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Full Length Research Paper

Chemical composition and biological activity of the volatile oils of *Hyptis spicigera* against *Trypanosoma brucei brucei, (Tbb)* found in Northern Nigeria

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The hydrodistillation of the fresh leaves of *Hyptis spicigera* gave a colourless volatile oil with yield of 0.65%.The volatile oil gave forty compounds on the basis of gas chromatography-mass spectroscopy (GC-MS) with low composition of cineole(4.11%) and caryophyllene (2.61%) while α-pinene (12.16%) β-pinene(9.47%) and α-phellandrene (10.19%) were predominant compounds. The biological activity of the volatile oils was evaluated *in vitro* for activity against *Trypanosoma brucei brucei* (Tbb) and was found to possess anti-trypanosoma activity *in vitro* in a dose dependent pattern at 0.5 µg/ml in 6 min. This activity showed the volatile oils from *H. spicigera* leaves to be a potential trypanocide.

Key words: *Hyptis spicigera,* gas chromatography-mass spectroscopy, *Trypanosoma brucei brucei*, trypanosome, volatile oils.

INTRODUCTION

Hyptis spicigera belongs to the family Lamiaceae. It is commonly known as Black beniseed, or Black sesame (Burkill, 1995). It is an erect aromatic herb, up to 1 m in height, with a terminal inflorescence in which the seeds are packed in quadruplets or more in the flowers. The plant possesses very tiny brown/black seeds that clustered in groups of fours, fives or even more, which are encased in each flower that make up the inflorescence (Dalziel, 1937) and the terminal inflorescence is dense cylindrical or ovoid with cylindrical spike up to 9 cm long (Burkill, 1995). The plant is found around Senegal to Western Cameroon, possibly native to Brazil, now widely naturalized in tropical Africa and Asia as well as Nigeria. It grows naturally in roadsides, waste and damp places as well as in cultivated farmlands (Burkill, 1995) as a weed. The seeds are used for oil production while the leaves are eaten as vegetables and spices. Generally, the whole plant is used in traditional stores to protect cowpea against damage by *Callosobruchus* species (Lambert et al., 1985). It is locally used as mosquito repellant by burning of the whole plant (Dallies, 1937).

H. spicigera plant is locally known as, “Bunsuru fadama” or “Dai fadama”by Hausas in Northern Nigeria. Part of the plant commonly used traditionally is the leaves. When the plant is crushed and applied to the head, it relieves head colds and headaches (Dalziel, 1937). The Bajju and Tyapp people of Southern Kaduna state, Nigeria, make use of the inflorescence (where the seeds are packed) to cure headaches by sniffing it.

Essential oils are volatile, natural, complex compounds characterized by a strong odour and are secondary metabolites formed by aromatic plants. They are usually obtained by steam or hydro-distillation methods first developed in the Middle Ages by Arabs. The essential oils (volatile oils) from these plants are known for their antisepticis. bactericidal, virucidal and fungicidal, and medicinal properties and their fragrance, which find uses in embalmment, preservation of foods and as antimicrobial, insecticidal, analgesic, sedative, anti-inflammatory,

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spasmolytic and locally anaesthetic remedies. Up till now, these characteristics of volatile oils have not changed much except that more about some of their mechanisms of action are known, particularly at the antimicrobial level. In nature, essential oils play an important role in the protection of the plants as antibacterials, antivirals, antifungals, insecticides and also against herbivores by reducing their appetite for such plants. They also may attract some insects to favour the dispersion of pollens and seeds, or repel undesirable others.

Essential oils have been largely employed for their properties already observed in nature, for example, in their antibacterial, antifungal and insecticidal activities. At present, approximately 3000 essential oils are known, 300 of which are commercially important especially for the pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries. Essential oils or some of their components are used in perfumes and make-up products, sanitary products, dentistry, agriculture, as food preservers and additives, and as natural remedies. For example, d-limonene, geranyl acetate or d-carvone are employed in perfumes, creams, soaps, as flavour additives for food, as fragrances for household cleaning products and as industrial solvents. Moreover, essential oils are used in massages as mixtures with vegetal oil or in baths but most frequently in aromatherapy. Some essential oils appear to exhibit particular medicinal properties that have been claimed to cure one or another organ dysfunction or systemic disorder (Silva et al., 2003; Hajhashemi et al., 2003; Perry et al., 2003).

Essential oils of these aromatic plants and spices have been tested for their potential as protective agents for human and/or livestock feeds. Many researchers have pointed out the use of essential oils from aromatic plants as the best way to control pests without leading to human and animal toxicity (Isman, 2000; Shaaya and Kostyukovysky, 2006). Essential oils are known to exhibit low toxicity to mammals, and the most important constituents are terpenoids and phenols found in plant essential oils which have minimal toxicity and have even been approved as flavouring agents in food (Isman, 2000; Shaaya and Kostyukovysky, 2006).

Trypanosoma brucei brucei (Tbb) causes African Trypanosomosis along with several other species of trypanosomes although Tbb has morphological similarities with *Trypanosoma evansi*, the latter differs from related species by the absence of kinetoblast DNA mini-circle (Hoare, 1972). Currently, 35 million people and 25 million cattle in Africa are at risk of contracting the disease which is fatal if untreated (Nok et al., 1996). Ironically, some registered trypanocides are frequently toxic, require lengthy administration, lack efficacy and are sometimes unaffordable for most of the patients (Hoet et al., 2004). In many African countries, plants have traditionally been used for centuries and are still being widely used to treat this illness and other parasitic diseases which may be due to limited availability and affordability of pharmaceutical products.

This paper reports the composition and biological activity of the volatile oils from the leaves of *H. spicigera* found in Zaria, Kaduna state, Northern, Nigeria against *T. brucei brucei*.

EXPERIMENTALS

Collection of the plant material

The fresh leaves (200 g) of *H. spicigera* were collected in Basawa Village, Zaria, Kaduna State, Northern, Nigeria on the 26th November, 2009. It was taxonomically identified and authenticated by Mallam U. S. Gallah of the Herbarium Section, Department of Biological Science, Ahmadu Bello University, Zaria, and a sample Voucher No. 528 was deposited at the Herbarium Section.

Extraction of the volatile oils

About 500 g of the fresh leaf parts was chopped into pieces and weighed into distillation flask fitted with condensers. Steam was supplied to the flask through a steam generator at constant flow. The essential oil which vaporizes with the steam was condensed into a collecting separatory funnel. The oil was separated by gravity and dried over anhydrous sodium sulphate.

GC-MS analysis

The gas chromatography-mass spectroscopy (GC-MS) of *H. spicigera* essential oil was analysed on a Shimadzu QP-2010 Instrument at 70 eV and 250°C. GC Column: ULBON HR-1 equivalent to OV-1, fused silica capillary –0.25 mm_50 M with film thickness-0.25 µ. The GC-MS was operated under the following conditions - the initial temperature was 60°C for 5 min and then heated at the rate of 5°C per minute to 250°C. Carrier gas (helium) flow was 2 ml per minute. The identification of components was based on comparison of their mass spectra with those present in the National Institute for Standard Technology computer data bank (NIST:2009s. LIB) (Adams, 2001).

Determination of parasitemia

The parasites were maintained in the laboratory by continuous passage in rats until it is required. The passage was done when parasitaemia was in the range of 16 to 32 parasites per field with infected blood containing 1×103 parasites was introduced intraperitoneally into healthy rats in 0.1 to 0.2 ml blood/physiological saline solution. The numbers of parasites were determined microscopically at magnification ×400 and Parasitaemia was monitored daily (Habila et al., 2010).

In-vitro test

The "drug incubation infectivity test" with wet and thick blood films method (Delespaux et al., 2008) to detect any motile trypanosomes was done in triplicates in a 96 well microtitre plate with 10 µl of infected blood was incubated with 5 ml of the volatile oil from 10, 5 and 0.2% (previously prepared) giving a final concentration of 22.8, 11.4 and 5.7 mg/ml, respectively. Parasitaemia was monitored between 1 to 10 min of incubation at 30°C. About 0.5 to1.0 µl of test mixtures were observed every one minute under a microscope ×400. One positive control (PC) was set up and allowed to stand for 2 h with the infected blood containing the parasites.

Table 1. Percentage composition of the hydro distilled essential oils from the leaf parts of *H. spicigera*.

Peak No.	Component	%
1	α-pinene	12.16
2	β-pinene	9.47
3	α-Phellandrene	10.19
4	Eucalyptol(Cineole)	4.11
5	γ –Terpinene	6.33
6	Bicyclo[4.1.0]hept-2-ene	7.60
7	Linalol	0.64
8	Octen-1-ol acetate	0.42
9	Bicyclo[3.2.1]oct-2-ene	0.29
10	Oxirane,2-(hexyn-1-yl)-3-methoxymethylene	0.50
11	3-Cyclohexen-1-ol	0.80
12	P-Cymen-8-ol	0.51
13	P-Menth-1-en-8-ol	0.48
14	7,7-Dimethyl-4-methylenebicyclo[4.1.0]heptan-3-ol	0.43
15	9-Decen-2-one,5-methylene	2.49
16	(4-Methyl-3-cyclohexen-1-yl)methanol	0.57
17	2-Oxabicyclo[2.2.2]octan-6-al	1.70
18	7-Oxabicyclo[4.1.0]heptanes	1.36
19	Bicyclo[7.2.0]undec-4-ene	7.92
20	-Ocimene	2.22
21	Aromadendrene	0.89
22	8-Methylenedispiro[2.0.2.5]	1.29
23	11-Tridecyn-1-ol	3.48
24	Caryophyllene	2.61
25	Bicyclo[3.1.0]hexan-3-ol	0.60
26	Octadecanal	0.24
27	3-Bromo-1-cyclodecene	0.28
28	2,4-Diisopropenyl-1-methyl-vinylcyclohexane	1.52
29	2,5-Dimethyl-3-vinyl-1,4-hexadiene	1.15
30	1-Isopropenyl-3-propenylcyclopentane	0.47
31	13-Hexyloxacyclopentane	0.47
32	Methyl heneicosanoate	0.26
33	7-Oxabicyclo[4.1.0]heptanes	2.41
34	Hexadecanoic acid phosphine	0.87
35	Cyclohexanone ethylene acetal	2.64
36	Dodecanoyl chloride	1.06
37	9-Octadecenoic acid-1,2,3-propanetriylester	2.91
38	N-Dodecanoyl chloride	3.27
39	Spiro[1,3-dioxolane-2,21-[6,7]diazabicyclo[3.2.2]non-6-ene	2.58
40	Pinene	0.05

RESULTS AND DISCUSSION

The hydro distillation of fresh leaves of *H. spicigera* gave a colourless essential oil of 0.65% yield. The value indicated a low content of the oil and is in the same range as those cited in the literature. Other workers have reported the yields of essential oils from other *Hyptis* species such as *Hyptis suaveoleas* (0.15%), *Hyptis pectinata* (0.60%), *H. spicigera* (0.12%) and *Hyptis lanceolata* (0.50%). From their findings, it showed that the volatile oils content of *H. spicigera* from Northern Nigeria is higher than those reported by these workers (Tchoumbougnang et al., 2005; Onayade *et al*., 1990).The chemical composition of essential oils of the *H. spicigera* was elucidated employing GC with MS. The GC-MS of the components are shown in Table 1, there

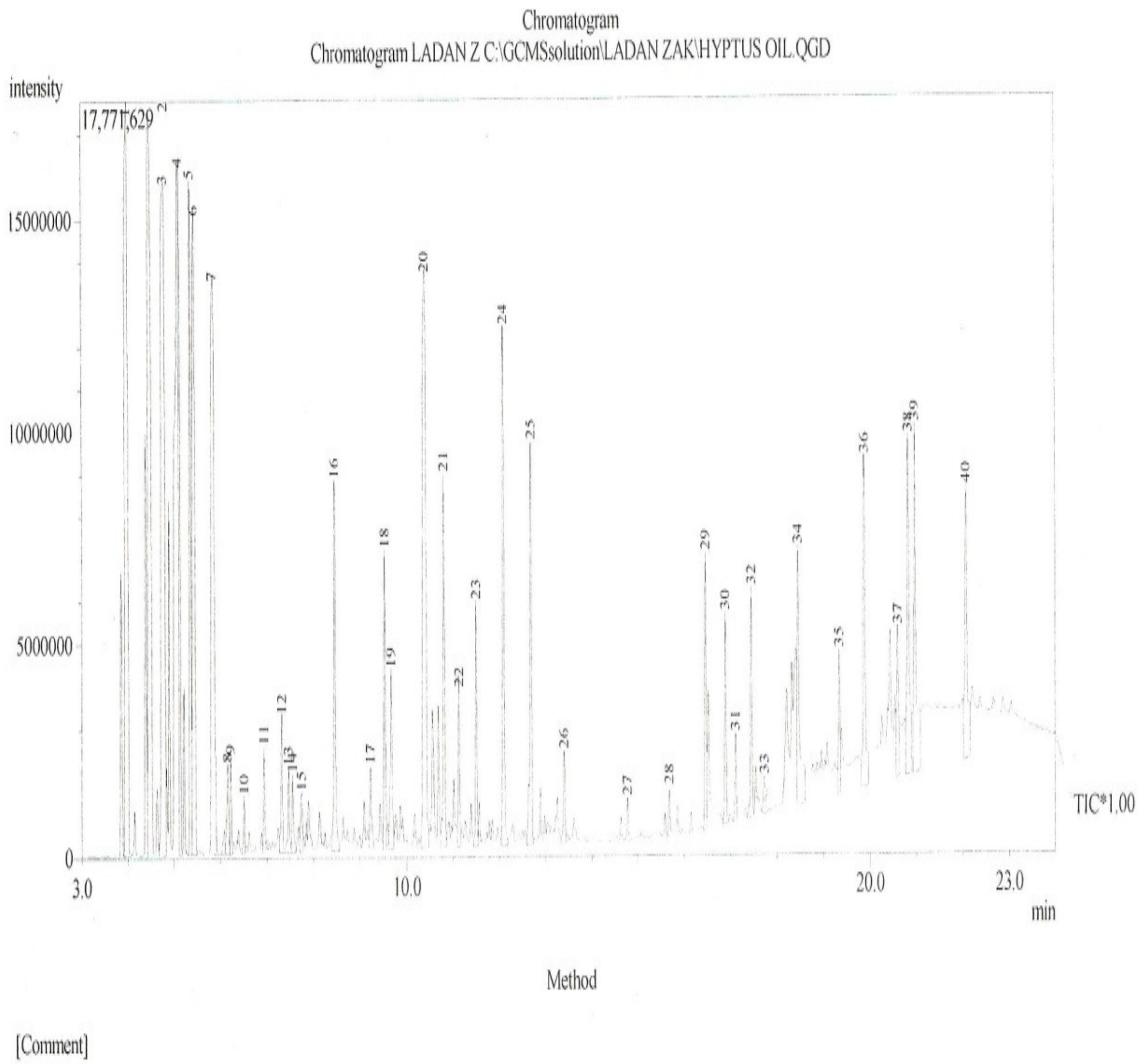

Figure 1. GC-MS of essential oils profile of *H. spicigera*.

were 40 compounds identified in *H. spicigera* volatile oil according to their order of elution on Ov-101 column.

The essential oil yields obtained (0.65%) from the leaf of *H. spicigera* had three main components (Figure 1): α-pinene (12.16%), α-phellandrene (10.19%) and β-pinene (9.47%), other compounds found in this essential oil were γ-Terpineol (6.33%), Bicyclo [4.1.0] hept-2-ene (7.60%), Bicyclo [7.2.0] undec-4-ene (7.92%). Other workers (Ngassoum *et al.*, 2007) have shown the essential oils of the flower of *H. spicigera* to contain two main components, namely: 1, 8-Cineole (24.0%) and (E) – caryophyllene (22.2%). Other compounds found in the essential oils were α-pinene (9.1%), β-pinene (5.7%), α-terpineol (8.3%) and linalol (8.4%). The essentials oils obtained from the leaves of *H.spicigera* in this study, showed low composition of Cineole (4.11%) Caryophyllene (2.61%) while α-pinene (12.16%), β-pinene (9.47%) and α-phellandrene (10.19%) were higher in the leaves than in the flowers as reported by Ngassoum et al. (2007). Flowers are highly scented and are attractive parts used by insects for cross-pollination. It is therefore expected that some high concentration of the essential oils may be found there than other parts of the plant.

Similarly, it has been reported (Onayade et al., 1990) that the essential oil from aerial parts of *H. spicigera* constituted mainly of sesquiterpene hydrocarbons (74%), β-Caryophyllene (68%) being the major component while α-pinene (5%) occurred in substantial quantity. Essential oils from the leaf of *H .spicigera* in this study has higher amount of α-pinene (12.16%) than in the aerial part (6.8%) as reported by Onayade et al. (1990). The difference in the amount of these secondary metabolites is probably a manifestation of the intrinsic variability of the species due to climatic and soil difference as well as ecotypes. Geographically, variation in essential oil

Table 2. Effect of volatile oil of *H.spicigera* on Trypanosome *(T.brucei brucei).*

Time(min)	Concentration (µg/ml)	No. of parasites cleared	No. of deaths (%)	Control (+ve)
0	0.1	0	0	5
2	0.2	3	60	5
4	0.3	4	80	5
6	0.5	5	100	5
8	0.7	5	100	5
10	0.9	5	100	5
12	1.0	5	100	5

composition indicated that the sesquiterpenes are mainly produced in the plant samples grown at lower latitudes (Onayade et al., 1990). Also, Tchoumbougnag et al. (2005) have shown the essential oil from the leaves of *H. spicigera* from Cameroon to contain four major component α-pinene (28.3%), β -Caryophyllene (19.1%), limonene (13.4%) and β-pinene (10.3%). The composition of these secondary metabolites from Cameroun is higher than the values obtained from *H. spicigera* specie found in Northern Nigeria for α-pinene (12.16%), Caryophyllene (2.61%) while β-pinene (9.47%) content compared favorably from the two countries. Several research teams have shown interest in the volatile compounds of *H. genus*, especially due to the potency of its photochemical (Campos et al., 2002).

Biological activity of the volatile oils of *H. spicigera* on trypanosome (*T. brucei brucei)*

Table 2 showed the *in vitro* activity of the volatile oil of *H. spicigera* against *T. brucei brucei* (Tbb). The potency showed a direct relationship with the concentration of the volatile oils prepared. Beyond 6 min at concentration of 0.5 µg/ml there was no more living trypanosomes, that is, all the trypanosomes were killed within six minutes at a concentration 0.5µg/ml. This showed that the optimum potency was achieved at a concentration of 0.5 µg/ml. The drop in number of the parasites over time was achieved at a dose of 1.0, 0.9, 0.7, 0.5, 0.3 0.2 and 0.1 µg/ml respectively. The concentration of 0.5 µg/ml was found to be more potent at 6 min in clearing all the parasites (*T. brucei brucei, Tbb*) that causes African Trypanosomosis along with several other species of trypanosomes. Recently (Habila *et al*., 2010) we reported that the essential oils from *Cymbopogon citratus Eucalyptus citriodora* and *Eucalyptus camalduelensis* were able to decrease cell number of *Tbb* and *T. evansi* in a dose dependent pattern, although *Tbb* has morphological similarities with *T. evansi*, the latter differs from related species by the absence of kinetoblast DNA mini-circle (Nok et al., 1996). Currently, 35 million people and 25 million cattle in Africa are at risk of contracting the disease which is fatal if untreated. Ironically, some registered trypanocides are frequently toxic, require lengthy administration, lack efficacy and are sometimes unaffordable for most of the patients (Hoet et al., 2004). From this study, the volatile oils of *H. spicigera* a potential source of trypanocides which can be exploited.

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REFERENCES

Adams RP (2001). Identification of essential oil components by Gas Chromatography/Quadruple Mass spectroscopy. Allured Publishing Corporation, Carol Stream, IL., USA, pp. 1-698.

Burkill HM (1995).The useful plants of West Tropical Africa,Second Ed. Royal Bot. Gardens Kew., 4: 88-144.

Campos IFP, Ferneira HD, Partes TA (2002). Proceedings of the first Latino-American Symposium on the Production of Medicinal, Aromatic and Condiment Plants. Acta Hortic., 569: 195-201.

Dalziel JM (1937). Useful plants of West Tropical Africa. Crown Agent for Overseas Governments, London, pp. 462-463.

Delespaux V, Geysen D, Van den Bossche P, Geerts S (2008). “Molecular tools for the rapid detection of drug resistance in animal trypanosomes.” Trends Parasitol., 24(5): 236–242.

Habila N, Agbaji AS, Ladan Z, Bello IA, Haruna E, Dakare MA, Atolagbe TO (2010). Evaluation of In Vitro Activity of Essential Oils against *Trypanosoma brucei brucei* and *Trypanosoma evansi*. J. Parasitol. Res., pp. 1-5.

Hajhashemi V, Ghannadi A, Sharif B (2003). Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of *Lavandula angustifolia* Mill. J. Ethnopharmacol., 89: 67–71.

Hoare CA (1972). “Classification and the Salivaria (Subgenus Trypanozoon). ”In The Trypanosomes of Mammals: A Zoological Monograph, Blackwell Scientific, Oxford, UK, pp. 60–79, 476–604.

Hoet S, Opperdoes F, Brun R, Adjakide V, Quentin–Leclercq J (2003). In vitro antitrypanosomal activity of orthnopharmacologically selected Beninese plants. J. Ethnopharmacol., 91(2004): 37–42.

Isman MB (2000). Plant essential oils for pest and disease management. Crop Protect., 19: 603-608.

Lambert J, Arnason JT, Philoge-Ane BJR (1985). Bruchid control with traditionally used insecticidal plants *Hyptis spicigera* and *Cassia nigricans*. Insect Sci. Appl., 6: 167-170.

Ngassoum MB, Tinkeu NSL, Ngatanko I, Tapondjou AL, Lognay G, Malaisse F, Hance T (2007). Chemical composition, Insecticidal effect and repellent activity of essential oils of three aromatic plants, alone and in combination towards *Sitophilus oryzae* L. *(Coleopteran; Curculionidae).* Nat. Prod. Comm., 2(12): 1229-1232.

Nok AJ, Williams IS, Onyenekwe PC (1996). “*Allium sativum*—induced death of African trypanosomes.” J. Parasitol. Res., 82: 634-637.

Onayade OA, Looma A, Scheffer JJ, Svendsen AB (1990). Composition of the leaf essential oil *Hyptis Spicigera* Lam. Flavor Fragrance J., 5: 101-105.

Perry NS, Bollen C, Perry EK, Ballard C (2003). Salvia for dementia therapy: Review of pharmacological activity and pilot tolerability clinical trial. Pharmacol. Biochem. Behav., 75: 651–659.

Shaaya E, Kostyukovysky M (2006). Essential oils: Potency against stored product insects and mode of action. Stewart Post Harv. Rev., 4:5

Silva J, Abebe W, Sousa SM, Duarte VG, Machado MIL, Matos FJA (2003). Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. J. Ethnopharmacol., 89: 277–283.

Tchoumbougnag F, Zollo PHA, Boyom FF, Ngegae MA, Bessiere JM , Menut C (2005). Aromatic plants of tropical Central Africa. XLVIII. Comparative study of the essential oils of four *Hyptis* species from Cameroun: *H. lancedata* Poit, *H. pectinata* (L) Poit, *Hyptis spicigera* Lam and *Hyptis suaveoleus* Poit. Flavour Fragrance J., 20: 340-343.