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Chemical Composition and Antimicrobial Activity of a New Chemotype of *Hyptis suaveolens* (Poit) from Nigeria

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Abstract: *Hyptis suaveolens* is one of the aromatic plants credited for substantial medicinal values in the tropics with three chemotypes previously reported in Nigeria. This study provided biological and chemical evidence for a new chemotype of *Hyptis suaveolens* in Lagos. Hydrodistillation of the dried leaves of the plant produced volatile oil with a yield of 0.31% and subsequent analyses by GC-MS identified 28 volatile compounds that accounted for 99.1% of the total oil composition. Although the oil was monoterpenoid dominated and has comparable levels of sabinene (25.8 vs. 13.2-30.1%), a-thujene (1.1 vs. 0.9-1.2%), and 4-terpineol (8.4-9.8 vs. 11.4%), it elicited a moderate level of α-pinene (4.7 vs. 1.8-13.6), higher levels of β-pinene (9.7 vs. 0-4.4%), limonene (2.3 vs. 0-0.8%), 1,8-cineole (4.8 vs. 0-1.2%), γ-terpinene (9.3 vs. 1.6-4.2%) and terpinolene (8.4 vs. 5.6-6.3) and the presence of new compounds: aromadendrene (0.3%), camphor (0.3%), germacrene B (0.4%) and himachalol (0.1%) when compared with the previous chemotypes. *In vitro*, the oil was found by agar diffusion assay to elicit antibacterial activity against *E. coli* ATCC25922, and *S. aureus* ATCC25923 and antifungal activity with *C. albicans* showing higher sensitivity (MFC = 53.3 μL/mL) and *Aspergillus niger* and *Trichophyton rubrum* displaying moderate to low sensitivity. Biological effect of the oil at sub-MIC on *E. coli* ATCC25922 was characterized by dose-dependent loss of outer membrane proteins. These findings provide evidence for a new chemotype of *Hyptis suaveolens* in Nigeria.

Key words: Antimicrobial activity, chemotype, *Hyptis suaveolens*, Nigeria, volatile oil

INTRODUCTION

Hyptis suaveolens is one of the aromatic and odoriferous plants belonging to the *Lamiaceae* family that are highly utilized for medicinal purposes and research in Nigeria and other endowed countries of the world (Iwu, 1993; Asekum and Ekundayo, 2000; Din *et al*., 1988; Azevedo *et al*., 2001; Belhamel *et al*., 2008; Malele *et al*., 2003). In Nigeria, the leaves of the plant are traditionally used as a remedy to many human diseases. They include fever, catarrh cold, stomachache, worm problem, cramps, convulsion, diabetes and skin diseases (Iwu *et al*., 1990; Iwu, 1993; Olivier-Bever, 1986). Several laboratory studies have also been carried out on *Hyptis suaveolens* and the leaf extracts of plant have been found to display anti-malarial, anti-bacterial, larvicidal, insecticidal, nematocidal, antioxidant, anticonvulsant and fungitoxic activity (Pandey *et al*., 1982; Oyedunmade, 1998; Amusan *et al*., 2005; Singh *et al*., 1992; Asekun *et al*., 1999; Chitra *et al*., 2009; Chukwujekwu *et al*., 2005). These pharmacological activities were actually reported for three chemotypes of *Hyptis suaveolens* between 1980 and 2009 (Iwu *et al*., 1990; Asekum and Ekundayo, 2000; Eshinlokun *et al*., 2005). Chemotype description of *Hyptis suaveolens* is in the context of its essential oil composition (i.e. principal component analysis) and chemometric analysis. Although, the three chemotypes described so far are monoterpenoid predominated based on the abundance of monoterpenes such as sabinene, α-pinene, p-cymene, 1, 8-cineole and 4-terpineol, these volatile compounds elicited chemovariations among the three chemotypes (Iwu *et al*., 1990; Asekun *et al*., 1999; Asekum and Ekundayo, 2000; Eshinlokun *et al*., 2005). The abundance of a few sequiterpenoids such as β-caryophyllene and β-bergamotene has also been reported for these chemotypes (Asekun *et al*., 1999; Asekum and Ekundayo, 2000; Eshinlokun *et al*., 2005). In many other countries of the world different chemotypes of *Hytis*

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suaveolens have also been reported. More than 3 chemotypes has been found in Brazil (Oliveira *et al*, 2005; Azevedo *et al*., 2001; Gottlieb *et al*., 1981), Asia (Din *et al*., 1988; Mallavarapu *et al*., 1993) and Australia (Peerzada, 1997). In Africa, Tonzibo *et al.* (2009) also reported 3 chemotypes of *H. suaeveolens* in Cote d'Ivoire, 1 chemotype was reported by Malele *et al. (2003*) in Tanzania, while chemotypes of this plant have also be scientifically evaluated in Mali (Sidibe *et al*., 2001), Cameroun (Ngassoum *et al*., 1999; Zollo Amvam *et al*., 1998), Aruba (Fun and Baerhiem, 1990) and USA (Ahmed *et al*., 1994). Latitude, altitude, soil composition, climate and genetic composition are the factors that have been implicated for chemotype variations in *Hyptis suaveolens* and other species of *Hyptis* as well as other aromatic herbs belonging to the *Lamiaceae* family (Azevedo *et al*., 2001; El-Hadj *et al*., 2010). Consequently, various bioactive compounds were recovered from the essential oil samples of this chemotypes with variations in yield, composition and pharmacological effects. In terms of the latter, Nigerian chemotypes were found to elicit antibacterial activity against *Bacilli sp* and antifungal activity against *Saccharomyces cereviasiae* and *Fusarium moniliforme* (Asekun *et al*., 1999). In Brazil, antifungi effects of *H. suaveolens* against *Aspergilli* were found (Morreira *et al*., 2010), similar to the findings of Malele *et al.* (2003) and Mallavarapu *et al.* (1993) for Tanzanian and Indian chemotypes. In recent times, essential oil research has paved way for the discovery of new chemotypes of *H. suaveolens* with unique phytoconstituents and bioactivity in countries where they have not been previously reported (Peerzada, 1997; Tonzibo *et al*., 2009) and in area where their abundance and medicinal values have been extensively reported (Santos *et al*., 2007). We recently, found a strain of *Hyptis suaveolens* growing obnoxiously as a weed along a bush path in Ibeshe, Lagos. The area is a coastal rural area, on the Atlantic coast at the extreme of southWestern Nigeria. Ibeshe is watered by the Lagos lagoon and Atlantic Ocean. *Plasmodium falciparium* malaria is endemic in this area (Salako *et al*., 2001; Afolabi *et al*., 2006) and *Hyptis suaveolens* was found via ethnobotanical survey as one the herbs used by the inhabitants to treat fever and malaria. (Iwalokun *et al*., 2009). A pilot study conducted on this plant revealed the ability of its fresh leaves to elicit repellent and knock down activity against Kisumu strain of *Anopheles gambiae* (Ajibaye *et al*., 2009), petroleum leaf extract of the plant also displayed antibacterial activity (Iwalokun *et al*., 2009) with a potency that was higher than that reported for one of the chemotypes by Asekun *et al.* (1999). Polyphenol composition and antioxidant activity of the ethanolic leaf extract of this plant has also been studied (Iwalokun *et al*.,2003).

Taken together the pharmacological activity history of *Hyptis suaveolens* from Ibeshe, we hypothesize a new chemotype of *Hyptis suaveolens* in Nigeria.

This study was carried out to identify and quantitate the phytoconstituents in the volatile oil fraction from the leaves of *Hyptis suaveolens* from Ibeshe. Antibacterial and antifungal activity of this essential oil was also investigated.

MATERIALS AND METHODS

Hyptis suaveolens collection and site: This study was conducted in 2009 in Lagos, Nigeria. Fresh specimen of the *Hyptis suaveolens* plant was collected at 11.00 h of March 18, 2009 along a bush path on Ibeshe island in Lagos. The island is seated on the Atlantic coast in the extreme southwestern corner of Nigeria and watered by the Lagos lagoon and salty Atlantic ocean. This area is endemic for malaria, which burdens over 10,000 inhabitants of the area who use herbal medicines and a number of allopathic medicines to treat the disease (Salako *et al*., 2001; Afolabi *et al*., 2006). The plant was taken to the department of Botany University of Lagos for authentication. Thereafter, a voucher sample of the plant was kept in the herbarium of the University.

Volatile oil extraction: The fresh leaves of the plant rinsed in sterile water to remove dirt from their surfaces, air dried at 40ºC for 3 days and ground into powder using a kitchen blender were hydrodistillated with the aid of a Clevenger apparatus for 6 h to obtain a yellowish volatile oil. The yield of the volatile fraction was calculated and used for antimicrobial assays in the range of 10-100 uL per mL of sterile water as previously adopted by Morreira *et al*. (2010). Prior to the assays, the obtained volatile oil was kept in a sealed glass tube at 4ºC.

Volatile oil phytoconstituent analysis: The volatile oil was analyzed for its phytoconstituents by Gas Chromatograph-Mass Spectrometric system (GC-MS, Shimadzu QP-5000, Kyoto, Japan). The mass spectrometer was operated at 70 ev ((m/z) ratio range of 50-550) at 230ºC (Adams, 1995).

The gas chromatography was equipped with a Flame Ionization Detector (FID) and fitted with a DB-5 fused silica column of dimension 30 m × 0.25 mm × 0.25 um (length x internal diameter x thickness) that was used to conduct injected oil sample at a programmed temperature of 70-250ºC ramped at a rate of 3ºC/min with initial and final temperatures held for 10 min each. One microlitre of the sample (1 uL oil in 1 mL of hexane) was injected at a split ratio of 1:20 using helium as the carrier gas (flow rate 0.9 mL/min). The injector and detector were programmed to function at 230 and 280ºC, respectively. The phytoconstituents eluted from the volatile oil sample

were identified by their retention index values (Schulz *et al*., 2003) and mass spectra comparison with those in the data bank (Mclafferty and Stauffer, 1989). Retention index values were obtained by co-injection of standard hydrocarbon mixture (C_9-C_{24}) as described by Van de Dool and Kratz (1963).

Microorganism and inoculum preparation: Viable bacterial cultures of *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC25923 on nutrient agar slant at 4ºC and fungal cultures of *Candida albicans* ATCC18804, *Aspergillus niger* ATCC 6275 and a local strain of *Trichophyton rubrum* (isolated from a patient with Tinea pedis) on potato dextrose agar slant at 4ºC were used for antimicrobial assays. They were obtained from the Microbiology Division of Nigerian Institute of Medical Research, Yaba-Lagos. An inoculum of each bacterial isolate was prepared by subculturing a loopful colony from the slant into 4 mL of Mueller Hinton broth, followed by incubation at 37ºC for 8 h. The resulting culture was serially diluted with fresh MH broth to achieve a turbidity that was equivalent to 1.0×10^6 cfu/mL. An inoculum from activated culture of *C. albicans* ATCC18804 grown in Sabouraud Dextrose Broth (SDB) at 28ºC for 16 h was also prepared by dilution using fresh SDB. The mould and dermatophyte were cultured on Sabouraud Dextrose Agar (SDA) for 8 days at 28ºC to allow mycella growth. They were then flooded with normal saline (0.9% NaCl) to obtain suspensions that were filtered using double-layered cheese cloth in sequence. The number of spores in each suspension was counted using haematocytometer and was subsequently adjusted by serial dilution with normal saline to 10^6 spores/mL (Morreira *et al*., 2010).

Determination of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC): MIC was determined by macrobroth dilution method using double strength MH broth for the bacterial isolates and Sabouraud broth for the fungal isolates according to Morreira *et al.* (2010). For each assay, 5 mL of MH broth or Sabouraud broth in a tube was inoculated with 1 mL of inoculum, followed by the addition of 4 mL of each of the volatile oil concentrations (20-80 µL/mL). The inoculated broth was mixed and then incubated at 37ºC (bacteria) or 28ºC for 24 h (*Candida albicans* ATCC18804) or 72 h (*Aspergillus niger*) without shaking. MIC was defined as the lowest concentration of the volatile required to completely inhibit visible bacterial and fungal growth after the incubation period.

An aliquot (1 mL) of the tubes displaying no visible growth of bacterial or fungal indicator strains tested was used to swab uniformly MH agar plates (bacteria) of SDA plates (fungi). The bacterial inoculated plates were incubated at 37ºC for 24 h, while fungal plates were incubated at 28ºC for 48 h (*Candida albicans* ATCC18804) and 72 h, respectively. MBC or MFC was defined as the lowest concentration of the volatile oil at which no growth occurred on the agar plate. Cultured tubes or plates lacking the volatile oil samples were used as negative control.

Effect of oil on membrane protein: An aliquot (1 mL) of *Escherichia coli* suspension in 5 mL of double strength MH broth was grown with the addition of the volatile oil at 10 and 20 µL/mL, respectively. The inoculated and oil treated tubes were mixed and then incubated at 37ºC for 24 h with shaking at 150 rpm for 12 h. Cultured tubes or plates lacking the volatile oil samples were used as negative control. The cells (control or test) were pelleted by centrifugation at 5000 rpm for 5 min at 0ºC. The bacterial cells were homogenized with 2 volumes of 2% SDS containing 0.5 mM PMSF at 60ºC to recover the cell envelope. Outer membrane protein fractions of the cell envelope were then extracted with 1.2% Triton X-100/5 mM $MgCl_2$ solution as described by Schnaitman (1974). The outer membrane protein fractions were transferred into a new tube and trichloroacetic acid solution was added to a final concentration of 10%. The tube was centrifuged at 10,000 rpm for 10 min after incubation on ice for 20 min. Concentration of protein was determined by using Bradford reagent. An aliquot (20 µL) of outer membrane protein solution at (190-215 µg/mL) was heated with equal volume of Tris-HCl (pH 6.8) containing β-mercaptoethanol (5%), SDS (10%) and glycerol (30%) and bromophenol blue (0.02%) at 95ºC for 5 min. The resulting protein preparation was subjected to SDS-Polyacrylamide gel electrophoresis for protein separation on 12.5% polyacrylamide gel using 5% acrylamide for stacking gel preparation (Schägger and Von Jagow, 1987; Kustos *et al*., 2007).The resolved proteins were stained with Coomassie blue and bands were visualized after photography.

RESULTS

The hydrodistillation of the dried and pulverized leaf of *Hyptis suaveolens* produced 0.31% yield of volatile oil. Further analysis of the oil identified 28 compounds that represented 99.1% of the oil composition (Table 1). The compounds, at concentrations comparable to those from the previously reported chemotypes include monoterpenoids such as α-thujene (1.1 vs. 0.9-1.2%), sabinene (25.8 vs. 13.2-30.1%), Borneol (0.5 vs. 0-0.4%) and 4-terpineol (8.1 vs. 9.8-11.4%) and sequiterpenoids such as β-caryophyllene (5.8 vs. 5.1-5.9%) and β-bergamotene (4.3 vs. 1.6-5.2%) (Table 1). However, higher levels of β-pinene (9.7 vs. 0-4.4%), limonene (2.3 vs. 0-0.8%), 1, 8-cineole (4.8 vs. 0-1.2%), γ-terpinene

Table 1: Compositional analysis of volatile oil from the leaves of a new *Hyptis suaveolens* (poit) chemotype and clomparison with previous chemotypes

Peak no.	Secondary metabolites	RI	% composition	% composition[@] LASU	OAU
1	α-Thujene	932	1.1	1.2	0.9
2	α-Pinene	940	4.7	13.6	1.8
3	3- Octenol	952	1.2	1.6	0.4
4	Sabinene	973	25.8	13.2	30.1
5	β-Pinene	980	9.7	0	4.4
6	Myrcene	990	0.3	0.5	1.8
7	α-Phallandrene	1003	0.7	0.5	0
8	p-Cymene	1021	1.4	11.7	1.1
9	Limolene	1027	2.3	0.8	0
10	1,8-Cineole	1030	4.7	0	1.2
11	β-Ocimene	1039	0.8	0	0
12	γ-Terpinene	1062	9.3	4.2	1.6
13	Fenchone	1070	0.2	0.1	0.6
14	Terpinolene	1085	8.4	6.3	5.6
15	Camphor	1140	0.3	0	0
16	Borneol	1155	0.5	0	0.4
17	4-Terpineol	1171	8.1	9.8	11.4
18	α-Longipinene	1357	0.2	0	0
19	β-Caryophyllene	1435	5.8	5.1	5.9
20	β-bergamotene	1438	4.3	5.2	1.6
21	Aromadendrene	1445	0.1	0	0
22	α-Humulene	1460	2.7	3.2	0.4
23	Germacrene - B	1510	0.4	-	-
24	Spathulenol1	572	0.4	0.2	0.1
25	Caryophyllene oxide	1585	4.9	4.5	0.5
26	Globulol	1590	0.1	0.2	0
27	Humulene epoxide	1610	0.5	0.4	0
28	Himachalol	1645	0.1	0	0
		Total	99.1	82.3	69.8

RI: Retention index value; [@]: Compositional analysis of previous chemotypes (Eshinlokun *et al*., 2005).

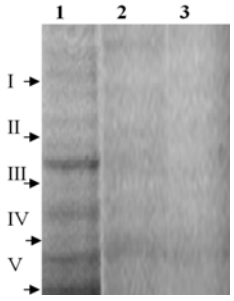

Fig. 1: Effect of *Hyptis suaveolens* volatile oil on outer membrane proteins of *escherichia coli* ATCC25922 Lane 1: Untreated *E. coli* (control); *E. coli* + 10 µL/mL oil); Lane 3: (*E. coli* + 20 µL/mL oil); Arrows indicate protein bands not distinct or seen in the oil treated *E coli* isolates; I: 70 kDa; II: 52 kDa; III: 45 kDa; 30 kDa; 13 kDa; [@]Molecular sizes of bands determined by electrophoretic mobility of standard proteins markers (results not shown)

(9.3 vs. 1.6-4.2%) and terpinolene (8.4 vs. 5.6-6.3) were found in the present oil sample compared to the previous ones. New compounds such as limonene α-longipinene (0.2%), aromadendrene (0.3%), camphor (0.3%), germacrene B (0.4%) and himachalol (0.1%) were also recovered from the oil samples, suggesting a new *Hyptis suaveolens* chemotype (Table 1). Data on the antimicrobial activity of the volatile oil are summarized in Table 2. The oil sample elicited antibacterial activity against *E. coli* ATCC25922 and *S. aureus* ATCC25923 with MIC (23.3 vs. 26.7+(5.8) µL/mL, p>0.05) and MBC

Table 2: Antimicrobial activity of the volatile oil

Antimicrobial parameter[@]	Microorganism: *E. coli* ATCC2592	*S. aureus* ATCC25923	*C. albicans* ATCC18804	*A. niger* ATCC6275	*Trichophyton rubrum*
MIC, uL/mL	23.3+5.8^{a}	26.7+5.8^{a}	33. 3+5.8^{b}	66.7+5.8^{c6}	56.7+5.8^{d}
MBC/MFC, uL/mL	36.7+11.6^{a}	46.7+15.3^{b}	53.3+11.5^{c}	83.3+5.8^{d}	66.7+5.8^{a}
p-value	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

Data are mean+standard deviation (SD) of three determinations. Figures per row with different superscripts are significantly different (p<0.05) (Duncan Multiple range test).[@]p<0.05 (MIC vs. MBC or MFC) Student's t-test, p<0.05 was considered to be significant

with MIC (23.3 vs. 26.7+(5.8) µL/mL, p>0.05) and MBC (36.7+11.6 vs. 46.7+15.3 µL/mL, p<0.05) that differed significantly (p<0.05). *E. coli* ATCC25922 and *A. niger* ATCC 6275 were the most and the least sensitive microorganisms tested. Bacteriostatic effect of the oil on *E. coli* ATCC25922 sub-MIC concentrations was characterized by dose-dependent loss of outer membrane proteins in the treated cultures compared to the untreated one (Fig. 1).

DISCUSSION

Essential oil producing aromatic herbs including *Hyptis* genus have increasingly become a focus of intensive research because of their multiple medicinal values, food and cosmetic values (Peerzada, 1997; Asekun *et al*., 1999; Chukwujekwu *et al*., 2005; Santos *et al*., 2007). In this study, we produced 0.35% yield of volatile oil from the dried leaves of a strain *Hyptis suaveolens* collected as an obnoxious weed along a bush path in Ibeshe, a coastal malaria endemic rural area of Lagos State in Nigeria. The oil yield obtained from this plant is higher than 0.26-0.29% yields found in previously reported chemotypes (Asekun *et al*., 1999; Asekum and Ekundayo, 2000; Eshinlokun *et al*., 2005). In *Hyptis suaveolens* and other *Hyptis* sp. from other tropical countries of the world oil yields comparable of higher than ours have been reported. This disparity in oil yield

could be due to the GC/MS system used, moisture level of the leaves and the chemotypic profile of *Hyptis suaveolens* strains analyzed. In this study, monoterpenoids and sequiterpinoids not reported for previous chemotypes in Nigeria were found. They include germacrene B, camphor, aromadendrene and himachalol. In addition, higher levels of previously reported monoterpenoids such as β-pinene, limonene, 1, 8 cineole, γ-terpinene and terpinolene were found, suggesting the description of a new chemotype of *Hyptis suaveolens* in the country. Chemotypic variation is one of the key phenotypic features among plants of the same genus with the *Lamiaceae* family and several of these features have reported for *Hyptis suaveolens* strains in Brazil, India, Australia and south-East Asia (Azevedo *et al*., 2001; Mallavarapu *et al*., 1993; Peerzada, 1997; Le Van Hac *et al*., 1996). Some of the monoterpenoids in the studied chemotype have also been found in chemotypes from other countries. They include garmacrene B and aromadendrene (Peerzada, 1997; Tonzibo *et al*., 2009), suggesting clonal similarity propagation among the chemotypes. The possibility of hybridization of genomic materials mediated by pollination cannot be excluded since this has been demonstrated *in vitro* (Martins and Polo, 2009).

Furthermore, we opine that the expression of newer monoterpenoids and higher levels of monoterpenoids such as γ-terpenene and terpinolene would enhance the pharmacological potency of the new chemotype compared to the previously reported ones. This is so because monoterpenoids such as γ-terpenene, 4-terpineol and terpinolene are the major antioxidant components in the volatile oil. They have been found to elicit better DPPH radical scavenging activity and ferric reducing potency than α-pinene found in higher levels in the previous chemotypes. Camphor and limonene are anti-pathogen membrane disruptors (Cowan, 1999; Warmington and Wyllie, 2000) and their presence in this chemotype is also expected to improve its antimicrobial activity by potency and spectrum. In this study, we found the new chemotype eliciting antibacterial activity against *Escherichia coli* ATCC25922 and *Staphyloccus aureus* ATCC25923 at significantly disparate MICs and MBCs, suggesting that it has bacteriostatic and bactericidal ability against these isolates. The lower sensitivity of the moulds compared to *C albicans* ATCC18804 may be due to vegetative conidial structure arising from sporulation and spore germination, which are not exhibited by our *Candida albican* isolate.

However, pattern of antifungal potency against the fungal isolates including *Trichophyton rubrum* and which depicts fungistatic and fungicidal ability is similar to that against the bacterial isolates except that it occurred at higher MICs and MFCs. The later implies that the present chemotype elicits better antibacterial activity than antifungal activity. Our findings agree with previously reported anti-infective profiles of essential oils from many aromatic herbs and spices including *Hyptis* sp. from other countries of the world. Examples of such chemotypes are *Myristica fragrans* (Singh *et al*., 2006), *Artemisia echegarayi* Hieron. (*Asteraceae*) (Laciar *et al*., 2009), *Pinus sp*. (Krauze-Baranowskaa *et al*., 2002) and *Hyptis suaveolens* from Australia and Brazil (Peerzada, 1997; Azevedo *et al*., 2001). In a work by Morreira *et al*. (2010), the oil of *Hyptis suaveolens* was found to elicit fungicidal activity against *A. flavus, A parasiticus, A. fumigatus, A. ochraceus and A.niger* at MIC and MFC of 40 and 80 uL/mL respectively. Malele *et al.* (2003) also reported antifungal activity at 500 and 1000 ug/mL against *Sacharomyces cerevisiae, Mucor sp*. and *Fusarium moniliforme* for chemotype from Tanzania. In one of the previously reported chemotypes in Nigeria, growth inhibitory activity against *Candida albicans, Pseudomonas aeroginosa, Bacillus cereus* and *Escherichia coli* at a relatively higher concentration of 5000 ug/mL, ascribing higher potency to the currently reporting chemotype. We also found the present chemotype to elicit anti-dematophyte activity against *Trichophyton rubrum* not reported for previous chemotypes (Iwu *et al*., 1990; Asekum and Ekundayo, 2000; Eshinlokun *et al*., 2005). Meanwhile, terpenoids have been reported as among the phytoconstituents in plants that can solubilize membrane proteins of cells, a mechanism of action plays a major in anti-cancer and anti-infective properties of such plants (Cowan, 1999; Trombeta *et al*., 2005). Thus, the observed loss of out membrane proteins in this study may be due to the monoterpenoids and sesquiterpenoids constituents in *Hyptis suaveolens*. In addition, being a new chemotype containing secondary metabolites not found in the previously reported chemotypes in Nigeria

Antibacterial activity against *E. coli and S. aureus* and antifungal activity of *Schinus molle* from Algeria has been attributed to its phallandrene and limonene (Belhamel *et al*., 2008).

Monoterpenes hydrocarbons such as a and b-pinene, caryophyllene and limonene and oxygenated monoterpenes such borneol and borneol acetate present in oils from Pinus plants such as *P. ponderosa, P. resinosa, and P. strobes* have been validated scientifically to be the key antibacterial agents in these plants (Trombeta *et al*., 2005). Monoterpenoids are lipophillic in nature with tendency to partition from an aqueous phase to form vesiculate structure. This structure may cause membrane expansion, alter membrane fluidity, disrupt membrane lipid-protein interaction and induce cell deaths. These sequence of events have been demonstrated by Trombeta *et al.* (2005) and in the work of Sikkema *et al*. (1994). It also explains the mechanism of antibacterial action

elicited by *Maleleuca alternifolia* (Warmington and Wyllie, 2000). A recent microarray study by Parveen *et al.* (2004) reported alterations in the expression of 793 genes including those involved in ergosterol biosynthesis, sterol uptake, cell wall structure and function and detoxification in *Saccharomyces cerevisiae* after 2 h of 0.02% α-terpenene exposure.

Several studies have implicated and attributed antimicrobial activity of essential oils to their ability to solubilize membrane proteins and disrupt phospholipid bilayer, employ their lipophillic property to permeate the pathogens they are exposed to and cause functional alterations of their intracellular proteins (Schnaitman, 1974; Regnier and Thang, 2004; Cowan, 1999; Trombeta *et al*., 2005). To investigate these anti-pathogenic mechanisms in our new chemotype, we exposed *E. coli* ATCC25922 to sub-MIC concentrations: 10 and 20 uL/mL of the volatile oil. The method used for recovering the cell envelop fraction was actually a modification of the protocol described by Lugtenberb *et al.* (1977). PMSF, being a protease inhibitor was included in the cocktail to complement inhibitory effect of higher temperature (i.e. 60ºC) to prevent degradation of Outer Membrane Proteins (OMPs) by cytoplasmic proteolytic enzymes in *E. coli* since this is a possibility (Schnaitman, 1974). OMPs of *E. coli* have been found to elicit a number of biochemical and immunological activities. They function as receptors, channels, porins for nutrient transport and drug secretion, virulence factors and immunological antigens (Trombeta *et al*., 2005; Warmington and Wyllie, 2000; Sikkema *et al*., 1994; Lugtenberb *et al*., 1977). A total of 35 different proteins having molecular weight ranging from 80-10 kDa with majority of the proteins having 30-45 kDa sizes have been reported to constitute the outer membrane proteome of *Escherichia coli*. Although lower number of OMPs were resolved in the study, dose-dependent loss of some of these proteins in *E .coli* cells exposed to sub-MIC concentrations of volatile oil were observed, suggesting that the oil is membranolytic in action, mediating OMP loss from the cell envelop of the cell. OMPs represent an important component of bacterial cell envelop and their alterations or loss could render them susceptible to antibiotics and compromise their pathogenicity (Trombeta *et al*., 2005). In our previous work, we found essential oil of *Ocimum gratissimum, another Lamiaceae to elicit* loss of haemagglutinin expression and reduction in O-LPS mannose, hence virulence among clinical *Shigella* isolates from Nigeria (Iwalokun *et al*., 2003).

Taken together, our findings and previous findings provide an indication that *Hyptis suaveolens* chemotype reported in this study is new and elicits a broad spectrum antibacterial (gram positive and gram -ve) and antifungal agent (yeast, mould and dematophyte) activity with mechanisms that involve outer membrane protein loss in *E. coli.* Therefore, this chemotype has tremendous potential for use in drug discovery, pharmaceutical, food and cosmetics industries in Nigeria.

AUTHORS CONTRIBUTION

IBA designed the study and prepare the initial draft of the manuscript. OA and O were involved in the antimicrobial assays and their interpretations. OA was involved in manuscript modification and analysis.

Competing interests: The authors hereby declare that there were no competing interests regarding the design, implementation and publication of this research work.

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