ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Identification of anticancer activity of phytoconstituents from mangrove

Sheela Devi A*, Joseph J, Bhuvaneshwari V

Department of Biotechnology, Karpaga Vinayaga College of Engineering and Technology, Chinna Kolambakkam - 603308, Tamil Nadu, India

Article History:

Received on: 24.09.2018
Revised on: 16.12.2018
Accepted on: 19.12.2018

Keywords:

Avicennia marina,
Anticancer,
Apoptotic cell death,
Cervical cancer,
Mangrove,
MTT assay

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.

* Corresponding Author

Name: A. Sheela Devi
Email: sheeladevi.kvcet@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v10i2.254

Production and Hosted by

IJRPS | https://ijrps.com

INTRODUCTION

Mangroves are an important ecological asset and economic resource of the coastal marine environment (Deshmukh & Balaji 1994). The stem bark of *Avicennia marina* (Mangrove) showed prominent anticancer activity against tumour cell lines (Prakash Sukhramani *et al*., 2013). The *A. marina* leaves contain bioactive compounds with antimutagenic and antileukaemic effects (Saeed Iriana *et al*., 2012).

Cancer is one of the most devastating diseases in both developing and developed countries. Due to a global increase in life expectancies, the incident s 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; Efferth 2009; Efferth 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

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 (Thiruvarur 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 (Harbone 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-thiazolyl)-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 (Baskić *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.

Salah *et al*., (1995); Del-Rio *et al*., (1997); Okwu (2004) revealed that flavonoids act as antioxidants, free radical scavengers and shows anticancer activity. Uma Maheswara Rao *et al*., (2014) reported that tannin possesses antioxidant property. Li & Wang (2003) stated the anticancer activity of tannin. Bashir & Mohammed (2000) revealed that tannins are potential metal ion chelators and biological antioxidants. Cowan (1999) resulted that alkaloids possess the anti-oxidising property and the anticancer activity of 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 Momtazi borojeni *et al*., (2011) according to their

Table 1: Anticancerous activity of phytoconstituents of *A. marina*

Concentration of phytoconstituents (µg/ml)	Percentage of the anticancerous activity (Mean ± S.D)			
	Alkaloids	Flavonoids	Saponin	Tannin
50	4.47±1.50	9.10±1.31	7.57±0.42	8.07±0.74
100	11.27±1.83	23.93±1.19	13.13±0.31	16.63±1.53
250	18.70±0.89	40.70±1.80	20.10±0.85	25.93±0.95
500	31.47±0.99	40.97±0.80	31.23±1.40	36.40±0.95
1000	46.20±1.11	51.80±1.15	44.40±0.70	48.90±1.40

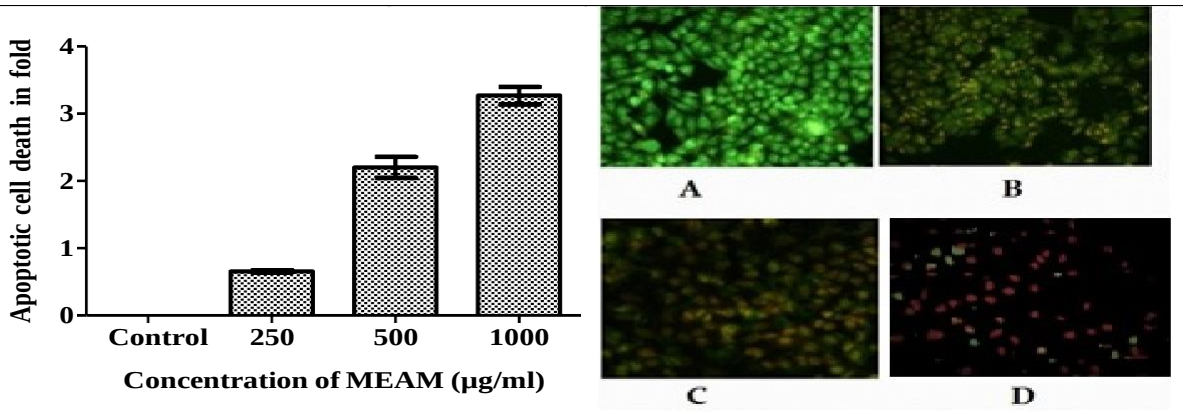

Figure 1: Apoptotic cell death effect of MEAM at different concentrations on cervical cancer cell. C: control (Hela cell line); B, C & D: Hela cell lines treated with 250, 500 & 1000 µg/ml of MEAM respectively. The cells were observed in 10x resolution by fluorescent microscopy and recorded for fluorescence 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).

Acknowledgement

The author A. Sheela Devi acknowledges the Science & Engineering Research Board (SERB), Department of Science & Technology, Government of India for financial assistance through this project (SB-EMEQ-123/2014). Also, we are grateful to the Department of Biotechnology, Karpaga Vinayaga College of Engineering and Technology, Chinna Kolambakkam, India for providing infrastructure for carrying out this research work.

REFERENCES

Alzeer, J, Vummidi, B, R, Arafeh, R, Rimawi, W, Saleem, H, Luedtke and Nathan, W. The influence of extraction solvents on the anticancer activities of Palestinian medicinal plants, Journal of Medicinal Plant Research, 2014, vol. 8, no. 9, pp. 408-415.

Deshmukh, S and Balaji, V. Conservation of Mangrove forest Genetic resources – A training manual, Itto-Crsard project, M.S.Swaminathan Research Foundation, Chennai, 1994, pp. 487.

Efferth, T. Cancer therapy with natural products and medicinal plants. Planta Med., 2010, vol. 76, pp. 1035-1036.

Efferth, T. Natural products pave their way in cancer therapy. Cancer Biol Ther., 2009, vol. 8, pp. 1869-1870.

Filip, A, Daicoviciu, D, Clichici, S, Mocan, T, Muresan, A and Postescu, I. D. Photoprotective effects of two natural products on ultraviolet B-induced oxidative stress and apoptosis in SKH-1 mouse skin. J Med Food. 2011, vol. 14, pp. 761-766.

Hait, W. N and Hambley, T. W. Targeted cancer therapeutics. Cancer Res., 2009, vol. 69, no. 1267, pp. 1263-1267.

Iriana, Leila Karamia, Ahmad Majdb, Sedigeh Mehrabiana, Mohammad Nabiunia and Mandana Salehia. Antimutagenic and anticancer effects of *Avicennia marina* leaf extract on *Salmonella typhimurium* TA100 bacterium and human promyelocytic leukaemia HL-60 cells, Saeed Science Asia, 2012, vol. 38, pp. 349-355.

Jawad Alzeer, Balayeshwanth, R. Vummidi, Rami Arafeh, Waleed Rimawi, Hatem Saleem and Nathan W. Luedtke. The influence of extraction solvents on the anticancer activities of Palestinian medicinal plants. Journal of Medicinal Plant Research, 2014, vol. 8, no. 9, pp. 408-415.

Lippert, T. H, Ruoff, H. J and Volm, M. Intrinsic and acquired drug resistance in malignant tumors. The main reason for therapeutic failure. Arzneimittel Forschung, 2008, vol. 58, pp. 261-264.

Petrelli, A and Giordano, S. From single- to multi-target drugs in cancer therapy: when aspecificity becomes an advantage. Curr Med Chem., 2008, vol. 15, pp. 422-432.

Prakash, S, Sukhramani and Piyush, M. Patel. Biological screening of *Avicennia marina* for anticancer activity, Der Pharmacia Sinica, 2013, vol.4, no. 2, pp. 125-130.

Raymond, E. Are natural products still of interest compared with moleculartargeted therapy for the treatment of cancer?. Clin Lung Cancer., 2004, vol. 5, no.5, pp. 267-313.

Siu, D. Natural products and their role in cancer therapy. Med Oncol., 2011, vol. 28, pp. 888-900.