



## A new chemical form of essential oil of *Hyssopus officinalis* L. (Lamiaceae) from Nigeria

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### ABSTRACT

Essential oil obtained by hydrodistillation from the air-dried leaves of *Hyssopus officinalis* L. (Lamiaceae) collected in Ajangbadi area, West of Lagos, Nigeria, was analyzed comprehensively for its constituents by means of gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The monoterpene hydrocarbons,  $\alpha$ -pinene (70.9%) and  $\beta$ -pinene (10.9%) are the dominant constituents of the oil of *H. officinalis*. A cluster analysis was performed for comparison and characterization of *H. officinalis* essential oil from Nigeria with other oils reported in the literature from different locations across the world, and reveals chemical variation in this species with at least 8 different chemotypes. The compositional pattern of Nigerian oil sample was being reported for the first time and represents another chemotype of the oil of *H. officinalis*.

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**Keywords:** GC-MS;  $\alpha$ -Pinene;  $\beta$ -Pinene; Chemotype

### INTRODUCTION

Herb hyssop (*Hyssopus officinalis* L., family Lamiaceae) is a brightly coloured herbaceous plant of the genus *Hyssopus* native to Southern Europe, the Middle East, and the region surrounding the Caspian Sea. The plant is commonly used by beekeepers to produce rich and aromatic honey (Busari, 2006). Herb hyssop leaves are used as an aromatic condiment. The leaves have a lightly bitter taste due to its tannins, and an intense minty aroma. Due to its intensity, it is used

moderately in cooking. The herb is also used to flavor liqueur. As a medicinal herb, hyssop has soothing, expectorant, and cough suppressant properties (Wyk and Wink, 2004). The  $\alpha$ -glucosidase inhibitory activity of this plant has been attributed to the presence of (7S,8S)-syringoylglycerol-9-O-(6'-O-cinnamoyl)- $\beta$ -D-glucopyranoside and (7S,8S)-syringoylglycerol 9-O- $\beta$ -D-glucopyranoside (Matsuura et al., 2004).

The present work provides information on the chemical constituents of *Hyssopus*

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*officinalis* grown in Nigeria, and furthermore to compare and contrast the Nigerian *H. officinalis* sample with chemotypes previously reported from other parts of the world. This is part of our extensive research aimed at the characterization of the chemical constituents and biological activities of Nigerian medicinal plants and herbs as they are made available (Ogunbinu et al., 2010; Ogunwande et al., 2010).

## MATERIALS AND METHODS

### Plant materials

The leaves of *H. officinalis* were obtained from Adaluko area of Ajangbadi, Afromedia, Lagos, Nigeria, in March 2009. The plant sample was identified by curators at the Herbarium of the Department of Botany and Microbiology, University of Ibadan and the Herbarium Headquarters, Forestry Research Institute of Nigeria (FRIN), Ibadan, where voucher specimens have been deposited.

### Isolation of the volatile oils

The air-dried plant sample was chopped and hydrodistilled for 4 h using a modified Clevenger-type apparatus. 700 g of the dried sample of the plant material were used for the hydrodistillation. The distilled oils were collected over water and stored in well capped bottles prior to analysis.

### Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis

The gas chromatography (GC) and the gas chromatography-mass spectrometry analysis follow the patterns previously described (Ogunbinu et al., 2010; Ogunwande et al., 2010).

### Identification of the constituents of the oil

Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear indices relative to the series of *n*-hydrocarbons, and on computer matching against commercially available spectral (Adams, 2005). Further identifications were also made possible by the use of self constructed spectral library built up from pure substances and components of known oils and MS literature data (Masada, 1975; Jennings and Shibamoto, 1980; Davies, 1990). Moreover, the molecular weights of all the identified substances were confirmed by gas chromatography-chemical ionisation mass spectrometry, using methanol as CI ionizing gas.

### Numerical cluster analysis

A cluster analysis was performed to determine the chemical relationships between the studied *H. officinalis* oil from Nigeria and the oils of this species reported in the literature from several other locations around the world. The 50 *H. officinalis* samples were treated as operational taxonomic units (OTUs). The percentage composition of eleven main essential oil components (pinocamphone, isopinocamphone, pinocarvone, germacrene D, limonene, methyl eugenol, 1,8-cineole, linalool,  $\alpha$ -pinene,  $\beta$ -pinene and bicyclogermacrene) were used to determine the chemical relationships between the different *H. officinalis* leaf oil samples by cluster analysis using the NTSYSpc software, version 2.2 (Rohlf, 2005). Correlation was selected as a measure of similarity, and the unweighed pair-group method with arithmetic average (UPGMA) was used for cluster definition.

## RESULTS

The hydrodistillation procedure afforded pale yellow oil in a yield of 0.24% (v/w). The percentage composition of the oil of *H. officinalis* is given in Table 1. Constituents were listed in order of elution from HP-5 capillary column. Sixty-three compounds were identified, accounting for 99.3% of total oil content. Monoterpenes (89.4%) constituted the bulk of the oil, highly represented by  $\alpha$ - and  $\beta$ -pinene (70.9% and 10.9% respectively). Apart from limonene (2.7%) and  $\alpha$ -campholenal (1.1%), all the

other monoterpene compounds occurred in amount less than 1%. Sesquiterpene compounds were less common in the oil.  $\beta$ -Caryophyllene (2.7%), caryophyllene oxide (2.1%), viridiflorene (1.1%),  $\alpha$ -guaiene (1.0%) and guaiol (1.0%) could be identified above 1%. It should be noted that compounds such as pinocarvone, pinocamphone and isopinocamphone that are widely reported as dominant constituents of the oils from other parts of the world, occurred in the Nigerian sample in less significant quantities.

**Table 1:** Essential oil composition of *Hyssopus officinalis*.

Constituents	LRI <sup>a</sup>	Percentage (%)
isopentyl isovalerate	876	tr
$\alpha$ -thujene	931	0.6
$\alpha$ -pinene	939	70.9
camphene	953	0.3
thuja-2,4(10)-diene *	954	0.2
benzaldehyde	961	tr
$\beta$ -pinene	980	10.9
myrcene	991	0.3
$\alpha$ -phellandrene	1008	tr
$\alpha$ -terpinene	1021	tr
<i>p</i> -cymene	1029	0.3
limonene	1034	2.7
1, 8-cineole	1037	0.2
( <i>Z</i> )- $\beta$ -ocimene	1043	tr
( <i>E</i> )- $\beta$ -ocimene	1054	tr
$\alpha$ -terpinene	1064	0.1
terpinolene	1091	0.3
linalool	1103	0.2
nonanal	1106	tr
$\beta$ -thujone	1114	tr
<i>exo</i> -fenchol	1117	tr
$\alpha$ -campholenal	1131	1.1
<i>trans</i> -pinocarveol	1144	tr
<i>cis</i> -verbenol	1150	tr

<i>trans</i> -pinocamphone	1160	tr
pinocarvone	1167	0.2
borneol	1170	tr
<i>cis</i> -pinocamphone	1178	tr
4-terpineol	1181	tr
<i>p</i> -cymen-8-ol	1188	tr
$\alpha$ -terpineol	1193	0.4
myrtenal	1196	0.2
verbenone	1215	tr
<i>trans</i> -carveol	1223	0.2
carvone	1249	tr
isobornyl acetate	1290	tr
<i>trans</i> -pinocarvyl acetate	1302	tr
$\alpha$ -terpinyl acetate	1355	0.3
neryl acetate	1370	tr
$\alpha$ -copaene	1378	0.3
geranyl acetate	1388	tr
$\beta$ -elemene	1394	tr
$\alpha$ -gurjunene	1412	tr
$\beta$ -caryophyllene	1421	2.7
$\beta$ -gurjunene	1430	tr
$\alpha$ -guaiene	1442	1.0
$\alpha$ -humulene	1458	0.3
$\gamma$ -muurolene	1478	tr
germacrene D	1483	tr
$\beta$ -selinene	1486	0.2
viridiflorene	1493	1.1
<i>trans</i> - $\gamma$ -cadinene	1515	tr
$\delta$ -cadinene	1525	0.2
spathulenol	1578	0.5
caryophyllene oxide	1583	2.1
guaiol	1595	1.0
humulene epoxide II	1609	tr
<i>epi</i> -10- $\gamma$ -eudesmol	1629	tr
$\beta$ -eudesmol	1651	tr
$\alpha$ -eudesmol	1655	0.2
bulnesol	1667	0.1
hexadecanal	1844	tr
hexahydrofarnesylacetone	1848	0.2
<b>Total</b>		<b>99.3%</b>

<sup>a</sup> Linear retention indices on HP-5 capillary column;

tr, Trace amount < 0.1%;

\* Correct isomer not defined

**Table 2:** Percentage compositions of some *Hyssopus officinalis* oils.

Principal Components	Authors
pinocamphone (1.4-46.0%), isopinocamphone (2.1-31.6%), pinocarvone (2.2-26.5%), germacrene D (9.7-16.7%), $\beta$ -pinene (0.7-10.8%)	Kerrola et al., 1994 <sup>a</sup>
$\beta$ -pinene (4.07-8.85%), pinocamphone (5.56-31.23%), isopinocamphone (39.21-55.17%), germacrene D (t-9.02%)	Jean et al., 1992 <sup>a</sup>
$\beta$ -pinene (11.09-11.24%), $\beta$ -phellandrene (7.88-12.67%), pinocamphone (14.87-29.16%), isopinocamphone (13.20-28.54%)	Venskutonis, 1995 <sup>a</sup>
pinocamphone (18.1%), isopinocamphone (17.4%)	Bourrel et al., 1995 <sup>a</sup>
pinocamphone (50.5%), isopinocamphone (17.9%), 1, 8-cineole (3.2%)	Bodrug et al., 1995 <sup>a</sup>
isopinocamphone (5-> 50%), pinocamphone (3- >50%),	Veres et al., 1997 <sup>a</sup>
isopinocamphone (43.29%), pinocamphone (16.79%), $\beta$ -pinene (16.31%)	Glamočlija et al., 2005
isopinocamphone (46.1%), pinocamphone (15.3%), germacren-D-11-ol (6.1%), elemol (5.2%)	Cvijovic et al., 2010
pinocamphone (14.1%), isopinocamphone (44.7%), germacren-D-4-ol (5.7%) and elemol (5.6%)	Mitic and Dordevic, 2001
$\alpha$ -pinene (7.3%), $\beta$ -pinene (5.3%), $\alpha$ -terpinene (9.4%), pinocamphone (46.7%), isopinocamphone (2.1%)	Sharma et al., 1963 <sup>a</sup>
$\beta$ -pinene (8.8%), pinocamphone (42.66%), isopinocamphone (30.88%)	Lawrence, 1995 <sup>a</sup>
Sample A: isopinocamphone (53.12%), $\alpha$ -terpineol (7.4%), pinocamphone (4.7%) Sample B: isopinocamphone (24.87%), pinocamphone (14.41%), elemol (8.55%), $\beta$ -pinene (7.81%)	Nazari et al., 2008
pinocamphone (1.3-64.9%), isopinocamphone (5.8-59.9%), pinocarvone (0.1-16.9%), $\beta$ -pinene (2.8-13.2%)	Chalchat et al., 2001
pinocamphone (4.4%), isopinocamphone (43.3%), limonene (12.2%) and $\beta$ -pinene (11.1%)	Mazzanti et al., 1998
pinocamphone (49.1%), $\beta$ -pinene (18.4%), isopinocamphone (9.7%)	Garg et al., 1999
1,8-cineole (48.2 and 39.6%), isopinocamphone (16.3 and 28.0%), $\beta$ -pinene (11.4 and 9.4%)	Tsankova et al., 1993 <sup>a</sup>
pinocamphone (34% and 18.5%), isopinocamphone (3.2% and 29%) and $\beta$ -pinene (10.5% and 10.8%), linalool (0.2% and 7.9%) and camphor (0.3% and 5.3%)	Fraternale et al., 2004
pinocarvone (36.3%), pinocamphone (19.6%), $\beta$ -pinene (10.6%), 1,8-cineole (7.2%) and isopinocamphone (5.3%)	Ozer et al., 2005
pinocamphone (1.72%), isopinocamphone (43.26%), limonene	Salvatore et al., 1997 <sup>a</sup>

(12.18%), methyl eugenol (4.01%) pinocamphone, camphor and $\beta$ -pinene *	Schultz and Stahl-Biskup, 1991
$\beta$ -pinene (9.60%), limonene (37.40%), methyl eugenol (38.30%)	Gorunovic et al., 1995 <sup>a</sup>
$\beta$ -pinene (16.82%), 1, 8-cineole (52.89%), myrcenol (3.1%)	Garcia et al., 1995 <sup>a</sup>
1,8-cineole with low amount of pinocamphone, isopinocamphone and pinocarvone *	Lopez et al., 2008
$\beta$ -pinene and limonene (1- >60%)	Veres et al., 1997 <sup>a</sup>
linalool (51.7%), 1,8-cineole (12.3%) and limonene (5.1%)	Mazzanti et al., 1998
linalool (49.00-51.65%), 1,8-cineole (11.92-14.91%), limonene (4.99-6.02%)	Salvatore et al., 1997 <sup>a</sup>
$\alpha$ -pinene (70.9%) and $\beta$ -pinene (10.9%), limonene (2.7%), pinocamphone and isopinocamphone (tr), pinocarvone (0.2%)	<b>Present Study</b>

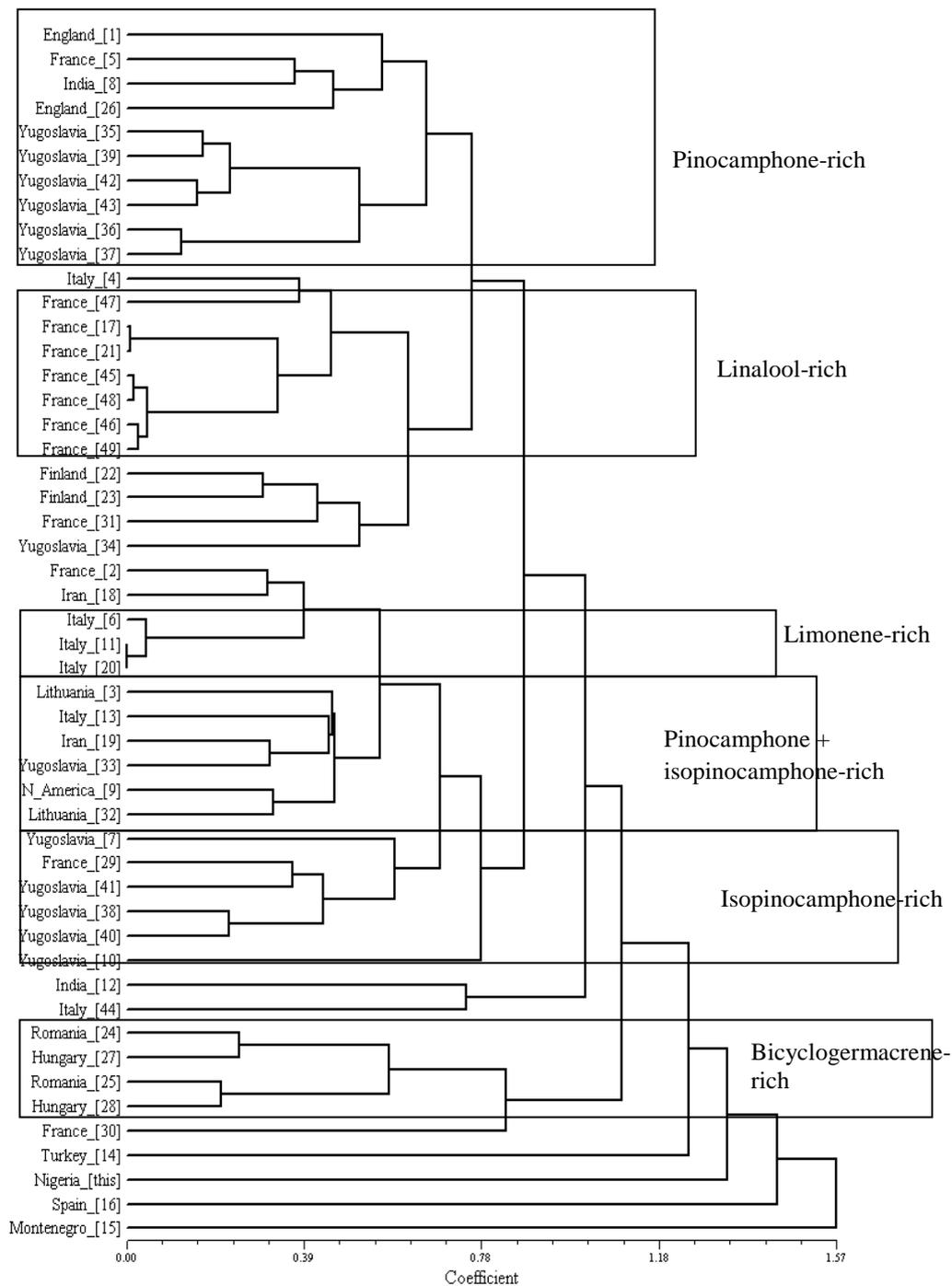
\* Quantitative data not available, <sup>a</sup> Cited in B.M. Lawrence, 2003

**Table 3:** Chemotypes of *Hyssopus officinalis* oils.

Chemotypes	Forms	References
Oils with abundance of pinocarvone	pinocarvone > isopinocamphone > germacrene D > $\beta$ -pinene	Kerrola et al., 1994 <sup>a</sup>
	pinocarvone > germacrene D > isopinocamphone > $\beta$ -pinene	
	pinocarvone > isopinocamphone > germacrene D > pinocamphone	
	pinocarvone > pinocamphone > $\beta$ -pinene > 1, 8-cineole	Ozer et al., 2005
Oils which are rich in pinocamphone	pinocamphone > isopinocamphone > $\beta$ -pinene > $\beta$ -phellandrene	Venskutonis, 1995 <sup>a</sup> , Lawrence, 1995, Chalchat et al., 2001, Bourrel et al., 1995 <sup>a</sup>
	pinocamphone > germacrene D > pinocarvone	Kerrola et al., 1994 <sup>a</sup>
	pinocamphone > germacrene D > $\beta$ -pinene	Kerrola et al., 1994 <sup>a</sup>
	pinocamphone > $\beta$ -pinene > isopinocamphone	Fraternale et al., 2004, Garg et al., 1999
	pinocamphone > $\alpha$ -terpinene > $\alpha$ -pinene > $\beta$ -pinene	Sharma et al., 1963 <sup>a</sup>
	pinocamphone > isopinocamphone > 1, 8-cineole	Bodrug et al., 1995 <sup>a</sup>
	pinocamphone > camphor > $\beta$ -pinene	Schultz and Stahl-Biskup, 1991

	isopinocampnone > pinocampnone > $\beta$ -phellandrene	Venskutonis, 1995 <sup>a</sup>
	isopinocampnone > pinocampnone > germacrene D	Kerrola et al., 1994 <sup>a</sup>
	isopinocampnone > germacrene D > $\beta$ -pinene	Jean et al., 1992 <sup>a</sup>
	isopinocampnone > pinocampnone > $\beta$ -pinene	Jean et al., 1992 <sup>a</sup> , Chalchat et al., 2001, Fraternala et al., 2004 and Glamoaliija et al., 2005
Oils with higher proportions of isopinocampnone	isopinocampnone > pinocarvone > germacrene D	Chalchat et al., 2001
	isopinocampnone > pinocarvone > $\beta$ -pinene	Chalchat et al., 2001
	isopinocampnone > limonene > methyl eugenol	Salvatore et al., 1997 <sup>a</sup>
	isopinocampnone > limonene > $\beta$ -pinene	Mazzanti et al., 1998
	isopinocampnone > $\alpha$ -terpineol > pinocampnone	Nazari et al., 2008
	isopinocampnone > pinocampnone > elemol	Nazari et al., 2008, Cvijovic et al., 2010
	isopinocampnone > pinocampnone > germacren-D-4-ol	Mitic and Dordevic, 2001, Cvijovic et al., 2010
	isopinocampnone > 1, 8-cineole > pinocampnone	Garg et al., 1999 <sup>b</sup>
	isopinocampnone > pinocampnone > pinocarvone	Garg et al., 1999 <sup>b</sup>
Oils containing large amount of linalool	linalool > 1, 8-cineole > limonene	Mazzanti et al., 1998, Salvatore et al., 1997 <sup>a</sup>
1, 8-Cineole rich oils	1, 8-cineole > $\beta$ -pinene > isopinocampnone or myrcenol	Garcia et al., 1995 <sup>a</sup> , Lopez et al., 1997 <sup>a</sup>
	1, 8-cineole > isopinocampnone > pinocampnone	Garg et al., 1999 <sup>b</sup>
Oil rich in methyl eugenol	methyl eugenol > limonene > $\beta$ -pinene	Gorunovic et al., 1995 <sup>a</sup>
$\beta$ -Phellanderene rich oil	$\beta$ -phellandrene > myrcene > germacrene D	Garg et al., 1999 <sup>b</sup>
$\alpha$ -Pinene rich oil	$\alpha$ -pinene > $\beta$ -pinene > limonene	<b>Present Study</b>

<sup>a</sup> Cited in Lawrence, 2003, <sup>b</sup> Cited in Garg et al., 1999



**Fig 1:** Cluster analysis of the oils of *Hyssopus officinalis*.

## DISCUSSION

The abundance of monoterpenoid compounds in the present oil sample is in agreement with previous literature reports. Previous authors (Schulz and Stahl-Biskup, 1991; Mazzanti et al., 1998; Garg et al., 1999; Chalchat et al., 2001; Mitic and Dordevic, 2002; Lawrence, 2003; Fraternali et al., 2004; Glamoalija et al., 2005; Ozer et al., 2005; Lopez et al., 2007; Nazari et al., 2008; Cvijovic et al., 2010) have shown that the oil composition of *H. officinalis* showed large variations in the relative concentration of its major components that can be related to genotype, location and climatic conditions, although the presence of the bicyclic monoterpene ketones, pinocamphone and isopinocamphone remains peculiar (Table 2).  $\alpha$ -Pinene as occurred in the present oil sample has not been described to be the major constituent of the oils of *H. officinalis* and its varieties. It is evident from the literature reports mentioned above and the present study that several chemotypic forms of the oils are discernible (Table 3). In the present oil sample from Nigeria, except for its  $\beta$ -pinene content, the major constituents of the oils of *Hyssopus* species, such as pinocamphone, isopinocamphone, 1, 8-cineole, pinocarvone, linalool and limonene, as reported by earlier researchers were either found in insignificant quantities or completely absent.

The cluster analyses of the principal components are depicted in Fig 1. There are some apparent clusters: pinocamphone-rich (1, 5, 8, 26, 35, 36, 37, 39, 42, 43), linalool-rich (17, 21, 45, 46, 47, 48, 49), limonene-rich (6, 11, 20), pinocamphone + isopinocamphone (3, 9, 13, 19, 32, 33), isopinocamphone-rich (7, 10, 29, 38, 40, 41), and bicyclogermacrene-rich (24, 25, 27, 28).

## Conclusion

It could be seen that the compositional pattern of the present oil sample does not fit into any of the clusters and is unique. This

may represent another chemotype of the essential oil of *H. officinalis*.

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