International Journal of Botany Studies
ISSN: 2455-541X, Impact Factor: RJIF 5.12
www.botanyjournals.com
Volume 1; Issue 3; March 2016; Page No. 30-32

In-Vitro antioxidant activities of methanolic extract of whole plant of *phyllanthus amarus* (Euphorbiaceae)

[1] Sangeeta Devi, [2] Davinder Kumar, [3] Muneesh Kumar

[1] Department of Botany Govt. College for Women, Parade Jammu, University of Jammu, India.
[2] Department of Botany Govt. Degree College Bhaderwah, University of Jammu, India.
[3] Department of Zoology Govt. Degree College Bhaderwah, University of Jammu, India.

Abstract
Antioxidants play an important role for health related factor. In normal condition, the levels of oxidants (i.e. free radicals) and antioxidants are balanced. *Phyllanthus amarus* belonging to family Euphorbiaceae is a small, erect, annual glabrous herb. The plant has been found to have anti-inflammatory, antihepatotoxic, antilithic, analgesic, hypotensive antispasmodic, antiviral, antibacterial, diuretic, antimutagenic, hypoglycaemic, etc activities.

Keywords: *Phyllanthus amarus*, *Euphorbiaceae*, Antioxidant, Total Phenolic Content, Total Flavonoid Content, Gallic acid

Introduction
Antioxidants play an important role for health related factor. Fruits and vegetables are the main source of naturally occurring antioxidants. Antioxidant compounds such as phenolic acids, polyphenols and flavonoids are found in plants.[1]Reactive oxygen species (ROS) are the free radicals such as superoxide anion radicals (O_2^-) and hydroxyl radicals (OH^-), as well as non-free radicals species (H_2O_2) and the singlet oxygen ($^1O_2$)[2] [2].
In normal condition, the levels of oxidants (i.e. free radicals) and antioxidants are balanced. Overproduction of these oxidants leads to oxidative damage of lipids, DNA, and proteins and and thus involve in many chronic and degenerative diseases, such as coronary heart disease, inflammation, stroke, diabetes mellitus, cancer, etc [3].
Phytochemicals from plants such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins act as antioxidants by scavenging free radicals & have therapeutic potential for free radical related diseases [4].
Phyllanthus amarus belonging to family Euphorbiaceae.[5] *Phyllanthus amarus*, is a small, erect, annual glabrous herb with 30-50 cm in length [6]. 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 colour, 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 [5, 7].
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 [5, 8]. The plant also has been found to have anti-inflammatory, antihepatotoxic, antilithic, analgesic, hypotensive, antispasmodic, antiviral, antibacterial, diuretic, antimutagenic, hypoglycaemic, etc activities [6].

Materials and methods
Collection of Plant Material
The indigenous plant *Phyllanthu amarus* Schum. Were collected from different locations of Bhopal (M.P.) region. The plants were acknowledged by a senior Botanist Dr. Tayaaf Safi Principal Gandhi P.R. 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* Schum was extracted according to (Harborne and Baxter., 1995) [9]. 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 activities
Total Phenolic Content (TPC) Estimation [10, 11]
The amount of total phenolic content was determined by the method as reported by Chun *et al*. (2003) using Folin Ciocalteu Reagent. Gallic acid was used as a standard and the total phenolic content was expressed as mg/g gallic acid equivalent (GAE). Different concentrations i.e. 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were also prepared in methanol. Concentrations of 0.1-1mg/ml of plant leaf extract were also prepared in methanol. 0.5ml of each extract sample was taken, mixed with 2.5ml of (a 10 fold) dilute folin Ciocalteu reagent and 2 ml of 7.5% sodium carbonate solution. The tubes were covered with paraffin and allowed to stand for 30 minutes at room temperature before the absorbance was read at 760nm spectrophotometrically using methanol as blank. All determinations were performed in triplicate.
The folin-Ciocalteu reagent was found to be sensitive to reducing compounds including polyphenols. It produced a blue

color upon reaction. This blue color was measured spectrophotometrically.

Total Flavonoid Content (TFC) Estimation [12, 13]

Total flavonoids were measured by a colorimetric assay method as reported by Zhishen *et al*. (1999). An aliquot of leaf extract sample and standard solution of rutin (1-100µg/lit) was added to a 75µl of $NaNO_2$ solution and mixed for 6 min, before adding 0.15ml $AlCl_3$ (100g/L). After 5 min, 0.5ml of 0.1M NaOH solution was added. The final volume was adjusted to 2.5ml with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510nm against the same mixture, without the leaf extract, as a blank. Total flavonoid content was determined as mg rutin/g dry weight (mg rutin/g DW), through the calibration curve of rutin. All samples were analysed in three replications.

Results and discussions

Table 1: Total phenolic contant of methanolic extract of *Phyllanthus amarus*

S. No.	Conc. of gallic acid(µg/ml)	Absorbance of gallic acid	Conc. of test sample (µg/ml)	Absorbance of test sample
1.	10	0.2	100	0.188
2.	20	0.315		
3.	30	0.382		
4.	40	0.455		
5.	50	0.514		
Total phenolic content- 6.714 µg/100 µg gallic acid equivalant				

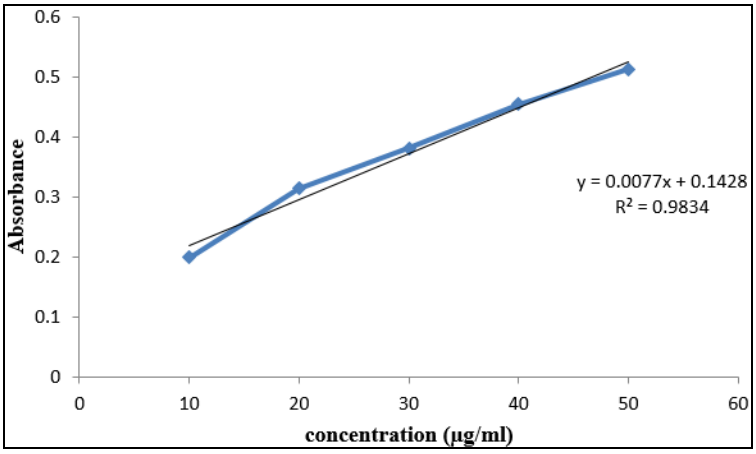

Fig 1: Graph represent regression curve of gallic acid

Table 2: Total Flavonoid contant of methanolic extract of *Phyllanthus amarus*

S. No.	Conc. of Rutine (µg/ml)	Absorbance of Rutine	Conc. of test sample (µg/ml)	Absorbance of test sample
1.	10	0.025	100	0.017
2.	20	0.032		
3.	30	0.044		
4.	40	0.058		
5.	50	0.066		
Total Flavonoid content- 5µg/100 µg rutine equivalant				

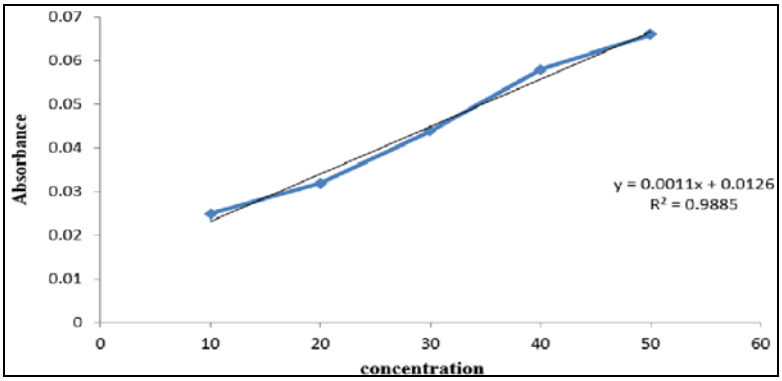

Fig 2: Graph represent regression curve of rutin

Antioxidants are chemical substances which cause delay in oxidation process by inhibiting the polymerization chain reaction initiated by free radicals [14]. Phytochemicals are naturally occurring antioxidants which are capable of mitigating the harmful effects of diseases related to oxidative stress.[15] Phenolic and flavonoids compounds have been reported as powerful antioxidants due to the presence of hydroxyl group in their structure [16].

On the basis of our results it is concluded that *Phyllanthus amarus S.* exhibit potent antioxidant property. The antioxidants act as defence mechanism that protects against oxidative damage, and include compounds to remove or repair damaged against diseases the present study provided valuable preliminary data which display its antioxidant capacity.

Conclusion

From the results obtained in the present study, it is concluded that a whole plant methanolic extract of *Phyllanthus amarus* contains phenolic and flavonoid compounds which exhibits antioxidant activity. This indicates that plant is a good source of natural antioxidant which may be helpful in prevention various diseases related to oxidative stresses. Therefore, isolation and identification on individual active compounds in *Phyllanthus amarus* and their *In vivo* antioxidant activities need to be investigated further.

Acknowledgement

Authors are beholden to Principal Govt. College for Women Parade Jammu, University of Jammu, India for providing laboratory facilities for this research work.

References

1. Jamuna S, Paulsamy S, Karthika K. Screening of *in vitro* antioxidant activity of methanolic leaf and root extracts of *Hypochaeris radicata* L. (Asteraceae) Journal of Applied Pharmaceutical Science. 2012; 02(07):149-154
2. Satheesh kumar D, Kottai Muthu A, Anton Smith A, Manavalan R. *In vitro* Antioxidant Activity of Various Extracts of whole Plant of Mucuna pruriens (Linn) International Journal of Pharm Tech Research. 2010; 2(3):2063-2070.
3. Battu GR, Kumar BM. *In-Vitro* Antioxidant Activity of Leaf Extract of *Aerva Lanata* Linn. International Journal of Pharma Sciences. 2012; 2(4):74-78
4. Yu-Ling HO, Shyh-Shyun HUANG, Jeng-Shyan DENG, Yaw-Huei LIN, Yuan-Shiun CHANG, Guan-Jhong HUANG. *In vitro* antioxidant properties and total phenolic contents of wetland medicinal plants in Taiwan Botanical Studies 2012; 53:55-66
5. Sonia Verma, Hitender Sharma, Munish Garg. *Phyllanthus Amarus:* A Review Journal of Pharmacognosy and Phytochemistry. 2014; 3(2):18-22
6. Kiran Divya, Rohilla Ankur, Rohilla Seema, Khan MU. Phyllanthus amarus: AN Ample Potential herb. IJRAP. 2011; 2(4):1096-1099.
7. OBIANIME AW, 2UCHE FI. The comparative effects of methanol extract of *Phyllanthus amarus* leaves and Vitamin E on the Sperm parameters of Male guinea pigs. J Appl. Sci. Environ. Manage. *March.* 2009; 13(1):37-41.
8. Joseph B, Raj SJ. An overview: Pharmacognostic Properties of Phyllanthus amarus Linn. International Journal of Phamacology. 2011; 7(1):40-45.
9. Harbrone SB, Baxter H. Phytochemical Dictionary. A handbook of bioactive compounds from plants Taylor and Francis, London, 1995.
10. Chun KO, Kim D, Lee CY. 'Superoxide Radical Scavenging Activity of the Major Polyphenols in Fresh Plums' Journal of Agriculture and Food Chemistry. 2003; 51:8067-8072.
11. Maurya S. Singh D. 'Quantitative Analysis of Total Phenolic Content in Adhatoda Vasica Nees Extracts' International Journal of Pharmtech Research. 2010; 4:2403-2406.
12. Zou YP, Lu YH, Wei DZ. 'Antioxidant Activity of a Flavonoid-rich Extract of *Hypericum perforatum. In-vitro*' J Sci. Food Agr. 2004; 52:5032-5039.
13. Zengin HW, Aktumsek A, Gular GO, Cakmak YS, Yildiztugay E. 'Antioxidant properties of methanolic Extract and fatty acid composition of centaurea urvillei dc. Subsp., rec.' Nat. Prod 2011; 5(2):123-132.
14. Farhan H, Rammal H, Hijazi A, Hamad H, Daher A, Reda M *et al. In vitro* antioxidant activity of ethanolic and aqueous extracts from crude *Malva parviflora* Grown in lebanon Asian J Pharm Clin Res. 2012; 5(3):234-238.
15. Saran Kumar Guptaa, Mitali Ghosala, Rajib Biswasb, Biman Krishna Sahab, Abhaya Prasad Dasb, Palash Mandala. Evaluation of in vitro antioxidant activity of methanolic extracts of some ferns from Mawsynram of Meghalaya, India INT J CURR SCI. 2014, 12:87-97
16. Jamuna S, Paulsamy S, Karthika K. Screening of *in vitro* antioxidant activity of methanolic leaf and root extracts of *Hypochaeris radicata* L. (Asteraceae) Journal of Applied Pharmaceutical Science. 2012; 02(07):149-154.