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Extraction, Compositional Analysis and Trypanocidal Activities of Essential Oils of Four Aromatic Plants Obtained From North-Eastern Nigeria

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Abstract:
The advantage of having in abundance aromatic plants in the North-eastern part of Nigeria was again utilized with the sole aim of harnessing their composition into useful discovery in the aromatic chemistry. To achieve this aim, the stem bark of Bosweillia dalziellii, the leaves of Ocimum americanus, Hyptis spicigera and Hyptis suaveolens were subjected to steam distillation for possible extraction of essential oils. The oils obtained were then analyzed using Gas Chromatography Mass Spectroscopy (GC-MS) technique. The results showed that 55 components were identified in the essential oil of Bosweillia dalzielii with α-pinene (18.515 %), isophthaldehyde (10.695 %) and β-pinene (5.641 %) as the major components of the essential oil. The essential oil of Ocimum americanus has a total 45 components in which terpinene-4-ol (14.507), copaene (7.438 %) and terpinene (6.178 %) are the predominant components. On the other hand α-pinene (30.536 %), β-pinene (15.840 %) and eucalyptol are the lead components in the essential oil of Hyptis spicigera out of 30 components identified. The GC-MS result for the essential oil of Hyptis suaveolens revealed that out of the 31 components identified carryophyllene (20.643 %), sabinene (16.711 %) and terpinolene (8.490 %) are the lead components. The various essential oils were tested for trypanacidal activities using trypanasoma congoles and the result obtained shows that essential oil of Hyptis spicigera inhibited the motility of the parasites in 60 and 30 minutes after the start of experiment, at concentrations of 25 and 50 µL/ml respectively. However complete inhibition of the motility of the parasites was noticed 90 minutes after the start of the experiment at same concentrations above when essential oils of Ocimum americanus and Hyptis suaveolens where introduced into the test tubes containing blood infested with trypanasoma congoles. The essential oil of Bosweillia dalzielii exhibited least trypanacidal activity having inhibition on the motility of the parasites at 120 minutes of the start of the experiment.

Keywords: *Essential oil, Trypanacidal, Components, Steam distillation*

1. Introduction

The high cost, increasing drug resistance, and side effects of current therapeutic approaches are forcing the scientists to explore alternative medicines, the traditional medicine, as an option to find new chemical entities for treatment of diseases. Among the alternative traditional approaches, various plant products classified as alkaloids, saponins, triterpenes, glycosides, and polyphenols among others have shown very promising anticancer properties in both *in vitro* and *in vivo*. There are more than one thousand plants which have been reported to possess significant anticancer properties (Mukherjee, 2003). Vincristine, vinblastine, colchicine, ellipticine, lepachol, taxol, podophyllotoxin, camptothecin, irinotecan, etoposide, and paclitaxel are classical examples of plant-derived compounds which are found to have wide applications in cancer therapeutics (Cragg and Newman, 2005).

Exploring natural plant products as an option to find new chemical entities as therapeutic agents is one of the fastest growing areas of research. Recently, in the last decade, essential oils (EOs) have been under study for their use in antimicrobial, antioxidant, antituberculosis and anticancer but little work has been done on in-vitro trypanocidal activity owing to their volatile nature that makes them difficult to handle especially when using them *in-vitro* analysis.(Nandini et al, 2014). EOs are the concentrated hydrophobic liquids with specific aroma produced by aromatic plants (Celiktas *et al*, 2007). These are also called volatile oils or ethereal oils and are the secondary metabolites present in lower amounts in various plant parts. The composition and other biological properties of the EOs depend on their constituents. The constituents may be terpenes, aromatic compounds and some other compounds of various origins. The constituents of the EOs have been classified on the basis of their chemical structures. EOs are considered more potent than their constituents (Ozkan and Erdo-gan, 2011) due to their synergistic and more selective effect. In addition, EOs from plants growing in varied environments differ in their composition and hence have different uses.

2. Material and Methods

The leaves of *Ocimum americanus, Hyptis suaveolens, Hyptis spicigera,* and the stem bark of *Bosweiliadalzielii* (each weighing 1 kg) obtained in the month of January 2015 at Girei Local Government Adamawa state North-eastern Nigeria and 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 passed through another vessel containing ice) for 2.5 h essential oils of the plants were collected over water and later kept at 4 ^{O}C until further required.

2.1 Methods

2.1.1. Gas chromatography Mass spectroscopy (GC-MS)

GC-MS analysis were 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) HP 5 ms, 5 % phenyl methyl silox: 469.56 x 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 2 min.

Injector temperature 230^0c carrier gas He, flow rate 1ml/min; the volume of the injected sample was 0.2µL of diluted oil in hexane, split less injection techniques, ionization energy 70ev, in the electron ionization (EI) mode, ion source temperature 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 (Ramzi et al., 2013).

3. Results and Discussion

The chemical composition of essential oils obtained from stem bark of *Bosweillia dalzielii,* leaves of *Ocimum americanus, Hyptis spicigera* and *Hyptis suaveolens* is presented in table 1. The GC-MS analysis of the essential oil obtained from the stem bark of *Bosweilia dalzielii* reveals the presence of 55. However, the major compounds were α-Pinene (18.515 %), isophthaladehyde (10.695 %), β-pinene (5.641 %), Acetonitrile, (3,5,5-trimethyl-2-cyclohexene-1-ylidene), (z) (4.598 %), 1,2-Naphthalenediol, 1,2,3,4-tetrahydro-1-methyl-, cis (4.439 %), 2H-1,3-Benzoxazine, octahydro-2-(phenylimino) cis (3.051) and 10,12- Tricosadioic acid, methyl ester (3.04 %), others are Cinnamyl carbanilate (2.904 %), 5-isopropylidene-4,6 dimethylnona-3,6,8-trien-2-ol (2.554 %), β-Elemenone (3.803 %), 5-isopropylidene-4,6-dimethylnona-3,6,8-trien-2-ol (2.554 %), 1,3-cyclopentadiene, 1,2,3,4-tetramethyl-5-methylin (2.298 %), 4,7-methano-1H-indene-5-ol, octahydro- (2.169%), 3-pentenimidic acid, 3-mehyl-N-phenyl-, methyl ester (2.108 %), Humulene (3.409%), Limonene (1.485 %), Bicyclo (3.2.0) nonane, 2-methyl-4,8,8- trimethyl-4-vinyl (1.430%), furan acetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo (1.226 %), α-C ubenene (1.137 %), and 1,3,8-p-menthatriene (1.017 %). Total of 22 compound contained 76.847 %, the remaining bulk of 83 compound covers only 23.153 %. Another result of the constituents of the essential oil obtained in Nigeria shows the major components being, α-Pinene (45.7 %) followed by α-Terpinene (11.5 %) (Kubmarawa et al, 2011). Therefore the predominance of monoterpenoids presented in the result of our work has concurred with the constituents of the oil of the genus reported by these authors.

Using GS-MS Technique, 45 components were detected in *Ocimun americanus* essential oil. Terpnene-4-ol (14.507 %) identified as marker compound. Other component of appreciable percentage include: copaene (7.438 %), terpinene (6.178 %), D-limonene (3.149%), Cis, β-terpineol (3.914 %), α-pinene(2.664 %), 4-piperidine carboxamide, 1,1-(2-hydroxybenzoyl)-(2.641 %), α-Bergamotene (2.598 %) phenol, 3 (1-methylethyl) (2.467 %), β-pinene (2.277%), 5-caranol, (2.123 %), 5-cadinene (2.062 %), Terpinolene (2.006%) α-Gurjunene (1.698 %), octen-1-ol, acetate (1.662 %), α-cubebene (1.442 %), Thujene (1.483 %), Cyclene (1.377 %) 2,2,5,6- Tetramethyl-1,3-oxathiane (1.344 %), α-caryophyllene (1.147 %), p-cymene (1.278 %), myrtenyl acetate (1.139%), isocaryophellene (1.041 %), and Bornyl acetate (1.025 %).

Similar research revealed that the essential oil of the leaves of *Ocimum gratissimum* the same family (Lamiceae) with *Ocimum americanus* from western Nigeria was reported to consist of *γ*- terpinene (52.86 %), Z-tert-butyl-4-hydroxy anisole (13.93 %), caryophyllene (10.37 %) and p-cymenene (7.16 %) as the major compounds while the essential oils of the seeds yielded α- pinene (48.19 %), caryophyllene (10.71 %), and 3-tert-butyl-4- hydroxyanisole (11.14 %) as the major compounds (Owokotomo *et al*, 2012). In another research carried on *Ocimum canum (Ocimum americanus)* leaves and flowers oils that was sourced from Burkina Faso, consisted mainly in 1,8-cineole (60.1 %) and cis, transpiperitol (68.5 %), respectively (Bassole *et al*, 2005). The variation observed in the chemical composition of the essential oils for the family Lamiceae obtained from different countries (Nigeria and Burkina Faso), implies that composition of essential oil differ within family and species of plants obtain from different locations.

The GC-MS for chemical composition of leaves of *Hiptis spicigera* essential oil show the present of 30 compounds. The major component was α-pinene (30.536 %) other component present in appreciable content being: β-pinene (15.840 %), Eucaiptol (4.378 %), caryophellene (3.082 %), Sabinene (2.849 %), Cis-Sabinol (2.788 %), Cis-verbenol (2.284 %), Benzene butyl (2.752 %), Cemene (2.284 %), limonene (1.930 %), alpha-campholene aldehyde (1.678 %), alpha-phellandrene (1.539 %), P-cymene (1.487 %), ϒ-Terpinene (1.435 %) 2-Pinene-10-ol (1.382 %), Eucarvone (1.190 %) 1,4-cycloheptadiene (1.084 %), alpha-thujene (1.058 %) and isovaleric acid, 2-methylbutyl ester (1.006 %). Therefore this GC-MS resulted in the identification of 30 compounds. In another work carried on *Hyptis spicigera* obtained from other locations such as Zaria Nigeria, has revealed the same major component but with different concentrations being, alpha-pinene (12.16 %), beta-pinene (9.47 %) and eucalyptol (4.378 %) (Ladan *et al,* 2011), alpha-

pinene (50.8 %), eucalyptol (20.3 %) and beta-pinene (18.3 %) as revealed by Takayama *et al*, (2011). Contrarily, Ngassoum reported the oil obtained from the same species having different lead compounds being: 1,8-cineol (24.0 %) and (E)-caryophellene (22.2 %) (Ngassoum *et al*, 2007). Monoterpene hydrocarbons (70.4 %) were the lead compounds followed by sesquiterpene hydrocarbons (22.6 %) as reported by (Barbara *et al*, 2010). In the same vein Koba *et al*, (2007*)* reported that the essential oil of the leaves of *Hyptis spicigera* obtained from Togo showed to have beta-caryophyllene (33.8 %), alpha-bergamoten (11.3 %) and alpha-caryophyllene (7.4 %), whereas 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 *Hyptis spicigera* (Bougnonou *et al*, 2013). 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 (Abdulrahaman *et al*, 2013).

The steam distillation of the leaves of *Hyptis suaveolens* produces essential oil which shows the presence of 31. The major component was caryophyllen (20.643 %), followed by Sabinene (16.711 %), Terpinolene (8.49 %), β-pineine (5.490 %), Germacrene B (5.280 %), alpha-Bergamotene (4.459 %), alpha-pinene (2.644), alpha-caryophyllene (2.160 %), Alloaromadendrene (1.447 %), ϒ-Terpine (1.403 %), Benzene, 1,2,4-trmethyl (1.614 %), pyrido [3,4-d]pyridazine-4,5 (3H,6H)-dione, 1-(2-furfuryl)-7-methyl- (1.377 %), 4-Terpineol (1.214 %) β-Elemene (1.182 %) and 1-octen-3-ol (1.021 %). Total of 16 compounds accounting for 75.135 %. Other researchers have reported the presence of major components of essential oil of *Hyptis suaveolens* cultivated as follows: in Italy; Sabinene (34 %), beta-Caryophyllene (11.2 %) and Terpinolene (10.7 %) were reported as the major components of the oil (Giovanni *et al*, 2012), 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 (Chatri *et al*, 2014). 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 (Sharma *et al*, 2007). 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 (Okonogi *et al*, 2005), whereas Fun and Baerhein reported the major compound found in the essential oil of the plant species are 1,8-Cineole (27-38 %), and Sabinene (12-18 %) (Fun and Baerhein, 2006). Brazilian *Hyptis suaveolens* essential oil was presented with Sabinene, Limonene, Bicyclogermacrene, beta-Caryophyllene and 1,8-Cineole as the major compounds of the oil (Azevedo *et al*, 2006).In the same vein analysis of essential oil obtained from Togo showed that beta-Caryophyllene (33.8 %), alpha-Bergamoten (11.3 %) and alpha-Caryophyllene (7.4 %) are the predominant compounds (Koba *et al*, 2007). From the above discussions, Caryophyllene is common in all the samples obtained from various locations except in the sample obtained from Brazil which has Limonene, Bicyclogermacrene and beta-Phellandrene. On the other hand Rimuene is another compound that has appeared only in oil sample from Indonesia.

Constituents				
	B. dal.	O. ame.	H. spi.	H. sua.
P-xylene	0.228	0.28		
β-Pinene	1.317	2.277	15.840	5.490
α-Pinene	18.515	2.664	30.536	2.644
Camphene	0.739	0.299	0.368	0.122
Camphor	0.153	0.623		
α-Campholene aldehyde	0.060		2.788	
Isopinocamphone	0.138			
(-)-(25,8Ar)-(camphorsulphurnyloxaridine)		0.882		
Cinnamyl carbanilate	4.439			
α-Phellandrene		0.663		0.575
β-Phellandrene	0.092			
β-Cubebene				
α-Cubebene	0.151	0.237	0.145	
β-Ocimene	0.88	0.079		
Ocimene (z)				
β-Sesquiphellandrene		1.031		
5-Caranol, (1s,3R,5s,6R)-(-)-				
(+)-2-Carene	0.150		1.449	0.505
3-Carene	0.475	0.388		0.804
(+)-3-Carene, 2-(acetylmethyl)-		0.051		
Terpinen-4-ol		14.507		1.214
Cis-β-Terpineol		3.914		
Terpinolene		2.006		8.496
ϒ-Terpinene				1.403
Terpinol, cis-β				
Humulen (VL)	5.602	0.458		
Humulene-1,6-diene-3-ol		0.809		

Humulene		0.053		
α-Caryophyllene	0.088	1.147	0.298	2.160
Caryophyllene	0.069	0.216	3.082	4.459
Caryophyllene oxide			0.786	2.893
Isocaryophyllene-11	0.288	1.041		17.750
Caryophyllene-(11)	0.213			
Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo	1.226			
Acetonitrile, (3,5,5-trimethyl-2-cycloxen-1-ylidene)-,(z)-	3.051			
Fumaric acid, isobutyl myrtenyl ester			0.151	
Fumaric acid, heptyl myrtenyl ester	0.676			
Fumaric acid, 3,5-dimethyl phenyl ethyl ester	0.393			
1,3,8-p-menthatriene	2.108	0.049		
P-mentha-2,5-diene-7-ol, cis				
P-mentha-2,5-diene-7-ol, trans				
β.-Elemenone	1.258			
Elemene				
Germacrene A				0.192
Germacrene B				5.280
Germacrene D		0.098	0.110	
Limonene	0.969	3.149	4.378	
D-Limonene				8.832
Copaene	1.403	8.277	0.390	0.211
Cyclone		1.377		
Piperitone				
(-)-Aristolene	0.115			
Thujopsene	0.087			
M-Cymene				
P-Cymene		1.278		
α-Thujene		1.483		
D-Verbenone			0.090	
α-Gurjunene		1.698		
β-Myrcene				0.536
3-Octanol				0.189
Borneol				0.495
Methylsalicylate				0.266
Santolina triene				0.178
10s, 11s-Himachala-3(12), 4-diene	0.089			
(-)-Isolongifolol, methyl ether	0.768			
Longifolene		0.086		
(-)- Isolongifolol, methyl ether	0.882			
Isolongifolene, 9,10-dehydro		0.074		
β-Linalool				
Nonanal				
Cadinene				
Eucalyptol			0.217	
Thiophene				
Isosafrole				
Terpinene		6.178		
Patcholene		0.431		
Mesitylene				
2-Hydroxy-4,5-dimethyl, acetophenone				
α-Bourbonene		0.097		
Eugenol				
Ylangene		0.729	0.128	
α-Bergamolene		2.669		
Thymol			0.572	0.057
Isobornyl formate	0.440			
Cadinene		2.126		

Bornyl acetate	0.132	1.025		
Fenchyl acetate		0.795		
Lendene				
Myrtenyl acetate		1.139		
ϒ-Eudesol				
Guaiol				
Levomenol				
Cyclopentene, 3-ethylidene-1-methyl-			1.006	
Biclo[3.1.0] hex-2-ene, 2-methyl-5-(1-methylethyl)-			1.058	
Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	5.641			
3-Cyclohexen-1-carboxylic acid, 4-methyl- ester				
Bicyclo[3.1.0] hexane, 4-methylene-			2.849	16.711
Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-				4.459
1H-Cyclo prop[e] azulene, decahydro-1,1,7-trimethyl-4-methylene-				1.447
2-Norbornene				
Cyclobuta [1,2.3,4] dicyclopentene, decahydro-3a-methyl-6-methylene-1-(1-methylethyl)-				1.182
1,3-cyclopentadiene, 1,2,3,4-tetramethyl-5-methylene	4.439			
Bicyclo [5.2.0] nonane,2-methylene-4,8,8-trimethyl-4-vinyl-	2.169			
Benzene, 1-methyl-4-(1-methylethyl)-			1.930	
Benzene, 1,2,3,4-tetramethyl-			2.284	
Benzene, 1-ethyl-2,4-dimethyl-	0.323		1.190	
Benzene, 1,2,3,5-tetramethyl	1.485		1.678	
Benzene, butyl-			2.752	
Benzene, 1,2,4-trimethyl	0.102		3.702	1.614
Benzene, 1-methyl-3-propyl			1.435	
Benzene, 1,3,5-trimethyl-				
2H-1,3-Benzoxazine, octahydro-2-(phenylimino)-cis	10.695			
1-(2-pyridyl) piperazine	2.794			
Benzyl cyclobautane				
1-Octen-3-ol				1.021
5-hydroxyanthranilic acid	0.872			
(-)-Globulol				
Octen-1-ol, acetate		1.662		
Alloaromadandrene				
2,2,5,6-Tetramethyl-1,3-oxathiane		1.344		
4-Piperidinecarboxamide, 1-(2-hydroxybenzoyl)-		2.641		
Sabinane				
Naphthalene			1.382	
Benzen, 1-methyl-3-(1-methylethyl)-				
1,3,6-Octatriene, 3,7-dimethyl-				
1,N-cyclohexadiene, 1-methyl-4-(1-methylethyl)-				
Aziridine, 1-phenyl-				
3-oxabicyclo[3.3.0]oct-7-ene-2-one-7-methyl-				
Cyclohexamethanol, 4-ethenyl-,α,α., 4-trimethyl-3-(1-methylethenyl)				
Pyrido [3,4-d] pyridazine-4,5-(3H.6H)-dione, 1-(2-furfuryl)-				1.377
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-				
3-pentenimidic acid, 3-methyl-N-phenyl-,methyl ester,	4.598			
1,3,5-Hexatriene, 3-methyl-,(2)-	0.064		1.084	
3-Cyclohexan-1-ol, 4-methyl-1-(1-methlethyl)-				
1,2,4-methanoazulene, decahydro-1,5,5,8a-tetramethyl	1.39			
Imidazo [4,5-e] [1,4] diazepine-5,8-dione, 2-chloro-1,4,6,7-tetrahydro-1,4-dimethyl-	1.430			
Isovaleric acid, butyl ester				

5-isopropylidene-4, 6-dimethylnona-3,6,8-trien-2-ol	2.298			
Phenol, 3-(1-methylethyl)-		2.467		
10,12-Tricosadiyoic acid, methyl ester	1.653			
Decane			0.206	0.246
1,3,5-Triazine-2,4-diamine,6 –chloro-4-N,N-triethyl	1.759			
1,2-Naphthalenediol, 1,2,3,4-tetrahydro-1-methyl-cis	1.017			
TOTAL	55	45	30	31

Table 1: Gas Chromatography Mass Spectroscopy Composition of various Essential oils

Sample/Conc.	**Survival of trypanosomes in minutes**						
D.dalzielli	0	30	60	90	120	150	180
25µL/ml	+++++	+++	+++	+	-ve	-ve	-ve
50µL/ml	+++++	+	+	-ve	-ve	-ve	-ve
H. spicigera							
25µL/ml	+++++	++	-ve	-ve	-ve	-ve	-ve
50µL/ml	+++++	-ve	-ve	-ve	-ve	-ve	-ve
H. suaveolens							
25µL/ml	+++++	+++++	++	-ve	-ve	-ve	-ve
50µL/ml	+++++	+++++	+	-ve	-ve	-ve	-ve
O. americanus							
25µl/ml	+++++	++++	+	-ve	-ve	-ve	-ve
50µL/ml	+++++	++	-ve	-ve	-ve	-ve	-ve
Control (0.9 % NaCl)	+++++	+++++	+++++	++	-ve	-ve	-ve

Table 2: In-vitro Antitry panocidal activitities of the isolated essentials oils

The *In-vitro* anti-trypanocidal Activities for Essential oil of various Plants are shown in table 2. The essential oil of *Boswelia dalziellii*, *Ocimium americanum*, *Hyptis spicigera* and *Hyptis suaveolens* were analysed for their *in-vitro* trypanocidal activity against *Trypanosoma congoles* at varying concentration of 25 and 50 µL/ml. Complete elimination of the parasites was first observed after 30 minutes in *H. spicigera* (50 µL/ml) and *V. cuspidata* (50 µL/mI). After 90 minutes, there was no motility observed in both the sample, however, motility continued in the control (0.9% NaCl solution) for 120 minutes.

A similar result was reported when the essential oil of *Carapa guianensis* (andiroba) and *Schinus molle* (aroeira) as reported by Baldissera et al 2013 in which the dose dependant reduction in parasite *Trypanosoma evansi* load was observed after 1hour and a significant reduction was observed after 3 hours at low concentration of 0.5% (Baldissera *et al*, 2013).

The essential oil extracted from traditional plants *Lippia sidoides* and *Lippia origanoides* indicated significant reduction in motility of the parasite *trypanosome cruzi*. These essential oils are reported to contain mainly monoterpenes and sesquiterpenes (Borges *et al* 2012). The leaves and rhizomes of *Hedychium coronarium* were also analyzed for antitrypanocidal activities using *Trypanosoma brucei*. The GC-MS results shows that caryophyllene oxide is the predominant compound of the rhizome while 1,8-cineol is predominant in the leaves extract. Their test on trypanosome reveals that the essential oil from the leaves was inactive against the parasite at concentration above 100µg/mL where as the essential oil from the rhizome showed a significant activity against the parasite at concentration of 65.77 µg/ml (Borges *et al*, 2012).

4. Conclusion

The essential oils of *Hyptis spicigera* and *Ocimum americanus* exhibited trypanocidal activities in which the parasite motility was seen to have stopped after 30 minutes and at concentration of 50 µL/ml and also 60 minutes at the concentrations of 25 and 50 µL/ml respectively. The major components of these essential oils are α-pinene (30.536 %), β-pinene (15.840 %), eucalyptol (4.378 %) for *Hyptis spicigera* and terpinen-4-ol (14.507 %), copaene (7.438 %), terpinene (6.178 %) for *Ocimum americanus.* Therefore these components in their concentrations could be responsible for the trypanocidal activities of the essential oils of these plants. In spite of some reports showing carryophyllene and α-pinene to have trypanocidal properties as revealed by Borges *et al* (2012) and as seen in the essential oil of *Hyptis spicigera* but not exhibited by the essential oil of *Bosweillia dalzielii* as shown in this work; therefore the only explanation to why the essential oils of *Bosweillia dalzielii* and that of *Hyptis suaveolens* are having low trypanocidal activities despite containing carryophyllene and α-pinene, perhaps could be related to the low concentrations of these compounds in the plants oil. On the other hand Ladan *et al* (2011) reported that *Hyptis spicigera* plants grow commonly by the roads side and as such cattle graze on them freely. It is therefore not a mere coincident but facts, that these cattle who graze on the plants rarely present symptoms of trypanosomiasis.

5. References

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