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PRELIMINARY PHYTOCHEMICAL ANALYSIS OF SELECTED MEDICINAL PLANTS OF NORTH EASTERN REGION OF INDIA

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Abstract: Objective: To evaluate the possible phytoconstituents present in fruits and aerial parts of *Coccinia indica* (L.) used as traditional medicine by the inhabitants of North Eastern region of India. **Methods:** Preliminary phytochemical analysis was carried out by various standard qualitative tests of different fractions of the ethanolic extracts, described by Harbone. **Result:** The phytochemical screening of the fruit extract of *Coccinia indica* showed presence of various secondary metabolites like flavanoids, pterocarpans, alkaloids, triterpenoids, phytosterols and saponin glycosides while aerial part extracts of *Coccinia indica* were enriched with alkaloids, phenolic compounds and tannins, phytosterols and triterpene, flavanoids, saponins, and coumarins in their respective fractions. **Conclusion:** The findings will provide the foundation for future research regarding the isolation, quantification and characterization of bio-active phytoconstituents from *Coccinia indica* along with their possible pharmacological potential *in-vivo* and *in-vitro*.

Keywords: *Coccinia indica*, Phytochemical screening, Secondary metabolites, Traditional medicine, Fractionation

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INTRODUCTION

North Eastern part of India has rich ancient heritage of traditional medicine [1]. From last two decades, the utility of medicinal plants have been phenomenally increased due to their vast chemical biodiversity as World Health Organization advocated traditional medicines as safe remedies [2]. The Silent valley of the North eastern hilly areas preserves the true bio-diversity of Indian Territory with more than 700 various species of traditional medicinal plants [3]. The conventional therapeutic experiences of an array of bioactive phytoconstituents from those species, over hundreds years are considered as valuable remedial recipe to treat various acute and chronic disorders. Among them, *Coccinia indica* (*C. indica*) of Cucurbitaceae family commonly known as Little gourd (English), Kunduri (Assamese) is a well explored folkloric medicinal plants among various tribes of Assam to treat diabetes along with various acute to chronic ailments [4].

C. indica is a perennial climber grows in dry hilly areas in tropical and sub-tropical climate. It is slightly bitter in taste and mild warming in action with calming, strengthening and anti-inflammatory property [5]. Classical Ayurvedic literatures describe its potentiality as to regulate the nervous system (Vata), venous system (Pitta) and arterial system (Kapha) essential to restore health [6]. It is used as folkloric medicine to treat diabetes, jaundice, asthma and various inflammatory conditions [7]. Recent pharmacological studies reveal it possess hypoglycemic, hypolipidemic and antioxidant and free radical scavenging properties [8-10].

Although, very few research reports had been cited on isolation of the phytoconstituents from *C. indica* but in detail literature regarding the phytochemical screening of the plant has not being reported. Hence, with this scientific background, an attempt had been made to explore the possible phytoconstituents present in different fractions of the ethanolic extracts of fruits and aerial parts of *C. indica* obtained from North Eastern region of India.

MATERIALS AND METHODS:

Collection and authentication of plant material:

The whole plant of *C. indica* was collected from Narengi, Kamrup district of Assam in June, 2017. The plants yield maximum phytoconstituents during the flowering and fruiting season in May-July; hence, they had been collected during that period.

The collected plant materials were washed and shade dried. The dried raw materials were pulverized to coarse powder (sieve no. 60) and stored in air tight container for extraction. The photographs of fruits and aerial parts of *C. indica* are given in figure 1.


Figure 1: Fruits and aerial parts of *C. indica* (L.)

Authentication:

The plant had been identified and authenticated by Prof. (Dr.) Partha Pratim Baruah, Professor and Head, Department of Botany of Guwahati University, Guwahati (specimen no.: 18395 dated 10.02.2018). The herbarium of *C. indica* (voucher specimen no. AdtU/Hb-01/2017) was preserved in the institution.

Preparation of the extracts:

100 gm each coarsely powdered raw material of fruit and aerial parts of the plants were extracted separately by cold maceration with ethanol for 3 days, with frequent shaking for first day and occasional stirring for the remaining days at room temperature. The process was repeated once more in order to increase the yield. The extracts were combined and filtered through Whatman No. 1 filter paper and concentrated by rotary flash evaporator at 40^0C temperature under reduced pressure and stored in deep freezer at -20^0C. The yield of ethanolic extracts of the fruits and aerial parts of *C. indica* were found 12.42% w/w and 16.18 % w/w respectively.

The concentrated ethanolic extracts of both the plants were subjected to fractionation with different solvents with increasing order of polarity (petroleum ether, chloroform, ethyl acetate, n-butanol and methanol)[11]. A flow chat of detailed method of extraction and fractionation is given in figure 2.

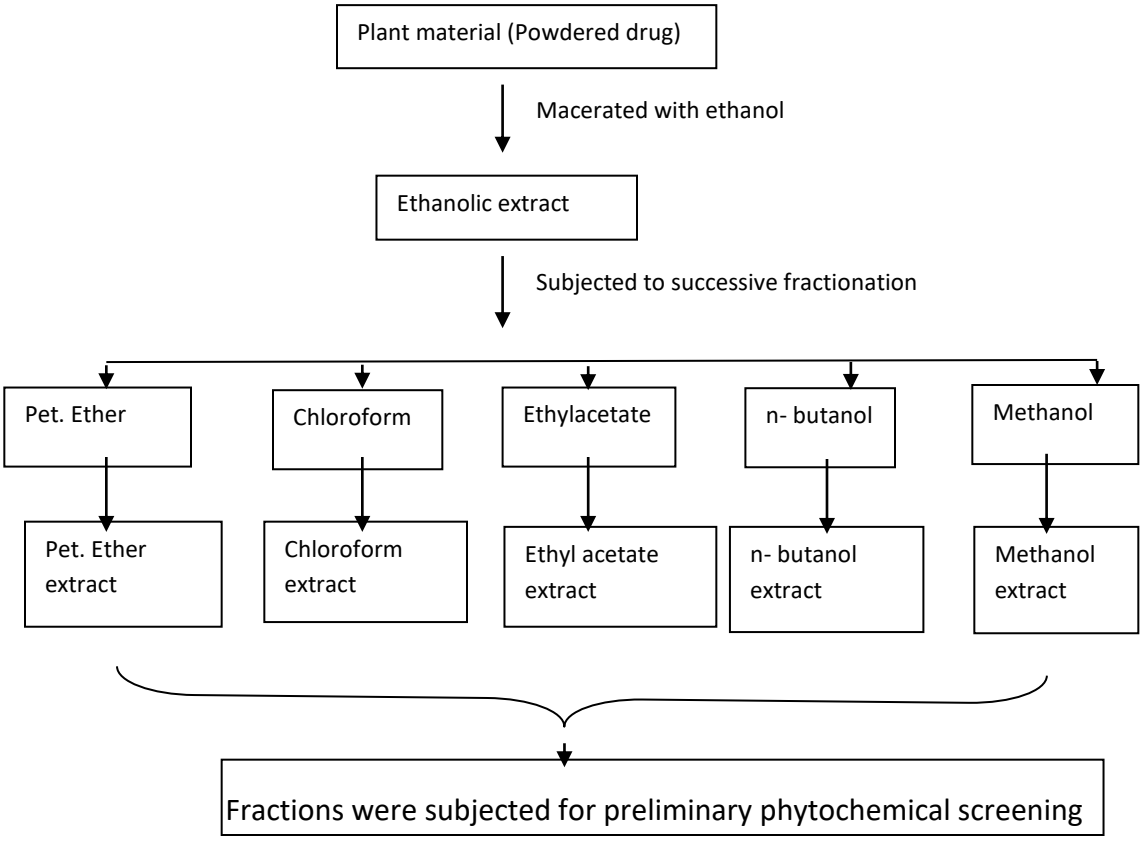

Figure 2: Schematic diagram of extraction and fractionation of fruits and aerial parts of *C. indica*

Preliminary phytochemical screening:

The crude fractions of *C. indica* were subjected to different qualitative phytochemical screening to identify the presence of various phytoconstituents as described by Harborne [12]. Various phytochemical examinations were carried out based on following tests.

1. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

i. Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

ii. Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

iii. Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

iv. Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

4. Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

5. Detection of saponins:

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

6. Detection of phytosterols

Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

7. Detection of phenols:

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

8. Detection of tannins:

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

9. Detection of flavonoids:

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

10. Detection of proteins and aminoacids

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

Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

11. Detection of diterpenes

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes

RESULT:

The crude ethanolic extracts of fruits and aerial parts of *C. indica* showed presence of diverse phytochemicals in their respective fractions. A total of 5 different types of fractions from individual extracts were prepared to test the availability of 10 phytoconstituents viz. alkaloids, phenols, flavanoids, saponins, phytosterols and triterpene, tannins, carbohydrates, proteins,

carboxylic acid and coumarins. Hence, in total 50 (5 X 10) qualitative test were performed for each extract. The results were tabulated in the table 1.

Table 1: Preliminary phytochemical screening of fruits and aerial parts of *C. indica*

Compounds	Ethanolic extracts									
	Pet. Ether		Chloroform		Ethyl acetate		n-butanol		Methanol	
	A	B	A	B	A	B	A	B	A	B
Alkaloids	-	-	++	+	++	+	+	-	-	-
Phenols	-	-	+	-	+	+	++	++	+++	++
Flavanoids	-	-	-	+	++	+	++	+	+++	++
Saponins	-	+	+	+	++	-	++	-	-	-
Phytosterols and Triterpene	+	+	+	+	++	++	++	+	+	-
Tannins	-	-	+	+	+	++	++	++	+++	++
Carbohydrates	-	-	-	-	+	+	+	++	+	++
Proteins	-	-	-	-	-	-	-	-	-	-
Carboxylic acid	-	-	-	-	-	-	-	-	++	-
Coumarins	-	-	-	-	+	-	++	+	++	++
Total	1	**2**	5	**5**	8	**6**	8	**6**	7	**5**

A: Fruits; **B: Aerial parts; (-) absent; (+) low; (++) average; (+++) high**

The ethyl acetate and n-butanol fractions of *C. indica* fruits showed maximum occurrence of phytoconstituents (8/10), followed by methanol extracts (7/10). The chloroform fraction showed presence of 5 different phytoconstituents. The petroleum ether extract showed presence of phytosterols and triterpenoid compounds. The phenolic compounds and tannins were present in all the extracts except petroleum ether. Alkaloids were present in chloroform,

ethyl acetate and n-butanol fractions where as carbohydrates, coumarins and flavanoid glycosides showed their occurrence in ethyl acetate, n-butanol and methanol extracts. Proteins were found to be absent in all the fractions. Presence of carboxylic acid was identified in methanol fraction only.

The ethyl acetate and n-butanol fraction of *C. indica* aerial parts showed maximum occurrence of phytoconstituents (6/10). The chloroform and methanol fractions were rich with 5 different types of phytoconstituents where as petroleum ether fraction was found to possess phenolic compounds and saponins. Flavanoids and tannins gave positive response for all the fractions except petroleum ether. Alkaloids were present in chloroform and ethyl acetate fractions. Carboxylic acid was absent in all the fractions. Carbohydrates were tested positive in ethyl acetate, n-butanol and methanol fractions where as coumarins were observed in n-butanol and methanol fractions. Phytosterols and triterpene were identified in chloroform, ethyl acetate and n-butanol fractions.

DISCUSSION:

C. indica posses wide range of therapeutic potential which may be associated with their bioactive secondary metabolites. Hence, an exhaustive phytochemical screening was performed to explore the possible phytoconstituents of both the plants with ten different fractions. Out of ten fractions, nine fractions were tested positive for phytosterols and triterpenoids where as tannins illustrated their existence in eight fractions. Phenolic compounds were present in seven fractions and flavanoid and saponin glycosides were observed in six fractions. Alkaloids, coumarins glycoside and carbohydrates were found in five fractions while proteins were tested negative in all the fractions.

Phytochemicals such as saponins are used traditionally as anti-diabetic, hypoglycemic, hypocholesteromic, and hemolytic drug where as tannins are being valued for anti-diarrheal, astringent property [13-15]. Antioxidant profiles of flavanoid glycosides are well established and associated with several CVS, CNS beneficial effects [16]. Alkaloids are very famous for 7their psychoactive potential along with anti-diabetic and cardio protective activity [17]. Steroids and triterpenoids showed hypocholesteromic, analgesic properties [18]. These observations cited on various phytochemicals support our findings on the usefulness of *C. indica*. Therefore, bioassay guided isolation, identification and characterizations along with in depth research to reveal the structure-activity relationship of these bioactive constituents are essential. Hence, we recommend further research on these plants to quantify and establish various pharmacological activities of these phytoconstituents.

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CONFLICT OF INTEREST:

We declare that we have no conflict of interest.

REFERENCES:

1. Ramila M, Manoharan A. Characteristics of pharmacognostical significance of *Erythrina variegate* var. and *Ficus racemosa* Linn. bark. Journal of Chemical and Pharmeutical Research. 2011; 3(6): 707-14.
2. Muraleedharannair JM, Johnson MA, Mony M, Zachariah MP, Solomon J. Phytochemical studies on Azolla pinnata R. Br., Marsilea minuta L. and Salvinia molesta Mitch.. Asian Pacific Journal of Tropical Biomedicine. 2011; 2: S26-29.
3. Shiddamallayya N, Azara Y, Gopakumar K. Hundred common forest medicinal plants of Karnataka in primary healthcare. Indian Journal of Traditional Knowledge. 2010; 9(1): 90-95.
4. Tamilselvan N, Thirumalai T, Elumalai EK, Balaji R, David E. Pharmacognosy of *Coccinia grandis*: a review. Asian Pacific Journal of Tropical Biomedicine. 2011; 2: S299-302.
5. Deokate UA,. Khadabadi SS. Pharmacology and phytochemistry of *Coccinia indica*. Journal of Pharmacognosy and Phytotherapy. 2011; 3(11): 155-59.
6. Khare CP. *Indian Medicinal plants*. New Delhi: Springer; 2009.
7. Ajay SS, Bhaskar VH, Teotia D . Quality standardization of *Coccinia indica* fruit. Asian Journal of Chemistry. 2009; 21(7): 5351-54.
8. Azad KAK, Akhtar S, Mahtab H. *Coccinia indica* in the treatment of patients with diabetes mellitus. Bangladesh Medical Research Council Bulletin. 1979; 5(2): 60-66.
9. Brahmachari HD, Augusti KT. Orally effective hypoglycemic principles from *Coccinia indica*. Journal of Pharmacy and Pharmacolology. 1963; 15(6): 411-12.
10. Gupta SS, Variyar MC. Experimental studies on pituitary diabetes. Iv. Effect of *Gymnema sylvestre* and *Coccinia indica* against the hyperglycaemic response of somatotropin and corticotropin hormones. Indian Journal of Medical Research. 1964; 52: 200-07.
11. Johnson M, Jalaja SA, Solomon J, Selvamony S, Babu A. Preliminary phytochemical studies on the methanolic flower extracts of some selected medicinal plnts from India. Asia.n Pacific Journal of Tropical Biomedicine. 2012; 2: S79-82.
12. HarboneJB. Phytochemical methods: a guide to modern techniques of plant analysis, Thompson Publishing IT, New York. 1998.

13. Mallick C, De Debasis, Ghosh D. Correction of protein metabolic disorders by composite extract of *Musa paradisiaca* and *Coccinia indica* in streptozotocin-induced diabetic albino rat: an approach through the pancreas. Pancreas. 2009; 38(3): 322-29.
14. Vaishnav MM, Gupta KR. A new saponin from *Coccinia indica* roots. Fitoterapia.1995; 66(6): 546-47.
15. Vaishnav MM, Jain P, Jogi SR, Gupta KR. Coccinioside-K, triterpenoid saponin from *Coccinia indica*. Oriental Journal of Chemistry. 2001; 17(3): 465-68.
16. Sarita K, Amol B, Smita D, Kavita S, Mukta A. Flavonoids: An overview. Journal of Pharmacy Research. 2009; 2(6), 1148-54.
17. Jeyaprakash K, Ayyanar M, Geetha KN, Sekar T. Traditiona uses of medicinal plants among the tribal people in Theni district (Westernghats), south India. Asian Pacific Journal of Tropical Biomedicine. 2011; S20-S25.
18. Arshad H, Shadma W, Aleza R, Sarfaraj H. Macroscopical, anatomical an physic-chemical studies on leaves of Coccinia indica Wight & Arn., growing wildly in eastern Uttar Pradesh rgion of India. Indian Journal of Natural Product Research. 2011; 2(1): 74-80.