Research Article CODEN: IJPRNK ISSN: 2277-8713
Mohan VR, IJPRBS, 2013; Volume 2(5):211-222 IJPRBS

INTERNATIONAL JOURNAL OF
PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

IN VITRO ANTIOXIDANT ACTIVITY OF *AMORPHOPHALLUS PAEONIIFOLIUS* (DENNST.) NICOLSON VAR. *CAMPANULATUS* (BLUME EX.DECNE) SIVADASAN

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Accepted Date: 28/09/2013; Published Date: 27/10/2013

Abstract: Antioxidant activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of the corm of *Amorphophallus paeoniifolius* have been tested using various antioxidant model systems viz., DPPH, hydroxyl, superoxide, ABTs and reducing power. The methanol extract of corm showed potent *in vitro* antioxidant activities. Ethanol extract of the plant showed strong DPPH radical scavenging activity. The maximum inhibitory concentration (IC_{50}) in all models viz, superoxide, DPPH, ABTs and hydroxyl radical were found to be 83.14, 34.50, 31.44 and 19.56µg/ml respectively at 1mg/ml concentration. The findings of the present study confirmed the presence of total phenolics and flavonoids and possess *in vitro* antioxidant activity.

Keywords: *in vitro* antioxidant activity, flavonoid, DPPH, ABTs

PAPER-QR CODE

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How to Cite This Article:

Mohan VR, IJPRBS, 2013; Volume 2(5):211-222

INTRODUCTION

Now a days, the role of free radicals in many ailments and diseases including inflammation, rheumatoid arthritis, cancer and cardiovascular diseases has been widely established [1]. Free radicals which have one or more unpaired electrons are produced during normal and pathological cell metabolites. Reactive oxygen species (ROS) react easily with free radicals to become radicals themselves. ROS are various forms of activated oxygen which include 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]. The antioxidant enzymes and free radical scavengers may provide a defensive mechanism against the deleterious acting of ROS.

Free radicals generated in the body can be removed by body's own natural antioxidant defenses e.g. glutathione, catalase etc. However, endogenous antioxidant defenses are not completely efficient. Therefore, dietary antioxidants are required to lessen the overall effect of antioxidant stress due to excessive free radicals occurring in our system[3]. Significant antioxidant properties have been recorded with phytochemicals that are necessary for the reduction in the occurrence of many diseases. Plants contain a wide variety of free radical scavenging molecules such as phenols, flavonoids, vitamins, terpenoids that are rich in antioxidant activity.

Natural antioxidants tend to be safer and they also possess antiviral, anti-inflammatory, anticancer, antitumour and hepatoprotective properties [4]. Therefore, the evaluation of antioxidant activity of various plant extracts is considered as an important step in the identification of their ability to scavenge the free radicals. The plant *Amorphophallus paeoniifolius* (Dennst). Nicolson var.*campanulatus* (Blume ex. Decne) Sivadasan commonly known as "Kaattu chenai" is a member of Araceae family. The corm is boiled in water with an equal amount of fresh leaves of tamarind. Then the peeled corm is cooked in tamarind. The prepared curry is taken as medicine once a day for seven to ten days to get relif from bleeding piles [5]. In view of its above medicinal properties, the present study was designated to evaluate the antioxidant activity of methanol extract of corm of *A. paeoniifolius* var. *campanulatus.*

Materials and Methods:

The corm of *Amorphophallus paeoniifolius* (Dennst). Nicolson var.*campanulatus* (Blume ex. Decne) Sivadasan were freshly collected from Thottamalai, Kanyakumari District, Tamil Nadu. The plant specimen was identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethnopharmacology unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of Plant extract:

Corms of the plant were dried in shade for one week, powdered and extracted with methanol using cold extraction in shaker for 48h at room temperature. The methanol extracts were concentrated in a rotary evaporator to obtain concentrated methanol extract which was then used for the estimation of total phenolic, flavonoid and the assessment of antioxidant activity.

Estimation of Total Phenolics:

Total phenolic contents were determined according to Lachman *et al*. [6]. 1 ml of sample extract was transferred into a 50ml volumetric flask and diluted approximately with 5ml distilled water. Then, 2.5 ml Folin-Ciocalteau reagent and 7.5ml of 20% (w/w) Na_2Co_3 were added and made up to 50ml with distilled water. It was agitated and left to stand for 2hrs. Absorbance of the sample was measured on the spectrophotometry at 765nm against a blank prepared with distilled water. Gallic acid was used for calibration. The results were expressed as Gallic acid equivalent (GAE) in g/100g.

Estimation of Flavonoids:

The total flavonoid content was determined according to Eom *et al*, [7]. An aliquot of 0.5 ml of sample (1mg/ml) was mixed with 0.1 ml of 10% aluminium chloride and 0.1 ml of potassium acetate (1M). In this mixture, 4.3 ml of 80% methanol was added to make 5ml volume. The mixture was vortexted and the absorbance was measured spectrophotometrically at 415nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

DPPH radical scavenging activity:

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the nonradical form DPPH-H [8].

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method [8]. Briefly, an 0.1mm solution of DPPH in methanol was prepared, and 1ml of this solution was added to 3 ml of the solution of all extracts in methanol at different concentration (125,250,500 &1000µg/ml). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbances were measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10UV: Thermo electron corporation).Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula.

DPPH scavenging effect (% inhibition) = $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in

presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Hydroxyl radical scavenging activity:

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell *et al*. [9]. Stock solutions of EDTA (1mM), FeCl3 (10mM), Ascorbic Acid (1mM), H_2O_2 (10mM) and Deoxyribose (10 mM), were prepared in distilled deionized water.

The assay was performed by adding 0.1ml EDTA , 0.01ml of $FeCl_3$,0.1ml H_2O_2, 0.36ml of deoxyribose, 1.0ml of the extract of different concentration (125, 250, 500 & 1000 µg/ml)dissolved in distilled water,0.33ml of phosphate buffer (50mM , pH 7.9), 0.1ml of ascorbic acid in sequence . The mixture was then incubated at 37^0C for 1 hour. 1.0ml portion of the incubated mixture was mixed with 1.0ml of 10%TCA and 1.0ml of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation is calculated by using the following equation

Hydroxyl radical scavenging activity= $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Superoxide radical scavenging activity:

The superoxide anion scavenging activity was measured as described by Robak and Gryglewski [10]. The superoxide anion radicals were generated in 3.0 ml of Tris – HCL buffer (16 mM, pH 8.0), containing 0.5 ml of NBT (0.3mM), 0.5 ml NADH (0.936mM) solution, 1.0 ml extract of different concentration (125, 250, 500 & 1000 µg/ml), and 0.5 ml Tris – HCl buffer (16mM, pH 8.0). The reaction was started by adding 0.5 ml PMS solution (0.12mM) to the mixture, incubated at 25^oC for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by using the following equation

Superoxide radical scavenging activity= $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Antioxidant Activity by Radical Cation (ABTs. +):

ABTs assay was based on the slightly modified method of Re *et al*. [11]. ABTS radical cation (ABTs) was produced by reacting 7mM ABTs solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTs Solution was diluted with ethanol to an absorbance of 0.70+0.02 at 734 nm.

After addition of 100µL of sample or trolox standard to 3.9 mL of diluted ABTs solution, absorbance was measured at 734 nm by Genesis 10s UV-VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC).

ABTs radical cation activity = $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Reducing Power:

The reducing power of the extract was determined by the method of Singh *et al.* [12] with minor modification to Oyaizu [13]. 1.0ml of solution containing 125, 250, 500 & 1000 µg /ml of extract was mixed with sodium phosphate buffer (5.0 ml, 0.2 M, pH6.6) and potassium ferricyanide (5.0ml, 1.0%): The mixture was incubated at 50°C for 20 minutes. Then 5ml of 10% trichloroacetic acid was added and centrifuged at 980gm (10 minutes at 5°C) in a refrigerator centrifuge. The upper layer of the solution (5.0 ml) was diluted with 5.0ml of distilled water and ferric chloride and absorbance read at 700nm. The experiment was performed thrice and results were averaged.

Results:

The total phenolics and flavonoid contents of methanol extract of *A. paeoniifolius* var. *campanulatus* corm were found to be 1.39g $100g^{-1}$ and 1.42g $100g^{-1}$respectively.The results of DPPH radical scavenging activity of the extract and the standard ascorbic acid were presented in figure 1. The extract exhibited potent radical scavenging activity. The IC_{50} value of the extract was comparable to the standard. Figure 2 showed the hydroxyl radical scavenging activity of methanol extract of *A. paeoniifolius* var. *campanulatus* corm and compared with ascorbic acid. It was observed that methanol extract of corm and ascorbic acid had more or less equal activity. At a concentration of 1000µg/ml, the hydroxyl scavenging activity of methanol extract of corm reached 68.19%, while at the same concentration, that of the ascorbic acid was 69.73%. Superoxide radical scavenging activity of methanol extract of *A. paeoniifolius* var. *campanulatus* corm extract is presented in Figure 3. The superoxide radical scavenging activity of corm extract was comparable to ascorbic acid. The ABTs radical cation scavenging potential of the methanol extract of corm of *A. paeoniifolius* var. *campanulatus* is presented in Figure 4. The percentage scavenging activity and IC_{50} of the investigated extract at 1 min of the reaction time was calculated. The highest percentage activity at 85.33% (1000µg/ml) was found for methanol extract of corm. Figure 5 shows the reducing capabilities of

the plant extract compared to ascorbic acid. The reducing power of methanol extract of corm of *A. paeoniifolius* var. *campanulatus* was very potent and the power of the extract was increased with quantity of sample. At a concentration of 1000µg/ml, reducing power of methanol extract of corm was 0.764%.The IC 50 values of DPPH, hydroxyl, superoxide and ABTs radical scavenging activity are shown in Figure 6.

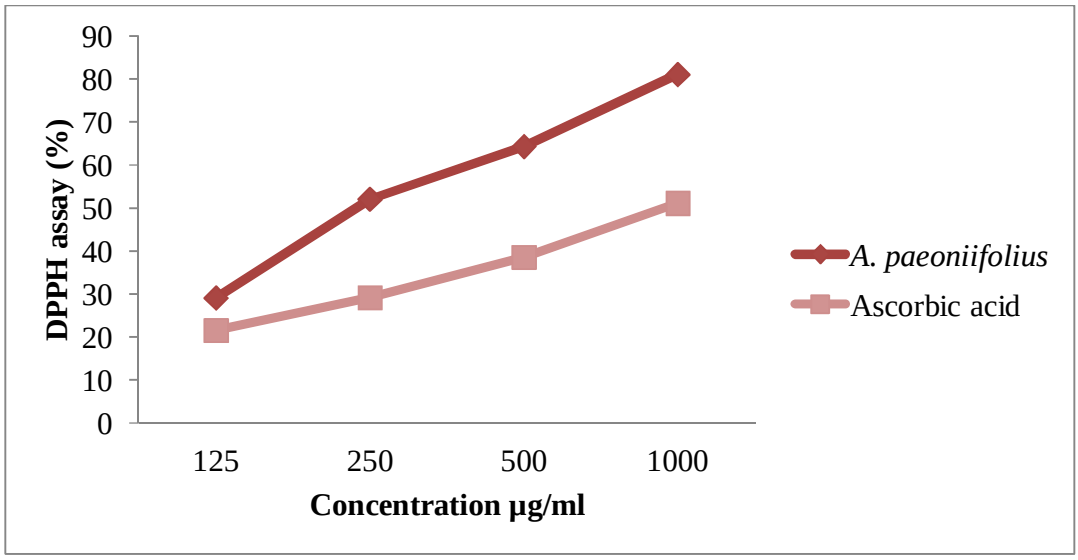

Figure 1: DPPH radical scavenging activity of methanol extract of *Amorphophallus paeoniifolius* var.*campanulatus*

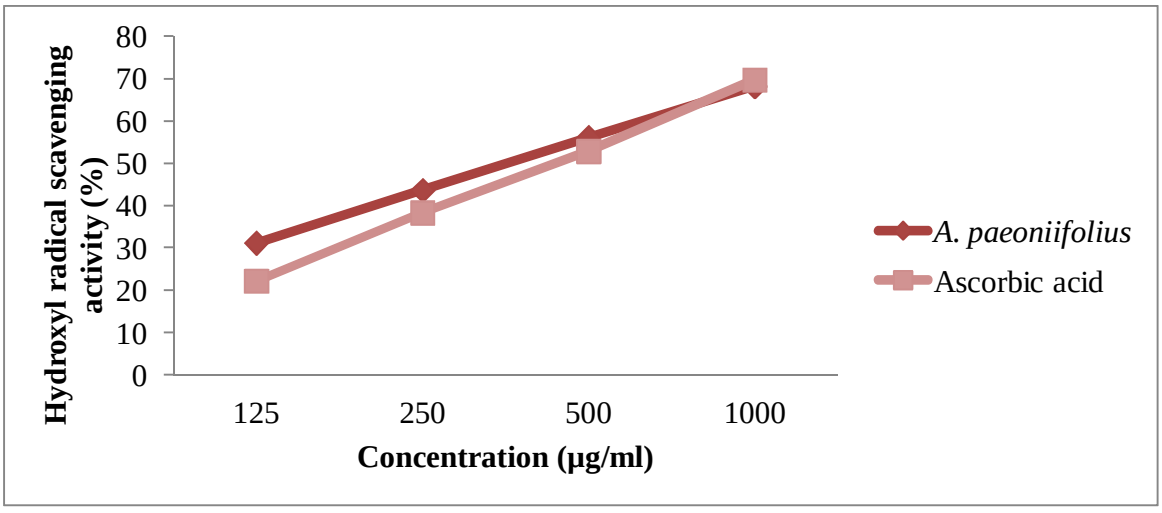

Figure 2: Hydroxyl radical scavenging activity of methanol extract of *Amorphophallus paeoniifolius* var.*campanulatus*

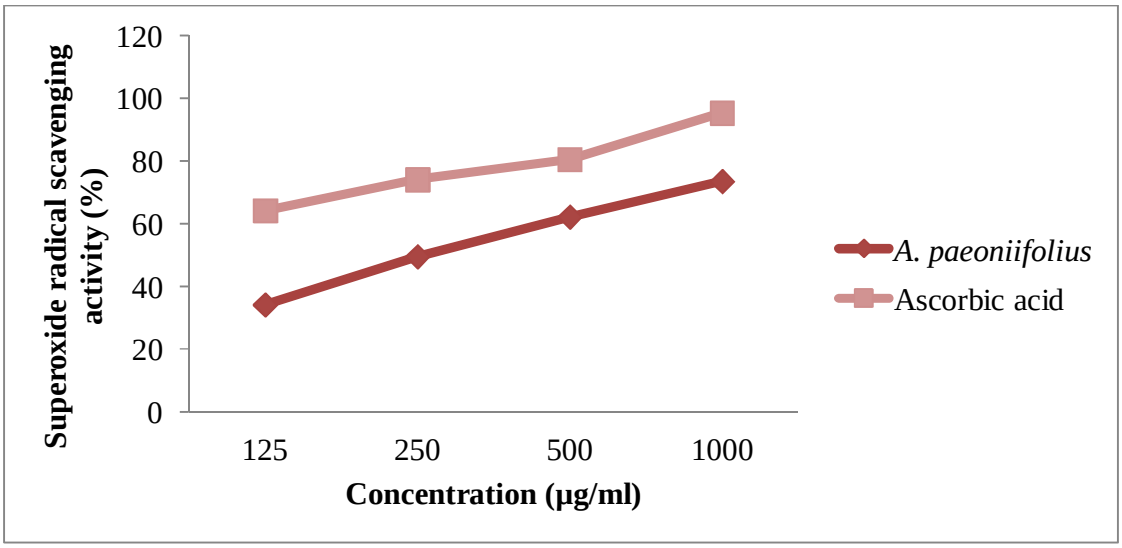

Figure 3: Superoxide radical scavenging activity of methanol extract of *Amorphophallus paeoniifolius* var.*campanulatus*

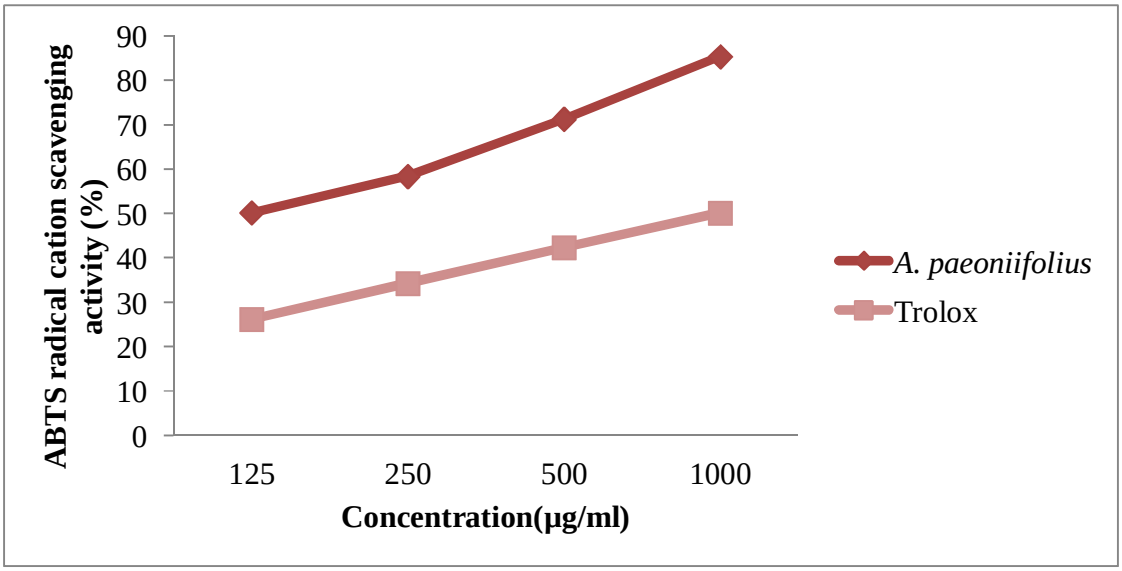

Figure 4: ABTs radical cation scavenging activity of methanol extract of *Amorphophallus paeoniifolius* var.*campanulatus*

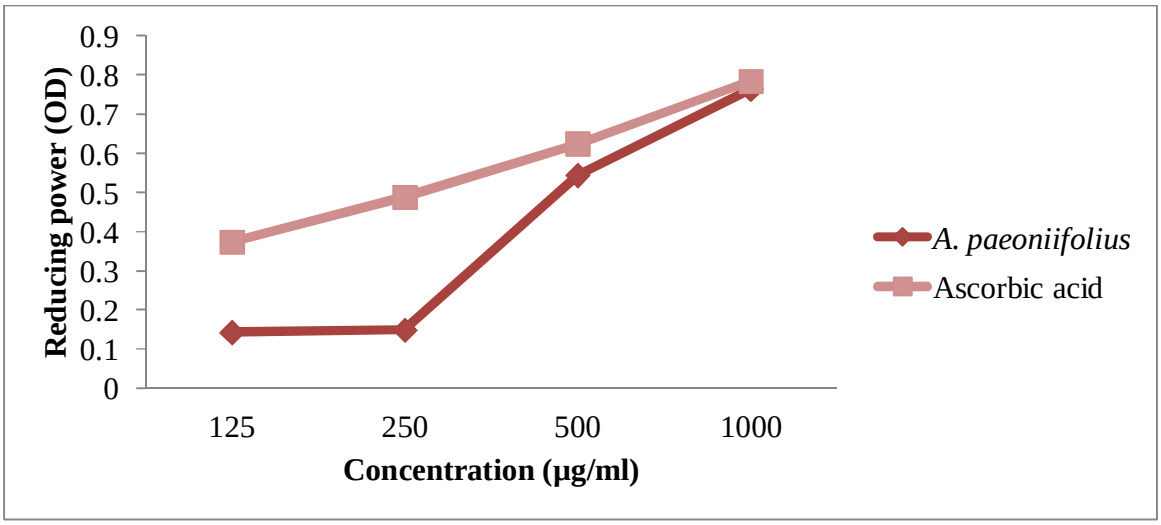

Figure 5: Reducing power ability of methanol extract of *Amorphophallus paeoniifolius* var. *campanulatus*

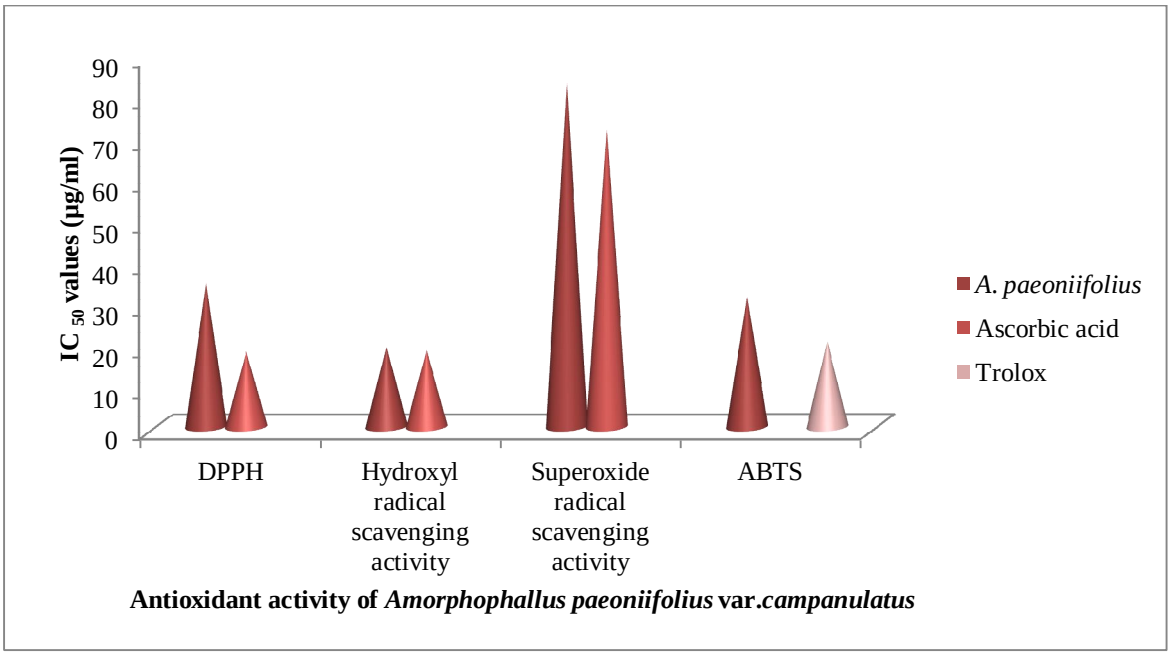

Figure 6: IC $_{50}$ values of methanol extract of *Amorphophallus paeoniifolius* var. *campanulatus*

Discussion:

Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals [14]. Phenolics have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory activities [15, 16].

Flavonoids, the major group of phenolic compounds reported for their antimicrobial, antiviral and spasmolutic activity. Flavonoids ability of scavenging hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals, which highlights many of the flavonoid health – promoting functions in organisms, which are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA. Flavonoids in human diet may reduce the risk of various cancers, as well as preventing menopausal symptoms. Flavonoids, on the other hand, are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anti-cancer activity [17, 18].

Amorphophallus paeoniifolius var. *campanulatus* extract exhibited potent *in vitro* antioxidant activity in DPPH radical scavenging assay, hydroxyl radical scavenging assay, superoxide free radical scavenging assay, ABTs radical cation scavenging activity and reducing power in comparison to the known antioxidants such as ascorbic acid and trolox.

The DPPH test provided information on the reactivity of test compounds with a stable free radical. Because of its odd electron, 2, 2- Diphenyl- picryl Hydrazyl radical (DPPH) gives a strong absorption band at 517 nm in visible spectroscopy. The efficacies of antioxidants are often associated with their ability to scavenge stable free radicals [19]. In the present study methanol extract exhibited more DPPH radical scavenging activity with IC_{50} value 34.56 µg/ ml comparable to ascorbic acid (18.26 µg/ml).

Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell [20]. The IC_{50} values were found to be 19.56 and 18.40µg/ml respectively for methanol extract of *A. paeoniifolius* var. *campanulatus* corm and ascorbic acid.

Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress [21]. Numerous biological reactions generate superoxide anions which are highly toxic species. The IC_{50} values were found to be 83.14 and 72.08 µg/ml respectively for methanol extract of corm and ascorbic acid.

The decolorization of ABTs radical reflects the capacity of an antioxidant species to donate electrons or hydrogen atoms to inactivate this radical species. The ABTs radical cation is generated from the

reaction of ABTs with potassium persulfate overnight in water [22]. The IC_{50} values of ABTs were found to be 31.44 and 20.67 µg/ml respectively for methanol extract of corm and trolox.

The reducing ability of a compound generally depends on the presence of reductants [23] which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom [24]. The reducing capacity of *A. paeoniifolius* var. *campanulatus* corm is a significant indicator of its potential antioxidant activity.

The present study was clearly indicated the methanol extract of *A. paeoniifolius* var. *campanulatus* corm showed strong antioxidant activity by inhibiting DPPH free radical scavenging, hydroxyl radical scavenging, superoxide radical scavenging and ABTs radical cation scavenging activities when compared with standard ascorbic acid and trolox. In addition, the methanol extract of corm was found to contain a noticeable amount of total phenolics and flavonoids, which play a major role in controlling antioxidants. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

Acknowledgement:

The second author, V.R. Mohan gratefully acknowledges and expresses his sincere thanks to University Grants Commisssion, New Delhi for providing financial assistance (Major Research Project: F39-429/2011 (HRP) dated 7 Jan 2011.

References

1. Vallo M, Rhodes CJ, Moncol J, Izaleovic M and Mazar M. free radicals, metals and antioxidants in oxidative stress- induced cancer. *Chem Biol Interact*. 2006; 160:1-40.

2. Adephapol AA, Jimoh FO, Afolayan AJ and Masika PJ. Antioxidant properties of the methanol extract of the leaves and stems of *Celtis Africana*. *Rec Nat Prod* 2009; 3:23-31.

3. Rajkumar V, Guha G and Kumar RA. Antioxidant and anti-neoplastic activities of *Picorhiza kurroa* extracts. *Food Chem Toxicol*. 2011; 49:363-369

4. Lim YY and Murtijaya J. Antioxidant properties of *Phyllanthus amarus* as affected by different drying methods. *Lebensm – Wiss. Technol.* 2007; 40:1664-1669.

5. Lalitharani S, Kalpana devi V, Tresina Soris P, Maruthupandian A and Mohan V.R. Ethnomedicinal plants used by Kanikkars of Agasthiarmalai Biosphere Reserve,Western Ghats. *J. of Ecobiotech.* 2011; 3:16-25

6. Lachman J, Hamouz K, Orsak M and Pivec V. Potato tubers as a signfcant source of antioxidant human nutrition. *Rostl. Vyr.* 2000; 46: 231-236.

7. Eom SH, Cheng WJ, Hyoung JP, Kim EH, Chung MI, Kim MJ, Yu CY and Cho DH. Far infra red ray irradiation stimulates

antioxidant activity in *Vitis flexuosa* Thunb. Berries. *Korean J. Med. Crop. Sci.* 2007; 15 : 31-323

8. Blois M.S. Antioxidant determination by the use of a stable free radical. *Nat* 1958; 181: 1199-1200.

9. Halliwell B, Gutteridge JMC and Aruoma OI. The deoxyribose method: a simple test to be assay for determination of rate constants for reaction of hydroxyl radicals. *Ana Biochem* 1987; 165: 215-219.

10. Robak J and Gryglewski RJ. Flavonoids are scavenges of superoxide anions. *Biochem. Pharmacol* 1988; 37:837-841

11. Re R, Pellegrini N, Proteggente A, Pannale A, Yang M and Rice- Evans C. Antioxidant activity applying an improved ABTs radical cation decolorization assay. *Free Radical Bio. Med.* 1999; 26: 1231-1237.

12. Singh R, Singh B, Singh S, Kumar N, Kumar S and Arora S. Investigation of ethyl acetate extract/fractions of *Acacia nilotica* wild. Ex. Del as potent antioxidant. *Re Nat Prod* 2009; 3: 131-138.

13. Oyaizu M. Studies on product of browning reaction prepared from glucose amine, *Jap J Food Chem* 1986; 44: 307-315.

14. Jorgensen LV, Madsen HL, Thomsen MK, Dragsted LO and Skibsted LH. Regulation of phenolic antioxidants from phenoxy radicals: An ESR and electrochemical study of antioxidant hierarchy. *Free Radical Res* 1999; 30: 207-220.

15. Arts IC and Hollman PC. Polyphenols and disease risk in epidemiological studies. *Am J Clin Nutr* 2005; 81: 317S-325S

16. Scallbert A, Manach C, Morand C, Remesy C and Jimenez L. Dietary poly phenols and the prevention of disease. *Crit Rev Food Sci Nutr* 2005; 45: 287-306.

17. Cushnie TPT and Lamb AJ. Antimicrobial activity of flavonoids. *Int J Antimicrob Agents.* 2005; 26:343-356.

18. De Sousa RR, Quiiroz KC, Souza AC, Gurgueira SA, Augusto AC and Miranda MA. Phosphoprotein levels, MAPK activities and NFKappaB expression are affected by fisetin. *J Enzyme Inhib Med Chem* 2007; 22: 439-444.

19. Basma AA, Zakania Z, Latha L and Saridhasan S. Antioxidant activity and phytochemical screening of the methanol extracts of *Euphorbia hirta* L. *Asian Pac J Trop Med* 2011; 4: 386-390.

20. Battu CR, Ethadi SR, Vede PG, Swathi PK, Chandrika K, Rao VA *et al*. Evaluation of antioxidant and anti-inflammatory activity of *Euphorbia heyneana* Spreng. *Asian Pac J Trop Biomed* 2011; $191-$194.

21. Naskar S, Islam A, Mozumdes UK, Saha P, Haldar PK, Gupta M. *et al*. *In vitro* and *in vivo* antioxidant potential of hydromethanolic extract of *Phoenix dactylijera* fruits. *J Sci Res* 2010; 2:144-157.

22. Mbaebie BO, Edeoga HO and Ajelayan AJ. Phytochemical analysis and antioxidant activities of aqueous bark extract of *Schoba*

lahtolic Jacq. *Asian Pac J Trop Biomed*. 2012; 118-124.

23. Dub PD, Tu YY and Yen GC. Antioxidant activity of water extract of Harng Iyur (*Chrysabthemum moifolium* Ramat). *Lebensm-Wiss Technol* 1999; 32: 269-277.

24. Gordon MH. The mechanism of the antioxidant action *in vitro*. In: *Food antioxidants*. Hudson BJF (ed).London. *Elsevier.* 1990; pp1-18.