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In-vitro Anticancer Activity and Essential oil Composition of *Tridax procumbens* (L.)

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ABSTRACT

The present study investigates the chemical composition of essential oil of leaves of *Tridax procumbens* (L.) and its *invitro* anticancer activity. Traditionally, the plant is used for malaria, stomachache, high blood pressure, hemorrhage and to prevent hair fall as well. It possesses antifungal, antibacterial, antiseptic, insecticidal, parasiticidal and hepatoprotective properties. The hydrodistilled essential oil of *T.procumbens* contains total of 18 components by GC-MS analysis. Dibutyl phthalate (19.29%), Trans-(α)-caryophyllene (9.55%), Biformeme (3.95%), p-cymen-7-ol (2.52%), 1,8-cineole (2.44%). And the minor compounds are trans-α- Bergamotol (1.78%), 2-α-pinene (1.62%), α-Selinine (1.49%), Caryophyllene oxide (1.39%), α-humulene (0.95%), The obtained essential oil was tested against Human breast cancer cell line (MCF-7) for its anticancer activity by MTT assay with different concentrations of essential oil (18.5-300 µg/ml). The result revealed that the essential oil showed concentration dependent activity against cell line. The IC_{50} value of MCF-7cell line was 96.6 µg/ml. This may be due to the presence of important terpenes present in the oil. There are only few reports are available for anticancer activity efficacy of this plant. Our findings confirm the anticancer potential of the *T.procumbens*.

Keywords: *T. procumbens,* Dibutyl phthalate, Trans-(α)-caryophyllene, Biformeme and MCF-7

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INTRODUCTION

In India most of the diseases are cured by herbal and its related products. Most of the cancer treating medicines is derived from plants. Due to advancement of medicinal field many commercial cancer drugs are discovered to cure various types of cancer with adverse side effects and unaffordable by low income people. Recently much research is focus on every possible plant based products like crude extracts, essential oil etc, has tested for anticancer studies. *Tridax procumbens* L. is a common medicinal plant belongs to Asteraceae family and is commonly known as "coat buttons" , it is native to tropical America and introduced to various parts of the world.[1,2] Traditionally the various parts of the *T.procumbens* used for reducing blood pressure, bronchial catarrh, malaria, stomach ache, diarrhea, dysentery, wound healing, conjunctivitis, inflammatory conditions and also prevents hair fall and check hemorrhage from cuts in India.[3,4] Moreover various solvent extracts as well as essential oil have been reported for many biological activities like antimicrobial, anti-inflammatory, antioxidant, wound healing, antidiabetic and hepatoprotective activities.[4-12] there are many chemical constituents were reported from the *T.procumbens* such as alkaloids, flavonoids, carotenoids, β- sitosterol, fumaric acid, quercitin, linoleic acid and myristic acids.[13-14] Almost all terpenes have biological activities in animals and play important role in human medicine. Hence there is a growing interest in terpenes in their role to cure diseases and provide scientific evidences. GC-MS is the best way to analyze the essential oil and extracts from the plants.[9] From the literature only few reports are available for anti cancer activity with limited cancer cell lines, so our present study focus the chemical composition present in the essential oil of *T.procumbens* fresh leaves and its anticancer activity against human breast cancer cell line (MCF-7) using MTT assay.

MATERIALS AND METHOD

Plant materials

Fresh leaves of *T.procumbens* were collected from home garden in pollachi between the periods of December to January 2017. The plant material was identified and authenticated by Department of Botany, NGM College, pollachi, Coimbatore, Tamilnadu. The voucher specimen (16CHE010) was preserved in the Chemistry department.

Isolation of essential oil

The essential oil was extracted from about 500g of fresh leaves and flowers by hydrodistillation using a Clevenger apparatus for 3hrs.The essential oil recovered was dried over anhydrous sodium

sulphate, the amount was measured and stored in a container and kept in freezer at 4 ^{0}C until GC-MS analysis.

Analysis of the essential oils

The GC-MS analysis of the essential oil was carried out on a Agilent system consisting of model 6890N gas chromatograph, a model 5975 inert mass selective detector (EIMS, electron energy 70eV, scan range 50-500 amu, and scan rate 2 scan per second), and a agilent chem station data system. The GC column was an HP-5 fused silica capillary with a (5% phenyl) – methyl poly siloxane stationary phase, filling thickness of 0.25 µm, a length of 30m, and an internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 7.07 and flow rate of 1.0ml/min. Inlet temperature was 220^o C and MSD detector temperature was 325 ^{0}C. The GC oven temperature program was used as follows: 85°C for 2 min, 85^o – 230^{o}C at 6^{o}C/min, 230^{o}C for 5 min, 230^o – 300^{o}C at 4^{o}C/min, ending with 10 min at 300^{o}C. The sample was dissolved in 10 ml of acetone: toluene (1:1) mixture. 1 µL injections using a split less injection technique was used. Identification of oil component was achieved based on their retention indices, and by comparison of their mass spectral fragmentation patterns with those reported in the literature and stored on the MS library [NIST database (G1036A, revision 0.01.00) / chem. station data system (0.02.275, version 2.0d)]

Anti cancer activity using MTT assay

Human breast cancer cell line (MCF-7) was obtained from National Centre for Cell Science (NCCS), Pune. The MCF-7 was grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS).

For screening experiment, the cells were seeded into 96-well plates in 100µl of respective medium containing 10% FBS, at plating density of 10,000 cells/well and incubated at 37^{0}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 respective medium containing 1% FBS. After 24 h, the medium was replaced with respective medium with 1% FBS containing the essential oil at various concentration (18.5µg/ml, 37.5 µg/ml, 75 µg/ml, 150µg/ml and 300 µg/ml) and incubated at 37^{0}C, 5% CO_2, 95% air and 100% relative humidity for 48h. Triplicate was maintained and the medium containing without oil served as control.

After 48h, 10µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4h. The medium with MTT 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.

Determination of IC_{50}

% cell inhibition was determined using the following formula and Nonlinear regression graph was plotted between % Cell inhibition and Log_{10} concentration and IC_{50} was determined using GraphPad Prism software.

% cell Inhibition = 100 – Abs (drug)/Abs (control) x100.

RESULTS AND DISCUSSION

Hydrodistilled essential oil was white color about 0.4% (v/w) of yield. It volatile composition was analysed by GC-MS. Total of 16 components were identified and represents 99% of the detected oil composition. The results were given in table 1. The major compounds of *T.Procumbens* was Dibutyl phthalate (19.29%), Trans-(α)-caryophyllene (9.55%) , Biformene (3.95%), p-cymen-7-ol (2.52%), 1,8-cineole (2.44%). The minor compounds are Z-α-trans Bergamotol (1.78%), 2-α-pinene (1.62%), α-Selinine (1.49%), Caryophyllene oxide (1.39%), α-humulene (0.95%), tau-cadinol (0.85%), Eicosane (0.80%), Quercetin 7,3',4'-trimethyl (0.78%), α- Amyrin (0.73%), Tricosane (0.71%), α- Elemene (0.67%), 9,12,15-octadecatrienoic acid (0.66%).

Whereas the essential oil of *T.procumbens L* showed that the oil contains 14 compounds and the major compound was α-pinene, 1, 3, 6-octatriene, Camphene, β-pinene, Sabinene, Phellandrene, L-limonene, β-ocimene, Trans-beta-ocimene, Trans-Caryophyllene, Gama-elemene, Spathulenol, Torreyol and Aromadendrene.[9] The flower essential oil contains (Z)-falcarinol (25.9%), followed by alpha-selinene (15.3%), limonene (8.3%) and zerumbone (4.3%). The DCM extract have reported to contains caryophyllene (62.97%), cedrene (11.85%), 3,7,11,15- tetramethyl-2-hexadecen-1-ol (6.43%), caryophyllene oxide (5.67%), trans-α-bergamotene (4.32%), and 3,5,11,15-tetramethyl-1-hexadecen-3-ol (3.10%) from hexane, and phytol (31.708%).[16] The exhaustive literature survey indicated that only few reports are available for essential oil composition of *T.procumbens*, and results revealed that α-pinene, β-pinene and caryophyllene was the major compounds in this plant. Our results showed similar compounds and some compounds are different such as Dibutyl phthalate, Biformeme, 1,8-cineole and p-cymen-7-ol. It is reported that the β-pinene generally accompany α-pinene in low quantities in the volatile extracts, essential oleoresins and oils of plants. Some specific studies have shown that the β-pinene, along with α-pinene and other terpenes are cytotoxic on cancer cells.[17] so an attempt was made to evaluate the anti cancer activity of *T.procumbens* against MCF-7 cell line (human breast cancer cell line) by MTT assay which measures the cell viability of *T.procumbens* essential oil and the result, was shown in the figure (2). The essential oil was diluted to 18.75µg/ml, 37.5µg/ml, 75µg/ml,

150µg/ml and 300µg/ml. The viability of cancer cells after incubation was calculated. The essential oil of *T.procumbens* showed significant cytotoxicity on MCF-7 cancer cell line with dose dependent pattern. The IC_{50} value of the essential oil of *T.procumbens* observed was 96.6µg/ml.

Table 1: Chemical composition of essential oil of *T.procumbens*

S. No	Compound	RT	AREA %
1	2-α-pinene	4.38	1.62
2	1,8-Cineole	5.43	2.44
3	p-Cymen-7-ol	6.51	2.52
4	α-Elemene	10.57	0.67
5	Trans (a)-caryophyllene	11.34	9.55
6	α- Humulene	12.24	0.95
7.	α-Selinene	13.19	1.49
8.	Caryophyllene oxide	16.04	1.39
9	Cadinol	17.22	0.85
10	Z-α-trans-Bergamotol	18.11	1.78
11	Eicosane	21.56	0.80
12	Biformene	24.56	3.95
13	Dibutyl phthalate	24.96	19.29
14	Tricosane	27.23	0.71
15	Quercetin 7,3,4-trimethyl	29.79	0.78
16	α-Amyrin	30.32	0.73
17	Epiabietol dehydro	32.22	1.80
18	9,12,15-Octadecatrienoic acid	38.62	0.66

CONCLUSION

The chemical composition of essential oil of *T.procumbens* grown in Western Ghats region was analyzed by GC-MS method. Total of 18 components were identified The major compounds of T.Procumbens was Dibutyl phthalate (19.29%), Trans-(α)-caryophyllene (9.55%), Biformene (3.95%), p-cymen-7-ol (2.52%), 1,8-cineole (2.44%). The *in-vitro* anticancer activity of essential oil of *T.procumbens* was evaluated for MCF-7 cell line by MTT assay. Essential oil showed concentration dependent activity on MCF-7 cell line. The IC_{50} value is 96.6µg/ml. From the result *T.procumbens* essential oil has significant anticancer activity this may attributed to the presence of important terpenes like α-pinene and β-pinene.

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