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Compositional Analysis and Antimycobacterium Tuberculosis Activity of Essential Oil of *Hyptis Suaveolens* Lamiceae Obtained from North-East Nigeria

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Abstract: ***Fresh leaves of Hyptis suaveolens were collected and pretreated for essential oil analysis with the sole aim of linking the ethnomedicinal uses of this plant by the people of Adamawa State Nigeria to its essential oil composition. The results obtained from the analysis shows that 68 compounds were present in varying concentration out of which 16 compounds have appreciable concentration making 75.135 % of the total abundance. The major compound being Caryophyllene (20.643 %) followed by Sabinene (16.711 %) and Terpinolene (8.490 %). The essential oil of this plant also exhibited anti mycobacterium tuberculosis activity when tested on strain 7H9/ADC with MIC of 3.13 %. This activity was compared with standard drug Rifampicin which also has MIC of 0.1 µg/ml.***

Keywords: *Hyptis suaveolens*, essential oil, antimycobacterium, tuberculosis, microbroth dilution

1. Introduction

The plant *Hyptis suaveolens* is an aromatic annual shrub distributed in tropical or subtropical region. It is usually applied in Asian food recipes as an appetizer because of its flavored essential oil. It was reported to be used for traditional medicine as an anticancer[1]. The leaves of *hyptis* plant have been utilized as sweat causing agent (sudatory), milk flow stimulant and as a cure for skin diseases[2]. The crude leaves extract is also used as a relief to excessive infant cry (infant colic) and gastric complications. Preparation made from the leaves and twig of *hyptis suaveolens* are used to relief spasm of involuntary muscles, to cure rheumatism, as anti-stress, as a cure for body inflammation, fertility enhancer and also applied on burns, wound and other skin complaints to prevent further infections[3]. Ethanolic extract from its leaves exhibited healing properties with a supportive role of antioxidant enzymes[4]. The decoction of the root parts is highly utilized and is reported to contain prunol, a natural source of virus Integrase strand transfer inhibitor[5]. Presence of sterols, flavonoids, alkaloids, saponins, terpenoids tanins in both organic and in-organic solvent extracts were also revealed by basic phytochemistry analysis, Thin layer Chromatography (TLC) and High Performance-TLC methods[6,7].

Studies are ongoing throughout the world in search for compounds that are biologically active with a low profile of side effects. Essential oils from plants usually show growth inhibition of various microorganisms including resistant strains of bacteria and fungi[8].

In this study steam distillation was used to extract essential oils from *Hyptis suaveolens* with the aim at determining the anti-mycobacterium tuberculosis and antioxidant activities.

2. Material

Plant Materials

Healthy leaves of *Hyptis suaveolens* were obtained in January 2015 and the site of collection was Sangere, Girei local government of Adamawa State North-east Nigeria. The plants was isolated and conserved for extraction.

3. Methods

Method of Essential Oil Extraction

The leaves of *hyptis suaveolens* (1 kg) were immediately subjected to extraction to avoid loss of some essential oils as a result of drying process, and using a modified type of steam distillation apparatus (in which the receiver end of the Steam distiller is passed through another vessel containing ice) for 2.5 h essential oils of the plant which has a yield of 0.4 % was collected over water and later kept at 4 ^{O}C until further required.

Gas Chromatography Mass spectroscopy (GC-MS)

GC-MS analysis was done on J and W Scientific gas chromatography directly couple to the mass spectrometer system (model GC Agilent technologies 7890A, Agilent technologies Inert MSD 5975C) HP 5 ms, 5 % phenyl methyl silox: 469.56 509. Capillary column (30M x 250µm) was used under the following condition: ovum temperature 50^0 c for 1 min, then 10^0c/min to 200^0c for 1min, and 20^0/min to 300^0 for 120 seconds.

Injector temperature was 230^0c, carrier gas was Helium, flow rate was set at 1ml/min; the volume of the injected sample was 0.2µL of oil in hexane solution, splitless injection techniques, lionization energy was 70ev in the electron ionization (EI) mode. Ion source temperature was 230^0c, scan mass range of m/z 60-335; the constituents of the essential oils were identified base on comparison of the

retention indices and mass spectra of most of the compound with data generated under identical experimental conditions by applying a two dimensional search algorithm considering the retention index as well as mass spectral similar with those of authentic compounds available in NBS75K and NIST08 Libraries.

The retention indices (RI) are in relation to a homologous series of n-alkanes on the GC column under the same chromatographic condition components. Relative concentration will be obtained by peak area normalization as describe by[9].

Antituberculosis activity of pure oil sample using Microbroth dilution technique

Dilution of the oils: One microliter of the oil was dissolved in 0.1 mI of 10% dimethylsulphoxide (DMSO) to give a concentration of 50% oil.

Organism preparation: Five hundred microliter of test organism *mycobacterium* bovis (BCG) freshly thawed stock was inoculated into 50 mI of sterile Middlebrook 7H9/ADC broth medium and incubated at 30^0C for 5-7 days. The optical density was measured at 650 nm wavelength. This in our work the OD WAS 0.2 and this is equal to 10^9 cfu/ml.

Sample screening for antituberculosis activity: Into each well of 96 microwell plate was transferred 50µl of sterile 7H9 broth starting from well 2 to 12. To each of the first well was added 100µl of 10% DMSO, 100 µl of 25 µg/ml solution of rifampicin (control drug) and 100 µl of the diluted oil sample. Using a multichannel pipette 50µl was carefully removed from well 1 to 2, mixed thoroughly and the process continued to well 11 from which 50µl was withdrawn and discarded.

The well were inoculated with 50 µl of diluted BCG culture and incubated at 30^0C for 7 days. The results were confirmed by adding tetrazolium dye after the incubating period. The wells where there was no color change were regarded as activity of test samples indicating inhibition of test organism. The last well where there was no growth is regarded as the minimum inhibitory concentration (MIC) of the sample and the result shown in **table 2.**

4. Results and Discussion

Essential Oil Composition

Figure-1 is the gas chromatography spectrum of essential oil of Hyptis suaveolens leaves extract. The mass spectrum reveals the presence of 68 compounds as presented in **table-1**; out of which 16 compounds were presented with appreciable percentage abundance in which Caryophyllene (20.643 %) has the highest concentration followed by Sabinene (16.711%) and Terpinolene (8.49 %). Other researches have also reported the present of major components of essential oil of *Hyptis suaveolens* cultivated in Italy as follows: Sabinene (34 %), beta-Caryophyllene (11.2 %) and Terpinolene (10.7 %), whereas beta-Caryophyllene (34.65 %), Germacrene (10.32 %), alpha-Bergamotene (6.56 %), Rimuen (6.46 %) and alpha-Copaene were shown to be the major compounds in essential oil of *Hyptis suaveolens* from Indonesia[10,11]. In India, a work carried on essential oil of *Hyptis suaveolens* reveals that, 1,8-Cineole (44.4 %) followed by beta-Caryophyllene, beta-Pinene and Camphene[12]. In a similar work by Okonogi *et al* on the essential oil of the same plant obtained from Northen Thailand revealed beta-caryophyllene, 1,8-Cineole and Phellandrene are the major compounds of the oil, whereas Fun and Baerhein reported the major compound of the essential oil of the plant species as 1,8-Cineole (27-38 %), and Sabinene (12-18 %)[13,14]. Brazilian source of *Hyptis suaveolens* essential oil was presented with Sabinene, Limonene, Bicyclogermacrene, beta-Caryophyllene and 1,8-Cineole as the major compounds of the oil[14]. In the same vein, analysis of essential oil obtained from Togo showed the predominace of beta-Caryophyllene (33.8 %), alpha-Bergamoten (11.3 %) and alpha-Caryophyllene (7.4 %)[16]. From the above discussions, Caryophyllene is common in all the samples obtained from various locations except in the sample obtained from Brazil which has Limone, Bicyclogermacrene and beta-Phellandrene. On the other hand Rimuene is another compound that has appeared only in oil sample from Indonesia. These results shows that differences in locations affect the composition of essential oil obtain from *Hyptis suaveolens.* However, some of the results are in line with our finding.

Anti-mycobacterium tuberculosis activity of the essential oil

After the evaluation of the essential oil of *Hyptis suaveolens* followed the anti- mycobacterium tuberculosis screening using 7H9/ADC strain of *mycobacterium bovi*. The result obtained are shown in **table-2** which shows that the essential oil of *Hyptis suaveolens* is active against the strain 7H9/ADC with MIC of 3.13 % although in comparison the control rifampicin drug was more active with MIC of 0.1 µg/ml. Dorman and Deans state that "the oils antimicrobial properties is related to the composition of the plants volatile oils, the structural configuration of the oils constituent compounds and their functional groups and potential synergistic interactions among the compounds"[17]. Therefore in our case the anti-mycobacterium activity observed in the essential oil of *Hyptis suaveolens* can be attributed to the presence of high percentage concentration of Caryophyllene, Sabinene, Terpinolene and to other minor compounds that may contribute to the antimicrobial activity.

5. Conclusion

The GC-MS analysis of the essential oil of leaves of *Hyptis suaveolens* source from Girei local government of Adamawa state, North-eastern Nigeria, indicate that the major compounds are caryophyllene (20.643 %), Sabinene (16.711 %) and Terpinolene (8.49 %). The essential oil of *Hyptis suaveolens* was active against 7H9/ADC strain of *mycobacterium bovi* as utilized in this study with MIC of 3.13 %. Therefore the result of this studies support the use of this plant in ethnomedicine as an alternative remedy for symptoms of tuberculosis; as such the essential oil of this plant may be a potential candidate for further studies to isolating the active compounds effective against *Mycobacterium tuberculosis.*

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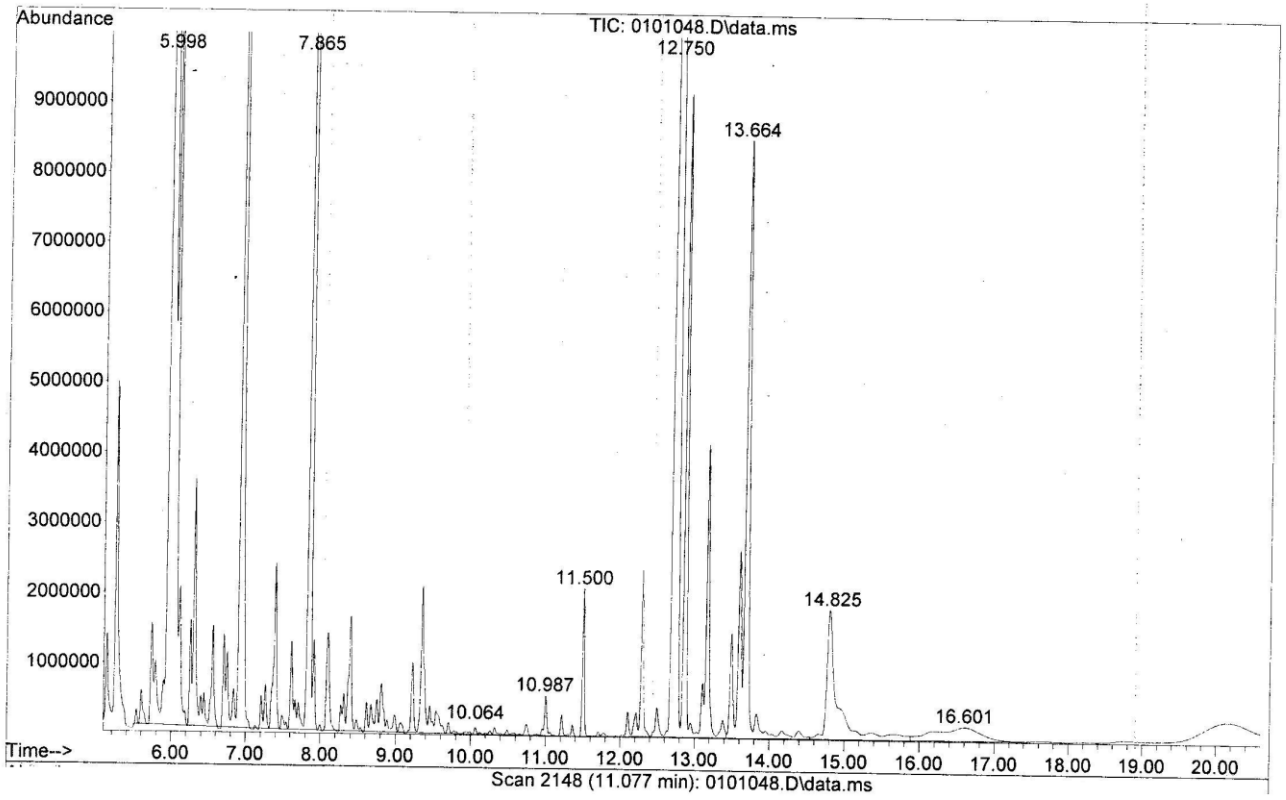

Figure 1: Gas chromatography (GC) spectrum of hyptis spicigera

Table 1: GC-MS Analysis of Hyptis suaveolens

S/No.	Constituents	RT(min)	% conc.	KI	MW
1.	Alpha.-phellandrene	5.131	0.575	902	136
2.	Alpha.-pinene	5.251	2.644	905	136
3.	Camphene	5.533	0.122	910	136
4.	Benzene, propyl-	5.597	0.337	811	120
5.	Benzene, 1-ethyl-2-methyl-	5.734	0.671	814	120
6.	Benzene,1-ethyl-2-methyl-	5.784	0.600	815	120
7.	Benzene, 1,2,3,-trimethyl-	5.896	0.344	817	120
8.	Bicyclo [3.1.0]-hexene, 4-methylene-	5.996	16.711	919	136
9.	β.-Pinene	6.052	5.490	921	136
10.	1-octen-3-ol	6.107	1.021	907	127
11.	β.myrcene	6.255	0.536	925	136
12.	Benzene, 1,2,4-trimethyl-	6.306	1.614	826	120
13.	3-octanol	6.398	0.189	939	130
14.	Decane	6.442	0.246	928	136
15.	3-Carene	6.556	0.804	931	136
16.	(+) -2-Carene	6.704	0.505	934	120
17.	Benzene, 1-ethyl-3-methyl-	6.749	0.509	834	134
18.	Benzene, 1-methyl-2-(1-methylethyl-)	6.838	0.295	936	136
19.	D-Limonene	6.936	8.832	938	134
20.	Benzene, 1,4-diethyl-	7.207	0.189	944	134
21.	Benzene, 1-methyl-3-propyl-	7.262	0.278	945	136
22.	ϒ-Terpimene	7.396	1.403	947	134
23.	Benzene, 1-methyl-2-propyl-	7.491	0.119	949	154
24.	3-cyclohexen-1-ol, 4-methyl-1(1-methylethyl)-	7.613	0.556	952	134
25.	Benzene, 2-ethyl-1,4-dimethyl-	7.664	0.167	953	134
26.	Benzene, 1-methyl-4-(-methylethyl)-	7.708	0.241	954	136
27.	Terpinolene	7.867	8.496	957	152
28.	Bicyclo [2.2.1] heptan-2-one, 1,3,3-trimethyl-	7.912	0.547	958	154
29.	1,6,-Octradien-3-ol, 3,7-dimethyl-	8.099	0.859	961	134
30.	1,3,8-P-menthatriene	8.274	0.137	965	134
31.	Benzene, 1,2,3,5-tetramethyl-	8.313	0.216	966	154
32.	Bicyclo [2.2.1] heptan-2-ol,1,3,3-trimethyl-	8.397	0.928	967	154
33.	2-Cyclohexen-1-ol,1-methyl-4-(1-methylethyl)-, trans	8.481	0.090	969	134
34.	1,3,8-P-menthatriene	8.614	0.156	972	132
35.	1H-indene,2,3-dihydro-4-methyl-	8.673	0.193	973	94
36.	Bicyclo [3.2.0] hept-6-ene	8.51	0.194	840	94
37.	Benzene, 2-ethyl-1,4-dimethyl-	8.810	0.390	1063	121
38.	Pyridine, 2 ethyl-5-methyl-	8.888	0.045	1055	148
39.	2-ethyl-2-methyl-1,3-dithiolane	8.985	0.104	998	134
40.	Benzene, 1-methyl-4-(1-methylethyl)-	9.072	0.095	981	154
41.	Borneol	9.022	0.495	984	154
42.	4-Terpineol	9.384	1.214	986	150
43.	Thymol	9.454	0.057	989	150
44.	Methylsalicylate	9.490	0.266	949	152
45.	Benzene, 1,3-dimethyl-5-(1-methylethyl)-	9.538	0.061	990	134
46.	Benzene, ethyl-1,2,4-trimethyl-	9.702	0.090	994	148
47.	6-Methyl-4-indanol	10.745	0.222	1014	142
48.	Naphthalene, 2-methyl-	11.002	0.123	1020	142
49.	Naphthalene, 1-methyl-	11.214	0.084	1024	142
50.	Santolina triene	11.361	0.800	1027	136
51.	Camphere	11.504	0.178	1030	136
52.	Copaen	12.100	0.211	1242	204
53.	Cyclobuta [1,2.3,4] dicyclopentene, decahydro-3a-methyl-6-methylene-1 (1-methylethyl)-, [Is-(1.alpha., alpha., 3b.β., 6a. β., 6b alpha alpha.,)]-	12.209	1.182	1244	204
54.	β-Elemene	12.290	0.274	1245	204
55.	Isocaryophyllene	12.493	17.750	1249	204
56.	Caryophyllene	12.750	4.459	1255	204
57.	Bicycle [3.1.1] hept-2-ene,2,6-dimethyl-6-(4-methyl-3-pentenyl)-	12.867	4.459	1257	204
58.	1H-cycloprop (e) azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR]-	12.945	0.116	1258	204
59.	1,6,10-Dodecatriene, 7,11-dimiethyl-3-methylene-, (E)-	13.104	0.401	1262	204
60.	Alpha.-caryophyllene	13.166	2.160	1263	204
61.	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-	13.377	0.157		204
62.	1H cyclopenta [1,3] cyclopropa [1,2] benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3as-(3a. alpha. 3b.β. 4.β., 7 alpha., 7a s)]-	13.489	0.802	1269	204
63.	1H-cycloprop [e] azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a. alpha.,	13.598	1.447	1271	204

	4a.β., 7. Alpha., 7a.β. 7b alpha)]-				
64.	Germacrene B	13.687	5.280	1273	204
65.	Germacrene A	13.829	0.192	1276	204
66.	Caryophyllene oxide	14.802	2.893	1296	220
67.	Bicyclo [3.1.1] hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	16.621	0.161	1332	204
68.	Pyrido [3,4-d] pyridazine-4,5(3H.6H)-dione, 1-(2-furfuryl)-7-methyl-	20.137	1.377	1302	257

Table 2: antimycobacterium bovi (7H9/ADC) of essential oil of Hyptis spicigera

% Conc.	25	12.5	6.25	3.125	0.45	0.28	0.14	0.07	0.035	MIC
Hyptis suaveolens	-	-	-	-	+	+	+	+	+	3.13%
Rifampicin	-	-	-	-	-	-	-	+	+	0.1µg/ml

KEY: (-) No growth in the well, means there is anti-TB activity.
(+) Growth in the wel,l means no anti-TB activity
Rifampicin (Control drug)