Available online on 15.07.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

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Evaluation of antioxidants and phytoactives in *Colocasia esculenta* (L.) Schott

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ABSTRACT

Colocasia esculenta is a perennial plant of araceae family and possess a large corm having a very large, heart-shaped leaves that are borne on thick stalks. The juvenile 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 that acts 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 ethanolic leaves extract of *Colocasia esculenta* showed the maximum antioxidant activity and phytoactives efficacy, Thus suggesting the sample to be as a potential therapeutic source of various diseases.

Keywords: *Colocasia esculenta*, Western Ghats, antioxidant, Phytoactives, Therapeutics

Article Info: Received 11 May 2019; Review Completed 22 June 2019; Accepted 28 June 2019; Available online 15 July 2019

Cite this article as:

Jyothi R, Srinivas Murthy KM, Evaluation of antioxidants and phytoactives in *Colocasia esculenta* (L.) Schott, Journal of Drug Delivery and Therapeutics. 2019; 9(4):411-414 **http://dx.doi.org/10.22270/jddt.v9i4.3077**

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INTRODUCTION

Colocasia esculenta is one of the significant staple food of various parts of Africa and Asia. It is usually known by the name 'taro'. It is a rich source of copper, iron, phosphorous, potassium, manganese, thiamine and also contains higher amounts of vitamin B-complex more than the whole milk (Soudy *et al.,* 2010). In addition, taro is especially useful to persons allergic to cereals and can be consumed by children who are sensitive to milk (Huang *et al.,* 2007) It is quite low in ascorbic acid and carotene, the amount of carotene is the same as that found in cabbage and twofold which is seen in potato (Wang, 1983). Taros have elevated quantity of β-carotene in the corm and will impart vitamin A and antioxidant property in the body. B-carotene differs only very slightly in terms of structure. They are very common in carotenoids and are antioxidants, as well as, possess various potential health benefits. As mentioned earlier, both can be converted into vitamin A by the body, though β-carotene has about twice the provitamin 'A' activity as α- carotene (Nip, 1997). Taro is one of the few non-animal sources of zinc (SPC (2006). Chemically, the plant Possess a range of biologically active phytoconstituents such as flavonoids, sterols, glycosides, and other micronutrients. (Prajapathi *et al.,* 2011).

Taro comprises of high protein than other root crops because of the presence of symbiotic soil bacteria in the root and rhizome region of taro, which fix atmospheric bacteria and raise nitrogen occurrence in the corm and leaves. The bacteria are used as plant growth enhancer due to release of growth hormone to the roots and distributed to the whole part of the plant. The free-living nature of the soil bacteria also helps the taro crop to grow at different environmental and ecologic conditions. Such properties have ecologic and economic significance to the environment (Lucy, 2004).Taro tubers provide a number of desirable nutritional and health benefits such as anticancer activity, phenolic acid and phytochemicals. (Rashmi *et al.,* 2018).The studies revealed that the aqueous extract of *C.esculenta* corm contain strong antioxidant activity. Thus the plant may be used for further phytochemical studies and its mechanism of action in order to develop effective, economical, safe and targeted antioxidant agents for the welfare of mankind (Yadav *et al* 2017). The Qualitative phytochemical screening of *Colocasia esculenta* tubers in methanolic and aqueous extract showed that alkaloids, glycosides, flavonoids, terpenes, saponins and

phenol are present. The results also revealed the absence of tannins, quinines and steroid in both the extracts. (Krishnapriya *et al*., 2017). The present study was focused on evaluating the antioxidant and phytoactives in the aqueous and ethanolic extracts of leaves extracts of *Colocasia esculenta.*

Fig.1: *Colocasia esculenta* sample from Western Ghats

MATERIALS AND METHODS

Specimen collection:

The healthy *Colocasia esculenta plant* samples collected from the Western Ghats of Karnataka were authenticated at Regional Ayurveda Institute for Metabolic Disorders, Govt. of India in order to confirm the plant species.

Preparation of extract:

The leaves were thoroughly washed in distilled water, and were shade dried for nearly 15 to 20 days at room temperature. Later they 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 Phytoactives

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 Carbohydrates

Molisch'stest: To 2ml of the extract, 1ml of a-napthol 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, and 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 Fats & Oils

1. 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.

2. 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.

Test for Flavonoids

Shinoda's Test:

1. The extract was treated with sodium hydroxide; formation of yellow color indicates the presence of flavones.

2. The extract was treated with concentrated Sulphuricacid, formation of yellow or orange color indicates flavones.

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 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 Steroids

Salkowski test: The extract was dissolve in chloroform and equal volume of conc. Sulphuric acid 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 Tannins

1. The little quantity of test solution was taken and mixed with basic lead acetate solution. Formation of white precipitate indicates the presence of tannins.

2. 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.

3. To the test extract, strong potassium dichromate solution was added, a yellow color precipitate indicates the presence of tannins and Phenolic compounds.

QUANTITATIVE ANALYSIS FOR ANTIOXIDANTS

Determination of Carbohydrate

100 mg of samples were hydrolyzed in a boiling tube with 5 ml of 2.5 N Hydrochloric acids 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 pipetted 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 hours and centrifuged at 7,000 rpm for 10 min .0.2 ml of extract was pipetted 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-Ciocalteau 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

The total phenolic content was determined according to the method described by25. Ten micro liter 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. 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 % $NaNO_2$ 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 as described by Gayatri *et al.,* 2011, Accordingly 4.3 mg 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 (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.

%Antioxidant activity =

$$\frac{\text{(Control Absorbance - Sample Absorbance)}}{\text{Control Absorbance}} \times 100$$

Determination of Total Reducing Power

The reducing power was determined according to the method of Oyaizu (30). 1ml of the extract (1mg/ml) was mixed with 1ml of 200mM of sodium phosphate buffer (pH-6.6) and1ml 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 plants 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 samples were estimated with the extraction using Ethanol and Water as solvents.

Table-1: Phytochemical activity in the extracts of *Colocasia esculenta*

Extract	Water	Ethanol
Alkaloids	Positive	Positive
Carbohydrates		
Molisch's Test	Positive	Positive
Fehlings Test	Positive	Positive
Benedicts Test	Negative	Negative
Tannins	Negative	Negative
Flavonoids Shinoda Test	Positive	Positive
Saponins	Negative	Negative
Fixed Oils Spot Test	Negative	Negative
Glycosides	Positive	Positive

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.

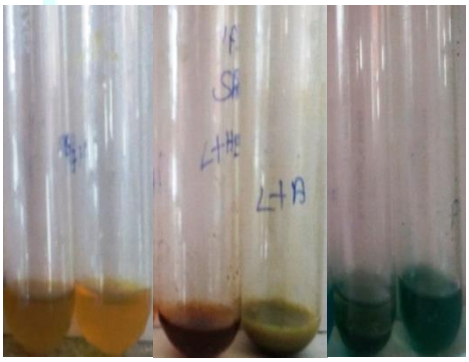

Figure 2: Phytoactives

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. The total flavonoids were estimated in both the extracts and found to be 31.0 mg/ml in ethanol and 29.0 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. The reducing power was estimated in both the extracts and found to be 700 mg/ml in ethanol and 800 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, anti mutagenic, anti carcinogenic 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. The total phenolics was estimated in both the extracts and found to be almost the same of about 26.5 mg/ml in ethanol and 26.2 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. DPPH was estimated in both the extracts and was found to be almost the same of about 46 mg/ml in Ethanol and 47.6 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. The total proteins were estimated in both the extracts and was found to be 199 mg/ml in ethanol and 180 mg/ml in water extract.

Carbohydrate Estimation

Carbohydrates are an essential compound of all organic life on this planet. Both plants and animals use carbohydrates as 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. The carbohydrates were estimated in both the extracts and was found to be 156.0 mg/ml in ethanol and 135.7 mg/ml in water extract.

CONCLUSION

C. esculenta is one of the important tuber crop and staple root grown for various purposes. Taro leaves provide a number of desirable nutritional and health benefits. In the present sample, Obtained from the Western Ghats of Karnataka, the leaves were screened for the antioxidant and photoactive potential in the aqueous and ethanol extracts. The studies revealed the significant antioxidant and phytochemicals in the ethanolic extract of the leaves.

ACKNOWLEDGEMENT

The authors express their sincere thanks to Chairman and Faculties of Department of Microbiology and Biotechnology. Many thanks to Shri.Gopala Krishna Bhat, Retd. Prof. Udupi, Mrs. Prasanna Bhat, Udupi, Dr.K.P.Sreenath, Retd. Prof. Bangalore University and Dr. Rama Rao, Regional Ayurveda Research Institute for Metabolic Disorders, Bengaluru for their timely support. Also thanks to Dr. V. Palani, Ms. Parvathy. S for their support throughout the work.

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