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GC–MS analysis for compound identification in leaf extract of *Lumnitzera racemosa* and evaluation of its *in vitro* anticancer effect against MCF7 and HeLa cell lines

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ABSTRACT

The aim of present study is to analyze and separate bioactive constitutes of methanol leaf extract from *Lumnitzera racemosa* by Gas chromatography–Mass spectroscopy (GC–MS) and further to test for *in vitro* anticancer activity on MCF 7 and HeLa cancer cells using MTT assay. GC–MS analysis of *L. racemosa* leaf extract showed the presence of different bioactive compounds mainly: furfurals and fatty acids. The bioactive compounds present in *L. racemosa* revealed significant cytotoxic activity against MCF 7 with IC_{50} value, 46.098 μg/mL and HeLa cells with IC_{50} value of 59.497 μg/mL. The leaf extracts of *L. racemosa* has high anticancer activity on MCF 7 cells in comparison with HeLa cell lines.

1. Introduction

Mangroves are specific group of salt tolerant plants which grow in the coastlines of tropic and sub tropic areas. Mangrove ecosystem is one of the highly diversified ecosystems and it tolerates adverse climatic conditions such as extreme salinity and temperatures. Mangroves play important role to balance ecosystem and act as a barriers to protect human life from tsunami, floods and provide shelter for many types of fauna and flora. The mangrove species produce a variety of compounds than terrestrial plants (Bandaranayake, 1998; Record et al., 2013). These plants are major source of phytochemicals and bioactive compounds used for design and development of new therapeutic compounds. The secondary metabolites produced from the plant extracts are being used in cancer treatment (Azam et al., 2016). Swamy and Sinniah (2015) reported that various drugs derived from plant sources are extensively used in pharmaceutical sector. The bioactive compounds like fatty acids, terpenoids and flavonoids are reported to be present in mangrove species (Nayak et al., 2018). Methanolic extract of bioactive compounds from mangroves has antioxidant, anticancer and anti-inflammatory activities and the major phytochemicals present in mangroves are flavonoids, triterpenoids and saponins (deMendonca et al., 2015). Mangrove plant species found to contain a variety of bioactive compounds which are effective against different human bacterial pathogens (Saravanan and Radhakrishnan, 2016). However, little work is available on the anticancer effect of *L. racemosa* leaf extracts and hence, the present article is focused on investigation of leaf extract for anticancer potential and also identification of bioactive constitutes present in the selected mangrove leaf extract.

2. Materials and methods

2.1. Sample preparation

The mature leaves from mangrove plant *L. racemosa* were collected from Nizampatnam mangrove vegetation. The leaves were washed under tap water, dried and then powdered. 50 g of powdered sample was soaked overnight in 100 ml methanol and then compounds extracted with soxhlet apparatus. The excess solvent was removed and then used as a test sample.

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2.2. Compound identification by GC–MS

The bioactive constitutes present in leaf extracts of *L. racemosa* were determined by Gas Chromatography (Agilent 6890 series) equipped with HP-5MS column mass spectrometer operated at initial column temperature of 30 °C and heated up to 300 °C at 10 °C /5min. Chromatographic conditions were: flow of 1.0 ml /min of high purity helium as carrier gas in split mode. The identification of the compounds in spectra was done based on retention time and integral area of peaks. The similarity of compounds matched with > 70% are listed based on NIST 5a library search (Kulkarni et al., 2015).

2.3. Anticancer activity by MTT assay

The methanol extract was tested for *in vitro* cytotoxicity using MCF 7 and HeLa cell lines by MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide) assay. To 100 μL of media, added 100 μL diluted plant extract followed by the addition of cell lines (6×10^5) into 96-well micro-titer, incubated at 37 °C for 48 h. MTT was added after incubation then left for 2 h until purple precipitate formed. The absorbance was measured at 520 nm. IC_{50} doses were calculated from a dose–response curve (Bhat, 2017).

3. Results and discussion

3.1. GC–MS analysis

GC–MS spectra of L*umnitzera racemosa* extract revealed the peaks that indicated the occurrence of different compounds (Fig. 1). The spectral fingerprint of compounds identified using the data library and molecular weight, the 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). The extracts of mangrove plants: *Acanthus ilicifolius, Excoecaria agallocha, Rhizophora apiculata* and *Rhizophora mucronate* had been reported for the presence of a wide range of bioactive compounds (Satyavani et al 2015). Raola and Chakraborty (2017) reported that the extracts of *Rhizophora mucranata* showed the presence of terpenoids, Olean-12-en-3-yl acetate which has the antimicrobial, antidiabetic, anti-inflammatory, antidepressant and anticancer activities. It was reported that six new diterpenes were isolated and extracted from mangrove plant *Ceriops tagal* having antitumor activity against different cancer cell lines (Yang et al., 2015). The bark extract of mangrove plant *Ceriops decandra* reported the presence of diterpenoids (Simlai et al., 2016).

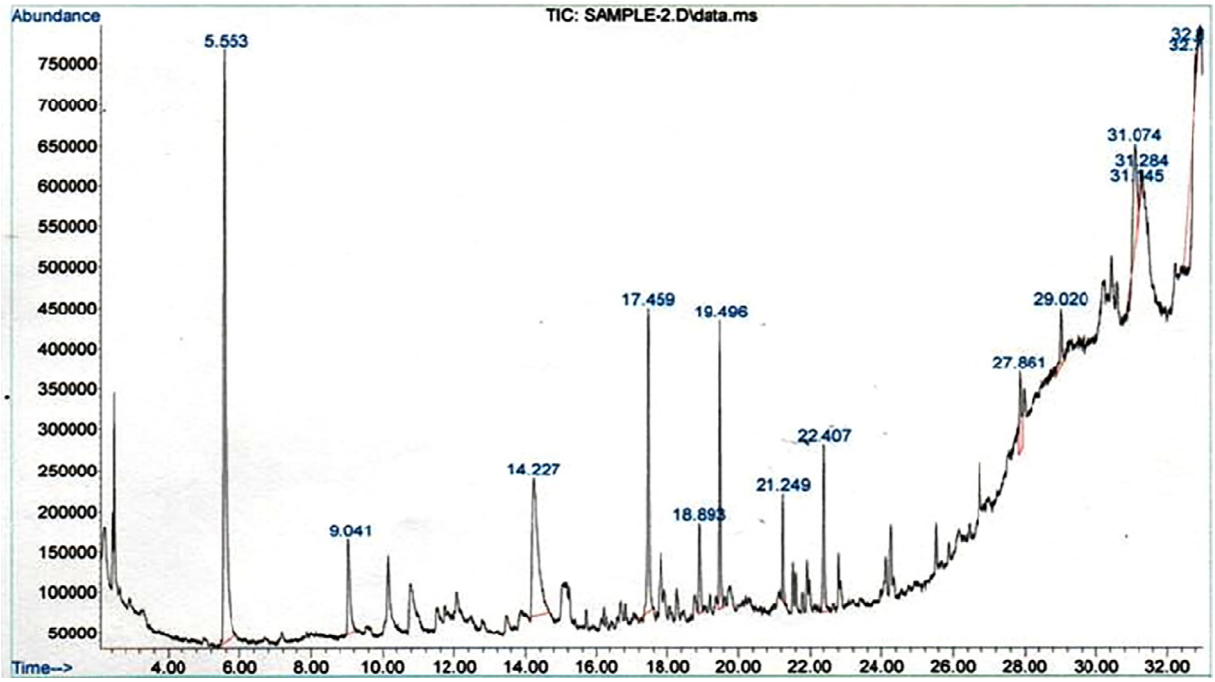

Fig. 1. GC–MS chromatogram represents the separated bioactive constitutes of *Lumnitzera racemosa* leaf extract.

Table 1
Bioactive compounds identified in leaf extract of *Lumnitzera racemosa.*

S.No	Retention time	Integrated peak area	Name of the compound	% Similarity matched with NIST05a Library	**Formula**	**Mol.Wt in g/mol**
1	5.568	34760228	Furfural	95	$C_2H_4O_2$	96.08
2	9.041	6029040	2-Furan Carboxaldehyde, 5-methyl	93	$C_6H_6O_6$	110.110
3	14.227	25100994	2-FuranCarboxaldehyde-5-(hydroxyl methyl)	70	$C_6H_6O_3$	126.111
4	19.496	5535591	Benzyle chloride	86	$C_6H_5CH_2Cl$	126.58
5	22.407	6138936	Hexa decanoic acid – methyl ester	99	$C_{17}H_{34}O_2$	270.457

3.2. Anticancer effect on MCF 7

Herbal medicine has significant role in the prevention of cancer and its treatment (Xiong et al., 2015). Anticancer study on MCF 7 cells showed that death rate of cancer cell lines increase with a rise in the concentration of *L.racemosa* leaf extract. 100 μg/ml of leaf extract of *L.racemosa* showed the maximum reduction in the MCF 7 cells viability (Fig. 2). The change in morphology of MCF 7 cell lines was clearly noticed in the microscopic images and with an increase in concentration of sample, the cells are found detached and also inhibited the growth of MCF 7 cells indicating that the cells are subjected to death (Fig. 3). It is also shown that there is the anticancer effect in the extracts *A. marina* on cell lines namely: HL60, MDA-MB 231 and NCI-H23 (Karami et al., 2012; Sukhramani and Patel, 2013). The effective antitumor effect has also been proved against the Hep G2, liver hepatocellular carcinoma cancer cell line from the methanolic leaf extract of *A. marina* (Shanthi et al., 2018). Mean and standard deviation (n = 6) are used to express cell viability data of both cell lines and ANOVA method is used to analyze data.

3.3. Anticancer effect on HeLa cells

In vitro HeLa cells slowly declined with an increase in concentration of *L. racemosa* leaf extract ranging from 100%, reduction of viability of HeLa cells to 43.05% at a concentration of 100 μg/mL (Fig. 4). After treatment with *L. racemosa* leaf extract theHeLa cells reduced in size as well as became detached from the surface as revealed through the HeLa cells morphology shown in Fig. 5. The extracts from *A. ilicifolius* had been reported for anticancer effect on HeLa and KB cells (Khajure and Rathod, 2011). The compound flavone from mangrove plant *Excoecaria agallocha* was also reported for anticancer effect against HeLa cells (Rajeswari et al., 2017). Similarly, Eswaraiah et al. (2019) reported the anticancer activity on MCF 7 and HeLa cells from *Avicennia alba* leaf extract. However, only very few species of mangroves reported for anticancer activity, till-date no reports have been found on *L. racemosa* leaf extracts for anticancer effect against MCF 7 and HeLa cells. Based on the results of the present study, it is confirmed that *L. racemosa* extract can be used as an alternative for development of molecules for anti-cancer therapy.

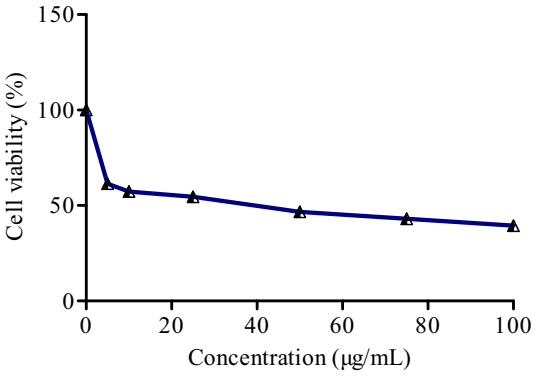

Fig. 2. Effect of different concentrations of *Lumnitzera racemosa* leaf extract on viability of MCF 7 Cell lines.

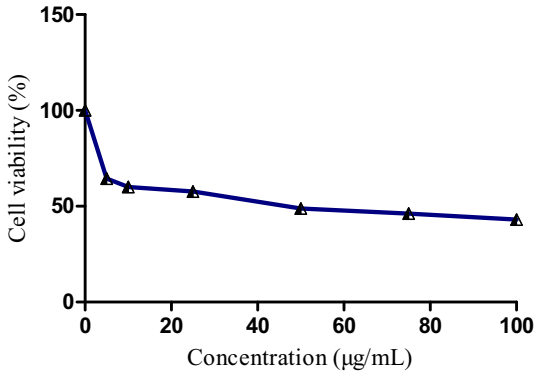

Fig. 4. Effect of different concentrations of *Lumnitzera racemosa* leaf extract on viability of HeLa cell lines.

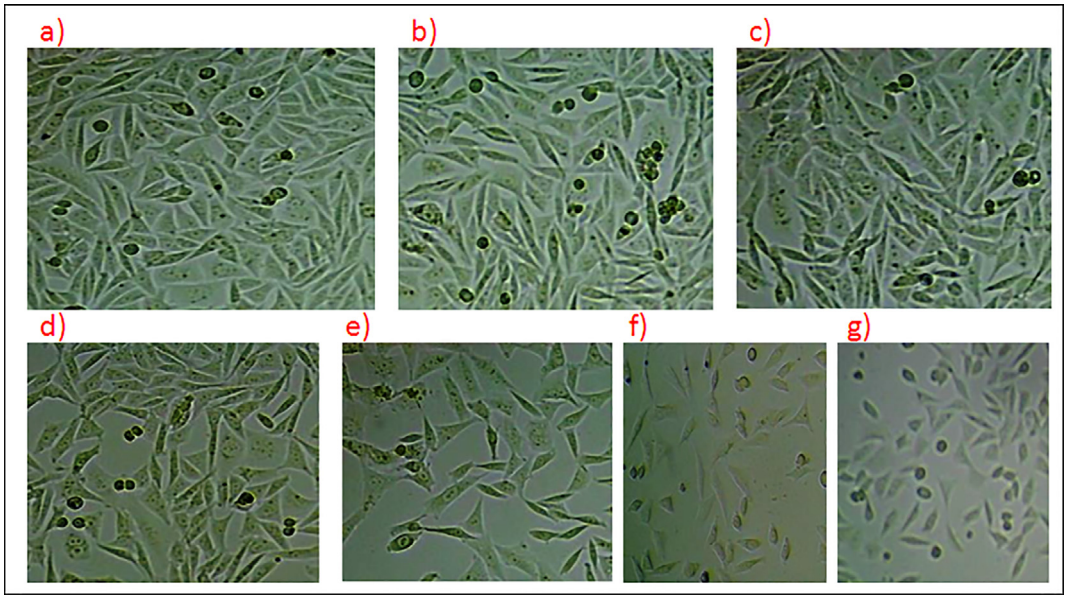

Fig. 3. The MTT assay of *Lumnitzera racemosa* against MCF 7 cell lines: a) Untreated MCF 7 cell lines and Fig. (3b-3 g) symbolized the concentrations of leaf extract i.e., 5 μg, 10 μg, 25 μg, 50 μg, 75 μg and 100 μg.

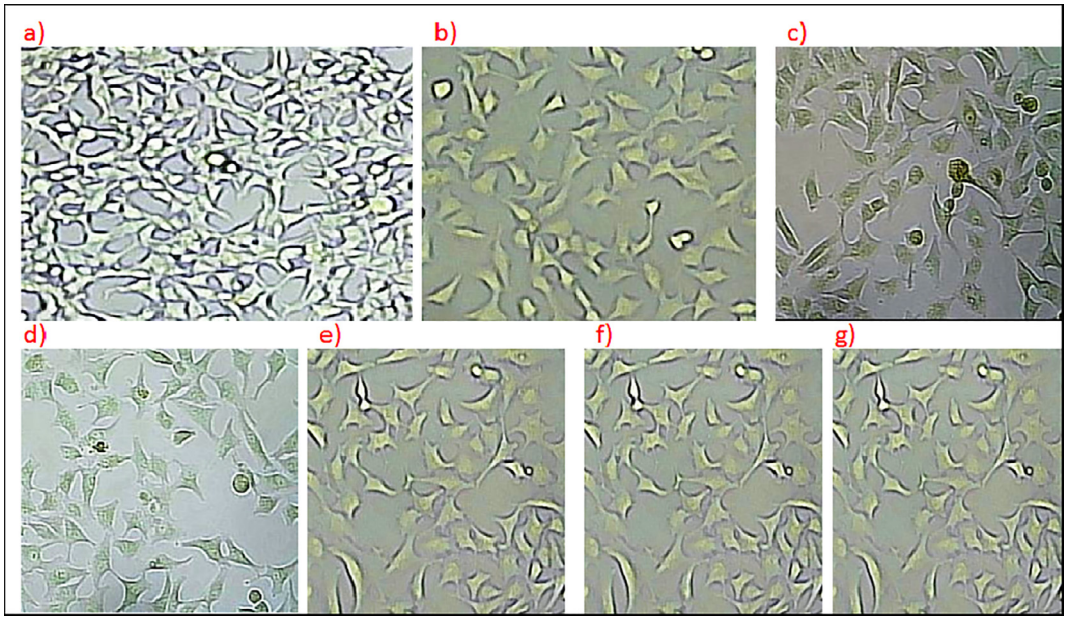

Fig. 5. The MTT assay of *Lumnitzera racemosa* against HeLa cell lines: a) Untreated HeLa cell lines and Fig. (5b-5 g) symbolized the concentrations of leaf extract i.e., 5 μg, 10 μg, 25 μg, 50 μg, 75 μg and 100 μg.

4. Conclusions

GC–MS analysis of mangrove plant *Lumnitzera racemosa* confirmed the presence of bioactive phytoconstituents. The extract of *L.racemosa* proved conclusively *in vitro* anticancer activity on MCF 7 and HeLa cells. These compounds from *L. racemosa* can be used in pharmaceutical industry for design and develop of novel lead drugs to treat cancer.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2019.01.014.

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