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ORA- Analytical study

Pharmacognostical, Pharmaceutical and Analytical Standardization of Shirishadi Arka through Physicochemical Evaluation, HPTLC Profiling, GC MS Characterization and Safety Assessment.

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ABSTRACT:

Background: Standardization of Ayurvedic formulations is essential to ensure their safety, quality, and reproducibility. *Shirishadi Arka* is an *Anubhuta Yoga* traditionally indicated in respiratory disorders such as *Shwasa* and *Kasa*. **Aim:** To prepare *Shirishadi Arka* and establish its pharmacognostical, pharmaceutical, and analytical standardization using modern scientific parameters. **Objectives:** To understand the preparation of *Shirishadi Arka* by method of distillation. To determine the analytical tests like physico-chemical parameters, and tests for heavy metals to assure to evaluate the pharmaceutical and analytical standardization. Gas chromatography-mass spectrometry analysis to investigate potential properties of *Shirishadi Arka*. **Material and Methods:** The formulation was prepared by classical distillation (*Arka Kalpana*) using *Shirisha* (*Albizia lebbeck*), *Musta* (*Cyperus rotundus*), and *Kantakari* (*Solanum xanthocarpum*). Pharmacognostical evaluation was carried out for authentication of raw drugs. Physicochemical parameters, microbial load, heavy metal analysis, pesticide residue were performed as per API guidelines. High Profile Thin Layer Chromatography (HPTLC) was employed for preliminary fingerprinting, while Gas Chromatography–Mass Spectrometry (GC–MS) analysis was conducted for phytochemical profiling. All experiments were performed in triplicate (n = 3), and results were expressed as mean ± standard deviation. Analytical methods were validated for precision and repeatability. **Results:** The formulation complied with API standards. Physicochemical parameters showed pH 7.00 ± 0.05, specific gravity 1.0033 ± 0.002, and total solid content 0.052 ± 0.003 % w/v. Microbial, heavy metal and pesticide residue analysis were within permissible limits. HPTLC analysis demonstrated distinct chromatographic patterns. GC–MS analysis identified 50 phytoconstituents, including fatty acids, terpenoids, and phytosterols such as n-hexadecanoic acid, γ-tocopherol, and β-sitosterol derivatives. %RSD values were within acceptable limits, indicating good reproducibility. **Conclusion**: The study establishes a comprehensive standardization profile of *Shirishadi Arka*. The presence of bioactive compounds with known anti-inflammatory and antioxidant properties supports its traditional use in respiratory disorders such as COPD (*Tamaka Shwasa*).

KEYWORDS: Analytical Study, COPD, GC-MS, HPTLC, Pharmacognostical Study, Pharmaceutical Study, *Shirishadi Arka*, *Tamaka Shwasa*

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1. INTRODUCTION

Ayurveda describes respiratory disorders under the broad spectrum of *Shwasa and Kasa Roga*, wherein *Kapha-Vata Dosha* vitiation predominates, affecting the *Pranavaha Srotas* (Respiratory system). COPD is characterized by progressive airflow limitation that is not fully reversible, associated with an abnormal inflammatory response of the lungs to noxious particles or gases. [1][2]. The breakdown of elastic fibres, so-called elastolysis, is one of the hallmarks of emphysema, an important phenotype contributing to COPD. [3] *Tamaka Shwasa* can be correlated to COPD as most of the signs and symptoms are similar. Classical texts advocate the use of herbal formulations possessing *Shwasahara* (Relieves dyspnoea)*, Kaphahara (Kapha-pacifying), Kasahara* (Antitussive), and *Rasayana* (Rejuvenation) properties for the management of chronic respiratory ailments. *Arka Kalpana* is a unique pharmaceutical dosage form in *Ayurveda*, prepared through the process of distillation, enabling the extraction of volatile and water-soluble phytoconstituents. *Arka* is prepared by combination of *Jala* and *Agni* (Under water and heat application) hence, it *is Laghupaki* (Easily metabolised)*,Vyavayi* (Rapidly diffusing before being digested) and *Vikasi* (Spreading) thus assimilates quickly in the body. [4] This dosage form offers advantages such as enhanced bioavailability, rapid absorption, longer shelf life, precise dosing, and improved patient compliance, aligning with modern pharmaceutics. However, there is limited scientific data on the pharmacognostical and analytical standardization of *Shirishadi Arka,* highlighting the need to ensure quality, safety, and reproducibility. Techniques like GC-MS play a crucial role in identifying bioactive constituents and understanding its potential in COPD (*Tamaka Shwasa*).

Aim of the study: To understand the efficacy of *Shirishadi Arka* in COPD (*Tamaka Shwasa*) through pharmaceutical analysis and Standardization by modern quality standards through comprehensive organoleptic, physicochemical, microbial analysis and GC-MS analysis.

Objective of study:

1. To determine organoleptic characteristics (Color, form, odor etc), physicochemical analysis and GC-MS Analysis.
2. To check for the safety of *Arka* with microbial limit test.
3. To analyze phytoconstituents of *Shirishadi Arka* in managing COPD (*Tamaka Shwasa*).

2. MATERIALS & METHODS:

Collection of raw drugs: All the raw drugs of *Shirishadi Arka* i.e., *Shirish (Albizia lebbeck (L.) Benth)*, *Musta (Cyperus rotundus Linn)* and *Kantakari (Solanum xanthocarpum Schrad and Wend)* were collected from the Pharmacy-ITRA, Jamnagar, Gujarat (GMP Certified Unit- license (Form 25-D) No.GA-2049), (FSSAI Reg. No. 20722028000726).

Supplier:

1. *Shirisha*- Raj Enterprises, Vadodara, Gujarat, 2. *Musta*- Natural Ayurved herbal company, Aligarh (UP), 3. *Kantakari*- A-Amritlal & company, Mumbai, Maharashtra

Batch no:

1. *Shirisha*- SH-001-04/25, 2. *Musta*- MU-001-04/25 3. *Kantakari*- KA-001-04/25.

All the raw materials were verified and authenticated at the Department of Pharmacognosy, ITRA, Jamnagar. After proper identification and assessment drugs were used for the *Arka* preparation.

Voucher specimen (herbarium) numbers of the drugs used in the study were properly retained and documented as follows:

1. *Shirisha* – ITRA/Phm/6779/2024–25
2. *Musta* – ITRA/Phm/6780/2024–25
3. *Kantakari* – ITRA/Phm/6781/2024–25

These voucher specimen numbers ensure traceability, authentication, and reproducibility of the plant materials used in the study.

Instruments used for the analysis:

1. GC-MS Analysis- GC–MS analysis was performed using Shimadzu (Model: GCMS-TQ8040). Calibration was carried out using perfluorotributylamine (PFTBA) as a tuning standard, and system suitability was verified by checking resolution, sensitivity, and peak symmetry.

2. Specific gravity- Specific gravity was determined using a calibrated pycnometer (25–50 mL capacity) at 25°C. The pycnometer was calibrated using distilled water of known density at the same temperature, and corrections were applied to account for temperature variations.

3. Tests for heavy metals- Atomic Absorption Spectrophotometer, Model: PerkinElmer Analyst 400, Detection limit -0.001–0.01 ppm. The instrument was calibrated using certified standard solutions of respective metals at different concentrations to generate calibration curves. Blank and quality control samples were analyzed to ensure precision and accuracy.

4. Tests for micro-organisms - It is done using sterile techniques in a laminar air flow cabinet, incubated in a temperature-controlled incubator (37 °C for bacteria, 25 °C for fungi), and observed under a compound microscope. The incubator was calibrated using a certified thermometer to maintain accurate temperatures (37°C for bacterial cultures and 25°C for fungal cultures).

5. pH Value- pH was measured by digital pH meter. The instrument was calibrated using standard buffer solutions of pH 4.0, 7.0, and 9.2 before each measurement to ensure accuracy and reliability.

6. Thin Layer Chromatography (TLC)- Silica gel 60 F254 aluminum plates that had been previously coated (Merck), and Camag twin trough chamber was used for development of plate and Relitech UV chamber was used for the detection of spots. The chamber was calibrated for wavelength accuracy (254 nm and 366 nm) using standard fluorescent markers to ensure proper detection of spots.

7. Pesticide residue- It was carried out by Gas Chromatography–Mass Spectrometry (GC–MS) and Liquid Chromatography–Mass Spectrometry (LC–MS) after suitable sample extraction and clean-up procedures. Instrument calibration was performed using certified multi-residue pesticide standards with matrix-matched calibration curves prepared at appropriate concentration levels prior to analysis.

Method of Preparation of *Shirishadi Arka*: *Shirishadi Arka* was prepared at the Pharmacy of ITRA following the API guidelines for *Arka Kalpana* (distillation). The raw drugs were cleaned, coarsely powdered, and soaked overnight in ten times the quantity of water. The soaked material was transferred to the *Arkayantra,* supplemented with the remaining water, and subjected to distillation. The initial and final fractions of the distillate were discarded, while the intermediate fraction containing the active principles was collected and pooled to obtain a uniform preparation. Six batches of *Shirishadi Arka* were prepared using the same procedure. [5]

Contents of *Shirishadi Arka*: It contains *Shirisha*, *Musta* and *Kantakari*. [6]

Pharmacognostical evaluation: As per the API standards, the drugs which were used in the formulation of *Shirishadi Arka was* identified and authenticated by the Pharmacognosy Laboratory, ITRA, Jamnagar. [10]

Table no 1: Contents of *Shirishadi Arka*

Name	Botanical Name	Family	Part use	Quantity
Shirisha	*Albizia lebbeck (L.) Benth*	Fabaceae	Stem bark	1 Part
Musta	*Cyperus rotundus Linn*	Cyperaceae	Rhizome	1 Part
Kantakari	*Solanum xanthocarpum Schrad and Wend*	Solanaceae	Whole plant	1 Part

Table no 2: Ayurvedic Parameters (*Rasa Panchaka*)

Drugs	*Rasa*	*Guna*	*Virya*	*Vipaka*	*Prabhava*
Shirish [7]	*Kashaya, Tikta, Madhura*	*Laghu, Ruksha, Tiksana*	*Anushna*	*Katu*	*Vishagna*
Musta [8]	*Tikta, Katu, Kashaya*	*Laghu, Ruksha*	*Sheeta*	*Katu*	*Kapha Vatashamaka*
Kantakari [9]	*Tikta, Katu*	*Laghu, Ruksha, Sara*	*Ushna*	*Katu*	*Deepana, Pachana, Kasa- shwashara, Jwarahara, Anilahara*

Fig 1 (a) Shirisha Twak

Fig 1 (b) Kantakari Panchanga

Fig 1 (c) Musta Kandmoola

Fig 1 (d) Overnight soaking of raw drugs Shirisha, Musta and Kantakari

Fig 1 (e) Preparation of Shirishadi Arka by distillation apparatus

Fig 1 (f) Shirishadi Arka stored in glass container

Figure no 1: Photographs of Preparation

Pharmaceutical Analysis of *Shirishadi Arka*: Qualitative Analysis consideration: Physicochemical analysis was conducted at pharmaceutical laboratory, ITRA, Jamnagar to find out the following parameters.

i. pH value: pH value was determined using digital pH meter. An electrode of pH meter was dipped in *Arka* sample, and the reading was noted. [11]

ii. Specific Gravity: Specific gravity is an important physico-chemical parameter used to assess the quality and purity of *Arka* preparations. It is defined as the ratio of the weight of a given volume of *Arka* to the weight of an equal volume of distilled water at a specified temperature, usually 25 °C. [12]

Method: A clean, dry specific gravity bottle (pycnometer) was weighed empty. It was then filled with distilled water and weighed, followed by filling with the *Arka* sample and weighing again. The weights obtained were used to calculate the specific gravity using the standard formula.

iii. Total Solid Content: Total solid content is an important quality-control parameter for *Arka* formulations, as it reflects the presence of dissolved and volatile principles extracted during the distillation process. It helps in assessing the strength, uniformity, and authenticity of the preparation. [13]

Method: A pre-weighed evaporating dish was filled with a measured amount of *Arka*, which was then evaporated to dryness on a water bath or at a regulated temperature for determination. The residue obtained was further dried to constant weight, cooled in a

desiccator, and weighed. The increase in weight represents the total solid content present in the *Arka*. The result was usually expressed as percentage w/v.

iv. Preliminary Phytochemical Study: Preliminary phytochemical screening was conducted to identify the presence of alkaloids, carbohydrates, glycosides, flavonoids, tannins, saponins, steroids, and amino acids using standard qualitative chemical tests. [14]

v. High Performance Thin Layer Chromatography (HPTLC): HPTLC analysis of catechin in *Shirishadi Arka* was carried out at Vasu Research Centre, Vadodara, using catechin as the reference marker. For standard preparation, 1 mg catechin was

dissolved in methanol and the volume was adjusted to 1 mL. For the test sample, 10 mL of *Shirishadi Ark* was evaporated to dryness, and the residue was dissolved in 1 mL methanol followed by filtration through a 0.45 µm syringe filter.

Chromatographic separation was performed on silica gel 60 F254 precoated aluminium plates using CAMAG Linomat 5. The mobile phase consisted of Toluene: Ethyl acetate: Formic acid: Methanol (6:3:0.1:1 v/v). After chamber saturation for 30 min, plates were developed up to 80 mm, dried at 100 ± 5°C, visualized at 254 nm, and scanned densitometrically at 278 nm for quantification.[15,16]

vi. Microbial Load: The microbial load of *Shirishadi Arka* was assessed to ensure its compliance with standard microbiological quality parameters and to confirm its safety for therapeutic use.

vii. Heavy Metals Test: Heavy metal analysis of *Shirishadi Arka* was carried out to determine the presence and permissible limits of toxic metals such as lead, arsenic, mercury, and cadmium, ensuring its safety and compliance with standard pharmacopeial guidelines.

viii. Pesticide Residue: All 34 Pesticides residues were tested as per Ayurvedic pharmacopeia of India Part 2, Volume 3. None of the pesticide residue were detected in the sample of *Shirishadi Arka* indicating compliance with suggested quality standard and safety for administration by inhalation.

ix. Gas Chromatography and mass spectrometry Analysis (GC-MS): GC–MS analysis of *Shirishadi Arka* was performed at CSIR–Central Salt and Marine Chemicals Research Institute (CSMCRI), Bhavnagar, Gujarat. Catechin was used as a comparative reference standard and analyzed under identical chromatographic conditions. Prior to analysis, the sample was filtered and subjected to solvent extraction to isolate volatile and semi-volatile constituents. The concentrated extract was analyzed using a GC–MS system equipped with an RTX-5 capillary column and helium as the carrier gas. Separation was achieved using a programmed temperature gradient, and mass spectra were recorded under electron impact ionization (70 eV). Compound identification was carried out by comparing the obtained spectra with the NIST library database. [17]

Validation of Analytical Methods: The analytical methods employed in the present study were validated for accuracy, precision, repeatability, and robustness using standard laboratory procedures. Precision of the analytical methods was assessed by performing all experiments in triplicate (n = 3), and the results were expressed as mean ± standard deviation. The low %RSD values obtained for physicochemical parameters indicated good precision and repeatability of the methods.

Reproducibility of the pharmaceutical procedure was evaluated by preparing six independent batches of *Shirishadi Arka*, which demonstrated minimal batch-to-batch variation. Instrument calibration was performed prior to analysis using certified standards and standard operating procedures. Robustness of the methods was ensured by maintaining controlled environmental and analytical conditions throughout the study.

3. RESULTS AND OBSERVATION:

Table no: 03 Batch-wise preparation details of *Shirishadi Arka*

Batch no.	Raw Drug Quantity (gm)	Water added (ml)	Distillation time (hrs)	Yield obtained (ml)	Yield (%)
B-1	150	1500	4.5	810	54%
B-2	151	1500	4.5	813	54.2%
B-3	152	1500	4.6	800	53.33%
B-4	150	1500	4.5	800	53.33%
B-5	152	1500	4.5	809	53.93%
B-6	152	1500	4.5	815	54.33%
		Mean ± SD		807.83 ± 6.43	53.85 ± 0.43%

Six batches of *Shirishadi Arka* were prepared under identical pharmaceutical conditions to assess reproducibility of the formulation. The yield percentage of all batches showed minimal variation with a mean yield of 53.85 ± 0.43%. The low %RSD value (0.80%) indicates good batch-to-batch consistency and reproducibility of the preparation method.

Pharmacognostical Evaluation: Organoleptic Parameters of Prepared Drug: It refers to the analysis of characters like Appearance, color, taste, odor. 2ml of prepared drug was taken and the identification was carried out on the basis of organoleptic features and microscopy of the prepared drug as mentioned in (Figure 2). Organoleptic characters of the prepared drug *Shirishadi Arka* were evaluated as per standard procedures described in API. The observations were recorded based on sensory perception.

Table no 04: Organoleptic Parameters

Parameters	Standard (API guidelines)	Observed in *Shirishadi Arka*
Appearance	Clear liquid	Clear, transparent liquid
Color	Colorless to pale	Colorless to pale green
Odor	Characteristic	Characteristic, slightly pungent
Taste	Characteristic	Slightly pungent and aromatic

Microscopic evaluation: Microscopic examination of the raw ingredients of *Shirishadi Arka* was carried out according to API guidelines to authenticate the constituent drugs. Microscopy of the finished product was not feasible due to its nature as a hydrodistillate lacking cellular structures. Diagnostic characters were documented and photomicrographs were recorded. Examination under 10× magnification revealed lignified stone cells, simple trichomes and cork cells of *Shirisha*; brown contents and starch grains of *Musta*; and stellate trichomes, epicarp cells, lignified pitted vessels, and starch grains of *Kantakari* (Figure 2).

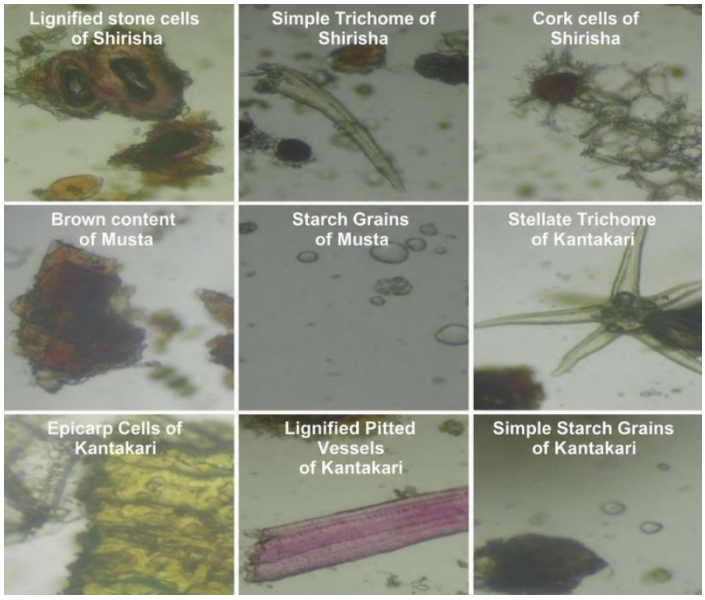

Figure no 2: Microphotographs of Raw drug

Pharmaceutico-analytical Study:

A. Physio Chemical Parameters: Physicochemical evaluation of *Shirishadi Arka* was carried out as per Ayurvedic Pharmacopoeia of India standards. The formulation exhibited a neutral pH (7.00 ± 0.05), specific gravity of 1.0033 ± 0.002, and total solid content of 0.052 ± 0.003 % w/v. All analyses were performed in triplicate (n = 3) and expressed as mean ± SD. The low SD values indicated good precision and reproducibility of the analytical methods. The absence of significant systematic or random errors ensured accuracy, precision, repeatability, and reliability. All parameters were found within acceptable limits, confirming the quality, purity, and consistency of the prepared *Shirishadi Arka* (Table 05).

Table No 05: Physio Chemical Parameters

Parameters	Permissible limits (API)	Result	%RSD
pH	5.0 - 7.0	7.00 ± 0.05	0.71%
Specific Gravity	0.990 – 1.02	1.0033 ± 0.002	0.19%
Total Solid Content	< 1.0% w/v	0.052 ± 0.003 % w/v	5.77%

B. Qualitative Phytochemical Analysis:

The qualitative phytochemical analysis of *Shirishadi Arka* revealed the presence of alkaloids, tannins, glycosides,

saponins, and flavonoids, while carbohydrates, phenols, diterpenes, and steroids were absent. Proteins and amino acids were not detected in the formulation. This preliminary phytochemical screening indicates that *Shirishadi Arka* contains bioactive constituents that contribute to its therapeutic potential as mentioned below in table no 6.

Table No 06: Qualitative Phytochemical Analysis

Name of test	Result
Alkaloids	+
Carbohydrates	-
Tannins	+
Glycosides	+
Phenols	-
Saponins	+
Flavonoids	+
Proteins and amino acids	
Diterpenes	-
Steroids	-

C. High Performance Thin Layer Chromatography:

HPTLC fingerprinting of *Shirishadi Arka* revealed the presence of multiple phytochemical constituents. The chromatogram of catechin standard showed a distinct peak at an Rf value of 0.26. A corresponding peak was observed in the chromatogram of *Shirishadi Arka* at the same Rf value (0.26), confirming the presence of catechin in the formulation.

Apart from the catechin peak, two additional peaks were observed at Rf values of 0.50 and 0.57, indicating the presence of other phytoconstituents extracted during the distillation process.

The peak area of the catechin standard was found to be 11382.9, whereas the corresponding peak area in *Shirishadi Arka* was 778.7. Quantitative estimation revealed the catechin content to be 0.034% w/v in the formulation.

Catechin is a naturally occurring flavan-3-ol known for its antioxidant, anti-inflammatory, antimicrobial and free radical scavenging activities. The detection of catechin in *Shirishadi Arka* supports the therapeutic utility of the formulation and may contribute to its pharmacological actions. Although the concentration detected was relatively low, its presence serves as an important phytochemical marker for quality control and standardization of the formulation.

Table No 07: HPTLC Rf values of *Shirishadi Arka*

Parameter	Catechin Standard	*Shirishadi Ark*
Rf Value	0.26	0.26, 0.50, 0.57
Peak Area	11382.9	778.7
Catechin Content	---	0.034%

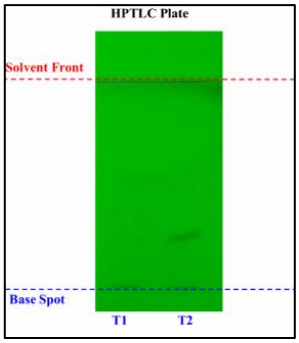

Figure No: 03 HPTLC image of *Shirishadi Arka* and Catechin standard

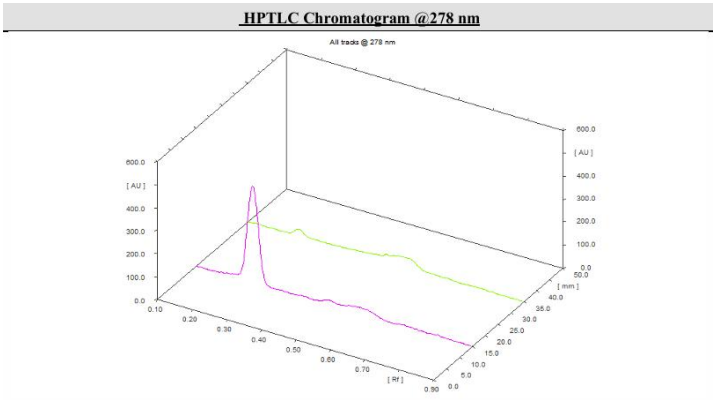

Figure No: 04 HPTLC Chromatogram of *Shirishadi Arka* and Catechin standard

D. Microbial Load for *Shirishadi Arka*:

Table no 08: Tests for specified Micro-organisms (Qualitative):

Sr. No	Bacteria	Limits	Results
1.	E coli	Absent / 100ml	Absent
2.	Enterobacter	Absent / 100ml	Absent
3.	S. aureus	Absent / 100ml	Absent
4.	P. aeruginosa	Absent / 100ml	Absent

Table no 09: Microbial limit test (Quantitative):

Sr. No	Count	Limits	Results
1.	Total Bacterial Count	30-300 cfu/ml	No growth
2.	Total Fungal Count	10-100 cfu/ml	No growth

*cfu=colony forming units

Table no 10: Test for Heavy metals

Sr. No	Parameter	Results	Permissible Limits (API)
1.	Lead (Pb)	Less than 0.1ppm	10 ppm
2.	Arsenic (As)	Less than 0.1ppm	3 ppm
3.	Cadmium (Cd)	Less than 0.1ppm	0.3 ppm
4.	Mercury (Hg)	Less than 0.1ppm	1 ppm

*ppm= parts per million

E. Tests for Heavy metals:

F. Pesticides analysis:

Table no 11: Pesticide residue

Parameter	Unit	Limits	Results	Method
Alachlor	mg/Kg	0.02	Absent	API part. II, Vol. III
Aldrin and Dieldrin (sum of)	mg/Kg	0.05	Absent	
Azinphos-methyl	mg/Kg	1.0	Absent	
Bromopropylate	mg/Kg	3.0	Absent	
Chlordane (sum of cis-, trans – and Oxythlordane)	mg/kg	0.05	Absent	
Chlorfenvinphos	mg/kg	0.5	Absent	
Chlorpyrifos	mg/kg	0.2	Absent	
Chlorpyrifos-methyl	mg/kg	0.1	Absent	
Cypermethrin (and isomers)	mg/kg	1.0	Absent	
DDT (sum of p,p-'DDT, o,p-'DDT, p,p-'DDE and p,p-'TDE	mg/kg	1.0	Absent	
Deltamethrin	mg/kg	0.5	Absent	
Diazinon	mg/kg	0.5	Absent	
Dichlorvos	mg/kg	1.0	Absent	
Dithiocarbamates (as CS2)	mg/kg	2.0	Absent	
Endosulfan (sum of isomers and Endosulfan sulphate)	mg/kg	3.0	Absent	
Endrin	mg/kg	0.05	Absent	
Ethion	mg//kg	2.0	Absent	
Fenitrothion	mg/kg	0.5	Absent	
Hexachlorocyclohexane isomers	mg/kg	0.3	Absent	
Lindane (-Hexachlorocyclohexane)	mg/kg	0.6	Absent	
Malathion	mg/kg	1.0	Absent	
Methidathion	mg/kg	0.2	Absent	
Parathion	mg/kg	0.5	Absent	
Parathion-methyl	mg/kg	0.2	Absent	
Permethrin	mg/kg	1.0	Absent	
Phosalone	mg/kg	0.1	Absent	
Piperonyl butoxide	mg/kg	3.0	Absent	
Pirimiphos-methyl	mg/kg	4.0	Absent	
Pyrethrins (sum of)	mg/kg	3.0	Absent	

Quintozene (sum of quintozene, pentachloroaniline)	mg/kg	1.0	Absent
Fenvalerate	mg/kg	1.5	Absent
Fonofos	mg/kg	0.05	Absent
Heptachlor (sum of Heptachlor and Heptachlorepoxide)	mg/kg	0.05	Absent
Hexachlorobenzene	mg/kg	0.1	Absent

G. GC-MS Analysis:

The GC-MS analysis of *Shirishadi Arka* revealed a total of 50 peaks within a runtime of 36.516 minutes, as shown in Table no 12. The chromatographic peaks and corresponding graph are visually represented in the adjacent image, with the peak areas calculated and presented through a graphical format for better interpretation.

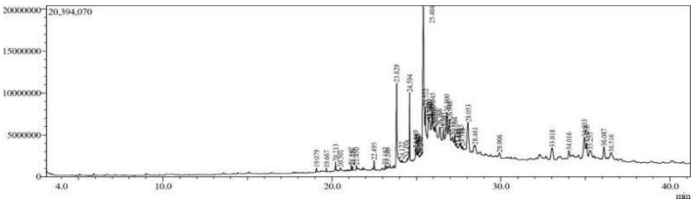

Fig. 05 *Shirishadi Arka* GC-MS Graph

Reference Standard:

Catechin was employed as a comparative reference standard in the GC–MS analysis of *Shirishadi Arka* to facilitate the identification, comparison, and validation of phytochemical constituents. It serves as a marker compound for standardization and quality assessment of the formulation. The GC-MS graph of Catechin is shown below in Fig. no 06.

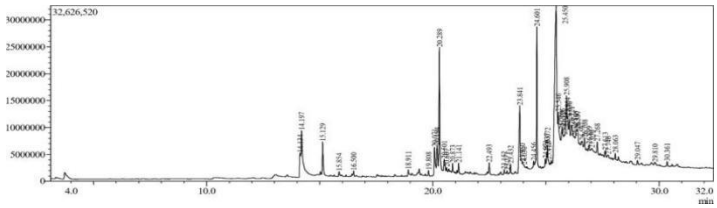

Fig. 06 Catechin GC-MS Graph

Table No 12: GC–MS Profile of *Shirishadi Arka* with Comparative Reference Standard Analysis

Peak#	R.Time	Area	Area%	Height	Height%	Name	Therapeutic Action
1	19.079	1036744	0.18	472153	0.40	Dodecanoic acid	Antimicrobial [18]
2	19.667	856163	0.15	398251	0.34	Diethyl Phthalate	-
3	20.213	1936071	0.34	970219	0.82	Dodecanoic acid, TMS derivative	Antimicrobial [18]
4	20.501	492864	0.09	234249	0.20	8-Carbomethoxyoctanoic acid, TMS	-
5	21.142	1129684	0.20	625872	0.53	1-Tetradecene	-
6	21.221	827634	0.14	441906	0.37	Cyclocolorenone	-
7	21.450	839911	0.15	424621	0.36	Tetradecanoic acid	-
8	22.495	2248705	0.39	1064022	0.90	Myristic acid, TMS	Anti-fibrotic [19]. Anti-inflammatory, Anti-oxidant [22]
9	23.152	967891	0.17	483654	0.41	2-Methyl-2-azatricyclo[4.3.1.0(3,8)]decane	-
10	23.280	1062510	0.19	319992	0.27	2-Tridecylfuran	-
11	23.829	31901036	5.57	9773561	8.24	n-Hexadecanoic acid	Anti-inflammatory [20]
12	24.122	462484	0.08	294693	0.25	1-Tetracosene	-
13	24.458	758207	0.13	344375	0.29	1-Nonacosanol, TMS	Anti- inflammatory [21]
14	24.594	15887204	2.77	8160045	6.88	Palmitic Acid, TMS derivative	Anti-inflammatory, Anti-oxidant [22]
15	24.949	4220945	0.74	1215040	1.02	1-Hexadecanol	-
16	25.033	1511068	0.26	706348	0.60	1,2-15,16-Diepoxyhexadecane	-
17	25.073	1724513	0.30	843324	0.71	9-Octadecenoic acid (Z)-, methyl ester	-

18	25.110	1184526	0.21	496050	0.42	9-Octadecenoic acid (Z)-, methyl ester	-
19	25.175	719146	0.13	212029	0.18	2-Hexyldodecanol, TMS derivative	-
20	25.240	816072	0.14	327174	0.28	Methyl tetradecanoate	-
21	25.404	102414928	17.87	18052917	15.22	cis-9-Hexadecenal	-
22	25.522	29165654	5.09	5989562	5.05	Octadecanoic acid	Anti- Inflammatory [23]
23	25.720	22537468	3.93	4636135	3.91	Ethyl tetradecyl ether	-
24	25.760	7610196	1.33	4266908	3.60	Dodecane, 1-iodo-	-
25	25.785	14172672	2.47	4183147	3.53	6,6-Dimethyl- vinylidenebicyclo[3.1.1]heptan	-
26	25.904	18345310	3.20	4283043	3.61	Oleic Acid, (Z)-, TMS	Anti-inflammatory, Anti-oxidant [22]
27	25.945	22717991	3.96	5347130	4.51	(9E,11E)-Octadecadienoic acid	-
28	26.041	12863105	2.24	3406432	2.87	Stearic acid, TMS derivative	Anti-inflammatory, Anti-oxidant [22]
29	26.117	25590866	4.47	3617230	3.05	Ethyl iso-allocholate	-
30	26.366	32190462	5.62	3265889	2.75	7-Hexadecenal, (Z)-	-
31	26.591	24614576	4.30	2787359	2.35	.gamma.-Tocopherol	-
32	26.710	7589681	1.32	2227993	1.88	18-Methyl-nonadecanol, trimethylsilyl ether	-
33	26.800	28309398	4.94	4833693	4.08	Stigmasta-5,22-dien-3-ol, acetate,(3.beta.)-	-
34	26.946	26459568	4.62	3966788	3.34	5-.alpha.-Androst-2-en-17-.beta.-ol, 17 methyl-	-
35	27.137	15434018	2.69	1810859	1.53	Bicyclo[9.3.1]pentadeca-3,7-dien-12-ol, 4,8,12	-
36	27.264	10553407	1.84	1941099	1.64	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	Anti-asthmatic. Anti-inflammmatory [24]
37	27.415	2819198	0.49	482399	0.41	1-Heptanol, 2,4-diethyl-	-
38	27.490	4039598	0.70	496408	0.42	11-(2-Cyclopenten-1-yl)undecanoic acid, (+)-	-
39	27.615	3113163	0.54	899100	0.76	Undec-10-ynoic acid, undec-2-en-1-yl ester	-
40	27.710	2087773	0.36	480300	0.40	Trichloroacetic acid, pentadecyl ester	-
41	28.053	21413138	3.74	3374664	2.85	.beta.-Sitosterol acetate	Anti-oxidant, Anti-inflammatory. Anti-asthmatic [25]
42	28.461	8215281	1.43	930316	0.78	9,19-Cyclolanost-24-en-3-ol, acetate, (3.beta.)-	-
43	29.906	2083896	0.36	447435	0.38	(1R,4aR,5S)-5-[(E)-5-Hydroxy-3-methylpent-3	-
44	33.018	10401231	1.82	1328459	1.12	Stigmasterol, TMS derivative	Immunomodulator, Anti-oxidant, Anti-inflammatory. [26]
45	34.016	4056360	0.71	873182	0.74	5,6-Dihydrostigmasterol, acetate	-
46	34.933	14847335	2.59	2326214	1.96	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-	-
47	35.058	7499652	1.31	1648061	1.39	5-.alpha.-Androst-2-en-17-.beta.-ol, 17-methyl-	-
48	35.255	7736713	1.35	819238	0.69	Cholestan-3-ol, (3.alpha.,5.beta.)-	-
49	36.087	8328472	1.45	1430434	1.21	.beta.-Sitosterol acetate	Anti-oxidant, Anti -inflammatory. Anti-asthmatic [25]
50	36.516	5274691	0.92	634552	0.54	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethy	-
		573069179	100.00	118594524	100.00		

Compounds were identified by comparing the obtained mass spectra with NIST library database and available published literature. Compounds showing high spectral similarity were considered for interpretation. The GC–MS analysis of catechin exhibited characteristic peaks corresponding to catechol, phloroglucinol derivatives and related phenolic compounds. However, no corresponding peak with similar retention time and mass spectral pattern of catechin or its major derivatives was observed in the GC–MS chromatogram of *Shirishadi Arka*, indicating that catechin was not detected in the *Arka* sample under the present experimental conditions.

The GC–MS chromatogram of *Shirishadi Arka* revealed the presence of fifty phytoconstituents, predominantly comprising fatty acids, fatty acid esters, long-chain alcohols, sterols and terpenoid derivatives. Major compounds identified by comparing the obtained mass spectra with NIST library database and published literature. Included n-Hexadecanoic acid, palmitic acid (TMS derivative), cis-9-hexadecenal, octadecanoic acid, oleic acid derivatives, γ-tocopherol, β-sitosterol acetate and stigmasterol derivatives.

Comparative GC–MS analysis of *Shirishadi Arka* and catechin reference standard revealed the presence of several common phytoconstituents, mainly fatty acids and their derivatives. Although catechin was not detected in *Shirishadi Arka*, the identified common compounds such as palmitic acid, oleic acid derivatives and furan compounds may be responsible for the pharmacological activity of *Shirishadi Arka* in respiratory disorders due to their reported anti-inflammatory and antimicrobial properties.

1. Entry:33676 Library: NIST23s.lib

SI:94 Formula: C16H32O2 CAS:57-10-3 MolWeight:256 RetIndex:1972; CompName:n-Hexadecanoic acid

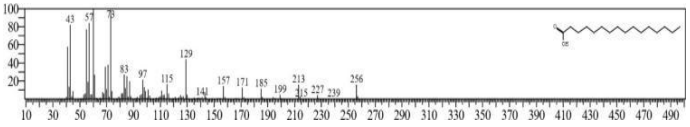

Figure no 07: n-Hexadecanoic acid

2. Entry:40660 Library:NIST23s.lib

SI:90 Formula:C19H40O2Si CAS:55520-89-3 MolWeight:328 RetIndex:2048

CompName:Palmitic Acid

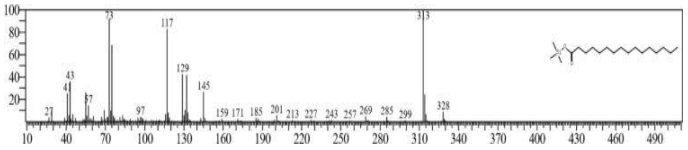

Figure no 08: Palmitic Acid

3. Entry:37945 Library:NIST23s.lib

SI:85 Formula:C19H36O2 CAS:1937-62-8 MolWeight:296 RetIndex:2107

CompName:9-Octadecenoic acid

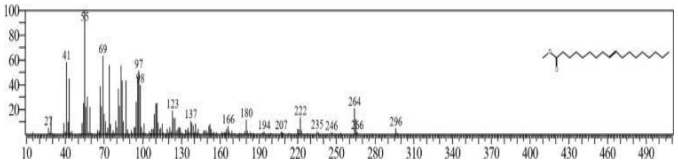

Figure no 09: 9-Octadecanoic acid

4. Entry:36500 Library:NIST23s.lib

SI:90 Formula:C18H34O2 CAS:112-80-1 MolWeight:282 RetIndex:2152

CompName:Oleic Acid

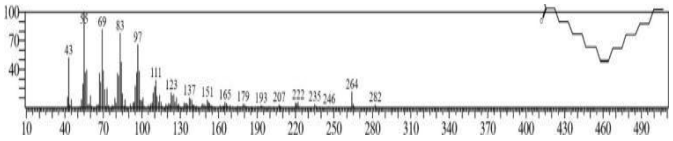

Figure no 10: Oleic acid

5. Entry:44949 Library:NIST23s.lib

SI:81 Formula:C29H48O CAS:83-48-7 MolWeight:412 RetIndex:3257

CompName:Stigmasterol

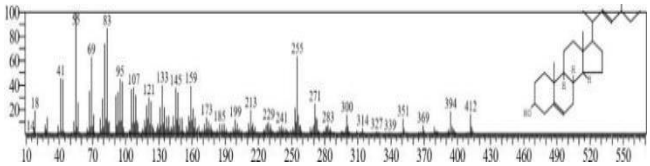

Figure no 11: Stigmasterol

6. Entry:45789 Library:NIST23s.lib

SI:87 Formula:C31H52O2 CAS:915-05-9 MolWeight:456 RetIndex:3468

CompName:.beta.-Sitosterol acetate

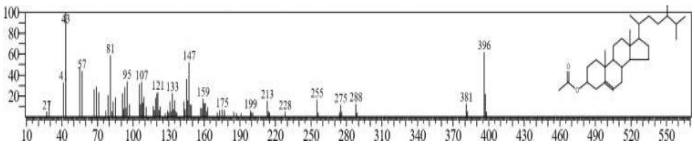

Figure no 12: beta.-Sitosterol acetate

5. DISCUSSION:

The batch-wise evaluation (Table 3) demonstrated minimal variation in yield percentage, confirming reproducibility of the pharmaceutical procedure. The low standard deviation and %RSD values suggest that the adopted distillation procedure is reliable, standardized, and capable of producing reproducible batches of *Shirishadi Arka* with minimal process variability.

Discussion on Physico-chemical parameters:

The physicochemical parameters mentioned in (Table 5) were found to be within acceptable limits prescribed in the API, indicating quality and consistency of the formulation. Pharmacognostical evaluation confirmed the genuineness and purity of the raw drugs, indicating the absence of adulterants and ensuring the authenticity of the formulation as shown in figure no. 02, Table no. 04. Physicochemical analysis mentioned in Table no. 05, revealed that *Shirishadi Arka* possessed a neutral pH of 7.00 ± 0.05 and low total solid content of 0.052 ± 0.003 % w/v, which is characteristic of *Arka Kalpana* preparations. The low total solid content suggests the presence of dissolved volatile principles rather than non-volatile residues, reflecting the suitability of distillation as a method for extracting therapeutically active constituents from the raw drugs. The specific gravity close to that of water (1.0033 ± 0.002) indicates the light nature of the preparation, which supports its rapid absorption and systemic availability, as described in Ayurvedic pharmaceutics.

Preliminary phytochemical screening mentioned in Table no. 06 demonstrated the presence of alkaloids, tannins, glycosides, flavonoids and saponins. These groups of phytoconstituents are well known for their pharmacological properties such as anti-inflammatory, antimicrobial, antioxidant and bronchodilatory actions. Their presence substantiates the classical indication of *Shirishadi Arka* in disorders like *Shwasa* and *Kasa*, which are pathophysiologically associated with airway inflammation, mucus hypersecretion and infection. Catechin is a flavonoid reported to possess antioxidant and anti-inflammatory activities. Detection of catechin in *Shirishadi Arka* provides an analytical marker for quality control and standardization as mentioned in table no. 07. The matching Rf value between standard and sample confirms authenticity of the phytochemical profile, while additional peaks reflect the polyherbal nature of the formulation as mentioned in table no. 07. The microbial analysis as shown in table no. 08 provided essential validation of safety. The absence of E. coli, Staphylococcus aureus, Pseudomonas aeruginosa and Enterobacter confirms the aseptic conditions during preparation, suggests its safety. [27] Microbial limit test mentioned in table no. 09 reported no bacterial or fungal growth suggests safety and stability of *Arka* according to standard limits mentioned in API. [27] Tests for heavy metals was done for Lead, Mercury, Arsenic, Cadmium suggested all these metals are present in less than 0.1 ppm and are under the permissible limits as described in API Standards as mentioned in table no. 10.

Also the sample had low contamination risk with pesticides, they are within the permissible limit as per API standards as mentioned in table no. 11 indicating superior quality of tested drug and suitable for administration with low risk of toxicity.

All these tests together suggested that *Shirishadi Arka* matched with API standards. In general safety and stability limits the formulation was found safe and stable within the evaluated quality control parameters.

Discussion on GC-MS analysis of *Shirishadi Arka*:

Although catechin was used as a comparative reference standard based on previously reported phytochemical studies of *Albizia lebbeck*, it was not detected in *Shirishadi Arka*. This

may be attributed to the non-volatile and polar nature of catechin [28], which limits its detection by GC–MS without derivatization. Furthermore, *Shirishadi Arka* is prepared by the process of *Ark Kalpana* (distillation), in which mainly volatile constituents are transferred into the distillate, whereas non-volatile polyphenolic compounds such as catechin may remain in the residue. The therapeutic activity of *Shirishadi Arka* in respiratory disorders may therefore be attributed to the presence of other bioactive constituents detected in the GC–MS analysis. Compounds such as n-hexadecanoic acid [20], oleic acid derivatives [22] and octadecanoic acid present in *Shirisha* are reported to possess anti-inflammatory and antimicrobial activities, which are beneficial in respiratory tract infections as mentioned in table no. 11. γ-Tocopherol and phytosterols such as β-sitosterol [25] and stigmasterol derivatives [26] present in *Kantakari* are known for their antioxidant, immunomodulator and anti-inflammatory properties, which may help in reducing airway inflammation as mentioned in table no. 11. Myristic acid present in *Musta* possess Anti-fibrotic [19], Anti-inflammatory and Anti-oxidant [22] properties as mentioned in table no. 11. The combined action of these bioactive molecules may contribute to the traditional use of *Shirishadi Arka* in respiratory conditions such as cough, asthma, COPD and bronchial disorders. Catechin was successfully detected and quantified by HPTLC, whereas it was not observed in GC-MS analysis, probably due to its non-volatile nature and poor suitability for direct GC-MS detection.

6. CONCLUSION:

The present study successfully established the pharmaceutical preparation, pharmacognostical authentication, and analytical standardization of *Shirishadi Arka*. The formulation complied with established quality parameters, confirming its safety, purity, and consistency. Pharmacognostical evaluation ensured the authenticity of raw materials, while physicochemical and microbiological analyses validated the stability and absence of contamination. Preliminary phytochemical screening, HPTLC profiling, and GC–MS analysis revealed the presence of multiple bioactive constituents, including fatty acids, phytosterols, and antioxidant compounds, which are known for their anti-inflammatory, antimicrobial, and immunomodulatory activities. These findings provide a scientific basis for the classical indication of *Shirishadi Arka* in respiratory disorders such as *Swasa* and *Kasa*.

The presence of various pharmacologically active constituents suggests that the therapeutic efficacy of the formulation is likely due to a synergistic effect of multiple compounds. The overall findings support the potential role of *Shirishadi Arka* in the management of COPD (*Tamaka Swasa*), thereby bridging traditional Ayurvedic knowledge with modern analytical validation.

Limitations of Study:

- The study was limited to preliminary phytochemical and analytical evaluation; detailed quantitative estimation of individual compounds was not performed.
- GC–MS analysis primarily detects volatile and semi-volatile compounds; non-volatile constituents such as certain polyphenols may not have been identified.
- The absence of advanced confirmatory techniques such as LC–MS limits comprehensive phytochemical profiling.
- Pharmacological activity was inferred based on reported properties of identified compounds; no in vitro or in vivo validation was carried out.

Clinical evaluation of *Shirishadi Arka* in patients with COPD (*Tamaka Swasa*) was not undertaken

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The authors declare this manuscript was written without the use of generative artificial intelligence tools. All the content, including text generation, data analysis and references was developed and reviewed by the author without assistance from AI technologies.

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