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Macroscopical, anatomical and physico–chemical studies on fruits of *Coccinia indica* Wight & Arn. (Cucurbitaceae)

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PEER REVIEW

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Comments

Study is based upon the very popular
medicinal plant. Article give the
detail information regarding the
various evaluation parameter of the
plant like ash values, extractive
values, microscopical parameter and
the chemical group present in the
plant which may be responsible of its
various pharmacological activities.
Details on Page S127

ABSTRACT

Objective: To rationalize the macroscopical, anatomical and physico–chemical studies on fruits of *Coccinia indica* Wight & Arn. (Cucurbitaceae).

Methods: The crude ethanolic extract of fruits of *Coccinia indica* Wight & Arn. (Cucurbitaceae) was using physico–chemical parameters, fluorescence analysis, and preliminary photochemical investigation (TLC, HPTLC, column chromatography).

Results: An attempt has been made to highlight this folk herbal medicine through present study which will assist in the identification of fresh as well as dried crude samples of fruits anatomically and physico chemically. TLC fingerprint profiling and fluorescence analysis of powdered fruits were also carried out and the salient qualitative and quantitative parameters are reported.

Conclusions: The present study will provide referential information for correct identification and help in checking adulteration in market samples used in the preparation of various herbal medicines. The present observation will also be helpful in macroscopical, anatomical and physico–chemical studies on fruits of *Coccinia indica* Wight & Arn. (Cucurbitaceae).

KEYWORDS

Coccinia indica, Ivy gourd, Kundru, Anatomy, Physico–chemical

1. 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 country[1]. 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 medicines[2]. Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune suppression and allergic reactions[3]. This situation forced to search for new antimicrobial substances. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants. Antimicrobials of plant origin have enormous therapeutic potential[4].

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They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials[5]. 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 body[6].

Coccinia indica (*C. indica*) belongs to the family Cucurbitaceae. It is growing wild throughout India and also cultivated in various parts of India. It is commonly known as kundru[7]. 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 hepatoprotective[8–13], anti-diabetic hypolipidemic[14–16], anti-bacterial[17] and anthelmintic activity[18], analgesic and antipyretic activity[19], wound healing activity[20], anti-inflammatory[21]. Though the plant has been reported for many biological activities, no scientific data available to identify the genuine sample.

The present investigation was therefore taken up to establish identity of fresh and dried fruits morphologically microscopically and physicochemically for the standardization of the drug.

2. Materials and methods

2.1. Collection and authentication

The fresh fruits of wildy growing plant *C. indica* were collected from the field areas of eastern Uttar Pradesh region during the month of September, 2012. For identification and taxonomic authentication, sample of plant material was given to National Vrakshayurveda Research Institute (NVRI) Jhansi, India. The text report from National Vrakshayurveda Research Institute, Jhansi, India confirmed that the authenticity of plant material sample was *C. indica* with voucher specimen no. NVRI-SOP-20932, 01-09-2012. The fresh fruits were used for the study of macroscopic and microscopical characters. Whereas collected plants were shade-dried and coarsely powdered. This coarse powder was used for the determination of ash values, extractive values and preliminary phytochemical investigation as per standard methods.

2.2. Macroscopic studies

2.2.1 Root

Root available in cut pieces with a few lateral roots, surface rough due to longitudinal striations and lenticels,

cylindrical, 2.5 cm in diameter, greyish–brown.

2.2.2. Stem

Slender, soft, 1.5 cm in diameter branched longitudinally grooved, glabrous, nodes swollen, whitish dots over external surface, a few tendrils attached with nodes grayish coloured externally and cream to light yellow Internally, fracture, fibrous, no odour taste.

2.2.3. Flower

Ebracteate, pedicellate, incomplete, unisexual, actinomorphic, penatamerous.

2.2.4. Male flower

Pedicel 8 cm long, subfiliform, calyx tube glabrous, broadly campanulate, 5 mm long linear corolla 2.5 cm long, white veined pubescent inside, glabrous outsides, segments 7.5 cm long triangular, acute ,stamina column glabrous, capitulum of anthers subglobose.

2.2.5. Female flower

Pedicel 2.5 cm long calyx and corolla as in males flower staminodes 3, subulate 3 cm long ovary fusiform, glabrous, slightly ribbed, sigma 3, bofid.

2.2.6. Fruit

A pepo, ovoid, glabrous, 4.5 cm long 2 cm thick greenish–brown to yellowish –brown with white linings; no odour and taste.

2.2.7. Seed

Somewhat obovoid 0.7 cm long and 0.3 cm wide rounded at apex much compressed, yellowish–grey. These structures are given below in Figure 1.



Figure 1. *C. indica*. a: whole plant; b: flower; c: fruits; d: leaves.

2.3. Microscopic

2.3.1. Root

The root shows 7 or more rows of thin walled cork cell

having lenticels at places; secondary cortex 5 layered oval to elliptical, tangentially elongated thin walled parenchymatous cells having groups of oval to rectangular, elongated stone cell in lower region.

2.3.2. Stem

Mature stem ridges and furrows shows a single layered epidermis composed of tabular cells externally covered with cuticle or the epidermis Interrupted at certain places due to formation o cork cell.

2.3.3. Leaf

Petiole shows single layered epidermis, consisting of flattened, tangentially elongated cells, covered externally with striated cuticle, cortex differentiated in to 6 layered collenchymas and 6 layered circular, thin walled

parenchymatous cells with conspicuous intercellular spaces. vascullar bundles bicollateral, arranged in single ring, usually seven and nine larger and two smaller.

2.3.4. Fruits

Epicarp single layered mesocarp composed of wide zone of thin-walled parrenchymatous cells differentiated in to two regions, outer 6 layered rectangular to polygonal smaller in size while inner region composed of oval to polygonal cells of larger size; a few fibro vascular bundles present in this region. These structures are given below in Figure 2 and Figure 3.

2.4. Extraction of plant materials

A total of 250 g coarse powder of air dried fruits of *C.*

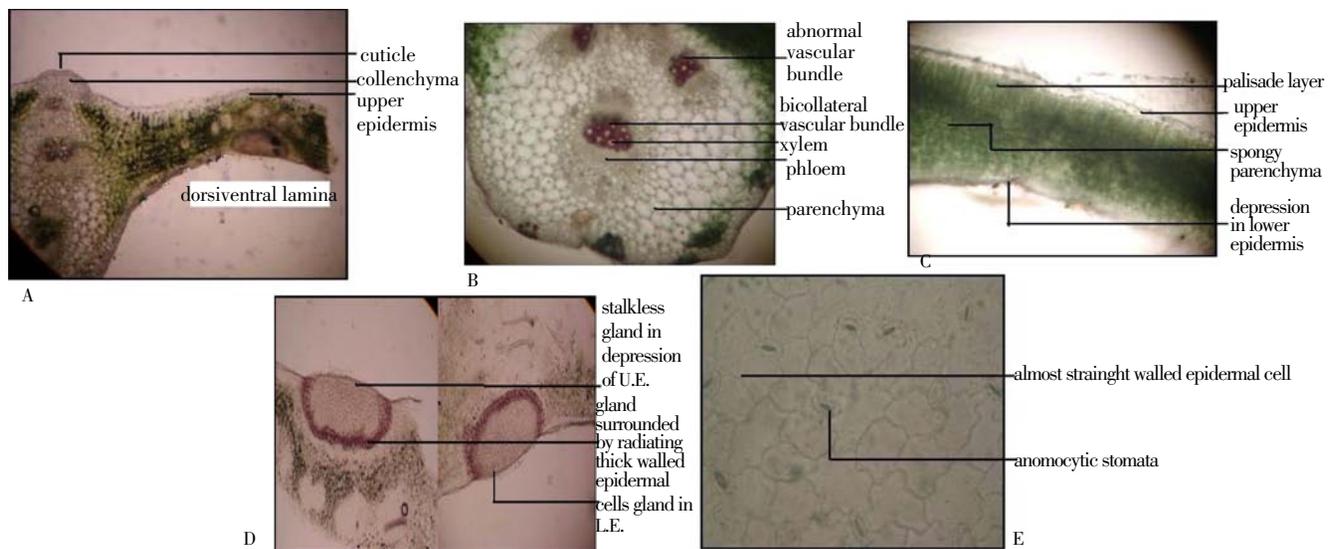


Figure 2. T.S. and surface preparation of leaf of *C. indica*.

A: 100×; B: 450×; C: Lamina of *Coccinia indica* leaf; D: Gland in upper and lower epidermis of lamina; E: Surface preparation of *C. indica* leaf.

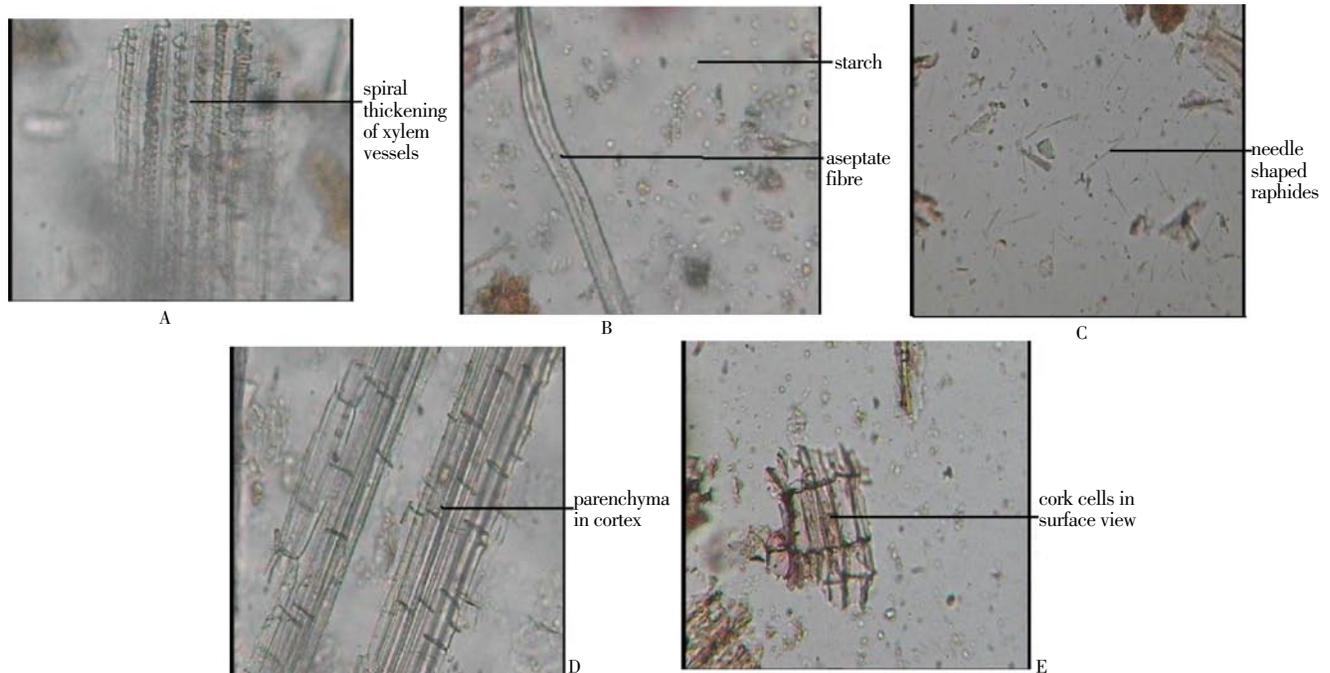


Figure 3. Powder study for aerial part of *C. indica*.

A: Spiral thickening of xylem vessels (100×); B: Starch grains and Fibres (450×), C: Needle shaped raphides(100×); D: Parenchyma in cortex (450×); E: Cork cells.

indica were packed in muslin cloth and subjected to soxhlet extractor for continuous hot extraction with petroleum ether and ethanol for 8 h separately. Then the each extracts were filtered and filtrate was evaporated to dryness. The percentage yield of the petroleum ether and ethanol extracts is given below in Table 1 respectively.

Table 1

Extraction of plant materials.

S. No.	Solvent	Weight of drug (g)	% Yield
1.	Pt. Ether (60%–80%)	250	2.248
2.	Ethanol (100%)	250	18.384

2.5. Physico–chemical parameters

Fruits *C. indica* such as total alcohol soluble extractive, water soluble extractive, ash value, acid insoluble ash, water–soluble ash, loss on drying, swelling index and foreign matter are presented in Table 2. The fluorescence analysis of the powdered drug of *C. indica* in various solvents and chemical reagents was performed under normal and UV light Table 3. The pH of 1% solution and 10% solution of powdered drugs was reported as 7.28 and 7.97 respectively.

Table 2Physico–chemical parameters of fruits of *C. indica*.

Loss on Drying	13
Total ash values	20.71
Acid insoluble ash value	1.71
Water soluble ash value	7.97
Water soluble extractive value	15.9
Ethanol soluble extractive value	18.384
Petroleum ether soluble extractive value	2.248
Swelling index	2
Foreign matter content	1.3

Table 3Fluorescence analysis of powdered fruits of *C. indica*.

S. No.	Solvent used	Observation under UV light (254 nm)	Observation under UV light (366 nm)
1.	NaOH in methanol	Light green	Yellowish brown
2.	NaOH in water	Light green	Dark brown
3.	Benzene	Fluorescent green	Reddish brown
4.	Acetone	Yellowish green	Orange
5.	Ethyl acetate	Yellowish green	Orange
6.	Chloroform	Light green	Creemish green
7.	Distilled water	Light green	Blackish brown
8.	Dil. HNO ₃	Light green	Bluish green
9.	Dil. H ₂ SO ₄	Light green	Dark green
10.	Con. HCL	Yellowish green	Yellowish brown

2.6. Fluorescence analysis

The fluorescence analysis of the powdered drug of *C. indica* in various solvents and chemical reagents was performed under normal and 254 nm and 366 nm UV light. The fluorescence analysis of the powdered drug of *C. indica* Observed the different colour under UV light is given in Table 3.

2.7. Preliminary photochemical investigation

Photochemical tests were done in plant extracts for the detection of presence of different chemical constituents such as; alkaloids, glycosides, flavonoids, essential oils, carbohydrates, proteins, tannins and other substances which are responsible for the biological activity. So the chemical tests are performed in the ethanolic extract of *C. indica*. For the detection of different chemical constituents are observed in the Table 4 given below respectively.

Table 4Data for the phytochemicals screening of powdered fruits of pet. ether and ethanolic extract of *C. indica* Wight Arn.

Tests	Pet.ether	Ethanolic extract
Carbohydrates		
Molish test	+ve	+ve
Fehling's test	+ve	+ve
Benedict's test	+ve	+ve
Protein		
Biuret test	–ve	+ve
Millon's test	–ve	+ve
Precipitation test	–ve	+ve
Alkaloids		
Mayer's test	–ve	+ve
Hager's test	–ve	+ve
Wagner's test	–ve	+ve
Dragendroff's test	–ve	+ve
Glycosides		
Keller – Killiani test	–ve	+ve
Baljet test	–ve	+ve
Steroids		
Salkowski test	–ve	+ve
Flavonoids		
Lead acetate	–ve	+ve
NaOH solution	–ve	+ve
Tannins		
5% FeCl ₃ solution test	–ve	+ve
Dil. iodine solution	–ve	+ve
Dil. HNO ₃	–ve	+ve
Saponins		
Foam test	+ve	+ve
Terpenoids		
Salowski test	–ve	+ve
Ethyl acetate and Dil. NH ₃ solution	–ve	+ve
Fatty acid and oils	–ve	–ve

2.8. Thin layer chromatography

“Their relative polarities which related to the type and number of functional groups present on a molecule capable of hydrogen bonding”

$$R_f = \frac{\text{(Distance travelled by solute front from origin line)}}{\text{(Distance travelled by solvent front from origin line)}}$$

Where R_f = Retention factor

The ethanolic extract of powdered of fruits of *C. indica*

Wight Arn was subjected to thin layer chromatography studies, to find the presence of number of compounds which support by the chemical test.

R_f value and colour of TLC spots, in solvent system of toluene: ethyl acetate and few drops of acetic acid (8.5 : 1.5: few drops). These TLC spots with R_f value and colour are in Table 5, and TLC plate in Figure 4 is given below.

Table 5

TLC finger printing of ethanolic extract of leaf of *C. indica* spots.

Extract	Solvent system	No. of spots	Colour of spots	R_f value
Ethanolic extract	Toluene : Ethylacetate : Acetic acid (8.5 : 1.5 : few drops)	6	Green	0.88
			Green	0.74
			Purple	0.62
			Pinkish	0.49
			Purple	0.29
			Purple	0.17

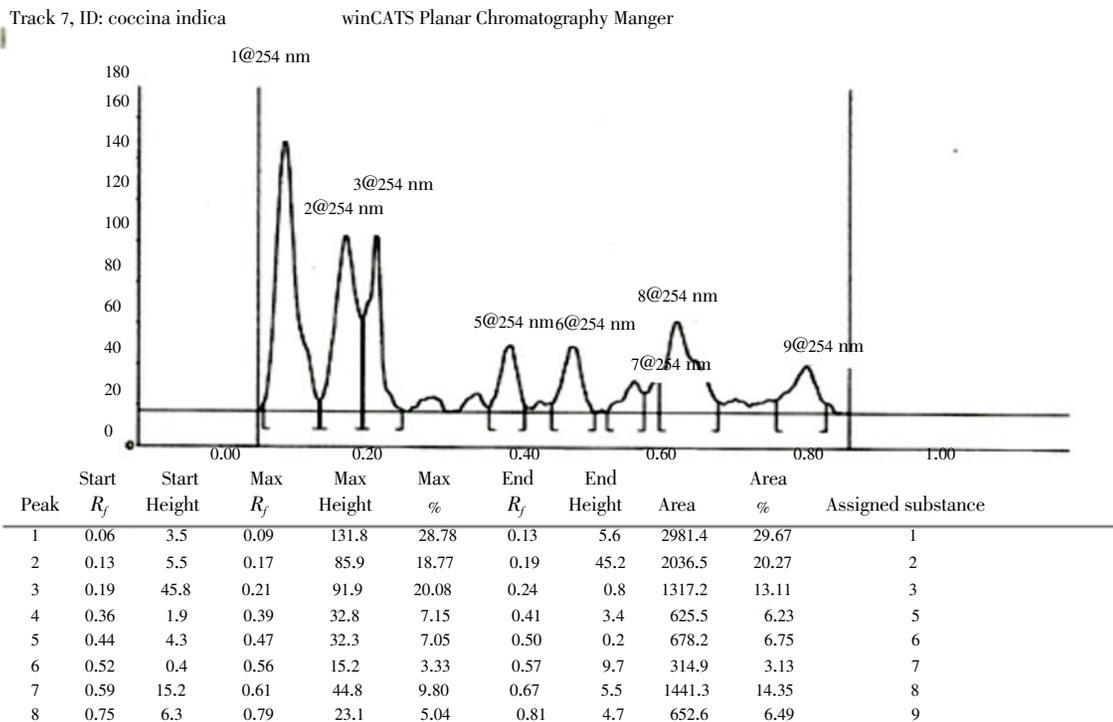
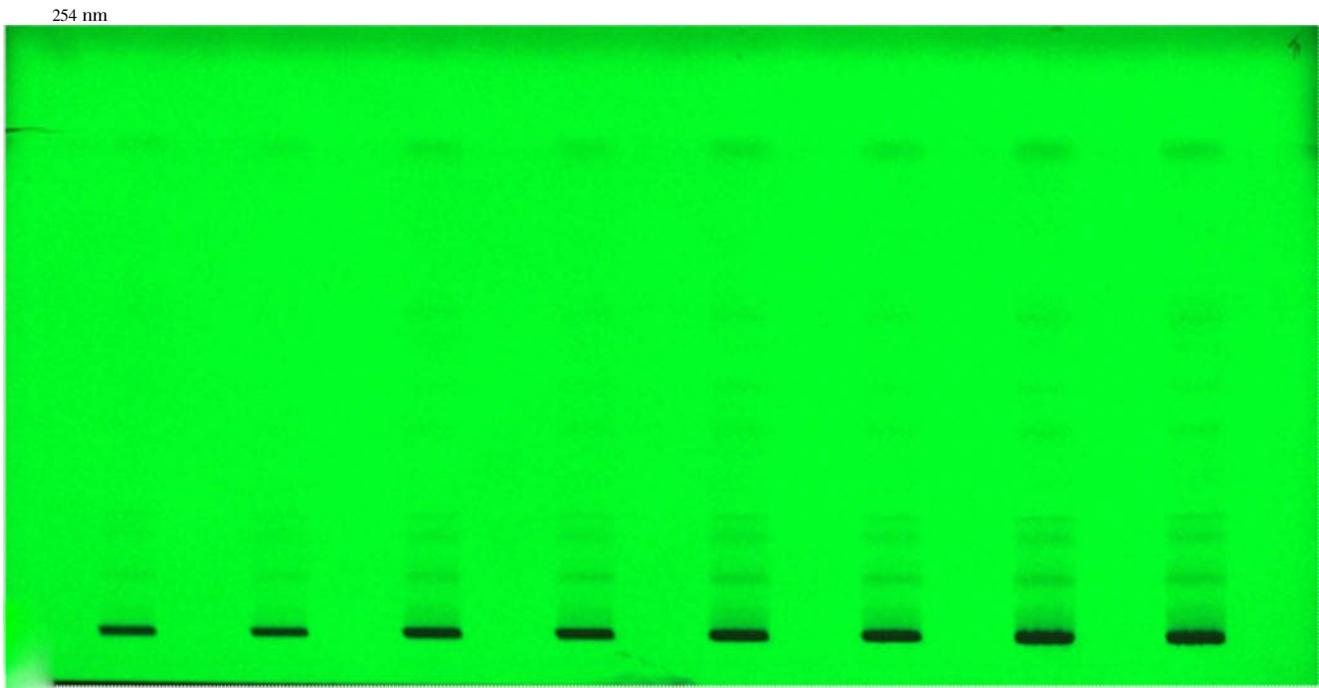


Figure 5. HPTLC finger printing and chromatogram of ethanolic extract on fruits of *C. indica*.

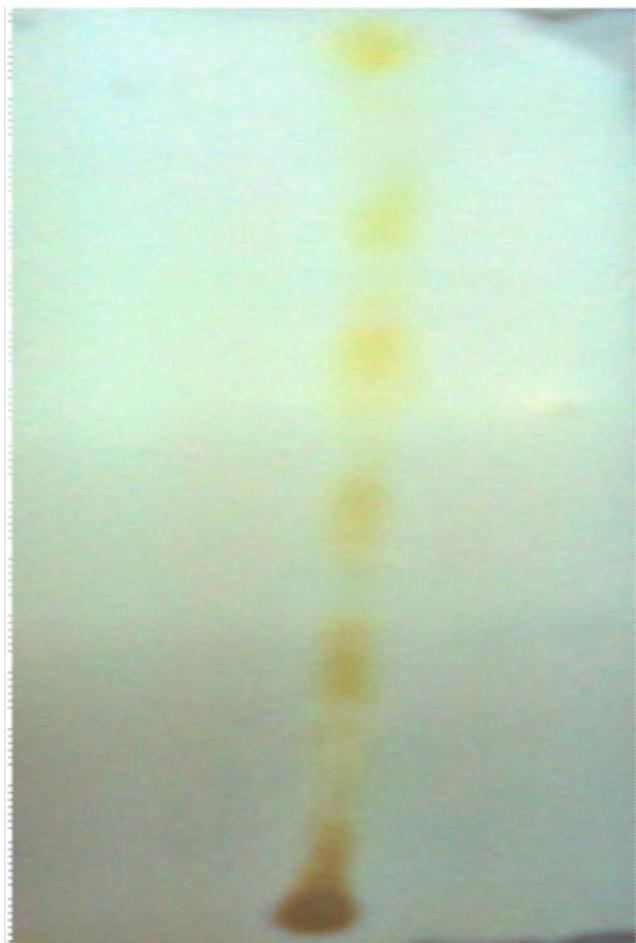


Figure 4. TLC finger printing of ethanolic extract on fruits of *C. indica*.

2.9. HPTLC finger printing

Ethanolic extract was developed on chromatographic plates with many ratios of different solvents and the best eluent mixture was used further for HPTLC profile to minimize errors in TLC pattern. The preliminary HPTLC studies revealed that the solvent system toluene: ethyl acetate and few drops of acetic acid (8.5:1.5: few drops) was ideal and gave well resolved sample peaks. Figure 5 HPTLC finger printing of ethanolic extract on fruits of *C. indica* given the spots of the chromatogram were visualized at 254 nm.

2.10. Column chromatography

The basic principle lying in the column chromatography is adsorption of component at solid-liquid interface. For good separation, the component of mixture should have different degree of affinity for the solid support. The component having strong adsorption for column material is held up while that component having less affinity moves down the column at faster rate as the elute passes through the column.

Column chromatography is separated into two categories depending on how the solvent flows down the column. If the solvent is allowed to flow down the column by gravity or percolation, it is called gravity column chromatography.

Table 6

Data of column chromatography ethanolic extract of *C. indica* Wight Arn. fruits.

Column	Fraction No.	Eluent	TLC Solvent system	Colour of fraction	No. of Spots	R_f value& Code
1.	1 (1-5)		<i>n</i> -Hexane	100	–	–
2.	2 (6-10)		<i>n</i> -Hexane:Toluene	98:2	–	–
3.	3 (11-15)		<i>n</i> -Hexane:Toluene	95:5	–	–
4.	3 (16-20)		<i>n</i> -Hexane:Toluene	95:5	–	–
5.	4 (21-25)		<i>n</i> -Hexane:Toluene	85:15	–	–
6.	4 (26-30)		<i>n</i> -Hexane:Toluene	85:15	–	–
7.	5 (31-35)		<i>n</i> -Hexane:Toluene	75:25	–	–
8.	5 (36-40)		<i>n</i> -Hexane:Toluene	75:25	–	–
9.	6 (41-45)		<i>n</i> -Hexane:Toluene	65:35	2	0.86,0.76
10.	7 (46-50)		<i>n</i> -Hexane:Toluene	55:45	2	0.86,0.76
11.	8 (51-55)		<i>n</i> -Hexane:Toluene	45:55	2	0.86,0.76
12.	9 (56-60)		<i>n</i> -Hexane:Toluene	35:65	2	0.86,0.76
13.	10 (61-65)		<i>n</i> -Hexane:Toluene	25:75	2	0.86,0.76
14.	11 (66-70)		<i>n</i> -Hexane:Toluene	15:85	2	0.86,0.76
15.	12 (71-75)		<i>n</i> -Hexane:Toluene	10-90	2	0.86,0.76
16.	13 (76-80)		Toluene	100	2	0.86,0.76
17.	14 (81-85)		Toluene :Ethyle acetate	98:2	2	0.86,0.76
18.	15 (86-90)		Toluene: Ethyle acetate	98:2	2	0.86,0.76
19.	16 (91-95)		Toluene: Ethyle acetate	98:2	1	0.76,V1
20.	17 (96-100)		Toluene: Ethyle acetate	98:2	1	0.76,V1
21.	18 (101-105)		Toluene: Ethyle acetate	98:2	1	0.76,V1
22.	19 (106-110)		Toluene: Ethyle acetate	98:2	1	0.76,V1
23.	20 (111-115)		Toluene: Ethyle acetate	98:2	1	0.76,V1
24.	21 (116-120)		Toluene: Ethyle acetate	98:2	1	0.76,V1

If the solvent is forced down the column by the air pressure, it is called flash chromatography. Data of column chromatography of ethanolic extract of *C. indica* Wight Arn. fruits is given below respectively in Table 6 and column chromatography in Figure 6 is given below.



Figure 6. Column of the ethanolic extract of *C. indica* Wight and Arn. fruits.

3. Results

As a part of standardization study, the macroscopically examination of drug was studied. The results showed greater extractive values in hot extraction, indicating the effect of elevated temperature on extraction. Percentages of the extractive values were calculated with reference to air-dried drug. The percent extractives in different solvents indicated the quantity and nature of constituents in the extracts. The extractive values are also helpful in estimation of specific constituents soluble in particular solvent.

4. Discussion

The fluorescence analysis of the powdered drug from the fruits of *C. indica* in various solvents was performed under normal and UV light. All the fruits extracts are examined in short UV (254 nm) and long UV (366 nm) to detect the

fluorescent compounds. Thin layer chromatography (TLC) is particularly valuable for the preliminary separation and determination of plant constituents. The chromatographic profile may serve as a characteristic fingerprint for qualitative evaluation of fruits.

It can be concluded that the present study on *C. indica* fruits can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant material available in market. This study is a substantial step and it further requires a long term study to evaluate therapeutic efficacy and toxicity of fruits.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The selected plant is one of the widely used plant throughout India and other sub continental region. As a traditional medicine, fruits have been used to treat leprosy, fever, asthma, bronchitis and jaundice. The fruit possesses mast cell stabilizing, anti-anaphylactic and antihistaminic potential. So in this respect the plant is very less studied for their phytochemical and pharmacognostical aspects. Sent article cover mentioned aspects

Research frontiers

Studies are performed on the dried and fresh fruits of *C. indica*. Macroscopical, Microscopical, physicochemical details and phytochemical details of pet ether and methanol extract are performed.

Related reports

The positive presence of some constituents like saponins

glycosides and other are also mentioned in some other studies. The various mentioned microscopical parameter like anomocytic stomata xylem vessels are also complies with other reports.

Innovations & breakthroughs

The article give the detailed information of the various phytoconstituents which are present in the plants. The various physicochemical parameter and HPTLC fingerprint are useful for its identification.

Applications

The various pharmacological studies can be performed on the basis of phytoconstituents mentioned in the reports. Morphological, microscopical, physicochemical details are helpful for the standardization of the plant,

Peer review

Study is based upon the very popular medicinal plant. Article give the detail information regarding the various evaluation parameter of the plant like ash values, extractive values, microscopical parameter and the chemical group present in the plant which may be responsible of its various pharmacological activities.

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