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

Runde M.[1], Kubmarawa D.[2]

[1, 2]Department of Chemistry, Modibbo Adama University of Technology, Yola Adamawa State, Nigeria

Abstract: ***Fresh leaves of Hyptis spicigera 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 69 compounds were present in varying concentration out of which 18 compounds have appreciable concentration making 80.582 % of the total abundance. The major compound being α-Pinene (30.536 %) followed by β-Pinene (15.840 %) and Eucalyptol (4.378 %).The essential oil of Hyptis spicigera also showed anti mycobacterium activity when tested on strain 7H9/ADC with MIC of 0.78 %. This activity was compared with standard drug Rifampicin which also has MIC of 0.1 µg/ml.***

Keywords: *Hyptis spicigera*, ethnomedicinal, anti-mycobacterium tuberculosis, minimum inhibitory, concentration (MIC)

1. Introduction

Our earliest human ancestors found plants to heal wounds cure diseases and ease trouble minds. People have long used indigenous medicinal plants, for treatment of various ailments dating back prehistory. Knowledge about the healing properties or poisonous effects of plants, mineral salts, and herbs accumulated from these earliest times to provide health and predates all other medical treatment.

Medicinal plant is defined as any plant which in one or more of its organ contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs[1]. This definition has set a difference between medicinal plants whose medicinal value have been tested and their medicinal constituents have been studied and plants that are regarded as medicinal but have not been studied scientifically. However, through trial and error, humans have learned that under certain condition, physiological effects of some secondary metabolites can have curative benefits or narcotic effects.

The medicinal effect benefited from plant materials typically resulted from the combination of secondary metabolites found in the plant. Unlike primary products like carbohydrates, protein, nucleic acids, chlorophyll and lipids, the medicinal actions of plants that are peculiar to particular plant species or groups are consistent with this idea as the combinations of secondary products in a particular plant are often taxonomically distinct[2,3].

Acknowledging the importance of plants and its medicinal value, extraction of essential oils were done using steam distillation method, hydro-distillation technique, dry/destructive distillation, cold press and supercritical fluid extraction. In this study steam distillation was used to extract essential oil from plant material of *Hiptis Spicigera*. In 2013, the medicinal values of various part of this plant as use by Lala community in Adamawa State Nigeria was reported[4]. Once the oils are obtained, the fundamental contribution of this research sides in their compositional determination and anti-mycobacterium tuberculosis (TB) activity analysis, as their chemical composition may differ even within same species. These variations might be as a result of the presence of different chemical composition, resulting from plant's adaptation to the surrounding environment, as well as its state of development[5].

A large number of medicinal plants contain essential oils that have extensive bioactivities. These oil plants are been utilized for their aromatic value as flavorings in foods and beverages and as fragrances in pharmaceutical products[6].

For centuries, oils are being use in various needs such as embalming process, medicinal and purification rituals. With the help of research we now know that the 'fragrant pharmacy' contains compounds with an extremely broad range of biochemical effects. There are about three hundred essential oils in general use today by professional practitioners[7].

2. Essential Oils (Volatile Oils)

The new encyclopedia, define essential oil as a term used to refer to highly volatile substance isolated by steam distillation from an odoriferous plant of a single botanical species. The oil bears the name of the plant from which it is derived. This definition based on the volatility and the process of isolation seems to be unsatisfactory, since many other plant metabolites such as fats, coumarins, anthraqunones and certain alkaloids are also distillable. As a result of that more accurate definitions have been proposed as follows:

Essential oil is define as odoriferous bodies of an oily nature obtained almost exclusively from vegetable sources, generally liquid (sometimes semi-solid or solid) at ordinary temperatures, and volatile without decomposition[8].

Essential oils are complex mixtures, constituted by terpenoid hydrocarbons, oxygenated terpenes and sesquiterpenes. They originate from the plant secondary metabolism and are responsible for their characteristic aroma[6].

Essential oils are derived mainly from aromatic plants while a few are obtained from animal sources e.g. musk and civet oils. Microorganisms like mosses, liver warts, sea weed and fungi also produce essential oils. Essential oils could be found in insects and some higher animals such as alligators, cats, and beaver. The oils are natural secretary products of plant metabolism and are stored in specialized structure such as glandular hairs on the epidermis, oil tube in the pericarp or isolated oil cell in the plant tissue and oil or resin ducts[9].

Essential oils are mainly made up by monoterpenes and sesquiterpenes whose main metabolic pathway is through mevalonate leading to sesquiterpenes, from methyl-erythritol leading to monoterpenes and the shikimic acid pathway en route to phenylpropenes[6].They are located in different parts of the plant, such as the root, stems, leaves flowers and even in seeds as in the case of anise (*Pimpinella Anisum*), coriander (*Coriandrum sativum*) and pepper (*Pipernigrum*), among others[6].

3. Material

Plant Material

Fresh leaves of *Hiptis spicigera* were collected in January 2015 at Sangere ,Girei local government Area of Adamawa State North-eastern Nigeria. The plants was isolated and conserved for extraction.

4. Methods

4.1 Essential Oil Extraction

The leaves of *hiptis spicigera* (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 distiller was pass through another vessel containing ice) for 2.5 h, the essential oil of the plant was collected over water and later kept at 4 ^{O}C until further required.

4.2 Gas Chromatography Mass Spectroscopy (GC-MS)

GC-MS analysis was performed on a J and W Scientific gas chromatography directly couple to the mass spectrometer system (model GC Agilent technologies 7890A, Agilent technologies Inert MSD 5975C), the Capillary column (30M x 250µm) was used under the following condition: ovum temperature 50^0 C for 1 min, then gradually raised at interval of 10^{0}C/min untill 200^0 C was reach then hold for 1min, and 20^0/min to 300^0 for 2 min.

Injector temperature was 230^{0}c and the carrier gas was Helium gas, the sample was then allowed to flow at the rate of 1ml/min; the volume of the injected sample was 0.2µL of diluted oil in hexane, splitless injection techniques was used in which ionization energy was 70ev in the electron ionization (EI) mode. Ion source temperature was 230^{0}C and the scan mass range was M/Z 60-335. Finally, 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[10].

4.3 Method of Antituberculosis Determination using Micro-Broth Dilution

The method of antimycobacterium tuberculosis activity was carried using micro-broth dilution technique as described in our previous work[11].

5. Results and Discussion

5.1 Oil Yield

The yield of essential oils of *Hyptis spicigera* leave extract varies from 0.23-0.3 %[12]. However , the essential oil yield observed in this work is 0.4 % which is higher than the result obtained by some researcher elsewhere like Zaria in Nigeria (0.1 %), Mali (0.1-0.3 %), Cameroon (0.12 %) whereas the result of Togo is higher (1.2 %)[,13,14,15,16,17].

5.2 GC-MS Compositional analysis

Fig.1 is the gas chromatography spectrum for the essential oil of *Hyptis spicigera*. The Mass spectrum reveals the presence of 69 compounds (table-I). The major component being alpha. Pinene (30.536%), followed by beta-pinene (15.842 %) then Limonene (4.378 %). Other component of appreciable concentration are: caryophyllene (3.082 %), sabinene (2.849 %), cis-sabinol (2.788 %),cis-verbinol (2.284 %), benzene butyl (2.752 %), cymene (2.284 %), limonene (1.930 %), alpha- campholene aldehyde (1.678 %), alpha-phellendrene (1.539 %),p-cymene (1.478 %), gama-terpinene (1.435 %), Naphthalene (1.382 %), eucarvone (1.90 %), alpha-thujene (1.058 %), and isovaleric acid, 2-methylbutyl ester (1.006 %). In another work carried on Hyptis spicigera obtained from Zaria, Nigeria has also revealed them same major component but with different concentrations as alpha-pinene (12.16 %), beta-pinene (9.47 %) and eucalyptol (4.378 %)[18]. Similar work showed monoterpenes as the major component of the essential oils of leaves of *Hyptis spicigera* which include: alpha-pinene (50.8 %), eucalyptol (20.3 %) and beta-pinene (18.3 %)[19]. Different lead compounds were observed for the same species being: 1,8-cineol (24.0 %) and (E)-caryophellene (22.2 %)[20]. In the same vein, the essential oil of the leaves of *Hyptis spicigera* obtained from Togo shows to have beta-caryophyllene (33.8 %), alpha-bergamoten (11.3 %) alpha-caryophyllene (7.4 %), alpha-pinene (16.9 %), sabinene (13.8 %) beta-pinene (9.6 %) and 1,8-cineol (3.8 %) were the major component of the oils of[12,15]. The variations observed in the essential oil of *Hyptis spicigera* obtained from different locations has further confirmed the report which says; "chemical composition of essential oils may vary even within same botanical species due to the presence of different chemotypes according to the plants adaptation to the surrounding environment, as well as its state of development"[5].

5.3 Anti-Tuberculosis activity of *Hyptis spicigera*

The anti-tuberculosis for essential oil of *Hyptis spicigera* is presented in table 2. The result shows a significant activity with minimum inhibitory activity (MIC) of 0.78 % compare to the control (0.14 mg/ml rifampicin). The anti-tuberculosis activity observed in this plant can be related to the presence of the lead compounds as recorded in our GC-MS result; alpha-pinene (30.536 %), beta-pinene (15.840 %) and eucalyptol (4.378 %). Other research also reported similar activities of essential oils such as oils of *citrus sinensis* peel in which the major component were reported to be mono terpenes (81.74 %) and sesquiterpenes (1.32 %)[21]. In another work pure samples of alpha-pinene (20 µL/ml), beta-pinene (20 µL/ml) and eugenol (10 µL/ml) were reported to have anti-microbial activity against various strains of gram positive and gram negative bacteria[22]. These reports have confirmed why the essential oil of *Hyptis spicigera* has anti-tuberculosis activity as revealed in our work.

6. Conclusion

The GC-MS analysis of the essential oil of leaves of *Hyptis spicigera* source from Girei local government Area of Adamawa state, North-eastern Nigeria, indicate that the major compounds are α-Pinene (30.536 %), β-Pinene (15.840 %) and Eucalyptol (4.378 %). The essential oil of *Hyptis spicigera* 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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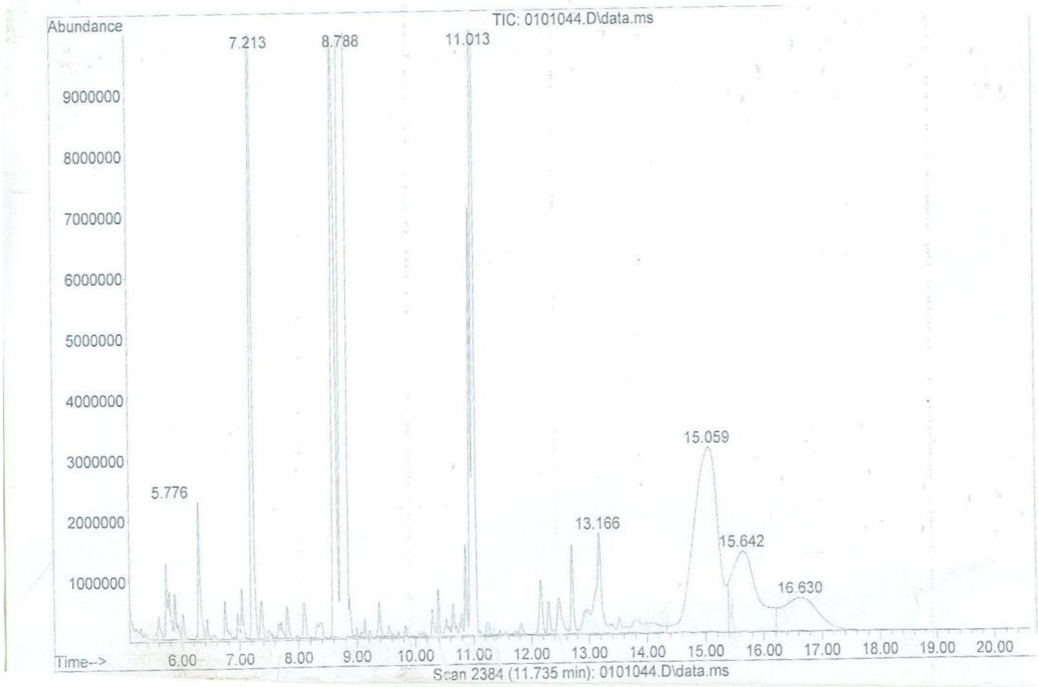

Figure 1: Gas chromatography (GC) spectrum of hyptis spicigera

Table 1: Result of GC-MS Analysis of *Hiptis spicigera*

S/No.	Constituents	RT(min)	Area %	KI	MW
1.	Bicyclo [3.1.0] hex-2-ene,2-methyl-5-(1-mrthylethyl)-	5.133	1.058	902	136
2.	Alpha-Pinene	5.299	30.536	905	136
3.	Camphene	5.536	0.368	910	136
4.	Benzene, butyl-	5.607	2.752	912	134
5.	Benzene, 1-ethyl-3-methyl-	5.732	0.904	814	120
6.	Benzene,1-ethyl-4-methyl-	5.784	0.754	815	120
7.	Benzene,1,3,5-trmethyl-	5.883	0.664	817	120
8.	Bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethy)-	5.954	2.849	919	136
9.	β.Pinene	6.045	15.840	920	136
10.	Tricyclo [2.2.1.02,6] heptanes, 1,7,7-trimethyl-	6.248	0.543	925	136
11.	Benzene,1,2,4-trimethyl-	6.301	2.215	826	120
12.	Decane	6.440	0.206	928	132
13.	Alpha.Phellandrene	6.520	1.539	930	136
14.	(+)-2-Carene	6.702	0.697	934	136
15.	Benzene, 1,2,4-trimethyl-	6.747	1.487	834	120
16.	Benzene, 1-methyl-4-(1-methylethy)-	6.836	1.930	936	120
17.	Limonene	6.914	4.378	938	136
18.	Eucalyptol	6.964	0.217	939	154
19.	Benzene, 1,2-diethyl-	7.207	0.405	944	134
20.	Benzene, 1-methyl-3-propyl-	7.260	1.435	945	134
21.	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethy)-	7.393	0.163	947	136
22.	Benzene, 1-methyl-2-propyl-	7.491	0.194	949	134
23.	Terpinol, Cis-β.-	7.616	0.259	952	154

24.	Benzene, 1-ethyl-2,3-dimethyl-	7.662	0.290	953	134
25.	Benzene, 1-methyl-2-(methylethy)-	7.705	0.339	954	120
26.	Benzene, 1-ethyl-2, 4-dimethyl-	7.811	1.190	956	134
27.	(+)-2-Carene	7.833	0.752	956	136
28.	2, 4-Cycloheptadiene-1-one, 2,6,6-trimethyl-	7.865	0.182	957	150
29.	Benzene, methyl (1-methylethyl)-	7.914	0.671	958	118
30.	Cyclopentene, 3-ethylidene-1-methyl-	8.003	1.006		108
31.	Benzene, 1-methyl-4-(1-methylethyl)-	8.094	0.385	961	132
32.	Butanoic acid, 3 methyl-, 2- methylbutylester	8.219	0.483	964	172
33.	1, 4-cyclohexadiene, 3-ethenyl-1,2-dimethyl	8.271	0.218	965	
34.	Benzene, 1,2,3,5-tetramethyl-	8.314	1.678	966	134
35.	Benzene, 1,2,3,5-tetramethy-	8.367	0.104	967	134
36.	2,4-cycloheptadiene1-one,2,6,6-trimethyl-	8.408	0.735	968	150
37.	Alpha-campholene aldehyde	8.504	2.788	970	152
38.	Benzene, 1,2,3,4-tetramethyl-	8.616	2.284	972	134
39.	Bicyclo [3.1.1]heptan-2-one, 6,6-dimethyl-, 9(ii)	8.685	0.394	873	152
40.	Bicyclo [3.1.0] hexan-3-o1, 4-methylene	8.740	0.889	974	152
41.	Bicyclo[3.1.0] hept-3-ene-2-o1, 4,6,6-trimethyl-	8.820	0.665	976	152
42.	1,3,5-Hexatriene,3-methyl-, (2)-	8.890	1.084		94
43.	Bicyclo[3.1.0] heptan-3-one,2,6,6-trimethyl-(1.alpha.. 2. B., 5. alpha)-	9.026	0.484	980	152
44.	Bicyclo[3.1.0] heptan-3-one,6,6-dimethyl-2-methylene	9.051	0.755	981	152
45.	1,3-cycloheptadiene	9.228	0.413		94
46.	3-cyclohexen -1-o1,4-methyl-1-(1-methylethyl)-	9.354	0.912	987	154
47.	Naphthalene	9.381	1.382	987	128
48.	Thymol	9.464	0.572	989	150
49.	Bicyclo[3.1.0] hept-2-ene-2-carboxade-hyde, 6,6-dimethy-	9.555	0.397	991	150
50.	Bicyclo[3.1.0] hept-2-ene-2-menthano1,6-dimethy-	9.592	0.089	991	152
51.	D-verbenone	9.739	0.090	994-150	150
52.	2-cycohexen-1-o1,2,-methyl-5-(1-methyletheny)-	9.942	0.183	998	152
53.	n-vaeric acid cis-3-hexeny ester	10.137	0.121	1002	184
54.	Fumaric acid, butyl myrtenyl ester	10.192	0.247	1353	306
55.	Hexyl n-valerate	10.233	0.239	1004	186
56.	2-cyclohexan-1-one,2-methyl-5-(1-methyletheny)-(s)-	10.266	0.331	1005	150
57.	Fumaric acid, isobutyl myrtenyl ester	10.368	0.151	1352	306
58.	Benzene, pentamethyl-	10.750	0.128	1015	148
59.	Naphthalene, 2-methyl-	11.006	0.122	1020	142
60.	Naphthalene, 1-methyl-	11.219	0.079	1024	204
61.	Ylangene	12.017	3.082	1240	204
62.	Copaen	12.102	0.145	1242	204
63.	Cyclobata [1,2:3,4] dicyclopentene, decahydro-3a-methyl-6-methylene-1-(1-methylethyl)-,[Is-(1.alpha., 3a alpha., 3bβ., 6a. β., 6b. alpha.)]-	12.216		1244	204
64.	Caryophyllene	12.698		1254	204
65.	Alpha.cubebene	13.071		1261	204
66.	Alpha.caryophellene	13.164		1263	204
67.	Germacrene D	13.492		1269	204
68.	1H-cyclopropa [a] naphthalene, 1a,2,3,5,6,	13.909		1278	204
69.	Caryophyllen oxide	13.824		1296	220

Table 2: Anti-mycobacterium tuberculosis (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
Hyptisspicigera	-	-	-	-	-	-	+	+	+	0.78%
Rifampicin	-	-	-	-	-	-	-	+	+	0.1µg/ml

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