Int. J. Chem. Sci.: 7(3), 2009, 1553-1562

EVALUATION OF *IN VITRO* ANTIOXIDANT ACTIVITY OF *AMORPHOPHALLUS CAMPANULATUS* (ROXB.) EX BLUME DECNE

K. G. SAHU*, S. S. KHADABADI and S. S. BHIDE

Govt. College of Pharmacy, AMARAVATI (M. S.) INDIA

ABSTRACT

Antioxidant activity of methanolic and aqueous extract of *Amorphophallus campanulatus* tuber was studied for its free radical scavenging property on different *in vitro* models e. g. - 1, 1-diphenyl-2-picryl hydrazyl (DPPH) method, nitric oxide method and reducing power method. The extracts showed good dose dependant free radical scavenging property in all the models. IC_{50} values for water and methanolic extract were found to be 59.91 and 99.40 µg/mL in DPPH method and 77.02 and 70.20 µg/mL in nitric oxide method. In reducing power method, aqueous extract shows more reducing power as compared to methanolic extract. Ascorbic acid was used as standard. It is concluded that the aqueous extract shows more antioxidant activity as compared to methanolic extract.

Key words : Antioxidant, *Amorphophallus campanulatus*, Free radical scavenging, Reductive ability

INTRODUCTION

Oxygen is vital for aerobic life process. However about 5% or more of the inhaled O_2 is converted to reactive oxygen species (ROS). A free radical (FR) can be defined as a chemical species possessing an unpaired electron. FR can be positively charged, negatively charged or electrically neutral[1]. When generation of ROS overtakes the antioxidant defense of the cells, the free radicals start attacking the cell proteins, lipids and carbohydrates and this leads to a number of physiological disorders[2]. Free radicals have been implicated in the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorders, neurological disorders and in the process of aging[3].

Antioxidants are chemical substances that donate an electron to the free radical and convert it to a harmless molecule. They may reduce the energy of the free radical or

* Author for correspondence; E-mail : kirtisahu789@rediffmail.com, Ph.: 09975444936;
C/o Sahu Anaj Bhandar, Malgujari pura, WARDHA - 442001 (M. S.) INDIA

suppress radical formation or break chain propogation or repair damage and reconstitute membranes.

Amorphophallus campanulatus (Roxb.) Blume. ex Decne (Synonym : Amorphophallus paeoniifolius; Araceae) commonly known as surana is a tuberous, stout, indigenous herb, 1.0-1.5 m in height. The tubers contain an active diastatic enzyme-amylase, betulinic acid, B-sitosterol, stigmasterol, B-sitosterol palmitate, lupeol, triacontane, amino acids, carbohydrates, saponin, thiamine, riboflavin, niacin and carotene. In ethno botanical studies, the plant is used for piles, splenomegaly, cough and rheumatism. The petiole is used in scorpion bite and dys-menorrhoea. The tubers find use in post delivery complaint, in migraine and in neck swelling[4]. In Ayurvedic medicine, *Amorphophallus campanulatus* is used to cure piles, deranged digestion and diseases of liver. In Unani medicine, sooran is given as vegetable in sluggish liver[5]. The plant tuber is reported to have analgesic activity[6]. The bioconversion of *Amorphophallus campanulatus* to citric acid by *Aspergillus niger* has also been studied[7].

Therefore the objective of present study has been directed to investigate the antioxidant activity of *Amorphophallus campanulatus* tuber through the DPPH scavenging, nitric oxide scavenging and reducing power method.

EXPERIMENTAL

Materials and methods

Chemicals

DPPH, N- (1-napthyl)-ethylenediamine, sodium nitroprusside, sulfanilamide, potassium ferricyanide and trichloroacetic acid were purchased from Sigma Aldrich. All other chemicals and solvents used were of analytical grade available commercially.

Preparation of extracts of *Amorphophallus campanulatus*

The tubers of *Amorphophallus campanulatus* were collected from local vegetable market of Wardha district, Maharashtra (India). Authentication has been done by Dr. Prabha S. Bhogaonkar, H. O. D., Department of Botany, Vidharbha Institute of Science and Humanities, Amravati, Maharashtra, (India). The collected tubers were cut into small pieces, dried and pulverized into coarse material. The coarse plant material was used for preparation of extracts. The extraction of powered tuber was done by successive extraction by using Soxhlet apparatus. 100 g of powdered tuber of *Amorphophallus campanulatus*

was extracted with petroleum ether (60-80^{0}C) in several batches by using Soxhlet apparatus

Successive extraction was done by using petroleum ether (60^{0}C-80^{0}C), chloroform, methanol, distilled water. All the extracts were stored in freeze. Methanol and aqueous extracts were used for antioxidant activity.

DPPH Radical scavenging[8]

Methanolic solution of each extract (10, 20, 40, 60, 80, 100 µg/ mL) was mixed with 400 µM DPPH (Sigma Aldrich) methanol solution at a ratio 1 : 3. The mixture was left in dark at room temperature for 90 minutes. The absorbance of the resulting solution was measured by spectrophotometer (Shimadzu 1700) at 517 nm. The capability of scavenging DPPH radical was then calculated by using following equation

Scavenging effect (%) = [1-Abs. of sample/Abs. of control] x 100

Nitric oxide method[9, 10]

Sodium nitroprusside (10 mM) in phosphate buffered saline was mixed with different concentrations of aqueous and alcoholic extracts of tubers of *Amorphophallus campanulatus* dissolved in methanol and incubated at room temperature for 180 minutes. The same reaction mixture without the extract of the sample but with equivalent amount of phosphate buffer served as the control. After incubation period, 0.5 mL Griess reagent {1% sulphanilamide, 2% H_3PO_4 and 0.1 % N-(naphthyl) ethylenediamine hydrochloride NEDA} was added to equivalent amount of sample. The absorbance of chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with NEDA was measured at 546 nm using UV Spectrometer (Shimadzu 1700).

Scavenging effect (%) = [1-Abs. of sample/Abs. of control] x 100

Determination of reducing power [9]

The reducing power of the extracts was determined according to the method of Oyaizu (1986). Various concentrations of the extracts (100- 500 µg/ mL) in 1.0 mL of deionized water were mixed with phosphate buffer (2.5 mL, 0.2M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). The mixture was incubated at 50^{0}C for 20 minutes; aliquots of trichloroacetic acid (2.5 mL, 10%) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared $FeCl_3$ solution (0.5 mL, 1%). The

absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Reducing power is given in ascorbic acid equivalent (ASE mL^{-1}) that shows the amount of ascorbic acid expressed in mM.

Statistical analysis

Tests were carried out in triplicate. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically determined by linear regression method using MS- window based graphpad instat software. Results are expressed as graphically/ mean± standard deviation. Dunnett test was performed. Experimental groups were compared with the standard.

RESULTS AND DISCUSSION

Free radicals of different forms are constantly generated for specific metabolic requirement and quenched by an efficient antioxidant network in the body. When the generation of these species exceeds the levels of antioxidant mechanism, it leads to oxidative damage of tissue and biomolecules, eventually leading to disease conditions, especially degenerative diseases. Many plant extracts and phytochemicals have been shown to have antioxidant / free radical scavenging properties.

Antioxidant activity of different extracts of *Amorphophallus campanulatus* tubers were determined by three different *in vitro* methods-

1. DPPH (1, 1-Diphenyl, 2-picryl-hydrazyl) antiradical activity
2. Nitric oxide (NO) scavenging activity
3. Reducing power

These methods are most popular *in vitro* assays for determination of antioxidant activity.

DPPH radical scavenging activity

In DPPH method, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity[11]. The obtained results of absorbance and % inhibition showed decrease in concentration of DPPH radical due to scavenging ability of extract and standard ascorbic acid, as a reference compound. The aqueous extract presented the better activity at all concentrations when compared to methanolic extract.

A 100 µg/mL of aqueous extract, methanolic extract and ascorbic acid exhibits 61.16%, 50.31% and 90.7% inhibition respectively and the IC_{50} values were found to be 59.91 µg/mL, 99.40 µg/mL and 8.53 µg/mL for aqueous extract, methanolic extract and ascorbic acid, respectively.

Nitric oxide radical scavenging activity

The nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite, which is inhibited by antioxidants by competing with oxygen to react with nitric oxide. Sulfanilamide is quantitatively converted to a diazonium salt by reacting with nitrite in acidic conditions (5% phosphoric acid). This diazonium salt coupled with N- (1-naphthyl)-ethylenediamine (NED); forming an azo dye that can be measured quantitatively at 542 nm[9, 10]. The results showed that the aqueous extract has higher % inhibition and lowest IC_{50} value as compared to methanol extract. The % inhibition and IC_{50} values of aqueous extract, methanol extract and ascorbic acid were 66.94 %, 62.97 % and 95.27 % and 70.20 µg/mL, 77.02 µg/mL and 10.82 µg/mL respectively.

Reducing power activity

For the measurement of the reducing ability, the reducing capability of a compound may serve as a significant indicator of its potential antioxidant. The reducing power of extracts increased with increasing concentration. The results showed that aqueous extract has higher reducing power as compared to methanol extract[12].

CONCLUSION

The results of the present study shows that the extract of *Amorphophallus campanulatus* tuber shows good antioxidant activity. The results of estimation of antioxidant activity of extracts prove its action on free radicals. The aqueous extract shows more significant antioxidant activity as compared to methanol extract.

Table 1 : Result of DPPH radical scavenging activity of each extract

Extract	Concentration (µg/ mL) and % inhibition						IC 50 (µg/mL)
	10	20	40	60	80	100	
Std. Ascorbic Acid	58.6 ± 0.28	65.4 ± 0.70	72.5 ± 0.42	77.1 ± 0.57	82.03 ± 0.20	90.7 ± 0.57	8.53 ± 0.21

Extract	Concentration (µg/ mL) and % inhibition						IC 50 (µg/mL)
	10	20	40	60	80	100	
Aqueous extract	31.74 ± 3.39**	34.25 ± 3.97**	44.01 ± 1.18**	50.07 ± 0.48**	51.24 ± 1.76**	61.1 ± 1.56**	59.91 ± 1.28
Methanolic extract	17.82 ± 1.20**	25.82 ± 0.51**	29.78 ± 0.41**	36.31 ± 0.62**	42.07 ± 0.41**	50.3 ± 0.52**	99.40 ± 0.46

Values are given as mean ± S. D. (n=3). ** Significant at $p < 0.01$, p-value was calculated by comparing with control by ANOVA followed by Dunnett's test, values are expressed as ± SEM.

Table 2 : Result of nitric oxide scavenging activity of each extract

Extract	Concentration (µg/ mL) and % inhibition						IC_{50} (µg/mL)
	10	20	40	60	80	100	
Std. Ascorbic acid	46.2 ± 1.1	56.51 ± 1.40	66.67 ± 2.51	77.61 ± 1.80	88.16 ± 1.68	95.27 ± 1.83	10.82 ± 1.02
Aqueous extract	22.02 ± 0.18**	26.78 ± 0.86**	31.73 ± 1.76**	41.4 ± 1.86**	56.98 ± 0.55**	66.94 ± 2.77**	70.20 ± 0.56
Methanolic extract	13.41 ± 1.55**	21.4 ± 1.83**	25.19 ± 2.55**	37.16 ± 1.41**	51.93 ± 2.68**	62.01 ± 1.32**	77.02 ± 1.48

Values are given as mean ± S. D. (n=3). **Significant at $p < 0.01$, p-value was calculated by comparing with control by ANOVA followed by Dunnett's test, values are expressed as ± SEM.

Table 3 : Result of radical scavenging activity of each extract by reducing power method

Concentration (µg/mL)	Absorbance		
	Std. Ascorbic acid	AQE	MEE
100	0.52 ± 0.04	0.20 ± 0.01**	0.07 ± 0.01**
200	0.82 ± 0.05	0.60 ± 0.02**	0.16 ± 0.01**
400	1.54 ± 0.06	0.81 ± 0.04**	0.29 ± 0.01**
600	1.70 ± 0.03	1.09 ± 0.04**	0.38 ± 0.01**
800	3.44 ± 0.03	1.11 ± 0.01**	0.45 ± 0.05**
1000	3.61 ± 0.03	1.27 ± 0.08**	0.86 ± 0.03**

Values are given as mean ± S. D. (n=3). **Significant at $p < 0.01$, p-value was calculated by comparing with control by ANOVA followed by Dunnett's test, values are expressed as ± SEM.

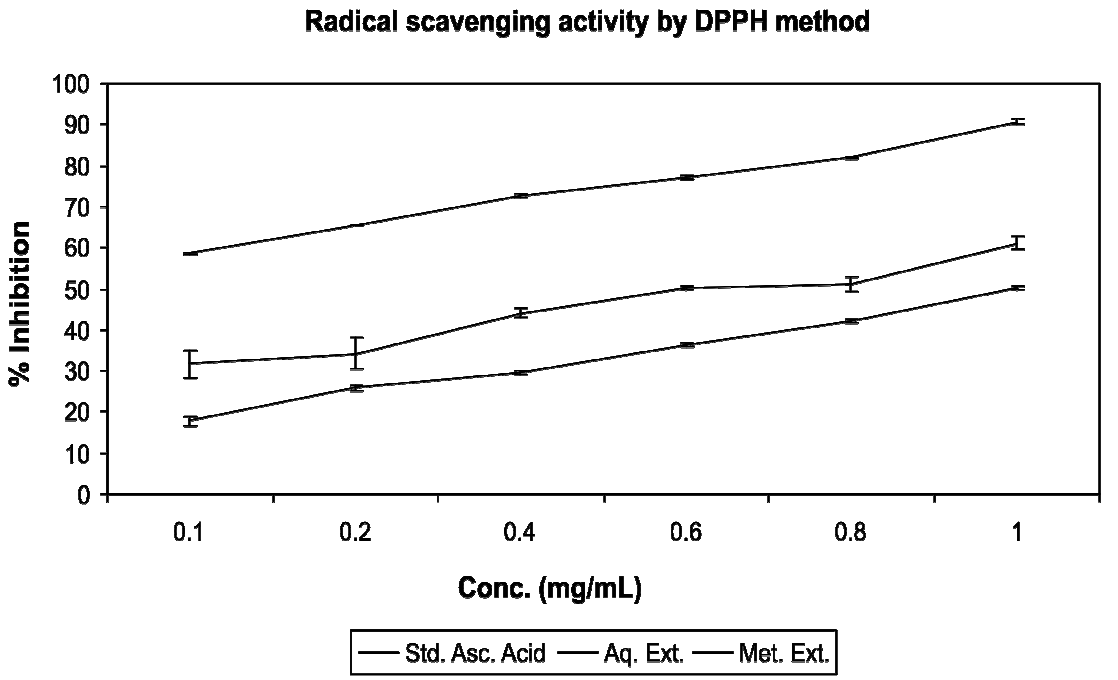

Fig. 1: Effect of extracts on accumulation of DPPH radical

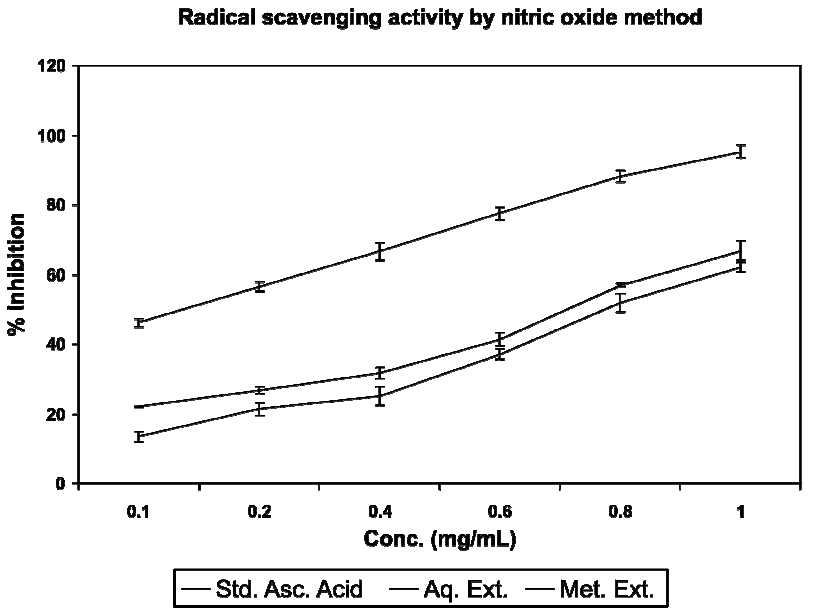

Fig. 2 : Effect of extracts on accumulation of nitric oxide radical

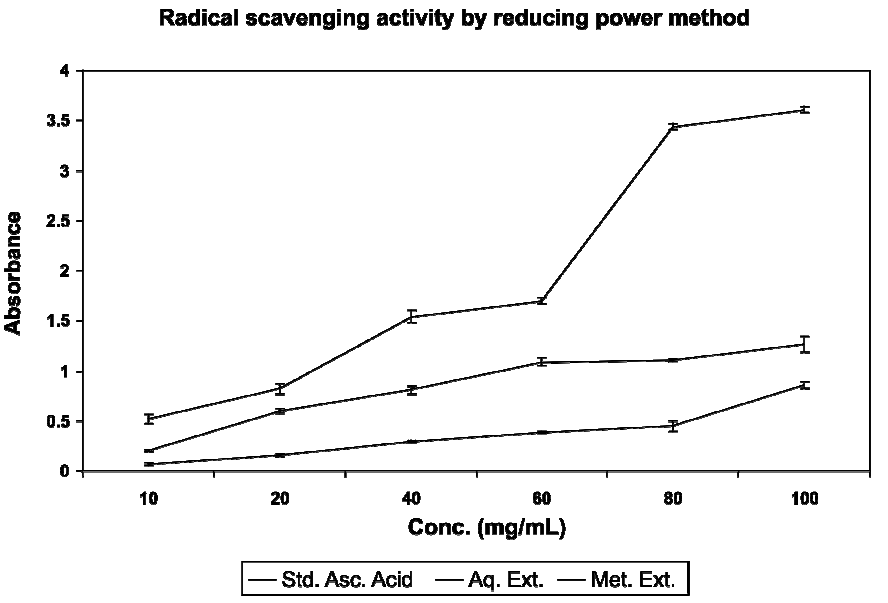

Fig. 3 : Radical scavenging activity by reducing power method

ACKNOWLEDGEMENT

The authors thank Dr. P. Y. Bhogaonkar, V. M. V., Amravati, who for authenticated the plant.

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Accepted : 10.04.2009