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Original Article

Anti-cancer Activities of Methanol Extract of *Rhizophora mucronata* against Breast Cancer MCF7 Cell Line

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ABSTRACT

Cancer is the uncontrolled growth and spread of abnormal cells, associated with regulation of cell cycle and apoptosis. Plant-derived compound is now considered as the most effective method of cancer treatment. The focus of the current research has been based on the identification of natural and synthetic compound that can be used in the prevention or the treatment of cancer. Methanolic extract of *Rhizophora mucronata* showed the presence of carbohydrates, phenol, alkaloid, amino acids, fat, flavonoids, glycosides, phenols, protein, saponins, sterols and tannins. Fourier-transform infrared spectroscopy (FTIR) confirmed the presence of primary, secondary amines, amides, alkanes, alkynes, aldehydes, saturated aliphatic, primary amines, alkyl halides, aliphatic amines, carboxylic acids, alkyl halides and alkyne. In 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay, at the concentration of 100 µg/mL of the extract, the viability of the cells was 90%. When increasing the concentration of the extract, the viability of the cells was decreased. At the concentration of 1,000 µg/mL, the viability of the cells was 50%. Tryphan blue dye exclusion assay was carried to perform the test for cell viability of the half maximal inhibitory concentration (IC_{50}) (1,000 µg/mL) value was found in the extract. The percentage of viability of Michigan Cancer Foundation-7 (MCF-7) cell line for control is 100%. Based on the result of present study, it can also be concluded that the effect on methanolic extract of *R. mucronata* has the anticancer activity potential in MCF-7 cell line.

Keywords: *Rhizophora mucronata*, Cancer, MCF7 cell line, FTIR, MTT, IC_{50}, Methanolic extract

INTRODUCTION

The Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments[2]. Many westerners have long regarded the Indian systems of medicine as a rich source of knowledge. In India, around 20,000 medicinal plants have been recorded; however traditional communities are using only 7,000–7,500 plants for curing different diseases[12]. Even today, majority of the medicines are prepared from the plant and animal products, minerals and metals and others. Major pharmaceutical industries depend on the plant products for the preparation of ayurvedic medicines.

Cancer is a group of diseases characterised by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. Cancer diseases are characterised by abnormal proliferation of cells. They constitute the second cause of mortality behind cardiovascular diseases in the developed countries and the third after infectious and cardiovascular diseases in the developing countries[6,17]. Cancer is a major public health problem worldwide with millions of new cancer patients diagnosed each year and many deaths resulting from this disease[10].

Cancer is fundamentally a disease of regulation of tissue growth. In order for a normal cell to transform into a cancer cell, genes which regulate cell growth and differentiation must be altered[7]. Genetic changes can occur at many levels, from gain or loss of entire

chromosomes to a mutation affecting a single Deoxyribonucleic acid (DNA). There are two broad categories of genes which are affected by these changes. Oncogenes may be normal genes which are expressed at inappropriately high levels, or altered genes which have novel properties. In either case, expressions of these genes promote the malignant phenotype of cancer cells. Tumour suppressor genes are genes which inhibit cell division, survival or other properties of cancer cells. Tumour suppressor genes are often disabled by cancer-promoting genetic changes. Typically, changes in many genes are required to transform a normal cell into a cancer cell[15].

Mangrove plants are used in tradition medicines, and their extracts have been proven activity against the plant, animal and human pathogens. Secondary metabolites from mangrove plant species like alkaloids, steroids, terpenoids and phenolics have been distinguishing from mangroves and have pharmacological, toxicological and ecological importance[4,5]. Alkaloid plays a major role in the formulation of numerous therapeutic agents. A wide range of biological effects had been reported for alkaloids, including emetic, anti-cholinergic, anti-tumour, diuretic, sympathomimetic, antiviral, antihypertensive, hypno-analgesic, antidepressant, mio-relaxant, antimicrobial and anti-inflammatory activities. However, natural compounds are generally complex, making it necessary to analyse their pharmacological activities.

Rhizophora mucronata (*Rhizophoraceae*) commonly known as an Asiatic mangrove, broadly distributed along the tropical and sub-tropical coastal regions. This mangrove plant has been reported to posses numerous medicinal properties. In countries like India, Burma and China, bark of *R. mucronata* was used as traditional medicine in the treatment of diarrhoea, blood in urine, dysentery, diabetes, fever, angina, haemorrhage and haematuria[13].

MATERIALS AND METHODS

Collection and Authentication of *Rhizophora mucronata* Leaves

The fresh leaves of *R. mucronata* were collected during November 2015 from Kurusadai Island (N0915′10.00″, E079°1325.00″), Gulf of Mannar Biosphere Reserve, Rameswaram and authenticated in the herbarium of CAS in Marine Biology, Annamalai University, Parangipettai, India. Fresh, healthy and uninfected leaves were washed with running tap water and spread out.

Preparation of Plant Extract

The leaves were air dried in the laboratory and made into powder by mortar and pestle. The methanol extracts were prepared with the help of supercritical fluid extraction. At the end, the extraction vessel was depressurised, and the extract was collected from the separation vessel and stored in airtight container.

In Vitro Anticancer Activity

Cell Culture

The MCF7 cell line was obtained from National Centre for Cell Science, Pune, India and maintained in Dulbecco’s Modified Eagles Medium containing 5% foetal bovine serum, 100 units/mL of penicillin and 100 µg/mL of streptomycin.

Cell growth was checked under the microscope to confirm that the cells are 90–100% confluent. Flasks were washed in 1 Phosphate-buffered saline (PBS), and trypsin was added to T-75 flasks and placed in the incubator at 37°C for 3–5 min to detach cells. Cells were resuspended, placed in 15 mL centrifuge tube and centrifuged for 5 min at 2,000 rpm at 4°C.

MTT Assay[18]

The MTT assay, based on the conversion of the yellow tetrazolium salt-MTT, to purple-formazan crystals by metabolically active cells, provides a quantitative determination of viable cells. Cells are plated on to 96 well plates at a cell density of 2×105 mL^{-1} per well in 100 µL of Roswell Park Memorial Institute medium (RPMI) 1,640 and allowed to grow in CO_2 incubator for 24 h (37°C, 5% CO_2). The medium was removed and replaced by fresh medium containing different concentrations of the sample for 48 h. The cells are incubated for 24–48 h (37°C, 5% CO_2). Then, 20-µL MTT stock solution (5 mg/mL in PBS) was added to each well and incubated for 5 h. The medium was removed and 200 µL DMSO was added to each well to dissolve the MTT metabolic product. Then the plate was shaken at 150 rpm for 5 min and the optical density is measured at 560 nm. Untreated cells (basal) were used as a control of viability (100%), and the results were expressed as % viability (log) relative to the control.

Tryphan Blue Dye Exclusion Assay[21]

The trypan blue dye exclusion assay was the most commonly utilised test for cell viability. In this assay, the cells were washed with HBSS (Hank's Buffered Salt Solution) and centrifuged for 10–15 min at 10,000 rpm. The procedure was repeated thrice. The cells were suspended in the known quantity of HBSS, and the cell count was adjusted to 2 × 106 cells/mL. The cell suspension was distributed into Eppendorf Tubes (0.1 mL containing 2 lakhs cells). The cells were exposed to drug dilutions and incubated at 37°C for 3 h. After 3 h, dye exclusion test, that drug-treated cell of equal quality were mixed with tryphan blue (0.4%) and left for 1 min. It was loaded in a haemocytometer, and viable and non-viable counts were recorded within 2 min. Viable cells do not obtain colour, whereas dead cells obtain colour. However, if kept longer, live cells also generate and obtain colour. The percentage of growth inhibition was calculated using the following formula:

Growth inhibition (%) = 100 - [(total cells - dead cells) / total cells] × 100

FTIR Analysis

FTIR spectroscopy of plant sample was relied on a Nickolet iS5, FTIR (St Joseph's college). Sample (10 mg) was mixed with 100 mg of dried potassium bromide (KBr) (Sigma) and compressed to prepare as a salt disc (10 mm diameter) for reading the spectrum further. Spectra were taken between wave numbers 4,000 and 100 cm^{-1}.

RESULTS

MTT (3-(4,5-Dimethyl-2-Thiaozolyl)-2,5-Diphenyl-tetrazolium Bromide) Assay

Growth inhibitory effect of *R. mucronata* on MCF7 cell line was determined using MTT assay. Methanolic extract of *R. mucronata* concentration which affects the viability of the cancer cells towards MCF7 cells was evaluated using the MTT assay (Figure 1). With increasing the concentration of the sample, the viability of the cells was decreased. In the concentration of 100 µg/mL of the extract, the viability of the cells was 90%. When increasing the concentration of the extract, the viability of the cells was decreased. At the concentration of 1,000 µg/mL, the viability of the cells was 50%.

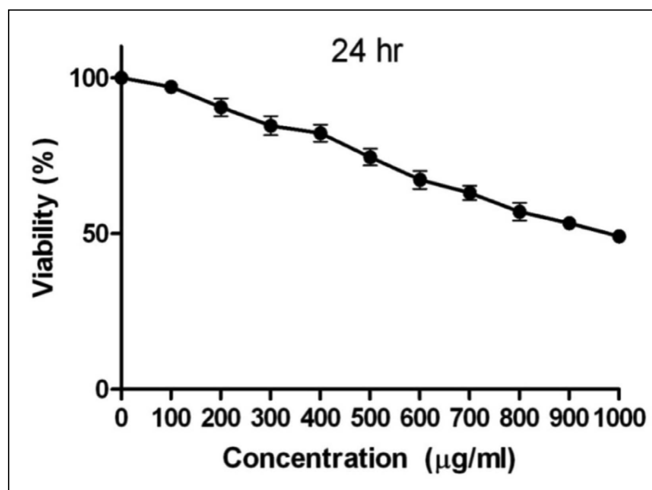

Figure 1: MTT assay of methanolic extract of *R. mucronata* against MCF cell line

Tryphan Blue Exclusion Assay

Methanolic extract was evaluated on the basis of 'Tryphan blue exclusion assay' for cell viability. Percentage viability of ethanolic extract of *R. mucronata* is shown in Figure 2. At the concentration of 1,000 µg/mL, the viability of the cells was 50%. Therefore, cytotoxic activity with the IC_{50} value of the drug is 1,000 µg/mL.

FT-IR

The FT-IR spectrum was used to identify the functional group of the active components based on the peak value

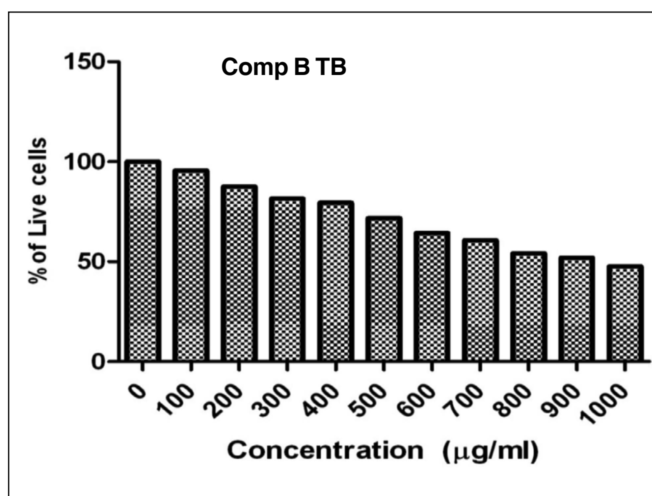

Figure 2: Tryphan blue exclusion assay of methanolic extract of *R. mucronata* against MCF cell line

Table 1: FTIR spectral qualities of fraction of *R. mucronata*

Sl. No.	Frequency (cm^{-1})	Bond	Functional Group
1.	3,963.83	–	Unknown
2.	3,762.50	–	Unknown
3.	3,432.82	N–H stretch	Primary, secondary amines, amides
4.	2,926.41	C–H stretch	Alkanes
5.	2,129.62	–C(triple bond)C– stretch	Alkynes
6.	1,924.47	–	Unknown
7.	1,723.26	C=O stretch	Aldehydes, saturated aliphatic
8.	1,632.79	N–H bend	Primary amines
9.	1,384.04	–	Unknown
10.	1,247.77	C–H wag (–CH_2X)	Alkyl halides
11.	1,055.51	C–N stretch	Aliphatic amines
12.	921.91	O–H bend	Carboxylic acids
13.	878.70	–	Unknown
14.	818.38	C–Cl stretch	Alkyl halides
15.	776.93	–	Unknown
16.	706.16	C–Br stretch	Alkynes

in the region of infrared radiation. The results of FT-IR peak values and functional groups were represented in Table 1. The FT-IR spectrum profile was illustrated in Figure 3. The FT-IR spectrum confirmed the presence of primary, secondary amines, amides, alkanes, alkynes, aldehydes, saturated aliphatic, primary amines, alkyl halides, aliphatic amines, carboxylic acids, alkyl halides and alkynes.

DISCUSSION

Breast cancer is the most prevalent cancer and the second cause of death among women around the world[19]. Breast cancer is a multifunctional disease which is also affected by environmental factors[8]. However, despite extensive research in the field of breast cancer, the factors contributing to the progression of the disease and its molecular mechanisms are not fully understood[16]. In the past, the most important treatment of breast cancer was surgery, chemotherapy, radiation or a combination of these methods. But in recent years, understanding of the molecular mechanisms involved in cancer pathways, leading to the creation of a new therapeutic approach, in which the most basic molecules that regulate metabolic pathways will be targeted[22].

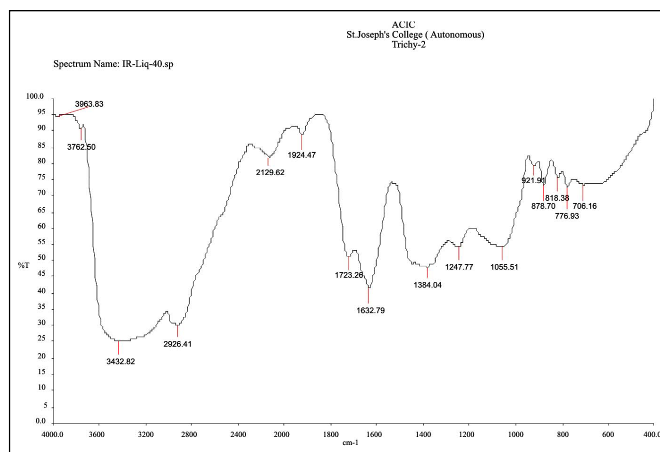

Figure 3: FTIR analysis of methanolic extract of *R. Mucronata*

Various chemical compounds with the aim of influencing on these molecules (such as fatty acid synthase) and decrease cancer cells viability are used but yet, herbal compounds are more noteworthy, because of their fewer side effects. Therefore, this study is planning to evaluate the effect of methanol extract of *Rhizophora mucroata* on MCF7 cell viability. The results of the present investigation are also supported by the others[1] who have used the different plant extracts to reduce chemical-induced carcinogenesis in their finding[9,11]. The results of the present study substantiate the anticarcinogenic and antioxidant activities of mangrove plants reported from our laboratory and others[20,23]. Mangroves have long been used in fisher-folk medicine to treat diseases[3,14].

CONCLUSION

Based on the result of the present study, it can also be concluded that effect on methanolic extract of *R. mucronata* has the anticancer activity potential in MCF7 cell line.

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