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Evaluation of *in vitro* Cytotoxicity of Essential Oil from *Clausena dentata* (Willd) Grown in Western Ghats Region, South India

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The present study examines the *in vitro* cytotoxicity of essential oil from *Clausena dentata* against the HeLa Cancer cell line and NIH 3T3 normal fibroblast by using methyl thiazol tetrazolium assay. The hydrodistilled essential oil of *C. dentata* was analyzed by GC-MS and results revealed that the chemical composition of essential oil of *C. dentata* contains 23 compounds. The major chemical compositions of oil were sabinene (19.70 %), β-caryophyllene oxide (17.90 %), *cis*-(R,2R)-hydroxy-2-methylindane (18.40 %) and ethanone 1,2-hydroxy-5-methylphenyl (7.85 %). The minor compounds were α-myrcene (3.48 %), spathulenol (2.44 %), isospathulenol (1.22 %), caryophyllene (1.08 %), curzerene (1.01 %), nerol (0.23 %), α-pinene (0.65 %), neryl acetate (0.47 %) *etc*. The *in vitro* cytotoxicity was investigated by methyl thiazol tetrazolium assay against HeLa and NIH-3T3 cell lines with different concentrations of essential oil (18.5-300 µg/mL). The results revealed that the essential oil showed concentration dependant activity against both employed cell lines. The IC_{50} value of HeLa and NIH3T3 were 75.3 and 99.83 µg/mL, respectively.

Keywords: *Clausena dentata*, Sabinene, β-Caryophyllene oxide, HeLa and NIH3T3 cell lines.

INTRODUCTION

According to World health organization (WHO), cancer is the second most major disease in the world. Now days 60 % of the available cancer drugs are derived from plant based pro-ducts, vinblastine and vincristine are very famous cancer drugs for long period. Still many commercial drugs are emerging for treating cancer with enormous side effects and unaffordable cost. Recently much research is focused on plant based products for anticancer property with less adverse effects, low cost and more efficiency. *Clausena dentata* (Wild) is a small tree belongs to Rutaceae found in Asia including China, Sri Lanka and India [1]. It is called kattukariveppilai in Tamil, due to its medicinal and nutritional value the local people of Valparai, Yercaud and Boda Hills are used for many purposes [2]. Traditionally the leaf juice is applied to the injured eye and leave paste is used for curing wounds [3,4]. The chemical composition of essential oil of the *C. dentata* in different part of the world were sabinene, borneol, δ-cadinol, β-bisabolol, biofloratriene α-pinene, β-amyrine β-caryophyllene and β-pinene [5-8]. The secondary metabolites like α-clausenan, rosesenan (α-clausenan), diclausenans A and B and furano terpenes [9]. Two coumarins were isolated and named dentatin and nordentatin from root bark of *C. dentata* by spectral identification [10]. Saponins and terpenoids were found in *C. dentata* plant extracts. The ethanol extract of this plant was evaluated for hepatotoxicity study in rats [11]. The essential oil has ovicidal, mosquito repellent and larvicidal activities [5,6]. From the literature survey, there are only few reports are available for its essential oil composition and its pharmacological activities. There is no report is available for *in vitro* cytotoxicity of essential oil. In this view, our present investigation was aimed to evaluate the chemical composition and *in vitro* cytotoxicity of essential oil of leaves of *C. dentata.*

EXPERIMENTAL

Fresh leaves of *C. dentata* were collected from Valparai hills between the periods of December-January 2017. The plant material was identified and authenticated by Department of Botany, NGM College, Pollachi, Coimbatore, India. The voucher specimen (16CHE009) was preserved in the Chemistry Department.

Isolation of essential oil: About 500 g of fresh leaves was subjected to hydrodistillation using Clevenger type apparatus for 3 h. The oil obtained was dried over anhydrous sodium sulphate and stored in a container and kept in freezer until GC-MS analysis.

GC-MS analysis: The gas chromatogram was recorded in Agilent make with GC 7890 with Mass detector 5975C with DB-5 column having 95 % polydimethylsiloxane with 5 %

phenyl group. For GC/MS detection, electron ionization with ionization energy of 70 eV was used. Helium gas (99.999 %) was used as the carrier gas at a constant flow rate of 1 mL/min and injection volume was 1 µL (split ratio 10:1). Injector temperature was 250 °C; ion-source temperature 260 °C. The oven temperature was programmed from 70 °C (isothermal for 2 min), with an increase of 25 °C/min, to 150 °C (hold 10 min), then 25 °C/min to 260 °C, ending with 40 min isothermal at 260 °C. Total run time was 59.6 min. Software adopted to handle mass spectra and chromatogram was a chemstation and compounds are identified from the NIST library match. Petroleum ether, ethyl acetate and methanol were purchased from Finar chemicals.

***In vitro* cytotoxicity activity by methyl thiazol tetrazolium assay:** Human cervical cancer cell line (HeLa) and mouse embryonic fibroblast (NIH-3T3) was obtained from National Centre for Cell Science (NCCS), Pune. The HeLa and NIH-3T3 cells were grown in eagles minimum essential medium (EMEM) containing 10 % fetal bovine serum (FBS) and NIH 3T3 fibroblasts were grown in dulbeccos modified eagles medium (DMEM) containing with 10 % FBS. For the screening experiment, the cells were seeded into 96-well plates in 100 µL of the respective medium containing 10 % FBS, at plating density of 10,000 cells/well and incubated at 37 °C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h prior to addition of essential oil. The essential oil was solubilized in dimethylsulfoxide and diluted in the respective medium containing 1 % FBS. After 24 h, the medium was replaced with respective medium with 1 % FBS containing the oil at various concentrations (12.5 to 300 µg/mL) and incubated at 37 °C, 5 % CO_2, 95 % air and 100 % relative humidity for 48 h. Triplicate was maintained and the medium containing without oil served as control. After 48 h, 10 µL of methyl thiazol tetrazolium (5 mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37 °C for 4 h. The medium with methyl thiazol tetrazolium was then flicked off and the formed formazan crystals were solubilized in 100 µL of DMSO and then measured the absorbance at 570 nm using micro plate reader [12]. Non-linear regression graph was plotted between % cell inhibition and $\log_{10}$ concentration and IC_{50} was determined using Graph pad prism software.

RESULTS AND DISCUSSION

GC-MS analysis: The essential oil from the leaves of *C. dentata* was analyzed by GC-MS. *C. dentata* essential oil was yellow in colour. A total of 23 compounds representing 92.3 % of the essential oil was identified. The major chemical compositions of oil were sabinene (19.70 %), β-caryophyllene oxide (17.90 %), *cis*-(R,2R)-hydroxy 2-methylindane (18.40 %) and car-2-en-4-one (7.85 %). The minor compounds were α-myrcene (3.48 %), spathulenol (2.44 %), isospathulenol (1.22 %), himoquinone D (1.38 %), caryophyllene (1.08 %), curzerene (1.01 %), nerol (0.23 %), α-pinene (0.65 %), neryl acetate (0.47 %), *etc*. The chemical composition of essential oil from pasirimalai forest region showed 12 components and major compounds were sabinene (28.57 %), borneol (14.62), δ-cadinol (12.49), β-bisabolol (15.56 %) and biofloratriene (18.54 %) [5]. Vietnamese plant leaf essential oil contains α-pinene (21.7 %), sabinene (18.35 %), β-amyrine (14.3 %) β-caryophyllene (8.9 %) and β-pinene are the major compounds [7]. Similarly, 14 different compounds were obtained by GC-MS analysis in which sabinene of 21.27 %, biofloratriene of 19.61 %, borneol of 18.34 % and β-bisabolol of 17.68 % were the major compounds [6]. The chemical composition of essential oil of *C. dentata* from Valparai Hills showed diffe-rent composition may be due to different climatic conditions, seasonal and geographical reasons [13].

***In vitro* cytotoxity:** We employed methyl thiazol tetrazolium (MTT) assay, a simple and reliable technique, which measures cell viability for screening the cytotoxic activity. The viability of cancer cells after incubation with different concen-

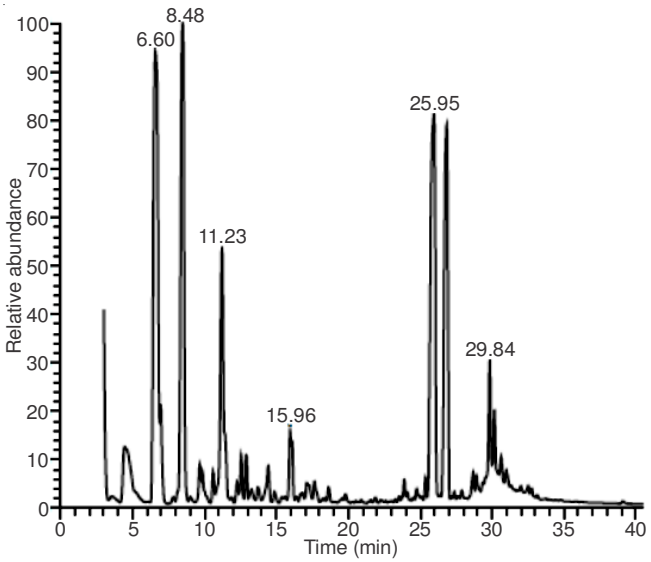

Fig. 1. GC-MS Chromatogram of essential oil of *C. dentata*

TABLE-1
GC-MS ANALYSIS OF VOLATILE COMPOSITION OF ESSENTIAL OIL OF *C. dentate*

S. No.	Compound name	RT	Composition (%)
1	Nerol	3.58	0.23
2	α-Myrcene	4.46	3.48
3	Sabinene	6.58	19.70
4	Propanal, 2-metyl-3-phenyl	6.96	0.65
5	β-Caryophyllene oxide	8.48	17.19
6	2,6-Dimetyl-2,4,6-octatrienedial	9.68	1.32
7	Neryl acetate	10.61	0.47
8	(+)-Car-2-en-4-one	11.23	7.85
9	Thymoquinone	12.56	1.38
10	Germacrene D	12.91	0.77
11	Cubedol	13.27	0.34
12	e-Cadinene	13.72	0.34
13	Caryophyllene	14.45	1.08
14	Hedycaryol	14.88	0.28
15	(+)Spathulenol	15.96	2.44
16	Humulene oxide	16.81	0.28
17	Isospathulenol	17.12	1.22
18	α-Cadinol	17.67	0.53
19	Ursuline	23.91	0.55
20	Phytol	25.37	0.33
21	*cis*-(1R,2R)-1-Hydroxy-2-methylindane	25.97	18.40
22	6-Methyl-4-indanol	26.85	14.45
23	Curzerene	28.68	1.01

tration of *C. dendata* essential oil are reported in Fig. 2. The effect of *C. dentata* essential oil on the viability of HeLa and NIH 3T3 cell lines showed concentration dependant activity. Data showed that incubation with different concentrations of oil affected the viability of human cervical cancer cell line (HeLa) and NIH 3T3 mouse embryonic fibroblasts. The IC_{50} values of both cell lines were 75.33 and 99.83 µg/mL respectively. The results revealed that the normal cell line NIH-3T3 requires more concentration compared to HeLa cancer cell line which showed that the essential oil of *C. dentata* is less toxic towards the normal cell line. The essential oil of *C. dentata* possesses anticancer activity. It may be attributed due to the presence of terpenes like caryophylline oxide, sabanine and pinene, *etc*. There is no report in the literature for cytotoxicity of *C. dentata* leave essential oil, this is the first kind of report against anticancer activity.

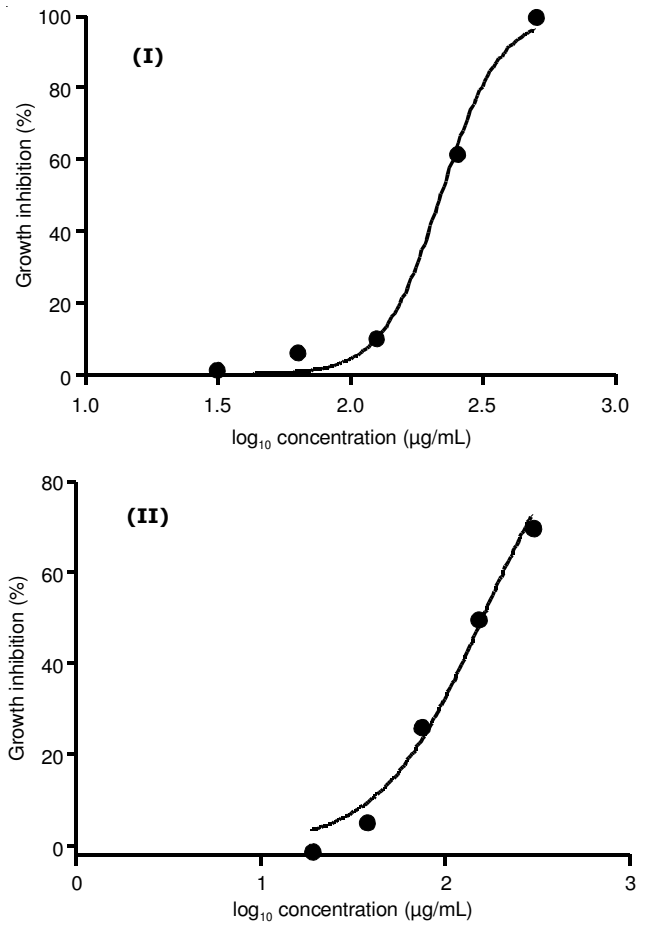

Fig. 2. Effect of essential oil of *C. dentata* against (I) HeLa cancer cell line and (II) NIH3T3 normal cell line

Conclusion

The chemical composition of essential oil of *C. dentata* grown in Western ghats region was analyzed by GC-MS method. A total of 23 components was identified. Sabanene (19.70 %), α-myrcene (3.48 %), β-caryophyllene oxide (17.90 %) and ethanone, car-2-en-4-one (7.85 %) were the major compounds in *C. dentata*. *In vitro* cytotoxicity activity of essential oil of *C. dentata* was evaluated against HeLa cancer cell line and NIH-3T3 by methyl thiazol tetrazolium assay. Essential oil showed concentration dependent activity on both cell lines. The IC_{50} value is 75.3 and 99.833 µg/mL respectively. From the results, *C. dentata* essential oil has significant anticancer activity this may attributed to the presence of important terpenoids.

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