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Phytochemical Screening of Crude Root Extracts of *Cephalandra indica* (Cucurbitaceae).

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ABSTRACT

The traditional system for treatment of various diseases based on the use of various plant parts of *Cephalandra indica*. The tuberous roots consist of various new bioactive molecules, which provide more folklore way to treat hypoglycemia, hypolipidimic and jaundice. Fresh juice of roots is used to treat diabetes. Considering the significance of it, the present study provides information for physicochemical parameters and HPTLC profile of hydroalcoholic extracts of roots of *Cephalandra indica*, so that the medicinal property can be explored properly.

Keywords: *Cephalandra indica* root, Physico-chemical, Fluorescence, Phytochemical, HPTLC.

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INTRODUCTION

Cephalandra indica Naudin (Synonym: *Coccinia grandis* Voigt, *Coccinia cordifolia*, *Coccinia indica* Wight & Arn.) is famous for its hypoglycemic and antidiabetic properties in Ayurvedic system of medicine [1]. Indian system of traditional knowledge that is, ayurveda is well known for its effective herbal treatments. *Cephalandra indica* is found throughout India in warm and humid conditions. There are about 7000 plant species found in India. It is slender prostrate or climbing herb [2]. Although most of them have a long history in folklore medicine; there is lack of scientific data on their efficacy and safety, esp. from human studies. The plant has also been used extensively in Ayurvedic and Unani practice in the Indian subcontinent [3]. It has long tuberous fleshy roots, smooth and green fruits. Fresh juice of roots is used to treat diabetes; tincture of leaves is used to treat gonorrhea, paste of leaves is applied to the skin diseases. Dried bark is a good cathartic. Leaves and stem are antispasmodic and expectorant. The fleshy green fruit is very bitter. Green fruit is chewed to cure sores on the tongue.

The present investigation has been undertaken with an objective to establish physicoparameter standards for cephalandra indica root, so that authentic plant material could be explored properly for its traditional claims.

MATERIAL AND METHODS

Plant material

The stem is a herbaceous climber or perennial slender climber with occasional adventitious roots forming where the stem runs along the ground. Both roots and stems are succulent with the length of the rootstock as long as 5 cm. The tendrils are long, elastic with coil-like springy character that can wrap around the host to the entire length. The leaves are classified as palmately simple with five lobes while the shape varies from the heart to pentagon form. The size of the leaves is approximately 5-10 cm in width and length. The flower is large and white about 4 cm in diameter and contains five long tubular petals. Ivy gourd is a dioecious plant of which male and female flowers grow separately. Female flowers have a two-lobed stigma while the male flowers have long (6 mm) filamentous stamens. At least two plants of different sexes must be present to form a viable seed. The ivy gourd fruit belongs to the berry type: oval and hairless with thick and sticky skin. The raw fruit is green in color and turns bright red when it is ripe. The mature fruit is usually from 25 to 60 mm long by 15–35 mm in diameter and contains several pale, flattened seeds (6-7 mm long)[4].

The roots were collected in the months of February and March 2012 from Cuttack, Odisha, India. The collected fresh roots were washed; shade dried and used to evaluate physicochemical parameters.The powder of dried root was used for the determination of ash values, extractive values and phytochemical investigations. All chemical and reagents used for testing were analytical grade obtained from S.D Fine chemicals, Mumbai (India).

Extraction

The powdered material was extracted successively with petroleum ether (60-80 ^{o}C), ethyl acetate, chloroform and hydroalcohol by using soxhlet apparatus. The solvent was removed under reduced pressure which gave deep yellow, pale white, and brown colored residue for petroleum ether, chloroform, and hydroalcoholic extract respectively. The extracts were concentrated under vacuum at 40-60 ^{o}C which yields a residue (25 w/w, 17.64 w/w, 14.4 % w/w) were stored in desiccators at room temperature.

Physico chemical parameters

The parameter was done to evaluate the percentage of total ash, water soluble acid insoluble ash were calculated as per Indian Pharmacopoeia[5].The total ash of the powdered root was tested for different inorganic constituents[6].Different extracts of the root were prepared for the study of extractive values[7]. Fluorescence analysis of powdered root was carried out by standared methods[8,9].

Preliminary phytochemical analysis

For the preliminary phytochemical analysis, 100 gm of dried powdered root drug was extracted with different solvents as per the polarity, petroleum ether, chloroform and hydroalcohol successively. The extracts were filtered in each step, concentrated, and the solvent was removed by rotary evaporator. The extracts were dried over desiccator and the residues were weighed. The presence and absence of different phytoconstituents *viz*. triterpinoids, steroids, alkaloids, sugars, tannins, glycosides and flavonoids etc, or the primary and secondary phytoconstituents were detected by usual prescribed methods[10,11].

High performance thin layer chromatography

Based on chemical test and thin layer chromatography of various extracts of root of the plant that hydroalcoholic root extract was found to have more number of phytoconstituents. So further attempt was taken to separate the individual components of hydroalcoholic root extract by HPTLC , CAMAG Linomat 5 taking Tolune: Acetone: Ethyle acetate:Formic acid. (40:30:20:2.5) as solvent system.

RESULTS

Behavior of root powder of *Cephalandra indica* with different chemical reagents were performed to detect the occurrence of phytoconstituents along with color changes under ordinary daylight by standard method which is tabulated in Table 1.

Table 1: Behaviour of root powder of *Cephalandra indica* with different chemical reagents.

Sl.no.	Acid/Reagents	Observation
1.	Powder as such	brown
2.	Powder + Picric acid	Yellowish brown
3.	Powder + Conc.nitric acid	Reddish brown
4.	Powder + Conc.hydrochloric acid	Light green
5.	Powder + Conc.sulphuric acid	Reddish black
6.	Powder + Glacial acitic acid	Yellowish brown
7.	Powder + 5% Ferric chloride solution	Yellowish green
8.	Aqueous	No change
9.	Powder + Sodium hydroxide(5N)	No change
10.	Powder + Potassium hydroxide(5%)	Brown
11.	Powder +Iodine/20	Blackish brown

Physico-chemical study

The percentage of total ash, acid-insoluble ash, water soluble ash, sulphated ash and different extractives are tabulated in Table 2 and 3.

Table 2: Ash values of *Cephalndra indica root.*

Sl.no.	Type of ash	% Yield (w/w)
1.	Total ash	13.55
2.	Acid insoluble ash	2.86
3.	Water soluble ash	6.39
4.	Sulphated ash	6.45

Table 3: Extractive values of *Cephalandra* indica root with different solvents.

Sl.no.	Types of solvent	% of Yield (w/w)	Colour of extractives
1.	Petroleum ether (60 -80 ^{o}C)	2.6	Deep yellow
2.	Chloroform	2.30	Whitish green
3.	Hydro alcohol	7.28	Dark brown

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Fluorescence characteristics

When physical and chemical parameters are inadequate as it often happens with the powdered drugs, the plant material may be identified from their adulterants on basis of fluorescence study of different extract and powdered drug which is tabulated in Tables 4 and 5.

Table 4: Florouecence analysis of different solvent extracts of cephalandra indica root under UV-Vis light.

Sl.no.	Extracts	Visible Light	UV	
			Short wave	Long wave
1.	Petroleum ether (60 -80 ^{o}C)	Deep yellow	Purple	Light red
2.	Chloroform	Pale white	Green	Yellow
3.	Hydro alcohol	Brown	Purple	Violet

Table 5: Flouresence analysis of root powder of cephalandra indica with different chemical reagents.

Sl.no.	Reagents	Colour in day light	UV	
			Short wave	Long wave
1.	Powder as such	Deep brown	Light brown	Brown
2.	Powder +1N NaOH in methanol	Brown	Green	Brown
3.	Powder +1N NaOH	Brown	Green	No change
4.	Powder + Ethanol	Brown	Green	Yellow
5.	Powder + HNO_3 + NH_3 solution	Brown	Green	Deep brown
6.	Powder + 50% HNO_3	Deep brown	Greenish yellow	No change
7.	Powder + 1N HCl	Green	Green	No change
8.	Powder + HCl	Pale green	Green	No change
9.	Powder + H_2SO_4	Radish black	Black	Yellowish green
10.	Powder + 50 % H_2SO_4	Deep red	Black	Yellowish green
11.	Powder + Glacial acid	Brownish green	Brown	Brown
12.	Powder +HNO_3	Deep brown	Deep green	Deep Brown

Preliminary phytochemical analysis

The preliminary phytochemical analysis of root extracts of petroleum ether (60-80°C), chloroform and hydroalcohol are tabulated in Table **6**.

Table 6: Qualitative phytochemical analysis of various extracts of cephalandra indica root.

Sl.no.	Type of constituents	Petrolium ether	Chloroform	Hydroalcohol
1.	Alkaloid	-	+	-
2.	Flavonoid	+	+	+
3.	Carbohydrate	+	+	+
4.	Mucilage	+	-	+
5.	Saponin	+	+	+
6.	Glycoside	+	-	+
7.	Volatile oil	-	+	+
8.	Amino acid	-	-	+
9.	Steroids	-	-	-
10.	Fats and lipids	-	-	-
11.	Tannins	-	-	-

High performance thin layer chromatography

HPTLC profile showed the separation of 8 different phytoconstituents having different retention factor. The results of HPTLC of hydroalcoholic root extract are shown in form of chromatogram 1, plate 1 and Table 7

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Table 7: HPTLC of hydroalcoholic root extracts.

Peak	Start R_f	Start Height	Maximum R_f	Maximum Height	Maximum %	End R_f	End Height	Area	Area %
1	0.01	7.1	0.06	601.6	47.59	0.08	264.7	10403.8	34.96
2	0.08	265.4	0.10	300.0	23.73	0.16	49.2	7377.6	24.79
3	0.21	47.6	0.25	76.1	6.02	0.29	29.2	3181.3	10.69
4	0.31	32.1	0.35	81.2	6.43	0.40	38.0	3498.1	11.75
5	0.54	29.8	0.55	36.3	2.86	0.59	22.9	1053.5	3.54
6	0.60	23.2	0.61	37.0	2.93	0.62	26.2	530.7	1.78
7	0.64	26.5	0.67	65.7	5.20	0.70	33.0	1775.7	5.97
8	0.72	33.0	0.75	66.2	5.24	0.81	2.2	1938.8	6.51

Solvent system-Tolune:Acetone:Ethyl acetate:Formic acid:(40:30:20:2.5) HPTLC-High performance thin layer chromatography

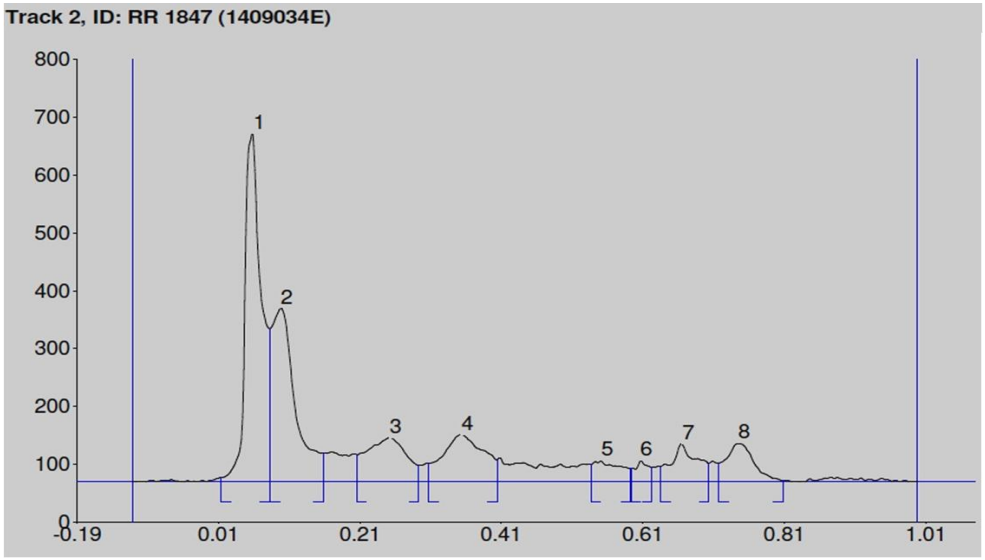

Figure 1(a): HPTLC chromatogram of hydroalcoholic root extract

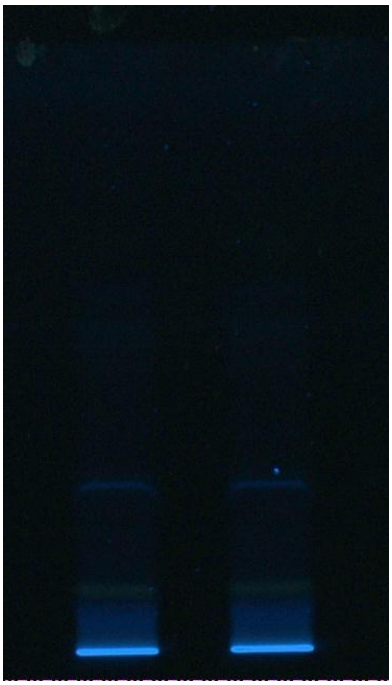

Figure 1 (b): HPTLC plate of hydroalcoholic root extract

DISCUSSION

The water soluble ash is almost half of total ash and twice of acid insoluble ash. The alcohol soluble extractive value is more than any other extractive value indicating the solubility of phytoconstituents in alcohol. The fluorescence analysis of powder and extract indicate that many fluorescent phytoconstituent present or adulterants. Preliminary phytochphytoconstituents present in different solvent extract. This also indicates that the hydroalcoholic extract have more number of phytoconstituent than any other extracts i.e. Flavonoids, Carbohydrates, Mucilage, Saponins, Glycosides, Volatile oils and Amino acids. These are few of the important physico-chemical characters of the root. HPTLC profile showed the separation of eight different phytoconstituents having different retention factor which may be the phytochemicals we got in preliminary phytochemical analysis of hydroalcoholic root extract.

CONCLUSION

Cephalandra indica is a famous plant for its safe antidiabetic property. It proved the insulin stimulatory effect of *C. indica* leaves from existing β-cells in diabeticrats. It possesses hypoglycemic, antidiabetic, hypolipidemic, hepatoprotective, larvicidal, anti-inflammatory, analgesic and antipyretic activities. It is found to be devoid of antitubercular properties. Various phytoconstituents reported in *C. indica* are cephalandrol, tritriacontane, lupeol, b-sitosterol, cephalandrine A, cephalandrine B, stigma-7-en-3-one, taraxerone and taraxerol. Terpenoids are found to be responsible for antidiabetic activity. Despite the broad use of *C. indica* in traditional medicine, very few systematic pharmacological and phytochemical studies are reported till date assessing its therapeutic properties. Now a days the standardization of crude drugs has become very important for identification and authentication of a drug. But due to certain problems the importance was not up to the mark.Thus, the lack of standardization technique fails to identify the drug from its originality which there by exploits the usage of drug from its Traditional System of medicine.

The plant *Coccinia grandis* is used widely for curing various diseases like diabetes and gives a helping hand to the Humans [12]. Thus a perfect protocol was designed for its Authentication and identification on the basis of Microscopy and chemical analysis. Thus the results were found to be significant and encouraging towards the goal for Standardization. The results of different ash values,extractive values determination, powder analysis with different reagents, fluorescence analysis , preliminary phytochemical screening and HPTLC profile have been done; will help in future for proper identification of *Cephalandra indica* in intact form or in a powdered form.

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