Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 5, 2012

Research Article

IN VITRO ANTI-OXIDANT ACTIVITY OF *AMORPHOPHALLUS CAMPANULATUS* TUBERS (ROXB.) BLUME

SEEMA FIRDOUSE[1]*, PARWEZ ALAM[1], RABIA BASRA[2], AMEENA AMREEN[3], NAYALA FIRDOUS [4]**

***[1,2,3,4]Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad, Andhra Pradesh, India, **Shadan college of pharmacy, Peerancheru, Hyderabad, Andhra Pradesh, India. Email. seemamiyna@gmail.com**

Received: 17 Sep, 2012, Revised and Accepted: 19 Oct, 2012

ABSTRACT

The Anti oxidant activity of *Amorphophallus campanulatus* tuber was evaluated by using different extracts. The extracts was prepared by using solvents like hexane. Chloroform, ethyl acetate, alcohol, hydro alcohol for the evaluation of nitric oxide, DPPH, reducing power and total anti oxidant activity.

Keywords: Anti oxidant activity, Nitric oxide, DPPH, Reducing power.

INTRODUCTION

Generally free radicals are produced in large amounts during metabolic disease conditions like atherosclerosis, urolithiasis, ulcers etc., they may cause damage to the organs and ultimately leads to fatal effects if the production of free radicals is not controlled.

When the normal level of anti-oxidant defense mechanism is not sufficient for the eradication of free radicals induced injury, administration of anti-oxidants has a protective role to play. Several anti-oxidants of plant origin are experimentally proved and used as effective protective agents against oxidative stress. They play an important role in major health problems such as cancer, cardio vascular diseases, rheumatoid arthritis, cataracts, Parkinson's disease, Alzheimer's disease and degenerative diseases associated with aging.[1,6]

There are various plants which are used to treat the diseases like ulcer, diabetes, epilepsy& cardio vascular diseases. One such plant is *Amorphophallus campanulatus* which was reported to cure diseases like diabetes, syphilis, piles, lung diseases and biliousness that are caused due to stress conditions. It contains various chemical constituents like steroids, alkaloids, flavanones, coumarins, reducing sugars, tannins and proteins. Hence an attempt has been made to evaluate the anti-oxidant property of various fractions of *Amorphophallus campanulatus* tuber viz. hexane, chloroform, ethyl acetate, alcohol and hydro alcohol by *in vitro* methods like ferrous ion induced lipid peroxidation, reducing power, total anti-oxidant activity, DPPH and nitric oxide scavenging activity.

MATERIAL AND METHODS

Collection of plant material

The *Amorphophallus campanulatus* tubers were procured from yucca enterprises Mumbai.

Preparation of extract [2]

The tubers of *Amorphophallus campanulatus* were washed well with water to remove its earthy matter and cut into small pieces, dried in shade and powdered coarsely. The powdered tubers of *Amorphophallus campanulatus* (1kg) were extracted exhaustively with different solvents based upon the increasing polarity like Hexane<Chloroform<Ethyl acetate<Alcohol <Hydro Alcohol in an aspirator bottle at room temperature for 72 hours. Nearly 80% of the solvent was removed by rotatory vaccum evaporator. The hydroalcoholic extract (1:1) were prepared in a similar manner. The Hexane, Chloroform, Ethyl Acetate, Alcohol, Hydroalcohol fractions were subjected to *in vitro* antioxidant studies.

Nitric Oxide Scavenging Activity

The nitric oxide scavenging activity of *Amorphophallus campanulatus* tuber was determined according to the method [3].Aqueous solution of sodium nitro prusside spontaneously generates nitric oxide (NO) at physiological pH, which interacts with oxygen to produce nitrate ions and which was measured colorimetrically. 3mL of reaction mixture containing 2mL of sodium nitro prusside (10mM) in phosphate buffered saline (PBS) and 1mL of various concentrations of the extracts were incubated at 37^{0}C for 4 hours. Control without test compound was kept in an identical manner. After incubation 0.5mL of griess reagent was added. The absorbance of the chromophore formed was read at 546nm. The percentage inhibition of nitric oxide generation was measured by comparing the absorbance values of control and those of test compounds. Curcumin (50,100 & 200µg) was used as standard. The percentage nitric oxide inhibition was calculated from the following formula. The results were tabulated in table 1 and represented in figure 1.

% Nitric Oxide Inhibition = [OD of control-OD of test/ OD of control] X 100

Determination of DPPH Scavenging Activity [4]

DPPH scavenging activity was measured by the spectrophotometric method. A stock solution of 25mg of DPPH (150µM) was prepared in 100mL of ethanol. To the 0.1 mL of extract of different concentrations, 1.9mL of DPPH was added. Control without test compound was prepared in an identical manner. In case of blank, DPPH was replaced by ethanol. The reaction was allowed to be completed in the dark for about 20 minutes. Then the absorbance of test mixtures was read at 517nm. The percentage inhibition was calculated and expressed as percent scavenging of DPPH radical. Curcumin (50,100 & 200µg) was used as standard. The percentage DPPH inhibition was calculated from the following formula. The results are tabulated in table 1 and represented in figure 2.

% DPPH Inhibition = [OD of control-OD of test/ OD of control] X 100

Determination Of Reducing Power

The reducing power of *Amorphophallus campanulatus* tuber was determined according to the method (Oyaizu, 1982). Extracts of different concentrations were prepared in 1mL of DMSO were mixed with 2.5mL of phosphate buffer (pH 6.6, 0.2M) & potassium ferric cyanide (2.5mL, 10%). The mixture was incubated at 50^{0}C for 20 minutes. Aliquot of TCA (2.5mL, 10%) were added to the mixture, which was then centrifuged at 1500 rpm for 10 minutes. The upper layer of reaction mixture was mixed with distilled water (2.5mL) and freshly prepared $FeCl_3$ solution (0.5mL, 0.1%). The absorbance was measured at 640 nm; increase in absorbance of the reaction mixture indicates the increase in reducing power. The results are tabulated in table 2.

Total Antioxidant activity[5]

The total anti-oxidant activity was evaluated by (Prieto et al., 1999). An aliquot of 0.1mL of sample solution/Vitamin E equivalent to

500µg was combined with 1mL of the reagent solution (0.6M H_2So_4, 28mM sodium phosphate and 4mM ammonium molybdate). In case of blank, 0.1mL of methanol was used in place of sample. The tubes were capped and incubated in a boiling water bath at 95^{0}C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695nm against blank. The anti-oxidant activity was expressed as equivalents of Vitamin E (µg/g). The results are tabulated in table 2.

RESULTS

Table 1: *In vitro* Anti-Oxidant Activity of *Amorphophallus campanulatus* tubers

Extracts	Concentration (µg)	Nitric Oxide (%Inhibition)	DPPH (%Inhibition)
Hexane	*62.5*	*51.31*	*29.30*
	125	*47.35*	*35.42*
	250	*33.85*	*34.56*
	500	*16.88*	*45.21*
	1000	*35.83*	*43.24*
	2000	*52.84*	*38.42*
Chloroform	*62.5*	*83.26*	*67.5*
	125	*23.58*	*35.8*
	250	*42.45*	*44.25*
	500	*62.98*	*51.02*
	1000	*52.52*	*54.16*
	2000	*48.43*	*62.85*
Ethyl acetate	*62.5*	*37.10*	*42.54*
	125	*48.24*	*35.21*
	250	*22.80*	*41.20*
	500	*19.27*	*42.50*
	1000	*22.28*	*39.48*
	2000	*21.62*	*36.45*
Alcohol	*62.5*	*52.3*	*36.12*
	125	*52.4*	*48.24*
	250	*39.69*	*45.24*
	500	*48.59*	*32.54*
	1000	*36.2*	*36.21*
	2000	*32.24*	*41.21*
Hydro Alcohol	*62.5*	*44.86*	*50.25*
	125	*83.4*	*48.25*
	250	*51.88*	*46.85*
	500	*28.49*	*61.25*
	1000	*44.22*	*54.32*
	2000	*38.84*	*62.48*
Curcumin	*50*	*50.00*	*49.20*
	100	*35.95*	*84.75*
	200	*20.57*	*97.12*

Table 2: Determination of Reducing power and Total Anti-Oxidant activity of *Amorphophallus campanulatus* tuber by in vitro method.

Extracts (62.5 µg)	Reducing Power (mg)	Total Anti-Oxidant (mg)
Hexane	0.32	0.78
Chloroform	0.40	1.17
Ethyl acetate	0.40	0.84
Alcohol	0.58	0.35
Hydro alcohol	0.56	0.56

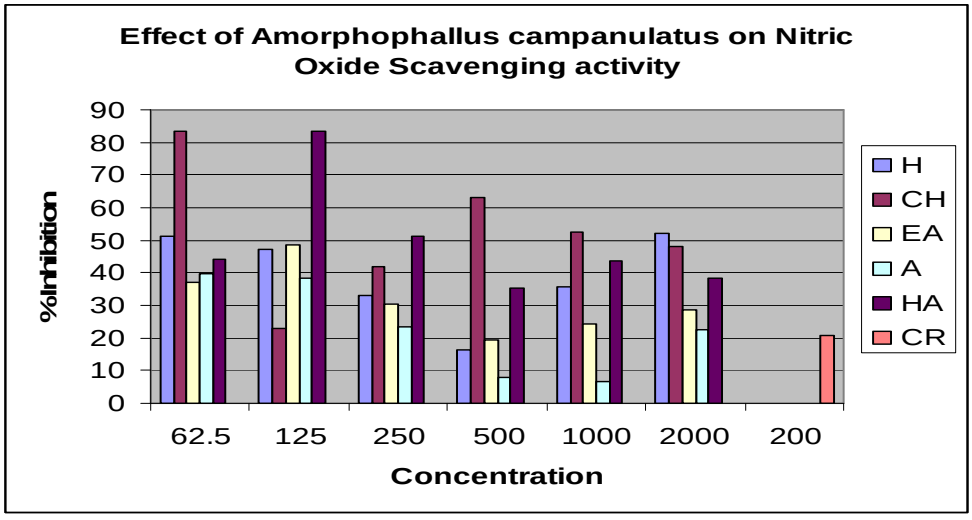

Fig. 1: Effect of *Amorphophallus campanulatus* tuber on Nitric Oxide Scavenging activity

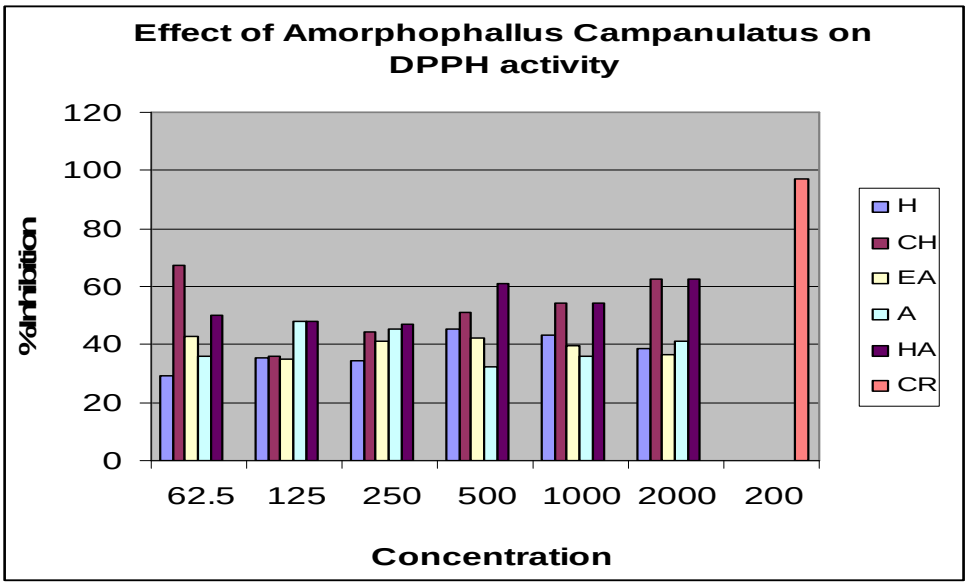

Fig. 2: Effect of *Amorphophallus campanulatus* on DPPH activity

DISCUSSIONS

In-Vitro antioxidant activity guided fractionation of *Amorphophallus campanulatus***.** The hydroalcohol and chloroform fractions of *Amorphophallus campanulatus* showed a dose dependent increase in nitric oxide scavenging property. About 28.5% inhibitions were observed by hydroalcoholic fraction at 500mg concentration, whereas chloroform extracts of *Amorphophallus campanulatus* tuber shows a biphasic response i.e., at 250mg concentration, about 45.24% of nitric oxide scavenging property was observed. But in ethyl acetate and alcohol the effect was increased as the dose decreases. The presence of sugars in hydroalcohol besides amino acids may be responsible for nitric oxide scavenging activity. From the result it is made clear that *Amorphophallus* also possess free radical scavenging activity through nitric oxide scavenging property also, and it is in the order hydroalcohol> chloroform>alcohol>Ethyl acetate> Hexane fractions.

The reducing power was shown by chloroform, hydroalcoholic and alcoholic fractions of *Amorphophallus campanulatus* whereas hexane and ethyl acetate fractions showed less activity than chloroform, hydroalcohol and alcohol. The reducing power is expressed in terms of ascorbic acid equivalents (AsEmg-1). The presence of amino acids in the alcoholic, chloroform and hydroalcoholic extract may be responsible for the reducing power. The chloroform, hydroalcoholic extract and various fractions of *Amorphophallus campanulatus showed* dose dependent response. In DPPH scavenging activity of curcumin also showed a dose dependent increase in DPPH activity.

CONCLUSION

The *in-vitro* antioxidant activity guided fractionation of tuber revealed that the alcoholic fractions showed more activity as compared to chloroform, ethyl acetate and hexane fractions. The hydro alcoholic fraction showed more percentage protection of nitric oxide and DPPH scavenging activity. The reducing power & total antioxidant activity also substantiates the above claim.

ACKNOWLEDGEMENT

The authors are thankful to honourable Dr.Venkateshwar Reddy, principal of Anwarul Uloom College of Pharmacy, New mallepally, Hyderabad for providing all the facilities to carry out research work.

REFERENCE

1. Nishigaki, I., Oku, H., Yagi, K., Suppressive effect of curcumin on lipid peroxidation induced in rats by CCl_4 or Cobalt 60 irradiation; *Journal of Clin & Nutri Biochem*; 13, 23, 1992.
2. J. B. Harborne, Phytochemical Methods – A Guide to Modern Techniques of Plant Analysis. Third edition. Page no. 3 -31.
3. *Green, L.C., D.A. Wagner, J. Glogowski, P.L. Skipper, J.S. Wishnok and S.R. Tannenbaum, 1982. Analysis of Nitrate and (^{15}N) nitrate in biological fluids.* Anal. Boichem*; 126; page.no. 131 – 138.*
4. M. R. Szabo, C. Idiţoiu, D. Chambre and A. X. Lupea, Improved DPPH determination for antioxidant activity spectrophotometric assay. *AAPS PharmSciTech Chemical Papers* Volume 61, Number 3 / June, 2007. Page no. 64-67.
5. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantification of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application of vitamin E. Anal. Biochem. (1999) 269: page no. 337-341.
6. Flohe, 'Free radical in biology', Academic press, New York, 5th ed, page no. 295, 1982.