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Research Article

Pharmacognostical, phytochemical and heavy metal studies on an ethno medicinal plant-*corallocarpus epigaeus* (rottl. &wild.) Clarke.

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ABSTRACT

Leaf, stem and tuber powder of *Corallocarpus epigaeus* were investigated for its pharmacognostical, phytochemical and heavy metal properties. Analytical value (loss on drying, total ash, acid insoluble ash, water soluble ash), solubility percentage, fluorescent analysis, extractive value using different solvents (petroleum ether, benzene, chloroform, acetone, ethanol, methanol and water), qualitative phytochemical analysis for detection of alkaloids, glycosides, flavonoids, tannins, phenols, proteins, amino acids, saponins and terpenoids. HPTLC studies of glycosides, flavonoids, phenolic compounds and heavy metal analysis for the accumulation of lead, copper and cadmium were studied. Analytical value, extractive value and solubility percentage exhibited marked difference between the leaf, stem and tuber powder of *C. epigeaus*. Fluorescent analysis does not differ among the selected plant parts under normal and UV light. Qualitative analysis of acetone and water extracts revealed the presence of secondary metabolites like alkaloids, glycosides, flavonoids, terpenoids, tannins, phenol, fats and fatty acids. HPTLC studies also confirmed the presence of glycosides, flavonoids and phenolic compounds. Heavy metals present in the plant parts are lower than the permissible level.

Key words: *Corallocarpus epigaeus*, pharmacognostical, Heavy metal studies.

INTRODUCTION

Corallocarpus epigaeus belonging to Cucurbitaceae family is a prostrate climbing perennial plant. The synonym of this plant is *Bryonia epigaea*. It is commonly called as Indian Bryonia and in tamil it is called as Kollan kovai or Akasagarooda[1]. It is found in Africa, Asia and temperate regions of the world. In India, it is distributed in Andrapradesh, Gujarat, Karnataka, Punjab, Rajasthan and Tamilnadu[2]. This plant belonging to a rare category[3] and is used by tribals of Rajasthan to cure various ailments related to digestive tracts like indigestion, constipation, abdominal pain, dysentery and typhoid. It is also used to cure skin diseases like wounds, tumors, boils, sunburns, cuts and injury[3]. Root of this plant contains a bitter glycoside; Bryonine is used as alterative and is specially used in syphilis, chronic dysentery, chronic mucus enteritis and rheumatism. It is a siddha remedy for chronic eczema[4]. The tuber of this plant has antirespiratory, anticancer and antimalarial properties. It is used for external application in conjunctivitis and chronic veneral complaints[5]. Tuber boiled with coconut oil is used to cure leprosy[6]. This plant is highly medicinal and there are few references found available in this plant regarding pharmacognosy and heavy metals. Therefore, the present study is focused on the pharmacognostical and heavy metal content of this plant.

MATERIALS AND METHODS

The fresh leaves, stem and tuber of *C. epigaeus* were collected from Pasumalai hills of Theni district, Tamilnadu, India (Plate 1 & 2). The collected plant parts were washed thoroughly with water, shade dried powdered and stored in an airtight container for further studies. The powder was successively extracted with different solvents like petroleum ether, benzene, chloroform, acetone, ethanol, methanol and water in a soxhlet apparatus.

Physicochemical analysis: Leaf, stem and tuber powder were subjected to physicochemical studies for determining the ash value, extractive value, solubility percentage and fluorescence analysis by the methods prescribed by Kokate[7].

Qualitative phytochemical studies: Leaf, stem and tuber powder were extracted with different solvents and subjected to screened for various secondary metabolites like alkaloids, glycosides, flavonoids, tannins, phenols, proteins, amino acids, saponins and terpenoids.

HPTLC Studies: Acetone and water extracts of leaf, stem and tuber powder were used for HPTLC studies to detect glycosides, flavonoids and phenolic compounds using CAMAG LINOMAT 5 instrument. Ethyl acetate-ethanol-water (8:2:12), Ethyl acetate-butanone-formic

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Plate 1 : *C. epigaeus*

Habit

Tuber

Table 1: Physico chemical analysis of leaf, stem and tuber powder of *C. epigaeus*

S.No	Parameter studied	Values expressed in % (W/W)		
		leaf	stem	tuber
1.	Loss on drying	86	83	82
2.	Total ash	2.6	3.2	3.6
3.	Acid insoluble ash	0.4	0.3	0.4
4.	Water soluble ash	1.0	0.9	0.8
5.	Solubility percentage			
	Alcohol	49	44	48
	Water	86	79	80

Table 2: Fluorescent behavior of leaf, stem and tuber powder of *C. epigaeus* with different solvents

S.no.	Treatment	Leaf		Stem		Tuber	
		Normal light	UV light	Normal light	UV light	Normal light	UV light
1.	Petroleum ether	Yellow	Cream	Orange	Orange	Cream	Pale cream
2.	Benzene	Orange	Orange	Orange	Pale cream	Orange	Pale cream
3.	Chloroform	Cream	Orange	Cream	Pale yellow	Pale cream	Orange
4.	Acetone	Cream	Pale cream	Cream	Cream	Pale cream	Orange
5.	Ethanol	Pale yellow	Pale yellow	Orange	Orange	Orange	Pale yellow
6.	Methanol	Deep orange	Orange	Orange	Deep orange	Orange	Pale yellow
7.	Water	Deep orange	Orange	Pale yellow	Pale yellow	Cream	Cream

acid-water (5:3:1:1) and Toluene-acetate-formic acid (4.5:4.5:1) were used as mobile phases for glycoside, flavonoid and Phenolic compounds respectively. The spray reagents for glycosides, flavonoids and phenolic compounds are Liebermann Burchard, 1% ethanolic aluminium chloride and fast green B salt reagent respectively. The standard references like Stevioside for

glycoside, Rutin for flavonoids and phenolic compounds for Quercetine were used for this study.

Heavy metal studies: The samples were digested in microwave digestion system using 10ml of HNO_3 (69%) for 10 min, 1ml of $HCLO_4$ (70%) for 5 min and 5ml of H_2O_2 (30%) for 10 min at 250W power setting. The digested samples were analyzed for metals like lead, chromium, cadmium and copper in double beam Atomic Absorption Spectrometer (Perkin Elmer Model Analyst 800). Standards used for all the heavy metals are manufactured by SRL, India.

RESULTS AND DISCUSSION

Pharmacognostical studies comprise the analytical value, extractive value, solubility percentage and fluorescent analysis. A detailed study of *C.epigaeus* exhibited marked difference between leaf, stem and tuber powder. The loss on drying is higher in leaf than stem and tuber.

Table 3: Extractive value (in percentage) of leaf, stem and tuber powder of *C.epigaeus*

Method of extraction	Solvent used	yield (in percentage)		
		Leaf	Stem	Tuber
Soxhlet extraction	Petroleum Ether	5.8%	5.7%	6%
	Benzene	4.2%	4.8%	6.8%
	Chloroform	4.2%	5.2%	6.8%
	Acetone	6.4%	4.6%	6.8%
	Ethanol	6.2%	8.6%	10.4%
	Methanol	6.8%	9.3%	9.6%
	Water	11.1%	12.1%	12.2%

Plate 2
Chromatogram of Glycosides using HPTLC

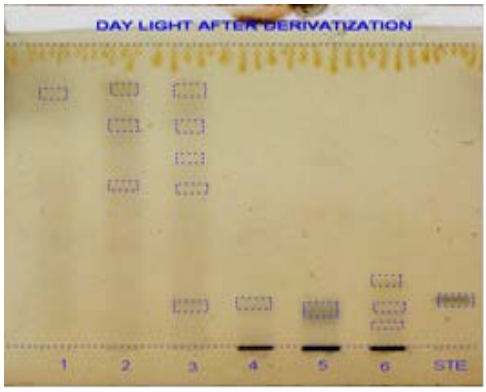
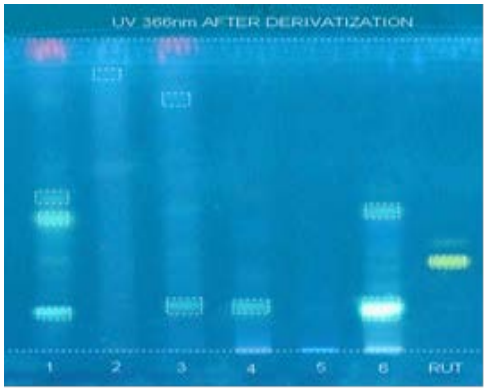


Plate 3
Chromatogram of Glycosides using HPTLC

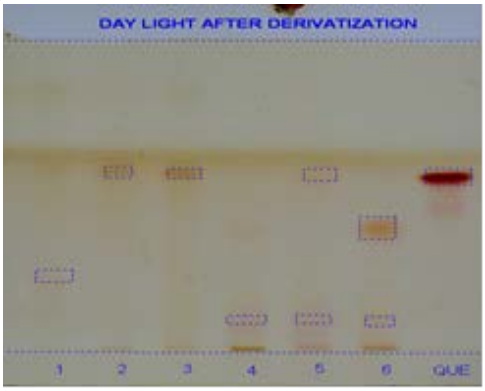


The total ash content is higher in tuber powder and the solubility percentage is higher in water than alcohol (Table 1). Fluorescent analysis of leaf, stem and tuber Powder, treated with different solvents does not show any marked difference (Table 2). The extractive value of water is higher than other solvents. These values will be helpful to identify the sample of genuine drug (Table 3).

A systematic study of a crude drug embraces, thorough consideration of primary and secondary metabolites derived as a result of plant metabolism. The secondary metabolites are usually responsible for the medicinal properties of the drugs. Water and acetone extracts showed the presence of most of the secondary metabolites like alkaloids, flavonoids, proteins, phenols, glycosides, and tannins. Fixed oils and terpenoids were absent in all the extracts studied. Petroleum ether and benzene extracts does not give any positive results. These studies revealed that by using this diagnostic features, one can identify this plant for further investigation. Similar results were obtained in the root and rhizome of this plant by Nisha shri *et al.*, (2010)[8]. With the advent of new analytical tools and sophisticated instrument technology, it is possible to suggest a practical quality assurance of a profile for a crude drug[9]. Physical evaluation of a crude drug could be of both qualitative and quantitative nature. Physical standards are to be determined for drugs, wherever possible[10]. HPTLC studies confirmed the presence of glycoside, flavonoid and phenols. Appearance of violet brown colour indicates the presence of glycoside, yellow and blue colour and reddish brown colour zone indicates the presence of flavonoid and phenolics respectively (Plate 2, 3&4). In the earlier report, Glycosides and phenolic compounds were absent in *C. epigaeus*[8]. In contrast, the present HPTLC studies on leaf, stem and tuber powder extracts of *C.epigaeus* confirmed the presence of five types of Glycosides, three types of Flavonoids and one type of Phenolic compound.

Medicinal plants are also known to contain trace metals which play a vital role as structural and functional components of metalloproteins, for the formation of bioactive constituents and enzymes in living cells. However, there is an inherent risk associated with many of these medicinal plants due to the presence of heavy metals. The accumulation of heavy metals can have middle term and long term health risks[11]. WHO recommends that medicinal plants which form raw

Plate 4
Chromatogram of Glycosides using HPTLC



Sample 1-acetone extract of leaf
Sample 2-acetone extract of stem
Sample 3-acetone extract of tuber
Sample 4-water extract of leaf
QUE- Quercetine Standard
Sample 5-waterextract of stem
Sample 6-water extract of tuber
STE- Stevioside Standard
RUT- Rutin Standard

Table 4: Variation in metal contamination pattern in soil and different parts of *C.epigaeus*

Name of the metals	Leaf	Stem	Tuber
Lead	0.1	Nil	Nil
Copper	1.0	0.65	0.25
Chromium	7.41	3.18	2.12
Cadmium	Nil	Nil	Nil

materials for the finished products may be checked for the presence of heavy metals, pesticides, bacterial or fungal contamination[12]. Heavy metal analysis revealed that lead is found only in leaf powder (0.1ppm) and copper and chromium were found in the leaf, stem and tuber powder (Table 4). Chromium is found to be present higher than copper but all the metals present were lower than the permissible level (Pb-10ppm, Cu-20ppm and Cr-50-200 µg). Cadmium was totally absent in all the samples studied. To conclude, the present investigations revealed that the therapeutic use of this plant species will not cause any heavy metal toxicity and may be beneficial to the users in case oral administration.

CONCLUSION

Pharmacognostical studies reported that the plant constituents are mostly water soluble and contains most of the secondary metabolites that are responsible for curing various ailments. Heavy metal studies also revealed that the plant is safe for oral consumption. Further quantitative phytochemical and pharmacological studies are undergoing to explore the medicinal efficacy of this plant.

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