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Assessment of Quality of "*Dhanyamla*": A Fermented Cereal Used in Ayurveda

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RLDSR and ERHSSE wrote the proposal. Authors RLDSR, WADDW and LDAMA involved in experimental part and prepared the manuscript. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Aim: *Dhanyamla* is a medicinal preparation having great therapeutic values which is described under the *Sandhana Kalpana* in Ayurveda texts. In the present study, an attempt was made to assess the quality of *Dhanyamla* by using standard protocols.
Methodology: Quality of the *Dhanyamla* was assessed by determination of pH, specific gravity, microbial counts, phytochemical screening and TLC-densitogram fingerprints.
Results: Specific gravity and the pH of *Dhanyamla* was 1.0068 and 4.13 at 30^{o}C respectively. Pathogenic microorganisms such as Coliforms, *Escherichia coli* and *Salmonella* were not found in *Dhanyamla*. However, aerobic and mesophilic organisms and few counts of *Staphylococcus aureus* were present. Phytochemical screening studies revealed the presence of tannins, flavonoids and saponins in the sample. TLC-densitogram fingerprint of *Dhanyamla* was comparable to the TLC-densitogram fingerprint of its standard mixture of raw materials.
Conclusion: Present study reveals the quality of *Dhanyamla* for the first time and observed

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parameters can be used as reference standard for quality control protocols in order to have a proper quality check over its preparation and processing.

Keywords: Dhanyamla; quality control; Ayurveda.

1. INTRODUCTION

Ayurveda is a traditional Indian Medical System being practiced for thousands of years. Out of the total of 422,000 flowering plants reported from the world, more than 50,000 are used for medicinal purposes. Plants contribute to our lives more than animals, mainly due to their extraordinary array of diverse classes of biochemicals with a variety of biological activities. Considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics of Ayurveda has been carried out and thereby numerous drugs have entered into the international pharmacopoeia [1]. World Health Organization indicated that primary health needs of countries in Africa, Asia and Latin America are met by traditional medicines. Such traditional medicines are adapted to industrialized countries as Complementary or Alternative Medicines (CAM) [2]. Ayurvedic system of treatment has been estimated to meet 70-80% of the healthcare needs of the world [3]. *Dhanyamla* is a medicinal preparation having great therapeutic values which is described under the *Sandhana Kalpana* in Ayurveda texts. *Sandhana Kalpana* is one of the best pharmaceutical preparations in Ayurveda practice since ancient time [4].

The term *Dhanyamla* is comprised of two discrete words, viz 'which gives an extensive meaning of 'fermented cereal'. Seeds of *Oryza sativa* L, *Macrotyloma uniflorum* (Lam.) Verdc., *Panicum sumatrense* Roth ex Roem. & Schult., *Paspalum scrobiculatum* L, *Trachyspermum involucratum* (Roxb.) Maire, pressed form of *Oryza sativa* L, puffed form of *Oryza sativa* L, rhizomes of *Zingiber officinale* Roscoe and fruits of *Citrus aurantifolia* (Christm.) Swingle are mixed with water and fermented to make *Dhanyamla* [5]. *Dhanyamla* can be administered internally and externally. Oral application of the drug enhances appetite and digestive power. It is also used in enema therapy and purgation therapy to reduce obesity [6]. Externally, *Dhanyamla* is used in massage therapy [7], fomentation (Pipe, Shower and Tub fomentation), *Shirodhara* and *Shiro Vasti* [8]. It improves blood circulation, immunity, skin complexion, relieves body ache and muscle spasms. In the present study, an attempt was made to assess the quality of *Dhanyamla* by using standard protocols.

2. MATERIALS AND METHODS

2.1 Preparation of *Dhanyamla*

All the ingredients needed for preparation of *Dhanyamla* were purchased from the Western Province of Sri Lanka and raw materials were authenticated (specimen no: DM 1-7) by the Senior Scientist, Botany Section, Bandaranayaka Memorial Ayurveda Research Institute, Nawinna, Maharagama, Sri Lanka.

A large deep earthen pot containing water was kept on hearth and boiled. All the ingredients mentioned in Table 1 were coarsely powdered and made into 9 bundles separately, using clean cloth bags. These bundles were put into the vessel, covered with a lid and heated gently and continuously in moderate fire, up to 30-40^{o}C temperature for 1 hour for consecutive period of 7 days. On the eighth day fermented liquid was taken out [9].

2.2 Organoleptic Properties

Organoleptic properties of *Dhanyamla* were evaluated in terms of taste, colour and odour [10].

2.3 Determination of Specific Gravity

Firstly, empty specific gravity bottle was weighed and then filled with distilled water and weighed. After that, specific gravity bottle was well dried and filled with *Dhanyamla* and weighed. Using following equation specific gravity of *Dhanyamla* was calculated.

Specific gravity of *Dhanyamla* = (Weight of *Dhanyamla*/ Weight of water).

2.4 Determination of pH

The pH of *Dhanyamla* was determined using the pH meter at 30ºC.

Table 1. Ingredients of *Dhanyamla* and their quantities

Plant ingredients	Part of the plant	Quantities mentioned in Sahasrayoga	Quantities used in the present study
Oryza sativa L. (Family: Poaceae)	Seed	7680 g	250 g
Pressed form of *Oryza sativa* L. (Family: Poaceae)	Pressed seed	7680 g	250 g
Macrotyloma uniflorum (Lam.) Verdc. (Family: Fabaceae)	Seed	1920 g	62.5 g
Puffed form of *Oryza sativa* L. (Family: Poaceae)	Puffed seed	1920 g	62.5 g
Panicum sumatrense Roth ex Roem. & Schult. (Family: Poaceae)	Seed	3072 g	100 g
Paspalum scrobiculatum L. (Family: Poaceae)	Seed	3072 g	100 g
Zingiber officinale Roscoe (Family: Zingiberaceae)	Rhizome	1536 g	50 g
Citus aurantifolia (Christm.) Swingle (Rutaceae)	Fruit	6144 g	200 g
Trachyspermum involucratum (Roxb.) Maire (Apiaceae)	Seed	1536 g	50 g

2.5 Development of Thin Layer Chromatography (TLC) Fingerprint and Confirmation of Presence of Active Ingredients

Sample and the standard mixture of raw materials (as mentioned in Table 1) extracted separately into dichloromethane, concentrated and spotted (10 µL from each) on a pre-coated TLC plate using dichloromethane, ethyl acetate and cychlo-hexane in a ratio of 4:1:0.5. Two densitograms were developed for the sample and the standard mixture of raw materials using a densitometer (CS – 9301PC, Shimadzu, Japan) at 366 nm.

2.6 Microbiological Limits

Limits of Aerobic plate count, Yeast and Moulds, Coliforms, Presumptive *Escherichia coli*, *Salmonella* and *Staphylococcus aureus* were determined according to the methods described in SLS standards [11-16].

2.7 Phyto-chemical Screening Studies [17]

2.7.1 Determination of the presence/absence of tannins

Sample 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.

2.7.2 Determination of the presence/absence of flavonoids

Sample 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.

2.7.3 Determination of the presence/absence of steroid glycosides

Sample was dissolved in equal volumes of acetic anhydride and $CHCl_3$. The mixture was transferred to a dry test tube and con. H_2SO_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.

2.7.4 Determination of the presence/absence of coumarins

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

2.7.5 Determination of the presence/absence of saponins

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

3. RESULTS AND DISCUSSION

The herbal drugs and medicinal plant products have been widely used for thousands of years in many parts of the world. Medicinal plants constitute a source of raw material for both traditional and modern systems of medicine. In recent few decades, growth and popularity of herbal medicine and plant products have taken a significant share of the healthcare. *Dhanyamla* is a light brown liquid with acidic odor and sour taste. Specific gravity and the pH of *Dhanyamla* was 1.0068 and 4.13 at 30^{o}C respectively. Therefore, *Dhanyamla* was acidic in nature. A similar study was carried out by Elmahood and Doughari [18] for *Kunun-zaki*, an indigenous fermented non-alcoholic beverage which is widely consumed in Nigeria. The pH of the tested samples of *Kunun-zaki* was in a range of 3.34 to 4.42. Table 2 represents the microbial counts in the *Dhanyamla.* In the present study, high content of yeast and moulds were detected. The low acidity value may have encouraged the growth of yeast and mould. The main components of cereals from which *Dhanyamla* is made are carbohydrates, proteins, vitamins and minerals and the chief product of fermentation is lactic acid and this leads to a decrease in pH value and an increase in acidity [19]. Pathogenic microorganisms such as Coliforms, *Escherichia coli* and *Salmonella* were not found in *Dhanyamla*. However, aerobic and mesophilic organisms and few counts of *Staphylococcus aureus* were present. Phytochemical screening studies revealed the presence of tannins, flavonoids and saponins in the sample.

Table 2. Microbial counts of *Dhanyamla*

Microbes	Microbial counts
Aerobic plate count, CFU/mL	1.3×10^5
Yeast and Moulds, CFU/mL	4.0×10^2
Coliforms, MPN/10 mL	Not detected
Presumptive *Escherichia coli*, MPN/10 mL	Not detected
Salmonella/25 mL	Absent
Staphylococcus aureus/mL	<10

As indicated in Fig. 1, TLC-densitogram fingerprint of *Dhanyamla* was comparable to the TLC-densitogram fingerprint of its standard mixture of raw materials which confirms the presence of all the ingredients listed in Table 1. Further, 10 prominent spots (R_f values: 0.09, 0.14, 0.23, 0.25, 0.28, 0.35, 0.61, 0.77, 0.88, and 0.89) were observed in the TLC fingerprints of both standard mixture of raw materials and *Dhanyamla*.

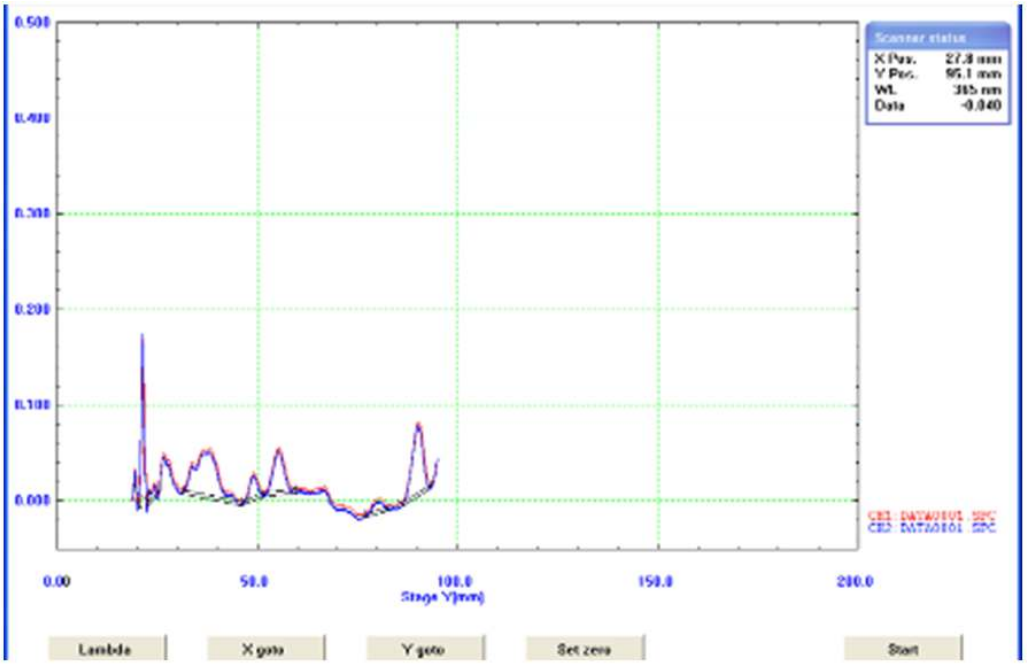

Fig. 1. TLC-densitogram fingerprint of *Dhanyamla* (in blue color) and its standard mixture of raw materials (in blue color) at 366 nm

4. CONCLUSION

Present study reveals the quality of *Dhanyamla* for the first time and observed parameters can be used as reference standard for quality control protocols in order to have a proper quality check over its preparation and processing.

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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