state) and the labels use for the six GT samples studied in the present work are as follows:

Name of location/(state)	Label
Moonar (Kerala)	MOON
Kodaikanal (Tamil Nadu)	TAN
Ootacamund (Tamil Nadu)	GT
Bengaluru (Karnataka)	TET
Kolkata (West Bengal)	KOL
Assam (Assam)	ASS

Chemical tests were carried out on the plant extract using standard procedures to identify the preliminary phytochemical screening following the methodology of Sofowara (1993), Trease and Evans (1989), and Harborne (1984).

GC-MS analysis was performed using Shimadzu QP 2010 plus mass analyzer. (Injection mode: Normal, column oven temperature: 100°C, injection temperature: 250°C). The relative percentage amount of each component was calculated by comparing average peak area to the total area. Individual components were identified using the database National Institute of Standards and Technology (NIST) containing more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known component stored in the NIST library. The name, molecular weight, and structure of the components of the test samples were ascertained. The biological activities of the components were based on Dr. Duke's phytochemical and ethnobotanical database created by Dr. Jim Duke of the agricultural research services/USDA.

RESULT AND DISCUSSION

Preliminary screening test of GT

Phytochemicals present in GT extracts, qualitatively detected using standard screening tests are presented in Table 1.

GC-MS analysis

Of the chromatograms recorded for the six GT samples, for illustration purpose, the chromatogram of "MOON" GT samples is shown in Fig. 1. Chemical structure of selected compounds of GT samples (percentage of area >1.00) is shown in Fig. 2.

The GC-MS analysis revealed the presence of several phytochemicals present in the GT samples (45 components in MOON tea, 40 in TAN, 26 in GT, 32 in TET, 34 in KOL, and 28 in ASS tea). Of these, the major components with peak area (%) >1 are considered in the present study and are listed in Tables 2-7.

Table 1: Phytochemicals screening results of GT extracts

S. No.	Phytochemical	Result (qualitative)
1	Tannin	−−−
2	Saponin	−−−
3	Steroids	+
4	Terpenoids	+++
5	Alkaloids	++
6	Amino acids	−−−
7	Glycoside	++
8	Polyphenols	+++
9	Protein	+++
10	Flavonoids	+++

−: Absence, +: Presence, ++, +++: Intensity of color, GT: Green tea

It can be observed from the chromatogram analysis using NIST library that the major phytochemicals identified, in all the GT samples are: Pyrogallol, quinic acid, caffeine (alkaloid), xanthine (alkaloid), palmitic

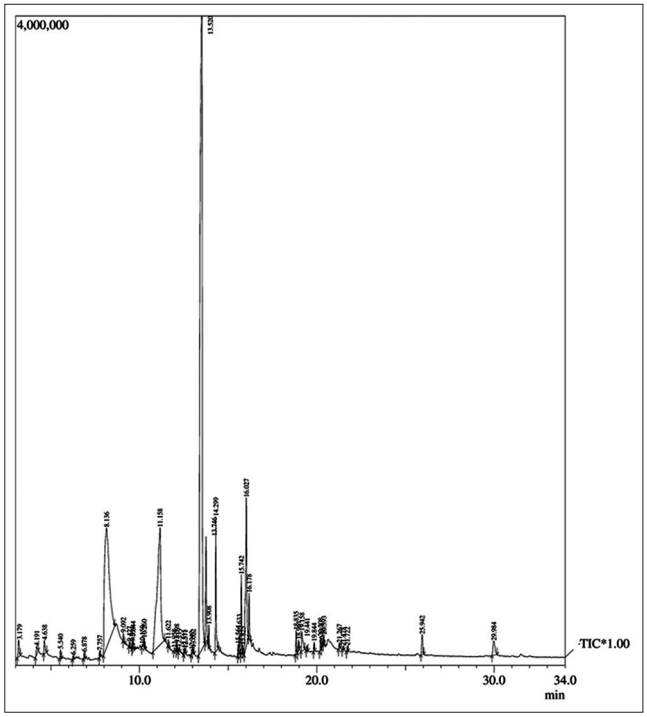

Fig. 1: Chromatogram of MOON green tea sample

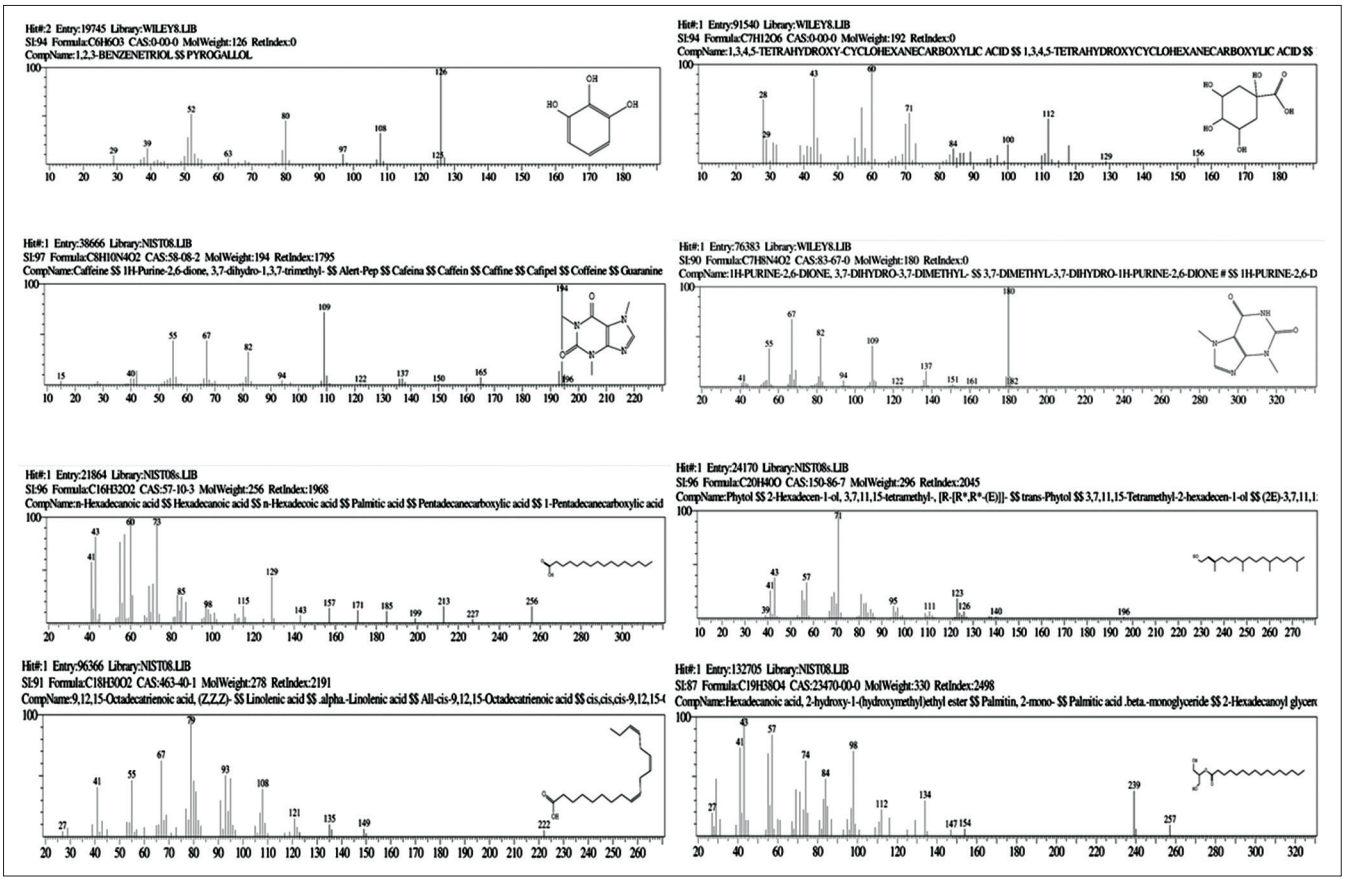

Fig. 2: Chemical structure of selected compounds of green tea samples (percentage of area >1.00)

Table 2: Peak number, Retention time, peak area (%), molecular formula, molecular weight, component name, component nature, bioactivity of MOON tea

Peak	Retention time	Area (%)	Molecular formula	Molecular weight	Name of the compound	Type	Therapeutic use
8	8.135	18.40	$C_6H_6O_3$	126.11	1,2,3-Benzenetriol	Pyrogallol	Antioxidant antiseptic fungicide, antidermatitic insecticide, candidicide
15	11.158	13.96	$C_7H_{12}O_6$	192.16	1,3,4,5-Tetrahydroxycyclo-hexanecarbonyl	Quinic acid	Antimicrobial, anti-inflammatory induces antioxidant
24	13.520	46.25	$C_8H_{10}N_4O_2$	194.19	Caffeine	Alkaloid	Both pro and antioxidant
25	13.746	3.00	$C_7H_8N_4O_2$	180.16	1H-Purine-2,6-dione, 3,7-Dihydro-3,7-Dimethyl (Theobromine)	Xanthine alkaloide	Vasodilation both pro- and antioxidant natural diuretic
27	14.299	2.90	$C_{16}H_{32}O_2$	256.42	*n*-Hexadecanoic acid	Palmitic acid	Antioxidant nematicide pesticide hemolytic anti-inflammatory hemolytic
30	15.742	1.34	$C_{20}H_{48}O$	296.53	Phytol	Diterpene	Antioxidant Antimicrobial, anticancer, diuretic
32	16.027	5.39	$C_{18}H_{30}O_2$	278	9,12,15-Octadecatrienoic acid (z, z, z)	α-linolenic acid	Antiarthritic, antihistaminic, anticoronary, antiandrogenis, antinematicide, anticancer, antibacterial

Table 3: Peak number, Retention time, peak area (%), molecular formula, molecular weight, component name, component nature, bioactivity of TAN tea

Peak	Retention time	Area (%)	Molecular formula	Molecular weight	Compound name	Type/ nature	Therapeutic use
9	8.127	16.28	$C_6H_6O_3$	126.11	1,2,3-Benzenetriol	Pyrogallol	Antioxidant antiseptic fungicide, antidermatitic insecticide, candidicide
15	11.022	9.64	$C_7H_{12}O_6$	192.16	1,3,4,5-Tetrahydroxycyclo-hexanecarbonyl	Quinic acid	Antimicrobial, anti-inflammatory induces antioxidant
20	13.480	57.17	$C_8H_{10}N_4O_2$	194.19	Caffeine	Alkaloid	Both pro and antioxidant
21	13.698	3.25	$C_7H_8N_4O_2$	180.16	1H-Purine-2,6-dione, 3,7-Dihydro-3,7-Dimethyl (Theobromine)	Xanthine, alkaloid	Vasodilation both pro-and antioxidant natural diuretic diuretic
23	14.289	1.53	$C_{16}H_{32}O_2$	256.42	*n*-Hexadecanoic acid	Palmitic acid	Antioxidant nematicide pesticide hemolytic anti-inflammatory hemolytic
34	19.141	1.19	$C_{18}H_{36}O_2$	284	Hexadecanoic acid	Palmitic acid, ethyl ester	Antioxidant, nematicide pesticide, hypocholesterolemic, hemolytic

Table 4: Peak number, Retention time, peak area (%), molecular formula, molecular weight, component name, component nature, bioactivity of GT tea

Peak	Retention time	Area (%)	Molecular formula	Molecular weight	Compound name	Type/ nature	Therapeutic use
7	8.141	28.36	$C_6H_6O_3$	126.11	1,2,3-Benzenetriol	Pyrogallol	Antioxidant antiseptic fungicide, antidermatitic insecticide, candidicide
11	11.139	1.67	$C_7H_{12}O_6$	192.16	1,3,4,5-Tetrahydroxy-cyclohexanecarboxyl	Quinic acid	Antimicrobial, anti-inflammatory induces antioxidant
14	13.564	56.48	$C_8H_{10}N_4O_2$	194.19	caffeine 1,3,7-Trimethyl-3, 7-dihydro-1H-Purine-2,6-dione	Alkaloid	Both pro and antioxidant
15	13.745	4.33	$C_7H_8N_4O_2$	180.16	1H-Purine-2,6-dione, 3,7-Dihydro-3,7-Dimethyl	Xanthine, Alkaloid	Vasodilation both pro-and antioxidant natural diuretic diuretic
16	14.299	2.11	$C_{16}H_{32}O_2$	256.42	*n*-Hexadecanoic acid	Palmitic acid	Antioxidant nematicide pesticide hemolytic anti-inflammatory hemolytic
20	16.024	2.07	$C_{18}H_{30}O_2$	278.	9,12,15-Octadecatrienoic acid (z, z, z)	Linolenic acid	Antiarthritic, antihistaminic, anticoronary, antiandrogenis, antinematicide, anticancer, antibacterial
23	19.134	1.03	$C_{16}H_{36}O_2$	284	Hexadecanoic acid	Palmitic acid, ethyl ester	Antioxidant, nematicide pesticide, hydrocholesterolemic, Haemolytic

acid, palmitic acid ethyl ester, (diterpene), α-linolenic acid. However, the samples TET and KOL contained a significant amount of alkane and fatty aldehydes. In addition, a variety of other chemical constituents were also present in all the samples in lesser fractions. The characteristic features of the major components identified are briefly discussed below.

Pyrogallol (1,2,3,-Benzenetriol)

It is a white solid but because of its sensitivity toward oxygen it appears brownish. It is one of the three isogeneric benzenetriols and can be prepared by heating the gallic acid. The pyrogallol moiety is important in making polyphenols as effective antioxidants [17].

Table 5: Peak number, Retention time, peak area (%), molecular formula, molecular weight, component name, component nature, bioactivity of TET tea

Peak	Retention time	Area (%)	Molecular formula	Molecular weight	Compound name	Type/ nature	Therapeutic use
7	7.859	1.62	$C_{14}H_{30}$	198.39	Tetradecane	Alkane	-
8	8.146	21.55	$C_6H_6O_3$	126.11	1,2,3-Benzenetriol	Pyrogallol	Antioxidant antiseptic fungicide, antidermatitic insecticide, candidicide
14	11.080	8.35	$C_7H_{12}O_6$	192.16	1,3,4,5-Tetrahydroxy-cyclohexanecarboxyl	Quinic acid	Antimicrobial, anti-inflammatory induces antioxidant
19	13.552	57.52	$C_8H_{10}N_4O_2$	194.19	Caffine 1,3,7-Trimethyl-3,7-dihydro-1H-Purine-2,6-dione	Alkaloid	Both pro and antioxidant
20	13.730	3.57	$C_7H_8N_4O_2$	180.16	1H-Purine-2,6-dione, 3,7-Dihydro-3,7-Dimethyl	Xanthine, alkaloid	Vasodilation both pro-and antioxidant natural diuretic diuretic
22	14.293	1.18	$C_{16}H_{32}O_2$	256.42	*n*-Hexadecanoic acid	Palmitic acid	Antioxidant nematicide pesticide hemolytic anti-inflammatory hemolytic
27	16.014	2.00	$C_6H_{26}O$	234	*Cis, cis, cis*-7,10,13-Hexadecatriental	Alcohol	-

Table 6: Peak number, Retention time, peak area (%), molecular formula, molecular weight, component name, component nature, bioactivity of KOL tea

Peak	Retention time	Area (%)	Molecular formula	Molecular weight	Compound name	Type/nature	Therapeutic use
9	8.128	17.41	$C_6H_6O_3$	126.11	1,2,3-Benzenetriol	Pyrogallol	Antioxidant antiseptic fungicide, antidermatitic insecticide, candidicide
17	11.024	10.10	$C_7H_{12}O_6$	192.16	1,3,4,5-Tetrahydroxy-cyclohexanecarboxyl	Quinic acid	Antimicrobial, anti-inflammatory induces antioxidant
22	13.490	59.79	$C_8H_{10}N_4O_2$	194.19	1,3,7-Trimethyl-3,7-dihydro-1H-Purine-2,6-dione	Caffeine	Both pro and antioxidant
23	13.70	2.23	$C_7H_8N_4O_2$	180.16	1H-Purine-2,6-dione, 3,7-Dihydro-3,7-Dimethyl	Xanthine, Alkaloid	Vasodilation both pro-and antioxidant natural diuretic diuretic
25	14.291	1.17	$C_{16}H_{32}O_2$	256.42	*n*-Hexadecanoic acid	Palmitic acid	Antioxidant nematicide pesticide hemolytic anti-inflammatory hemolytic
29	16.016	2.40	$C_6H_{26}O$	234	*Cis, cis, cis*-7,10,13-Hexadecatriental	Fatty aldehydes	-

Table 7: Peak number, Retention time, peak area (%), molecular formula, molecular weight, component name, component nature, bioactivity of ASS tea

Peak	Retention time	Area (%)	Molecular formula	Molecular weight	Compound name	Type/ nature	Therapeutic use
1	3.189	2.13	-	-	5-Methyl-5,6-dihydro-2 (1H)-pyridinone	Unknown	-
3	4.185	1.98	-	-	1-(N, N-Dimethyl) aminopropyne	Unknown	-
4	4.640	2.12	$C_6H_8O_4$	144.12	4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydro-6-methyl	Flavonoid fraction	antimicrobial and anti-inflammatory
12	11.441	31.46	$C_7H_{12}O_6$	192.16	1,3,4,5,-tetrahydroxy-cyclohexanecarboxyl	Quinic acid	Antimicrobial, anti-inflammatory induces antioxidant
16	13.547	42.75	$C_8H_{10}N_4O_2$	194.19	1,3,7,-trimethyl-3, 7-dihydro-1H-purine-2,6-dione	Coffeine alkaloid	Both pro and antioxidant
17	13.849	4.16	$C_7H_8N_4O_2$	180.16	1H-Purine-26-Dione, 3,7-Dihydro-3,7-dimethyl	Xanthine, alkaloid	Vasodilaton both pro-and antioxidant natural diuretic diuretic
18	14.306	3.58	$C_{16}H_{32}O_2$	256.42	*n*-Hexadecanoic acid	Palmitic acid	Antioxidant nematicide pesticide hemolytic anti-inflammatory hemolytic
23	16.026	1.50	$C_{18}H_{30}O_2$	278.	9,12,15-Octadecatrienoic acid (z, z, z)	Linolenic acid	Antiarthritic, antihistaminic, anticoroanary, antiandrogenis, antinematicide, anticancer, antibacterial

Quinic acid (1,3,4,5-tetrahydroxy-cyclohexanecarbonyl)

It is found in various plant products and not produced in human body. It stimulates the antioxidant mechanism of other compounds and increases the efficiency of antioxidant activity.

Coffeine (alkaloid)

It is a while crystalline xanthine alkaloid, bitter in taste, and a metabolic stimulant. It is used medically and recreationally to reduce fatigue and to reduce drowsiness coeffective consumption is associated with a lower overall risk of cancer. Coffeine is one of the more efficient non-enzymatic antioxidants. Coeffeine directly acts as an antioxidant, independent of flavonoids, by binding on to the hydroxyl radical, and makes it inert. This means that caffeine is not only a metabolic stimulant; it is also a protective antioxidant. The alkaloid caffeine and its catabolic products theobromine and xanthine exhibit both antioxidant and prooxidant properties [18].

Table 8: Major health beneficial phytochemicals of GT samples analyzed by GC-MS

Compounds	Peak area (%) of GT samples					
	MOON	TAN	GT	TET	KOL	ASS
Pyrogallol	18.40	16.28	28.36	21.55	17.61	–
Quinic acid	13.96	9.64	1.67	8.35	10.10	31.46
Caffeine (alkaloid)	46.25	57.17	56.48	57.52	59.79	42.75
Xanthine (alkaloid)	3.00	3.25	4.33	3.57	2.23	4.16
Palmitic acid	2.90	1.53	2.11	1.18	1.17	3.58
Palmitic acid	-	1.19	1.03	-	-	-
Phytol ethyl ester	1.34	-	-	-	-	-
α-linolenic acid	5.39	-	2.07	-	-	-
Total	91.24	89.06	96.05	92.17	90.9	83.45

-:Not detected, GC-MS: Gas chromatography-mass spectroscopy, GT: Green tea

Phytol (deterpene)

It is a key acidic diterpene alcohol that is a precursor of vitamins E and K1, and an antioxidant and a preventive agent against exoxido-induced breast carcinogenesis [19]. Phytol is the product of chlorophyll mechanism in plants and so is available abundantly in nature. It is also known to inhibit the growth of *Staphylococcus aureus* and to block teratogenic effects of retinal phytol exhibits antibacterial activity by causing damage to cell membranes and leakage of potassium ions from bacterial cells [20]. Phytol demonstrated a strong antioxidant effect *in-vitro* by removing hydroxyl radicals and nitric oxide as well to prevent the formation of thiobarbituric acid reactive substances [21].

Theobromine [^{1}H-purine-2,6-dione,3,7-dihydro-3,7-dimethyl]

It is xanthine alkaloid found in chocolate, tea plants, kola nut, and guarana white powder, and is a close relative of caffeine. It has similar, but lesser, effect as coeffine in human nervous systems. It exhibits both pro and antioxidant effects.

Palmitic acid (n-hexadecanoic acid)

It is the most fatty acid (saturated) found in plants and microorganisms. It is a major component of the oil from palm trees hence the same.

Palmitic acid ethyl ester (hexadecanoic acid)

It is a neutral, lipid soluble form of the free acid. It has earlier been reported as a component in alcohol extract of leaves *Kigelia pinnata* [22]. It exhibits antioxidant and anti-carcinogenic effects.

α-Linolenic acid (9,12,15-octadecatrienoic acid, (z,z,z))

It is an essential omega-3 fatty acid found in seeds and leafy green vegetables. It is a member of the group of essential fatty acid so called because it cannot be synthesized in the body and can be acquired only through diet. Preliminary research has found that the α-linolenic acid is related to a lower risk of cardiovascular risk. It is more susceptible to oxidation and will become rancid more quickly [23] have reported that α-linolenic acid was more efficient antioxidant than α-eleostearic acid against oxidative DNA damage.

The GC-MS analyzed major phytochemicals in the GT samples that possess antioxidant, antibacterial, and other health benefits are short-listed and presented in Table 8. It can be observed from Table 8 that all the commercial GT samples contained a very high percentage of phytochemicals, some of them, with wide spectrum beneficial properties. The highest percentage of composition of ~96% was exhibited by GT tea and a lowest value of ~83% by ASS tea. These amazing high composition of bioactive phytochemicals emphasizes the fact that the GT is the super food.

CONCLUSION

GC-MS analysis was carried out to identify major phytochemicals with health beneficially potential in six commercial GTs of India. The results revealed that the GT samples contained a very high percentage of various phytochemicals, all with specific as well as multiple health improving properties.

REFERENCES

1. Chin YW, Balunas MJ, Chai HB, Kinghorn AD. Drug discovery from natural sources. AAPS J 2006;8:E239-53.
2. Pcerangeli G, Vital G, Rivera W. Antimicrobial activity and cytotoxicity of *Chromolaena odorata* (L.f) king and rabinson and *Uncaria perrottetii* (A. Rich) Merr extracts. J Med Plants Res 2009;3(7):511-8.
3. Sermakkani M, Thangapandian V. GC-MS analysis of *Cassia itallica* leaf methanol extract. Asian J Pharm Clin Res 2012;5(2):90-4.
4. Marston A. Role of advances in chromatographic techniques in phytochemistry. Phytochemistry 2007;68(22-24):2786-98.
5. Konovalova O, Gergel E, Herhel V. GC-MS analysis of bioactive components of *Shepherdia argentea* (Pursh.). Nutt. from Ukranian Flora. Pharm Innov J 2013;2(6):7-12.
6. Kalaivani CS, Sathish SS, Janakiraman N, Johnson M. GC-MS studies on *Andrographis paniculata* (Burm. f.) Wall. Ex nees – A medicinally important plant. Int J Med Arom Plants 2012;2(1):69-74.
7. Praveenkumar P, Kumaravel S, Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. Afr J Biochem Res 2010;4(7):191-6.
8. Chibani S, Berhail-Boudouda H, Kabouche A, Aburaj T, Kabouche Z. Analysis of the essential oil of *Ferula communis* L. from Constantine Algeria. Int J Med Aromat Plants 2011;1(2):41-4.
9. Sangeetha J, Vijayalakshmi K. Determination of bioactive components of ethyl acetate fractions of *Punica granatum* rind extract. Int J Pharm Sci Drug Res 2011;3(2):116-22.
10. Ghannadi A, Dezfuly N. Essential oil analysis of the leaves of Persian true myrtle. Int J Med Aromat Plants 2011;1(2):48-50.
11. Venkataraman B, Samuel LA, Saradhi MP, Rao BN, Vamsi N, Krishna A, *et al*. Antibacterial, antioxidant activity and GC-MS analysis of *Eupaturium odoratum*. Asian J Pharm Clin Res 2012;5(2):99-106.
12. Namita P, Mukesh R, Vijay KJ. *Camellia sinensis* (Green tea): A review. Glob J Pharm 2012;6(2):52-9.
13. Hamilton-Miller JM. Antimicrobial properties of tea (*Camellia sinensis* L.). Antimicrob Agents Chemother 1995;39(11):2375-7.
14. Graham HN. Green tea composition, consumption, and polyphenol chemistry. Prev Med 1992;21(3):334-50.
15. Kulandhaivel M, Palaniswamy M. *In vitro* antimicrobial activity of *Camellia sinensis* and *Myristica fragrans* against *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans*. Int J Pharm Biol Arch 2012;3(3):604-9.
16. Sinija VR, Mishra HN. Green tea: Health benefits (Review). J Nutr Environ Med 2008;17(4):232-42.
17. Mitsuhashi S, Saito A, Nakajima N, Shima H, Ubukata M. Pyrogallol structure in polyphenols in involved in apoptosis induction on HEK 293T and K562 cells. Molecules 2008;13(12):2998-3006.
18. Azam S, Hadi N, Khan NU, Hadi SM. Antioxidant and prooxidant properties of caffeine, theobrumine and xanthine. Med Sci Monit 2003;9(9):BR325-30.
19. Yu FL, Gapor A, Bender W. Evidence for the preventive effect of the polyunsaturated phytol side chain in tocotrienols on 17beta-estradiol epoxidation. Cancer Detect Prev 2005;29(4):383-8.
20. Inoue Y, Hada T, Shiraishi A, Hirose K, Hamashima H, Kobayashi S. Biphasic effects of geranylgeraniol, teprenone, and phytol on the growth of *Staphylococcus aureus*. Antimicrob Agents Chemother 2005;49(5):1770-4.
21. Patricio Santos CC, Salvadori MS, Mota VG, Costa LM, Almeida AA, Oliveira GA, *et al*. Antinociceptive and antioxidant activities of phytol *in-vivo* and *in-vitro* models. Neuroscience Journal, 2013;2013:1-9.
22. Grace OM, Light ME, Lindsey KL, Moholland DA, Staden JV, Jader AK. Antibacterial activity and isolation of antibacterial compounds from fruit of the traditional compounds from fruit of the traditional African medicinal plant *Kigelia africana*. S Afr J Bot 2002;68:220-2.
23. Pal M, Ghosh M. Prophylactic effect of a-linolenic acid and a-eleostearic acid against MeHg induced oxidative stress, DNA damage and structural changes in RBC membrane. Food Chem Toxicol 2012;50:2811-8.