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Physicochemical and Phytochemical Analysis of *Colocasia esculenta*: a promising nutritional Food plant

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Abstract

Colocasia esculenta(family Araceae) is a green leafy vegetable that is a rich source of proteins, carbohydrates, dietary fibers, and vitamins as well as minerals like iron, potassium, sodium, zinc, etc. The aim of study to analyze the physicochemical and phytochemical characteristics of *Colocasia esculenta* in aqueous and methanolic extracts. Ash value and extractive value of the drug was determined as per standard procedure. Using Soxhlet equipment the extract was prepared from powdered leaves and tubers of *Colocasia esculenta* for 20 hours using water and methanol as solvents. The percentage yield of methanol extract of Leaves and tubers was found to be 12.4 & 5.1% while aqueous extract was 15.2 & 20 % respectively. Phytochemical analysis of *Colocasia esculenta* extract revealed the presence of alkaloids, glycosides, flavonoids, terpenoids, saponins, and phenols. It was found that the phytochemical constituents are very much enriched in the *Colocasia esculenta* extract and can be used for the development of new formulations. Thus, it can be concluded that *Colocasia esculenta* is a staple food and have a significant number of phytochemicals, thus recommended for the pharmaceutical industry.

Keywords: *Colocasia esculenta*, Physicochemical, Phytochemical

Introduction

Nature is always a shining example of the long-standing phenomena of symbiosis. All the biotic and abiotic factors are interconnected. Plants are necessary for man's survival. Nature has offered a vast array of cures to treat all of humanity's diseases. As a result of man's inquisitive inclination, drug knowledge has gathered over thousands of years, and we now have numerous effective techniques for assuring a healthy status.

Taro (*Colocasia esculenta* Linn.) is a vegetative propagated tropical root having its origin in Southeast Asia. It occupies 9th position among world food crops with its cultivation spread across Africa. Taro tubers are important sources of carbohydrates as an energy source and are used as staple foods in tropical and subtropical countries. It is largely produced for its underground corms and contains 70–80% starch. There are numerous root and tuber crops grown in the world. Taro is one such crop grown for various purposes. It is an erect herbaceous perennial root crop widely cultivated in the tropical and subtropical world belonging to the genus Colocasia in the plant family Araceae **(Macharia WM, et.al 2014).** To meet global healthcare needs, traditional medicine systems have played a very important role. Indian System of Medicine is one of the largest and oldest systems constitutes all the medicines that have their origin in India. Plants have played a very vital role in sustaining and improving the deity of human life and have served humans well as important components of medicines, seasoning, beverages, cosmetics, and dyes. Due to this fact, the focus on plant research has increased all over the world. Here are any herbs that are used to treat liver, cardiovascular, digestive, Metabolic, and central nervous system (CNS) disorders. Medicinal plants or herbal drugs and their extracts containing isolated compounds have showcased a wide spectrum of biological activities **(Pawar, H.A., et.al 2018)** of such plant with wide applications is C. esculenta. The herb has been known since ancient times for its curative properties and has been used for the treatment of various ailments including asthma, arthritis, diarrhea, internal hemorrhage, neurological disorders, and skin disorders. **(Meredith, P. A. et.al)** The juice of the c. antiquarian corm is widely used for the treatment of body achesand baldness.

Plant profile

Colocasia esculenta (taro root) is a perennial herb (family Araceae) with yellow-orange flowers and large heart-shaped leaves found in the Central Valley of California. It is native to eastern Asia. It favors grasslands, wetlands, and bog and marsh habitats. It spreads via seeds and vegetative corms and is sometimes cultivated as a crop. Seeds are dispersed by water and agricultural activities.

Plant description

Classification:

Kingdom **:** Plantae.
Division **:** Tracheophyta
Class **:** Liliopsida.
Oder : Alismatales.
Family **:** Araceae.
Genus **:** Taro.
Species **:** *Colocasia esculenta.*
Binomial name: *Colocasia esculenta* L.

Materials and Methods

Dried *Colocasia esculenta* was size reduced to mesh size #40 and used for physicochemical analysis. The powder is stored in a closed container and utilized for extraction. The dried powder of *Colocasia esculenta* leaves & tuber was standardized for physicochemical parameters as per standard methods **(Khandelwal, 2008)**

Physicochemical evaluation of crude drug

The following procedures were used to determine the different ash values and extractive values of *Colocasia esculenta* powder.

Determination of ash values

The purpose of determining ash values is to discover adulterants that are exhausted and sandy or earthy particles. It can also be used to detect chemical components using water-soluble and acid-insoluble ash.

Total ash:

The total ash was calculated by incinerating the fine powder of crude drug (2 g) in a tarred silica crucible at 450 °C until the carbon was completely removed. After that, the ash was allowed to cool before being weighed. The weighted value of ash and powdered crude drugs were used to calculate the percentage of total ash.

Water soluble ash:

The ash obtained from the total ash process was combined with 25 ml of water to determine the water-soluble ash value. The mixture was filtered, collected, and weighed on the filter paper. The water-soluble ash value was calculated by subtracting the weighed amount of insoluble matter from the weighed amount of ash. The percentage of water-soluble ash value was calculated using this weighted quantity.

Determination of extractive values

Alcohol soluble extractive value: In a stoppered conical flask 5 g of coarsely powdered was macerated with 100 ml of ethanol for twenty-four hours shaking regularly during the first six hours and allowing it to stand for eighteen hours. To avoid solvent loss, it was quickly filtered, and 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish and dried at 105 °C to a consistent weight. The proportions of alcohol-soluble extractive values were calculated using the air-dried crud drugs as a reference.

Water soluble extractive value: In a stopped conical flask 5 g of coarsely powdered was macerated with 100 ml of chloroform-water for twenty-four hours shaking regularly during the first six hours and allowing it to stand for eighteen hours. To avoid solvent loss, it was quickly filtered, and 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish and dried at 105 °C to a consistent weight. The proportions of water-soluble extractive values were calculated using the air-dried crud drugs as a reference.

Loss on drying: The approach provided was used to calculate the loss on drying. A measured amount of dried powder was poured into a weighed petri dish. The petri dish was placed in the oven and weighed at various intervals at 105° C until two consecutive weighs did not deviate by more than 0.25 mg, indicating the drug's final loss of moisture. The percentage loss on drying was estimated using the formula below.

LOD (%) = (Weight of porcelain dish with the drug at time 0-Weight of porcelain dish after 6 h)/(Weight of porcelain dish at time 0-Weight of empty porcelain dish)

Table 1: Quantitative standards.

Parameter	Results
Total ash	Not more than 9 %
Acid insoluble ash	Not more than 1 %
Water soluble ash	Not more than 6.5%
Water soluble extractive value	Not less than 11.2%
Ethers soluble extractive value	Not less than 14.4%
Alcohol soluble extractive value	Not less than 12 %
Moisture content	Not more than 4.5%

Phytochemical Screening of *Colocasia esculenta*

Qualitative phytochemical screenings of leaves & tuber of *Colocasia esculenta* were performed to investigate the presence of major chemical classes of components e.g. alkaloids, flavonoids, glycosides, tannins, phenols, and steroids using the following qualitative chemical tests **(Kokate, 1991).**

Test for alkaloids

A little quantity of the solvent-free extract was filtered after being agitated with a few drops of dil. hydrochloric acid. Mayer's reagent (cream ppt), Hager's reagent (yellow ppt), Wagner's reagent (reddish brown ppt), and Dragendroff's reagent (reddish brown ppt) were used to test the filtrate for the presence of alkaloids (orange brown ppt).

Tests for carbohydrates

A little amount of the extract was diluted in 4 ml of distilled water and filtered separately. Molisch's and Fehling's tests were used to determine the carbohydrate present in the filtrate.

Molisch's test: 2-3 drops of 1 percent alcoholic alpha-naphthol solution were added to the filtrate and 2 ml of Conc. Sulphuric acid was poured from side of the test tube. The presence of carbohydrates was shown by the appearance of a brown ring at the intersection of two liquids.

Fehling's test: Extract was stored in the water bath A and B Fehling solutions were mixed. The presence of reducing sugars was visible in the brick-red precipitate.

Tests for glycosides

Another portion of the extract was hydrolyzed with hydrochloric acid for a few hours in a water bath and the hydrolysate was tested for the presence of various glycosides using Legal's and Bontrager's tests.

Legal's test: 1 ml pyridine and a few drops of sodium nitroprusside solutions were added to the hydrolysate, which was then made alkaline with sodium hydroxide solution. The presence of glycosides was indicated by the appearance of a pink-to-red tint.

Bontrager tests: The chloroform layer was removed from the hydrolysate after it w treated with chloroform. An annual amount of weak ammonia solution was added to this. The ammonia layer turns pink, indicating that glycosides are present.

Test for saponins

With 20 ml of distilled water, the extract was dissolved and agitated for 15 minutes. The presence of saponins was demonstrated by the creation of a 1 cm layer of foam over time.

Tests for flavonoids

With sodium hydroxide: 1 ml sodium hydroxide solution was added to the extract. Anthocyanins are found in blue to violet colors, Flavanones are found in yellow to orange colors and flavones are found in yellow.

Concentrated sulphuric acid: Concentrated sulphuric acid was added to the extract. The presence of anthocyanin is indicated by a yellow-orange, while the presence of flavones is indicated by an orange-to-red tint.

Shinoda test: Extract was dissolved in ethanol and magnesium turnings were added to perform Shinoda's test concentration of hydrochloric acid was added to this combination. The presence of flavonoids is indicated by a change in hue from magenta to purple.

Test for mucilage

Small amounts of the extract were added individually to 25 ml of pure alcohol and filtered while constantly stirring. The precipitate was dried in the air and analyzed for the presence of mucilage as well as its swelling qualities.

Test for phytosterol

The extract was heated in a solution of alcoholic potassium hydroxide until it was completely saponified. Ethyl ether was used to dilute the mixture and extract it. The ether layer was evaporated, and the residue was examined for phytosterol .

Liebermann-Burchard test: The residue was dissolved in a few drops of diluted acetic acid, followed by 3 ml of acetic anhydride and a few drops of concentrated sulphuric acid. The presence of phytosterol was shown by the presence of a bluish-green tint.

Test for phenolic compounds and tannins

Small amounts of the extract were separated in water and tested for the presence of phenolic compounds and tannins using the reagents listed below.

Dilute Ferric chloride solution (5%)-Violet color, 1% solution of gelatine containing 10% sodium chloride - White ppt, 10% lead acetate solution - White ppt.

Estimation of phytochemical standards

Estimation of total phenolic content

Total phenolic contents (TPC) in the leaves & tuber extracts were determined by Folin–Ciocalteu colorimetric method as described by **Singleton et al** with some modifications. The standard gallic acid solution was prepared by dissolving 10 mg of it in 10 mL of methanol (1 mg/mL). Various concentrations of gallic acid solutions in methanol (20, 40, 60,80, and 100 µg/mL) were prepared from the standard solution. To each concentration, 5 mL of 10% Folin–Ciocalteu reagent (FCR) and 4 mL of 7% Na_2CO_3 were added making a final volume of 10 mL. Thus, the obtained, blue-colored mixture was shaken well and incubated for 30 min at 40°C in a water bath. Then, the absorbance was measured at 760 nm against blank. The FCR reagent oxidizes phenols in plant extracts and changes into a dark blue color, which is then measured by UV-Vis spectrophotometer. All the experiments were carried out in triplicates, and the average absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve.

Estimation of total flavonoids content

Total flavonoid contents in the extracts were determined by aluminum chloride colorimetric assay. A stock solution (4 mg/mL) of quercetin was prepared by dissolving 4 mg of quercetin in 1 mL of methanol. This standard solution was diluted serially to make various concentrations of 0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL, and 1 mg/mL solutions. 1 mL quercetin of each concentration was added to the test tube containing 4 mL of distilled water. At the same time, 0.3 mL of 5% $NaNO_2$ was added to the test tube, and 0.3 mL of 10% $AlCl_3$ after 5 min. Then, 2 mL of 1 M NaOH was added to the mixture after 6 min. The volume of the mixture was made to 10 mL by immediately adding 4.4 mL of distilled water. The flavonoid content was expressed as quercetin equivalents using the linear equation based on the calibration curve. Stock solutions of 4 mg/mL concentration in methanol of the extracts were prepared, and they were diluted serially to make different concentrations (0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL, and 1 mg/mL) solutions. A similar procedure as described for quercetin was followed for the extracts also, and the absorbance was measured by spectrophotometer at 510 nm. Readings were taken in triplicate, and the average value of absorbance was used to calculate the total flavonoid content. The flavonoid content was expressed as quercetin equivalent (mg QE/g) using the linear equation based on the standard calibration curve. **(Singleton et al)**

Results and Discussion

Organoleptic characters of Colocasia esculenta

Table 2: Organoleptic characters of *Colocasia esculenta*

S. No	Parameters	Colocasia leaves	Colocasia tuber
1	Odor	Sweet smell	Sweet smell
2	Color	Greenish	Whites
3	Taste	Sweet	sweet
4	Consistency	Solid – powder	Solid-powder

Table 3. The percentage yield of extract preparation.

S. No	Content	Solvent	Color	Type of extract	Consistency	% Yield
1	*Colocasia esculenta* Leaves	Aqueous	Greenish Brown	Crude	Solid	15.2
2		Methanol	Brown	Crude	Solid	12.4
1	*Colocasia esculenta* tuber	Aqueous	Whites	Crude	Solid	20.4
2		Methanol	whites	Crude	Solid	5.10

The extraction of the crude drug of Colocasia esculenta was carried out as per the methodology given in Ayurvedic Pharmacopoeia's . The percentage yield of crude extract was found to be more in methanol when compared to that of the aqueous solvent. The percentage yield of methanol extract is found to be 5% & 21%. This shows that ethanol diffuses and solubilizes more phytochemical constituents when compared to distilled water.

Physicochemical parameters

The physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, loss on drying, and foreign and pH of the aqueous

Table.4.Physicochemical evaluation of *Colocasia esculenta*

S. No	Parameters	Result	
		Leaves	Tuber
1	Total ash	9 %	16.33 %
2	Acid insoluble ash	1 %	9.5 %
3	Water soluble ash	6. 5%	12.5 %
4	Water soluble extractives	11.2 %	18.4 %
5	Alcohol soluble extractives	12.0 %	1.6 %
6	Ether soluble extractives	14.4 %	2.4 %
7	Loss on drying	4.50 %	2.5 %
8	pH (aqueous solution)	6.8	6.7

Phytochemical screening:

The phytochemical constituents such as alkaloids, tannins, phenols, saponins, and carbohydrates are identified in both aqueous and methanol extracts of *colocasia esculenta*. Also, flavonoids, saponins, and alkaloids are present in methanol extract but not found in aqueous extract. So, the methanolic extract is used for the further research.

Table 5: Phytochemical screening of *Colocasia esculenta*

Name of tests	Leaves		Tuber	
	Aqueous extract	Methanolic extract	Aqueous extract	Methanolic extract
Test for Alkaloids				
Dragendroff's	++	++	++	++
Mayer's	++	++	++	++
Hagers	++	++	++	++
Wagners	++	++	++	++
Test for Flavonoids				
With sodium hydroxide	+	+	+	+
With conc. sulphuric acid	+	++	++	++
Shinoda	++	+	+	+
Test for Tannins				
$FeCl_3$	-	-	-	-
Lead acetate	-	-	-	-
Test for Phenols				
Lead acetate	+	+	+	+
Gelatin test	-	+	+	+
Test for Saponins				
Foam test	++	++	++	++
Test for Terpenoid				
Salkowski	+	+	+	+
Test for Carbohydrates				
Molisch's test	+	+	+	-
Fehling's test	+	-	+	-

Absence (-), Presence (+), Good amount (++)

Estimation of phytochemical constituents

The major phytochemical constituents present in this Colocasia crude drugs are believed to be total flavonoid &total phenol. The presence of total flavonoid, gallic acid equivalent for total phenol, and ascorbic acid can be used to identify these phytochemical constituents. The total phenol was estimated by the Folin-denis method and the Folin-ciocalteu method, respectively. The flavonoid was estimated by a colorimetric assay. The amounts of total flavonoids & total phenol were found to be 6.88±0.005 & 19.25±0.004 in leaves aqueous extract, and 3.15±0.004 & 3.85±0.001 of the tuber aqueous extract. When methanol extract is compared to aqueous extract, the number of phytochemical phenolic constituents was found to be higher in methanol extracts. While flavonoid compounds are higher in aqueous extract.

Table 6: Estimation of phytochemical standards in *Colocasia esculenta* extract.

	Herbal drug name	Solvent used	Total flavonoid (mg)	Total phenolic (mg)
1	*Colocasia esculenta leaves*	Aqueous	6.88±0.005	19.25±0.004
2		Methanol	1.79±0.009	325±0.003
1	*Colocasia tuber*	Aqueous	3.15±0.004	3.85±0.001
2		Methanol	0.14±0.001	115±0.005

Values are expressed as a mean standard deviation; * per 100 g of dry crude drugs.

Conclusion

Colocasia esculenta showed a better extractive value in methanol than water suggesting that the phytoconstituents would be more concentrated in methanolic extract. The methanolic extract of *Colocasia esculenta* showed the presence of Protein, alkaloids, flavonoids, tannins, phenols & saponins. It was found that the phytochemical constituents are very much enriched in the *Colocasia esculenta* extract and can be used for the development of new formulations.

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