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A STUDY TO EVALUATE THE PRESERVATIVE ACTION OF NAGAKESARA ARKA IN TRIPHALA KWATHA

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ABSTRACT

Introduction: Primary dosage forms are basic preparations whose shelf life is a challenge for the practice. Kwatha is a basic primary preparation which is easily prone to contamination and can be marketed only by the addition of suitable preservatives to increase the shelf life. So addition of preservatives is being practiced to prolong the shelf life of kwatha but use of chemical preservatives are harmful to body and even have carcinogenic effects. Hence there aroused a need to find natural preservatives to benefit the mankind. Arka which is a water distillate consists of essential substances from the crude drug and has longer shelf life comparatively. Nagakesara arka possess anti- microbial and anti- oxidant properties and economically cheaper and easily available drug and triphala kwatha being useful in many purposes, in this study an attempt was made to elucidate the preservative action of nagakesara arka in triphala kwatha. **Materials and methods**: Includes preparation of nagakesara arka, triphala kwatha and conduction of analytical and microbiological study to see the preservative action using SDA and MHA media. **Observations**

and Results: Study follows observations over microbial growth of the sample on daily basis where nagakesara arka showed preservative action for 25 days. *Aspergillus niger* was the fungal growth seen on 26th day. **Discussion**: Nagakesara arka owing to its pH, chemical constituents and other properties preserved the triphala kwatha for a stipulated period of time. **Conclusion**: From the study, it was concluded that the nagakesara arka preserved triphala kwatha without any microbial contamination for 25 days which was added in the concentration of 15%

Keywords: Nagakesara arka, Triphala kwatha, Preservative, Microbiological study

INTRODUCTION

Bhaishajya Kalpana is the branch of Ayurveda which deals with the different processes involved in the preparation of different drug formulations. In ancient times, Vaidya himself used to prepare medicines and most of those were to be consumed immediately. With the growing industrialization and globalization, the scenario has been changed to acceptance according to the modern lifestyle. Different Samskara were used to convert drugs into different dosage forms like Sharkara Paka, Avaleha Kalpana, etc. to improve the stability and durability of the formulations. Kwatha is one such preparation possesses short shelf life or it should be consumed immediately after its preparation which is easily prone to contamination and can be marketed only by the addition of suitable preservatives to increase the shelf life. Preservatives being used these days are proven to have carcinogenic and allergy inducing properties **[1]**. So there arises a need to find a natural preservative.

Arka Kalpana is a unique method of preparation explained by Ravana in his book Arka Prakasha. Arka is a liquid preparation obtained by distillation of certain liquids or of drugs soaked in water using Arka yantra (distillation apparatus) or any other convenient modern distillation apparatus **[2]**. Arka is one which does not undergo decomposition easily and possess longer shelf life.

The drug Nagakesara identified as *Mesua ferrea* Linn belongs to Guttiferae family, part used is flower. Chemically it possess messuaferrone- A, messuaferrone- B, mesuol, flavanoids, alpha Copaene, glycosides **[3]**. Chaturjataka Arka which is mentioned in the fourth Shataka of Arka Prakasha stated as Deepaka, Pachaka, Vishapaha in nature **[4]**. Nagakesara arka being colourless liquid contains volatile

content that forms as droplets, has aromatic characteristic odour and it is Kashaya rasa pradhana in nature. By considering these properties, an attempt is made to elicit the preservative action of Nagakesara arka in Triphala kwatha as Triphala kwatha is being used most commonly, has many therapeutic actions and can be prepared by drugs which are easily available.

MATERIALS AND METHODS

It is classified into following sections

1. Preparation of Nagakesara arka
2. Preparation of Triphala kwatha
3. Preparation of sample
4. Analytical study
5. Preparation of media
6. Microbiological study

Raw drugs for the preparation of Nagakesara arka and Triphala kwatha were procured from C K Kumaran Memorial (CKKM) Pharmacy, Tripunithura, Kerala and authentication was also done from the same.

Preparation of Nagakesara arka [5]: The preparation of Nagakesara arka was done under aseptic conditions by following of 1:3 (drug: water) ratio and by considering volume/ volume (v/v) measurement. Dried drug of the mentioned species was cleaned, coarsely powdered (sieve number 44) and used for arka preparation. Initially the mentioned quantity of coarse powder of nagakesara was taken in a round bottom flask and soaked with sufficient quantity of water just enough to soak the drug (200ml) and kept overnight. Next day morning, remaining quantity of water (100ml) was added and using Arka yantra (distillation apparatus) heating was started. The heat given was 600^0 initially; once it started boiling during the procedure temperature gradient was maintained between 400^0- 600^0. Initial few drops of Nagakesara arka were discarded as it may not contain therapeutically essential substances and the process of distillation of Nagakesara arka was continued till 30% of the distillate was collected.

Preparation of Triphala kwatha: Kwatha churna of Triphala [Amalaki (*Emblica officinalis* Gaertn), Haritaki (*Terminalia chebula* Retz) and Vibhitaki (*Terminalia bellerica* Roxb)] was taken one part in a sterile stainless steel vessel and added with four parts of water. This was kept for boiling by maintaining the temperature. Study follows 1:4 (drug: water) ratio, w/v measurement for the preparation of Triphala kwatha and when the kwatha reduced to 1/4th, it was filtered through a clean cloth.

Preparation of sample: To the 50ml of Triphala kwatha, 7.5ml of Nagakesara arka was added in the concentration of 15% based on the previous research study **[6]**.

Analytical study: It includes organoleptic and physico- chemical parameters of Nagakesara arka and Triphala kwatha. pH of sample was done on the day of preparation and on the day of spoilage.

Analysis of the following parameters was done based on the references available in the CCRAS protocol **[7]**.

1. Morphological evaluation- organoleptic characters like appearance, colour, odour, taste

2. Physico- chemical parameters- pH, specific gravity, viscosity, total suspended solids, refractive index, volatile oil estimation.

The above mentioned analytical parameters were carried out for three times and the average reading or value was taken as a result.

Preparation of media [8]: For this study, Sabouraud Dextrose Agar (SDA) medium, Mueller Hinton Agar (MHA) medium were used to see the fungal and bacterial growth respectively.

a) Preparation of Sabouraud Dextrose Agar (SDA) medium: Dextrose 40g, beef extract 5g, casein peptone 5g were taken and dissolved in 1000ml distilled water and pH was adjusted to 5.6 ± 0.2 and 15g of agar was added to it and mixed. Then the media was autoclaved for 20 minutes at 121°C.

b**) Preparation of Mueller Hinton Agar (MHA) medium**: Beef extract 2g, acid hydrolysate of casein 17.50g, starch 1.50g, agar 17.00g were taken and dissolved in distilled water and made up to 1000ml and pH was adjusted to 7.3± 0.2 and added with 7.5g of agar and mixed. Then media was autoclaved at 121°C for 20 minutes. Then 10-12ml of the media was poured into petri dishes and allowing it to set in a sterile area for further use.

Microbiological study: This study was carried out in the Microbiology Laboratory of the department of Roganidana evam Vikriti Vigyana, Sri Dharmasthala Manjunatheshwara College of Ayurveda & Hospital, Hassan. Every day new plates were used from both the media (SDA and MHA). Streaking method was adopted on both media using inoculum loop to test the microbial contamination and plates were kept overnight with respective temperature environment to assess the growth on the next day. Microscopic view of the sample was checked on the day of microbial growth. All above mentioned procedures were done in aseptic precautions for better result.

Fig 1(a): Nagakesara coarse powder

Fig 1(b): Distillation process

Fig 1(c): Nagakesara arka

Figure 1 (a-c): Preparation of Nagakesara arka

Fig 2(a): Kwatha churna of triphala

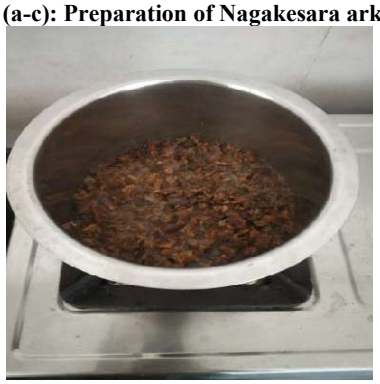

Fig 2(b): Process of boiling

Fig 2(c): Triphala kwatha

Figure 2(a-c): Preparation of Triphala Kwatha

Figure 3: Preparation of sample

Figure 4: Preparation of media

Figure 5: Streaking of sample on plate

Figure 6: Growth on SDA media after 25 days

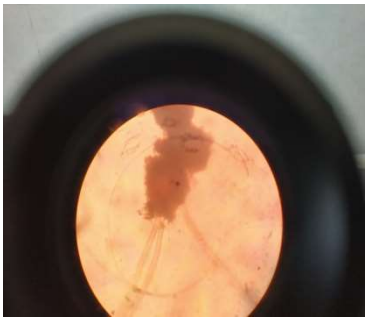

Figure 7: Microscopic view

OBSERVATIONS AND RESULTS

Nagakesara arka was colourless liquid with an aromatic characteristic odor and appreciated a layer of volatile content as oily droplets above the surface which possessed kashaya rasa (bitter taste) on taste and pH of 4.09 **(Table 2)**.

Triphala kwatha was brown liquid possessed characteristic odour, tikta rasa pradhana kashaya rasa (prominent bitter taste with slight astringent taste) on taste and pH of 3.14 **(Table 3)**.

Microbiological study:

The study was conducted by keeping the sample in sterile autoclaved glass bottle in normal room temperature for storage. SDA and MHA are the two media used to see the growth of fungus and bacteria respectively. Streaking method was adopted using petri dish. After streaking in the plates by adopting aseptic precautions, SDA plates were kept in normal room temperature between 30^0- 35^0 Celsius for 24- 48 hours to observe the growth; MHA plates were kept in incubator by maintaining the temperature between 35^0 – 41^0 Celsius for 24 hours to see the growth **(Table 4)**.

The microbial load was assessed by assessing the growth over the media plates. To elucidate the stability of the sample and of the microbial contamination, microscopic view of the sample was done. In the microscopic view, motile microbes, suggestive of contamination were observed. *Aspergillus niger* was the fungus found when viewed under microscope. It is one of the most common species of the genus Aspergillus belongs to Trichocomaceae family **[9]**. It is commonly known to cause food contaminations or food spoilage. No growth was observed in the plates of MHA media.

Monitoring of colour, odour, appearance and pH parameters were done for the sample on the day of preparation and after spoilage **(Table 5)**.

Table 1: Observations during preparation of Nagakesara arka

Parameters	Nagakesara Arka
Drug quantity (v/v)	100ml (45.37g)
Water	300ml
Proportion (drug: water)	1:3
Initial temperature	60^0
Maintained temperature gradient	40^0- 60^0
Starting time	1:45pm
Time of first drop	2:12pm
Ending time	4:25pm
Distillate obtained	90ml
% obtained	30%

Table 2: Observations during the preparation of Triphala kwatha

Drugs used	Kwatha Churna of Amalaki, Haritaki, Vibhitaki
Proportion (drug: water)	1:4 (w/ v)
Quantity of drugs	360g (120g each)
Quantity of water	1440ml
Reduction	$1/4^{th}$
Temperature (degree Celsius)	88^0- 95^0 C

Table 3: Analytical parameters of Nagakesara arka and Triphala kwatha

Sl. No	Particulars	Nagakesara arka	Triphala kwatha
1	Appearance	Clear liquid with a layer of less volatile content that forms as droplets	Brown liquid
2	Colour	Colorless liquid	Brown
3	Odour	Aromatic characteristic odor	Characteristic odour
4	Taste	Kashaya rasa pradhana	Tikta rasa pradhana kashaya rasa
5	pH	4.09	3.14
6	Specific gravity	1.0019	1.0608
7	Viscosity (Pa.s)	0.0062	0.0137
8	TSS (Total Suspended Solids) mg/l	0.1	17
9	Refractive index	1.34	1.36
10	Volatile oil estimation (%)	0.05%	-

Table 4: Observations and Results during Microbiological study

Sample	Date of preparation	Date of spoilage	Number of days stayed	SDA medium		MHA medium	
				Growth date	Organism	Growth date	Organism
Nagakesara arka in Triphala kwatha added in 15% concentration	8/10/2020 (pH: 2.79)	3/11/2020 (pH: 2.90)	25 days	3/11/2020	*Aspergillus niger*	No growth	No growth

Table 5: Observations of the sample for stability

Parameters	Sample	
	Day of preparation	After spoilage
Colour	Brown	Dark brown
Odour	Kwatha Nagakesara odour	Fruity odour
Appearance	Liquid	Thicker mucous liquid
pH	2.79	2.90

DISCUSSION

Nagakesara possesses different properties like anti- microbial, anti- oxidant which have positive result for the study **[10, 11]**. In classical Ayurveda treatises also many references are available which explains the gunaadi karma of drug nagakesara. Owing to these properties, this study was taken up to assess the extent or days of preservative action of nagakesara drug in its arka form by extracting volatile content to check the probable preservative action in triphala kwatha.

Nagakesara arka was prepared by taking 1:3 ratio of drug and water considering v/v measurement. During the preparation, size reduction of drug was done by making coarse powder of the drug which helps to increase the

surface area of the drug for the active principles to be dissociated into water. So soaking was done overnight to make the drug soft and this helps in release of essential volatile principles while boiling as the drug nagakesara has volatile content. Next day distillation process was started by adding remaining quantity of water. As the amount of water used for the preparation was less, precaution has to be taken not to char the drug and not to collect the distillate more than the calculated quantity as it may not contain therapeutically potent or essential substances. Arka obtained finally was a colourless liquid possessed characteristic aromatic odor with a layer of volatile content and had kashaya rasa in predominance because of the higher concentration of constituents present in the drug. Yavakuta churna (coarse powder) used for the preparation of Triphala kwatha which facilitates the proper absorption of water soluble principles followed 1:4 ratio of drug and water that reduced to 1/4th of the total volume. Triphala kwatha obtained was brown in color, possessed characteristic odor with tikta pradhana kashaya rasa.

Nagakesara arka had pH of 4.09 suggestive of highly acidic nature where more acidic nature also act as preservative and the efficacy, absorption, irritability also depends on pH of a drug. A more acidic pH result in lesser oxidation suggestive of acidic nature reduces the growth of micro-organisms **[12]**. Triphala kwatha had pH of 3.14, which was also acidic in nature; this also influences on the rate of oxidation. Specific gravity of nagakesara arka and also the triphala kwatha was near to the value one suggestive of these had specific gravity that was similar to water. Viscosity of nagakesara arka was similar to that of water as it is a distillate of water. Viscosity of triphala kwatha was 0.0137 as it was a filtrate of water along with drugs. Total suspended solids for arka was 0.1mg/l as it was a distillate it would only have water soluble active principles along with volatile principles extracted from the raw drug. But the total suspended solid of triphala kwatha was 17 suggestive of more solid particles suspended in the final filtrate liquid. Refractive index of both arka and kwatha suggests that they had low viscosity and density. Nagakesara arka contained volatile matters in the form of essential oil.

Microbiological study was conducted to test the microbial contamination of the sample and to check the number of days of preservative action of nagakesara arka in triphala kwatha. Arka showed preservative action for 25 days without any microbial contamination. The drug is a rich source of essential oil; this could have also impacted on the reduction and the components present in

nagakesara arka could have also influenced over the rate of microbial growth. Nagakesara arka was highly acidic in nature and the sample showed the pH of 2.79 on the day of preparation which is also acidic in nature, this could have also influenced to reduce the rate of oxidation as the acidic nature helps in reducing the growth of microbes. On spoilage the sample acquired fruity odour, dark brown in colour which forms thicker mucous liquid due to the influence of fungal growth.

CONCLUSION

By this study it was concluded that the Nagakesara arka showed preservative action for 25 days in Triphala kwatha without any microbial contamination owing to its properties which was added in the concentration of 15%. *Aspergillus niger* was the fungal growth observed which is a common food spoilage organism is the most common species of the genus Aspergillus. This study extends scope for many research works to be taken up on the same path.

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