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ORIGINAL ARTICLE

In vitro antioxidant and cytotoxic potential of whole plant of *Phyllanthus maderaspatensis Linn*

Vilas B. Ghawate*[1], Varsha S. Jadhav[1], Devidas G. Bachhav[2]
1. Department of Pharmacognosy, Mula education society's college of Pharmacy, Sonai, India (MS).
2. S.P.H College of Pharmacy, Malegaon, Nashik, India (MS)
Corresponding author's E-mail id- ghawatevilas@gmail.com

ABSTRACT
The natural products from plants are safe as compared to synthetic medicine. The aim of present study was to determine the In vitro antioxidant and cytotoxic activities of different extracts of Phyllanthus maderaspatensis plant. In vitro antioxidant activity of Phyllanthus maderaspatensis Linn extract was evaluated by using DPPH assay method. Invitro cytotoxic effect of Phyllanthus maderaspatensis extracts on BRL-3A cell was studied by using MTT assay. Among the three extracts studied the methanolic extracts of Phyllanthus Maderaspatensis aerial plants showed the highest antioxidant activity by DPPH method with IC50- 3.3 ± 0.17 which is comparable the standard Ascorbic acid (IC50- 2.8 ± 0.08) and the pet ether & aqueous extracts of Phyllanthus Maderaspatensis showed moderate antioxidant activity of DPPH method. In assessment of In vitro cytotoxic studies the methanolic extract showed CTC50 values was 95.6 ± 2.7. In preliminary phytochemical studies methanolic extract shows the presence of alkaloids, glycosides, and tannins. The antioxidant and cytotoxic activity of methanolic extract of Phyllanthus maderaspatensis may be due to the presence of these secondary metabolites.
Keywords: *Phyllanthus maderaspatensis Linn,* Cytotoxic activity, Phytochemicals, DPPH, Anti-oxidant

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INTRODUCTION

The medicinal plants contain, primary, secondary metabolites like flavonoids, alkaloids, Phenolic, tannins, glycosides and steroids they are acting as medicine in single or combination dosage form. The plant oriented drugs are remaining an importance especially in developing countries to use in treatment of diseases. Approximately 60-80% peoples were using or following traditional medicines for treating the disorders. [1] The scientific research was done to investigate plants possess biological activities. *Phyllanthus*-an important medicinal species. Among 700 species distributed worldwide [2], about 24 species occur wild in India and some ornamental exotics are planted in gardens [3]. In general the genus is strongly astringent, diuretic, cathartic and used for treatment of liver disorders, gonorrhea and genitourinary disorders [4]. *Phyllanthus Maderaspatensis* (Euphorbiaceae) is a traditional herbaceous medicinal plant. Leaves are expectorant, diaphoretic. The seeds have bad taste and are carminative, laxative tonic to liver, diuretics useful in bronchitis ear ache, griping opthalmia and ascites [5]. In south India the infusion of leaves is given for headache [6]. The plant shown to be effective in protecting Paracetamol induced liver damage [7, 8].

The current study was designed to evaluate the antioxidant and cytotoxic activity of *Phyllanthus Maderaspatensis.*

MATERIAL AND METHODS

Collection and Identification of Plant Material

The whole plant of *Phyllanthus maderaspatensis* was gifted by M/s. Natural Remedies, Bangalore in form of coarse powder.

Extraction of Plant Material

The coarse powder of the whole plant material was shade dried. Successive extraction was carried out with petroleum ether and methanol respectively by hot percolation process. The marc obtained after methanol extraction was subject to cold maceration using water for 7 days. The extract obtained was concentrated and the yield value of pet ether of *P. maderaspatensis* (PEPM), methanol extract of *P. maderaspatensis* (MEPM) and aqueous extract of *P. maderaspatensis* (AEPM) was found to be 1.9, 4.1 and 3.2 % respectively.

Chemicals and Reagents:

Several chemicals and glassware's have been used to perform this study. The methanol solvent, DPPH (2, 2-diphenyl-1-picryl-hydrazyl), Acetic acid, Petroleum ether were used for study.

Qualitative chemical tests (Chemical color reactions)

The various extracts of *P. maderaspatensis* were tested for the presence of phytoconstituents such as carbohydrates, alkaloids, steroids and sterols, glycosides, saponins, flavonoides, tannins, proteins and amino acids employing standard methods [9-11].

Antioxidant Assay

DPPH Method

21mg of pet ether, methanol and aqueous extracts of *Phyllanthus maderaspatensis* and was dissolved using DMSO to obtain a solution of 21mg/ml concentration and further diluted to appropriate concentrations (500, 125, 62.5, 31.25 15.625, 7.5, 3.9 and 1.95 µg/ml) before transferring to 96 well microtitre plate. To 200µl of DPPH (100µM) solution, 10µl of the test extract (1.95-125µg/ml) was added separately in wells of microtitre plate. The plates were incubated at 37°C for 30 min and the absorbance of each solution was measured at 490nm against corresponding blank, using ELISA reader (Compaq). Ascorbic acid (21 mg/ml in DMSO solution with strengths of 1.95-125µg/ml was used as the positive control.

IC_{50} value is the concentration of sample required to inhibit 50% DPPH free radical. The data obtained were used to determine the concentration of the sample required to scavenge 50% of the DPPH free radical (IC_{50}). The % of the inhibition was plotted against the concentration and IC_{50} obtained from the fitted arm. [12]

MTT Assay

Cytotoxicity studies of plant extracts using established cell line (BRL-3A) using by MTT assay (tetrazolium assay). The monolayer cell culture was trypsinised and the cell count was adjusted to $3x10^5$ cells / ml using medium containing 10% newborn calf serum. To each well of 96 well micro titre plates, 0.1ml of diluted cell suspension was added. After 24 hours, when the monolayer is formed the supernatant is flicked and 100µl of different extracts of various concentration to the cells in microtitre plates. Positive control with cells treated only with plain medium and 2% calf serum were maintained. The plates were then incubated at 37°C for 1 hour in 5% CO_2 atmosphere air. The drug solution in the wells was discarded and 50µl of MTT was added to each well. The plates were gently shaken and incubated for 3 hours at 37°C in 5% CO_2 atmosphere air. The supernatant was removed and 50µl of propanol was added and the plates were gently shaken to solubilize the formed formazen. The absorbance was measured using a micro titer reader at a wavelength of 540nm. [13]

RESULTS AND DISCUSSION

Preliminary Phytochemical Studies

The results from preliminary phytochemical analysis revealed that steroids are present in pet ether extract of *Phyllanthus maderaspatensis*. The methanol and aqueous extracts showed the presence of multiple constituents like carbohydrates, alkaloids, glycosides, flavonoids tannins.

Table 1. Phytochemical analytical test of various extracts of *P. maderaspatensis*.

Sr. No	Phytochemical constituents	Extracts of *P. maderaspatensis*		
		Pet Ether	Methanol extract	Aqueous extract
01	Carbohydrate	-	+	+
02	Alkaloids	-	+	+
03	Steroids and Sterols	+	-	-
04	Glycosides	-	+	+
05	Flavonoids	-	+	+
06	Tannins	-	+	+
07	Saponins	-	-	-

***In vitro* antioxidant activity by DPPH method**

This method depends on the reduction of the purple DPPH by accepting on electron or hydrogen radical to become stable diamagnetic molecules with discoloration. The degree of discoloration indicates the free radical scavenging potentials of the antioxidant compounds or extracts in terms of hydrogen donating ability [14]. DPPH free radical scavenging activities of the different extracts of *Phyllanthus maderaspatensis* are shown in table-2. Methanol extract (IC_{50}- 3.3 ± 0.17) exhibited considerably higher DPPH radical scavenging activity when compared is pet ether extract (IC_{50}- 26.8 ± 1.7) and aqueous extract (IC_{50}- 42.0 ± 0.16). MEPM showed prominent free radical oxygen scavenging capacity with IC_{50} value of 3.3 ± 0.17 and can be considered equipotent to ascorbic acid, which had IC_{50} value of 2.7 ± 0.08 in DPPH method. The antioxidant activity of methanolic extract of *Phyllanthus maderaspatensis* may be due to presence of flavonoids.

Table 2. Antioxidant potential of various extracts of *P. maderaspatensis* in DPPH inhibition model (*in vitro* study)

Treatment	IC_{50} (µg/ml) DPPH method
PEPM	26.8 ± 1.7
MEPM	3.3 ± 0.17
AEPM	42.0 ± 0.16
Ascorbic acid	2.8 ± 0.08

Cytotoxic activity:

Cytotoxicity of extracts of *P. maderaspatensis* were determined by MTT assay on BRL-3-A cell lines. The results of the cytotoxic activity of crude extracts are summarized in Table-3. The methanolic extract of *P. maderaspatensis* show 79% growth inhibition at concentration 250 ug/ml (CTC_{50}-95.6±2.7) and the pet ether extract shows 55% growth inhibition at concentration 250ug/ml (CTC_{50}-233.8±12). Cytotoxic potential was observed in methanolic extract *P. maderaspatensis.* The methanolic extract shows the antioxidant and cytotoxic effect may due to the presence of flavonoids and Polyphenols present in extracts.

Table 3. Effect of Pet ether and methanol extract of *P. maderaspatensis* in MTT assay (cytotoxic evaluation).

TREATMENT	Concentration (µg/ml)	% growth inhibition	CTC_{50}
Control	-	-	-
PEPM	250	55	233.8 ± 12
	125	11	
MEPM	250	79	95.6 ± 2.7
	125	18	

CONCLUSION

The whole plant of extract of *P. maderaspatensis* has antioxidant and cytotoxic activities. In future phytochemical screening of fraction may be required to identify antioxidant and cytotoxic compounds in the P. *maderaspatensis* plant extracts.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this research.

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