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Original Research Article

Bioactive Components of Tea Plant (*Camellia sinensis* (L.) O. Kuntze) From Mambilla Plateau, Taraba State - Nigeria

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ABSTRACT

Tea is the most widely consumed drink after water. It is of tremendous therapeutic, nutritional, and economic importance to consumers. There is documented information on the phytochemical composition of tea plants. There seems to be a dearth of information on the bioactive components of the plant in the Mambilla plateau. Therefore, this study explores tea clones' bioactive components in the Mambilla plateau, Taraba State, Nigeria. Leaves of *Camellia sinensis* were collected from the six original clones (CBB36, C68, C143, C236, C318 and C1212) in the Mambila plateau. The tea plant leaves collected were washed and air dried at room temperature for two weeks before powdering using an electric blender. The powdered leaves were poured into an airtight bottle and stored in a dark room. Bioactive compounds in the leaves were extracted by cold maceration and analysed using the GC-MS. The number of compounds identified followed the pattern: clone 68 (25) >clone 1212 (23) > clone 143 (22) >clone236 (17)> clone BB36 (14)> clone 318 (6). It was also observed that all the leaves of the tea clones analysed contained caffeine (clones BB36, 318, 1212, 236, 68) though at varying levels, except clone 143. Also, phenolic compounds like phenol, 2,4-dimethyl- (0.068 mg/ml - 0.419 mg/ml), phenol, 3,5-dimethyl-(1.430 mg/ml - 2.806 mg/ml), phenol, 2-ethyl (0.318 mg/ml) and phenol, 3-methyl- (2.953 mg/ml) were detected in leaves of *C. sinensis*. Several bioactive compounds were identified in the different clones of tea plants that were investigated, and some may be of therapeutic and nutritional importance to consumers.

Keywords: Bioactive contents, Caffeine, Green tea, Camellia sinensis, Phytochemical profiling, Mambilla Plateau

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Introduction

The tea plant (*Camellia sinensis*) is an aromatic beverage and a member of the *Theaceae* family native to East Asia.¹ The plant is also grown in Europe^{2,3}, India⁴ and some African countries.⁵⁻⁷ It comprises many useful constituents or compounds with therapeutic benefits.²⁻⁷ The tremendous therapeutic, nutritional, and economic importance of the tea plant makes it an incredibly imperative industry in Nigeria,⁷ Europe and Asian countries^{3,4,8} should strengthen large-scale cultivation of it. Interestingly, the medicinal properties of *Camellia sinensis* have been scientifically validated to treat cardiovascular disease, obesity, immune enhancement, fatigue and sluggishness.³⁻⁵ There is documented information on the phytochemical composition of green tea in Nigeria⁶ and other countries^{3,4} but none on bioactive components in Taraba State to the best of our knowledge.

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Based on the literature review, there seems to be a dearth of information on the bioactive components of the plant in the Mambilla plateau, Taraba State, Nigeria. Against this backdrop, there is a need for updated information to assess the bioactive contents of the tea plant in the Mambilla plateau, Taraba State, Nigeria. Therefore, this study seeks to investigate the bioactive components of tea clones in the tea plantation of Mambilla Beverages Nigeria Limited, Taraba State, Nigeria.

Materials and Methods

Location and sample collection

Mambilla Plateau, Taraba State, is located at coordinates range of 7°20'N and 11°43'E.⁹ Leaves of *Camellia sinensis* were collected from the six original clones (CBB36, C68, C143, C236, C318 and C1212) of the tea plantation of Mambilla Beverages Limited Nigeria. These tea plant leaves were collected in July 2022 with voucher number MBNL2022-0046. Tea plant leaves were collected, washed with clean water and air-dried at room temperature for two weeks before powdering using an electric blender (Qlink-Q15L40). The powdered leaves were poured into an airtight bottle and stored in a dark room.

Bioactive compound identification and quantification of tea plant leaf

Tea plant leaves (CBB36, C68, C143, C236, C318 and C1212 clones in Kakara tea plant plantation) were extracted by cold maceration and

subjected to Gas Liquid Mass Spectrometric analysis at Kembiz Scientific and Laboratories Nigeria Limited, Abuja, Nigeria, using a previously described method.⁴

GC-MS analysis and compound identification

GC-MS analysis was carried out on Shimadzu 2010 plus comprising AOC-20 I auto samples and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: Column diameter is 0.32 mm, column length is 30m, column thickness 0.50 µm, operating in electron impact mode at 70 e V; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml/min and an injection volume of 0.5µL was employed (Split ratio of 10:1) injector temperature 270°C; ion-source temperature 200°C. The oven temperature was programmed from 40°C (isothermal for 2 min), with an increase of 8°C /min, to 150°C, then 8°C/min to 250°C, ending with a 20 min isothermal at 280°C. Mass spectra were taken at 70eV, a scan interval of 0.5 seconds and fragments from 40 to 450 Da. The total GC running time was 51.25 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total area. The software adopted to handle mass spectra and chromatograms was Turbo Mass ver 5.20.

The Gas chromatography-mass spectrometer (GC-MS) result was interpreted using the National Institute Standard Technology (NIST) database, which housed over 62,000 patterns. The unknown component spectrum was compared with the known ones stored in the NIST library⁴.

Statistical analysis

All data from this study were analysed using descriptive statistics using SPSS (Statistics Package for Social Sciences) version 21.0. Data were presented in simple percentages. To describe the responses, basic statistics like frequencies were computed.

Results and Discussion

The findings from this study revealed bioactive constituents that are of medicinal value and are summarised in Table 1 to Table 6.

Table 1: Bioactive compound identified in leaves of CBB36 and the quantities

S/	Compound	Quantity(mg/mL)
Ν	-	
1	Terpineol	0.0650
2	Eucalyptol	0.0240
3	3-Cyclohexen-1-ol, 1-methyl-4- (1-	0.0390
	methylethyl)-	
4	α-Phellandrene	0.0470
5	Cyclohexene, 1-methyl-4-(1-	0.0590
	methylethylidene)-	
6	Cyclopentene, 1,2,3-trimethyl-	0.0420
7	Phenol, 2,4-dimethyl-	0.0680
8	7-Oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-	0.0850
	methylethyl)-	
9	Hexane, 1-chloro-5-methyl-	0.1610
10	4-Nitrobenzoic acid, 3-pentyl ester	0.0570
11	Theophylline, 8-amino-	2.2900
12	Caffeine	2.2900
13	5-Aminosalicylic acid, O,O'-bis(trimethylsilyl)-	15.7900
14	Bis[di(trimethylsiloxy)phenylsiloxy]trimethylsi	78.9830
	loxyphenylsiloxane	

Results from the GC-MS analysis identified different chemical compounds in the tea leaves in varying quantities, and the chromatograms are presented in Figure 1 and Figure 2. The number of compounds identified followed the pattern: clone 68 (25) >clone 1212 (23) > clone 143 (22) >clone236 (17) > clone BB36 (14) > clone 318 (6). The variations in the bioactive contents in various clones may be due to the differences in the various clones cultivated in the Mambilla Plateau.

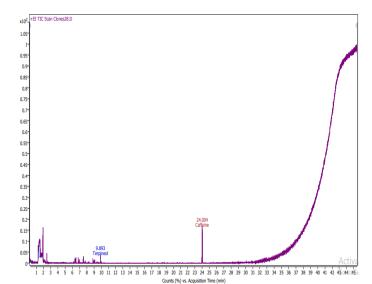


Figure 1: Chromatogram showing bioactive compounds in tea plants (terpineol, caffeine)

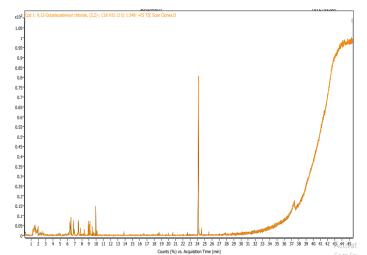


Figure 2: Chromatographic profile of 9,12-Octadecadienoyl chloride, (Z,Z)- in tea plant leaves of clone 143

However, this is subject to confirmation through further research. It was also observed that all the leaves of the tea clones analysed contained caffeine (clones BB36, 318, 1212, 236, 68) though at varying levels, except clone 143. Undoubtedly, there are different tea plant species today with varying bioactive compounds and quantities; 2,3,6 agree with this current study in Mambilla Plateau, Taraba State, Nigeria. The essence of planting different tea plant clones is to select the highestperforming clone with enrichment in bioactive compounds. However, no variety embodies these attributes, thus the importance of the breeding programme. For a good selection of the tea plant clones, attention should be given to the best clones with a richness of bioactive compounds.10 Bioactive compounds like caffeine in their different quantities (0.47 - 21.750 mg/mL) were identified from different tea plant leaves in the Mambilla Plateau, North-eastern Nigeria. These findings agree with previously documented research in Nigeria, where caffeine ranged from 1.00- 1.29%,7 Qingdao, China, between $14.71{\pm}0.23-18.33{\pm}0.46$ g/kg 8 and other countries, where caffeine or caffeic acid were detected in leaves of C. sinensis.^{3,11} In past years, tea leaves with caffeine have been reported among "Yunkang-10 cultivars" in Yunnan Province, China,¹⁰ "Shuchazao cultivars",¹² wild tea plants,¹³ "Longjing-43 cultivars", ¹⁴ "Tieguanyin cultivars", ¹⁵ "Biyun cultivars" ¹⁶ and "Huangdan cultivars".¹⁷ These published findings are similar to this current study in Mambilla Plateau, North-eastern Nigeria in which caffeine was detected in majority of C. sinensis leaves. Caffeine is used to relieve/prevent drowsiness, improve cognitive performance, treat apnea, and prevent premature infant breathing disorder.18 In this study, different bioactive compounds and their quantities like theophylline, 8amino-acid (2.7530 - 2.290 mg/mL), 5-Aminosalicylic acid, O,O'bis(trimethylsilyl)- (15.790 mg/mL), 12-Dimethylamino-10oxododecanoic acid (8.640 - 18.2080 mg/mL) were detected in leaves of C. sinensis cultivated in Mambilla Plateau, Taraba State. These findings are similar to those obtained from tea cultivars across different countries^{4,17,} where bioactive compounds were detected in C. sinensis leaves. Also, phenolic compounds like phenol, 2,4-dimethyl- (0.068 -0.419 mg/mL), phenol, 3,5-dimethyl- (1.430 - 2.806 mg/mL), phenol, 2-ethyl (0.318 mg/mL) and phenol, 3-methyl- (2.953 mg/mL) were detected in leaves of C. sinensis from various clones. Phenolic compounds were also detected in Italian tea ("Camelia d'Oro"),³ Yunan green tea leaves,¹⁹ Chinese tea,²⁰ white tea²¹ and commercial green tea in Spain²², and these documented reports mentioned above are similar to results obtained in this current study in Mambilla Plateau. Phytoconstituents of the tea plant were responsible for its biological activities such as anticancer, antihypertensive, analgesics, cardiac stimulants, immune stimulants, and antioxidants decrease.²³⁻²⁶ These roles or pharmacological relevance are derived from individuals who consumed *C. sinensis*. Also, in this research carried out in the Mambilla Plateau, different quantities of ethyl acetate, triterpenes and γ -terpinene were detected in leaves of *C. sinensis*. The bioactive compound named γ terpinene was detected in leaves of *C. sinensis* in tea cultivars from India⁴, and similar bioactive compounds observed in this current study agree with these findings. The γ -terpinene is used as a fragrance (sweet aromatic and lime smell), food flavouring, antifungal, antioxidant, slowing down the progression of diabetes and Alzheimer's disease, and other pharmacological importance.^{3,4}

S/N	Compound	Quantity(mg/mL)
1	Phthalic acid, cyclobutyl tridecyl ester	0.3730
2	Benzene, [(methylsulfinyl)methyl]-	0.3960
3	1-Methyl-2-phenylcyclopropane	0.3970
4	Phenol, 3,4-dimethyl-	0.4190
5	Fenchol, exo-	0.6310
6	Cyclohexene, 3-methyl-6-(1-methylethylidene)-	0.6390
7	1,4-Methano-1H-Cyclopropa[d]pyridazine, 4,4a,5,5a-tetrahydro-6,6	0.6680
8	2-Cyclopenten-1-one, 2,3,5-trimethyl-4-methylene-	0.6840
9	1,3-Hexadiene, 2,5-dimethyl-	0.8580
10	Benzenemethanol, 4-methyl-α-(1-methyl-2-propenyl)-, (R*,R*)-	1.1710
11	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-	1.4000
12	p-Cresol	1.7860
13	3-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-	2.0090
14	γ-Terpinene	2.1200
15	Bicyclo[3.3.1]non-6-ene-3-carboxylic acid	2.6260
16	Eucalyptol	2.7530
17	Phenol, 3,5-dimethyl-	2.8060
18	7-Oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)-	3.6850
19	Terpineol	3.9360
20	Silane, dimethoxymethyl-	5.5220
21	Hexane, 1-chloro-5-methyl-	6.5340
22	Santolina triene	7.1920
23	5-Decene, 4-ethynyl-, (E)-	12.6170
24	Caffeine	17.9260
25	5.7-Dodecadiyn-1.12-diol	20.5490

Table 2: Bioactive compound identified in leaves of C68	and the quantities
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	Table 3: Bioactive compound identified in leaves of C143 and the quantities	
S/N	Compound	Quantity (mg/mL)
1	4-Deoxypyridoxine, 2TMS derivative	0.5150
2	Phenol, 3,5-dimethyl-	1.4300
3	7-Oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)-	1.4510
4	Terpineol	2.1130
5	9,12-Octadecadienoyl chloride, (Z,Z)-	2.2990
6	: 1,3,6-Heptatriene, 2,5,5-trimethyl-	2.3600
7	Theophylline, 8-amino-	2.7530
8	Butanoic acid, methyl ester	2.7690
9	Santolina epoxide	2.8690
10	Hexane, 1-chloro-5-methyl-	3.0660
11	trans-β-Ocimene	3.2620
12	2-Pentanol, 3-chloro-2-methyl-	3.4010
13	Santolina triene	3.5260
14	Fenchol, exo-	3.9800
15	Bicyclo[3.1.0]hexane, 6-isopropylidene-1-methyl-	4.0480
16	Tridecanoic acid, methyl ester	5.1140
17	endo-Borneol	7.0050

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18	p-Cresol	7.0290
19	4-Carene, (1S,3R,6R)-(-)-	8.0750
20	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-	8.3130
21	3-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-	11.7260
22	Eucalyptol	12.8980

S/N	Compound	Quantity (mg/mL)	
1	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-	0.6530	
2	p-Cresol	0.7570	
3	1,6-Dideoxydulcitol	0.7980	
4	3-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-	0.9860	
5	γ-Terpinene	1.0430	
6	Eucalyptol	1.2890	
7	1,3,5-Cycloheptatriene, 1-methoxy-	1.3030	
8	Benzene, 2-methoxy-1-(2-nitroethenyl)-3-(phenylmethoxy)-	1.3400	
9	7-Oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)-	1.8050	
10	Terpineol	2.5860	
11	2-Adamantylamine, TBDMS derivative	2.6120	
12	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	3.6770	
13	trans-3-Caren-2-ol	3.7780	
14	Bicyclo[3.3.1]non-6-ene-3-carboxylic acid	4.4610	
15	12-Dimethylamino-10-oxododecanoic acid	8.6400	
16	Caffeine	20.0070	
17	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	44.2630	

Table 5: Bioactive compound identified in leaves of C318 and the quantities

S/N	N Company d	
S/N	Compound	Quantity (mg/mL)
1	Caffeine	0.4750
2	Methylamine, N,N-dimethyl-	15.8910
3	Ethyl Acetate	17.2880
4	1,6-Dideoxydulcitol	17.6810
5	Quinolin-2(1H)-one, 3,4,5,6,7,8-hexahydro-3-dimethylaminomethyl-	18.2080
6	Acetic acid, ethoxy-, ethyl ester	30.4570

S/N	Compound	Quantity (mg/mL)
1	Benzene, tert-butyl-	0.2320
2	Phenol, 2-ethyl-	0.3180
3	4a,8a-(Methaniminomethano) naphthalene-9,11-dione, 10-phenyl-	0.3210
4	trans-Arbusculone	0.3320
5	2H-Pyrazol-3-ol, 5-furan-2-yl-	0.4860
6	1,3,5-Cycloheptatriene, 1-methoxy-	0.6980
7	Benzene, 4-ethenyl-1,2-dimethyl-	0.8060
8	Fenchol	0.8250
9	1,3,6-Heptatriene, 2,5,5-trimethyl-	0.8990
10	Glycine, 2-cyclohexyl-N-(but-3-yn-1-yl)oxycarbonyl-, heptyl ester	0.9880
11	Cyclohexene, 3-methyl-6-(1-methylethylidene)-	1.2700
12	1,3-Hexadiene, 2,5-dimethyl-	1.3800
13	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-	2.4590
14	Phenol, 3-methyl-	2.9530
15	3-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-	3.6150
16	γ-Terpinene	3.8250
17	Eucalyptol	4.9590
18	Terpineol	7.3380
19	7-Oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)-	8.8790
20	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	9.2060
21	1,3,4-Thiadiazolium, 2,3-dihydro-4-methyl-5-phenyl-2-thioxo-, hydroxide, inner salt	12.1860
22	cis-Chrysanthenyl formate	14.2760
23	Caffeine	21.7500

Conclusion

In conclusion, the research revealed that leaves of different clones of *C. sinensis* cultivated in the Mambilla Plateau are rich in bioactive compounds as expressed by the various clones investigated. These phytoconstituents have shown diverse pharmacological activities, such as managing diabetes and Alzheimer's, as antioxidants, cardiac stimulants, etc. The bioactive compounds may be of therapeutic and nutritional importance to consumers, and further explorative studies are needed.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original. Any liability for claims relating to this article will be borne by us.

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