IJPSR (2026), Volume 17, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 13 March 2026; received in revised form, 28 April 2026; accepted, 19 June 2026; published 01 July 2026

COMPARATIVE ARJUNIC ACID ANALYSIS BY HPTLC STUDY OF ARJUNA KWATHA PREPARED BY USING VARIOUS KWATHA TECHNIQUES

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Keywords:

Arjuna Kwatha, Arjunic acid, HPTLC, Soxhlet extraction, Pressure cooker method, Ayurvedic pharmaceutics

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ABSTRACT: Kwatha Kalpana is a fundamental pharmaceutical dosage form in Ayurveda, widely used as a base for multiple formulations. Variations in preparation techniques such as water proportion, heating duration, and extraction methods may significantly influence phytochemical composition and therapeutic efficacy. The present study aimed to comparatively evaluate the effect of different Kwatha preparation techniques on the qualitative and quantitative presence of Arjunic acid in Arjuna Kwatha using High-Performance Thin Layer Chromatography (HPTLC). Authenticated Arjuna bark was processed and Kwatha was prepared using four techniques: conventional method (16 and 8 parts water), time-based method (1–4 hours boiling), Soxhlet extraction (60°C, 80°C, 100°C), and pressure cooker method (30 minutes). A total of ten batches were analyzed. Pharmaceutical parameters such as time, fuel consumption, and yield were recorded. HPTLC fingerprinting and quantification were performed using Arjunic acid as a marker compound. Results showed maximum Arjunic acid content in Soxhlet extraction at 100°C (0.0181%), followed by the conventional method with 8 parts water (0.0166%). Time-based heating showed gradual increase in marker content, while the pressure cooker method provided moderate yield with minimal time and energy consumption. The study concludes that Kwatha preparation technique significantly influences quantitative marker content while maintaining qualitative phytochemical consistency.

INTRODUCTION: *Kwath Kalpana* (Decoction) is the most famous and widely used Kalpana. It is the source of most of the secondary dosage forms in *Bhaishjya Kalpana.* (Pharmaceutical Science) Also, it is the mostly for the purpose of various pharmaceutical procedures. *Kwatha* is an important pharmaceutical dosage form in the treatment of various diseases whether it is prepared by single or multi-drug combination. It can be used as Anupana (Adjuvant) or Sahapna form also.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.17(7).2116-25

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.17(7).2116-25

Preparation is the most important and essential aspect of *Bhaishjya Kalpana* with fulfilment of appropriate standard operating procedures. *Kwatha kalpana* is mentioned in various Ayurveda classics but there is a difference of opinion regarding the addition of water, reduction of water, etc. Although ancient seers are mentioned these methods in their respective contexts, it's quite difficult to correlate the purpose of pharmaceutical procedures. Addition of water as per the consistency of drug is specially mentioned by *Sharangdhara* in the context of *Snehakalpana* [1].

Most of the time consistency is not identified properly and hence water is added as per the need. Also, due to time - saving approach of the various pharmacist, the quantity of water added and to be reduced is always compromised.

Similarly, difference of temperature, quantum of heating, vessel to be used, the Particle size of *Kwath Dravyas* are other factors that may question its potency. Thus, there need a quality check in order to optimize the *Kwatha* process for respective drugs. So, the study is conducted to observe the difference in the quality of Kwatha by using different techniques of Kwatha by HPTLC study.

For this purpose, Arjuna (*Terminalia arjuna* Roxb.) Dravya is selected. Arjuna is famous drug in Ayurved and widely used for therapeutic purpose. As the Arjuna *Twak* (Bark) [2] has been used for pharmaceutical purposes, its consistency is not categorised which could be between *Madhyam* (Moderate*)* and *Kathin* (Hard*)* Dravya.

So, this study will compare the difference in quality of *Kwatha* by adding two different proportion of water *i.e*. 16 part [3] and 8 parts [4] by conventional method of *Kwatha* preparation. Alternative techniques which can optimize *Kwatha* quality may be breakthrough for saving time and fuel.

In order to follow uniformity among all the batches, eight-part water is used in alternative techniques (Methods) of *Kwatha*. So, present study taken three alternative techniques of *Kwatha* preparations.

First is the time-based method, second is Soxhlet method [5] and third is pressure cooker method. The analysis of Arjuna Kwatha quality was assessed by the presence of *Arjunic acid* [6] which is the main marker for Arjuna with that of conventional method.

Materials:

Equipment for *Kwatha* Preparation: For Group A and B Technique: Stainless-steel vessel of having capacity of 5 lit (Length – 20cm: Depth - 20cm: Diameter -18cm) was taken for Kwatha preparation, LPG gas (Commercial LPG at 2.92kN/m2 (30gf/cm^2). Fuel consumption of LPG gas cylinder was calculated as per consumption in minutes (189gm per hour).

For Group C Technique: Soxhlet Apparatus of capacity 3lit (Extractor 1000ml F. B Flask 3000ml Allihn condenser 300mm Rating watt – 500). Calculation of units consumed for soxhlation were done by the energy consumed (units per hour) in one hour (60 minutes) is equal to heater power i.e. 500W. For Group D Technique: Pressure cooker of ISI (IS – 2347), CM/L -3707063 (German steel) having capacity of 5 lit (Length – 20cm, Depth – 17cm, Diameter - 23cm)

Collection and Authentication of Arjuna: Arjuna stem was collected from the Yavatmal region of Maharashtra, India, in the month of December 2020 (Latitude 20°21′36.1692″ N; Longitude 78°08′08.5668″ E). Sample was collected from single tree only. It was authenticated on 22/12/2020: by Government of India, Botonical survey of India, Koregaon Road, Pune, India. (Voucher specimen number: NOBSI/WRC/100-1/Tech/2020/114) deposited at ASS, Ayurved College, Panchvati, Nashik. Well dried Arjuna bark was pulverized to fine powder. It was then sifted with mesh size# 100

FIG. 1: SELF COLLECTED ARJUNA BARK

Categorization of *Kwatha* Techniques: Preparation of *Kwatha* were done by different techniques which were categorized under four major groups i.e. Conventional method (Group A), Time Based method (Group B), Soxhlet method (Group C) and pressure cooker method (Group D). Total 10 batches were prepared by these techniques and codified for the practical purpose.

TABLE 1: CODIFICATION OF ARJUNA KWATHA PREPARED BY FOUR TECHNIQUES

Groups	Techniques/Methods	Procedures used in *Kwath*	Experiment numbers	Code used
A	Conventional Method	1:16 times of water	1	AC1
		1:8 times of water	2	AC2
B	Time based Method	1 hr. boiling	3	A1H
		2hrs. boiling	4	A2H
		3 hrs. boiling	5	A3H
		4hrs. boiling	6	A4H
C	Soxhlet Method	Boiling @60°C	7	AS60
		Boiling @ 80°C	8	AS80
		Boiling @ 100°C	9	AS100
D	Pressure cooker Method	30 min boiling in Pressure Cooker	10	AP30

Methods of Preparation:

Group A: *Arjuna Kwatha* were prepared by *Arjuna Kalka* (Paste) (250gms) in two batches by using two different proportion of water *i.e.* 16 parts (4000 ml) and 8 parts (2000 ml) and boiled on mild heat to reduced $1/8^{th}$, and $1/4^{th}$ respectively (Total 2 batches).

Group B: *Arjuna Kwatha* were prepared using four different time duration using *Arjuna kalka* (250G) and 8 Parts (2000ml) of distilled water and boiled. First batch were boiled for 1 hr only (A1H). Similarly, another three batches were prepared by boiling the *Kwatha* for 2 hrs (A2H), 3 hrs (A3H) and 4 hrs (A4H) respectively (Total 4 batches).

Group C: In this method, three batches of *Kwatha* were prepared by Soxhlet extraction technique using *Arjuna Kalka* (250G) and distilled water (2000ml) at three different temperatures i.e. 60°C, 80°C and 100°C respectively (Total 3 batches).

Group D: In this method, pressure cooker was used for *Kwatha* preparation. *Arjuna kalka* was then taken into pressure cooker and 8 Parts (2000ml) distilled water was added into it and boiled at low temperature for 30 mints (One batch).

Temperature of *Kwatha* belongs to group A, B and D were maintained between 91°C to 100°C. Frequent stirring were done in the groups except Soxhlet extraction and pressure cooker method in order to maintain uniform temperature of mixture and avoid excessive heating at base. Glass mercury thermometer having range from 0 to 110 degree Celsius (100mm long) were used for temperature noting at every 30 minutes in classical (A) and time based method (B).

All the 10 samples of *Kwatha* were filtered with muslin cloth and stored in PET bottles.

TABLE 2: HPTLC QUANTIFICATION AND FINGERPRINTING OF ARJUNA

Chromatographic Conditions	
Application Mode	CAMAG Linomat 5 - Applicator
Filtering System	Whatman filter paper No. 1
Stationary Phase	MERCK - TLC / HPTLC Silica gel 60 F254 on Aluminum sheets
Application (Y axis) Start Position	10 mm
Development End Position	80 mm from plate base
Band Length	8 mm
Distance Between Tracks	15 mm
Sample Application Volume	12.0 µL
Standard Application Volume	12.0 µL
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	30 minutes
Mobile Phase (MP)	Chloroform : Methanol (9 : 1 v/v)
Visualization	@ 254 nm
Quantification Wavelength	205 nm

Preparation of Test Solution: Accurately weighed 1 g of Arjuna Kwatha sample was taken in a conical flask. It was refluxed for 30 minutes with 15 mL of Methanol consecutively for 3 times. It was then Filtered with the help of Whatman filter paper No. 1 and concentrated the combined extracts in an evaporating dish on water bath. Thereafter, made up the volume up to 10 mL with Methanol. Test solution thus obtained used for HPTLC fingerprinting.

Preparation of Standard Solution: Accurately weighed 11.7 mg of standard Arjunic acid taken into 2 mL volumetric flask, dissolved in Methanol and made up the volume up to 2 mL with Methanol. From this stock solution, 0.1 mL of solution pipetted into 2 mL volumetric flask and made up volume up to 2 mL with Methanol. The Standard solution thus obtained used for HPTLC fingerprinting

OBSERVATIONS AND RESULT:

TABLE 3: TIME AND FUEL REQUIRED FOR ARJUNA KWATHA

Sr. no.	Group Name	Quantity of Kalka (gm)	Quantity of water (ml)	Obtained volume (ml)	Total time in minutes	Total fuel used (in gms)/ Units of electricity
1	AC1	250	4000	450	510	1606.5
2	AC2	250	2000	470	240	693
3	A1H	250	2000	1400	60	189
4	A2H	250	2000	1000	120	378
5	A3H	250	2000	850	180	567
6	A4H	250	2000	450	240	756
7	AS60	250	2000	2000	480	4
8	AS80	250	2000	1850	375	3.075
9	AS100	250	2000	1840	450	3.650
10	AP30	250	2000	2000	30	94.5

AC1 **AC2**

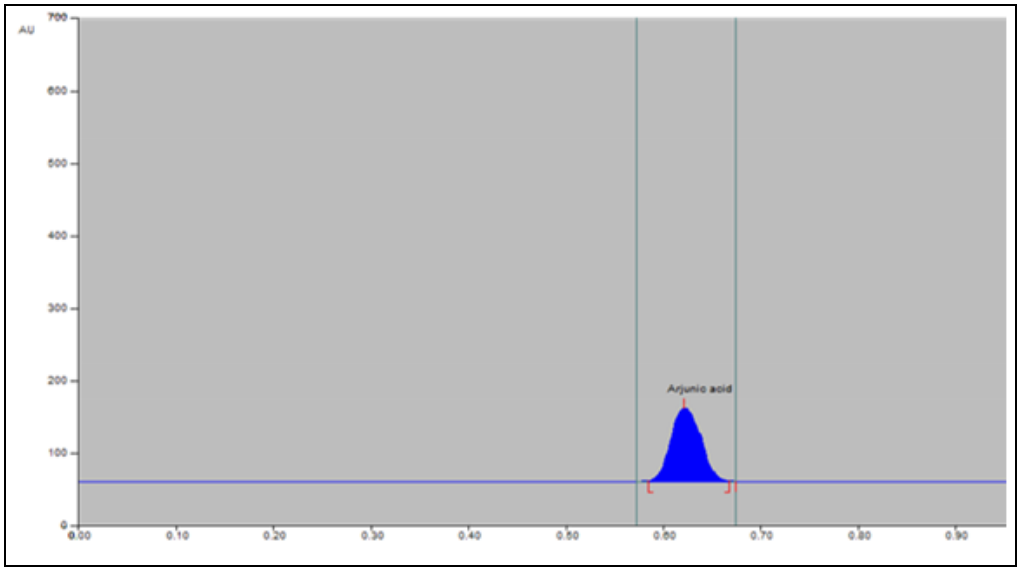
FIG. 2: ARJUNA KWATHA BY GROUP A METHOD

A. A1H **B. A2H** **C. A3H** **D. A4H**

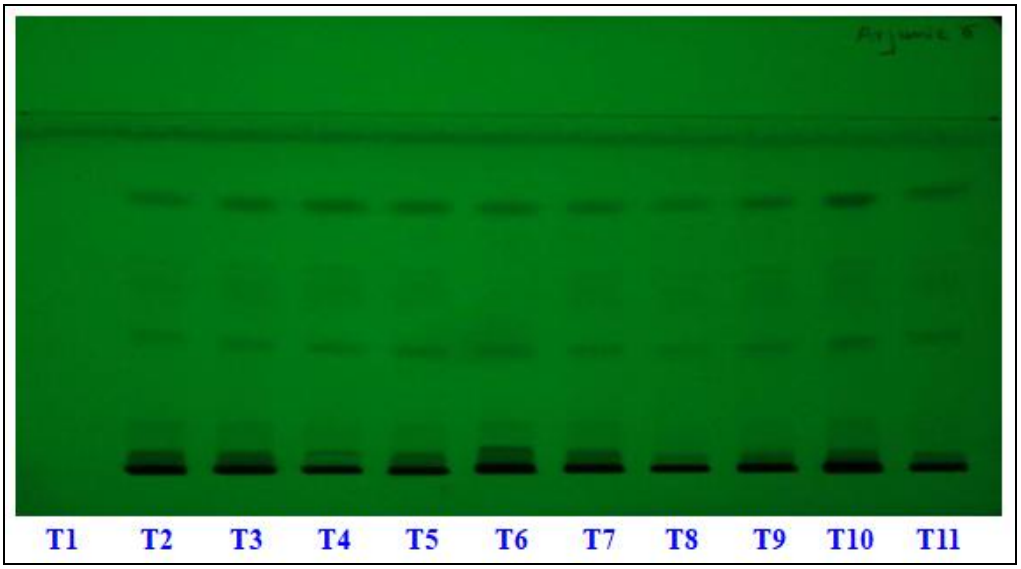
FIG. 3: ARJUNA KWATHA BY GROUP B METHOD

AS60 **AS80** **AS100**

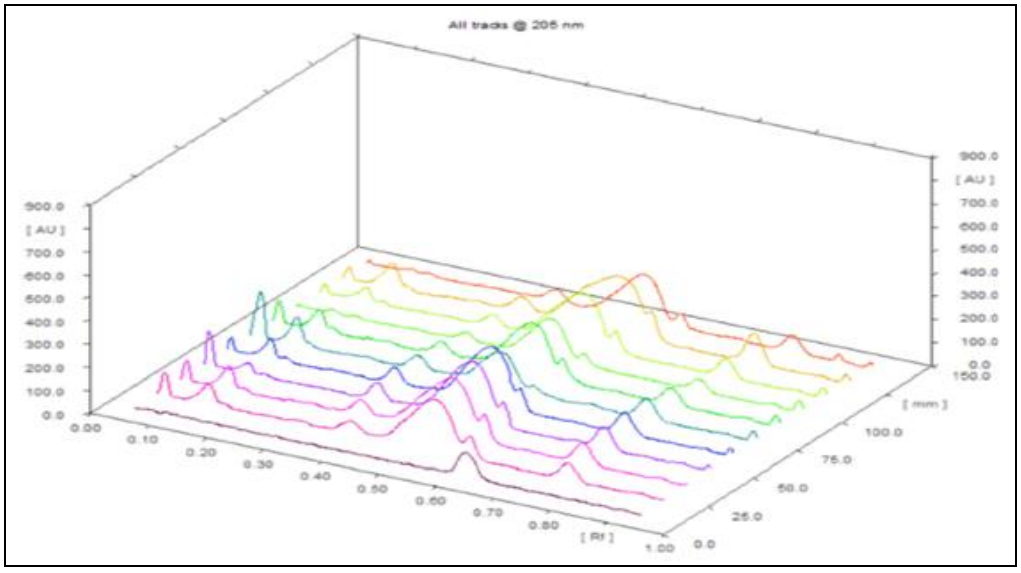
FIG. 4: ARJUNA KWATHA BY GROUP B METHOD



A. 2D CHROMATOGRAM OF ARJUNIC ACID @ 205 NM



B. HPTLC PLATE OF ARJUNIC ACID IN VARIOUS SAMPLE

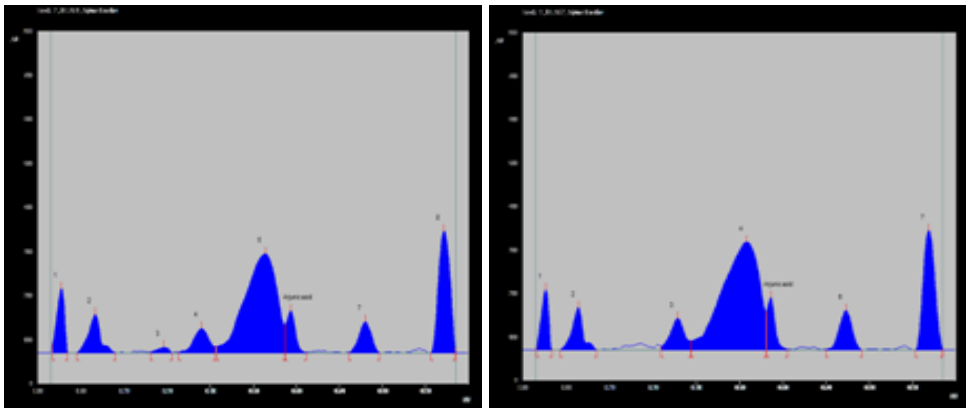


C. ALL TRACTS @205

FIG. 5: HPTLC STUDY OF ARJUNA KWATHA: COMPARATIVE QUANTIFICATION OF ARJUNIC ACID IN ARJUNA KWATHA SAMPLES

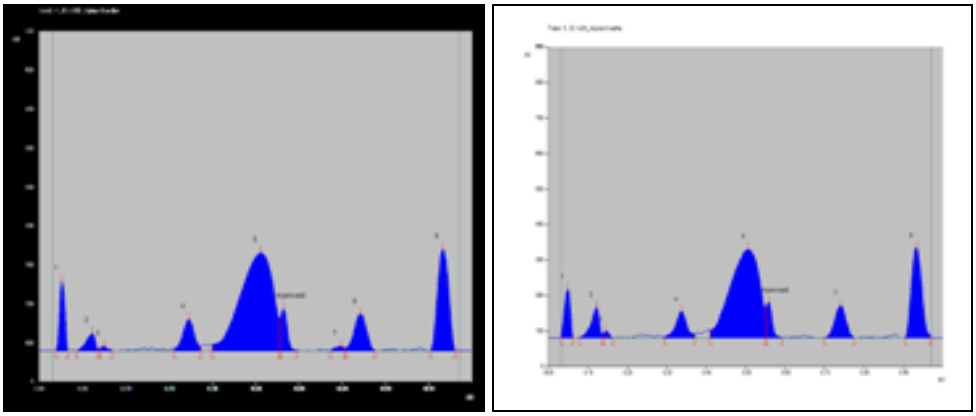
TABLE 4: HPTLC QUANTIFICATION OF ARJUNIC ACID IN ARJUNA KWATHA SAMPLES

Sample	Weight (mg)	Area	Arjunic Acid (%)
Arjunic acid	0.29mg	2851.2	-------
AC1	5114	1601.9	0.0150
AC2	5064	1758.2	0.0166 %
A1H	5082 mg	743.4	0.0070 %
A2H	5064 mg	1207.6	0.0114 %
A3H	5091 mg	1328.3	0.0125 %
A4H	5158 mg	1531.1	0.0142 %
AS60	5083 mg	962.9	0.0090 %
AS80	5105 mg	1540.9	0.0144 %
AS100	5033 mg	1909.8	0.0181 %
AP30	5042 mg	1008.3	0.0096 %

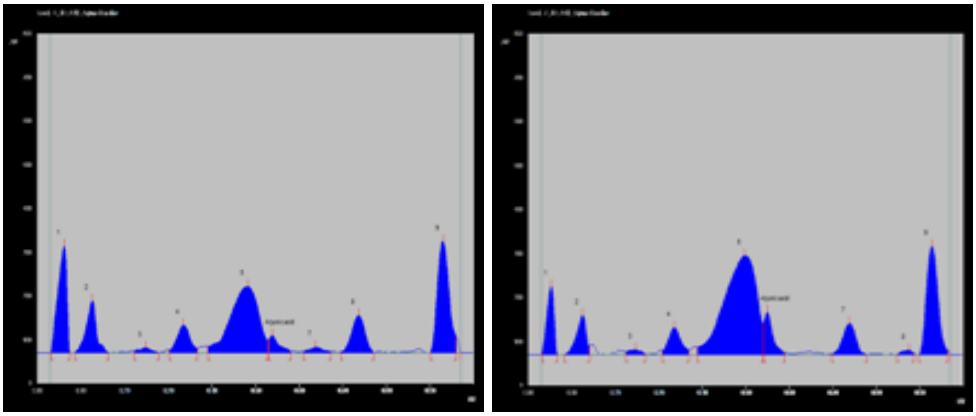


A. 2D CHROMATOGRAM OF AC1 **B. 2D CHROMATOGRAM OF AC2**

FIG. 6: 2D CHROMATOGRAM OF ARJUNA KWATHA BY GROUP A METHOD @ 205 NM

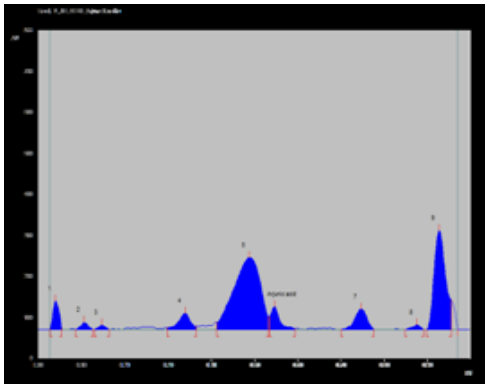


A. 2D CHROMATOGRAM OF A1H **B. 2D CHROMATOGRAM OF A2H**

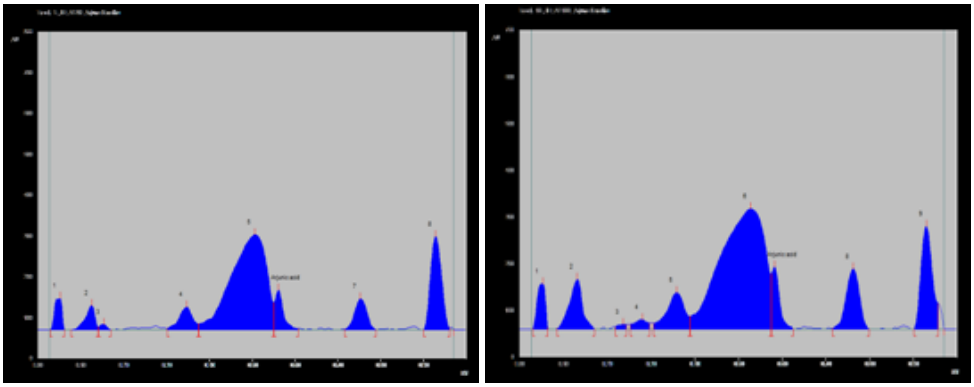


C. 2D CHROMATOGRAM OF A3H **D. 2D CHROMATOGRAM OF A4H**

FIG. 7: 2D CHROMATOGRAM OF ARJUNA KWATHA BY GROUP B METHOD @205NM

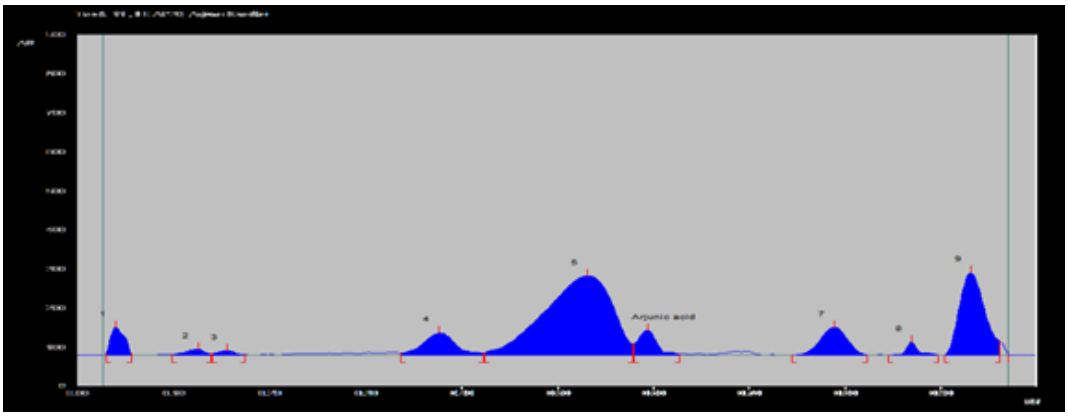


A. 2D CHROMATOGRAM OF AS60



B. 2D CHROMATOGRAM OF AS80 **C. 2D CHROMATOGRAM OF AS100**

FIG. 8: 2D CHROMATOGRAM OF ARJUNA KWATHA BY GROUP C METHOD @205NM



2D CHROMATOGRAM OF AP30

FIG. 9: 2D CHROMATOGRAM OF ARJUNA KWATHA BY GROUP D METHOD @205NM

TABLE 5: R_F VALUE OF ARJUNA KWATHA SAMPLE @205N

Spot No.	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
1.(0.05)	--	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
2.(0.14)	--	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
3.(0.25)	--	--	--	--	--	0.25	0.25	--	--	0.25	--
4.(0.28)	--	0.28	--	--	--	--	--	--	--	0.28	--
5.(0.36)	--	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
6.(0.52)	--	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52
7 0.60 (Arjunic acid)	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
8.(0.64)	--	--	--	--	--	0.64	--	--	--	--	--
9 (0.70)	--	--	--	0.70	--	--	--	--	--	--	--
10 (0.75)	--	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
11 (0.88)	--	--	--	--	--	--	0.88	0.88	--	--	0.88
12 (0.93)	--	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93

Denotes presence of spot in the sample, T1: Arjunic acid Standard; T2: AC1, T3: AC2, T4: A1H, T5: A2H, T6: A3H, T7: A4H, T8: AS60, T9: AS80, T10: AS100, T11: AP30

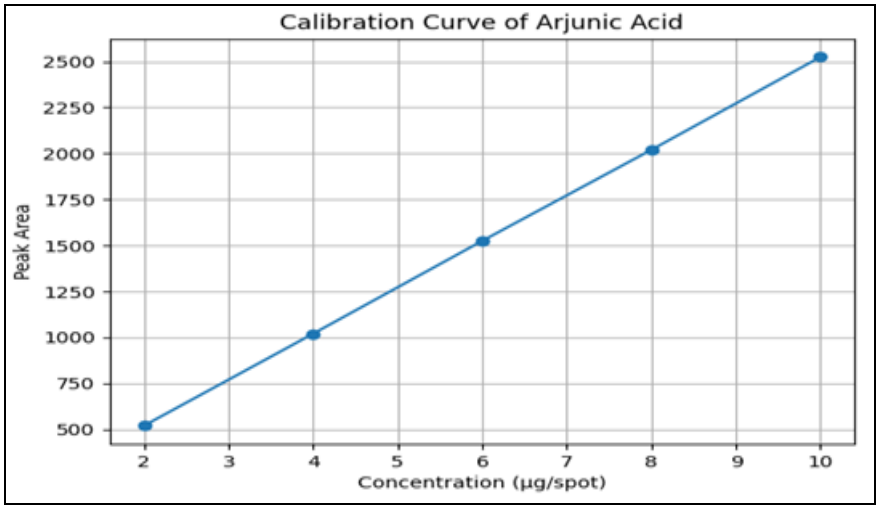


FIG. 10: CALIBRATION CURVE OF ARJUNIC ACID

TABLE 6: R_F VALUES OBSERVED IN ARJUNA KWATHA

Sample names	No of Spots	R_f Values
Arjunic acid	01	0.60
AC1	08	0.05,0.14,0.28,0.36,0.52,0.60,0.75,0.93
AC2	07	0.05,0.14,0.36,0.52,0.60,0.75,0.93
A1H	08	0.05,0.14,0.36,0.52,0.60,0.70,0.75,0.93
A2H	07	0.05,0.14,0.36,0.52,0.60,0.75,0.93
A3H	09	0.05,0.14,0.25,0.36,0.52,0.60, 0.64,0.75,0.93
A4H	09	0.05,0.14,0.25,0.36,0.52,0.60,0.75,0.88,0.93
AS60	08	0.05,0.14,0.36,0.52,0.60,0.75,0.88,0.93
AS80	07	0.05,0.14,0.36,0.52,0.60,0.75,0.93
AS100	09	0.05,0.14,0.25,0.28,0.36,0.52,0.60,0.75,0.93
AP30	08	0.05,0.14,0.36,0.52,0.60,0.75,0.88,0.93

DISCUSSION: Standardization of pharmaceutical processing is essential because variations in water proportion, duration of heating, temperature, and extraction system can significantly alter phytochemical yield.

The previous research study reported that the field sample of Arjuna bark which was collected according to the classical guidelines, showed organoleptic and physicochemical values fulfilled pharmacopeial standards [7]. This establishes the authenticity and suitability of the selected sample for pharmaceutical evaluation **Fig. 1.**

The particle size of Kwatha Churna is not very clearly mentioned in the ancient classics of Bhaishjyakalpana. Ayurvedic Pharmacopeia of India mentioned the sieve size of Kwatha Churna as 22# [8], present study was planned to study on the principle of size reduction, coarse material requires a longer extraction time and for the smaller the particle size, the more rapid the extraction. Smaller mesh size was chosen to report the result of this particular size of Kwatha churna.

Among various extraction techniques, Soxhlet extraction is very commonly practiced as it is more resembles Kwatha due to the hot continuous extraction. It required very little solvent and temperature regulation can be easily achieved with uniform percolation. The present study aimed to save time and reduce the processing cost; hence this method is selected with water as a solvent for extraction.

The Pressure cooker principle is based on increasing the boiling point of water with an increase in pressure, hence very less energy is required to cook. Also, the loss of nutrients is less, due to close procedure.

In Group A of Conventional Method, comparison between AC1 (16 parts water) and AC2 (8 parts water) revealed that AC1 required significantly more time (510 min) and fuel (1606.5 g) compared to AC2 (240 min; 693 g fuel).Despite prolonged heating and higher water volume, AC1 showed lower Arjunic acid content (0.0150%) compared to AC2 (0.0166%).

The higher marker concentration and reduced energy consumption make AC2 pharmaceutically and economically preferable however in group B, which was the time-based method (A1H–A4H), Arjunic acid percentage increased progressively with duration of boiling i.e. A1H (1 hr): 0.0070% , A2H (2 hrs): 0.0114%,A3H (3 hrs): 0.0125% and A4H (4 hrs): 0.0142%. Although A4H showed higher marker concentration, the fuel consumption also increased proportionally. Interestingly, A3H and A4H exhibited similar numbers of phytochemical spots in HPTLC, suggesting that beyond three hours, qualitative composition stabilizes, while quantitative increase is marginal. Hence, an optimized duration (around 3–4 hours) may balance efficiency and energy utilization.

In Group C, at Soxhlet Extraction Method among three batches, Arjunic acid concentration was AS60: 0.0090%, AS80: 0.0144% and AS100: 0.0181%. AS100 demonstrated the highest marker content among all ten samples. Higher temperature may improve solubility and diffusion rate of phytoconstituents, which explains higher yield at 100°C. HPTLC fingerprinting showed increased number of spots in AS100, suggesting better extraction of diverse phytochemicals. Energy consumption analysis revealed AS80 required comparatively less electricity and time than AS60 and AS100, indicating that while AS100 offers maximum marker yield, AS80 may represent a cost-effective compromise. In group D, which was the pressure cooker method (AP30) required only 30 minutes and minimal fuel (94.5 g), making it highly energy-efficient. Arjunic acid content (0.0096%) was higher than A1H but lower than conventional AC2 and Soxhlet AS100 methods. The closed high-pressure environment likely enhances rapid extraction, even with shorter duration. Although quantitative marker content was moderate, qualitative fingerprinting revealed comparable phytochemical spots with other groups. This indicates that pressure extraction can produce pharmaceutically acceptable Kwatha within significantly reduced time.

A prominent and consistent band was observed at Rf 0.60 in all tracks, which corresponds to the reference standard of Arjunic acid. The uniform presence of this band across all samples confirms the distribution of the marker compound irrespective of the preparation technique. The band was sharp, symmetrical, and free from interference, suggesting good specificity of the chromatographic method. Six common Rf values (0.05, 0.14, 0.36, 0.52, 0.75, 0.93) were observed in all batches, suggesting stable core phytochemical composition irrespective of processing variation.

Notably, the absence of overlapping peaks at Rf 0.60 across all samples confirms the selectivity of the method for Arjunic acid. The clear resolution of the marker band from adjacent peaks indicates that the chosen mobile phase system is suitable for effective separation. Ayurvedic classical texts emphasize the importance of *Samskara* (processing) in modifying drug potency. This study scientifically validates that pharmaceutical technique significantly alters marker concentration in Kwatha preparations. Moderate water proportion (8 parts), controlled heating duration, and optimized temperature enhance extraction efficiency. Although Soxhlet extraction yielded maximum Arjunic acid, it deviates from classical Kwatha preparation principles. Therefore, AC2 or optimized time-based method (3–4 hours) may be recommended for practical Ayurvedic pharmacy settings which is almost same fuel consumption. Also, these findings provide a scientific basis for optimizing Kwatha preparation and contribute toward evidence-based standardization of *Arjuna Kwatha* in Ayurvedic pharmaceutics.

Present study offers preliminary parameters for different techniques of Kwatha preparation, still the study lacks in quantitative method validation, also due to varying types of Kwatha techniques, there is no common pharmaceutical basis across samples, study also lacks to replicate HPTLC quantification and laboratory parameters cannot justifies its pharmacological correlation with marker differences.

CONCLUSION: Kwatha preparation techniques significantly affects quantitative recovery of Arjunic acid while maintaining qualitative phytochemical integrity. Soxhlet extraction at 100°C yielded maximum marker concentration, whereas the conventional 8-part water method provided a pharmaceutically balanced and energy-efficient approach aligned with classical guidelines. Standardization of water proportion, heating

duration, and extraction conditions is essential for ensuring reproducible quality in Arjuna Kwatha. Further studies need to correlate marker concentration with pharmacological activity to establish clinical relevance.

ACKNOWLEDGEMENTS: The authors sincerely thank Vasu pharmacy, Vadodara, Gujrat for their analytical assistance and ASS Ayurved College, Nashik, Panchvati for Pharmaceutical assistance. The authors are thankful to Botanist Dr. Chavhan sir, for authentication of the plant material used in this study.

CONFLICTS OF INTEREST: No conflict of interest.

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How to cite this article:
Dongre S and Pande S: Comparative arjunic acid analysis by HPTLC study of arjuna kwatha prepared by using various kwatha techniques. Int J Pharm Sci & Res 2026; 17(7): 2116-25. doi: 10.13040/IJPSR.0975-8232.17(7).2116-25.