



Research Article

BASIC ORGANOLEPTIC AND PHYTOCHEMICAL STUDY ON ESSENTIAL INGREDIENTS OF MAHADALU ANUPANA (A SRI LANKAN TRADITIONAL RECIPE)

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ABSTRACT

Mahadalu anupana is one of the most common *Dalu anupana* (fluid vehicles for medicine which is prepared by tender leaves and some other additives) of the traditional medical system in Sri Lanka and this *Anupana* is one of *Anupana* mentioned under *Chandra kalka*. *Mahadalu anupana* is used in many ailments with or without *Kalka* in traditional medical system. This research work is mainly based on the main twelve ingredients of the formulation of *Mahadalu anupana* without the additives (sugar, ghee, orange juice, breast milk, sandalwood and *Strychnos potatorum*) which has mentioned in the authentic text of *Vatika prakaranaya/ Deshiya beheth guli kalka potha* in Sri Lankan traditional medicine. In this study, organoleptic properties and selected phytochemical studies were performed for future standardization of drug. Phytochemicals are bioactive compounds obtained from the plants and has therapeutic potential. The organoleptic properties indicated of this *Anupana* as follow; colour: brownish green, odour: strong aromatic, taste: astringent and the texture were liquid. The phytochemical analysis study was done for the water extract of *Mahadalu anupana*. This study has revealed that the presence of alkaloids, tannins, flavonoids, saponins, terpenoids, glycosides and carbohydrates qualitatively in main ingredients of *Mahadalu anupana* but steroids and proteins were not reported. It was concluded that the *Mahadalu anupana* (without additives) were rich in phytochemicals with medicinal significant and this study results can be utilized in future consistency studies of *Mahadalu anupana*.

INTRODUCTION

The word *Anupana* is that a fluid vehicle which takes with or after medicine intake. *Anupana* is one of the crucial factors which help in absorption, administration assimilation as well as efficacy of the drug. According to the authentic text of *Saramgadharu Samhitha*, after administration of medicine with *Anupana*, it spreads very quickly in the body likewise when a drop of *Thaila* (oil) spreads in water.^[1]

Mahadalu anupana is one of the important *Anupana* mentioned under the *Anupana* of *Chandra kalka* in the Sri Lankan authentic text of *Vatika prakaranaya/Deshiya beheth guli kalka potha*.^[2]

The formulation is one of the most common *Dalu anupana* of traditional medical system in Sri Lanka. According to the formulation it contains twelve main ingredients of and seven additive ingredients in its composition. Tender leaves of *Dehi* (*Citrus aurantifolia*), *Dodam* (*Citrus sinensis*), *Nika* (*Vitex negundo*), *Elabatu* (*Solanum surattense*), *Yakinaran* (*Atalantia ceylanica*), *Heennaran* (*Citrus reticulata*), *Adatoda* (*Adhatoda vasica*), *Olinda* (*Abrus precatorius*), *Kuppameniya* (*Acalypha indica*), tender leaves and seeds of *Kumburu* (*Caesalpinia bonduc*) and bulb of *Sudulunu* (*Allium sativum*) have been mentioned as the main ingredients while bee honey, sugar, ghee, orange juice, breast milk, sandalwood and *Ingini* (*Strychnos potatorum*) have been mentioned as the additive drugs in this formula.

Although several research studies have been reported the pharmacological properties and clinical efficacy of *Mahadalu anupana*, but none of them has done organoleptic properties and phytochemical screening study for this formula. The phytochemicals

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are defined as bioactive nutrient plant chemicals and may provide desirable health benefits. The lack of quality assurance emphasizes the need for standardization of *Mahadalu anupana* and according to standard methods.

A standardized preparation according to the accurate standard methods confirms the higher security of the product and also it raises the level of reliability of the people those who are interested in herbal preparations.^[3]

Therefore, this study was carried to analyse the main ingredients of *Mahadalu anupana* of *Chandra kalka* (without additives) according to organoleptic and selected phytochemical parameters which can be help to understand the therapeutic efficacy and standardization of the drug in future studies.

Methodology

Collection of Ingredients

Tender leaves of *Dehi* (*Citrus aurantifolia*), *Dodam* (*Citrus sinensis*), *Nika* (*Vitex nigundo*), *Batu* (*Solanum indicam*), *Yakinaran* (*Atlanta ceylanica*), *Heennaran* (*Citrus reticulate*), *Adathoda* (*Adatoda*

vesica), *Olinda* (*Abrusp recatorious*), *Kuppameniya* (*Acalypa indicum*), tender leaves and seeds of *Kumubu* (*Caesalpinia bonduc*) and bulbs of *Sudulunu* (*Allium sativum*) were collected in the month of October 2020 from the low country dry zone in *Hambantota* district in Sri Lanka and authenticated was done by the unit of *Dravyaguna vingnana*, Institute of Indigenous Medicine, University of Colombo, Sri Lanka.

Preparation of *Mahadalu Anupana*

All collected Ingredients adulterants were removed and raw drugs were cleaned with tap water and the shade dried for one day. These shade dried ingredients were chopped with mortar and pestle and four bundles were prepared by using four pieces of cotton cloth. Bundles were placed in the *Swedayanthra* (steamer) and steamed under the low heat (The procedure is called as the process of *Wandu thembum*). Then the bundles were kept out from the *Sweda yanthra* when the aromatic odour came out and allowed to release the heat. Finally the bundles were squeezed to collect the juice and the squeezing process was repeated for two times (Figure 1).



Figure 1: Preparation of *Mahadalu anupana*

Preparation of freeze dried powder of *Mahadalu anupana*

Prepared juice of *Mahadalu anupana* (100ml) was directed to the freezer dryer and obtained a power. The obtained freeze dried powder (10g) was stored in a labelled air tight glass vial and kept in refrigerator at 4°C (Figure 2).



Figure 2: Freeze dried powder

Preparation of water extract of *Mahadalu anupana*

10g of freeze dried powder was mixed with 25ml of distilled water and mixed properly until powder was totally dissolved. It was shaken by an electric shaker at 160rpm for 24 hours for better extraction. The obtained water extract was stored in a labelled air tight glass vial and kept in refrigerator at 4°C.

Determination of organoleptic parameters of *Mahadalu Anupana*

- I. Colour– A sample of was examined under daylight and the colour was reported
- II. Odour– A sample was inhaled and the strength of the odour and the odour sensation was determined
- III. Taste– A sample of was tasted and reported
- IV. Texture– A sample of was touched and reported

Phytochemical Screening

Basic ingredients of *Mahadalu anupana* subjected to the following tests with respective control for the presence of various phytoconstituents like, alkaloids, Tannins, flavonoids, saponins, terpenoids, sterols, glycosides, carbohydrates and proteins.

Determination of Alkaloids

To determine the Alkaloids, Mayer's reagent test, Wagner's test and Hager's test were done. At the commencement of the test three water extracts of samples of 2 ml each were taken. The three samples were then tested for each reagent separately.

Mayer's Test

Two drops of Mayer's reagent were added to the sample solution and observed for cream precipitate at the bottom of the test tube.^[4]

Wagner's Test

Few drops of Wagner's reagent were added to the sample solution and observed for reddish brown precipitate at the bottom of the test tube.^[5]

Hager's Test

Few drops of Hager's reagent were added to the sample solution and observed for yellowish precipitate at the bottom of the test tube.^[6]

Determination of Tannins

Braymer's test was done to confirm the presence of Tannins.

2ml sample of the water extract of test drug was dissolved in 2ml of distilled water and the solution was taken. Then 2-3 drops of 10% FeCl₃ were added and observed for dark greenish (condensed tannins) or dark bluish (hydrolysable tannins) colour change of the solution.^[7]

Determination of Flavonoids

For the Flavonoid test, three tests were performed separately.

Lead acetate test

2ml of the water extract of test drug was mixed with 1ml of 10% lead acetate and observed for yellow precipitate.^[8]

Alkaline reagent test

Few drops of NaOH was added to a 2ml sample of the water extract of test drug and observed for intense yellow colour of the solution. Then few drops of dilute HCl were added to the yellowish solution and observed for the disappearance of the yellow colour.^[8]

Shinoda test

Few drops of concentrated HCL acid were added to 2ml of water extract of test drug and then few pieces of Magnesium were added. It was observed for pinkish colour change of the solution.^[9]

Determination of Saponins

To determine Saponins, Foam test was done.^[8] 2ml of the water extract of test drug was mixed with 5ml of distilled water. Then the solution was shaken vigorously and observed for stable foam of honey comb appearance.

Determination of Terpenoids

Salkowski test was done to confirm the presence of Terpenoids.^[10]

2ml sample of water extract of test drug was mixed with 2ml Chloroform and then it was followed by the addition of 3ml of concentrated H₂SO₄ acid along the side of the test tube. This was kept for a while

without shaking. Then observed the solution for reddish brown ring in the interface.

Determination of Sterols

Liebermann-Burchard test was done to confirm the presence of Sterols.^[4]

2ml sample of water extract of test drug was shaken with chloroform in a test tube and few drops of acetic anhydride was mixed with the solution. Then this was boiled in a water bath and rapidly cooled in iced water. After that 2ml of concentrated H₂SO₄ acid was added along the wall of the test tube. It was observed for the formation of a brown ring at the junction of two layers and turning the upper layer to green.

Determination of Glycosides

Keller Kiliani test was done to confirm the presence of Cardiac Glycosides.^[11]

2ml of water extract of MA was mixed with 2ml of glacial acetic acid, one drop of 5% FeCl₃ and 2ml of concentrated H₂SO₄ acid along the side of the test tube and kept for a while without shaking. Then it was observed for reddish brown ring in the interface.

Determination of Carbohydrates

Two tests were done to confirm the presence of Carbohydrates.

Benedict's test

3ml of benedict's qualitative reagent was added to the 2ml sample of water extract of test drug and boiled the

solution for about 2 minutes. Then observed whether the solution progress in the colours of blue, green, yellow, orange and finally to brick red precipitate.

Fehling's test

1ml of Fehling's A solution was added to 1ml sample of water extract of test drug and then Fehling's B solution was added. After that it was boiled for 2 minutes and then observed whether the solution progress in the colours of blue, green, yellow, orange and finally to brick red precipitate.^[12]

Determination of Proteins

Two tests were done to verify the presence of Proteins.

Biuret test^[11]

2ml of 1% NaOH solution and few drops of 1% CuSO₄ solution were added to a 2ml sample of water extract of test drug. (Biuret reagent) The solution was observed for purple colour.

Ninhydrin test^[13]

Few drops of Ninhydrin reagent was added to 2ml sample of water extract of test drug and heated for about 2 minutes. Then it was observed for violet or bluish colour appearance of the solution.

OBSERVATIONS AND RESULTS

Organoleptic parameters

The results of organoleptic parameters were tabulated in Table no.1.

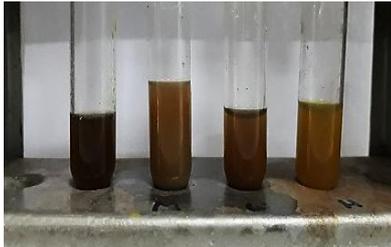
Table 1: Results of organoleptic parameters

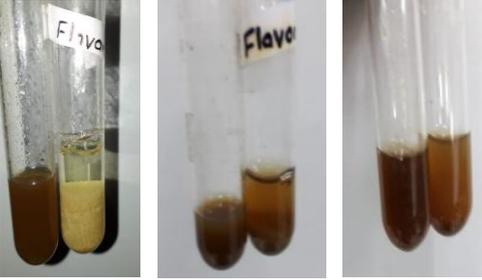
Organoleptic parameter	Results
1. Colour	Brownish green
2. Odour	Strong aromatic
3. Taste	Astringent
4. Texture	Liquid

Phytochemical screening

Tested results of selected phytochemical analysis are mentioned in Table 2.

Table 2: Results of phytochemical analysis

Phytochemical Compound	Test	Results	Results
Alkaloids	Mayer's test (M)	++	 <p>Control M W H</p>
	Wagner's test (W)	++	
	Hager's test (H)	+	

Tannins	Ferric Chloride test (F)	++	 Control F
Flavonoids	Lead acetate test (L) Alkaline reagent test (A) Shinoda test (S)	+ + -	 CL CA CS
Saponins	Foam test (F)	+ (weakly)	 Control F
Terpenoids	Salkowski test	+	 Control S
Steroids	Liebermann-Burchard test (L)	-	 Control L

DISCUSSION

Mahadalu anupana of *Chandra kalka* is one of the most effective *Dalu anupana* which is commonly used in both Ayurveda and traditional medical practitioners in Sri Lanka. Even though *Mahadalu anupana* has shown effective results in practice, there are no any sufficient standardization and quality control profiles till to date. The organoleptic

parameters revealed that the *Mahadalu anupana* (without additives) is brownish green in color, strong aromatic in odour, astringent in taste and liquid in touch morphologically. The results obtained from phytochemical screening revealed that the constituents of alkaloids, flavonoids, tannins, saponins, terpenoids, glycoside and carbohydrates were present

in the water extract of basic ingredients of *Mahadalu anupana* (without additives). These results revealed that the therapeutic potential of *Mahadalu anupana*, which was used in thousands of years. Also this study discovered that there were no any steroids or proteins in the water extract of tested drug.

CONCLUSION

It was concluded that phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, saponins, terpenoids, glycosides and carbohydrates which indicates the presence of medicinally important constituents in the formulation and need further studies to validate clinically importance of this phytochemicals in *Mahadalu anupana* and standardization of the drug with use of these studied phytochemicals.

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