Identification of anticancer activity of phytoconstituents from mangrove

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**ABSTRACT**

Medicinal plants from the marine ecosystem are a rich source of medicinal plants having a potential for miracle drugs. It is clear from the observation that the local inhabitants of Pichavaram mangrove forest have excellent knowledge about the phytomedicine. Thus they have developed their traditional system of utilizing these mangroves for medicinal purposes. Cancer is the name given to a collection of related and multistep disease. In all types of cancer, a few of the body's cells begin to divide without stopping and spread into the surrounding. It is developed by environmental, physical, chemical, metabolic and genetic factors. In this study, methanol was used to prepare an extract from *A. marina* leaves and screened for anticancer activity. The phytoconstituents like alkaloids, flavonoids, saponin and tannin present in the extract were quantified, and anticancer activity of the same was identified. Further, the apoptotic cell death effect of methanol extract on the Hela cell line was determined. The flavonoids of *A. marina* showed higher anti-cancer activity on Hela cells followed by tannin, alkaloids and saponin. The result of the apoptotic cell death effect of *A. marina* may provide an effective therapeutic strategy against cervical cancer. Finally, it is concluded that the extract of *A. marina* exhibited anticancer activity.

Cancer is one of the most devastating diseases in both developing and developed countries. Due to a global increase in life expectancies, the incidence of cancer and related mortality rates are dramatically increasing. Treatment options are typically expensive and unavailable in developing countries. New and widely-available drugs are therefore needed to provide treatment options. Natural products have provided some of the most important cancer chemotherapeutics (Raymond 2004; Effert 2009; Effert 2010; Filip et al., 2011; Siu 2011).

The extraction of drug candidates from natural product sources requires a proper selection of plant, extraction method, and screening method for discovering bioactive molecules (Jawad Alzeer et al., 2014).

Generally, at present used anticancer drugs are highly toxic, costly, and resistance mechanisms pose a significant problem (Lippert et al., 2008; Petrelli & Giordano 2008; Hait & Hambley, 2009). Thus there is a continuing need to identify new

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drugs that are more effective, widely available and less toxic.

MATERIALS AND METHODS

Collection and authentication of A. marina

Fresh leaves of A. marina were collected from different places such as Muthupet (Thiruvurur district) and Pichavaram (Cuddalore district), Tamil Nadu, India. They were authenticated at Arignar Anna Siddha Central Research Institute, Arumbakkam, Chennai, Tamil Nadu, India. After washing with distilled water, the leaves were shade dried, powdered and used for further work.

Preparation of methanol extract of A. marina (MEAM)

The MEAM was prepared and lyophilized to remove the solvent completely. Then it was stored at 4°C in airtight bottles and used for further studies (Harbome 1973). The percentage yield for MEAM was calculated at 8.7%.

Quantitative analysis of bioactive constituents present in A. marina

Alkaloids, flavonoids, saponin and tannin were extracted from the leaves of A. marina by Harborne (1973), Boham & Kocipai - Abyazan (1994), Obadoni & Ochuko (2001), and Onwuka (2005) respectively. The content of these phytoconstituents was found to be 13.87%, 5.25%, 4.83% and 14.52% respectively. Further, they were subjected to find out anticancer activity on a cervical cancer cell by 3-(4, 5-Dimethyl-2-thiazoly)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay (Mosmann 1983). The test was performed in triplicates and mean values calculated.

Determination of apoptotic cell death by fluorescence microscopic analysis

Apoptotic cell death was performed by acridine orange/ethidium bromide (AO/EB) double staining method (Baksić et al., 2006). The cells used are hela cell lines which were derived from cervical cancer. The concentration of MEAM such as 500, 1000 µg and 2500 µg was used in this experiment. The final suspension was observed under the fluorescent microscope (Labomed - Carl zeiss Lens with blue filter Olympus India). The level of apoptotic cell death was quantified using image J software (Version 2.1).

RESULTS AND DISCUSSION

Anticancer activity of alkaloids, flavonoids, saponin and tannin from A. marina

The percentage of the anticancerous activity of alkaloids, flavonoids, saponin and tannin at a concentration of 25, 50, 100, 250 and 500 µg/ml on Hela cell lines was given in Table 1. The result showed that flavonoids of A. marina exerted higher anti-cancer activity on Hela cells followed by tannin, alkaloids and saponin.


The above results strongly recommended for consideration of A. marina as a valuable source for the study of isolation, identification and characterization of potential bioactive compounds with the anticancer property. Finally, there is a need to explore this area further to know the potentiality of the A. marina towards the development of new medicines against cancer.

Apoptotic cell death effect of MEAM

The apoptotic cell death effect of MEAM at a concentration of 250, 500, and 1000 µg/ml on Hela cell line was found to be 0.66±0.01, 2.20±0.16 and 3.27±0.13 respectively. It was compared with untreated (control) Hela cell line. The results were shown in Figure 1.

Apoptosis is programmed cell death and is usually characterised by a distinct set of morphological hallmarks, including membrane blebbing, cytoplasmic and nuclear shrinkage, and Nuclear DNA fragmentation in cells due to endonuclease activation (Levine et al., (1991); Reed (1999). Also, chromatin condensation, reduction of cell volume and formation of apoptotic bodies (Dejan Baskic et al., 2006).

The present study also indicates that a significant level of nucleus changes occurred by staining with dual fluorescent dyes. The live cells existed in fluorescent green colour, and the dead cells treated with MEAM existed in red colour. The control cells had healthy spherical green with the yellow nucleus. The treated cells by MEAM at a concentration of 250 and 500 µg/ml showed apoptotic cells and severe nuclear change. At 1000 µg/ml dose treated cells showed nuclear condensation and also fragmentation of the nuclear bodies. The morphology was observed as shrink end small spherical and dislodged structure.

The results obtained from this study are comparable with the results reported earlier by Montazi borojeni et al., (2011) according to their
Table 1: Anticancerous activity of phytoconstituents of A. marina

<table>
<thead>
<tr>
<th>Concentration of phytoconstituents (µg/ml)</th>
<th>Percentage of the anticancerous activity (Mean ± S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alkaloids</td>
</tr>
<tr>
<td>50</td>
<td>4.47±1.50</td>
</tr>
<tr>
<td>100</td>
<td>11.27±1.83</td>
</tr>
<tr>
<td>250</td>
<td>18.70±0.89</td>
</tr>
<tr>
<td>500</td>
<td>31.47±0.99</td>
</tr>
<tr>
<td>1000</td>
<td>46.20±1.11</td>
</tr>
</tbody>
</table>

Figure 1: Apoptotic cell death effect of MEAM at different concentrations on cervical cancer cell.
C: control (Hela cell line); B, C & D: Hela cell lines treated with 250, 500 & 1000 µg/ml of MEAM respectively. The cells were observed in 10x resolution by fluorescent microscopy and recorded for fluorescence quantification using Image J image pixel analysis software. Green cells represent live cells and red cell represents dead cells. Values represent mean ± SD of triplicate experiments. The maximum activity was observed at 1000µg/ml of MEAM.

studies the A. marina leaves were induced apoptosis in a dose-dependent manner on human breast cancer cell line (MDA-MB 231). This was inconsistent with a study by Luke Esau et al., (2015). They reported that the ethyl acetate extract of leaves of A. marina induced apoptosis on the breast cancer cell, in specific 200 µg/ml of the extract showed a significant increase in apoptosis from 20% to 45% on breast cancer cell (breast adenocarcinoma - MCF-7).

CONCLUSION

The current project revealed that the leaf extract of A. marina contains bioactive phytoconstituents that kill on the human cervical cancer cell line, which was analysed by MTT assay. Finally, in our experience, the results of the apoptotic cell death effect of MEAM showed a positive result. The activity of the above-mentioned mangrove plant is due to the presence of the anticancerous compound. Thus the results of the study suggest that it can serve as a candidate for the development of anti-cancer herbal agents against cervical cancer (Hela cells).

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