PHARMACOGNOSTIC STUDIES AND PHYTOCHEMICAL SCREENING OF AERIAL AND ROOT PARTS OF CYANOTIS TUBEROSA (ROXB.) SCHULT. & SCHULT.F. - AN ETHNOMEDICINAL HERB

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ABSTRACT
Cyanotis tuberosa (Roxb.) Schult. & Schult.f is traditionally used as ethnomedicine for curing several health problems in India. Objective of this study is to evaluate and compare the pharmacognostic, preliminary phytochemical and physicochemical properties between the crude drugs obtained from aerial and root parts of this herb. Leaf epidermal cells are polygonal in shape, cell wall outline straight. Stomata are of tetracytic type. Stomatal index is 18.56. Palisade ratio is 15.79. Only non-glandular, unicellular trichomes were found in both surfaces of the leaf. Phytochemical groups like alkaloids, anthraquinones, tannins, glycosides, etc. have been detected in both parts of the herb. Histochemical localization test revealed the presence of tannins, alkaloids, glycosides, lignin, etc. in different tissue zones of root. In aerial part, moisture content, total ash and acid insoluble ash are of 11.70±0.17%, 14.72±0.53% and 97±0.017%, respectively. In root part, values of moisture content, total ash and acid insoluble ash are 9.08±0.33%, 15.79±0.25% and 6.56±0.22%, respectively. Extractive values of root part in different solvents like water, methanol, ethanol, benzene and petroleum ether are 17.44±2.62%, 7.61±0.95%, 5.33±0.44%, 1.88±0.21% and 1.33±0.33%, respectively. In aerial part, extractive values for those solvents are 7.04±0.36%, 5.83±0.16%, 5.33±0.16%, 2.5±0.50%, 1.66±0.66%, respectively. Foaming index of powdered drugs of root and aerial parts is 250 and 200, respectively. Swelling index is 6.67±0.16ml for root and
it is 6.16±0.16ml in case of aerial portion. The diagnostic features recorded here in this study will help in proper identification of this ethnomedicinal herb in its fresh as well as dried form.

**KEYWORDS:** Cyanotis tuberosa, Foliar micromorphology, Phytochemical screening, Physicochemical analysis.

**INTRODUCTION**

Medicinal plants have been playing a noteworthy role in primary health care of human beings and their domesticated animals since the dawn of our civilization. There is a long-standing history regarding the uses of plants as a natural source of treatment and therapies for human health in many indigenous societies. The practices of traditional medicine are based on hundreds of years of belief and observations. In health care programmes of developed as well as developing countries, herbal medicines have now drawn more attention and are considered as a promising choice over modern synthetic drugs. According to WHO report, about 80% of the rural population in developing and under-developed countries of the world still depend upon herbal drug for their primary health care. As herbal medicine is less expensive, more efficacious and has very minimum or no side effects in comparison to modern medicine. In any traditional system of medicine, fresh or dried plant parts are usually used in preparation of herbal formulations. Correct knowledge of such crude drugs is very important aspect in preparation, safety and efficacy of the herbal products. So, documentation and standardization of the raw materials used in herbal medicine is very essential aspect for the worldwide acceptance of this system of medicine. Pharmacognosy is a simple and reliable tool which provides complete information about the crude drugs for its proper identification. Pharmacognostic standardisation, physicochemical analysis and phytochemical screening are globally accepted aspects considered here in identification and authentication of the genuine plant materials. More than 3000 plant species are commonly used in Indian systems of medicine and many of them are constantly being screened for their biological activity. It has also been found that pharmacognostic authentication of certain portion of such medicinal plants has been completed. The scientific evaluation of ethnomedicinal plants is now being done thoroughly covering various aspects of study like efficacy of crude drug, chemistry of active principles, different pharmacognostic parameters including micromorphology, anatomy, physical constants, etc. Foliar micromorphology and vegetative anatomy are now considered as effective tools for identification and taxonomical characterization of various groups of vascular plants. Importance of epidermal characters...
in general and those of trichomes in particular, and comparative wood anatomy are widely recognised in taxonomic consideration of angiosperms including medicinal plants.\textsuperscript{[9-15]}

Stomatal structure, its ontogeny and other epidermal features have also been considered as diagnostic characters for identification of the members of many angiospermic families.\textsuperscript{[16-18]}

Different members of the family Commelinaceae have been studied anatomically by the earlier researchers highlighting the leaf epidermal micromorphology and root anatomy.\textsuperscript{[19-22]}

Pharmacognostic studies of medicinal plants have been carried out earlier by many workers for proper identification of the crude drugs obtained from respective medicinal plants.\textsuperscript{[23-28]}

However, no such work has been done earlier to study the pharmacognostic standard of the crude drugs obtained from different parts of this selected medicinal plant. In this context, present study has been undertaken to evaluate the pharmacognostic as well as phytochemical and physicochemical properties of \textit{Cyanotis tuberosa} (Roxb.) Schult. & Schult. f., an ethnomedicinal plant.

\textbf{MATERIAL AND METHODS}

\textbf{Material}

\textit{Cyanotis tuberosa} (Roxb.) Schult. & Schult. f. (Commelinaceae) (Fig.1).

\textbf{Common English name:} Greater Cat Ears; \textbf{Local name:} Kasmul; \textbf{Tribal Name:} Hodo-Jereng-arak.

\textbf{Botanical characters}

A creeping herb with tuberous fasciculated roots. Stems stout, 20-80 cm long, villous, rooting at the nodes. Leaves sessile; radical leaves ensiform or narrowly oblong, 15-32 × 0.9-1.5 cm, villous; sheath 2.5-3.5 cm, villous; cauline ones linear-oblong, 6-12 × 0.6-1.2 cm; sheath 1-1.8 cm long. Flowers bluish-purple, in densely villous, spiciform cymes. Spathes ovate-lanceolate, 1.5-1.8 cm long, acute; bract 1-1.3 cm long. Sepals 6-7 mm long, densely villous. Corolla-tube narrow; lobes ovate, subacute. Stamen 6, all fertile; filaments spirally twisted, densely bearded above. Capsules ellipsoid, pubescent, 3-3.25 mm long. Seeds 2mm, oblong-lanceolate, brown.
Flowering and fruiting time
July-September.

Medicinal uses
Root part is used in treatment of continued fevers and worm infestation in cattle by the tribal people of eastern India. Root paste is administered two spoonful twice a day for 15 days to treat liver problem and menstrual disorder by the Santal tribe.

Aerial part of this herb is used by the tribal people in North Eastern India as leafy vegetable.

Collection of plant material
Plant samples of the investigated species were collected from the forest areas of Illambarazar, Birbhum district, West Bengal, India. The plant species has been identified and authenticated with the help of different standard floras. The voucher specimen has been deposited at the Visva-Bharati Herbarium, Department of Botany, Visva-Bharati, Santiniketan, West Bengal for future reference.

Study of foliar micromorphology
Leaf samples were cleared following the Bokhari’s method. The cleared leaf samples were then mounted on the slide with a drop of 10% glycerine and 1% aqueous safranin and observed under compound light microscope.

Vegetative anatomy
Free hand sections of root of the selected plant were cut, stained suitably following safranin-light green staining schedule and observed under compound light microscope.
Preliminary phytochemical screening

Aqueous, methanol, ethanol, benzene and petroleum ether extracts were obtained from the dried powdered samples of aerial and root parts of the selected herb by cold maceration technique. The extracts were then screened for detection of different phytochemical groups by chemical colour reaction tests following standard methods.\(^{[37-40]}\)

Histochemical study

Transverse sections of the root were laid out in several glass slides; one to two drops of different reagents (Wagner's, Dragendorff's, Mayer's, Lugol’s, Millon’s, 1% lead acetate, Phloroglucinol, Ferric chloride, etc.) were added to the sections and kept for few minutes to allow the specific reaction between reagents and phytochemicals present in the cells. Then observed under compound light microscope to detect different phytochemical groups localized in different tissue zones in the respective sections.\(^{[36-40]}\)

Physicochemical evaluation

Physicochemical parameters of the powdered plant samples like moisture content, ash value (total ash, acid insoluble ash, water soluble ash and sulphated ash), extractive value, swelling index and foaming index were determined as per guidelines of Indian Pharmacopoeia and WHO.\(^{[41,42]}\) The fluorescence characters of the powdered plant samples treated with different chemical reagents were observed under visible and long UV light (366 nm).\(^{[43]}\)

RESULTS

Foliar micromorphology

General description along with measurements of the epidermal cells, stomata, trichomes are given below.

Epidermis- Leaf epidermal cells are polygonal and anticlinal walls of the cells are straight in both adaxial and abaxial leaf surfaces. Size of the epidermal cells on adaxial surface is 177.61± 4.80 µm × 98.56± 3.52 µm and it is 106.26± 4.56 µm × 80.08± 3.90 µm on the abaxial leaf surface. Frequency of the epidermal cells is 76.66± 1.09/mm\(^2\) on the adaxial surface and it is 198.75±2.85/mm\(^2\) on the abaxial surface. Palisade ratio is 14.95± 0.38 (Table 1; Fig. 2).
Table 1: Foliar epidermal cell characters of the investigated plant.

<table>
<thead>
<tr>
<th>Leaf surface</th>
<th>Cell shape and outline</th>
<th>Cell measurement (length × breadth) µm</th>
<th>Cell frequency (No./mm²)</th>
<th>Palisade ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper</td>
<td>Straight and polygonal</td>
<td>177.61±4.80×98.56±3.52</td>
<td>76.66±1.09</td>
<td>14.95±0.3</td>
</tr>
<tr>
<td>Lower</td>
<td>Straight and polygonal</td>
<td>106.26±4.56×80.08±3.90</td>
<td>198.75±2.85</td>
<td></td>
</tr>
</tbody>
</table>

Stomatal complex- Stomata are of tetracytic type and distributed exclusively in the abaxial surface of the leaf, i.e., leaves are hypostomatic. Size of the stomata is 57.80±0.81 µm × 32.91±0.23 µm. Stomatal index is 17.66±0.50 and frequency of the stomata is 49.5±1.28/mm² (Table 2; Fig. 3).

Table 2: Stomatal features of the investigated plant.

<table>
<thead>
<tr>
<th>Leaf surface</th>
<th>Stomatal type</th>
<th>Stomatal measurement (length × breadth) µm</th>
<th>Stomatal index (%)</th>
<th>Stomatal frequency (No./mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lower</td>
<td>Tetracytic</td>
<td>57.80±0.81×32.91±0.23</td>
<td>17.66±0.50</td>
<td>49.5±1.28</td>
</tr>
</tbody>
</table>

Trichomes- Trichomes are non-glandular, unicellular with pointed tips and present in both surfaces of the leaf. Size of the trichomes of adaxial surface is 1871.74±43.51 µm × 15.44±0.79 µm and it is 1812.29±70.67 µm × 15.67±0.93 µm in case of abaxial surface trichomes. Frequency of trichomes is 16.62±1.78/mm² and 19.88±1.55/mm² in adaxial and abaxial surfaces, respectively. Trichome index is 1.46±0.93 in adaxial surface and it is 2.05±0.79 in abaxial surface (Table 3; Fig. 4).

Table 3: Trichome features of the investigated plant.

<table>
<thead>
<tr>
<th>Leaf surface</th>
<th>Type</th>
<th>Trichome measurement (length×width) µm</th>
<th>Trichome frequency (No./mm²)</th>
<th>Trichome index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper</td>
<td>Non glandular, unicellular</td>
<td>1871.74±43.51×15.44±0.79</td>
<td>16.62±1.78</td>
<td>1.46±0.93</td>
</tr>
<tr>
<td>Lower</td>
<td>Non glandular, unicellular</td>
<td>1812.29±70.67×15.67±0.93</td>
<td>19.88±1.55</td>
<td>2.05±0.79</td>
</tr>
</tbody>
</table>

Root anatomy

The fascicular root of this plant has swollen and un-swollen parts and their anatomical features are given below.

i. Un-swollen part of the root

Transverse section of the un-swollen root part is nearly circular in outline and shows the following features (Fig. 5a).
Exodermis - It is composed of large cells, 1-2 cell layered.

Cortex- It is massive, parenchymatous, 9-12 cell layer broad, differentiated into three distinct zones. First zone is of 1-3 cells thick, present beneath the exodermis, known as outer cortex. Cells are smaller in size, thick walled, irregularly arranged. Cells in the middle cortex are rounded, larger as well as smaller in size, moderately thick walled. Larger cells are present in 2-4 layers on the outer side of middle cortex and smaller cells are in 4-5 layers on the inner side of it. Cortex is terminated by a layer of barrel shaped cells known as endodermis. The endodermal cells are differentially thickened with lignin and suberin on its both radial side walls and inner tangential wall, conferring the cell wall a U-shaped thickening.

Pericycle- It is uniseriate layer, composed of elongated, narrow parenchymatous cells, present just beneath the endodermis.

Vascular system- Xylem and phloem are arranged in separate patches on different radius. Xylem strand is exarch with three protoxylem elements towards periphery and one very larger metaxylem towards centre of the root. Number of xylem strands is 9 (polyarch), arranged alternately with the phloem patches. Phloem is scanty.

Pith- It is present at the centre, composed of isodiametric, thick walled parenchymatous cells with few thin walled cells.

ii. Swollen part of the root

Transverse section of the swollen root part is nearly circular in outline and shows the following features (Fig. 5b).

Exodermis -It is composed of large cells, 1-2 cell layered thick.

Cortex- It is more massive than the cortex of un-swollen root portion. It is of 18-21 cell layer broad and differentiated into three distinct zones like that of un-swollen root cortex. The outer cortex is 3-4 cell layered and middle cortex is 7-8 layers broad. Cells of the inner part of middle cortex are rhomboidal in shape, thin walled, arranged radially in 8-10 cell layers. Rest of the tissue organization of cortex is similar with that of un-swollen part of the root.

Pericycle - It is single cell layer and is similar with unswollen part of the root.
Vascular system- Xylem and phloem tissue organization is very similar with vascular system of un-swollen part of the root, except the number of xylem strands which is 7-8 in number. Each xylem strand consists of one metaxylem and four protoxylem elements.

Pith- It is very narrow here. In mature root, pith is hollow due to disintegration of the central cells and a ring of lignified cells at the periphery of pith was observed.

**Preliminary phytochemical screening of the powdered plant sample**

Phytochemical screening of different solvent extracts of root and aerial part of the investigated plant showed presence of the different phytochemical groups in varying degrees. The phytochemical groups like alkaloids, tannins, glycosides, reducing sugars, carbohydrates, saponins and proteins have been detected in aqueous extract of root powder. Whereas aqueous extract of aerial part revealed positive test only for the reducing sugars, saponins, tannins and glycosides. In methanolic and ethanolic extracts of root, alkaloids, anthraquinones, tannins, glycosides, saponins, lignin, reducing sugars, carbohydrates and proteins have been detected. Methanolic and ethanolic extracts of aerial part showed similar result with that of root part except the saponin group which was detected only in root extracts. The benzene extract of root showed presence of alkaloids, saponins, proteins and tannins and no phytochemical groups have been detected in benzene extract of the aerial part. Petroleum ether extracts of root and aerial part showed presence of similar phytochemical groups as detected in benzene extracts. In both solvent extracts of root part of the investigated plant, alkaloids, saponins, tannins and proteins have been detected except anthraquinones, steroids and flavonoids (Table 4; Fig. 6, 7).

<table>
<thead>
<tr>
<th>Test for</th>
<th>Test/ reagents</th>
<th>Nature of changes</th>
<th>AE</th>
<th>ME</th>
<th>EE</th>
<th>BE</th>
<th>PE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>RP</td>
<td>AP</td>
<td>RP</td>
<td>AP</td>
<td>RP</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Mayer's reagent</td>
<td>White cream ppt.</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Wagner’s reagent</td>
<td>Orange brown ppt.</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Dragendroff’s reagent</td>
<td>Orange brown ppt.</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>Fehling’s reagent</td>
<td>Brick red</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Benedict’s reagent</td>
<td>Brick red</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Molish’s test</td>
<td>Violet ring</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>Borntrager’s test</td>
<td>Pink colour</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>1% Lead acetate</td>
<td>White ppt.</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
### Proteins

<table>
<thead>
<tr>
<th>Test for</th>
<th>Test/ reagents</th>
<th>Histological location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer’s reagent</td>
<td>Pith and cortical cells</td>
</tr>
<tr>
<td></td>
<td>Wagner’s reagent</td>
<td>Pith cells, pericycle</td>
</tr>
<tr>
<td></td>
<td>Dragendorff’s reagent</td>
<td>Few pith cells, pericycle</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>Fehling’s reagent</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Benedict’s reagent</td>
<td>Few cells of pith</td>
</tr>
<tr>
<td>Glycoside</td>
<td>10% NaOH</td>
<td>Few cells of pith</td>
</tr>
<tr>
<td>Saponins</td>
<td>1% Lead acetate</td>
<td>Phloem zone</td>
</tr>
<tr>
<td>Proteins</td>
<td>Millon’s reagent</td>
<td>Pith cell</td>
</tr>
<tr>
<td></td>
<td>Lugol’s reagent</td>
<td>Pith cell, endodermal cell</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Ninhydrin reagent</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>10% Ammonium hydroxide</td>
<td>Intercellular spaces of cortex</td>
</tr>
<tr>
<td></td>
<td>10% Lead acetate</td>
<td>Cortex</td>
</tr>
<tr>
<td></td>
<td>5% Ferric chloride</td>
<td>Metaxylem</td>
</tr>
<tr>
<td>Lignin</td>
<td>Phloroglucinol</td>
<td>Vascular bundle</td>
</tr>
</tbody>
</table>

AE- Aqueous extract; ME-Methanolic extract; EE-Ethanolic extract; BE-Benzene extract; PE-Petroleum ether extract; RP-Root part; AP-Aerial part; ‘+’= Positive, ‘-’ = Negative.

### Histochemical study

In histochemical study, different phytochemical groups like tannins, proteins, alkaloids, glycosides, lignin, etc. have been detected which are localised in different tissue zones of the root. Vascular bundles and cortical zone of the root were found most active sites where most of the phytochemical groups are localized (Table 5).

#### Table 5: Histochemical localization test of the root part.
Physicochemical analysis

For physicochemical characterization of the powder plant samples moisture content, total ash content, acid insoluble ash, water soluble ash, sulphated ash, extractive value, swelling index and foaming index were determined and results were presented (Table 6). In aerial part, moisture content is 11.70±0.17% and it is 9.08±0.33% in root part. In aerial part, values of total ash, acid insoluble ash, water soluble ash and sulphated ash were found to be 14.72±0.53%, 1.97±0.017%, 1.79±0.12% and 5.67±0.33%, respectively. In root part, values of total ash, acid insoluble ash, water soluble ash and sulphated ash were estimated to be 15.79±0.25%, 6.56±0.22%, 1.34±0.15% and 7.67±0.33%, respectively. Percentage yield of individual solvent extracts of the plant parts (extractive values) varies according to the nature of the solvent. It was found that water soluble extractive value is highest among the five solvent extracts of this plant. In root, extractive value for water solvent was 17.44±2.62% and it was 7.04±0.36% in case of aerial part. Extractive value for petroleum ether was found lowest among the values of all five solvent extracts. Extractive value of petroleum ether was 1.33±0.33% in case of root and it was 1.66±0.66% for aerial part. In root part, extractive values of other solvents like methanol, ethanol and benzene were 7.61±0.95%, 5.33±0.44% and 1.88±0.21%, respectively. In case of aerial part, methanol, ethanol and benzene soluble extractive values were 5.83±0.16%, 5.33±0.16% and 2.5±0.50%, respectively. Foaming index was found to be 250 in root part and it was 200 in aerial portion of the plant. Swelling index was 6.67±0.16ml and 6.16±0.16ml in root and aerial part of the plant, respectively (Table 6).

Table 6: Physicochemical properties of the dried powder of root and aerial parts.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Physicochemical parameters</th>
<th>Average value of aerial part</th>
<th>Average value of root part</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Moisture content(% w/w)</td>
<td>11.70±0.173</td>
<td>9.08±0.331</td>
</tr>
<tr>
<td>2.</td>
<td>Ash value(% w/w)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Total ash</td>
<td>14.72±0.539</td>
<td>15.79±0.253</td>
<td></td>
</tr>
<tr>
<td>b. Acid insoluble ash</td>
<td>1.97±0.017</td>
<td>6.56±0.223</td>
<td></td>
</tr>
<tr>
<td>c. Water soluble ash</td>
<td>1.79±0.121</td>
<td>1.34±0.156</td>
<td></td>
</tr>
<tr>
<td>d. Sulphated ash</td>
<td>5.67±0.333</td>
<td>7.67±0.333</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Extractive value (% w/w)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Water soluble extract</td>
<td>7.04±0.369</td>
<td>17.44%±2.62</td>
<td></td>
</tr>
<tr>
<td>b. Methanol soluble extract</td>
<td>5.83±0.166</td>
<td>7.61%±0.953</td>
<td></td>
</tr>
<tr>
<td>c. Ethanol soluble extract</td>
<td>5.33±0.166</td>
<td>5.33%±0.44</td>
<td></td>
</tr>
<tr>
<td>d. Petroleum ether extract</td>
<td>1.66±0.666</td>
<td>1.33%±0.33</td>
<td></td>
</tr>
<tr>
<td>e. Benzene extract</td>
<td>2.5±0.500</td>
<td>1.88%±0.21</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Foaming index</td>
<td>200</td>
<td>250</td>
</tr>
<tr>
<td>5.</td>
<td>Swelling index</td>
<td>6.16±0.166</td>
<td>6.67±0.166</td>
</tr>
</tbody>
</table>
Fluorescence analysis

The drug powder treated with different chemical reagents gives characteristic colour when seen under UV light (365 nm) and it is compared with colour observed under visible light. In some cases marked differences in colour have been observed when powdered plant samples treated with different chemical reagents were seen under UV light (365 nm) (Table 7; Fig. 8,9,10,11).

Table 7: UV fluorescence study of the dried powder of root and aerial parts.

<table>
<thead>
<tr>
<th>Material and treatment</th>
<th>Aerial part</th>
<th>Root part</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In visible light</td>
<td>In UV light (366nm)</td>
</tr>
<tr>
<td>Powder as such</td>
<td>Greyish green</td>
<td>Fluorescent green</td>
</tr>
<tr>
<td>Paper with powder</td>
<td>Yellowish green</td>
<td>Dark pink</td>
</tr>
<tr>
<td>Treated with 50% Nitric acid</td>
<td>Orange</td>
<td>Red rose</td>
</tr>
<tr>
<td>Treated with Methanol</td>
<td>Olive green</td>
<td>Fluorescent orange</td>
</tr>
<tr>
<td>Treated with 5% KOH</td>
<td>Dark chocolate</td>
<td>Saddle brown</td>
</tr>
<tr>
<td>Treated with Ethanol</td>
<td>Olive green</td>
<td>Fluorescent reddish orange</td>
</tr>
<tr>
<td>Treated with Acetone</td>
<td>Olive green</td>
<td>Reddish orange</td>
</tr>
<tr>
<td>Treated with 1N HCl</td>
<td>Dark green</td>
<td>Dark coffee</td>
</tr>
<tr>
<td>Treated with 1N NaOH</td>
<td>Yellowish brown</td>
<td>Dark reddish maroon</td>
</tr>
<tr>
<td>Treated with Antimony trichloride</td>
<td>Greenish brown</td>
<td>Red rose</td>
</tr>
<tr>
<td>Treated with 80% H₂SO₄</td>
<td>Coffee colour</td>
<td>Dark green</td>
</tr>
</tbody>
</table>

Fig. 2: A portion of upper epidermis.
Fig. 3: A portion of lower epidermis with stomata.

Fig. 4: Non-glandular trichome.

Fig. 5a: T.S. of un-swollen root part.
Fig. 5b: T.S. of swollen root part.

Fig. 6: Chemical colour reaction test (root powder).

Fig. 7: Chemical colour reaction test (leaf powder).

Fig. 8: Spot test of leaf powder under visible light.
DISCUSSION

Present study reveals some of the characters obtained from foliar micromorphology, root anatomy, preliminary phytochemical screening and physicochemical evaluation, which are found very distinct and can be used as marker in identification of the herb *C. tuberosa* in its fresh as well as dried form.

The foliar epidermal cell characters are successfully employed in resolving quite a number of taxonomic problems at different levels of the plant taxa. In *C. tuberosa*, leaf epidermal
cells are polygonal with straight wall found in both adaxial and abaxial leaf surfaces. In previous studies, almost similar type of epidermal cells were observed in different members of Commelinaceae including other species of *Cyanotis* where cells are polygonal, rectangular to isodiametric in shape, straight walled. In present study, frequency of foliar epidermal cells per sq mm is higher in adaxial surface than abaxial surface that confirms the observations made by previous workers in other members of the Commelinaceae.

Characters of the stomata do have immense taxonomic as well as pharmacognostic value in proper identification of different plant taxa including medicinal plants. In the family Commelinaceae, tetracytic type of stomata is predominant and sometimes hexacytic type has also been observed in some members of this family. Here in *C. tuberosa*, stomata are strictly of tetracytic type. Stomatal index is used in many of the cases as marker character for taxonomic identification of plant species as well as the leaf drugs obtained from respective species. In present investigation, stomatal index is 17.66± 0.50 which may be regarded as distinct feature of this medicinal plant.

Foliar trichome features are now considered as one of the valuable taxonomic markers and successfully employed in proper identification of the plant species. Epidermal trichomes of the investigated plant are non-glandular and unicellular type, present on both surfaces of the leaf. In previous studies, both glandular and non-glandular types with bicellular to multicellular trichomes were found in different species of the genera *Commelina* and *Aneilema* of the family Commelinaceae. Here long, non-glandular and unicellular type of foliar trichome of *C. tuberosa* has been found distinct as such type has not been observed in any of the members of this family so far studied.

Chemical analysis and biological assay are considered as very important aspects in pharmacognostic evaluation of medicinal plants. Preliminary phytochemical screening is useful in prediction of the nature of crude drugs and also valuable for detection of phytoconstituents present in it. The important phytochemical groups detected both in aerial and root parts of this plant were alkaloids, anthraquinones, saponins, tannins, glycosides, etc. In histochemical study, it was observed that vascular bundles and cortical zone are very active sites where alkaloids, saponins, proteins and tannins have been localized in greater amount. Therapeutic properties of all these phytochemicals have scientifically been studied well and documented. In tannins, various bioactive compounds isolated from a number of medicinal plants have been studied for their biological activities like anti-tumour, anti-
inflammatory, anti-bacterial, anti-diarrhoeal and also in case of some sexual diseases.\cite{40,47-48} Different groups of alkaloids have pharmacologically been investigated and they have found effective in a wide range of biological activities like CNS stimulatory, anthelminthic, anti-hypertensive, anti-malarial, anti-diabetic, antirheumatic, anti-cancerous, anti-inflammatory, antiviral, etc.\cite{40,48} A number of studies have identified the phytochemical groups like glycosides, anthraquinones and saponins as effective agents for tumour, viral and bacterial infection, inflammatory diseases, artherosclerosis, platelet-aggregation, hypertension, fatigue, hepatoprotection, immunosuppression, etc.\cite{49-51} Presence of pharmacologically active above mentioned phytochemical groups in this investigated herb highlights its prospect to be a potent candidate for its further phytochemical and biological activity studies. It has clearly been seen that root part of this herb is more potent than aerial part as greater number of biologically active phytochemical groups have been detected in root which basically support the traditional claims of using root part of this plant as phytomedicine in treating the helminthiasis, liver disorder, inflammation and sexual diseases.

In Pharmacognosy, physicochemical characters help in setting standard for a crude drug and are successfully employed in detection of adulterants and improper handling of the crude drug.\cite{41} In this study, physicochemical constants of the crude drugs obtained from root and aerial parts of the investigated plant, have been reported for the first time here. Moisture content of a crude drug is an important parameter in respect of its shelf life because insufficient drying favours the growth of molds and microorganisms which ultimately spoil the biomass and active principles of the drugs. It has also been established that moisture content is directly related to maintain the stability and quality of crude drugs.\cite{2,41} In this study, a noticeable difference was observed between moisture contents of aerial (9.08 ± 0.33\%) and root parts (11.70±0.17\%) of this plant. Among the physical constants ash value is considered as an important tool in appraisement of purity and identity of a crude drug, and also considered as an indicator for mineral constituents of the crude drug or medicinal plant. In this medicinal herb, the total ash content was found greater in root (15.79±0.25\%) than aerial part (14.72±0.53\%) which indicates that root part consists of more amount of inorganic minerals like carbonate, oxalate, phosphate including silica and siliceous earthy matters.\cite{41,52} Interestingly, a marked difference between contents of acid insoluble ash of root part (6.56±0.22\%) and aerial portion (1.97±0.017\%) was observed. It is possibly due to presence of greater amount of siliceous matter and metallic salts in root tissue than that of aerial part.\cite{52} The water soluble ash content is estimated by measuring the amount of ash soluble in
water which includes mostly the phosphate salts and some oxalate and carbonate salts. In aerial part, water soluble ash value is 1.79±0.12% that is slightly higher than the root part (1.34±0.15%). In case of sulphated ash, sulphuric acid is added to the drug powder before its incineration and during normal ignition of plant samples they get fixed in stable sulphate form. Sulphation of the drug sample helps in complete combustion of it also. Sulphated ash value highlights the total inorganic impurities present in the crude drug in the form of sulphated salts.[41,52-53] Here, sulphated ash content is greater in root (7.67±0.33%) than aerial portion (5.67±0.33%) which further indicates that more amount of inorganic salts including siliceous matter present in root than aerial part of this medicinal herb. Values for various parameters of ash observed in root and shoot parts of this plant are different and distinct from one another which can be used as marker in proper identification of the crude drug obtained from C. tuberosa and for quality control of it.

Extractive value plays an imperative role to evaluate the nature of chemical constituents present in crude drug and also help in estimation of specific constituent extracted out in particular solvent.[2,41-42] Values of the extractable matters vary according to the polarity of solvent and purity of the crude drug. In this study, water was found to be the best extractive solvent as it extracted out highest yield of the phytoconstituents from the plant materials. There is a marked difference between water extractive values of aerial part (7.04±0.36%) and root part (17.44 ±2.62%) which indicates that water soluble active constituents in root (such as tannins, sugars, plant acids, mucilage, glycosides, inorganic compounds, etc.) are present in greater amount than aerial part. In petroleum ether, both aerial and root parts of the plant showed lowest extractive value which indicates that comparatively lesser number of extractable phytochemical groups (phytosterols, fixed oils, fats, waxes, etc.) have been leached out from both parts. Alcoholic solvents (methanol and ethanol) gave moderate results for those two parts of the investigated plant which give the indication that polar chemical groups such as phenols, steroids, glycosides, flavonoids, alkaloids and other secondary metabolites are present in moderate amount in the alcoholic extracts. Here, percentage yield of extracts was found abundant in aqueous solvent for both root and shoot parts, though preliminary phytochemical screening of the alcoholic extracts of this plant indicates that relatively more major phytochemical groups were extracted out in ethanol and methanol solvents. So, it can be concluded that aqueous- alcoholic solvent is the best option among the solvents taken here for extraction of maximum number of phytochemicals which will further be exploited for various biological activity studies.
Foaming index is higher in root powder, that is 250 in comparison to the aerial portion (200). It highlights that root part is more potent in respect of its saponin content than the aerial portion.\cite{42} Swelling property of the plant sample is due to presence of polysaccharides like gum, mucilage, pectin, starch and hemicellulose. Pectin has specific therapeutic uses, rest other polysaccharides are used as pharmaceutical aids.\cite{42} In root part of \textit{C. tuberosa}, swelling index is 6.67±0.16 ml which is slightly greater than the swelling index of aerial portion (6.16 ± 0.16 ml). So, root part contains bit more amount of polysaccharides which may have greater therapeutic as well as pharmaceutical importance than the aerial part of this plant.

The fluorescence analysis of the drug powder is also used as a finger print for proper identification of crude drugs from their adulterants when physical and chemical parameters of the crude drugs felt inadequate.\cite{41} Fluorescence phenomenon exhibited by plant powder is primarily due to its chemical composition. The same material treated with various chemical reagents appears with different colours in different wavelength of light. Here, methanol treated aerial portion powder appears olive green colour in visible light, but it fluoresces orange colour under UV light which is quite distinct from its colour observed under visible light. Similarly, methanol treated root powder appears pinkish brown colour in visible light and it changes to fluorescent blue colour under UV light.

**DIAGNOSTIC CHARACTERS OF THE INVESTIGATED PLANT**

i. Foliar epidermal cells are polygonal and anticlinal walls of the cells are straight.

ii. Stomata are of strictly tetracytic type; stomatal index- 17.66±0.50.

iii. Non-glandular, unicellular trichomes are present on both surfaces of the leaf.

iv. In aerial part: Total ash content- 14.72±0.539%, water soluble ash- 1.79±0.121% and acid insoluble ash- 1.97±0.017%.

v. In root part: Total ash content- 15.79%±0.253%, water soluble ash- 1.34±0.156% and acid insoluble ash- 6.56±0.223%.

vi. UV fluorescence character

Aerial part- methanol treated drug powder gives olive green colour in visible light and it fluoresces orange colour under UV light.

Root part- methanol treated drug powder appears pinkish brown colour in visible light and it shows fluorescent blue colour in UV light.
CONCLUSION

The diagnostic characters generated from this study will be useful in proper identification of the crude drug obtained from *C. tuberosa* and they will also help in quality assurance of it. A wide range of therapeutically important phytochemical groups have been detected in alcoholic (ethanol and methanol) extracts of both root and aerial parts of this medicinal herb which support its traditional uses for various health conditions. Moreover, aqueous and alcoholic extracts showed highest extractive values for both parts of the plant which indicate that hydro-alcoholic extract can be used in search of novel bioactive compounds and in carrying out their biological activity studies. Further scientific study of the selected extract fractions of *C. tuberosa* can lead to the new drug development and can establish this valuable herb as a potential source of phyto-medicines.

ACKNOWLEDGEMENT

Authors are thankful to the Department of Botany (DST-FIST and UGC-DRS supported), Visva-Bharati, Santiniketan for administrative support. CHR is thankful to the UGC for financial assistance in the form of a major research project [Ref. No.-42-972/2013(SR)].

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