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Standardization of Sri Lankan market samples of *Rubia cordifolia* Linn.

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ABSTRACT
Rubia cordifolia Linn (Family: Rubiaceae); is a common medicinal plant and an essential ingredient for Ayurvedic and traditional medicinal preparations. It is commonly sold under the trade name 'Manjistha'. In the present study, an attempt was made to establish quality control parameters for *R. cordifolia* market samples found in Sri Lanka by a). physico-chemical evaluation b). screening of major phytochemical classes and c). development of Thin Layer Chromatography fingerprints. Physico-chemical parameters such as moisture content (10.62 ± 0.06%), hot water extractable matter (15.55 ± 0.51%), hot ethanol extractable matter (6.23 ± 0.05%), cold water extractable matter (9.66 ± 0.11%), cold ethanol extractable matter (2.34 ± 0.0%), total ash content (6.63 ± 0.16%), acid-insoluble ash content (0.71 ± 0.07%), and water-soluble ash content (2.93 ± 0.02%) were determined. *R. cordifolia* hot water and hot ethanol extracts showed the presence of phenolic compounds, alkaloids, flavonoids, saponins, steroid glycosides, coumarin and tannins. Thin Layer fingerprint profile was developed to *R. cordifolia* methanolic extract. Therefore, the present study helps to establish quality control parameters for *R. cordifolia* market samples found in Sri Lanka.

Keywords: *Rubia cordifolia,* Physico-chemical, Phytochemical, Thin Layer Chromatography.

1. Introduction
Ayurvedic Pharmacopoeia recorded more than 300 medicinal plants that are commonly used in Ayurvedic system of medicine. The knowledge of chemical compounds present in a plant helps the scientists to understand the mode of action of the drug [1]. It has been observed that there is a wide dissimilarity and variation in clinical results obtained by the use of crude drugs obtained from different geographical regions [2]. According to Ayurvedic basic texts and in traditional preparations, *Rubia cordifolia* Linn (Family: Rubiaceae) is a common ingredient of herbal formulations such as Manjishthadi kvatha (Laghumanjishthadi and Mahamanjishthadi), Pinda taila, Vipadikahara grita taila, Ashwagandharista, Aravindasava, Chandanasava, Ushirasava, Jatyadi ghrita, Manjishthadi taila and Khadiradi gutika.
R. cordifolia is commonly named as 'Indian Madder' and sold under the trade name 'Manjistha'. It is a straggling perennial with very long cylindrical, flexuose roots with a red cortex and very long flexible, tough, white barked cylindrical stem. Stems often many yards long, rough, grooved, becoming slightly woody at the base [3-4].
R. cordifolia is used both externally as well as internally. The roots of Manjishtha are used for medicinal purposes. Externally, Manjishtha is highly recommended in skin diseases associated with oedema and oozing. Especially, chronic skin diseases, wounds are responding very well for Manjishtha kvata and grita. The root is sweet, bitter, acrid, heating, alexiteric, antidysenteric, antipyretic, analgesic, anthelmintic, improves the voice and the complexion and cures inflammation, leucoderma, erysipelas, ulcers [3].
Propagation of *R. cordifolia* is not sufficient to the demand for drug manufacturing processes in Sri Lanka and almost depends on the imported material. Therefore, confirmation of its identity, quality and purity should be made before developed the drug. At present, various chemical methods and thin layer chromatographic analysis of the plant materials are used to estimate the active constituents in the crude drugs as the materials which received to the market for drug manufacturing are not in suitable condition.

However, no scientifically controlled studies were conducted to check the quality control parameters of *R. cordifolia* imported samples. Therefore, present study was conducted to establish quality control parameters of *R. cordifolia* market samples found in Sri Lanka by a). physico-chemical evaluation b). screening of major phytochemical classes and c). development of Thin Layer Chromatography fingerprints.

2. Materials and Methods

2.1 Plant material

R. cordifolia market samples were collected from three locations of Western province, Sri Lanka. Plant materials were identified (RC: 2013-01) and authenticated by the Head, Department of Materia Medica, Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka. Samples were washed, shade dried, crushed and powdered by using a domestic grinder. All samples were kept in air tight containers until used.

2.2 Determination of extractable matter using *Rubia cordifolia* (WHO, 2000) [5]

2.2.1 Hot water extractable matter

Accurately weighed 4.0 g sample of *R. cordifolia* was placed in a glass stoppered conical flask. Water (100 mL) was added to the flask and it was weighed to obtain the total weight, including the flask. Then, the flask was shaken well and allowed to stand for 1 h. A reflux condenser was attached to the flask and boiled gently for 1 h and then it was cooled and weighed. The weight was readjusted to the original total weight by adding required amount of water. The flask was shaken well and filtered rapidly through a dry filter paper (90 mm Diameter Whatman®). After that, 25 mL of the filtrate was transferred to a tared flat bottomed dish and evaporated to dryness on a water bath. Then the dish was dried at 105 °C for 6 h and cooled in a dessicator and weighed. The content of extractable matter (in mg/g) air dried material was calculated.

2.2.2 Cold water extractable matter

Accurately weighed 4.0 g of coarsely powdered air dried sample of *R. cordifolia* was placed in a glass stoppered conical flask, macerated with 100 mL of water for 6 h, shaken frequently, and then allowed to stand for 18 h. It was filtered rapidly with qualitative filter paper (90 mm Diameter,Whatman®). After that, 25 mL of the filterate was transferred to a tared flat bottomed dish and evaporated to dryness on a water bath. Finally, dried at 105 °C for 6 h, cooled in a desiccator for 30 min, then weighed without delay. The content of extractable matter in water was calculated as mg per g of air dried material.

2.2.3 Hot ethanol extractable matter

Accurately weighed 4.0 g of coarsely powdered sample of *R. cordifolia* was placed in a glass stoppered conical flask. Ethanol (95%, 100 mL) was added to the flask and it was weighed to obtain the total weight, including the flask. Then, the flask was shaken well and allowed to stand for 1 h. A reflux condenser was attached to the flask and boiled gently for 1 h, and then it was cooled and weighed. The weight was readjusted to the original total weight by adding required amount of ethanol. The flask was shaken well and filtered rapidly through a dry filter paper (90 mm Diameter Whatman®). After that, 25 mL of the filtrate was transferred to a tared flat bottomed dish and evaporated to dryness on a water bath. Then the dish was dried at 105 °C for 6 h in an oven, cooled in a dessicator and weighed. The content of extractable matter (in mg/g) air dried material was calculated.

2.2.4 Cold ethanol extractable matters

Accurately weighed 4.0 g of coarsely powdered air dried sample of *R. cordifolia* was placed, in a glass stoppered conical flask, macerated with 100 mL of ethanol (95%) for 6 h, shaken frequently, and then allowed to stand for 18 h. It was filtered rapidly with qualitative filter paper (90 mm Diameter,Whatman®). After that, 25 mL of the filterate was transferred to a tared flat bottomed dish and evaporated to dryness on a water bath. Finally, dried at 105 °C for 6 h, cool in a desiccator for 30 min, then weighed without delay. The content of extractable matter in ethanol was calculated as mg per g of air dried material.

2.3 Determination of physico-chemical parameters using *Rubia cordifolia*

Physico-chemical parameters were determined according to methods described in the guidelines of WHO (2000) [5].

2.3.1 Determination of Moisture Content

The powdered material (1 g) was placed in an aluminium moisture dish and dried to a constant weight in an oven at 100-105 °C. The weight loss of the sample was calculated as

$$\% \text{ Moisture Content} = \frac{\text{Weight loss}}{\text{Weight of Sample}} \times 100$$

2.3.2 Total ash Content

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

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

2.3.3 Acid-insoluble ash Content

2 M HCl (25 mL) was added to the crucible containing the total ash, covered with a watch glass and boiled gently over a flame for 5 min. 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 ashless 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.

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

2.3.4 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 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 constant weight. The weight of this residue was

subtracted from the weight of total ash and the content of water soluble ash calculated.

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

2.4 Preparation of extracts using *Rubia cordifolia* for screening of phytochemical compounds

Phytochemical analysis was performed for water extracts and ethanolic extracts (cold and hot) for samples according to the method described by Fansworth (1996) [6] to find the presence or absence of phenolic compounds, flavanoids, steroid glycosides, tannins, coumarins, alkaloids and saponins with some modifications.

2.4.1 Presence /Absence of Phenolic compounds

Two or three drops of 1% $FeCl_3$ solution was added to 2 mL portions (1%) of each extract. Phenolic compounds produce a deep violet colour with ferric ions.

2.4.2 Presence /Absence of Saponins

The extract is taken in a test tube with a small amount of water and shaken vigorously for one minute and observed for formation of rich lather, which is stable for more than ten minutes.

2.4.3 Presence/Absence of Flavanoids

The extract was dissolved in methanol (50%, 1-2 mL) by heating. Then metal magnesium and 5-6 drops of concentrated hydrochloric acid (HCL) were added. The solution turns red when flavonoids are present.

2.5 Development of Thin Layer Chromatography (TLC) fingerprints for *Rubia cordifolia:* Methanol (50 ml) was added to 4.0 g of the sample and stirred well for 30 min. Then filtered through a funnel and evaporated the filtrate using a rotovapour (Buchi, B-480) and then redissolved the residue in 20 mL methanol. Each extract (2 and 4 µL) was spotted on TLC plates.

Absorbent	:	Silica gel-GF_{254}
Solvent system	:	Ethyl acetate: Dichloromethane: Cyclohexane (0.5:3.5:1 v/v/v).

Detection

Visualization	:	Vanillin – sulphuric acid reagent was sprayed to the TLC plate and heated at 105 ^oC for 5 min.
Scanning	:	Densitometer (CS – 9301PC, Shimadzu, Japan) at 254 nm (before spraying)

3. Results and Discussion

The results of the physico-chemical and phytochemical studies are tabulated in Table 1 and 2. The quality control parameters for the raw materials were established with the help of several official determinations based on physicochemical parameters. Results revealed that the total ash content of *R. cordifolia* was 6.63 ± 0.16%. The results obtained in the present study for the ash values were almost similar with that of Devi Priya and Siril (2013) [7]. Controlled incineration of crude drugs results in an ash residue consisting of inorganic materials (metallic salts and silica) and this value varies within fairly wide limits and it is therefore an important parameter for the purpose of evaluation of crude drugs. More direct contamination, such as by sand or earth, is immediately detected by the ash value and the results were observed slightly higher due to contamination or unwanted parts of the drug [8]. In physicochemical parameters of the present study, slight variations were shown in hot water, cold water, hot ethanol and cold ethanol extractable values of in each three samples. Different extractive values determine the amount of active ingredients in a given amount of medicinal plant material when extracted with solvents [8].

According to the results, amounts both hot and cold water extractable were higher than that of ethanol extractable matter. Similar results were observed with *Averrhoa carambola* fruits [9] and *Mallotus philippensis* leaves and fruits [10].

The general requirement for the moisture content in crude drugs is less than 14%. The excess moisture can result in the breakdown of important constituents by enzymatic activity and which may encourage the growth of yeast and fungi during storage [11]. According to Jayanthy and co-workers (2013) [12], the value of moisture content was high in the plants collected in the rainy season and the seasonal variation is associated with the vegetative and reproductive stages of the plant, it has direct influence with the variation in chemical constituents of the plants also. The study revealed that *R. cordifolia* contain 10.62% moisture content and was not high and that indicates less chances of microbial degradation of the drug during storage and more suitable to medicinal preparations.

Table 1: Physico-chemical parameters of *Rubia cordifolia* market samples found in Sri Lanka

Physico-chemical parameters	Amount in percentage (Dry weight basis)
Moisture content	10.62 ± 0.06
Hot water extractable matter	15.55±0.51
Hot ethanol extractable matter	6.23 ± 0.05
Cold water extractable matter	9.66 ± 0.11
Cold ethanol extractable matter	2.34 ± 0.0
Total ash content	6.63 ± 0.16
Acid-insoluble ash content	0.71 ± 0.07
Water-soluble ash content	2.93 ± 0.02

Values are expressed as mean ± SEM., n=6

Table 2: Phytochemical classes of *Rubia cordifolia* market samples found in Sri Lanka

Phytochemical Classes	Presence or Absence of Phytochemical Classes			
	(HWE)	(HEE)	(CWE)	(CEE)
Phenolic compounds	√	√	√	√
Saponins	√	√	√	×
Flavanoids	√	√	√	×
Steroid glycosides	√	√	√	√
Tannins	√	√	√	√
Coumarin	√	√	√	√
Alkaloids	√	√	×	×

HWE – Hot Water Extract, HEE - Hot Ethanol Extract, CWE - Cold Water Extract, CEE - Cold Ethanol Extract
(√ : presence, ×: absence)

Preliminary phytochemical screening was performed for establishing the profile of extract for its nature of chemical composition. In phytochemical study, both hot water and hot ethanolic extracts showed presence of phenolic compounds, alkaloid, flavonoids, saponins, steroid glycosides, coumarin and tannins. Few phytochemical classes could be able to identify in the cold ethanol extract. Phenolics are present ubiquitously in all the plants that are partly responsible for health benefits including as antioxidants, anticancer and against other cardiovascular complications [13].

Slight variation in physicochemical and phytochemical results may be due to several factors such as different geographical conditions, edaphic factors, environmental conditions, period of cultivation and harvesting, method of collection, source of irrigation and fertilizers, age of the plant, powdering method, and extraction method [14]. Apart from the physicochemical parameters provided in Ayurvedic Pharmacopoeia, the data for moisture content, pH value and phytochemical study evolve can be considered as viable parameters, which will go a long way for prescribing dependable standards to the raw drugs [14]. Further, Thin Layer Chromatography fingerprint and chemical compounds present in the three samples of *R. cordifolia* are similar. Hence, the present study has been helped to given out the parameters to determine the quality of market samples of *R. cordifolia*.

4. Conclusion

Results of the present study with physico-chemical evaluation, screening of major phytochemical classes and Thin Layer Chromatography fingerprints help to establish quality control parameters for *R. cordifolia* market samples found in Sri Lanka.

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