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PATRA ARKA AS A PRESERVATIVE IN *TRIPHALA KWATHA*

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ABSTRACT

Introduction: Primary dosage forms are the important basic preparations whose shelf life is a challenge for the practice. *Kwatha* is one such 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 like sodium benzoate, etc are harmful to body and even have carcinogenic effects. Hence there aroused a need to find natural preservatives. *Arka* which is a water distillate consists of essential substances from the crude drug and has longer shelf life comparatively. *Patra arka* possess anti- bacterial, anti- fungal and anti- oxidant properties and economically cheaper and easily available drug and *triphala kwatha* being useful in many purposes, an attempt was made in this study to elucidate the preservative action of *patra arka* in *triphala kwatha*. **Materials and methods**: Includes preparation of *patra 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 *patra arka* showed preservative action for 21 days. *Aspergillus niger* was the fungal growth seen on 22nd day. **Discussion**: *Patra 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 *patra arka*

preserved *triphala kwatha* without any microbial contamination for 21 days which was added in the concentration of 15%

Keywords: *Patra arka*, Preservative, *Triphala kwatha*, Microbiological study

INTRODUCTION

Bhaishajya Kalpana is the branch of Ayurveda mainly focuses on the different processes involved in the preparation of different drug formulations. References regarding the usage of herbal preparations are available right from the Vedic period. Acharya Charaka is the first one to coin the term '*Kalpa'* for the pharmaceutical preparations and mentions that *kalpana* should have *Vishesha Shakti,* quoted as "*Shakti Vishesha Kalpanartha Cha Kalpanam*" **[1]**. *Kwatha kalpana* is one such preparation mentioned in the classics, being used for both internal and external purpose. It is said to be consumed instantly as it falls under *Panchavidha kashaya kalpana* and the shelf life of *kwatha* in general can be taken as 24 hours **[2]**. So to extend its shelf life usage of preservatives is commonly observed for marketing but the use of chemical preservatives on long term has carcinogenic and toxic effects over the body **[3]**. Hence there is a need to find out the natural preservative which is beneficial to mankind on its usage.

Arka Kalpana is a unique preparation where water soluble active principles and volatile oils are extracted from a drug through the method of distillation **[4]**. As this preparation is a product of distillation, do not undergo decomposition easily and stays for one to two years.

Patra botanically identified as *Cinnamomum tamala* Nees, Lauraceae family and part used is leaves. *Patra arka* is a colourless liquid with characteristic aromatic odour possess *katu rasa* (pungent taste) with little tingling sensation on taste. In Arka Prakasha, *Chaturjataka arka* is said as *Vishapaha* **[5]**; *patra arka* being one among *Chaturjataka arka* and owing to its properties an attempt was made in this study to evaluate the preservative action of *patra arka* in *triphala kwatha* as *triphala kwatha* is easy to prepare with feasibility in availability of drugs and most commonly used *kwatha* in many therapeutic procedures.

MATERIALS AND METHODS

It is divided into following sections

1. Preparation of *Patra 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 *patra arka* and *triphala kwatha* were procured from C K Kumaran Memorial (CKKM) Pharmacy, Tripunithura, Kerala. Authentication of drugs was also done from the same.

Preparation of *Patra arka* [6]: The preparation of *patra arka* was carried out under aseptic conditions by following of 1:3 (drug: water) ratio and using volume/ volume (v/v) measurement. Dried drug of the specified species was cleaned, coarsely powdered (sieve number 44) and used for *arka* preparation. Initially the mentioned quantity of coarse powder of *patra* 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 portion of water (100ml) was added and the *Arka yantra* (distillation apparatus) was set and heating was started. The heat given was 600^0 initially; once it started boiling temperature gradient was maintained during the procedure between 400^0- 600^0. Initial few drops of *patra arka* were discarded as it may not contain therapeutically potent substances and the process of distillation of *patra arka* was continued till 30% of the distillate was collected.

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

Preparation of sample: To the 50ml of *triphala kwatha*, 7.5ml of *patra arka* was added in the concentration of 15% based on the previous research study **[7]**.

Analytical study: It includes organoleptic and physico- chemical parameters of *patra 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 following the references available in the CCRAS protocol **[8]**.

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

2. Physico- chemical parameters- pH, specific gravity, viscosity, refractive index, total suspended solids, 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 [9]: 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 1210C.

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 department of Roganidana evam Vikriti Vigyana, Microbiology Laboratory of 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 to assess the growth on the next day. Microscopic view of the sample was checked on the day of microbial growth and all above mentioned procedures were done in aseptic precautions for better result.

Fig 1(a):*Patra* coarse powder

Fig 1(b): Distillation process

Fig 1(c): *Patra arka*

Figure 1(a-c): Preparation of *Patra 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*

Fig 3: Preparation of sample

Fig 4: Preparation of media

Fig 5: Streaking of sample on plate

Fig 6: No growth on SDA media till 21 days

Fig 7: Growth on SDA media after 21 days

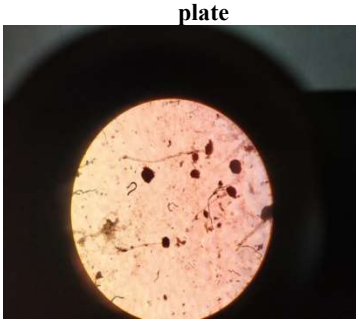

Fig 8: Microscopic view

OBSERVATIONS AND RESULTS

Patra arka was colourless liquid with an aromatic characteristic odour and noticed a layer of volatile content on the surface which possessed *katu rasa* (pungent taste) with little tingling sensation on taste and pH of 4.69 **(Table 2)**.

Triphala kwatha prepared was brown in colour possessed characteristic odour with *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 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 and 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 as it is one of the most common species of the genus Aspergillus belongs to Trichocomaceae family **[10]**. It is commonly known to cause food contaminations or food spoilage. No growth was observed in the plates of MHA media **(Table 5)**.

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

Table 1: Observations during the preparation of *Patra arka*

Parameters	*Patra Arka*
Drug quantity (v/v)	100ml (17.64g)
Water	300ml
Proportion (drug: water)	1:3
Initial temperature	60^0
Maintained temperature gradient	40^0- 60^0
Starting time	9:20am
Time of first drop	9:40am
Ending time	12:10pm
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 *Patra arka* and *Triphala kwatha*

Sl. No	Particulars	*Patra arka*	*Triphala kwatha*
1	Appearance	Transparent liquid comparatively less volatile content	Brown liquid
2	Colour	Colorless liquid	Brown
3	Odour	Aromatic characteristic odor	Characteristic odour
4	Taste	*Katu rasa pradhana* with little tingling sensation	*Tikta rasa pradhana kashaya rasa*
5	pH	4.69	3.14
6	Specific gravity	1.0009	1.0608
7	Viscosity (Pa.s)	0.0060	0.0137
8	TSS (Total Suspended Solids) mg/l	0.1	17
9	Refractive index	1.34	1.36
10	Volatile oil estimation (%)	0.03%	-

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
***Patra arka* in *Triphala kwatha* added in 15% concentration**	**8/10/2020 (pH: 2.75)**	**30/10/2020 (pH: 2.69)**	**21 days**	**30/10/2020**	***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 patra* odour	Fruity odour
Appearance	Liquid	Thicker mucous liquid
pH	2.75	2.69

DISCUSSION

The drug *patra* possess anti-bacterial, anti- fungal and anti- oxidant properties which have positive impact for the study **[11, 12, 13]**. In the classics many references are available which explains the *gunaadi karma* of the drug *patra*. Owing to these properties, this study was taken up to assess the extent or days of preservative action of *patra* drug in its *arka* form by extracting volatile content to check the probable preservative action in *triphala kwatha*.

Patra arka was prepared by following 1:3 ratio of drug and water considering v/v measurement. During the preparation, size reduction of drug was done into coarse powder which helps to increase the surface area of the drug for the active principles to be dissociated into water. Hence soaking was done overnight to make the drug soft and this helps in release of essential volatile principles while boiling as the drug *patra* has volatile content. Next day distillation process was started by adding remaining amount 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 *katu rasa* in predominance with little tingling sensation because of the higher concentration of constituents present in the drug.

Yavakuta churna (coarse powder) of *triphala kwatha* was used for the preparation 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*.

Patra arka had pH of 4.69 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 **[14]**. *Triphala kwatha* had pH of 3.14, which was also acidic in nature; this also influences on the rate of oxidation. Specific gravity of *patra 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 *patra 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. *Patra 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 *patra arka* in *triphala kwatha*. *Arka* showed preservative action for 21 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 *patra arka* could have also influenced over the rate of microbial growth. *Patra arka* was highly acidic in nature and the sample showed the pH of 2.75 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 *patra arka* showed preservative action for 21 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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