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Article

Chemical Composition of Essential Oil of *Cananga odorata* (Lam.) Hook. F. & Thomson Leaves and Its Biological Activities

Krishnasamy Vimaladevi [1]*, Madhiyan Selladurai [2], Kathirvel Poonkodi [1], Velliangiri Prabhu [1], Raman Mini [1] and Balasubramaniyam Manojkumar [1]

[1] Post Graduate Department of Chemistry, Nallamuthu Gounder Mahalingam College, Pollachi-642001, Tamil Nadu, India

[2] Department of Chemistry, Nallamuthu Gounder Mahalingam College, Pollachi-642001, Tamil Nadu, India

***** Corresponding Author: k.vimala83@gmail.com (Krishnasamy Vimaladevi)

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Abstract: In the current study, the chemical constituents of leaf essential oil from *Cananga odorata* (Lam.) Hook. F. & Thomson were identified by GC/MS analysis. A total of 25 components (91.1 %) in the essential oil were identified. The major compounds present in the oil were *trans*-caryophyllene (19.7 %), ocimene (13.2 %), E, E-α-farnesene (10.3 %), phenylmethyl ester (5.6 %), farnesyl acetone (5.1 %), t-muurolol (4.2 %), farnesol (3.7 %), β-elemene (3.1 %), α-cadinol (3.0 %), copaene (2.9 %), benzyl benzoate (2.3 %), *trans*-farnesol (2.1 %). The essential oil is rich in sesquiterpenoids including sesquiterpenes hydrocarbon (42.3 %), monoterpene hydrocarbons (18.1 %), and in addition to that oxygenated compound representing (30.7 %). The Antioxidant efficiency of essential oil of *C. odorata* leaves was performed by DPPH radical method together with BHA as standard. The essential oil showed concentration-dependent antiradical activity with EC_{50} value of 19.5±1.25 µg/mL, when compared with standard BHA which showed EC_{50} value of 1.10±0.55 µg/mL. The *in vitro* anticancer potential of essential oil was evaluated by using MOLT-3 cell line by MTT assay. The *C. odorata* essential oil exhibited significant anticancer activity with IC_{50} value of 44.22 µg/mL. From the results, *C. odorata* essential oil has significant antioxidant and anticancer properties which can be utilised as a natural supplement as antioxidant and anticancer agents in pharmaceutical and food industries since it is available throughout the year.

Keywords: *Cananga odorata*, essential oil, GC/MS, DPPH, MOLT-3 cell line, and MTT assay.

Introduction

The plant *Cananga odorata (Lam.)* Hook. F. & Thomson is a fast-growing tree, native of Southeast Asia, invasive plant in America, China, India, and Africa [1]. It belongs to the family Annonaceae, *Cananga* is a small genus, consists of only two species viz. *C. odorata* and *C. latifolia* are distributed throughout the tropical and subtropical regions of the world [2,3]. *C. odorata* is commonly known as kattuchempakam in tamil, Apurva chempakame in telugu, kenenga, chenanga and ylang-ylang in Malaysia [4]. These blooms of *C. odorata* are well known as the source of ylang-ylang or Cananga oil, a matter widely used in the perfume industry [5]. Previous reports in the literature have shown that ylang-ylang essential oil contains monoterpene, terpene, and sesquiterpene alcohols, sesquiterpene hydrocarbons, acetate, benzoate, and phenol and have a traditional therapeutic use [6].

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The bark is used in the treatment of stomach problems and as a laxative by Tonga and Samoa. At Java, fresh flowers were used to treat asthma and malaria [7]. It is also used for treating headaches, eye injuries, and gout. The bark of the plant is used to treat rheumatism, mucosity, ophthalmitis, ulcers, and fevers [8,9]. Fresh flowers are prescribed as carminative and as a treatment for asthma, and an infusion of flowers is used to prevent skin itching. Fruits and seeds are used in the treatment of fever. It is used in aromatherapy and is thought to be beneficial for the treatment of depression, afflicted respiration, high blood pressure, anxiety, and as an aphrodisiac [10,11]. Recent studies have shown a wide range of bioactivities evidenced by essential oils and *C.odorata* extracts which include antimicrobial, antibiofilm, anti-inflammatory, vector, insect repellent, antidiabetic, anti-fertility, and anti-melanogeny [12]. However, there are a few outcomes for *in vitro* anticancer and antioxidant activities of the essential oil of *C. odorata* leaves. The present study is intended to evaluate the chemical composition of essential oil from the leaves of *C. odorata* and its *in-vitro* anti-oxidant and *in vitro* anticancer activities.

Materials and methods

Plant material

Fresh leaves of *C.odorata* (2 kg) were collected near Udumalpet (10.6419°N, 77.2659°E.) in Tamil Nadu, Southern India, between September and October 2020. The plant sample was identified and authenticated by Dr. P. Sathishkumar, Assistant Professor, Department of Botany, Nallamuthu Gounder Mahalingam College, Pollachi, and the voucher specimen (PCH015) was preserved in the Chemistry department.

Isolation of essential oil from *C. odorata* leaves

About 500 g of fresh leaves were taken in a round bottom flask and hydrodistillated using Clevenger type apparatus for 4h. The essential oil was dried over anhydrous sodium sulphate (Merck) until the last traces of water were removed and then stored in a container at 4°C before GC/MS analysis. The extraction of the essential oil process was repeated (4 times) for the required amount of oil for further analysis.

Gas chromatography-Mass spectrometry analysis

GC/MS analysis of the chemical composition of *C. odorata* was performed using thermo GC-trace ultra-version: 5.0 coupled with thermo MS DSQ II instrument. The compounds were separated on DB-35, MS capillary standard non-polar column (30 x 0.25 mm), film thickness 0.25 µm. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 70°C and held for 2 minutes and the temperature of the oven was raised to 260°C for 10 minutes and raised 6°C per minute and the final temperature was 300°C for 10 minutes. The sample was dissolved in 1 mL of acetone and injected with splitless mode. The mass spectra were recovered over a range of 50-500 amu with electronic impact ionization energy of 70 eV, while the temperature of the injector and the MS transfer line was set at 260°C and 300°C respectively.

The components were identified by comparison of their mass spectra with those of the National Institute of Science and Technology (NIST) mass spectral library version 2.0g, as well as on the comparison of their retention time either with those of authentic compounds or with their literature value [13]. Quantification was done by an external standard method using calibration curves generated by running GC analysis of representative compounds.

Determination of *in-vitro* antioxidant activity

The antioxidant activity was measured using the 2,2 -diphenyl-1-picryl-hydrazyl (DPPH) radical reduction assay, according to the method of Brand-Williams [14,15]. The hydrogen atom donating ability of the essential oil of *C. odorata* leaves were determined by the decolourization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). It produces violet/ purple colour in methanol solution and fades to shades of yellow colour in the presence of antioxidants. 0.1 M solution of DPPH in methanol was prepared and 1.0 mL of this solution was added to the essential oil solution in water at different concentrations (10, 20, 40, 60, 80, and 100 µg/mL). After 30 minutes, the absorbance was measured at 517 nm. The lower absorbance of the reaction mixture indicates higher free radical activity. The control experiment

was also carried in the same manner with distilled water in place of the oil. Butylated Hydroxyanisole (BHA) was used as standard. Radical Scavenging Activity was calculated using the following equation:

Scavenging effect % = $[(A_0 - A_1)/A_0] \times 100$

Where A_0 was the control and A_1 was the absorbance of the essential oil.

In-vitro anticancer activity

An *in vitro* anticancer activity was performed for the essential oil as per standard procedure using MTT assay [16]. MOLT-3 cell lines are employed for *in vitro* anticancer activity and cell viability was calculated. The culture medium from the MOLT-3 monolayer was replaced with a fresh medium. Test sample in duplicates was added to the cells. After incubation at 37±1°C for 18 h, MTT was added in all the wells and incubated for 4 h. After incubation, DMSO was added to the wells and read at 570 nm using a microplate reader. Cytotoxicity was calculated by using the formula.

Cytotoxicity = [(Control — Treated)/ Control] * 100

Statistical analysis

All experiments were repeated at least thrice. The results were expressed as Mean ± Standard deviations. For IC_{50} and EC_{50} values are calculated by using the sigmoidal dose-response formula in origin Pro 8.5 software.

Results and discussion

The pale yellow essential oil yield of *C. odorata* leaves was 0.9 % (w/w) and the actual average yield of essential oil was determined as 0.85 g from approximately 2000 g of the *C. odorata* leaves. The principal compounds identified in the essential oil of fresh leaves of *C. odorata* by GC/MS analysis. A total of 25 compounds were identified (Table 1), which accounts for 91.1 % of total oil. Six monoterpenoids including monoterpene hydrocarbons (18.1 %), eight sesquiterpenoids including sesquiterpenes hydrocarbon (42.3 %), and in addition to that oxygenated compounds representing (30.7 %) were identified.

The major compounds present in the essential oil were *trans*-caryophyllene (19.7 %), ocimene (13.2 %), E, E-α-farnesene (10.3 %), phenyl-methyl ester (5.6 %), farnesyl acetone (5.1 %), t-muurolol (4.2 %), farnesol (3.7 %), β-elemene (3.1 %), α-cadinol (3.0 %), copaene (2.9 %), benzyl benzoate (2.3 %), *trans*-farnesol (2.1 %), the minor compounds were cubenol (1.9 %), α-cubebene (1.9 %), γ-elemene (1.9 %), 2-ethyl-3-methylthiirane 1,1-dioxide (1.6 %), limonene (1.3 %), geraniol (1.3 %), α-myrcene (1.3 %), (-) caryophyllene oxide (1.1 %), camphene (0.9 %), 1,4-bis (ethynyl) butane (0.9 %), sabinene (0.8 %), α-thujene (0.6 %), muurolene (0.4 %). From the collection of literatures, a very few results were found on the GC/MS analysis of the essential oil from *C. odorata* leaves. A total of 23 components were reported from the essential oil of *C. odorata* leaves grown in China, the major components were linalool (21.08 %), linalool acetate (16.4 %), α-pinene (12.73 %), eugenol (8.86 %), α-terpineol acetate (7.7 %), isobornyl acetate (3.56 %), α-terpineol (3.46 %) and camphor (3.23 %) [17]. However the major component of essential oil was quite different from China, but the essential obtained in the present study showed *trans*-caryophyllene, ocimene, and α-farnesene are major compounds whereas linalool, linalool acetate, and α-pinene are the main constituents of Chinese origin. The leaf essential oil from *C. odorata* in Australia contains β-caryophyllene (34.2 %), α-humulene (6.3 %), sabinene (19.6 %), germacrene D (4.3 %), myrcene (3.2 %) and the minor compounds are linalool (1.8 %), δ-cadinene (1.2 %), α-thujene (2.6 %), γ-terpinene (2.3 %), α-terpinene (1.1%) [18]. The essential obtained from African origin contains β-caryophyllene (26.3 %), α-pinene (14 %), germacrene D (11.7 %), α-humulene (6.3 %), β-pinene (4.3 %) and some of the minor compounds were δ-cadinene (3.0 %), α-copaene (2.9 %), linalool (1.9 %), β-elemene (1.4 %), myrcene (0.6 %) [19]. The obtained results are almost similar in Australia and African origin. However, there were variations in their compositions; this may be due to climatic and geographical variations [20].

Table 1. Chemical composition of essential oil of *C. odorata* leaves

S. No.	Compounds	R.T	RI estimated	RI reported	% Composition
1	α-Thujene	3.9	915	918	0.6
2	Camphene	4.8	938	935	0.9
3	Sabinene	5.9	963	965	0.8
4	α-Myrcene	6.5	980	988	1.3
5	Phenylmethyl ester	6.8	1019	-	5.6
6	Limonene	7.6	1020	1018	1.3
7	Ocimene	8.2	1035	1032	13.2
8	Geraniol	8.5	1252	1249	1.3
9	β-Elemene	9.6	1334	1335	3.1
10	Copaene	10.3	1370	1374	2.9
11	*trans*-Caryophyllene	11.6	1425	1417	19.7
12	γ-Elemene	12.5	1430	1434	1.9
13	E,E-α-Farnesene	13.1	1500	1505	10.3
14	(-) Caryophyllene oxide	15.6	1595	1582	1.1
15	Cubenol	17.8	1625	1618	1.9
16	t-Muurolol	18.4	1634	1640	4.2
17	α-Cubebene	20.9	1644	1638	1.9
18	α-Cadinol	22.1	1648	1652	3.0
19	Farnesol	23.2	1652	1688	3.7
20	Benzyl benzoate	23.6	1732	1739	2.3
21	*trans*-Farnesol	24.2	1740	1742	2.1
22	1,4-bis (Ethynyl) butane	28.5	1756	-	0.9
23	2-Ethyl-3-methylthiirane 1,1-dioxide	30.2	1768	-	1.6
24	Muurolene	39.4	1779	1779	0.4
25	Farnesyl acetone	44.1	1911	1913	5.1
	Total identified				91.1
	Monoterpene hydrocarbons (S.No: 1-4,6,7)				18.1
	Sesquiterpenes hydrocarbons (S.No: 9-13,17,21 ,24)				42.3
	Oxygenated compounds (S.No: 5,8,14-16,18-20,22,23,25)				30.7

***In-vitro* antioxidant activity**

The present study examines the antioxidant efficiency of essential oil of *C. odorata* leaves by DPPH assay and it showed a concentration-dependent antiradical activity and was given in Table 2 by inhibiting DPPH radical with EC_{50} value of 19.5 ± 1.25 µg/mL. BHA was used as a standard with EC_{50} value of 1.10 ± 0.55 µg/mL. DPPH is a purple colored free-radical which in reaction to the plant-based product changes to the yellow-colored stable compound and the extent of the reaction depends on the hydrogen releasing capacity of the antioxidant [21,22]. So the essential oil of *C. odorata* can stop the chain reactions of free radicals by forming stable compounds.

From the reported results the ethyl acetate extract of the stem bark of *C. odorata* was revealed to exhibit the maximum % of DPPH inhibition (79 %) when compared to other investigations [23]. Meanwhile, the antioxidant property of that methanol extract of *C. odorata* leaves were studied using ferric ion reducing power assay, indicated overall 290.0 ± 13.1 % ferric reducing power around 0.5 µg /mL[24]. Another study using DPPH assay of the essential oil from *C. odorata* flowers showed IC_{50} value of 86.56 µg/mL and

Table 2. *In-vitro* antioxidant activity of essential oil of *C.odorata* leaves for DPPH assay

Conc. (µg/mL)	DPPH· % inhibition
10	33.16±0.38
20	52.52±0.37
40	65.84±0.37
60	73.61±0.51
80	78.16±0.24
100	81.05±0.28
EC_{50} (µg/mL)	19.50±1.25
Standard	BHA
EC_{50} (µg/mL)	1.10±0.55

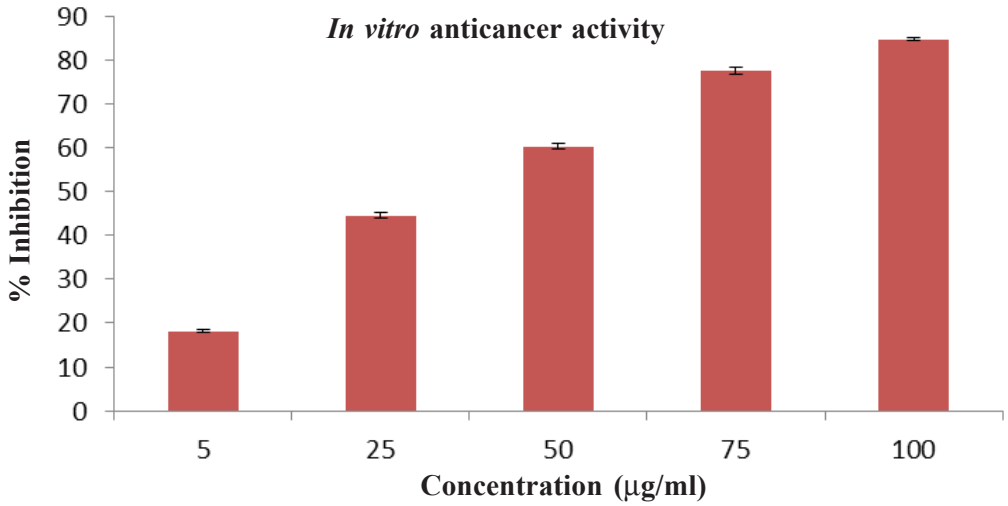

Figure 1. Percentage of cell inhibition at various concentrations

Ascorbic acid as a standard [25]. There is no report on *in vitro* antioxidant activity of essential oil from *C. odorata* leaves, the results revealed that the leaf essential oil possesses potent antioxidant activity compared to stem and flowers.

In vitro anticancer activity

The current study revealed the *in vitro* anticancer potential of *C. odorata* essential oil was tested by MOLT-3 cell line by using MTT assay. The viability of the blood cancer cells after incubation with different concentrations of essential oil from *C. odorata* leaves were carried out. The incubation with different concentrations of essential oil of the sample (5, 20, 50, 75, 100 µg/mL) affected the viability of the cancer cell line (MOLT-3). The graph was plotted between % cell inhibition and concentrations from which IC_{50} value was calculated and shown in Fig. 1. Essential oil of *C. odorata* leaves showed the anticancer activity on the MOLT-3 cancer cell line in dose-dependent pattern. At lower levels, cancer cell decomposition is minimal (25 µg/mL), while almost all cancer cells break down at 100 µg/mL. The IC_{50} was calculated to be 44.22 µg/mL. From the result, the essential oil extracted from *C. odorata* leaves have potent *in vitro* anticancer property. Previously *in vivo* antitumor growth was tested using flower essential oil against EAC treated mice which inhibited the tumor growth [26]. This is the first kind of report against *in vitro* anticancer activity of essential oil of *C. odorata* leaves grown in South India.

Conclusions

The chemical composition of essential oil of C.

odorata leaves was analyzed by GC/MS method. A total of 25 components were identified. The *in vitro* anticancer and *in vitro* antioxidant activities of essential oil of *C. odorata* leaves were evaluated for MOLT-3 cell line by MTT assay and DPPH assay respectively. The essential oil showed concentration-dependent activity. From the results, the essential oil of *C. odorata* leaves has significant anticancer and antioxidant activities due to the complex mixture of various terpenes present in the essential oil could act as a potent anticancer agent. It was concluded that *C. odorata* essential oil has significant antioxidant and anticancer properties which can be utilised as natural supplements as antioxidant and anticancer agents in pharmaceutical and food industries since the plant is available throughout the year. Further exploration is required not only *in vitro* but *in vivo* as well, to verify and establish their activity for their future use in the battle against cancer.

Conflicts of Interest

The authors declare no conflicts of interest in this work.

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