



Research Article

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Role of Madhuka Pushpa in Sandhana Kalpana; microbiological pharmaceutico-analytical study

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Abstract

Introduction: Sandhana kalpana are unique alcoholic formulations using natural sources of drugs as ingredients. Flowers of Madhuka (*Madhuka indica* Gmel) (wet/dry) are added as fermentative initiators in many such asva- arishta preparations. But exact role of these flowers was not well studied or documented scientifically. Hence a study designed to prove evidences for these facts. **Materials and Methods:** Fresh and dry sample of test drug was taken and microbiological study was conducted to explore their microflora. Three samples of Madhukasava was prepared as per classical references, adding fermentative initiators as fresh flowers, dried flowers and not adding any flowers and a comparative analytical study of these samples conducted. **Results:** Microflora extraction delineated that dry flowers contain more yeast colonies than that of fresh flowers. Pharmaceutico- analytical study has shown better parameters from the point of total sugar, total solids and total alcohol among fresh and dry flower groups. **Conclusion:** Overall result emphasizes the fact that Madhuka flowers have definite role in sandhana kalpana and dried ones with more yeast colonies have proved better than fresh flowers.

Keywords: Sandhana kalpana, Madhuka (*Madhuka indica* Gmel), Madhukasava, Microflora, Pharmaceutico- analytical).

INTRODUCTION

The science of Ayurveda advocates utilisation of various sources of natural products, like plant, mineral and of animal origin for therapeutic as well as maintenance of health^[1]. Turning these drugs into medicaments of different dosage forms to meet the therapeutic requirements is a wonderful contribution to the mankind^[2]. Among these dosage forms Sandhana kalpana is a unique form, in which drugs are made into acidic and Alcoholic formulations. These Asavaristas (fermented products) possess self-generated alcohol which acts as a natural preservative; apart from this these are unique for their higher stability, palatability and greater clinical outcome^[3]. This particular pharmaceutical preparation involves multiple steps with addition of various drugs at different stages of preparation. The whole approach is built upon traditional wisdom.

Sandhana is a process of fermentation, when the *drava-dravya* (*Kwatha*, *Swarasa* or any other liquid preparation), *Madhuradravya* (Jaugary, honey or sugar), *Prakshepaka dravyas* (fine powder of aromatic/spicy drugs and Sandhana dravya (Dhatakpushpa and Madhukapushpa as initiators of fermentation) are put together in an inert vessel (mud pot), sealed for a specific period of time to facilitate the process of fermentation^[4]. Asava and Arista are the two major products of this process.

Role of Sandhana dravya (fermentation agents) of natural origin (Dhataki and Madhuka flowers) is a beautiful scientific concept involved in this process as natural fermentation initiators^[5]. Researchers suggest the micro flora of these flowers having a great role in this process.

Madhukapushpa (flowers of *Madhuka indica* Gmel) commonly known as Mahua flowers are edible fragrant cream coloured flowers collected out of *Madhuka indica* Gmel. found in Western Ghats^[6]. These flowers are said to be rich source of sugars along with other essential nutrients and vitamins^[7]. Since ancient times Madhuka flowers were used as natural fermentative agents in different asava and aristas. But exactly the role of these flowers in Sandhana Kalpana is not yet explored. The recent texts such as Indian Materia Medica Vol I^x (K.M.Nadkarni), Illustrated Bhaishajya Kalpana Vijnana (Dr. P. V. N. R. Prasad) mention the presence of yeast in Madhukapushpa^[8].

Hence with this background a study is designed to elucidate the role of Madhukapushpa (*Madhuka indica* Gmel.) in Sandhana Kalpana w.s.r. to their microbiological and pharmaceutico-analytical topic.

MATERIALS AND METHODS

Flowers of Madhuka (*Madhuka indica* Gmel.) are collected in flowering season from Udupi district, cleaned properly from extraneous matter, authenticated using floras and botanists opinion, and sample deposited at SDM Centre for Research in Ayurveda and Allied sciences. (Voucher No. 15070101). Some flowers are shade dried. Both fresh and dry flowers were used for the study. Microbiological study of both fresh and dry flower of Madhuka flowers (Yeast cell culture, and estimation). d) Pharmaceutical study includes preparation of 3 samples Madhukasava. e) Comparative analytical study of 3 samples of Madhukasava

Microbiological study

Total aerobic microbial count of test drug was carried out by plate count method^[9]. The place of work was cleaned in laminar air flow using 70% ethanol and switched on the UV for 20 minutes. One gram of *Madhuca indica* flowers were mixed with 10 ml of sterile BSCPS to make dilution 10^{-1} . After cooling Sabouraud dextrose agar medium, added one ml of diluted sample into petridish containing the media. Plates were gently rotated in a circular motion to achieve uniform distribution of the sample and allow the media to solidify. Incubated all petridishes for 5 days at 25° C in BOD incubator. Experiment was carried out in duplicate for fresh and dry samples of *Madhuka indica* Gmel. Number of colonies counted using digital colony counter^[9].

Pharmaceutical study

Three samples of *Madhukasava* were prepared according to classical references. S₁ using fresh flowers of Madhuka, S₂ adding dried flowers

whereas S₃ without adding flowers of Madhuka. Quantity of ingredients used for the preparation of Madhukasava, are displayed in Table 1^[10-12]. Observations were recorded at three stages of its preparation i.e. at the initial stage, after the onset of fermentation and after completion of fermentation.

Analytical study

Comparative analytical study of three samples of Madhukasava (S₁, S₂, S₃) carried out in SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. Organoleptic characters of three samples documented. P_H, refractive index, specific gravity, total solids, total sugar, reducing sugar, non reducing sugar, alcoholic content, total acidity were measured as per standard methodology^[13,14].

OBSERVATIONS AND RESULTS

Results of microbiological study are displayed in Table 1 and figure 1. Dried and fresh flowers of *Madhuca indica* were found contaminated with colonies of yeast. Fresh flowers have shown less colonies of yeast, whereas dried flowers have shown indefinite colonies of yeast.

Three samples of Madhukasava a polyherbal compound was prepared as per classical references, and proper fermentation features observed on 45th day of its initiation (among s₁ and s₂). However these three samples were filtered, and taken for further study. Organoleptic characters observed have been displayed in Table 2. Comparative analytical test reports on these three samples displayed in Table 3.

Table 1: Ingredients of *Madhukasava*

S No	Ingredients	Latin name	Part used	Qty used for preparation
1	Madhukapushpa	<i>Madhuka indica</i> Gmel	Flower	0.500 Kg
2	Vidanga	<i>Embelia ribes</i> Burm. F.	Fruit	0.250 Kg
3	Chitraka	<i>Plumbago zeylanica</i> Linn	Root	0.125 Kg
4	Bhallataka	<i>Semicarpus anacardium</i>	Fruit	0.125 Kg
5	Manjishta	<i>Rubia cordifolia</i>	Stem	0.015 Kg
6	Jala	<i>Water</i>	Liquid	1.500 litre
7	Madhu	<i>Honey</i>	Liquid	0.0625 litre
8	Ela	<i>Elattaria cardamomum</i>	Seed	0.004 Kg
9	Mrinala	<i>Nymphaea stellata</i> Willd	Stem	0.004 Kg
10	Chandana	<i>Santhalam album</i> Linn.	Pith	0.004 Kg
12.	Agaru	<i>Aquilaria agallocha</i> Roxb	Heartwood	0.004 Kg

Table 2: Microbiological study of *Madhuka indica* Gmel. flowers

S No	Sample name	Dilution	Number of Colonies (NOC)		CFU/g
1	Fresh flower of <i>Madhuca indica</i>	1/10 (10^{-1})	4	9	6.5×10^1
2	Dry flower of <i>Madhuca indica</i>	1/10 (10^{-1})	INC	INC	INC

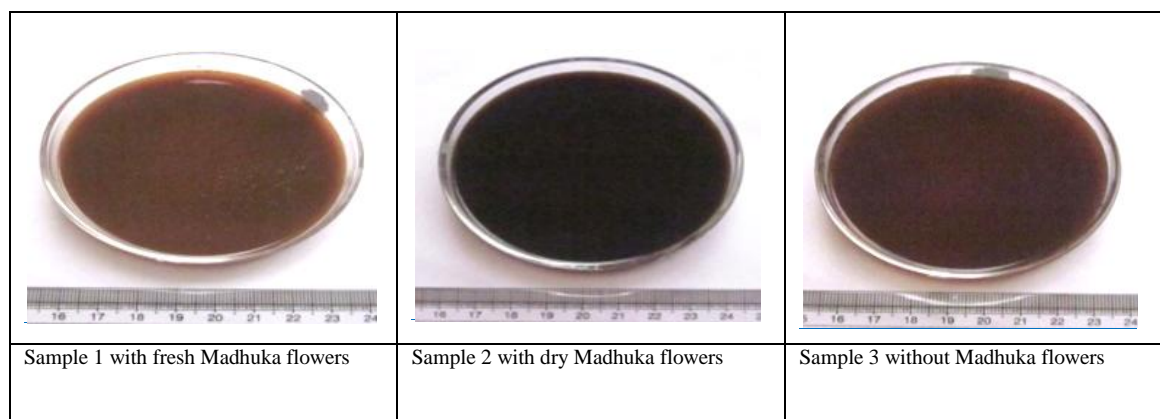
CFU-Colony Forming Units, INC-Indefinite Number of Colonies

Table 3: Organoleptic characters of 3 samples of *Madhukasava*

Sample	Quantity	Colour	Odour	Taste	Consistency
S ₁	520 ml	Orange Brown	Strong alcoholic	Sour + Sweet	Thin
S ₂	570ml	Dark Brown	Strong peiasant alcoholic	Sour + Sweeter+Astringent	Thin
S ₃	490 ml	Purplish Brown	Very mild alcoholic	Very sour	Thick

(S₁-with fresh Madhuka flowers, S₂-with dry Madhuka flowers and S₃-without flowers)**Table 4:** Analytical study results of 3 samples of *Madhukasava*

Parameter	Results n = 3 %w/w		
	S1	S2	S3
Ph	4.5	4	4.5
Refractive Index	1.35183	1.35333	1.34033
Specific Gravity	1.0265	1.0262	1.0162
Total Solids	10.575	15.698	5.265
Total Sugar	4.519	5.175	-
Reducing Sugar	4.155	4.369	-
Non Reducing Sugar	0.369	0.805	-
Alcoholic content (Fresh)	2.6428	2.5	1.642
Total Acidity	0.354	0.375	0.363

(S₁-with fresh Madhuka flowers, S₂-with dry Madhuka flowers and S₃-without flowers)**Figure 1:** *Madhukasava* study samples

DISCUSSION

Science of Ayurveda, is a treasure trove of medicaments to live in a healthy way and to eradicate disease. Sandhana-kalpanas are among unique alcoholic formulation using natural sources of drugs as ingredients as well as fermentative initiators. Since ancient times Madhuka flowers were used as natural fermentative agents in different asava and aristas. To find out their exact role in Sandhana-karma this study was planned.

To understand microbiological flora of the flowers, study of Fresh and Dry flowers were carried out using standard methodology. Fresh flowers have shown less colonies of microbial growth; whereas dried flowers have shown indefinite colonies of microbial colonies. Thus dried flowers have shown more microbial existence and justified the ancient wisdom behind the use of dried flowers of *Madhuka indica* Gmel.

Madhukasava is a poly herbal self generated alcoholic compound mentioned in treatise of Ayurveda. According to the proposed work Madhukasava is prepared in three ways. Sample S₁ fresh flowers of

Madhuka, Sample S₂ adding dried flowers of Madhuka and S₃ without adding flowers of Madhuka. On forty fifth day when the jars seals were opened S₁ and S₂ samples showed proper classically said completion of fermentation features, but third one S₃ sample didn't show proper features of completion. The sample S₁ (with ingredient Fresh flowers of Madhuka) have shown strong alcoholic odor with orange brownish color and sour with sweet taste. In this sample few prakshepaka dravyas were floating on surface. Sample S₂ (with dried flowers of Madhuka) have shown pleasant, strong alcoholic odor with dark brown colour with sweet, sour and astringent taste. In this sample very few Prakshepaka dravyas were floating on surface which were less compared to S₁ Sample S₃ without the flowers of Madhuka was purplish brown in appearance with mild alcoholic odour and was very sour in taste with very thick consistency. Prakshepaka dravyas were more in quantity, floating on the surface of this sample compared to other two samples.

Analytical parameters of three sample of Madhukasava have given us valuable results which help us to understand the efficacy of Madhukapushpa in the fermentation process and outcome. P^H is a measure of quality of fermented product [15]. In this study the P^H of all

the three samples have not shown much difference and all were in a P^H range of 4 to 4.5.

Refractive index ^[16] is a measure of density of the media compared to that of distilled water. In this study all three samples have shown minimum difference among the refractive indices.

Specific Gravity ^[17] is the ratio of the density of a substance to the density of a refractive substance, for the same given volume. S₁ and S₂ have not shown much variation among these values (1.0265 and 1.0262 respectively). Whereas S₃ (sample without adding Madhuka flowers) has shown 1.0162 a low specific gravity. Thus flowers of Madhuka have role in solubility of ingredients.

Total solids ^[18] indicate the amount of active constituents, extractable in aqueous media. Sandhana Kalpana facilitates many chemical / Biochemical reactions with breakage and reunion of the bonds, so that all phytochemical constituents of the mixture found to be dissolved in uniform status. These suspended /dissolved particles of herbs may have their contribution to total solids. In this study Sample S₁ with fresh flowers of Madhuka had shown 10.575 value, which is obviously less compared to Sample S₂ with dry flowers of Madhuka total solid value 15.698. Sample S₃ without any flowers of Madhukas has shown its value 5.265 which in turn represents its less solubility/ suspension of the compounds.

Total sugar ^[19] is a measurement of sucrose and reducing sugars, which represents the measurement of sugar content of Asavaristas. In this sample S₁ with fresh flowers of Madhuka has total sugar value 4.519 and Sample S₂ with dry flowers of Madhuka has value of total sugar as 5.175 where as S₃ without any flowers of Madhuka has shown nil value of total sugars.

Reducing sugars ^[20] include all monosaccharide and galactose, glucose, fructose, ribose and xylose are some reducing sugars. As the corolla of Madhuka flowers are rich source of reducing sugars like fructose, maltose etc in their fermentation products they have shown their constant values. Reducing sugar value of sample S₁ with fresh flowers of Madhuka was 4.155 and that of sample S₂ with dry flowers of Madhuka was 4.369.

Asavaristas are fermented products containing self generated alcohol. But a value 12% or beyond that value among the self generated alcoholic preparations is not allowed by the legal Act ^[21]. In this study alcohol content of the samples were under limit with minimum difference among three samples. Total acidity of all samples were within a range of 0.352% w/w to 0.375% w/w. Thus Madhuka flowers have shown precise role in proper fermentation which has been reflected in their analytical study.

CONCLUSION

The fragrant flowers of Madhuka (*Madhuka indica* Gmel. of *Sapotaceae*) are used in *Sandhanakalpana* (Fermented alcoholic preparations) in Indian system of medicine. Microflora extraction of fresh and dry Madhuka flowers revealed that Dry Madhuka flowers contain more yeast colonies than that of fresh flowers. Pharmaceutical Study confirmed the fact alleged by Ancient Acharyas that there is definite role of Madhukapushpa in Sandhana Kalpana (fermentation procedure). Comparative analytical study of three samples of Madhukasava classically prepared (S₁-with fresh Madhuka flowers, S₂-with dry Madhuka flowers and S₃-without flowers) has shown supportive facts for the study. Samples S₁ and S₂ (Madhukasavas with fresh and dry flowers) given better analytical standards than that of S₃

(asava without adding Madhukapushpa: Total sugar-0%w/w, Total solids-5.265%w/w). From the point of total solids count(S₁- 10.575% w/w, S₂-15.698% w/w) and total sugar count (S₁-4.519% w/w,S₂- 5.175% w/w) , dried flowers have shown better analytical parameters than that of fresh flowers. Thus whole study has emphasized the fact that Madhukapushpa has a definite role in Sandhana Kalpana and dried flowers will have better efficacy compared to fresh one.

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