Biochemical Communication

Biosci. Biotech. Res. Comm. 9(1): 15-18 (2016)

BBRC Bioscience Biotechnology Research Communications

# *Phyllanthus amarus* augments the serum antioxidant capacity and invigorates the blood in experimental mice

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## ABSTRACT

The presence of antioxidant molecules in plants is well documented and there is increasing demand for natural antioxidants over synthetic additives in food and pharmaceutical industries. *Phyllanthus amarus* is a broad spectrum medicinal plant that has received global recognition and is known to contain certain antioxidant chemicals, but knowledge on the impact of such chemicals on in vivo antioxidant defense capacity is still accumulating. This present study, therefore, investigated changes in serum total antioxidant capacity (TAC) and associated levels of oxidative assault (Malondialdehyde, MDA) in mice administered graded amounts of *Phyllanthus amarus* ethanolic leaf extracts. Forty (40) adult Swiss albino mice, weighing between 20-30g were randomly divided into four groups (n=10/group) for the investigation. Group 1: Control (given placebo - normal saline); Group 2: Experimental (administered 150mg/kg/d of *P. amarus* ethanolic leaf extract, respectively). Each group was so treated for 7days and then observed for another 14days. After the 7-day treatment and 14-day observation periods, the mice were sacrificed (n=5/group/each day) under chloroform anaesthesia usually after an overnight fast. Whole blood was there after collected and centrifuged to obtain serum sample for the biochemical assay of total antioxidant capacity (TAC) and malondialdehyde (MDA) using documented methods. Results show that administration of *P. amarus* for 7 days and 14 days observation thereafter, significantly ($p<0.05$) increased total antioxidant capacity (TAC) administered at (150mg/kg/d: 0.29±0.0mM and 0.19±0.05mM,300mg/kg/d :0.30±0.04mM and 0.23±0.03mM, 450mg/kg/d : 0.29±0.01mM and 0.25±0.06mM) with associated reductions in the levels of malondialdehyde (150mg/kg/d : 26.33±3.51µM and 30.67±6.66µM,300mg/kg/d: 23.33±2.50µM and 27.67±3.72 µM, and 450mg/kg/d: 28.67±6.66µM and 31.67±3.51µM) when compared with control values (TAC = 0.13±0.06mM and 0.09±0.02mM, MDA= 38.00±6.16µM and 40.00±1.53µM). Data indicate that crude ethanolic leaf extract of *P. amarus* improved antioxidant defense capacity and invigorated the blood of experimental mice. This vitalizing property may be due to *P. amarus* antioxidant phytocompounds already identified. The bioavailability and antioxidant boosting capacity of these chemical ingredients are hereby demonstrated in experimental mice.

**KEY WORDS:** *PHYLLANTHUSAMARUS*, TOTAL ANTIOXIDANT CAPACITY, MALONDIALDEHYDE, BLOOD, OXIDATIVE ASSAULT

ARTICLE INFORMATION:

*Corresponding Author: simacoakpos@yahoo.com
*Received 21st December, 2016*
*Accepted after revision 20th Feb, 2016*
*BBRC Print ISSN: 0974-6455*
*Online ISSN: 2321-4007*
*Thomson Reuters ISI SCI Indexed Journal*
*NAAS Journal Score : 3.48*

*Online Contents Available at:* http//www.bbrc.in/

## INTRODUCTION

Herbs have been investigated for their antioxidant properties (Gazzaneo *et al*., 2005) and medicinal plants containing active chemical constituents with high antioxidant activity play important role in prevention of various degenerative diseases (Lukmanul *et al*., 2008). Antioxidants can abstract lone electron from free radical molecules such as reactive oxygen species, ROS and help humans to control these harmful substances. ROS, are usually produced in the body by chemical reactions which occur during normal or pathological cellular processes (Lakenbrink *et al*., 2000 and Onyesom *et al*., 2015).

Excess formation of these ROS can overwhelm body defense and cause oxidative stress. Oxidative stress plays significant roles in processes of ageing and pathogenesis of numerous diseases like diabetes, cancer, neurodegenerative and respiratory tract disorders (Anderson *et al*., 2000). Halliwell (1996), opined that the sum of endogenous and food derived antioxidants represents thetotal antioxidant capacity of a system. The role of antioxidant is to detoxify reactive oxygen intermediates in the body (Delay, 1993). Therefore,improved antioxidant status can minimize oxidative stress and associated damages. This delays or decreases the risk of developing free radical induced diseases.

Protective antioxidants bestowed by many plant extracts and products make these agents promising therapeutic drugs for free radicals induced pathologies. *Phyllanthus amarus* (*P. amarus*) is a medicinal plant of the family Euphorbiacae. It has about 800 species which are found in tropical and subtropical regions of the world including Nigeria (Mazumder*etal*., 2006). *Phyllanthus amarus* is used as a chemoprotective agent (Kumar and Kulta, 2005), and it has been observed to exhibit hypoglycaemic property (Kussuya *et al.,* 2003). Recently we have demonstrated that crude ethanolic leaf extract of *P. amarus*restored renal dysfunction associated with *P. berghei* malarial parasite- induced oxidative stress in experimental mice (Onyesom *et al*., 2015). *P.amarus* has been reported to contain antioxidant phytocompounds, but this study, however, investigated the bioavailability and impact of these substances in graded crude ethanolic leaf extract on serum total antioxidant capacity and associated levels of oxidative stress in experimental mice.

## MATERIALS AND METHODS

### HARVESTING AND PREPARATION OF PLANT EXTRACT

Fresh whole plants of wild type *Phyllantusamarus* growing in uncultivated farmland in Abraka, Ethiope East Local Government Area of Delta State, Nigeria were obtained in May, 2015 and authenticated (No: FHI: 109728) in the Herbarium Unit, Forest Research Institute of Nigeria, Ibadan. Crude ethanolic leaf extract of the harvested plant was prepared as earlier described (Onyesom *et al*., 2015).

### ANIMAL GROUPING AND EXTRACT ADMINISTRATION

Forty (40) adult Swiss albino mice of mixed sexes weighing between 20-30g were assigned into four (4) groups (n=10/group). Group 1: Control (was given placebo – normal saline), Groups 2, 3 and 4: Experimental (were administered placebo – 150, 300 and 450mg/kg/d of *P. amarus* ethanolic leaf extract, respectively). The graded doses of extract were prepared and administered as already documented (Onyesom *et al.,* 2015).

### ANIMAL SACRIFICE AND COLLECTION OF SPECIMEN

After 7 days of extract administration and another 14 days of observation, the mice were fasted overnight and sacrificed (n=5 mice per each time) the next day under chloroform anaesthesia.Whole blood was collected by heart puncture and centrifuged (Cent 80D, Serico, China) to obtain serum which was used for the biochemical analyses of total antioxidant capacity, TAC and malondialdehyde, MDA levels in blood.

Serum TAC was determined by the Trolox Equivalent Antioxidant Capacity (TEAC) method described by Miller *et al*., (1993) and MDA level was estimated by assessing amount of Thio Barbituric Acid Reacting Substances (TBARS) (Ohkawa *et al*., 1979).

### STATISTICAL ANALYSIS

Data were assessed by the one way analysis of variance (ANOVA) and Dunnett's post hoc test using SPSS software package version 20. Level of significant difference was established at $p<0.05$.

## RESULTS AND DISCUSSION

The results obtained from the investigation into the total antioxidant capacity and malondialdehyde levels in serum of mice administered with graded crude ethanolic leaf extract of *Phyllanthus amarus*are presented in Table 1.

Table 1 showed the data on total antioxidant capacity, TAC as well as malondialdehyde, MDA levels in experiment mice administered graded doses of crude ethanolic leaf extract of *Phylanthus amarus*.

Table 1: Total antioxidant capacity, TAC and levels of malondialdehyde, MAD in serum of experimental mice administered varying doses of crude ethanolic leaf extract of *Phyllanthus amarus*

| | TAC(mM) | | MDA(µM) | |
|---|---|---|---|---|
| Group | 7 Days | 21 Days | 7 Days | 21 Days |
| 1. | 0.13±0.06[a] | 0.09±0.02[a] | 38.33±4.73[a] | 40.33±1.53[a] |
| 2. | 0.29±0.05[b] | 0.19±0.05[b] | 26.33±3.51[b] | 30.67±6.66[b] |
| 3. | 0.30±0.04[b] | 0.23±0.03[b] | 23.33±2.50[b] | 27.67±3.72[b] |
| 4. | 0.29±0.01[b] | 0.25±0.06[b] | 28.67±6.66[b] | 31.67±3.51[b] |

*Values are expressed as Mean±SD for n=5 mice. Values that bear another superscript on a column differ significantly (p<0.05).*

*Group 1 = Control (given placebo– normal saline)*

*Group 2 = Experimental (treated with 150mg/kg P. amarus)*

*Group 3 = Experimental (treated with 300mg/kg P. amarus)*

*Group 4 = Experimental (treated with 450mg/kg P. amarus)*

*TAC = Total antioxidant capacity*

*MDA = Malondialdehyde*

The administration of varying doses (150, 300 and 450mg/kg/d) of *Phyllanthus amarus* crude ethanolic leaf extract to experimental mice significantly ($p$<0.05) increased serum total antioxidant capacity, TAC, but reduced ($p$<0.05) levels of malondialdehyde, MDA (an oxidative stress / lipid peroxidation biomarker) after 7days of administration and another 14 days of observation when compared with control values at the 5% probability level.

Herbs have been investigated for their antioxidant properties (Gazzaneo *et al*., 2005). Medicinal plants having active chemical constituents with good antioxidant property play significant role in prevention of various (degenerative) diseases (Lukmanul *et al*., 2008). Natural antioxidants from plant sources are potent, safe and harmless because of their low toxicity reports (Calixto*etal*., 1998; Ogbonon *etal*., 2008; Onocha and Ali, 2010).

This study assessed total antioxidant capacity and levels of oxidative damage in serum of mice administered crude ethanolic leaf extract of *Phyllanthus amarus*. The estimation of malondialdehyde, MDA levels was used to ascertain the levels of oxidative damage because MDA is one of the final products of polyunsaturated fatty acids (PUFAs) peroxidation in cells. An increase in free radicals causes overproduction of MDA. Hence, malondialdehyde level is commonly used as oxidative stress biomarker.

The results (Table 1) indicate that *Phyllathus amarus* crude ethanolic leaf extract administered to experimental mice in apparent good health for seven days and observation for another fourteen days thereafter induced an increase in total antioxidant capacity resulting in decreased levels of malondialadehyde. These changes were found to be significant ($p$<0.05) when compared with control values. Therefore, oral administration of the *P. amarus* leaf extract served as a factor that improved antioxidant defense and significantly reduce oxidative stress.

The phytochemicals identified in theleaf of *Phyllanthus amarus* include flavonoids, tannins, saponins, alkaloids, terpenoids, gycosides and anthroquinones(Faremi *et al*., 2008; Onyesom *et al*., 2015). Flavonoids from this plant have been shown to possess several pharmacological properties such as anti-inflammatory (Joy and Kuttan, 1998; Kassuya *etal*., 2003; Adeneye, 2006) and antioxidant activities (Wampa *et al*., 2012).

Total antioxidant capacity, TAC of systems include the summation of both endogenous and food - derived antioxidants which involve some enzymes such as superoxide dismutase, catalase and glutathione peroxidase, and arrays of small macromolecules like ascorbic acid, tocopherol, carotene, reduced glutathione (Halliwell, 1996).

Antioxidants interact with free radicals including reactive oxygen species ROS – which are usually produced by the body as a result of chemical reactions during normal cellular processes (Lakenbrink *et al*., 2000) in order to terminate their cell damaging activities. So, increased activities of ROS can lead to oxidative stress are known to play significant role in the process of ageing and pathogenesis of numerous diseases like diabetes, cancer, neurodegenerative diseases and respiratory tract disorders (Anderson *et al*., 2000).Improved antioxidant status therefore, helps to minimize oxidative damage and this could delay or decrease the risk of developing many age related free - radical induced diseases.

Evidence indicates that *Phyllanthus amarus* crude ethanolic leaf extract contributes to the improvement of antioxidant defense which invigorated the blood and provide health benefits at low, moderate and high doses.

## CONCLUSION

On the whole, crude ethanolic leaf extract of *P. amarus* was observed to improve antioxidant defense, reduced oxidative stress and invigorated the blood in experimental mice. This ability could be due to the bioactivities of identified phytochemicals especially phenolics (flavonoids and tannins) which have been observed to display significant antioxidant activity (Ettebong *et al*., 2012; Sen and Batra, 2013). As a corollary, this study hereby confirms the bioavailability and bioactivity of *P. amarus* antioxidant phytochemicals. The antioxidant phytochemicals (flavonids and tannins) should be purified and further studied in order to identify the chemical compounds that possess the invigorating property.

## ACKNOWLEDGEMENTS

We gratefully acknowledge the services of Affamefune Biomedical Consult, Abraka, Delta State.

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