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

Vani, M. and ‘Dr. T. Manikandan

PG & Research Department of Botany, Arignar Anna Government Arts College, Villupuram-605 602, Tamil Nadu, India

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 extracts 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 110°C and maintained at this temperature 2 minutes.

At the end of this period the oven temperature was rose up to 280°C at the rate of an increase of 6°C/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 ascertainment 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
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 mucronata* showed cytotoxicity against human gastricadenocarcinoma (AGS) cells (Panjaitan and Suprajitno, 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 ethanol extract of mangrove plant *Avicennia germinans* leaves contain the bioactive compounds namely: 9,12-dihydroxypropyl ester, (Z)-hexadecanoic acid, ethyl ester; 9,10-Octadecadienoic acid; Tetratriacontane (alkane).
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<sub>50</sub> value of 414.70 (±0.055) µg/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<sub>50</sub> values clearly indicated, the anticancer activity of Acanthus ilicifolius leaf extract is high in-comparison with MCF-7 cell line. These compounds from A. ilicifolius can be used in pharmaceutical industry for design and develop of novel lead drugs to treat cancer.

**REFERENCES**


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