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Standardization of Jala Peenisa Choornam Used for Peenisa Rogam

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Authors' contributions

This work was carried out in collaboration between all authors. Authors YMPKM, KS and HMUIM carried out the standardization of the drug and performed the statistical analysis. Authors PS and TT designed the study, wrote the protocol and wrote the first draft of the manuscript. All authors managed the analyses of the study and the literature searches, read and approved the final manuscript.

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ABSTRACT

Indigenous Medicine of Sri Lanka consists of Ayurveda, Siddha and Unani Medicines. Herbal drugs are commonly used in Siddha Medicine. Peenisa Rogam is one of the common diseases of neck and above. Jala Peenisa choornam (JPC) mentioned in Siddha literature for the treatment of Jaladosham (Acute rhinitis) contains powder of *Caesalpinia bonducella* seeds, *Curciuma longa* rhizome and *Nigella sativa* seeds in 2:1:1 w/w ratio. There is no validated scientific data on the efficacy of JPC on Peenisa Rogam. Therefore, this study was aimed to standardize the JPC in terms of phytochemicals, physico-chemicals and antioxidants as total polyphenolic content (TPC) before evaluating the efficacy. *C. bonducella* seeds, *C. longa* rhizome and *N. sativa* seeds were cleaned, dried, powdered separately and blend together in 2:1:1 w/w ratio to obtain JPC and used for the standardization. TPC was determined by Folin-Ciocalteu method.
The results revealed that the physico-chemical parameters: moisture, total ash, acid-insoluble ash, water soluble ash, water extractable matter and ethanol extractable matter are 2%, 4.98±0.001%,

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0.28±0.002%, 2.40±0.003%, 9.50±0.01% and 8.63±0.007% on dry weight basis respectively and the presence of phytochemicals: steroids, terpenoids, flavonoids, cardiac glycosides and saponins (3.6 mg/g). TPC was 2.16±0.07 mg gallic acid eqiv./g of JPC.
In conclusion, the low acid-insoluble ash and moisture content indicates the good quality of this drug. The presence of phytochemicals in this plant determines the bioactivity. The phytochemicals present and high TPC content of JPC indicates the value for using this herbal drug as a nutraceutical.

Keywords: Standardization; Jala Peenisa choornam; Peenisa Rogam.

1. INTRODUCTION

Indigenous Medicine of Sri Lanka consists of Ayurveda, Siddha and Unani Medicines. Siddha Medical system is popular in Northern and Eastern part of Sri Lanka. In Siddha Medicine, most of the herbs are used in the preparation of Siddha drugs to treat various human diseases. Peenisa Rogam is one of the common diseases in Siroroga Maruththuvam (Diseases of neck and above) [1]. The signs and symptoms of Peenisa Rogam affect nose and para-nasal sinuses. Choornam is one of the internal medicines contains single or a mixture of herbs. Jala Peenisa choornam (JPC) was mentioned in Siddha literature for the treatment of all types of Peenisam including Jaladosham (acute rhinitis) [2]. JPC contains the powder of *Caesalpinia bonducella* (English: fever nut; Tamil: Kalachchi seeds; Sinhala: Wael kumburu) seeds, *Curcuma longa* (English: Turmeric; Tamil: Manjal; Sinhala: Kaha) rhizome and *Nigella sativa* (English: black seed; Tamil Karuncheeragam; Sinhala: Kaluthuru) seeds in a 2:1:1 w/w ratio respectively. These 3 ingredients are individually used in the treatment of Peenisam as a powder [3-9]. There is no validated scientific data for the efficacy of JPC on Peenisa Rogam. Therefore, this study was aimed to standardize the JPC by phytochemical screening, physico-chemical determination and antioxidants as total polyphenolic content (TPC) before evaluating the efficacy.

2. MATERIALS AND METHODS

2.1 Preparation of Jala Peenisa Choornam

All three plant parts (*Caesalpinia bonducella* seeds, *Curcuma longa* rhizome and *Nigella sativa* seeds) were purchased from Ayurvedic Pharmacy and authenticated in Unit of Siddha medicine. The outer covering of dry seeds of *Caesalpinia bonducella* was removed. The kernel of the seeds was ground, sieved and kept in an air tight container. *Curcuma longa* rhizome and *Nigella sativa* seeds were washed, dried under shade and ground separately, sieved and kept in air tight containers. *Caesalpinia bonducella* seed powder (110 g), *Curcuma longa* rhizome (55 g) and *Nigella sativa* seed powder (55 g) were blended together to prepare the JPC.

2.2 Determination of Physico-chemical Parameters of JPC

Physico-chemical parameters were determined according to the methods described in WHO guidelines [10].

2.3 Determination of Moisture Content

JPC (25 g) was taken in a round bottomed flask and filled with 50 mL of saturated toluene in the flask and the Dean-Stark arm fixed to the round bottomed flask. The Dean-Stark apparatus was heated for 8 hours until constant volume of moisture obtained. It was allowed to cool and the volume of moisture trapped in the arm was measured.

$$\text{Moisture content (\% by mass)} = \frac{\text{V. d}}{\text{M}} * 100$$

V – Volume of water collected in receiving tube (mL)

d – Density of water (g/cm^3)

M – Mass of the sample used (g)

2.4 Determination of Water Extractable Matter (Hot Extraction)

Accurately weighed JPC (4 g) was taken in a glass stoppered conical flask. Water (100 mL) was added to the flask and weighed to obtain the total weight. The conical flask was shaken well and allowed to stand for 1 hour. It was refluxed gently for 1 hour, cooled and weighed. The weight was readjusted to the original weight by adding required amount of water. The contents were shaken well and filtered through a dry filter

paper. From the water extract 25.0 mL was transferred to a tared flat bottomed dish, evaporated to dryness on a water bath, dried at 105°C for 6 h in an oven and cooled in a desiccator for 30 minutes. The weight was taken. The experiment was carried out in triplicate.

The content of water extractable matter (in mg/g) JPC was calculated as follows:

$$\%\ \text{water extractable matter} = \frac{\text{Weight of residue}}{\text{Weight of JPC}} \times 100$$

2.5 Determination of Ethanol Extractable Matter (Cold Extraction)

Accurately weighed JPC (4 g) was taken in a glass stoppered conical flask. It was macerated with 95% ethanol (100 mL) for 6 h with frequent shaking and allowed to stand for 18 h. The contents were filtered rapidly through a dry filter paper; 25.0 mL of filtrate was transferred to a tared flat bottomed dish, evaporated to dryness on a water bath and dried at 105 °C for 6 h in an oven. The dishes were cooled in a desiccator for 30 minutes and the weight was taken. The experiment was carried out in triplicate.

The content of ethanol extractable matter (in mg/g) JPC was calculated as follows:

$$\%\ \text{Ethanol extractable matter} = \frac{\text{Weight of residue}}{\text{Weight of JPC}} \times 100$$

2.6 Determination of Total Ash Content

JPC (2 g) was accurately weighed and spread evenly in 3 tarred crucibles. It was ignited and placed in the Muffle furnace at 550°C for 4 hours to obtain constant weight. The crucibles were allowed to cool in a desiccator for 30 minutes and weighed. The content of total ash (in mg/g) of JPC was calculated as follows:

$$\%\ \text{Total ash} = \frac{\text{Weight of ash}}{\text{Weight of JPC}} \times 100$$

2.7 Determination of Acid-insoluble Ash Content

Diluted hydrochloric acid (7%, 25 mL) was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 minutes. The watch glass was rinsed with 5 mL of hot water and the rinsed contents were added to the crucible. The acid-insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate became neutral (pH=7). Filter paper containing acid-insoluble material was transferred to the original crucible, dried on a hot plate and ignited at 450°C for 2 hours to obtain constant weight. The residue was allowed to cool in a desiccator and weighed. The content of the acid-insoluble ash (in mg/g) of air-dried material was calculated as follows:

$$\%\ \text{Acid} - \text{insoluble ash} = \frac{\text{Weight of ash}}{\text{Weight of JPC}} \times 100$$

2.8 Determination of Water Soluble Ash Content

Water (25 mL) was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 minutes. The watch glass was rinsed with 5 mL of hot water and added to the crucible. The water insoluble matter was collected on an ashless 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 a constant weight at 450 °C for 15 min. The water soluble ash content was calculated using the following equation.

$$\%\ \text{Water soluble ash} = \frac{\text{Weight of total ash} - \text{weight of water insoluble residue}}{\text{Weight of JPC}} \times 100$$

2.9 Phytochemical Screening of JPC

The qualitative tests for alkaloids, saponins, steroids, terpenoids, flavonoids, tannins and cardiac glycosides and the quantification of saponins was performed according to method [11] H.O. Edeoga et al. [4].

2.10 Total Polyphenolic Content (TPC)

TPC was quantified according to Folin-Ciocalteu method [12]. JPC (15 g) was extracted with methanol and the solvent evaporated. The extract, 766 mg from the original extract (2.9 g) was dissolved in 766 µL of Dimethyl sulfoxide (DMSO). From this solution, 5 mg/mL concentration of stock solution was prepared by diluting with distilled water. Then the serial dilution was prepared (0.15625 - 2.5 mg/mL)

from the stock solution. Gallic acid (7.8125 -1000 µg/mL) was used as the standard (1 mg/mL in distilled water). 10% Na_2CO_3 solution and 10 times diluted 2 X Folin-Ciocalteu (FC) reagents were used. FC reagent (110 µL) was added to the micro plate and 20 µL of samples and standards also added to it. Pre-plate reading was taken at 765 nm. 10% Na_2CO_3, 70 µL was added to each plate and incubated at room temperature for 30 minutes. The absorbance was taken at 765 nm. Water was taken as the blank [13,14].

3. RESULTS AND DISCUSSION

3.1 Physico-chemical Parameters

The physico-chemical analysis of plant drugs is important to maintain the quality as well as to detect the adulteration. The total ash consist both organic and inorganic materials and determines the purity and quality of the drugs. The acid-insoluble ash indicates the presence of materials like soil and sand and other foreign materials including the inorganic material from the plants. A high acid-insoluble ash value indicates the contamination, substitution or adulteration during preparation of the drug. Since the total ash and acid-insoluble ash contents are within the standard values indicates the good quality and purity of this drug.

Physico-chemical analysis results are listed in Table 1.

The extractable matter values of water and ethanol indicates the amount of active ingredients in given amount of plant material when extracted with respective solvents. According to the results higher amount of extractable matter was resulted in water when compared to ethanol. Moisture is a critical parameter for the herbal drugs to prevent from microbial contamination. The moisture content (2%) of JPC indicates there is less chance of contamination by microbes.

Table 1. Physico-chemcial parameters of JPC

Physico-chemical parameters	% (dry weight basis)
Moisture content	2.00
Total ash	4.98±0.001
Acid-insoluble ash	0.28±0.002
Water soluble ash	2.40±0.003
Ethanol extractable matter	8.63±0.007
Water extractable matter	9.5±0.01

Data were represented as mean±SD; n=3

The quantitative determination of some phytochemicals is useful to determine the efficacy of the crude drugs and determine the bioactivity. Phytochemical screening revealed the presence of steroids, terpenoids, flavanoids, cardiac glycosides and saponins in JPC.

3.2 Total Polyphenolic Content (TPC)

Calibration curve for gallic acid is given in Fig. 1.

Total Polyphenolic Content of JPC is 2.16±0.07 mg gallic acid eqiv./g of JPC.

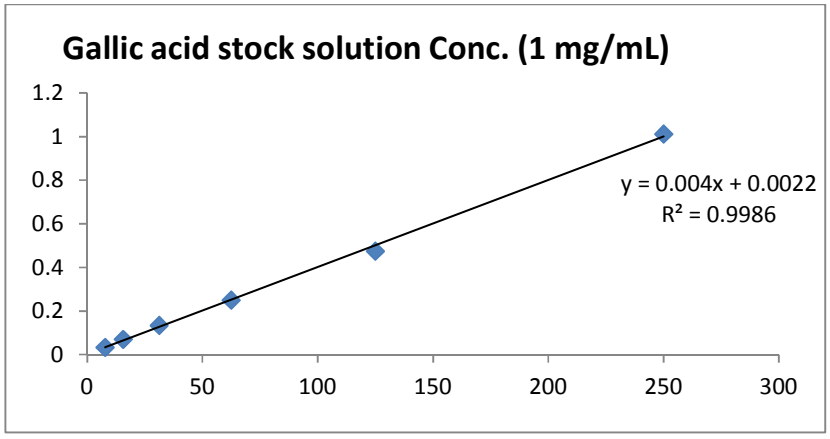

Fig. 1. Calibration curve of gallic acid

4. CONCLUSION

In conclusion, the low acid-insoluble ash and moisture content indicates the good quality of this drug. The presence of phytochemicals in this plant determine the bioactivity. The phytochemicals present and high TPC content (2.16±0.07 mg gallic acid eqiv./g of JPC) of JPC indicates the value for using this herbal drug as a nutraceutical.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

DISCLAIMER

The abstract of this work was presented and published in the conference "National Research Conference and Exhibition on Indigenous Medicine 2017 [NRCEIM 2017]".
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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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