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Biochemical and phytochemical analysis of *colocasia esculenta* (L.) Schott tubers

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Abstract
Colocasia esculenta (L.) Schott belongs to the family Araceae. Nutritional value and preliminary phytochemical analysis of *Colocasia esculenta* dried tubers were determined. The tubers is used as a vegetable and considered as a good source of carbohydrate, protein and starch. Nutritional analysis showed that moisture content is (56.8%), ash content (1.22%), carbohydrate (3000 mg/100gm), protein (824 mg/100gm) and starch (2700 mg/100gm) in dry tubers. The tubers can be consumed by baking, roasting, steaming or boiling. It can be fried, preserved, dried, made in to flour or consumed in many other ways. Phytochemical analysis of revealed the presence of alkaloids, glycosides, flavonoids, terpenoids, saponins and phenols. The tubers are applied locally to painful rheumatic joints, to treat tuberculosis and pulmonary congestion. Alkaloids are also used in medicine for reducing headache and fever. Glycosides are naturally cardioactive drugs used in the treatment of congestive heart failure. Flavonoids are free radical scavengers, super antioxidants and potent water soluble which prevent oxidative cell damage and have strong anti-inflammatory action. Thus it can be concluded that *Colocasia esculenta* is a staple food and also have significant amount of phytochemicals, thus recommended for pharmaceutical industry.

Keywords: *colocasia esculenta*, biochemical, schott tubers

Introduction
Traditional knowledge of medicine has long been used since ages for curing various human ailments. About 60-80% world populations still rely on plant based medicines. Through the traditional Indian systems of medicine has a long history of use, yet they lack adequate scientific knowledge (Shrivastava *et al*., 2010) [8]. Through phytochemical screening one could detect the various important compounds which could be used as the base of modern drugs that curing various diseases (Nilofer *et al*., 2013) [3].

Plant – derived substances have recently become of great interest owing to their versatile applications. The medicinal importance of plant is due to the presence of chemical constituents like alkaloids, glycosides, resins, volatile oils, gums, tannins etc. These compounds are synthesized by primary or rather secondary metabolism of living organisms (Yadav and Agarwala 2011) [13]. Phytochemicals may have biological significance, for example carotenoids or flavonoids, but are not established as essential nutrients. The systematic screening of plant species (fig1) with the purpose of discovering new bioactive compounds is a routine activity in many laboratories (Parekh *et al*., 2006) [4].

The nutritional composition of taro tuber like other root crops is high in carbohydrate and protein but low in fat. It is a good source of potassium and provides moderate level of phosphorus. Taro tuber (fig1a) is a good source of minerals and the small granule size of its starch helps to increase the bioavailability of its nutrients due to efficiency of digestion and absorption (Standal 1970) [10]. Taro tuber has been reported to have 70–80% (dry weight basis) starch with small granules (Jane *et al*., 1992) [1]. Taro starch, in view of its small granule size, has also been used for industrial applications (Wang 1983) [12]. Taro starch is easily digestible, the starch grains are fine and very small, it has hypoallergenic nature (Kochhar 1998) [2] and also the starch is gluten free. For supplying nutrients, the tuber may be considered as a good source of carbohydrates and potassium. Taro also contains greater amounts of vitamin B-complex than whole milk (Soudy *et al*., 2010) [9].

The historic use and importance of taro can explain the reason for its significant implications in human health. Taro tubers are rich in starch and the tubers contain anthocyanins, cyanidin 3-glucoside. In common with flavonoids, the related anthocyanins are reputed to improve blood circulation by decreasing capillary fragility (Wagner *et al*., 1985) [11] to improve eyesight, to act as potent antioxidants, to act as anti-inflammatory agents, and to inhibit human cancer cell growth (Youdim *et al*., 2000) [14]. It is a well-known fact that traditional systems of medicines have always played important role in meeting the global healthcare needs. They are continuing to do so at present and shall play major role in future as well (Pritha Chakraborty *et al*., 2015) [6].

Fig 1: *Colocasia esculenta* (l.) Schott

Fig 1a: Tubers of *Colocasia esculenta*

Materials and methods

Collection of plant material

The fully mature tubers of *Colocasia esculenta* were collected from Kasargod district, Kerala during September 2016. The tubers were washed with distilled water and dried at room temperature. The dried tubers was manually grind to a fine powder. Fine powder of tubers was used for the physiochemical analysis.

Nutritive analysis

Moisture content

Fresh weight of the sample was determined and placed the sample in a hot air oven initially for one hour at 100ºC and then the dry weight of the sample become constant.

$$\text{Moisture content \%} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Total ash

About 5g of powdered tubers was accurately weighed and taken separately in silica crucible, which was previously ignited and weighed. The powder was spread as a fine layer on the bottom of crucible. The powder was incinerated gradually by increasing temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to air dried powder.

Extraction and estimation of total soluble carbohydrates

Extraction

200 mg sample dried tubers powder was suspended in 1:5(w/v) hot 70% ethanol and extracted for 10 minutes. The pellet was re-extracted twice with equal volumes of 70% of ethanol. The ethanol extracts were clarified by centrifugation, pooled and concentrated to 1-2 ml evaporations in vacuum. The concentrated ethanol extracts was diluted to 50 ml with distilled water.

Estimation

The total soluble carbohydrate from dried tubers powder were extracted and estimated by the anthrone reagent method of Yemm and Wills (1954) using glucose as a standard at 660 nm using a spectrometer. The average values were expressed on percentage on dry weight basis.

Extraction and estimation of total proteins extraction

Extraction

200mg sample of dried tubers powder were taken and was suspended in 1:5% (W/V) phosphate buffer. The extract was removed by centrifugation at 3000 rpm for 10 minutes. The pellet was washed with phosphate buffer twice and the defatted meal was washed with 100 ml of cold 10% trichloro acetic acid (TCA) and centrifuged at 3000 rpm for 15 minutes. The procedure was repeated, the resulting TCA-washed pellet was suspended in NaOH solution.

Estimation

The proteins separated were estimated following the method of Lowry *et al.,* (1951) after TCA precipitation, as described earlier. The values were expressed as percentage on dry weight basis.

Extraction and estimation of starch

Extraction

Air- dried tubers sample was suspended in 1:5 (W/V) hot 80% ethanol and extracted for minutes at 90˚C. The pellet was re-extracted twice with equal volume of hot 80% ethanol. The ethanol extracts were clarified by centrifugation, pooled and concentrated to 1-2 ml by evaporations in vaco. The concentrated ethanol extract was diluted to 50 ml with glass distilled water.

Estimation

From suitable aliquots of the above extract, total soluble carbohydrates were estimated by the anthrone reagent method using glucose as a standard at 620 nm in a spectronic 20D spectrometer. The values were expressed as percentage on dry weight basis.

Preliminary phytochemical screening

Preparation of plant extract

Each 15 gm. of air dried powder were taken in 50 ml of methanol and water. Plugged with cotton wool and then kept on a rotary shaker at 199-220 rpm for 48 hours. The solvent were evaporated to the final volume one-fourth of the original volume and stored at 4°C in air tight containers. The plant extract used for phytochemical analysis.

The condensed extracts were used for preliminary screening of phytochemical such as alkaloid, glycosides, carbohydrates, flavonoids, terpenes, saponins, phenols, tannins, quinones, and steroids.

Chemicals

Methanol, Ethanol, Mayer's Reagent, Glacial Acetic Acid, Ferric Chloride, Concentrated Sulphuric Acid, Concentrated Hydrochloric Acid, Molish Reagent, Ammonia, Chloroform, Distilled Water, iodine.

Test for Alkaloids
To reveal the presence of alkaloids, few drops of Mayer's reagent (potassium mercuric iodide) reagent were added to the extract, cream colour precipitate visualises the presence alkaloids.

Test for Glycosides
To 2 ml of extract, add 1 ml of glacial acetic acid, few drops of 5 % $Fecl_3$ and Conc. H_2SO_4 were added reddish brown colour at the junction of two layers and upper layers appears bluish green visualises the presence of glycosides.
To 2.3ml of extract, few drops of Molisch reagent (α-napthol) was added, shaken well and Conc. H_2SO_4 was added from the sides of the test tube, violet ring formation at the junction of two layers visualises the presence of carbohydrates.

Test for Flavonoids
To the 1 ml of extract few drops of 10% Conc. H_2SO_4 was added and followed by adding 1ml of ammonia, formation of greenish yellow precipitate visualises the presence of flavonoids.

Test for Terpenes/Terpenoids
To 2ml of extract, 5ml of chloroform and 2 ml of Conc. H_2SO_4 was added. Reddish brown colourations of interface visualize the presence of terpenes (Harborne 1973).

Test for Saponins
To 2ml of extract add water and shaken vigorously for frothing presence visualize saponins.

Test for Phenols
To 1ml of extract add alcohol and few drops of ferric chloride solution is added for the formation of greenish yellow visualize the presence of phenols.

Test for Tannins
To 1ml of extract, 1ml of 5% $Fecl_3$ was added which visualize by the presence of greenish black precipitate.

Test for Quinones
To 2ml of extract add Conc. HCl by formation of green colou rvisualises the presence of quinones.

Test for Steroids
To 2ml of extract, 1ml of chloroform and drop of glacial acetic acid was added, followed by heating and add Conc. H_2SO_4 which visualises by the presence of orange colour (Liebermann Burchard test, Salkowski test and Liebermann's reaction).

Results

Table 1: Moisture content and total ash value of dried tubers of *Colocasia esculenta*.

Parameter Analysed	*C.esculenta* tubers
Moisture content	56.8 %
Total ash	1.22%

Table 1A: Nutritional composition of dried tubers of *Colocasiaesculenta*.

Parameter Analysed	*C.esculenta* tubers
Carbohydrates	3000 mg/100gm
Protein	824 mg/100gm
Starch	2700 mg/100gm

The above table 1 and table 1A showed that tubers of *Colocasia esculenta* contain moisture content (56.8%), total ash value (1.22%), carbohydrate (3000 mg/gm), protein (824mg/gm) and starch (2700 mg/100gm).

Table 2: Phytochemical constitutents of dried tubers of *Colocasia esculenta.*

Phytochemical components	Methanolic extract	Aqueous extract
Alkaloids	++	++
Glycoside	+	+
Flavonoids	+	+
Terpenes	+	+
Saponins	++	+
Phenol	+	+
Tannins	-	-
Quinones	-	-
Steroid	-	-

Absence (-), Presence (+), Fairly good amount (++)

Qualitaive 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.
Alkaloids and saponins are significantly present in the methanolic extract of *Colocasia esculenta* tubers. Glycoside, flavonoids, terpenes and phenols are also noted. And this study also revealed the absence of tannins, quinines and steroids in methanolic extract.
It was indicated that aqueous extract of tubers shoes significant presence of alkaloids. Glycosides, flavonoids, terpenes, saponins and phenols are moderately present. Tannins, quinines and steroids are absent in the same extract.

Discussion
Plants are important sources of potentially bioactive constituents for the developments of new chemotherapeutic agents. The first step towards this goal is the nutritional profile and phytochemical screening. Phytochemicals are nonnutritive plant chemicals that have protective or disease preventive properties; they are found generally in plants. Phytochemicals can have complementary and overlapping action including antioxidants, modulation of detoxification enzymes and reduction of inflammation, modulation of steroid metabolism, antibacterial and antiviral effects in humans (Nilofer *et al.*, 2013) [3].
Colocasia esculenta tubers contain moisture content (56.8%), ash content (1.22%), carbohydrate (3000mg/gm), protein (824mg/gm) and starch (2700mg/gm). It contains high nutritive value.

In my findings tubers of *Colocasia esculenta* contain various chemical components such as alkaloids, glycosides, terpenoids, flavonoids, saponins and phenols in methanolic extract. Similar result were reported by (Chandra *et al*., 2012) [19] except in the case of saponins.

The plant species could use alkaloids to protect themselves against herbivores. Because of the life style plants are unable to avoid their predators. They could also be used as a natural source of insecticides and fungicides. Researchers also revealed that alkaloids help biologically in storage of waste nitrogen, cationic balancing and protection against parasites (Ting 1982) [21]. Alkaloids are also used in medicine for reducing headache and fever. These are attributed for anti-bacterial and analgesic properties (Shi *et al*., 2004) [20]. Terpenoids represent a diverse class of molecules that are related to therapeutic properties including anti-cancer, anti-parasitic, anti-microbial, anti-allergic, anti-spasmodic, anti-hyperglycemic, anti-inflammatory and immunomodulatory properties (Barre *et al*., 1997, Habtemariam *et al*., 1993, Scortichini *et al*., 1991**)** [16, 18, 22]. Phenolic compound with strong antioxidant activity have been identified in edible members of Araceae family and are of interest to food manufactures as consumers moves toward functional foods with specific health effects. Phenolic compounds are considered to be the most important antioxidants of plant materials. They constitute one of the major groups of compounds acting as primary antioxidants or few radical terminators. Glycosides are naturally cardioactive drugs used in the treatment of congestive heart failure and cardiac arrhythmia (Brian *et al*., 1985) [17]. The presence of glycosides indicates that they may be potent in curing cardiac insufficiency, coughs and circulatory problems. Also, they may act as good sedatives and have antispasmodic properties (Sule *et al*., 2010) [23]. Flavonoids are group of polyphenolic compounds which influence the radical scavenging, inhibition, of hydrolytic and oxidative enzymes and also act as anti-inflammatory agent (Frankel 1995) [24]. The biological functions of flavonoids apart from its antioxidant properties include protection against aggregation, microbes, ulcers, hepatoxins, viruses and tumors (Barakat *et al*., 1993) [15]. Saponins were found in *Colocasia esculenta* tubers shows natural tendency to ward off microbes makes them good candidates for treating fungal and yeast infections.

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