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Evaluation of *In-Vitro* Anticancer Activity from *Rhizophora Mucronata Mangrove* Leaf Extracts against Lung Cancer Cell Line

Aksha Sharen, P Sivaperumal, Lakshmi T*, Anitha Roy

Department of Pharmacology, Saveetha Dental College & Hospitals,
Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

ABSTRACT

Introduction: Herbal medicines play an important role in cancer prevention. Mangroves have been used worldwide due to the presence of various bioactive metabolites in it. The specific medicinal properties of Rhizophora mucronata have been an interest in preventing breast cancer. Mangroves Rhizophora mucronata belongs to the family Rhizophoraceae. The leaf extract reveals various roles in folk remedies to treat various diseases. The aim of the study is to determine anticancer activity of Rhizophora mucronata mangroves leaf extract against lung cancer cell line

Methodology: The Rhizophora mucronata mangrove leaf samples were collected. The collected sample was washed and then dried for and turned into fine powder. A 25g dried powdered Rhizophora mucronata sample was mixed with 100ml of Ethanol and allowed to place for 24 hours at ambient temperature. Then the mixture was passed through Whatman filter paper then the filtrate was centrifuged at 3000rpm for 10min and further filtered by 0.45µm syringe micro filter. At last, the solvents are evaporated via vacuum rotary evaporator until samples obtain in powder form. Then the sample was stored in a shadowy aluminium container at 4°C for further analysis. The proliferation of MCF-7 cells was assessed by MTT assay.

Results: The present study demonstrated the anticancer activity of Rhizophora mucronata mangrove leaf extract against lung cancer cell line and the result is graphically represented.

Conclusion: The obtained result established the anticancer activity of Rhizophora mucronata mangrove leaf extract against lung cancer cell lines.

Key words: *Mangrove* plant, *Rhizophora mucronate*, Bioactive compounds, Lung cancer cell line

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Corresponding author: Lakshmi T
e-mail ✉ : lakshmi@saveetha.com
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INTRODUCTION

Ayurveda is one of the oldest health systems in India. In Sanskrit, Ayu means life and Veda means knowledge or science. Ayurvedic medicine is based on six darshanas; it includes logic of Samkhya, Nyaya vaisesika systems of natural philosophy. Ayurveda is the most comprehensive and practical medical science that revives support of the public. Ayurvedic medicine remained stagnated and even suppressed. However, it survived against the adversity of time, especially during the Mughal and British period [1,2]. *Rhizophora mucronata* is a slow-growing, much-branched, evergreen tree growing up to 27 metres tall, with a bole 50 - 70 cm in diameter. The tree produces numerous stilt roots from the base. The tree is an important local source of tannins and fuel, mainly gathered from the wild but also semi-cultivated in some areas. Traditional Use of *Rhizophora mucronata* is the bark of this plant is known as an astringent. It has traditionally been used in the treatment of diabetes, diarrhoea, nausea, haematuria, haemorrhages, and angina [3,4]. The traditional use of several *mangrove* plants including R. mucronata has recently been reviewed [5,6]. The most use of this plant is anti-cancer activity [7-9]. The ethanol bark extract was found to have high activity against the Newcastle disease, vaccinia, encephalomyocarditis and Forest viruses. Also, the ethanol flower extract showed good activity in human health. The bark of *Rhizophora mucronata* was the most promising Anticancer agent [10].

Rhizophora mucronata leaf extracts exhibit best antiviral and anti-cancer natural agents. The natural compounds used in both traditional and modern therapies for human health improvement with fewer side effects [11,12]. Leaf extracts show novel drug molecules to combat the threat of human diseases [13-15]. The screening of plant extracts is an innovative method to find therapeutically active compounds in many plant species [16,17]. The aim of the study is to evaluate the anti-cancer activity from *Rhizophora mucronata mangrove* leaf extracts against lung cancer cell lines.

MATERIALS AND METHODS

Sample collection and preparation

The *Rhizophora mucronata mangrove* leaf samples were collected from the Parangipettai, Tamilnadu. The collected sample was washed thoroughly with tap water then was dried in shadow for 48 hrs and further oven dried for 24hrs and turned into fine powder by mortar and pestle.

Preparation of extraction

25g of dried powdered *Rhizophora mucronata* sample was mixed with 100ml of Ethanol and allowed to place for 24 hours at ambient temperature. Then the mixture was passing through Whatman filter paper (No.4) then the filtrate was centrifuged at 3000rpm for 10min and further filtered by 0.45µm syringe micro filter. At last, the solvents are evaporated via vacuum rotary evaporator until samples obtain in powder form. Then the sample was stored in a shadowy aluminium container at 4ºC for further analysis.

MTT Assay

The proliferation of MCF-7 cells was assessed by MTT assay Safadi et al., (2003). MCF-7cells were plated in 48 well plates at a concentration of 2x104 cells/well 24 hours after plating, cells were washed twice with 500µl of serum-free medium and starved by incubating the cells in serum-free medium for 3 hours at 37ºC. After starvation, cells were treated with *Rhizophora mucronata* extract in different concentrations for 24 hours. At the end of treatment, the medium from control and *Rhizophora mucronata* extract treated cells were discarded and 200µl of MTT containing DMEM (0.5 mg/ml) was added to each well. The cells were then incubated for 4h at 37ºC in the CO2 incubator.

The MTT containing medium was then discarded and the cells were washed with 1x PBS. The crystals were then dissolved by adding 200µl of solubilization solution and this was mixed properly by pipetting up and down. Then the formed crystals were dissolved in dimethyl sulfoxide (200µl) and incubated in dark for an hour. Then the intensity of the color developed was assayed using a Micro ELISA plate reader at 570 nm. The number of viable cells was expressed as the percentage of control cells cultured in serum-free medium. Cell viability in control medium without any treatment was represented as 100%. The cell viability is calculated using the formula: % cell viability=(A570 nm of treated cells/A570 nm of control cells)×100.

Morphology study

Based on MTT assay we selected the optimal doses (300µg/ml) for further studies. Analysis of cell morphology changes by a phase contrast microscope. 3×104cells were seeded in 6 well plates and treated with R. mucronata extract (500µg/ml for MCF-7 cells) for 24h. At the end of the incubation period, the medium was removed, and cells were washed once with a phosphate buffer saline (PBS pH 7.4). The plates were observed under a phase contrast microscope.

Statistical analysis

All data obtained were analysed by Student's-t-test using MS-Excel, represented as mean ± SD for triplicates. The results were computed statistically (SPSS/10 Software Package; SPSS Inc., Chicago, IL, USA) using one-way ANOVA. The level of statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

It is shown in the results that the *Rhizophora mucronata* tested in this investigation possess anticancer activity. *Mangrove* leaf extract have high phenolics and flavonoid contents and also, high antioxidant activity with a low IC50. Also, there are strong positive and significant correlations between DPPH radical scavenging and phenolics and flavonoid contents. As the phenolic compounds and flavonoids are the main contributors of anticancer activity in these *mangrove* leaf extract (Figure 1).

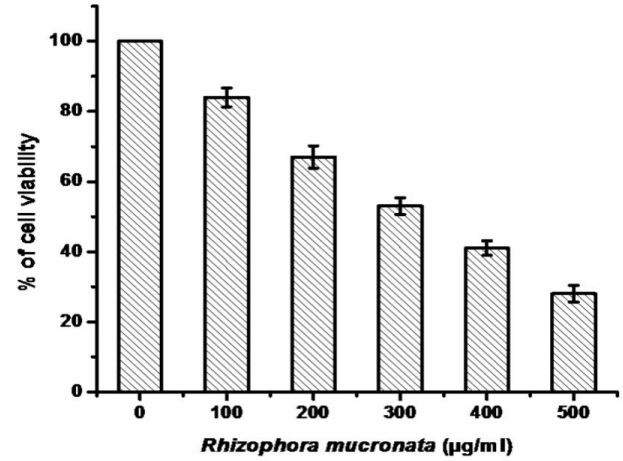

Figure 1: showing anticancer activity of *Rhizophora mucronata mangrove* leaf extract against lung cancer cell line. X-Axis represents the concentration in µl and Y-Axis represents the percentage of cell viability.

The contribution of new and novel products from potential bioactive plants or their extracts for disease treatment and prevention is still vast, despite the overshadowing by recent synthetic chemistry as a method of drug discoveries and drug productions [17,18]. Moreover, plant derived drugs like vinblastine, vincristine, taxol, and camptothecin had led to greatest extent within the vicinity of antitumor upon where the drugs were reported to improvise the chemotherapy of some cancers [19]. Plants contain almost unlimited capacity to generate compounds that fascinates researchers in the quest for new and novel chemotherapeutics The persistence search for new Anticancer compounds in plant medicines and traditional foods is a realistic and promising strategy for its prevention. Numerous compounds found in plants with

anticancer properties are such as alkaloids, phenylpropanoids, and terpenoids. Therefore, in this study *Rhizophora mucronata mangrove* leaf extract was evaluated as a new anticancer agent by using MTT assays. Plants used in folk and traditional medicines have been accepted as leads for therapeutic drug development in modern medicine. *Rhizophora mucronata mangrove* leaf extract was chosen for this study due to its use as a wound healing agent among the natives of Africans and as therapeutic agent in other parts of the world [20–22]. Hence this study the cytotoxicity was evaluated in vitro. Studies have observed the presence of many bioactive compounds in the methanolic extracts of this plant including tannins, alkaloids, steroids, saponins, terpenoids, and flavonoids which exhibit various biological activities [23-26]. These compounds are present in several food items and hold great potential as drug candidates due to their safety, low toxicity and wide acceptance amongst the public [27–29].

The focus of the current research has been based on the identification of natural and synthetic compounds that can be used in the prevention or the treatment of cancer [30,31]. 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 [32-35]. 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. The limitations can be constrained by global climate change and terrestrial for its vegetation. The future scope is to create awareness and better knowledge about the anticancer properties of *Rhizophora mucronata* as found that high dietary intake of natural phenolics is strongly associated with longer life expectancy, reduced risk of developing some chronic diseases, various types of cancer, diabetes, obesity, improved endothelial function and reduced blood pressure.

CONCLUSION

In conclusion, the obtained result established the preliminary phytochemical analysis of *Rhizophora mucronata* leaf. The outcome of the existing study encourages carrying out further compounds identification present in the extract. Large screening is required for the evaluation of natural products. This provides a way for the future pipeline in drug discovery. Thus, *Rhizophora mucronata* possesses various pharmaceutical properties in the field of herbal medicine.

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CONFLICT OF INTEREST

The authors declare that there was no conflict of interest.

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