Anticancer potential from *Rhizophora mucronata* plant leaf associated *Streptomyces* species against the breast cancer cell line

ABSTRACT

INTRODUCTION: *Rhizophora mucronata* is a small to medium sized evergreen tree growing to a height of about 20 to 25 meters (approximately 66 to 82 feet), on the banks of river. These mangroves have anticancer, antioxidant, antifungal, and viral activities which enhances its existence. *Streptomyces* species are the largest genus of Actinobacteria. They are gram positive and are found in soil, decaying vegetation and mangrove leafs. The breast cancer cell lines are used to test the anticancer potential.

AIM: Aim of the study was to assess the antibacterial activity from Rhizophora mucronata, against breast cancer cell line.

MATERIALS AND METHODS: Collection of *Rhizophora mucronata* (a mangrove species) followed by *Streptomyces* from mangrove leaf, preparation of extract with characterisation features positive for *Streptomyces* and MTT assay to check the anticancer potential.

RESULTS AND DISCUSSION: The *Rhizophora mucronata* was identified by colour of aerial mycelium, soluble pigments, Spore chain morphology. Further, the Arabinose enzyme production was done and estimated also completed. The potential antimicrobial activity from the *Streptomyces* metabolites was done. Zone of inhibition and MIC were calculated.

CONCLUSION: *Rhizophora mucronata* mangrove plant leaf associated Maine *Streptomyces* shows good anti cancer activity. Further detailed calculations and future study is possible using the species.

Keywords- *Rhizophora mucronata, Streptomyces* species, Anticancer potential, Novel drug

INTRODUCTION

Mangroves are defined as woody trees and shrubs that grow in marshy areas(1). These plants have adapted themselves morphologically and physiologically to the habitat which has salinity, high tidal inundation as well as high wind velocity complex. Plants belonging to this genus are very effective in producing phytochemicals and metabolites, thus have high medicinal potential. Mangroves are tremendously used in the arena of traditional medicine. The leaves, roots, and bark of these mangroves are used for the treatment of hemorrhages, angina as well as hematuria(2).

Rhizophora mucronata has various benefits as antiviral and antibacterial, cytotoxic, analgesic and diuretic activities(3). Mangrove plants are halophyte plants seen in tropical and subtropical areas in some parts of the world. Different chemical compounds and metabolites associated with the plants are extracted for various properties(4).

Streptomyces is the largest genus of actinobacteria, belonging to the type of the genus of the family Streptomycetaceae(5). These are gram-positive bacteria, belonging predominantly to the soil and decaying vegetation. Streptomycetes are characterized by various complex secondary metabolism. They produce over two-thirds of the clinically useful antibiotics from their natural origin. In recent years, the service by the biotechnology researchers have begun using Streptomyces species for heterologous expression of proteins(6).

Cancer is the deadly disease that affects different organs, and is identified by the unchecked proliferation of abnormal cells that invade other healthy tissue, associated with various regulation of cell cycles and apoptosis processes(7). The treatment is primarily confined to the chemotherapy process. Besides being an expensive process, chemotherapy is known for various severe side effects to the patient's body, making treatment problematic eventually. For medicinal chemists, the primary goal still remains hidden with the discovery and identification of various chemotherapeutic agents derived from natural products(8). Despite numerous researches from past decades and effective treatment for deadly disease cancer is still lacking, therefore there is a great need for newer compounds having anticancer potential including the cell selective activities with reduced adverse effects. Secondary metabolites derived from various flora like that of Rhizophora mucronata have opened new avenues for the development of novel therapeutic agents., Plant derived compound is now considered as the most effective and crucial method. Research scientists have identified many other crucial anticancer molecules from fungal endophytes of mangrove plants(9). Many researchers were attracted to marine mangrove fungi because of their diversity, which may lead to the discovery of several novel natural products to society. With the remarkable advancements that occurred in the spectroscopic techniques, along with the separation methods and microplate-based sensitive in vitro assays, the natural product exploration of mangrove fungi has attracted special attention regarding novel and unexplored chemical substances associated with it.

Breast cancer cell lines have been widely used for the process of breast cancer modelling which encompassess a panel of diseases using distinctive phenotypic associations. Hence, the main aim of the study is to evaluate the anticancer potential from Rhizophora mucronata plant leaf associated Streptomyces species, against the breast cancer cell line(10). Further, our team has extensive knowledge and research experience that has translated into high quality publications(11–15),(16),(17),(18),(19),(20),(21),((13,22,23),(24–28),(29),(30). Aim of the study was to assess the antibacterial activity from *Rhizophora mucronata*, against breast cancer cell line.

MATERIALS AND METHODS

The sediment sample was collected from the Pichavaram~~Pitchavaram~~ coast of Tamil Nadu. The collected sample was sun dried for 48 hours and turned into fine powder by mortar and pestle. Isolation of Streptomyces from mangrove leaves. preparation of extract was done and characterization of features positive for *Streptomyces*. MTT assay to check the anticancer potential. The study was done in the Blue Lab, Saveetha Dental College and Hospital.

RESULTS

The results obtained confirmed that *Rhizophora mucronata* associated *Streptomyces* were isolated from the sediment samples. They were confirmed by the features as colour of the aerial mycelium as white, soluble pigment present and spiral spore chains. In case of assimilation of carbon source- inositol, mannitol, arabinose, rhamnose, sucrose and raffinose were positive. This is illustrated in Table 1. Various cell wall amino acids were present including LL-DAP and glycine as well as cell wall type as 1. Table 2 illustrates the same features of the cell wall. Colour of aerial mycelium is considered to be white (figure 1) and spore chain as spiral (figure2) are certain positive features.

MTT assay was done for various drug concentrations. In the control for 24hours the cell viability was maximum. When the drug concentration increases the cell viability decreases as the breast cancer cell line dies eventually. The results illustratedresults was illustrated in Figure 3 & 4 depicts the cell viability before and after the addition of secondary metabolites.

Figure 1: White colour of aerial mycelium of *Streptomyces*

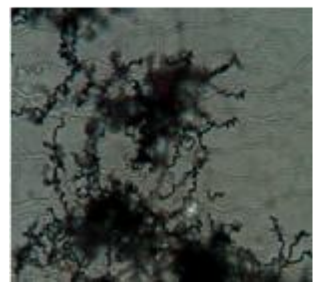


Figure 2: Spiral spore chain

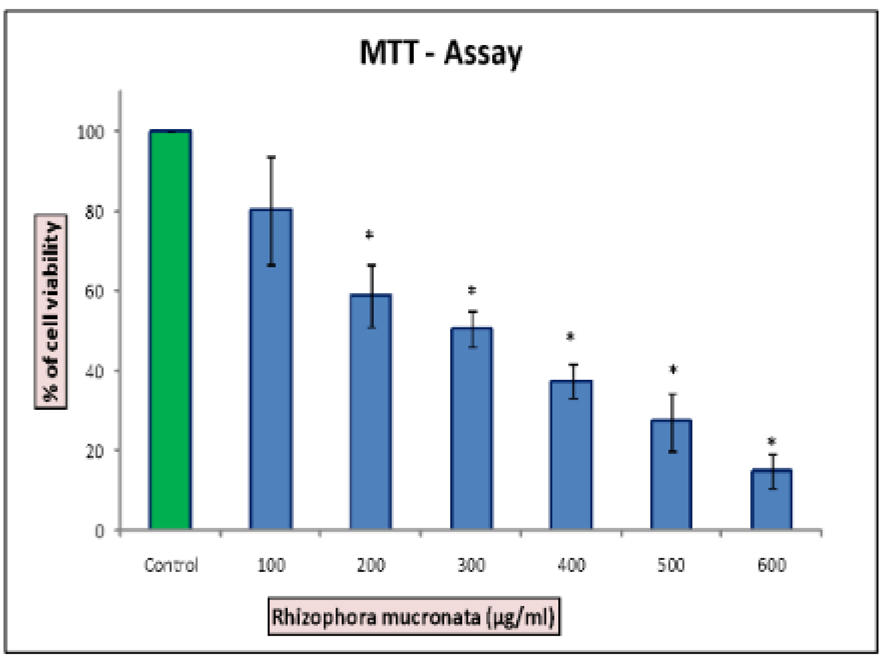
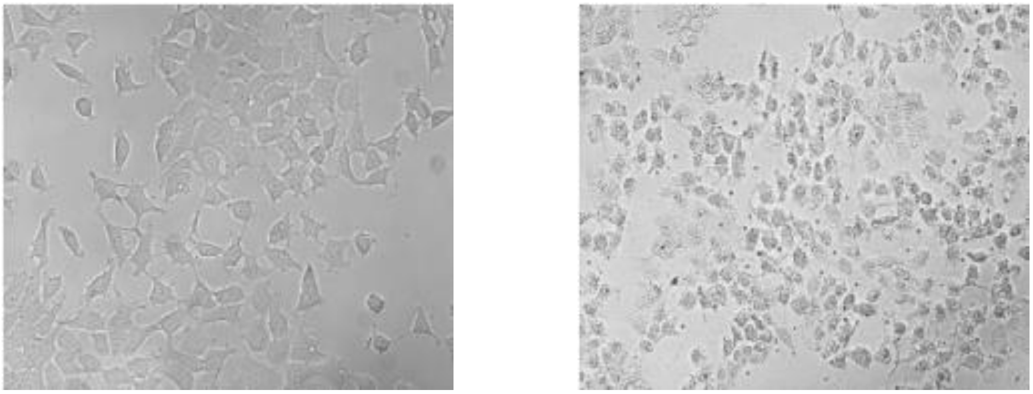


Figure 3: Demonstration of Drug concentration in MTT assay



Control Treated

Figure 4: Figure depicting the cell viability before and after the addition of secondary metabolites

Table 1: Conventional Identification of Mangrove associated *Streptomyces* species

FEATURES	**PRESENCE/ ABSENCE**
Colour of aerial mycelium	White
Melanoid pigment	-
Soluble pigment	+
Reverse side pigment	-
Spore chain	Spiral
ASSIMILATION OF CARBON SOURCE	
Arabinose	+
Xylose	-
Inositol	+
Mannitol	+
Fructose	-
Rhamnose	+
Sucrose	+
Raffinose	+

Table 2: Cell wall and sugar pattern analysis of *Streptomyces* sp

INDEX	STREPTOMYCES	
Cell wall type	1	
Cell wall amino acids	LL-DAP	+
	MesoDAP	-
	Glycine	+
Cell wall sugar	Arabinose	-
	Galactose	-

DISCUSSION

Marine actinobacterial *Rhizophora mucronata* showed potential anticancer activity against all other anticancer studies when compared to other studied organisms. It can be comprehended from various studies that there is still a very little understanding of diversity in this genus. The results confirmed that *Rhizophora* sp associated with marine~~associated marine~~ *Streptomyces* (Table 1) metabolites have good anti cancer potential. The *Streptomyces* species were verified by the white color of the aerial mycelium. In addition to that, there were positive readings for soluble pigments, arabinose, inositol, mannitol, rhamnose and there were negative readings for melanoid pigment, reverse side pigment, xylose and fructose. Marine populations represent reservoirs of novel bioactive metabolites with diverse groups of chemical structures. Therapeutic strategies and the present use of marine natural products components, its future direction and limitations are discussed by Khalifa et al., (31). Actinobacteria are still~~Actinobacteria still~~ a source of novel antibiotics (32). There are more than 22,000 known microbial secondary metabolites, 70% of which are produced by actinomycetes, 20% by fungi, 7% by *Bacillus* spp. and 1–2% by other bacteria (33). Some of these bioactive compounds are antimicrobial agents, whereas dibutyl phthalate and di-(2-ethylhexyl) phthalate have been reported to be cathepsin B inhibitors (34). Discodermolide, bryostatins, sarcodictyin, and eleutherobin are among the most effective anticancer drugs produced mainly by marine bacteria (35,36). In the exploration of marine-derived actinomycetes as sources of antitumor compounds, lucenta mycins~~lucentamycins~~ A-D, which are 3-methyl-4-ethylidene proline-containing~~3-methyl-4-ethylideneproline-containing~~ peptides were isolated from *Nocardiopsis lucentensis* (strain CNR-712). Lucentamycins A and B exhibited significant in vitro cytotoxicity against HCT-116 human colon carcinoma using MTS assay with IC_{50} = 0.20 and 11 μM, respectively (37).

CONCLUSION

Mangrove fungi are considered to be a ubiquitous source of novel bioactive metabolites with the potential to display anticancer properties as a major property. The phenomenon is greatly observed in mangrove fungal metabolites, as they show potent anticancer activity via different mechanisms of action such as apoptotic cell death, the inhibition of kinase proteins involved in signal transduction pathways and the destruction of cell wall protein. Although many metabolites demonstrated moderate cytotoxic activities against cancer cell lines, only a few displayed superior activity than the standard anticancer drugs. It can be suggested that the rational derivatization of secondary metabolites may provide molecules with better activity against a wide range of breast cancer cell lines. In addition, the identified secondary metabolites with broad-spectrum anticancer activity need to be investigated to establish their mechanisms of action and to develop as novel anticancer therapeutic agents in future(27) (31) (32) (33) (34) (35) (36) (37) (38) (39) (40) (41) (42) (43) (44). Rhizophora mucronata mangrove plant leaf associated Streptomyces shows good anti cancer activity. Further detailed calculations and future study is possible using the species.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used~~use products~~ in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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