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PRESERVATIVE ACTION OF ELA ARKA IN TRIPHALA KWATHA- MICROBIOLOGICAL STUDY

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ABSTRACT

Introduction: Kwatha is a preparation which is easily prone to contamination and can be marketed only by the addition of suitable preservatives to increase the shelf life where the commonly used preservatives like sodium benzoate, etc. are proven to have carcinogenic and toxic effects over the body. So there aroused a challenge to find a natural preservative. Arka preparation is a product of distillation that does not undergo decomposition easily and stays for longer duration. Triphala is a combination of three fruits used in the treatment of several diseases due to its various pharmacological activities. Hence an attempt was made in this study to evaluate the preservative action of ela in the arka form in triphala kwatha. **Materials and methods:** Includes preparation of ela arka, triphala kwatha, sample and the conduction of microbiological study to see the preservative action using SDA and MHA media. **Observations and results:** The microbial load was assessed by assessing the growth over the media plates. Study includes observations over microbial growth of the sample on daily basis where the ela arka showed preservative action for 34 days. *Aspergillus niger* was the fungal growth observed on 35th day. **Discussion:** Drug ela possess different properties which have positive result for the study. Ela arka owing to its pH, chemical components and essential oil concentration preserved

the triphala kwatha for a stipulated period of time. **Conclusion:** From this study, it was concluded that ela arka was stable and preserved the triphala kwatha without any microbial contamination for 34 days which was added in the concentration of 15%.

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

INTRODUCTION

Preservatives are substances which are commonly added to various foods and pharmaceutical products to prolong their shelf life. They have been used since prehistoric times. A preservative is a substance or a chemical which is added to the products includes food products, beverages, pharmaceutical drugs, paints, biological samples, cosmetics, wood and many other products to prevent decomposition by microbial growth or by undesirable chemical changes **[1]**. Commonly used chemical preservatives are proven to have carcinogenic and allergy inducing properties **[2]**. So there arises a need to find a natural preservative.

Kwatha is the liquid preparation mentioned in Ayurveda Pharmaceutics, where it can be used for both internal and external purposes. In classics, Acharya have mentioned different ratio of drug and water depending on the nature of the drugs used for the preparation of kwatha **[3]**. It is said to be consumed instantly in its fresh form itself as it falls under Panchavidha kashaya kalpana and the shelf life of kwatha in general is said to be 24 hours **[4]**. As it has to be consumed instantly, adding of preservatives to prolong its shelf life for marketing for longer duration is practiced judiciously.

Arka 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 **[5]**. It is a water distillate which has longer shelf lifeand it is a preparation where through distillation method, essential oils from herbal drugs are extracted **[6]**.

Ela (*Elettaria cardamomum* Maton) is a well-documented aromatic condiment in Indian medicine and tradition belongs to Zingiberaceae family which is one among the Chaturjataka **[7]**. Arka of chaturjataka is said as vishapaha in nature mentioned in Arka Prakasha. Description of ela arka is mentioned in the text of Arka Prakasha written by Ravana. Ela arka possess tikta (bitter) rasa, katu (pungent) vipaka, pitta-kaphahara (alleviates pitta and kapha) and deepana (digestive) action, indicated in

mutrakricchra (dysuria). Components present in ela arka are D-limonene, cyclohexene methanol, naphthalene, etc. which are proven anti- microbial in nature. Triphala kwatha which is most commonly used has many therapeutic actions and can be prepared by drugs which are easily available. Owing to these, in this study an attempt was made to prepare ela arka and to evaluate its preservative action in triphala kwatha.

MATERIALS AND METHODS

It is divided into following sections

1. Preparation of ela arka
2. Preparation of triphala kwatha
3. Preparation of sample
4. Preparation of media
5. Microbiological study

The raw drug for the preparation of ela arka and triphala kwatha were ordered and procured from C K Kumaran Memorial (CKKM) Pharmacy, Tripunithura, Kerala and authentication was also done from the same.

Preparation of ela arka [8]: The preparation of ela arka was done under aseptic conditions by following the ratio of 1:3 (drug: water) and 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 ela 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 the Arka yantra (distillation apparatus) was set and heating was started. The heat given was 60^0 initially; once it started boiling temperature gradient was maintained between 40^0- 60^0 during the procedure. Initial few drops of ela arka were discarded as it may not contain therapeutically essential substances and the process of distillation of ela arka was continued till 30% of the distillate was collected.

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

Preparation of sample: To the 50ml of triphala kwatha, 7.5ml of ela arka was added in the concentration of 15% based on the previous research study **[9]**.

Preparation of media [10]: For this study to see the fungal and bacterial growth, Sabouraud Dextrose Agar (SDA) medium, Mueller Hinton Agar (MHA) medium were used 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 121^0C.

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). To test the microbial contamination, streaking method was adopted on both media using inoculum loop 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): Ela coarse powder

Fig 1(b): Distillation process

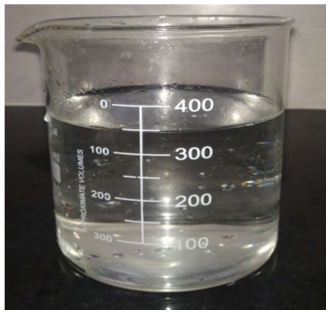


Fig 1(c): Ela arka

Figure 1: (a to c): Preparation of Ela arka

Fig 2(a): Kwatha churna of triphala

Fig 2(b): Process of boiling

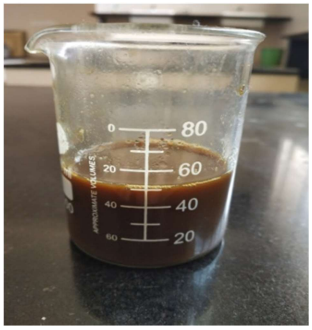


Fig 2(c):Triphala kwatha

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

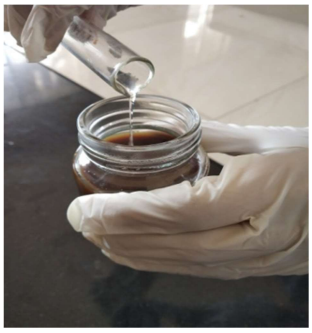
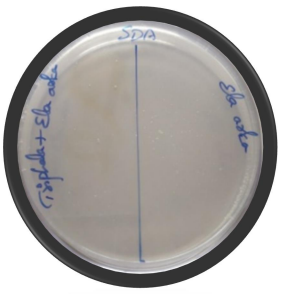


Figure 3: Preparation of sample

Figure 4: Preparation of media

Figure 5: Streaking of sample on plate



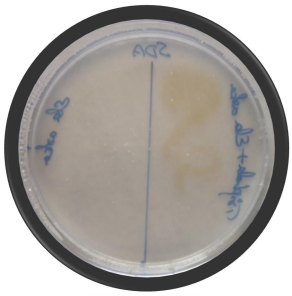


Figure 6: 24 to 48 hours- no growth on SDA media

Figure 7: 35th day- growth

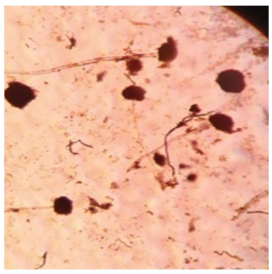


Figure 8: Microscopic view

OBSERVATIONS AND RESULTS

Obtained ela arka was colorless liquid with an aromatic characteristic odor had a layer of volatile content which forms oily droplets above the surface possessed katu rasa (pungent taste) with strong tingling sensation on taste and had pH of 2.75 **(Table 1)**.

Triphala kwatha obtained was brown liquid possessed characteristic odor, tikta rasa pradhana kashaya rasa (prominent bitter taste with slight astringent taste) on taste and had pH of 3.14 **(Table 2)**.

Microbiological study: The study was conducted by keeping the sample in sterile autoclaved glass bottle in normal room temperature for storage. Two media used include SDA and MHA 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 3)**.

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 which was found when viewed under microscope. It is one ofthe most common species of the genus Aspergillus belongs to Trichocomaceae family **[11]**. It is commonly known to cause food contaminations or food spoilage. No growth was observed in the plates of MHA media.

Monitoring of color, odor, appearance and pH parameters were done for the sample on the day of preparation and after spoilage **(Table 4)**.

Table 1: Observations during preparation of ela arka

Parameters	Ela arka
Drug quantity (v/v)	100ml (54.15g)
Water	300ml
Proportion (drug: water)	1:3
Initial temperature	60^0
Maintained temperature gradient	40^0- 60^0
Starting time	2:30pm
Time of first drop	2:41pm
Ending time	4:45pm
Distillate obtained	90ml
% obtained	30%

Table 2: Observations during 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: 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
Ela arka in triphala kwatha added in 15% concentration	8/10/20 (pH: 2.70)	12/11/20 (pH: 2.01)	34 days	12/11/20	*Aspergillus niger*	No growth	No growth

Table 4: Observations of the sample for stability

Parameters	Sample	
	Day of preparation	After spoilage
Color	Brown	Dark brown
Odor	Kwatha ela odor	Fruity odor
Appearance	Liquid	Thicker mucous liquid
pH	2.70	2.01

DISCUSSION

Study includes the arka of ela to see the effect as a preservative in triphala kwatha. Ela possess different properties which have positive result for the study like antimicrobial and antioxidant properties. In classics also many references are available that explains the gunaadi karma of ela drug. Owing to these properties, this study was taken up to assess the extent or days of preservative action of ela drug in its arka form by extracting volatile content to check the probable preservative action in triphala kwatha.

Ela arka was prepared by taking 1:3 ratio of drug and water considering v/v measurement. 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 and it was used for the preparation of arka. It is advised to soak the powdered drugs for sometime before boiling as the drug is soaked; the tissue swells up because of the cell wall of drug takes up the liquid **[12]**. So soaking was done overnight to make the drug soft and this helps in release of essential volatile principles while boiling as the drug is rich in volatile content in varied proportions. Next day remaining amount of water was added and kept for distillation process. 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 colorless liquid possessing characteristic aromatic odor with layer of volatile content which forms oily droplets above the surface.

Use of yavakuta churna (coarse powder) for the preparation of triphala kwatha facilitates the proper absorption of water soluble principles. In this study, 1:4 ratio of drug and water was used and reduced to $1/4^{th}$ of the total volume. Triphala kwatha obtained was brown in color, possessing characteristic odor.

Ela arka had pH of 2.15 suggestive of highly acidic nature, more acidic nature also act as preservative. Also the efficacy, absorption, irritability depends on pH of a drug. It also influences on the rate of oxidation. More acidic pH results in lesser oxidation suggestive of acidic nature reduce the growth of micro-organisms **[13]**. Triphala kwatha had pH of 3.14, which was also acidic in nature; this also influences the rate of oxidation.

Microbiological study was conducted to test the microbial contamination of the sample and to check the number of days of preservative action of ela arka in triphala kwatha. Ela arka showed preservative action for 34 days without any microbial contamination. As the ela arka was highly acidic in nature this could have reduce the rate of oxidation thus acidic nature helps in reducing the growth of microbes. The drug is a rich source of essential oil; this could have also impacted on the reduction and the components present in ela arka could have also influenced over the rate of microbial growth.

CONCLUSION

In this research work an attempt was made to evaluate the efficacy of ela arka as a preservative in triphala kwatha and to check the duration of its preservative action. Ela arka was stable for 34 days without any microbial contamination which was added in the concentration of 15%. *Aspergillus niger* was the fungal growth observed which is a common food spoilage organism.

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REFERENCES

[1] Russell NJ, Gould GW. Food Preservatives. Springer Science & Business Media; 2003 Jul 31.

[2] Aledwany A Z, Basal W T, Al-Senosy N K, Issa A M. Evaluation of the Cytotoxicity, Cell Cycle Perturbations and Apoptotic Induction in Human Normal and Cancer Liver Cell Lines Exposed to Potassium Nitrate and Sodium Benzoate. Egyptian Academic Journal of Biological Sciences, B. Zoology. 2018 Jun 1; 10(1): 105-18.

[3] Mishra Dwarakaprasad. Sharan-gadhara Samhita. First edition. Varanasi: Chaukhamba Sanskrit Series; 2010: 322.

[4] Acharya Sharangadhara, Shastry P. Sharangadhara Samhita with Deepika and Gudarthadipika commentary, 7th ed. Varanasi: Chaukhambha Orientalia; 2002: 145.

[5] Government of India Ministry of Health & Family Welfare. The Ayurvedic Formulary of India. First English edition. New Delhi. The Controller of Publications Civil Lines, Delhi; 2000: Part II.41.

[6] Rakshitha D, Hussain Gazala. Preliminary pharmaceutico analytical study of trisugandhaarka. IJAAR. 2020; 4(11): 1331-36.

[7] Rakshitha D, Balagopalan Devika, Hussain Gazala. A Review on Chaturjataka. IJRAR. 2020; 7(4): 510-19.

[8] Government of India Ministry of Health & Family Welfare. The Ayurvedic Formulary of India. Second edition. New Delhi. The Controller of Publications Civil Lines, Delhi: Part I.27.

[9] Devika Balagopalan. Pharmaceutico Analytical Evaluation of Chaturjataka Arka as a preservative for Triphala Kashaya. Sri Dharmasthala Manjuna-theshwara College of Ayurveda and Hospital, Hassan. 2019: 25.

[10] Pooja B. Modification of Kasisadi Churna to Varti, its physicochemical analysis and invitro study against *Candida albicans*. Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan. 2020: 73.

[11] Schuster E, Dunn-Coleman N, Frisvad J C, Van Dijck P W. On the safety of *Aspergillus niger*– a review. Applied Microbiology and

Biotechnology. 2002 Jan; 59(4): 426-35.

[12] Devika Balagopalan. Pharmaceutico Analytical Evaluation of Chatur-jataka Arka as a preservative for Triphala Kashaya, Sri Dharm-asthala Manjunatheshwara College of Ayurveda and Hospital, Hassan. 2019: 60.

[13] Oladipo I C, Adeleke D T, Adebiyi A O. The effect of pH and chemical preservatives on the growth of bacterial isolates from some Nigerian packaged fruit juices. Pakistan Journal of Biological Sciences: PJBS. 2010 Jan 1; 13(1): 16-21.