



## Assessment of Antioxidants, Phytoactives in Extracts of *Colocasia esculenta*

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### ABSTRACT

*Colocasia esculenta* is a robust perennial plant belonging to Araceae family with a large corm having a very large, heart-shaped leaves that are borne on thick stalks. The young leaves of this plant are rich in Vitamins C, B1, B2, Riboflavin and Niacin. The tubers contain amino acids and are rich in starch. This multipurpose perennial plant is used as a traditional medicine acting as anti-cancer agent, anti-hepatotoxic agent, antimicrobial agent, anti-diabetic agent. This shows the necessity for the study of *Colocasia esculenta*. In this study, the herbal extracts are distilled using aqueous and ethanol solvents for the antioxidant activity and phytoactives assessment in order to prove the efficiency of *Colocasia esculenta* role as a potential natural antioxidant. Future studies will reveal the comparison of *Colocasia esculenta* collected from various regions on their available antioxidant enzymes activity that concludes the biological action of the selected species in therapeutics.

**Keywords:** *Colocasia esculenta*, Extraction, Phytoactives, Natural antioxidant, Antioxidant enzymes

### INTRODUCTION

'Taro' is the common name of genus *Colocasia*, often described as 'Elephant's ear' is an evergreen, perennial plant that produces a cluster of leaves with a long, erect petioles, growing from 40 – 200cm tall from a large, tuberous rootstock. This species is considered as a very important, staple food crop in many parts of the tropics, with a long history of cultivation. It prefers humid, lowland tropical regions.

### SCIENTIFIC CLASSIFICATION:

Kingdom: Plantae  
Order: Alismatales  
Family: Araceae  
Subfamily: Aroideae  
Tribe: Colocasiodeae  
Genus: *Colocasia*  
Species: *C. esculenta*

### BOTANICAL STUDY:

*Colocasia esculenta* contains 2-3 leaves or more which are green in color. They are ovate, 20-50 cm long, apex short acuminate, 4 lateral veins; petiole of 20-90 cm long with solitary inflorescences. Spathe constricted of 20 cm long with tube green in color, 4 cm long, 2.2 cm wide, long-ellipsoid in shape. Spadix is of 10 cm long, flowers unisexual, female portion is 3-3.5 cm long, 1.2 cm wide, conic, slender; male portion 4-4.5 cm long, 7 mm wide, cylindrical. It is often established in low lying areas along streams and river banks.



**Figure 1:** *Colocasia esculenta*

### Medicinal values

- The plant possess antibacterial properties
- The extract of leaves is drunk to promote menstruation
- The extract is taken to relieve stomach problems and to treat cysts
- The sap of the leaf stalk is used in treating conjunctivitis
- The plant is used to treat wounds.
- The leaves are rich in nutrients such as Calcium, Phosphorous and Iron; minerals and vitamins like C, Riboflavin, Thiamine, Niacin.
- Fresh edible leaves of the selected plant are rich source of protein, ascorbic acid, fibres.

With all the above medicinal properties, the plant also contains calcium oxalate crystals which are responsible for causing an extremely unpleasant sensation similar to needles being stuck into the mouth and tongue if they are eaten, hence, the leaves can be neutralized by drying or cooking thoroughly or by steeping it in water.

### PHYTOACTIVES:

Pharmacological importance of plants has been gaining attention due to zero toxicity and less expense. Potential components from various parts of the plants have been identified and their extract is used for the formulation of drugs. Modern clinical drugs includes the use of natural extract's origin which possess the presence of phytoactives such as Alkaloids, Glycosides, Proteins, Carbohydrates, Lipids, Fats and Oils because of which they are known for the lower incidence of the adverse effects caused by the drugs.

### MATERIALS AND METHODS

#### Specimen collection:

The plant samples were collected from Shettyhalli village, Sakleshpura Taluk, Hassan district, Karnataka and authenticated in Regional Ayurveda Institute for Metabolic Disorders, Govt. of India in order to confirm the plant species. The leaves as the sample were excised from the collected plant.



Figure 2: PLANT AUTHENTICATION



Figure 3: COLLECTED PLANT SPECIES

#### PLANT EXTRACTION:

The leaves were subjected to extraction using water and ethanol as solvents at room temperature using Soxhlet apparatus where in the collected extracts were stored at 4 °C until use.

#### QUALITATIVE ANALYSIS OF PHYTOACTIVE:

##### ➤ Test for Alkaloids

**Dragendorff's test:** To the 1 ml of extract, 1 ml of Dragendorff's reagent (potassium bismuth iodide solution) was added. An orange-red precipitate indicated the presence of alkaloids.

**Wagner's test:** To the 1 ml of extract 2 ml of Wagner's reagent (iodine in potassium iodide) was

added. The formation of reddish brown precipitate indicated the presence of alkaloids.

➤ **Test for Saponins:** Small quantity of alcoholic and aqueous extracts were taken separately and to this 20 ml of distilled water was added and was shook well vertically in a graduated cylinder for 15 minutes. Layer of foam indicated the presence of Saponins.

➤ **Test for Glycosides:**

**Legal's test:** The extract was dissolved in pyridine and to this sodium nitropruside solution was added to make it alkaline. The change from pink to red colour shows the presence of glycosides.

**Baljet's test:** To 1ml of the test extract, 1ml of sodium picrate solution was added and the yellow to orange color reveals the presence of glycosides.

➤ **Test for Carbohydrates**

**Molisch's test:** To 2ml of the extract, 1ml of  $\alpha$ -naphthol solution was added, followed by concentrated sulphuric acid through the side of the test tube. Purple or reddish violet color at the junction of the two liquids reveals the presence.

**Fehling's test:** To 1ml of the extract, equal quantities of Fehling solution A and B, were added, upon heating formation of a brick red precipitate indicates the presence of sugars.

**Benedict's test:** To 5ml of Benedict's reagent, 1ml of extract solution was added, boiled for 2 minutes and cooled. Formation of red precipitate shows the presence of sugars.

➤ **Test for Tannins**

the little quantity of test solution was taken and mixed with basic lead acetate solution. Formation of white precipitate indicates the presence of tannins. ii) To 1ml of the extract, ferric chloride solution was added; formation of a dark blue or greenish black color product shows the presence of tannins. The little quantity of the extract was treated with potassium ferric cyanide and ammonia solution. A deep red color indicates the presence of tannins. iii) To the test extract, strong potassium dichromate solution was added, a

yellow color precipitate indicates the presence of tannins and Phenolic compounds.

➤ **Test for Flavonoids**

**Shinoda's Test:** i) The extract was treated with sodium hydroxide; formation of yellow color indicates the presence of flavones. ii) The extract was treated with concentrated  $H_2SO_4$ , formation of yellow or orange color indicates flavones.

➤ **Test for Steroids**

**Salkowski test:**

The extract was dissolve in chloroform and equal volume of conc.  $H_2SO_4$  was added. Formation of bluish red to cherry color in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

➤ **Test for Fats & Oils**

A thick section of extract was placed on glass slide. a drop of Sudan Red III reagent was added. After 2 min. wash with 50% alcohol and was mounted in glycerin, Observed under microscope. Oil globules appear red. ii) Little amount of extract was placed on the filter paper and allowed to stand for 15 minutes. A greasy spot observe due to presence of fats.

## QUANTITATIVE ANALYSIS FOR ANTIOXIDANTS:

### DETERMINATION OF CARBOHYDRATE

100 mg of sample was hydrolyzed in a boiling tube with 5 ml of 2.5 N HCl in a boiling water bath for a period of 3 hours. It was cooled to room temperature and solid sodium carbonate was added until effervescence ceases. The contents were centrifuged and the supernatant was made to 100 ml using distilled water. From this 0.2 ml of sample was pipette out and made up the volume to 1 ml with distilled water. Then 1.0 ml of phenol reagent was added followed by 5.0 ml of sulphuric acid. The tubes were kept at 25-30°C for 20 min. The absorbance was read at 490 nm.

### DETERMINATION OF PROTEIN

The dried and powdered samples were extracted by stirring with 50 ml of 50% methanol (1:5 w/v) at 25 °C for 24 hrs. and centrifuged at 7,000 rpm for 10 min. 0.2 ml of extract was pipette out and the volume was made to 1.0 ml with distilled water. 5.0 ml of alkaline copper reagent was added to all the tubes and allowed

it to stand for 10 min. Then 0.5 ml of Folin-Ciocalteu reagent was added and incubated in dark for 30 min. The intensity of the colour developed was read at 660 nm .

#### DETERMINATION OF TOTAL PHENOLICS

Ten micro litre aliquots of the extracts (2 mg/2 ml) was taken in test tubes and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as tannic acid equivalents.

#### DETERMINATION OF TOTAL FLAVONOID CONTENT

The flavonoid content was determined by the use of a slightly modified colorimetric method described. A 0.5 ml aliquot of appropriately (2 mg/2 ml) diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5 % NaNO<sub>2</sub> solution. After 6 min, 0.15 ml of 10% aluminium chloride solution was added and allowed to stand for 6 min, and then 2 ml of 4% sodium hydroxide solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus water blank. The analysis was performed in triplicate and the results were expressed as rutin equivalent.

#### DPPH FREE RADICAL SCAVENGING ASSAY:

The radical scavenging activity of selected root extracts was determined. 4.3mg of DPPH (2, 2-Diphenyl -1- picrylhydrazyl) was dissolved in methanol (6.6ml) to prepare 0.3mM DPPH solution and it was protected from light by covering the test tubes with aluminium foil. DPPH (150µl) was added to 3ml of methanol and absorbance was noticed immediately at 516nm for control reading. Different concentrations of test samples i.e 25 µl, 50 µl, 100 µl, 150 µl, 200 µl and 250 µl were taken and each of the samples was diluted with methanol up to 3ml, to it 150 µl DPPH was added. The samples were kept in dark for 15 min after which the optical density was observed at 516nm using methanol as blank.

$$\text{S\% Antioxidant activity} = \frac{(\text{Control Absorbance} - \text{Sample Absorbance})}{\text{Control Absorbance}} \times 100$$

#### DETERMINATION OF TOTAL REDUCING POWER

1ml of the extract (1mg/ml) was mixed with 1ml of 200mM of sodium phosphate buffer (pH- 6.6) and 1ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and 1ml of 10% trichloroacetic acid (w/v) was added. The mixture was centrifuged at 2000rpm for 10min. The upper layer solution (2.5ml) was mixed with 2.5ml of double deionised water and 1ml of fresh ferric chloride solution (0.1%). The absorbance was measured at 700nm. A higher absorbance indicated a higher reducing power.

#### RESULTS AND DISCUSSION

The leaves of the plant from araceae family were subjected to qualitative and quantitative analysis and the results were tabulated.

#### PHYTOCHEMICAL ANALYSIS

Phytochemicals are bioactive non-nutrient plant compounds that provide a wide range of activities in treating the diseases/disorders. They protect the plants from harmful agents such as insects and microbes as well as stressful/extreme temperatures. The phytochemical constituents present in the plant sample were estimated with the extraction of Ethanol and Water as solvents.

EXTRACT	Water	Ethanol
<b>ALKALOIDS</b> (WAGNER'S TEST)	+	+
<b>CARBOHYDRATES</b> MOLISCH'S TEST	+	+
FEHLINGS TEST	+	+
BENEDICTS TEST	-	-
<b>TANNINS</b>	-	-
<b>FLAVONOIDS</b> SHINODA TEST	+	+
<b>SAPONINS</b>	-	-
<b>FIXED OILS</b> SPOT TEST	-	-
<b>GLYCOSIDES</b> BORNTRAGERS TEST	+	+

On the whole, the *Colocasia esculenta* extracts showed positive results in the analysis of phytochemicals where the saponins and oils were absent in the extract and tannins were absent; all the other phytochemicals showed their presence in both the extracts which proved the ability of the plant extracts to perform wide range of defense mechanisms.

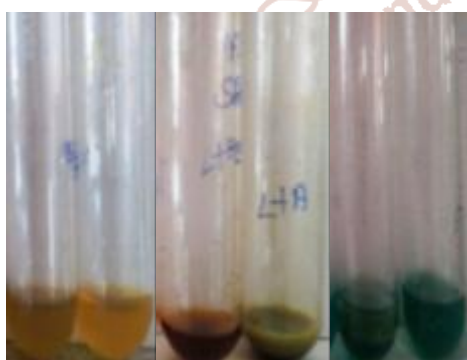
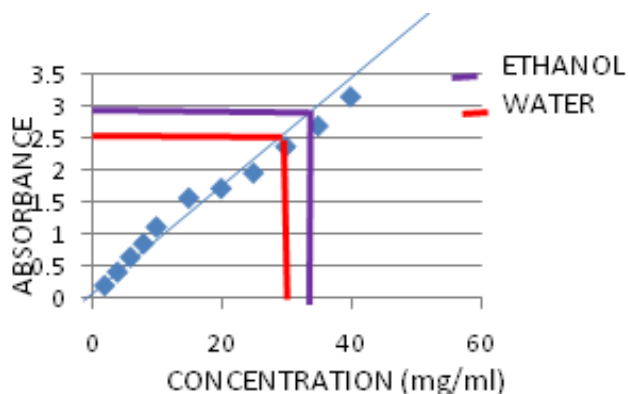


Figure 4: PHYTOACTIVES

**QUANTITATIVE ANALYSIS:  
TOTAL FLAVONOIDS:**

Some flavonoids have inhibitory activity against organisms that cause plant diseases. Flavonoids are the most important plant pigments for flower coloration, producing yellow or red/blue pigmentation in petals designed to attract pollinator animals. Flavonoids are generally secreted by the root of the host plant.

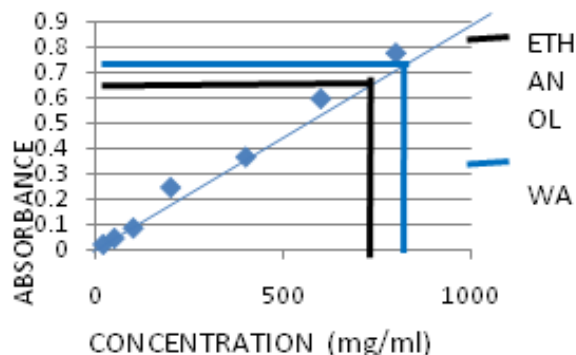


Graph 1: Graph showing the Total Flavonoids

From the graph, the total flavonoids were estimated in both the extracts and found to be 30.6 mg/ml in ethanol and 28.7 mg/ml in water extract.

**REDUCING POWER ASSAY:**

It is a measure of antioxidant activity to reduce other substances, or the environment. and reduce to other substances means addition of hydrogen and removal of oxygen and also gain of electron.

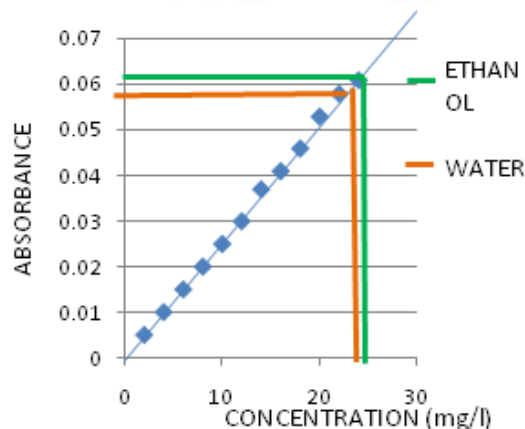


Graph 2: Graph showing Reducing power

From the graph, the reducing power was estimated in both the extracts and found to be 700 mg/ml in ethanol and 802 mg/ml in water extract.

**DETERMINATION OF TOTAL PHENOLIC CONTENT:**

Phenolic compounds are plant substances which possess in common an aromatic ring bearing one or more hydroxyl groups. There are about 8000 naturally occurring plant phenolics and about half of these numbers are flavonoids. Phenolics possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic as well as ability to modify the gene expression. Phenolics are the largest group of phytochemicals that account for most of the antioxidant activity in plants or plant products.



Graph 3: Graph showing the Total Phenolics

From the graph, total phenolics was estimated in both the extracts and found to be almost the same of about 26mg/ml in ethanol and 25 mg/ml in water extract.

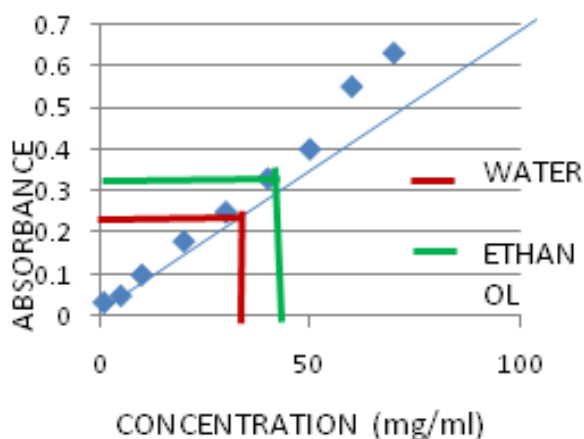
From the graph, total proteins were estimated in both the extracts as 199mg/ml in ethanol and 182 mg/ml in water extract.

**DPPH RADICAL SCAVENGING:**

DPPH is a free radical with red color which turns into yellow color after scavenging at the specific absorbance. The degree of discoloration indicates the scavenging potential of the antioxidant compounds in terms of hydrogen donating ability.

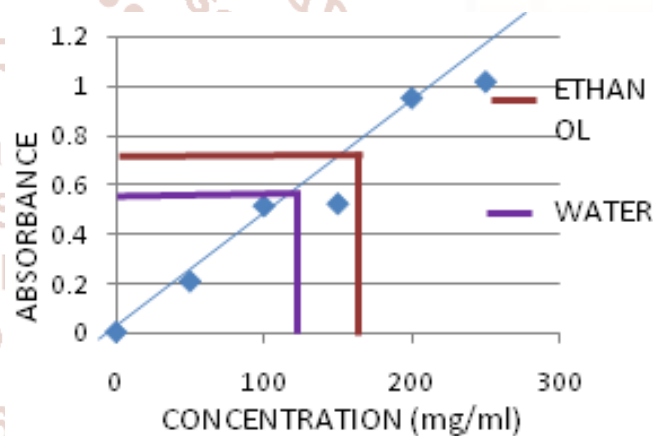
**CARBOHYDRATE ESTIMATION:**

Carbohydrates are an essential compound of all organic life on this planet. Both plants and animals use carbohydrates as a primary source of energy, which keeps the body functioning at the most basic level. Carbohydrates also fulfill other needs by helping in the synthesizing of other chemicals and providing structure for cells within the body. Carbohydrates also serve as antigens, substances which trigger the creation of antibodies for the immune system. Other carbohydrates provide hormones, such as follicular stimulating hormone (FSH), which aids in ovulation, and glycoprotein, which aids in cell-to-cell interaction such as those between antigens and antibodies.



**Graph 4: Graph showing the DPPH activity**

From the graph, DPPH was estimated in both the extracts and found to be almost the same of about 46 mg/ml in ethanol and 40.6 mg/ml in water extract.



**Graph 6: Graph showing the Total Carbohydrates**

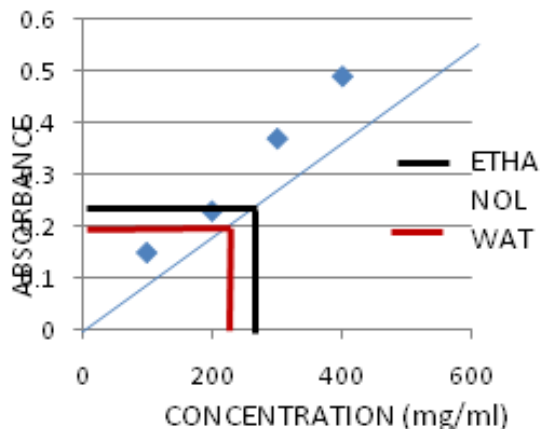
From the graph, total proteins were estimated in both the extracts as 155.7 mg/ml in ethanol and 134.6 mg/ml in water extract.

**PROTEIN ESTIMATION:**

Proteins are the most complex and abundant of the macro molecules. Within cells, many proteins function as enzymes in the catalysis of metabolic reactions

**CONCLUSION:**

In the selected plant, the antioxidant and phytochemicals were found to be maximum, which paves way for the antioxidant enzymes activity in order to prove the therapeutic importance. This leaves extract was found to possess inhibitory effect against three microbes. Further investigations on this leaves extract can reveal various biological mechanisms present in them.



**Graph 5: Graph showing the Total Proteins**

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