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Identification of bioactive compounds in leaf extract of *Avicennia alba* by GC-MS analysis and evaluation of its in-vitro anticancer potential against MCF7 and HeLa 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, *Avicennia alba* and the leaf extract of *Avicennia alba* was tested for cytotoxicity against MCF 7 and HeLa cell lines. The spectral properties (GC-MS) of each separated compound were determined and found the different compounds namely terpenoids, di-terpene alcohols, tri-terpenes and phenolic compounds. GC-MS analysis proved that the leaf extract of *Avicennia alba* contain a high content of phenolic compounds. The methanol extract showed potent cytotoxicity against Human breastadeno carcinoma (MCF7) and HeLa cell lines, the viability of cancerous cells is reduced to 44.68% for MCF 7 and 35.89% for HeLa cells. The high anticancer activity was found against HeLa cell lines than MCF 7 cell lines.

1. Introduction

The plant investigation has opened up a new respective biopharma 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 tropic and sub-tropic 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, barks and flowers of mangrove species which are used for treatment of different human diseases (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: alkaloids, steroids, phenols, and terpenoids 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 cancer (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: *Acanthus illicifolia, 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 *Avicennia alba* and hence, the present study focused on identification of different bioactive compounds from the leaf exacts of

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Avicennia alba and further it is evaluated for cytotoxicity effect against MCF 7 and HeLa cell lines.

2. Materials & methods

2.1. Extraction of bioactive compounds

Collect fresh and healthy leaf sample of *Avicennia alba* from Nizampatnam mangrove sanctuary, Guntur, Andhra Pradesh, India. The leaves are washed with tap water to remove dust and other organic matter present on surface of leaves and then airs dried under shade at room temperature, and then make it into the fine powder with pestle and mortar. The powdered sample used for extraction of bioactive molecules. The quantity 100 g of powder sample of *Avicennia alba* was mixed with 200 ml of methanol and then the test sample was subjected extraction with Soxhlet apparatus for 72 h. After completion of extraction, the solvent was distilled off and concentrated was air dried (Patel, 2017).

2.2. GC-MS analysis

The analysis of compounds in crude extract of *Avicennia alba* was performed by Gas Chromatography (Agilent 6890 series GC -MS) equipped with HP-5MS column (diameter length 30 m; diameter 0.25 mm; film thickness 0.25 μm) mass spectrometer programmed at temperature 30 °C – 280/300 °C with hold time 5 min with rate 10 °C/min. The conditions of chromatography are column flow rate was 1.2 ml/min, injection mode: split and carrier gas was Helium 99.999%. The compounds were identified by GC-MS spectra with mass library search (NIST based AMDIS software) with their relative retention indices (Kulkarni et al., 2015).

2.3. MTT assay

Cytotoxicity of methanolic leaf extract with different concentrations was assessed on MCF 7 and HeLa cell lines using (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide) (MTT) assay by mitochondrial succinate dehydrogenase, then followed by 48 hrs incubation. Assay plates were read using spectrophotometer at 520 nm. Data generated were used to plot a dose-response curve of which the concentration of extract required to kill 50% of cell population (IC_{50}) was determined. Calculations: % viability = (OD of test material/OD of control) × 100 and % Inhibition = 100- (% viability) (Bhat, 2017).

3. Results and discussion

3.1. GC-MS analysis

GC-MS chromatogram of methanolic leaf extract of *Avicennia alba* showed 12 peaks indicating presence of 12 compounds (Fig. 1). The mass spectral fingerprint of each compound can be identified from the data library. From the chromatogram peaks, found different bioactive leads which includes: terpenoids, silicones, phenolic compounds and fatty acid and the detailed of each compound was listed in Table 1. Among all, one unknown compound was found i.e., 3- (3-Fluoroanilino)-1-(3-nitrophenyl)-1-propanone (7.954%). This compound was not reported previously and it is first time reported from mangrove plant *Avicenia alba.* In previous studies reported that the presence of butanoic acid and pentyl ester along with other 19 different compounds in the methanolic extract of marine mangrove, *Rhizophora apiculata* and the extracts from this mangrove is also proved for anti-inflammatory and anticancer activity (Prabhu and Guruvayoorappan, 2012). 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

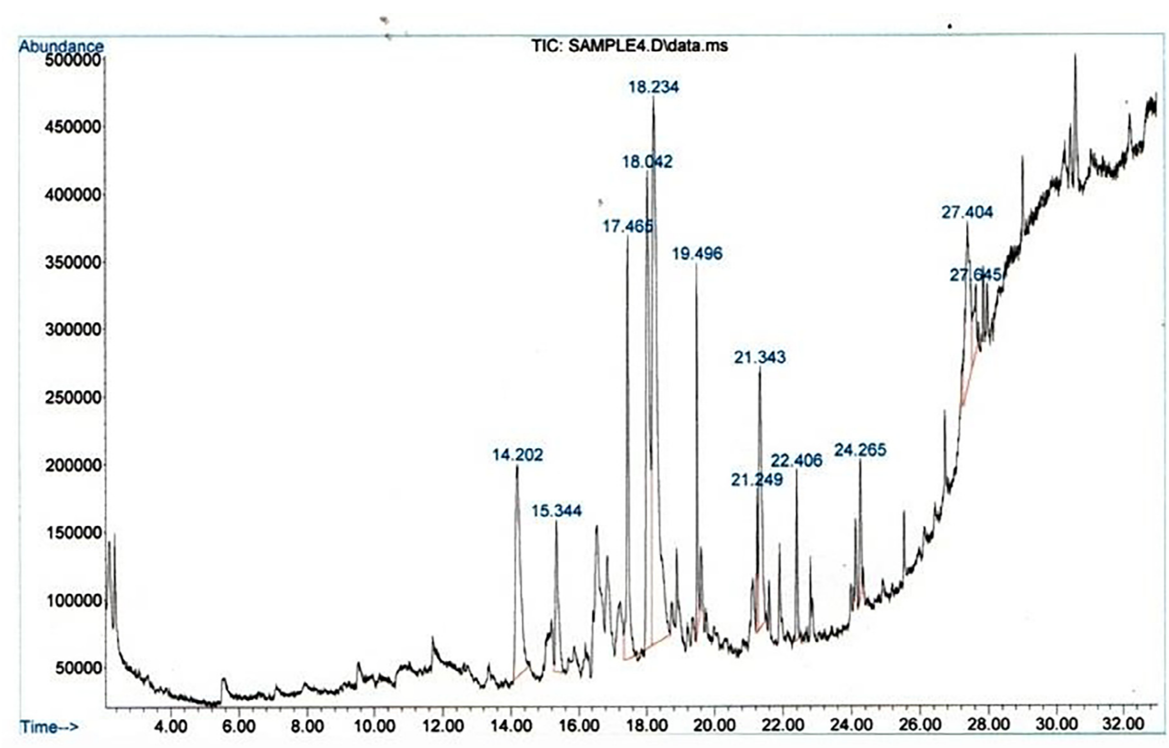

Fig. 1. GC-MS Chromatographic profile of methanol Extract of leaves of *Avicennia alba*.

Table 1
Bioactive compounds detected from Methanol extract of *Avicennia alba*.

Peak No	Retention time (Min)	Compound name	% Total	Mol. Formula	Mol. Wt
1	14.202	Catechol borane	10.888%	$C_6H_4BO_2$	118.906 g/mol
2	15.344	2-Methoxy -4- vinyl phenol	4.573%	$C_9H_{10}O_2$	150.18 g/mol
3	17.465	Cyclohepta Siloxane, Tetra deca methyl	9.317%	$C_{14}H_{42}O_7Si_7$	519.078 g/mol
4	18.042	Neophytadiene (7,11,15 Trimethyle -3- methylene -1- hexadecene	16.989%	$C_{20}H_{38}$	278.52 g/mol
5	18.234	Hexadecanoic acid	29.499%	$C_{16}H_{32}O_2$	256.43 g/mol
6	19.496	Docosanoic acid (Behenic acid)	4.079%	$C_{21}H_{44}COOH$	340.59 g/mol
7	21.249	3,7,11,15 Tetra methyl -2-hexadecene-1-ol	1.481%	$C_{20}H_{40}O$	296 g/mol
8	21.343	3- (3-Fluoro anilino)- 1- (3-nitrophenyl) -1- Propanone	7.954%	$C_{15}H_{13}FN_2O_3$	288.274 g/mol
9	22.406	Hexa decanoic acid – methyl ester	2.386%	$C_{17}H_{34}O_2$	270.457 g/mol
10	24.265	Phytol	2.337%	$C_{20}H_{40}O$	296.539 g/mol
11	27.404	Alpha amyrin	8.219%	$C_{30}H_{50}O$	426.73 g/mol
12	27.645	Docosanoic acid, Methyl ester	2.277%	$C_{23}H_{46}O$	354.619 g/mol

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). 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 methanol 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.

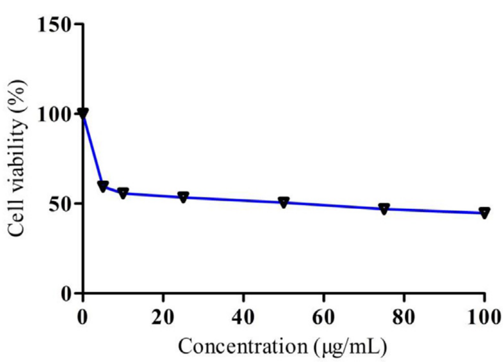

Fig. 2. Cytotoxic activity of *Avicennia alba* leaf extract against MCF-7 cell lines.

3.2. Anticancer activity against MCF 7 cell lines

The anticancer activity of methanol leaf extract from mangrove plant *Avicennia alba* was tested against MCF 7 and HeLa 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 100 μg/ml where the viability of cells lowers down to 44.68%. The extract obtained from leaf showed IC_{50} value of 57.02 (±0.03) μg/mL at 48 h on MCF-7 cell line. The inhibition of viable cell count of MCF 7 cell lines from the *Avicennia alba* leaf extract as represented in the Fig. 2. The study proved that

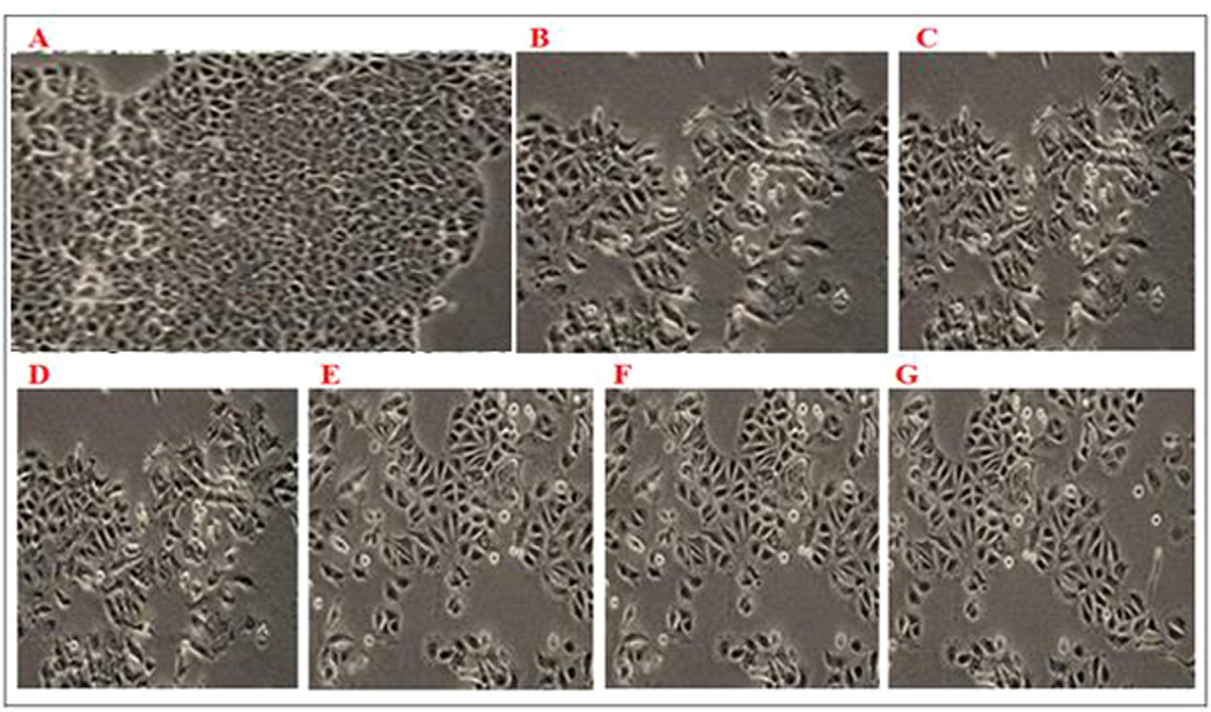

Fig. 3. The MTT assay of *Avicennia alba* against MCF 7 cell lines: A) Untreated MCF-7 cell lines and B-G represents the different concentrations leaf extract i.e., 5 μg, 10 μg, 25 μg, 50 μg, 75 μg and 100 μg.

after treatment with the leaf extract of *Avicennia alba* 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 methanol leaf extract of *Avicennia marina* exhibited significant anticancer activity (Thatoi et al., 2016).

3.3. Anticancer activity against HeLa cell lines

The MTT assay showed HeLa cells viability reduced with the increase in the concentration of leaf extract of *Avicennia alba.* The methanol extract showed significant activity at the concentration, 5 μg/mL. The HeLa cells viability reduced from 100% to 35.89% at concentration, 100 μg/mL. The extract obtained from leaf showed IC_{50} value of 44.30 (±0.04) μg/mL at 48 h on HeLa cell line. From the Fig. 4, it was clearly observed that the cell viability of HeLa cell lines reduced by the change in concentration of *Avicennia alba* leaf extract. For both cell lines, the cell viability data are expressed as mean and standard deviation (n = 6) and analyzed using one-way analysis of variance (ANOVA). A difference was considered statistically significant when $p \leq 0.05$. The morphological studies confirm that after treatment with the leaf extract, cells reduce in size to smaller and detached from the surface (Fig. 5). Khajure and Rathod in (2011) reported that the ethyl acetate extracts of *Acanthus ilicifolius* have the potent anticancer effect against the HeLa and KB cell lines. However, till-date no report is found on *Suaeda nudiflora* for anticancer potential against the MCF 7 and HeLa cells. Hence, the findings of this study proved that the extracts from this mangrove species could be developed as lead drug molecules.

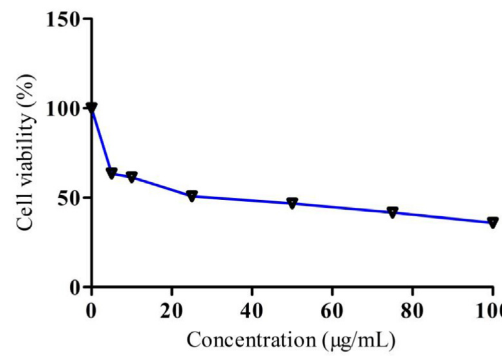

Fig. 4. Cytotoxic activity of *Avicennia alba* leaf extract against HeLa cell lines.

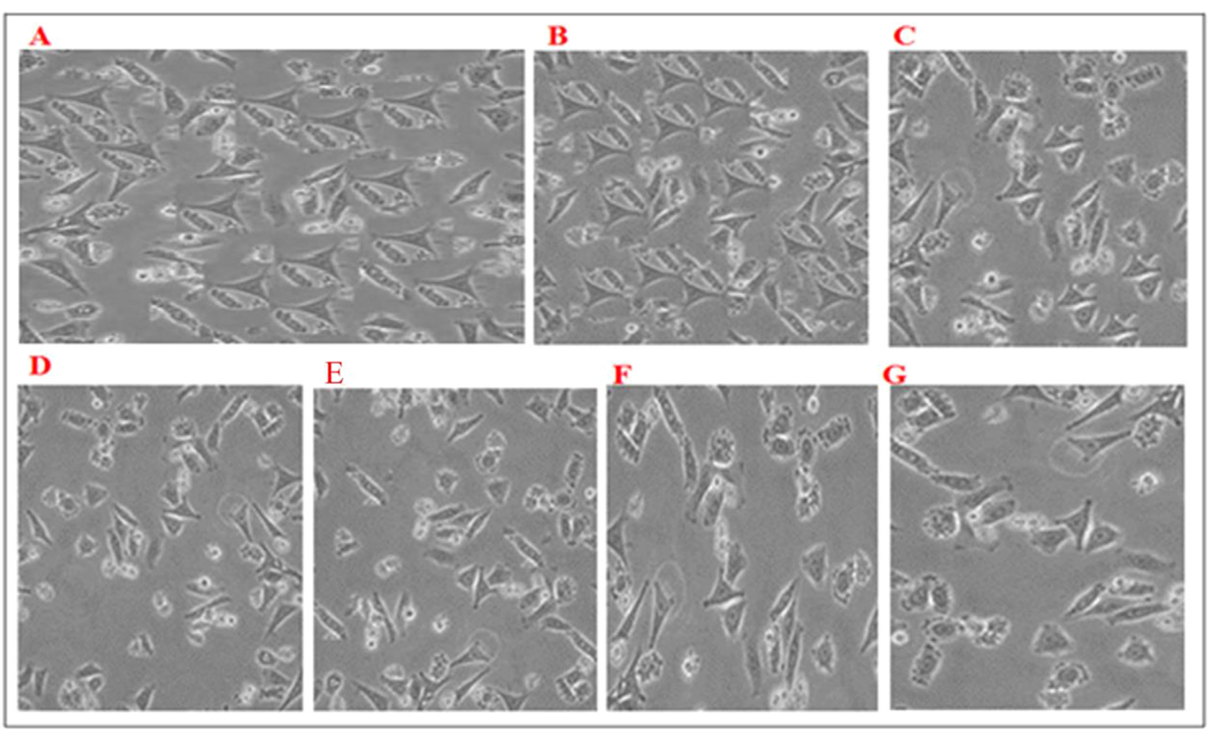

Fig. 5. The MTT assay of *Avicennia alba* against HeLa cell lines: A) Untreated HeLa cell lines and B-G represents the different concentrations leaf extract i.e., 5 μg, 10 μg, 25 μg, 50 μg, 75 μg and 100 μg.

4. Conclusions

The findings of present study revealed that the mangrove plant *Avicennia alba* 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 *Avicennia alba* leaf extract is high in-comparison with MCF 7cell line. Further studies will be carried In-vivo in the laboratory animal models for evaluation of the bioactive leads.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflicts of interest.

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