Phytochemical screening and cytotoxic effect of methanolic leaf extract of *Rhizophora apiculata* blume against Michigan Cancer Foundation-7 breast cancer cell lines

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ABSTRACT

**Introduction:** *Rhizophora apiculata* is an important traditional medicinal plant used in many people in Asia and Africa for curing of both infectious and non-infectious diseases. **Objective:** The present study was aimed to evaluate the cytotoxic effect and observation of cell morphology changes of breast cancer (Michigan Cancer Foundation-7 [MCF-7]) cell lines of after the treatment with the methanolic leaf extract of *R. apiculata*. **Materials and Methods:** Cytotoxicity of the methanolic leaf extract of *R. apiculata* was tested against MCF-7 cell lines by using MTT assay and to find the cell morphology of MCF-7 cells upon exposure to the methanolic leaf extract of *R. apiculata* by using phase contrast microscope. **Results:** The results showed that the methanolic leaf extract of *R. apiculata* with different concentrations (25, 50, 100, and 200 μg/mL) had shown significant concentration-dependent anticancer (cytotoxic) activity against MCF-7 breast cancer cell by showing 48.27% of cell viability with half maximal inhibitory concentration (IC$_{50}$) values of 140.97 μg/ml, comparing with standard tamoxifen by showing 80.80% of cell viability with IC$_{50}$ values of 61.88 μg/ml. **Conclusion:** From this study, it can be concluded that the methanolic leave extract of *R. apiculata* could be serve as a potential source of plant-derived, anticancer agents for the development of therapeutic anticancer drugs and further study has been focused on the isolation of bioactive anticancer compounds present in the methanolic leaf extract of *R. apiculata* by bioactivity-guided fractionation.

**KEY WORDS:** *Rhizophora apiculata*, Michigan cancer foundation-7, Cytotoxicity, Cell morphology, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, Anticancer agents

INTRODUCTION

Cancer is one of the foremost deadly diseases and public health burdens in both developed and developing countries. Cancer is the continual and unregulated proliferation and differentiation of individual cells in a multicellular organism results from the defects in the fundamental regulatory mechanism at molecular and cellular levels.[1,2] The International Agency for Research on Cancer announced that the incidence of mortality and prevalence of all major types of cancer, among the world shown that there were 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer in 2012 worldwide and by year 2030, it is estimated that there will be 26 million new cancer cases and 17 million cancer deaths per year.[3,4] Breast cancer is the second leading cause of cancer deaths among women in the United States, chemotherapy commonly used strategies in treatment of breast cancer, due to adverse side effects, ranging from nausea to bone marrow failure and development of multidrug resistance.[5,6] hence, discovery of natural compounds from plants may be an alternative source for cancer treatment, The plant based drugs have less toxic and more potent anticancer agents that are comparable to the drugs available in the market.

Medicinal plants occupy a premier position in human history as an ultimate source of phytocompounds having high therapeutic value. It is found that 40% of all medicines derived from natural sources and out of the 25% are from plant sources. Medicinal plants have various advantages over chemical products because plant-derived compounds are more tolerant and non-toxic to the normal human cells.[7] Several studies have been reported that plant-
derived secondary metabolites such as flavonoids, flavones, anthocyanins, and lignans, coumarins shown anticancer or antiproliferative activity by inhibiting various stages of tumorigenesis and associated inflammatory-related diseases. There are several medicinal plants are considered to possess anti-inflammatory and antitumor properties. Actaea racemosa belongs to the family Ranunculaceae used for ovaritis and liver cancer. Bacopa Monnieri belongs to the Scrophulariaceae family shown anticancer activity by inducing apoptosis and Bidens pilosa belongs to the Asteraceae family shown the antitumor activity on various cancer cell lines.

*Rhizophora apiculata*, belongs to the family Rhizophoraceae, it has been traditional use by many people in Asia and Africa for treatment of infectious bacterial, viral, and fungal diseases. Mingzhe G and Hongbin X reported that various extracts and fractions of the stem of *R. apiculata* indicate butanol fraction that possesses the highest strongest scavenging activities against DPPH, ABTS, and hydroxyl radicals. Ravikumar et al. reported that *R. apiculata* contain secondary metabolites such as flavonoids, tannin, saponins, catachin, anthraquinone, and phenolic group, among them, catachin was the predominant constituent of mango tannins Cichewicz et al. reported that anthraquinone which is a bioactive constituent of the *R. apiculata* was recognized to shown in vitro antitumor property against four human cancer cell lines. The present study was aimed to evaluate the qualitative phytochemical analysis and investigate the antitumor activity of methanolic leaf extract of *R. apiculata* against human breast cancer (michigan cancer foundation-7 [MCF-7]) cell lines in accordance to the observable changes of cell morphology on exposure to the extract.

**MATERIALS AND METHODS**

**Materials**

Roswell Park Memorial Institute (RPMI), 1640 with L-glutamine, was purchased from GibcoBRL (Life Technologies, Grand Island, NY, USA); dimethylsulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma Chemical Co. (St. Louis, MO, USA), phosphate buffered saline (PBS) (pH 7.4). All reagents and solvents used are of analytical grade.

**Plant Material Collection**

The fresh leaves of *R. apiculata* were collected from Pichavaram, Chidambaram, Tamil Nadu, India, during the month of March 2015. The plant was authenticated by Dr. Subramanian, Professor, Department of Botany, Annamalai University, Tamil Nadu, India.

**Preparation of Plant Extracts**

The fresh leaves of *R. apiculata* were properly washed with distilled water, shade dried, and coarse powdered. Powder weighing 250 g was methanol by a hot percolation method using a Soxhlet apparatus. The successive methanolic extract was concentrated in a rotavapor according to the boiling temperatures of the solvents to dryness to obtain organic solvent crude extracts. The obtained methanolic (8.2 g) extracts were evaluated for phytochemical analysis and in vitro, the cytotoxic effect on breast cancer (MCF-7) cell lines.

The methanolic leaf extract of *R. apiculata* was solubilized with 0.1% DMSO resulting in concentration 1 mg/ml in Dulbecco’s Modified Eagle Medium culture medium. These preparations were filtered by 0.2 μm filters, and these extracts were used for the evaluation of anticancer activities.

**MCF-7 (Breast Cancer Cell Line) Cell Culture**

MCF-7 cell lines were obtained from American type culture collection (ATCC, Manassas, VA, USA) and maintained with RPMI 1640 with L-glutamine supplemented with 10% fetal bovine serum, 100 μg/ml streptomycin, and 100 U/ml penicillin (Invitrogen), maintained at 37°C in a 5% CO2 incubator.

**Cell Viability Assay**

The viability of cells was assessed by MTT assay (Mosmann) using MCF-7 cell line. The assay is based on the reduction of a soluble yellow tetrazolium salt to insoluble purple formazan crystals by metabolically active cells. Only live cells are able to take up the tetrazolium salt. The enzyme (mitochondrial dehydrogenase) present in the mitochondria of the live cells is able to convert internalized tetrazolium salt to formazan crystals, which is purple in color. Then, the cells were lysed and dissolved in DMSO solution. The color developed is then determined in an ELISA reader at 570 nm.

The cells were plated separately in 96 well plates at a concentration of 1 × 104 cells/well. After 24 h, cells were washed twice with 100 μl of serum-free medium and starved for an hour at 37°C. After starvation, cells were treated with different concentrations of, namely 25, 50, 100, and 200 μg/ml of methanolic leaf extract of *R. apiculata* for 24 h. At the end of the treatment period, the medium was aspirated, and serum-free medium containing MTT (0.5 mg/ml) was added and incubated for 4 h at 37°C in a CO2 incubator. The MTT containing medium was then discarded, and the cells were washed with PBS (200 μl). The crystals were then dissolved by adding 100 μl of DMSO, and this was mixed properly by pipetting up and down. The spectrophotometrically absorbance of the purple-blue formazan dye was measured in a microplate reader at 570 nm (Biorad 680).
Percentage of viability = \( \left( \frac{O.D \text{ of test}}{O.D \text{ of control}} \right) \times 100 \)

**Statistical Analysis**

All the results were expressed as the mean ± standard error of mean of triplicate analysis \((n = 3)\). The statistical analysis was carried out by one-way ANOVA followed by Dunnett’s test and anticancer activity of methanolic leaf extract of *R. apiculata* evaluated as half maximal inhibitory concentration \((IC_{50})\) values (defined as the concentration of the compound required to inhibit cell proliferation by 50%) using GraphPad Prism software version 6.0. \( *P \leq 0.05, **P \leq 0.01, ***P \leq 0.001 \) represent a significant difference compared with the control group.

**RESULTS AND DISCUSSION**

The Cytotoxic Effect of Methanolic Leaf Extract of *R. apiculata* on MCF-7 cell Lines

The anticancer effect of the methanolic leaf extract of *R. apiculata* was evaluated on MCF-7 cancer cell lines with different doses, namely 25, 50, 100, and 200 \(\mu\)g/ml by microtube tetrazolium bromide assay and effective dose \((IC_{50})\) was calculated by dose-response curve using GraphPad Prism software version 6.0. The cytotoxic activity of methanolic leaf extract of *R. apiculata* on MCF-7 cancer cell is shown in Table 1 and Figure 1. As shown in Table 1 and Figure 1, a dose-dependent significant anticancer activity of methanolic leaf extract of *R. apiculata* was reported at 200\(\mu\)g/ml by showing 51.73 % of cell viability with the significant \(IC_{50}\) of 140.97 \(\mu\)g/ml. Tamoxifen standard anticancer drug shown 80.79% of cell viability with an \(IC_{50}\) value of 61.88 \(\mu\)g/ml. Prabhu et al.[15] reported that methanolic extract of *R. apiculata* shown the antimetastatic activity in metastatic tumor-bearing animals, due to the high content of pyrazole, 4-pyrrolidinyl, ketone derivatives, and thiazolidinediones found in the methanolic extract. Several secondary metabolites such as alkaloids, terpenoids, polyphenolic compounds, and coumarins found in medicinal plants shown anticancer activity, these plant-derived secondary metabolites are promising source for prevention of cancer.[16]

**Effect of Methanolic Leaf Extract of *R. apiculata* on Morphological Changes of MCF-7 Cell Lines**

The morphological changes of the MCF-7 cell lines up on the treatment with methanolic leaf extract of *R. apiculata* observed under the phase contrast microscope. As shown in Figure 2, from the microscopic observation revealed that methanolic leaf extract of *R. apiculata* shown the promising anticancer activity compared with untreated (control) cells. Cell viability of MCF-7 decreased with by dose depended increasing in the concentration of methanolic leaf extract of *R. apiculata*, at high concentration 200 \(\mu\)g/ml, MCF-7 cell lines will became shrunken and lose its contact with the surface of microwell plate.

A number of novel plant-derived drugs from plant secondary metabolites have been used in the treatment of cancer.[17] The plant-derived anticancer drugs such as Vinblastine, Vincristine, Taxol, and Camptothecin were reported to for treatment of cancer. Medicinal plants used in folk and traditional medicine have been used as a lead for development of medicine for curing of diseases. Based on traditional important in curing the inflammatory and skin related disease *R. apiculata* for chosen for development of anticancer agents. From

**Figure 1**: Dose-dependent effect of methanolic leaf extract of *Rhizophora apiculata* on Michigan Cancer Foundation-7 (breast cancer cell line) proliferation. Values are expressed as mean ± standard error mean. \(*P < 0.05, **P < 0.01, ***P < 0.001\) represent significant difference compared with control group by student’s \(t\)-test \((n = 3)\)

**Table 1**: Cytotoxic effect of methanolic leaf extract of *R. apiculata* on MCF-7

<table>
<thead>
<tr>
<th>Dose ((\mu)g/ml)</th>
<th>O.D. at 570 nm</th>
<th>% cell viability</th>
<th>IC(_{50}) ((\mu)g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Control</td>
<td>1.39</td>
<td>1.39</td>
<td>1.37</td>
</tr>
<tr>
<td>25</td>
<td>1.22</td>
<td>1.23</td>
<td>1.25</td>
</tr>
<tr>
<td>50</td>
<td>1.03</td>
<td>0.981</td>
<td>0.99</td>
</tr>
<tr>
<td>100</td>
<td>0.798</td>
<td>0.771</td>
<td>0.791</td>
</tr>
<tr>
<td>200</td>
<td>0.672</td>
<td>0.664</td>
<td>0.684</td>
</tr>
<tr>
<td>100 (tamoxifen)</td>
<td>0.266</td>
<td>0.260</td>
<td>0.269</td>
</tr>
</tbody>
</table>

*R. apiculata*: *Rhizophora apiculata*, MCF-7: Michigan cancer foundation-7, SD: Standard deviation, \(IC_{50}\): Half maximal inhibitory concentration
the significant cytotoxic activity of methanolic leaf extract of R. apiculata on MCF-7 cell lines, it was observed that methanolic leaf extract contain maximum number secondary metabolites such as flavonoids, phenolic compounds, alkaloids and terpenoids which is responsible for the anticancer activity.[18]

CONCLUSION

From the present study, it was concluded that the methanolic leaf extract of R. apiculata shown a significant cytotoxic effect against MCF-7 breast cancer cell lines, therefore methanolic leaf extract of R. apiculata used for the treatment of breast cancer progression. Further this, a study has been focused on the isolation of bioactive anticancer compounds present in the methanolic leaf extract of R. apiculata by bioactivity-guided fractionation and investigations of mechanisms involved in the anticancer property of methanolic extract of R. apiculata.

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REFERENCES