International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 05-08-2020; Accepted: 20-08-2020: Published: 06-09-2020
www.botanyjournals.com
Volume 5; Issue 5; 2020; Page No. 77-80

Microbiological characterisation of *Woodfordia fruticosa* Kurz. *Madhuca indica* Gmel. Flowers and sediment used as fermentation initiators

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Abstract
Introduction: *Sandhana Kalpana* is a distinctive dosage variety in which acidic and alcoholic fermented preparations are formulated. Under fermentation preparations liquid media like decoction, expressed juices etc., sweetening agents, additives, fermentation initiators like *Woodfordia fruticosa* Kurz. And *Madhuca indica* Gmel. Or sediment are bring together in an inert vessel and sealed for precise time duration to make the process of fermentations. To initiate or potentiate the fermentation process numbers of components are required which serve as natural carries of the fermenting organism. From these, fermentation initiator is one of the main drugs for fermentation and sediment of previous fermentation also can use, but precise quality of these flowers and sediment was ailing studied. Therefore this microbiological study carried out to know the role of these flowers, sediment and ancient perception behind it.
Aim & objectives: To assess the role of *Woodfordia fruticosa* Kurz., *Madhuca indica* Gmel. Flowers and Sediment in fermentation process w.s.r. to their microbiological flora in *Kharjurasava*
Material & Methods: *Kharjurasava* was prepared as per reference from Yogaratnakar by adding *W. fruticosa* flowers and another by adding *M. indica* flowers as fermentation initiator. Total aerobic microbial count of drugs in *Kharjurasava* and Sediment was carried out by serial dilution agar plate technique.
Observation & Results: *Kharjurasava* prepared with *W. fruticosa* flowers and their Sediment have shown more number of colonies of yeasts, where *Kharjurasava* prepared with *M. indica* flowers and their Sediment have shown few colony forming units of yeasts.
Conclusion: Microbiological assessment for yeast cells has shown that *W. fruticosa* and their Sediment has more number of yeast colonies than that of *M. indica* and their Sediment.

Keywords: *Sandhana Kalpana, Kharjurasava, Woodfordia fruticosa* flowers, *Madhuca indica* flowers, sediment, microbiological study

Introduction

Ayurveda is a conventional Indian remedial system being practiced for thousands of years which advocate utilisation of various sources of natural products for therapeutic purposes. The Ayurvedic system of medicines became prosper because it had its concomitant materia medica which had been developed to progressive level. The Ayurveda contains many different methods and dosage forms of formulations they all are intended for different restorative effects and to preserve the potencies of herbs in different approach [1]. Along with these dosage forms *Sandhana Kalpana* is a distinctive dosage variety where acidic and alcoholic fermented formulations are prepared. In this process self-generated alcohol is formed by source medium used in pharmaceutical procedures [2]. These preparations occupy special position in pharmaceutics on account of their supremacy to added preparations it is having longer shelf life, rapid assimilation and greatest bioavailability [3]. Whereas, liquid media like decoction, expressed juices etc., sweetening agents, additives, fermentation initiators like *Woodfordia fruticosa* Kurz. And *Madhuca indica* Gmel. Flowers or sediment are bring together in an inert vessel and sealed for precise time duration to make the process of fermentations. *Asava* and *Arishta* are two important formulations of this practice [4]. To initiate or potentiate the fermentation process numbers of ingredients are essential which provide as usual carries of the fermenting organism. E.g. *W. fruticosa* flowers, *M. indica* flowers, Sediment, Yeast (*Saccharomyces cerevisiae*) and rarely *Areca catechu* Linn. Fruit, *Ziziphus sativus* Gaertn. Bark and *Acacia nilotica* Linn. Bark used [5]. The well-known fermentation initiator in Ayurveda is *W. fruticosa* flowers but its use had commenced since the time of *Ashtanga Hridaya* [6]. Charaka had not mentioned any fermentation initiator but describes the properties of *W. fruticosa* [7] whereas *Acharya* Sushruta mentioned Sediment after fermentation used as fermenting agent [8]. References of *M. indica* flowers as fermentation initiator are found in Sharangdhara *Samhita* [9].

As maintained by texts *W. fruticosa* flowers contains natural yeasts, which can indulge high sugar attention and are distinctly able to bring about the fermentation process [10]. These flowers were termed as *Madakari*, *Sandhaniya* in the treatise of Ayurveda [11] and highly tanniferous [12]. But the exact role of these flowers in fermentation process is not yet analyzed. However, the effect of *W. fruticosa* flowers in

Mustakarishta reveals that the dried *Woodfordia fruticosa* flowers are best fermentative agents [13].

As same since ancient times *M. indica* flowers were used in different fermentation preparations as a fermentative initiator. Which are commonly known as *Mahua* flowers. These flowers are supposed to be rich spring of sugars along with other necessary nutrients and vitamins [14]. The use of dried *Madhuca indica* flowers in *Madhukasava* has exposed role in fermentation process [15].

Acharya Sushruta mentioned Sediment after fermentation used as fermentation initiators. But after completion of fermentation, in Sediment how much quantity of yeasts remains is not explored yet and the references are said the Sediment of previous fermentation can be used for same new fermentation and it can be used again and again [16]. Now days in Ayurvedic Pharmaceuticals commercially prepared the *Asava-Arishta* and they are practice of using yeast as fermentation initiator due to already available of yeast inoculums which multiply rapidly by division in a short time, easy initiation of fermentation, fast alcohol production, short acidity, low cost and reducing of batch size. A study on the outcome of adding of yeast reveals that the arrival and end of fermentation method in the samples containing yeast were quick [17].

For that reason this trial study has been designed to provide scientific proof for the traditional wisdom behind the use of these flowers (*Woodfordia fruticosa* and *Madhuca indica*) and Sediment in fermentation procedure in one formulation *Kharjurasava*. Hence as a part of study here an attempt has been done to explore micro flora of *W. fruticosa* and *M. indica* flowers. With this background study was designed to elucidate the role of *W. fruticosa* flowers, *M. indica* flowers and Sediment in fermentation process with reference to their microbiological study.

Material and Methods

Dhataki pushpa (*Woodfordia fruticosa* Kurz.) were collected from herbal medicinal garden Mahatma Gandhi Ayurved College Hospital and Research Centre, Salod (H), Wardha Maharashtra, India. *Madhuka pushpa* (*Madhuca indica* Gmel.) were collected from local farm of Nagpur district, Maharashtra, India. Both these flowers were authenticated from Foundation of Revitalisation of local health Tradition (FRLHT), Centre for Conservation of Natural Resources (CCNR), Bangalore, India. *Kharjurasava* was prepared by using two methods and altering fermentation initiator in each one, as per reference from Yogaratnakar by adding *W. fruticosa* flowers and other by adding *M. indica* flowers as fermentation initiators.

Microbiological study

Different *Kharjurasava* prepared by adding *W. fruticosa* and *M. indica* flowers were assessed for microbiological study. The sediments of these respective *Kharjurasava* were also assessed for microbiological study. Total aerobic yeast colonies count of drugs was carried out by serial dilution agar plate technique [18].

Procedure

1. Potato dextrose agar (21.45gm) and Agar agar powder (6gm) was dissolved in 550ml of distilled water and boiled it up to homogenous mixture.
2. Then, test tubes prepared with adding 9ml of distilled water in each test tubes and swabs prepared for enclosed to test tubes, Petri plates are swap in paper, micropipette, micro tip, spreader etc. kept for autoclave at 121.5°c for 1 hrs.

Pour plating

3. After 1 hr all equipments were removed from autoclave.
4. Laminar air flow was cleaned with 70% ethanol and started the UV before 20 minutes.
5. All Petri plates were labelled with sample number and dilution.
6. In each Petri plate by opening lid slightly, 20 ml of warm dilution was poured with measuring cylinder and plates were smoothly rotated in a circular motion and swirling action with the help of spreader to complete uniform circulation of the sample and then media was let for 8 hrs for solidify.
7. Then, serial dilution was done by labelled dilution blank (test tube) as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8}.
8. 8) One ml of *Kharjurasava* prepared with *W. fruticosa* flowers sample was mixed in test tube with 9ml of sterile distilled water to make dilution 10^{-1} similar to that *Kharjurasava* prepared with *M. indica* flowers sample prepared. Followed by one gram of *Kharjurasava* with *W. fruticosa*-Sediment sample mixed in test tube with 9ml sterile distilled water to make 10^{-1} similar to that *Kharjurasava* with *M. indica*-Sediment sample prepared, initial dilution was done by adding 1 ml of the sample into 9ml dilution blank labelled 10^{-1} thus dilution the original sample 10 times.
9. From the first dilution, 1 ml of the suspension was transferred to the dilution blank 10^{-2} was done with a sterile and fresh 1 ml pipette diluting the original suspension to 100 times(1/10x1/10=1/100 or 10^{-2}).
10. From the 10^{-2} suspension, 1ml of suspension was transferred to 10^{-3} dilution blank with a fresh sterile pipette, thus diluting the original sample to 1000 times(1:1000 or 10^{-3}).
11. This procedure was repeated till the original sample has been diluted 10,000,000(10^{-8}) times using every time a fresh sterile pipette.
12. Experiment was carried out in triplicate for each dilution.
13. The contents were mixed to each plate by rotating with spreader gently to distribute the cells throughout the medium.
14. The plates were allowed to solidify.
15. These plates were incubated in an inverted position for 5 days at 37°c in BOD incubator.
16. Quantity Colonies forming unit was counted using digital colony counter.

Observation and Results

Microbiological study

Results of microbiological study are displayed in Table 1 and Figure 1 (1.a to 1.d). KAD-I (*Kharjurasava* prepared with *W. fruticosa* flowers), KAD-II (*Kharjurasava* with *W. fruticosa*-Sediment), KAM-I (*Kharjurasava* prepared with *M. indica* flowers) and KAM-II (*Kharjurasava* with *M. indica*-Sediment) samples were taken one ml and grown in Potato dextrose agar and Agar agar powder medium. These Petri dishes were incubated for five days at 37°c in BOD incubator. Total yeast count shown following information,

Kharjurasava prepared with *W. fruticosa* flowers and *W. fruticosa*-Sediment have shown more number of colonies of yeasts, where *Kharjurasava* prepared with *M. indica* flowers and *M. indica*-Sediment have shown few colony forming units of yeasts. Figure 2 (2.a and 2.b)

Table 1: Microbiological study of total yeast count of- KAD-I, KAD-II and KAM-I, KAM-II samples

Sr. No.	Name of Samples	Dilution	Number of colonies (NOC) CFU/g
1.	KAD-I	8/10 (10^{-8})	$20x10^8$
2.	KAD-II	8/10 (10^{-8})	$4x10^8$
3.	KAM-I	8/10 (10^{-8})	$9x10^8$
4.	KAM-II	8/10 (10^{-8})	$1x10^8$

CFU-Colony Forming Units, KAD-I – *Kharjurasava* prepared with *W. fruticosa* flowers, KAD-II - *Kharjurasava* with *W. fruticosa*-Sediment, KAM-I – *Kharjurasava* prepared with *M. indica* flowers, KAM-II- *Kharjurasava* with *M. indica*-Sediment

Yeast colonies of *W. fruticosa*, *M. indica* flowers and their Sediments

Fig 1.a: (KAD-I) with *W. fruticosa* flowers

Fig 1.b: (KAD-II) with *W. fruticosa*-Sediment

Fig 1.c: (KAM-I) with *M. indica* flowers

Fig 1.d: (KAM-II) with *M. indica*-Sediment

Samples of *Kharjurasava* prepared with *W. fruticosa* and *M. indica* flowers

Fig 2.a: (KAD-I) *Kharjurasava* with *W. fruticosa* flowers

Fig 2.b: (KAM-I) *Kharjurasava* with *M. indica* flowers

Discussion

Ayurvedic medication is all set to regain its lost fame Because of its preventive and curative nature, less side effects and holistic approach [19]. With advances in Ayurveda drug manufacturing, innovative instruments/procedures are being developed to help man power as well as large scale manufacturing of medicines within desired time limit [20]. However fermentation is a classical procedure which is still unchanged even in this modern era except the use of different fermentation agent. Fermentation process is among famous Ayurvedic formulations using expected origin of drugs as constituent also fermentative initiators. The well-known fermentation initiator in Ayurveda is *W. fruticosa* flowers were used and since time *M. indica* flowers were also used as ordinary fermentative agents in diverse *Asava* and *Arishtas* [21]. And also *Acharya* Sushruta mentioned Sediment as fermentation initiator. The *Kharjurasava* is itself of unique herbal self-generated alcoholic formulation mentioned in Yogaratnakar [22]. Because there are no sweetening agents like jaggery, sugar or honey etc. According to the projected effort *Kharjurasava* is prepared in two ways. The samples of KAD-I prepared with *W. fruticosa* flowers and KAM-I prepared with *M. indica* flowers.

However, onset of fermentation was found same in KAD-I sample and KAM-I sample i.e. on fifth day. On sixty one day after the jars seals were released. KAD-I and KAM-I samples showed correct classically said attainment of fermentation and all tests were passed. The sample KAD-I (with *Woodfordia fruticosa*) has shown strongly alcoholic odour with light brown colour, sour with sweet taste and additives settled down. Where in KAM-I (with *Madhuca indica*) has shown pleasant, strongly alcoholic odour with dark brown colour with sweet and sour taste and additives settle down.

To understand microbial flora of these flowers and Sediment of *Kharjurasava* without adding any sweetening agents to identify how much quantity of yeasts are produced naturally with this aim study were carried out by using standard methodology. Total aerobic yeast colonies count of *Kharjurasava* samples was carried out by plate count method and this procedure was done with serial dilution method. On 5^{th} day, incubated plates of *W. fruticosa* flowers have shown more amounts of colonies of microbial growth ($20x10^8$); whereas *M. indica* flowers shown less colonies of microbial colonies ($9x10^8$). In with *W. fruticosa*-Sediment have shown more amount of colonies of microbial growth($4x10^8$) compared to *M. indica*- Sediment **(**$1x10^8$) which shown less amount of microbial colonies. Thus *Kharjurasava* prepared with *W. fruticosa* flowers and their Sediment have shown more microbial existence than *Kharjurasava* prepared with *M. indica* flowers and their Sediment which validated the ancient wisdom behind the utilization of *W. fruticosa* flowers and Sediment. Thus *W. fruticosa* and *M. indica* have shown specific function in proper fermentation which has been imitate in their microbiological study.

Conclusion

Pharmaceutical preparation of *Kharjurasava* and time duration for fermentation was near about same. In analytical

study variation in alcohol percent was observed which is little more in KAM-I (6.75%) compared to KAD-I (6.41%). The *W. fruticosa* flowers were used as commonly precious source of fermentation process and *M. indica* are edible fragrant flowers also used in fermentation process as a fermentation initiator in Indian system of medicine. Microbiological study has shown that *Kharjurasava* prepared with *W. fruticosa* flowers and their Sediment has more number of colonies of microbial growth and then of *M. indica* and their Sediment. As a result of entire study has give emphasis to the fact of dried *W. fruticosa* flowers and their Sediment has a distinct role in fermentation process than the *M. indica* flowers.

References

1. Paranjpe P, Indian Medicinal Plants-Forgotten Healers, A Guide to Ayurvedic herbal medicine, Chaukhambha Sanskrit Pratishthan, Varanasi, 2005, p.23
2. Pargunde S, Thakkar J, Basaiye R. A review on Sandhana Kalpana, J Ayurpub. 2017; 2(2):414-420.
3. Muralidhar R, Chaudhary A, Ravishankar B, Dey S, Prajapati PK. A comparative pharmaceutico-pharmacoclinical study of different samples of Shirisharishta and its Shwasahara effect. AYU, 2004; 7:45-9.
4. Angadi R. A text book of Bhaishajya Kalpana Vijnana, Chaukhambha Surbharti Prakashana, Varanasi, 2016, p.287.
5. Harpreet K, Harish K, Wijayanthamala, Asava and Arishta under umbrella of Sandhana Kalpana, Int J Res: Ayurveda Pharm, 2015, 6(6).
6. Murthy KR Srikantha. Editor, Ashtanga Hrudayam of Vagbhata. Chikitsasthana, 8/64-67, 1st ed, Varanasi: Chaukhambha Orientalia, 2009, 2:316.
7. Agnivesha Charaka Samhita. Vol-I Sutra Sthana 27/188, English Translation by Sharma RK and Dash B, Reprint edition, Chaukhambha Sanskrit series office, Varanasi, 2014, p.525.
8. Sharma RA, editor, Sushruta Samhita of Sushruta. Chikitsasthana 10/6, 1st edn. Varanasi: Chaukhambha Orientalia, 2012, 2:262.
9. Vidyasagar Parasurama Shastri, editor, Sharangdhara Samhita of Sharangdhara. Madhyama Khanda, 10/44-46; 7th edn, Varanasi: Chaukhambha Orientalia, 2008, p. 237.
10. Iqbal A, Farrukh A, Mohammed O. Modern Phytomedicine, Turning Medicinal plants into Drugs. Weinheim: Wiley VCH, 2006, p.30-4.
11. Vohra A, Satyanarayanaa T. Phytase production by the yeast Pichila anomala, Biotech Lett, 2001; 23:551-554.
12. Sharma PV. Dravyaguna-vijnana. Vol II. Varanasi: Chaukhambha Bharati Academy, 2009, p. 472-474.
13. Admani M, Kumar KS, Mallya SV. Pharmacognostic characterisation of flowers Woodfordia fruiticosa Kurz. (Dhataki Pushpa) used as fermentation initiators. Journal of Ayurvedic and Herbal Medicine. 2015; 1(1):09-12
14. Khare CP. Indian Medicinal Plants- An illustrated Dictionary. New Delhi; Springer (India) Pvt Ltd, 2007, p.191.
15. Suma Mallya V, Julient Elizabeth Castelino, MB Seema, Role of Madhuka pushpa in Sandhana Kalpana; microbiological pharmaceutico-analytical study, Journal of Scientific and Innovative Research. 2017; 6(2):68-72.
16. Chaudhary A, Singh N, Dalvi M, Wele A. A Progressive review of Sandhana Kalpana (Biomedical fermentation): An advanced innovative dosage form of Ayurveda, AYU. 2011; 32(3):408-17.
17. Shingadiya R, Dhruve K, Shukla VJ, Prajapati PK, Standard manufacturing procedure and quality parameters of Kanakabindvarishta. International Journal of Herbal Medicine. 2015; 3(1):33-36.
18. Aneja KR. Experiments in Microbiology Plant Pathology and Biotechnology, 4th edition, New Age International (P) Limited, Publishers, New Delhi, 2007, p.g.7.
19. Zumsteg IS. Plants and Traditions used in Prenatal and Postnatal Care in Minahasa, North Sulawesi, Indonesia (Doctoral dissertation, Master Thesis, Institute of Systematic Botany, University of Zurich).
20. Rathi B, Wanjari A, Rajput D, Khan M, Rathi R. Pharmaceutical standardization of Chyawanprash prepared by two different methods. Journal of Indian System of Medicine. 2018; 16(4):179
21. Das C, Ghosh G, Das D. Ayurvedic liquid dosage form Asava and Arishta: An overview. Indian J Pharm Edu Res. 2017; 51(2):169-76.
22. Shastri S, Yogaratnakar, Vidyotini Hindi Commentary, Chaukhambha Sanskrit Sansthana, Varanasi, reprint ed., 2005, p.382.