International Journal of Pharmacy and Pharmaceutical Science

ISSN Print: 2664-7222
ISSN Online: 2664-7230
IJPPS 2022; 4(2): 15-24
www.pharmacyjournal.org
Received: 02-08-2022
Accepted: 04-09-2022

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Preparation, standardization of babbularishta and its comparison to marketed formulations

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DOI: https://doi.org/10.33545/26647222.2022.v4.i2a.56

Abstract
Arishtas are regarded as unique and valuable therapeutics due to their efficacy, stability and desirable features with additional benefit of longer shelf life. It is prepared using decoction of herbal drugs and contains self-generated alcohol. In the present study different marketed brands and in-house formulation of Babbularishta were thoroughly evaluated for their organoleptic characteristics and physicochemical parameters, to establish a routine procedure for standardization of these Ayurvedic formulations. As there is no evidence of TLC fingerprinting has been found reported, an attempt was made to design proper TLC fingerprinting of Babbularishta. Acacia Arabica is an active component of Babbularishta and it's mainly used to treat respiratory problems and also improves digestive power. 5-10% v/v alcohol was generated after fermentation of traditionally prepared Babbularishta formulation.

Keywords: TLC, HPTLC, ayurveda, standardization, babbularishta, gallic acid

Introduction
A large proportion of the world's population, particularly in developing and underdeveloped countries, still relies heavily on traditional medicine. Medicinal plants are a valuable and inexpensive source of unique phytochemicals, which are frequently used in the development of drugs to treat various diseases. The use of plants and plant products in medicine is becoming more popular because herbal medicines are inexpensive, widely available, and of natural origin, with higher safety margins and few or no side effects [1].
Standardization is an important criterion for ensuring quality control of herbal drug [2]. The standardization of herbal medicines is the process of defining a set of standards or features, consistent parameters, and definite quantitative and qualitative values that guarantee quality, effectiveness, safety, and repeatability. It is the method of creating and approving technical standards. The technique of prescribing a collection of qualities demonstrated by certain medications is determined through testing and observation, leading to the development of specific criteria. Therefore, standardization is a tool in the process of quality control [3]. "Evaluation" of a drug means confirming its identity as well as determining its quality, purity, and detecting the nature of adulteration [4]. An overview of fermentation as per Ayurvedic literature and its application in modern-day medicines with various aspects of manufacturing at industrial scale is presented [5].
Babbularishta is a fermented herbal preparation that is effective against a wide variety of ailments. It comprises of 5 to 10 percent alcohol produced by itself. For its effect, this alcohol serves as a medium [6].
Babbula is the primary herbs in Babbularishta (Indian gum Arabic tree). This plant includes several phytochemicals, including flavonoids, glycosides, polyacetylenes, alkaloids, thoipenes, phytosterols and polypeptides. Because of the presence of qualities such as vasoconstrictor, anti-bacterial, anti-oxidant, anti-inflammatory, spasmogenic, anti-platelet aggregators, antiseptic, and expectorant, these phytochemicals make herbs effective against a variety of illnesses. Its proven anti-inflammatory and anti-oxidant qualities are applied to treat acute and chronic bronchitis. This lowers mucus production and liquefies aberrant discharge, protecting the mucosa lining of the bronchi and bronchioles from harm. This is also recognized for its antibacterial capabilities, which aid in eradicating the germs and bacterial infection responsible for bronchitis. These qualities are also known to treat asthma, the common cold, and cough.

Anti-bacterial, antiseptic, anti-oxidant, anti-inflammatory, etc. medications are known to treat many skin illnesses, including allergic dermatitis and eczema. This traditional remedy also aids intestinal function. The syrup is made from babul and a variety of medicinal plants, including Dhataki, Krushna (Pippali), Jatiphala, Kankola, Ela (Cardamom), Tvak, Nagkesara, Lavanga, and Marica [7].

In the present study, Babbularishta was prepared in wide mouth porcelain jar with and without the addition of years. Assessment of the effect was made on the basis of the total time taken for the onset and completion of fermentation process and on the basis of organoleptic, physico-chemical and phytochemical characters.

Materials and Methods

Collection, identification and authentication of raw materials

The herb i.e. Babbula plant collected from the local region of sangli and other herbal drugs were procured form Welankar Ayurvedic Store, Sangli. In table no.1 shows the required herbal drugs which are used for the preparation of Babbualrishta formulation. Two different brands Babbularishta were purchased from Sankalpa Ayurveda and in-house formulate laboratory scale. Babbula plant and other ingredients were authenticated by Mr. M. D. Wadmare sir, Dept. of Botany, Smt. Kasturbai Walchand College, Sangli.

Table 1: Composition of babbularishta formulation

Sr. No	Ingredients	Latin names	Part used	Quantity by reference	Quantity used
1	Babbula API	*Acacia Arabica*	Stem bark	9.600 kg	2.400 kg
2	Jala for decoction Reduced	Water	Distilled	49.152 l 12.288 l	12.288 l 3.072 l
3	Guda	jaggery	-	4.8 kg	1.2 kg
4	Dhatki	*Woodfordia fructicosa*	Flower	768 g	192 g
5	Pippali	*Piper longum*	Fruit	96 g	24 g
6	Jatiphala	*Myristica fragrans*	Seed	48 g	12 g
7	Kankola	*Piper cubeba*	Fruit	48 g	12 g
8	Ela	*Elettaria cardamommum*	Seed	48 g	12 g
9	Tvak	*Cinnamomum zeylanicum*	Stem bark	48 g	12 g
10	Patra (tejpatra)	*Cinnamomum tamala*	Leaf	48 g	12 g
11	Nagakesara	*Mesua ferrea*	-	48 g	12 g
12	Lavanga	*Syzygium aromaticum*	Flower	48 g	12 g
13	Marica	*Piper nigrum*	Fruit	48 g	12 g

Preparation of babbularishta by fermentation method

All the required raw material was taken as per pharamacopoeial quantity. The Coarse powder of Babbula bark was added with required amount of water and soaking for overnight. Then it was boiled in a wide mouth vessel to a quarter part. Decoction (kashayam) was formed after filtration. Jaggery was added to this kashayam and stirred until completely dissolved before being filtered again. Then filtrate was transferred into fermenting vessel. Now, fine powders of the other components were added to the vessel and mixed to create a homogenous mixture. The porcelain jar was tightly sealed with cotton cloth and kept for fermentation for 30 days. After the fermentation process is completed, the decoction was filtered by muslin cloth and stored in clean air tight bottle for maturation. The detailed observations during preparation are shown in table no.2 and 3 [6].

Table 2: Details of preparation of kwath

Sr. No.	Parameters	Result
1	Quantity of Bharad taken	2.400 kg
2	Quantity of water taken	12.288 L
3	Total time of soaking	12 hr
4	Temperature maintained after ½hr of heating	90-95°C
5	Kwath obtained	3.072 L
6	Weight of residue after filtration	2.800 kg
7	Total time taken for preparation	4.5 hr

Table 3: Details of preparation of babbularishta

Sr. No.	Parameters/Stage	Result
1	Kwath used	3.072 L
2	Guda added	1.2 kg
3	Dhataki pushpa added	192 gm
4	Initiation of fermentation	On 5th day
5	Completion of fermentation	In 30 days
6	Quantity of Babbularishta obtained	2.950 L
7	Time taken for maturation	30 days

Standardization of different marketed brands and in-house formulation of babbularishta

Organoleptic properties: All the different brands and in-house formulation of Babbularishta were evaluated organolepticaly for their colour, odour and taste. The results are shown in table-4 [6].

Physicochemical evaluation: Physicochemical Evaluation *viz*. pH, Phenolic content, total solids, alcohol content, specific gravity etc. were determined as per the procedure given in Ayurvedic Pharmacopoeia of India [6].

1) Determination of Total phenolic content

The absorbance of standard tannic acid solutions is measured at 725 nm using a UV-visible spectrophotometer (Jasco, V550), and the standard curve is plotted. The phenolic content is given as tannic acid equivalent. Sample Babbularishta was treated to determine the total phenolic content using the Folin-Ciocalteau technique [6, 8].

2) Determination of total solid content

Solid contents were determined by heating the products until the solvent was removed. A 50 mL of a formulation was taken in evaporated dish which was previously weighed and heated on water bath until the residues were apparently dry, dried at 105º for 3 hrs. to constant weight and solid contents were calculated [6, 9].

3) Specific Gravity

The specific gravity of each formulation was determined in triplicate using 10 ml specific gravity bottles and the following formula -

Specific gravity of liquid (formulation) = (weight of 10 ml of liquid /10) / (weight of 10 ml of water/10) [6, 10].

4) Determination of pH

The digital pH meter was used to check the pH of formulation. pH was noted for all brands of Babbularishta after opening the bottle and 7 days and 14 days after opening the bottle. The results have been shown in the table-10 [6, 11].

5) Determination of Reducing and non-reducing sugars

Clarifying reagent

Solution 1: Dissolve 21.9 g of zinc acetate and 3 ml of glacial acetic acid in purified water and make the volume to 100 ml.

Solution 2: Dissolve 10.6 g of potassium ferrocyanide in water and make up to 100 ml.

Reducing sugars: The suitable amount of the sample was taken and neutralize with sodium hydroxide solution (10% in water). Evaporate the neutralized solution to half the volume on a water bath at 50 °C to remove the alcohol. Cool the solution add 10 ml of the clarifying solution 1 followed by 10 ml of the clarifying solution 2. Mix, filter through a dry filter paper and make up the volume to 100 ml. Take 10 ml of the Fehling's solution and from a burette and add sugar solution (above prepared sample) in a drop wise manner and heat to boiling over the hot plate (maintained at 800) until the mixture of Copper (Fehling's solution) appears to be nearly reduced. Add 3-5 drops of 1% methylene blue and continue the titration till the blue colour is discharged. Note down the readings and calculate the percentage of glucose.

Non-reducing sugars: The suitable amount of sample was taken and neutralize with sodium hydroxide solution (10% in water). Evaporate the neutralized solution to half the volume on a water bath at 50 °C to remove the alcohol. Cool the solution add 10 ml of the clarifying solution 1 followed by 10 ml of the clarifying solution 2. Mix, filter through a dry filter paper. To the Filter add 15 ml of 0.1 N hydrochloric acid. Cover with stopper and heat to boiling for two minutes. Add phenolphthalein and neutralize with sodium hydroxide solution (10%). Transfer to 100 ml volumetric flask and make the volume to 100 ml and perform the titration as done for the reducing sugars. Calculate the percentage of the total sugars. Subtract the percentage of the reducing sugars from the sugars to obtain non-reducing sugars [6, 9].

6) Determination of alcohol content

25 ml of preparation were mixed separately with about 100 ml of water and saturated with sodium chloride. Then 100 ml of hexane was added and the mixtures and vigorously shacked for 2 to 3 minutes and allowed to stand for 15 to 20 minutes. Lower layers were run into the distillation flask, the hexane layer was washed by shaking vigorously with about 25ml of sodium chloride solution, allowed to separate and washed liquors were run into the first saline solution. Mixed solutions were made just alkaline with 1M sodium hydroxide using solid phenolphthalein as indicator. Little pumice powder and 100 ml of water were added. Mixture was distilled and not less than 90 ml of distillates were collected into 100 ml volumetric flasks and made up the volume with distilled water. Specific gravities of both the mixtures were determined and alcohol contents were calculated from the table [6, 12].

Phytochemical Screening

Active Phytoconstituents like carbohydrates, alkaloids, glycosides, tannins and flavonoids were identified in marketed formulations and Laboratory formulation of Babbularishta [13].

Thin Layer Chromatography (TLC)

Formulations were subjected to chromatographic separation on silica gel. TLC fingerprint profiles of standard gallic acid, caffeic acid and entire three Babbularishta were compared and confirmed by thin layer chromatography.

Sample preparation

- Preparation of gallic acid solution (standard 1): 1mg of gallic. acid dissolved in 1 ml methanol.
- Preparation of caffeic acid solution (standard 2): 0.1 mg of caffiec. acid dissolved in 1 ml methanol.

15µl of solution as prepared above (test solution) and 5 µl each of gallic acid (std 1) and caffeic acid (std 2) solutions applied separately on TLC plate and plate was developed at distance of 8 cm using toluene: ethyl acetate: formic acid: methanol as mobile phase in ratio of 3: 3: 08: 0.2. (v/v/v/v). After development, plate was allowed to air dry and examined under ultraviolet light (366nm). Rf values were calculated. Same procedure was repeated in triplicate. Results are tabulated in Table 6 and 7 [6].

High Performance Thin Layer Chromatography (HPTLC)

Estimation and Identification of Marker Constituent Gallic Acid from Babbularishta

The samples were spotted in the form of bands of width of 4 mm with space between bands of 3.8 mm, with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium plate 60 F_{254} (5 cm ×10 cm) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 5 mm × 0.45 mm and scanning speed of 20 mm/sec was employed.

The linear ascending development was carried out in 10 cm×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using Toluene: Ethyl Acetate: Acetic Acid: Methanol (3: 3: 0.8: 0.2 v/v) as mobile phase. The optimized chamber saturation time for mobile phase was 15 min. The length of chromatogram run was 8 cm and development time was approximately 20 min. TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 271 nm for all developments operated by WINCATS software version 1.4.2 [6].

Results and Discussion

Babbularishta formulation was prepared as per procedure and formula given in the Ayurvedic Pharmacopoeia of India. Laboratory prepared Babbularishta was considered as standard (LB). Figure 9 shows images which were captured during various stages of preparation of Babbularishta.

While preparing a Kwath, the colour of mixture became light reddish brown after ½ hr and later turned dark brown after 2 hrs. Before onset of fermentation, the colour of kwath was unchanged and the Dhataki pushpa were floating on the surface. In china clay jar, fermentation process commenced after 05 days and effervescence along with the hissing and bubbling sound was noticed. The lid of jar was tightly closed and jar was kept in clean dry place for one month till the fermentation process is complete.

After completion of fermentation process, ready Babbularishta was kept for a period of one month for maturation. No frothing upon the surface of liquid was observed during this period.

- **Completion fermentation:** In 30 days
- **Quantity of Babbularishta obtained:** 2.950 L
- **Time taken for maturation:** 30 days

Organoleptic evaluation

Organoleptic characteristics of prepared Babbularishta formulation revealed that the taste of the formulation was slightly bitter, alcoholic and the appearance was liquid with suspended particles.

Table 4: Organoleptic Properties of Babbularishta

Sr. No.	Formulations	Appearance	Colour	Taste	Odour
1	LB	Liquid	Dark reddish brown	Slightly Bitter, Alcoholic	Aromatic
2	MB 1	Liquid	Brown	Sweet alcoholic	Aromatic
3	MB 2	Liquid	Brown	Slightly sweet, alcoholic	Aromatic

Physicochemical evaluation

Phytochemical Screening

All the three samples were subjected to qualitative phytochemical investigation. Table no.5 Shows results of phytochemical testing of laboratory prepared Babbularishta and both marketed products. It showed presence of alkaloid, flavonoid, tannin, volatile oil, carbohydrate etc.

Table 5: Phytochemical screening

Phytoconstituents	LB	MB 1	MB 2
Alkaloid	+	+	+
Glycoside (anthraquinone & coumarin)	_	_	_
(cardiac & saponin)	+	+	+
Flavonoid	+	+	+
Carbohydrate	+	+	+
Tannins and phenolic compound	+	+	+
Proteins and amino acid	+	+	+
Steroids	+	+	+
Volatile oil	+	+	+

Physicochemical studies

Prepared Babbularishta was found to be dilute, having suspended particles whereas marketed product was clear and concentrated liquid. Self-generated alcohol itself act as preservative and improve stability of formulation, no addition of external preservative is essential. Prepared product showed more solid content of 64.93% w/v than marketed arishta, which was 36.93%w/w (MB1) and 61.04 (MB2). Specific gravity of all products was found to be within range i.e. 1.05-1.10. Alcohol content of all three formulations was found to be within range 5-10% v/v. Alcohol content in prepared arishta was 7.2081% v/v while in that of marketed arishta was 6.4202% v/v for MB1 and 7.232%v/v for MB2. Both marketed and Prepared formulation give positive tests for tannin, volatile oil, alkaloid, carbohydrate and flavonoid (table No.2). All remaining parameters were found to be within the normal range which are given in table No.10.

Thin layer chromatography (TLC)

TLC fingerprint profiles of standard gallic acid, caffeic acid and entire three Babbularishta were compared.

Standard gallic acid and caffeic acid were identified and confirmed by thin layer chromatography. Table no. represents results of TLC fingerprint profiles which shows Rf value of both gallic acid and caffeic acid matches with prepared babbularisha and two marketed arishtas.

Table 6: RF value of standard caffeic acid and formulations

Sr. No.	Sample	RF value from TLC fingerprinting
1	Caffeic acid	0.57
2	LB	0.58
3	MB 1	0.57
4	MB 2	0.58

Table 7: Rf value of standard gallic acid and formulations

Sr. No.	Sample	RF value from TLC fingerprinting
1	Gallic acid	0.44
2	LB	0.44
3	MB 1	0.45
4	MB 2	0.46

High Performance Thin Layer Chromatography (HPTLC): Estimation and Identification of Marker Constituent Gallic Acid from Babbularishta.

- Quantitative estimation of gallic acid was done by using Toluene: ethyl acetate: formic acid: methanol (3: 3: 0.8: 0.2) v/v/v/v as mobile phase. The formulation contains 0.02 to 0.10 percent w/v of gallic acid. The polynominal regression data of gallic acid was interpreted separately for its linearity at 1000-5000 µg/ml with regression coefficient of (r^2) 0.9985.
- Amount of gallic acid in test solution (formulations) was calculated from the calibration curve of gallic acid.

Table 8: linearity of gallic acid by HPTLC

Track	Sample volume in µl	Amount fraction in ng	Rf value	Mean area (AU)	±S.D	%RSD	Correlation coefficient (r^2)
1	1	1000	0.50	7614.4	3.605551	0.047352	0.9985
2	2	2000	0.52	14068.4	3.464102	0.024623	
3	3	3000	0.52	21770.2	1.732051	0.007956	
4	4	4000	0.52	29990.5	3.464102	0.011551	
5	5	5000	0.52	37339.6	1.732051	0.004639	

Calibration curve of gallic acid

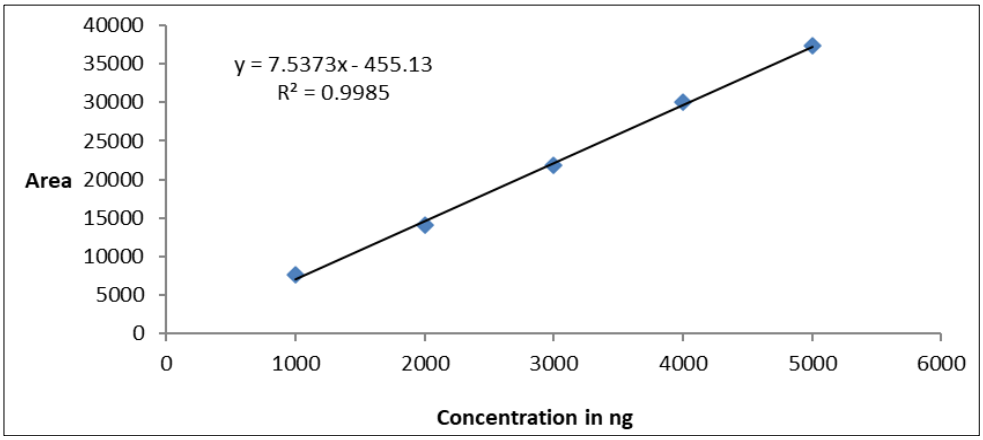


Table 9: Quantitative estimation of gallic acid in formulations by hptlc

Sample	Sample volume (µl)	Mean area (AU)	±S.D	% R.S.D	Concentration in ng	Rf Value	% w/v gallic acid	Normal Range
LB	5 µl	31148.9	3.605551	0.011575	4193.180	0.50	0.084	0.02 -0.10 % w/v
MB 1	5 µl	30345.1	3.605551	0.011882	4086.533	0.51	0.082	
MB 2	5 µl	33237.5	3.923009	0.011803	4470.293	0.49	0.089	

HPTLC Chromatograph Samples

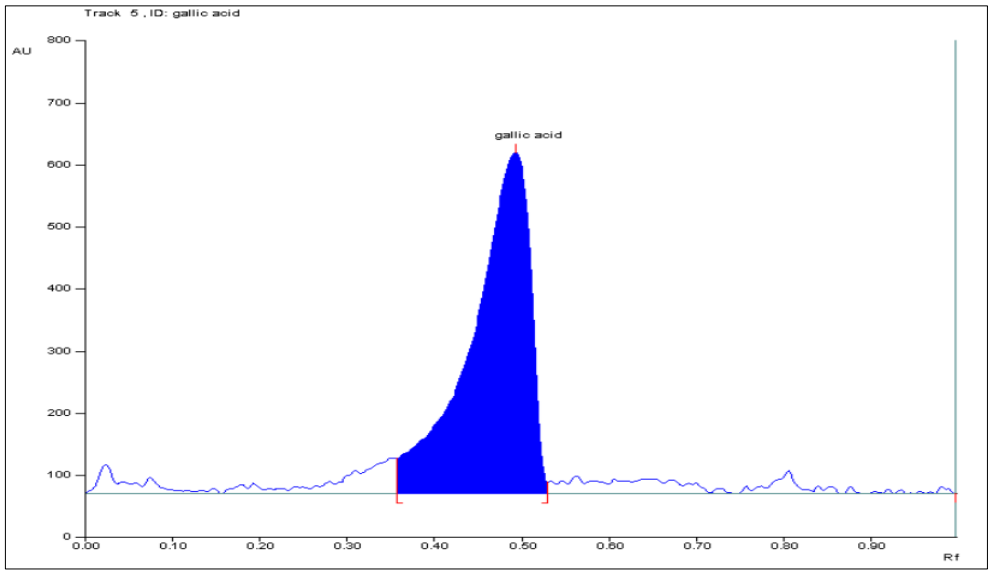


Fig 1: HPTLC fingerprint Chromatograph of track 1 (STD) i.e. Gallic acid

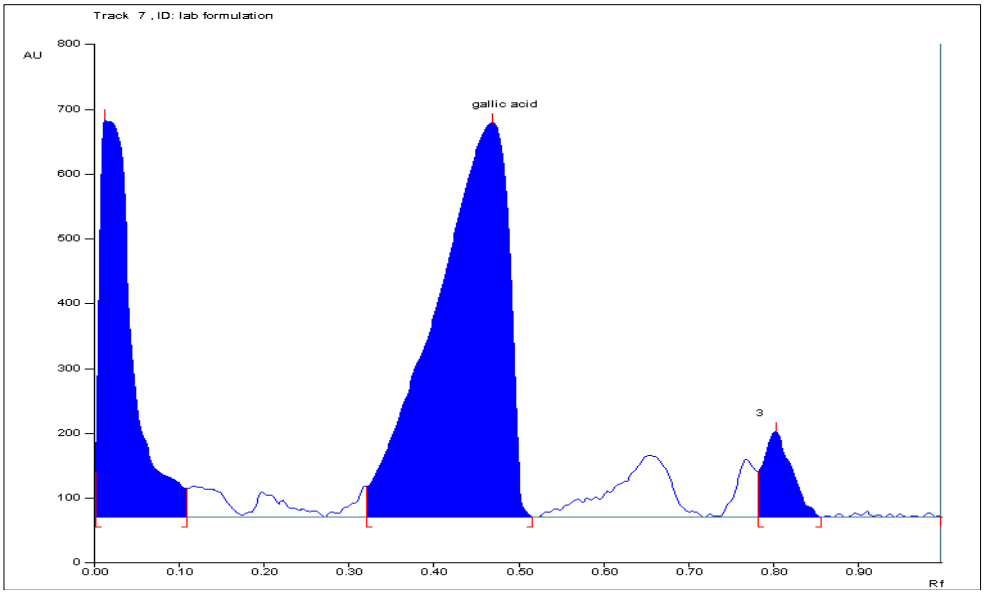


Fig 2: HPTLC fingerprint Chromatograph of track 2 (LB)

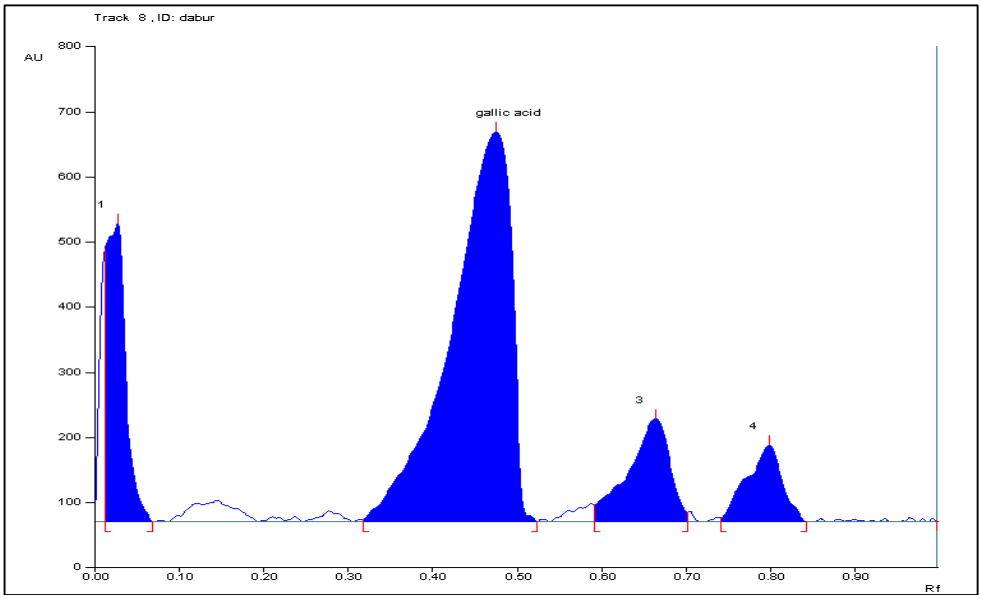


Fig 3: HPTLC fingerprint Chromatograph of track 3 (MB 1)

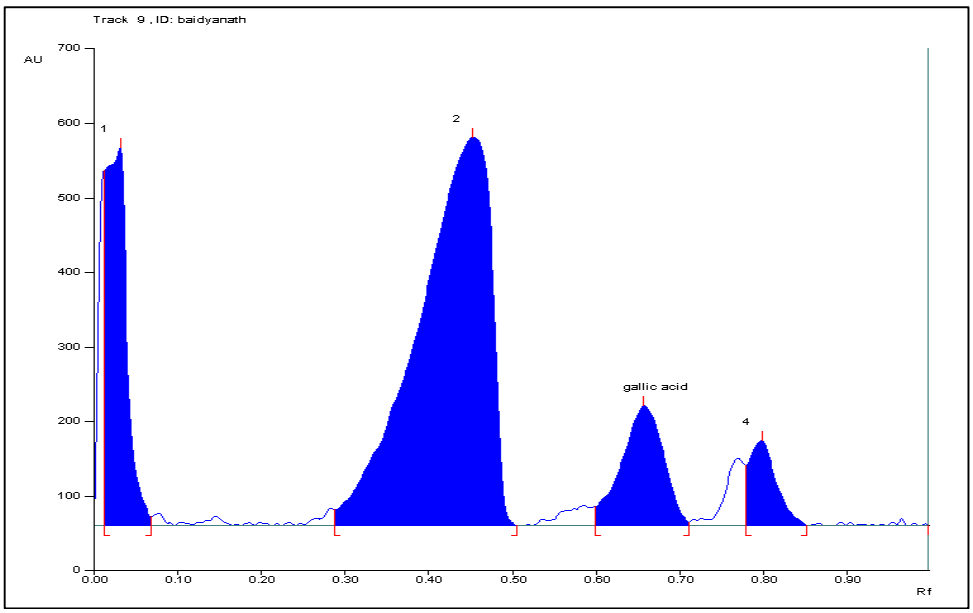


Fig 4: HPTL fingerprint Chromatograph of track 4 (MB 2)

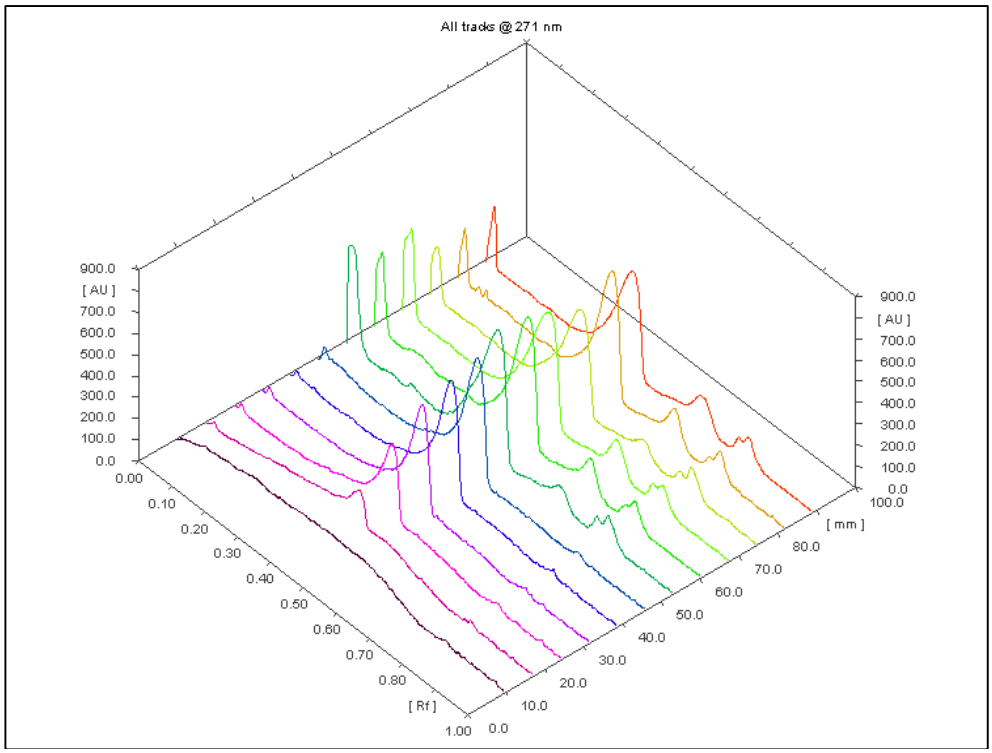
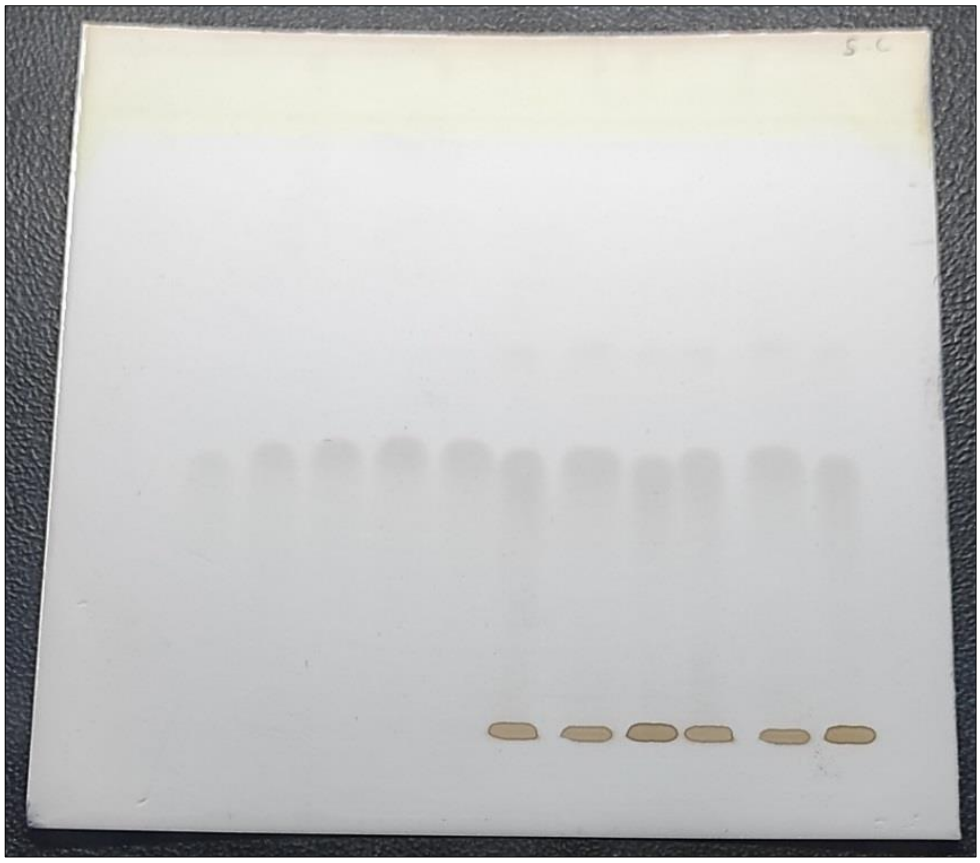


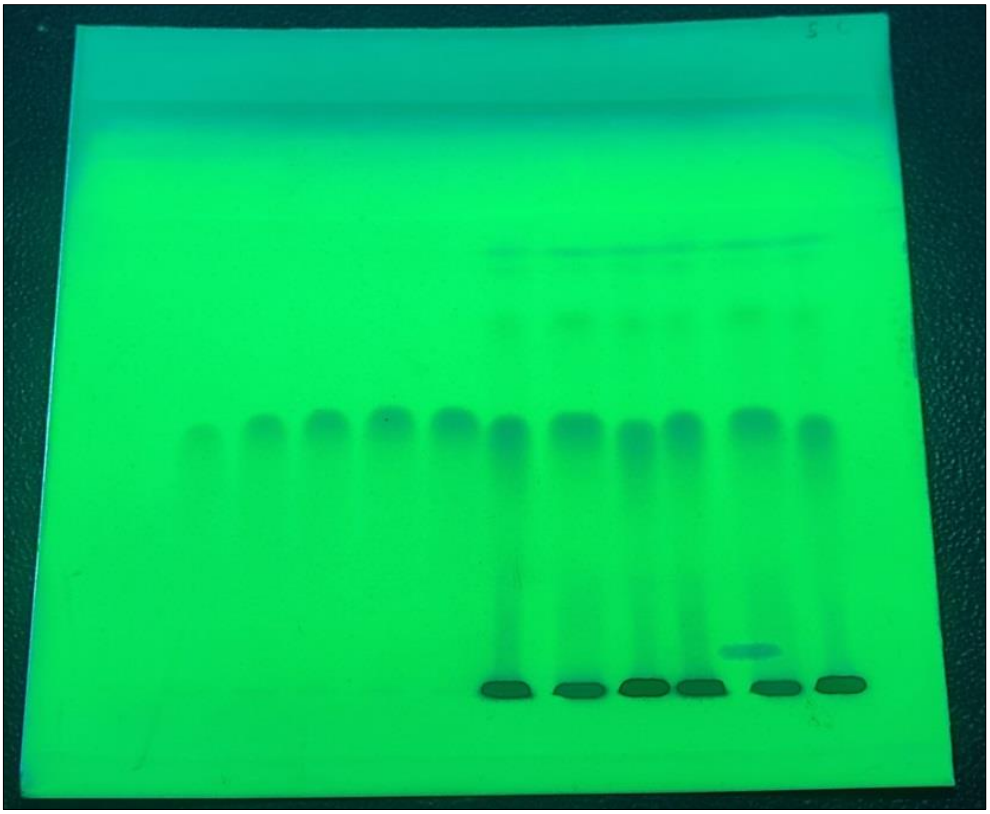
Fig 5: 3D Densitogram (All tracks scanned at 271 nm)

HPTLC photo documentation: All spots were clearly observed under visible light and UV region (254 nm) and having Rf values are 0.52.



1 2 3 4 5 6 7 8 9 10 11 12

Fig 6: HPTLC Chromatograph of samples under visible light



1 2 3 4 5 6 7 8 9 10 11 12

Fig 7: HPTLC Chromatograph of samples under UV light

Track 1: Blank (Methanol)
Track 2: Gallic Acid in Methanol (1 µL, 1000 ng/band)
Track 3: Gallic Acid in Methanol (2 µL, 2000 ng/band)
Track 4: Gallic Acid in Methanol (3 µL, 3000 ng/band)
Track 5: Gallic Acid in Methanol (4 µL, 4000 ng/band)
Track 6: Gallic Acid in Methanol (5 µL, 5000 ng/band)
Track 7: Laboratory Formulation (5 µL)
Track 8: MB 1 Formulation (5 µL)
Track 9: MB 2 Formulation (5 µL)
Track 10: Laboratory Formulation (5 µL)
Track 11: MB 1 Formulation (5 µL)
Track 12: MB 2 Formulation (5 µL)

Table 10: Standardization of prepared and marketed babbularishta

Sr. No.	Parameters	Laboratory Babbularishta	Marketed Babbularishta 1 (MB 1)	Marketed Babbularishta 2 (MB 2)	Standard values
1	Phenolic content% w/v equivalent to tannic acid	0.197	0.201	0.215	187-0.208% w/v
2	Total solids% w/v	64.93	36.93	61.04	NLT 16.5% w/v
3	Specific gravity gm/cm³	1.009	1.08	1.10	1.05-1.10 gm/cm³
4	pH	4.41	4.37	4.50	4.0-4.50
5	Alcohol content	7.2081	6.4202	7.232	5-10%v/v
6	Reducing sugar	7.60	9.33	9.98	NLT 4.20%w/v
7	Non-reducing sugar	0.71	0.86	0.73	NMT 0.80%W/V
8	Detection of methanol	Absent	Absent	Absent	Methanol absent
9	Viscosity	2.3458	2.4812	3.6329	-
10	Estimation of gallic acid by HPTLC	0.084	0.082	0.089	0.02-0.10%w/v

TLC fingerprinting of samples

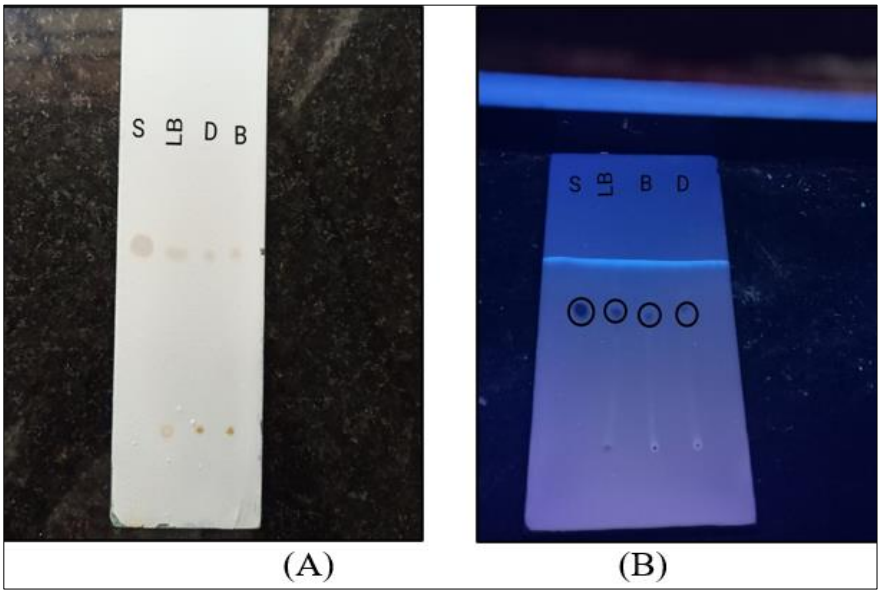


Fig 8: (A) TLC of standard Caffeic acid (S), laboratory prepared Babbularishta (LB) and marketed formulations MB 1 (D), MB 2 (B) (B) TLC of standard Gallic acid (S), laboratory prepared Babbularishta(LB), marketed formulations MB 1(D), MB 2 (B) under UV light 366nm

Images captured during preparation of babbularishta

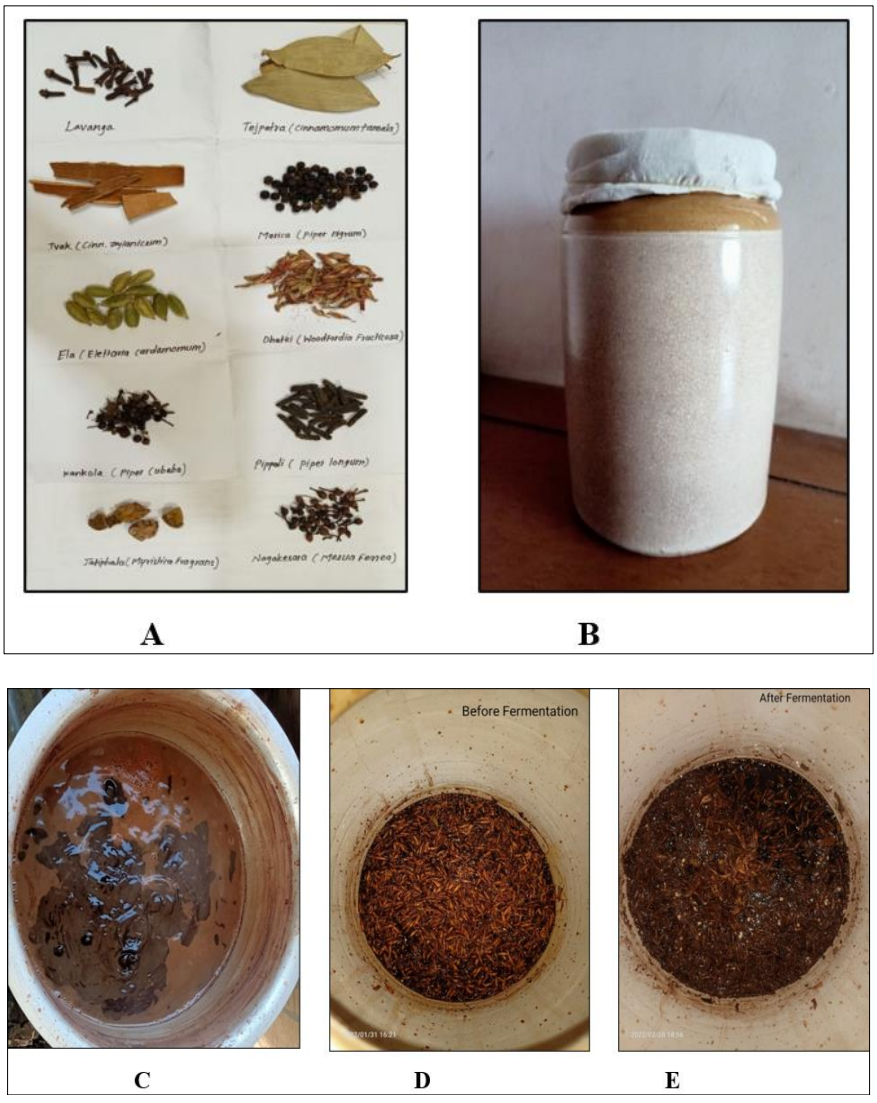


Fig. 9: A) Herbal raw material, B) Tightly closed container placed undisturbed (1 month) for fermentation, C) Decoction of Kwath, D) Arishta in container before fermentation, E) Arishta in container after fermentation

Conclusion

Arishta and ASAVA are considered as one among best formulation in Ayurveda as they possess self-generated alcohol which acts as self-preservative. By following the classical method of preparation, arishta of good quality can be obtained. All the results of raw materials were consistent with standard values given in their respected monographs. Babbularishta was successfully prepared on lab scale and Organoleptic characters fulfills the standards of fermented Ayurvedic preparation i.e. Sandhana preparations. Preliminary phytochemical screening revealed the presence of Alkaloids, Tannin, Flavonoids, carbohydrates and Proteins. Considering quality assessment and standardization, physicochemical evaluation was performed and its values were analyzed to set the standard. Parameters studied for standardization of prepared and marketed products were found within acceptance limits, except Phenolic content were found 0.215%w/v i.e. slightly higher than normal range. TLC fingerprinting was developed in which it was found that all three sample of Babbularishta showed better separation. Estimation of Gallic acid was successfully done by using HPTLC method in all three Babbularishta formulations. Thus, TLC and HPTLC can be consider as the basic tool for the quality control measures of Babbularishta to improve their acceptance. By utilizing modern technique and tools we can standardize these formulations to minimize these gaps, which will improve global use of Ayurvedic formulation.

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