

PHYTOCHEMICAL ANALYSIS AND *IN VITRO* CYTOTOXIC EFFECT OF *PHYLLANTHUS MADRASPATENSIS* L.

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ABSTRACT

Drug discovery from natural sources have become important in the treatment of cancer and indeed over the last few decades, newer clinical application of plant metabolites and derivatives have been attempted for combating cancer. In the present work *Phyllanthus maderaspatensis* L. belonging to the family Euphorbiaceae is selected and subjected to phytochemical analysis and *in vitro* cytotoxicity studies. *In vitro* experiments were carried out against Ehrlich Ascites Carcinoma (EAC) cells employing Trypan Blue method. It is noticed that the cytotoxic effect was dose dependent, 96.03% of cell death was observed at 1000µg/ml. Further in-depth studies can result in a safe and efficacious anticancer drug from this plant source.

Keywords: *Phyllanthus maderaspatensis*; Lupeol; Phyllanthin; Ehrlich Ascites Carcinoma (EAC) cell

INTRODUCTION

Medicinal plants have been contributing for the management of cancer since time immemorial. With a view to develop an eco-friendly herbal drug for cancer, many phytoconstituents and their derivatives were subjected to clinical and preclinical studies. Based on literature review it is observed that many of the Euphorbiaceae members such as *Phyllanthus urinaria*¹, *Mallotus philippensis*² and *Tragia plukenetii*³ were screened for their anticancer potentials against various cancers and the results were encouraging this prompted us to select common Euphorbiaceous plant available in SASTRA campus for screening against EAC cell lines. *Phyllanthus maderaspatensis* L. (Euphorbiaceae) a common weed in SASTRA University campus. This taxon is an erect herb distributed in many places ranging from South Africa to Sri Lanka. *Phyllanthus* species are used since ancient times in different systems of medicine, particularly for treating liver disorders and urinary tract infections⁴. Extract of aerial parts of *Phyllanthus maderaspatensis* are used as a hepatoprotective agent⁵, also useful in headache, bronchitis, ear ache, ophthalmia, griping, cough, ascites, incipient, blindness, sores, ulcers, stomachache, inflammations, intestinal spasms, gonorrhoea, antimicrobial and viral infections^{6,7}. Traditional healers use this plant in treating fever and burns. The drug is reported for its chemoprotective⁸ anti-edematous, anti-dysenterial, laxative, carminative, diuretic and immunomodulatory effects⁹. *P. maderaspatensis* contains essential oil, maderin, mucilage and β - Sitosterol. Seeds of *P. maderaspatensis* contain long chain fatty acids and β - sitosterol. Defatted seed cake contains mucilage, which yields galactose, arabinose, rhamnose and aldobionic acid¹⁰, niruriside¹¹, phyllanthin, hypophyllanthin¹² and cinnamoyl sucrose acetate¹³.

MATERIALS & METHODS

Plant Collection

Whole plant materials of *P.maderaspatensis* were collected from in and around SASTRA University campus, Thanjavur, Tamil Nadu, identified in the department of Centre for Advanced Research in Indian Systems of Medicine (CARISM), SASTRA University, Thanjavur, and authenticated with herbarium specimen of Rapinat Herbarium, St. Joseph's College, Trichy. The plants were cleaned, shade dried and coarsely powdered.

Preparation of plant Extract

The course powdered plant material was extracted by cold maceration with n-hexane for 48 hours and then extracted using methanol for 48 hours¹⁴. The extracts were concentrated under the vacuum in rotary evaporator and stored in an airtight container and refrigerated.

Preliminary phytochemical analysis

The n-hexane extract and methanol extracts were subjected to preliminary phytochemical screening as per standard textual procedure and results are tabulated.

HPTLC fingerprint analysis

HPTLC fingerprint analysis was carried out to identify lupeol and phyllanthin

Standard preparation:

Standards were prepared by dissolving 10 mg of lupeol and phyllanthin in 10 ml of chloroform and methanol respectively.

Sample preparation

100 mg of methanol extract of *P.maderaspatensis* diluted with 10 ml of MeOH and used for the identification of lupeol and phyllanthin.

Chromatographic parameters and conditions

TLC was run using mobile phases 1) hexane: ethyl acetate (8:2) for lupeol and 2) hexane: acetone: ethyl acetate (74:12:8) for phyllanthin.

In vitro cytotoxicity was carried out using Trypan blue exclusion method¹² using various concentrations of the extracts (50, 100, 250, 500, 1000 µg/ml) and the % cytotoxicity was calculated using standard formula.

RESULTS AND DISCUSSIONS



Fig. 1: *Phyllanthus maderaspatensis* L.

Preliminary phytochemical analysis

The data of the preliminary phytochemical screening of hexane and methanol extracts were given in the Table No. 1 and noted the presence of flavonoid compounds, tannin, triterpenoids, carbohydrates and proteins in both extracts.

High Performance Thin Layer Chromatographic Finger Prints

In the present study a HPTLC method is reported to ensure the presence of lupeol content in various fractions of *Phyllanthus*

maderaspatensis. Under the conditions the most suitable mobile phase consisted of hexane: ethyl acetate (8:2) which gave best resolution of free lupeol at R_f 0.54. Lupeol band observed, confirmed the presence of lupeol in the extract.

Phyllanthin was identified in methanol extract of *P. Maderaspatensis* in the mobile phase which consisted of hexane/ acetone/ ethyl acetate (74/12/8). The phyllanthin is visible in both reference and test solution tracks at (R_f 0.24).

Table 1: Preliminary phytochemical screening of hexane and methanol extracts of *P. maderaspatensis*

Compound	Test	Hexane extract	Methanol extract
Alkaloids	Mayer's test	-	-
	Wagner's test	-	-
	Hager's test	-	-
Vitamin - C	Sodium nitro-pruside test	-	-
Proteins and Amino acids	Millon's test	-	+
	Ninhydrin test	-	+
Carbohydrates	Glycoside test	-	+
	Molish test	-	+
	Benedict's test	-	+
	Fehling's test	+	+
Flavanoids	Zinc chloride test	-	+
	Alkaline test	-	+
Triterpenoids	Libermann-Buchard test	+	+
	Salkowski test	-	+
Tannins	Gelatin test	+	+

+ indicates Positive.- indicates Negative.

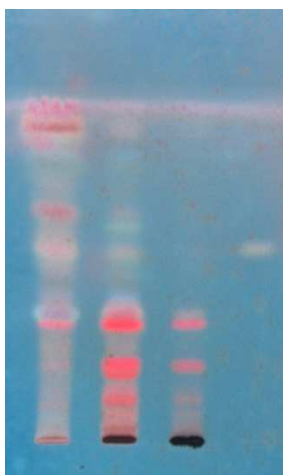


Fig. 2: HPTLC - Lupeol

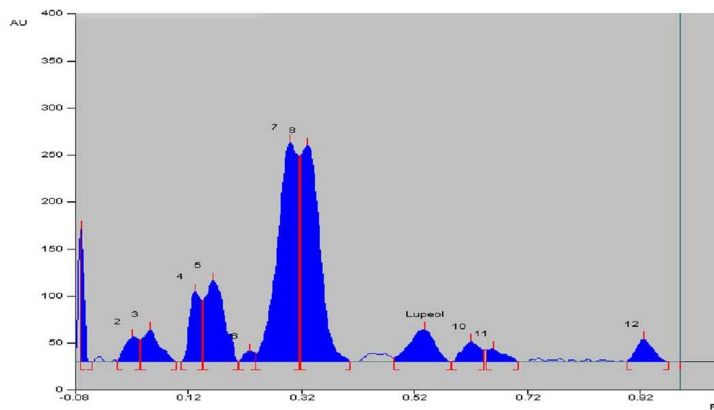


Fig. 3: Chromatogram for lupeol



Fig. 4: TLC for Phyllanthin

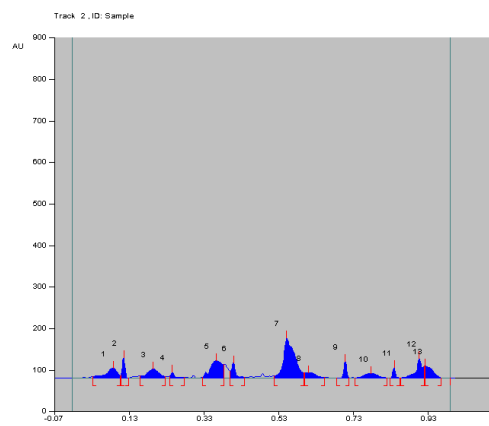


Fig. 5: HTLC chromatogram

In Vitro Cytotoxic effect of methanol extract of against EAC cell lines using Trypan blue method

Plant extract under study was tested for their cytotoxic potentials against Ehrlich Ascites Carcinoma cell lines using trypan blue method. The results revealed that methanolic plant extract possess cytotoxicity against EAC cells lines 93.62 % of cell death was observed at 1000 µg/ml. The cytotoxicity effect of the methanolic plant extract was dose dependent. Maximum cytotoxicity effect was observed at the dose level 1000 µg/ml.

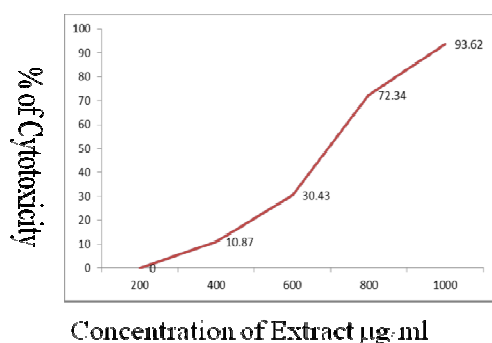


Fig. 6: In vitro cytotoxic studies of *P. maderaspatensis*

As a part of the apoptosis process there must be a considerable loss of membrane integrity and there by the cells will permit the dye to enter. In the present work the extract might have had considerable membrane damaging effect which might have permitted trypan blue to enter cancerous cells causing death of cancer cells.

DISCUSSION

Present study suggested that the test drug might have activated apoptosis. Presence of hepatoprotective molecule phyllanthin is very interesting feature observed in the present study. This molecule can help not only in reverting back the hepatic marker enzymes such as AST, ALT, ALP and GCT but also can contribute towards the inhibition of tissue necrosis, organ destruction and cell injury. Altered metabolic conditions are often encountered in malignancy due to these abnormal variations in liver marker enzymes, which can be corrected by the presence of phyllanthin in the extract. Flavone like luteolin is a proven antioxidant and can help in providing enhanced antioxidant mechanism and contribute towards the prevention of cancer cell growth^{15&16}.

CONCLUSION

Preliminary phytochemical analysis was carried to find out the major chemical constituents present in the *P. Maderaspatensis* the drug under study. Methanol extract contained tannins, triterpenoids, flavonoids, proteins and carbohydrates. Trypan blue method was

employed to assess the cytotoxic potential of the plant extract under study. The results of *in vitro* cytotoxicity study depicted that the methanol extract of *Phyllanthus maderaspatensis* possess good cytotoxic potentials at higher concentration (93.62% of inhibition observed in 1000 µg/ml). The activity might be due to the presence of secondary metabolites such as flavone and phyllanthin present in the plant extract. Flavanoids are known to implicate signal transduction in cell proliferation and thereby prevent cancer. The preliminary data obtained in the present work suggested that further in depth studies can result in the development of a novel new non-toxic anticancer herbal drug from this traditional source.

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REFERENCES

- Huang ST, Wang CY, Yang RC, Chu CJ and Pang JHS. *Phyllanthus urinaria* increases apoptosis and reduces telomerase activity in human nasopharyngeal cells. *Forsch Komplementmed* 2009;16:34-40.
- Tanaka R, Nakata T, Yamaguchi C, Wada S, Yamada, and Tokuda T H Potential Anti-Tumor-Promoting Activity of 3 α -Hydroxy-D:A-friedooleanan-2-one from the Stem Bark of *Mallotus philippensis*. *Planta Med.* 2008; 74(4): 413-416.
- Muthuraman MS, Dorairaj S, Rangarajan P and Brindha P Antitumor and antioxidant potential of *Tragia plukenetii* R.Smith on Ehrlich ascites carcinoma in mice. *African Journal of Biotechnology.* 2008. Vol. 7 (20): 3527-3530.
- Calixto JB, Santos ARS, Filho VC, Yunes RA A review of the plants of the genus *Phyllanthus*: their chemistry, pharmacology, and therapeutic potential. *Medicinal Research Reviews.* 1998.Vol. 18: 225-258.
- Asha VV, Sheeba MS, Suresh V and Wills PJ Hepatoprotection of *Phyllanthus maderaspatensis* against experimentally induced liver injury in rats. *Fitoterapia.* 2007. Vol. 78(2): 134-14.
- Karthikeyan M, Mathews JA, and Annamalai A Antibacterial Activity of *Phyllanthus Maderaspatensis* Leaves Extract. *Pharma Science Monitor.* 2011. IC Vol. 4 (1):1542-1551.
- Munshi A, Mehrotra R, Panda SK Evaluation of *Phyllanthus amarus* and *Phyllanthus maderaspatensis* as agents for post exposure prophylaxis in neonatal duck hepatitis B virus infection. *J Med Virol.* 1993 Vol. 40(1):53-8.
- Munshi A, Mehrotra R, Ramesh R, Panda SK Evaluation of anti-hepadnavirus activity of *Phyllanthus amarus* and *Phyllanthus maderaspatensis* in duck hepatitis B virus carrier Pekin ducks. *J Med Virol.* 1993. Vol. 41(4):275-81.
- Sharma SK, Arogya SM, Bhaskarmurthy DH, Agarwal A, Velusami CC Hepatoprotective activity of the *Phyllanthus* species on tert-butyl hydroperoxide (t-BH)-induced

- cytotoxicity in HepG2 cells. Pharmacognosy Magazine. 2011. Vol. 7(27):229-33.
10. Komuraiah A, Krishna B, Rao K N, Ragan AV, Raju S and Singaracharya M A Antibacterial studies and phytochemical constituents of South Indian *Phyllanthus* species. African Journal of Biotechnology. 2009, 8: 4991-4995.
 11. Schmelzer GH, Fakim AG, Arroo RR and Lemmens HM J. 2008. PROTA Foundation .p.426.
 12. Steward N, Martin R, Engasser JM and Goergen JL. A new methodology for plant cell viability assessment using intracellular esterase activity. Plant Cell Reports,1999, 19:171-176.
 13. Qian-Cutrone J, Huang S, Trimble J, Li H, Lin P, Alam F, Klohr MSE, Kadow K. F J Nat. Prod. 1996. Vol. 59: 196-199.
 14. Tripathi AK, Verma RK, Gupta AK, Gupta MM and Khanuja SPS Quantitative Determination of Phyllanthin and Hypophyllanthin in *Phyllanthus* Species by High-performance Thin Layer Chromatography. Phytochem. Anal. 2006.Vol. 17: 394-397.
 15. Chang CC, Kishore P H, Lee SS "Chemical investigation of *Phyllanthus maderaspatensis* by hyphenation of liquid chromatography-solid phase extraction-nuclear magnetic resonance spectroscopy" The 11th Asian Symposium on Medicinal Plants, Spices and Other Natural Products (ASOMPS XI), 2003. Abstract pc-PP-009, Kunming, China.
 16. Harborne JB Phytochemical methods: A guide to modern techniques of plant analysis. 2nd edn, Chapman and Hall, New York, 1973; pp. 88-185.
 17. Ahmed KKM, Rana AC, Dixit VK *Calotropis* species (Asclepiaceae) A comprehensive review. Pharm. Mag. 2005. Vol. 1: 48-52.
 18. Lhinhatrakool T, Sutthivaiyakit S 19-Nor- and 18, 20-Epoxy-cardenolides from the leaves of *Calotropis gigantea*. J. Nat. Prod. 2006. Vol. 69:1249-1251.