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Comparative Gas Chromatography-Mass Spectrometry Analysis of the Leaves of Four *Cola* Species

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ABSTRACT: This study aimed to identify, quantify and compare the phytochemical constituents present in the ethanolic leaf extracts of four *Cola* species - *Cola acuminata*, *Cola rostrata*, *Cola parchycarpa*, and *Cola nitida*, using Gas Chromatography-Mass Spectrometry (GC-MS). The analysis revealed varying numbers of distinct peaks: 77 for *C. acuminata*, 47 for *C. rostrata*, 43 for *C. parchycarpa*, and 45 for *C. nitida*, indicating diverse phytochemical profiles among the species. In *C. acuminata*, 14-Pentadecenoic acid (5.93%) was the dominant compound, with a retention time of 33.746 minutes. Conversely, Cyclohexane, 1,1'-(1,4-butanediyl) bis- (0.11%) was the least abundant, with a retention time of 29.038 minutes. For *C. rostrata*, n-Hexadecanoic acid dominated the extract (26.65% at 27.600 minutes), while 3-Eicosene, (E)- was the least prevalent (0.10% at 30.310 minutes). In *C. parchycarpa*, Dodecane, 2,6,10-trimethyl- was most abundant (10.12% at 8.958 minutes), and Octane, 1-bromo- was least abundant (0.23% at 30.324 minutes). For *C. nitida*, Heptadecane, 2,6,10,14-tetramethyl was dominant (9.44% at 8.958 minutes), whereas β-Myrcene was the least compound (0.39% at 6.301 minutes). Long chain hydrocarbons such as dodecane, tridecane and hexadecane appear in multiple *Cola* species. These findings contribute to the phytochemical database of *Cola* species, established their chemotaxonomic relationship, and suggests potential pharmaceutical and nutraceutical applications of *Cola* leaves.

Keywords: *C. acuminata, C. rostrata, C. parchycarpa, C. nitida,* GC-MS

Introduction

GC-MS is the best technique used to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds. Hence, Gas chromatography (GC) and Mass spectroscopy (MS) associated with particular detection techniques have become a sophisticated means for analysis of various compounds (Vinodh *et al.,* 2013).

Cola, a tropical African genus of the family Sterculiaceae, comprises about 125 species. *Cola* species are evergreen, mostly small or moderately sized trees although a few grow to 25 metres. The most commonly used are *Cola acuminata* and *Cola nitida;* this is due to their great economic importance (Lovejoy, 1980). Their fruits are sessile, placed at the end of a short peduncle, from which they radiate in star-shaped fashion. In *C. nitida*, there are two cotyledons and the seeds readily split into half while in *C. acuminata*, where there are three or four cotyledons, sometimes as many as six, the seed splits into a corresponding number of pieces (Keay, 1958). *Cola rostrata* and *Cola parchycarpa* are commonly called monkey kola (Keay, 1989). Monkey kola is a common name given to a number of minor relatives of the *Cola* species that produce edible tasty fruits. The fruit pulp or aril, which is the edible part of the fruit, varies in colour, from yellow in *C. rostrata* to white in *C. parchycarpa.* The seeds of monkey kola species are not edible unlike the seeds of the *Cola nitida* and *Cola acuminata* which are known for their masticatory and stimulating nuts (Bosch *et al.,* 2002).

At present, nearly 80% of the world populations relies on plant-based drugs for their health care needs (Sermakkani and Thangapandian, 2012). Most scientific literature on phytochemistry and GC-MS of *Cola* species was done on the nuts of these plants. These nuts or edible fruit pulp of these *Cola* species are mostly consumed as food by humans and other animals, and in some other cases are sold by farmers to get income. Hence, the need to explore other bioparts of the *Cola* plant for its

medicinal and industrial use, leaving the edible bioparts for consumption and income generation. In this study, the leaves of these four selected *Cola* species were comparatively analysed for their bioactive constituents using the GC-MS technique.

Materials and methods

Sample collection and preparation: The leaves of *C. acuminata, C. rostrata, C. parchycarpa* and *C. nitida,* were collected in different homestead from Amaoba community in lkwuano Local Government Area, of Abia State, Nigeria. The collected plant materials were identified in the taxonomic unit of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. Voucher specimen was deposited in the Department herbarium. The leaves were air dried for 14 days and the dried samples were ground into powder with the aid of a blender, sieved and stored in air tight container bottles. The bottles for *C. acuminata, C. rostrata, C. parchycarpa,* and *C. nitida* were labeled sample A, B, C, and D respectively and thereafter taken to the laboratory for analysis.
Extraction of powdered samples: The powdered samples were subjected to Soxhlet extraction method. 500ml clean boiling flask was dried in oven at 105-110 ℃ for about 30 min. This flask was transferred into a dessicator and allowed to cool. 10 g of the sample was weighed and poured into the Soxhlet thimble. The extraction thimble was plugged lightly with cotton wool to aid filter the extract. The boiling flask was then filled with about 300 mL of ethanol. The Soxhlet apparatus was assembled and allowed to reflux for about 4 hours at a temperature of 60 ℃. The thimble was then removed with care, thereafter the mixture was poured into a volumetric flask and allowed to cool. The content in the volumetric flask was then transferred into a rotatory evaporator to separate the solvent from the extract.
Extraction of phytochemicals: 1 g of the extract was weighed and transferred in a test tube and 25ml of ethanol was added. The test tube was allowed to react in a hotplate at 60 ℃ for 90 min. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20 mL of ethanol, 10 mL of cold water, 10 mL of hot water and 3 mL of hexane, which was all transferred to the funnel. These extracts were combined and washed three times with 10ml of 10% v/v ethanol aqueous solution. The solution as dried with anhydrous sodium sulphate and the solvent was evaporated. The sample was solubilized in 1000 µL of pyridine of which 200 µL was transferred to a vial for analysis.
Quantification by GC-MS: The analysis of phytochemical was performed on a BUCK M910 Gas chromatography equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min. The initial temperature was set at 50 °C with increasing rate of 3 °C/min and holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliter of the prepared 1% of the extracts diluted with acetonitrile was injected in a split less mode. Relative quantity of the chemical compounds present in each of the extracts was expressed as percentage based on peak area produced in the chromatogram.
Identification of chemical constituents: Bioactive compounds extracted from different extracts were identified based on GC retention time on HP-5MS column and matching of the spectra with computer software data of standards (Replib and Mainlab data of GC–MS systems).

Results

The GC-MS chromatogram of the ethanolic leaf extract of *C. acuminata*, *C. rostrata, C. parchycarpa* and *C. nitida* revealed distinct peaks of 77, 47, 43 and 45 respectively. In *Cola acuminata* leaf, 14-pentadecenoic acid was most abundant compound with the highest retention time of 33.746 minutes and % of total 5.93 while cyclohexane, 1,1'-(1,4-butanediyl) bis- was the least abundant compound with retention time 29.038 minutes and % of total 0.11, cyclodecene had the least retention time of 5.427 minutes and % of total 0.12 (Table 1).

Table 1: GC-MS analysis of ethanolic leaf extract of *Cola acuminata*

S/N	Retention Time (minutes)	% of Total	Identified Compound in *Cola acuminata*
1	5.427	0.12	Cyclodecene
2	6.965	0.18	Undecane
3	9.633	0.51	Dodecane
4	12.326	0.13	Tridecane

Table 1: GC-MS analysis of ethanolic leaf extract of *Cola acuminata* (contd.)

S/N	Retention Time (minutes)	% of Total	Identified Compound in *Cola acuminata*
5	13.376	0.26	9-Eicosene, (E)-
6	14.665	0.73	Cyclotetradecane
7	14.944	1.40	Tetradecane
8	15.831	0.18	Cyclotetradecane
9	16.545	0.25	cis-1,4-Cyclohexanediamine, N-methyl
10	17.405	1.95	2,4-Di-tert-butylphenol
11	17.665	0.57	Undecanoic acid, 10-methyl-, methyl ester
12	19.144	2.60	Dodecanoic acid
13	19.563	0.39	9-Octadecene, (E)-
14	19.811	0.44	Hexadecane
15	21.542	0.24	1-Dodecene
16	23.421	0.67	Tetradecanoic acid
17	24.000	0.39	9-Eicosene, (E)-
18	24.211	0.27	1-Octadecanesulphonyl chloride
19	27.488	2.37	n-Hexadecanoic acid
20	28.035	0.68	Cetene
21	28.203	0.16	Methoxyacetic acid, 2-tetradecyl ester
22	29.038	0.11	Cyclohexane, 1,1'-(1,4-butanediyl) bis-
23	29.249	0.18	9-Octadecenoic acid, (E)-
24	29.576	0.12	Methyl stearate
25	29.738	4.72	trans-13-Octadecenoic acid
26	29.891	0.61	cis-Vaccenic acid
27	29.969	2.53	Octadecanoic acid
28	30.094	0.70	Cyclohexane, 1-(1,5-dimethylhexyl) -4-(4-methylpentyl)-
29	30.157	0.53	9-Eicosene, (E)-
30	30.242	2.68	1-Docosene
31	30.307	0.92	1-Octadecanesulphonyl chloride
32	30.345	0.40	1-Eicosene
33	30.394	1.28	Cyclohexadecane, 1,2-diethyl-
34	30.459	0.64	Cycloeicosane
35	30.493	0.41	2-Methyl-Z,Z-3,13-octadecadienol
36	30.591	2.64	Oleic Acid
37	30.660	1.05	Oleic Acid
38	30.751	1.89	cis-Vaccenic acid
39	30.788	2.19	Cyclohexane, 2-propenyl-
40	30.844	0.82	5-Eicosene, (E)-
41	30.889	1.48	3-Eicosene, (E)-
42	30.924	1.04	Ethanol, 2-(tetradecyloxy)-
43	30.967	1.35	9-Eicosene, (E)-
44	31.103	4.19	Octadec-9-enoic acid
45	31.178	1.55	1-Dodecanol, 2-hexyl-
46	31.215	1.75	2-Piperidinone, N-[4-bromo-n-butyl]-
47	31.264	1.67	Cycloeicosane
48	31.300	1.46	3-Eicosene, (E)-
49	31.343	1.46	Cyclohexadecane, 1,2-diethyl-
50	31.376	1.67	3-Eicosene, (E)-
51	31.429	1.01	1-Docosene
52	31.482	4.68	1-Docosene
53	31.523	1.73	3-Eicosene, (E)-
54	31.621	1.50	1-Docosene
55	31.932	1.11	Cyclotetracosane
56	32.021	0.36	Dodecane, 1,2-dibromo-
57	32.063	0.36	Bis (2-ethylhexyl) phthalate
58	32.437	0.75	1-Octadecene
59	32.469	0.54	3-Eicosene, (E)-
60	32.616	0.37	trans-13-Octadecenoic acid

Table 1: GC-MS analysis of ethanolic leaf extract of *Cola acuminata* (contd)

S/N	Retention Time (minutes)	% of Total	Identified Compound in *Cola acuminata*
61	32.740	1.80	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester
62	32.878	1.02	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester
63	32.919	0.63	2-Piperidinone, N-[4-bromo-n-butyl]-
64	32.962	0.46	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester
65	33.046	0.96	Oleic Acid
66	33.089	0.67	Erucic acid
67	33.183	1.24	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester
68	33.235	0.80	2-Piperidinone, N-[4-bromo-n-butyl]-
69	33.297	0.71	9-Octadecenal, (Z)-
70	33.397	2.22	2- Chloropropionic acid, hexadecyl ester
71	33.435	1.05	Octacosane
72	33.503	2.58	Squalene
73	33.566	0.84	cis-13-Octadecenoic acid
74	33.615	2.09	Oleic Acid
75	33.673	0.99	9-Octadecenoic acid (Z)-, 2,3-droxypropyl ester
76	33.695	1.47	13-Octadecenal, (Z)-
77	33.746	5.93	14-Pentadecenoic acid

In *Cola rostrata* leaves, n-hexadecanoic acid was the most abundant compound with the retention time of 27.600 minutes and % of total 26.65 while 3-Eicosene, (E)- was the least abundant with retention time of 30.310 minutes and % of total 0.10. 1-Docosene had the highest retention time of 32.437 minutes and % of total 0.17 while Dodecane had the least retention time of 9.634 minutes and % of total 0.15 (Table 2).

Table 2: GC-MS analysis of ethanolic leaf extract of *Cola rostrata*

S/N	Retention Time (min)	% of Total	Identified Compound in *Cola rostrata*
1	9.634	0.15	Dodecane
2	14.662	0.43	5-Tetradecene, (E)-
3	14.945	0.61	Tetradecane
4	16.492	0.35	Cyclododecane
5	17.404	1.86	2,4-Di-tert-butylphenol
6	17.663	0.42	Undecanoic acid, 10-methyl-, methyl ester
7	19.221	6.89	Dodecanoic acid
8	19.268	2.53	Dodecanoic acid
9	19.566	1.41	9-Octadecene, (E)-
10	19.812	0.96	Hexadecane
11	20.815	0.22	Cyclohexane, 1,1'-(1,4-butanediyl) bis-
12	21.243	0.23	Cyclotetradecane
13	21.546	1.59	1-Dodecene
14	22.063	0.31	Heptadecane
15	22.273	0.22	Tridecanoic acid, 12-methyl-, methyl ester
16	22.904	0.26	1,2,4-Butanetriol, trinitrate
17	23.518	3.01	Tetradecanoic acid
18	24.006	0.53	1-Octadecene
19	24.214	0.24	Heptadecane, 2-methyl-
20	24.880	0.23	1-Octadecene
21	25.557	0.18	Trifluoroacetic acid, pentadecyl ester
22	26.479	0.25	Hexadecanoic acid, methyl ester
23	26.977	1.64	9-Hexadecenoic acid
24	27.600	26.65	n-Hexadecanoic acid
25	27.735	9.11	n-Hexadecanoic acid
26	28.044	3.54	1-Octadecene
27	28.209	0.94	Heptacosane, 1-chloro-
28	28.594	0.46	Heptadecanoic acid
29	28.703	0.57	Butyl eicosyl ether
30	28.854	1.50	trans-13-Octadecenoic acid
31	29.255	0.14	6-Octadecenoic acid, methyl ester, (Z)-

Table 2: GC-MS analysis of ethanolic leaf extract of *Cola rostrata* (contd.)

S/N	Retention Time (min)	% of Total	Identified Compound in *Cola rostrata*
32	29.826	3.45	9-Octadecenoic acid
33	30.005	0.68	Octadecanoic acid
34	30.091	0.28	Hexadecanoic acid, 1,1-dimethylethyl ester
35	30.245	0.56	1-Docosene
36	30.310	0.10	3-Eicosene, (E)-
37	30.599	1.59	cis-Vaccenic acid
38	30.791	0.74	1-Docosene
39	31.103	1.67	cis-Vaccenic acid
40	31.249	2.67	Palmitoleic acid
41	31.382	2.66	2-Piperidinone, N-[4-bromo-n-butyl
42	31.483	6.06	1-Docosene
43	31.621	2.84	1-Docosene
44	31.739	2.04	1-Docosene
45	31.821	4.09	Erucic acid
46	32.063	2.97	Diisooctyl phthalate
47	32.437	0.17	1-Docosene

In *Cola parchycarpa* leaves, dodecane, 2,6,10-trimethyl- was the most abundant compound with the retention time of 8.958 minutes and % of total 10.12 while octane, 1-bromo- was the least abundant compound with retention time of 30.324 minutes and % of total 0.23. 1-hexacosene had the highest retention time of 34.217 minutes and % of total 1.79 while methylene chloride had the least retention time of 6.365 minutes and % of total 0.64 (Table 3).

Table 3: GC-MS analysis of ethanolic leaf extract of *Cola parchycarpa*

S/N	Retention Time (minutes)	% of Total	Identified Compound in *Cola parchycarpa*
1	6.365	0.64	Methylene chloride
2	6.496	1.19	Oxirane, (chloromethyl)-
3	6.848	2.36	Benzene, 1,4-dichloro-
4	7.131	0.24	S-(Propoxythiocarbonyl)thiohydroxylamine
5	7.199	0.34	o-Cymene
6	7.946	0.51	Hexane, 2,2,4-trimethyl-
7	8.113	3.13	Oxalic acid, isobutyl nonyl ester
8	8.256	1.11	Decane, 3,6-dimethyl-
9	8.382	3.67	Dodecane, 2,6,10-trimethyl-
10	8.534	3.43	Nonane
11	8.643	1.21	Decane
12	8.699	1.66	Octane, 2-methyl-
13	8.958	10.12	Dodecane, 2,6,10-trimethyl-
14	9.118	2.25	Carbonic acid, nonyl vinyl ester
15	9.176	3.55	Decane
16	9.272	3.90	Heptadecane, 2,6,10,14-tetramethyl
17	9.339	5.62	Decane, 2-methyl-
18	9.485	1.36	Dodecane, 2,6,11-trimethyl-
19	9.542	1.49	Carbonic acid, nonyl vinyl ester
20	9.692	5.95	Heptadecane, 7-methyl-
21	9.806	2.71	Dodecane, 2,6,11-trimethyl-
22	10.026	1.68	2,6-Dimethyldecane
23	10.101	2.90	2,6-Dimethyldecane
24	12.027	0.56	5-Dodecene, (Z)-
25	12.262	2.04	Dodecane
26	15.109	2.11	Tridecane
27	17.625	1.56	1-Hexadecanol
28	17.830	1.57	Hexadecane
29	20.414	1.07	Pentadecane
30	20.974	9.65	2,4-Di-tert-butylphenol
31	22.691	3.35	1-Pentadecene

Table 3: GC-MS analysis of ethanolic leaf extract of *Cola parchycarpa* (contd.)

S/N	Retention Time (minutes)	% of Total	Identified Compound in *Cola parchycarpa*
32	22.859	0.50	Hexadecane
33	27.259	4.06	1-Octadecene
34	30.022	1.19	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester
35	30.257	3.19	Cyclohexadecane, 1,2-diethyl-
36	30.433	0.68	Indazol-4-one, 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro-
37	30.324	0.23	Octane, 1-bromo-
38	31.665	0.65	Ethyl Oleate
39	31.814	1.80	1-Docosene
40	31.980	0.29	Bromoacetic acid, octadecyl ester
41	32.970	0.78	1-Docosene
42	34.008	1.89	Bis(2-ethylhexyl) phthalate
43	34.217	1.79	1-Hexacosene

In *Cola nitida* leaves, Heptadecane, 2,6,10,14-tetramethyl was the most abundant compound with the retention time of 8.958 minutes and % of total 9.44 while .beta.-myrcene was the least abundant compound with the least retention time of 6.301 minutes and % of total 0.39. 5.alpha.-cholest-8-en-3-one, 14-methyl- had the highest retention time of 36.021 and % of total 2.23 (Table 4).

Table 4: GC-MS analysis of ethanolic leaf extract of *Cola nitida*

S/N	Retention Time (minutes)	% of Total	Identified Compound in *Cola nitida*
1	6.301	0.39	.beta.-Myrcene
2	6.365	0.66	Benzene, 1,2,4-trimethyl-
3	6.496	1.01	Oxirane, (chloromethyl)-
4	6.849	1.52	Benzene, 1,4-dichloro-
5	6.956	0.71	1,3-Cyclohexadiene, 1-methyl-4-(1- methylethyl)-
6	7.201	1.30	p-Cymene
7	7.949	0.49	Hexane, 2,2,5-trimethyl-
8	8.160	4.07	.gamma.-Terpinene
9	8.258	1.15	Decane, 3,6-dimethyl-
10	8.382	3.58	Dodecane, 2,6,11-trimethyl-
11	8.536	3.28	Decane
12	8.645	1.02	Tridecane
13	8.700	1.55	Undecane, 3,7-dimethyl-
14	8.958	9.44	Heptadecane, 2,6,10,14-tetramethyl
15	9.120	2.12	Octane, 3,5-dimethyl-
16	9.178	3.39	Decane, 2,3,5,8-tetramethyl-
17	9.273	3.48	Heptadecane, 2,6,10,14-tetramethyl
18	9.339	5.13	Tetradecane
19	9.486	1.30	Undecane, 4,7-dimethyl-
20	9.543	1.30	Carbonic acid, nonyl vinyl ester
21	9.806	8.00	Undecane
22	9.917	0.59	Heptadecane, 2,6,10,14-tetramethyl
23	10.026	1.56	2,6-Dimethyldecane
24	10.101	2.64	Decane, 2,4-dimethyl-
25	12.027	0.51	5-Dodecene, (Z)-
26	12.262	1.81	Dodecane
27	15.109	1.89	Tridecane
28	17.625	1.65	Cetene
29	17.829	1.47	Tridecane
30	18.346	0.65	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4 Z,9S*)]
31	20.413	1.01	Pentadecane
32	20.974	9.27	2,4-Di-tert-butylphenol
33	22.690	3.16	Z-8-Hexadecene
34	22.860	0.50	Hexadecane
35	27.258	3.94	1-Octadecene
36	30.022	1.18	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester

Table 4: GC-MS analysis of ethanolic leaf extract of *Cola nitida* (contd)

S/N	Retention Time (minutes)	% of Total	Identified Compound in *Cola nitida*
37	30.257	3.01	1-Octadecene
38	30.433	0.62	6-(Trifluoromethoxy)-N-(trimethylsilyl)-1,3-benzothiazol-2-amine
39	31.666	0.50	Ethyl Oleate
40	31.814	1.54	1-Docosene
41	32.969	0.63	1-Docosene
42	34.008	1.50	Bis(2-ethylhexyl) phthalate
43	34.216	1.11	Tetradecanoic acid, 2-hydroxy-, methyl ester
44	35.582	2.11	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-
45	36.021	2.23	5.alpha.-Cholest-8-en-3-one, 14-methyl-

Table 5; presents a comparative analysis of the GC-MS result of the leaves of the four *Cola* species. *Cola acuminata* had the highest number of identified compounds (77 compounds). *Cola rostrata* had the highest most abundant single compound; n-Hexadecanoic acid with 26.65 % of total and the least abundant compound; 3-Eicosene, (E)- with 0.10 % of total. Compound with the highest retention time was observed in *Cola nitida*; 5.alpha.-Cholest-8-en-3-one, 14-methyl- (36.021 mins).

Table 5: Comparative GC-MS analysis of the leaves of four *Cola* species

***Cola* species**	**Number of Identified Compounds**	**Most abundant Compound (% of Total)**	**Least Abundant Compound (% of Total)**	**Compound with the Least Retention Time**	**Compound with the Highest Retention Time**
Cola acuminata	77	14-Pentadecenoic acid (5.93)	Cyclohexane, 1,1'-(1,4-butanediyl) bis- (0.11)	Cyclodecene (5.427mins)	14-Pentadecenoic acid (33.746 mins)
Cola rostrata	47	n-Hexadecanoic acid (26.65)	3-Eicosene, (E)- (0.10)	Dodecane (9.634 mins)	1-Docosene (32.437 mins)
Cola parchycarpa	43	Dodecane, 2,6,10-trimethyl- (10.12)	Octane, 1-bromo- (0.23)	Methylene chloride (6.365 mins)	1-Hexacosene (34.217 mins)
Cola nitida	45	Heptadecane, 2,6,10,14-tetramethyl (9.44)	.beta.-Myrcene (0.39)	.beta.-Myrcene (6.301 mins)	5.alpha.-Cholest-8-en-3-one, 14-methyl- (36.021 mins)

Discussion

The leaves of *C. acuminata* contained higher number of bioactive compounds when compared with others while *C. parchycarpa* had the least number of bioactive compounds. This may suggest a higher potential for pharmacological and industrial applications due to the variety of bioactive compounds in *C. acuminata*. But in the research conducted by Niemenak *et al* (2008) they confirmed that the seed of *C. nitida* presented significantly higher (26 mg/g FW on average) levels of catechin than *C. acuminata* and *C. anomala* (13 mg/g FW on average). They also revealed that Caffeine content was highest in *C. nitida* (≈14 mg/g FW), followed by *C. acuminata* (≈11 mg/g FW) and *C. anomala* (≈7 mg/g FW).

14-Pentadecenoic acid which was contained more in the leaf of *C. acuminata* is an odd chain fatty acid which is clinically relevant as it helps to stem chronic cardio metabolic, liver and inflammatory diseases (Venn-Watson *et. al.,* 2020; Dornan *et. al.,* 2021). 1-Docosene (also known as Behenic acid) which was present in *Cola rostrata* and *Cola acuminata* has smoothing and moisturizing properties which makes it a valuable ingredient in lotions, creams and lipsticks. Its ability to act as a thickening agent and emulsifier also contributes to product stability and texture (Gunstone *et. al.,* 2017). Dodecanoic acid (also present in *C. rostrata*) can be used in baking, confectionery and ice cream for its thickening and stabilizing properties, also used in soaps, shampoos and lotions for its cleansing and moisturizing properties (Martins *et. al.,* 2006). Oleic acids which include Octadec-9-enoic acid present in *C. rostrata* also demonstrates positive influence on weight management and its beneficial effect on blood lipids and insulin levels (Kris-Etherton *et. al.,* 1995).

Undecane acid which is present only in *C. nitida* leaf (Table 4) can be used as a monomer for the synthesis of polyesters, offering enhanced properties like heat resistance and biodegradability (Wang *et. al.,* 2018).

Tetradecane is used as flavoring agent in food, it also enhances texture, palatability and shelf life (FAO, 2019). Gamma-terpinene acid possesses a unique citrus-like aroma and taste, making it a potential flavouring agent in food and beverages (Adams and McGinty, 2008). It can be used in cosmetics and fragrances, biomaterials, pharmaceuticals, and also has neuroprotective properties (Li *et al*., 2025).

Cola rostrata had the highest concentration of a single compound (n-Hexadecanoic acid) (Table 5), a saturated fatty acid known for anti-inflammatory and antioxidant activity (Sharma *et al.,* 2016). These compounds occur in trace amounts and may still contribute significantly to aroma or pharmacological synergy despite low concentrations. For example, β-myrcene is known for its analgesic and anti-inflammatory properties (Surendran *et al.,* 2021). High retention times typically indicate high molecular weight or lower volatility. Compounds like 5α-Cholest-8-en-3-one are steroids with potential hormonal or membrane-stabilizing activity. Short retention time compounds, such as Methylene chloride and β-myrcene, are volatile and contribute more to aroma or early physiological interactions. Comparatively, the four species exhibit both similarities and differences in their phytochemical composition. These similarities suggest phylogenetic relationships and shared metabolic pathways among species of the genus Cola, while differences in compound distribution may reflect genetic variation, environmental factors and ecological adaptation.

The number of identified compounds in the ethanolic leaf extract of *C. acuminata*, *C. rostrata*, *C. parchycarpa* and *C. nitida* were 77, 47, 43 and 45 respectively. However, the concentration of the compounds identified in the leaves of these four *Cola* species varied significantly. *Cola acuminata* emerges as the most chemically diverse, while *Cola rostrata* contains the most concentrated dominant compound. The presence of these valuable bioactive compounds in the leaves of these *Cola* species shows that they could be employed in the nutraceuticals, pharmaceuticals and food industries. Further bioactivity-guided studies should focus on *Cola acuminata* leaf due to its rich chemical profile.

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