Journal Of Harmonized Research (JOHR)

Journal Of Harmonized Research in Pharmacy
5(4), 2016, 230-236

ISSN 2321 – 0958

Original Research Article

*IN VITRO*ANTIOXIDANT ACTIVITIES OF *PHYLLANTHUSAMARUS* HERBS

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Abstract: In this study, measure antioxidant potential of *P. amarus* by DPPH radical scavenging activity, Superoxide radical scavenging activity and Hydroxyl radical scavenging activity method using ether, aqueous and ethanol extract in different propagation also reference compound as Ascorbic Acid. In DPPH radical scavenging activity, ethanol extract show higher percentage inhibition 93.50% at 250µg/mL. Higher percentage inhibition of all three activities *i.e.* DPPH, SOD and hydroxyl at 250 µg/mL by ethanol extract is 96.50 % with reference compound *i.e.* Ascorbic Acid-97.00%.

Keywords: - *Phyllanthus amarus*, Antioxidant, DPPH, Hydroxyl Ion.

Introduction: Oxidative stress as elevated levels of free radicals or other reactive oxygen species (ROS) which can produce either direct or indirect impairment to the body[1]. Several Reactive Oxidative Species found in literature such as superoxide anions, hydrogen peroxide, hydroxyl, nitric oxide and peroxynitrite radicals which have play an important role in oxidative stress related to the pathogenesis of numerousdiseases[2].Natural antioxidants exhibit a wide range of pharmacological activities, and have been shown to have anticancer, anti-inflammatory and anti-aging properties[3, 4].*Phyllanthusamarus*herb is widely found in sub- continental parts of India since ancient time is most commonly used in Ayurvedic system of medicines in problem of stomach[5]. *Phyllanthus amarus* was also reported various pharmacological activities *i.e.*antiamnesic[7], antibacterial[8-11], anticancer[12-16], anti-diarrhoeal[17-19], antifugal[20-21], analgesic[22-25], antioxidant[26-30]antiplasmodial[31-33]. The aim of this study, investigated *in vitro* antioxidants potential of *Phyllanthus amarus* by various methods.

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Received on: November 2016
Accepted after revision: December 2016
Downloaded from: www.johronline.com

Materials and Methods

Chemicals: DPPH (1,1-diphenyl, 2-picrylhydrazyl), NBT (nitro blue tetrazolium), NADH (nicotinamide adenine dinucleotide

phosphate reduced), PMS (phenazinemethosulphate), ferrous chloride ($FeCl_2$), streptozocin, glibenclamide, epinephrine, thiobarbituric acid (TBA), reduced glutathione (GSH) were purchased from SD fine chemical with analytical grade.

Collection and Preparation of Plant Material: - *Phyllanthus amarus* Herb were collected from the local areas of Bhopal region of Madhya Pradesh and authenticated by Dr. Neelam Tripathi, Botanist, University Teaching Department, Sri Satya Sai University of Medical Sciences, Sehore, M.P under accession no. 15021. The leaves of *P. amarus* were dried at room temperature and ground into powder. About 1000 g of the powdered leaves was extracted in 1L of cold sterile distilled water maintained on a mechanical shaker.

***In Vitro* Antioxidant Activities:** Antioxidant potential of *Phyllanthus amarus* leaf extract was measured by different assays method. In each of these assays, ascorbic acid was used as a reference substrate. The ability of the extract to scavenge or inhibit free radicals was expressed as percentage inhibition and was calculated using the following formula:

$$Percentage\ inhibition = \frac{(A_0 - A_t)}{A_0} \times 100$$

DPPH Radical Scavenging Activity: The percentage of antioxidant activity (AA %) of each substance was assessed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Katalinic *et al*. Free radical scavenging activity of different extracts of *Phyllanthus amarus* herb were measured by 1,1-diphenyl-2-picryl hydrazyl (DPPH). In brief, 0.5 ml of 0.1 mM DPPH solution was prepared in ethanol was performed. This solution (1 ml) was added to 3 ml of different extracts in ethanol at different concentrations. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 100 min of reaction using a UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan). The mixture of ethanol (3.3 mL) and sample (0.5 mL) serve as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). The scavenging activity percentage (AA %) was determined[34].

Superoxide Radical Scavenging Activity (SOD): Super oxide anion radical scavenging activity principally based on conversion of Nitroblue Tetrazolium (NBT) into NBT diformazan via superoxide radical. SOD utilizes the highly water-soluble tetrazolium salt and that produces a water-soluble formazan dye upon reduction with a superoxide anion. The rate of the reduction with O_2 is linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD. In brief, superoxide anions were generated in nonenzymatic phenazinemethosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system through the reaction of PMS, NADH, and oxygen. It was assayed by the reduction of NBT in the presence of different concentrations (1-250 µg/ml) of the extract. The reaction was initiated by adding 0.75 mL of PMS (120 µM) to the mixture. The absorbance was measured at 560 nm by using a spectrophometer (UV-1700, Shimadzu, Kyoto, Japan) following 5-minute incubation at room temperature. Ascorbic acid was used as standard comparator. Percentage inhibition of superoxide radical formation (Scavenging of Superoxide radicals) was determined[35].

Hydroxyl radical scavenging activity: Hydroxyl radical is one of the potent reactive oxygen species that reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell. HRS assay is used to find the scavenging activity of free hydroxyl radicals (which damage the body cells) like hydrogen peroxide in the presence of different concentrations of plant sample. The hydroxyl radical scavenging activity of *Phyllanthus amarus* extract was measured according to a modified method by Eswar

Kumar *et al.* The reaction mixture contained 60 µL of 1.0 mM $FeCl_2$, 90 µL of 1 mM 1, 10-phenanthroline, 2.4 mL of 0.2 M Phosphate buffer (pH 7.8), 150 µL of 0.17 M hydrozen peroxide (H_2O_2), and 1.5 mL of different concentrations of the extract (20-300 µg/mL). H_2O_2 was added at the start of the reactions. After incubation at room temperature for 5 min. absorbance of the mixture was measured by using a spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan) at 560 nm[36].

Table 1. Percent inhibition of DPPH oxidation by *P. amarus* extract at different concentration

Sr. No.	Conc. (µg/ml)	Ether Extract (Mean±SEM)		Aq. Extract (Mean±SEM)		EtOH Extract (Mean±SEM)		Ascorbic acid (Mean±SEM)	
1	1	11.33	3.72	12.83	2.64	22.67	1.75	32.33	1.51
2	5	17.17	4.26	16.83	2.64	32.33	1.51	40.83	2.64
3	10	29.67	5.32	28.00	4.86	40.67	3.01	47.50	5.01
4	25	45.17	6.05	38.50	5.09	48.67	4.41	63.00	3.41
5	50	61.00	4.77	53.83	8.42	63.50	3.08	80.33	3.01
6	100	70.17	3.43	70.67	5.13	80.00	3.16	93.50	3.67
7	250	83.00	4.73	91.83	4.92	93.50	3.67	98.67	1.97

Each value represents mean ± SEM of n=6 observations

Table2.Percent inhibition of superoxide radical by *P. amarus* extract at different concentration

Sr. No.	Conc. (µg/ml)	Ether Extract (Mean±SEM)		Aq. Extract (Mean±SEM)		EtOH Extract (Mean±SEM)		Ascorbic acid (Mean±SEM)	
1	1	10.17	3.43	11.83	1.33	22.33	1.37	24.33	2.50
2	5	22.00	1.79	18.00	2.10	33.83	1.72	34.33	2.34
3	10	34.50	2.07	28.00	2.10	40.67	3.01	49.17	3.97
4	25	44.50	2.07	37.67	2.16	45.83	1.72	62.00	2.19
5	50	60.33	3.39	55.83	3.31	64.50	3.39	73.17	2.86
6	100	70.50	3.62	72.83	2.32	80.00	2.28	86.67	2.58
7	250	84.50	2.81	93.67	3.44	91.67	2.42	97.00	3.41

Each value represents mean ± SEM of n=6 observations

Table 3.Percent inhibition of hydroxyl radical oxidation by *P. amarus* extract at different concentration

Sr. No.	Conc. (µg/ml)	Ether Extract (Mean±SEM)		Aq. Extract (Mean±SEM)		EtOH Extract (Mean±SEM)		Ascorbic acid (Mean±SEM)	
1	1	17.50	1.87	18.67	2.50	16.67	2.58	17.50	1.87
2	5	32.17	1.94	31.17	1.47	30.33	1.63	30.33	1.03
3	10	47.50	3.39	43.50	2.43	39.17	1.47	47.50	1.87
4	25	60.83	4.62	61.83	2.64	46.50	1.05	61.50	1.38
5	50	73.67	2.73	70.67	1.97	58.50	2.88	73.17	2.86
6	100	83.83	2.79	83.83	2.64	73.33	3.44	86.00	2.00
7	250	94.33	2.50	95.00	1.90	96.50	1.87	97.00	3.41

Each value represents mean ± SEM of n=6 observations

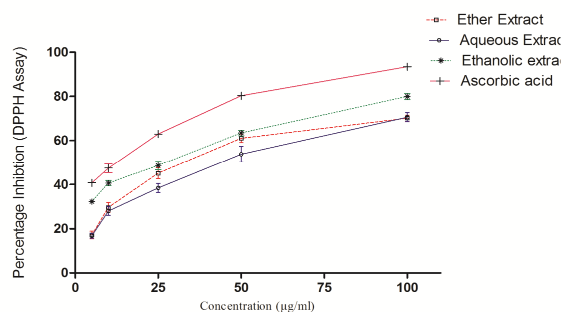

Figure 1(a). *In vitro* antioxidant assay with DPPH of *P. amarus* extracts. Each point represents mean ± SEM of n=6 observations.

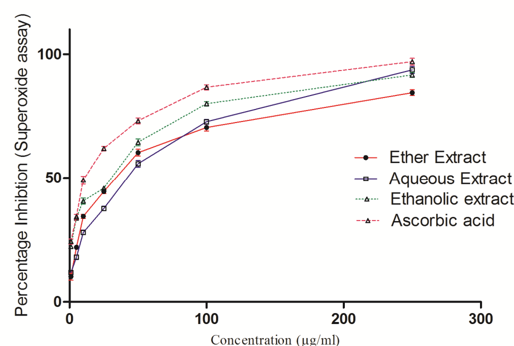

Fig. 1 (b).*In vitro* antioxidant superoxide scavenging assay of *P. amarus* extracts. Each point represents mean ± SEM of n=6 observations.

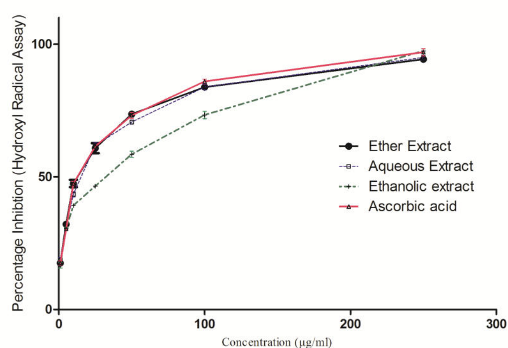

Fig. 1 (c).*In vitro* antioxidant hydroxyl radical scavenging assay of *P. amarus* extracts. Each point represents mean ± SEM of n=6 observations

Results and Discussion

DPPH Radical Scavenging Activity: DPPH radical scavenging activity of ethanolic, ether and aqueous leaf extract of *P. amarus* is presented in Figure 1 (a). The DPPH assay revealed that all the extracts of *P. amarus* possess potent antioxidant activity. The activity was found to be concentration dependent with linear increased in the concentration of 10-100 µg/ml. The ether extract produced 11.33 % inhibition at 1 µg/ml which increased in concentration dependent manner and reached maxima at 250 µg/ml. Similarly, aqueous extract produced 12 % inhibition of DPPH oxidation at 1µg/ml and reached maxima at 250 µg/ml. The ethanolic extract was found to produce marked inhibition at all the concentrations employed and was higher than other extracts used. The maximal inhibition was found to be at 100 µg/ml. Finally it was comparable to that of Ascorbic acid that was employed as reference standard. In conclusion the ethanolic extract was found to possess potent concentration dependent antioxidant activity, which was comparable to that of ascorbic acid.

Superoxide Radical Scavenging Activity (SOD): Figure 1 (b) shows dose superoxide radical scavenging assay revealed that all the extracts of *P. amarus* possess potent antioxidant activity. The activity was found to be concentration dependent with linear increased in the concentration of 10-100 µg/ml. All the extracts produced comparable results at all concentrations used with about 20 % inhibition at 1 µg/ml which increased in concentration dependent manner and reached maxima at 250 µg/ml. This was found to be comparable to that of Ascorbic acid that was employed as reference standard. In conclusion, in this assay all the extracts exhibit potent concentration dependent antioxidant activity, which was comparable to that of ascorbic acid.

Hydroxyl radical scavenging activity: The hydroxyl radical scavenging assay revealed that all the extracts of *P. amarus* possess potent antioxidant activity. The activity was found to be concentration dependent with linear increased in the concentration of 1-250 µg/ml. All the extracts produced comparable results at

all concentrations used with about 50 % inhibition at 1 μg/ml which increased in concentration dependent manner and reached maxima at 150 μg/ml. This was found to be comparable to that of Ascorbic acid that was employed as reference standard. In conclusion, in this assay all the extracts exhibit potent concentration dependent antioxidant activity, which was comparable to that of ascorbic acid (Figure1(c)).

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