PHARMACOGNOSTIC STUDIES AND PHYSICOCHEMICAL ANALYSIS OF LEAVES AND STEMS OF COCCINIA INDICA WIGHT AND ARN

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A B S T R A C T

The Leaves and stems of Coccinia indica are reported to have good medicinal values in traditional system of medicine. Coccinia indica belonging to family Cucurbitaceae is distributed all over India, it is commonly known as Ivy gourd and the leaves, roots, fruits and bark has been used for various disorders in traditional and folk medicine. The present study highlights the pharmacognostical as well as phytochemical studies including parameters such as macroscopic, microscopic characters, physical evaluation and preliminary phytochemical studies of the leaves and stems. These observations will help in the pharmacognostical identification and standardization of the drug in the crude form and also to distinguish the drug from its adulteration.

INTRODUCTION

Today, about 40% doctors, especially in India and China have reverted to increasing use of indigenous drugs and natural medicines. Steadily, a sizable 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.1,2

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.3

The major part of traditional therapy involves the use of plant extracts and their active constituents. Following the advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades, advances in phytochemistry and in identification of plant compounds, effective against certain diseases have renewed the interest in herbal medicines.4

Coccinia indica (Synonym: Coccinia grandis, Coccinia cordifolia) family Cucurbitaceae commonly called little gourd or Rantondli in Marathi, Bimba in Sanskrit and Kandutikibel in Hindi. It is indigenous to Bengal and other parts of India.5

Coccinia indica grows abundantly all over India, Tropical Africa, Australia, Fiji and throughout the oriental countries. The plant has also been used extensively in Ayurvedic and Unani practice in the Indian subcontinent.5

The plant is used as cathartic, antispasmodic, expectorant, Also used in treatment of diabetes, wounds, ulcer, inflammation, fever, asthma, cough, etc. The leaves are used as anthelmintic, antispasmodic, expectorant, and antiulcer. Fruits are used as laxative, antiemetic, anti-inflammatory, antieptic. Roots are used as cathartic, antiarthritic, hypoglycemic.5-14 Though the plant has been reported for many biological activities but less scientific data is available to identify the genuine sample. Thus, the present investigation was therefore done to establish identity of drug morphologically, microscopically & physicochemically for standardization of the drug.

MATERIAL AND METHOD

Collection & authentication

The leaves and stems of Coccinia indica were collected in the month of June from Satara district. The leaves and stems are authenticated by Mr. J. Jayanthi, (Scientist ‘C’ and H. O. O.) Botanical Survey of India, Pune. (V. No.- KUVCO61). The fresh leaves were used for study of macroscopic, and anatomical characters. Collected plant material was shade dried and coarsely powdered. This coarse powder was used for determination of extractive values, ash value, LOD and preliminary phytochemical investigation.
Pharmacognostic Studies And Physicochemical Analysis of Leaves And Stems of Coccinia Indica Wight And ARN

Preparation of Extract
Air dried leaves and stems coarse powder packed in thimble of filter paper and subjected to soxlet extractor for continuous hot extraction by using petroleum ether, chloroform, methanol & water. Each extract were filtered & evaporated to dryness.

Macroscopic Characteristic
The macroscopy of leaves and stems were studied according to standard methods.15,16

Microscopic characteristics
For microscopy hand section of leaf & stem was taken, stained & mounted following usual micro-techniques.17

Physical Evaluation
The ash values, extractive values and loss on drying were performed according to the official methods prescribed in Indian pharmacopeia.18 Fluorescence analysis was carried out according to the method of Chase and Prati19 and Kokoski.20

Phytochemical Screening
The pulverized dried leaves and stems powder were extracted with petroleum ether, chloroform, and methanol and water. The behavior of powder with various chemical reagent and preliminary chemical tests for various extracts were also carried out according to the standard procedures described by Kokate21 and Horborne.22

Fluorescence Analysis
Many drugs fluorescence when their powder is exposed to ultraviolet radiation. It is important to observe all materials on reaction with different chemical reagents and preliminary chemical tests for various extracts were also carried out according to the standard procedures described by Kokate21 and Horborne.22

RESULT AND DISCUSSION

Macroscopic Characteristic of Leaves and stems of Coccinia Indica
It is perennial herbs with tuberous rootstock producing annual stems up to several meters long, which is found spreading on ground and twilling around the tree and supports around it.

Leaves are 5-10cm long and broad, lamina bright green above, paler beneath, surface studded and sometimes rough with papillae, obtusely 3 to 5 angled or sometimes deeply 5- lobed, the lobes broad, obtuse or acute, apiculate, more or less sinuate-toothed; petioles 2-3.2 cm. long, cylindrical. Palmately 5-nerved from a cordate base, often with circular glands between the nerves near the petiole and nervules usually ending in glandular distant denticulations; delicately venous beneath, ovate or orbicular, simple and slender tendrils.

Stem are slender, soft, 0.3-1.5cm in diameter, branched, longitudinally grooved, glabrous nodes, swollen, whitish dots over external surface, a few tendrils attached with nodes, grayish colored externally and cream to light yellow internally, fracture, fibrous; no odour and taste.

Microscopic characteristics of leaves of coccinia indica
The T.S. of leaf consists of Midrib and Lamina.

Midrib:- The midrib shows slight projection on the upper side and lower side was wider. It consist of single layered epidermis, on either side, upper epidermis composed of single layer tabular cells externally covered with striated cuticle. Below the upper epidermis 3-4 layers of well developed more or less isodiametric collenchymatous tissues were observed bordering the epidermal cells on the dorsal side and 3-5 layers on the ventral side.

Vascular bundles are bicolateral, three in number. One vascular bundle is present on ventral side which is larger and two on dorsal side which are smaller forming a prominent ridge, xylem is well developed and the phloem consists of strands of sieve tubes and small celled parenchyma. The space between the endodermis and collenchymatous layer is filled with cortical parenchyma.

Lower epidermis consisted of single layered irregular shaped cells with smooth cuticle and just above the lower epidermis 2-3 layers of parenchymatous cells followed by the layers of collenchymatous cells were present. Prismatic calcium-oxalate crystals were found in the midrib region inside collenchymatous cells.

Lamina:- Dorsiventral structure with single layered upper and lower epidermis with a layer of polygonal cells having wavy walls, externally covered with striated cuticles. Epidermal cells show almost straight walls and anomocytic stomata in surface view; below upper epidermis palisade single layered which is discontinuous where the glands occur in the depressions of the upper epidermis.

Rest part of the lamina is filled with loosely arranged parenchymatous cells having large intra cellular spaces (Spongy Parenchyma), few cells contains calcium oxalate crystals and no lower palisade cells were observed.
Microscopic characteristics of stem of Coccinia indica

Stem consists of a layer of rectangular cells of upper epidermis which is followed by two to three layers of collenchymatous cells. Then is a layer of cells of endodermis followed by two to three layers of collenchymatous cells and scattered vascular bundles. Vascular bundle consists of metaxylem, protoxylem and phloem. Metaxylem is towards the periphery and protoxylem towards the centre. Starch is present in cortex region.

Physical evaluation

The Loss on Drying, Ash Values likes (Total Ash, Acid insoluble ash, Water soluble ash), Loss on drying, Pet. Ether soluble extractive, Chloroform soluble extractive, Methanol soluble extractive, Water soluble extractive of leaf powder are given in table-1.

Table No.1 Result of physical evaluation of Leaves and Stems of Coccinia indica

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Physical Constants</th>
<th>Leaves</th>
<th>Stems</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ash Value (% w/w)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Total Ash</td>
<td>26.8</td>
<td>26</td>
</tr>
<tr>
<td>2.</td>
<td>Acid Insoluble Ash</td>
<td>6.4</td>
<td>2.2</td>
</tr>
<tr>
<td>3.</td>
<td>Water Soluble Ash</td>
<td>13.8</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>Loss on Drying (% w/w)</td>
<td>17.2</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Extractive Values (% w/w)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pet. ether soluble extractive</td>
<td>0.11</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Chloroform soluble extractive</td>
<td>0.31</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Methanol soluble extractive</td>
<td>1.27</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td>Aqueous soluble extractive</td>
<td>1.84</td>
<td>1.50</td>
</tr>
</tbody>
</table>

Phytochemical screening

The extracts of leaves and stems were subjected to phytochemical screening for the presences of types of phytoconstituents. The extracts of leaves and stems were found to contain carbohydrates, alkaloids, glycosides, flavonoids, tannins and sterols.

Table N 2 Results of Qualitative Chemical Investigation of Leaves and Stem extracts

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Name of the test</th>
<th>Leaf extract</th>
<th>Stem extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Test for sterols</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Test for Triterpenoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Test for carbohydrates</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Test for flavonoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Test for alkaloids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Test for saponins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Test for tannins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Tests for proteins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>Test for amino acids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>Test for fatty acids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11.</td>
<td>Test for volatile oils</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fluorescence analysis

The fluorescence characteristics of powdered drug were studied under U.V. light after treating with different chemical reagents.

Table No.3 Result of Fluorescence analysis of Coccinia indica powder

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Fluorescence Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
</tr>
<tr>
<td></td>
<td>254nm</td>
</tr>
<tr>
<td>NaOH in Methanol</td>
<td>Light green</td>
</tr>
<tr>
<td>NaOH in water</td>
<td>Light green</td>
</tr>
<tr>
<td>50% Hydrochloric acid</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>50% Sulphuric acid</td>
<td>Light green</td>
</tr>
<tr>
<td>50% Nitric acid</td>
<td>Light green</td>
</tr>
<tr>
<td>Pet. Ether</td>
<td>Light green</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Light green</td>
</tr>
<tr>
<td>5% Iodine</td>
<td>Light green</td>
</tr>
</tbody>
</table>

DISCUSSION

1. The leaves and stems were collected from Satara district, Maharashtra region and authenticated. The leaves were subjected for Pharmacognostic investigation which includes determination of physical constants such as ash value, extractive values, loss on drying and fluorescence analysis. The powder of leaves and stems shows fluorescence at 254nm and 366nm.

2. Macroscopic and microscopic characteristics of the leaf were studied. The microscopic study shows that it contains midrib and lamina portion. The lamina shows upper and lower epidermis, spongy parenchyma, palisade cell layer while midrib portion shows upper and lower epidermis, collenchyma, vascular bundles, anomocytic stomata and covering trichomes etc.

3. Macroscopic and microscopic characteristics of the stem were studied. The microscopy of stem consists of upper epidermis which is followed by two to three layers of collenchymatous cells. Then is a layer of cells
of endodermis followed by sclerenchymatous cells consisting of pericycle. The general cortex consists of parenchymatous cells and scattered vascular bundles. Vascular bundle consists of metaxylem, protoxylem and phloem. Metaxylem is towards the periphery and protoxylem towards the centre. Starch is present in cortex region
4. The leaves and stems were subjected to extraction by using pet. Ether, chloroform, methanol and water and these extracts were subjected to phytochemical investigation.
5. Phytochemical investigation of leaves extracts of Coccinia indica shows presence of steroids, glycosides, carbohydrates, alkaloids, flavonoids, etc. While Phytochemical investigation of stem extracts of Coccinia indica shows presence of steroids, glycosides, carbohydrates, alkaloids, flavonoids, tannins, etc.

CONCLUSION
As there is less information available on pharmacognostical work on leaves and stems of Coccinia indica Wight & Arn, hence the present work is undertaken to produce some pharmacognostical standards. It serves as an important source of information to ascertain the identity and to determine pharmacognostical standards. It serves as an important source of information to ascertain the identity and to determine therapeutic diagnostic tools for the evaluation of Anthelmintic Activity of Coccinia indica (fruits). J. Chem. Pharm. Res., 2011, 3(1):488-491.
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