Macroscopical, anatomical and physico-chemical studies on leaves of *Coccinia indica* Wight & Arn., growing wildly in eastern Uttar Pradesh region of India

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*Coccinia indica* Wight & Arn. of family Cucurbitaceae, called Ivy Gourd in English and Kundru or Bimb in Hindi, is a wildly growing climber or trailing weed in eastern Uttar Pradesh region of India. Its leaves are traditionally used for treatment of various diseases like wounds, ulcers, inflammation, skin diseases, fever, asthma, cough, diabetes and anemia. 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 leaves anatomically and physicochemically. TLC fingerprint profiling and fluorescence analysis of powdered leaves were also carried out and the salient qualitative and quantitative parameters are reported. These studies 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 differentiating the leaves of this species from closely related species of same genus and family.

**Keywords**: *Coccinia indica*, Ivy Gourd, Kundru, Anatomy, Physico-chemical, Cucurbitaceae, Wild, Medicinal Plant.

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**Introduction**

Search for eternal health and longevity and to seek remedy to relieve discomfort prompted man to develop diverse ways and means of health care. The early man explored his immediate natural surroundings, tried many things like plants, animals and minerals and developed a variety of therapeutic agents. The knowledge gathered by generations was either documented or passed on to the posterity and this practice was generally termed as Traditional Medicine. Plants are also appreciated in pharmaceutical research as the major resource for new medicine and a growing body of medical literature supports the clinical efficacy of herbal treatments. Today, about 40% doctors, especially in India and in China (the Mystic Orient) have reverted to increasing use of indigenous drugs and natural medicines. Steadily, a sizeable section of scientists in biological, biochemical and biomedical discipline have embarked on research on medicinal plants, which are the staple sources of many indigenous drugs. The World Health Organization (WHO) estimates that about 80% of the population living in the developing countries relies almost exclusively on traditional medicine for their primary health care needs. In almost all the traditional medicines, the medicinal plants play a major role and constitute the backbone of the traditional medicines. Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained thereof. To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentication. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. According to the World Health Organization (WHO, 1998), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken.

*Coccinia indica* Wight & Arn., belonging to the family Cucurbitaceae grows wildly throughout India and to a limited extent also cultivated in various parts. It is commonly known as Kundru. The whole plant is traditionally used for various medicinal purposes and leaves are used in Indian folk medicine for treatment of number of ailments including diabetes, wounds, ulcers, inflammation, eruptions of skin, fever, asthma and cough. Scientific investigations have shown that the
crude extract possesses hepatoprotective, antioxidant, anti-inflammatory, anti-nociceptive, anti-diabetic, hypolipidemic, antibacterial and antitussive activities. Though the plant has been reported for many biological activities, no scientific data is available to identify the genuine sample. The present investigation was therefore, taken up to establish identity of fresh and dried leaves morphologically microscopically and physicochemically for the standardization of the drug.

**Materials and Methods**

**Collection and authentification**

The fresh leaves of wildly growing plant *C. indica* were collected from the field areas of eastern Uttar Pradesh region during the month of February, 2009. For identification and taxonomic authentication, sample of plant material was given to National Botanical Research Institute (NBRI), Lucknow, India which confirmed the authenticity of plant specimen. (voucher specimen no. NBRI-SOP-202 receipt no. and date 19/72, 24-02-09). The fresh leaves were used for the study of macroscopic and anatomical characters. Collected plants were shade-dried and coarsely powdered. This coarse powder was used for the determination of ash values, extractive values. Preliminary phytochemical investigation was done as per standard methods.

**Preparation of leaves extract**

Air dried leaves (100 g) coarse powder was packed in four pouches of muslin cloth and subjected to Soxhlet extractor for continuous hot extraction with distilled water, ethanol, petroleum ether and chloroform for 8 h, separately. Each extracts were filtered and filtrate was evaporated to dryness. The percentage yield of the water, ethanol, petroleum ether and chloroform extracts was 23.8, 7.03, 2.45 and 4.15%, respectively.

**Macroscopic and microscopic studies**

The macromorphology of the leaves were studied according to standard methods. For anatomical studies hand section of the leaf was taken, stained and mounted following usual microtechniques and representative diagrams were taken with the help of inverted microscope for photodocumentation (Leitz, Japan).

**Physicochemical analysis**

Physicochemical analysis i.e. alcohol (90% ethanol) and water soluble extractive values, fluorescent analysis, total ash, acid-insoluble ash, water-soluble ash, swelling indices and foreign matter was done as per standard methods. Calibrated digital pH meter was used to measure the pH of 1 and 10% aqueous extracts and also loss on drying was noted.

**Preliminary phytochemical screening**

Preliminary phytochemical screening for the detection of various chemical constituents was carried out by using standard procedures described by Harborne and Khandelwal.

**Thin layer chromatography and high performance thin layer chromatography (HPTLC)**

Thin layer chromatography of the ethanol and chloroform extracts was carried out in various solvents at 30°C using silica gel G as adsorbent and the Rf values were determined. The same mobile phase was used for the HPTLC profiles of these extracts.

**Results**

**Macroscopic/morphological characters**

It is climbing perennial herbs with tuberous rootstock producing annual stems up to several meters long, which is found spreading on ground and twilling around the trees and supports around it (Plate 1). Leaves are triangular or pentagonal in shape. Margin is dentate, upper surface glabrous and attachment of petiole and major vein branching occurs. Apex obtuse, petioles 1-3 cm long and tendrils are unbranched. Flowers monoecious, solitary, rarely in axillary clusters of 2-3, pedicels 15-50 mm long, corolla lobes white, ovate, hypanthium 10-15 mm long. Fruit is slimy in touch, pulpy and ovoid to ellipsoid shaped. It is green in color when young which turns to scarlet red when it ripens, 2.5-5 cm long and 1.3-2.5 cm in diam., glabrous, pulp red. The fruit possesses numerous seeds which are oblong, 6-7 mm long, margins thickened.

Leaves are simple, subflashing, alternate, ovate, palmately 3 to 5 lobed with obtuse apex, ranging from 5-8 cm long and 3-6.5 cm wide. It shows reticulate venation with glabrous surface, dentate margin and cordate base. Petiole stout, cylindrical, smooth, 1.2-3.0 cm long, slightly flashy. The leaves have bright green upper surface and pale-green underneath, with characteristic odour and astringent taste.

**Microscopic characters**

**Midrib region**

Microscopical study shown, transverse section passing through midrib protrudes at the lower side
and flat at the upper side. Single layered upper and lower epidermis was observed with straight walls. Epidermis was covered with thick cuticle bearing few short glandular trichomes. Below the upper epidermis single layered palisade cells were observed with spongy mesophyll cells and in between were the xylem and phloem vessels. Xylem and phloem are arranged in ring. Xylem ring present towards the center and is surrounded by phloem ring. Small collenchymatous patch lied under the upper epidermis and 1-3 layers of well developed collenchymatous cells were on the lower side. Vascular bundles were semicircular, vessels arranged in radial rows, bicollateral, three, one centrally located was bigger in size, and two lying under the upper epidermis were smaller in size (Plate 2).

**Petiole**

Transverse section of petiole shows single layered epidermis, consisting of flattened, elongated cells with covering of cuticle. Under the epidermis 2-5 layered collenchymatous and 2-6 layered circular, thin walled, chlorenchymatous cells with intracellular spaces were observed. Bicollateral vascular bundles were arranged in a single ring. Some bundles were capped by one or two layered, thick walled, lignified, polygonal pericyclic sclerenchyma. In centre very wide pith was observed which was composed of large parenchymatous cells (Plate 3).

**Physico-chemical parameters**

The physicochemical characters of powdered leaves of *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 1. The fluorescence analysis of the powdered drug of *C. indica* in various solvents and chemical reagents was performed under normal and Ultra Violet (UV) light (Table 2). The pH of 1% solution and 10% solution of powdered drugs was reported as 7.23 and 6.98, respectively.
Preliminary phytochemical screening

The preliminary phytochemical investigation of the aqueous, ethanol, petroleum ether and chloroform extracts of leaves showed the presence of phytosterols, flavonoids, terpenoid saponis, carbohydrates, tannins, glycosides alkaloids proteins organic acids (Table 3).

Thin layer chromatography and high performance thin layer chromatography (HPTLC)

Thin layer chromatography of the ethanol and chloroform extracts was carried out using Toluene:Ethyl acetate (8.5:1.5) as mobile phase, respectively and the Rf values were recorded (Table 4). The visualizing reagent employed was anisaldehyde-sulphuric acid reagent to effect visualization of the resolved spots (Figure 1). TLC and HPTLC finger printing studies on ethanol extract showed presence of various phytoconstituents with their respective Rf values. The 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 (8.5:1.5) was ideal and gave well resolved sample peaks. The spots of the chromatogram were visualized at 366 nm (Figure 2).

Discussion

As a part of standardization study, the macroscopical examination of leaf was studied. Macroscopical evaluation is a technique of qualitative...
evaluation based on the study of morphological and sensory profiles of drugs. The macroscopical characters of leaf of plant can serve as diagnostic parameters. The extractive value, ash value, loss on drying and fluorescent analysis of the leaf extracts have been carried out. 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. The fluorescence analysis of the powdered drug from the leaves of *C. indica* in various solvents was performed under normal and UV light. All the leaf 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

### Table 2—Fluorescence analysis of powdered leaf of *Coccinia indica*

<table>
<thead>
<tr>
<th>Solvent used</th>
<th>Observation under UV light (254 nm)</th>
<th>Observation under UV light (366 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOH in methanol</td>
<td>Light green</td>
<td>Yellowish brown</td>
</tr>
<tr>
<td>NaOH in water</td>
<td>Light green</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Benzene</td>
<td>Fluorescent green</td>
<td>Reddish brown</td>
</tr>
<tr>
<td>Acetone</td>
<td>Yellowish green</td>
<td>Orange</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Yellowish green</td>
<td>Orange</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Light green</td>
<td>Creamish green</td>
</tr>
<tr>
<td>Distilled water</td>
<td>Light green</td>
<td>Blackish brown</td>
</tr>
<tr>
<td>Dil. HNO₃</td>
<td>Light green</td>
<td>Bluish green</td>
</tr>
<tr>
<td>Dil. H₂SO₄</td>
<td>Light green</td>
<td>Dark green</td>
</tr>
<tr>
<td>Conc. HCL</td>
<td>Yellowish green</td>
<td>Yellowish brown</td>
</tr>
</tbody>
</table>

### Table 3—Qualitative analysis of phytochemicals in *Coccinia indica* leaf extracts

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Pet. ether</th>
<th>Chloroform</th>
<th>Ethanolic</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterols</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins and amino acid</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acidic compounds</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ Present - Absent

### Table 4—Thin layer chromatography of leaf extracts of *Coccinia indica*

<table>
<thead>
<tr>
<th>Test extract</th>
<th>Solvent system</th>
<th>Number of spots</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>Toluene: Ethylacetate (8.5:1.5)</td>
<td>6</td>
<td>0.07, 0.15, 0.31, 0.69, 0.76, 0.99</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Toluene: Ethylacetate (8.5:1.5)</td>
<td>5</td>
<td>0.07, 0.15, 0.72, 0.79, 0.99</td>
</tr>
</tbody>
</table>

Under UV 366nm

Fig. 1—TLC finger printing of ethanolic and chloroform extract of leaf of *Coccinia indica*

**CF-313 Coccinia indica (leaf) extract**

Fig. 2—HPTLC fingerprinting of ethanolic extract of leaf of *Coccinia indica* scanned at wavelength 366 nm
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Conclusion

It can be concluded that the present study on C. indica leaf 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 leaf.

plant constituents. The chromatographic profile may serve as a characteristic fingerprint for qualitative evaluation of leaf.

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