Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Suppl 1, 2014

Full Proceeding Paper

IN -VITRO ANTI OXIDANT AND ANTITUMOR STUDIES ON *TERMINALIA CATAPPA* BARK

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Received: 15 Nov 2013, Revised and Accepted: 06 Dec 2013

ABSTRACT

Plant research has been intensified worldwide in the recent times. A large number of medicinal plants and their purified constituents were proved to have immense therapeutic potentials and have been reported to exhibit antioxidant activity. In this study, attempts are made to evaluate the antioxidant and antitumor potentials of aqueous extract of *Terminalia catappa* bark. The anti tumor study was carried out against Ehrlich Ascites Carcinoma Cell line. *In-Vitro* antioxidant activity was investigated using DPPH assay, superoxide anion scavenging assay, nitric oxide (NO) scavenging and metal ion chelating assays. For assessing In-vitro antitumor activity, cytotoxicity of the extract against Ehrlich Ascites Carcinoma cell line was studied using Trypan blue dye exclusion method and MTT assay. The results of the present study clearly depicted that aqueous extract of *Terminalia cata*ppa bark has potent antioxidant and antitumor activities.

Keywords: Antioxidant activity, Antitumor activity, EAC cell line, *Terminalia catappa*

INTRODUCTION

Free radicals play a major role in the development of chronic and degenerative diseases such as cancer, arthritis, autoimmune disorders, aging, cardiovascular and neurodegenerative diseases [1-5]. Imbalance in the formation and neutralization of free radicals generates a condition called oxidative stress, which affect the cell membranes and major biomolecules [6-11].

Cancer is the third leading cause of death worldwide, preceded by cardiovascular and infectious diseases. Oxidative stress, unhealthy lifestyle, hormonal imbalance and chronic infections are the major causes of cancer. Free radical scavenging activities of endogenous and exogenous antioxidants prevent and repair the damages caused by ROS and RNS. Thus antioxidants enhance the immune defence and thereby reduce the incidence of cancer and other degenerative diseases.

The search for herbal based drugs and dietary supplements has been increased in the recent years, because of better curative potential and without side effects. Tropical almond which is botanically equated as *Terminalia catappa* has been reported to have many pharmacological activities. It was reported earlier that various extracts of leaves and bark of *T.catappa* possess anticancer, anti-HIV reverse transcriptase [12] hepato-protective [13] as well as anti-inflammatory [14], antihepatitis [15], anti-diabetic [16] and aphrodisiac [17] properties. Hence in this study, attempts are made to evaluate the *In-vitro* antioxidant and anticancer potentials of *T.catappa* bark.

MATERIALS AND METHODS

Collection of Plant Material

The bark of *T.catappa* were collected from Mannargudi, TamilNadu. It was identified and authenticated in the Centre for advanced research in Indian system of Medicine, SASTRA University, Thirumalaisamudhram, Tamil Nadu, and authenticated by comparing with the voucher specimen deposited at Raphinet Herberium St. Joseph's College, Trichy (Acc No RHF 13456047)

Preparation of the Plant Extract

The powdered material of bark (200g) was extracted with distilled water. The aqueous extract was evaporated to dryness and stored in a refrigerator till the time of use.

Cell Lines

Ehrlich ascites carcinoma (EAC) cells were obtained from a recognised cancer research centre, Trissur, Kerala and they were maintained by intra peritoneal inoculation of $1x10^6$ cells /mouse.

In vitro Antioxidant Assay

In-vitro antioxidant activity of the bark of *T.catap*pa was evaluated through DPPH radical scavenging assay [18], reducing power assay [19], Nitric Oxide Scavenging activity [20], superoxide scavenging activity [21].

In vitro Cytotoxicity

In-vitro cytotoxicity of the extract was evaluated through Trypan blue test [22] and MTT assay [23].

RESULTS

In Vitro Antioxidant Assay of aqueous extract of *T. catappa* bark

From the Table 1it is evident that free radical scavenging activity of the extract is dose dependent which increases with increasing concentration.

In vitro cytotoxic effect of aqueous extract of *T. Catappa* bark against EAC Cell lines (Trypan Blue dye exclusion method)

The aqueous extract of *T. catappa* bark was found to be more cytotoxic against EAC cells (Table 2).The effect was dose dependent.1000 µg/ml exhibited 57.9% cytotoxicity.

In vitro cytotoxic effect of aqueous extract of *T. Catappa* bark against EAC Cell lines (MTT ASSAY)

The extract was found to be cytotoxic against EAC cells. A dose level of 250 µg/ml showed 57.37% cytotoxicity.

Table 1: *In vitro* Antioxidant Assay of aqueous extract of *T. catappa* bark

S. No.	Test	Concentration of *T. catappa* bark (µg/ml)				
		50	100	250	500	1000
1	DPPH	11.36	22.12	30.71	39.85	65.62
2	Reducing Power Assay	20.13	31.23	39.24	45.86	57.14
3	Nitric Oxide	11.38	17.14	30.86	46.67	55.92
4	Superoxide	9.15	17.26	39.12	45.71	61.46

Table 2: *In vitro* cytotoxic effect of aqueous extract of *T. catappa* bark against EAC Cell lines (Trypan Blue dye exclusion method)

S. No.	Concentration of Plant Extract (µg/ml)	No. of viable Cells	Viable Cells (%)	No. Dead cells	Dead cells (%)
1	50	94	89.52	11	10.48
2	100	89	76.72	27	23.28
3	250	74	70.47	31	29.53
4	500	66	60.55	43	39.45
5	1000	40	42.10	55	57.9

Table 3: *In vitro* cytotoxic effect of aqueous extract of *T. catappa* bark against EAC Cell lines (MTT ASSAY)

Conc. (µg/ml)	OD-1	OD-2	OD-3	Avg	% Cytotoxicity
Control	0.307	0.312	0.297	0.305	-
10	0.291	0.297	0.263	0.283	7.21
25	0.261	0.259	0.243	0.254	16.72
50	0.210	0.198	0.164	0.190	37.70
100	0.171	0.193	0.168	0.177	41.96
250	0.116	0.143	0.132	0.130	57.37

DISCUSSION

In the present study, the aqueous extract of *T. catappa* bark has been evaluated for it's antioxidant and anticancer potentials. At present chemotherapy is considered as the most efficient approach for cancer treatment. Even though it significantly improves symptoms and the quality of life of cancer patients, only modest increase in survival rate can be achieved. As a palliative care, many cancer patients use herbal therapies. Medicinal plants are well known for their immunomodulatory and antioxidant activities by enhancing both non specific and specific immunity [24, 25]. Plants contain phytochemicals with strong antioxidant activities which may prevent and control cancer and other diseases by protecting the cells from the deleterious effects of the 'free radicals'.

In the present study, various concentrations of the extract of the test drug (50,100, 250,500 and 1000 µg/ml) showed free radical scavenging potential, among which 1000/ µg/ml of the extract showed maximum radical scavenging activity. The antioxidant activity of the extract was compared with the standard Ascorbic acid (200 µg/ml).

Varieties of medicinal plants have been reported to be effective in various types of malignant (cancer) and benign tumors of humans and experimental animals. In the present study, various concentrations of the extract showed cytotoxic activities against EAC cells which was evaluated through Trypan blue dye exclusion method and MTT assay method. Percentage of dead cells was found to be increasing when the concentration of the extract was 1000 µg/ml (Table 1). From the MTT assay, it was evident that the cytotoxicity of the extract was dose dependent. Maximum cytotoxicity was produced with 250 µg/ml of the extract.

Present findings, clearly depicted the anti-tumor and antioxidant potentials of the aqueous extract of *T. catappa* bark.

CONCLUSION

The data of the present study clearly suggested that the aqueous extract of *T. catappa* bark possess potent antioxidant and anticancer activities. The results of the present study suggest that a potent herbal anticancer drug can be developed from this source, after conducting further in-depth research.

REFERENCES

1. Valko M, Izakovic M , Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem. 2004; 266:37-56.
2. Valko M, Leibfritz D, Moncola J, Cronin MD. Free radicals and antioxidants in normal physiological functions and human disease. Review. Int J Biochem Cell Biol. 2007; 39: 44-84.
3. Parthasarathy S, Santanam N, Ramachandran S, Meilhac O. Oxidants and antioxidants in atherogenesis: An appraisal. J Lipid Res.1999;40: 2143-57.
4. Frei B. Reactive oxygen species and antioxidant vitamins. Linus Paulin Institute. Oregon State University. 1997;http://lpi.oregonstate.edu/ f-w97/reactive.html
5. Chatterjee M, Saluja R, Kanneganti S. Biochemical and molecular evaluation of neutrophil NOS in spontaneously hypertensive rats. Cell Mol Biol 2007; 53: 84-93.
6. Droge W. Free radicals in the physiological control of cell function, Review. Physiol Rev. 2002;82: 47-95.
7. Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease. Review. Crit Rev Food Sci Nutr 2004;44: 275-95.
8. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev 2007;87:315-424.
9. Genestra M. Oxy radicals, redox-sensitive signalling cascades and antioxidants. Review. Cell Signal 2007;19:1807-19.
10. Halliwell B. Role of free radicals in neurodegenerative diseases: therapeutic implications for antioxidant treatment. Drugs Aging 2001;18: 685-716.
11. Young I, Woodside J. Antioxidants in health and disease. J Clin Pathol 2001;54: 176-86.
12. Tan GT, Pezzuto JM, Kinghorn AD, Hughes SH. Evaluation of natural products as inhibitors of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase. J Nat Prod 1991; 54: 143-154.
13. Lin CC, Chen YL, Lin JM and Julie, T. Evaluation of antioxidant activity of *Terminalia catappa*. Am J Chin Med 1997;25: 153-161.
14. Lin CC, Chen, YL.. Effects of punicalin on carrageenan- induced inflammation in rats. Am J Chin Med 1999; 27: 371-376.
15. Chen PS and Lin TC. Folk medicine *Terminalia catappa* and its major tannin components are effective in ovary cells. Cancer letters, 2000. 52 : 115-122.
16. Nagappa AN, Thakurdesai PA , Venkat Rao , N and Singh, J. Anti diabetic activity of T*erminalia catappa* Linn fruits . J Ethnopharmacol 2003; 88: 45-50.
17. Ratsnasooriya WD and Dharmasuri ,MG. . Effects of *Terminalia catappa* seeds on sexual behavior and fertility of male rats. Asian J Androl 2000 ;2: 213 – 226.
18. Gyamfi, MA, Yonamine M and Aniya Y. Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally-induced liver injuries, Gen Pharmacol 1999;32(6): 661–667.
19. Yıldırım A., Mavi A., Kara AA. Determination of Antioxidant and Antimicrobial Activities of *Rumex crispus* L. Extracts. J Agric Food Chem 49: 4083-4089, 2001
20. Sreejayan and M.N. Rao, 1997. Nitric oxide scavenging by curcuminoids. J. Pharm. Pharmacol., 49: 105-107.
21. Halliwell, B., Gutteridge, J.M.C. 1985. Free radicals in biology and medicine. Oxford, UK: Oxford University Press.

22. Gothoskar SV, Ranadive KJ. Anticancer screening of SAN-AB, An extract of marking nut *Semicarpus anacardium,* Indian J Exp.Biol. 1971; 9:372-375.
23. Scudiero DA, Shoemaker RH, Paul KD. Evaluation of soluble terazolium formazan assay for cell growth and drug sensitivity in clusters using human and other tumor cell lines. Cancer Res 1988; 48:4827-4833
24. Agarwala Sk, Chatterjee S, Misra Sk . Immunepotentiation activity of a poly herbal formulation 'Immu-21' (research name). Phytomedica, 2001; 2: 1-22.
25. Lin YL, Juan IM, Chen YL, Liang YC. Lin JK. Composition of polyphenols in fresh tea leaves and associations of their oxygen radical with anti-proliferative actions in fibroblast cells. J Agric Food chem., 1996;44: 1387-1394.