

## Aromatic Plants growing in Nigeria: Essential Oil Constituents of *Cassia alata* (Linn.) Roxb. and *Helianthus annuus* L.

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**Abstract:** The volatile constituents identified from the leaves of two Nigerian plants are being reported. The oil samples were obtained from the studied plant species by hydrodistillation using a Clevenger apparatus and then subsequently analyzed for their constituents by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS). The quantitatively significant constituents of the leaf oil of *Cassia alata* (Linn.) Roxb., (Fabaceae) were 1, 8-cineole (39.8%),  $\beta$ -caryophyllene (19.1%) and caryophyllene oxide (12.7%). Limonene (5.2%), germacrene D (5.5%) and  $\alpha$ -selinene (5.4%) constituted the other significant compounds present in the oil. The sunflower oil, *Helianthus annuus* L., (Asteraceae) was rich in  $\alpha$ -pinene (16.0%), germacrene D (14.4%), sabinene (9.4%) and 14-hydroxy- $\alpha$ -muurolene (9.0%).

**Keywords:** *Cassia alata*; *Helianthus annuus*; 1,8-cineole;  $\beta$ -caryophyllene; caryophyllene oxide; germacrene D

### 1. Introduction

Essential oils are volatile and liquid aroma compounds from natural sources, usually plants. Essential oils are not oils in a strict sense, but often share with oils a poor solubility in water. Essential oils often have an odor and are therefore used in food flavoring and perfumery. Essential oils are usually prepared by fragrance extraction techniques such as distillation (including steam distillation), cold pressing, or extraction (maceration). Essential oils are distinguished from aroma oils (essential oils and aroma compounds in an oily solvent), infusions in a vegetable oil, absolutes, and concretes. Typically, essential

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oils are highly complex mixtures of often hundreds of individual aroma compounds. Essential oils possess some therapeutic properties such as anesthetic (e.g. peppermint), anti-asthmatic (e.g. *Cupressus lusitanica*), antimicrobial (e.g. *Eucalyptus camaldulensis*), anti-venomous (e.g. *Ocimum gratissimum*), carminative (e.g. *Piper nigrum*, *Carrota dauca*, *Zingiber officinalis*, *Ocimum gratissimum*) and anti-hypertension (e.g. *Hyssopus officinalis*) e.t.c. [1]

*Cassia alata* (L.) Roxb., (syn. *Senna alata* L) is an erect tropical, annual herb with leathery compounded leaves. It belongs to the Fabaceae family. It grows up to about 8m tall and can be found in diverse habitats. This perennial shrub has erect waxy yellow spikes that resemble fat candles before the individual blossoms open. The large leaves are bilateral-symmetrical opposed and fold together at night. The fruit is a pod, while the seeds are small and square [2]. Extracts from the leaves of this species has shown several pharmacological properties such as antimicrobial and antifungal activities [3-7], antiseptic [8], anti-inflammatory and analgesic [9] and anti-hyperglycemic [10]. It has also shown therapeutic [11] and anti-ageing activities [12]. The plant is a source of chrysoeriol, kaempferol, quercetin, 5,7,4'-trihydroflavanone, kaempferol-3-O- $\beta$ -D-glucopyranoside, kaempferol-3-O- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranoside, 17-hydrotetracontane, n-dotriacontanol, n-triacontanol, palmitic acid ceryl ester, stearic acid, palmitic acid [13]. Several other flavonoids [14-16] and anthraquinones [17-19] have been isolated from the plant. There is only a report on the constituents of its volatile oil [20].

The genus *Helianthus* is comprised of 51 species and 19 subspecies with 14 annual and 37 perennial species found spread all over the world. The sunflower, *Helianthus annuus* L., is counted among most important oil crops on the world [21]. It belongs to the Asteraceae family. Sunflower oil has excellent nutritional properties. It is practically free of significant toxic compounds and has a high concentration of linoleic acid. This polyunsaturated fatty acid is an essential fatty acid not synthesized by humans, and is a precursor of  $\gamma$ -linolenic and arachidonic acids [22]. The sunflower is valuable from an economic, as well as from an ornamental point of view. Every part of the plant may be utilised for some economic purpose. Sunflower oil is used for cooking, margarine, salad dressings, lubrication, soaps, and illumination. A semi-drying oil is used with linseed and other drying oils in paints and varnishes. Decorticated press-cake is used as a high protein food for livestock. Kernels are eaten by humans raw, roasted and salted, or made into flour. Poultry and cage birds are fond of raw kernels. Flowers yield a yellow dye. Plants are used for fodder, silage and green-manure crop. Hulls provide filler in livestock feeds and bedding. The plant is known to be a source of bioactive sesquiterpene lactone, Helivypolide G [23], bisnorsesquiterpenes annuionones A-C and the helinorbisabone [24], 24 $\alpha$ -Methyl-5 $\alpha$ -cholest-7-en-3 $\beta$ -ol [25], the germacranolide, Annuithrin [26], the heliangolide, niveusin B and its ethoxy derivative [27].

The present work provides information on the chemical constituents of the volatile oils of the leaves of *Cassia alata* and *Helianthus annuus* growing in Nigeria. 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 [28].

## 2. Materials and Methods

### 2.1. Plant Materials

Fresh leaves of *C. alata* were collected at Abaranje Town, a suburb of Lagos, Nigeria, in March 2009; while those of *H. annuus* were obtained from plants cultivated at the Flower Garden in front of the Federal Government College, Ijanikin, Lagos, Nigeria, in January 2009. The plant samples were identified by Messrs Ugbuga and Shosanya at the Herbarium Headquarters, Forestry Research Institute of Nigeria (FRIN), Ibadan, where voucher specimens (FHI 108824 and FHI 108827) respectively have been deposited.

The plant samples were air-dried for two weeks under laboratory shade prior to extraction of the oil samples.

## 2.2. Isolation of the Volatile Oils

The air-dried plant samples were chopped and hydrodistilled for 4 h using a Clevenger-type apparatus. Between 350 and 400g of the dried samples of each of the plant materials were used for the hydrodistillation. The essential oils were collected separately and stored in well capped bottles prior to analysis. The yields were 0.10% (v/w) and 0.08% (v/w) respectively for *C. alata* and *H. annuus*.

## 2.3. Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS)

GC analysis was accomplished with a HP-5890 Series II instrument equipped with a HP-Wax and HP-5 capillary columns (both 30m x 0.25mm, 0.25  $\mu$ m film thickness), working with the following temperature program: 60 °C for 10 min, rising at 5 °C/ min to 220 °C. The injector and detector temperatures were maintained at 250 °C; carrier gas was nitrogen (2mL/min); detector dual, FID: split ratio was 1:30. The volume injected was 0.5  $\mu$ L (10% hexane solution dilution). The identification of the components was performed by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (LRI) relative to the series of *n*-hydrocarbons. The relative proportions of the oil constituents were percentages obtained by FID peak-area normalization without the use of response factor.

GC-EIMS analysis was performed with a Varian CP-3800 gas-chromatograph equipped with a HP-5 capillary column (30m x 0.25 mm; film thickness 0.25  $\mu$ m) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperature were 220 °C and 240 °C respectively; oven temperature programmed from 60°C to 240 °C at a rate of 3 °C/min.; carrier gas was helium at a flow rate of 1mL/min.; injection volume was 0.2  $\mu$ L (10% hexane solution); split ratio was 1:30. Mass spectra were recorded at 70 eV. The acquisition mass range was 30-300 *m/z* at a scan rate of 1 scan/sec.

GC-CIMS have been measured on the same instrument as GC-EIMS. The experimental conditions are exactly the same as GC-EIMS (in order to obtain the same retention times). The only difference is the use of HPLC-grade methanol introduced in the dedicated reservoir. The CI or EI analysis is controlled by the software of the instrument. It is possible to perform a complete GC-EIMS run, or a complete GC-CIMS run or we also can switch between a mode to the other how many times we desire and at any time during the run. During the GC-CIMS mode the valve of the methanol reservoir is opened by the software and vapours of methanol are introduced into the ion trap. Here molecules of methanol are ionized by the electrons emitted by the heated filament and the new-formed ions are able to protonate the essential oil components. The resulting spectrum contains only the quasimolecular peak  $[M+1]^+$  (sometimes a few other weak lines), so it is possible to know the molecular weight of the unknown.

## 2.4 Identification of the constituents

Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing with their linear indices relative to the series of *n*-hydrocarbons, and on computer matching against commercially available spectral libraries (NIST 98 and Adams) [29]. Further identifications were also made possible by the use of home-made library mass spectra built up from pure substances and components of known oils and MS literature data [30-32]. Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS using MeOH as CI ionizing gas.

## 3. Results and Discussion

Fifteen out of twenty-five constituents of *C. alata* oil were identified in trace amount (i.e. < 0.1%). The oil was dominated by mono- and sesquiterpene compounds (48.7% and 47.9 % respectively). The results are given in Table 1. The oil was rich in 1, 8-cineole (39.8%),  $\beta$ -caryophyllene (19.1%) and caryophyllene oxide (12.7%). There were significant proportions of bicyclogermacrene (5.4%), germacrene D (5.5%) and limonene (5.2%). There are only two reports in the literature on the essential oil of *Cassia*

species. The essential oil obtained by hydrodistillation of leaves of *C. alata* collected in Gabon [20] was found to contain linalool (23.0%), borneol (8.6%) and pentadecanal (9.3%) as the major constituents. The antioxidant activity of the oil was also reported to be low compared to that of butylated hydroxytoluene (BHT). The chemical compositions of the flower and leaf essential oil of *C. fistula* L. growing in Egypt [33] showed that forty-four compounds were identified representing 92.6% and 90.7% of the total oil contents, respectively. The main components of the flower oil were (*E*)-nerolidol (38.0%), 2-hexadecanone (17.0%) and heptacosane (12.8%), while the composition of the leaf oil was characterized by the abundance of phytol (16.1%) together with the hydrocarbons, tetradecane (10.5%) and hexadecane (8.7%).

**Table 1.** Essential oil constituents of *Cassia alata*

Compounds <sup>a</sup>	LRI <sup>b</sup>	%	Compounds <sup>a</sup>	LRI <sup>b</sup>	%
( <i>E</i> )-2-hexenal	854	3.3	germacrene D	1483	5.5
tricyclene	926	t	( <i>E</i> )- $\beta$ -ionone	1487	t
benzaldehyde	961	t	bicyclogermacrene	1497	t
$\alpha$ -phellandrene	1008	3.7	$\alpha$ -selinene	1498	5.4
$\alpha$ -terpinene	1021	t	<i>n</i> -pentadecane	1500	t
<i>p</i> -cymene	1029	t	$\alpha$ -bulnesene	1506	1.0
limonene	1034	5.2	$\delta$ -cadinene	1525	t
1,8-cineole	1037	39.8	caryophyllene oxide	1583	12.7
$\beta$ -elemene	1394	t	<i>n</i> -hexadecane	1600	t
$\beta$ -caryophyllene	1421	19.1	humulene epoxide II	1609	t
( <i>E</i> )-geranyl acetone	1457	t	tetradecanal	1614	t
$\alpha$ -humulene	1458	t	$\alpha$ -cadinol	1656	4.2
( <i>E</i> )- $\beta$ -farnesene	1461	t	<b>Total</b>		<b>99.9%</b>

<sup>a</sup> compounds eluted from HP-5 capillary column; <sup>b</sup> Linear retention indices on HP-5 capillary column; t: Trace amount < 0.1%

It should be noted that the major compounds mentioned in the two previous studies i.e. linalool, borneol, pentadecanal, (*E*)-nerolidol, 2-hexadecanone, heptacosane, tetradecane and phytol were conspicuously absent from the present oil sample. Moreover, the dominant compounds in this study were not reported previously to be part of the constituent of *C. alata*.

$\alpha$ -Pinene (16.0%), germacrene D (14.4%), sabinene (9.4%) and 14-hydroxy- $\alpha$ -muurolene (9.0%) were the dominant constituents of *H. annuus*. Overall, 69 different volatile compounds were identified in the oil (Table 2). Other significant compounds include limonene (3.7%),  $\beta$ -bourbonene (3.6%), hexadecanal (3.1%) and isobornyl acetate (3.0%). From a previous report on the volatile oil of *H. annuus* [34], the main constituents of the seeds of Carlos cultivar were the monoterpene hydrocarbon,  $\alpha$ -pinene (53.6%); followed by the monoterpene alcohol *cis*-verbenol (16.7%) and the sesquiterpene hydrocarbon  $\beta$ -gurjunene (7.2%), while in the seeds of Florom cultivar, the principal constituent were also  $\alpha$ -pinene (43.1%),  $\beta$ -gurjunene (13.0%), *cis*-verbenol (7.2%), and the aldehyde  $\alpha$ -campholenal (4.4%). In another investigation [35], the leaves oil was reported to comprised mainly of  $\alpha$ -pinene (28.2 and 29.2%), sabinene (23.5 and 23.2%), limonene (11.1 and 12.3%), isobornyl acetate (8.0 and 7.8%) and germacrene D (8.2 and 8.8%) respectively for the Florom 350 and Carlos cultivars. In addition, the capitula oils were dominated by  $\alpha$ -pinene (74.5 and 70.7%) and sabinene (11.2 % and 12.1%), respectively for the Florom 350 and Carlos cultivars. It could be seen that the dominant compounds in the two previous reports [34, 35] were also the compounds occurring in higher proportions in our sample, except for its quantitative content of 14-hydroxy- $\alpha$ -muurolene which has not been reported previously to be a significant compound in the oil. These results showed homogeneity among the major constituents of the oil of *H. annuus* between Nigeria and Italian samples.

**Table 2.** The volatile compounds of *Helianthus annuus*

Compounds <sup>a</sup>	LRI <sup>b</sup>	%	Compounds <sup>a</sup>	LRI <sup>b</sup>	%
( <i>E</i> )-2-hexenal	854	0.3	dodecanal	1409	t
tricyclene	926	t	$\beta$ -caryophyllene	1420	2.6
$\alpha$ -thujene	931	0.1	$\beta$ -gurjunene	1432	2.2
$\alpha$ -pinene	939	16.0	aromadendrene	1441	0.4
camphene	953	2.3	geranyl acetone	1455	0.3
benzaldehyde	961	t	$\alpha$ -humulene	1457	1.2
sabinene	976	9.4	<i>cis</i> -muurolo-4(14),5-diene	1462	0.4
$\beta$ -pinene	980	2.2	$\gamma$ -muurolene	1477	t
myrcene	991	0.3	germacrene D	1482	14.4
$\delta$ -terpinene	1020	t	( <i>E</i> )- $\beta$ -ionone	1486	0.8
<i>p</i> -cymene	1028	2.2	valencene	1493	0.2
limonene	1033	3.7	bicyclogermacrene	1495	0.3
benzene	1045	t	<i>trans</i> - $\beta$ -guaiene	1503	t
acetaldehyde					
( <i>E</i> )-ocimene	1052	t	germacrene A	1504	0.2
$\alpha$ -terpinene	1063	0.2	tridecanal	1510	0.1
<i>cis</i> -sabinene	1070	t	cubebol	1515	0.9
hydrate					
terpinolene	1090	t	$\delta$ -cadinene	1525	0.2
<i>trans</i> -sabinene	1099	t	<i>cis</i> -calamenene	1540	0.1
hydrate					
nonanal	1104	0.1	( <i>E</i> )- <i>trans</i> -nerolidol	1566	1.1
$\alpha$ -campholenal	1127	t	( <i>Z</i> )-3-hexenyl benzoate	1571	0.2
<i>trans</i> -pinocarveol	1141	t	germacrene D-4-ol	1575	1.2
<i>trans</i> -verbenol	1144	0.1	spathulenol	1577	0.5
borneol	1167	0.2	caryophyllene oxide	1582	1.4
4-terpineol	1179	0.2	guaiol	1595	0.2
naphthalene	1181	t	<i>epi</i> -10- $\gamma$ -eudesmol	1621	0.2
safranal	1201	t	caryophylla-4(14),8(15)-dien-5- $\alpha$ -ol	1637	0.3
isobornyl acetate	1287	3.0	$\tau$ -cadinol	1642	1.3
<i>trans</i> -pinocarvyl acetate	1299	t	pentadecanal	1719	0.2
undecanal	1307	t	14-hydroxy- $\alpha$ -muurolene	1780	9.0
eugenol	1358	t	cedren-13-ol-acetate *	1789	0.8
$\alpha$ -copaene	1377	0.1	hexadecanal	1844	3.1
$\beta$ -bourbonene	1385	3.6	hexahydrofarnesylacetone	1848	0.5
$\beta$ -cubebene	1391	0.2	tricosane	2300	0.2
$\beta$ -elemene	1393	0.2	pentacosane	2500	0.2
methyl eugenol	1403	t	<b>Total</b>		<b>90.1%</b>

<sup>a</sup> compounds eluted from HP-5 capillary column; <sup>b</sup> Linear retention indices on HP-5 capillary column; tr Trace amount < 0.1%;

\* Tentative identification

In conclusion it should be noted that each plant species has its own compositional pattern and are different from other. This data provides information on the essential oil constituents of the plant samples collected in Nigeria, which is readily unavailable initially.

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