Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [9] August 2023 : 06-10

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Preparation & Standardization of Tulasi Arka: An Ayurvedic Formulation

Anjali[1*], Vijayalaxmi M[2], Shailaja U[3].
1; 2; 3: Department of Kaumarabhritya Sri Dharmasthala Manjunatheswara College of Ayurveda & Hospital, Hassan, Karnataka.
*Corresponding Author: anj3126@gmail.com

ABSTRACT
Tulasi (Ocimum sanctum L) belongs to the family of Lamiaceae and it contains abundant source of volatile oils. Arka a transparent liquid prepared through the process of distillation of specific drugs or liquids by using Arkayantra for soaking. Tulasi arka contains Tulasi, Katu-Tikta Rasa, KatuVipaka and Ushna Viryaand Laghu-Ruksha Guna. This combination is useful in treating pratishyaya (common cold) in children. Ayurvedic formulation standardization is an mandatory aspect in establishing the chemical profile, quality assurance and biological activity for herbal drugs production. Therefore, the aim of this study was to standardize Tulasi arka using a standardized testing protocol for Ayush Drugs. Physicochemical, proximate and chemical studies, such as the assay for essential oils, refractive index, optical rotation, viscosity, total acidity, and microbial contamination, were conducted according to an well-established methodology. Chemical and physical tests for indicating quality were conducted and the standard values were recorded for Tulsi arka. Authentication of herbal preparation and quality assurance was confirmed through standardization tests performed on Tulsi arka.
***Key words**: Tulasi arka; Distillation process; Quality parameters; Standardization; Volatile Oil.*

Received 10.04.2023 Revised 19.05.2023 Accepted 29.07.2023

INTRODUCTION

Tulasi, a renowned medicinal herb in Ayurveda, has a wide range of applications. Known as the 'queen of herbs'[1], Tulasi has been revered as a sacred medicinal plant [2] since ancient times and continues to be highly valued in the present era. However, when it comes to administering medicines to children, palatability becomes a crucial factor. This becomes challenging when prescribing bitter-tasting Ayurvedic formulations such as swarasa, choorna, vati, kashaya, etc., as children often struggle to take them. To address the issue of palatability and acceptability in children, the medicine is planned to be administered orally in the form of arka.

Tulasi possesses the qualities of katu and tikta rasa, katuvipaka, laghu-ruksha guna and ushna virya [3]. It pacifies the kapha and vata doshas i.e. the predominant doshas included in pratishyaya (childhood rhinitis). The oral administration of Tulasi arka facilitates the vilayana (dispersion) of kapha due to the vyavayi and vikasiguna (dispersive and penetrative properties) of arka. Hence, the oral administration of Tulasi arka in Pratishyaya was planned.

To determine the quality and purity of any drug or formulation standardization is must. hence, the present study is aimed to prepare Tulasi arka and analyze its analytical parameters according to standard protocols. The objective was to establish a monograph for Tulasi, providing comprehensive information about its characteristics and standardization.

MATERIAL AND METHODS

In the present study, Tulasi arka was prepared and standardized based on its physical, physicochemical properties, and phytochemical analysis. Additionally, microbial contamination and total fungal count were conducted to ensure compliance with the permissible limits set by AYUSH and WHO standards [4].

Method of Preparation:

Arka, the medicinal liquid preparation, was meticulously prepared using the distillation process with the aid of a specialized apparatus called Arka Yantra. This involved distilling water-soaked raw drugs, where

the volatile constituents released during the process mixed with water vapor and were subsequently condensed and collected.

In case when fresh and wet drugs are utilized for Arka preparation, then the same has to be slashed into small pieces. In the case of dry drugs, they are first transformed firstly in the form of coarse powder followed by soaking in water, and then thoroughly mixing the same by hand. The resulting mixture is then placed in a stainless-steel container and allowed to sit for approximately 24 hours. Afterward, the contents, along with water, are transferred to the still for the distillation process. This step serves for drugs softening and helps in facilitating the release of volatile principles release through distillation. In Arka Prakasha, the detailed instructions provided for adding the water quantity in a precise manner [5] and the recommended soaking duration for different categories of drug, ensuring the optimal extraction of medicinal properties by Ravan [6].

Atyantakathinadravyas: To prepare Arka from these ingredients, the dry drugs are transformed into a coarse powder. This powder is then soaked in double the quantity of water and left in a shaded area until the water evaporates and the drug becomes soft. Next, an equal amount of water is added to the softened drug mixture. The entire mixture is then exposed to both sunlight and moonlight for a duration of 8 praharas, equivalent to twenty four hours. Further the same is transferred to Arka yantra for Arka extraction.

Ardradravya: The term 'Ardra' refers to wetness, and Arka can be prepared in two forms: Sarasa and Nirasa. Sarasa indicates the presence of juice in the drugs. To prepare Arka from Sarasa drugs, a churna (powder) is created by using the stems (naala) of the said drugs. Subsequently, adding water in one twentieth part to the churna and subsequent heating for a duration of 1 muhurtha (48 minutes). On the other hand, Nirasa denotes drugs that are juiceless, preparation the Arka of the said drugs adding water in one twentieth part and subsequent soaking of the mixture for 3 hours i.e. 1 prahara. In the case of Pallavadravya, which refers to leaves, the Arka preparation involves adding water in the quantity of one hundredth part to the leaves than subsequent soaking for 24 minutes. Afterward, the soaked mixture is transferred to the Arka Yantra for further processing and extraction.

Dravadravya – For preparation of Arka from liquid drugs, there is no need of adding water. Filling of drugs in Arka yantra is done for preparation of Arka.

Process Completion: The Arka preparation completion process is indicated through the emergence of dark smokes from the exhaust. Once this phenomenon is observed, it is important to refrain from any additional effort for heating and collecting the remaining condensate. This serves as a clear indication that the extraction process has reached its conclusion. Typically, the obtained distillate volume is approximately three fourth of the volume of the mixture of water and drug drawn for distillation initially. This proportion provides a general guideline for estimating the yield of the final Arka product.

Final product: The resulting Arka is a distillate suspension in water, exhibiting a subtle haziness. Specific characteristics of the crude drugs used for the process of distillation contribute to distinct color and aroma of the Arka. Thus, each Arka formulation possesses its unique hue and fragrance, reflecting the nature and properties of the herbs involved.

Prashastha Arka:

Arka is expected to possess a heightened fragrance compared to its individual constituent dravyas when taken separately. When stored in jeernasthimrittikapatra (an earthenware vessel), the color of the Arka should remain consistent, resembling that of the original drug. Furthermore, when transferred to other vessels, the Arka should evoke imagery reminiscent of a Shankha, Kunda, or Indu. In terms of taste, when the Arka is placed on the tongue, it should impart the distinct flavor of the constituent drug itself, maintaining the essence and identity of the herb in the liquid form.

Tulasi arka was meticulously prepared under aseptic conditions, with the ratio of 1:5 (drug : water). Fresh & cleaned leaves of Tulasi were carefully selected and coarsely powdered. A round bottom flask is used for placing the coarse powder for subsequent soaking with an appropriate amount of water for ensuring complete saturation of drug. This mixture was left to steep overnight, allowing for the extraction of the medicinal properties. The following morning, the remaining quantity of water required for the distillation process was added to the flask. The Arkayantra, a specialized apparatus for distillation, was carefully set up, and the process of heating was initiated. Throughout the procedure, temperature control was maintained through a controlled temperature gradient. During the initial stages of distillation, initial few drops of Tulasi arka should be discarded as it may not contain therapeutically essential substances. Distillation process should be continued until the collection of approximately 30% of the distillate. Arka collected was then carefully stored in a sterile, airtight glass bottle, ensuring its preservation. To evaluate the quality of the Tulasi arka, various organoleptic characters such as appearance, taste, color, and odor were assessed. Additionally, physicochemical parameters including pH, viscosity, total suspended solids,

specific gravity, refractive index, and estimation of volatile oil were analyzed following standard protocol outlined in the CCRAS guidelines [7].

Table No.1 summarizes the observations during the Tulasi Arka preparation. Table No.2 provides a brief summary of analytical test parameters.

Shelf life of Arka : 12 months from the date of preparation. (Rule 161-B of D & C Act)

Dose: API: 10 to 20 ml / day in 2 divided doses.

AFI: 12 to 24 ml / day in 2 divided doses.

Standardization means confirming the identity of a drug and determination of its quality and purity. The standardization of herbal drug is the outcome of standardized and therapeutically effective Ayurveda formulations for further usage [8]. CCRAS guidelines have been proposed for the different dosage forms [9]. So this study attempts to prepare *tulasi arka* and carrying out its analytical study for further documentation.

Organoleptic characters:

Color, odor and taste of sample are noted using sensory organs

Volatile matter:

10 ml of the Tulasi arka sample was subjected to extraction twice using 20 ml of n-hexane. The portion of the sample soluble in hexane was carefully transferred into a pre-weighed china dish. The hexane solvent was then evaporated at room temperature, allowing for the removal of any residual liquid. The resulting weight difference observed in the china dish was used for calculation of volatile matter content existing in the Tulasi arka sample.

Specific gravity:

The specific gravity bottle got properly disinfected firstly with acetone followed by ether. Afterward, the bottle was dehydrated and then the weight of the same was recorded. Next, the sample solution is left so as to label at the room temperature. Followed by pouring test liquid taking care to avoid any spillage, sealed with the stopper, and any excess liquid on the exterior of the bottle was carefully removed. Then weight is recorded. The procedure was repeated using distilled water in lieu of sample solution.

Refractive index:

A water droplet was placed on the prism and calibrated drive knob in a manner that the boundary line traverses the separatrix certainly at the centre. The reading was recorded. The refractive index of distilled water is 1.33206 at the temperature of 29˚C. The difference between the recordings and 1.3320 provides an error of the instrument. If the recording is lower than 1.3320, than the inaccuracy or error is negative (-) and the correction is positive (+) and vice-versa. One drop of sample is used for determination of refractive index. In case of any correction the same should be included in the measured recording for obtaining precise refractive index. The test samples reg=fractive index was recorded at 29˚C.

pH Determination:

Preparation of buffer solutions: Standard buffer solution: 100 ml quantity of distilled water was used for dissolving the tablet of pH 9.2, 7 and 4.

pH Determination: 10 ml volume was prepared by diluting 1 ml of sample with distilled water. The said composition was blended followed by filtration. The experiment was performed using the said filtrate. After switching on the instrument half an hour duration wait time was there for warming up the pH meter. Firstly there was an addition *of solution having* pH value as 4 and subsequently the same was altered to 4.02 at the temperature of 30°C using a knob. Further to this solution with pH 7 was added and knob was used to adjust the pH meter at 7. Next to this solution with pH 9.2 was added and pH reading was recorded without using knob. Lastly the sample solution was added and the readings were recorded. The same process was repeatedly done for four times and the average readings were recorded.

Total Acidity:

In a titeration flask one gram of sample was dissolved in 75 ml of CO_2 free water. The same has to be blended properly and titerated against standard sodium hydroxide solution after that four to six drops of phenolphthalein was added till the appearance of pink color at least for 10 seconds.

% Acidity = 0.23 X . V/M

V = Corrected volume of 0.05 NaOH used

M = sample weight in grams

Boiling Point:

A test tube serves as an excellent boiling tube. A capillary tube with a broken half is used as both a boiling stone and a vapour trap. The capillary tube's closed end remains at atmospheric pressure, aiding in the determination of a narrow boiling point range. The boiling tube (consisting of liquid sample inside) with caterpillar tube was placed closed end up. The vapour pressure increases due to the heating of liquid sample and will continue to increase until it labels the surrounding atmosphere pressure. As the vapour pressure equates the vapour inside the capillary tube results into small stream of bubbles eliminating from

the open end. The said reason of this elimination of bubbles is reaching of liquid to the boiling point. When heating is stopped the temperature of sample also comes down because of the reduction in temperature of heating bath. When both the heating bath and the sample reach the temperature exact to that of the boiling point, the vapour pressure of the liquid within the capillary tube becomes equal to the pressure of atmosphere above the boiling tube. Consequently, the bubbles stream also stops as the vapour pressure of the liquid now matches the atmospheric pressure. This indicates the attainment of boiling point. Thermometer can now be used to record this characteristic temperature.

Viscosity:

A U tube viscometer is used for filling the sample according to the expected liquid viscosity so that when the capillary is kept vertically and the test liquid attains the specific temperature the fluid level filling marks should stand within 0.2 mm in the viscometer. The liquid is sucked or blown to the specified height of the viscometer and the time taken for the sample to pass the two marks is measured. Viscosity is measured using the following formula:

$$\eta_1 = \rho_1 t_1 / \rho_2 t_2 * \eta_2$$

η_1: Viscosity of sample

η_2 : Viscosity of water

t_1 and t_2 : Time taken for the sample and water to pass the meniscus

ρ_1 and ρ_2 : Density of sample and water

The given sample of Tulasi arka has been standardized as per standard testing protocol. The results of standardization parameters are represented in respective Table No-2.

Preparation of Casein Soyabean Digest Agar Medium (CSDAM):

A combination of five grams of soya peptone, fifteen grams of casein peptone and five grams of sodium chloride was dissolved in 990 ml of distilled water, subsequently the pH was adjusted to 7.3 (+/-) 0.2 and a final volume of 1000 ml was made. At last fifteen grams of agar was added to the media and 20 minutes autoclavation was done at a temperature of 121°C. Results of the same are shown in Table-3.

Preparation of Sabaud's Dextrose Agar:

Dissolving forty grams of dextrose, five grams of casein peptone and five grams of beef extract in 990 ml of distilled water followed by pH adjustment to 5.6 (+/-) 0.2 and subsequently making the total volume as 1000 ml. Lastly 15 grams of agar was added to media and autoclavation was done for twenty minutes at a temperature of 121°C.

Preparation of Buffered Sodium Chloride Peptone Solution (BSCPS) pH 7.0:

3.56 grams of Potassium dihydrogen phosphate, 1 gram of peptone, 4.3 grams of sodium chloride and 7.23 grams of disodium hydrogen phosphate was dissolved in 990 ml of distilled water followed by pH adjustment to 7 and subsequently making the total volume as 1000 ml. Autoclavation of this buffer solution was done for twenty minutes at a temperature of 121°C. Results of the same are depicted from Table-4.

RESULTS AND DISCUSSION

Arka Kalpana holds significant importance and is considered the best among the five types of Kashaya Kalpana, as mentioned in Arka Prakasha. Distillation process can be linked to Arka Kalpana. Distillation involves separating the components of a liquid by vaporizing it; passage of vapours through a cold surface, and converting them back into liquid status. Arka Prakasha asserts that Arka possesses greater potency compared to other Kalpanas. This is attributed to its absence of doshas (impurities) and its specific qualities. Due to its heightened potency, faster absorption, and easy palatability, Arka is particularly suitable for pediatric use. Additionally, Arka has a longer shelf life, allowing its storage for a longer duration. During the preparation of Kashaya, the yield is only one fourth of the initial water quantity used. However, in the case of Arka, the yield is approximately three forth of the initial water quantity. Arka is obtained through a simple distillation process [10], eliminating the need for preservatives required in the preparation of Kashaya.

Table. No 1: Showing Observations during *Tulasi arka Preparation*

Observation	*Tulasiarka*
Drug quantity (v/v)	400 ml (216.6g)
Water	1200 ml
Proportion (Drug : Water)	1:5
Temperature gradient	Between 40^0-60^0
Starting time for preparation	3:30 pm
Closing time for preparation	5:45 pm
Distillate obtained	150 ml
% obtained	30%

Table No.2: Organoleptic characters and Standardisation

Parameter	*Results n = 3% w/w*
	Tulasi arka
Color	Colorless
Odour	Pleasant, sweet
Taste	Pungent
Volatile matter (%)	0.12
Specific gravity	0.9856
Refractive index	1.33267
pH	7.0
Total acidity	0.02
Boiling point	100^0C
Viscosity	1.06

Table No-3: Tulasi Arka Microbial load analysis

S. No.	Dilutions	Number of Colonies(NOC)		CFU/ml
1	Direct	0	0	0

Table No.4: Total Fungal Count of Tulasi Arka

S. No.	Dilutions	Number of Colonies (NOC)		CFU/ml
1	1/10(10^1)	0	0	0
2	1/100 (10^2)	0	0	0
3	1/10000(10^4)	0	0	0

CONCLUSION

Tulasi Arka obtained was a transparent liquid with a characteristic aromatic fragrance, mainly composed of volatile oils. Various physico-chemical parameter was performed and analyzed. The pH of Tulasi Arka shows its highly acidic nature, attributed to the presence of volatile matters in the form of essential oil, as the herb itself is rich in volatile oils. Other parameters were also analyzed to get better insights of its properties.

The preparation of Tulasi Arka is simple, and due to its favorable properties, it can be utilized in the treatment of numerous diseases. The formulated Arka was evaluated for its Arka parameters, phytochemical properties, and antimicrobial properties. All the quality control Arka parameters fell within the acceptable limits. The microbiology parameters, such as the total aerobic count and total fungal count, complied with the standards set by the Indian Pharmacopoeia.

REFERENCES

1. Sastry, J. L. N. (2012). Dravyaguna Vijnaana. Reprint edition; Varanasi: Chaukhambha Orientalia; Vol. II, p.527.
2. Indian Medicinal Plants (2006). Arya VaidyaSala, Kottakkaled, Chennai: Orient Longman Private Limited, Vol. 2, p.360.
3. Rakshitha D., Devika Balagopalan, Hussain Gazala (2020). A Review on Chaturjataka, IJRAR, 7(4): 510-19.
4. Sastry, J. L. N. (2012). Dravyaguna Vijnaana. Reprint edition; Varanasi: Chaukhambha Orientalia; Vol. II, p.528.
5. Government of India Ministry of Health & Family Welfare (2000). The Ayurvedic Formulary of India, First English edition, NewDelhi. The Controller of Publications Civil Lines, Delhi, PartII, p.41.
6. Ravana (1990). Arka Prakasha with Mukunda Ram Pandit Hindi Commentary, ArkaPrakasha, Mumbai, Ganga Vishnu Srikrishnadas Prakashana, 59.
7. CCRAS, Laboratory Guide for the Analysis of Ayurveda and Siddha Formulations (2000). First edition, New Delhi.
8. CCRAS. (2022). Department of AYUSH, Ministry of Health and Family Welfare. Government of India, Ministry of Health & Family Welfare. The Ayurvedic Formulary of India, Second edition, New Delhi.
9. The Controller of Publications Civil Lines, Delhi: Part I, p.27.
10. Devika Balagopalan (2019). Pharmaceutico Analytical Evaluation of Chaturjataka Arka as a preservative for Triphala Kashaya, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan, p.60.

CITATION OF THIS ARTICLE

Anjali, Vijayalaxmi M, Shailaja U. Preparation & Standardization of Tulasi Arka: An Ayurvedic Formulation. Bull. Env. Pharmacol. Life Sci., Vol 12[9] August 2023: 06-10.