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

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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].
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 authentification

The fresh fruits of wildly 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, swithish dots over external surface, a few tendills 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, penatameronous.

##### 2.2.4. Male flower

Pedicel 8 cm long, subfiliform, calyx tube glabrous, broadly campunlate, 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.

#### 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 of 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.
vascular 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.
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 2
Physico-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 3
Fluorescence 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 | Creamish green |
| 7.     | Distilled water | Light green | Blackish brown |
| 8.     | Dil. HNO3 | Light green | Bluish green |
| 9.     | Dil.H2 SO4 | Light green | Dark green |
| 10. | Con.HCL | Yellowish green | Yellowish brown |

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

| Tests | Pet.ether | Ethanolic extract |
|-------|-----------|-------------------|
| Carbohydrates | +ve | +ve |
| Molish test | +ve | +ve |
| Fehling’s test | +ve | +ve |
| Benedict’s test | +ve | +ve |
| Protein | -ve | +ve |
| Biurest test | -ve | +ve |
| Millon’s test | -ve | +ve |
| Precipitation test | -ve | +ve |
| Alkaloids | -ve | +ve |
| Bayer’s test | -ve | +ve |
| Wagnar’s test | -ve | +ve |
| Dragendorff’s test | -ve | +ve |
| Glycosides | -ve | +ve |
| Keller – Killiani test | -ve | +ve |
| Baljet test | -ve | +ve |
| Steroids | -ve | +ve |
| Salkowski test | -ve | +ve |
| Lead acetate | -ve | +ve |
| NaOH solution | -ve | +ve |
| Tannins | -ve | +ve |
| 5% FeCl3 solution test | -ve | +ve |
| Dil. iodine solution | -ve | +ve |
| Dil. HNO3 | -ve | +ve |
| Saponins | +ve | +ve |
| Foam test | +ve | +ve |
| Terpenoids | -ve | +ve |
| Salowski test | -ve | +ve |
| Ethyl acetate and Dil.NH3 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*.
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

| Column Fraction No. | Eluent | TLC Solvent system | Colour of fraction | No. of Spots | $R_f$ value& Code |
|---------------------|--------|--------------------|--------------------|--------------|------------------|
| 1.                  | 1 (1–5)| $n$-Hexane        | 100                | –            | –                |
| 2.                  | 2 (6–10)| $n$-Hexane:Toluene| 98:2               | –            | –                |
| 3.                  | 3 (11–15)| $n$-Hexane:Toluene| 95:5               | –            | –                |
| 4.                  | 3 (16–20)| $n$-Hexane:Toluene| 95:5               | –            | –                |
| 5.                  | 4 (21–25)| $n$-Hexane:Toluene| 85:15              | –            | –                |
| 6.                  | 4 (26–30)| $n$-Hexane:Toluene| 85:15              | –            | –                |
| 7.                  | 5 (31–35)| $n$-Hexane:Toluene| 75:25              | –            | –                |
| 8.                  | 5 (36–40)| $n$-Hexane:Toluene| 75:25              | –            | –                |
| 9.                  | 6 (41–45)| $n$-Hexane:Toluene| 65:35              | 2            | 0.86,0.76        |
| 10.                 | 7 (46–50)| $n$-Hexane:Toluene| 55:45              | 2            | 0.86,0.76        |
| 11.                 | 8 (51–55)| $n$-Hexane:Toluene| 45:55              | 2            | 0.86,0.76        |
| 12.                 | 9 (56–60)| $n$-Hexane:Toluene| 35:65              | 2            | 0.86,0.76        |
| 13.                 | 10 (61–65)| $n$-Hexane:Toluene| 25:75              | 2            | 0.86,0.76        |
| 14.                 | 11 (66–70)| $n$-Hexane:Toluene| 15:85              | 2            | 0.86,0.76        |
| 15.                 | 12 (71–75)| $n$-Hexane:Toluene| 10–90              | 2            | 0.86,0.76        |
| 16.                 | 13 (76–80)| $n$-Hexane: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* 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 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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