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SECONDARY METABOLITES AND NUTRITIONAL VALUE PROFILING OF *COLOCASIA ESCULENTA*

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ABSTRACT

Colocasia esculenta is a green leafy vegetable which is the rich source of proteins, carbohydrates, dietary fibers and vitamins as well as minerals like iron, potassium, sodium, zinc etc. It is also recognized for its color, flavor and therapeutic value. Green leafy vegetables play an important role in management of various diseases because of it's inability to cause side effects. The present study was designed to investigate the nutritional value, antioxidant activity, haemolytic activity, antibacterial properties and phytochemical components of the *Colocasia esculenta*. Qualitative analysis indicates the presence of alkaloids, saponins, phenols and coumarin. The leaf extract showed presence of high antioxidant and haemolytic activities. The plant extract exerted a better antifungal activity as compared to antibacterial activity. The present study revealed that, the vegetable is good source of macro-nutrients and micro-nutrients. Hence regular consumption of these vegetables can meet the nutritional requirements to overcome the micronutrient malnutrition at minimum cost.

KEYWORDS: Phytochemicals, Antioxidants, Haemolytic, Nutritional value

INTRODUCTION

Colocasia esculenta is one of the few major staple foods where both the leaf and underground parts are important in the human diet [1,2]. It has commonly known as Alu. Opara reported that leaves of *Colocasia* are an excellent source of carotene, potassium, calcium, phosphorous, iron, riboflavin, thiamine, niacin, vitamin A, vitamin C and dietary fiber. Its edible corms and leaves are traditionally used for hepatic ailments. The large green leaves often described as 'elephant ear' and they can reach up to 1-2 m high during growth.

The starchy, tuberous root is the main edible part of the crop; however the leaves are also used as a leafy vegetable. *Colocasia esculenta* leaves have been reported to be rich in nutrients including minerals and vitamins such as calcium, phosphorous, iron, vitamin C, thiamine riboflavin and niacin. *Colocasia esculenta* and *Colocasia fontanesii* are the vegetables which belong to family Araceae. These vegetables are found mostly in moist areas. *Colocasia esculenta* found in all seasons but *Colocasia fontanesii* is common in rainy season.

Scientific Classification:

Kingdom - Plantae

Order –Alismatales

Family – Araceae

Subfamily – Aroideae

Tribe – Colocasiodeae

Genus – *Colocasia*

Species – *C. esculenta*

MATERIALS AND METHODS

Collection of plant material

Collection of plant material was done during July to August 2015 from the Kelye village, Ratnagiri.

Preparation of extract

Aqueous extract and methanolic extract of plant leaves are used for the analysis.

Aqueous extract:

The fresh leaf material of *Colocasia esculenta* collected were washed thoroughly with running tap water and air dried under shade. After complete shade drying, the plant material was ground and the powder was kept in small plastic air-tight containers with paper labeling. 1 gm of the ground material was mixed in 100 ml of distilled water, boiled at 80-100 °C for 10 minutes and was filtered through Whatman No.1 filter paper. The filtrate was used for the phytochemical screening.

Methanol extract of plant:

Methanolic extracts of 10% of leaves, bark, flower and root were prepared by using same procedure of aqueous extract.

Determination of nutritional value

Carbohydrate content was determined by DNSA method. Protein content was determined by Folin-Lowry's method. Vitamin C content was determined by titration using DCPIP indicator. Moisture content was determined by oven dried method and fiber content was determined by acid base digestion method.[3,4]

Sodium and potassium contents were analyzed by using flame photometer (Elico, India) and zinc and iron content was analyzed using atomic absorption spectroscopy (Model no. SL 168, Elico, India).

Phytochemical screening

Confirmative tests for various phytochemicals such as saponin, tannin, phlobatannin, alkaloids, flavonoids, quinones, anthraquinones, phenols, terpenoides, coumarins, cardiac glycosides and anthocyanins are done using different chemical tests.[4-7]

Quantitative phytochemical analysis

Alkaloid, total saponinin, total phenol and total flavonoid content was determined by standard methods.[7,8,9]

Determination of antioxidant activity.[3,6,10]

Antioxidant activity was determined by using DPPH radical scavenging assay. For this 0.2 mM DPPH was used as standard. Antioxidant activity was calculated as % free radical scavenging activity, given by:

Control O.D–Sample O.D/Control O.D* 100

Determination of haemolytic activity

% Haemolytic activity was determined by using method of Malagoli [8]. Sterile PBS was used as negative control i.e. blank and 0.1% triton X was used as positive control. % Haemolytic activity was calculated using the formula:

Sample O.D- Blank O.D/O.D of positive control*100

Antimicrobial properties of methanolic extracts

Agar cup method was used for study of antimicrobial properties of *Colocasia esculenta*. Both bacterial and fungal cultures are used for this study. Methanolic extract of green leafy vegetables was used to study antimicrobial properties. *Candida albicans, E.coli, S.aureus, Pseudomonas aeruginosa, S.pyogenes, S.typhi, Klebsiella pneumoniae, S.paratyphi B, B. subtilis, M. furfur, S. faecalis* and *Penicillium chrysogenum* were used as test cultures. Cultures were procured from Microbial Culture Collection of Department of Microbiology, Gogate Jogalekar College, Ratnagiri. The test cultures were grown overnight in nutrient broth and used for determination of antimicrobial activity of selected plant extracts.

Antimicrobial activity of standard antibiotic *i.e.* Streptomycin (30µg/ml) was also determined in parallel experiment as a positive control and respective solvents as negative control.

Thin layer Chromatography of extracts

Thin layer chromatography was carried out for the separation of plant compounds responsible for antioxidant and antimicrobial properties [5]. Chloroform: glacial acetic acid: methanol: water (6:2:1:1) was used as solvent system. The spots obtained after developing the plates were further analysed.

Characterization of separated compounds

Characterization of spots obtained by TLC was done by using UV visible spectroscopy (Chemito Spectrascan UV 2600, India) and FTIR spectroscopy (Perkin Elmer, UK).

RESULTS

Nutritional value analysis

Nutritional value analysis of *Colocasia esculenta* is evaluated in Table 1. Vitamin C content was higher than the carbohydrate, protein and dietary fibers while potassium content was highest *i.e* 2.4 ppm as compared to the other minerals. It contains high amount of moisture. However the carbohydrate content in *Colocasia esculenta* is quite low. The protein content is

also found to be on the lower side. The vegetable has a very high Vitamin C content and hence is a good source of the vitamin through diet. The high moisture content provides a greater activity of water soluble enzymes and coenzymes needed for metabolic activities of these leafy vegetables. The analysis shows presence of high moisture content. Though nutritionally beneficial, the moisture content reduces the storage time or shelf life of the vegetable. So has to be cooked when fresh. High amount of fibre and also high content of the different minerals ions indicates that *Colocasia esculenta* is rich in micro nutrients.

Table 1: Nutritional value profile of *Colocasia esculenta*

Parameters	gm/100gm
Carbohydrate	10
Protein	12
Vitamin C	66.6
Moisture	88.9
Dietary Fibers	45
Potassium	2.40 ppm
Sodium	4.50 ppm
Iron	0.11 ppm
Zinc	0.006 ppm

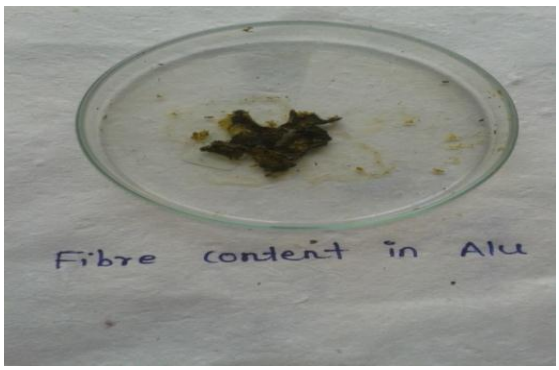


Figure 1: Dietary fiber content in *Colocasia esculenta*

Qualitative phytochemical screening

Colocasia esculenta show presence of saponins, alkaloids, phenols and coumarin (Table 2). Phlobatannins, tannins, flavonoids, terpenoids, anthraquinones, quinones, cardiac glycosides and anthocyanins are completely absent in *Colocasia esculenta*. Phytochemicals can have complementary and overlapping action including antioxidants, modulation of detoxification enzymes, stimulation of immune system, reduction of inflammation, modulation of steroid metabolism, antibacterial and antiviral effects in humans. The presence of these secondary metabolites has contributed to its medicinal value as well as physiological activity.

Table 2: Phytochemical screening of *Colocasia esculenta*

Phytochemical	Observation
Saponin	+
Phlobatannin	-
Tannin	-
Flavonoid	-
Alkaloid	+
Terpenoid	-
Anthraquinone	-
Phenol	+
Quinones	-
Coumarin	+
Cardiac glycosides	-
Anthocyanin	-

(2a) (2b) (2c) (2d)

Figure 2: Phytochemical tests for *Colocasia esculenta* shows presence of (2a) saponins, (2b) phenols, (2c) alkaloids and (2d) coumarin

Quantitative phytochemical analysis

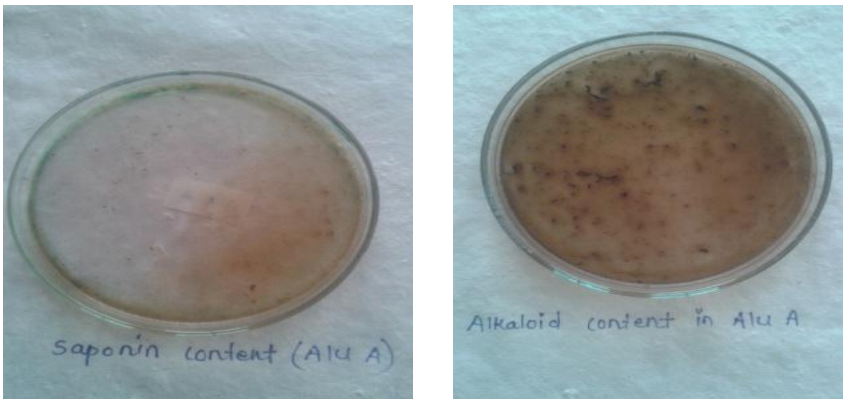
Results of quantitative analysis of phytochemicals are evaluated in Table 3. *Colocasia esculenta* shows high amount phenols and saponins as compared to other phytochemicals. Phenol and saponin concentration is more as compared to the alkaloid content. Saponins are structurally diverse compounds and have been observed to kill protozoans and molluscs, have antioxidant activity, to impair the digestion of protein and the uptake of vitamins and minerals in the gut, to cause hypoglycemia, and to act as antifungal and antiviral.

Varied biological activities of phenolic acids have been reported. Increase in bile secretion, reduction in blood cholesterol and lipid levels and antimicrobial activity against some strains

of bacteria such as *Staphylococcus aureus* are some of biological activities of phenolic acids. Phenolic acids possess diverse biological activities, for instance, antiulcer, anti-inflammatory, antioxidant, cytotoxic and antitumor, antispasmodic and antidepressant activities.

Table 3: Quantitative phytochemical analysis of *Colocasia esculenta*

Phytochemical	Percentage (%)
Alkaloids	05
Phenols	17
Saponin	17



(3a) (3b)

Figure 3: (3a) Saponin and (3b) alkaloid content in *Colocasia esculenta*

Antioxidant activity

Colocasia esculenta showed increase in antioxidant activity as concentration of extract increases (Figure 4). *Colocasia esculenta* show higher antioxidant activity than the standard ascorbic acid. Antioxidants regulate various oxidative reactions naturally occurring in tissues and are evaluated as a potential anti-aging agent. Hence, antioxidants can terminate or retard the oxidation process by scavenging free radicals, chelating free catalytic metals and also by acting as electron donors. Antioxidants have been widely used as food additives to provide protection from oxidative degradation of foods and oils. Hence, antioxidants are used to protect food quality mainly by the prevention of oxidative deterioration of constituents of lipids.

Colocasia esculenta show 30% antioxidant activity for 0.1 mg/ml, 46% for 0.2 mg/ml, 54% for 0.4 mg/ml, 66% for 0.6 mg/ml, 78% for 0.8 mg/ml and 82% for 1.0 mg/ml concentration.

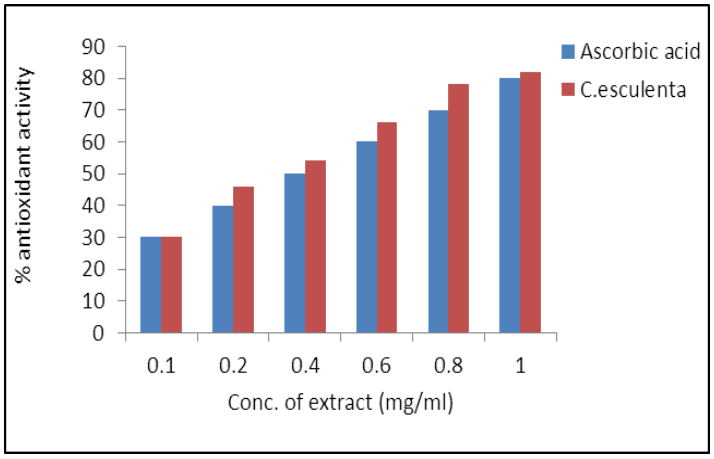


Figure 4: Graph of concentration of extract (mg/ml) against % antioxidant activity of *Colocasia esculenta*

Haemolytic activity

Haemolytic activity of leafy vegetables increased with increasing concentration (Figure 5). *Colocasia esculenta* does not show haemolytic activity at 50 µg/ml and 100 µg /ml concentration, while at 1000 µg/ml concentration, it shows highest haemolytic activity. All the vegetables exhibited very low haemolytic effect toward human erythrocytes. However, these extracts showed dose dependant increase in haemolytic activity. The result of this study concludes that the aqueous extracts from the vegetables are non/less toxic to the human erythrocytes.

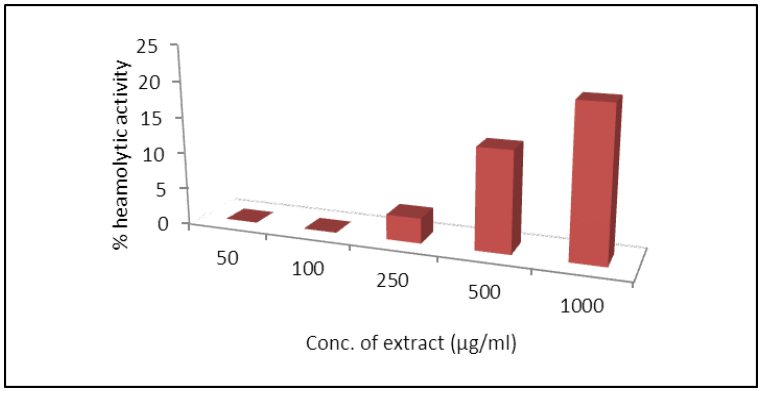


Figure 5: Graph of concentration of extract of *Colocasia esculenta* against % haemolytic activity of *Colocasia esculenta*

Antimicrobial properties

Out of the 12 selected test organisms only 3 organisms are sensitive to methanolic extract of *Colocasia esculenta* (Table 4). *Colocasia esculenta* show highest zone of inhibition against

Candida albicans as compared to *S. aureus* and *S. pyogenes*. Thus, most of the test organisms were resistant to the plant extracts.

Table 4: Antimicrobial activity of *Colocasia esculenta*

Test organism	Zone of inhibition (mm)
E.coli	-
S. aureus	13
S. pyogenes	13
K. pneumoniae	-
S. feacalis	-
B. subtilis	-
S. typhi	-
Penicillium	-
C. albicans	16
M. furfur	-
S. paratyhi B	-
P. aeruginosa	-

Thin layer chromatography

Thin layer chromatography for *Colocasia esculenta* extract showed 2 different spots of orange and green colour as shown in Figure 6. Rf value for orange spot was 0.60 and for green spot, Rf value was 0.80. The methanolic extract thus shows presence of two compounds which may be the plant pigments.

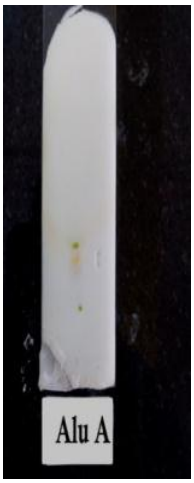


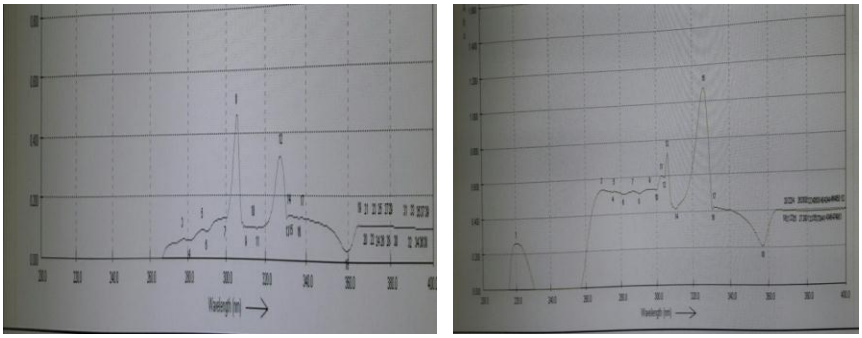
Figure 6: Thin layer chromatography profile for *Colocasia esculenta*

Characterization of separated compounds

UV visible spectroscopy and FTIR spectroscopy are used for the characterization of separated compounds.

a) UV visible spectroscopy

UV spectrum of orange spot of *C. esculenta* show λ max at 326 nm and green spot show λ max at 327.4 nm and it was very close to 316 nm which was of standard betulin in methanol extract.

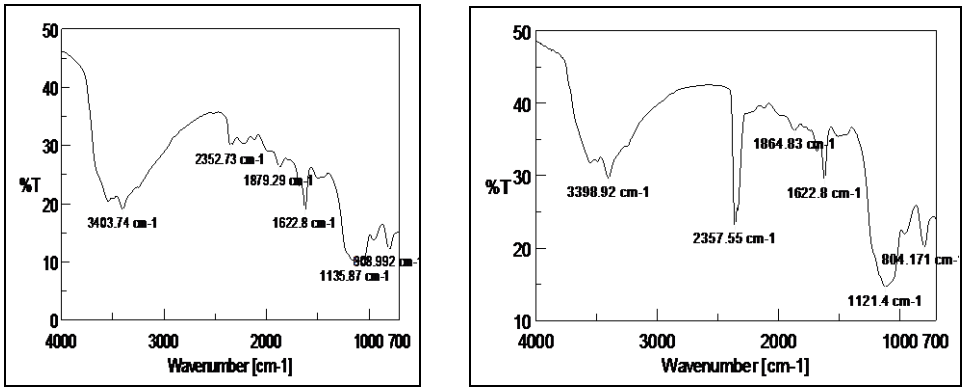


(7a) **(7b)**

Figure 7: (7a) UV visible spectrum of orange spot of *Colocasia esculenta* shows λ max at 326 nm and (7b) UV visible spectrum of green spot of *Colocasia esculenta* shows λ max at 327.4 nm

b) FTIR spectroscopy

FTIR spectrum for orange band of *Colocasia esculenta* show peak values at 3403.74, 2352.73, 1879.29, 1622.8, 1135.8 cm^{-1}. This indicates presence of functional groups like alcohols, phenols, carboxylic acid, acid anhydride, nitro compounds, alcohols, carboxylic acids, etc. FTIR spectrum for green band of *Colocasia esculenta* show peak values at 3398.92, 2357.55, 1864.83, 1622.8, 1121.4 cm^{-1}. This indicates presence of functional groups like alcohols, phenols, 1° and 2° amines, amides, aliphatic amines etc.



(8a) **(8b)**

Figure 8: (8a) FTIR spectrum for orange spot of *Colocasia esculenta* & (8b) FTIR spectrum for green spot of *Colocasia esculenta*

From this study, we can conclude that *Colocasia esculenta* is a good source of macro-nutrients and micro-nutrients. Regular consumption of the vegetable can meet the nutritional requirement to overcome the micronutrient malnutrition at minimum cost.

Total phenolic content had positive correlation with antioxidant capacity. It was observed that the leaf extract contain high level of phenolic content that might have accounted for strong activity observed against DPPH radicals.[11] Thus the finding of this study suggests that plant leaves could be a potential source of natural antioxidant that would have great importance as therapeutic agent. The consumption of these may play a role in preventing human diseases in which free radicals are involved such as cancer, cardiovascular diseases and aging.[12]

The phytochemical contents of the leafy vegetables serve as supplements for food and have the potential to improve the health status of its users through their anti-microbial properties. The phytochemical screening of plants shows that they are rich in alkaloid, flavonoids. It has been noted that the presence of phytochemical compounds in these plants are responsible for the observed biological activities including antibacterial, antiviral, and anti-diabetic properties of these plants.

Our data indicates that the green leafy vegetables studied are potential sources of secondary metabolites and methanolic extracts possess good antioxidant activity. The presence of secondary metabolites that are biologically important and contributes to its medicinal value and thus can be a potential source of useful drugs. Further studies are needed to evaluate the *in vivo* potential of these extracts in animal models and also isolation and characterization of the active antioxidant compounds. Determination of the antioxidant compounds in plant extracts will help to develop new drug supplement for antioxidant therapy.

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