

ANTIOXIDANT ACTIVITY AND ANTIBACTERIAL SCREENING OF TUBERS OF *AMORPHOPHALLUS KONKANENSIS* AND *AMORPHOPHALLUS BULBIFER* (ARACEAE)

CHIDANAND C. SHETE¹, SURYAKANT S. WADKAR¹, NIKHIL B. GAIKWAD¹, KUMAR S. PATIL^{2*}

¹Department of Botany, Shivaji University, Vidyanagar, Kolhapur, 416004, (MS) India, ^{2*}Department of Botany, Smt. K. W. College, Sangli 416416, (MS) India
Email: kspatilbiotech@yahoo.com

Received: 24 Sep 2014 Revised and Accepted: 25 Oct 2014

ABSTRACT

Objective: Antioxidant activity (AOA) and antibacterial screening of tubers of unexplored *Amorphophallus konkanensis* Hett., Yadav & Patil (AKT) and *Amorphophallus bulbifer* (Roxb.) Bl. (ABT).

Methods: Antioxidant activity was evaluated by using ferric reducing antioxidant power (FRAP), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, hydroxyl (OH•) radical, nitric oxide (NO•) radical scavenging activities. Phenolics were determined by total phenolic content (TPC) assay. Antibacterial screening of different solvent extracts of tubers was tested against Gram positive and Gram negative bacterial strains by using the agar diffusion method. HPLC analysis was carried out for active phenolic compounds.

Results: Tubers were extracted with three different solvents (ethanol, acetone and water). Acetone extracts of AKT and ABT exhibited the highest phenolic content 29.37±0.83 and 20.62±1.04 mg GAE/g. The AKT and ABT acetone extracts possess highest free radical scavenging activity towards FRAP (0.632 & 0.586 O. D), DPPH (85.78% & 82.68%), hydroxyl (OH•) radical (82.43% & 80.26%), nitric oxide (NO•) radical (81.34% & 80.8%). The phenolic compounds tannic acid, gallic acid, quercetin, p-coumaric acid, catechin were identified by the HPLC method in acetone extracts. Among the different extracts, acetone extract of both the species of *Amorphophallus* showed significant antibacterial activity against all Gram positive and Gram negative bacterial strains tested.

Conclusion: The results indicated that AKT and ABT can be considered as good sources of natural antioxidant for medicinal, commercial, nutraceutical and functional food applications. Antibacterial efficacy shown by these plants provides a scientific basis for their traditional uses in remedies.

Keywords: Antioxidant, Antibacterial, Phenolics, HPLC, *Amorphophallus konkanensis*, *Amorphophallus bulbifer*.

INTRODUCTION

A free radical is a molecule with one or more unpaired electrons in the outer orbital. These free electrons are referred to as oxidizing agents [1]. Many of the free radicals such as superoxide radical (O₂^{•-}), hydroxyl radical (OH•), peroxy radical (ROO•) and nitric oxide radical (NO•) attack biological molecules, such as lipids, proteins, enzymes, DNA and RNA, leading to cell or tissue injury associated with aging, atherosclerosis, carcinogenesis [2].

Antioxidants are able to retard, delay or prevent oxidation process [3]. The potential of antioxidant constituents of plant materials for the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists and food manufacturers as consumers move toward functional foods with specific health effects [4]. Number of plant species possess natural antioxidants with high antioxidant activity and investigations on these were initiated based on their uses in traditional medicines [5].

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi or protozoan. A wide range of natural compounds are used as antimicrobials [6]. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. Most of which are secondary metabolites, of which at least 12,000 have been isolated. A number were estimated to be less than 10% of the total. Such a secondary metabolites plays a very important role in plant defense mechanism [7]. The World Health Organization estimated that 80% of the population in developing countries still relies on traditional medicines, mostly plant drugs for their primary health care needs. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated [8]. Hence, there is an urgent need to study the screening of antimicrobial properties of herbs, which will be helpful in the treatment of several diseases caused by microorganisms [9, 10].

Amorphophallus species are mainly used as a vegetable and as an ingredient in ayurvedic preparations [11]. 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 [12]. The tuberous roots of the plant also have tonic, stomachic and appetizer properties [13]. The tuber contains flavonoids, phenols, coumarins, terpenoids, sterols, tannins, steroids, alkaloids and sugars like glucose, galactose and rhamnose [14, 15].

The purpose of this study was to evaluate *Amorphophallus konkanensis* and *Amorphophallus bulbifer* as new potential sources of phenolic compounds having the natural antioxidants and antibacterial activity.

MATERIAL AND METHODS

Plant material and sample preparation

Fresh tubers of *Amorphophallus konkanensis* Hett., Yadav & Patil (AKT) and *Amorphophallus bulbifer* (Roxb.) Bl. (ABT) (Family: Araceae) were collected from Ratnagiri and Amboli in Western Ghats of Maharashtra, India respectively. The tubers were cleaned, chopped and kept in hot air oven at 40°C for 48h for drying. The dried AKT and ABT were made to a fine powder using mortar pestle. Tubers were extracted using solvents, acetone, ethanol and water at solvent to powder ratio of 1: 10. Weighed amount of each sample was extracted in known volume of the solvent for 24 h with shaking on a rotary shaker. Each extracted material was filtered through Whatman filter paper No. 1. These extracts were again dried and concentrated by evaporating the solvent completely in a water bath at the range of boiling points of solvents. The dried extracts were re suspended in respective solvents [16] and stored at 4°C. These extracts used to test the total phenolic content (TPC), Ferric reducing antioxidant power (FRAP), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, (OH•) radical, (NO•) radical.

Total phenolic content (TPC)

The TPC of AKT and ABT extracts in four different solvents was determined spectrophotometrically using the Folin-Ciocalteu assay of Singleton and Rossi with some modifications [17]. An aliquot of 100µl 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 stand at 25°C for 5 min before adding 2 ml sodium carbonate (15%) solution in distilled water. After 90 min at room temperature, absorbance was measured spectrophotometrically at 765 nm. This was compared to a standard curve of gallic acid concentrations and expressed as mg of gallic acid equivalents per g (mg GAE g⁻¹) of dry powder.

Antioxidant activity

Ferric reducing antioxidant power assay (FRAP)

The FRAP assay was carried out as previously described [18]. The various aliquots of AKT and ABT extract (0.5-2 mg/ml) concentrations were allowed to react with 2.5 ml sodium phosphate buffer (200 mM, pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min, then 2.5 ml trichloroacetic acid (10% w/v) was added.

Then 5 ml of the above reaction solution was combined with 5 ml distilled water and 1 ml ferric chloride (0.1%). The absorbance was measured spectrophotometrically at 700 nm and compared to Butylated Hydroxy Anisole (BHA) standard; any increase in absorbance is synonymous of an increase in reducing power.

DPPH radical scavenging activity

Antioxidant activity of AKT and ABT extracts was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay [19]. The different concentrations (0.5-2 mg/ml) of AKT and ABT extracts were allowed to react with 5 ml methanolic DPPH (0.1 mM) solution. The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark. The absorbance was measured spectrophotometrically at 517 nm. A control sample with no added extract was also analyzed and the results were expressed as radical scavenging activity (% RSA).

$$\% \text{ RSA} = \frac{[A(\text{control}) - A(\text{sample})] \times 100}{A(\text{control})}$$

Where, A = absorbance at 517 nm.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined according to method of Klein *et al* [20]. The different concentrations (0.5-2 mg/ml) of AKT and ABT extracts were allowed to react with 100µl of phosphate buffer (0.1M, pH 7.4), 1 ml of Fe-EDTA solution (0.13% ferrous ammonium sulphate & 0.26% EDTA), 0.5 ml EDTA(0.018%) and 1 ml Dimethyl Sulphoxide (0.85% v/v) in phosphate buffer (0.1M pH 7.4) were added to these mixtures. 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 1 ml ice cold TCA (17.5% w/v). Finally 3 ml Nash reagents (150g 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 colour 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).

$$\% \text{ RSA} = 1 - \frac{A(\text{sample})}{A(\text{control})} \times 100$$

Where A = absorbance at 412 nm

Nitric oxide radical scavenging activity

The nitric oxide radical scavenging activity was determined by using Griess reagent [21] with some modifications. The various aliquots of AKT and ABT extract concentration (0.5-2 mg/ml) were allowed to

react with 400µl sodium nitroprusside (10 mM), 250µl Griess reagent (1% sulphanilamide, 2 % H₃PO₄ & 0.1 % naphthylethylene diamine dihydroxy chloride) and incubated at 25°C for 1 hr. Finally 2 ml distilled water was added and absorbance was taken at 546 nm on spectrophotometer. Radical scavenging activity of nitrite oxide generated is measured by comparing the absorbance values of control and test preparations. The antioxidative potential of both extracts was evaluated as showed for the DPPH assay. The absorbance of all the assays was measured on Thermo Scientific-Chemito UV-2100.

Antibacterial activity

Test organisms

Four bacterial strains were used in the present study were Gram positive: *Staphylococcus aureus* (NCIM 2802), *Bacillus subtilis* (NCIM 2045); and Gram negative: *Klebsiella pneumoniae* (NCIM 2883) and *Salmonella typhi* (NCIM 2501). Bacterial cultures were first incubated at 37°C±0.1°C for 24 h in the nutrient broth (Mueller-Hinton). The bacterial suspensions were prepared and adjusted by comparison against 0.5 of the MacFarland turbidity standard (5×10⁷ cells/mL) tubes, and further that was standardized in our laboratory by a turbidity measure at 420 nm wavelength prior to use for antibacterial testing. The test organisms were sub cultured at 37°C for 24 h and maintained on nutrient agar media.

Screening of antibacterial activity

Sensitivity of different bacterial strains to various extracts was measured in terms of zone of inhibition using agar diffusion assay [22]. The plates containing Mueller-Hinton agar media were spreaded with 0.2 ml of the inoculums of different bacterial cultures. Wells were cut out from agar plates using a sterilized stainless steel borer and filled with 0.1 ml (500µg) of the extract.

The plates were inoculated with different bacterial cultures and incubated at 37°C up to 24 h and diameter of the resultant zone of inhibition was measured in mm. The bacteria with a clear zone of inhibition were considered to be sensitive. Chloramphenicol (2 µg/well) was used as a positive control. For each combination of extract and the bacterial strain, the experiment was performed in duplicate and repeated thrice.

Phytochemical analysis

Phytochemical analysis involves the qualitative analysis of plants. The preliminary qualitative tests have been attempted in AKT and ABT to find out the presence or absence of certain bioactive compounds. Different chemical tests were performed by using different extracts of tubers (ethanol, acetone and water) for phytochemical screening of compounds like, the phenolics, flavonoids, tannins, alkaloids, coumarins, sterols, triterpenoids and saponins using standard procedures to identify the constituents [23, 24].

HPLC analysis

HPLC was done using a Hitachi LaChrome chromatograph fitted with a reversed phase column (Column- C18; 5 mm, 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 0.5 ml/min. The injection volume for all samples was 100 µL [25](Biswas et al, 2013). The phenolic compounds were analyzed 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 performed in triplicate (n=3). Values representing the effective concentration of investigated extracts that because 50% of inhibitions (EC₅₀ value) were determined by linear regression analysis of obtained RSA. Analysis of variance was performed 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)

The yield of ethanolic, acetic, aqueous extracts of AKT and ABT is found to be 16.35%, 23.06%, 11.42% and 12.92%, 19.58%, 10.14% respectively. Typical phenolics that possess antioxidant activity have been characterized as phenolic acids and flavonoids [26]. Phytochemical investigation of *Amorphophallus campanulatus* revealed the presence of phenols and flavonoids [14]. The amount of TPC determined in different solvent extracts of AKT and ABT 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 AKT and ABT shows higher phenolic contents 29.37 ± 0.83 and 20.62 ± 1.0 mg GAE/g, respectively.

TPC was influenced by the solvent used for extraction [27]. It is generally believed that plants which are having more phenolic content show good antioxidant activity that is there is a direct correlation between total phenolic content and free radical scavenging activity [28].

Table 1: Total phenolic contents in AKT and ABT extracts

Extracts	Total phenolics (mg GAE/g)	
	AKT Extracts	ABT Extracts
Ethanolic	17.25 ± 1.10	14.32 ± 0.92
Acetonic	29.37 ± 0.83	20.62 ± 1.04
Aqueous	9.88 ± 1.31	8.64 ± 1.17

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

Ferric reducing antioxidant power assay (FRAP)

Antioxidant compounds cause the reduction of ferric (Fe^{3+}) form to the ferrous (Fe^{2+}) form because of their reductive capabilities. Prussian blue colored complex is formed by adding FeCl_3 to the ferrous (Fe^{2+}) form. Therefore, reduction can be determined by measuring the formation of Per's Prussian blue at 700 nm [18, 29]. In this assay, yellow color of the test solution changes to green or blue color being dependent upon the reducing power of antioxidant samples.

A higher absorbance indicates a higher ferric reducing power. Different AKT and ABT extracts showed increased ferric reducing power with the increased concentration (Fig. 1). The reducing power of AKT extracts decreased in the order of acetone (0.632 O. D) > ethanol (0.491 O. D) > water (0.212 O. D) and of ABT extracts, acetone (0.586 O. D) > ethanol (0.429 O. D) > water (0.167 O. D) respectively for higher concentration (2mg/ml). Fe^{3+} reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action [18, 30]

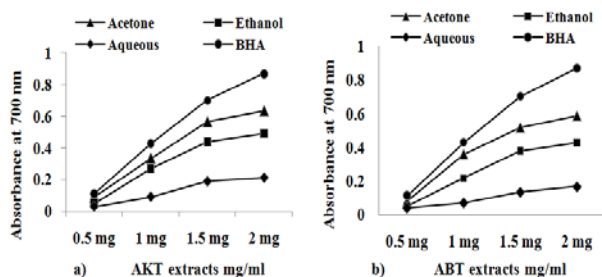


Fig. 1: Ferric reducing antioxidant power of a) AKT extracts b) ABT extracts

DPPH radical scavenging activity

One mechanism by which antioxidants inhibit oxidation is by quenching reactive species through hydrogen or electron donation [31]. 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 an antioxidant in the sample results in the 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 [32]. The radical scavenging effects of AKT and ABT extracts are shown in Fig. 2. All the assessed extracts were able to reduce the stable, purple colored DPPH radical reduction. From the analysis of Fig. 2, we can conclude that the AKT and ABT acetone extracts shows highest scavenging effects on DPPH radicals (85.78 ± 0.46 & 80.68 ± 1.02 % respectively at conc. 2mg/ml) and increased with the concentration increase.

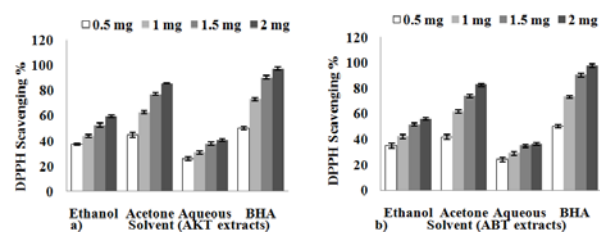


Fig. 2: DPPH radical scavenging activity of a) AKT extracts b) ABT extracts

Hydroxyl radical scavenging activity

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

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 [20]. The activity of the extracts is attributed to their hydrogen-donating ability [33].

Data in Fig. 3 showed the OH^\cdot radical scavenging activity of the different AKT and ABT extracts. Results clearly indicated that all the extracts exhibited antioxidant activity and it was dose dependent acetone > ethanol > water. Acetone extracts showed the strongest (AKT- 82.43 ± 1.01 % & ABT- 80.26 ± 1.1 % at conc. 2mg/ml) OH^\cdot radical scavenging activity. These results show that the potential scavenging abilities of phenolic substances might be due to the active hydrogen donor ability of hydroxyl substitution.

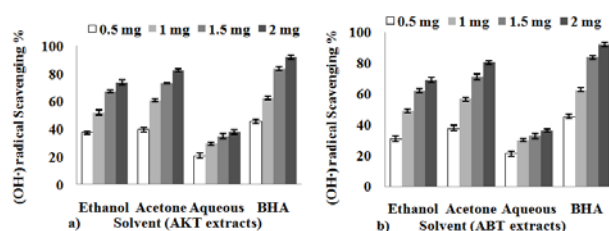


Fig. 3: Hydroxyl radical scavenging activity of a) AKT extracts b) ABT extracts

Nitric oxide radical scavenging activity

Nitric oxide is generated in reaction forms chromophore with Griess reagent which was measured 546 nm. Absorbance decreases with colour intensity shows nitric oxide scavenging activity. Nitric oxide (NO) is a potent pleiotropic mediator of physiological process such as a smooth muscle relaxant, neuronal signaling, inhibition of

platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities [34].

Data in Fig. 4 clearly indicated that AKT and ABT extracts inhibited nitric oxide radical in a dose dependent manner as acetone > ethanol > water. The highest Nitric oxide radical scavenging activity was recorded in acetone extract (AKT-81.34±1.03% & ABT-80.8±1.97% at conc. 2mg/ml).

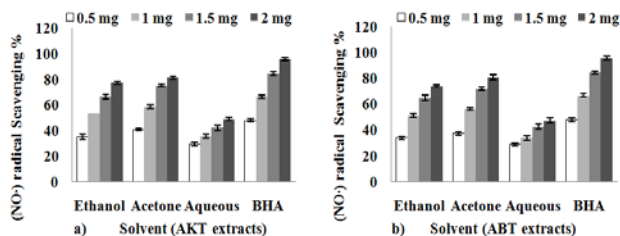


Fig. 4: Nitric oxide radical scavenging activity of a) AKT extracts b) ABT extracts

In Table 2, EC₅₀ values are presented (acetone and ethanol extracts) for DPPH, hydroxyl, nitric oxide radical scavenging effects of AKT and ABT extracts obtained from different solvents. The correlation of TPC with FRSA of AKT and ABT is shown in Table 3. The results indicate both AKT and ABT extracts demonstrated a strong and highly significant correlation of TPC with antioxidant activity for FRAP, DPPH, hydroxyl, nitric oxide respectively. These data indicate that phenolic compounds are potent scavenger of free radicals as well as reducing agents. Similar relation was reported in earlier studies [5, 35].

Table 2: EC₅₀ values (mg/ml) of AKT and ABT extracts

Extracts	DPPH Radicals (EC ₅₀)	Hydroxyl Radicals (EC ₅₀)	Nitric Oxide Radicals (EC ₅₀)
AKT Extracts			
Ethanol	2.70	1.88	1.92
Acetone	1.21	1.50	1.47
ABT Extracts			
Ethanol	3.02	2.28	2.04
Acetone	1.37	1.69	1.7

EC₅₀ (mg/ml): effective concentration at which 50% of DPPH radicals, (OH·) radicals, (NO·) radicals are scavenged.

Table 3: Correlation between total phenolics and FRSA

Methods of FRSA	Total Phenolics			
	AKT Extracts		ABT Extracts	
	Acetone	Ethanol	Acetone	Ethanol
Ferric reducing antioxidant power (FRAP)	0.951	0.939	0.926	0.955
DPPH Radicals Scavenging activity	0.976	0.997	0.966	0.984
Hydroxyl Radicals Scavenging activity	0.963	0.975	0.977	0.962
Nitric Oxide Radicals Scavenging activity	0.959	0.987	0.976	0.980

Antibacterial screening

Antibacterial potency was assessed by the presence or absence of inhibition zones. Antibacterial activities of ethanol, acetone and aqueous extracts of AKT and ABT against different Gram positive and Gram negative bacterial strains were summarized in Table 4. The results showed that, acetone and ethanol extracts of both AKT and ABT having the antibacterial activity against all selected bacterial strains viz. *Bacillus subtilis* (NCIM 2045), *Staphylococcus*

aureus (NCIM 2802), *Salmonella typhi* (NCIM 2501), *Klebsiella pneumoniae* (NCIM 2883). Aqueous extracts of both the tubers showed poor inhibitory activity against all bacterial cultures except *Staphylococcus aureus* (NCIM 2802).

Acetone extracts showed significant antibacterial activity as compared to the ethanolic extract of both the tubers. All the extracts of both the tubers showed significant inhibitory zone against *Staphylococcus aureus* (NCIM 2802).

Table 4: Antibacterial activity of AKT and ABT extracts

Test organisms	AKT extracts			ABT extracts		
	ethanol	acetone	aqueous	ethanol	acetone	aqueous
Gram positive						
<i>Bacillus subtilis</i>	+	++	-	+	++	-
<i>Staphylococcus aureus</i>	+	+++	+	++	++	+
Gram negative						
<i>Salmonella typhi</i>	+	++	-	+	++	-
<i>Klebsiella pneumoniae</i>	++	++	-	+	++	-

(+) zone diameter less than 15 mm, (++) zone diameter 15 - 20 mm, (+++) zone diameter >20 mm, (-) absence of inhibition zone

Phytochemical analysis

The qualitative phytochemical analysis of ethanol, acetone and aqueous extracts of AKT and ABT was presented in Table 5.

All phytochemical compounds viz. phenolics, flavonoids, tannins, alkaloids, coumarins, sterols, triterpenoids and saponins were found in acetone extracts of tubers of both the species. Aqueous extracts of both AKT and ABT showed the presence of phenolics, flavonoids and tannins only while ethanolic extracts both species of tubers do not contain sterols and triterpenoids. Ethanol extracts of AKT and ABT showed the presence of alkaloids and coumarins respectively.

Phytochemicals present in AKT and ABT extracts may be responsible for the antibacterial activity. The results of the present study reveal the fact that the organic solvent extracts (ethanol and acetone extracts) exhibited greater antimicrobial activity because the antimicrobial principles were extracted only through the organic solvent medium [36, 37]. The present study supports the claimed uses of *Amorphophallus* tubers in the traditional system of medicine.

HPLC analysis

Acetone extracts of both AKT and ABT showed significant antioxidant activity. Therefore, during HPLC analysis, we selected

acetone extracts for evaluation of phenolic compounds. HPLC chromatograms of AKT and ABT extracts are shown in Figure 5. HPLC analysis is the best way for chemical characterization [38, 39]. The present study also established HPLC fingerprint for the active phenolic acids that can serve as antioxidants and antibacterial compounds.

Polyphenols are present in rich amount in several plants and many of them possess antioxidant, anti-inflammatory, antibacterial and several other therapeutic properties [40].

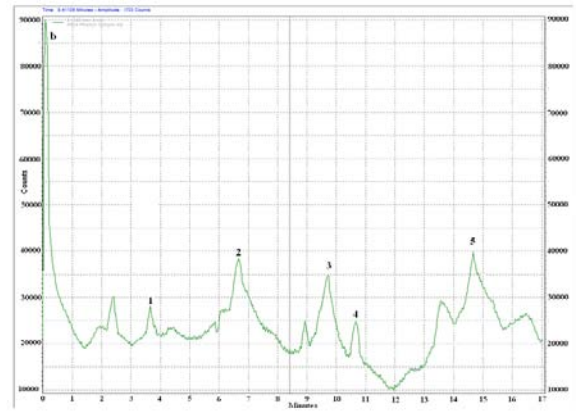
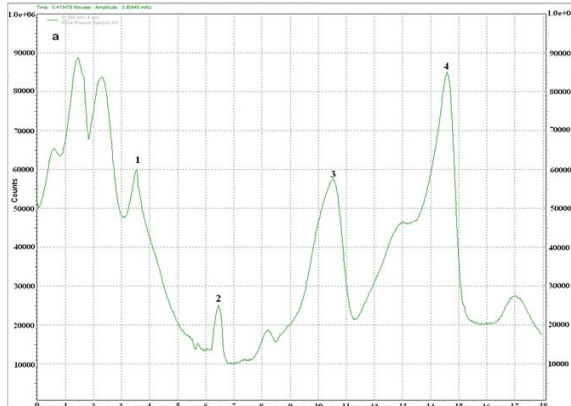


Fig. 5: HPLC chromatogram for acetone extract of a) AKT: 1) tannic acid 2) gallic acid 3) quercetin 4) p-coumaric acid, b) ABT: 1) tannic acid 2) gallic acid 3) catechin 4) quercetin 5) p-coumaric acid.

The reducing property of phenolics can influence the redox potential of microbial growth causing growth inhibition [41]. HPLC analysis of the acetone extracts of both AKT and ABT showed the presence of tannic acid, gallic acid, p-coumaric acid and quercetin. Catechin was observed only in ABT.

Table 5: Qualitative analysis of phytochemicals in AKT and ABT extracts

Phytochemicals	AKT extracts			ABT extracts		
	ethanol	acetone	Aqueous	ethanol	acetone	aqueous
Phenolics	+	++	+	+	++	+
Flavonoids	+	++	+	+	+	+
Tannins	+	+	+	+	+	+
Alkaloids	+	+	-	-	+	-
Coumarins	-	++	-	+	++	-
Sterols	-	+	-	-	+	-
Triterpenoids	-	+	-	-	+	-
Saponins	+	+	-	+	+	-

+ Present – Absent

CONCLUSION

In conclusion, present investigation on *A. konkanensis* and *A. bulbifer* tubers from India, indicate that they have antioxidant and antibacterial activity because of presence of significant high level of phenolic compounds and other phytochemicals. Acetone is the best solvent not only for extracting phenolic compounds showing high radical scavenging activities but also showed good antibacterial property. The results of HPLC profiling of acetone extracts justify that the isolated phenolics exhibit significant free radical scavenging activity. *A. konkanensis* and *A. bulbifer* tubers showed significant inhibitory activity against both Gram positive and Gram negative bacterial cultures i. e. wide spectrum activity. The anti-bacterial action of various extracts of both the tubers may show their potential as antibacterial herbal remedies. Therefore, AKT and ABT can be considered as excellent sources of natural antioxidant and antibacterial for medicinal, commercial, nutraceutical and functional food applications.

CONFLICT OF INTERESTS

Declared None

ACKNOWLEDGEMENT

The authors are grateful to the Head, Department of Botany, Shivaji University, Kolhapur, Principal, Smt. K. W. College, Sangli, and to the Department of Botany of the same college for providing all the necessary facilities to complete the work successfully.

REFERENCES

- Halliwell B. Free Radicals, Antioxidants and human disease: curiosity, cause, or consequence? *Lancet* 1994;344:721-4.
- Chen K, Geoff W, Richard NB, Youngping B. Antioxidant activities of extracts from five anti-viral medicinal plants. *J Ethnopharmacol* 2005;96:201-5.
- Halliwell B. Antioxidants and human diseases: a general introduction. *Nutr Rev* 1997;55:44-52.
- Javanmardi J, Stushnoff C, Locke E, Vivanco JM. Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. *Food Chem* 2003;83:547-50.
- How YL, Yau YL. Evaluation of antioxidant activities of the methanolic extracts of selected ferns in malaysia. *Int J Env Sci Dev* 2011;2(6):442-7.
- Roy S, Dutta CM, Paul SB. Antibacterial activity of Araceae: an overview. *Int J Res Ayur and Pharm* 2013;4(1):15-7.
- Umamaheswari A, Shreevidya R, Nuni A. *In vitro* Antibacterial activity of *Bougainvillea spectabilis* leaves extracts. *Advan Biol Res* 2008;2:01-05.
- Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. Natural plant chemicals: Sources of industrial and medicinal. *Mater Sci* 1985;228:1154-60.
- Ikegami F, Fujii Y, Ishihara K, Satoh T. Toxicological aspects of Kambo medicines in clinical use. *Chem Biol Int* 2003;145:235-50.
- Izzo AA. Drug interactions with St. John's Wort (*Hypericum perforatum*): A review of the clinical evidence. *Int J Clin Pharmacol Ther* 2004;42:139-48.

11. Angayarkanni J, Ramkumar KM, Poornima T, Priyadarshini U. Cytotoxic activity of *Amorphophallus paeoniifolius* tuber extracts in vitro. Am Eurasian J Agric Environ Sci 2007;2(4):395-9.
12. Yusuf M, Chowdhury JU, Yahab MA, Begum J. Medicinal plants of Bangladesh, BCSIR Laboratories, Bangladesh; 1994. p. 2.
13. Chopra RN, Chopra IC, Handa KL, Kapur LD. Indigenous Drugs of India (2nd Edition), U. N. Dhur and Sons Private Ltd., 15, Bankim Chattrjee street, Calcutta; 1958.
14. Nataraj HN, Murthy RLN, Ramachandra SS. In vitro quantification of flavonoids and phenolic content of suran. Int J Chem Tech Res 2009;1:1063-7.
15. Yadu ND, Ajoy KG. Pharmacognostic evaluation and phytochemical analysis of the tuber of *Amorphophallus paeoniifolius*. Int J Pharm Res Dev 2010;2(9):44-9.
16. Sule A, Ahmed QU, Samah OA, Omar MN. Bacteriostatic and bactericidal activities of *Andrographis paniculata* extracts on skin disease causing pathogenic bacteria. J Med Plants Res 2011;5(1):7-14.
17. Jagtap UB, Panaskar SN, Bapat VA. Evaluation of antioxidant capacity and phenol content in Jackfruit (*Artocarpus heterophyllus* Lam.) fruit pulp. Plant Foods Hum Nutr 2010;65:99-104.
18. Ekrem K, Ercan B, Emrah D, Fatih T, Ilhami G. Antioxidant activity of *Melissa officinalis* leaves. J Med Plants Res 2011;5(2):217-22.
19. Lee HC, Kim JH, Jeong SM, Kim DR, Ha JU, Nam KC. Effect of far infrared radiation on the antioxidant activity of rice hulls. J Agric Food Chem 2003;51(15):4400-3.
20. Murthy KN, Chidambara, Rajasekaran T, Giridhar P, Ravishankar GA. Antioxidant property of *Decalepis hamiltonii* Wight and Arn. Indian J Exp Biol 2006;44:832-37.
21. Marcocci L, Packer L. Antioxidant assay of *Ginkgo biloba* extract EGB 761. Methods Enzymol 1994;234:462-75.
22. Kaur GJ, Arora DS. Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi*. BMC Complement Alternat Med 2009;9:30-4.
23. De S, Dey YN, Ghosh AK. Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of *Amorphophallus paeoniifolius* (Araceae). Int J Pharm Biomed Res 2010;1(5):150-7.
24. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. Nirali Prakashan 2009;6(6):16-7.
25. Biswas Nirupam, Balac Pauline, Narlakanti Sai Kishore, Haque MD Enamul, Hassan MD Mehedi. Identification of phenolic compounds in processed cranberries by HPLC method. J Nutr Food Sci 2013;3(1):1-5.
26. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, et al. Antioxidant activity of plant extracts containing phenolic compounds. J Agri Food Chem 1999;47(10):3954-62.
27. Zhou K, Yu L. Antioxidant properties of bran extracts from Trego wheat grown at different locations. J Agri Food Chem 2004;52:1112-7.
28. Biglari F, Alkarkhi AFM, Easa AM. Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. Food Chem 2008;107:1636-41.
29. Chung YC, Chang CT, Chao WW, Lin CF, Chou ST. Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus subtilis* IMR-NK1. J Agric Food Chem 2002;50:2454-8.
30. Dorman HJ, Bachmayer O, Kosar M, Hiltunen R. Antioxidant properties of aqueous extracts from selected Lamiaceae species grown in Turkey. J Agric Food Chem 2004;52:762-70.
31. Singh N, Rajini PS. Free radical scavenging activity of an aqueous extract of potato peel. Food Chem 2004;85:611-6.
32. Ozcelik C, Lee JH, Min DB. Effects of light, oxygen and pH on the absorbance of 2, 2-diphenyl-1-picrylhydrazyl. J Food Sci 2003;68:487-90.
33. Chourasiya RK, Jain PK, Jain SK, Nayak SS, Agrawal RK. In-vitro antioxidant activity of *Clerodendron inerme* Gaertn leaves. Res J Pharm Biol Chem Sci 2010;1:119-23.
34. Nagulendran KR, Velavan S, Mahesh R, Hazeena BV. In Vitro antioxidant activity and total polyphenolic content of *Cyperus rotundus* rhizomes. Electron J Chem 2007;4(3):440-9.
35. Sultana B, Anwar F, Pirzybyiski R. Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica* and *Eugenia jambolana* Lam. Trees Food Chem 2007;104:1106-14.
36. Krishna KT, Ranjini CE, Sasidharan VK. Antibacterial and antifungal activity of secondary metabolites from some medicinal and other common plant species. J Life Sci 1997;2:14-9.
37. Natarajan E, Senthilkumar S, Francis Xavier T, Kalaiselvi V. Antibacterial activities of leaf extracts of *Alangium salviifolium*. J Trop Med Plants 2003;4:9-13.
38. Bauer R, Tittel G. Quality assessment of herbal preparations as a precondition of pharmacological and clinical studies. Phytomed 1996;2:193-8.
39. Springfield EP, Eagles PKF, Scott G. Quality assessment of South African herbal medicines by means of HPLC fingerprinting. J Ethnopharmacol 2005;101:75-83.
40. Singh S, Srivastava R, Choudhary S. Antifungal and HPLC analysis of the crude extracts of *Acorus calamus*, *Tinospora cordifolia* and *Celestrus paniculatus*. J Agric Tech 2010;6(1):149-58.
41. Jay JM. Modern Food Microbiology. 5th Ed. Chapman and Hall: New York; 1996.