

Research Article

Physicochemical and Phytochemical Screening of Aqueous and Ethanolic Extracts of *Costus Pictus* D. Don and *Enicostema Littorale* Blum.

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Abstract

The hypoglycaemic and antidiabetic activity of *Costus pictus* and *Enicostema littorale* plant material is well reported in experimental diabetes in rats and is mainly because of secondary metabolites present in it. The present investigation was carried out for standardization of herbal extracts of said plant material employing Phyto and physicochemical screening of aqueous and ethanolic extracts of *Costus pictus* D. Don leaves and *Enicostema littorale* Blum collected from this area. The study includes estimation of physicochemical parameters viz. moisture, crude protein, crude fibre, ash values, ether extract, nitrogen free extract, extractability in water and ethanol, pH and gross energy. Some essential elements like calcium, phosphorus and salts for nutritive value were also tested. The preliminary screening for presence of various phytochemical constituents in extracts was focused.

The extracts analysed were found positive for various phytoconstituent in both the plant material, responsible to execute hypoglycaemic characters in medicinal use of their herbal preparations. The generated information of the present investigation will provide the data for reference which is helpful in the correct identification and authentication of this medicinal plant for future use in drug discovery process.

Keywords: *Costus pictus*, *Enicostema littorale*, physicochemical, phytochemistry, hypoglycaemia, drug discovery

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Introduction

Medicinal plants have been a major source of treatment for human diseases since time immemorial and exhibit their properties due to secondary metabolites present in it. One fourth of the world population i.e. 1.42 billion people are dependent on traditional medicines, particularly plant drug for curing their ailments [1]. *Costus pictus*, locally known as 'insulin plant', a member of Costaceae family is used as a munching dietary supplement for the treatment of diabetes in Southern India [2], and abundantly seen in konkan area of Maharashtra. *Enicostema littorale* is another herb of family Gentianaceae used for hypoglycemic activity found in many parts of India such as Gujarat [3], Osmanabad and Latur district of Maharashtra [4]. Herbal medicines are promising choice over modern synthetic drugs. They show minimum/no side effects and are considered to be safe. Generally herbal formulations involve the use of fresh or dried plant parts or their extracts. Correct knowledge of such crude drugs is a very important aspect in preparation, safety and efficacy of the herbal product. The process of standardization and understanding their activity can be achieved by stepwise pharmacognostic studies [5]. Standardization is a system to ensure that every packet of medicine that is sold has the correct amount and will induce its therapeutic effect [6]. For the useful application of the plant parts in modern medicine, physico-chemical and phytochemical standardization is very important [7]. Determination of extractive values, ash residues and active components (saponin, alkaloids & essential oil content) plays a significant role for standardization of the indigenous crude drugs [8], so that medical benefits of the plants under screening may be used properly and scientifically and reach to the larger populations of the world to consider significantly for drug development. Considering the hypoglycaemic and antidiabetic activity of the *C. pictus* leaves and *E. littorale* as a whole plant, the present investigation was carried out to evaluate the physicochemical parameters and phytochemical constituents in aqueous and ethanolic extracts.

Materials and Methods

Collection of Plant Material

Costus pictus D. Don plant (**Figure 1**) leaves were collected from the Government controlled nursery gardens of “Maharashtra Nature Park, Dharavi, Mumbai”, whereas whole plant of *Enicostema littorale* Blume (**Figure 2**) was collected from College campus and nearby areas of Veterinary and Animal Sciences, Udgir of Maharashtra State. Plant material thus collected were washed thoroughly and dried under shade, segregated, pulverized by a mechanical grinder and passed through mesh sieves to obtain powder. Both the plants under study were identified and authenticated by a Botanist at “Blatter Herbarium” from the Department of Botany, Saint Xaviers College, Fort, Mumbai.



Figure 1 *Costus pictus* whole plant



Figure 2 *Enicostema littorale* whole plant

Preparation of Aqueous and Ethanolic Extracts

Aqueous extract was obtained following standard ayurvedic method (API, Part-I, Volume VI, Appendix- 6, page 247) of *Kvatha* preparation. For this, powdered plant material was mixed in its 16 times volume of water and boiled to reduce its one-fourth on medium gas flame. Residue left in bowl was filtered through the muslin cloth and filtrate so obtained was once again passed through Whatman filter paper No.1 to get extract.

For ethanolic extract, powdered herbs were subjected to Soxhlet's Extraction Apparatus as per standard procedures using 70% ethanol. The flask was placed on heating mantle till the colourless solvent started returning back to reservoir. Content thus obtained was transferred to a dry and clean petri dish and kept on water bath till complete evaporation of the solvent resulting into semisolid material.

Physicochemical, Nutrient Profile and Proximate analysis

The physico-chemical properties, nutrient profile and proximate analysis of the herbs under study were determined at Animal Feed Analytical and Quality Assurance Laboratory, Veterinary College and Research Institute, Nammakkal, Tamilnadu, and at Department of Animal Nutrition, Bombay Veterinary College, Parel Mumbai; as per methods described in Ayurvedic Pharmacopoeia of India, Part 1 (Reference API /APPENDIX III / PAGE NO 156 / 3.3) following standards recommended by World Health Organization. The flowing analysis was done in the current evaluation

Moisture content/ Loss on drying (Gravimetric determination)

One gram of drug sample was taken in a pre weighed dried tarred evaporating dish. It was dried in an oven at 105°C. Drying was continued and weight was taken at one hour interval until difference between two successive weighing corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference. The dish was then taken out, the weight loss i.e. moisture content (loss on drying) was calculated and expressed as %.

Determination of crude protein

Crude protein was estimated using Macro kjeldahl method. About 3 g weighed sample was transferred in a Kjeldahl's flask and poured 25 cc of conc. H₂SO₄ in it. Added 1 g of anhydrous Na₂SO₄ and a crystal of copper sulphate (9:1) and about pinch of selenium powder(digestion mixture) in that and kept this flask in digestion chamber (Temp. about 200°C) for 2-3 hours till contents become perfectly colourless or clear. Removed the flask from digestion chamber and allowed to cool. Transferred all the contents of Kjeldahl's flask in to volumetric flask and made the volume up to the mark adding water. About 20 cc of 2 % boric acid solution in receiving flask and added 2-3 drops of Kjeldahl's indicator in it. As soon as the steam pressure developed, kept the receiver flask under the tip of condenser and started the cold water circulation. Pipette out 10 cc of aliquot in the funnel of the distillation unit and added 10 cc of 45% NaOH in it. Distillate such prepared was titrated against 0.1N HCl till green to violet or pink colour appeared and note down this burette reading as end point. Putting recorded data in formulae, calculated the crude protein % for both the plant material in the study.

Determination of crude fiber

Weigh about 3 g of the material and extract the fat for about 8 hrs. Transfer the sample in to tall spoutless beaker and add 200 cc of 1.25 % H₂SO₄ in this beaker connect a flask with reflex condenser and heat so that the contents begin to boil. Continued the boiling for exactly 30 minutes. Filter the content of a beaker in a conical flask through a muslin cloth (18 threads/sq.cm) held over the glass funnel. Washed the residue on cloth with boiling water till residue gets free from acid. Test with blue litmus paper. Transfer the material from muslin cloth back to the original beaker. Add 1.25 % NaOH 200 cc upto the mark and heat to boil for exactly 30 minutes. Filter the content of beaker through the same muslin cloth on the conical flask. Wash the residue with boiling water till residue gets free from alkali. Test with red litmus paper. Dip the muslin cloth with residue in petroleum ether to remove the fat for 5 minutes. Transfer the residue to a clean dry silica basin. Dry it at 100 ± 5°C in hot air oven for 2-3 hours, cool and weigh. Ignite the residue in muffle furnace at 600°C for 30 minutes. Cool and weigh. After getting all estimated values, crude fiber % was calculated using standard formula.

Ether extracts

Weigh about 2-5 gm moisture free sample and transfer it in a filter paper/thimble. Plug the mouth of the thimble with fat free absorbent cotton. Introduced the thimble with sample in to the Soxhlet's extractor assembly. Filled an

extractor with petroleum in Soxhlet's apparatus and plugged the tip of the condenser with cotton. Place the apparatus on a heater plate at 40°C and extracted approximately for 8 hours [about 250 cycles]. After extraction was over, removed the thimble with the material from the extractor. Assembled an apparatus again and heat it on the heater plate to recover all the ether from the receiver flask. Disconnect the receiver flask, wiped the outside of the flask thoroughly with a clean dry cloth to remove the film of moisture and dust and dry it in a hot air oven at 100°C. for 1 hour, cool it in a desiccator and weigh. Recorded the observed data and ether extract was calculated using recommended formula.

Total Ash

One gram accurately weighed sample was taken in a pre weighed dried crucible. It was incinerated in a muffle furnace up to 450° C. The crucible was taken out, self-cooled and weighed immediately. From the weight of the ash, the ash value was derived with reference to the air dried drug. It was calculated and expressed as % w/w.

Acid Insoluble Ash

The ash of the sample as procured above was taken with 25 ml dilute hydrochloric acid in a 100 ml of beaker, boiled for few minutes and cooled. It was then filtered through 41 No. Whatman filter paper (ashless) and washed with distilled water repeatedly till it becomes chloride free. The filter paper in the glass funnel, along with its residue, was kept for drying in the oven. The dried paper along with the residue was shifted to a pre weighed crucible, kept in muffle furnace and incinerated. After cooling it was weighed and the acid insoluble ash content was calculated from the weight of residue obtained and expressed as % w/w.

Water Soluble Extractive Value

Five gm. of the sample was weighed accurately. To it 100 ml of distilled water was added and kept covered overnight. It was stirred intermittently during first 6 hours. Next day, it was filtered. 25 ml of the filtrate was accurately measured with a pipette and transferred to the already weighed beaker. The beaker was placed on a water bath for evaporation of the water. After evaporation of the water it was dried in an oven, allowed cooling and weighed immediately. From the weight of the residue obtained, the percentage of water soluble extractive was calculated and expressed as % w/w.

Alcohol Soluble Extractive Value

The method adopted for this experiment was same as that of water-soluble extract but by using ethanol instead of water. Percentage of ethanol soluble extract was calculated and expressed as % w/w.

Determination Nitrogen Free Extract

The value of nitrogen free extract was obtained by subtracting the sum of moisture, crude protein, ether extract, crude fiber and ash from 100.

Determination Calcium

The calcium was determined volumetrically by titrating the solution with standard KMnO_4 . For this, 10 ml aliquot was pipetted out from the stock ash solution into 250 ml beaker. Diluted it up to 100 ml by distilled water and added 10 ml saturated solution of Ammonium oxalate and 2-3 drops of 0.2 % alcoholic solution of methyl red indicator in it. Mixed the solution thoroughly with glass rod. The solution turns red as the acid been extracted with HCl in the preparation of the acid soluble ash. The solution which is acidic in nature is neutralized by adding dilute 5 % Ammonium hydroxide solution drop by drop to just reach the pH range in the alkaline medium. Boil the content of the beaker for 10-15 minutes and cool it. Washed the precipitate with hot distilled water and filter through filter paper No. 40 to make it chloride free. The filtrate was then tested with 1 % AgNO_3 solution to detect presence of chlorides.

Transferred the filter paper to original beaker in such a way that outer surface of filter paper sticks to inner wall of beaker. Then dissolve the precipitate with jet of distilled water. Added 10 ml of conc. H_2SO_4 to the original beaker.

Heated the beaker till the contents started boiling. Titrated the solution (when it was hot) against N/10 KMnO_4 solution. The pink colour appearing after running KMnO_4 solution and recorded the volume of N/10 KMnO_4 required for titration. After getting the analyzed values, % of Calcium was calculated employing standard formula.

Determination Phosphorus

Phosphorus was estimated by volumetric method following standard protocol. The phosphorus is converted to "Phospho – ammonium – molybdate" by addition of ammonium molybdate. The precipitate is then dissolved in excess of standard alkali. The quantity of alkali required to dissolve the precipitate is indirectly found out by back titrating with standard HCl or HNO_3 .

Determination Salt

The salt content was determined based on its refractive index. Refractive index was determined by passing a light through a prism into a sample and measuring the light bends and establishing the critical angle.

Determination of pH

One gm of drug to be examined was taken with 100 ml of distilled water. The suspension was kept for about 4 hrs, during this period it was subjected to a magnetic stirrer for 2 minutes, several times. Reading was noted down with the help of digital pH meter. To check the accuracy of pH meter, the buffer solution of tablet BDH7 and BDH4 were used. The pH meter was standardized with the help of distilled water. Then the sample was measured for pH.

Determination of Gross Energy

The Gross Energy (GE) was measured with an adiabatic calorimeter bomb. Benzoic acid was used, as a standard, for calibration of the calorimeter system with a known caloric value (26.4 MJ/Kg). In order to establish the exact amount of the sample and primer to mix to obtain a complete combustion, some other samples from the same material were analysed. Variable quantities of water were added to simulate silage samples with a DM content between 14 and 39%, and polyethylene, with a predetermined caloric value (46.4 MJ/Kg), was added as a primer, the thus obtained samples were analysed and the value of energy was corrected by deducting the caloric value of the fuse and that obtained from the primer, the latter being calculated by multiplying the weight of the primer with its caloric value to get the Gross energy.

Preliminary Phytochemical Screening

The aqueous and 70% ethanolic extract of *C. pictus* leaves and *Enicostema littorale* plant material were subjected to preliminary phytochemical screening as per [9], at Dravyagun Vibagh, Institute of Research and Post Graduate Studies in Ayurveda, Kharghar, Navi Mumbai and it revealed the presence and absence of various phytochemical constituents, as under.

Determination of alkaloids

Two tests were employed for determination of alkaloids in both the extracts prepared us under.

- *Dragendroff's Test:* Extracts were dissolved individually in dilute Hydrochloric aci and filtered. Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.
- *Mayer's Test:* One ml portions of each extract was acidified with 2-3 drops of 1M Hydrochloric acid and treated with 4-5 drops of Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow or white coloured precipitate or turbidity indicates the presence of alkaloids

Determination of flavonoids

Shinoda test-The extracts were dissolved in methanol (50%, 1-2 ml) by heating. To an alcoholic solution of each of

the extract, three pieces of magnesium chips were added followed by a few drops of concentrated hydrochloric acid. Appearance of an orange, pink or red to purple colour indicates the presence of flavonoids.

Determination of glycosides

Molisch's test-Two ml portion of each extracts were shaken with 10 ml of water, filtered and filtrate was concentrated. To this 2-3 drops of Molisch's reagent was added, mixed and then 2 ml of concentrated sulfuric acid was placed carefully through the side of the test tube. Reddish violet ring appear, indicating the presence of glycosides.

Determination of carbohydrates

- *Benedict's test*- Extracts were shaken with 10 ml of water, filtered and filtrate was concentrated. To this 5ml of Benedicts reagent solution was added and boiled for 5 minutes. Formation of brick red coloured precipitate indicates the presence of Carbohydrates.
- *Anthron test test*- Extract was shaken with 10 ml of water, filtered and filtrate was concentrated. To this 2 ml of anthrone reagent solution was added. Formation of green or blue color indicates presence of carbohydrates.

Detection of proteins

Xanthoproteic Test- The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Detection of reducing sugar

Fehlin's test- To a test tube 1 ml each a Fehling's A and B solutions were added and mixed. To this 2 ml of plant extract was added and heated on a boiling water bath for 10 minutes. Formation of brick red or orange precipitate indicates the presence of reducing sugar/ carbohydrates.

Determination saponins

Foam Test- 0.5 ml of each extracts were shaken with 2 ml of water. Foam produced persists for ten minutes indicates the presence of saponins.

Determination resins

Acetone Test- One ml of each extracts was dissolved in acetone and the solutions were poured in distilled water. Turbidity indicates the presence of resins.

Determination of steroids

Salkowski reaction- Each extracts were shaken with chloroform, to the chloroform layer sulphuric acid was added slowly by the side of test tube. Formation of red colour indicated the presence of steroids.

Determination of tannins

Ferric chloride test- A small quantity of the each extracts were boiled with water and filtered. Two drops of ferric chloride was added to the filtrate. Formation of a blue-black or green blackish colour in the presence of ferric chloride precipitate was taken as evidence for the presence of tannins.

Determination of triterpenoids

Sulphuric acid test-Two mg of dry extract was dissolved in acetic anhydride, heated to boiling cooled and then 1ml of concentrated sulphuric acid was added along the side of the test tube. Formation of a pink colour indicates the presence of triterpenoids.

Determination of Quinones:

Sodium hydroxide test-To extract, sodium hydroxide was added. Formation of green, blue or red colour indicated the presence of quinone.

Results and Discussion

The physico-chemical, Nutrient Profile and Proximate parameters of the coarsely grind leaves of *C. pictus* and Whole plant of *E. littorale* course powder are tabulated as **Table 1**. Whereas, the results of Preliminary phytochemical screening for various functional groups is presented in **Table 2**.

Table 1 Physico-chemical, Nutrient Profile and Proximate analysis of the herbs

Sr. No	Parameters studied	Values obtained for herbs under study	
		<i>Costus pictus</i>	<i>Enicostema littorale</i>
1.	Moisture	07.93 %	08.69 %
2.	Crude Protein	14.21 %	06.54 %
3.	Crude Fibre	20.05 %	20.79 %
4.	Ether Extract	05.18 %	02.04 %
5.	Total Ash	13.99 %	34.93 %
6.	Sand & Silica (Acid Insoluble Ash)	03.00 %	31.35 %
7.	Water soluble extractive	11.52 %	09.84 %
8.	Alcohol soluble extractive	06.08 %	02.64 %
9.	Nitrogen Free Extract	54.66 %	19.67 %
10.	Calcium	01.90 %	01.10 %
11.	Phosphorus	00.36 %	00.12 %
12.	Salt	00.46 %	00.29 %
13.	pH	05.70	06.23
14.	Foreign matter	Nil	Nil
15.	Gross energy	3640 kcal/kg	2474 kcal/kg

Table 2 Results of Phytochemical screening of extracts of plant material

Sr. No	Chemical Constituent	Test Applied	<i>Costus pictus</i>		<i>Enicostema littorale</i>	
			Aq. extract	Et. extract	Aq. extract	Et. extract
1.	Alkaloids	Dragondroffs	+	+	+	+
		Meyers	+	+	+	+
2.	Flavanoids	Shinoda test	+	+	+	+
3.	Glycosides	Molisch's test	+	+	+	+
4.	Carbohydrates	Benedict's	+	+	+	+
		Anthron test	+	+	+	+
5.	Proteins	Xanthoproteic	+	+	+	+
6.	Reducing Sugars	Fehling's test	+	+	+	+
7.	Saponins	Foam test	+	+	+	-
8.	Resins	Acetone test	+	-	-	-
9.	Steroids	Sulkowitch	+	+	+	+
10.	Tannin	Ferric chloride test	-	-	+	+
11.	Triterpenoides	Sulphuric acid	+	+	+	+
12.	Quinones	Sodium hydroxide	+	+	-	-

The plant kingdom has lots of hidden secrets, such as their complex compounds or active principles present in it which is thought to be responsible for their therapeutic efficacy. Knowledge about Physico-chemical, Nutrient Profile, Proximate parameters and phytoconstituent plays vital role in development of new chemotherapeutic agents. Current investigation revealed the promising results for consideration of *Costus pictus* and *Enicostema littorale* blume in drug discovery process.

Physicochemical parameters of whole plant powder of *E. littorale* were estimated based on the methods recommended by World Health Organization (WHO) [10]. As appearant from Table 2, the fiber content in present investigation of *costus pictus* and *E. littorale* were 20.05 % and 20.79 % respectively, which indicate the more fiber content is good and essential for diabetic patients, as stated by George [11]. The less value of moisture content of drugs as obtained in the present study could prevent content bacterial, fungal or yeast growth through storage [12]. Ash values are basically used to find out quality, authenticity and purity of unsophisticated drug. The extractive values observed in current assay are valuable to estimate the chemical constituents present in the crude drug and furthermore assist in evaluation of definite constituents soluble in a particular solvent [13].

Calcium, phosphorus and salts are required for the overall development of the bones in healthy individual. Basically, phosphorus plays a central *role* in *energy* and cell metabolism. It is present in every living cell in the nucleic acid fraction. A liberal supply of *Ca* and *P* is essential for normal physiological activities. Prominent supply of proteins, vitamins, salts, minerals element in body tissues is vital for normal health and so also in individuals with diabetes mellitus [14]. It is observed that *C. pictus* contains 1.90 % calcium, 0.36 % phosphorus and 0.46 % salts. Whereas, *E. littorale* whole plant extract contained 1.10 % calcium, 0.12 % phosphorus and 0.29 % salts in extract, which may act as dietary and mineral support to the diabetic patient.

Energy homeostasis is a well-regulated process that depends on the coordination between feeding behaviour and energy expenditure. The pancreas has the important role of maintaining energy balance in the body, as it is responsible for the secretion of insulin and glucagon. Insulin and glucagon are two counter regulatory hormones that control the systemic concentration of glucose, a metabolic intermediate used by cells as the primary source of energy. If glucose homeostasis is thrown off balance, a diabetic state develops. As the plant *C. pictus* and *E. littorale* contains gross energy value of 3640 kcal/kg and 2474 kcal/kg, respectively which could be defender to maintain the energy requirement of the diabetic patient and thus might be helping in combating diabetes mellitus.

As seen in **Table 2**, the preliminary phytochemical screening of aqueous and ethanolic extracts of both the plant material indicated the presence of alkaloids, saponins, flavonoids, steroids, tannins, proteins, quinines, reducing sugar, phenols etc. However, the tannin was absent in both and resin in ethanolic extract of *C. pictus*. Whereas, quinones and resins were negative for aqueous and ethanolic extracts of *E. littorale*. The presences of these constituents may possibly be responsible for the biological activities of *Enicostemma littorale*.

Conclusions

As there is not enough evidence for detailed physicochemical and phytochemical evaluation on *Costus pictus* and whole plant of *Enicostemma littorale* Blume is reported. Therefore present work is taken up in the view to primarily standardize the herb in accordance to parameters of World Health Organization (WHO) Guidelines and standard laboratory procedures. In the present investigations, both the plant material was studied thoroughly for their organoleptic characters; physicochemical characters and important active constituents to understand their quality, safety and medicinal properties. The generated information of the present study will provide data which is helpful in the correct identification and authentication of plant material as well as to predict the scope for Bioactivity-guided fractionation, isolation and purification of new chemical entity from the plant for pharmacological evaluation to cater them in to novel drug for the treatment.

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