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# IN VITRO EVALUATION OF ANTIOXIDANT POTENTIAL OF ETHANOLIC EXTRACT OF *PHYLLANTHUS AMARUS*- A MAGICAL HERB

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## ABSTRACT

For use of herbal medicines all over the globe, it is important to evaluate antioxidant activity. Antioxidant activity of ethanolic extract of *Phyllanthus amarus* were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and hydrogen peroxide scavenging activity. The results of present study showed potent antioxidant activity of ethanolic extract of plant extract.

**KEYWORDS:** Antioxidant, *Phyllanthus amarus,* DPPH, $H_2O_2$

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## INTRODUCTION

Due to adverse effects of antibiotics on the host including hypersensitivity, immune-suppression and allergic reactions much attention is now focused on plant extracts with biologically active compounds used in traditional herbal medicine. India has a very long, safe and continuous usage of many herbal drugs in the officially recognized alternative systems of health viz. Ayurveda, UnaniSiddha, Homeopathy and Naturopathy. *Phyllanthus amarus* (PA) is widespread throughout the tropics and subtropics in sandy regions as a weed in cultivated and waste lands and also found through the roads, valleys, on the riverbanks and near lakes. This planta common arable weed of disturbed ground in southern Florida, the Bahamas, the West Indies and tropical America and is naturalized in the old world tropics [1]. Phyllanthus, so aptly called the wonder plant perhaps has more useful properties to offer than mankind could ever use [2].This herb finds its use worldwide for treating problems of stomach, urinogenital system, liver, kidney and spleen. It plays a significant role in Ayurveda, an Indian system of medicine, and is used to treat jaundice, gastropathy, diarrhoea, dysentery, fevers, ulcers and wounds [3]. Measurement and use of plant antioxidant has become indispensible for scientific research and industrial purposes. Biological compounds with antioxidant properties contribute to the protection of cells and tissues against deleterious effects of reactive oxygen species and other free radicals [4]. In the present study antioxidant activity of ethanolic extract of PA was evaluated by DPPH and $H_2O_2$.

## MATERIALS AND METHODS

### *1.1. Preparation of plant extract*

*Phyllanthus amarus* whole plant was obtained from the authenticated ayurvedic dealer and identified by the experts of Botany Department, Jiwaji University, Gwalior (India). The shade dried plant was pulverized. The powder of the plant was socked in 75% ethanol for 10 days with vigorous shaking. Extract was filtered and lyophilized in freeze drier to furnish ethanolic extract.

### 1.2. *Free radical scavenging activity by DPPH assay*

The free radical scavenging activity of *Phyllanthus amarus* was measured by 1, 1-diphenyl-2-picryl-hydrazil (DPPH•) using the method of Blois [5] in triplicate. Briefly, a 0.1 mM solution of DPPH dye in ethanol was prepared and 1 ml of this solution was added to 3 ml of PA solution in water at different concentrations (10-50 µl/ml) respectively. Vitamin C used as a standard atsame concentration. The mixture was shaken vigorously and allowed to stand at room temperature for 20 minutes. Then the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Lower absorbance values of the reaction mixture indicated higher free radical scavenging activity.

**Calculation**

$$\text{DPPH• Scavenging effect (\%)} = [(Ao - A1/Ao) \times 100]$$

***Where Ao is the absorbance of the control and A1 is the absorbance in test sample.***

### *1.3. Hydrogen peroxide scavenging activity*

The hydrogen peroxide scavenging activity of PA was determined using the method of Ruch et al. [6]. Sample with different concentration (10-50 µl/ml and 100-500 µg/ml)) of both extracts was added to 3.4 of 0.1 M phosphate buffer (pH 7.4) and mixed with 0.6 ml of 43 mM hydrogen peroxide. After 10 min, the absorbance at 230 of the reaction mixture was recorded. For each concentration, mixture without sample was used ascontrol and mixture without $H_2O_2$ was used as blank and percentage inhibition was calculated.

Calculation

$$\%\ \text{inhibition}\ [H_2O_2] = [(A0-A1)/A0] \times 100$$

***Where A0 was the absorbance of the control, and A1 was the absorbance of the sample. Ascorbic acid was taken as standard.***

**Table 1**
**Free radical scavenging activity at various concentrations of PA ethanolic extract**

| Concentration | DPPH Inhibition (%) | |
|---|---|---|
| (µg/ml) | Ascorbic acid | *Phyllanthus amarus* |
| **10** | 70.9 ± 3.91 | 60.8 ± 3.36 |
| **20** | 77.4 ± 4.27 | 70 ± 3.86 |
| **30** | 80.2 ± 4.43 | 72 ± 3.98 |
| **40** | 84.6 ± 4.67 | 74.8 ± 4.13 |
| **50** | 88.3 ± 4.88 | 75.9 ± 4.19 |

***Values are mean ± SE of triplicate determinations.***

**Table 2**
***H2O2 Scavenging activity of Phyllanthus amarus ethanolic extract***

| Concentration | Inhibition (%) | |
|---|---|---|
| (µg/ml) | Ascorbic acid | *Phyllanthus amarus* |
| **10** | 26 ± 1.43 | 28.17 ± 1.55 |
| **20** | 35 ± 1.93 | 29 ± 1.60 |
| **30** | 48 ± 2.65 | 40 ± 2.21 |
| **40** | 62 ± 3.42 | 58 ± 3.20 |
| **50** | 85 ± 4.69 | 80.6 ± 4.45 |

***Values are mean ± SE of triplicate determinations.***

## RESULTS

To evaluate the antioxidant activity of natural compounds, DPPH assay is widely used as a free radical. Antioxidant activity of PA using DPPH dye is shown in Table1.PA at different doses, i.e. 10–50µg/ml showed free radical scavenging activity in a dose dependent manner. Maximum percentage inhibition of DPPH radicals by the antioxidant activity of PA using DPPH dye was about 75.9% at a concentration of 50µg/ml. Ascorbic acid, as a standard drug showed about 88.3% inhibition of the DPPH radicals at 50µg/ml. $H_2O_2$ scavenging activity of PA was dose dependent as demonstrated in table 2. The maximum $H_2O_2$ scavenging activity of PA at 50µg/ml is 80.6% which is comparable with ascorbic acid 50µg/ml**.**

## DISCUSSION

Plants are the source of free radical scavengers like polyphenols, flavonoids and phenolic compounds which help in the prevention and repairing damages caused by ROS [7],. At low or moderate concentrations, generally reactive oxygen species (ROS) exert beneficial effects on cellular responses and immune function but at high levels, a deleterious process can damage cell structures, including lipids, proteins, and DNA.The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ, or externally supplied through foods. The DPPH assay is used to determine antioxidant potential of natural compounds. It is reduced from a purple compound to a light yellow compound by electrons from oxidant compounds. Reaction of DPPH with hydroxyl groups involves a homolytic substitution of one of the phenyl rings of DPPH yielding 2-(4-hydroxyphenyl)-2-phenyl-1- picryl hydrazine as a major product. The concentration of DPPH at the end of a reaction will depend on the concentration and structure of the compound being scavenged [8-10].Thus, the ability of the test samples to quench this radical is a measure of its antioxidative ability. Strong antioxidant activity of the *Phyllanthus amarus* was found which was nearly same when compared to ascorbic acid as standard. Plant exhibited antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals [11-13].The occurrence of alkaloids, flavonoids, hydrolysable tannins, lignans, phenolics and polyphenols in the extract may be responsible for anti-oxidative action in biological system, acting as scavengers of singlet oxygen and free radicals. The beneficial effects derived from phenolic compounds can be attributed to their antioxidant activity. This is also supported by the findings of other authers who worked on *Tephrosia purpurea* Linn., *Viscum album, Caesalpinia pulcherrima* and *Crocus sativus* [14-16].

## CONCLUSION

This data provides a scientific explanation of presence of potent antioxidant potential of Phyllanthus *amarus*, which will be helpful in use of this plant for medicinal purpose globally.

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