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Phenolic contents and antioxidant capacity of *Amorphophallus commutatus* and *Amorphophallus paeonifolius*

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Abstract

Tubers of *Amorphophallus* species are used as an ingredient in ayurvedic formulations. The tuberous roots of the species of *Amorphophallus campanulatus* Bl. are used traditionally for the treatment of piles, abdominal pain, tumors, enlargement of spleen, asthma and rheumatism, beside stomachic and appetizer properties. In this investigation the tubers of *Amorphophallus commutatus* (Schott) (ACT) and *Amorphophallus paeonifolius* (Dinnst.) (APT) were extracted with three different solvents (ethanol, acetone and water) and screened for total phenolic content and antioxidant activity. Acetone extracts of ACT and APT exhibited the highest phenolic content 17.66±0.44 and 15.90±0.25 mg GAE/g respectively. The compounds that were identified by the HPLC included tannic acid, gallic acid, quercetin, p-coumaric acid and catechin. The ACT and APT acetone extracts also possesses highest antioxidant activity towards Ferric reducing antioxidant power (0.554 & 0.541 O.D), 1, 1-diphenyl-2-picrylhydrazyl (79.4% & 74.52%), hydroxyl radical scavenging activity (66.45% & 64.29%) and nitric oxide radical scavenging activity (67.5% & 59.11%). Positive correlation was observed between total phenolic content and various antioxidant activities indicated that such extracts could have good applications in nutraceutical and other pharmaceutical formulations.

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Introduction

Antioxidants are able to retard, delay or prevent oxidation process (Halliwell, 1997). The potential of the antioxidant constituents of plant materials for the maintenance of good health, protection from coronary heart diseases and cancer, is of tremendous interest among scientists and food manufacturers as consumers are more inclined to consume functional foods with specific health effects (Javanmardi *et al*., 2003). Number of plant species possess natural antioxidants with high antioxidant activity and investigations on these were initiated based on their uses in traditional medicines (How and Yau, 2011). These are phenolics and flavonoids from tea, wine, fruits, vegetables and spices which have been already exploited commercially either as antioxidant additives or as nutritional supplements (Patel *et al*., 2010).

Tubers of many *Amorphophallus* species is used as vegetable and in ayurvedic formulations (Angayarkanni *et al*., 2007) as these have restorative, carminative, stomachic and tonic effects (Chopra *et al*., 1958), in piles, acute rheumatism, abdominal tumors, boils, asthma and enlargement of spleen (Yusuf *et al*, 1994). The tuber contains flavonoids, phenols, coumarins, terpenoids, sterols, tannins, steroids, alkaloids and sugars like glucose, galactose and rhamnose (Nataraj *et al*., 2009; Yadu and Ajoy, 2010).

The objective of this investigation is to evaluate *Amorphophallus commutatus* and *Amorphophallus paeonifolius* as new potential sources of natural antioxidants, phenolic compounds and to demonstrate a possible relationship between phenolic contents and antioxidant activity in these species.

Materials and Methods

Plant material and sample preparation

Fresh tubers of *Amorphophallus commutatus* (Schott) and *Amorphophallus paeonifolius* (Dinnst.) Nicolson (Family: Araceae) were collected from Gaganbawda and Amboli in Western Ghats of Maharashtra, India respectively. The tubers were cleaned, chopped and air dried at 40^0C for 48 h. The dried ACT and APT were pulverized. These were then extracted using acetone, ethanol and water as solvent for 24 h with shaking on rotary shaker (120 r.p.m.). The pulverized mass was added in a ratio of 1: 10 (w/v) in each of the solvents. The extracted materials were filtered through Whatman filter paper

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No. 1 and concentrated by evaporating the solvent completely in water bath at the range of boiling points of the solvents. The dried extracts were redissolved in definite quantity of respective solvents and stored at 4°C for further studies (Sule *et al,* 2011) unless otherwise specified.

Total phenolic content (TPC)

The total phenolic content of ACT and APT extracts in four different solvents were determined spectrophotometrically using the Folin-Ciocalteu assay (Jagtap *et al*., 2010) with slight modifications. An aliquot of 100 µl of each extract was mixed with 2 ml of Folin- Ciocalteu reagent which was previously diluted 10-fold with distilled water. The solutions were allowed to rest at 25°C for 5 min before adding 2 ml sodium carbonate (15%) solution and again rested for 90 min at room temperature. Absorbance was measured spectrophotometrically at 765 nm. These were compared using standard gallic acid.

Ferric reducing antioxidant power assay (FRAP)

The FRAP assay was performed as described by Ekrem *et al.* (2011). The various aliquots of ACT and APT extract (0.5-2 mg/ml) concentrations were allowed to react with 2.5 ml sodium phosphate buffer (200 mM, pH 6.6) and 2.5ml potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min, followed by addition of 2.5 ml trichloroacetic acid (10% w/v). Then 5 ml of above reaction solution was mixed with 5 ml distilled water and 1ml ferric chloride (0.1%). The absorbance was measured spectrophotometrically at 700 nm and compared with standard Butylated Hydroxy Anisole (BHA). Any increase in absorbance is synonymous of an increase in reducing power.

DPPH radical scavenging activity

Antioxidant activity of the ACT and APT extracts was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay (Lee *et al.*, 2003). The different concentrations (0.5-2 mg/ml) of ACT and APT extracts were allowed to react with 5 ml methanolic DPPH (0.1 mM) solution. The mixture was shaken vigorously and left to rest at room temperature for 30 min in dark. The absorbance was measured at 517 nm. A control sample with no added extract was also analyzed and the results were expressed as percent radical scavenging activity (% RSA).

$$\%RSA = (A_{control} - A_{sample})100 / A_{control}$$

Where, A = absorbance at 517 nm.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined according to method of Murthi *et al.* (2006). The different concentrations (0.5-2 mg/ml) of ACT and APT extracts were allowed to react with 100µl of phosphate buffer (0.1 M, pH 7.4), 1ml of Fe –EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml EDTA(0.018%) and 1ml Dimethyl sulfoxide (0.85% v/v) in phosphate buffer (0.1 M pH 7.4). The reaction was initiated by adding 0.5 ml ascorbic acid (0.22%). These reaction mixtures were incubated at room temperature for 15 min. The reaction was terminated by the addition of 1ml ice cold TCA (17.5% w/v). Finally 3 ml Nash reagent (150 g ammonium acetate, 3 ml glacial acetic acid and 2 ml acetyl acetone were mixed and raised to 1L with D.W) was added and left at room temperature for 15 min for colour development. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm. A control sample with no added extract was also analyzed and the results were expressed as radical scavenging activity (RSA).

$$\% RSA = 1 - (A_{sample} / A_{control}) X 100$$

Where A = absorbance at 412 nm

Nitric oxide radical scavenging activity

The Nitric oxide radical scavenging activity was determined by the method of Marcocci and Packer (1994) with slight modifications. The various concentrations (0.5-2 mg/ml) of ACT and APT extracts were allowed to react with 400µl sodium nitroprusside (10 mM), 250 µl Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylene diamine dihydroxy chloride) and incubated at 25°C for 1 hr. Finally 2 ml distilled water was added and absorbance was measured at 546 nm. Radical scavenging activity of the nitric oxide generated, was measured by comparing the absorbance values of control and test preparations. The antioxidative potential of both extracts were evaluated in the same manner as per the DPPH assay. The absorbance of all the assays was measured on Thermo Scientific-Chemito UV-2100.

HPLC analysis

HPLC was done using a Hitachi LaChrome chromatograph fitted with a reversed phase column (Column- C18; 5mm, 250×4.6 mm) and a UV detector set at 240 nm. The column was operated at room temperature. Separations were carried out in a liquid feed pumping system by using acetonitrile

(70%) and water (30%) as a mobile phase with a flow rate of 0.5ml/min. The injection volume for all samples was 100 µL. The phenolic compounds were analysed by matching the retention time and their spectral characteristics against those of standards.

Statistical analysis

In this study, three analyses of each sample were made and each experiment was carried out in triplicate (n=3). Values representing the effective concentration of investigated extracts that because 50% of inhibitions (EC_{50} value) were determined by linear regression analysis of obtained RSA. Analysis of variance was carried out for all data at $p<0.05$ using Graph Pad software (GraphPad InStat version 3.00, GraphPad Software, San Diego, CA, USA) with n>3.

Results and Discussion

Total phenolic content (TPC)

Phenolics that possess antioxidant activity have been characterized as phenolic acids and flavonoids (Kahkonen *et al*., 1999). Previous phytochemical investigation of *Amorphophallus companulatus* revealed the presence of the compounds (Nataraj *et al*., 2009). The yield of ethanol, acetone, aqueous extracts of ACT and APT found to be 13.61%, 17.53%, 9.73% and 10.43%, 16.32%, 8.29% respectively. The amount of TPC determined in different solvent extracts of ACT and APT is shown in Table 1. Results revealed that acetone was the best solvent for extracting phenolic compounds followed by ethanol and water. Acetone extracts of ACT and APT showed more phenolic contents (17.66±0.24 and 15.90±0.04 mg GAE/g, respectively) as compared with other extracts. It was reported earlier that the total phenolic content was influenced by the solvent used for extraction (Zhou and Yu, 2004). It is generally believed that plants which are having more phenolic content show good antioxidant activity and there is a direct correlation between total phenolic content and antioxidant activity (Biglari *et al.*, 2008).

Table 1. Total phenolic content in ACT and APT extracts

Extracts	Total phenolics (mg GAE/g)	
	ACT Extracts	APT Extracts
Ethanolic	11.57 ± 0.29	10.55 ± 0.34
Acetone	17.66 ± 0.32	15.90 ± 0.41
Aqueous	5.41 ± 0.31	4.76 ± 0.17

Each value is expressed as mean ± SE (n = 3).

Ferric reducing antioxidant power assay (FRAP)

Antioxidant compounds have the capability to reduce ferric (Fe^{3+}) to the ferrous (Fe^{2+}) form. Reduction can be determined by measuring the formation of Perl's Prussian blue at 700 nm (Ekrem *et al*., 2011). A higher absorbance indicates a higher ferric reducing power. Different ACT and APT extracts showed increased ferric reducing power which was directly proportional to concentration of extracts. The reducing power of ACT extracts decreased in the order of acetone (0.554 O.D) > ethanol (0.427 O.D) > water (0.144 O.D) and of APT extracts, acetone (0.544 O.D) > ethanol (0.359 O.D) > water (0.128 O.D) respectively. Ferric ion (Fe^{3+}) reduction is often used as an indicator of electron-accepting ability, which is an important mechanism of phenolic antioxidant action (Ekrem *et al*., 2011; Dorman *et al.*, 2003).

1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

Antioxidants inhibit oxidation by quenching reactive species through hydrogen or electron donation (Singh and Rajini, 2004). The DPPH assay measures this capacity by monitoring the decrease in absorbance of DPPH radical as it reacts with the antioxidant, marked by the color change from purple to yellow.

The presence of antioxidant in the sample leads to disappearance of DPPH radical chromogens, which can be detected spectrophotometrically at 517 nm. This method is sensitive to light, oxygen, pH, and type of solvent used (Ozcelik *et al*., 2003).The radical scavenging effects of ACT and APT extracts are represented in Table 2. Acetone and ethanol extracts significantly reduce the stable, purple colored DPPH• radical while aqueous extract was comparatively less significant. Acetone extracts of ACT and APT showed highest scavenging effects on DPPH radicals (79.4 and 74.52% respectively at 2 mg/ml) and it increased with the increasing concentration of the extracts.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was estimated by generating the hydroxyl radicals using ascorbic acid-iron EDTA complex. The hydroxyl radicals formed by the oxidation react with dimethyl sulfoxide (DMSO) to yield formaldehyde, which provides a convenient method for their detection by treatment with Nash regent.

Hydroxyl radical is an extremely reactive species formed in biological systems and has been implicated as highly damaging in free radical pathology, capable of damaging almost every molecule found in living cells. This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity

Table 2. Antioxidant activity of ACT and APT extracts at various concentration

racts (mg/ml)	DPPH Radicals %		Hydroxyl Radicals %		Nitric Oxide Radicals %	
CT Extracts	Acetone	Ethanolic	Acetone	Ethanolic	Acetone	Ethanolic
0.5	30.44±1.76	18.52±1.30	15.13±1.69	10.53±1.56	31.07±1.49	19.14±1.48
1.0	45.25±1.33	28.7±1.22	31.58±1.46	19.74±1.49	41.79±1.21	30.75±1.32
1.5	62.73±1.36	45.72±1.25	54.61±1.35	32.24±1.53	56.43±1.26	46.29±1.70
2.0	79.4±1.52	64.47±1.24	66.45±1.31	43.42±1.73	67.5±1.33	55.57±1.38
PT Extracts						
0.5	28.47±1.06	27.2±1.29	12.86±1.52	11.43±0.91	28.75±0.99	16.43±0.92
1.0	45.3±1.05	39.7±1.18	30.0±0.93	20.71±1.30	32.5±1.23	31.07±1.02
1.5	64.7±1.00	52.08±1.30	48.57±1.07	31.43±0.97	51.25±1.13	45.87±0.93
2.0	79.4±1.04	60.3±1.00	64.29±1.21	40.71±1.18	59.11±0.80	50.89±1.34

Each value is expressed as mean ± SE (n = 3).

Table 3. EC_{50} values (mg/ml) of ACT and APT extracts

Extracts	DPPH Radicals	Hydroxyl Radicals	Nitric Oxide Radicals
ACT Extracts			
Ethanolic	3.18	4.38	3.46
Acetone	2.23	2.69	2.56
APT Extracts			
Ethanolic	2.96	4.93	3.68
Acetone	2.29	2.64	3.14

EC_{50} (mg/ml): effective concentration at which 50% of DPPH radicals, (OH·) radicals, (NO·) radicals are scavenged

(Murthy *et al.*, 2006). The activity of the extracts is attributed to their hydrogen-donating ability (Chourasiya *et al*., 2010).

Hydroxyl radical scavenging activity of the different ACT and APT extracts clearly indicating that all the extracts exhibited antioxidant activity (Table 2) except aqueous extract which was less significant. Acetone extracts showed the strongest (ACT-66.45% and APT-64.29% at 2 mg/ml) hydroxyl radical scavenging activity. This indicate that the potential scavenging ability might be due to active hydrogen donor ability of hydroxyl substitution.

Nitric oxide radical scavenging activity

Nitric oxide generated in reaction forms chromophore with Griess reagent which was measured at 546 nm. Absorbance decreased with colour intensity indicating the scavenging activity. It is a diffusible free radical which plays many roles as effectors molecule in diverse biological systems including pleiotropic mediator of physiological process such as smooth muscle relaxant, neuronal signaling and inhibition of platelet aggregation and regulation of cell mediated toxicity (Nagulendran *et al.*, 2007).

Acetone and ethanol extracts of ACT and APT significantly inhibited nitric oxide radical in a dose dependent manner. The highest Nitric oxide radical scavenging activity was recorded in acetone extract (ACT-67.5% and APT-59.11% at 2 mg/ml) (Table 2). Table 3 shows the EC_{50} values (acetone and ethanol extracts) for DPPH, hydroxyl and nitric oxide radicals. The significant correlation of TPC with antioxidant activity of ACT (R^2= 0.952-0.998) and APT (R^2= 0.941-0.998) was observed. The results indicate, that both ACT and APT extracts could be significantly correlated with total phenolic content having antioxidant activity for FRAP, DPPH, hydroxyl, nitric oxide respectively. These data suggest that phenolic compounds are powerful scavenger of free radicals as well as reducing agents. Similar relation was reported in earlier studies (How and Yau, 2011; Sultana *et al*., 2007).

HPLC analysis

HPLC analysis of acetone extracts are presented in Figure 1 and 2. Earlier HPLC analysis was carried out for similar chemical characterization (Bauer and Tittel, 1996; Springfield *et al.*, 2005). The present study also established HPLC fingerprint for the active phenolic acids that can act as antioxidants. The HPLC analysis of the acetone extracts of both ACT and APT showed the presence of tannic acid, gallic acid and quercetin. Catechin was observed only in ACT while p-coumaric acid in APT extracts. Polyphenols were observed to be present in significant amount in several plants and many of them possess antioxidant, anti-inflammatory and several others therapeutic

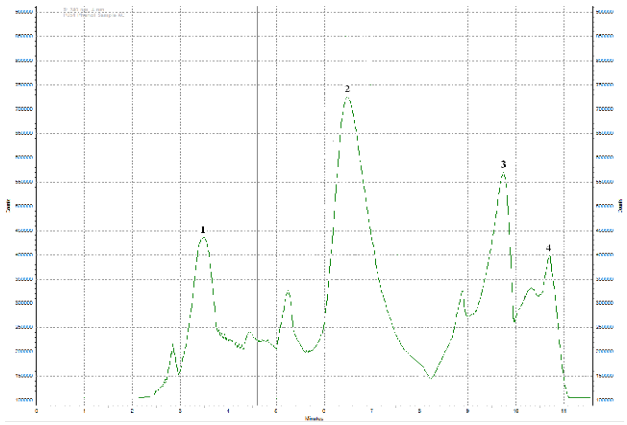

Figure 1. HPLC chromatogram for acetone extract of ACT 1) tannic acid 2) gallic acid 3) catechin 4) quercetin

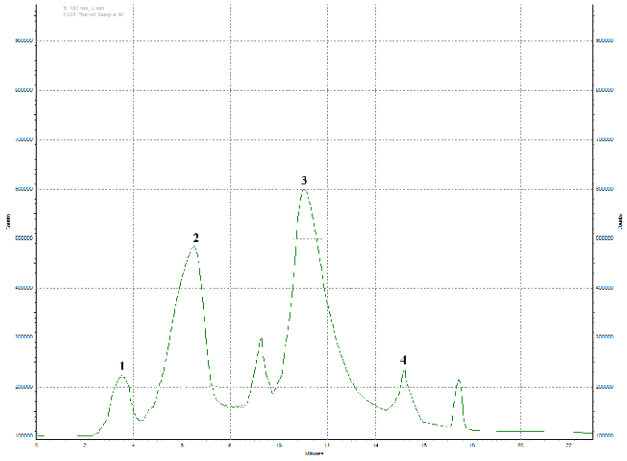

Figure 2.HPLC chromatogram for acetone extract of APT 1) tannic acid 2) gallic acid 3) quercetin 4) p-coumaric acid

properties (Singh *et al*., 2010).

Conclusion

This investigation on acetone extracts of tubers of *A. commutatus* and *A. paeonifolius* indicate that these are good sources of phenolic compounds having high levels of antioxidant properties and high radical scavenging activities. These findings are substantiated by the HPLC profiling of ACT and APT. Therefore, ACT and APT can be considered as good sources of natural antioxidant for medicinal, and functional food applications.

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