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Three Brands of Chinese Green Tea Confer Immunity and Attenuate Susceptibility to Malaria Infection on a Long Term

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Abstract
The study evaluated the phytochemical compounds; the antioxidant and anti-malarial activity of Chinese green tea (GBTI9593, TD659 and XH609) extract fractions and the synergistic interactions of the bioactive constituents. Antioxidant activity was measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity of the extract, based on the scavenging of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical using ethanol extract, methanol fraction, and water fraction of Chinese green tea in 4 levels of concentration (25, 50, 75, 100µg/ ml). Gas chromatography – Mass spectrometry (GC-MS) was used to extract samples of the Chinese green tea into different components with different retention times. Malaria susceptible respondents (10) in number who have used the tea in the last 3 years were interviewed and their experiences regarding the tea were noted. The results of GC-MS analysis showed that at least 9, 12 and 8 bioactive compounds were present in the methanol extract of GBTI9593, TD659 and XH609 respectively. These compounds were identified through mass spectrometry (MS) attached to gas chromatogram (GC). XH609 had the highest value of 0.094%, ascorbic acid was 0.146% and garlic acid was 0.203% at 25µg/ml compared to GBTI9593 and TD659. These results reveal that the extracts of green tea XH-609 could act as electron donor and could also react with free radicals by converting them to more stable products and terminating the radical chain reaction. Thus the in-vitro studies clearly indicate that the methanol extract of these Chinese tea show significant antioxidant activity and also a better source of natural antioxidant, which might be helpful in preventing the process of various oxidative stresses implicated in the onset of malaria infection.
In conclusion, the extract and fractions of Chinese green tea GBTI9593, TD659 and XH609 have potential anti-malarial and antioxidant properties.
Keywords: Chinese, tea, antioxidant, GBT19593, TD659, malaria

INTRODUCTION

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans (a group of single-celled microorganisms) belonging to the *Plasmodium* type. Malaria causes symptoms that include fever, fatigue, vomiting, and headaches. In severe cases it can cause yellow skin, seizures, coma, or death. Symptoms usually begin ten to fifteen days after being bitten. If not properly treated, people may have recurrences of the disease months later. In those who have recently survived an infection, reinfection usually causes milder symptoms. This partial resistance disappears over months to years if the person has no continuing exposure to malaria. (WHO, 2014 and Caraballo 2014).

The rapid increase in the resistance and multi resistance to many drugs used at the moment makes it necessary to find and test new drugs or new molecules with antimalarial properties (De Ridder *et al*., 2008). The difficulty of creating efficient vaccines as well as adverse side effects of existing antimalarial drugs highlight the urgent need for novel, well tolerated antimalarial drugs both for prophylaxis and treatment of malaria (Ramazani *et al.*, 2010).

Many drugs used in conventional medicine were originally derived from plants. Salicylic acid is a precursor of aspirin that was originally derived from white willow bark and the meadowsweet plant (*filipendula ulmaria*). (L. Maxim) (Raskin, 1992). Quinine and Artemesinin are antimalarial drugs derived from *Cinchona pubescens* Vahl bark and Artemisa annua L. plant respectively.

The use of herbs to treat disease is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care. Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become increasingly more in recent years as scientific evidence about the effectiveness of herbal medicine has become more widely available.

The association between tea consumption, especially green tea, and human health has long been appreciated (Weisburger JH *et al*.,2000). Tea has been cultivated for centuries, beginning in India and China. Today, tea is the most widely-consumed beverage in the world, second only to water. Hundreds of millions of people drink tea, and studies suggest that green tea (*Camellia sinesis*) in particular has many health benefits. Antonella et al (2012) has reported the in vitro antimalarial activity of Artemisia annua herbal tea. Akande et al (2015) also reported the anti malarial activity of Chinese green tea in vivo among other reports. However, there has not been report of acquired immunity against malaria on a long term basis by drinking green tea.

Chinese herbal/patent medicine (CHM/CPM) has long been an integral part of Asian culture and practices. The long time increase in Asian immigration to North America, particularly to New York, has brought about a rise in the sale and consumption of Chinese medicines among Asians as well as non-Asians in this region (Ernst 2004). These products are used in everything from teas and sports bars to spa treatment and herbal ''medicines''. Although, CHM/CPM may not pose a problem when used appropriately, misused, mislabeled and inappropriate preparations may lead to serious health consequences (Chan 2003).

Green tea is made from unfermented leaves and contains the high concentration of powerful antioxidants called polyphenols. Antioxidants are substances that fight free radicals, damaging compounds in the body that change cells, damage DNA, and even cause cell death. Free radicals contribute to the aging process, as well as the development of a number of health problems, including susceptibility to malaria, cancer and heart disease. Antioxidants, such as polyphenols in green tea, can neutralize free radicals and may reduce or even help prevent some of the damage they cause. The processing preserves natural polyphenols in green tea with respect to the health-promoting properties. As green tea is fermented to Oolong and then to black tea, polyphenol compounds (catechins) in green tea are dimerized to form a variety of the aflavins, such that these teas may have different biological activities.

Green tea is a popular neutraceutical as an antioxidant. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals, and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage (Halliwell and Gutteridge., 1985). Catechins are hypothesized to help protect against these diseases by contributing, along with antioxidant vitamins (i.e., vitamins C and E) and enzymes (i.e., superoxide dismutase and catalase), to the total antioxidant defense system (Abdel-Raheim *et al*., 2009).

Despite the available evidence on the anti malarial activities of Chinese green tea, no concrete evidence so far has suggested their usefulness as malaria immunity conferring agent.

MATERIALS AND METHODS

Chemicals and Reagents

Chemicals and reagents used were of analytical grade.

Herbal Mixture Collection

The Chinese herbal tea produced by China tea (Human), An Hua Tea Factory, was sourced from Guangzhuo, China.

Herbal mixture preparation

The three different samples purchased were extracted using the infusion method, 200g of the first sample GBTI9593 (A) ,150g of second sample TD659 (B), 200g of third sample XH609(C) were weighed into 3 clean bowls, 550cl of hot water was added and was left to infuse for 3days. After the third day, the samples were filtered into different stainless plates and placed on the hot plate to concentrate. A portion of the concentrate was then weighed and made up to 20ml with distilled water.

Phytochemical screening (Qualitative analysis)

Chemical constituents of the aqueous extracts were screened and identified by the methods described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

The presences of the following active constituents were tested for: Tannins, saponin, steroids, alkaloids, flavonoids, terpenoids, phenol, cardiac glycosides and anthraquinone.

ANTIOXIDANT PROPERTIES

Reducing power determination

About 1ml of sample was measured into a testube,1ml of distilled water was added to 2.5ml of potassium ferricynide, the mixture was incubated for 30mins at 50^0C and 2.5ml of 10% TCA was added to the mixture and incubated again for 10mins and later centrifuged. 2.5ml of the centrifuged mixture was weighed, 2.5ml of distilled water and 0.5ml of 1% ferric chloride was added, the mixture was incubated for another 10mins and the absorbance was read at 700nm.

DPPH radical scavenging activity assay

The free radical scavenging activity of the extract, based on the scavenging of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was estimated according to the procedure described by (Cuendet *et al.,* 1997 and Burits and Bucar, 2000).

Nitric oxide scavenging activity assay

The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO Under aerobic condition, NO reacts with oxygen to produce stable products (nitrate and nitrite), which can be determined using Griess reagent. The absorbance of the chromophore that formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with

Naphthylethylenediaminedihydrochloride can be immediately read at 550 nm. A 4 ml sample of plant extract or standard solution of different concentrations (25, 50, 75, 100 µg/ml) were taken in different test tubes and 1 ml of Sodium nitroprusside (5 mM in phosphate buffered saline) solution was added into the test tubes. They were incubated for 2 hours at 30 °C to complete the reaction. A 2 ml sample was withdrawn from the mixture and mixed with 1.2 ml of Griess reagent (1% Sulphanilamide, 0.1% naphthylethylenediaminedihydrochloride in 2% H3PO4). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthylethylenediamine was measured at 550 nm (Alisi *etal.,*2008). Ascorbic acid was used as standard. The percentage (%) inhibition activity was calculated from the following equation: % inhibition = [(A0 - A1)/A0] x 100 Where, A0 is the absorbance of the Control and A1 is the absorbance of the extract or standard.

Total Antioxidant Capacity

Total antioxidant capacity determination: The total antioxidant capacity of the extracts was determined using the method of (Prieto *et al*., 1999)

Mineral content analysis

The dried powdered samples were first digested with nitric acid and per chloric acid and then the aliquots were used for the determination of sodium, potassium, calcium, magnesium, phosphorus, iron, copper, zinc, lead and manganese content. Phosphorous was determined by spectrophotometer while sodium and potassium were determined by flame photometer (Khalil and Mannan, 1990). Iron, copper, zinc, manganese, calcium, lead and magnesium were determined by atomic absorption spectrophotometer (A.O.A.C., 1990).

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY ANALYSIS

The samples were extracted using N-hexane and thereafter concentrated and subjected to further analysis (Vuong et al., 2010 and Akande et al., 2015).

GC-MS machine

The bioactive constituents analysis was done using GC-MS (model Agilent technologies 7890A GC system, model detector (agilent technologies 5975c vlmsd), ejector (agilent technologies 7683b series), column(HP5MS with length 30 m and internal diameter 0.320 µm, thickness of column, 0.25 µm Mobile phase used was helium gas. Temperature programmes for the analysis was initial temperature (80^{o}C) to hold for 20mins, it was increased by 10^{o}C/min to 24^{0}C to hold for 6mins.

STATISTICAL ANALYSIS

The results were expressed as mean ± standard deviation. Statistical analysis was carried out using Excel package. Student's test was used for comparing the different concentrations obtained. A difference of $p < 0.05$ was considered significant.

RESULTS

Table 1: Qualitative phytochemical screening of the Chinese herbal teas

TEST	GB/T19598 (A)	TD-659 (B)	XH 609 (C)
Saponin	+	-	-
Tannin	+	-	+
Alkaloids	+	+	+
Terpenoids	+	+	+
Phlobatannins	-	+	+
Steriods	+	+	+
Phenols	+	+	+
Flavanoids	+	-	+
Cardiac glycoside	+	+	+

- = Not detected

+ = Present

Table 2; Quantitative analysis of phytochemicals present in Chinese green tea

	GB/T19598	TD659	XH-609
Total phenol (mg/100g)	25.68±2.15	37.94±2.78	44.96±2.51
Tannin (mg/100g)	0.85±0.26	-	6.36±0.79
Total flavonoid (mg/100g)	11.17 ±1.57	-	14.71±1.16
Phlobatanin (mg/100g)	-	54.91±3.15	56.05±3.54
Saponin %	55.0 ± 2.0	-	-

Values represented as Mean ± Standard deviation (N=2)

Quantitative analysis of phytochemicals in Chinese tea shows highest concentration of phenol in XH-609 and lowest in GB/T19598 but absent in TD-659. Percentage of saponins was high in GB/T19598 and absent in TD-659 and XH-609 (Table 2).

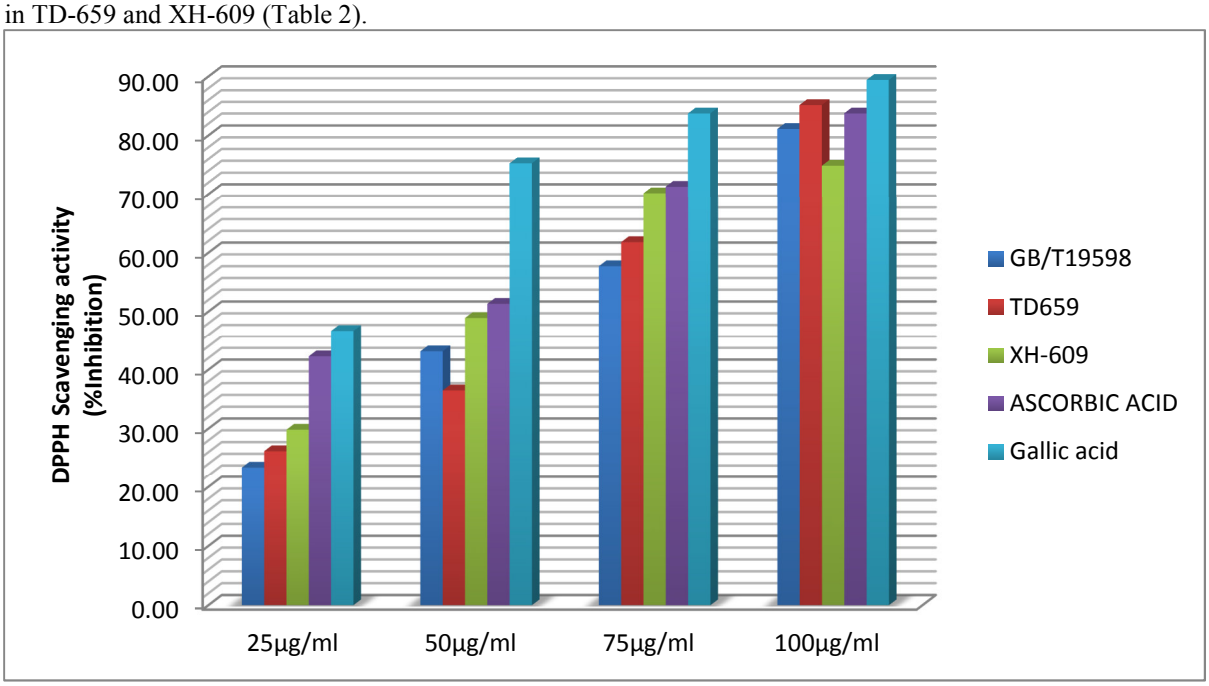


Fig 1: DPPH scavenging activity of three tea samples

At 25um/ml DPPH scavenging activity GB/T19598 shows concentration lower than 42.29% inhibition of ascorbic standard, 46.71% inhibition of garlic acid and also lower at DPPH scavenging activity of 100um/ml with 81.12% inhibition compared to 83.78% of ascorbic acid and 89.43% of garlic acid. TD—659 shows lower percentage of inhibition at 100um/ml. ascorbic acid of DPPH scavenging activity, whole XH-609 was lowest in both.

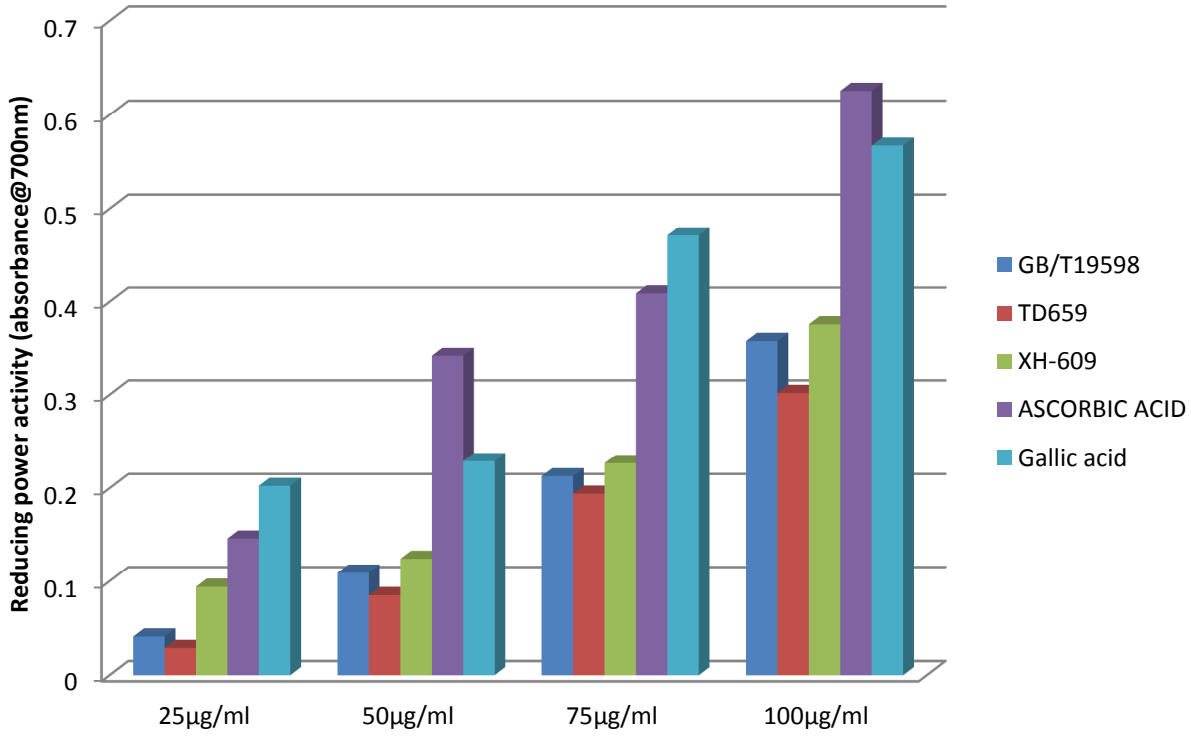


Fig 2: Reducing power activity of the samples

At 25(g/ml), XH609 had the highest value of 0.094%, ascorbic acid was 0.146% and garlic acid was 0.203%. The lowest reducing capacity was observed in TD-659 and GBT19598.

Table 3; Mineral contents (mg/100g)

Samples	Cd	Ni	Cr	Pb	Zn
GB/T19598	ND	0.05	0.13	0.05	2.05
TD659	ND	0.18	0.30	0.23	1.73
XH-609	ND	ND	0.23	ND	2.20

ND = Not detected

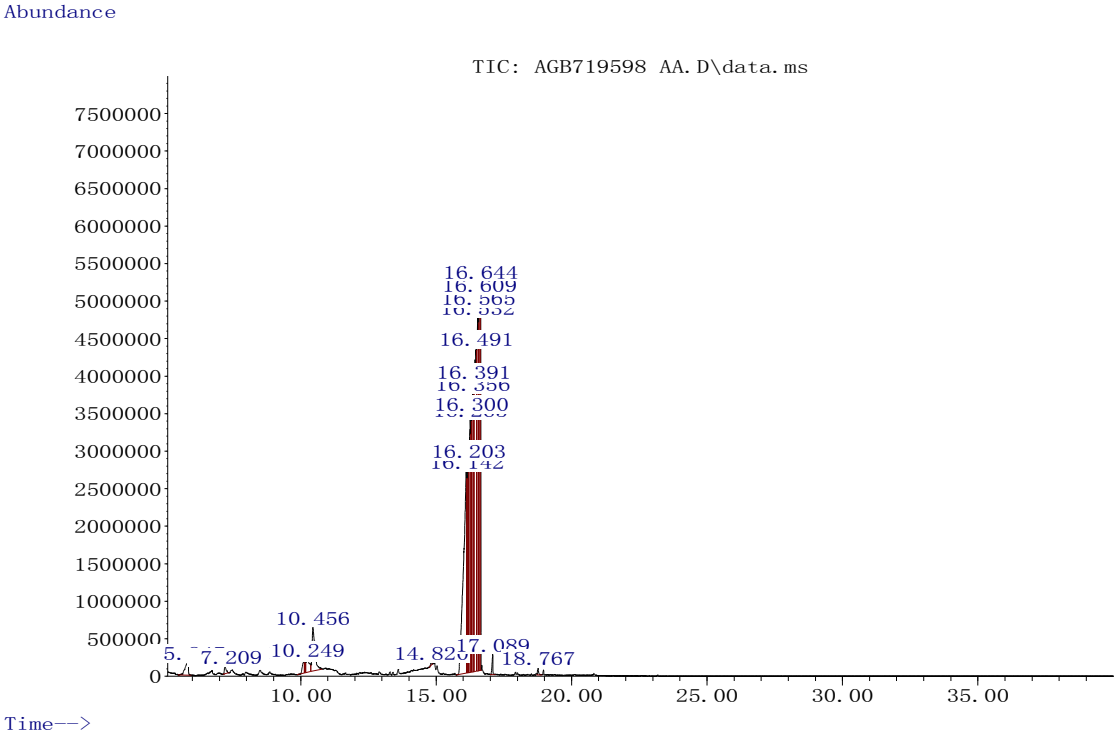


Figure 3: Chromatogram of Chinese tea (AGB719598) using GC-MS

Table 4: Bioactive constituents of Chinese tea (AGB719598) using GC-MS

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	5.848	0.97	C:\Database\NIST08.L			
			4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-	20368	028564-83-2	78
			4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-	20367	028564-83-2	56
			4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-	20366	028564-83-2	50
2	7.210	0.28	C:\Database\NIST08.L			
			Benzofuran, 2,3-dihydro-	9282	000496-16-2	68
			Benzofuran, 2,3-dihydro-	9283	000496-16-2	62
			Benzenemethanol, .alpha.-(1-phenyl aminoethyl)-	80469	042787-62-2	47
3	10.122	0.44	C:\Database\NIST08.L			
			1,2,3-Benzenetriol	10978	000087-66-1	97
			1,2,3-Benzenetriol	10981	000087-66-1	94
			1,2,3-Benzenetriol	10986	000087-66-1	91
4	10.151	0.29	C:\Database\NIST08.L			
			1,2,3-Benzenetriol	10978	000087-66-1	94
			1,2,3-Benzenetriol	10981	000087-66-1	91
			1,2,3-Benzenetriol	10986	000087-66-1	87

5 10.248 1.03 C:\Database\NIST08.L
1,2,3-Benzenetriol 10978 000087-66-1 95
1,2,3-Benzenetriol 10981 000087-66-1 93
1,2,3-Benzenetriol 10986 000087-66-1 74

6 10.454 2.95 C:\Database\NIST08.L
3,5-Dimethyl-1-dimethylphenylsilyl 102718 1000307-90-6 70
oxybenzene
Benz[a]anthracene, 7-ethyl- 102881 003697-30-1 53
Benz(a)anthracene, 12-ethyl- 102882 018868-66-1 53

7 14.820 0.07 C:\Database\NIST08.L
Propanoic acid, 2-methyl-, 2-ethyl 59356 035061-61-1 27
hexyl ester
1-Cyclohexene-3-thione 6377 201139-65-3 18
Ethanone, 1-(6-methyl-7-oxabicyclo 27248 015120-94-2 14
[4.1.0]hept-1-yl)-

8 16.113 13.86 C:\Database\NIST08.L
Caffeine 55116 000058-08-2 97
Caffeine 55119 000058-08-2 96
Caffeine 55120 000058-08-2 95

9 16.142 2.78 C:\Database\NIST08.L
Caffeine 55116 000058-08-2 97
Caffeine 55119 000058-08-2 96
Caffeine 55120 000058-08-2 95

10 16.188 4.48 C:\Database\NIST08.L
Caffeine 55119 000058-08-2 97
Caffeine 55118 000058-08-2 95
Caffeine 55120 000058-08-2 95

11 16.205 1.99 C:\Database\NIST08.L
Caffeine 55116 000058-08-2 97
Caffeine 55119 000058-08-2 96
Caffeine 55120 000058-08-2 95

12 16.268 8.52 C:\Database\NIST08.L
Caffeine 55119 000058-08-2 97
Caffeine 55118 000058-08-2 97
Caffeine 55116 000058-08-2 95

13 16.302 3.14 C:\Database\NIST08.L
Caffeine 55116 000058-08-2 97
Caffeine 55119 000058-08-2 96
Caffeine 55118 000058-08-2 95

14 16.354 8.22 C:\Database\NIST08.L
Caffeine 55116 000058-08-2 97
Caffeine 55119 000058-08-2 96
Caffeine 55118 000058-08-2 95

15 16.394 4.62 C:\Database\NIST08.L
Caffeine 55119 000058-08-2 97
Caffeine 55116 000058-08-2 95
Caffeine 55120 000058-08-2 95

16 16.491 16.06 C:\Database\NIST08.L
Caffeine 55119 000058-08-2 97
Caffeine 55120 000058-08-2 95
Caffeine 55118 000058-08-2 95

17 16.531 9.42 C:\Database\NIST08.L
Caffeine 55120 000058-08-2 97
Caffeine 55118 000058-08-2 97
Caffeine 55119 000058-08-2 96

18 16.565 5.09 C:\Database\NIST08.L
Caffeine 55119 000058-08-2 97
Caffeine 55116 000058-08-2 95
Caffeine 55118 000058-08-2 95

19 16.611 9.64 C:\Database\NIST08.L
Caffeine 55119 000058-08-2 97
Caffeine 55118 000058-08-2 95
Caffeine 55116 000058-08-2 95

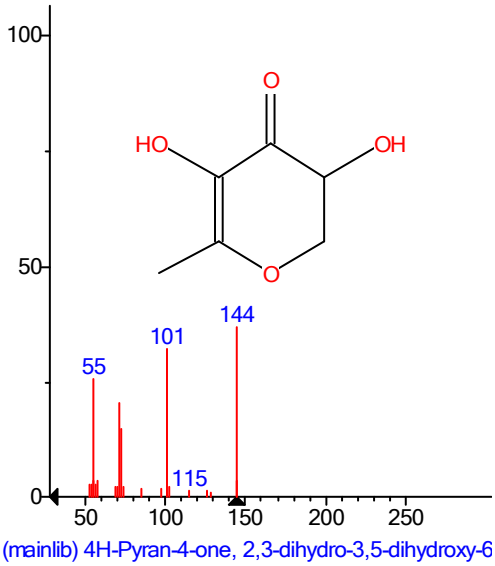
20 16.645 5.56 C:\Database\NIST08.L
Caffeine 55119 000058-08-2 97
Caffeine 55116 000058-08-2 95
Caffeine 55118 000058-08-2 95

21 17.092 0.40 C:\Database\NIST08.L
n-Hexadecanoic acid 102726 000057-10-3 99
n-Hexadecanoic acid 102724 000057-10-3 95
n-Hexadecanoic acid 102725 000057-10-3 92

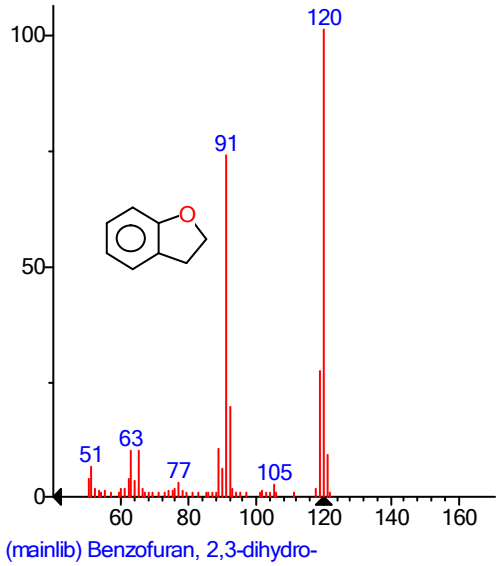
22 18.768 0.18 C:\Database\NIST08.L
9,12,15-Octadecatrienoic acid, (Z, 119802 000463-40-1 97
Z,Z)-
Methyl 8,11,14-heptadecatrienoate 119800 1000336-35-1 93
9,12,15-Octadecatrien-1-ol, (Z,Z,Z 108923 000506-44-5 93
)-

Table 5:Total Percentage Concentrations of bioactive constituents in Chinese green tea AGB719598

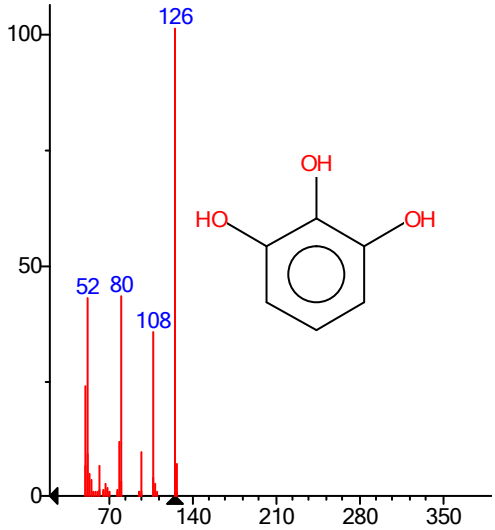
peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	5.847	70	133	148	BB 2	171967	14762779	6.03%	0.968%
2	7.209	358	371	393	BV	100437	4290939	1.75%	0.281%
3	10.122	836	880	882	BV 3	149740	6739152	2.75%	0.442%
4	10.149	882	885	891	VV 6	151060	4448229	1.82%	0.292%
5	10.249	891	902	924	VV 10	172904	15735997	6.43%	1.032%
6	10.456	924	938	996	VV 3	584210	44961552	18.36%	2.949%
7	14.820	1687	1701	1708	PV 9	37116	1028868	0.42%	0.067%
8	16.112	1863	1927	1929	BV 2	2688997	211331605	86.30%	13.860%
9	16.142	1929	1932	1934	VV	2677397	42437184	17.33%	2.783%
10	16.187	1934	1940	1941	VV	2853124	68320360	27.90%	4.481%
11	16.203	1941	1943	1945	VV	2823482	30308707	12.38%	1.988%
12	16.265	1945	1954	1957	VV	3328838	129846617	53.02%	8.516%
13	16.300	1957	1960	1961	VV	3427976	47876457	19.55%	3.140%
14	16.356	1961	1969	1971	VV 3	3700563	125368607	51.19%	8.222%
15	16.391	1971	1976	1977	VV	3854873	70390318	28.74%	4.616%
16	16.491	1977	1993	1994	VV 3	4306550	244887446	100.00%	16.060%
17	16.532	1994	2000	2003	VV 2	4698598	143649845	58.66%	9.421%
18	16.565	2003	2006	2008	VV	4838411	77640258	31.70%	5.092%
19	16.609	2008	2014	2017	VV 2	4989203	147055681	60.05%	9.644%
20	16.644	2017	2020	2024	VV	5178833	84847985	34.65%	5.565%
21	17.089	2079	2098	2110	BV 2	270944	6142402	2.51%	0.403%
22	18.767	2383	2391	2410	VV 4	97593	2710032	1.11%	0.178%



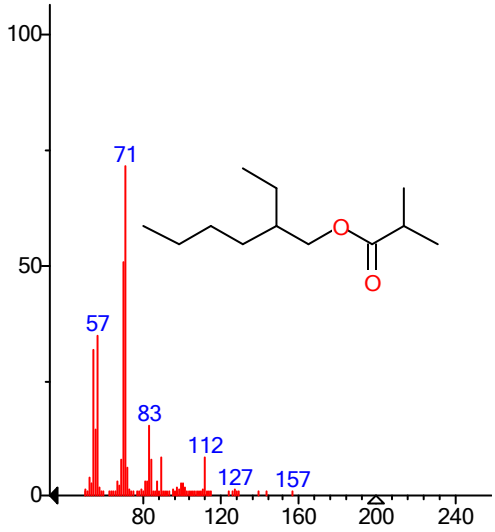
(mainlib) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6



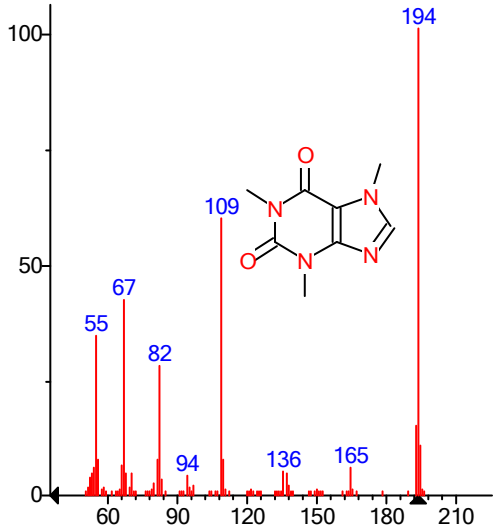
(mainlib) Benzofuran, 2,3-dihydro-



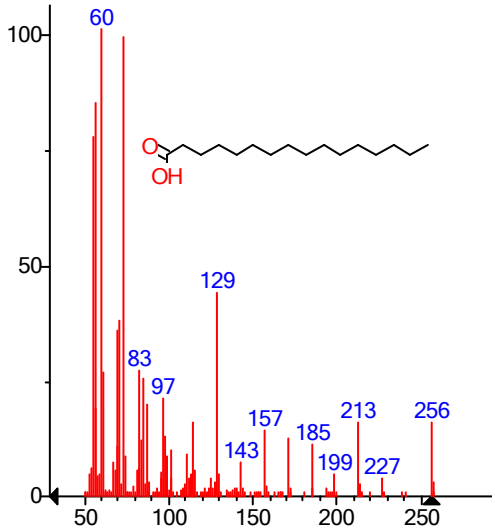
(replib) 1,2,3-Benzenetriol



(mainlib) Propanoic acid, 2-methyl-, 2-ethylhexyl ester



(replib) Caffeine



(replib) n-Hexadecanoic acid

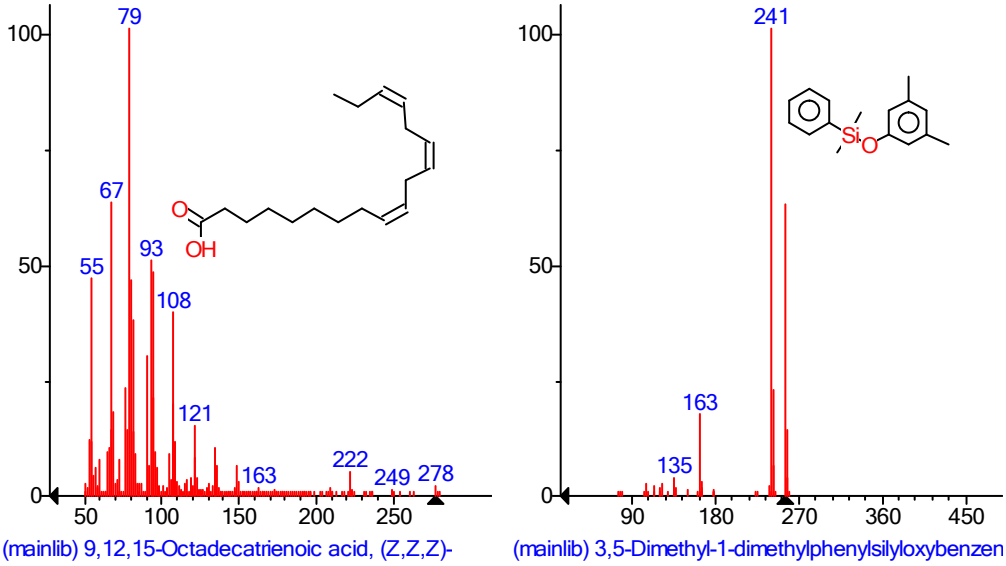


Figure 4: Elucidated structures of bioactive constituents common to the three Chinese green tea (AGB719598, C-XH609 and TD-59)

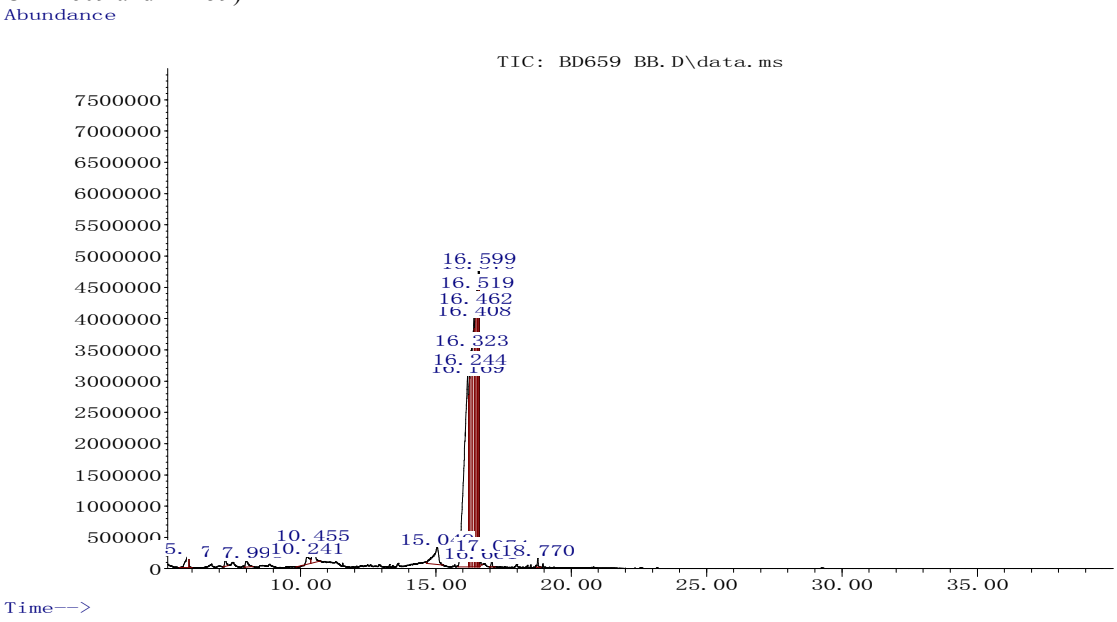


Figure: Chromatogram of Chinese tea (BD 659) using GC-MS

Sample : B-D659

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	5.865	1.09	C:\Database\NIST08.L			
			4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-	20368	028564-83-2	64
			4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-	20367	028564-83-2	50
			4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-	20366	028564-83-2	45
2	5.882	0.22	C:\Database\NIST08.L			
			4H-Pyran-4-one, 2,3-dihydro-3,5-di	20367	028564-83-2	86

hydroxy-6-methyl-
4H-Pyran-4-one, 2,3-dihydro-3,5-di 20368 028564-83-2 72
hydroxy-6-methyl-
4H-Pyran-4-one, 2,3-dihydro-3,5-di 20366 028564-83-2 56
hydroxy-6-methyl-

3 7.215 0.37 C:\Database\NIST08.L
Benzofuran, 2,3-dihydro- 9283 000496-16-2 62
N-Benzyl-2-phenethylamine 67984 003647-71-0 50
N-Benzyl-2-phenethylamine 67983 003647-71-0 50

4 7.999 0.52 C:\Database\NIST08.L
1,2-Benzenediol, 3-methoxy- 18105 000934-00-9 95
1,2-Benzenediol, 3-methoxy- 18108 000934-00-9 91
1,2-Benzenediol, 3-methoxy- 18109 000934-00-9 90

5 10.242 0.73 C:\Database\NIST08.L
1,2,3-Benzenetriol 10981 000087-66-1 93
1,2,3-Benzenetriol 10978 000087-66-1 93
1,2,3-Benzenetriol 10986 000087-66-1 58

6 10.454 1.79 C:\Database\NIST08.L
3,5-Dimethyl-1-dimethylphenylsilyl 102718 1000307-90-6 52
oxybenzene
Phenol, 4,4'-(1-methylethylidene)b 102804 000079-97-0 50
is[2-methyl-
Benz(a)anthracene, 12-ethyl- 102882 018868-66-1 50

7 15.049 2.82 C:\Database\NIST08.L
Cycloheptanone, 3-(3,3-dimethylbut 56495 040564-95-2 25
yl)-
4-Methyl-5-imidazolemethanol 6212 1000238-64-2 25
2,5-Dimethylcyclohexanol 12431 003809-32-3 18

8 16.170 24.04 C:\Database\NIST08.L
Caffeine 55116 000058-08-2 97
Caffeine 55119 000058-08-2 96
Caffeine 55118 000058-08-2 95

9 16.245 7.02 C:\Database\NIST08.L
Caffeine 55116 000058-08-2 97
Caffeine 55119 000058-08-2 96
Caffeine 55118 000058-08-2 95

10 16.325 10.21 C:\Database\NIST08.L
Caffeine 55116 000058-08-2 97
Caffeine 55119 000058-08-2 96
Caffeine 55118 000058-08-2 95

11 16.411 14.02 C:\Database\NIST08.L
Caffeine 55119 000058-08-2 97
Caffeine 55118 000058-08-2 95
Caffeine 55116 000058-08-2 95

12 16.462 10.29 C:\Database\NIST08.L
Caffeine 55119 000058-08-2 97
Caffeine 55118 000058-08-2 95
Caffeine 55116 000058-08-2 95

13 16.519 11.54 C:\Database\NIST08.L
Caffeine 55119 000058-08-2 97
Caffeine 55120 000058-08-2 95
Caffeine 55118 000058-08-2 95

14 16.571 9.35 C:\Database\NIST08.L
Caffeine 55119 000058-08-2 97
Caffeine 55116 000058-08-2 95
Caffeine 55120 000058-08-2 95

15 16.599 5.21 C:\Database\NIST08.L
Caffeine 55119 000058-08-2 97
Caffeine 55118 000058-08-2 97
Caffeine 55116 000058-08-2 95

16 16.662 0.14 C:\Database\NIST08.L
Theobromine 44940 000083-67-0 64
Purine-2,6(1H,3H)-dione, 1-(2-ethe 97335 1000272-53-5 50
nyloxyethyl)-3,7-dimethyl-
Theobromine 44941 000083-67-0 49

17 17.074 0.37 C:\Database\NIST08.L
n-Hexadecanoic acid 102726 000057-10-3 99
n-Hexadecanoic acid 102724 000057-10-3 95
Tetradecanoic acid 81211 000544-63-8 84

18 18.768 0.28 C:\Database\NIST08.L
9,12,15-Octadecatrienoic acid, (Z, 119801 000463-40-1 99
Z,Z)-
9,12,15-Octadecatrien-1-ol, (Z,Z,Z 108923 000506-44-5 95
)-
7,10,13-Hexadecatrienoic acid, met 108848 056554-30-4 94
hyl ester

Area Percent Report

: BD659 BB.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	5.863	76	136	137	BV	6 188098	15007934	4.53%	1.090%
2	5.882	137	139	155	VB	2 188894	2985750	0.90%	0.217%
3	7.215	359	372	393	BV	3 122627	5052506	1.53%	0.367%
4	7.998	495	509	547	PB	3 107774	7143976	2.16%	0.519%
5	10.241	830	901	925	BV	9 130586	10082921	3.05%	0.732%
6	10.455	925	938	983	VV	4 320692	24593235	7.43%	1.786%
7	15.049	1670	1741	1774	VV	8 285210	38769047	11.71%	2.816%
8	16.169	1875	1937	1944	VV	3046562	330949450	100.00%	24.035%
9	16.244	1944	1950	1953	VV	2 3195659	96634991	29.20%	7.018%
10	16.323	1953	1964	1965	VV	3488911	140620131	42.49%	10.213%
11	16.408	1965	1979	1981	VV	3958967	193083542	58.34%	14.023%
12	16.462	1981	1988	1991	VV	2 4144046	141639641	42.80%	10.287%
13	16.519	1991	1998	2002	VV	2 4419859	158959221	48.03%	11.545%
14	16.570	2002	2007	2010	VV	4733785	128760659	38.91%	9.351%
15	16.599	2010	2012	2017	VV	4777334	71733917	21.68%	5.210%
16	16.661	2017	2023	2029	PV	2 69006	1921047	0.58%	0.140%
17	17.074	2079	2095	2108	BB	2 227477	5160653	1.56%	0.375%
18	18.770	2383	2391	2407	VB	3 146294	3823732	1.16%	0.278%

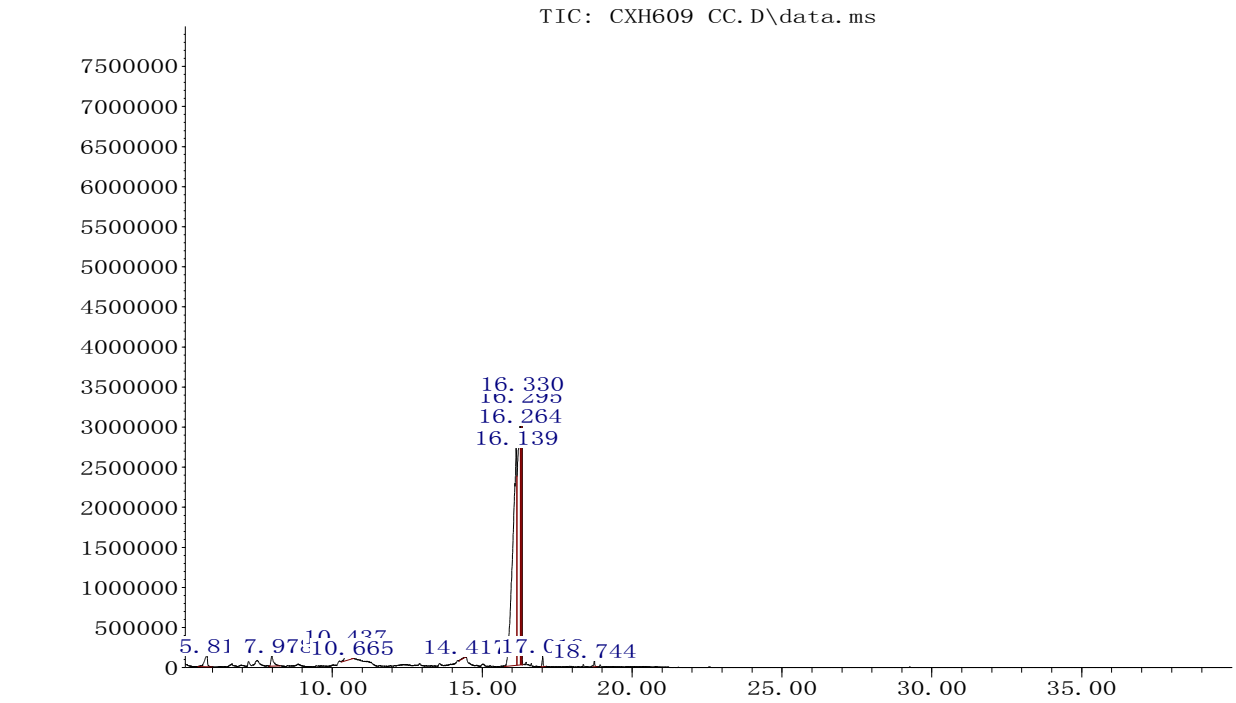


Figure 5: Chromatogram of Chinese tea (C-XH 609) using GC-MS

Table 6: Bioactive constituents of Chinese tea (CXH609) using GC-MS

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	5.819	1.71	C:\Database\NIST08.L			
			4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-	20367	028564-83-2	72
			4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-	20368	028564-83-2	64
			4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-	20366	028564-83-2	56
2	7.976	1.26	C:\Database\NIST08.L			
			1,2-Benzenediol, 3-methoxy-	18105	000934-00-9	94
			1,2-Benzenediol, 3-methoxy-	18109	000934-00-9	91
			1,2-Benzenediol, 3-methoxy-	18108	000934-00-9	91
3	10.437	2.37	C:\Database\NIST08.L			
			1,2,3-Benzenetriol	10978	000087-66-1	89
			1,2,3-Benzenetriol	10981	000087-66-1	50
			1,2,3-Benzenetriol	10986	000087-66-1	43
4	10.666	0.05	C:\Database\NIST08.L			
			1,2,3-Benzenetriol	10978	000087-66-1	93
			1,2,3-Benzenetriol	10981	000087-66-1	90
			1,2,3-Benzenetriol	10986	000087-66-1	90
5	14.419	0.10	C:\Database\NIST08.L			
			Propanoic acid, 2-methyl-, 2-ethyl hexyl ester	59356	035061-61-1	27
			N-Methylallylamine	610	000627-37-2	25
			Butanoic acid, pentyl ester	29657	000540-18-1	22

6 16.142 43.06 C:\Database\NIST08.L
Caffeine 55119 000058-08-2 96
Caffeine 55118 000058-08-2 95
Caffeine 55120 000058-08-2 95

7 16.262 29.35 C:\Database\NIST08.L
Caffeine 55119 000058-08-2 96
Caffeine 55118 000058-08-2 95
Caffeine 55120 000058-08-2 95

8 16.296 13.53 C:\Database\NIST08.L
Caffeine 55116 000058-08-2 97
Caffeine 55119 000058-08-2 96
Caffeine 55118 000058-08-2 95

9 16.330 7.82 C:\Database\NIST08.L
Caffeine 55119 000058-08-2 96
Caffeine 55120 000058-08-2 95
Caffeine 55118 000058-08-2 95

10 17.017 0.46 C:\Database\NIST08.L
n-Hexadecanoic acid 102726 000057-10-3 99
n-Hexadecanoic acid 102724 000057-10-3 95
n-Hexadecanoic acid 102725 000057-10-3 91

11 18.745 0.30 C:\Database\NIST08.L
9,12,15-Octadecatrien-1-ol, (Z,Z,Z 108923 000506-44-5 98
)-
7,10,13-Hexadecatrienoic acid, met 108848 056554-30-4 94
hyl ester
9,12,15-Octadecatrienoic acid, met 130796 000301-00-8 91
hyl ester, (Z,Z,Z)-

Table 7: Total Percentage Concentrations of bioactive constituents in Chinese green tea CXH609

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	5.817	74	128	154	BB	5 138812	10378856	3.97%	1.708%
2	7.978	489	505	552	BV	2 124099	7645736	2.92%	1.258%
3	10.437	911	935	973	VV	6 170222	14379413	5.50%	2.366%
4	10.665	973	975	982	VV	4 17758	319843	0.12%	0.053%
5	14.417	1597	1631	1634	PB	7 9729	589431	0.23%	0.097%
6	16.139	1859	1932	1936	PV	2 2716798	261643189	100.00%	43.060%
7	16.264	1936	1953	1955	VV	4 2980252	178349703	68.17%	29.352%
8	16.295	1955	1959	1963	VV	3 3219989	82223004	31.43%	13.532%
9	16.330	1963	1965	1971	VB	3393937	47527575	18.17%	7.822%
10	17.019	2072	2085	2097	BV	2 133693	2775581	1.06%	0.457%
11	18.744	2379	2387	2400	VB	4 74741	1795067	0.69%	0.295%

DISCUSSION

This study was designed to evaluate the phytochemical compounds, the antioxidant and anti-malarial activity of Chinese herbal green tea (GBTI9593, TD659 and XH609) extract and its fraction.

Several compounds were identified in the three different samples that were analysed. The compounds are known to have different functions and effects on consumption. Some of the drugs are stimulants which could be toxic when taken in excess. Stimulants are psychoactive drugs that induce temporary improvements in either mental or physical functions or both. Caffeine, theobromine, 1,2benzenediol and benzofuran are the active stimulants in the Chinese tea samples.

Caffeine is a central nervous system (CNS) stimulant of the methyl xanthine class. It is the world's

most widely consumed psychoactive drug, but unlike many other psychoactive substances is legal and unregulated in nearly all parts of the world. There are several known mechanisms of action to explain the effects of caffeine. The most prominent is that it reversibly blocks the action of adenosine on its receptor and consequently prevents the onset of drowsiness induced by adenosine. Caffeine also stimulates certain portions of the autonomic nervous system.

Qualitative phytochemical screening of the Chinese herbal teas sample GBT19598 tested positive to the presence of Saponins, tannins, alkaloids, terpenoids, steroids, flavonoids, cardial glycosides, phenols and negative for phlobatanin. Sample TD659 tested positive to alkaloid, terpenoids, steroid, cardial glycosides, phenol, phlobatanin and negative to tannis, saponins and flavonoid. Sample XH-609 tested positive to tanins, alkaloid, terpenoids, steroid, flavonoids, cardial glycosides, phenol, phlobatanin, and negative to saponins as shown in Table 1. A synergistic relationship amongst phytochemicals has been adduced to be responsible for the overall beneficial effect derivable from plants (Liu, 2004). Steroids and phlobatannins were found to be present in all the plants. Quantitative analysis in Chinese tea shows highest concentration of phenol (44.9mg/100g) was found in XH-609 and lowest in GB/T19598 (25.6mg/ 100g) but absent in TD-659. Percentage of saponins is high in GB/T19598 (55%) and absent in TD-659 and XH-609 which is in accordance with the results of Rath *et al* (2004), Mueller *et al* (2004) and Antonella De Donno *et al* (2012) that artemisinin itself is poorly soluble in water, but its solubility may be improved by the presence of other plant constituents with amphiphilic properties such as flavonoids, glucosides or saponins which are found in the herbal tea. It has been found that some of these investigated plants contained steroidal compounds. It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones (Okwu, 2001).

The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, alkaloids protect against chronic diseases. Saponins protect against hypercholesterolemia and antibiotic properties. Steroids and triterpenoids show the analgesic properties. Saponins could also elicit an anti plasmodial effect due to erythrocyte lysis, nevertheless the solubility of saponins in the herbal tea preparation, an aqueous solution, is expected to be very low. This has been confirmed by reports on metabolic profiles of *A. annua* teas, which do not report the presence of substantial quantities of saponins (Carbonara *et al*., 2012 and Liu *et al*., 2010).

The antioxidant capacities of the plant extracts largely depend on the composition of the extracts and conditions of the test system. The antioxidant capacities are influenced by many factors, which cannot be fully described with one single method. Therefore, it is necessary to perform more than one type of antioxidant capacity measurement to take into account the various mechanisms of antioxidant action (Wong, Leong and Koh, 2006). Reducing power, nitric oxide, DPPH, total antioxidant capacity were analysed. Nitric oxide is a potential pleiotropic mediator of various physiological processes such as smooth muscle relaxation, neuronal signalling, and inhibition of platelet aggregation and regulation of cell-mediated toxicity. At 25um/ml nitric oxide scavenging activity GB/T19598 shows concentration lower than 42.29 % inhibition of ascorbic standard and higher at nitric oxide scavenging activity of 100um/ml with 83.89 % inhibition. TD-659 and XH-609 shows lower percentage of inhibition at 25um/ml and higher percentage at 100um/ml Ascorbic Acid of nitric oxide scavenging activity. DPPH radical is used as the model system to investigate the scavenging activities of several natural compounds (Bhaskar*et al.,* 2007). DPPH is scavenged by antioxidants through the donation of proton forming the reduced DPPH which can be quantified by its decrease of absorbance (Chowdhury *et al.,* 2011). Radical scavenging activity increased with increasing percentage of the free radical inhibition (Bhaskar *et al.*, 2007).

At 25um/ml DPPH scavenging activity GB/T19598 shows concentration lower than 42.29 % inhibition of ascorbic standard, 46.71% inhibition of garlic acid and also lower at DPPH scavenging activity of 100um/ml with 81.12 % inhibition compared to 83.78% of ascorbic acid and 89.43% of garlic acid. TD-659 shows lower percentage of inhibition at 25um/ml and higher percentage at 100um/ml Ascorbic Acid of DPPH scavenging activity, while XH-609 was lowest in both.

The reducing capacity of a sample is regarded as a significant indicator of its potential antioxidant activity. The reducing power values of the chinese herbal tea extracts (µg/ml) are presented in Figure 2. At 25µg/ml, XH609 had the highest value of 0.094%, ascorbic acid was 0.146% and garlic acid was 0.203%. These results reveal that the extracts of green tea XH-609 could act as electron donor and could also react with free radicals by converting them to more stable products and terminating the radical chain reaction (Yen & Chen, 1995).

This is in agreement with the in vivo studies of Yokozawa *et al* (2002) and Skrzydlewska *et al* (2002) showing that green tea catechins increase total plasma antioxidant activity. (Yokozawa *et al*., 2002, Skrzydlewska *et al*., 2002). Intake of green tea extracts also increases the activity of superoxide dismutase in serum and the expression of catalase in the aorta; these enzymes are implicated in cellular protection against

reactive oxygen species (Skrzydlewska *et al*., 2002, Negishi *et al*., 2004). This action is combined with direct action on oxygen species by a decrease in the nitric oxide plasma concentration (Yokozawa *et al*., 1999). Malondialdehyde, a marker of oxidative stress, also decreases after green tea intake (Yokozawa *et al*., 2002, Yokozawa *et al*., 1999).These results suggest that catechins could have a direct (antioxidant) or indirect (increase of activity or expression) effect. Since catechins can act as antioxidants *in vitro*, they might prevent the oxidation of other antioxidants, such as vitamin E. However, ingestion of green tea catechins does not modify the plasma status of vitamins E and C *in vivo* (Skrzydlewska *et al*., 2002, Tijburg *et al*., 1997 and Alessio *et al*., 2003). Nevertheless, one study reported that catechins increase vitamin E concentration in low-density lipoprotein (Tijburg *et al*., 1997). and in this way could protect low-density lipoprotein against peroxidation(Yokozawa *et al*., 2002). The endoplasmic reticulum and mitochondria release oxygen. This oxygen gets converted into hydrogen peroxide, which in turn releases reactive oxygen species molecules. These reactive oxygen species molecules can lead to damage of DNA, RNA, oxidize proteins (enzymes, histones), oxidize lipids and can also activate cell suicide.

On the other hand, the lowest reducing capacity was observed in TD-659 and GB/T19598. The GC-MS result shows presence of bioactive compound in (Table 6) 4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl Benzofuran, 2,3-dihydro1,2,3-Benzenetriol 3,5-Dimethyl-1-dimethylphenylsilyl oxybenzene, Caffeine, nn-Hexadecanoic acid 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-Methyl 8,11,14-heptadecatrienoate 9,12,15 Octadecatrien-1-ol, (Z,Z,Z)-.4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl- had the lowest retention time of 5.848mins with 78% quality, 9,12,15-Octadecatrien-1-ol, (Z,Z,Z)- had the highest retention time and quality percentage, 18.768mins and 93% respectively in sample GB/T19598(A). Sample BD-659(B) 4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl- had the lowest retention time of 5.882mins with 86%, 9,12,15-Octadecatrienoic acid)- had the highest retention time and quality percentage, 18.768mins and 99% respectively. 4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl- had the lowest retention time of 5.819mins with 72% quality. 9,12,15 Octadecatrien-1-ol, (Z,Z,Z)- had the highest retention time and quality percentage, 18.745mins and 99% respectively in Sample XH609(C).

The results of mineral analysis of both varieties are presented in (Table 3). The three samples varieties contained variable quantities of minerals. It was found that GB/T19598 had relatively higher concentration of Zn (1.73mg/100g), as compared to the concentration of these minerals ,GB/T19598 had low values in Ni(0.05mg/100g) ,Cr(0.13mg/100g), Pb(0.05mg/100g). While TD659 had somewhat higher values of Zn (2.05mg/100g), but lower values in Ni(0.18mg/100g), Cr(0.30mg/100g), Pb(0.23mg/100g), while the third sample XH609 had high value of Zn(2.20mg/100g),low value of Cr(0.23mg/100g) but no values were detected in Pb and Ni. Generally, in all three samples Cd was not detected in all. The data on mineral analysis revealed that the investigated varieties appear to be a rich sources of zinc, but had lower values of Ni, Cr and Pb. These varieties can effectively contribute towards the daily recommended dietary allowances (RDA) for all groups.

CONCLUSION

In conclusion, the extract and fractions of Chinese green tea GBTI9593, TD659 and XH609 have potential anti-malarial and antioxidant properties. Intake of green tea can stop all these degenerative changes by inhibiting the action of the reactive oxygen species molecule. Polyphenols and flavonoids found in green tea help boost our immune system, making our health stronger in fighting against malaria infection. Therefore, drinking tea may be able to prime the body's immune system against these agents by teaching disease-fighter immune cells to recognize and remember alkylamines.

In addition, these teas also contain phytochemical constituents that have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. Although caffeine content of the tea were confirmed high we believe the effects- beneficial and perhaps adverse are being modulated by other bioactive constituents in the tea which we believe act in synergy. The long term anti malaria effect of these tea and their availability, affordability and general acceptance by Nigerian populace make them good candidates for malaria prophylaxis and treatment.

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