Research Article

Antioxidant Activities of Some Tuberous Plant Leaves

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ABSTRACT

The present study reports antioxidant potential of leaves of four vegetables, *Allium sativum, Beta vulgaris, Colocasia esculenta* and *Amarphophyllus captulentus*. Different *in vitro* assays were carried out to determine antioxidant potential of their leaf extracts. Total polyphenols and total flavonoids content of these samples were also analyzed. The results showed that ethanolic leaf extract of *Allium sativum* and *Beta vulgaris* possessed higher free radicals scavenging activities (In term of IC_{50}) as compared to other extracts. Identification and purification of bioactive compounds showed that out of four tested samples, *Allium sativum* and *Beta vulgaris* leaves have more phytochemicals (CGA, CA, RU, Quer and Kaem) than the other two. In term of total flavonoids content the highest value was observed in leaf extract of *Colocasia esculenta* and *Allium sativum.* Above data showed that *Allium sativum* and *Beta vulgaris* have more antioxidant properties and hence may be considered for potential use as nutraceuticals.

Keywords: Phytochemicals, Antioxidant potential, Nutraceuticals, IC_{50} value, Flavonoids, Free radical scavenging activity.

INTRODUCTION

Free radicals are fundamental to any metabolic process and represent an essential part of aerobic life and our metabolism. These are continuously produced by body's normal use of oxygen such as respiration and some cell mediated immune functions. Naturally, there is a dynamic balance between the amount of free radicals generated in the body and antioxidants to quench or scavenge them and protect the body against their deleterious effects. The cause of majority of disease conditions are being considered to be primarily due to the imbalance between pro-oxidant and antioxidant homeostasis. The harmful effects of free radicals result in oxidative stress on the tissue and plays an important role in the genesis of various pathological conditions such as ageing process, asthma, anemia, arthritis, inflammation, ischemia, Parkinson's disease and dementia etc.[1] . Antioxidants are radical scavengers, which protect the human body against oxidative stress[2,3]. An antioxidant is natural substance that significantly delays or prevents oxidation of substrate[4]. The phenolic compounds present in plant are responsible for their antioxidant activity. Besides phenolic compounds, flavonoids that are derived from many fruit and vegetables show antioxidant capacity[5,6]. Biological systems have evolved with endogenous defence mechanisms to help protect against free radical induced cell damage. The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) serve as primary line of defence in destroying free radicals. They require micronutrient as cofactors such as selenium, iron, copper, zinc and manganese for optimum catalytic activity and effective antioxidant defence mechanisms[7-9]. Antioxidant principles from natural resources possess multifacetedness in their multitude and magnitude of activities and provide enormous scope in correcting the imbalance between pro-oxidant and antioxidant. Hence, there is no doubt that phytochemicals deserve a proper position in the therapeutic armamentarium. The present study aims to evaluate the antioxidant potential of leaves of *Allium sativum*, *Colocasia esculenta*, *Amarphophyllus campantulatus* and *Beta vulgaris* to explore their potential use as nutraceuticals.

MATERIALS AND METHODS

Chemicals

Gallic acid, Quercetin and Bovine serum albumin (BSA) were purchased from Sigma-Aldrich, St. louis, USA. Ascorbic acid and Folin Ciocalteau's phenol reagents was the product of E. Merk, Mumbai, India. Nitro blue tetrazolium (NBT), 2,2-Di (4-*tert*octylphenyl)-1-picrylhydrazyl (DPPH), thiobarbituric acid (TBA), Phenazinemethosulphate (PMS), nicotinamide adenine dinucleotide (NADH), potassium ferricyanide, trichloroacetic acid, ferric chloride and sodium dodecyl sulphate were purchased from SRL, India. All the other reagents and chemicals used were of analytical grade.

Collection of samples

Plant sample were collected from Ayodhya and Naveen Mandi Faizabad areas of UP, India. The leaves of plants sample were washed under running tap water and cut in to small pieces. The samples were dried under sun shade condition, powered and stored at 4°C in polythene bags till further processing.

Extraction

Twenty grams of powdered plant leaves (20 g) were extracted with 95% ethanol (100 ml) at room temperature in orbital shaker to ensure complete extraction. The extracts were filtered through Whatman

no.4 filter paper. This process is repeated until the supernant turned colourless. Now the total volume of supernatant was nearly 800-1000 ml. Ethanol was removed from the supernatants on a rotary evaporator at 50°C, to yield thick and viscous residues as ethanolic crude extracts. These extracts were used for determination of antioxidant activity using DPPH, superoxide anion radical scavenging activity, reducing power and ferric ion chelation activity.

Antioxidant Assay

TPC was measured using the method of Ragazzi and Veronese[10]. The TPC was reported as mg of gallic acid equivalent (GAE) /g of dry weight. Total flavonoids were estimated as described by Oyaizu[11] and expressed as mg Quercetin/g sample. Free radical scavenging activity (FRSA) of the extracts was measured by using DPPH stable radical according to Yen and Duh[12]. Superoxide anion radical scavenging assay was based on the capacity of the extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) by the method of Nishikimi[13]. Reducing power (RP) of the extracts was determined using a slightly modified ferric reducing-antioxidant power assay[14]. Reducing power was expressed as ascorbic acid equivalents (1ASE = 1mM ascorbic acid). The ASE value is inversely proportional to reducing power. Ferric thiocyanate assay was done by method of Tsuda[15]. HPLC analysis was done by Lin-Chin[16].

Statistical analysis

Value from *in-vitro* antioxidant activities shown in are the mean± standard deviation (SD) of three determinants. The statistical analysis was done by software Prism.

RESULTS

Total phenolic content

Phenolic content showed variations among the tested plant leaves as well as in different organic solvents (ethanol and methanol) (Table 1). The TPC content in ethanolic and methanolic extracts of four plant samples ranged from 19.68 to 25.37 mg/g of GAE and 14.89 to 24.25 mg/g of GAE, respectively. In ethanolic and methanolic leaf extracts, the highest TPC content was found in *B. vulgaris (*25.37 mg/g of GAE) whereas lowest value was present in *C. esculenta* (15.90 mg/g of GAE). In both ethanolic and methanolic leaf extracts, the increasing orders of TPC content are as follow: *C. esculenta* < *A. sativum* < *A. captulentus* < *Beta vulgaris.* The above data showed that the extent of TPC content in ethanolic extract was higher than methanolic extracts. It may be concluded that ethanol is better solvent than methanol for extraction purpose. Hence, further studies were performed with ethanolic extracts.

Total flavonoids content

Flavonoids as one of the most diverse and wide spread group of natural compound are probably the most important natural phenols[17]. Total flavonoid content among the tested samples ranged between 0.109 to 0.338 mg Quercetin/g. The highest flavonoid content was present in *C. esculenta* (0.338 mg Quercetin/g) leaf whereas lowest flavonoid content was determined in *B. vulgaris* (0.109 mg Quercetin/g) leaf. The moderate value of flavonoid content was present in *A. sativum* and *A. captulentus* leaf (Table 2).

Free radical scavenging assay by DPPH

DPPH radical is commonly used as substrate to evaluate antioxidant activity. Antioxidants react with DPPH, which is a stable free radical and convert it to 1,1-diphenyl-2-picryl hydrazine[18] . The degree of decolourization of the purple colored solution of DPPH indicates the scavenging potential of the antioxidant compound. The antioxidant activity assays by DPPH of plant samples are given in Table 3. The value of IC_{50} of ethanolic leaf extracts ranged between 7.6 to 67.48 mg/ml. The lowest value of inhibitory concentration was present in *A. sativum* (7.6 mg/ml) while highest value was in *A. captulentus* (67.48 mg/ml). The decreasing order of antioxidant activities are as follows: *A. sativum* >*B. vulgaris* > *C. esculenta* > *A. captulentus*. The ARP value of different ethanolic leaf extracts was found in the following order: *A. sativum* > *C. esculenta* > *A. captulentus* > *B. vulgaris.*

Superoxide anion radical scavenging activity (SOD)

Superoxide anion radical scavenging activity of plant extracts are presented in Table 3. The IC_{50} of extracts ranged from 10.24 to 178.00 mg/ml. *B. vulgaris* leaf had lowest inhibitory concentration (10.24mg/ml). The orders of superoxide anion radical scavenging activity was as follows: *B. vulgaris* > *A. sativum* > *A.captulentus* > *C. esculenta*.

Reducing power (RP)

The reducing powers of four plants leaf are presented in Table 3. The RP values of leaf extract of *A. sativum*, *C. esculenta*, *A.captulentus* and *B.vulgaris* were 7.73, 3.46, 4.29 and 3.33 ASE/ml, respectively. These data suggest that *A. sativum* leaf extract had more reducing power than the other three leaf extracts. The orders of reducing power activity were as follows: *A. sativum* > *A. captulentus* > *C. esculenta* > *B. vulgaris.*

Ferric thiocyanate activity (FTC)

FTC is used to measure the production of peroxide compounds at the initial stage of oxidation while TBA test is used to measure the secondary product such as aldehyde and ketone[19]. Maximum FTC activity was observed in *A. sativum* leaf extract (1.60 mg/ml) and minimum with *A. captulentus* (12.48 mg/ml). Based on IC_{50} values, the order of FTC activity of the plant extract was as follows: *A. sativum* leaf > *Beta vulgaris* > *C. esculenta* > *A. captulentus*.

Isolation and purification of extracts by HPLC

Phenolic compounds were analyzed by using HPLC. The ethanolic plant extracts contained a variety of phenolic compounds. By comparing the retention time of these

compounds with those of standards, five phenolic compounds were identified (Table 4). HPLC analysis of leaf extract of *A. sativum* and *B. vulgaris* showed maximum number of phenolic compound. In both plant samples chlorogenic acid (CGA) (0.3757 and 0.12481 mg/100g of DW), caffeic acid (CA) (0.21499 and 0.57754 mg/100g of DW), rutin (RU) (0.18272 and0.12639 mg/100g of DW), qucertin (Quer) (0.20693 and 0.06862 mg/100g of DW) and kaempferol (Kaem) (0.21985 and 0.04366 mg/100g of DW, respectively) were present. *C.esculentam* leaf contained CGA (1.38782 mg/100g of DW) and CA (0.28416mg/100g of DW) out of five phenolics. *A. captulentus* showed presence of CGA (0.57145 mg/100g of DW), CA (0.45093 mg/100g of DW), RU (0.15757 mg/100g of DW) and Quer (0.21896 mg/100g of DW) but Kaem was not detected.

Table 1: TPC content of ethanolic and methanolic extracts of four plant leaves

Plant	TPC (mg/g of GAE)	
	Ethanolic Extract	Methanolic Extract
Allium sativum	19.68± 2.20	17.58± 1.29
Colocasia esculenta	15.90±2.11	14.89±1.31
Amarphophyllus captulentus	20.14±1.48	19.24±2.50
Beta valgaris	25.37±1.31	24.25±2.41

Values are mean ± SD of three replicates

Table 2: Total flavonoids content of ethanolic leaf extract of four plant samples

Plant	Total flavonoids content (mg Quercetin/g)
Allium sativum	0.321±0.52
Colocasia esculenta	0.338±0.34
Amarphophyllus captulentus	0.318±0.18
Beta vulgaris	0.109±0.22

Values are mean ± SD of three replicates

Table 3: Antioxidant activity of ethanolic plant leaf extracts

Plant	FRSA (IC_{50})	EC_{50}	ARP	RP (ASE/ml)	SOD (IC_{50})	FTC (IC_{50})
Allium sativum	7.6±0.61	330.43	0.302	7.73±0.54	18.35±1.46	1.60±0.58
Colocasia esculenta	26.20±4.79	1139.13	0.088	3.46±0.12	178.00±05.45	10.09±0.15
Amarphophyllus captulentus	67.48±2.29	2933.91	0.034	4.29±1.45	20.67±2.16	12.48±0.95
Beta vulgaris	24.36±0.46	1059.13	0.094	3.33±0.51	10.24±2.13	08.43±0.47

Values are mean ± SD of three replicates; IC_{50}: Inhibitory concentration; EC_{50}: Efficiency concentration; RP: Reducing power; SOD: Superoxide anion radical scavenging Activity; FTC: Ferric thiocyanate assay

Table 4: Quantitative estimation of phenolic compound in different plant leaves using HPLC

Plant	CGA mg/100g of DW	CA mg/100g of DW	RU mg/100g of DW	Quer mg/100g of DW	Kaem mg/100g of DW
Allium sativum	0.3757	0.21499	0.18272	0.20693	0.21985
Colocasia esculenta	1.38782	0.28416	ND	ND	ND
Amarphophylluscaptulentus	0.57145	0.45093	0.15757	0.21896	ND
Beta vulgaris	0.12481	0.57754	0.12639	0.06862	0 .043660

CGA- Cholorogenic acid; CA- Caffic acid; RU- Rutin; Quer- Quercetin; Kaem-Kaempferol; ND- Not detected; DW- Dry weight

DISCUSSION

Free radical oxidative stress play important role in the pathogenesis of a wide variety of clinical disorders, resulting usually from deficient natural antioxidant defenses. Potential antioxidant therapy, therefore, should include either natural free-radical scavenging antioxidant enzymes or agents which are capable of augmenting the activity of these enzymes. Reactive oxygen species (ROS) received considerable attention in the recent past because of its role in several pathological conditions including cancer, diabetes, arthritis, aging and atherosclerosis. ROS produces hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and superoxide anion radical which can interact with proteins (lactoferrineon) in the presence of transition metal ions to yield a highly reactive oxidizing species, the hydroxyl radical[20].

In our study, antioxidant effects of leaf extract of *A. sativum, B. vulgaris, C. esculenta* and *A. captulentus* were measured through a variety of biological parameters. Because of the complex nature of phytochemicals, the antioxidant activities of plant extracts cannot be evaluated by a single method. Therefore, commonly accepted assays, including non-enzymatic methods under *in vitro* conditions were employed to evaluate the total antioxidant potential of plant extracts. Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants. Typical phenolics that possess antioxidant activity have been characterized as phenolic acid and flavonoids[21]. These belong to a class of antioxidant agents which act as free radical terminators[22]. Flavonoids compounds possess a broad spectrum of chemical and biological activity including radical scavenging activity due to the presence of hydroxyl groups. The antioxidant activities observed can be ascribed both to mechanisms exerted by phenolic compounds and also to synergistic effects of different phytochemical. *B. vulgaris* showed significantly higher amount of TPC content followed by *A. captulentus, A. sativum* and *C. esculenta.* The results showed that ethanolic extract of plant sample have more TPC content in comparison to methanolic extract meaning thereby that ethanol is a better solvent for extraction of TPC. Hence ethanolic extracts were selected for further studied of antioxidant activity. Phenolic acid have repeatedly been implicated as natural antioxidants in fruits and vegetables for example, caffeic acid, ferulic acid and vanillic acid are widely distributed in plant kingdom[23]. Rosmarinic acid, an important phytochemicals has been found to be a potent active substance against human immunodeficiency virus type 1(HIV-1)[24]. The functional and nutritional values of vegetables protein are affected by polyphenolic compounds. It enhances the nutritional value of food stuffs and contributes to the sensory and organoleptic properties (colour, taste, astringency) of fruits and vegetables[25].

DPPH method is one of the most popular methods to determine antioxidant activity under *in vitro* condition[26]. DPPH is relatively stable nitrogen cantered free radical that easily accepts an electron or hydrogen. It reacts with suitable reducing agent and as a result, the electron become paired and the solution losses its colour depending on the number of electron taken up[27]. The high reductive potentials indicate that the plant have redox properties which allow them to act as reducing agents, hydrogen donors or oxygen quenchers. There are different methods for estimation of antioxidant activities but the most widely used methods are those that involve generation of free radical species which are then neutralized by antioxidant compounds. In our studies ethanolic extract of *A. sativum* exhibited a significant dose-dependent inhibition of DPPH activity. IC_{50} values of *A. sativum* and *B. vulgaris* leaf extracts showed maximum antioxidant activity in comparison to other two. *A. captulentus* showed significant amount of TPC content, but not that much antioxidant activity. There was little or no correlation between antioxidant activity and total phenolic contents in case of *A. captulentus* (Table 1 & 3). It may be possible that many other factors also play an important role in determining antioxidant activities**.** But in case of other plants sample TPC is directly correlated with antioxidant activity.

Superoxide radicals are known to be very harmful to cellular components. They are precursors of many ROS[28]. In addition, the superoxide radical is biologically important because it can be decomposed to form a stronger oxidative species such as singlet oxygen and hydroxyl radicals[29]. Superoxide has also been observed to directly initiate lipid peroxidation[30]. Superoxide anion derived from dissolved oxygen reduces NBT. Antioxidants are able to inhibit the blue NBT formation[31]. In our study *B. vulgaris* and *A. sativum* showed maximum SOD activity in comparison to other plants extract. The results suggest that the *B. vulgaris* and *A. sativum* plant extract is a more potent scavenger of superoxide radical. Hence, the super oxide radical scavenging capacity of an extract may serve as a significant indicator of its potential antioxidant activity.

Several studies demonstrated that the reducing power in plant extracts is highly correlated with their antioxidant activities[32]. The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity. It is used to measure the reductive ability of antioxidant and it is evaluated by the transformation of Fe(III) to Fe(II) in the presence of the sample extract[33]. The reducing power of antioxidant have been attributed to various mechanism such as prevention of chain initiation, binding of transition metal ion catalysis, decomposition of peroxides, prevention of continued proton abstraction and radical scavenging activities[34]. The reducing ability of a compound generally depends on the presence of reductants[35] which exhibit antioxidative potential by breaking the free-radical chain, donating a hydrogen atom. In the present study, reducing power of four plant samples showed similar pattern as shown in case of FRSA. *A. sativum* and *A. captulentus* showed significantly more reducing power activity comparison to other plants extract. As far as overall parameters are concerned *A. sativum* and *B. vulgaris* emerged as most potent candidates for further studies of their role as nutraceuticals.

In FTC assay the pattern of IC_{50} value were same as in the case of DPPH. *A. sativum* and *B. vulgaris* showed the maximum antioxidant activity in comparison to other plant extracts. It is mainly due to the presence of high content of phenolic compound in both plant extracts.

CONCLUSION

It is well-known that ROS have a significant positive correlation with several diseases such as ageing, atherosclerosis, inflammatory injury, cancer, and cardiovascular disease. The results obtained by us are noteworthy, not only with respect to the antioxidant

activities of the ethanolic extract of *A. sativum* and *B. vulgaris*, but also with respect to its content of a variety of phenolic compounds. On the other hand, the results confirm the feasibility of assessing radical scavenging activity of specific phytochemicals using the HPLC. This technique could allow rapid detection of natural antioxidants present in plant samples. Accordingly, five specific and excellent antioxidants were detected and identified. Plant sample containing higher phenolic compound showed maximum antioxidant activity. HPLC analysis of the four tested plant leaves clearly indicate that samples having more number of phenolic compounds shows higher antioxidant activity. So we can conclude that higher antioxidant activity of plant extracts was mainly due to presence of good quantity of phenolic compounds. Thus, the leaf extracts of *A. sativum* and *B. vulgaris* having number of phenolic compounds and can be used in the development of nutraceuticals, in various herbal formulation and health care product.

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