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Standardization and Analytical Profiling of *Babula* Fruit (Acacia Arabica Willd.) Using Physicochemical, Phytochemical and Hptlc Screening

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ABSTRACT

Background: *Acacia arabica* Willd. (syn. *Acacia nilotica*), commonly known as *Babula*, is a well-documented medicinal plant used in Ayurveda, Unani, and folk medicine for treating various conditions including inflammation, infections, gastrointestinal disorders, and bone-related ailments. However, scientific validation through standardized analytical studies is essential for quality assurance and pharmacological relevance.

Objective: The present study was conducted to evaluate the physicochemical properties, phytochemical constituents, and HPTLC fingerprint profile of *Babula* (*Acacia arabica* Willd.) fruit to support its traditional use and ensure quality control.

Methods: Physicochemical parameters (pH, loss on drying, ash values, and extractives) were evaluated as per standard protocols. Methanolic extract was used for preliminary phytochemical screening and was tested for alkaloids, flavonoids, tannins, saponins, and steroids. HPTLC was performed using methanol extract with a mobile phase of toluene:ethyl acetate:formic acid (7:3:0.3 v/v) and visualized at 254 nm, 366 nm, and 540 nm.

Results: Physicochemical analysis showed pH 4.57, loss on drying 7.62%, ash value 10.53%, acid insoluble ash 1.25%, alcohol soluble extractive 20.39%, and water-soluble extractive 15.78%. Phytochemical screening detected flavonoids, tannins, steroids, and saponins; alkaloids were absent. HPTLC revealed 4, 12, and 4 distinct peaks at 254 nm, 366 nm, and 540 nm respectively, providing a characteristic fingerprint of the plant material.

Conclusion: The combined physicochemical, phytochemical, and HPTLC tests of *Babula* (*Acacia arabica* Willd.) fruit confirm its traditional uses and help ensure its quality. These results support using Acacia arabica in herbal medicine and help develop it into reliable herbal products.

KEYWORD: *Babula*, *Acacia arabica* Willd., physicochemical, Phytochemicals, HPTLC

INTRODUCTION

Medicinal plants have long been integral to traditional healthcare systems, particularly in Ayurveda, Unani, and folk medicine, due to their therapeutic potential and minimal side effects. *Babula* (commonly referring to *Acacia nilotica*), known for its wide range of pharmacological activities, has been used traditionally to treat various ailments such as inflammation, infections, gastrointestinal disorders and bone related disease. To establish scientific credibility and ensure the quality, safety, and efficacy of such herbal drugs, comprehensive studies involving physicochemical, phytochemical, and chromatographic profiling are essential.

The present study aims to explore the physicochemical parameters, which include moisture content, ash values, and extractive values that help assess the purity and quality of the raw drug. In addition, phytochemical screening was carried out to identify the presence of bioactive constituents such as alkaloids, flavonoids, tannins, saponins, and glycosides, which contribute to the therapeutic potential of the plant. Furthermore, High-Performance Thin Layer Chromatography (HPTLC) was employed as an advanced analytical tool for the development of a fingerprint profile, aiding in the authentication and standardization of the plant material.

This integrated approach ensures a robust quality control framework for *Babula* (*Acacia arabica* Willd.), supporting its use in evidence-based herbal medicine and paving the way for its potential pharmaceutical applications.

Vernacular Names[1]:

Table No.1: Vernacular Names of *Babula*

No.	Region	Names
1.	Bengali	*Babla, Babul.*
2.	English	Indian gum, Arabic tree, *Babula* tree
3.	Gujrati	*Babaria, baval, Kaloabaval*
4.	Hindi	*Babul, Kikar.*
5.	Kannad	*Gobbli, Karijali.*
6.	Tamil	*Kaluvelamaram, Karuvelam.*
7.	Malayalam	*Karivelan, Karuvelum.*
8.	Marathi	*Babhul, Vedibabul.*
9.	Punjabi	*Sak*
10.	Telugu	*Nallatumma, Tuma.*

AIM

To study Physicochemical, Phytochemical and HPTLC data of *Babula* (*Acacia arabica* Willd.) Fruit.

OBJECTIVE

1. To generate physicochemical parameters of *Babula* (*Acacia arabica* Willd.) Fruit.
2. To generate preliminary phytochemical parameters of *Babula* (*Acacia arabica* Willd.) Fruit.
3. To generate HPTLC of *Babula* (*Acacia arabica* Willd.) Fruit.

MATERIAL AND METHODS

The study was performed at Pharmacognosy laboratory of Upgraded department of *Dravyaguna Vijnana*, Government Ayurveda College, Vadodara and Vasu research centre, Vadodara.

The plan of work was as following:

1. Collection of plant material
2. Physicochemical study
3. Phytochemical study
4. HPTLC

1. Collection of plant material:

Fruit of *Babula* (*Acacia arabica* Willd.) was collected from forest area of Junagadh (21.52° North latitude and 70.47° East longitude) Gujarat after proper identification. After proper drying, Fruit was converted into powder form in Government Ayurveda pharmacy, Vadodara, Gujarat.

2. Physico-chemical analysis[2]:

Assessment of the parameter such as pH, loss on drying, ash value, Acid insoluble ash, water soluble extractive and alcohol soluble extractive was carried out by following standard procedure recommended by Ayurvedic Pharmacopoeia of India and other standard texts.

Table No. 2: physicochemical parameters of *Babula* (*Acacia arabica* Willd.) Fruit

No.	Parameters	Results
1.	pH	4.57
2.	LOD (%w/w)	7.62 %
3.	Ash value (%w/w)	10.53 %
4.	Acid insoluble ash (%w/w)	1.25 %
5.	Alcohol soluble extractive (%w/w)	20.39 %
6.	Water soluble extractive (%w/w)	15.78%

3. Phytochemical analysis[3]:

By performing various qualitative tests, one can get an idea about the type of Phyto constituents present in the sample. Hence the powder of samples subjected for following tests. Preparation of plant extracts is as shown in below table no. 3.

Table No. 3: Steps for preparation of Plant extracts[4]

Step 1	The samples of powder taken 5 gm and was extracted with methanol (100ml).
Step 2	Kept it overnight. Initially occasional shaking up to 6 hours was performed and then kept aside for settle down.
Step 3	After 24 hours, it was filtered, and alcoholic extract was collected
Step 4	Similarly, water extracts of sample were prepared and collected
Step 5	After that, qualitative tests were done by using appropriate extracts

Phytochemical analysis was performed by following the standard protocol given in Ayurvedic Pharmacopoeia of India.[5]. As like - Alkaloid - Dragendorff's reagent, Saponin - Foam test, Flavonoids, Tannin, Steroids. Observations are mentioned in table no. 4

Observations:

Table No. 4: Phytochemical Screening of *Babula* (*Acacia arabica* Willd.) Fruit

No.	Phytoconstituents	Results
1.	Alkaloids	-
2.	Flavonoids	+
3.	Steroids	+
4.	Tannins	+
5.	Saponin	+

- Absent, + Present

4. High Performance Thin Layer Chromatography (HPTLC)

Principle:

The separation of the components of a mixture is due to their different affinities for a stationary phase such as solid or liquid and their differential solubility in a moving phase such as a liquid or gas.

Steps involved in HPTLC-

1. Selection of chromatographic layer
2. Sample and standard preparation
3. Layer pre-washing
4. Layer pre-conditioning experimental study
5. Application of sample and standard
6. Chromatographic development
7. Detection of spots
8. Scanning and documentation

- **Preparation of Test solution**:

  Weight approximately 1 g of sample in a 250 mL reflux flask. Add 20 mL of Methanol and reflux it for 20 minutes on water bath. On completion of time, remove the reflux flask from the water bath and allow to cool. Filter with the help of Whatman filter paper no.1. Use the Test Solution thus obtained for HPTLC fingerprinting.

- **Preparation of Spray reagent [Anisaldehyde – sulphuric acid reagent]:**

  0.5 mL Anisaldehyde is mixed with 10 mL Glacial acetic acid, followed by 85 mL Methanol and 5 mL Sulphuric acid (98%).

Chromatographic Conditions:

Table No.3.1: Chromatographic Conditions in HPTLC

Application mode	CAMAG Linomat 5 – Applicator
Filtering System	Whatman filter paper No. 1
Stationary Phase	MERCK – TLC/HPTLC Silica gel 60 F_{254} on Aluminium sheet
Application (Y axis) Start position	10 mm
Development End Position	80 mm from plate base
Sample Application Volume	8 µL
Distance Between Tracks	0 mm
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	30 minutes

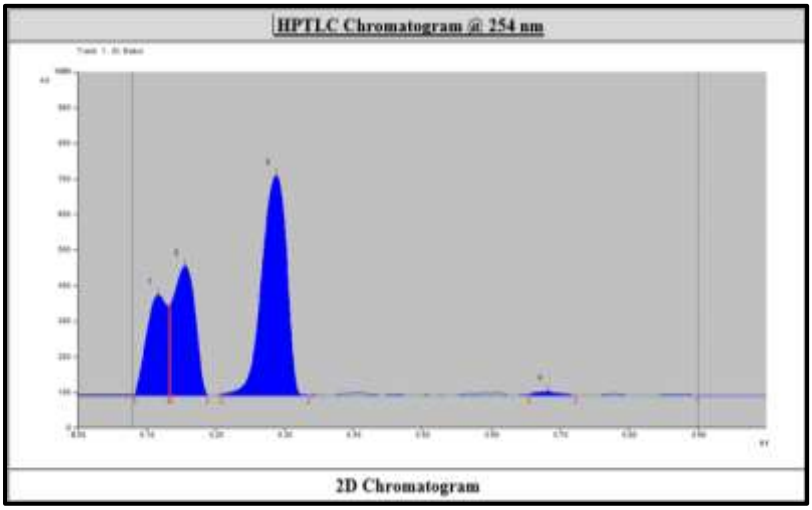
Mobile Phase (MP)	Tolune: Ethyl Acetate: Formic acid (7:3:0.3 v/v)
Visualization	@254nm, @366nm and @540nm (After derivatization)
Spray reagent	Anisaldehyde- Sulphuric acid reagent
Derivatization mode	CAMAG – Dip tank for about 1 minute
Drying Mode, Temp. & Time	TLC Plate Heater Preheated at 100 ±5 ^{0}C for 3 minutes

Observations:

Table No.3.2.: Observations of HPTLC

Observed under UV light	R_f value, area %		No. of spots
254 nm	**Rf**	0.12, 0.16, 0.29, 0.68	4
366 nm	**Rf**	0.08, 0.12, 0.22, 0.23, 0.33, 0.35, 0.39, 0.46, 0.59, 0.65, 0.74, 0.86	12
540 nm	**Rf**	0.12, 0.29, 0.65, 0.74	4

Plate no:1. HPTLC Chromatogram and photo under UV light 254 nm



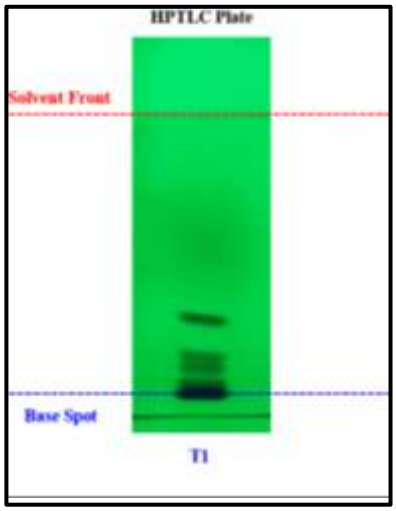
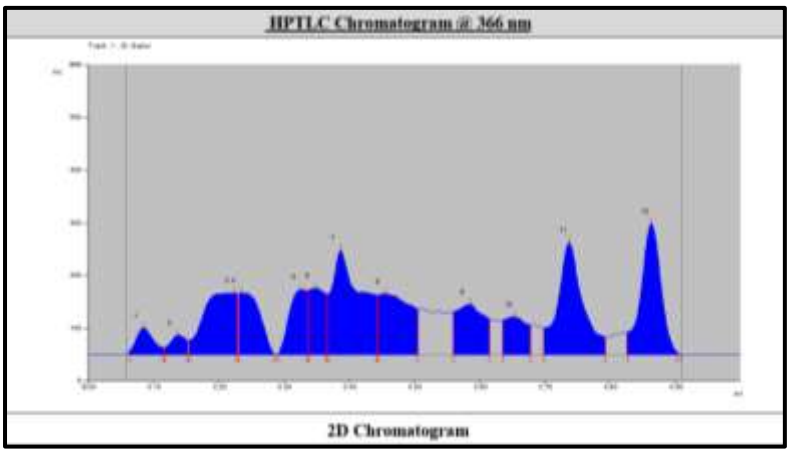


Plate no:2. HPTLC Chromatogram and photo under UV light 366 nm



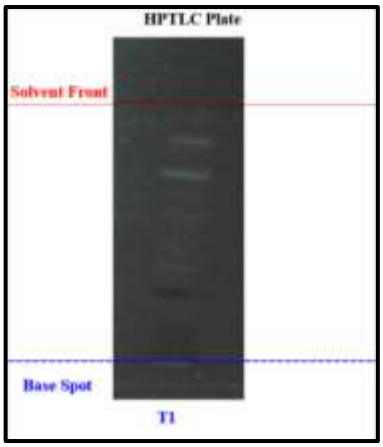
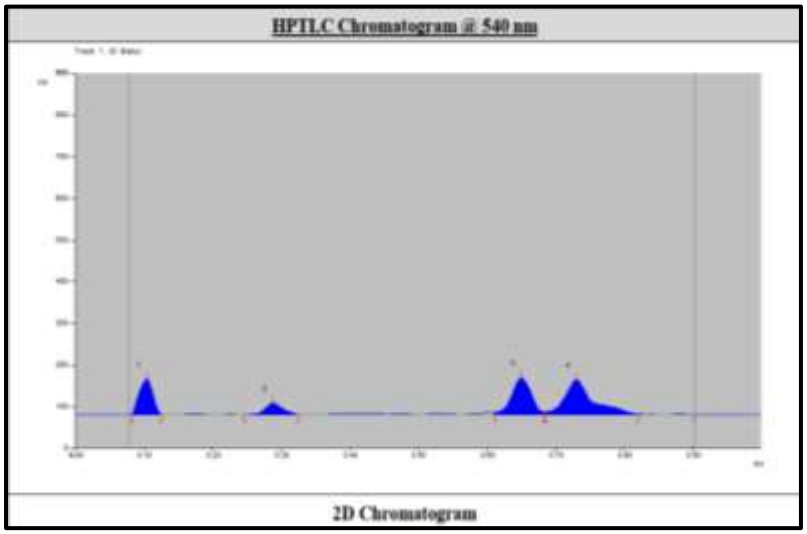


Plate no:3. HPTLC Chromatogram and photo under UV light 540 nm



CONCLUSION

The comprehensive evaluation of *Babula* (Fruit of *Acacia arabica* Willd.) through physicochemical, phytochemical, and HPTLC analyses establishes a scientific basis for its quality control and standardization. Phytochemical screening confirmed the presence of key bioactive compounds, including flavonoids, steroids, saponins, and tannins. Phytochemical analysis revealed presence of flavonoid, steroid, saponin, and tannin. Physicochemical parameters showed pH (4.57), loss on drying (7.62 %w/w), ash value (10.53 %w/w), acid insoluble ash (1.25 %w/w), water soluble extractive (1.25 %w/v) and alcohol soluble extractive (20.39 %w/w). In HPTLC analysis 4 peaks, 12 peaks and 4 peaks were observed at 254nm, 366nm and 540nm, respectively for Fruit of *Babula* (*Acacia arabica* Willd.). These findings support its traditional uses and promote its potential for development into safe and effective herbal formulations.

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