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Antioxidant Analysis and Phytochemical Screening of *Colocasia Esculenta* Leaf Extract

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Abstract:

Aim: To analyse and understand the antioxidant activity and phytochemicals present in the *Colocasia esculenta* leaf extract.

Materials and Methods: The *Colacacea esculenta* leaves were taken from Indian Institute of Horticulture Research (IIHR), Bangalore. Free radical scavenging activities of the extract was assessed using FRAP, DPPH and ABTS scavenging assay. Phytochemical screening was carried out on the aqueous, methanolic, acetone and petroleum ether extract of the leaves.

Results: The antioxidant property of leaf extracts were confirmed by their excellent free radical scavenging activity. The phytochemical analysis of all the four extracts showed the presence of alkaloids and proteins.

Conclusion: Various phytochemicals present in the *Colocasia esculenta* leaf extracts may contribute to the free radical scavenging and other pharmacological activities.

Key words: Antioxidant, *Colocasia esculenta*, Medicinal plant, Phytochemicals.

INTRODUCTION:

Plants play a significant role in maintaining human health and enhancing the quality of life since long and have been beneficial to human because of their potential uses as alternative remedies for the treatment of many infectious diseases and also served as valuable components of medicine, seasonings, beverages, cosmetics, and dyes [1]. They have always been used as a common source of medicaments, may be either in traditional preparations or as pure active principles forms which are referred to be Medicinal plants [2]. The medicinal plant extracts have wide range of scope in the biological activities thus forming the basis for the drug discovery [3]. Extracts from plant source are used in the treatment of cardiovascular diseases, central nervous system, liver and other metabolic disorders [1]. The ethno botany provides a rich sources for the drug discovery and drug development. The emphasis on the use of the plants is on the treatment than the prevention of diseases [4].

One such medicinal plant that is known to us as a potential medicinal herb is *Colocasia esculenta* Linn, commonly known as Taro. *Colocasia esculenta* Linn. is a tall herb, tuberous or with a stout short caudex, flowering and leafing together. Various parts of *Colocasia esculenta* are traditionally used to treat many diseases, is a green leafy vegetable which is rich in proteins, carbohydrates and vitamins and microminerals like iron, potassium, zinc etc., [5]. It is commonly known as "Taro" in (English), Arvi, Kachalu (Hindi), Alupam, Alukam (Sanskrit) and also known as Arum esculentum L. and *Colocasia antiquorum* Schott which belongs to the Araceae family which is an annual herbaceous plant. The plant is known for its medicaments of various ailments like asthma, arthritis, diarrhea, internal hemorrhage, neurological disorders and skin diseases [1]. The parts used mainly are Leaves and Corms. The juice extract of corm of *Colocasia esculenta* is use for baldness, stimulant, expectorant, used to arrest arterial hemorrage and as a body pain reliever [1]. The taro tuber is rich in carbohydrates, proteins however low in the fat content. The starch which is gluten free present in the Taro are fine and small which can be easily digested. It is also noted that the Vitamin B-complex present in the Taro is greater than the whole milk [6]. The objective of the present study was to assess the free radical scavenging activities Ferric Reducing Antioxidant Power (FRAP), 2,2-diphenyl-1- picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay identifying the phytochemicals present in the methanolic, acetone, petroleum ether, water extract of *Colocasia esculenta* leaves by qualitative analysis.

MATERIALS AND METHODS

Collection of Sample

The *Colocasia esculenta* leaves were bought from the fields of Indian Institute of Horticulture Research (IIHR), Hessaragatta Road, Bangalore. It was authenticated by a Botanist, from the Department of Botany, Bangalore University, Bangalore.

Sample Preparation

Colocasia esculenta leaves were washed thoroughly with distilled water and were cut into small pieces. Then the leaves were dried at room temperature for 48 h. The dried leaves were then crushed using a mixer to a fine powder and stored at room temperature for further analysis. Approximately 20 g of powder was obtained from 100 g leaves.

Extraction

The leaf powder was extracted using distilled water, methanol, acetone and petroleum ether. It was then filtered using Whatmann No.1 filter paper to obtain a clear filtrate. The resulting filtrate was used for phytochemical analysis. Methanolic extract was used for analysing free radical scavenging activity.

Estimation of Free radical scavenging activity

DPPH free radical scavenging activity

The radical scavenging assay of the plant extract was done using 2,2-diphenyl-1- picrylhydrazyl (DPPH) method, which was the most widely accepted radical scavenging assay. Different concentrations of the sample (100-500 µg) were taken in different test tubes and the volume in each test tube was made upto 0.1 ml with methanol. To all the tubes, 3 ml of DPPH solution (0.3 mM) was added and incubated in dark condition at room temperature for 30 min [7]. After incubation the absorbance was read at 517 nm spectrophotometrically with methanol as a blank. All determinations were done in triplicates. Percentage of inhibition of the DPPH radical was calculated according to the following equation. Inhibition of DPPH (%) = (Ac - As/Ac) X 100 Where, Ac = Absorbance of control As = Absorbance of samples (or) standard.

Estimation of antioxidant property by ABTS assay

Free radical scavenging capacity of the extract was estimated using the stable ABTS radical [8]. $ABTS^{\cdot+}$ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12-16 h before use. $ABTS^{\cdot+}$ solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After the addition of 5 µl of plant extract to 3.995 ml of diluted $ABTS^{\cdot+}$ solution, the absorbance was measured at 30 min after the initial mixing. An appropriate solvent blank was run in each assay. All the measurements were carried out at least three times. Percent inhibition of absorbance at 734 nm was calculated using the formula, $ABTS^{\cdot+}$ scavenging effect (%) = ((AB–AA)/ AB) × 100 (2), where, AB is absorbance of ABTS radical + methanol; AA is absorbance of ABTS radical + sample extract/standard. The IC_{50} value for the sample was determined using the straight line equation obtained from the graph.

Estimation of antioxidant property by FRAP assay

Free radical scavenging capacity of the extract was estimated using the reducing power assay [9]. Different concentrations of the sample (100-500 µg) were taken in the test tubes and the volume in each test tube was made up to 0.1 ml with methanol. To all the tubes, 2.5 ml of 0.2 M phosphate buffer (pH-6.6) and 2.5 ml of 1% Potassium ferricyanide was added and incubated at 50ºC for 20 min. Later, 2.5 ml of 10% Tri-chloroacetic acid was added. The samples were centrifuged at 3000 rpm for 10 min if turbidity is observed. To 2.5 ml of the upper layer, 2.5 ml of distilled water and 0.5 ml of 0.1% Ferric chloride solution was added. The absorbance was read at 700 nm spectrophotometrically with methanol as a blank. A graph was plotted with concentration of sample versus absorbance. The IC_{50} value for the sample was determined using the straight line equation obtained from the graph. This result indicates that increase in absorbance of the reaction mixture indicates increase in reducing power.

Phytochemical analysis

The extracts were subjected to preliminary phytochemical screening by different qualitative chemical tests using standard procedures for several classes of natural products [10,11]. Mention name of the parameters carried out in phytochemical analysis section.

RESULTS AND DISCUSSION

DPPH Scavenging activity

Free radical scavenging capacity of *Colocasia esculenta* leaf extracts was evaluated with their ability to scavenge DPPH free radicals. From the Figure 1, the IC_{50} value of the sample was found to be 2.89 µg. Studies have revealed that methanolic extract of the *Colocasia esculenta* leaves has shown higher antioxidant activity as compared to tuber extracts [12]. It was observed that the leaf extract contain high level of phenolic content that might have accounted for strong activity observed against DPPH radicals [13]. The free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution. The use of DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry [14].

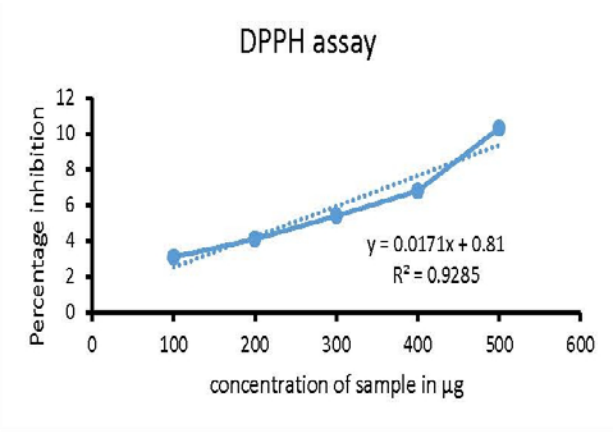

Figure 1: DPPH Scavenging activity

Estimation of antioxidant property by ABTS assay

Figure 2 shows the ABTS radical scavenging potential of *Colocasia esculenta* in a concentration dependent mode. *Colocasia esculenta* leaf extracts showed maximum inhibition of 79.1% at the concentration of 500 µg. The IC_{50} value for the methanolic extract of *Colocasia esculenta* leaves was found to be 251.41 µg. 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 $ABTS^{\bullet}$ [15]. 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 an 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 systems [16].

Ferric Reducing Anti-oxidant power FRAP assay

From Figure 3, the IC_{50} value for the *Colocasia esculenta* methanolic leaf extracts was found to be 248.88 µg. FRAP estimates the reducing ability of antioxidants against the free radical effect of reactive oxygen species. In this assay, the presence of antioxidant function as an electron donor reductant causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}) and measured by direct electron donation. *Colocasia esculenta* is associated with high antioxidant activity which have excellent reduction potential to react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}) and hence act as natural antioxidants. 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 activity [17].

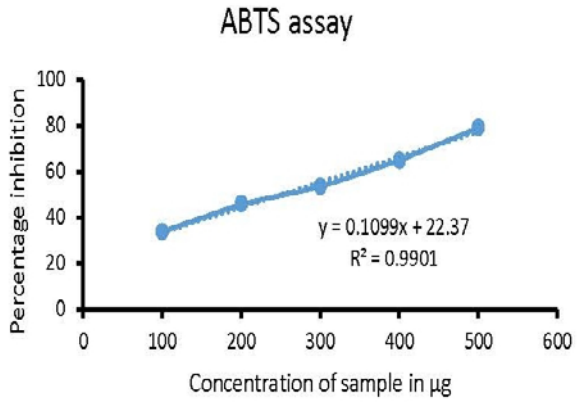

Figure 2 Estimation of antioxidant property by ABTS assay

Phytochemical analysis on the methanolic extract of *Colocasia esculenta* leaf

Table – 1 shows the results of phytochemical screening of *Colocasia esculenta* leaf extracts and the results reveal the presence of alkaloids, tannins and phenols, oils, steroids, amino acids and proteins. Plants possessing carbohydrates are well known to exert a beneficial action on immune system by increasing body strength and they are valuable as dietary supplements. *Colocasia esculenta* was found to possess tannin which has amazing stringent properties. They are known to hasten wound healing and healing of inflamed mucous membranes [18] whereas steroids are responsible for cholesterol reducing property [19]. Steroids also help in regulating immune system [20]. Several alkaloids isolated from natural herbs exhibit anti-proliferation and anti-metastasis effects on various types of cancers both *in vitro* and *in vivo* [21]. Natural alkaloids also possess anti-inflammatory and antioxidant properties as well as anti-depressive and anti-convulsing efficacy [22].

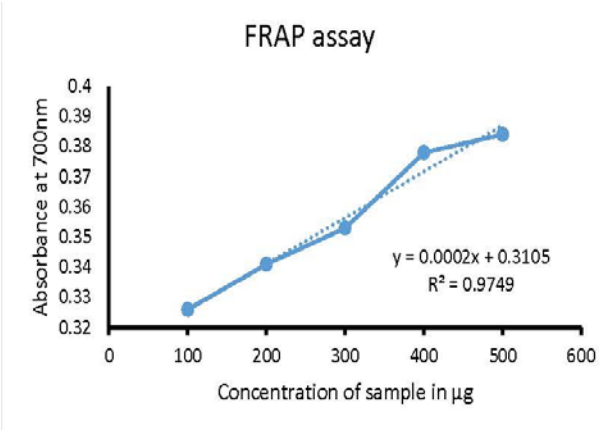

Figure 3 Estimation of antioxidant property by FRAP assay

Table 1 Phytochemical analysis on the methanolic extract of *Colocasia esculenta* leaf

Phytochemicals	Methanolic extract	Petroleum ether	Acetone extract	Aqueous extract
Alkaloids	+	+	+	+
Flavonoids	-	-	-	-
Tannins and Phenols	+	+	+	-
Saponins	-	-	-	+
Oils	+	+	+	-
Anthraquinone	-	-	-	-
Coumarins	-	+	+	+
Steroids	+	+	+	-
Terpenoids	-	-	-	+
Carbohydrates	-	-	-	+
Amino Acids	+	-	+	+
Proteins	+	+	+	+

CONCLUSION

Natural antioxidants are widely distributed in medicinal plants. Antioxidants derived from medicinal plants are increasing notably for their ability to prevent the pathological consequences caused by free radicals and also for their various nutrition function and health benefits. The present findings conclude the *Colocasia esculenta* leaf extracts possess strong antioxidant potential. Hence the plant can be exploited further for phytochemical investigation and their mode of action in order to develop safe, efficient and targeted antioxidants for the benefit of mankind.

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