

Note

Chemical composition and antimicrobial activity of the essential oil from the rhizome of *Canna indica* Linn

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The essential oil of the rhizome of *Canna indica* Linn. (yellow flower variety) has been isolated by hydrodistillation and analysed by GC and GC/MS. Forty-three compounds representing 95.32% of the oil have been identified. The sesquiterpene hydrocarbons and their derivatives form the major part (52.56%). The major terpene/terpenoid constituents of the essential oil of rhizome are γ -eudesmol (9.79%), δ -cadinol (6.33%), γ -selinene (5.23%) and luciferin (5.05%). Surprisingly, certain fatty acids are also found to be present in noticeable amount, palmitic acid (8.53%) being the main. The oil showed good antibacterial activity against *Staphylococcus aureus* but mild activity against *Bacillus subtilis*.

Keywords: *Canna indica*, *Canna orientalis*, essential oil composition, γ -eudesmol, palmitic acid

Canna indica Linn. (syn. *Canna orientalis* Roscoe) belongs to Cannaceae family. It is a commonly occurring plant in India but much less explored. Several varieties (red, orange, pink or yellow flowers) are grown as ornamental plant in the gardens all over India. Known as *Sabbajala* in Hindi, it is called *Devakuli* in Sanskrit, *Sudarshan* in Himachal and *Keli* in some parts of Northern India. It is also known as Indian shoot or Canna lily. Stem 0.9-1.2 m; leaves 15-45 cm by 10-20 cm, lanceolate to ovate, oval or almost orbicular, caudate acuminate, veins arching, sheath open above. Flowers are rather distant, 5-6.5 cm long, bracts 1.3-2.5 cm, oblong and membranous^{1,2}. Yellow flowers have a shining colour. Seeds are very many, globose, black and shining². Rhizomes and leaves of this plant are used as food material in Myanmar³. The root is given as a demulcent and stimulant and used as a diaphoretic and diuretic in fevers and dropsy². Total phenol content and flavonoid content have been

analysed in crude methanolic extracts of red and yellow varieties of *C. indica*. Red Canna flowers were found to serve as a potential source of natural antioxidant and natural food colorant⁴. The antioxidant activity and the polyphenol and flavonoids content of methanolic extracts of plant seeds have also been investigated⁵. The benzene and methanol extracts of the various parts of *C. indica* have been reported to show antinociceptive and anthelmintic activities⁶. The aqueous extract of *C. indica* root, which contains total phenolic compounds, is found to increase the amount of glucose transporter isoforms for treating diabetes⁷. The water extract of rhizome of *C. indica* has been reported to have HIV-1 reverse transcriptase inhibitory activity⁸; fractions derived from *C. indica* root significantly inhibit the activity of certain other enzymes as well⁹. Six non-polar compounds from petroleum ether extract of *C. indica* rhizome have been detected by GC/MS¹⁰. Starch isolated from rhizome of this plant has been hydroxypropylated and characterized¹¹. Some properties of Canna starch have been investigated by Perez and Lares¹². Recently a patent has appeared for hair preparations containing *C. indica* extract¹³.

No work on the study of essential oil from rhizomes has so far been reported. The present study concerns the isolation of the rhizome oil from the yellow flower variety and analysis of the isolated oil by GC and GC/MS along with an antibacterial evaluation against certain bacteria.

Results and Discussion

The yield of essential oil obtained from rhizomes of *Canna indica* was 0.52% w/w. Forty-three constituents were identified representing 95.32% of the oil. They are summarized in **Table I**.

The remarkable observation is that whereas the terpenes and terpenoids constitute 81.33% of the oil, the fatty acid and their esters, too, are present in noticeable amount (13.84%). Among the identified 34 terpenes and terpenoids, 16 are monoterpene hydrocarbons and derivatives (including 11 oxygenated monoterpenes) representing 21.63% of the total essential oil. The major monoterpenes and derivatives are 1-terpinen-4-ol (4.60%), α -fenchyl acetate (3.26%), 1,8-cineole (3.17%) and β -pinene

Table I — Percentage composition of the rhizome oil of *Canna indica*

Compd	RI	Percent
hexanal	806	0.05
furfural	832	0.04
heptanol	892	0.06
α -pinene	933	0.73
camphene	943	0.48
β -pinene	972	3.13
1,8-cineole	1028	3.17
γ -terpinene	1052	0.71
α -terpinolene	1066	0.22
<i>cis</i> -sabinene hydrate	1077	0.19
β -linalool	1092	0.63
fenchol	1102	0.46
<i>trans</i> -pinocarveol	1140	0.40
1-terpinen-4-ol	1177	4.60
α -fenchyl acetate	1221	3.26
bornyl acetate	1268	0.52
2,3-pinandiol	1280	0.08
isobornyl acetate	1291	2.56
unidentified	1304	0.60
unidentified	1341	1.30
geosmin	1376	0.49
β -caryophyllene	1422	2.00
α -caryophyllene	1455	4.78
γ -selinene	1484	5.23
selina-3,7(11)-diene	1507	1.72
<i>trans</i> -nerolidol	1541	3.23
carotol	1588	2.72
caryophyllene oxide	1598	4.96
δ -cadinol	1612	6.33
γ -eudesmol	1626	9.79
9-cedranone	1639	2.43
tridecanoic acid	1654	2.14
α -acorenol	1665	2.49
myristic acid	1708	1.83
luciferin (latia)	1765	5.05
palmitic acid	1968	8.53
dibutyl phthalate	2037	0.85
manool	2056	2.75
unidentified	2076	0.65
methyl linoleate	2092	0.63
geranyl linalool	2103	2.75
unidentified	2118	0.49
dodecenyl succinic anhydride	2159	0.98
oleic acid	2171	0.33
stearic acid	2197	0.38
unidentified	2228	1.29
geranyl geraniol acetate	2296	0.50
unidentified	2331	0.35
4,8,13-duvatriene-1,3-diol	2391	1.14

RI = Retention index relative to *n*-alkanes on the DB-5 column.

(3.13%). Monoterpenes have their own biological importance¹⁴⁻¹⁶. 1-Terpinen-4-ol has been reported to possess antihypertensive effects¹⁷ and to suppress inflammatory mediator production by activated human monocytes¹⁸. α -Fenchyl acetate is mainly applied in herbal-pine fragrances. It is used in combination or as a support for bornyl acetate and isobornyl acetate. The compound in alcohol form is used as solvent, organic intermediate, odorant and in flavouring¹⁹. 1,8-Cineole is known as a stimulating expectorant in cases of chronic bronchitis. It is also used in room sprays, lotions and in all kinds of cosmetic preparations. Its cardiovascular effects in normotensive rats have also been reported²⁰. β -Pinene is used among the lube-oil additives²¹. The presence of geosmin (0.49%) is novel. It is among the rarely occurring monoterpenoids. Species use it in their chemical communication system. In fact, it is geosmin that is responsible for the musty off-flavour of dry beans²²; its presence has also been reported in green coffee²³.

Sesquiterpene hydrocarbons and their derivatives are the major components (52.56%) of the oil, though numerically they are only fourteen, including 10 oxygenated sesquiterpenes. The prominent among them are γ -eudesmol (9.79%), δ -cadinol (6.33%), γ -selinene (5.23%) and luciferin (5.05%), caryophyllene oxide (4.96%), α -caryophyllene (4.78%), and *trans*-nerolidol (3.23%). Sesquiterpenes, in general, are quite useful^{14,24}. γ -Eudesmol is useful as a termite repellent²⁵. δ -Cadinol is reported to have significant cytotoxicity against certain human tumour cell lines²⁶, while the γ -selinene containing fraction in certain essential oils is recently reported to be having strong superoxide anion-scavenging capability²⁷. Luciferin (Latia) is probably responsible for yellow shining colour of flowers. Presence of latia luciferin in fresh water snails is well known²⁸. Caryophyllene oxide, used as a preservative in food, drugs and cosmetics, is also an antifungal against dermatophytes²⁹. α -Caryophyllene, also known as α -humulene, has anti-inflammatory effects³⁰. *trans*-Nerolidol is used in perfumery and flavouring³¹. Presence of 9-cedranone (2.43%) is novel. Earlier its presence has been reported in *Marchantia convoluta* leaves during a study on cytotoxicity to human liver and lung cancer cells³². Another rare compound identified is dodecenyl succinic anhydride (DDSA) that constituted 0.98% of the oil. It is used as a curing agent for epoxy resins³³. Presence of DDSA has been reported among the floral fragrances of *Rosa hybrida*³⁴.

Another important observation is the presence of diterpenes in good quantity (7.14%). In all, four diterpenes were identified. All are oxygenated diterpenes, the major being manool and geranyl linalool (2.75% each). Presence of geranyl linalool is comparatively rare. Species use it in chemical communication systems. Its presence has been reported in jasmine oil³⁵. A novel observation has been the presence of 4,8,13-divatriene-1,3-diol (1.14%). It is a monocyclic diterpene. Its presence has recently been reported in *Scapania verrucosa*³⁶. 4,8,13-Divatriene-1,3-diol, isolated from green tobacco leaves, increases tobacco budworm egg laying³⁷.

The essential oil of *Canna indica* rhizome was tested for its antibacterial properties against certain important bacteria. It showed a moderate inhibitory activity against the Gram(+) *Staphylococcus aureus* and mild activity against the Gram (+) *Bacillus subtilis* and no activity against the Gram(-) *Escherichia coli* (Table II).

Plant Material

The authentic rhizomes were collected from Himachal Pradesh (India) in January 2009, with the authenticity further verified by Forest Research Institute (FRI), Dehradun. A voucher specimen has been deposited in the Herbarium of the Plant Medicine Section of the Chemistry Department of Gurukula Kangri University under the registry no. 20/15. The rhizomes were washed with luke-warm water and dried in shade for a couple of months.

Experimental Section

Isolation of essential oil

The oil was obtained by hydrodistillation using a Clevenger type apparatus. The lighter than water transparent brownish oil was re-extracted with diethyl ether and dried over anhydrous Na₂SO₄. A light brown oil having sharp camphoraceous odour (yield 0.52% w/w, d₃₀: 0.9102 g/mL) was obtained.

Table II — Antibacterial activity of essential oil from rhizome of *Canna indica*

Microorganism	Inhibition zone (mm)	
	Essential oil	Standard
<i>Staphylococcus aureus</i> (+)	12	32
<i>Bacillus subtilis</i> (+)	4	51
<i>Escherichia coli</i> (□)	Nil	36
Standard was Chloramphenicol, 30 µg/disc		

Gas Chromatography

The essential oil sample was subjected to GC analysis. Perkin-Elmer Gas Chromatograph Clarus 500 equipped with DB-5 MS (60 m × 0.32 mm; film thickness 5 µm) was used. Oven temperature was kept at 65°C for 4 min and programmed to 250°C at a rate of 4°C/min and kept constant at 250°C for 20 min. Carrier gas: nitrogen; flow rate: 1.5 mL/min; split ratio: 1:10; injection port temperature: 240°C; detector temperature: 250°C. The percentage compositions were computed electronically from the GC peak areas.

Gas Chromatography/Mass Spectrometry

GC/MS analysis of the oil was carried out on Shimadzu GC-2010, GCMS-QP 2010 equipped with similar column and operated under similar conditions as GC. Carrier gas: helium; flow rate: 24 cm/s (linear velocity); split ratio: 1:5, detector voltage: 1 KV; ion source temperature: 200°C. The oil components were identified by comparing their retention indices and mass fragmentation pattern with those of standards and MS library (NIST 12 and NIST 62).

Antimicrobial studies

A collection of three microorganisms was used that included the Gram (+) bacteria *Staphylococcus aureus* and *Bacillus subtilis* and the Gram (-) *Escherichia coli*. The test pathogenic bacteria were maintained on nutrient agar medium (NAM) slants at 4°C. Paper disc diffusion method was used to determine inhibitory zone of pure essential oil and the chloramphenicol standard (30 µg/disc) for antibacterial studies.

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