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In vitro Antioxidant potential of methanolic extract of whole plant of *Phyllanthus amarus* Schum (Euphorbiaceae)

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Abstract
Phyllanthus amarus belonging to family Euphorbiaceae is an important herb used for the treatment of various diseases such as diarrhoea, dysentery, dropsy, jaundice, intermittent fevers, urinogenital disorders, scabies etc. The objective of present investigation was to evaluate the antioxidant potential of *Phyllanthus amarus*. Antioxidant potential was determined by DPPH free radical scavenging activity using ascorbic acid as standard. The result showed that methanolic extract of whole plant of *Phyllanthus amarus* possess antioxidant property (IC_{50} 41.61 µg/ml) compared to that of the standard ascorbic acid (IC_{50} 12.93 µg/ml).

Keywords: *phyllanthus amarus*, DPPH, IC_{50}

Introduction
Phyllanthus amarus belonging to family Euphorbiaceae [1]. *Phyllanthus amarus*, is a small, erect, annual glabrous herb with 30-50 cm in length [2]. It comprises slender, leaf-bearing branchlets, distichous leaves. The leaves are subsessile elliptic-oblong, obtuse with rounded base. Flowers of the plant are found with 5 white sepals and apical acute anther and are yellowish, whitish or greenish in color, auxillary. Male flowers are found in groups of 1-3 whereas females are solitary. Fruits are depressed-globose like smooth green capsules and fruiting pedicels present underneath the branches. Seeds are trigonous, pale brown with longitudinal parallel ribs on the back.
Phyllanthus amarus has been used traditionally in several health problem such as diarrhoea, dysentery, dropsy, jaundice, intermittent fevers, urinogenital disorders, scabies and wounds over 2000 years. The plant also has been found to have anti-inflammatory, antihepatotoxic, antilithic, analgesic, hypotensive, antispasmodic, antiviral, antibacterial, diuretic, antimutagenic, hypoglycaemic, etc activities.
Oxygen is most important element for all living organism to survive. Some oxygen are univalently reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals in normal metabolic and physiological processes. All these radicals known as reactive oxygen species (ROS). Reactive oxygen species (ROS) play an important role in oxidative stress related to the pathogenesis of many chronic & degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases etc.

Material and Method
Collection of Plant Material
The Indigenous plants were collected from different locations of Bhopal (M.P.) region in the month of Sept.-Oct. 2012 and Jan.-Feb. 2013. The Plant *Phyllanthus amarus* Schum was selected on the basis of ethnomedicinal value for further study. The plants were acknowledged by a senior Botanist Dr. Tayaaf Safi Principal Gandhi P.R. College Bhopal and herbarium deposited at Safia Science College Bhopal.

Preparation of Extract
Plant material was washed with water and then allowed to dry in shade for about 3 to 4 weeks. Dried plant materials were grinded by using the electronic grinder. The powder of the whole plants of *Phyllanthus amarus* S. was extracted according to (Harborne and Baxter., 1995) [8]. The dried plants sample was powered and filed into the soxhlet using petroleum ether and methanol respectively. Almost all the chlorophyll and lipid was deposited on the side of the flask and removed carefully. The extracts were stored in refrigerator till any further use.

Antioxidant Activity
DPPH free Radical Scavenging Assay
Principle
The scavenging reaction between (DPPH) and antioxidant (H-A) can be written as:

$$(DPPH^{\cdot}) + (H\text{-}A) \longrightarrow DPPH\text{-}H + (A)$$
(Purple) (Yellow)

Preparation of Standard Ascorbic Acid Solution
Various solution of the ascorbic acid was prepared in 90% methanol to obtain different concentration (10-100µg/ml). 200µM solution of DPPH in methanol was prepared and 1.5ml of this solution was added to 1.5ml of methanolic ascorbic acid solution to different concentration and incubated for 30 min (at room temperature) in dark. After 30 minutes, the absorbance of each solution of ascorbic acid was taken against methanol (as blank) at 517nm.

Preparation of Test Solution

Various solution of plant extract was prepared in 90% methanol to obtain different concentrations (10-100µg/ml). 200µM solution of DPPH in methanol was prepared and 1.5ml of this solution was added to 1.5ml of methanolic extract solution of different concentration and incubated for 30 min (at room temperature) in dark. After 30 minutes, the absorbance of each solution of ascorbic acid was taken against methanol (as blank) at 517nm

Preparation of Control Solution

For control, 1.5ml of methanol was mixed with 200µM DPPH solution and incubated for 30 min at room temperature in dark. Absorbance of the control was taken after 30min against methanol (as blank) at 517 nm.

The antioxidant activity of plant leaf extract and ascorbic acid were calculated by using the following formula in terms of % of inhibition:

% Inhibition = [(Ac 515 nm- At 515 nm/ Ac 515 nm) x 100].

Where,

Ac = Absorbance of control

At = Absorbance of ascorbic acid/ methanoli plant extract.

Results and Discussion

Antioxidants react with DPPH, a stable free radical, which gets reduced to DPPH-H consequently, the absorbance gets decreased. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extract in terms of hydrogen donating ability.

The obtained results of the methanolic extracts *Phyllanthus amarus* plant are shown below. The scavenging activity of methanolic extract of whole plant of *Phyllanthus amarus* was found (IC_{50} 41.61µg/ml). The scavenging effect was compared to that of the standard ascorbic acid with IC_{50} value 12.93 µg/ml.

Table 1: % Inhibition data of DPPH free radical scavenging assay by ascorbic acid

S. No	Conc.(µg/ml)	Absorbance (Control), Ac	Absorbance (Test), At	% Inhibition
1.	2	0.70	0.51	27.143
2.	4		0.485	30.714
3.	6		0.458	34.571
4.	8		0.424	39.429
5.	10		0.395	43.571
6.	12		0.362	48.286
7.	14		0.336	52
8.	16		0.302	56.857

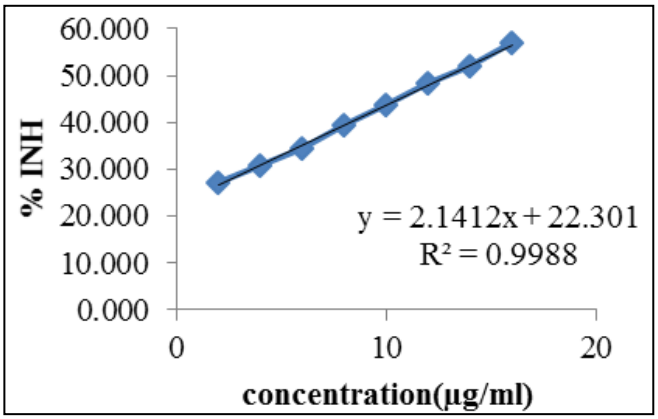
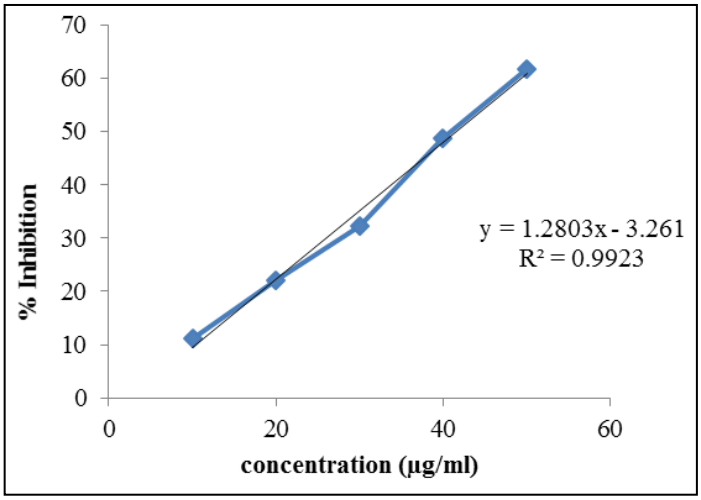


Fig 1: Standard curve of ascorbic acid. Graph representing regression curve of ascorbic acid by DPPH assay method

Table 2: % Inhibition of DPPH by methanolic extract of *Phyllanthus amarus* Schum

S. No	Conc. (µg/ml)	Absorbance (Control),A_c	Absorbance (Test),A_t	% Inhibition	IC_{50} (µg/ml)
1	10	0.478	0.425	11.09	41.61
2	20		0.373	21.97	
3	30		0.324	32.22	
4	40		0.245	48.74	
5	50		0.183	61.72	



Graph 2: Showing regression curve of methanolic extract of *Phyllanthus amarus* Schum by DPPH assay method

Conclusion

In the present study DPPH free radical scavenging activity of methanolic extract of whole plant of *Phyllanthus amarus* was evaluated. The scavenging activity of the plant methanolic extract through DPPH radicals was investigated using ascorbic acid as standard and was found to be (IC_{50} 41.61µg/ml). The current results suggested that the *Phyllanthus. amarus* methanolic extract has potent antioxidant effects. The antioxidant property of the plant can be use in the treatment of various diseases. Further studies are needed for the isolation and characterization of antioxidant compounds.

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