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ORA-Analytical Study

Pharmaceutical and Analytical Standardization of *Ajamoda Arka* with *Yavakshara* through Physicochemical Evaluation, HPTLC Profiling, GC–MS Characterization and Safety Assessment.

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ABSTRACT:

Background: Digestive disorders such as *Vataja Udarashoola* (abdominal colic) are commonly encountered in pediatric clinical practice. In *Ayurveda*, these conditions are attributed to *Agnimandya*, *Ama* accumulation and vitiation of *Vata–Kapha Dosha*, resulting in impaired digestion and abdominal discomfort. Classical Ayurvedic formulations such as *Ajamoda Arka* and *Yavakshara* are indicated in the management of the above conditions owing to their *Deepana, Pachana* and *Vatanulomana* properties. **Objective:** The objective of this work is to analytically standardize *Ajamoda Arka* with *Yavakshara* by testing its organoleptic, physicochemical, chromatographic, microbial properties, Heavy metal and Pesticides following standard guidelines. **Materials and Methods:** The preparation of *Ajamoda Arka* is done according to *"Arka Kalpana"* given in AFI. *Yavakshara* prepared using AFI guidelines is mixed with distilled *Ajamoda Arka* in a specific ratio. "Organoleptic characters", "clarity", "pH", "specific gravity", "refractive index", "viscosity", "volatile matter", "total acidity", HPTLC, GCMS, "Heavy metals", "Pesticides" were done using API guidelines. **Results:** *Ajamoda Arka* with *Yavakshara* was clear, colorless and aromatic with salty taste. Physicochemical parameters showed pH 6.34, specific gravity 0.9710, refractive index 1.32507 and viscosity 1.01, indicating stability of the formulation. Volatile matter was 0.13%. Microbial analysis revealed absence of bacterial and fungal growth, confirming microbiological safety within permissible limits. HPTLC analysis reported characteristic bands analogous to α-phellandrene and β-pinene. GC–MS chromatogram revealed several constituents including 9-Octadecenoic acid derivatives, 'cis-9-Hexadecenal, terpen' and 'glycidyl palmitate', indicating presence of anti-inflammatory bioactive compounds. Heavy metals were within permissible limits and all tested pesticides were negative, confirming the safety of the formulation. **Conclusion:** The present study establishes preliminary analytical standards and chromatographic fingerprints for *Ajamoda Arka* with *Yavakshara*. The results of this pharmaceutical study can serve as criteria for 'quality control' and 'standardization parameter' for future research. However, further studies can be enriched with safety and stability evaluation for shelf life.

KEYWORDS: *Ajamoda Arka,* Analytical standardization, Ayurveda, GC-MS, HPTLC, Heavy metals, Pesticides, *Yavakshara.*

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1. INTRODUCTION

Ayurveda, the age-old system of life wisdom, is entering growing acceptance worldwide. Hence, it is pivotal to establish proper quality evaluation and standardization of its formulations. Digestive diseases such as *Vataja Udarashoola* (Abdominal colic) are most constantly encountered in pediatric clinical practice. This primarily corresponds to '*Agnimandya'* (impaired digestion), accumulation of '*Ama'* (metabolic toxin) and vitiation of *Vata* and *Kapha doshas*, leading to disabled digestion and immersion processes. [1-2] Classical Ayurvedic phrasings similar as '*Ajamoda Arka', 'Yavakshara',* and *'Takra'* are described for the operation of these digestive diseases. [3] *Ajamoda Arka* is a distilled liquid medication explained under *Arka Kalpana*, prepared from *Ajamoda*, which possesses *Deepana*, *Pachana*, and *Vatanulomana.* [4] *Arka* was named in the present study considering its felicity for *Bala Chikitsa*. babies retain a delicate constitution (*sukumara deha*), and hence, to be treated with sweet, less piercing mild cures rather mixed with '*ksheera'* are preferred. Administration of heavy or strong phrasings may lead to unwanted complications. *Arka*, being a *mrudu* (mild) and *laghu* (light) [5] thus safe and well permitted in infantile age group also. Because it is a water-grounded excerpt, it is easy to administer, quick to initiate action and is readily absorbed by the body. *Yavakshara* is a classical *Kshara Kalpana* prepared from the ash of *Yava* indicated for its *Lekhana*, *Shoolahara* and *Anulomana.* [6] The medication of *Yavakshara* involves burning *Yava* to ash and also rooting it with water, which produces an alkaline substance rich in potassium mariners. This alkaline nature helps in digesting *Ama*, relieving abdominal distension, and correcting the perturbed movement of *Vata*. Using *Ajamoda Arka* along with *Yavakshara* is grounded on their reciprocal conduct. *Ajamoda Arka* acts as a digestive and carminative, while *Yavakshara* aids in detoxification and balances *Vata* and *Kapha*, thereby supporting better digestion. When this combination is taken with *Takra (*buttermilk) as *Anupana*, the effect is further bettered. *Takra* enhances digestion, helps immersion, and prevents heaviness or bloating after taking the drug. [7] It is important to ensure the safety, quality. For this, evaluation of organoleptic characters, pH, specific gravity, physicochemical parameters, chemical profiling, microbial Analysis, Heavy metal and pesticides is necessary. Thus, this study focuses on preparing *Ajamoda Arka* mixed with *Yavakshara*, to be administered with *Takra* as *Anupana,* and on assessing its stability and utility in managing digestive diseases.

2. STUDY DESIGN AND METHODOLOGY:

Voucher specimen authentication

Ajamoda Arka was procured from GMP certified SDM Pharmacy, Udupi, with GMP certification number AUS 783 and specimen number (AJD013) was prepared as per the AFI (Ayurvedic Formulary of India). *Yavakshara* was procured from GMP certified Vyas Pharmacy, with GMP certification number GA/1696 And specimen number (00163) was prepared as per the AFI (Ayurvedic Formulary of India).

Table 1: *Rasapanchaka* of *Ajamoda arka* with *Yavakshara*

Parameter	Observation
Rasa	*Lavana, Kashaya, Katu*
Guna	*Laghu, Snigdha, Tikshna*
Veerya	*Ushna*
Vipaka	*Katu*
Karma	*Kapha-Vata Shamak, Deepana, Shoolaprashamana*
Gandha	Characteristic pungent odor
Varna	Colorless

Preparation of *Ajamoda Arka with yavakshara*: *Ajamoda* was procured from a GMP-certified *Ayurvedic* pharmacy and authenticated using classical *Dravya Pariksha* parameters. For the preparation of *Arka*, the powdered drug was soaked in eight times its quantity of water and kept overnight. The

mixture was then transferred to an *Arka Yantra*, where controlled heating was applied. The vapours containing volatile and aromatic constituents were condensed through the cooling chamber and collected as *Ajamoda Arka*, a clear distilled herbal extract. After completion of distillation and cooling, *Yavakshara* was added to the freshly prepared *Ajamoda Arka* in the prescribed proportion and mixed thoroughly to ensure uniform dissolution. The final formulation was stored in airtight, amber-colored glass containers to preserve its potency and prevent volatilization.

Method of Analytical standardization of *Ajamoda Arka*: In the current study, Analytical parameters including organoleptic characters, clarity, volatile matter, 'Specific gravity', 'Refractive index', 'Ph', 'viscosity', 'total acidity', 'HPTLC profiling', GCMS', [8-9] 'microbial load analysis', [10] 'Heavy metals and Pesticides' [9] were carried out. All tests were performed based on standards prescribed in the Indian Pharmacopoeia, *Ayurvedic* Pharmacopoeia of India, and WHO guidelines.

Organoleptic characters: Organoleptic characteristics of *Ajamoda arka* with *Yavakshara* was documented using sensory characteristics like eye, nose and tongue.

Clarity test: Visual inspection was done under a bright light source and viewed against black and white background with swirling action.

Volatile matter: 20 ml of *n*-hexane was extracted twice into two 10 ml samples. A pre-weighed china dish was filled with hexane soluble portion and evaporated to room temperature. The 'weight difference' was observed, and the percentage of volatile matter was calculated.

Specific gravity: The 'specific gravity' bottle was cleaned with acetone followed by ether, and the 'weight' of the dried bottle was noted. The sample was cooled to room temperature and carefully filled with the test liquid. The stopper was inserted and the surplus liquid was removed, and the final weight of the bottle was noted. The same procedure was repeated with distilled water instead of sample solution.

Refractive index: To determine the 'refractive index' of the sample, a drop of water was placed on the prism. After that, the 'drive knob' was turned to ensure that the line intersected the separatrix at the center and recorded the reading. The error of instrument was calculated by comparing this refractive index with that of standard refractive index of water (33°C is 1.33157). If the reading obtained was below 1.3315, this implied that the error was negative and that positive correction was to be made. On the other hand, if the reading obtained was higher than 1.3315, this implied that the error was positive and that negative correction was to be made. After that, a drop of arka was placed on the prism to find its refractive index. Any necessary corrections were applied to the measure reading to ensure accuracy of refractive index. All readings were taken at a standard temperature of 33°C to ensure precision. This process was followed to determine accurate refractive index of the test samples.

Determination of pH

Preparation of buffer solutions: One tablet each of pH '4', '7' and '9.2' were dissolved in '100 ml' of distilled water to obtained standard buffer solution.

To determine the pH of *Ajamoda arka* with *Yavakshara*, '10 ml' sample was taken and mixed it with distilled water to make a '100 ml' solution and stirred well. This mixture was then filtered to get a clear filtrate. First, the 'pH meter' was warmed up for 30 minutes. Then, it was calibrated using a 'pH 4' solution, adjusting the knob to get a precise reading of 4.02 at room temperature, which was 30°C. Next, 'pH 7' solution was introduced and adjusted the meter to read exactly 7. After that, 'pH 9.2' solution was added and the reading was checked without making any adjustments. Finally, the reading of the sample solution was noted. This process was repeated

four times and the average of readings was taken as the final result to ensure the accuracy.

Viscosity: The sample was put into a U-tube viscometer and allowed to reach the desired temperature. The time it took for the liquid to move between the two marks was noted after it was drawn to the indicated level. The viscosity ascertained by comparing the density of sample and flow time of the water.

Total Acidity: Total acidity was calculated by dissolving One-gram sample in 75 ml of CO_2-free water and titrated with 0.05 N NaOH using phenolphthalein as indicator until it changed to a persistent pink color for 10 seconds.

HPTLC

20 ml of n-hexane was used to extract 10 ml of the *Ajamoda arka* sample with *Yavakshara*. Using a Linomat 5 TLC applicator, 3, 6, and 9 microliters (µl) of the extract were applied to a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm. Petroleum ether: Dichloromethane (3:7) was used to develop the plate. Following short UV and long UV visualization, the developed plates were derivatized with vanillin sulfuric acid, observed under white light, and scanned under UV 254 nm, 366 nm, and 620 nm (post derivatization). Rf, the spots color, and the 'densitometric scan' were noted.

Preparation of Casein Soya bean Digest Agar Medium (CSDAM): Casein peptone (15 g), Soya peptone (5 g) and Sodium Chloride (5 g) were taken and dissolved in 900 ml of distilled water and adjusted to a 'pH of 7.3±0.2' and finally the volume was made up to 1000 ml. Then 15 g of agar was added to the medium and autoclaved at 121°C for 20 minutes.

The media was cooled around 45-55˚C. 20 ml of this solution was poured onto the sterile Petri plates. 1 ml of the *Ajamoda arka* with *Yavakshara* was directly poured onto the medium in the plate and swirled for uniform distribution. The plates were incubated overnight at 37℃ and observed after 48 h.

Preparation of Casein Soya bean Digest Agar Medium (CSDAM): After dissolving 15 g of casein peptone, 5 g of soy peptone, and 5 g of sodium chloride in 900 ml distilled water, the pH was fixed to 7.3±0.2, and the volume was made up to 1000 ml. Lastly, autoclave the media for 20 minutes at 121℃ after adding 15 g of agar.

Preparation of Buffered Sodium Chloride Peptone Solution (BSCPS) pH 7.0: "Potassium dihydrogen phosphate" (3.56 grams), disodium hydrogen phosphate (7.23 grams), sodium chloride (4.3 grams), and peptone (1.0 grams) were dissolved in 900 milliliters of distilled water. The volume was increased to 1000 ml and the adjusted the pH to 7.0. After that, then the buffer solution was autoclaved for 20 minutes at 121℃.

Preparation of Sabouraud Dextrose Agar: "Sabouraud Dextrose Agar" was prepared by dissolving 40 g of dextrose, 5 g of beef extract, and 5 g of casein peptone in 900 ml of distilled water, adjusting the pH to 5.6±0.2, and adding 1000 ml of water. Lastly, autoclave the media for 20 minutes at 121℃ after adding 15 g of agar.

Preparation of Buffered Sodium Chloride Peptone Solution (BSCPS) pH 7.0: Potassium dihydrogen phosphate (3.56 g), disodium hydrogen phosphate (7.23 g), sodium chloride (4.3 g), and peptone (1.0 g) were dissolved in 900 ml of distilled water to create the Buffered Sodium Chloride Peptone Solution (BSCPS) pH 7.0. The volume was increased to 1000 ml to adjust the pH to 7.0. After that, the buffer solution was autoclaved for 20 minutes at 121℃.

GCMS:

Gas Chromatography-Mass Spectrometry (GC-MS) was employed to analyze the chemical composition of the sample. The sample was injected into the GC system equipped with a suitable capillary column. Separation of compounds was achieved based on their retention times under controlled

temperature programming. The compound identification by matching the mass spectra with reference libraries and confirming via CAS registry numbers was done by Mass spectrometer. Quantification was done by measuring the peak areas, and relative abundance percentage of the total peak area was calculated.

Heavy Metals Test: Heavy metal analysis of *Ajamoda Arka* with *Yavakshara* was performed using Atomic Absorption Spectroscopy (AAS) after acid digestion of the sample. The sample was analyzed for heavy metal contamination of Lead (Pb), Arsenic (As), Mercury (Hg) and Cadmium (Cd) using calibrated solutions. The concentrations of tested heavy metals were found less than the permissible limit indicating no risk of contamination and compliant with recognized safety parameters as per Ayurvedic pharmacopeia of India and WHO standards.

Pesticides: Pesticide residue analysis of *Ajamoda Arka* with *Yavakshara* was carried out by Gas Chromatography-Mass Spectrometry and Liquid Chromatography-Mass Spectrometry after suitable sample extraction and clean-up procedure. All 34 tested 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 *Ajamoda arka* with *Yavakshara* indicating compliance with suggested quality standard and safety for oral administration.

Instrumentation

HPTLC Instrumentation: HPTLC was analyzed using Linomat-5 applicator, CAMAG double trough development chamber, CAMAG visualization chamber, and "CAMAG TLC Scanner 4" controlled by winCAT software (version 1.4.6).

GC-MS Instrumentation: For GC–MS analysis, 10 mL sample was extracted with 2 mL chloroform, and 1 µL extract was injected into the GC–MS system. The analysis was conducted using Shimadzu GC–MS QP 2010 SE with helium as carrier gas and a capillary column (40 m length, 0.22 mm internal diameter). Compounds were identified based on retention time and mass spectral data.

3. RESULTS:

The given sample of *Ajamoda arka* with *Yavakshara* has been standardized as per standard testing protocol. The results of standardization parameters are represented in respective Tables (Table 2 – Table 6).

Table 2: Results of standardization parameters of *Ajamoda arka* with *Yavakshara*

Parameter	*Ajamoda arka* with *Yavakshara*
Color	Colorless
Odor	Aromatic, pleasant
Taste	Salty
Clarity test	Free from visible particles and growth
Volatile matter (%)	0.13%
Specific gravity	0.9710
Refractive index	1.32507
pH	6.34
Viscosity	1.01
Total acidity	0.02

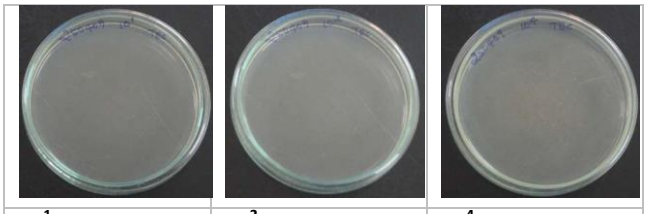
The observed physicochemical parameters were within the acceptable range described for distillate preparations in the Ayurvedic Pharmacopoeia of India, indicating conformity with pharmacopeial standards.

Table 3: Microbial load - Direct method of sample *Ajamoda arka* with *Yavakshara*

Sl. No.	Dilution	Number of Colonies (NOC)		CFU/ml
1	Direct	0	0	0

Table 4: Total bacterial count with dilution of *Ajamoda arka* with *Yavakshara*.

Sl. 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



10^1	10^2	10^4

Figure 1: Total bacterial count with dilution of *Ajamoda arka* with *Yavakshara.*

Table 5: Total Fungal Count with dilution of *Ajamoda arka* with *Yavakshara*

Sl. 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

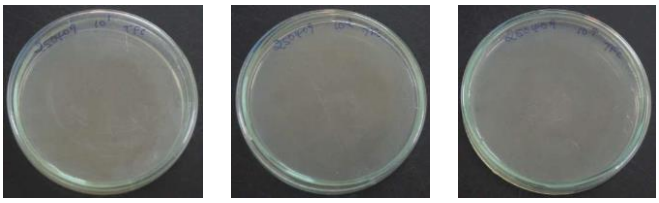


Fig 2a- 10^1 Fig 2b- 10^2 Fig 2c- 10^4

Figure 2: Total Fungal Count with dilution of *Ajamoda arka* with *Yavakshara*

Interpretation: The absence of microbial colonies in all dilutions (Figure 1 and Figure 2) indicates microbial counts below detectable limits, confirming microbiological quality of the formulation.

HPTLC

Table 6: R_f values of *Ajamoda arka* with *Yavakshara*

Short UV	Long UV	After derivatisation
0.42 (Green)	-	-
-	0.70 (F. blue)	-

*D – dark; L – light; F – fluorescent

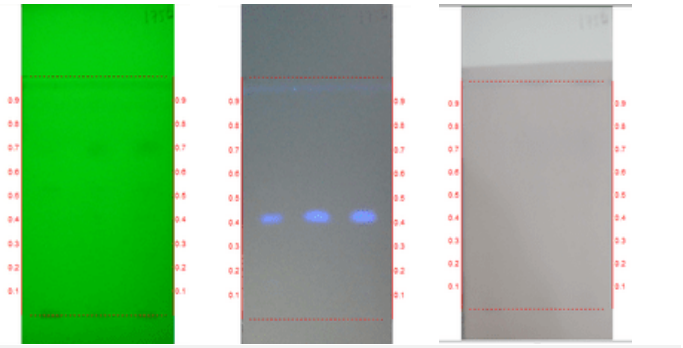
Solvent system – Pet. ethar: Dichloromethane (3:7)

R_f of alpha phellandrene - 0.6, R_f of beta pinene - 0.8

Track 1 – *Ajamoda arka* with *Yavakshara* – 3µl

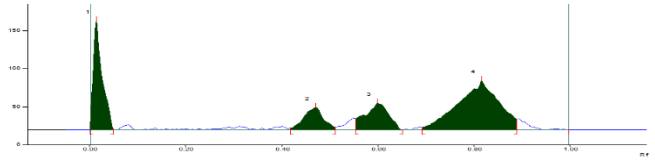
Track 2 – *Ajamoda arka* with *Yavakshara* – 6µl

Track 3 – Ajamoda arka with Yavakshara – 9µl



Short UV Long UV Post derivatisation (VSA)

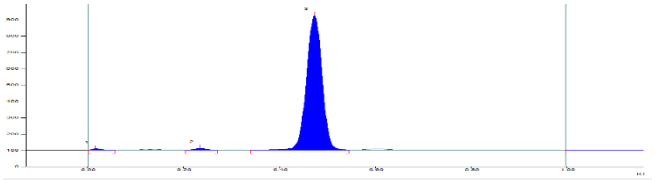
Figure 3: HPTLC photo documentation of Hexane fraction of *Ajamoda arka* with *Yavakshara*



Track 3, ID: Ajamoda arka with Yavakshara

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	142.5 AU	52.72 %	0.05 Rf	0.6 AU	1882.6 AU	24.12 %
2	0.42 Rf	2.3 AU	0.47 Rf	29.0 AU	10.74 %	0.51 Rf	3.4 AU	851.0 AU	10.90 %
3	0.55 Rf	14.5 AU	0.60 Rf	34.6 AU	12.81 %	0.65 Rf	0.1 AU	1208.5 AU	15.49 %
4	0.69 Rf	3.2 AU	0.82 Rf	64.2 AU	23.73 %	0.89 Rf	14.2 AU	3861.8 AU	49.49 %

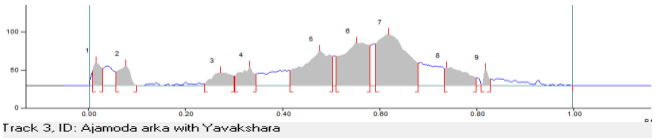
Fig 2a. At 254nm



Track 3, ID: Ajamoda arka with Yavakshara

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.6 AU	0.02 Rf	10.3 AU	1.21 %	0.06 Rf	0.0 AU	141.5 AU	0.72 %
2	0.20 Rf	1.3 AU	0.24 Rf	12.0 AU	1.42 %	0.27 Rf	0.1 AU	224.1 AU	1.15 %
3	0.34 Rf	0.9 AU	0.47 Rf	825.5 AU	97.37 %	0.55 Rf	2.8 AU	19158.7 AU	98.13 %

Fig.2b. At 366nm



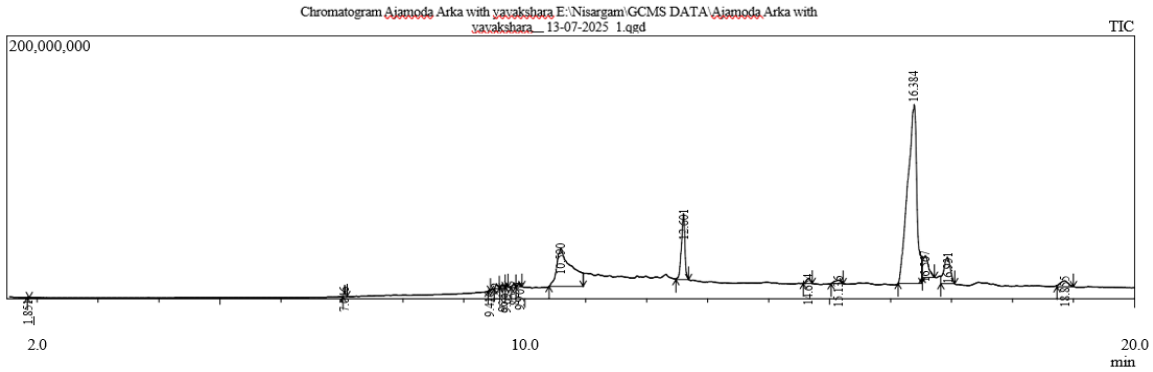
Track 3, ID: Ajamoda arka with Yavakshara

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	18.9 AU	0.02 Rf	30.4 AU	9.70 %	0.03 Rf	22.3 AU	362.8 AU	3.78 %
2	0.06 Rf	18.3 AU	0.08 Rf	26.2 AU	8.39 %	0.10 Rf	0.1 AU	454.8 AU	4.74 %
3	0.24 Rf	2.5 AU	0.27 Rf	17.2 AU	5.49 %	0.30 Rf	12.7 AU	491.7 AU	5.12 %
4	0.30 Rf	12.8 AU	0.33 Rf	24.5 AU	7.82 %	0.35 Rf	15.2 AU	502.3 AU	5.23 %
5	0.42 Rf	19.6 AU	0.48 Rf	45.7 AU	14.60 %	0.50 Rf	38.4 AU	1795.8 AU	18.70 %
6	0.51 Rf	38.4 AU	0.55 Rf	56.3 AU	17.98 %	0.58 Rf	52.8 AU	2139.5 AU	22.28 %
7	0.59 Rf	54.7 AU	0.62 Rf	67.6 AU	21.60 %	0.68 Rf	29.8 AU	2950.0 AU	30.72 %
8	0.73 Rf	22.7 AU	0.74 Rf	23.8 AU	7.61 %	0.80 Rf	10.3 AU	773.6 AU	8.06 %
9	0.81 Rf	2.0 AU	0.82 Rf	21.3 AU	6.81 %	0.83 Rf	7.9 AU	133.4 AU	1.39 %

Fig 2c. At 620nm

Figure 4: Densitometric scan of *Ajamoda arka* with *Yavakshara*

GCMS:-



Peak Report TIC

Peak#	R.Time	Area	Area%	Height	CAS#	Name
1	1.851	485839	0.02	546327	105-57-7	Ethane, 1,1-diethoxy-
2	7.046	11432548	0.46	8574273	499-75-2	Phenol, 2-methyl-5-(1-methylethyl)-
3	9.416	2032972	0.08	1226450	21964-51-2	1,15-Hexadecadiene
4	9.532	10420903	0.42	3463470	18835-32-0	1-Tricosene
5	9.648	5701355	0.23	1691894	629-96-9	1-Eicosanol
6	9.697	1249434	0.05	1013643	18435-45-5	1-Nonadecene
7	9.827	8117221	0.33	3135027	7501-44-2	Glycidyl palmitate
8	9.904	6071076	0.25	2812805	112-62-9	9-Octadecenoic acid (Z)-, methyl ester
9	10.590	493218434	19.92	29377657	56219-4-6	cis-9-Hexadecenal
10	12.601	201012580	8.12	50146593	7501-44-2	Glycidyl palmitate
11	14.654	17054815	0.69	3856317	58594-45-9	13-Octadecenal, (Z)-
12	15.136	18400871	0.74	2719143	2423-10-1	9-Octadecenal, (Z)-
13	16.384	1410612864	56.96	136024610	5431-33-4	9-Octadecenoic acid (Z)-, oxiranylmethyl ester
14	16.567	103329993	4.17	16456792	2463-2-7	11,14-Eicosadienoic acid, methyl ester
15	16.931	146981597	5.94	20219444	7501-44-2	Glycidyl palmitate
16	18.855	40333483	1.63	4547991	506-43-4	9,12-Octadecadien-1-ol, (Z,Z)-
		2476455985	100.00	285812436		

Figure 5: GC-MS chromatogram of *Ajamoda arka* with *yavakshara*

The GC–MS chromatogram revealed sixteen peaks corresponding to various organic compounds with retention times ranging from 1.851 to 18.855 minutes, with a total peak area of approximately 2.48 billion (2,476,455,985), indicating the total constituents detected in the sample. The major compound identified was 9-Octadecenoic acid (Z)-, oxiranylmethyl ester (CAS 5431-33-4) at a retention time of 16.384 minutes, contributing 56.96% of the total peak area. This was followed by cis-9-Hexadecenal (CAS 56219-4-6) at 10.590 minutes with 19.92% peak area. Glycidyl palmitate (CAS 7501-44-2) was detected at three different retention times (9.827, 12.601, and 16.931 minutes) with a combined peak area of approximately 14.39%. Other notable compounds identified include 11,14-Eicosadienoic acid, methyl ester (4.17%), 9-Octadecenoic acid (Z)-, methyl ester (0.25%), 9,12-Octadecadien-1-ol (Z) (1.63%), 13-Octadecenal (Z) (0.69%), and 9-Octadecenal (Z) (0.74%). Minor constituents present in trace amounts (<1%) included Ethane, 1,1-diethoxy- (0.02%) and Phenol, 2-methyl-5-(1-methylethyl)- (0.46%).

The observed physicochemical parameters were found to be within the acceptable limits prescribed for distillate preparations in the Ayurvedic Pharmacopoeia of India, indicating that the formulation complies with pharmacopeial standards.

Pesticides:

Table 7: Pesticides

Parameter	Unit	Limits	Results	Method
Alachlor	mg/Kg	0.02	Absent	
Aldrin and Dieldrin (sum of)	mg/Kg	0.05	Absent	API part. II, Vol.
Azinphos-methyl	mg/Kg	1.0	Absent	III

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

Table 8: Heavy metals

Parameter	Unit	Limits	Results	Method
Lead	mg/Kg	10	<0.1	API Part. II, Vol. III
Arsenic	mg/Kg	3	<0.1	
Mercury	mg/Kg	1	<0.1	
Cadmium	mg/Kg	0.3	<0.1	

The sample was analyzed for heavy metal contamination of Lead (Pb), Arsenic (As), Mercury (Hg) and Cadmium (Cd) and they were found to be within the permissible range indicating no risk of contamination.

4. DISCUSSION:

Ajamoda Arka is a classical *Ayurvedic* distillate widely used in the management of digestive disorders such as *Ajeerna, Agnimandya*, and *Vataja Udarashoola* (abdominal colic) due to its *Deepana, Pachana* and *Vatanulomana* properties. *Ajamoda Arka* is widely used in clinical settings, but little is known about its chromatographic characterization and analytical standardization including pesticides and heavy metal contamination. Such analytical studies are essential to ensure quality, safety and efficacy of herbal formulations. Therefore, the present study was undertaken to establish analytical reference parameters for *Ajamoda Arka* with *Yavakshara*, based on classical principles of *Arka Kalpana* along with contemporary analytical techniques.

In the present study, *Ajamoda Arka* was prepared using the classical distillation method in *Arka Yantra*, which facilitates extraction of both volatile and water-soluble phytoconstituents. Classical *Ayurvedic* texts such as *Arka Prakasha* describe *Arka* preparations as potent formulations with longer shelf life, improved stability and lower chances of microbial contamination compared to decoctions or infusions. [3]

Previous studies on *Shatapushpa Arka* and *Supachya Arka* have reported their therapeutic utility in the management of abdominal colic. [11-12] However, comprehensive analytical profiling of *Arka* formulations is still limited. Therefore, the aim of the present study is to evaluate the physicochemical parameters and chromatographic profile of *Ajamoda Arka* with *Yavakshara.*

The pH of *Ajamoda Arka* with *Yavakshara* was slightly acidic to neutral. This may support stability of the formulation and prevent microbial growth. It is also observed that the specific gravity of the formulation is near the value of water. This is characteristic of Arka preparations which are light, aqueous distillates containing volatile bioactive phytoconstituents.

The refractive index of the formulation is 1.3315 at 33°C. This indicates the presence of volatile substances in solution and is an important parameter for the identification of the formulation. Estimation of volatile matter confirms the essential oil fractions, which contributes to the therapeutic activity of *Ajamoda* in relieving abdominal discomfort, flatulence and colic.

The total acidity of the formulation indicated a mildly acidic nature, which may support digestive processes and enhance enzymatic activity. These physicochemical parameters collectively suggest the stability and acceptable quality of the prepared formulation.

HPTLC analysis of the hexane extract demonstrated a characteristic chromatographic pattern with multiple spots under UV detection at 254 nm and 366 nm, along with coloured bands after derivatization with vanillin–sulphuric acid reagent (Fig. 3). These findings indicate the presence of various phytochemicals like terpenoids, phenolics and other aromatic compounds, which contribute to the pharmacological properties of *Ajamoda*.

The present study didn't involve marker-based standardization. However, the chromatographic spots observed in the HPTLC profile correspond with literature reports of α-phellandrene and β-pinene, which are known volatile constituents of *Ajamoda (Apium graveolens)*. Thymol was reported in traces in the same spots however not considered as ideal primary marker for *Apium graveolens*. This indicates the probable presence of characteristic volatile phytoconstituents of *Ajamoda* in the formulation.

The HPTLC densitometric scan (Fig. 4) of *Ajamoda Arka* with *Yavakshara* showed major peaks at Rf 0.48, 0.55, 0.62, 0.67 and 0.74, with the highest peak area observed at Rf 0.62 (30.72%). Previous chromatographic studies on *Ajamoda* (*Apium graveolens*) have reported the presence of characteristic volatile constituents such as α-phellandrene (Rf

≈ 0.6) and β-pinene (Rf ≈ 0.8). In this research, peaks observed around Rf 0.59–0.62 and Rf 0.81–0.82 are similar with previous research values, indicating the possible presence of similar monoterpene constituents in the formulation. Thus, the Rf pattern obtained in this study is comparable with earlier reported HPTLC profiles of *Ajamoda*, supporting the chromatographic fingerprint and identity of the formulation. [13]

GC–MS analysis further revealed the presence of several phytoconstituents within the retention time range of 10.59–16.38 minutes, including alcohols, esters, fatty acids and terpenoid compounds (Fig.5) Cis-9-hexadecenal was identified amongst these constituents. Glycidylpalmitate and 11, 14-Eicosadienoic acid methyl ester were other important components observed. Similar compounds have been reported in plant extracts and are known to add to the aroma profile and biological properties of herbal formulations. Further, previous GC–MS analysis of *Apium graveolens* extracts have attributed its antimicrobial and antioxidant activities to various bioactive components present in it. [14, 15] Thus supporting the claim of the presence of biologically active compounds in *Ajamoda*-based preparations.

The physicochemical parameters obtained in this study (Table 2) indicate the stability and purity of the drug. Microbial parameters (Tables 3, 4, and 5; Figures 1, 2) indicate that there was no bacterial and fungal contamination within permissible limits. Similarly, HPTLC fingerprinting of the sample was used to confirm the presence of characteristic phytoconstituents markers of *Ajamoda* and develop a profile for the drug preparation.

The sample had low contamination risk with pesticides and heavy metals within the permissible limit as per API standards indicating superior quality of tested drug and suitable for oral administration with low risk of toxicity as per Ayush standards (Table 8).

Overall, the present study provides baseline analytical data for the preliminary standardization of *Ajamoda Arka* with *Yavakshara*, which may serve as reference data for quality assessment and future pharmacological investigations.

5. CONCLUSION:

The present study provides preliminary analytical standardization of *Ajamoda Arka* with *Yavakshara* using classical preparation methods supported by modern analytical techniques. Physicochemical parameters, HPTLC fingerprinting, and GC-MS analysis, microbial analysis, heavy metals, pesticides residue analyzed serve as baseline quality control standards for the formulation. The identified phytoconstituents may serve as marker compounds for arka containing *Apium graveolens*. The results obtained can serve as reference data for quality assurance and scientific standardization of the formulation in Ayurvedic pharmaceutics and future researches.

Abbreviations

AFI – Ayurvedic Formulary of India

API – Ayurvedic Pharmacopoeia of India

As – Arsenic

BSCPS – Buffered Sodium Chloride Peptone Solution

CAS – Chemical Abstracts Service

Cd – Cadmium

CFU – Colony Forming Units

cm – Centimeter; m – Meter; µm – Micrometre

CSDAM – Casein Soya Bean Digest Agar Medium

GC–MS – Gas Chromatography–Mass Spectrometry

g – Gram; kg – Kilogram; mg – Milligram

GMP – Good Manufacturing Practice

Hg – Mercury

HPTLC – High Performance Thin Layer Chromatography

L – Litre; mL – Millilitre; µL – Microlitre

min – Minutes

NaOH – Sodium Hydroxide

NOC – Number of Colonies

Pb – Lead

pH – Potential of Hydrogen

Rf – Retention factor

*RT – Retention Time

UV – Ultraviolet

VSA – Vanillin Sulphuric Acid

WHO – World Health Organization

°C – Degree Celsius

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