



**PHYTOCHEMICAL ANALYSIS AND INVITRO ANTHELMINTIC
ACTIVITY OF HYDRO-ALCOHOLIC AND AQUEOUS EXTRACT OF
COLOCASIA ESCULENTA (RHIZOME PART)**

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ABSTRACT

The objective of the present work was to study phytochemical and anthelmintic study of the plant *Colocasia esculenta* that belongs to family "Araceae". The folklore reports that the corm is used as, astringent, thermogenic, irritant, anti-inflammatory, antihaemorrhoidal, haemostatic, expectorant, carminative, digestive, appetiser, stomachic, anthelmintic, liver tonic, aphrodisiac rejuvenating and tonic. They are useful in haemorrhoids, vomiting, cough, bronchitis, asthma, anorexia, dyspepsia, flatulence, colic constipation, helminthiasis hepatopathy, dysmenorrhoea seminal weakness. The roots are ophthalmic and are useful in ophthalmia amenorrhoea. **Aim:** The aim of the study is to investigate phytochemical screening of Hydro-alcoholic and aqueous extract of powdered rhizome and to inspect for the anthelmintic study

Results: The percentage yield of Hydro-alcoholic and Aqueous extract of powdered rhizome was found to 7.33 % w/w and 4.15% w/w. And from the phytochemical screening it showed the presence of alkaloids, glycosides, amino acids, protein, saponins. The fluorescence analysis of the drug was observed in day light and ultra violet light using powder drug. In fluorescence analysis the powder drug was treated with different reagent which showed different colour in U.V light. Aqueous extract showed more significant anthelmintic activity but can be comparable to standard albendazole drug. **Conclusion:** The preliminary phytochemical screening showed a wide range of phytoconstituent known through it's screening. The aqueous extract shows more significant anthelmintic activity as compared to Hydro-alcoholic but can comparable to the standard drug albendazole.

KEYWORD: *Colocasia esculenta*, Medicinal use, Hydro-alcoholic, Aqueous extract, Phytochemical screening.

INTRODUCTION

Plants are hidden treasures of nature. Nature has provided us medicines through plants. Many of the plants are still unknown/Still unexplored of their medicinal value. The plant *Colocasia esculenta* is a tropical plant grown primarily for its edible corms, the root vegetables most commonly known as taro. It is known by many local names as It is commonly called as taro (English); alavi, patarveliya (Gujarati); arvi, kachalu (Hindi); alu (Marathi); alupam, alukam (Sanskrit); and sempu (Tamil)^[1] and often referred to as "elephant ears" when grown as an ornamental plant. Linnaeus originally described two species which are now known as *Colocasia esculenta* and *Colocasia antiquorum*^[2] of the cultivated plants. Taro is commonly refers to the plant *Colocasia esculenta*. It occurs throughout India and is cultivated worldwide.^[1,2]

Plant Profile

Colocasia esculenta Linn is a tall herb (Figure-1) and is a perennial, tropical plant primarily grown as a root vegetable for its edible starchy corm. This plant and its root is generally called taro.^[3] Its leaves are simple, size is up to 40 × 24.8 cm and is of dark green above and light green beneath. The leaves are with a stout, erect petiole up to 1.2-m long rarely longer with a triangular sinus cut one-third to half way to petiole, with a dull, not polished surface above, paler or colored beneath, but rarely glaucous.^[3] The Spadix is shorter than the petiole and much shorter than the spathe with flowering parts up to 8 mm in diameter and its appendix is much shorter than the inflorescence. The Peduncle shorter than the petiole. The female portion is at the fertile ovaries intermixed with sterile white ones. Neuters grow above the females, and are rhomboid or irregular oblong in shape. The male portion is above the neuter.^[3] The Female inflorescence short, male inflorescence long, cylindrical, usually interposed neuters between the two. Appendix erect, elongate-conical or fusiform, subulate or abbreviate. Male flowers are of 3-6 androus. Female flowers have 3-4 gynous and ovary is of ovoid or oblong.^[3] Stem is slightly swollen at the base of the leaf-sheaths, arising from a hard tapering rhizome or in cultivated forms a tuberous rhizome suckers and stolons sometimes present. The rhizome of this plant has different shapes and sizes.



Figure-1: Plant *Colocasia esculenta*.



Figure-2: Rhizome part.



Figure-3: Small chips of rhizome Part.



Figure-4: Extraction of rhizome of *C. esculenta*.

Scientific Classification

The scientific classification of this plant is as Kingdom- Plantae, Clade -Angiosperms, Clade- Monocots, Order – Alismatales, Family – Araceae, Genus – *Colocasia*, Species- *Colocasia esculenta* and It is commonly called as taro (English); alavi,; arvi, alu (Marathi); alupam; and sempu (Tamil), kachalu (Hindi); alukam (Sanskrit) patarveliia (Gujarati).

Uses

The pressed juice of the petiole is styptic, and may be used to arrest arterial haemorrhage. It is sometimes used in ear ache and otorrhoea, and also as stimulant and rubefacient^[3] and also in internal haemorrhages. The Leaf juice is stimulant, expectorant, astringent, appetizer, and otalgia.^[3] The juice expressed from the leaf stalks with salt is used as an absorbent in cases of inflamed glands and buboes. The Cooked vegetable contains mucilage and found to be an effective nervine tonic. By the decoction of the peel is given as a folk medicine to cure diarrhoea. It increases body weight, prevents excessive secretion of sputum in asthmatic

individuals. The juice of the corm is used in cases of alopecia.^[3] Internally, it acts as a laxative, demulcent, anodyne, galactagogue^[3] and is used in cases of piles and congestion of the portal system; also used as an antidote to the stings of wasps and other insects. The corm is used by people of the Munda tribe as a remedy for body ache. Taro's primary use is the consumption of its edible corm and leaves. The leaves are rich in vitamin A and C, nutrients including minerals such as phosphorous, calcium, iron, riboflavin, thiamine and niacin.^[4] It is also sold as an ornamental aquatic plant. The corms, which have a light purple colour due to phenolic pigments are roasted, baked or boiled, and the natural sugars give a sweet nutty flavour. The starch is easily digestible, and since the grains are fine and small it is also used as a baby food. *Colocasia esculenta* fresh edible leaves are rich sources of protein, dietary fibre, ascorbic acid hence it can be used as a dietary food supplements. The leaf juice of the plant also finds its application in scorpion sting, in snake bite, in food poisoning from plant origin, etc. The plant also finds its application in ayurveda identified ailments viz. vata and pitta, constipation, alopecia, stomatitis, haemorrhoids and general weakness.^[5-6] Pharmacologically *Colocasia esculenta* is found to possess hepatoprotective activity against liver injury in rats which were experimentally induced. *Colocasia esculenta* is reported to possess hypoglycemic activity due to the presence of cyanoglucoside. The plant also possesses hypolipidemic and antihyperlipidemic activity due to the presence of arabinogalactan and mono and digalactocyl diacylglycerols. The plant gets its antifungal property due to presence of cystatin. The leaves of the plant have been found to exhibit antihelminthic, Antibacterial activity, anti-diabetic and anti-inflammatory activity.^[7-12]

Chemical constituents

Mainly leaves contain calcium oxalate, fibres, minerals (calcium, phosphorus) and starch, vitamin A, B, C^[13] etc. Phytochemically, these also contain flavones, apigenin, luteolin, and anthocyanins. *Colocasia esculenta* tubers contain globulins accounting for 80% of the total tuber proteins, belonging to two unrelated globulin families. The total amino acids recorded in the tubers are in the range of 1,380-2,397 mg/100 g. The lysine concentration was relatively low. The starch content of the flour varies from 73-76% and the starch yields are in the range of 51-58%. The nitrogen content in the flours varies from 0.33-1.35%. The starch contains 0.23-0.52% lipid and 0.017-0.025% phosphorus in the form of phosphate monoester derivatives. Corm contains starch, mucilage, dihydroxysterols, fat, calcium oxalate, vitamin B, iron etc.^[14] Besides starch the tubers contain natural polysaccharide with 56% neutral sugars and 40% anionic components. Steamed corms contain 30% starch and 3% sugar. From

the tubers β -sitosterol and stigmasterol, nonacosane and cyanidin 3-glucoside was found. In addition, five novel aliphatic compounds tetracos-20-en-1, 18-diol; 25-methyl triacont-10-one; octacos-10-en-1, 12-diol; pentatriacont-1, 7-dien-12-ol and 25-methyl-tritriacont-2-en-1, 9, 11-triol, along with nonacosane and cyanidin 3-glucoside. The corm contains betulinic acid, β -sitosterol, stigmasterol, triacotane, lupeol, and β -sitosterol palmitate and Besides these, glucose, galactose, sharp crystal of calcium oxalate, rhamnose and xylose are also present.^[15]

MATERIALS AND METHODS

The different drugs and chemicals were used for the different experimental study. The Mayer's, Hager's, Barfoed's, Benedict's and millon's reagent were purchased from S.D. Fine Chemical, Mumbai. The solvents petroleum ether, Chloroform was purchased from Hi Media Laboratories Pvt. Ltd., Mumbai. Methanol and Petroleum ether was purchased from Qualigens chemicals Mumbai. And all others chemicals, solvents and reagents were of analytical grade and procured from authorized dealer.

Plants collection, Identification and processing

The rhizome of the plant (Figure-2) *Colocasia esculenta* was collected from adjoining area of Barpali (Dist-Bargarh, Odissa) in the month of July-2018. The plant was identified by Botanist Prof. (Dr.) Santosh Kumar Dash, Retired Professor and H.O.D, P.G Dept. of Biosciences, C.P.S, Mohuda, Berhampur, Ganjam, Odisha. The corm was washed properly with water to remove the mud or dust if any; initially it was dried in sunlight for an hour and shade dried completely. Also all the foreign matters like dead or destructed part were removed precautionary. The dried corm was cut into small chips (Figure-3) by means of wood grinder and was sieved to get the coarse powder.

Preparation of the extracts

- (a) Hydro-alcoholic
- (b) Distilled Water

Hydro-alcoholic extract

The shade dried course powder of *Colocasia esculenta* (200 gm) was packed well in soxhlet apparatus (Figure.4) and was subjected for continuous hot extraction with 70% ethanol and 30% distilled water for 8 hours at 50°C for five days. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the

solvent completely and to obtain a semisolid product. The dried product was kept in the desiccators till experimentation. The obtained then extract was weighed and percentage yield was calculated in terms of air-dried powdered crude material.

Aqueous extract

The shade dried course powder of *Colocasia esculenta* (200 gm) was packed well in soxhlet apparatus and was subjected to continuous hot extraction with distilled water for 8 hours at 50°C for five days. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the distilled water completely and to obtain a semisolid product. The dried product was kept in the desiccators till experimentation with the use of anti-microbial agents as it has the presence of water which has the chance of microbial contamination. The obtained extract was weighed and percentage yield was calculated in terms of air-dried powdered crude material. The yield and % yield of both Hydro-alcoholic and aqueous extracts of powdered rhizome of *Colocasia esculenta* was reported.

Table-1: % Yield values of Hydro-alcoholic and aqueous extracts of *Colocasia esculenta*.

| Sl. No. | Extracts | % Yield (w/w) |
|---------|-------------------------|---------------|
| 1. | Hydro-alcoholic Extract | 7.33 |
| 2. | Aqueous Extract | 4.15 |

QUALITATIVE PHYTOCHEMICAL ANALYSIS^[16]

Both Hydro-alcoholic and aqueous extracts obtained by the powdered rhizome of *Colocasia esculenta* was subjected to various qualitative tests for the identification of different phytoconstituents present in it. The constituents present in different extracts of *Colocasia esculenta* are summarized.

Table No-2: Qualitative Phytochemical analysis of Hydro-alcoholic and aqueous extracts of *Colocasia esculenta*.

| Phytochemical test | Hydro-alcoholic Extract (30:70) | Aqueous Extract |
|---------------------------------------|---------------------------------|-----------------|
| Alkaloid test | | |
| Mayer's test | +ve | +ve |
| Wagner's test | +ve | -ve |
| Hager's test | +ve | +ve |
| Dragendorff's test | +ve | +ve |
| Carbohydrates & glycosides | | |
| Molish's test | +ve | +ve |
| Fehling's test | +ve | -ve |
| Barfoid's test | -ve | -ve |

| | | |
|--------------------------------------------|-----|-----|
| Benidict's test | +ve | -ve |
| Borntrager's test | +ve | +ve |
| Saponins | | |
| Foam test | -ve | +ve |
| Proteins & amino acid | | |
| Millon's test | +ve | +ve |
| Biuret's test | +ve | -ve |
| Ninhydrin test | +ve | +ve |
| Phenolic compounds & flavonoids | | |
| Ferric chloride test | -ve | -ve |
| Lead acetate test | +ve | -ve |
| Alkaline test | +ve | +ve |
| Phytosterol : | | |
| Liebermann-Burchard's test | -ve | -ve |

(+): Present (-): Absent

Table No – 3: Powder analyses with chemical reagent.

| Reagents | Colour observed (Naked eye) |
|-----------------------------------------------|-----------------------------|
| Powder as such | Light Brown |
| Powder + conc. HCl | Light yellow |
| Powder + conc. HNO ₃ | Yellowish brown |
| Powder + conc. H ₂ SO ₄ | Dark brown |
| Powder + glacial acetic acid | brown |
| Powder + 5% KOH sol. | Light brown |
| Powder + 5% NaOH sol. | Light brown |
| Powder + FeCl ₃ | Yellowish green |
| Powder + picric acid | Yellow |
| Powder + ammonia | Light brown |

Table No – 4: Fluorescence analysis of powder drug.

| Reagent | Colour observed (naked eye) | Colour observed (U.V short wave length) | Colour observed (U.V long wave length) |
|--------------------------------------------|-----------------------------|-----------------------------------------|----------------------------------------|
| Powder as such | Light Brown | Light Brown | Dark brown |
| Powder + 1N NaOH in methanol | Yellowish brown | Light green | Dark brown |
| Powder + NaOH in water | Light brown | Light brown | Dark brown |
| Powder + 50% HCl | yellowish brown | Light green | Black |
| Powder +50% H ₂ SO ₄ | Light brown | Light green | Black |
| Powder +50% HNO ₃ | Light brown | green | Dark brown |
| Powder + Pet.ether | Light brown | Light brown | Dark brown |
| Powder + Chloroform | brown | green | Dark brown |
| Powder + Picric acid | yellow | green | Black |
| Powder + 5% FeCl ₃ | Yellow green | green | Black |
| Powder + 5% iodine sol ⁿ | Dark green | Dark green | Black |
| Powder + Methanol | Light brown | Dark brown | Dark brown |
| Powder + HNO ₃ +NH ₃ | Light brown | Yellow green | Dark brown |

DETERMINATION OF BIOLOGICAL (ANTHELMINTIC) ACTIVITY^[17]

The anthelmintic study was done by using one in-vitro species adult earthworms *Pheretima posthuma*. Earthworms were collected near the swampy water in our locality. The average size of the round worm was 5-7 cm; average size of the earthworm was 8-9 cm. These earthworms were identified and services of veterinary practioner were utilized to confirm the identity of worms. The suspensions of various extracts were prepared in 2% gum acacia solution to obtain 1, 2.5 and 5% concentrations. Solutions of similar concentrations of the standard drug albendazole were also prepared in distilled water.

Two ml of each concentration of various extracts of *Colocasia esculenta* and standard drug albendazole were diluted to 10 ml separately with normal saline and poured in petridishes. 2ml of 2% gum acacia solution was diluted to 10ml with normal saline to serve as control. Six earthworms of nearly equal size were placed in each Petridis at room temperature. Time was recorded at the time of releasing the earthworms to each concentration. The time taken (minutes) for the complete paralysis and death were recorded. The mean paralysis time for each sample was recorded. The anthelmintic activity was evaluated on adult Indian earthworm *Pheritima posthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed by fading away of their body colour.

Table 5: Anthelmintic effect of *Colocasia esculenta* extracts.

| Group | Concentration of Extract (%) | Time in minutes (Mean \pm SEM) | |
|-------------------------|------------------------------|----------------------------------|------------------------|
| | | Paralysis time(Min) | Death time(Min) |
| Albendazole (standard) | 10 mg/ml | 15min,12 sec \pm 17 | 19min,15 sec \pm 48 |
| | 30 mg/ml | 13min,26 sec \pm 12 | 17 min,26 sec \pm 12 |
| | 50 mg/ml | 11 min,48 sec \pm 14 | 15min,48 sec \pm 14 |
| Hydro-alcoholic extract | 15 mg/ml | 30min,16 sec \pm 17 | 35min,15 sec \pm 48 |
| | 30 mg/ml | 26min,26 sec \pm 12 | 33 min,26 sec \pm 12 |
| | 50 mg/ml | 25 min,48 sec \pm 14 | 32 min,48 sec \pm 14 |
| Aqueous extract | 15 mg/ml | 12min,19 sec \pm 17 | 18min,15 sec \pm 48 |
| | 30 mg/ml | 12min,26 sec \pm 12 | 16 min,26 sec \pm 12 |
| | 50 mg/ml | 10 min,48 sec \pm 14 | 13min,14 sec \pm 10 |
| Control | - | - | - |

Results are expressed as mean \pm SEM from six observations, *Control worms were alive upto 24 hrs. of observation*, N/A= No Activity shown within 24 hours.

RESULTS AND DISCUSSION

The percentage yield of Hydro-alcoholic and aqueous extract were found to 7.33% w/w and 4.15%w/w (Table-1). The preliminary phytochemical screening on the leaf extract was carried out by subjecting the different extracts to qualitative test for the identification of various plant constituents. It showed the presence of alkaloids, glycosides, amino acids, saponins and Phenolic compounds (Table-2) but don't shows the presence of Phytosterol. The fluorescence and Powder analysis was observed in naked eye, U.V short and long wave length. The results were shown in the (Table-3, 4).

The results (as shown in Table-5) depict the time taken for paralysis and death of earthworms after the treatment with the test extracts at the selected concentrations. The data revealed that the aqueous extract has a better wormicidal effect than Hydro-alcoholic extract with compared with the standard drug, Albendazole. Further study is required to find out the novel phytoconstituents responsible for anthelmintic action against various helminthes.

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