Polyphenol analysis and Antitumor activity of Crude extracts from Tegmen of Artocarpus heterophyllus.

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Citation

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Abstract

Artocarpus heterophyllus are rich sources of the isoprenylated phenolic compounds, including flavonoids. In this study, crude extracts from the tegmen of A. heterophyllus were tested in vitro fortheir antitumor activity. Total polyphenol content of the extracts ranged from 97.33 to 117.75 mg gallic acid equivalent (GAE) /g extract depending on the solvent used and extraction time applied. Among the three solvent extracts, methanol extract showed maximum polyphenol content at 2 hr extraction time followed by ethanol and butanol respectively. The methanolic extract showed maximum cytotoxicity on HEp2 cells up to 1:4 dilution. Cytotoxic changes observed was cell aggregation, cell rounding and cell death. The overall result indicates the promising baseline information for the potential uses of crude extract from the tegmen of A. heterophyllus as an antitumor agent.

INTRODUCTION

Many medicinal and food plants contain large amounts of antioxidants other than Vitamin C, Vitamin E and Carotenoids. The antioxidative effects are mainly due to Phenolic acids, flavonoids, and phenolic diterpenes. Natural products are reportedly beneficial to physiological health. Various favonoids and non-favonoids have been reported as showing radical scavenging activity (Sawa et al., 1999). Phenolic acids constitute a large group of naturally occuring organic compounds with a broad spectrum of pharmaceutical activities. It was found that they possess not only antioxidant but also antiviral and antibacterial properties. The antioxidant activity of phenolics is generally combined with hydroxyl groups on their molecules (Perchellet, 1989 and Dragsted, 1998). These natural antioxidants can exert considerable protection, in humans, against aging and cancer caused by free radicals, and can replace synthetic antioxidants such as butylated hydroxyanisole (BHA) and buylated hydroxytoluene (BHT), which are suspected to have toxic and carcinogenic effect on humans (Beier and Oertli, 1983, Afek et al., 1986, Zaat et al., 1987 and Laks and Pruner, 1989).Employing different long term experimental tumorigenesis protocols, several studies have demonstrated the cancer preventive effects of polyphenolic antioxidants (Hocman, 1989 and Yang, 1997). The cancer chemopreventive effects of polyphenolic antioxidants is specifically important since environmental pollutants, radiation and physical stress exhibit the ability to produce enormous amount of free radicals which cause many diseases, including tumor promotion and cancer (Agarwal, 1993).

Moracea is large family comprising sixty genera and nearly 1400 species, including important group such as Artocarpus, Morus, and Ficus. Artocarpus heterophyllus or Jackfruit (family of Moraceae) is a monoecious evergreen tree that is grown in several tropical countries. It produces a large pear or barrel-shaped fruit that can grow up to 90 cm long, 50 cm thick and having a weight of 20 kg. Several individual fruits, covered with fleshly and juicy perianths, are found under the spiny surface. The seed is large, oblong and has a slimy membranous testa and a brown tegmen. A.heterophyllus is widely distributed in tropical region and has been used as traditional folk medicine against inflammation, malarial fever and so on. In addition, the function of Artocarpus heterophyllus in human health such as pulp and seed for tonic; root for diarheae, fever; wood for muscular contraction; leaves for activating milk in women and animal, anti-syphilis, vermifuge; leaf ash for ulcers and wound. Moraceae plants including A. heterophyllus are rich sources of the isoprenylated phenolic compounds, including flavonoids.

MATERIALS AND METHODS

PREPARATION OF PLANT EXTRACT

About 25 g of the inner thin membranous brown tegmen of the Jack fruit seed was and taken in a conical flask and immersed in 100 ml of organic solvent namely ethanol, Methanol and Butanol. It was incubated at room temperature at different time intervals like 2, 12 and 24 hr at 150 rpm. After incubation time, the suspension was filtered and the solvent was evaporated. The extract was concentrated to dryness and dissolved in 0.25% Dimethyl Sulphoxide (DMSO, Merck) to the concentration of 100 mg / ml.

DETERMINATION OF TOTAL POLYPHENOL CONTENTS

Total polyphenol contents in the extracted powder from the tegmen were determined by the Folin-Ciocalteu colorimetric method (Ough and Amerine, 1988 and Kumazawa et al., 2002). Various solvent extracts of the sample (1 mg/ml) were mixed with 1 ml of the Folin-Ciocalteu reagent and 1 ml of 10% Na_2CO_3, and the absorbance was measured at 760 nm after 1 hr incubation at room temperature. The standard curve was prepared using 50 to 250 mg/ml solutions of gallic acid. Total polyphenol contents were expressed as mg GAE/ g of extract.

ANTITUMOR ASSAY

The antitumor assay was performed on human laryngeal epithelioma (HEp2) cells obtained from King Institute of Preventive Medicine, Chennai, India. The cells were grown in 24 well plate (Falcon) in Eagle's Minimum Essential Medium (Hi Media) supplemented with 10% fetal bovine serum (Gibco Laboratories) and 1% antibiotics (streptomycin, penicillin-G, kanamycin, amphotericin B, Hi Media). The cell suspension (105 cells / ml) was seeded in every well and incubated at 37oC for 48 hr in 5% CO_2 for the formation of confluent monolayer. The monolayer of cells in 24 well plates was exposed to various dilutions of the methanolic extract. The cell viability was measured using MTT assay as described by Mosmann (1983) using MTT (5 mg / ml) and DMSO. Cell control was maintained throughout the experiment and the assay was performed in triplicates.

RESULTS

The extractive yield of extracts from the tegmen of Artocarpus heterophyllus seed using ethanol, methanol, and butanol as solvents were 9.42, 14.44 and 4.78 % respectively, on dry weight basis. Total polyphenolic content ranged from 97.33 to 117.75 mg GAE/g of extract (Table 1). Among the three solvent extracts, methanol extract showed maximum polyphenol content at 2 hr extraction time followed by ethanol and butanol respectively.

Figure 1

Table 1. Total polyphenol content and extractive values of crude extracts from the tegmen of

Extraction solvent	Extractive yield (%)	Extraction time (hr)	Total polyphenol (mg GAE/ g extract)
Ethanol	9.42	2	101.15
		12	107.9
		24	114.21
Methanol	14.44	2	117.75
		12	111.42
		24	115.45
Butanol	4.78	2	97.33
		12	99.27
		24	106.65

The tumor cell suppression potential of methanolic extract of the sample on HEp2 cells was recorded. The cytotoxicity of extracts on HEp2 cells was measured using MTT assay. The methanolic extract showed maximum cytotoxicity on HEp2 cells up to 1:4 dilution and the activity started decreasing with increase in dilution (Figure 1). Cytotoxic changes observed was cell aggregation, cell rounding & cell death (Figure 2).

Figure 2

Figure 1. Cytotoxicity of methanolic extracts from tegmen of on human laryngeal epithelioma cells (HEp2).

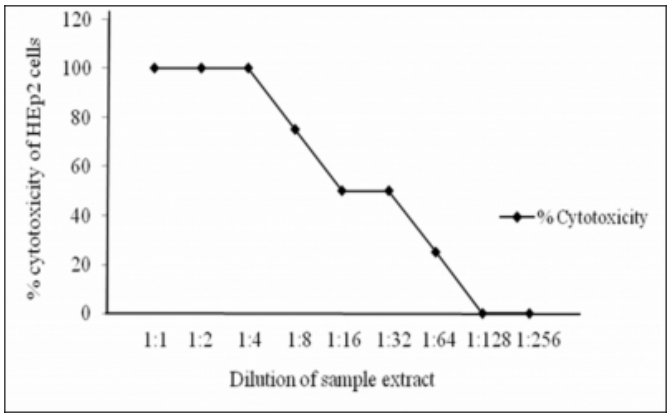
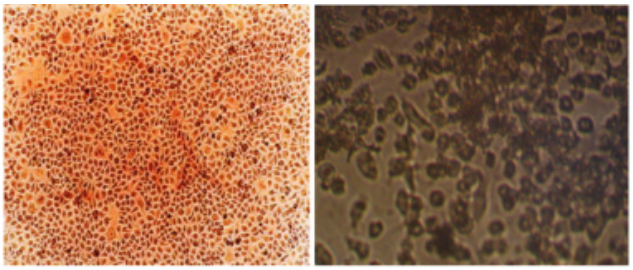


Figure 3

Figure 2. Antitumor activity of methanolic extracts from tegmen of Artocarpus heterophyllus using Hep2 cells (cancer cell line).



A. HEp2 cells, magnification 10X B. HEp2 cells intoxicated with methanolic extracts, magnification 10X

DISCUSSION

The extractive yield of sample was higher in methanol, compared to ethanol and butanol. The yield obtained decreased with decrease in polarity. Since methanol has high polarity, it could dissolve both the polar and non polar compounds in it.

The methanol extracts of sample inhibited nearly 100 % of HEp2 cells up to 1:4 dilution of the crude extract and started decreasing with increase in dilution. Arpornsuwan and Punjanon (2006) reported that the methanolic extract of M. citrifolia fruit was much more effective on breast cancer cells and neuroblastoma cells. Mayalen Zubia (2009) also reported that crude extracts of B. bifurcata, C. tamariscifolia, Desmarestia ligulata, Dictyota dichotoma and H. siliquosa exhibited strong cytotoxic activities against three different tumor cells lines (Daudi, Jurkat and K562).

The extractive value, total polyphenolic content and antitumor activity was at its peak in methanolic extract indicating that most of the active components are extracted with methanol. Cytotoxic changes observed was cell aggregation, cell rounding and cell death. The overall results indicates the promising baseline information for the potential uses of the methanol extracts of tegmen of Artocarpus heterophyllus seed as an antitumor agent.

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References

1. Afek, U., A. Sztejnberg, and S. Carmely, 1986. 6,7–Dimethoxycoumarin, a citrus phytoalexin conferring resistance against Phytophthora gummosis. Phytochemistry., 25:1855–1856.
2. Arpornsuwan, and Punjanon T., 2006. Tumor cell selective antiproliferative effect of the extract from Morinda citrifolia fruits. Phytother Res., 20: 515-517.
3. Hocman, G., 1989. Prevention of cancer: vegetables and plants. Comp. Biochem. Physiol., 93B: 201–212.
4. Agarwal, R., Katiyar, S. K., Khan, S. G., and Mukhtar, H., 1993. Protection against ultraviolet B radiation-induced effects in the skin of SKH-1 hairless mice by a polyphenolic fraction isolated from green tea. Photochem. Photobiol., 58: 695-700.
5. Beier, R.C. and E.H. Oertli, 1983. Psoralen and other linear furocoumarins as phytoalexins in celery. Phytochemistry., 22:2595–2597.
6. Dragsted, L.O., 1998. Natural antioxidants in chemoprevention. Arch. Toxicol., 20 (suppl.): 209–226.
7. Kumazawa, S., Taniguchi, M., Suzuki, Y., Shimura, M., Kwon, M.S. Kumazawa, M. Taniguchi, Y. Suzuki, M. Shimura, M.S Kwon, T. Nakayama, 2002. Antioxidant activity of polyphenols in carob pods. J. Agric. Food Chem., 50: 373– 377.
8. Laks, P.E. and M.S. Pruner, 1989. Flavonoid biocides: structure/activity relations of flavonoid phytoalexin analogues. Phytochemistry 28:87–91.
9. Mayalen, Z, Marie S.F., and Veronique, K, 2009. Antioxidant and antitumoural activities of some Phaeophyta from Brittany coasts. Food chemistry., 116: 693-701.
10. Mosmann T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods., 65: 55-63.
11. Ough, C.S. and Amerine, M.A., 1988. Methods for analysis of musts and wines. Wiley & Sons, New York, USA, 196-221.
12. Perchellet, J.P. and Perchellet,E.M., 1989. Antioxidants and multistage carcinogenesis in mouse skin. Free Radic. Biol. Med., 7: 377–408.
13. Sawa, T., Nakao, M., Akaike, T, Ono, K. and Maeda, H., 1999. Alkylperoxyl radical scavenging activity of various flavanoids and other polyphenolic compounds: implications for the anitumor promoter effect of vegetables. J. Agri. Food Chem., 47: 397-402.
14. Yang, C.S., Lee, M.J., Chen, L. and Yang, G.Y., 1997. Polyphenols as inhibitors of carcinogenesis. Environ. Health Perspect., 105 (suppl. 4): 971–976
15. Zaat, S.A.J., A.A.N. van Brussel, T. Tak, et al., 1987. Flavonoids induce Rhizobium leguminosarum to produce nodDABC gene-related factors that cause thick, short roots and root hair responses on common vetch. Journal of Bacteriology., 169:3388–3391.

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