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Phytochemical Variation of a Poly-herbal Formula According to Its Preparation Method: Qualitative & Quantitative Analysis

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Authors' contributions

This work was carried out in collaboration among all authors. Author RLDSR designed the concept, planned the experimental protocols, wrote the first draft of the manuscript and literature searches. Author RHSKDS contributed to the literature searches. Author LDAMA supervised the experimental protocols and finalised the draft. Author HGSGW managed the collection of raw material and the preparation of drugs. All authors read and approved the final manuscript.

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ABSTRACT

Madhyama Rasnadi decoction, one of the poly-herbal decoctions used in Ayurveda medicine is especially indicated for inflammatory conditions. The literature provides three different preparation methods of this decoction viz; Sri Lankan Traditional method (Method 1) and methods described in the texts Sharangadhara Samhita (Method 2) and Bhaishajya Ratnavali (Method 3). The aim of this study was to analyse and compare the phytochemical profiles of these three preparation techniques. Phytochemical profile analysis was carried out by (i) investigation of the extractable matter in 1 ml of decoction, (ii) development of Thin Layer Chromatography profiles and (iii)

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qualitative/quantitative determination of major phytoconstituents. Results revealed that the extractable matter of methods 1, 2 and 3 was 310±0 mg/ml, 420±0 mg/ml and 180±0 mg/ml, respectively. Differences (in terms of the number of spots and intensity) were observed in TLC fingerprint profiles, and phytochemicals such as phenols, tannins, flavonoids, saponins, alkaloids and terpenoids were present in all three types of decoctions. Gallic acid and quercetin were taken as standards to express the results of polyphenolic and flavonoid contents, respectively. Total polyphenolic contents of decoctions prepared according to methods 1, 2 and 3 were 121.68±0.60, 178.40±0.56, 86.20±0.25 mg gallic acid equivalents/g extract respectively. Total flavonoid contents of decoctions prepared according to methods 1, 2 and 3 were 69.45± 0.80, 129.30±0.65, 52.64± 0.50 mg quercetin acid equivalents/g extract respectively. In conclusion, more phytochemicals are concentrated on the decoction that made of method 2. The study opens more vistas of clinical applicability of *Madhyama Rasnadi* decoction, where further randomised case-control studies are needed.

Keywords: Anti-oxidant properties; herbal decoctions; Madhyama Rasnadi decoction; phytochemicals; TLC profiles.

1. INTRODUCTION

Madhyama Rasnadi is a poly-herbal decoction consists of 13 medicinal plants. This decoction is especially indicated for *Sama Roga* (inflammatory conditions), *Vata Roga* (nervous diseases) and *Shula* (pains) [1]. The ingredients of the decoction are given in Table 1 [1].

According to literature, there are three different preparation techniques for *Madhyama Rasnadi* decoction including Sri Lankan Traditional method (Method 1), methods described in the texts Sharangadhara Samhita (Method 2) and Bhaishajya Ratnavali (Method 3) respectively (Table 2) [2]. The current study was focused on analysing and comparing the phytochemical profiles of these three preparation techniques.

2. MATERIALS AND METHODS

2.1 Collection of Raw Materials

The raw materials were collected from Western Province, Sri Lanka and authenticated by a Scientist at Department of Botany, Bandaranayake Memorial Ayurveda Research Institute (BMARI), Nawinna, Maharagama, Sri Lanka. The voucher specimens (MR 1 to MR 13) were kept in Department of Ayurveda, Institute of Indigenous Medicine, University of Colombo, Sri Lanka.

Table 1. Ingredients of *Madhyama Rasnadi* decoction

Botanical name	Family	Part used	Proportion in grams (g)
Alpinia calcarata (Andrews) Roscoe	Zingiberaceae	Rhizome	1
Ricinus communis L.	Euphorbiaceae	Root	1
Asparagus racemosus Willd.	Liliaceae	Root	1
Barleria prionitis L.	Acanthaceae	Root	1
Tragia involucrata L.	Euphorbiaceae	Root	1
Justicia adhatoda L.	Acanthaceae	Root	1
Tinospora cordifolia (Willd.) Hook. f. & Thomson	Menispermaceae	Stem	1
Cedrus deodara (Roxb. ex D.Don) G.Don	Coniferae	Wood	1
Aconitum heterophyllum Wall. ex Royle	Ranunculaceae	Root	1
Terminalia chebula Retz.	Combretaceae	Pericarp	1
Cyperus rotundus L.	Cyperaceae	Rhizome	1
Kaempferia galanga L.	Zingiberaceae	Rhizome	1
Zingiber officinale Roscoe	Zingiberaceae	Rhizome	1

Table 2. Three different preparation techniques for *Madhyama Rasnadi* decoction [1,3,4]

Methods	Form of the ingredients	Amount of the ingredients	Amount of water	Final amount
Sri Lankan traditional method (Method 1)	Crude	Total weight of the ingredients: 60 g (Ratio of the ingredients- 1:1 w/w)	1920 ml	1/8 (240 ml)
Method described in the text Sharangadhara Samhita (Method 2)	Coarse powder	Total weight of the ingredients: 48 g (Ratio of the ingredients- 1:1 w/w)	768 ml	1/8 (96 ml)
Method described in the text Bhaishajya Ratnavali (Method 3)	Powder	Total weight of the ingredients: 25 g (Ratio of the ingredients- 1:1 w/w)	200 ml	1/4 (50 ml)

2.2 Preparation of Decoctions

The raw materials were washed with water to remove all unwanted foreign matter and air-dried under room temperature (27°C - 30°C for 7 days) and stored in an airtight container for further use. The decoctions were prepared according to the three different techniques given in Table 2 at the Bhaishajya Laboratory, Institute of Indigenous Medicine, University of Colombo, Sri Lanka.

(i) **Method 1:** Raw materials (60 g) in crude form added into an earthen pot containing 1920 ml of water. The mixture is reduced to 1/8th of its original volume (240 ml) on mild fire and filtered.
(ii) **Method 2:** Coarse powder (48 g) of raw materials put into an earthen pot and added 768 ml of water. The mixture is reduced to 1/8th of its original volume (96 ml) on mild fire and filtered.
(iii) **Method 3:** Raw materials (25 g) in powder form put into a glass beaker and 200 ml of water was added. The mixture is reduced to 1/4th of its original volume (50 ml) on mild fire and filtered.

2.3 Comparison of Chemical Profiles of *Madhyama Rasnadi* Prepared in Three Different Techniques

Chemical comparison carried out by (a) quantification of extractable matter (b) development of TLC fingerprints (c) phytochemical screening (d) quantification of total polyphenols and (e) quantification of total flavonoids.

2.3.1 Determination of extractable matter

A petri dish was accurately weighed and poured 10 ml of decoction from method 1. Then, the petri dish was kept on a boiling water bath. Once the evaporation was completed, the petri dish was kept at 105°C for 4 h and taken the weight. The weight difference was considered as the extractable matter for 10 ml of method 1. This procedure was repeated thrice for method 1 and the same procedure was carried out for method 2 and method 3.

2.3.2 Development of Thin Layer Chromatography (TLC) fingerprint

Each decoction (30 ml) was added to a separatory funnel containing dichloromethane (15 ml), mixed well and kept for separation. Then the dichloromethane layer was separated. This method was repeated thrice, and combined dichloromethane fractions were evaporated to dryness under vacuum and re-dissolved in 5 ml of dichloromethane. Finally, 5 µl from each dichloromethane fraction was spotted on a TLC plate.

2.3.3 Qualitative phytochemical evaluation

Phytochemical screening was conducted to test the presence or absence of phenols, flavonoids, tannins, alkaloids, saponins, steroids, terpenoids, monoterpenes, sesquiterpenes and cardiac glycosides according to the standard protocols described by Yadav and Agarwala [5], Ranasinghe and co-workers [6] and Kulathunga and co-workers [7]. In addition to phytochemical screening, total polyphenolic and flavonoid contents were quantified in *Madhyama Rasnadi* decoction using *in vitro* assays.

2.3.4 Quantitative determination of total polyphenolic content

Known concentrations (0-100 µg/mL; n=3) of extract or gallic acid (0.1 mL) were diluted with distilled water (0.9 mL). To each tube, 5 mL of a

ten-fold diluted solution of Folin-Ciocalteu reagent was added and mixed. Each tube was then mixed with 4 mL of saturated sodium carbonate solution, and well shaken. After 2 h, the absorbance of each reaction mixture was measured at λ 765 nm. Gallic acid was considered as the standard for the calibration curve. The total phenolic content was calculated by the calibration curve of gallic acid, and the results were explained as mg of gallic acid equivalents per g of the extract (mg gallic acid/g extract) [8].

2.3.5 Quantitative determination of total flavonoid content

The total flavonoid content was evaluated using the Dowd method, as previously described [9]. In this experiment, 5 mL of 2% $AlCl_3$ in methanol was mixed with the same volume of the extract, or quercetin in known concentrations (0-100 µg/mL; n =3). After 10 min, the absorbance of the reaction mixture was measured at λ 415 nm. Quercetin acid was used as the standard for the calibration curve. The total flavonoid content was calculated based on the calibration curve of quercetin and the results were expressed as mg of quercetin equivalents per g of extract (mg quercetin/g extract).

3. RESULTS AND DISCUSSION

Among the three different preparation methods, the highest amount of extractable matter was found in Method 2 (420±0 mg/ml) followed by Method 1 (310±0 mg/ml) and Method 3 (180±0 mg/ml). When the sizes of the particles are smaller, there is a more chance to interact with water. Similarly, when the ingredients are exposed to heat more lengthy time, there is a tendency to increase more extractable matter that is extractable. In Methods 1 and 2, ingredients are exposed to heat until the final water volume reduces $1/8^{th}$ compared to original water volume. In contrast, in Method 3, ingredients are exposed to heat until the final water volume is reduced $1/4^{th}$ compared to original water volume. Therefore, in comparison to Method 3, ingredients of Methods 1 and 2 were exposed to the heat for a lengthier time. Compared to Method 1, the size of the particles is smaller in Method 2. Although the fine powder was used in Method 3, the time that the particles were exposed to heat was shorter. Therefore, the use of coarse powder and the exposing of the ingredients to heat for comparatively a long time may be the reasons for the highest extractable matter in Method 2.

TLC fingerprint profiles are used to check the differences in phytochemical constituents in plant extracts. Differences in TLC fingerprint profiles of *Madhyama Rasnadi* decoction prepared in three different methods are illustrated in Fig. 1. Significant differences were observed in three preparation techniques under 254 nm and 366 nm. A similar pattern was observed for Methods 1, 2 and 3 when the TLC fingerprint profiles were observed under 254 nm. However, the spots observed from Method 1 were more intense than that of Methods 1 and 2. In contrast, one prominent spot was observed on both TLC fingerprints of Methods 2 and 3, which was not presented in the TLC fingerprint of Method 1.

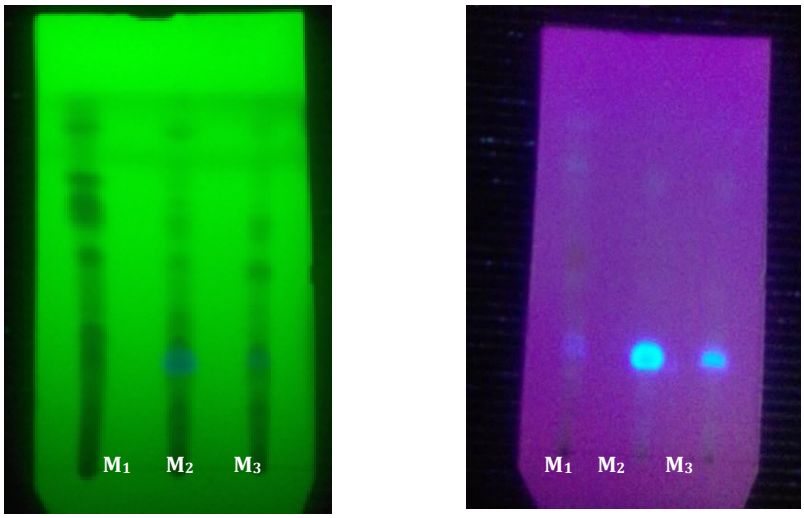
Plants produce secondary metabolites which serve as a defense mechanism for various disease conditions such as diabetes, gastritis and inflammatory joint diseases [10]. In the present study, phenols, tannins, flavonoids, saponins, alkaloids and terpenoids were present in all three types of decoction preparation techniques (Table 1). However, saponins, alkaloids and terpenoids were prominent in decoction prepared according to method 2. Secondary metabolites such as terpenoids [11], tannins [11], alkaloids [12,13] and phenols [14] exhibit anti-inflammatory actions while terpenoids and tannins possess analgesic activity [11]. In general, anti-inflammatory drugs exhibit analgesic activity. Therefore, the presence of these phytochemicals in the decoction justifies the use of *Madhyama Rasnadi* decoction in inflammatory disorders such as *Amavata* (rheumatoid arthritis), *Urustambha* (stiffness of thighs).

Total polyphenolic contents of decoctions prepared according to methods 1, 2 and 3 were 121.68±0.60, 178.40±0.56, 86.20±0.25 mg gallic acid equivalents/g extract respectively. Folin Ciocalteu reaction is an antioxidant assay, based on electron transfer, which measures the reductive capacity of an antioxidant [15]. Polyphenols of medicinal plants exhibit a strong free radical scavenging ability as demonstrated by using free radical scavenging assays such as Diphenyl-1-Picrylhydrazyl (DPPH) and Oxygen Radical Absorbance Capacity (ORAC). This may due to the ability of (a)scavenging the free radicals and (b)modulate the function of mitochondria which constitutes the major cellular source of reactive oxygen species [16] by polyphenols.

Table 3. Phytochemical screening of *Madhyama Rasnadi* decoction prepared in three different methods

Phytochemicals	Method 1	Method 2	Method 3
Phenols	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Saponins	+	++	+
Alkaloids	+	++	+
Terpenoids	+	++	+

+: Moderate Amount, ++: High Amount



(a) at 254 nm **(b) at 366 nm**

Fig. 1. TLC fingerprint profiles *Madhyama Rasnadi* decoction prepared for Method 1 (M_1), Method 2 (M_2) and Method 3 (M_3) at 254 nm (a) and 366nm (b)

Total flavonoid contents of decoctions prepared according to methods 1, 2 and 3 were 69.45± 0.80, 129.30±0.65 and 52.64±0.50 mg quercetin acid equivalents/g extract respectively. In general, this colourimetric assay is based on the quantification of yellow colour produced by the interaction of flavonoids with $AlCl_3$ reagent. Flavonoids are polyphenolic compounds that are categorised, according to chemical structure: flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcone [17]. The highest amount of polyphenols and flavonoids were presented in *Madhyama Rasnadi* decoction prepared according to method 2 followed by method 1 and 3, respectively.

Moreover, scientific evidence is available to prove the capability of antioxidants to inhibit inflammation [18,19]. Both phenols and flavonoids have antioxidant properties and play a significant role in inflammatory conditions. *Madhyama Rasnadi* decoction is also rich in phenols and flavonoids, which may contribute for its potent against anti-inflammatory disorders. Antioxidant potential of phenolic compounds depends on the number and arrangement of the hydroxyl groups and the extent of structure conjugation [20,21]. For flavonoid compounds, O-dihydroxy groups in the B-ring, the presence of a C 2/3 double bond in conjunction with 4-oxo in the C-ring, and 3- and 5-hydroxy groups and the 4-oxo function in the A and C-rings are associated with the antioxidant activity [22].

4. CONCLUSION

This study is claimed to be the first report on the phytochemical comparison of three different preparation techniques of *Madhyama Rasnadi* decoction. It can be suggested that among three preparation methods, the method described in Sharangadhara Samhita is the most effective method in terms of quantification of total

polyphenolic content and flavonoid content, high amounts of saponins, alkaloids and terpenoids. The study opens more vistas of clinical applicability of *Madhyama Rasnadi* decoction, where further randomised case-control studies are needed.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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