

# Standardization of *Sarasvatha Choorna*: Used as a Remedy for Dementia

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**Abstract:** Sri Lanka has been recognized as one of the fastest aging populations from the developing countries. There is a large senior population in the country. Since age is the biggest risk factor for Dementia, the need to create awareness and management is an increasing necessity. *Sarasvatha Choorna* is one of the polyherbal preparation used in Ayurveda for the management of Dementia which consists of 12 medicinal plants. In the present study, an attempt was made to standardize *Sarasvatha Choorna* by using standard protocols. Standardization was carried out by determination of total ash, water soluble ash, acid insoluble ash, microbial counts, heavy metals, phytochemical screening and development of TLC-densitogram fingerprints. Results revealed that 10.6±0.0% of total ash, 8.4±0.0% of water soluble ash 0.65±0.01% of acid insoluble ash 11.0±0.1% of cold ethanol extractable matter, 13.9±0.1% hot ethanol extractable matter, 13.9±0.1% cold water extractable matter and 20.2±0.2% hot water extractable matter were present in the *Sarasvatha Choorna*. Pathogenic microorganisms such as *Coliforms*, *Escherichia coli* and *Salmonella* were not found and heavy metal concentrations of *Sarasvatha Choorna* were well below the recommended upper limits for the tested heavy metals. Phytochemical screening studies revealed the presence of flavonoids, steroid glycosides and coumarins in both water and ethanol extracts of *Sarasvatha Choorna*. Presence of the raw materials in the *Sarasvatha Choorna* was confirmed by TLC fingerprints. Present study reveals the quality of *Sarasvatha Choorna* for the first time and quality control parameters resulted from this study can be used as a reference standard for quality control of *Sarasvatha Choorna*.

**Keywords:** *Sarasvatha Choorna*, Dementia, Standardization, Quality Control

## 1. Introduction

Sri Lanka has been recognized as one of the fastest aging populations from the developing countries. There is a large senior population in the country [1]. Since age is the biggest risk factor for Dementia, the need to create awareness and management is an increasing necessity. Dementia is a combination of several symptoms that are associated with the declining abilities of the brain and its functions [2]. There may be a decline in thinking, memory, cognition, language skills, understanding and judgment. *Sarasvatha Choorna* is one of the polyherbal preparation used in Ayurveda for the management of Dementia [3] which consists of 12 medicinal

plants and rock salt (Table 1).

The quality assessment of herbal formulation is a vital importance in order to justify their acceptability in modern system of medicine. Methods of standardization should take into consideration all aspect that contribute to the quality of the herbal drugs, namely authentication of the sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test, etc [4]. Therefore, in the present study, an attempt was made to standardize *Sarasvatha Choorna* by using standard protocols. Standardization of *Sarasvatha Choorna* was carried out by determination of total ash, water soluble ash,

acid insoluble ash contents, microbial counts, heavy metals, phytochemical screening and development of TLC-densitogram fingerprints.

**Table 1.** *Ingredients of Sarasvatha Choorna.*

Ingredients	Part of the plant used
<i>Saussurea lappa</i> Sch.Bip	Stem
<i>Withania somnifera</i> Linn	Root
<i>Apium graveolens</i> Linn	Seed
<i>Cuminum cyminum</i> Linn	Seed
<i>Carum carvi</i> Linn	Seed
<i>Zigiber officinale</i> Linn	Rhizome
<i>Piper nigrum</i> Linn	Seed
<i>Piper longum</i> Linn	Fruit
<i>Cissampelos Pereira</i> Linn	Creeper
<i>Evolvulus alsinoides</i> Linn	Whole plant
<i>Acorus calamus</i> Linn	Rhizome
<i>Bacopa monieri</i> Linn	Leaf
Rock salt	Crystals

## 2. Materials and Methods

### 2.1. Preparation of Sarasvatha Choorna

Dried powders of *Saussurea leppa*, *Withania somnifera*, *Apium graveolens*, *Cuminum cyminum*, *Carum carvi*, *Zigiber officinale*, *Piper nigrum*, *Piper longum*, *Cissampelos Pereira*, *Evolvulus alsinoides* and rock salt were taken in equal quantities. Powdered *Acorus calamus* was taken equal to that of the total quantity and the entire mixture is soaked in the juice of *Bacopa monieri*.

Finally, the pulp was dried completely.

### 2.2. Investigation of Physico-chemical Parameters of *Sarasvatha Choorna*

Physico-chemical parameters such as total ash, water soluble ash, acid insoluble ash contents and extractable matter (ethanol and water) of *Sarasvatha Choorna* were determined according to the WHO guide lines [5].

#### 2.2.1. Total Ash Content

The material (2 g) was accurately weighed, in a previously ignited and tarred crucible. The material was spread in an even layer and ignites it by gradually increasing the heat to 500-600 °C using muffle furners until it turned into white ash, indicating the absence of carbon. The crucible was cooled in desiccators and weighed. The content of total ash in the dried material was calculated as:

$$\% \text{ Total Ash} = \frac{\text{Total Ash Weight} \times 100}{\text{Weight of Sample}}$$

#### 2.2.2. Acid insoluble Ash Content

Acid (2M HCl, 25 mL) was added to the crucible containing the total ash, covered with a watch glass and

boiled gently for 5 min using a hot plate. The watch glass was rinsed with 5 mL of hot water and the rinsed contents added to the crucible. The acid insoluble matter was collected on an ash less filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the acid insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight.

$$\% \text{ Acid Insoluble Ash} = \frac{\text{Acid Insoluble Ash Weight} \times 100}{\text{Weight of Sample}}$$

#### 2.2.3. Water Soluble Ash Content

Water (25 mL) was added to the crucible containing the total ash and boiled for 5 min. The water insoluble matter was collected on an ash less filter paper and washed with hot water. The filter paper containing the water insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The weight of this residue was subtracted from the weight of total ash and the content of water soluble ash calculated.

$$\% \text{ Water Soluble Ash} = \frac{\text{Total Ash Weight} - \text{Water Insoluble Residue} \times 100}{\text{Weight of Sample}}$$

### 2.3. Microbiological Limits

Limits of Aerobic plate count [6], Yeast and Moulds [7], Coliforms [8], presumptive *Escherichia coli* [9], *Salmonella* [10] and *Staphylococcus aureus* [11] were determined according to the methods described in SLS standards.

### 2.4. Heavy Metal Analysis

Quantitative determination of Arsenic [12], Mercury [13], Cadmium [14] and Lead [12] were carried out according to relevant methods described in AOAC methods.

### 2.5. Phyto-chemical Screening Studies

Phytochemical screening was carried out using ethanol and water extracts of the drug [15].

#### 2.5.1. Determination of the Presence/Absence of Tannins

Extract was diluted with water and added to diluted ferric chloride solution. Blackish blue or green blackish color in the presence of ferric chloride was taken as an indication for tannins.

#### 2.5.2. Determination of the Presence/Absence of Flavonoids

Extract was dissolved in methanol (50 %, 1 - 2 ml) by heating. Then metal magnesium and 5 - 6 drops of con. HCl were added. Appearance of a red color was taken as confirmation of flavonoids.

#### 2.5.3. Determination of the Presence/Absence of Steroid Glycosides

Extract was dissolved in equal volumes of acetic anhydride and  $\text{CHCl}_3$ . The mixture was transferred to a dry test tube and con.  $\text{H}_2\text{SO}_4$  acid was introduced to the bottom of the

tube. Formation of a reddish brown or violet – brown ring at the interface of the two liquids was taken as an indication for steroids.

#### 2.5.4. Determination of the Presence/Absence of Coumarins

Coumarins form a yellow color with 1% KOH in absolute ethanol. 1 mL of portions of 1% extract in test tubes was treated with 3-4 drops of 1% KOH in absolute ethanol.

#### 2.5.5. Determination of the Presence/Absence of Saponins

Extract was mixed with 5 mL of distilled water in a test tube and it was shaken vigorously. Formation of a stable foam was taken as an indication for the presence of saponins.

#### 2.6. Development of Thin Layer Chromatography (TLC) Fingerprint and Confirmation of Presence of Active Ingredients

Sample, standard mixture of raw materials in a ratio of 1:1 w/w (as mentioned in Table 1) and individual raw materials extracted separately into dichloromethane, concentrated and spotted (5 µL from each) on a pre-coated TLC plate

Solvent system: Methanol: Ethyl acetate: Dichloromethane: Cyclohexane (0.2:0.8:3:6 v/v/v).

Spray reagent: Vanillin sulphate and heated at 100°C for 5 m

### 3. Results and Discussion

It is very important that a system of standardization is established for every herbal medicine in the market because the scope for variation in different batches of medicine is enormous. Plant material may vary in its phytochemical content and therefore in its therapeutic effect according to different places of collection, with different times in a year for collection, with collection at the same time and places but in different years and with different environmental factors surrounding the cultivation of a particular medicinal plant. Adding to this variability is the fact that in herbal medicine several plants may be used together in the same preparation. This means that there should be a quality control test for the entire preparation to ensure quality of the product [16]. Physico-chemical parameters of *Sarasvatha Choorna* were given in Table 2. Total ash is particularly important in the evaluation of purity and quality of a plant. The total ash usually consists of carbonates, phosphates, silicates, and silica, which include both physiological ash and non physiological ash. The physiological ash comes from the mineral components of the plant itself. However, the plant may contain foreign matter adhered to it by contact with the soil and sand. This foreign matter is called non-physiological ash. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the leafy vegetable for marketing [17]. Water soluble ash is that part of the total ash content, which is soluble in water. It is a good indicator of the water soluble salts in the plant. Acid insoluble ash indicates contamination with silica, for

example, earth and sand. In the present study, very low amount of acid insoluble ash content indicates the purity of *Sarasvatha Choorna* [18]. Extractive values are representative of the presence of the polar or nonpolar compounds in a plant material. Higher hot water extractable matter implies that most of the active compounds in *Sarasvatha Choorna* dissolve in the hot water better than ethanol (both cold and hot extracts).

Table 2. Physico-chemical parameters of *Sarasvatha Choorna*.

Physico-chemical parameters	Percentage (%) in dry weight basis
Total ash	10.6±0.0
Water soluble ash	8.4±0.0
Acid insoluble ash	0.65±0.01
Ethanol extractable matter (cold)	11.0±0.1
Ethanol extractable matter (hot)	13.9±0.1
Water extractable matter (cold)	18.8±0.0
Water extractable matter (hot)	20.2±0.2

Values are presented as Mean ± SEM, n=6

Pathogenic microorganisms such as Coliforms, *Escherichia coli* and *Salmonella* were not found in *Sarasvatha Choorna*. However, aerobic and mesophilic organisms and few counts of *Staphylococcus aureus* were present in the sample (Table 3). Heavy metal concentrations of *Sarasvatha Choorna* were Pb (0.73 ppm), Cd (0.06 ppm), Hg (0.10 ppm) and As (< 0.1 ppm). Further heavy metal concentrations of *Sarasvatha Choorna* were well below the recommended upper limits for heavy metals in Canada (Pb: 10 ppm, Cd: 0.3 ppm, Hg: 0.2 ppm and As 5 ppm). Phytochemical screening studies revealed the presence of flavonoids, steroid glycosides and coumarins in both water and ethanol extracts of *Sarasvatha Choorna*. In addition, saponins was present in the water extract.

Table 3. Microbial counts of *Sarasvatha Choorna*.

Microbes	Microbial counts
Aerobic plate count, CFU/g	$9.0 \times 10^5$
Yeast and Moulds, CFU/g	$8.5 \times 10^{10}$
Coliforms, MPN/g	Not detected
Presumptive <i>Escherichia coli</i> , MPN/g	Not detected
<i>Salmonella</i> /25 g	Absent
<i>Staphylococcus aureus</i> /g	<100

Presence of *Saussurea lappa*, *Withania somnifera*, *Apium graveolens*, *Cuminum cyminum*, *Carum carvi*, *Zigiber officinale*, *Piper nigrum*, *Piper longum*, *Cissampelos Pereira*, *Evolvulus alsinoides*, *Acorus calamus* and *Bacopa monieri* in the *Sarasvatha Choorna* was confirmed by TLC fingerprint profiles.

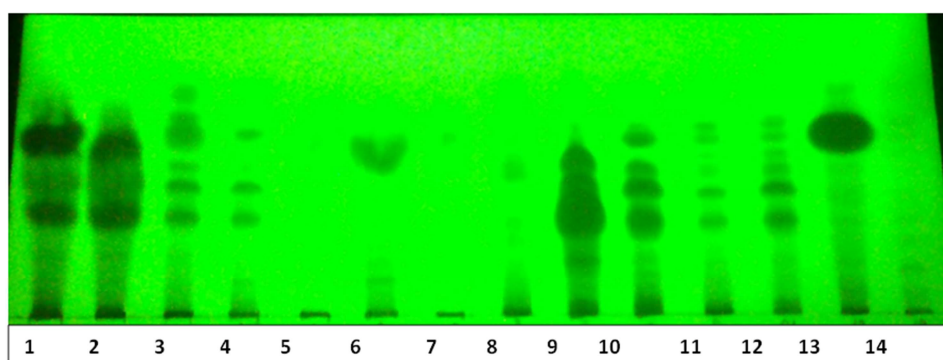


Fig. 1. TLC fingerprint profile of the (a) *Sarasvatha Choorna*, (b) standard mixture of raw materials and (c) individual plant materials at 254 nm.

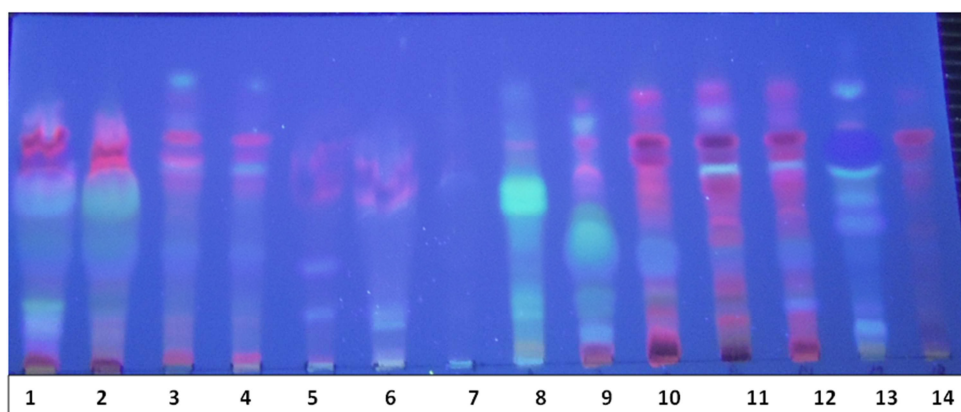


Fig. 2. Fig 1. TLC fingerprint profile of the (a) *Sarasvatha Choorna*, (b) standard mixture of raw materials and (c) individual plant materials at 366 nm.

1. *Sarasvatha Choorna*
2. standard mixture of raw materials
3. *Saussurea leppa*
4. *Withnia somnifera*
5. *Apium graveolens*
6. *Cuminu cyminum*
7. *Carum carvi*
8. *Zigiber officinale*
9. *Piper nigrum*
10. *Piper longum*
11. *Cissampelos Pereira*
12. *Evolvulus inoides*
13. *Acorus calamus*
14. *Bacopa monieri*

## 4. Conclusion

Present study reveals the quality of *Sarasvatha Choorna* for the first time and quality control parameters resulted from this study can be used as reference standard for quality control of *Sarasvatha Choorna*.

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