Progress in Essential Oils

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Calamus Oil

Acorus calamus L. (also known as sweet flag) is a perennial, aromatic herb, one form of which is native to marshy regions in northern temperature zones of Europe, where it grows wild along the banks of rivers and ponds, as well as along the edges of swamps. This is the triploid, or European, form of *A. calamus* var. *calamus*. A diploid form, which can be found in Canada and the United States, is *A. calamus* var. *americanus* (Raf.) Wulff, while a tetraploid form, which is native to Central Asia, India and Himalayan countries, is *A. calamus* var. *angustata* Engl. (syn. *A. calamus* var. *angustatus* Bess.).

Ploidy refers to the number of sets of chromosomes found in the biological cells' nucleus. The base plants are diploids, which contain two homologous copies of each of the chromosomes from the male and female. Triploids contain three sets of chromosomes and are generally sterile. Tetraploids contain four sets of chromosomes and, as such, grow more vigorously than triploids or diploids.

Stahl and Keller (1981) reported that the oil content of diploid rhizomes of Canadian and U.S. origins was 4.7–6.0%, the oil content of triploid European rhizomes was 1.7–4.0% and the tetraploid Asian rhizomes possessed an oil content of 4.8–8.7%.

Diploid Rhizome Oils

Diploid rhizomes of *A. calamus* collected from the Rondines region (Quebec, Canada) were steam distilled to yield an oil in 1.05%. This oil, which was analyzed by Garneau et al. (2008) using GC-FID and GC/MS, was found to possess the following composition:

α-pinene (0.1%)
camphene (0.2%)
β-pinene (0.2%)
camphor (0.1%)
decanal (0.1%)
octyl acetate (0.1%)
2,3-dimethoxytoluene (0.2%)
bornyl acetate (0.5%)
2-epi-α-funebrene (0.2%)
β-elemene (0.3%)
7-epi-α-cedrene (0.1%)
(Z)-isoeugenol (0.2%)
β-funebrene (1.6%)
α-cedrene (0.5%)
decyl acetate (0.1%)
β-cedrene (1.0%)
α-cedrene isomer[*] (0.1%)
trans-α-bergamotene (0.8%)
prezizaene isomer[*] (0.8%)
(E)-isoeugenol (0.1%)
prezizaene (1.7%)
zizaene (0.2%)
acoradiene[a] (0.4%)
(E)-β-farnesene (1.1%)
methyl (Z)-isoeugenol (0.6%)
acora-3(10),14-diene (0.4%)
β-acoradiene (0.4%)
α-neocallitropsene (0.1%)
germacrene D (0.4%)
γ-curcumene (0.2%)
ar-curcumene (0.6%)
5-epi-aristolochene (0.5%)
6-epi-shyobunone (3.1%)
hinesene (0.2%)
bicyclogermacrene (0.2%)
α-muurolene (0.2%)
isogermacrene A (0.2%)
cuparene (0.2%)
shyobunone (13.3%)
δ-cadinene (0.5%)
β-sesquiphellandrene (2.1%)
isoshyobunone (1.3%)
(E)-nerolidol (1.5%)
spathulenol (0.7%)
germacrene D-4-ol (0.1%)
vulgarone A (0.3%)
preisocalamenenediol (18.0%)
sesquithuriferol (0.3%)
T-muurolol (0.1%)
α-cadinol (0.8%)
4-epi-acorenone (0.7%)
acorenone (14.2%)
6α-hydroxygermacra-1(10),4-diene (0.2%)
khusinol (0.1%)
acora-7(11),9-dien-2-one (0.6%)
isocalamenenediol (3.1%)
calamenenediol[a] (0.5%)
eudesma-3,11-dien-2-one[a] (0.1%)
cryptoacorone (7.5%)
epi-acorone (1.0%)
acorone isomer[*] (0.9%)
acorone isomer[*] (2.1%)

[*]correct isomer not identified; atentative identification

Trace amounts (<0.05%) of myrcene, limonene, nonyl acetate, methyl eugenol, 4,5-di-epi-aristolochene and (E)-isoelemicin were also identified in this oil.

Triploid Rhizome-Leaf Oils

Although calamus oil is normally obtained from the rhizomes, Menghini et al. (1998) showed that the leaf oil of triploid *A. calamus* contained both α- and β-asarone.

Venskutonis and Dagilyte (2003) collected rhizomes and leaves at their different growing phases from the Rumšiškės area (Central Lithuania) and dried them at ambient temperature in the shade. When dried, the leaves and rhizomes were separately ground and hydrodistilled to yield a leaf oil in 0.56–1.05% yield, depending upon their growing phase, and 1.20% rhizome oil. Analysis of the triploid rhizome oil using GC-FID and GC/MS revealed that it possessed the following composition:

limonene (0.1%)
linalool (1.0%)
camphor (5.1%)
borneol (0.1%)
geranyl acetate (0.1%)
β-elemene (0.2%)
1,7-di-epi-β-cedrene (1.7%)
β-cadinene (1.1%)
β-gurjunene (1.3%)
prezizaene 2 (2.2%)
α-humulene + methyl (Z)-isoeugenol (0.8%)

shyobunone + α-selinene (4.9%)
shyobunone* (7.8%)
δ-cadinene (0.2%)
6,11-oxido-acor-4-ene (2.9%)
germacrene D-4-ol (0.7%)
spathulenol (1.4%)
caryophyllene oxide (1.4%)
β-asarone (2.3%)
T-muurolol + T-cadinol (0.4%)
α-cadinol (1.3%)
calacorene hydrate 1 (1.0%)
calacorene hydrate 2 (1.1%)
α-asarone (0.2%)
acorenone (20.9%)
calamenenediol* (12.8%)
acorone (2.0%)

*correct isomer not identified

A trace amount (<0.1%) of (Z)-isoelemicin was also characterized in this oil.

The range in composition of the oils produced from leaves harvested at different growth stages over a four year period was also reported by Venskutonis and Dagilyte to be as follows:

limonene (t–1.9%)
(Z)-β-ocimene (0–1.9%)
linalool (0.1–1.6%)
camphor (0.2–1.1%)
borneol (0.3–0.8%)
citronellol (0–1.7%)
methyl citronellate (0–1.2%)
δ-elemene (0.8–1.7%)
geranyl acetate (0.7–1.7%)
β-elemene (0.4–0.6%)
1,7-di-epi-β-cedrene (0–t)
β-caryophyllene (4.0–8.1%)
β-gurjunene (0.2–0.5%)
α-humulene + methyl (Z)-isoeugenol (3.2–8.7%)
germacrene D (0.3–0.6%)
cis-β-guaiene (0.6–1.0%)
bicyclogermacrene (2.2–5.3%)
trans-β-guaiene (0.1–0.7%)
shyobunone* (0.6–1.1%)
δ-cadinene (3.4–4.6%)
6,11-oxido-acor-4-ene (1.8–3.2%)
germacrene B (1.2–1.9%)
(Z)-isoelemicin (0.5–1.2%)
germacrene D-4-ol (0.8–1.7%)
spathulenol (0.4–1.1%)
caryophyllene oxide (0.2–0.7%)
β-oplopenone (0.7–1.6%)
β-asarone (27.4–45.5%)
T-muurolol + T-cadinol (0.3–0.7%)
α-cadinol (0.3–2.2%)
α-asarone (2.7–6.2%)
calamusenone (3.1–6.0%)
acorenone (1.1–3.9%)
isocalamenenediol* (0–t)
acorone (0.1–0.3%)

*correct isomer not identified

Dusek et al. (2007) examined the morphological and chemical variations in triploid *A. calamus* found in the Czech and Finnish gene bank collections. They found that the oil content and β-asarone contents in the Czech rhizomes ranged from 1.30–2.92% and 11.3–21.3%, respectively. In contrast, the oil and β-asarone contents of the Finnish rhizomes ranged from 1.15–1.87% and 9.7–11.5%, respectively.

Wilczewska et al. (2008) collected triploid *A. calamus* rhizomes from the surroundings of Lake Tajty (Wrony village, near Gizcko, Poland), air-dried them and subjected the chopped rhizomes to hydrodistillation to produce an oil in 0.05% yield. The oil, which was analyzed using GC-FID and GC/MS, was compared with the headspace of the ground dried rhizomes. The rhizome oil was found to possess the following constituents:

α-pinene (0.2%)
camphene (1.2%)
β-pinene (0.1%)
limonene (0.5%)
(Z)-β-ocimene (0.4%)
(E)-β-ocimene (0.1%)
linalool (1.1%)
camphor (3.7%)
borneol (0.2%)
terpinen-4-ol (0.3%)
α-terpineol (0.2%)
bornyl acetate (0.3%)
β-elemene (0.3%)
1,7-di-epi-β-cedrene (1.1%)
isocaryophyllene (2.5%)
β-caryophyllene (1.2%)
β-gurjunene (1.2%)
trans-α-bergamotene (1.0%)
aromadendrene (1.1%)
β-humulene (1.2%)
α-humulene (1.2%)
methyl (Z)-isoeugenol (0.2%)
β-acoradiene (0.2%)
ar-curcumene (0.2%)
β-selinene (6.1%)
α-selinene (0.4%)
shyobunone* (10.5%)
β-sesquiphellandrene[a] (1.0%)
δ-cadinene (1.2%)
6,11-oxido-acor-4-ene (0.3%)
α-calacorene (0.3%)
germacrene B (0.3%)
β-calacorene (0.1%)
(E)-nerolidol (2.1%)
spathulenol (0.6%)
(Z)-isoelemicin (0.2%)
(Z)-asarone (10.4%)
elemicin (3.5%)
acorenone (14.6%)
(E)-asarone (7.7%)
8α,11-elemenediol (0.3%)
acorone (1.3%)
isoacorone (0.2%)

*correct isomer not identified

Trace amounts (<0.05%) of hexanal, tricyclene, benzaldehyde, sabinene, myrcene, δ-3-carene, p-cymene, γ-terpinene, terpinolene, *cis*-β-terpineol, *trans*-carveol, citronellol, geraniol, methyl citronellate and α-copaene were also found in this oil. The headspace analysis is not included in this review as the same compounds were characterized in different quantitative amounts.

Tetraploid Rhizome-Leaf Oils

Rana et al. (2008) collected rhizomes and leaves of *A. calamus* from the Imphal region (Manipur, India). Fresh rhizomes and leaves were washed, cut into small pieces and subjected to hydrodistillation. The tetraploid rhizome oil had an oil content of 0.8%, while that of the leaf oil was 0.27%. The results of the analysis of these two oils can be seen in **T-1**. Trace amounts (<0.05%) of α-pinene, β-pinene, myrcene, α-terpinene, p-cymene, 1,8-cineole, (Z)-β-ocimene, γ-terpinene, *cis*-sabinene hydrate, terpinolene, *trans*-sabinene hydrate, *cis*-p-mentha-2,8-dien-1-ol, an allo-ocimene isomer, camphene hydrate, borneol, α-terpineol and aristolone were also characterized in the rhizome oil. The leaf oil also contained some other trace compounds such as *trans*-p-mentha-2,8-dien-1-ol, citronellal, (Z)-4-decenal, decanal, methyl citronellate, 4-vinylguaicol, δ-elemene, asaronaldehyde, phytone, heneicosane and tricosane.

Wild *A. calamus* rhizomes were collected from six locations in Uttarkhand (Champawat, Pittoraagarh, U.S. Nagar, Dehradun, Chamoli and Almora) by Kumar et al. (2009). Analysis of these oils revealed that they were very similar; the range of constituents characterized in this oil was as follows:

α-pinene (t–0.2%)
camphene (t–1.0%)
β-pinene (0–0.3%)
myrcene (0–t)
p-cymene (0–0.1%)
β-phellandrene (0–t)
1,8-cineole (0–t)
linalool (t–0.3%)
6-campholenol (0–0.3%)
borneol (0–t)

myrtenol (0–t)
geraniol (0–0.2%)
bornyl acetate (0–0.1%)
α-copaene (0–t)
geranyl acetate (0–t)
methyl eugenol (0–0.1%)
β-caryophyllene (t–4.4%)
methyl (Z)-isoeugenol (t–4.2%)
dehydro-aromadendrene (0–1.6%)
spathulenol (0–1.7%)
γ-muurolene (0–3.2%)
α-calacorene (0.1–1.1%)
germacrene D-4-ol (0–1.7%)
caryophyllene oxide (0–1.7%)
(Z)-asarone[a] (74.8–91.6%)
(E)-asarone[b] (1.1–4.9%)

[a]also known as β-asarone; [b]also known as α-asarone

Fresh rhizomes of *A. calamus* were collected from different regions in the vicinity of Bhimtal (Nainital District, Uttarakhand, India), from which oils were produced by hydrodistillation in 3–5% yield by Joshi et al. (2012). Analysis of the oil by GC-FID and GC/MS revealed that it possessed the following composition:

limonene (0.2%)
linalool (0.4%)
terpinen-4-ol (0.1%)
methyl eugenol (0.2%)
β-caryophyllene (0.2%)
β-gurjunene (0.2%)
methyl (Z)-isoeugenol (2.6%)
spathulenol (1.1%)
β-bisabolene (4.1%)
δ-cadinene (1.1%)
(Z)-isoelemicin (1.7%)
germacrene D-4-ol (1.2%)
(Z)-asarone (74.8%)
(E)-asarone (5.6%)

Trace amounts (<0.1%) of camphene, (Z)-β-ocimene, γ-terpinene, camphor, α-copaene, longifolene and khusinol were also characterized in this oil.

T-1. Comparative percentage composition of the rhizome and leaf oils of tetraploid *Acorus calamus*

Compound	Rhizome oil	Leaf oil
camphene	0.2	t
benzaldehyde	-	0.1
sabinene	0.1	t
limonene	t	0.3
(E)-β-ocimene	0.6	0.4
linalool	0.1	2.3
camphor	0.7	t
terpinen-4-ol	0.1	-
bornyl acetate	0.1	0.1
β-elemene	0.1	0.1
methyl eugenol	0.1	t
β-caryophyllene	-	0.4
β-gurjunene	0.1	t
α-humulene	t	0.1
methyl (Z)-isoeugenol	4.2	0.9
germacrene D	-	0.1
methyl (E)-isoeugenol	-	0.1
shyobunone	4.4	2.2
δ-cadinene	0.3	0.1
kessane	0.1	-
α-calacorene	0.2	0.2
elemol	0.1	0.1
elemicin	0.1	0.1
β-calacorene	0.1	-
(Z)-isoelemicin	1.7	0.6
β-asarone	76.5	84.7
α-cadinol	0.2	0.1

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Nigella sativa Oil

Nigella sativa oil is obtained by steam distillation of the seeds of *N. sativa* L., a member of the Ranunculaceae family that is grown both in the Middle East and Asia, particularly India. *Nigella sativa* is an annual, erect herbaceous plant with branched stems that grows to the height of 30–60 cm. It has finely divided pinnated leaves and hermaphrodite violet blue to pale blue flowers. The fruit comprises a large inflated oblongal capsule, within which is found three to seven united follicles containing numerous 3–4-mm-long black striped sees, which are pungent and bitter when unripe, but are characteristically aromatic, almost carrot-like, when mature (Guzman and Siemonsma, 1999).

Nigella sativa is sometimes known as black cumin, or black caraway, even though it is not a member of the Apiaceae family. The seeds have a long history of use in traditional medicine in the Middle East and Asia, particularly India. In addition, the seeds are used as a spice in the Middle East. They contain 25–35% fixed oil, 30–35% carbohydrates, 15–20% protein and 0.3–0.6% essential oil. Summaries of the biological effects of *N. sativa* can be seen in the reviews by Paarakh (2010) and Naz (2011).

Burits and Bucar (2000) used GC/MS only to characterize the composition of water-distilled oils obtained from the hexane extracts of seven different seed sources of *N. sativa*. A summary of the range of constituents can be seen as follows:

α-pinene (0.2–2.0%)
camphene (0–0.1%)
β-pinene (0.1–1.8%)
sabinene (0.1–1.2%)
myrcene (0–0.4%)
α-terpinene (t–0.1%)
limonene (0.3–3.3%)
β-phellandrene (0–0.1%)
1,8-cineole (t–0.2%)
γ-terpinene (0–1.1%)
p-cymene (7.1–15.5%)
terpinolene (t–0.1%)
2-heptanal (0–0.4%)
α-thujone (t–0.2%)
trans-sabinene hydrate (0–0.2%)
longipinene (0.2–1.7%)
camphor (0.1%)
linalool (0.1–0.4%)
cis-sabinene hydrate (0–0.2%)
longifolene (1.3–8.0%)
bornyl acetate (0.5–0.8%)
2-undecanone (0–0.5%)
terpinen-4-ol (2.0–6.6%)
borneol (0–0.5%)
carvone (0–1.1%)
thymoquinone (27.8–57.0%)
2-tridecanone (0–0.4%)
(E)-anethole (0.3–2.0%)
p-cymen-8-ol (0–0.8%)
p-anisaldehyde (0–0.1%)
thymol (0.1–0.3%)
carvacrol (5.8–10.8%)

t=trace (<0.05%)

A hydrodistilled oil of a concentrated petroleum ether extract of Iranian *N. sativa* seeds was analyzed by Nickavar et al. (2003) using GC/MS only. The compounds reported to be identified in this oil were as follows:

nonane (1.7%)
α-thujene (2.4%)
3-methylnonane (0.3%)
α-pinene (1.2%)
1,3,5-trimethylbenzene† (0.5%)
sabinene (1.4%)
β-pinene (1.3%)
myrcene (0.4%)
decane (0.4%)
α-phellandrene (0.6%)
p-cymene (14.8%)
limonene (4.3%)
1-methyl-3-propylbenzene† (0.5%)
γ-terpinene (0.5%)
1-ethyl-2,3-dimethylbenzene† (0.2%)
fenchone (1.1%)
terpinen-4-ol (0.7%)
p-cymen-8-ol (0.4%)
methyl chavicol (1.9%)
dihydrocarvone* (0.3%)
carvone (4.0%)
thymoquinone (0.6%)
anisaldehyde (1.7%)
(E)-anethole (38.3%)
carvacrol (1.6%)
α-longipinene (0.3%)
tetradecane (0.2%)
longifolene (0.7%)
myristicin (1.4%)
hexadecane (0.2%)
dillapiole (1.8%)
apiole (1.0%)

*correct isomer not identified; †contaminants from the petroleum ether used to extract the seeds

Black cumin seeds that were purchased from a local market in Gorakhpur (Uttar Pradesh, India) were hydrodistilled by Singh et al. (2005) to produce an oil in 1.2% yield. Analysis of this oil using GC/MS only revealed that it possessed the following composition:

α-thujene (10.0%)
α-pinene (3.3%)
thuja-2,4(10)-diene (0.1%)
camphene (0.1%)
sabinene (1.3%)
β-pinene (3.8%)
α-terpinene (0.5%)
p-cymene (36.2%)
limonene (1.8%)
γ-terpinene (0.2%)
cis-sabinene hydrate (0.2%)
terpinolene (0.1%)
trans-sabinene hydrate (0.2%)
linalool (0.1%)
terpinen-1-ol (0.2%)
camphor (0.1%)
terpinen-4-ol (2.4%)
p-cymen-8-ol (0.1%)
α-terpineol (0.1%)
4,5-epoxy-1-isopropyl-4-methyl-1-cyclohexene† (0.9%)
carvone (0.2%)
thymoquinone (11.3%)
bornyl acetate (0.4%)
(E)-anethole (0.6%)
2-undecanone (0.1%)
thymol (0.1%)
carvacrol (2.1%)
α-longipinene (1.5%)
longicyclene (0.1%)
longifolene (6.3%)
β-caryophyllene (0.1%)
2-tridecanone (0.1%)
epi-zonarene (0.1%)
pima-8(14),15-diene (0.2%)

† questionable identity

A trace amount (<0.05%) of 1,8-cineole was also characterized in this oil.

Benkaci-Ali et al. (2005) compared the composition of the oils of the seeds of *N. sativa* collected from Medea (Algeria) produced either by hydrodistillation or by microwave distillation. Using both GC-FID and GC/MS as their method of analysis, the compositions of both oils can be seen in **T-2**.

Trace amounts of δ-3-carene, *trans*-sabinene hydrate, butyl octadecanoate, (Z)-docos-13-enoic acid (eracic acid), octacosane, nonacosane and tricontane were reported to be identified in the hydrodistilled oil, while *trans*-p-menth-2-en-1-ol, *trans*-sabinene hydrate,

T-2. Comparative percentage composition of *Nigella sativa* seed oils produced by two distillation methods

Compound	Water-distilled oil	Microwave-distilled oil
2-methylisovaleraldehyde†	0.1	t
nonane	0.2	t
α-thujene	-	2.3
α-pinene	-	0.5
isocumene†	0.2	0.1
thuja-2,4(10)-diene	0.2	-
sabinene	0.1	0.8
β-pinene	t	1.4
myrcene	-	0.1
α-phellandrene	t	0.1
α-terpinene	4.2	0.4
p-cymene	0.3	30.7
limonene	-	2.4
phenylacetaldehyde	0.1	0.1
γ-terpinene	t	2.6
cis-sabinene hydrate	-	0.1
terpinolene	t	0.1
p-cymenene	0.2	0.1
trans-sabinene hydrate	-	0.2
linalool	0.2	0.2
undecane	0.9	-
p-mentha-1,3,8-triene	t	0.2
trans-pinocarveol	-	0.1
camphor	-	0.1
cis-β-terpineol	-	0.2
karahanaenone	t	1.0
pentylbenzene†	0.1	-
1-(1,4-dimethyl-3-cyclohexen-1-yl)-ethanone†	3.9	-
terpinen-4-ol	-	2.3
naphthalene	0.4	0.5
p-cymen-8-ol	0.2	0.3
methyl chavicol	0.6	0.1
trans-dihydrocarvone	-	2.7
isodihydrocarveol	-	0.5
trans-carveol	-	0.1
cis-carveol	0.1	t
octanoic acid	0.2	-
cuminaldehyde	0.9	0.3
carvone	1.8	1.4
thymoquinone	-	13.5
α-terpinen-7-al†	1.4	-
bornyl acetate	-	1.1
isobornyl acetate	-	0.9
2-undecanone	-	0.3
thymol	12.1	0.1
carvacrol	0.2	2.8
methyl geranate†	0.2	0.1
methyl decanoate	0.2	-
α-longipinene	0.3	1.3
longicyclene	-	0.1
α-copaene	-	0.6
β-cubebene	t	0.3

T-2. Comparative percentage composition of *Nigella sativa* seed oils produced by two distillation methods (Cont.)

Compound	Water-distilled oil	Microwave-distilled oil
methyl eugenol	0.8	-
longifolene	-	6.0
dodecanal	t	0.1
β-caryophyllene	0.1	0.7
γ-himachalene	t	0.1
6-methyl-γ-(E)-ionone†	-	0.4
curcumene*	-	0.3
4-methyldiphenyl†	-	0.2
2-tridecanone	t	0.2
pentadecane	-	0.2
β-bisabolene	-	0.2
6-methyl-α-ionone†	-	0.2
δ-cadinene	t	0.2
citronellyl butyrate†	-	0.2
(Z)-nerolidol	12.3	0.1
thymohydroquinone	-	0.9
(E)-α-tetradecenal†	0.6	0.4
hexadecane	-	0.3
tetradecanal	-	0.1
dillapiole	0.2	t
T-cadinol	0.1	-
caryophyllene acetate	0.4	-
heptadecane	0.2	0.2
isolongifoliol	-	0.6
(E,Z)-farnesol	0.1	-
(E)-cyclododecene†	-	0.1
β-bisabolol†	0.7	-
octadecane	1.0	0.2
tetradecanoic acid	1.4	-
(E,E)-farnesyl acetate	0.7	-
rimuene†	0.2	-
nonadecane	0.5	0.1
methyl hexadecanoate	0.1	-
pimara-8(14),15-diene	-	0.4
(Z)-9-tetradecenyl formate†	-	0.3
hexadecanoic acid	15.6	1.8
hexadecyl butyrate	-	0.3
(E)-7-tetradecenol†	0.6	-
methyl linoleate	0.4	-
linoleic acid	29.5	1.0
oleic acid	-	0.9
3 α-hydroxy-manool†	0.2	-
tricosane	0.3	-
tetracosane	t	0.1
pentacosane	t	0.1
hexacosane	t	0.1
heptacosane	t	0.2
squalene	0.2	-

t=trace (<0.05%)
†identity requires corroboration

thujopsene, *trans*-α-bergamotene, α-guaiene and dodecyl butyrate were reported as trace components of the microwave-distilled oil. Tetrahydrolinalyl acetate, which does not occur in nature, was also reported as a trace constituent of the hydrodistilled oil.

Benkaci-Ali et al. (2006) reported the compositions of microwave-distilled oils of *N. sativa* seeds from Algeria, Syria, Jordan, Iran and Ethiopia. The analyses, which were performed using GC-FID and GC/MS, can be seen in **T-3**.

Pourmortazavi and Hajimirsadeghi (2007) reported that an oil produced from Iranian black cumin seed by hydrodistillation possessed the following composition:

α-pinene (2.8%)
β-pinene (3.7%)
myrcene (1.0%)
p-cymene (5.6%)
o-cymene (0.1%)
limonene (10.6%
γ-terpinene (45.7%)
cuminaldehyde (12.7%)
cuminyl alcohol (6.4%)
styrallyl alcohol† (3.5%)

†incorrect identification

A supercritical fluid CO_2 extract of the same batch of black cumin seeds at 45°C and 200 atmospheres was found to contain:

α-pinene (0.8%)
β-pinene (1.5%)
myrcene (0.6%)
o-cymene (7.8%)
γ-terpinene (38.0%)
cuminaldehyde (11.5%)
styrallyl alcohol† (25.6%)

†incorrect identification

The main components characterized in this oil look incorrect; however, there are possibilities that *N. sativa* could exist in more than one chemotypic form.

An oil produced from *N. sativa* seeds (known locally in Iran as 'Zireh Siah') that were obtained from a traditional farm in Kerman Province (southern Iran) was the subject of study by Jalali-Heravi et al. (2007). The components of the oil, which was produced in 2.66% yield, were characterized by GC-FID and GC/MS as follows:

α-thujene (0.1%)
α-pinene (4.0%)

camphene (0.3%)
sabinene (1.1%)
β-pinene (6.2%)
myrcene (1.2%)
δ-3-carene (0.1%)
α-terpinene (0.3%)
p-cymene (8.3%)
limonene (1.4%)
(Z)-β-ocimene (1.1%)
(E)-β-ocimene (0.3%)
γ-terpinene (24.4%)
terpinolene (0.9%)
thujone* (0.1%)
linalool (0.2%)
α-fenchyl alcohol (0.1%)
2,3,4,5-tetramethyl-2-cyclopenten-1-ol† (0.1%)
terpinen-4-ol (0.6%)
p-cymen-8-ol (0.1%)
anethemol† (0.2%)
2-methyl-3-phenylpropanal† (13.2%)
4-hydroxy-4-methylacetophenone† (0.1%)
bornyl acetate (0.7%)
2-caren-10-al† (0.8%)
cuminyl alcohol (0.3%)
1-phenyl-1-butanol† (0.9%)
isopropylbenzyl alcohol† (0.3%)
geranyl acetate (0.2%)
isopropylbenzyl acetate† (5.9%)
β-caryophyllene (0.5%)
thuja-2,4-(10)-diene† (14.0%)
ar-curcumene (0.1%)
β-selinene (0.2%)
β-bisabolene (0.2%)
myristicin (1.0%)
elemicin (0.1%)
caryophyllene oxide (0.1%)
dillapiole (0.2%)

*correct isomer not identified: †incorrect identification

In addition, the authors reported characterizing the following trace (<0.05%) constituents: tricyclene, 1-methylethylidene-cyclohexane,* dehydro-1,8-cineole, α-phellandrene, a cresol isomer, *trans*-sabinene hydrate, geranial, α-pinene oxide, *cis*-sabinene hydrate, p-mentha-1,3,8-triene, terpinen-1-ol, an isomer of allo-ocimene, octa-1,3,6-triene, 4-acetyl-1-methylcyclohexane,* p-mentha-1,5,8-triene, an isomer of p-menth-2-en-1-ol, *trans*-pinocarveol, terpinolene epoxide, *trans*-verbenol, camphor, 1,4-dimethyl-3-cyclohexenyl methyl ketone,* *cis*-limonene oxide, *trans*-limonene oxide, borneol, 1-(1,2,3-trimethyl)-cyclopent-2-enyl)-ethanone,* cumyl methyl ether,* dill ether, α-terpineol, myrtenol, myrtenal, a carveol isomer, chrysanthenyl acetate isomer, o-cumenol, geraniol, p-menth-2-en-7-ol, 2(3,3-dimethylcyclohexadiene)-ethanol,* perillyl alcohol, phellandral, methyl

T-3. Comparative percentage composition of microwave-distilled oils of *Nigella sativa* from five different origins

Compound	A	S	J	I	E
α-thujene	16.5	6.5	12.4	11.4	10.7
α-pinene	-	2.1	3.2	t	1.9
camphene	0.1	-	t	-	-
sabinene	7.5	-	1.7	0.2	-
β-pinene	0.2	-	3.8	1.4	-
α-terpinene	-	-	0.8	t	-
p-cymene	36.5	73.0	36.8	64.6	66.5
limonene	-	-	0.2	-	-
γ-terpinene	1.9	0.4	3.0	0.9	1.0
cis-sabinene hydrate	0.1	-	-	-	-
p-cymenene	0.2	-	-	t	t
terpinolene	-	0.6	t	0.9	0.5
trans-sabinene hydrate	2.2	-	1.2	-	-
α-thujone	0.1	-	-	-	-
p-mentha-1,3,8-triene	0.4	t	0.2	t	t
trans-limonene oxide	t	-	-	0.3	t
camphor	0.1	t	t	-	0.2
karahanaenone	0.7	0.2	0.5	-	-
terpinen-4-ol	0.6	0.3	0.7	0.3	0.3
p-cymen-8-ol	-	-	6.2	-	-
α-thujenal†	0.1	-	-	-	-
trans-dihydrocarvone	-	-	-	1.0	-
isodihydrocarveol	-	0.7	-	-	0.4
cis-sabinene hydrate acetate	-	0.1	0.1	0.1	t
cuminaldehyde	-	t	t	0.1	-
carvone	0.1	t	0.1	0.1	t
thymoquinone	14.7	6.2	18.4	9.7	9.6
carvenone†	-	-	-	-	1.9
trans-sabinene hydrate acetate	-	0.2	0.1	0.1	t
geranial	-	0.2	-	-	0.2
(E)-anethole	-	-	-	t	0.2
bornyl acetate	0.5	0.5	-	0.2	-
isobornyl acetate	-	-	0.2	0.3	-
2-undecanone	0.2	t	-	-	-
carvacrol	1.9	-	0.3	-	0.6
α-longipinene	1.1	0.5	0.7	0.2	0.2
carvacryl acetate	-	0.4	t	0.3	-
longicyclene	0.1	t	-	-	-
α-copaene	0.2	-	-	t	-
β-patchoulene†	0.1	-	-	-	-
isolongifolene	0.4	-	-	-	-
β-longipinene	-	-	0.1	t	t
longifolene	3.1	1.7	2.4	0.4	0.9
dodecanal	0.1	-	-	-	-
β-caryophyllene	0.1	0.1	0.2	t	0.1
cuminyl acetate	-	0.1	-	-	-
cis-thujopsene	0.1	-	-	-	-
(E)-isoeugenol	-	0.1	-	-	-
α-humulene	0.1	0.1	t	-	-
6-methyl-α-ionone†	0.1	-	-	-	-
γ-muurolene	0.2	-	-	-	-
2-tridecanone	0.1	-	-	-	-
β-bisabolene	0.1	-	-	t	-
δ-cadinene	0.1	-	0.2	0.1	-

T-3. Comparative percentage composition of microwave-distilled oils of *Nigella sativa* from five different origins (Cont.)

Compound	A	S	J	I	E
γ-dehydro-ar-himachalene†	0.1	-	-	-	-
citronellyl butyrate	0.1	-	-	-	-
thymohydroquinone	0.3	0.5	0.3	t	0.7
tetradecanal	0.2	-	-	-	-
himachalol	-	-	0.2	-	-
2-pentadecanone	0.1	-	-	-	-
isolongifolol	0.2	t	-	-	-
pimara-8(14),15-diene	0.1	t	t	t	-
methyl linoleate	0.2	0.1	0.3	t	-

*correct isomer not identified
†questionable identification
A=Algerian seed; S=Syrian seed; J=Jordanian seed; I=Iranian seed; E=Ethiopian seed

thymol, α-copaene, β-bourbonene, sinularene,* 2,7,7-trimethyl-bicyclo[2,2,1]-hept-2-ene,* *trans*-α-bergamotene, a β-farnesene isomer, germacrene D, zingiberene, (Z)-γ-bisabolene, δ-cadinene, (Z)-α-bisabolene, ledol, spathulenol, apiole and anymol* were also found in this oil. This reviewer has placed an asterisk against the trace components that are probably misidentified.

The conditions for supercritical fluid CO_2 extraction of *N. sativa* seeds were examined by Rao et al. (2007). They found that the highest yield (31.7%) could be obtained at 50°C, 400 bar and a volume of 100 mL of CO_2.

Salomi et al. (1991 and 1992) and Worthen et al. (1998) reported that the oil and some purified constituents of *N. sativa* seeds possessed anti-tumor activity.

Edris (2009) confirmed the fact that thymoquinone, the main component of *N. sativa* oil, showed great potential as a chemopreventive anti-cancer agent.

A volatile concentrate obtained from the hexane extract of *N. sativa* by Hamrouni-Sellami et al. (2008) was analyzed using GC/MS only. The components characterized in this volatile concentrate were:

α-pinene (1.4%)
α-thujene (7.2%)
β-pinene (1.8%)
sabinene (0.7%)
α-phellandrene (0.1%)
myrcene (2.1%)
limonene (0.1%)
1,8-cineole (1.9%)
β-ocimene* (18.5%)
γ-terpinene (1.2%)
p-cymene (53.1%)
terpinolene (0.1%)
1-octen-3-ol (6.5%)
linalool (0.1%)
terpinen-4-ol (0.4%)
α-terpineol (0.1%)
thymol (1.8%)

*correct isomer not identified

Seeds of *N. sativa* that were cultivated in Poland were purchased from Kawon market (Gostyn, Poland) and subjected to hydrodistillation by Wajs et al. (2008). Analysis of the oil produced in 1.7% yield by GC-FID and GC/MS revealed that it contained:

α-thujene (7.2%)
α-pinene (2.0%)
sabinene (0.8%)
β-pinene (2.1%)
myrcene (0.4%)
α-phellandrene (0.2%)
p-cymene (60.2%)
limonene (1.3%)
γ-terpinene (12.9%)
terpinolene (0.6%)
trans-sabinene hydrate (0.5%)
trans-4-methoxythujone (4.0%)
camphor (0.1%)
trans-verbenol (0.3%)
terpinen-4-ol (0.9%)
trans-dihydrocarvone (0.7%)
carvone (0.2%)
cis-chrysanthenyl acetate (0.1%)
bornyl acetate (0.1%)
carvacrol (3.0%)
α-longipinene (0.1%)
longicyclene (0.4%)
2-tridecanone (0.1%)
β-selinene (0.1%)
γ-cadinene (0.1%)
apiole (0.1%)
(Z,E)-farnesal (0.2%)

Trace amounts (<0.05%) of camphene, (Z)-β-ocimene, (E)-β-ocimene, *cis*-sabinene hydrate, β-thujone, borneol, α-terpineol, thymoquinone, thymol, cyclosativene, α-copaene, longifolene, allo-isolongifolene, (Z)-β-farnesene, myristicin, β-bisabolene, thymohydroquinone, caryophyllene oxide and 2-pentadecanone were also found in this oil.

Benkaci-Ali et al. (2010) compared the compositions of soxhlet extraction of *N. sativa* seeds from four locations in Algeria (Ouargla, Timimoun, Adrar and Médéa) using both hexane and isopropanol as solvents. Each extract was subjected to steam distillation to isolate the volatiles, which in turn were analyzed using both GC-FID and GC/MS. The range of components characterized in each of the volatiles were as follows:

α-thujene (0–1.4%)
camphene (0–0.1%)
sabinene (0–0.2%)
β-pinene (0–0.7%)
α-terpinene (0–0.1%)
p-cymene (4.0–19.9%)
γ-terpinene (0–1.0%)
m-cymene (0–0.1%)
terpinolene (0–0.9%)
linalool (0–0.1%)
nonanal (0–0.2%)
p-mentha-1,3,8-triene (0–0.1%)
karahanaenone (0–0.5%)
terpinen-4-ol (0–1.9%)
p-cymen-8-ol (0–0.4%)
myrtenal (0–1.0%)
myrtenol (0–0.6%)
trans-dihydrocarvone (0–1.8%)
cuminaldehyde (0–0.4%)
carvone (0–4.0%)
thymoquinone (15.2–46.7%)
trans-sabinene hydrate acetate (0–0.1%)
(E)-2-decenal (0–0.2%)
neothujyl acetate (0–0.2%)
decanol (0–0.4%)
(E)-anethole (0–0.9%)
bornyl acetate (0–0.3%)
cuminyl alcohol (0–0.5%)
thymol (0–0.4%)
2-undecanone (0–0.2%)
(E,Z)-2,4-decadienal (0–1.2%)
carvacrol (0.7–9.5%)
(E,E)-2,4-decadienal (0–2.6%)
β-longipinene (0.1–0.8%)
eugenol (0–0.1%)
γ-nonalactone† (0–0.1%)
α-copaene (0–0.8%)
β-longipinene (0–0.1%)
longifolene (0–5.9%)
isocaryophyllene (0–0.1%)
β-caryophyllene (0–0.1%)

α-himachalene (0–0.1%)
methyl (Z)-isoeugenol (0–0.2%)
cis-muurola-4(14),5-diene (0–0.9%)
6-methyl-(E)-γ-ionone† (0–0.9%)
2-tridecanone (0–0.9%)
β-bisabolene (0–0.1%)
γ-cadinene (0–0.3%)
δ-cadinene (0–0.9%)
citronellyl butyrate† (0–0.1%)
calacorene* (0–0.1%)
thymohydroquinone (18.2–53.6%)
isoeugenyl acetate* (0–0.2%)
longiborneol acetate (0–0.3%)
(E,Z)-farnesol (0–0.9%)
tetradecanoic acid (0–2.4%)
(Z,E)-farnesyl acetate (0–0.2%)
isopropyl myristate (0–0.3%)
methyl hexadecanoate (0–1.2%)
pimara-8(14),15-diene (0–0.7%)

*correct isomer not identified; †questionable identification

Oils produced from seeds (1 g) of *N. sativa* that were purchased at a local market in Germany were micro-hydrodistilled to produce oils that were analyzed using GC-FID and GC/MS by Zawirska-Wojtasiak et al. (2010). The oils, which were found to be similar, were found to possess the following constituent ranges:

α-pinene (0.2–1.2)[a]
δ-2-carene (0.1–1.2)
β-pinene (0.1–0.7%)
p-cymene (9.7–11.8%)
limonene (0.2–0.7%)
linalool (0.1–0.3%)
terpinen-4-ol (0.2–0.4%)
carvone (0.3–1.6%)
(E)-anethole (0.1–0.5%)
thymoquinone (1.0–4.4%)
carvacrol (0.4–3.5%)

[a]mg/g volatiles

The authors also used chiral GC with an Rt-βDEXsm column to determine the enantiomeric ratios of six of the constituents. They were as follows:

(1R,5R)-(+)-α-pinene (25.3–63.3%):(1S,5S)-(-)-α-pinene (36.7–74.7%)
(1R,5R)-(+)-β-pinene (32.8–62.3%):(1S,5S)-(-)-β-pinene (37.7–67.2%)
(4R)-(+)-limonene (78.2–94.1%):(4S)-(-)-limonene (5.9–21.8%)
(3S)-(+)-linalool (52.0–75.3%):(3R)-(-)-linalool (24.7–48.0%)
(4S-(+)-terpinen-4-ol (5.9–87.7%):(4R)-(-)-terpinen-4-ol (12.3–94.1%)
(4S)-(+)-carvone (12.2–14.3%):(4R)-(-)-carvone (85.7–87.8%)

Steam distillation of *N. sativa* seeds that were purchased from a Maiduguri market (Borno State, Nigeria) to yield an oil in 0.4% was examined for its major components by Adamu et al. (2010) using GC/MS only. The components characterized in this oil were as follows:

α-thujene (62.3%)
α-pinene (2.3%)
β-pinene (2.5%)
p-cymene (45.7%)
terpinen-4-ol (4.8%)
thymoquinone (30.8%)
carvacrol (4.5%)
longifolene (4.2%)

Fractionation of *N. sativa* oil by Bourgou et al. (2011) combined with GC/MS and 2-dimensional NMR resulted in the characterization of four new monoterpenes. They were *cis*-sabinene hydrate methyl ether, *trans*-sabinene hydrate methyl ether, 1,2-epoxy-p-menth-4-ene and 1,2-epoxy-p-menth-4(8)-ene. The authors showed that *cis*-sabinene hydrate methyl ether was most effective in inhibiting oxidative stress in human stain fibroblast cells. The interesting point of this study was that it suggested that biological activity showed geometric stereoselectivity.

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