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Studies on Phytochemical Composition and Anticancer Potential of Methenolic Leaf Extract of *Tecomella Undulata*

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Contents

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Abstract

The methanolic leaf extract of *Tecomella undulata* were tested for their anticancer potential against MCF-7, breast cancer cell line. The extracts Tecomella undulata were found to inhibit the growth of MCF-7, breast cancer cell lines. The methanolic leaf extracts of *Tecomella undulata* showed a remarkable inhibition in the maximum concentrations of 150 & 300µg/mL to an extent of 99.5% of cell growth. The lower concentration of the extract 18.75µg/mL showed 1.2%, 37.5µg/mL is 10.55% while 75µg/mL inhibited 96.7% of the cell growth. The IC_{50} value for the methanolic leaf extracts of *Tecomella undulata is* 49.01µg/mL. The regression value is 0.9998µg/mL.

Keywords: *Tecomella undulata*, MCF-7

1. Introduction

The plants are the natural reservoir of medicinal agents almost free from the side effects normally caused biosynthetic chemicals. The World Health Organization(WHO) estimates that herbal medicine is still the mainstay of about 75-80% of the world's population, mainly in the developing countries for primary health care because of better cultural acceptability, better compatibility with the human body, and lesser side effects. The over-use of synthetic drugs with impurities resulting in higher incidence of adverse drug reactions has motivated mankind to go back to nature for safer remedies. Due to varied locations where these plants grow, coupled with the problem of different vernacular names, the WHO published standards for herbal safety to minimize adulteration and abuse (Doughari, *et al* 2009).Secondary metabolites are the classes of compounds which are known to show curative activity against several ailments in man, and therefore could explain the use of traditional medicinal plant for the

treatment of some illnesses. There are a number of chemical compounds (phenolic compounds, alkaloids, terpenoids, steroids, quinones, saponins, etc.,) with complex structures and with more restricted distribution than primary metabolites. They are not indispensable for the plant that contains them; at least their metabolic functions have not been discovered yet.

A Cancer cell also has the character of immortality even *in vitro* whereas normal cells stop dividing after 50-70 generations and undergoes a programmed cell death (Apoptosis). Cancer cells continue to grow invading nearby tissues and metastasizing to distant parts of the body. Metastasis is the most lethal aspect of carcinogenesis (Block, 1991A and Block, 1991B).

2. Materials and Methods

Collection of plant materials

The leaves of *Tecomella undulata* plants were collected from Coimbatore district and dried in shade. These leaves were then powdered and stored in air tight container at room temperature until further use.

Preparation of plant extract

10g of air dried powder were taken in 100mL of methanol. Plugged with cotton wool and then kept on arotary shaker at 190-220rpm for 24hours. After 24hours the supernatant were collected and the solvent were evaporated to make the final volume one-fourth of the original volume and stored at 40c in air tightcontainer.

Gas Chromatography Analysis

Gas Chromatography (GC) analysis was carried out using Varian 3800 gas chromatography equipped with mass selective detector coupled to front injector type 1079. The chromatography was fit with VF 5 MS capillary column (30 m × 0.25 mm). The injector temperature was set at 240 ºC, and the oven temperature was initially be at 70 ºC then programmed to 300º C at the rate of 10 ºC / minute and finally held at 300 ºC for 10 min. Helium was used as carrier gas with the flow rate of 1.51mL/min. The percentage of composition of extract was calculated by GC peak areas.

GC- MS Analysis

Gas Chromatography coupled with mass spectroscopy was performed using Varian 3800 gas chromatography equipped with Varion 1200 L single quadruple mass spectrometer. GC conditions were the same as reported for GC analysis and the same column was used. The mass spectrometer operated in the electron impact mode at 70eV. Ion source and transfer line temperature was maintained at 250 ºC. The compounds was identified based on comparison of their retention indices (RI), retention time (RT) and mass spectra

In Vitro Cytotoxicity Assay

The human breast cancer cell line (MCF 7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37^0 C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. 100µL per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h, the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) and diluted to twice the desired final maximum test concentration with serum free medium. Additional four, 2 fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulted the required final sample concentrations. Following drug addition the plates were incubated for an additional 48 h at 37^0 C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations **(**Mosmann. T, 1983).

MTT Assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

% cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC50 was determined using Graph Pad Prism software **(**Monks, *et al.*, 1991**).**

3. Results and Discussion

GC-MS Analysis

GC-MS analysis of methanolic leaf extracts of *Tecomella undulata* (Table-1 & Fig-1) showed the presence of 30 different compounds with various percentages. Out of these 30 compounds 2-Methoxy-4-vinylphenol (16.70%) were found to be maximum (in terms of percentage) followed by 2, 3-Dihydro-benzofuran (16.45%). Of this Glycerine-1,3-dimyristate, 2-O-trimethylsilyl- (0.71%) is found to be very least among the 30 compounds. The graphical representations of these compounds were given in (Fig.1). Various peaks were observed with regular interval depending the presence of the compounds. Variations among the peaks were observed with respect to the presence (%) of compounds. The retention time of the compounds varied between the amounts of compound present in the extract. The presence of these compounds may be the reason for the enormous medicinal properties of the plant. The compounds like Cyclohexanone, 2,2-dimethyl- (CAS) (2.44%), à-D-Glucopyranoside, O-à-D-glucopyranosyl-(1.fwdarw.3)-á-D-fructofuranosyl (1.38%), 2-Propenoic acid, 3-phenyl-, methyl ester (1.26%), Benzaldehyde, 2-hydroxy-4-methyl- (1.37%), 1,9-Octadecadiene, and 1-methoxy-, (?,Z)- (CAS) (0.74%) were also observed in minimum quantity or in slight trace.

The other compounds such as á-D-Glucopyranose, 1,6-anhydro- (1.08%), 9-Hydroxy-1-methyl-1,2,3,4-tetrahydro-8h-pyrido(1,2-a)pyrazin-8-one (1.19%), 10-Heptadecen-8-ynoic acid, methyl ester, (E)- (CAS) (0.87%), Neophytadien (2.23%) and 3,7,11,15-Tetramethyl-2 hexadecen-1-ol (1.24%) were also found to be present in GC-MS analysis of *Tecomella undulata.* Out of 30 compounds present in the extract only very few compounds are found to be more in terms of percentage and the rest were either very limited or slight trace has been observed.

Table 1: The chemical composition of methanolic leaf extract of *Tecomella undulata*

Peak	R .T	Probability	Molecular Formula	Area%	Name
1	4.94	35.25	C5H10S	1.47	Thietane, 2,4-dimethyl- (CAS)
2	6.13	25.03	C8H16O2	5.00	2-Propyl-tetrahydropyran-3-ol
3	8.20	79.93	C8H14O	2.44	Cyclohexanone, 2,2-dimethyl- (CAS)
4	10.23	42.81	C8H8O	16.45	2,3-Dihydro-benzofuran
5	11.50	16.80	C18H32O16	1.38	à-D-Glucopyranoside, O-à-D-glucopyranosyl-(1.fwdarw.3)-á-D-fructofuranosyl
6	12.15	55.13	C9H10O2	16.70	2-Methoxy-4-vinylphenol
7	13.55	41.55	C10H10O2	1.26	2-Propenoic acid, 3-phenyl-, methyl ester
8	15.20	10.60	C8H8O2	1.37	Benzaldehyde, 2-hydroxy-4-methyl-
9	15.64	14.31	C19H36O	0.74	1,9-Octadecadiene, 1-methoxy-, (?,Z)- (CAS)
10	16.82	38.08	C12H22O11	8.20	à-D-Glucopyranoside, á-D-fructofuranosyl (CAS)
11	18.07	44.93	C6H10O5	1.08	á-D-Glucopyranose, 1,6-anhydro-
12	18.43	48.77	C9H12N2O2	1.19	9-Hydroxy-1-methyl-1,2,3,4-tetrahydro-8h-pyrido(1,2-a)pyrazin-8-one
13	18.95	36.92	C18H30O2	0.87	10-Heptadecen-8-ynoic acid, methyl ester, (E)- (CAS)
14	19.79	28.00	C20H38	2.23	Neophytadien
15	20.45	7.06	C17H32O2	0.76	E-11-Methyl-12-tetradecen-1-ol acetate
16	20.82	15.56	C20H40O	1.24	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
Peak	**R .T**	**Probability**	**Molecular Formula**	**Area%**	**Name**
17	21.64	11.02	C10H14O2	1.95	(5S*,6R*)-5,6-Dimethyl-3,4,5,6-tetrahydro-2H-cyclopenta[b]pyra n-7-one
18	22.81	58.42	C17H34O2	0.95	Pentadecanoic acid, 14-methyl-, methyl ester (CAS)
19	23.09	58.77	C10H12O3	1.67	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol
20	23.82	64.49	C16H32O2	2.62	Hexadecanoic acid (CAS)
21	25.72	36.97	C19H38O4	1.36	Hexadecanoic acid, 2,3-dihydroxypropyl ester (CAS)
22	26.34	55.56	C20H40O	2.37	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-(CAS)
23	27.81	23.93	C26H44O5	2.83	Ethyl iso-allocholate

24	28.20	26.49	C21H36O4	2.50	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-
25	29.11	31.55	C29H48O	6.26	Stigmasterol
26	30.88	41.77	C28H20N6 NiO6	2.05	Nickel-1,5,9,13-tetraaza-2,6,10,14tetrabenzo-4,12-dinitro-cyclohexadec-4,8,12,16-tetra-ene
27	32.28	8.17	C17H16O4	4.20	Benzoic acid, 4-methyl-, [4-(methoxycarbonyl)phenyl]methyl ester
28	33.77	23.21	C34H68O5 Si	0.71	Glycerine-1,3-dimyristate, 2-O-trimethylsilyl-
29	33.98	8.53	C26H24O4	1.48	7á,9á:8à,10à-Bis(dimethylmethylenedioxy)-7,8,9,10 Tetrahydrobenzo[a]pyrene
30	38.21	41.74	C30H50	6.67	Squalene

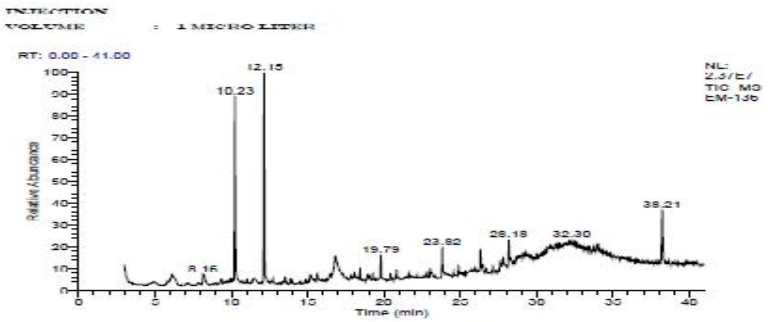

Figure 1: The GC-MS analysis of methanolic leaf extract of *Tecomella undulata*

Anticancer studies

The methanolic leaf extract of *Tecomella undulata* were tested for their anticancer potential against MCF-7, breast cancer cell line. The extracts Tecomella undulata were found to inhibit the growth of MCF-7, breast cancer cell lines **(Table-2; Fig. 3 & 4 ;)**. The methanolic leaf extracts of *Tecomella undulata* showed a remarkable inhibition in the maximum concentrations of 150 & 300µg/mL to an extent of 99.5% of cell growth. The lower concentration of the extract 18.75µg/mL showed 1.2%, 37.5µg/mL is 10.55% while 75µg/mL inhibited 96.7% of the cell growth. The Ic_{50} value for the methanolic leaf extracts of *Tecomella undulata is* 49.01µg/mL. The regression value is 0.9998µg/mL.

Table 2: The anticancer activity of methanolic leaf extract of *Tecomella undulata* using MCF-7 cell line

Plant extract conc (µg/mL)	% inhibition (µg/mL)	IC_{50} (µg/mL)	R^2 (µg/mL)
18.75	1.13122	49.01	0.9998
37.5	10.55807		
75	96.75716		
150	99.01961		
300	100		

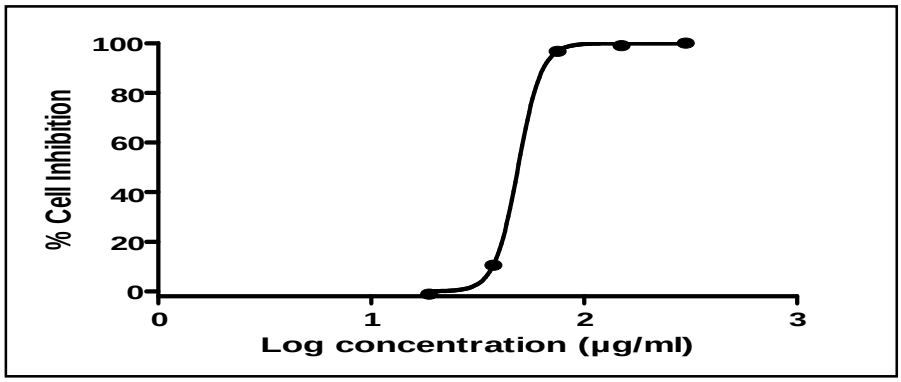

Figure 3: The % of growth inhibition of cancer cell line against methanolic leaf extract of *Tecomella undulata*

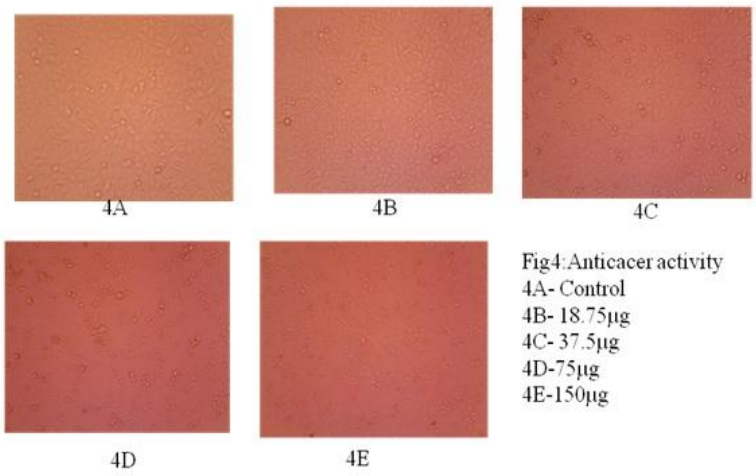

Figure 4: The anticancer activity of different concentration of methanolic leaf extract of *Tecomella undulata* using MCF-7

4. Conclusion

The wide spectrum of antibacterial, antioxidant and anticancer activity may be due to the presence of the chemical compound identified in *Tecomella undulata* through GC-MS analysis. The present study confirms the valuable chemicals present in the plants and further thorough studies may bring out the real potential of these widely used medicinal plants in the preparation of antibiotic, antioxidant and anticancer drugs.

5. References

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