Pharmacognostic standardization of medicinally important

Convolvulus microphyllus (stem)

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ABSTRACT

The present study pertains to pharmacognostical, phytochemical and toxicological investigation of the medicinal important plant C. microphyllus (stem). Standardization was carried out in terms of macroscopical, microscopical characters, toxicological parameters and chromatographic analysis of C. microphyllus (stem). Different standard methods were adopted to carry out the investigation. The study will provide referential information for the correct identification of the crude drug. Foreign organic matter, extractive value, ash value, insoluble ash and loss on drying were found to be 0.0257% w/w, 15.12% w/w, 11.1% w/w, 4.92% w/w and 7.26% w/w. The heavy metal analysis of arsenic was found to be 0.12 ppm, Lead 0.39 ppm, Mercury 0.01 ppm, Cadmium 0.02 ppm. These physicochemical data and phytochemical analysis of Convolvulus microphyllus is useful for further studies for pharmacological screening. In future this study will be helpful for qualitative & quantitative analysis of phytoconstituents for isolation of newer molecule.

Keywords: Convolvulus microphyllus, Pharmacognostic standardization, Phytochemical investigation

INTRODUCTION

Herbs are staging a comeback and herbal ‘renaissance’ is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been prized for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while [1]. Shankhpushpi consists of the whole plant of Convolvulus pluricaulis syn; Convolvulus microphyllus. It belongs to family Convolvulaceae. Convolvulus pluricaulis is a common plant in southern India, in Chindwara, Madhya Pradesh, India. In Gonda Uttar Pradesh, India, the leaves are recommended for depression and mental disturbance [2]. Its stem is Slender, cylindrical, about 1-2 mm in thickness with clear hairy nodes and internodes; light green. Transverse section of stem shows single layered epidermis, covered with thick cuticle; at places unicellular hairs present; cortex differentiated in two zones, 2-3 upper collenchymatous and 1-2 lower parenchymatous layers, both having round to oval, elongated, thin-walled cells[3]. Plant have shown the presence of glycosides, coumarins, flavonoids and alkaloids. Shankhpushpi, (the alkaloid) has been identified as active principle contains B. sitosterol glycoside, Hydroxy Cinnamic acid and Octacosanol tetracosane [4-6]. Convolvulus pluricaulis is used to treat various disorders related to nervous weakness, problems like insomnia, mental as well as physical fatigue, loss of memory etc. C. microphyllus also used as a brain tonic, antihypertensive, improves memory, antiulcer, in bronchitis asthma and treatment of epilepsy [7-
In the present study pharmacognostical, phytochemical and toxicological investigation of the *C. microphyllus* (stem) were carried out to develop a marker tool for the identification of the genuine plant material.

**EXPERIMENTAL SECTION**

**Processing of plant material**
The plant material *C. microphyllus* (stem) was procured from Global Herbs, New Delhi (approved vendor). Powdered stem was preserved in air tight container.

**Plant extracts, chemicals and reagents**
Powdered drug was extracted in a soxhlet apparatus with n-hexane 150 ml to defat the drug filtered and concentrated the methanolic extract in vaccum. All the chemicals and reagents used were of analytical grade.

**Standard analytical parameters development**
Microscopic studies, macroscopic evaluation, physic-chemical analysis like foreign matter, ash value, fluorescence analysis, extractive value and moisture content were performed according to the standard procedures.

TLC was performed in the solvent system Ethyl acetate: Toluene: Acetic acid (5:4:1) and visualized under UV at 366nm.

**Toxicological Parameters**
Various toxicological studies like total viable count, microbial load determination, heavy metal analysis, pesticidal residue and aflatoxin determination were carried out using standard methods.

**RESULTS AND DISCUSSION**

**Macroscopic characteristics**
Macroscopic identity of a medicinal plant material is based on shape, size, colour, taste, surface characteristic, texture, fracture characteristic and appearance of cut surface (W.H.O. Guidelines, 1998). For each particular morphological group, a particular systemic examination can be carried out (Table 1).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Stem</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Color</td>
<td>Light green</td>
</tr>
<tr>
<td>2.</td>
<td>Odor</td>
<td>characteristic agreeable</td>
</tr>
<tr>
<td>3.</td>
<td>Taste</td>
<td>Bitter</td>
</tr>
<tr>
<td>4.</td>
<td>Size</td>
<td>0.1 cm in thickness</td>
</tr>
<tr>
<td>5.</td>
<td>Shape</td>
<td>Cylindrical</td>
</tr>
<tr>
<td>6.</td>
<td>Touch</td>
<td>Plain</td>
</tr>
<tr>
<td>7.</td>
<td>Fracture</td>
<td>hairy nodes with internodes</td>
</tr>
</tbody>
</table>

The macroscopic and organoleptic properties such as colour, odour, taste, size shape, touch and fracture have been observed and were found as per the specifications.

**Foreign Matter Analysis**
Foreign matter means the material not coming from the original plant source and also includes insects and other contamination. The results were recorded in the form of % w/w (table 2).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Wt. of drug (g)</th>
<th>Wt. of drug after removal of foreign matter</th>
<th>Wt of foreign matter</th>
<th>% foreign matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>10.003</td>
<td>10.000</td>
<td>0.003</td>
<td>0.0197</td>
</tr>
<tr>
<td>2.</td>
<td>10.221</td>
<td>10.219</td>
<td>0.002</td>
<td>0.0287</td>
</tr>
<tr>
<td>3.</td>
<td>10.128</td>
<td>10.125</td>
<td>0.003</td>
<td>0.0287</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td>0.0257</td>
</tr>
</tbody>
</table>

The result of foreign matter of *Convolvulus microphyllus* was observed 0.0257 % (w/w with respect to air dried crude drug).
Extractive values
The amount of an extract that a drug yields in a particular solvent is often an approximate measure of the amount of certain constituents that the drug contains. The drug should be extracted with different solvents in order of their increasing polarity to get the correct and dependable values. Generally petroleum ether, alcohol and water extractives are taken into consideration for fixing the standard of a drug. The values are recorded in (Table 3).

Table 3: Water soluble extractive value of Convolvulus microphyllus

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Volume taken (ml)</th>
<th>Weight of empty petriplate (A)</th>
<th>Weight of petriplate after drying (B)</th>
<th>LOD value</th>
<th>B – A</th>
<th>WSE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>51.0877</td>
<td>51.2279</td>
<td>7.26</td>
<td>0.1402</td>
<td>15.12</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>51.0850</td>
<td>51.2249</td>
<td>7.26</td>
<td>0.1399</td>
<td>15.09</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>51.0865</td>
<td>51.2267</td>
<td>7.26</td>
<td>0.1402</td>
<td>15.12</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15.12</td>
</tr>
</tbody>
</table>

The results of Water soluble extractive of Convolvulus microphyllus (stem) was observed 15.12 % (w/w with respect to air dried crude drug).

Ash Values
This parameter can be used for the determination of inorganic materials, such as carbonates, silicates, oxalates and phosphates. Ash value is an important characteristic of a drug and with the help of this parameter we can detect the extent of adulteration as well as establish the quality and purity of the drug. The acid insoluble ash consists mainly of silica and high acid insoluble ash thereby indicating the contamination with earthy materials. The water-soluble ash is used to estimate the amount of inorganic elements. The results of ash values are given in (Table 4 and 5).

Table 4: Results of total ash values:

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Weight of drug (gm)</th>
<th>Weight of empty crucible (gm)</th>
<th>Weight of crucible + wt. of ash (gm)</th>
<th>Weight of ash (gm)</th>
<th>% total ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>20.9118</td>
<td>21.1284</td>
<td>0.2166</td>
<td>11.38</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>20.9110</td>
<td>21.1277</td>
<td>0.2167</td>
<td>11.36</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>20.9120</td>
<td>21.1285</td>
<td>0.2165</td>
<td>11.37</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.37</td>
</tr>
</tbody>
</table>

The result of total ash of Convolvulus microphyllus (stem) was observed 11.37 % w/w with respect to air dried crude drug.

Table 5: Results of acid insoluble ash

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Weight of drug (gm)</th>
<th>Weight of empty crucible (gm)</th>
<th>Weight of crucible + wt. of ash (gm)</th>
<th>Weight of ash (gm)</th>
<th>% acid insoluble ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>20.9118</td>
<td>21.0054</td>
<td>0.0936</td>
<td>4.92</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>20.9110</td>
<td>21.0045</td>
<td>0.0935</td>
<td>4.91</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>20.9115</td>
<td>21.0049</td>
<td>0.0936</td>
<td>4.92</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.92</td>
</tr>
</tbody>
</table>

The result of acid insoluble ash of Convolvulus microphyllus (stem) was observed 4.92 % (w/w with respect to air dried crude drug).

Loss on drying
Take 2 or 5 or 10g of sample (coarse powder) in a dry and weighed dish (glass or aluminium). Then place in an hot air oven at 105±5°C for 5 hrs, cool to room temperature in a desiccator and weigh. After that continue drying and weighing at half an hour intervals till difference between two successive weighing corresponds to not more than 0.1% of weight of sample. Results are given in (Table 6).

Table 6: Results of Loss on drying

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Wt. of drug + dish (before drying) (g) (A)</th>
<th>Wt. Of drug + dish (after drying) (g) (B)</th>
<th>A – B (g)</th>
<th>Loss on drying (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47.3289</td>
<td>47.1823</td>
<td>0.1466</td>
<td>7.26</td>
</tr>
<tr>
<td>2</td>
<td>47.3280</td>
<td>47.1814</td>
<td>0.1466</td>
<td>7.26</td>
</tr>
<tr>
<td>3</td>
<td>47.3279</td>
<td>47.1812</td>
<td>0.1467</td>
<td>7.27</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td>7.26</td>
</tr>
</tbody>
</table>
The result of loss on drying of *Convolvulus microphyllus*(stem) was observed 7.26 % (w/w with respect to air dried crude drug).

**Toxicological Parameters**

**Microbial content determination**

Medicinal plant material generally carry a great number of bacteria and moulds, often originating in the soil, while a large range of bacteria and fungi form the naturally occurring micro flora of herbs, aerobic spore forming bacteria frequently predominate. There are some microbes seen in the plant materials that are pathogenic to the human beings, e.g. *Escherichia coli, Salmonella, Pseudomonas, Staphylococcus aureus* and other type of yeast and moulds.(Table 7).

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Microbial load</th>
<th>Result (per gm)</th>
<th>specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total Aerobic count</td>
<td>150000</td>
<td>1250000</td>
</tr>
<tr>
<td>2.</td>
<td><em>Enterobacteriaceae</em></td>
<td>NAD</td>
<td>1000</td>
</tr>
<tr>
<td>3.</td>
<td><em>E.coli</em></td>
<td>NAD</td>
<td>10</td>
</tr>
<tr>
<td>4.</td>
<td><em>Salmonella sp.</em></td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>5.</td>
<td><em>Staphylococcus aureus</em></td>
<td>NAD</td>
<td>100</td>
</tr>
<tr>
<td>6.</td>
<td>Yeasts</td>
<td>NAD</td>
<td>100</td>
</tr>
<tr>
<td>7.</td>
<td>Moulds</td>
<td>2000</td>
<td>10000</td>
</tr>
<tr>
<td>8.</td>
<td><em>Bacillus Cereus</em></td>
<td>NAD</td>
<td>1000</td>
</tr>
<tr>
<td>9.</td>
<td><em>Pseudomonas Aeruginosa</em></td>
<td>NAD</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 7: Results of microbial load

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Type of heavy metal</th>
<th>Max. residual limits (ppm)</th>
<th>Acid blank values (ppm)</th>
<th>Sample (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Arsenic (As)</td>
<td>1.00</td>
<td>6.234</td>
<td>0.12</td>
</tr>
<tr>
<td>2.</td>
<td>Lead (Pb)</td>
<td>3.00</td>
<td>19.55</td>
<td>0.39</td>
</tr>
<tr>
<td>3.</td>
<td>Mercury (Hg)</td>
<td>0.1</td>
<td>0.343</td>
<td>0.01</td>
</tr>
<tr>
<td>4.</td>
<td>Cadmium (Cd)</td>
<td>0.5</td>
<td>0.845</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 8: Results of heavy metals

**Table 9: Phytochemical screening of *Convolvulus microphyllus***

<table>
<thead>
<tr>
<th>Extract constituents</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td></td>
</tr>
<tr>
<td>Molisch Test</td>
<td>+</td>
</tr>
<tr>
<td>Fehlings Test</td>
<td>+</td>
</tr>
<tr>
<td>Benedict Test</td>
<td>+</td>
</tr>
<tr>
<td>Pentose sugar</td>
<td>+</td>
</tr>
<tr>
<td>Tollens phlorogucinol Test</td>
<td>+</td>
</tr>
<tr>
<td>Cobalt Chloride Test</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td></td>
</tr>
<tr>
<td>Cardiac Glycoside</td>
<td>Keller killians Test +</td>
</tr>
<tr>
<td></td>
<td>Legal Test +</td>
</tr>
<tr>
<td></td>
<td>Libermann Test +</td>
</tr>
<tr>
<td></td>
<td>Baljet Test +</td>
</tr>
<tr>
<td>Anthraquinone Glycoside</td>
<td>Bronitorgers Test -</td>
</tr>
<tr>
<td></td>
<td>Mod. Brontrager -</td>
</tr>
<tr>
<td>Saponin Glycoside</td>
<td>-</td>
</tr>
<tr>
<td>Cyanogenetic Glycoside</td>
<td>-</td>
</tr>
<tr>
<td>Coumarin Glycoside</td>
<td>+</td>
</tr>
<tr>
<td>Tamins &amp; Phenols</td>
<td></td>
</tr>
<tr>
<td>5% FeCl₃</td>
<td>-</td>
</tr>
<tr>
<td>Gelatin Test</td>
<td>-</td>
</tr>
<tr>
<td>Chlorogenic acid Test</td>
<td>-</td>
</tr>
<tr>
<td>PPT. Test.</td>
<td></td>
</tr>
<tr>
<td>Lead acetate</td>
<td>-</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>-</td>
</tr>
<tr>
<td>Dil.HNO₃</td>
<td>-</td>
</tr>
<tr>
<td>Pot. Dichromate</td>
<td>-</td>
</tr>
<tr>
<td>Ferric chloride</td>
<td>+</td>
</tr>
<tr>
<td>10% AgNO₃</td>
<td>-</td>
</tr>
<tr>
<td>Pot.permanganate</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
</tr>
<tr>
<td>Zinc chloride Test</td>
<td>-</td>
</tr>
<tr>
<td>Shinoda Test</td>
<td>+</td>
</tr>
</tbody>
</table>
The microbial count in *Convolvulus microphyllus* was determined and it was observed that the bacterial growth was almost nil and some mould growth was observed.

**Determination of Trace Metals**

Contamination of medicinal plant materials with arsenic and heavy metals can be attributed to many causes including environmental pollution and traces of pesticides. The method of determination is left to the analyst. Nevertheless, the determination must be consistent and sensitive enough to allow comparison with a reference material. (Table 8).

The heavy metal analysis of arsenic was found to be 0.12 ppm, Lead 0.39 ppm, Mercury 0.01 ppm, Cadmium 0.02 ppm

**CONCLUSION**

The present study pertains to pharmacognostical, phytochemical and toxicological investigation of the medicinal plants. The standardization was according to International standardization criteria. The Qualitative as well as Quantitative analysis of the raw material has been investigated through which we came to know the active constituents present in that and in how much quantity. From this study we have found that all the results were as per specification and the product is therapeutically effective and is safe for mankind. If we use these type of modern techniques for standardization of the products as well as raw materials then ayurveda can proved to be a boon to mankind as compared to Allopathic drugs, because ayurveda has very less side effects as compared to allopathy drugs which shows sometimes serious side effects.

**Acknowledgement**

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**REFERENCES**