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GC–MS ANALYSIS FOR COMPOUND IDENTIFICATION IN LEAF EXTRACT OF *ACANTHUS ILICIFOLIUS* AND EVALUATION OF ITS *IN VITRO* ANTICANCER EFFECT AGAINST MCF-7 CELL LINES

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ABSTRACT

The current study focused on GC-MS analysis for identification of bioactive compounds in leaf extract of mangrove plant, *Acanthus ilicifolius* leaf extract was tested for cytotoxicity against MCF-7 cell lines. The spectral properties (GC-MS) of each separated compound were determined and found the different compounds namely aziridine, alcohols, phenolic and fatty acid compounds. GC-MS analysis proved that the leaf extract of *Acanthus ilicifolius* contain a high content of ethanol compounds. The ethanol extract showed potent cytotoxicity against Human breast adenocarcinoma (MCF-7) cell lines, the viability of cancerous cells is reduced to 52.37% for MCF-7 cells. The high anticancer activity was found against Michigan Cancer Foundation (MCF-7) cell lines.

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INTRODUCTION

The plant investigation has opened up a new respective bio-pharma research. The mangrove plants were used in development of potential antioxidants. Mangroves are specific group of salt tolerant plants that grow within the coast regions of tropical and sub-tropical along the coastlines. Mangroves have been used in folk medicine for treatment of several diseases (Saranraj and Sujitha, 2015; Prabhu and Devaraj, 2016). The mangrove plants contain many bioactive compounds and medicines these compounds were obtained from the roots, leaves, fruits and flowers of mangrove species which are used for treatment of different human diseases namely (lung, stomach, colorectal, liver and breast cancer) (Reddy and Grace, 2016). Cancer is one of the major causes of death in the world, and it starts with the damage of DNA caused by genetic mutations (Kooti *et al*., 2017; Valastyan and Weinberg, 2011). Cancer is a painful disease and fighting against this disease is very important for public health. The advancements in phytochemical research of herbal products proved that the plant extracts are used as popular sources for treatment of cancer. The therapeutic molecules from natural sources being biodegradable are preferred over the synthetic molecules because of their comparative safe and effective nature (Ahmad *et al*., 2016). Plant derived novel Bioactive and secondary metabolites such as vincristine, vinblastine, etoposide, paclitaxel, camptothecin, topotecan, and irinotecan are reported for the treatment of cancer (Azam *et al*., 2016). Many researchers worked on the analysis of bioactive molecules from mangrove forest plants due to their demand of therapeutic applications. The mangrove plants contain secondary metabolites namely: Flavonoids, tannins and phenols and these compounds studied from the extracts mangroves and have toxicological and pharmacological importance (Piyusha *et al*., 2012; Philip *et al*., 2009). In recent years, GC-MS technique is well proved for analysis of different bioactive compounds, from the plant extracts (Dineshkumar and Rajakumar, 2016). The many mangrove species were identified which are rich in antioxidants, these compounds used in treatment of anticancer (Das *et al*., 2015). Some studies, reported that the leaf extracts of mangrove plant *Phoenix paludosa* contain bioactive compounds and have been reported for

cytotoxicity and antioxidant activity (Samarakoon *et al*., 2016). The mangrove plant species namely: *Avicennia alba, Excoecaria agallocha* and *Rhizophora apiculata* contains the bioactive molecules having anticancer and antioxidant activity (Satyavani *et al*., 2015; Miranti *et al*., 2018). Till date no reports are found on the anticancer activity from the leaf extracts of *Acanthus ilicifolius* and hence, the present study focused on identification of different compounds from the leaf exacts of *Acanthus ilicifolius* and further it is evaluated for cytotoxicity effect against Michigan Cancer Foundation (MCF-7) cell lines.

MATERIAL AND METHODS

Preparation of the leaf extract

The leaves of *Acanthus ilicifolius* L. *(Acanthaceae)* were collected freshly from Alapakkam area, Cuddalore district, Tamilnadu during the month of January 2018. Plant specimens were authenticated by Dr. T. Manikandan, Professor, Department of Botany, Arignar Anna Government Arts college, villupuram, Tamilnadu. The fresh leaves of *Acanthus ilicifolius* were washed thoroughly, cut into small pieces, dried under shade completely at room temperature. Dried materials were ground into coarse powder and stored in air tight for further works. The powdered leaf samples (100g each) was immersed separately in different solvents including ethanol. The cold percolation was carried out for three times in solvents (300ml each) with increasing polanty to ensure exhaustive extraction. After 72hrs, the extracts were filtered through whatman filter paper No-1 and were concentrated under reduced pressure at 40˚C using rotary vaccum evaporator. This was stored in cold condition from 2˚C to 8˚C for further use in subsequent experiments, (Petal, 2017).

GC-MS analysis: The GC –MS analysis was carried out using a Thermo GC-Trace ultra ver: 5.0. Gas chromatograph was equipped and coupled to a mass detector Thermo MS DSQ II containing DB35 –MS , 30mx 0.25 mm ID x 0.25 µm df capillary column. The instrument was set to an initial temperature of 110ºC and maintained at this temperature 2 minutes. At the end of this period the oven temperature was rose up to 280 ºC at the rate of an increase of 6ºC/min and maintained for 9 minutes. Injection port temperature was ensured as 250 ºC and Helium flow rate as 1ml/min and ionization voltage of 70eV. The samples (1µl) were injected in split mode as 10:1. Mass spectral scan range was set as 45 – 450 (m/z). The time at which each component eluted from the GC column was termed as Retention time (RT). The eluted component was detected in the Mass detector. The spectrum of the unknown components were compared with that of known components listed in the NIST library and ascertains the name, molecular weight and structure of the components of the test materials in GC-MS study (kulkarni *et al*., 2015).

MTT ASSAY: The ethanol extract was tested for in vitro cytotoxicity using MCF-7 cell lines using 3- (4,5 dimethylthiozol-2-yl)- 2,5 diphenyl tetrazolium bromide solution (MTT) Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, (MTT, Sigma) solution (5mg/ml) was added to each well and incubated at 37°C for 4hrs. The medium was removed and formazan, was dissolved in acidified isopropanol (0.4N HCl). The amount of MTT formazan is directly proportional to the number of living cells and was determined by measuring the optical density (OD) at 570nm and 630nm using a ELISA reader (biorad, USA). The concentration of the crude extract that killed 50% of the cells (IC50) was calculated. The results were analysed and photographs were taken using an Epifluorescent microscope at 400X magnifications. All determinations were performed in triplicate (Bhat, 2017).

RESULT AND DISCUSSION

GC-MS chromatogram of ethanol leaf extract of *Acanthus ilicifolius* showed 30 peaks indicating presence of 30 compounds (Fig. 1). The mass spectral fingerprint of each compounds identified using the data library and molecular weight, molecular formula and compound names are listed in (Table 1). Previously it has been reported that the methanolic leaf extract of mangrove plant, *Avicennia marina* contain different molecules (Almardeai *et al*., 2017).

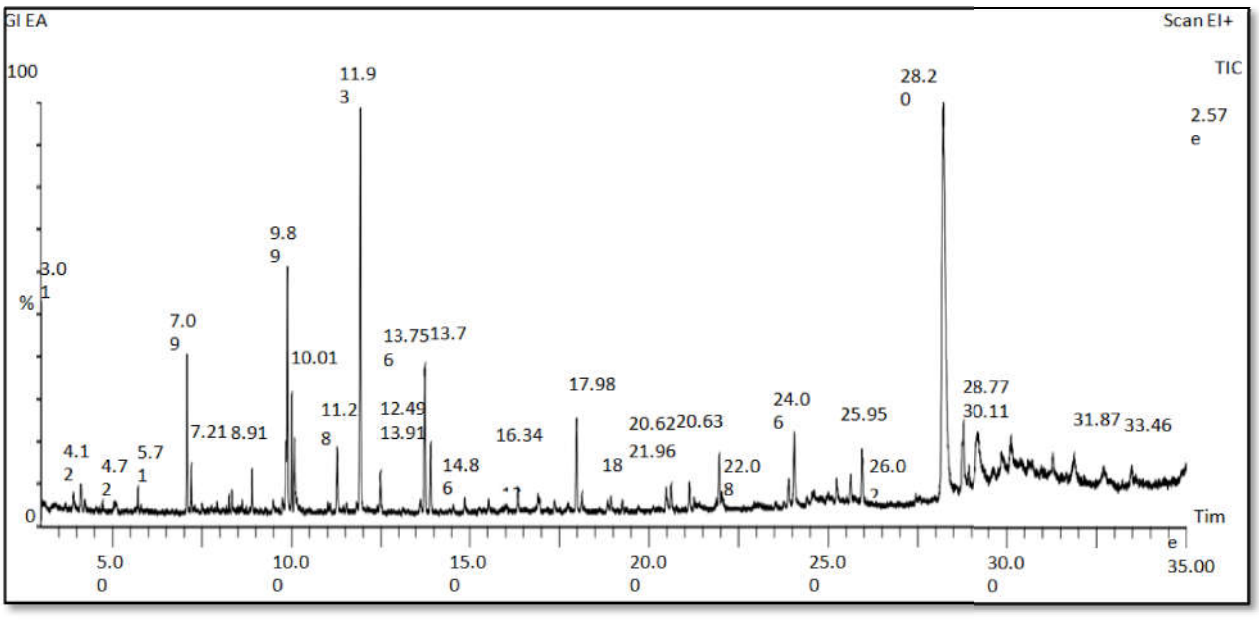

Fig. 1. GC-MS Chromatographic profile of ethanol extract of leaves of *Acanthus ilicifolius*

Table 1. Bioactive compounds detected from ethanol extract of *Acanthus ilicifolius*

Peak No	Retention time (Min)	Compound name	% Total	Mol. Formula	Mol. Wt
1	5.975	Aziridine, 1,2,3-trimethyl-, trans-	0.739	C5H11	85.15g/mol
2	14.828	3-O-Methyl-d-glucose	1.630	C7H14O6	194.18g/mol
3	21.276	n-Hexadecanoic acid	2.143	C16H32O2	256.42g/mol
4	21.681	5,8,11-Eicosatrienoic acid, (Z)-, TMS derivative	0.202	C20H34O2Si	378.66g/mol
5	21.961	Hexadecanoic acid, ethyl ester	0.216	C18H36O2	284.5g/mol
6	24.077	Phytol	0.185	C20H40O	296.5g/mol
7	24.437	9,12-Octadecadienoic acid (Z,Z)-	0.815	C18H32O2	280.4g/mol
8	24.562	trans-13-Octadecenoic acid	2.338	C18H34O2	282.5g/mol
9	24.787	Dieldrin	0.287	C12H8Cl6O	380.9g/mol
10	25.012	Octadecanoic acid	1.356	C18H36O2	285.5g/mol
11	27.473	Hexadecanoic acid, 1-(hydroxymethyl)-1,2- ethanediyl ester	0.351	C35H68O5	568.91g/mol
12	27.908	Gitoxigenin	0.176	C23H34O5	390.5g/mol
13	28.008	Hexadecanoic acid, 2-bromo-	0.201	C16H31BrO2	335.32g/mol
14	28.253	Azafrin	0.208	C27H38O4	426.6g/mol
15	28.618	Cholest-5-en-3-one	0.227	C27H44O	384.6g/mol
16	29.184	21-Hydroxyprogesterone, trifluoroacetate	10.030	C21H30O3	330.5g/mol
17	29.294	21-Hydroxyprogesterone, trifluoroacetate	9.397	C21H30O3	330.5g/mol
18	29.794	9,12,15-Octadecatrienoic acid, 2,3- dihydroxypropyl ester, (Z,Z,Z)-	0.539	C21H36O4	352.5g/mol
19	29.864	Glycidyl oleate	0.830	C21H38O3	338.5g/mol
20	30.114	2-Ethylbutyric acid, eicosyl ester	2.316	C26H52O2	396.68g/mol
21	30.354	Hexadecanoic acid, 1-(hydroxymethyl)-1,2- ethanediyl ester	1.924	C35H68O5	568.91g/mol
22	30.614	Hexa-t-butylselenatrisiletane	0.794	C24H54SeSi3	505.9g/mol
23	30.814	Demecolcine	0.311	C21H25NO2	371.4g/mol
24	30.929	Olean-12-ene-3,15,16,21,22,28-hexol, (3á,15à,16à, 21á,22à)-	0.258	C30H50O6	506.7g/mol
25	31.430	Ursodeoxycholic acid	0.217	C24H40O4	392.6g/mol
26	31.475	17-(2-Hydroxy-1,5-dimethyl-hex-4-enyl)-4,4,10,13, 14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16, 17-tetradecahydro-1H-cyclopenta[a]phenanthrene	0.454	C30H50O	426.71g/mol
27	31.700	Trilostane	0.206	C20H27NO3	329.4g/mol
28	32.785	10,12,14-Nonacosatriynoic acid	0.335	C29H46O2	426.7g/mol
29	32.900	8,14-Seco-3,19-epoxyandrostane-8,14-dione, 17- acetoxy-3á-methoxy-4,4-dimethyl-	0.923	C24H36O6	420.5g/mol
30	33.670	9,19-Cyclolanostan-3-ol, 24-methylene-, (3á)-	49.228	C33H54O2	482.8g/mol

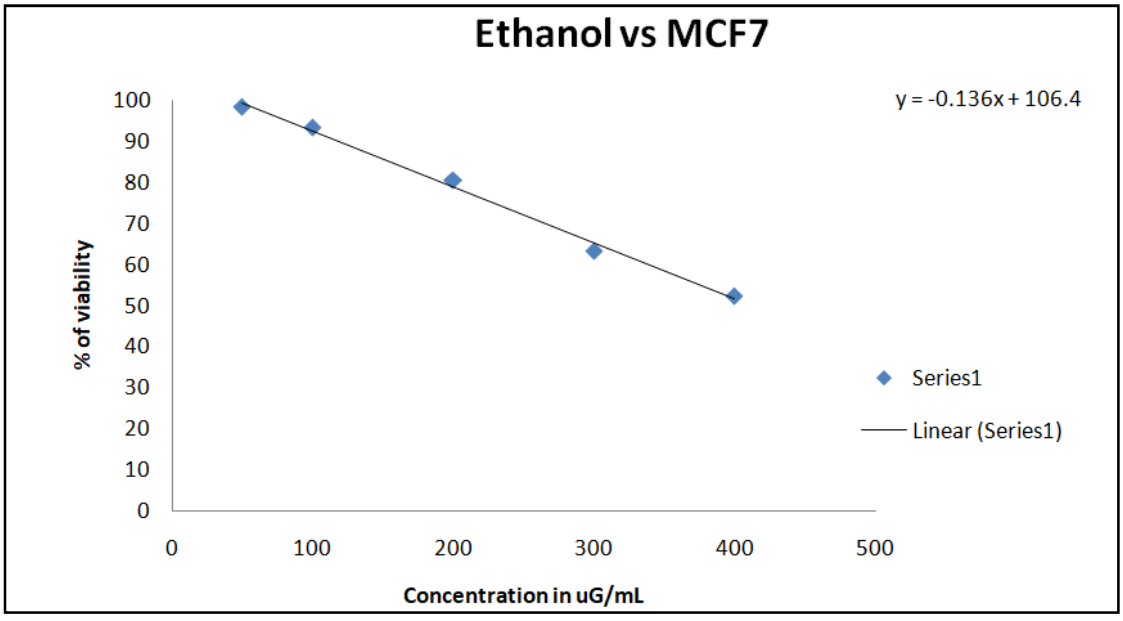

Fig. 2. Cytotoxic activity of *Acanthus ilicifolius* leaf extract against MCF-7 cell lines

The phytol was found in leaves of mangrove plant *Rhizophora mucronata* and this compound has role in decrease of the cell aging and cholesterol and acts as anticancer agent and also controls blood glucose. Phytol extracted from *Rhizophora mucranata* showed cytotoxicity against Human gastricadeno carcinoma (AGS) cells (Panjaitan and Suprajitnob, 2018). The studies of Ramalingam and Rajaram (2018) proved that the extracts from mangrove plant *Rhizophora apiculate* showed the presence of phenolic compounds, which are actively involved in anticancer activity. The phenolic compounds from *R. apiculate* inhibited the growth and induce the apoptosis through ROS generation against A549, lung cancer cells. It was reported that the ethanolic extract of mangrove plant *Avicennia germinans* leaves contain the bioactive compounds namely: 9-eicosene; 9, 10-anthracenedion (aromatic organic compound); Tetracosane (alkane); Tetratriacontane (alkane).

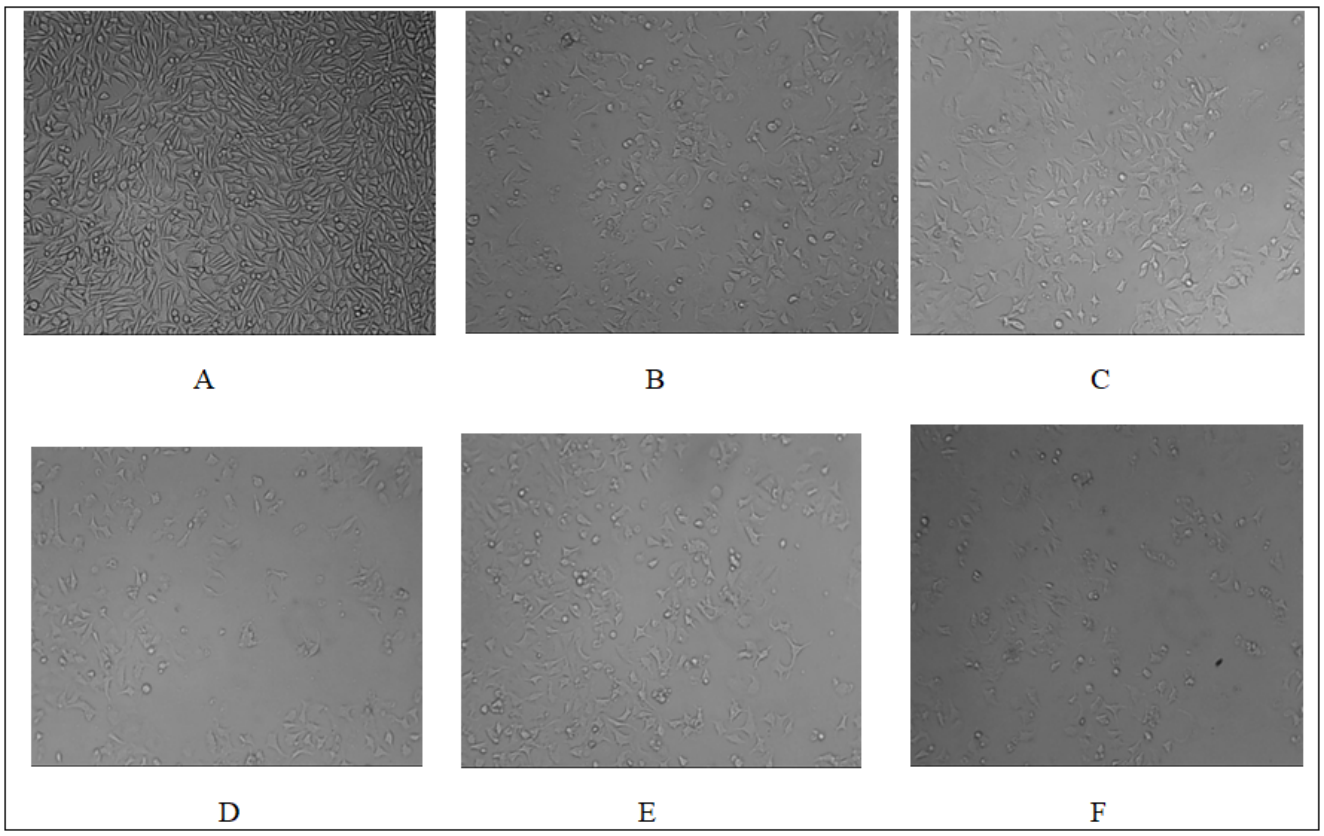

Fig. 3. The MTT assay of Acanthus *ilicifolius* against MCF-7 cell lines: A) Untreated MCF-7 cell lines and B-G represents the different concentrations leaf extract i.e., 50 µg, 100 µg, 200 µg, 300 µg, and 400µg

The compound Tetracosane has anticancer property against AGS, MDA-MB-231, HT-2918 and NIH 3T3 cell lines (Subathra and Mohideen, 2018). The studies from Kumar *et al*. (2013) reported that the ethanol extracts of *Ceriops decandra* leaves contain different phytochemical constituents identified as triterpenes, Clionasterol and Squalene and other compounds are Lupeol, Stigmast-5-en-3-ol and Diolein (Kumar *et al*., 2013). These studies provide, evidence that the mangrove species contains high potential bioactive compounds having anticancer and anti-tumor properties.

Anticancer activity against MCF 7 cell lines: The anticancer activity of ethanol leaf extract from mangrove plant *Acanthus ilicifolius* was tested against MCF-7 cell lines by MTT assay. The MTT assay results revealed that the cell line viability of treated cells decreased gradually with increase of the sample concentration. The maximum reduction of cell lines was found at the concentration of 400 mg/ml where the viability of cells lowers down to 52.37%. The extract obtained from leaf showed IC_{50} value of 414.70 (±0.055) lg/mL at 72 hrs on MCF-7 cell line. The inhibition of viable cell count of MCF 7 cell lines from the *Acanthus ilicifolius* leaf extract as represented in the Fig. 2. The study proved that after treatment with the leaf extract of *Acanthus ilicifolius* cell size is slowly reduced by the change of concentration of the sample and further the cells are detached from the surface (Fig. 3). The mangrove plant *Avicennia marina* extract reported for their anticancer effect against the cell lines: HL60, MDA- MB 231, and NCI-H23. The bioactive compound flavonoid enhances the anticancer activity and kills the human promyelocytic leukaemia cells by apoptosis mechanism. The ethanol leaf extract of *Acanthus ilicifolius* exhibited significant anticancer activity (Thatoi *et al*., 2016).

Conclusion

The findings of present study revealed that the mangrove plant *Acanthus ilicifolius* leaf extracts could be used as a potential alternative for development of bioactive leads in the treatment of cancer. The IC_{50} values clearly indicated, the anticancer activity of *Acanthus ilicifolius* leaf extract is high in-comparison with MCF-7cell line. These compounds from *A.ilicifolius* can be used in pharmaceutical industry for design and develop of novel lead drugs to treat cancer.

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