Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 4, 2012

Research Article

ANTIOXIDANT AND ANTICANCER ACTIVITIES OF *NYCTANTHES ARBOR-TRISTIS*

T D SANDHYA KUMARI*[1], T D SUDHA MADHURI[2], M A SINGARA CHARYA*[3] AND K SUBBA RAO[1]

[1]Centre for Biotechnology, IST, JNTU-Hyderabad, Hyderabad 500085, A.P., India, [2]Department of Microbiology, Kakatiya University, Warangal 506009, A.P., India, [3]Department of Biochemistry, Andhra University, Visakhapatnam 500085, A.P., India.
Email: mascharya@gmail.com

Received: 04 July 2012, Revised and Accepted: 11 Aug 2012

ABSTRACT

Traditional medicine with a long history has played a prominent role in treating diseases throughout the world. India became a herbal hub. In India, several medicinal plants were found to possess anticancer agents since plants contain significant amount of phytochemicals that are antioxidant which can be used for the prevention and treatment of cancer. In the present study, the antioxidant activity by DPPH free radical scavenging assay and anticancer activity by MTT reduction cytotoxicity assay was investigated on MDA-MB 231 Breast Cancer Cell Lines. The dried leaf (DLM), fruit (DFM) and stem (DSM) of *Nyctanthes arbor-tristis* extracted in methanol were used for this assay and the results revealed that DFM at 15µg/ml conc. exhibited strong inhibition of cancerous cell growth with 46%, whereas 71% inhibition was observed with DLM at 30µg/ml conc. and DSM at 30µg/ml conc. showed 82% inhibition. The maximum anti-oxidant activity obtained with DFM at 1000mg/ml conc. is 93.8% whereas the least activity observed is 27.8% at 1.0 mg/ml concentration with DSM.

Keywords: MTT, *Nyctanthes arbor-tristis*, MDA-MB 231, DU-145, Cytotoxicity, Radical Scavenging Assay.

INTRODUCTION

Cancer is one of the major human diseases that cause much suffering and expenditure. About 12.7 millions of cancer cases and 7.6 million deaths were recorded worldwide in 2008[1]. The Egyptians were the first to observe tumors arising in different parts of the body in 3500 BC and in 2000 BC in the classical manuscripts of the Ramayana it was recorded as a disease. Scientific enquiries were first made by Sir Percival Pott in 1775 AD and since then several scientists have been trying to discover the factors responsible for cancer. However an acceptable universal model of a cancer cell against therapeutic attack has yet to be discovered[2]. Generally the treatment of cancer is done by chemotherapy, but its overwhelming side effects[3] and highly toxic nature increased the popularity of herbal medicine usage. Since times immemorial plants have been used for treatment of cancer[4]. The discovery and development of anticancer agents from natural plant sources started in the year 1950[5]. About 35,000 different plant samples were collected by National Cancer Institute from 20 countries in which 1, 14, 000 extracts were screened for anticancer activity[6]. Now-a-days the anticancer drug of natural origin available in the market is 60%[7]. The use of natural products by breast cancer patients is 50%[8]. The major incidence of breast cancer was reported in Pakistan accounting for 38.5% out of which 43.7% were detected in advanced stages[9]. 23% of breast cancer cases and 14% of breast cancer deaths were observed during the year 2008[10]. For cell signaling processes and bacterial intra cellular killing free radicals play an essential role which is derived from molecular oxygen. Antioxidants which help in defence mechanisms are usually required to reduce the free radical damage[11]. Free radicals cause oxidative damage related to aging and diseases like cancer, cirrhosis and diabetes.[12]

Today much attention is being paid to treatment of anticancer activity with natural products. Owing to the strong antimicrobial and other pharmacological properties of the plant, the main objective of this study is antioxidant and anticancer activities of *Nyctanthes arbor-tristis*. It is an ever green shrub or a small tree, about 10 m tall, with shiny and scabrous leaves, sweetly scented flowers and cordate fruit. The plant is distributed throughout the world. It has been used in folk medicine for antibilious, gynecological troubles, and hepatoprotective activity. In this study the antioxidant and anticancer activities of methanolic crude extracts of leaf, fruit and stem are being reported through invitro evaluation.

MATERIALS AND METHODS

Chemicals: Reagent Grade chemicals were used for this assay.

Media: 10% MEM possessing L-glutamine (4 mmol/L), 10% fetal bovine serum (FBS), streptomycin (100 µg/mL) and penicillin (100 units/mL). It is incubated in a humidified atmosphere containing 5% CO_2 with 95 % air at 37°C.

Cell Lines: MDA-MB 231 (Breast Cancer Cell Lines free of pathogens) were purchased from American Type Culture Collection.

Collection of Plant Material

Nyctanthes arbor-tristis plant parts were collected from areas in and around Hyderabad, A.P. The collected plant material was botanically authenticated by Botanical Survey of India, Coimbatore, No. BSI/SRC/5/23/2011-12/Tech. 1443.

Extraction Procedure

The plant materials were extracted through Soxhlet extraction[13].

Antioxidant Activity

DPPH Free Radical Scavenging Assay

According to Adam and Piotrowska[14] method, using stable free radical DPPH, the antioxidant activity was measured by hydrogen donation or radical scavenging abilities. Generally, 1.8 ml of methanolic DPPH solution (0.5mM) was added to 200µl aliquots of different extracts with different concentrations (1.0, 10.0, 100.0, 1000.0 mg/ml). The reaction mixture was allowed to stand for an incubation period of 30 minutes at room temperature. By using UV-VIS spectrophotometer the absorbance of test solutions, control solution consisting of DPPH and Methanol and a blank without the extract were measured at 517 nm. The standard used in the assay was ascorbic acid (Vit. C) with varying concentrations. By comparing the test results with control (not treated with extract) the DPPH percentage inhibition was calculated by using the formula,

$$\text{DPPH radical scavenging activity} \quad \text{or} \quad \% \text{ Inhibition} = \left\{ \frac{A_{Control} - A_{sample}}{A_{Control}} \right\} \times 100$$

Where, $A_{control}$ is the absorbance of DPPH and methanol solution without extract and A_{sample} is the absorbance of extract. The values are represented in Table 1.

Table 1: Effect of different concentrations of methanol extracts by free radical scavenging assay

Plant Extract	Antioxidant Activity			
	Scavenging Effect of Phenolic Crude (%)			
	1.0 mg/ml	10 mg/ml	100 mg/ml	1000 mg/ml
Dried Leaves Methanol	27.8	30.4	68.0	70.8
Dried Stem Methanol	54.6	55.6	69.9	89.0
Dried Fruit Methanol	51.0	59.0	86.0	93.8

Anti Cancer Activity

The Breast Carcinoma Cells MDA-MB 231 (aggressive) were grown in Minimum Essential Medium (MEM) with 10% FBS supplemented with antibiotics and treated with test compounds at 10 µM concentration for a period of 48 h and cytotoxicity was measured by standard MTT assay.

MTT Reduction Cytotoxicity Assay

A colored formazan product is formed by the reduction of MTT which were taken up by the cells with the detection of the readings using spectrophotometry (λmax = 562 nm). Based on the mitochondrial respiratory function, the MTT is reduced, by measuring the number of viable cells in the culture. MDA MB-231 Breast cancer cells were treated with various concentrations (0, 10, 20 and 30 µg) of the compound for 48h. At the end of the treatments, media was aspirated, washed with Dulbecco's phosphate buffered saline (DPBS) and incubated with 20µl of 5 mg/ml MTT solution in 500 µl of culture medium for 1 h at 37°C. In order to solubilize the cells, 500 µl of formazan DMSO was used. By using automated TECAN multimode reader the absorbance was measured at 562 nm. The experiment is conducted in triplicates by comparing the test results with the control in which the drug is omitted[15]. The values are represented in Tables 2 & 3.

Table 2: Effect of different concentrations of methanol solvent against MDA MB-231 breast cancer cell lines

	Conc.	R1	R2	R3	Average	% of Cell Viability	STDEV
	Control	**1.056**	**1.079**	**1.27**	**1.135**	**100**	**0.117478**
Solvent Control	5µl methanol	1.05	1.16	1.02	1.076667	94.86049927	0.073711
	10µl methanol	1.04	1.1	1.06	1.066667	93.979442	0.030551
	20µl methanol	1.08	1.094	0.99	1.054667	92.92217327	0.056439
	30µl methanol	0.91	0.89	0.892	0.897333	79.06020558	0.011015

Table 3: Effect of different concentrations of crude methanol extracts of *Nyctanthes arbor-tristis* against MDA MB-231 breast cancer cell lines

Crude Plant Extract	Conc.	R1	R2	Average	% of Cell Viability	STDEV
	Control	**1.0843**	**1.0317**	**1.058**	**100**	**0.010**
Dried Leaf Methanol	10µg	0.89726	0.86281	0.880035	83.17911	2.302441
	20µg	0.83467	0.83727	0.83597	79.01418	0.173769
	30µg	0.75854	0.76174	0.76014	71.84688	0.21387
Dried Stem Methanol	10µg	0.92846	0.89151	0.909985	86.00992	2.469527
	20µg	0.93741	0.88177	0.90959	85.97259	3.71866
	30µg	0.85832	0.87703	0.867675	82.01087	1.25047
Dried Fruit Methanol	5µg	0.61068	0.59328	0.60198	56.89792	1.162917
	10µg	0.51037	0.51991	0.51514	48.68998	0.637599
	15µg	0.4958	0.47847	0.487135	46.04301	1.158238

[a] Each value represents mean value ± standard deviation.

RESULTS & DISCUSSION

In this study, fruit, leaf and stem methanol extracts of *Nyctanthes arbor-tristis* were tested for invitro anti oxidant and anticancer activities.

The antioxidant activity of dried fruit methanol of *Nyctanthes arbor-tristis* by DPPH free radical scavenging assay showed 93.8% scavenging effect of phenolic crude at 1000 mg/ml conc. and a moderate value of 69.9% with dried stem methanol at 100.0 mg/ml conc. and 27.8% least value was observed with dried leaves methanol at 1.0 mg/ml conc. Based on the antioxidant activities the anti cancer activity by MTT assay was carried out on MDA MB-231 cancer cell lines by MTT assay and found that of all the extracts, dried fruit methanol was most active with 46% of inhibition against the cancer cells at 15µg/ml conc. Moderate activity was observed at 30µg/ml conc. with 71% inhibition of dried leaf methanol extract and least inhibitory activity was observed at 10µg/ml conc. with 86% inhibition of MDA MB – 231 cancer cell lines. A high degree of inhibition against human breast cancer cell lines (MDA-MB 231) was observed with NAT **dried fruit methanol** and the **IC_{50} values** were calculated to be **9.72µg** and **13.8µg**. The **phytochemicals** isolated from NAT **dried fruit methanol** are **glycosides, tannins, phenols and steroids** and are predicted to be responsible for this anticancer activity.

Many plant extracts possessing anti-oxidant principles were reported to have anti cancer nature. Based on this, it was intended to work on this plant and the present study showed that there is a reduction in the cancer cell number count after the usage of dried fruit methanol extract. The results obtained proved that *Nyctanthes arbor-tristis* plants possess anti-cancer nature against MDA MB-231 breast cancer cell lines.

CONCLUSION

The methanolic dried fruit extract of *Nyctanthes arbor-tristis* plant was found to be a great source of active principles for potent inhibition of cancer cell lines. In future the plant can be considered to be an important pharmacophore. Further investigations on fruit extract are required to study the mechanism involved in the inhibitory action against breast cancer cell lines.

ACKNOWLEDGEMENT

Authors are extremely obliged to Dr. Srigiridhar Kotamraju, Scientist, Mr. K. Koteswara Rao & Mr. Karnewar Santosh CSIR, IICT,

Hyderabad, Mr. M. Ramprasad & Mr. Shiva Krishna, Research Scholars, Dept. of Microbiology, Kakatiya University, Warangal.

REFERENCES

1. GLOBOCAN 2008 (available at http://www.globocan.iarc.fr).
2. C.D. Varghese; Anticancer Activity of Selected Medicinal Plants; Sep., 1993.
3. Modha J, Modha N; International Conference for Ayurveda Studies, Jamnagar, Gujarat, India: Gujarat Ayurveda University; Role of Ayurveda in the management of cancer; 2007.
4. Hartwell JL; Plants used against cancer: a survey, Lawrence, MA. Quarterman Publications; 1982; 438-39.
5. Reddy L, Odhav B, Bhoola KD; Natural products for cancer prevention: a global perspective, Pharmacology & Therapeutics; 2003; 99:1-13.
6. Shoeb M; Cytotoxic compounds from the Genus *Centaurea*; PhD Thesis, Aberdeen, UK, The Robert Gordon University; 2005.
7. Cragg GM, Newman DJ, Snader KM; Natural products in drug discovery and development; J Nat Prod. 1997; 60: 52-60.
8. Richardson MA; Biopharmacologic and herbal therapies for cancer: research update from NCCAM; J. Nut. 2001131:3037S–3040S.
9. Soliman AS, Samad S, Banerjee M, Chamberlain RM , Robert M, Chamberlain , Aziz Z; Brief Continuing Medical Education (CME) Module Raises Knowledge of Developing Country Physicians International Electronic J. Health Education; 2006; 9:31-41.
10. Ahmedin Jemal DVM, Freddie Bray, Melissa M. Center, Jacques Ferlay, Elizabeth Ward, David Forman. Global Cancer Statistics, A Cancer Journal for Clinicians, WILEY Online Library, February 4, 2011; Vol.1 Issue 2; 69-90.
11. Ashwini.M, Nisha Lather, Shivaji Bole, Vedamurhy AB, Sam Balu; Invitro Antioxidant and Anti-inflammatory activity of *Coccinia grandis;* IJPPS, 2012; Vol.4, Issue 3, 239-242.
12. Vijaya kumarArul Doss, P.T.Kalaichelvan; Invitro antimicrobial and antioxidant activity screening of Andrographis Paniculata leaf ethanolic extract in Tamilnadu; 2012, Vol.4, Issue1, 227-229.
13. Walter, H; Ecology of Tropical and Sub Tropical Vegetation, Oliver and Boyd-Edinburgh, UK, 1971; 539.
14. Matkowski A, Piotrowska M; Antioxidant and free radical scavenging activities of some medicinal plants from the Lamiaceae, *Fitoterapia;* 2006; 77:346-53.
15. Srigiridhar Kotamraju, Carol L. Willams, and Balaraman Kalyanaraman; "Statin-Induced Breast Cancer Cell Death: Role of Inducible Nitric Oxide and Arginase-Dependent Pathways"; Cancer Res August 1, 2007; *67;* 7386.