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COMPARATIVE ACCOUNT OF TRADITIONALLY FERMENTED BIOMEDICINE FROM AYURVEDA: MUSTAKARISHTA

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ABSTRACT
Mustakarishta is an Ayurvedic preparation belonging to class of formulations commonly known as Arishtas. Mustakarishta is widely used in cases like dyspepsia, digestive impairment and gastro-enteritis with piercing pain. Information on the qualitative and quantitative parameters of Mustakarishta to guarantee the quality and the safety of the product to the consumer is less; many of these parameters vary according to the method of preparations. With this aim in the recent study three different methods were performed for the preparation of Mustakarishta i.e. Mustakarishta prepared by using traditional earthen pot (MP) method, using steel container (MS) and by using the wooden container (MW). These all formulations were analyzed for various qualitative and quantitative parameters according to WHO guidelines and the results were compared with the marketed formulation. With the change in method of preparation a considerable variations were observed in the parameters and the results of ME were found to be most significant.

Keywords: Ayurvedic preparation, Alcohol content, Fermentation, Mustakarishta, Standardization.

INTRODUCTION

Ayurvedic system of medicine is accepted as the oldest written medical system came into existence in about 900 BC; which is more effective in certain cases than the modern therapies.[1] India has an ancient heritage of traditional medicine. With the emerging interest in the world to adopt and study the traditional system, the evaluation of the rich heritage of the traditional medicine is essential.[2] Ayurvedic medicines are of various types so as to meet the diverse requirement in the treatment of human illness. Aristas (fermented decoctions) and Asavas (fermented infusions) are considered as unique and valuable therapeutics in Ayurveda. Due to their medicinal value, sweet taste and easy availability, people are prone to consume higher doses of these drugs for longer periods. The manufacture and sale of Arishta and Asava occupies an important place in the ayurvedic pharmaceutical industry. Information on the quantitative parameters of Asava and Arishta to guarantee the quality and the safety of the product to the consumer is less. Therefore, establishing quality and standard parameters like alcohol level, pH, acid value and other constituents of these preparations are highly significant. Mustakarishta is one of the ancient liquid oral formulations prescribed in Ayurveda for dyspepsia, digestive impairment and gastro-enteritis type of disorders.[3, 4] The objective of this study was to determine the level of alcohol, acidity and pH in commercially available Mustakarishta and Mustakarishta prepared by three different fermentation methods to establish a routine procedure for standardization of this Ayurvedic preparation. Though traditional knowledge in literature a well as in practice exists about Mustakarishta but there was little effort to documents on variations occurring due to different techniques of preparations. The objective of this paper was to compare the variations occurring in standardization parameters through the traditional practices and modern techniques, which bring out the technological details. Finally, scope for futuristic development in the arena of microbial fermentation and biomedical applications based on the tools of modern scientific investigations is discussed.

MATERIALS AND METHODS

Collection of Plant Materials

During the research work, at the very first step it was necessary to identify the raw material required for the preparation of Mustakarishta. Therefore, the plant materials were procured from local Market from Mankarnika Ayurvedic Aushdhalaya, Gandhi Peth, Pune in the month of March, 2011 and Authentication was confirmed by Department of Pharmacognosy Marathwada Mitra Mandal's College of Pharmacy, Pune by correlating their macromorphological characters with those given in literatures the Authentication numbers of the plant material used were provided in Table 1.

Preparation before Fermentation

In the preparation of Mustakarishta, the rhizomes of Mustaka (*Cyperus rotundus*), the fruits of Ova (*Trachyspermum ammi*), the rhizomes of Sunth (*Zingiber officinale*), the fruits of Marica (*Piper longum*), the flower buds of Lavang (*Syzygium aromaticum*), the seeds of Methi (*Trigonella foenum-graecum*), the roots of Citraka (*Plumbago zeylanica*), the fruits of Jira (*Cuminum cyminum*) and the flowers of Dhataki pusp (*Woodfordia fruticosa*) were used. All above raw materials of Pharmacopoeial quality were first cleaned and rinsed in water to get rid of dirt. The ingredient numbered 1 (According to the Table 1), (Kvatha Dravya – a filtered decoction obtained by boiling coarse powder of drug into water) of the formulation composition was crushed and passed through the sieve number 44 to obtain coarse powder. The ingredients numbered 5 to 11 (Prakasepa Dravya – fine powder of drugs added to decoction) of the formulation composition were powdered individually and passed through the sieve number 85 to obtain fine powder. The specified amount of water was added to the

Kvatha Dravya, and soaked overnight, heated to reduce 1/4th and filtered through muslin cloth to obtain Kvatha (Decoction). The ingredient number 3 of the formulation composition was added to the Kvatha (decoction), which was allowed to dissolve in it and was filtered through the muslin cloth. The filtrate was transferred to a cleaned container and the dhataki pusp along with other finely powdered Praksepa Dravya were added to it.[5]

Table 1: Formulation Composition
The various raw materials used for the formulation of Mustakarishta

Sr. No.	Ingredients	Biological Source	Voucher No.	Parts used	Quantity used
1.	Mustaka(Musta API)	*Cyperus rotundus* (Cyperaceae)	MCR-1	Rhizomes	02.40kg
2.	Jala for decoction	Water		-	12.28 L
3.	Guda	Jaggery		-	03.07 L
4.	Dhataki	*Woodfordia fruticosa* (Lythraceae)	MWF-2	Flowers	03.60 kg
5.	Yamani	*Trachyspermum ammi* (Apiaceae)	MTA-3	Fruits	192.00 g
6.	Visvabhesaja (sunthi)	*Zinhgiber officinale* (Zingiberaceae)	MZO-4	Rhizomes	24.00g
7.	Marica	*Piper longum* (Piperaceae)	MPL-5	Fruits	24.00g
8.	Lavang	*Syzygium aromaticum* (Myrtaceae)	MSA-6	Flower buds	24.00g
9.	Methi	*Trigonella foenum graecum*	MTF-7	Seeds	24.00g
10.	Vahni(citraka)	*Plumbago zeylanica* (Plumbaginaceae)	MPZ-8	Roots	24.00g
11.	Jiraka(sveta jiraka)	*Cuminum cyminum* (Apiaceae)	MCC-9	Fruits	24.00g

Method of Preparation

In the present study, Mustakaristha was prepared by using three different equipments for fermentation process i.e. traditional earthen pot (ME), using steel container (MS) and by using the wooden container (MW). The earthen pot intended for fermenting the medicine was tested for weak spots and cracks and similarly a lid, a steel container and a wooden container were also chosen. The internal surface of the pot, the lid and other containers were wiped with a clean dried cloth and the cow's ghee was smeared on that surface to prevent oozing out of the contents when poured and kept for fermentation, after then the containers were passed under the process of Dhupana (the process of fumigating a pot or a vessel with the prescribed drugs)[5] in the presence of Pipali churna. When all containers were ready, the sweetened and flavoured drug extract was poured into the pot, in the steel container and in the wooden container up to 3/4th of the capacity. After completion of this preparation the final solution was stored in the same respective vessels for the process of fermentation by sealing the mouth of the container. Sealing was done by winding around a long ribbon of cloth smeared with clay on one surface. These fermentation vessels were shifted into a pit in the soil and left undisturbed for a month and then opened.[6]

The fermented material was filtered through a clean muslin cloth and was packed in air tight containers and allowed for maturation. Finally, the Mustakarishta formulation prepared by using traditional earthen pot (ME) method, using steel container (MS) and by using the wooden container (MW) were used for the evaluation of the quantitative parameters as per WHO guidelines. Among the above mentioned ingredients the major one was *Cyperus rotundus* (Mustaka) which as per the Ayurveda, have the curative and medicinal uses for treating fevers, digestive system disorders and dysmenorrhea. Therefore, the raw material was subjected to standardization.[3,5]

Characterization of Formulations

Preliminary Analysis

Organoleptic evaluation was carried out to assess the colour, odour and taste of these formulations and the results were compared with the marketed formulation.

Preliminary Phytochemical Investigations

The qualitative chemical tests were carried out for the identification of the nature of phytoconstituents present in these formulations.

Determination of Alcohol Content

Ethanol content by distillation and specific gravity 25ml of the preparation being examined was transferred, accurately measured at 24.9° to 25.1°, to the distillation flask. It was diluted with 150ml of water and to it, a little pumice powder was added. It was distilled and not less than 90ml of the distillate was collected into a 100-ml volumetric flask and diluted to volume with distilled water at 24.9° to 25.1°. Relative density at 24.9° to 25.1° was determined and alcohol content was reported.[7]

Determination of Total Solid Content

The method was used to determine the solids concentration in the formulation. The unfiltered sample was vigorously shaken and rapidly transferred to a tared platinum evaporating dish with the help of pipette. The pipette was rinsed with demineralized water to ensure transfer of all particulate matter to the evaporating dish. The sample was evaporated at as low a temperature as possible until the solvent was removed and heated on water bath until the residue was apparently dried. It was transferred to an oven and dried to constant weight at 105^0 C as per stated in the monograph, then cooled in desiccator and immediately weight was taken.[8] The total solid contents was calculated by using following formula:

$$\text{Total solids (mg/L)} = \frac{1000}{\text{mL sample}} \times \text{mg residue}$$

Determination of pH

The pH of different formulations in 1% w/v and 10% w/v of water soluble portions were determined using Digital pH meter which was calibrated using Buffer tablets of pH 4.00 and pH 7.0[9].

Determination of Specific gravity and Viscosity

The specific gravity of liquid is weight of given volume of the liquid at the specific temperature compared with the weight of an equal volume of water at the same temperature, all weighing been taken in normal condition. The procedure consisted of use of a pycnometer of 25 ml

capacity, which was cleaned, dried and weighed. It was filled up to the mark with water at the required temperature and weighed. The pycnometer was next filled up to the mark with the sample, filtered, at the same temperature and weighed. The specific gravity was determined by dividing the weight of the sample expressed in gram by the weight of the water, expressed in gram. While the viscosity was determined by using Ostwald's Viscometer.[10] All the results were expressed in SEM by performing statistical analysis.

RESULT

Physicochemical Description

Table 2 narrate the results of the physicochemical constants of main raw material Mustaka which was found to be within the limit; The values were 6.47 ± 0.15 % for total ash, 5.00 ± 0.09% for water soluble ash and 4.58 ± 0.11 % for acid soluble ash value; which were within fairly wide limit.

Table 2: Physicochemical Properties

Sr. No.	Test	Result (%)
1.	Ash Value:	
	Total Ash	6.47 ± 0.15
	Water Soluble Ash	5.00 ± 0.09
	Acid Insoluble Ash	4.58 ± 0.11
2.	Extractive Value:	
	Water Soluble	4.43 ± 0.03
	Alcohol Soluble	8.16 ± 1.85
3.	Moisture Content	0.10 ± 0.00

Preliminary Analysis of Formulations

The Organoleptic evaluation of Pot, Steel, Wooden and Marketed formulation revealed that the all formulations were dark brown in colour, with aromatic odour and bitter taste. The results of this evaluation were mentioned in Table 3, which were compared with the marketed formulation and the results were found to be most significant.

Preliminary Phytochemical Description

The all formulations were subjected to preliminary phytochemical screening for the presence of types of phytoconstituets. The all formulations were found to contain Carbohydrates, Amino acids, Flavonoids, Alkaloids and Tannins. The results of the Preliminary Phytochemical Screening were expressed in Table 4.

Determination of Alcohol content

The alcohol content was determined to evaluate the quantity of the alcohol present in the formulations, which were prepared with different techniques since it was also in accordance with pertinent regulations.[8] The results of alcohol content were found to be for MP (7.74 ± 0.08) %v/v, MS (14.13 ± 0.00) %v/v, for MW (4.49 ± 0.09) %v/v and for standard Marketed formulation it was (7.17 ± 0.05) %v/v; this elaborated the use of traditional system as a better one. (Table 4)

Determination of Total Solid Content

In the results of total solid content, it was found that MP showed 49.8 ± 0.10 mg/lit, MS 49.73 ± 0.14 mg/lit, MW 28.93 ± 0.63 mg/lit and for Marketed formulation it was found to be 40.4 ± 0.20 mg/lit of solid content which signified the resemblances of MP with marketed formulation. (Table 4)

Determination of pH

The pHs of the formulations were found to be 3.32±0.00 for MP, 3.21±0.00 for MS, 2.98±0.00 for MW and 3.17±0.00 for Marketed formulations. (Table 4)

Determination of Specific gravity and Viscosity

The specific gravity was found to be MP (1.09± 0.01) g/ml, MS (1.1±0.01) g/ml, MW (1.02± 0.01) g/ml and Marketed (1.07 ±0.00) g/ml and the viscosity was found to be MP (329± 3.60) mPa.s, MS (251.3± 0.66) mPa.s, MW (138 ±4.72)mPa.s and Marketed (90± 3.60)mPa.s. Thus the results were found to be comparable and variation was insignificant. (Table 4)

Table 3: Organoleptic Evaluation

Sr. No	Parameter	Pot	Steel	Wooden	Marketed Formulation
1.	Colour	Dark brown	Dark brown	Dark brown	Dark brown
2.	Odour	Aromatic	Aromatic	Aromatic	Aromatic
3.	Taste	Bitter	Bitter	Bitter	Bitter

Table 4: Phytochemical Investigations

Sr. No.	Parameters	Marketed Formulation	ME	MS	MW
1.	Carbohydrates	+	+	+	+
2.	Amino acids	+	+	+	+
3.	Glycosides	-	-	-	-
4.	Flavonoids	+	+	+	+
5.	Alkaloids	+	+	+	+
6.	Tannin	+	+	+	+

Table 5: Physicochemical Characterizations

Sr. No.	Test	MP	MS	MW	Marketed
1.	Alcohol content (% v/v)	7.74 ± 0.08	14.13 ± 0.00	4.49 ± 0.09	7.17 ± 0.05
2.	Total solid content (w/v)	49.8 ± 0.10	49.73 ± 0.14	28.93 ± 0.63	40.4 ± 0.20
3.	pH	3.32±0.00	3.21±0.00	2.98±0.00	3.17±0.00
4.	Specific Gravity (g/ml)	1.09 ± 0.01	1.1 ± 0.01	1.02± 0.01	1.07 ±0.00
5.	Viscosity (mPa.s)	329 ± 3.60	251.3 ± 0.66	138 ±4.72	90 ± 3.60

DISCUSSION

The history of development of pharmaceutical dosage forms can be traced back to Charak Samhita, the first systematic documentation of Ayurveda. Arishtas are the unique dosage forms discovered by Ayurveda and is supposed to have indefinite shelf life and it was said that the "older the better it is".[6] The residue remaining after incineration of plant material is the ash content or ash value, which simply represents inorganic salts, naturally occurring in crude drug or adhering to it or deliberately added to it, as a form of adulteration. The total ash method is employed to measure the total amount of material remaining after ignition. This includes both 'physiological ash' which is derived from the plant tissue itself, and 'non-physiological ash', which is the residue of the extraneous matter adhering to the plant surface. Acid-insoluble ash is a part of total ash and measures the amount of silica present, especially as sand, siliceous earth. Water-soluble ash is the water soluble portion of the total ash. These ash values are important quantitative standards. The ash content of the crude drug signifies that the sample of crude drug was of good quality without any adulterant or substitution as well as useful for preparation of formulations; the results of Ash values signify the content of inorganic material mainly the content of metallic salts and silica present in the raw material. The results signify the nature of the phytoconstituents present in Mustaka. Insufficient drying favors spoilage by molds and bacteria and makes possible the enzymatic destruction of active principles. Not only the ultimate dryness of the drug is important, equally important is the rate at which the moisture is removed and the condition under which it is removed thus the determination of moisture content also provide the method of preparation of drug. The results of drug signify that the drug was properly dried and properly stored. The organoleptic evaluation of the formulations are simplest and quickest tool to standardize the formulation, the results of these evaluation were mentioned in Table 3, which were compared with the marketed formulation and the results were found to be most significant. The preliminary phytochemical investigations of these formulations were performed and compared with the standard which showed the presence of alkaloids, flavanoids type of major secondary metabolites which revealed their potent therapeutic activity. The alcohol content was determined to evaluate the quantity of the alcohol present in the formulations, which were prepared with different techniques since it was also in accordance with pertinent regulations.[8] During the manufacturing process, when the earthen pot, steel container, and wooden container all these were ready, the prepared drug decoction was poured into it up to 3/4th of the capacity. The unfilled space provides room for fermenting liquid when it rises up due to frothing and evolving large amount of gases. Otherwise the medium may damage the container and flow out. So, the inoculum has to be added to initiate the fermentation. In the preparation of alcoholic medicaments in Ayurvedic systems such as in the case of Mustakarishta the inoculums of yeasts comes from the dhataki pushpa, which contain the wild species of yeast. These flowers are nectariferous and highly tanniferous. The flowers were added and the contents were stirred well to distribute the inoculums of yeast. The yeast multiplies rapidly within a short period of time. As the process and environment for fermentation process was the same, the variations were produced only because of changes in the fermentation vessels. During manufacturing procedure and storage, it was observed that change in the vessel causes relative difference in the content of alcohol. The term 'total solid' is applied to the residue obtained when the prescribed amount of the preparation is dried to constant weight under the specified condition. This parameter was important for the pharmacokinetic and pharmacodynamic activity of drug because of the bioavailability condition. In the result for total solid content, it was signified the resemblances of MP with marketed formulation. Ayurvedic system plays a vital role in maintaining internal environment, buffer system and in homeostasis of the body[8], hence the contribution from Ayurvedic formulation is much more in this case; where pH of the formulation is an important parameter and this may change quality, purity and compatibility of the formulation. Specific gravity and viscosity are responsible for the flow property of the formulation which affect the patient compliance and stability of the formulation. The results were found to be comparable and variation was insignificant, which revealed that all the formulations had more or less the same consistency and flow rate.

CONCLUSION

The results reveals that the Arishta prepared by using traditional techniques showed superiority over the modern techniques of preparation, minor modification in the procedure will lead to the change in the qualitative and quantitative parameters of the formulation, which cannot be approved for Arishta.

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