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Research Article

Extraction, Qualitative and Quantitative Determination of Secondary Metabolites of *Coccinia Indica* Fruits

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ABSTRACT

In the last few years, there has been an exponential growth in the field of herbal medicine and gaining popularity both in developing and developed countries because of their natural origin and less side effects. Medicinal plants continue to be an important therapeutic aid for alleviating ailments of humankind. *Coccinia Indica* (*C. indica*, cucurbitaceae) is found in warmer and humid part of India. It is also known as Kundru, Bimbi, Lindora. The various extracts of fruit, root juice & leaves of the plant have been reported to be anti-diabetic, dysentery, vomiting, mouth ulcers and bronchitis, asthma and gastrointestinal disturbance. The phytochemicals of this plant include saponin, flavonoid, glycosides and polysaccharides, xyloglucan, taraxerol, carotenoids, cryptoxanthin. The aim of the present study is to examine *C. indica* fruits for phytochemical profile. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by Folin Ciocalteu reagent method and aluminium chloride method respectively. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, fixed oil and fats. The total phenolics content of fruits ethanolic extract was (4.3524 mg/100mg), followed by flavonoids (5.0900mg/100mg). The present study concluded that the crude extract of *C. indica* is a rich source of secondary phytoconstituents which impart significant antioxidant potential. This work also contributes significantly to support the claim about the use of this herb in folk medicines. Further investigation regarding isolation and purification of a number of phytoconstituents from fruits, leaves, stem, flowers and seeds of *C. indica* may yield optimal combinations of therapeutic alternates.

Keywords: *Coccinia indica*, Qualitative analysis, Quantitative analysis, TPC, TFC, Folin Ciocalteu

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INTRODUCTION

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country¹. Herbal medicine is still the mainstay of about 75%-80% of the whole population and the major part of traditional therapy involves the use of plant extract and their active constituents. Following the advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades, advances in photochemistry and in identification of plant compounds, effective against certain diseases have renewed the interest in herbal medicines². The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins and phenol

compounds, flavonoids, resins, fatty acids, gums which are capable of producing definite physiological action on body³. *Coccinia indica* (Bimba, kanduri, kundru Cucurbitaceae) is famous for its hypoglycemic and antidiabetic properties in Ayurvedic system of medicine. It is found in warmer and humid part of India⁴. *C. indica* grows abundantly all over India, Tropical Africa, Australia, Fiji and throughout the oriental countries^{5,6}. It is also known as *Cephalandra indica*⁷. Plant contains resins, alkaloids, fatty acids, flavonoids and proteins as chief chemical constituents. Aspartic acid, Glutamic Acid, Asparagine, Tyrosine, Histidine, Phenylalanine And Threonine, Valine, Arginine are also found. The methenolic extract of fruit contains alkaloids, steroids, tannins, saponins, ellagic acid, phenols, glycosides, lignans and triterpenoids⁸. Roots contains Triterpenoid, saponinococcinoside, Flavonoid glycoside ombuin 3-o- arabinofuranoside, Lupeol, β -amyrin, and β -sitosterol and Stigmast-7-en-3-one⁹⁻¹¹. The whole plant is traditionally used for various medicinal purposes. Leaves of this plant are used in Indian folk medicine for treatment of

number of ailments including diabetes, wounds, ulcers, inflammation, in eruptions of skin, fever, asthma and cough. Earlier scientific investigation of *C. indica* showed that the crude extract has hepatoprotective¹²⁻¹⁷, anti-diabetic hypolipidemic¹⁸⁻²⁰, anti-bacterial²¹ and anthelmintic activity²², analgesic and antipyretic activity²³, wound healing activity²⁴, anti-inflammatory²⁵. Though the plant has been reported for many biological activities, no scientific data available to identify the genuine sample. The aim of this work was to determine the quality (types), quantity (amount) of bioactive compounds of fruits of *C. indica*.

MATERIALS AND METHODS

Plant materials

The fruits of *C. indica* were collected in the month of September and October 2018 from Bhopal, Gwalior and Sehore district MP and were authenticated by the Head of the Botany Department, Govt. M.L.B Girls College, Bhopal. Vouchers specimens were deposited at the herbarium of the college. The collected fruits were washed, chopped; shade dried and was pulverized with mechanical pulverizer for size reduction. It was then passed through mesh 40 and the fine powder was collected and used for the experiment and preparation of extract.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SigmaAldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

Extraction

Coarse powder of air dried fruits of *C. indica* were packed in muslin cloth and subjected to successive soxhlet extraction for continuous hot extraction with petroleum ether, chloroform and ethanol for 8 hrs separately. Then the each extracts were filtered. The extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts²⁶.

Qualitative phytochemical analysis of plant extract

The *C. indica* extracts obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate^{27, 28}. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Test for carbohydrates

Molisch's test: In a test tube containing extract of drug, added two drop of freshly prepared 20% alcoholic solution of α -naphthol and mixed concentrated sulphuric acid along the sides of the test tube. If carbohydrate present purple color or reddish violet color produce at the junction between two liquids.

Benedict's test: In a test tube containing extract of drug add benedict's solution, mix well, boiled the mixture vigorously for two minutes and then cooled. Formation of red precipitate due to presence of carbohydrates.

Barfoed's test: The barfoed's solution added to 0.5 ml of solution under examination, heated to boil. Formation of red precipitate of copper oxide was indicated the presence of carbohydrates.

Anthrone test: To the two ml of anthrone test solution, add the extract of drug. A green or blue colour indicated the presence of carbohydrate.

Test for alkaloids

Dragendorff's Test: Few mg of extract of the drug dissolved in 5 ml of water added 2 M hydrochloric acid until an acid reaction occurred; 1 ml of dragendorff's reagent (potassium bismuth iodide solution) was added an orange red precipitate indicated the presence of alkaloids.

Wagner's test: Acidify the extract of drug with 1.5 % v/v of hydrochloric acid and added a few drop of Wagner's reagent (iodine potassium iodide solution). Formations of reddish brown precipitate indicated the presence of alkaloids.

Mayer's Test: Two ml of extract solution was treated with 2 - 3 drops of Mayer's reagent was added (potassium mercuric iodide solution) formation of dull white precipitate indicated the presence of alkaloid.

Hager's Test: Extract of the drug solution was treated with 3 ml of Hager's reagent (saturated solution of picric acid) formation of yellow precipitate confirmed the presence of alkaloids.

Test for glycosides

Legal's test: Extract solution dissolved in pyridine then sodium nitroprusside solution was added to it and made alkaline. Pink red colour indicated the presence of glycosides.

Baljet's test: To the drug extract, sodium picrate solution was added, yellow to orange colour was indicated the presence of glycosides.

Borntrager's test: Few ml of dilute sulphuric acid solution, the test solution of extract was added. It was filtered and the filtrate was boiled with ether or chloroform. Then organic layer was separated to which ammonia was added, pink, red or violet colour was produced in orange layer confirmed the presence of glycosides.

Keller Kiliani test: Methanolic extract was dissolved in glacial acetic acid containing trace of ferric chloride one ml concentrated sulphuric acid was added carefully by the side of the test tube. A blue colour in the acetic acid layer and red colour at the junction of the two liquid indicated the presence of glycosides.

Test of saponins

1 ml of alcoholic extract was diluted with 20 ml distilled water and shaken in graduated cylinder for 15 minutes. One cm layer of foam indicated the presence of saponins.

Test for flavonoids

Shinoda test: In the test tube containing alcoholic extract of the drug added 5 - 10 drops of dil. hydrochloric acid followed by the small piece of magnesium. In presence of flavonoids a pink, reddish pink or brown color was produced.

Test for tannins

To the sample of the extract, ferric chloride solution was added appearance of dark blue or greenish black colour indicated the presence of tannins.

To the sample of extract, potassium cyanide was added, deep red colour was confirmed the presence of tannins.

To the sample of extract, potassium dichromate solution was added, yellow precipitate was produced.

Test for protein and amino acid

Biuret's test: To 2 - 3 ml of the extract of drug added in 1 ml of 40 % sodium hydroxide solutions and 2 drops of 1 % copper sulphate solution mix thoroughly, a purplish - violet or pinkish - violet colour produced that indicates the presence of proteins.

Ninhydrin's test: Two drops of freshly prepared 0.2 % ninhydrin reagent was added to the extract and heated to boiling for 1 - 2 min. and allow cooling. A blue colour developed that indicating the presence of proteins, peptides or amino acids.

Xanthoprotein test: To the extract in a test tube, add conc. nitric acid. A white precipitate was obtained and upon heating turns to yellow and cool the solution carefully. Added 20 % of sodium hydroxide solution in excess orange colour indicated presence of aromatic amino acid.

Millon's test: The small quantity of extract of the drug dissolved in distilled water added 5 - 6 drop of millon's reagent. A white precipitate was formed which turned red on heating, indicated the presence of proteins.

Lead acetate test: The extract was taken and two ml of 40 % sodium hydroxide solution was added and boiled, glacial acetic acid was added and cooled than added 1 ml of lead acetate solution, gray black precipitate was formed which indicated presence of sulphur containing amino acid.

Test of fats or fixed oils

Using sodium hydroxide: The extract was mixed in one ml 1 % of copper sulphate solution then added 10 % sodium hydroxide solution a clear blue solution was obtain which showed glycerin present in sample.

Using sodium hydrogen sulphate: The extract was taken in test tube added a pinch of sodium hydrogen sulphate pungent odour was formed which showed glycerin present in sample.

Saponification: Four ml of 2 % sodium carbonate solution was taken and the extract was added. Shaked vigorously and boiled. A clean soapy solution was formed cooled and added few drops of conc. HCl and observed that fatty separate out and float up.

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TPC and TFC are determined. Extracts obtained from flower of *C. indica* plant material of subjected to estimate the presence of TPC and TFC by standard procedure.

Total phenol determination

The total phenolic content was determined using the method of Olufunmiso *et al*²⁹. A volume of 2ml of each extracts or standard was mixed with 1 ml of Folin Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso *et al* [29]. 1 ml of 2% AlCl₃ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer. The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

RESULTS AND DISCUSSIONS

The crude extracts so obtained after each of the successive soxhlet extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The percentage yield of extraction is very important in phytochemical extraction in order to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from the fruits of the plants using petroleum ether, chloroform, and ethanol as solvents are depicted in the Table 1.

Table 1: Results of percentage yield of fruits extracts

Plant Name	Percentage yield (%)		
	Pet. ether	Chloroform	Ethanol
<i>C. indica</i>	3.5	4.3	6.3

The results of qualitative phytochemical analysis of the crude powder of fruits of *C. indica* are shown in Table 2. Ethanolic extracts of fruits sample of *C. indica* showed the presence of alkaloids, terpenoids, flavonoids, phenols, tannins, carbohydrate, glycosides, saponins and Phytosterols, chloroform extracts show the presence of glycosides, terpenoids, saponins but in petroleum ether extracts all phytoconstituents was absents.

Table 2: Phytochemical evaluation of *Coccinia indica* fruits

Test	Petroleum ether	Chloroform	Ethanol
Alkaloid	-	-	+
Glycoside	-	+	+
Terpenoids	-	+	+
Flavonoids	-	-	+
Phenols	-	-	+
Tannins	-	-	+
Carbohydrates	-	-	+
Saponins	-	+	+
Phytosterols	-	-	+
protein and amino acids	-	-	-
Fixed oil and fats	-	-	-
Resin	-	-	-

The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. TPC of ethanolic and chloroform extract of *C. indica* fruits showed the content values of 4.3524 and 0.0435 respectively. But petroleum ether extracts of *C. indica* fruits have no phenolic content. The total flavonoids content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. The total flavonoids estimation of ethanolic and chloroform extracts of

fruits of *C. indica* showed the content values of 5.0900 and 0.0125 respectively. The above results showed that chloroform extract contain less phenolic and flavonoids content than the alcoholic extract. It may due to the solubility of principle contents presence be higher in case of alcoholic solvent, thus it has been accepted that it is a universal solvent for the extraction of plant constituents. Results are provided in Table 3.

Table 3: Estimation of total phenolics and total flavonoids content in *Coccinia indica*

S. No	<i>Coccinia indica</i> Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	Petroleum ether	-	-
2.	Chloroform	0.0435	0.0125
3.	Ethanol	4.3524	5.0900

CONCLUSION

The present study concluded that this medicinal plant viz. *Coccinia indica* is a promising source of various activities and may be efficient as preventive agents in the pathogenesis of some diseases. However, the strength of the existing data is not enough to suggest a reasonable mode of action for antioxidant effects. Further phytochemical studies are also required to isolate and characterize active ingredients that are responsible for its antioxidant activity and to explore the existence of synergism if any, among the compounds.

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