

PHARMACEUTICAL SCIENCES



Received on 23 September, 2016; received in revised form, 19 December, 2016; accepted, 20 March, 2017; published 01 April, 2017

ASSESSMENT OF ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL SCREENING OF COLOCASIA ESCULENTA CORM

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Keywords:

Colocasia esculenta, Corm, Antioxidant, Phytochemical

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ABSTRACT: Colocasia esculenta corm aqueous extract was assessed for its in vitro antioxidant capacity and free radical scavenging potential by seven different assays, viz. total phenolic content, total flavonoid content, total flavonoi content, reducing power estimation as well as 1,1-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO) and 2,2'-azino-bis(3-ethylbenzothiazoline-6sulphonic acid) (ABTS) radical scavenging activities. Results reveal that the total phenolic, total flavonoid and total flavonol content present in C. esculenta corm extract were found to be 68.25 ± 17.52 mg of GAE (Gallic Acid Equivalent)/g, 26.70 ± 40.18 mg of QE (Quercetin Equivalent)/g and $1.40 \pm$ 53.99 mg of QE/g of dry extract respectively. Reducing power of the extract was found to be concentration dependent as it increase with increase in concentration and it was maximum at the highest evaluated concentration of 200 µg/ml. Significant antioxidant efficacy of C. esculenta corm was further confirmed by IC₅₀ values of DPPH, NO and ABTS radical scavenging assays as they were found to be 74.34 \pm 12.17 µg/ml, 65.52 \pm 7.76 µg/ml and 132.29 \pm 14.13 µg/ml respectively. Results were compared with the reference, ascorbic acid. Phytochemical screening of the aqueous extract of C. esculenta corm also validated its antioxidant potential by showing the presence of certain phytochemicals viz. tannins, saponins, flavonoids, steroids, carbohydrates, proteins and glycosides supposed to be responsible for its antioxidant efficacy. Thus, C. esculenta corm may be explored for developing a novel antioxidant agent.

INTRODUCTION: Imbalance between the generation and neutralization of Reactive Oxygen Species (ROS) causes cell damage. ROS also plays a vital role in the origin and development of several health issues like diabetes, etc. Hence, antioxidant compounds which could protect the cellular organelles from oxidative damage are generally used either to control the generation of ROS or to scavenge them.



DOI: 10.13040/IJPSR.0975-8232.8(4).1758-64

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (4).1758-64

Recently, the use of natural antioxidants has gained importance because of their indigenous origin, strong efficiency to trap free radicals, perceived safety, potential therapeutic value and long shelf life ¹. Thus, phytochemical screening of medicinal plants used in Indian Traditional System of Medicine is the need of the hour in order to identify the natural antioxidants to protect the system from various diseases. The present study is a forwarding step in this direction to discover and develop novel natural antioxidants with improved efficacy and lesser toxicity. Furthermore, this information of biologically active phytochemical could be exploited for synthesizing valuable and efficient antioxidant agents as well ^{2,3}.

Colocasia esculenta is a herbaceous perennial plant, selected for the study, is of "Araceae" family and commonly known as 'Taro' in English and 'Arbi' or 'Khuyya' in Hindi. In India, it is locally cultivated and used as vegetable. Its starchy root as fleshy corm (tuber) and wide leaves, both are edible. In addition to starch the corms known to contain substantial amounts of protein, vitamin C, thiamine, riboflavin, niacin and dietary fiber. C. esculenta is reported to display antidiabetic 4, antiinflammatory ¹⁵, antioxidant ⁶ and anticancer activities ⁷. Studies on the chemical constituents of C. esculenta have reported the presence of pelargonidin-3-glucoside, cyanindin-3-rhamnoside, cyanidin-3-glucoside, orientin, isoorientin, isovitexin, vitexin X"-O-glucoside and luteolin 7-O-glucoside ^{8, 9}.

The aim of the present study was therefore, to evaluate antioxidant efficacy of *C. esculenta* corm extract *in vitro* and correlate this activity with the presence of screened phytoconstituents by standard chemical methods.

MATERIAL AND METHODS:

Plant material: Corms of *C. esculenta* were purchased from the local market of Allahabad, U.P., India and were authenticated by Prof. Satya Narayan, Taxonomist, Department of Botany, University of Allahabad, Allahabad, U.P., India. A voucher specimen has been submitted to the University herbarium.

Extract preparation: Corms of *C. esculenta* (1 kg) were peeled off, washed well, air dried and finally boiled in distilled water. The extract was filtered through Whatmann filter paper No. 45 and the filtrate was concentrated and lyophilized to obtain a dry dark brown solid material (9.7g) which was stored at -40 °C for further investigations

Chemicals: All the chemicals and solvents used were of high purity (99%). Gallic acid, folin-Ciocalteu reagent, sodium carbonate, hydrochloric acid, quercetin, aluminium chloride, sodium acetate. ascorbic acid. 1,1-diphenyl-2picrylhydrazyl (DPPH), sodium nitroprusside (SNP), sulphanilamide, naphthylenediamine dihydrochloride (NED), sodium chloride, potassium chloride, disodium hydrogen phosphate phosphate, dihydrate, dihydrogen potassium

phosphoric acid, potassium ferricyanide, trichloroacetic acid (TCA), dihydrogen sodium phosphate dihydrate, ferric chloride, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), potassium persulfate, ethanol and methanol were purchased from Sigma Aldrich, New Delhi, India.

Determination of total phenolics: Total phenolic content was determined using Folin-Ciocalteu reagent ¹⁰. Gallic acid was used for standard curve. The total phenolic content was expressed as mg of Gallic Acid Equivalents (GAE) per g of dried extract.

Determination of total flavonoids: Total flavonoid content was determined by aluminum chloride method ¹¹. Quercetin was used for standard curve. The total flavonoid content was expressed as mg of Quercetin Equivalent (QE) per g of dried extract.

Determination of total flavonols: Total flavonol content was determined by sodium acetate method ¹². Quercetin was used for standard curve. The total flavonols content was expressed as mg of Quercetin Equivalent (QE) per g of dried extract.

Reducing power assay: Reducing power was evaluated according to Oyaizu, 1986 ¹³. Different concentrations of dried extract (50-200 μg/ml) were suspended in distilled water and mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% K₃Fe(CN)₆. The mixture was incubated at 50°C for 20 minutes. Thereafter, 2.5 ml of 10% TCA was added to the mixture and centrifuged at 3000 rpm for 10 minutes. The upper layer of this solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl₃ (0.5 ml, 0.1%). Ascorbic acid was taken as standard. The absorbance of dried extract and standard was measured at 700 nm.

DPPH radical scavenging assay: DPPH radical scavenging assay was performed according to the method of Liyana-Pathirana & Shahidi, 2005 ¹⁴. 0.1 mM DPPH solution was made in methanol and 1 ml of this solution was mixed with 1 ml of different dried extract concentrations (50-200 μg/ml) in methanol. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes.

Ascorbic acid was taken as standard. The absorbance of dried extract and standard was measured at 517 nm. The ability of dried extract and standard to scavenge DPPH radical was calculated by the following formula:

DPPH radical % Inhibition = $\{(Ac - As)/Ac\} \times 100$

Where, Ac and As are the absorbance of the control and sample, respectively.

NO radical scavenging assay: Scavenging of NO radical was determined by the method of Green et al, 1982¹⁵. Reaction started by incubating 0.5 ml SNP solution (10 mM in Phosphate Buffered Saline) with different concentrations of dried extract (10-50 μg/ml) at 25 °C. After 150 minutes, 0.25 ml of sulphanilamide and 0.25 ml of NED were added. The mixture was allowed to stand for 30 minutes. Appearance of pink colour occurs. The absorbance of dried extract and standard was measured at 540 nm. The ability of dried extract and standard to scavenge NO radical was calculated by the following formula:

NO radical % Inhibition = $\{(Ac - As)/Ac\} \times 100$

Where, Ac and As are the absorbance of the control and sample, respectively.

ABTS radical scavenging assay: Scavenging of ABTS radical was determined by the method of Re *et al.*, 1999 ¹⁶. The ABTS twas pregenerated by mixing 7 mM ABTS stock solution and 2.45 mM potassium persulfate. The solution was incubated for 12 hours in the dark at room temperature until the reaction was complete and the absorbance was stable. 1 ml of above solution was mixed with 1 ml of different concentrations of dried extract/standard (25-125 μg/ml). After 6 minutes of incubation, absorbance was measured at 734 nm. The ability to scavenge ABTS radical was calculated by the following formula:

ABTS radical % Inhibition = $\{(Ac - As)/Ac\} \times 100$

Where, Ac and As are the absorbance of the control and sample, respectively.

Phytochemical screening: Dried extract was subjected for preliminary phytochemical screening by different qualitative chemical tests using

standard procedures for several classes of natural products ¹⁷⁻¹⁸.

Statistical analysis: All assays were carried in triplicate and results were expressed as Mean \pm Standard Deviation.

RESULTS AND DISCUSSION:

Total phenolics, flavonoids and flavonols: Table 1 represents the measures of total phenolics, flavonoids and flavonols present in C. esculenta corm extract. Result reveals that dry extract of C. esculenta corm contains 68.25 ± 17.52 mg GAE/g of total phenolics, 2.67 ± 40.18 mg QE/g of total flavonoids and 1.40 ± 53.99 mg QE/g of total flavonols. High content of total phenolics, flavonoids and flavonols in the extract validate that they can be used as potent dietary sources of natural antioxidants as well as in pharmaceutical formulations. Since, polyphenols are natural antioxidants responsible for the antioxidant activity of plants, therefore, the obtained amount of total polyphenols present in the extract indicates the extent of their antioxidant activity. Plants rich in phenolics are increasingly being used in the food they impede industry because degradation of lipids, improve the quality and nutritional value of food due to their hydroxyl groups which confer scavenging ability ¹⁹.

The polyphenolic compounds present in plant have a great diversity of compounds, such as flavonoids, tannins, etc. Flavonoids also comprises number of compounds like anthocyanins, flavanols, flavonols, flavones etc ²⁰. As antioxidants, flavonoid have been reported to be able to interfere with the biochemical pathway involved in the generation of reactive oxygen species (ROS), in addition to quenching of free radicals and chelating of redox active metals ²¹. Results illustrate that total phenolics, flavonoids and flavanols are present in significant amount in *C. esculenta* corm. Their presences in addition to their multifaceted actions make the *C. esculenta* corm plant extract a potent candidate for exploring as an antioxidant.

TABLE 1: TOTAL PHENOLICS, FLAVONOIDS AND FLAVONOLS OF *C. ESCULENTA* CORM EXTRACT

Dry Extract	Total	Total	Total
	phenolics	flavonoids	flavonols
	(mg GAE/g)	(mg QE/g)	(mg QE/g)
C. esculenta (corm)	68.25 ± 17.52	26.70 ± 40.18	1.40 ± 53.99

Reducing power estimation: Fig. 1 shows the reducing power of C. esculenta corm extract and ascorbic acid, taken as standard. Results reveal that the reducing power was increased in a concentration dependent manner and the maximum reducing power was at the concentration of 200 μ g/ml for each sample. The graphical presentation clearly indicates that the absorbance values of C. esculenta corm and reference, ascorbic acid are 0.161 ± 0.02 and 0.276 ± 0.07 at the concentration of 200μ g/ml at 700 nm respectively. Since, antioxidants possess the ability to donate an electron to free radicals for their neutralization, therefore, the reducing power may serve as a major indicator of potential antioxidant capacity 22 .

In this assay system, the presence of antioxidant which serves as an electron donor reductant causes the reduction of the Fe³⁺/ferricyanide complex to the ferrous form (Fe²⁺) and measured by direct electron donation. *C. esculenta* corm is associated with significant antioxidant efficacy because of its high concentration of polyphenolics which have excellent reduction potential to react with potassium ferricyanide (Fe³⁺) to form potassium ferrocyanide (Fe²⁺) and hence act as natural antioxidants.

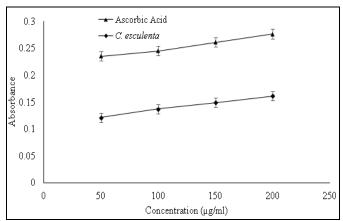


FIG. 1: REDUCING POWER OF C. ESCULENTA CORM EXTRACT AND ASCORBIC ACID

DPPH radical scavenging activity: Fig. 2 demonstrates the DPPH radical scavenging activity of *C. esculenta* corm extract and ascorbic acid, taken as standard. Results depict that the scavenging ability of *C. esculenta* corm and ascorbic acid was concentration dependent and was found to be maximum at a concentration of 200 μ g/ml with 19.35 \pm 0.82 and 92.01 \pm 1.59% inhibition, respectively validating thereby the

significant antioxidant potential of C. esculenta corm in terms of DPPH radical scavenging activity. DPPH radical scavenging activity of C. esculenta corm was found to be $74.34 \pm 12.17 \,\mu\text{g/ml}$ in terms of IC₅₀ values. However, the standard, ascorbic acid showed the lower IC₅₀ value of $9.23 \pm 0.25 \,\mu\text{g/ml}$ in case of DPPH free radical. DPPH radical is generated very quickly in methanol producing violet colour. This free radical gets reduced to DPPH-H (diphenylhydrazine) in the presence of natural antioxidants giving rise to colourless solution. Discoloration occurs due to the decreasing quantity of DPPH radicals in the environment 23 . The discoloration of the DPPH therefore reflects the radical scavenging activity of the sample.

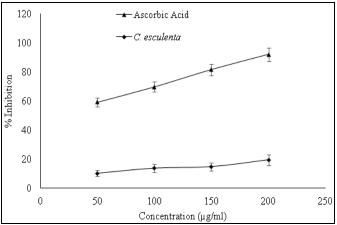


FIG. 2: DPPH RADICAL SCAVENGING ACTIVITY OF C. ESCULENTA AND ASCORBIC ACID

NO radical scavenging activity: Fig. 3 exhibits the NO radical scavenging activity of C. esculenta corm extract as well as of ascorbic acid taken as standard, in a concentration dependent manner. C. esculenta corm extract was found to effectively reduce the generation of nitric oxide from sodium nitroprusside. C. esculenta corm extract exhibited maximum NO radical inhibition of $42.56 \pm 0.50\%$ at the concentration of 50 µg/ml showing thereby significant radical scavenging activity. Whereas, ascorbic acid as usual showed a relatively higher inhibition of $63.63 \pm 1.03\%$ at the same evaluated concentration. Moreover, the IC₅₀ values obtained in this case for C. esculenta corm was found to be $65.52 \pm 7.76 \, \mu \text{g/ml}$ showing thereby substantial nitric oxide radical scavenging activity. However, as expected, ascorbic acid again showed the lower IC_{50} value of 12.92 \pm 0.52 µg/ml in case of NO free radicals also.

Nitric oxide radical (NO') is known to be a ubiquitous free radical distributed in tissues or organ systems and have a vital role in neuromodulation or neurotransmitter in the central system. In this assay, SNP nervous decomposed in the aqueous solution physiological pH 7.4 and produced NO radical. This NO radical reacts with oxygen to produce nitrate and nitrite, which was determined by using Griess reagent ²⁴.

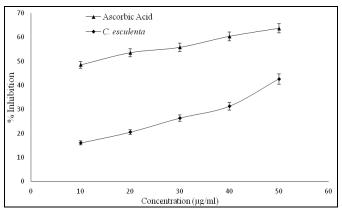


FIG. 3: NO RADICAL SCAVENGING ACTIVITY OF C. ESCULENTA AND ASCORBIC ACID

ABTS radical scavenging activity: Fig. 4 shows the ABTS radical scavenging potential of C. esculenta corm and ascorbic acid in a concentration dependent mode. C. esculenta corm showed maximum inhibition of $47.06 \pm 1.41\%$ at the concentration of 125µg/ml. Based on their percentage inhibition, C. esculenta corm was again found to be associated with higher ABTS radical scavenging activity also. However, ascorbic acid shows maximum inhibition of $94.13 \pm 0.16\%$ at the same concentration. In case of ABTS radical scavenging activity IC₅₀ value of *C. esculenta* corm was found to be $132.29 \pm 14.13 \,\mu\text{g/ml}$ whereas, the standard, ascorbic acid showed the lower IC₅₀ value of 66.62 ± 0.91 µg/ml in case of ABTS free radicals. Proton radical scavenging is an important attribute of antioxidants. ABTS, a protonated radical, has characteristic absorbance which decreases with the scavenging of the proton radicals resulting into decolorization of ABTS •+ 25.

The ABTS radical monocation is generated by oxidation of ABTS with potassium persulfate and it gets reduced in the presence of hydrogen-donating antioxidants. The efficacy of such antioxidants depends upon both the concentration of antioxidant

and duration of reaction on the inhibition of the radical cation absorption. This assay has several advantages for the determination of antioxidant activity in a number of ways. First, the chemistry involves the direct generation of the ABTS radical monocation with no involvement of intermediary radical. Second, it is a decolorization assay; thus the radical cation is pre-formed prior to addition of antioxidant test systems. Third, it is applicable to both aqueous and lipophilic systems¹⁵.

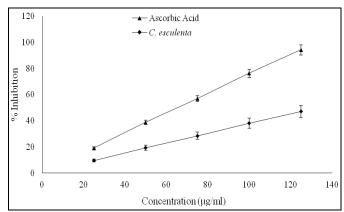


FIG. 4: ABTS RADICAL SCAVENGING ACTIVITY OF C. ESCULENTA AND ASCORBIC ACID

Phytochemical analysis: Table 2 shows the results of phytochemical screening of *C. esculenta* corm and the results reveal the presence of saponins, steroids, carbohydrates and glycosides in high amounts in *C. esculenta* corm extract. *C. esculenta* corm extract also showed the presence of tannins, flavonoids and proteins in considerable amounts.

C. esculenta corm was found to possess tannins which have amazing stringent properties. They are known to hasten the healing of wounds and inflamed mucous membranes ²⁶. Flavonoids are also present in C. esculenta as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity. It also helps in managing oxidative stress as well ²⁷. Although, both tannins and flavonoids, are in higher concentration in C. esculenta corm and hence validates the high extent of antioxidant efficacy of C. esculenta corm.

Plants rich in saponins possess the unique property of coagulating red blood cells, whereas steroids are responsible for cholesterol reducing property ²⁸.

Steroids also help in regulating the immune response $^{29, 30}$. Both saponins and steroids are present in *C. esculenta* corm and their concentrations were also significant.

Plants containing carbohydrates and glycosides are known to exert a beneficial action on immune system by increasing body strength and hence are valuable as dietary supplements. Glycosides also have vast therapeutic efficacy as they are found in almost every medicinal plant. *C. esculenta* corm also shows the presence of carbohydrates and glycosides.

TABLE 2: PHYTOCHEMICAL SCREENING OF C. ESCULENTA CORM EXTRACT

Phytoconstituents	Presence/Absence	
	in C. esculenta	
	corm	
Saponins	++	
Carbohydrates (Molisch's Test)	++	
(Fehling's test)	++	
Proteins (Biuret test)	+	
(Xanthoproteic test)	-	
Tannins	+	
Steroids (Salkowski's test)	++	
Flavonoids	+	
Glycosides (Molisch's Test)	++	
(Liebermann's test)	-	
(Salkowski's test)	++	
Phlobatannins	-	
Terpenoids	-	
Anthocyanins	-	
Leucoanthocyanins	-	
Coumarins	-	
Emodins	-	
Anthraquinones (Borntager's test)	-	
Alkaloids (Hager's test)	-	
(Wagner's test)	-	
(Mayer's test)	-	

CONCLUSION: Since, natural antioxidants derived from plant sources contribute notably in the prevention of pathological consequences caused by free radicals therefore, the present findings, conclude that the aqueous extract of *C. esculenta* corm possesses strong antioxidant potential. Hence, this promising medicinal plant may be exploited further for phytochemical investigation and their mode of action in order to develop safe, economical, effective and targeted antioxidant agents for the benefit of mankind.

ACKNOWLEDGEMENT: The first author, Manjulika Yadav is grateful to UGC (University

Grants Commission), New Delhi, Govt. of India, for financial assistance in the form of fellowship.

CONFLICT OF INTEREST: The authors of this article declare no conflict of interest in this study.

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How to cite this article:

Yadav M, Kushawaha DK, Chatterji S and Watal G: Assessment of antioxidant activity and phytochemical screening of *Colocasia esculenta* corm. Int J Pharm Sci Res 2017; 8(4): 1758-64.doi: 10.13040/JJPSR.0975-8232.8(4).1758-64.

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