STANDARDIZATION AND QUALITY CONTROL OF CENTELLA ASIATICA LINN. (GOTUKOLA) DRIED POWDER AND CAPSULES

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ABSTRACT

Centella asiatica L. (Gotukola) is one of the potential medhya rasayana medicine identified in ancient Ayurveda textual references. Standardised Gotukola powder capsules, the test medication were used as a part of a study in memory assessing. Although, Gotukola products are widely available in global market as cognitive boosters, evidence based supported document with scientific validity is very limited. Hence, the major objective of the current study is to standardize Gotukola powder and the finished product. The raw materials of Gotukola were thoroughly cleaned, grinded and passed through the mesh No 180 and 80 sieves respectively. Then fine powder obtained was filled into capsules without any exipients or preservatives under very stringent hygienical conditions at a GMP certified pharmaceutical premises. The raw materials and the finished product were screened for physical identity, phytochemical analysis and TLC as per the standard methods. In findings foreign matter was not more than 2.0 percent. Total moisture content, total ash and acid insoluble ash were respectively in the ranges of 8.9 %, 16.4 % and 3.6%. In TLC fingerprints, observed bands at Rf=0.4 and 0.3 which are corresponding to that of reference marker compounds of authenticated standard sample of Centella asiatica. The values were within the range of standard reference. Hence the finished product was suitable for clinical use.

KEYWORDS: Centella asiatica, memory, cognitive abilities standardization.
INTRODUCTION

Charaka samhita, one of the oldest Ayurvedic classics among the existing described a separate group of drugs which is designated as Medhya Rasayana. These medicines produce Medhya effect (intellectual promoting). These drugs constitute an important class of medicines in Ayurveda and are proved to produce beneficial effect on medha. The term Mandukaparni (Gotukola) is found in groups of Prajasthapana Mahakashaya, Vayasthapana Mahakashayaya and Tiktaskandha in Charaka Samhita. *Centella asiatica* (L.) is a perennial creeper plant belonging to the family of Umbellifere. It is found throughout Sri Lanka, India and in most tropical and sub tropical countries. *Centella asiatica* (L.) is one of the main herbs used in Ayurveda for treating nervine disorders and rasayana therapy.[1] It is also well documented in textual references in Ayurveda widely used as blood purifier, in memory enhancement, other cognitive domains and promoting longevity.[1, 2, 3] Although, *Centella asiatica* products are widely available in global market as cognitive boosters, evidence based supported document with scientific validity is very limited. But the mere fact is that there is no single evidence based scientifically proven medication that has been manufactured or used in indigenous medical institutions in Sri Lanka. The major objective of the current study is to standardise Gotukola powder and the finished product. Therefore, development of clinically proven, standardised Ayurvedic formulations would definitely enhance the image and prime necessities of primary healthcare delivery system of Ayurveda in Sri Lanka, and indeed, worldwide.

MATERIALS AND METHODS

Botanical drug used

Raw materials of *Centella asiatica* were collected as bulk samples from a cultivator who grows gotukola almost organically off Bandaragama area in western province. Used parts - Whole plant were used.

Authentication

Raw materials were authenticated by the Department of Dravyaguna, Institute of indigenous Medicine, University of Colombo. In addition, voucher specimen was further authenticated at the Bandaranayake Memorial Ayurveda Research Institute, Nawinna. Some of the identified active ingredients of *Centella asiatica*. 
Water - Distilled water was used as the sole medium for the preparation of extracts and standard laboratory investigations. **Chemicals** - All the chemicals used were conformed to the laboratory standards.

**Chromatographic materials**
Thin Layer Chromatography:- Chromatography papers, 250 μm thickness, Whatmann, aluminium backing and silica gel coating were used for identification of raw materials of *Centella asiatica* and extracts.

**Preparation of TLC**
The hot extraction and cold extraction were spotted at the origin of the TLC plate. The plate was run in the solvent system of hexane and acetone, in the ratio of 6:1, in the TLC chamber.

**Methods for standardization**
Standardization process was designed as per the criteria and Standard Operational Procedures followed in pharmaceutical industry and Good Laboratory Practices.

**Ethical clearance**
The project proposal was submitted to the Ethics Committee of the Institute of indigenous Medicine, University of Colombo, Rajagiriya and the ethical clearance was obtained prior to study. (Registration No – ERC 14/34).
Final drug preparation
The raw materials were thoroughly cleaned and all physical impurities were removed manually. Then whole plant was washed in tap water several times until the fine sandy particles were removed. Then shade dried in trays. All the raw materials were stored in air tight containers for the prevention of moisture, contamination with micro organisms and excessive heat which affect the quality of final preparation. All the raw materials were grinded at the Pharmacy of the Department of DravyaGuna vignana, Institute of indigenous Medicine. Then the grinded plant materials were passed through the mesh No 180 and 80 sieves respectively and a fine powder was obtained. The powder material was kept in air tight containers until fill into capsules.

Capsules preparation
A zero number capsules were used. The capsules were filled with finely powdered raw material without any exipients or preservatives. They were filled under very stringent hygienical conditions at a GMP certified pharmaceutical premises by Astron Ceylon Ltd. They were repacked again into ninety capsules packs for the convenient usage of individual participant.

Quantitative Physico-chemical analysis
Determination of foreign matters
A weight of 500 g of Centella asiatica Linn. Sample was weighed and spread out in a form of thin layer. The foreign matters were detected by inspection with naked eyes and by the use of a magnifying glass (×6). They were separated and percentage of foreign matter was calculated.

Determination of Moisture content/ loss on drying at 105°C
Loss on drying was determinate by accurately weighing of three samples of 2 g of each in petri dishes and drying in an oven at 105°C till three constant weights were achieved. The weight was calculated and the difference and moisture content was calculated and expressed as a percentage.

Determination of total ash content
Accurately weighed three samples of 2 g each air dried powder samples were taken into porcelain crucibles and the material was spread into even layer and ignited to a gradually increasing heat up to 550°C in a muffle furnace until it became white, indicating the absence
of carbon. Then the crucibles were cooled in a desiccator and weighed. Percentage of total ash was calculated with reference to the air dried samples.

**Determination of acid insoluble ash content**

Twenty milliliters of dilute hydrochloric acid was added to the porcelain crucible containing the total ash, covered with a watch glass and boiled gently for ten minutes. Then watch glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ash less filter paper and washed with hot water until the filtrate was neutral. The filter papers containing the insoluble matter was transferred to the original crucible, dried in an oven and ignited to constant weight. Obtained residue was allowed to cool in a desiccator for 30 minutes, and weighed without delay. The percentage of acid insoluble ash was calculated with reference to air dried sample.

**Determination of water soluble ash content**

Twenty five milliliters of water was added to the porcelain crucible containing the total ash and boiled for ten minutes. Then the insoluble matter was collected on an ash less filter paper, washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C till obtaining constant weight. Then the weight of this residue was subtracted in mg from the weight of total ash. Finally, the percentage of water soluble ash was calculated with reference to air dried sample.

**Determination of extractable matter**

Accurately weighed 2.5 g of powdered air dried sample was placed in a round bottom flask. Then it was shaken using a mechanical shaker for six hours. After that it was allowed to stand for 18 hours. The solvent was filtrated carefully not to loose any solvent and 20 ml of solvent was transferred to the petri dish and these dishes were kept in a water bath to evaporate the water. After evaporating the water left the sample was dried at 105°C for 6 hours in an oven. Then it was cooled in a desiccator for 30 min and weighed. The percentage of each extractable matter in solvents was calculated as follows. The solvents which were used to detect extractability of raw materials were as mentioned below Water, 95 % ethanol, Chloroform, Hexane.

**Qualitative chemical tests**

**Test for alkaloids:** A number of three drops of dilute HCl were added to 2 ml of the filtrate sample and a few drops of Meyer’s reagent were added. The change was observed and noted.
Test for tannins
A few drops of ferric chloride reagent were added to the water extract and the change was observed and noted.

Test for saponins
A small amount (0.1 g) of extracted powder was vigorously shaken with 2 ml of distilled water in a test tube for 30 seconds. The test tube was left to stand for 20 minutes. The formation of persistent frothing was observed and noted.

Test for sugars
Three millilitres of Benedict’s solution was added to 1 ml of test solution. Then it was mixed and boiled for 2 minutes over a mild flame.

Test for flavonoids
A one gram of powder was extracted with 10 ml of 95% ethanol for 15 minutes on a boiling water bath. Small pieces of metal magnesium and a few drops of concentrated HCl acid were added to the filtrate. The change was observed and noted.

Finished product were analysed as follows.
Uniformity of weight
Disintegration test
Total ash
Acid insoluble ash
Water soluble ash
Loss on drying
TLC identification

Disintegration test
The test was done by using the tablet disintegration apparatus (Pharma Test PTZS, Make West Germany). A number of six capsules were used for this test. Capsules were placed in six tubes of plastic 80-100 mm long containers, which was immersed in water at 36-38°C (normal body temperature) and it was constantly raised and lowered down in such a manner that the complete up and down movements were repeated 30 times per minute through a distance of 75 mm. The time taken to disintegrate the capsules was observed and noted.
Preparation of hot extraction
Weight of 5 g of coarsely powdered material of *Centella asiatica* was measured and taken in to a round bottom flask (500 ml). A volume of 100 ml of hexane was added in to the flask and flask was connected to the Soxhlet apparatus. The powdered drug was refluxed for about 5 hours.

Preparation of cold extraction
Weight of 1 g of coarsely powdered material of *Centella asiatica* was measured and taken in to a volumetric flask. A volume of 20 ml of hexane was added in to the flask and covered. The mixture was shaken frequently up to 6 hours and was made to stand for 18 hours. Then the mixture was filtered and concentrated in a rotary evaporator.

RESULTS AND DISCUSSION
Fine powder shows fragments epidermal cells polygonal in surface view with stomata, palisade cells, spiral vessels, rosette crystals of calcium oxalate, simple grains measuring 8-16 micron in diameter. Shows an epidermis of tangentially elongated cells on both surfaces, larger on upper surface, covered by striated cuticle, mesophyll 2-3 layered of palisade cells, somewhat isodiametric spongy parenchyma; rosette crystals of calcium oxalate present in few cells.
Stomatal Index on Upper surface 10 -12
Lower surface 14-19

Quantitative parameters
Foreign matter – not more than 2.0 percent

Table 1: Determination of proximate analysis for powder samples and the finished product of *Centella asiatica*

<table>
<thead>
<tr>
<th></th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Finished product mean value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on drying</td>
<td>8.9 %</td>
<td>8 %</td>
<td>8.5 %</td>
<td>8%</td>
</tr>
<tr>
<td>Total ash</td>
<td>16.4 %</td>
<td>16 %</td>
<td>15 %</td>
<td>16%</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>3 %</td>
<td>3.6 %</td>
<td>3 %</td>
<td>3%</td>
</tr>
<tr>
<td>Alcohol-soluble extractive</td>
<td>11 %</td>
<td>10.5 %</td>
<td>10 %</td>
<td>10%</td>
</tr>
<tr>
<td>Water-soluble extractive</td>
<td>18 %</td>
<td>18.9 %</td>
<td>19 %</td>
<td>18%</td>
</tr>
</tbody>
</table>
Table 2. Determination of qualitative analysis for different extractions of *Centella asiatica*.

<table>
<thead>
<tr>
<th>Phytochemical analysis</th>
<th>Hot extraction</th>
<th>Cold extraction</th>
<th>Alcoholic extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugars – Fehling’s test and Benedict’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids – Mayer’s test</td>
<td>+</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Flavonoids</td>
<td>+</td>
<td>_</td>
<td>+</td>
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<tr>
<td>Glycosides</td>
<td>_</td>
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</tbody>
</table>

Thin Layer Chromatography (TLC)

Observed bands at Rf ≈ 0.4 and 0.3 which are corresponding to that of reference marker compounds of *Centella asiatica* (Linn.)

Finished product analysis

Uniformity of weight = 0.4856 g ± 10% per cap

Disintegration test – mean 6.5 minutes

(Finished product specifications were conformed to the standards of British Pharmacopoeia)

DISCUSSION

Results noted in quantitative analysis were conformed as per the standards references. In findings foreign matter was not more than 2.0 percent. Total moisture content, total ash and acid insoluble ash were respectively in the ranges of 8.9 %, 16.4 % and 3.6 %. In the qualitative analysis for phytochemicals the major components were detectable in extractions. In TLC fingerprints, observed bands at Rf ≈ 0.4 and 0.3 which are corresponding to that of reference marker compounds of authenticated standard sample of *Centella asiatica*. The values were within the range of standard reference. Hence the finished product was suitable for clinical use.

Water soluble extractable value showed a slight decrease to the normal range and it may be due some factors related to seasonal or demographic variations. Also glycosides were observed negative in dry extractions although it had been found in wet samples in some other studies. Detailed studies on TLC and HPTLC are needed to identify the variations of different samples from demographical and seasonal changes to develop optimal standards of *Centella asiatica* confined to Sri Lanka.
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