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Research Article

PHYTOCHEMICAL SCREENING OF GARHWAL HIMALAYA WILD EDIBLE TUBER *COLOCASIA ESCULENTA*

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ABSTRACT
The present study aimed at evaluating the nutritional profile, successive value, thin layer chromatography and phytochemical screening of *Colocasia esculenta*. It is an important medicinal plant in India which is used in traditional medicine. *Colocasia esculenta* tubers contain nutrients such as ash value (total ash 4.80%), moisture 56.52%, crude fat 0.80% and crude fiber 7.5%, including minerals and vitamins such as calcium, phosphorous, iron, vitamin C, thiamine, riboflavin and niacin. Its edible corns and leaves are traditionally used for hepatic ailments. Leaf juice of this plant is applied over scorpion sting or in snake bite as well as it is used in food poisoning of plant origin. Ayurveda identified ailments viz. vata and pitta are supposed to be pacified by the leaf juice and so also the constipation, stomatitis, alopecia, hemorrhoids as well as general weakness.
Key Words: Nutritional value, Successive value, Thin Layer Chromatography and Phytochemical Screening.

INTRODUCTION

Colocasia esculenta is herbaceous perennial plant belonging to the Araceae family however the leaves are also used as a leafy vegetable. They contain β-carotene, iron and folic acid, which protects against anemia and are important source of proteins and vitamins. Its edible corns and leaves are traditionally used for hepatic ailments[1]. Leaf juice of this plant is applied over scorpion sting or in snake bite as well as it is used in food poisoning of plant origin. Its corn is used as an abortifacient to treat tuberculous ul¬cers, pulmonary congestion, crippled extremities, fungal abscesses in animals, and as an anthelmintic. Its foliage is used as a styptic and poultice. The stem sap is used by the Warao as a treatment for wasp stings. Poi, a fermented product made from corm shavings, is used to improve muscle tone by bathing the sickly person in it and allowing the poi to dry on the body[2, 3]. Other secondary metabolites found in *Colocasia esculenta* include alkaloids, alkyl-resorcinols, glycosides, phenolics, saponins, sterols, essential oils, resins, numerous sugars and organic acids[4, 5]. Ayurveda identified ailments viz. vata and pitta are supposed to be pacified by the leaf juice and so also the constipation, stomatitis, alopecia, hemorrhoids as well as general weakness[6]. It possess hypoglycemic efficacy due to the presence of cyanoglucoside[7]. A high level of reactive metabolites increases the level of hepatotoxicity with increased level of protein adducts formation, mitochondrial dysfunction and oxidative stress[8]. The development of new drugs consists of a variety of single steps leading from the discovery of pharmacological effects in cell and animal models to the assessment of toxicity and finally to the demonstration of efficacy and safety in humans. In the field of investigational toxicology, different models have been established to assess the toxicity of a compound/newly developing drug in an early stage[9].

MATERIAL AND METHODS

Plant Material

The fresh parts of tuber of *Colocasia esculenta* was collected from adjoining area of Khanda village Dist- Pauri, Uttarakhand in the month of September-November 2011. The plant was authenticated by botanist Dr. R. D. Guar, Department of Botany H. N. B. Garhwal (A Central University) Srinagar Garhwal, Uttarakhand India.

Preparation of plant Extract

The plant material was separated into its selected parts (tuber and leaf) air dried ground to moderately fine powder and Soxhlet extracted with increasing polarity solvent (Petroleum ether, chloroform, ethyl acetate, acetone, methanolic, ethanolic and water)[10]. Each extract was evaporated to dryness under reduce pressure using rotary evaporator. The coarse powder of tuber and leaf was subjected to successive hot continuous extraction with various solvent each time before extracting with next solvent the powdered material will be air dried (weight of crude extract 100gm). The various concentrated extracts were stored in air tight container for further studies.

Nutritional & Mineral assay

The edible portion of tubers was analyzed for moisture, ash, fat[11]. Fiber as per method reported in AOAC. Total nitrogen was analyzed by microkjeldhal method[12]. And for crude protein the value was multiplied by 6.25. Total carbohydrates were obtained by subtracting the value moisture, crude protein, crude fat crude fiber and ash from 100%[13]. The total energy value equal to addition of fat, protein and sugars calorie, each gram of fat give 9 kcal, protein and sugar give 4 kcal energy. The minerals analyzed were Potassium using atomic absorption spectrophotometer, Calcium and Phosphorus by flame photometer. Ascorbic acid in tubers was estimated[14].

Successive value

Accurately weighed 500gm coarse and air dried drug material were subjected to hot successive continuous extraction in Soxhlet apparatus with different solvents with increase in polarity petroleum ether, benzene, chloroform, methanol, ethanol and finally with water. The extracts were filtered in each step concentrated and the solvent was removed by vaccum distillation. The extracts were dried in the vacuum dessicator and the residues were weighed[15]. Which contain maximum chemical compound are these categories as depend upon solvent nature and types.

Detection of chemical compound through TLC

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. Thin layer chromatography is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material usually silica gel, aluminium oxide, or cellulose. This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Thin Layer Chromatographic plates are prepared by spreading silica gel G on glass plate using Distill water as solvent these plates are activated in oven at $110^{O}C$ for half hour. All six extracts are applied separately and run in different solvent system of varying polarity. These plates are developed in Iodine chamber, UV chamber and spraying reagent for different spot of constituent chemical[16].

Phytochemical analysis

Preliminary phytochemical analysis extract was prepared by weighing and the dried powdered tuber was subjected to hot successive continuous extraction with different solvents as per the polarity petroleum ether, benzene, chloroform, methanol, ethanol and finally with water. The extracts were filtered in each step concentrated and the solvent was removed by vacuum distillation. The extracts were dried over desiccators and the residues were weighed. The presence or absences of the primary and secondary phytoconstituents were detected by using standards methods[17].

RESULT AND DISCUSSION

Plants are important source of potentially bioactive constituents for the development of new chemotherapeutic agents. The first step towards this goal is the nutritional profile, TLC analysis, successive extraction and phytochemical screening. The results of nutritional profile, TLC analysis, successive extraction and phytochemical screening as table 1, 2, 3 and 4, 5.

Nutritional value

The level of nutrients such as crude protein, carbohydrates, crude fiber, and ash content (3.4%, 26.98%, 7.50% and 4.8%) and also minerals as calcium, magnesium, potassium and phosphorus (0.83, 0.94, 1.24 and 0.58 mg/gm) respectively.

Successive value

Colocasia esculenta tubers contain significant value 36.59%, 33.59% and 5.37% against water, methanolic and ethanolic solvent with 500gm plant sample.

Phytochemical screening

The phytochemical screening for the presence of glycosides, flavonoids, phenols, resin and tannins. This analysis revealed that, the tubers contained higher value of fat, protein, fiber and minerals as compared to the cultivated tubers with carrot and 500 gm tubers contain sufficient amount of nutrients, required per day by a person.

CONCLUSION

The tubers of *Colocasia esculenta* contain phytoconstituents like alkaloids, steroids, fats & fixed oil, flavonoids, tannins, proteins and carbohydrates. The TLC results of the petroleum ether extract and methanol extract show that at least three different phytoconstituents were present in each extract of *Colocasia esculenta* tuber. More detailed study must be done for farther isolation leading to the pure compounds.

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Table 1 Nutritional value of *Colocasia esculenta* plant tuber.

Nutrients	Value	Nutrients	Value
Moisture (%)	56.52 ± 0.10	Insoluble ash (%)	2.46 ± 0.10
Ash (%)	4.80 ± 0.15	Soluble ash (%)	2.54 ± 0.10
Crude fat (%)	0.8 ± 0.20	Na (mg/100gm)	0.65± 0.12
Crude fibre (%)	7.50 ± 0.14	Ca (mg/100gm)	0.83 ± 0.13
Total nitrogen (%)	0.54 ± 0.05	Mg (mg/100gm)	0.94± 0.15
Total protein (%)	3.40 ± 0.08	K (mg/100gm)	1.24 ± 0.20
Carbohydrate (%)	26.98± 0.10	P (mg/100gm)	0.58 ± 0.02
Organic matter (%)	95.20± 0.15		

Table 2 Observations of thin layer chromatographic (TLC) studies of tuber of *Colocasia esculenta*, W: C: M, (Water: Chloroform: Methanol, 10:64:28-36).

Extract	Mobile phase	No. of spot	Rf. value	hRf. value
Pet. Ether Extract	(C:M:W) 64:30:10	1	(0.50)	(50)
Benzene Extract	(C:M:W) 64:30:10	1	(0.44)	(44)
Chloroform Extract	(C:M:W) 64:30:10	1	(0.44)	(44)
Methanolic Extract	(C:M:W) 64:28:10	7	(0.16,0.30,0.38,0.53, 0.57,0.75,0.80)	(16,30,38,53, 57,75,80)
	(C:M:W) 64:30:10	8	(0.16,0.30,0.38,0.53, 0.57,0.64,0.75,0.80)	(16,30,38,53, 57,64,75,80)
Ethanolic Extract	(C:M:W) 64:28:10	6	(0.36,0.42,0.45, 0.51,0.57,0.58)	(36,42,45, 51,57,58)
	(C:M:W) 64:30:10	7	(0.36,0.42,0.45, 0.51, 0.54,0.57 0.58)	(36,42,45,51, 54,57,58)
Water Extract	(C:M:W) 64:30:10	2	(0.51,0.52)	(51,52)

Table 3 Extractive values of *Colocasia esculenta* plant tuber.

Method of extraction	Values of three replicates (%w/w)	Mean (% w/w) ± SEM
Cold maceration:		
1) Water soluble	(28.90, 29.75 & 29.20)	29.28 ± 0.20
2) Alcohol soluble	(3.85, 3.63 & 3.48)	3.65 ± 0.12
Hot Extraction:		
1) Pet. Ether soluble	(0.40, 0.67 & 0.52)	0.53 ± 0.05
2) Benzene soluble	(2.40, 2.59 & 2.19)	2.39 ± 0.18
3) Chloroform soluble	(0.90, 1.25 & 1.06)	1.07 ± 0.34
4) Methanol soluble	(33.40,33.86 & 33.53)	33.59 ± 0.50
5) Ethanol soluble	(5.12, 5.47 & 5.53)	5.37 ± 0.85
6) Water soluble	(36.75, 36.39 & 36.64)	36.59 ± 0.90

Table 4, Phytochemical screening of wild edible tuber *Colocasia esculenta* (+) – Present, (-) – Absent,

Test	Pt. ether Extract	Benzene Extract	Chloroform Extract	Methanolic Extract	Ethanolic Extract	Water Extract
Carbohydrates/ glycosides						
(1) Molish test	(-)	(-)	(+)	(+)	(+)	(+)
(2) Fehling test	(-)	(-)	(+)	(+)	(+)	(+)
(3) Benedict test	(-)	(-)	(+)	(+)	(+)	(+)
Alkaloid						
(1) Mayer's test	(-)	(+)	(-)	(+)	(+)	(+)
(2) Dragondroff test	(+)	(-)	(-)	(-)	(-)	(-)
Flavonoids						
(1) Shinoda/pew	(-)	(-)	(-)	(+)	(+)	(+)
(2) Ammonia	(-)	(-)	(-)	(+)	(+)	(+)
Saponins	(-)	(-)	(-)	(-)	(-)	(+)
Tannins						
(1) Pyrogoll & catechol	(-)	(-)	(-)	(+)	(+)	(-)
(2) Gallic acid	(-)	(-)	(-)	(-)	(-)	(-)
Unsaturated sterol/triterpenes						
(1) Liebermann Burchard test	(+)	(+)	(+)	(+)	(+)	(+)
(2) Salkowiskis test	(+)	(+)	(+)	(+)	(+)	(+)
Resin	(-)	(-)	(-)	(-)	(-)	(-)
Phenolics compound						
(1) Ferric chloride	(-)	(-)	(-)	(+)	(+)	(-)
Protein and amino acid						
(1) Xanthoprotien	(-)	(-)	(-)	(+)	(+)	(+)

Table 5 Qualitative estimation of *Colocasia esculenta* tuber amino acid screening.

Amino acid test	*Colocasia esculenta* tuber
L- Hydroxy proline	(.)
DL Serine	(-)
DL Iso-leucine	(-)
DL Valine	(+)
DL-2-Aminobutyric acid	(+)
L-Ornithin	(-)
L-Cystein hydroxyl	(+)
DL-Nor-leucine	(-)
DL-Tryptopham	(-)
DL-Alanine	(+)
L-Glutamic acid	(-)
Glycine	(-)
L –Proline	(-)
L- Arginine	(+)
DL – Aspartic acid	(-)
L –Cystein hydroxychloride	(+)
L- Histidine	(-)
L – Leucine	(-)
L –Lysine monochloride	(+)
DL – Methionine	(-)
DL – β-Phenyl alanine	(-)
DL – Threonine	(+)
L – Tyrosine	(-)
3-C-3-4Dihydroxy phenyl	(-)

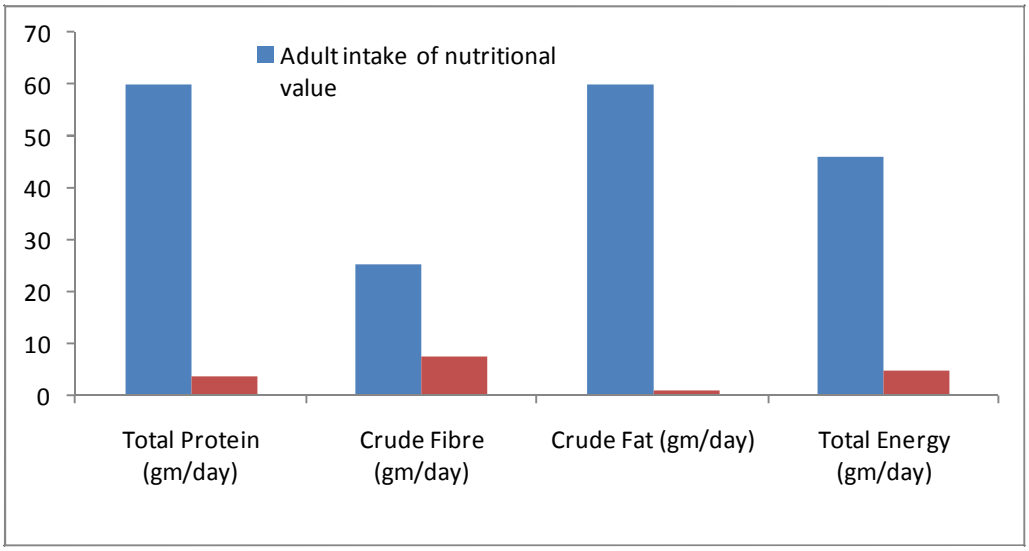

Figure 1.1 Comparison of per day intake of nutrients by Adults with the nutrients present in the tuber of *Colocasia esculenta*.

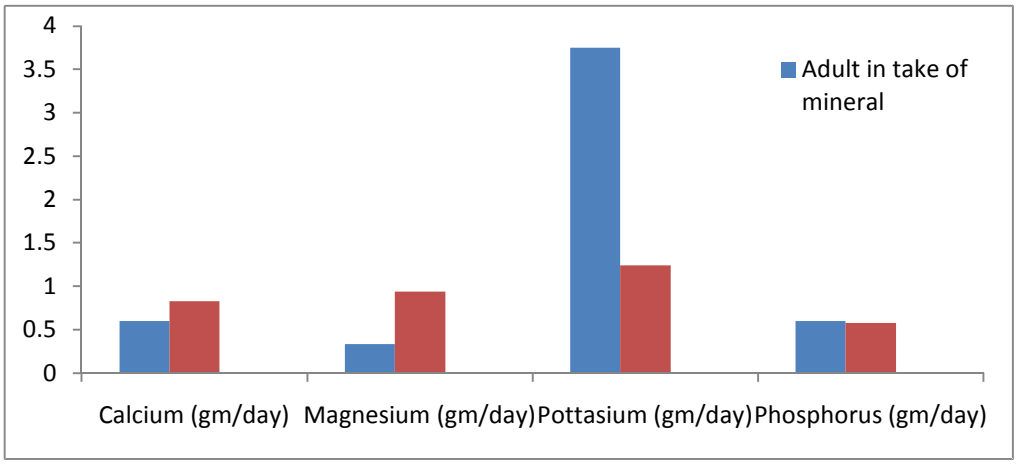

Figure 1.2 Comparison of per day intake of minerals by Adults with the mineral present in the tuber of *Colocasia esculenta*.

Figure 2.1 Thin layer chromatography qualitative analyses of six fractions against *Colocasia esculenta* plant tuber extract.

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