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Original Article

Pharmacognostic, Phytochemical and HPTLC Profiling of Vibitaki Phala Beeja (*Terminalia bellerica* Fruit Seed)

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ABSTRACT

Background: *Terminalia bellirica* (Gaertn.) Roxb., often referred to as Vibhitaki, is a significant member of the Combretaceae family of medicinal plants and a component in the traditional Ayurvedic remedy Triphala. There is few thorough standardized research on the fruit seed, despite its widespread therapeutic use. **Objectives:** To establish pharmacognostic, phytochemical, TLC, and HPTLC criteria for *Terminalia bellirica* fruit seeds. **Materials and Methods:** In accordance with the standards of the Ayurvedic Pharmacopoeia of India, authenticated fruit seeds were evaluated both macroscopically and microscopically. Methanolic, petroleum ether, and aqueous extracts were subjected to phytochemical screening. The phytochemical composition of the extracts was characterized using Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) studies. **Results and Discussion:** Starch grains, sclereids, stone cells, vascular strands, calcium oxalate crystals, and elongated epidermal hairs were among the distinctive characteristics identified by both macroscopic and microscopic investigations. Alkaloids, glycosides, flavonoids, triterpenoids, saponins, tannins, carbohydrates, and proteins were found through phytochemical screening; steroids and starch were not. The existence of potassium, iron, sulphate, and chloride was verified by inorganic analysis. Different phytochemical bands with different polarities were visible on TLC. With prominent peaks at R_f 0.22 and 0.73 in petroleum ether extract and R_f 0.29, 0.41, and 0.67 in methanolic extract, HPTLC profiling produced distinctive fingerprints. **Conclusion:** In order to support *T. bellirica* fruit seed authentication, quality control, and next phytopharmaceutical research, the study develops diagnostic pharmacognostic and chromatographic standards.

Keywords: HPTLC, Macroscopy, Phytochemical evaluation, Vibitaki phala beeja.

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INTRODUCTION

Nature is the main source of medical remedies (Oke *et al.*, 2002). It is believed that two thirds of the population in many underdeveloped nations relies on medicinal plants for basic medical care (Farnsworth *et al.*,1991). Compared to manufactured pharmaceutical products, herbal medicine is becoming more and more popular because of its safety, effectiveness, and therapeutic potential (Abraham *et al.*, 2014). *Terminalia bellerica* Often referred to as Bibhitaki, it is a member of the Combretaceae family. '*Bheeta*' means fear. '*Vibheeta*' means lack of fear and *Vibhitaki* means the fruit that takes away the fear of disease. The Latin phrase "terminus" or "terminalis" (ending), which refers to the habit of leaves being crowded or borne on the tips of branches, is the source of the generic name "Terminalia." Triphala, a combination of three myrobalan fruits, is a rasayana (rejuvenation) preparation compound that is significant in both Tibetan and Indian traditional remedies (Peterson *et al.*, 2017). Plants are a rich source of medications and produce a wide range of bioactive compounds. It is possible to separate and identify the herbal goods as possible forms of medication (Amrithpal *et al.*, 2011).

While many researches have been studied in terms of their botanical and chemical composition, there remains a lack of systematic standardization, particularly in terms of pharmacognostical and phytochemical analysis. To address this gap, the current investigation focuses on studying the fruit Seed of *Terminalia bellerica* Roxb. with the aim of establishing specific botanical and chemical standards. These standards will serve to facilitate the identification of crude drugs and to detect any potential adulteration. Additionally, further research in this

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area will significantly contribute to ensuring the quality and consistency of finished herbal products.

MATERIALS AND METHODS

Ethical statement

Since the study doesn't have any involvement of animals or human participants, there was no need of permission from Institutional Ethical Committee or else Institutional Animal Ethical Committee.

Statistical analysis

As the study doesn't require any statistical analysis as it doesn't involve any comparison of the groups, the statistical analysis wasn't performed.

Pharmacognostic parameters and Phytochemical evaluation

Market samples of the fruit seed was collected and authentication of the sample was done in FRLHT, Bengaluru. Followed by that the macroscopic and microscopic evaluation of the plant was done. Later, phytochemical analysis of three extract i.e. methanolic, pet-ether and aqueous including organic and inorganic compounds were assessed. Furthermore, Thin Layer Chromatography (TLC) was performed on Pet-ether and Methanolic extract. All the above said procedures were performed according to Figure 1 depicts the outline of the procedure from collection of medicinal plant to *in vitro* study (API GoI, 2001).

High Performance Thin Layer Chromatography

Take 50 mg of the extract in 10 mL volumetric flask and the extracts were dissolved with suitable solvents up to the mark [Chloroform, methanol, water, pet ether] and this solution was filtered through 0.45 micro millipore filter paper using the syringe filter 20µL,10 µL,5 µL aliquots of each of the extracts were separately applied on Silica gel 60 F254 precoated HPTLC plates,10x10 cm and 5x5 cm (Merck, Darmstadt, Germany) with the help of Camag Linomat-V applicator and eluted the plate to a distance of 2 cm at room temperature (25°C) in solvent system -

1) *Terminalia bellirica* [pet. ether extract]- hexane - 6: ethyl acetate - 2.5: methanol - 0.5.

2) *Terminalia bellirica* [methanolic extract]- ethyl acetate - 7.5: toluene - 2: acetic acid - 0.2: methanol - 0.5

The Camag Linomat-V automated TLC applicator was used to apply the sample solution to a 6 mm wide band, and the nitrogen flow allowed for a syringe delivery speed of 150 nl/s. Following sample application, plates were developed in a Camag twin through glass tank that had been pre-saturated with the mobile phase for 20 min in order to perform detection and quantization. The Camag horizontal developing chamber (10 cm x 10 cm) was used to develop the plate horizontally at ambient temperature. After half an hour, the plate was inspected. under UV light at 254 and 366 nm in a Camag UV cabinet, and the HPTLC fluorescence image was recorded. A Camag TLC scanner III equipped with winCATSV1.2.3 software was used to do the matching digital scanning profiling.

RESULTS

Authentication

The whole drug i.e *Vibitaki phala majja* (fruit seed of *Terminalia bellarica*) was authenticated, the fruit is drupe, fleshy, obovoid and sub-globose, grey in color, with velvety or wooly hairs and thick hard wall, 1- seeded and surrounded by a green tissue. Seed - 1.5cm x 1 cm, yellowish color, oblongoid with taste of groundnut.

Macroscopic examination

The macroscopic evaluation of the whole drug and the powder form was evaluated and the result of the both are depicted in Table 1and Figure 2 shows the macroscopy of *Vibitaki* fruit seed.

Microscopic examination

Transverse section of the fruit seed showed pericarp with epicarp consisting of one layer of epidermal cells inner tangential and upper portions of radial wall is thick. Mesocarp consists of 2-3 layers of collenchyma, followed by a broad zone of parenchyma in which fibres and sclereids in groups and vascular bundles scattered, fibres with peg like out growth and simple pitted walls. Sclereids of various shapes and sizes but mostly elongated. Tannins and raphides in parenchyma, endocarp consists of thick-walled sclereids of various shapes and sizes, mostly elongated. Epidermal surface view reveals polygonal cells, uniformly thick walled, several of them divided into two by a thin septum, starch grains simple rounded or oval in shape, found in plenty in almost all cells of mesocarp.

Powder microscopy

The powder microscopy of the *Vibitaki* fruit seed revealed the presence of vittae, sclereids, endosperm and mesocarp with lignified parenchymatous cells. There was also presence of starch grains and stone cells. Calcium oxalate crystals were also present, one of the notable things were that the epidermal cells elongate to form hair like protuberance with swollen base.

Phytochemical analysis

Among the organic phytochemicals all the three extracts revealed the presence of alkaloids, glycosides, flavonoids, triterpenoids, saponins, tannins, carbohydrates and proteins in methanolic and aqueous extract while it had shown absence of flavonoids in pet-ether extract. Steroids and starch were absent in all the three extracts. The test results are given in the Table 2.

The Inorganic tests were performed for the *Vibitaki* fruit seed and the results showed the presence of potassium, iron, sulphate

and chloride. While calcium, magnesium, sodium and nitrates. Table 3 shows the inorganic result of *Vibitaki churna* (powder of *Terminalia bellarica*)

Thin Layer Chromatography (TLC)

Pet- ether extract exhibited comparatively higher R_f values, confirming the predominance of non-polar compounds. Methanolic extracts showed lower to intermediate R_f values, indicating polar phytochemicals. Different spot colors indicate the distinct constituents reacting differently under the visualization reagent or UV visualization. The TLC profile confirms the diversity of the *Vibitaki* phala majja (fruit seed of *Terminalia bellarica*).

HPTLC

In Table 4, peak 1 at 0.22 R_f shows a highest peak intensity of 97.5 AU with 37.78%, suggesting a major phytoconstituent. Peak 5 also showed a maximum area coverage of 35.38% with again a major constituent. All the other R_f values 0.45, 0.55 and 0.69 suggests of minor to moderate constituents and the all the higher R_f values suggestive of non-polar phytochemicals. In Table 5, the peak 4 at 0.70 R_f with area coverage of 32.96% suggests that a major phytochemical is involved in this particular area. While in Table 6, the methanolic extract at 254 nm suggests that there is possibility of mixture of both polar and non-polar compounds at different R_f values, while in 0.29 R_f at peak 1 shows 40.25% area coverage it also shows an area coverage of 26. 72% at peak 4 with 0.67 R_f. In Table 7, the methanolic extract at 366 nm suggests major phytoconstituents at two different places, especially at 0.41 R_f with maximum percentage while one more at 0.53 R_f with maximum area coverage.

The 2D images of the HPTLC is shown in the Figure 3 regarding the Pet-ether and Methanolic extract in 254 nm and 366nm UV-visualisation.

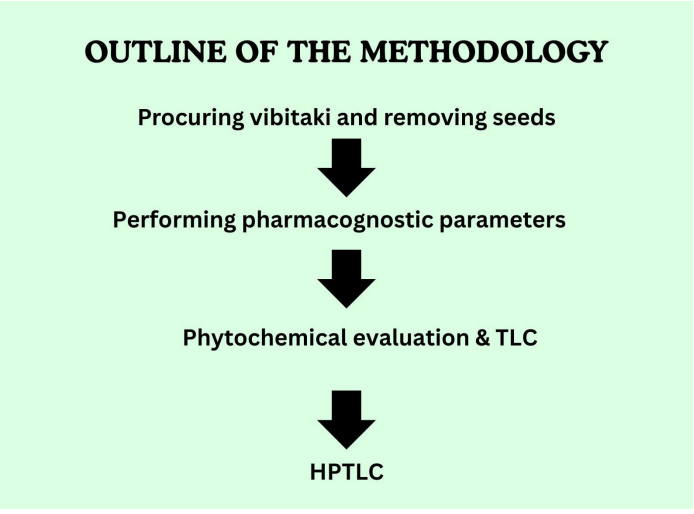


Figure 1: Outline of methodology.

DISCUSSION

For thousands of years, plants have been used to maintain human health and enhance quality of life. They are also useful ingredients in medicines, seasonings, drinks, cosmetics, and pharmaceuticals. Since ancient times, the plants have been utilized in medicine. One of the earliest Vedas, the Rigveda, mentioned using plants as medicine. An extensive list of therapeutic plants can be found in ancient medical systems such as Ayurveda. The Charaka Samhita lists about 500 plants with vegetable origins, whereas the Sushruta Samhita lists 573. However, thorough pharmacological and taxonomical research is necessary for these species.

The majority of the crude medications used in Indian medical systems are derived from plants. Standards must be established in order to regulate, verify the plant's identity, and verify its quality prior to usage. Therefore, a thorough pharmacognostic evaluation is a crucial precondition. Along with this if phytochemical analysis, TLC and HPTLC profiling is performed then the plant's useful part will have some validation so that the identification of the correct specimen will be easier for the preparation of the various formulation and prevent adulteration of the drug.

In previous research, on fruit of *Terminalia bellarica* the fruit had presence of calcium oxalate crystals, stone cells, fibres, starch grains, xylem and hairs were also present (Kumari *et al.*, 2017). This confirms that the seed and the fruit are having almost similar structure as per this study. In the microscopic study on leaves, calcium oxalate crystals and starch granules were also present (Menpara *et al.*, 2014). This suggests that the structures in almost the fruit, seed and the leaves are almost similar.

Further in previous researches, the phytochemical analysis of seeds revealed the presence of phenolics, glycosides, terpenoids, tannins and carbohydrates, while the present study had the presence of alkaloids, flavonoids and protein (Hazra, 2019). Although in majority of the phytochemical analysis both the study matched but there are few phytochemicals such as alkaloids, flavonoids and protein that were present in the current study which was missing in the previous study. This may be due to the soil, place and time of collection. And one more close proximity is that there was absence of steroids in both the studies.

Table 1: **Macroscopic examination of the Whole drug and the powder.**

Features	Whole drug	*Churna* (Powder)
Colour	Yellowish Brown	Smooth
Shape and size	Oblongoid 1.5cm in length and 1cm across	--
Taste	Taste of Ground Nut	Creamish white
Surface	Ridged	Characteristic
Odour	Characteristic	Groundnut taste

Table 2: Phytochemical analysis of three different extract of *Terminalia bellarica* fruit kernel.

Constituents	Tests	*Terminalia bellirica* fruit kernel		
		Methanolic extract	Pet- ether extract	Aqueous
Alkaloids	Mayer's reagent	Present	Present	Present
	Wagners test	Present	Present	Present
Glycosides	Molisch's test	Present	Present	Present
	Bontrager's test	Present	Present	Present
Flavonoids	Ferric chloride test	Present	Absent	Present
	Lead acetate test	Present	Absent	Present
Triterpenoids	Salkowski test	Present	Present	Present
Saponins	Foam test	Present	Present	Present
Tannins	Ferric chloride test	Present	Present	Present
Steroids	Salkowski's test	Absent	Absent	Absent
Carbohydrates	Molisch's test	Present	Present	Present
	Benedicts test	Present	Present	Present
Protein	Biuret test	Present	Present	Present
Starch		Absent	Absent	Absent

Table 3: Inorganic phytochemical analysis of *Terminalia bellarica*.

Tests	*Terminalia bellirica* Fruit Kernel
Calcium	-
Magnesium	-
Sodium	-
Potassium	+
Iron	+
Sulphate	+
Chloride	+
Nitrates	-

Table 4: Showing Results of TLC of Pet-Ether Extracts.

Sl. No.	R_f Value	Spot Colour
1.	0.75	Blue
2.	0.24	Bluish White
3.	0.69	Red

Table 5: Showing Results of TLC of Methanolic Extracts.

Sl. No.	R_f Value	Spot Colour
1.	0.60	White
2.	0.29	Blue
3.	0.45	Bluish white

TLC analysis of the seeds had revealed various phytoconstituents in different polarity. The pet-ether had shown different compounds with higher R_f values, indicating non-polar constituents. While the methanolic extract showed different compounds with lower R_f values, indicating polar constituents. The presence of distinct coloured spots confirmed spots confirms the heterogeneity of extracts and supported the presence of bioactive secondary metabolites.

Several phytoconstituents with different polarity and concentrations were found in the pet-ether and methanolic extracts of *Terminalia bellirica* fruit seed, according to the HPTLC fingerprint analysis. While the methanolic extract displayed notable polar contents at R_f 0.29, 0.41, and 0.67, the pet-ether extract showed significant non-polar chemicals with dominating peaks at R_f 0.22 and 0.73. Flavonoids, tannins, phenolics, terpenoids, and sterols are examples of UV-active and fluorescent bioactive metabolites that were detected at both 254 and 366 nm. The unique HPTLC fingerprint profile could be a useful tool for plant material identification, quality assurance, and standardization. In the previous researches there was comparatively lower number of phytochemicals and peak area present when compared to the study (Hazra, 2019). This suggests the place, time and soil also plays a major role in the plant's quality.

The study has given us a data regarding the phytochemical, Pharmacognostic and HPTLC profiling had given us the presence of various secondary metabolites in the seeds of *Vibitaki*. Although, the study is suggesting us that the plant has medicinal properties, still a proper quantification of the individual phytochemical is needed so as to establish the presence various other secondary metabolites. Furthermore, GCMS analysis and LCMS analysis of the seed extract should be made so as to finalise the phytochemicals (Gurudevi *et al.*, 2020).

Table 6: **Results of HPTLC Pet-ether extract at different UV-visualization.**

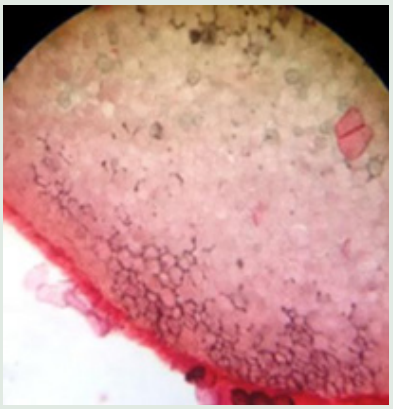
Results of HPTLC of Pet - Ether extract at 254nm									
Peak	Start position	Start Height	Max position	Max Height	Max %	End position	End Height	Area	Area %
1	0.17 R_f	0.3 AU	0.22 R_f	97.5 AU	37.78%	0.26 R_f	4.0 AU	2489.2 AU	34.59%
2	0.45 R_f	16.3 AU	0.51 R_f	25.8 AU	10.01%	0.53 R_f	16.5 AU	1257.2 AU	17.47%
3	0.55 R_f	1.6 AU	0.56 R_f	28.7 AU	11.14%	0.57 R_f	16.5 AU	362.0 AU	5.03%
4	0.64 R_f	18.4 AU	0.65 R_f	52.1 AU	20.19%	0.66 R_f	19.7 AU	541.9 AU	7.53%
5	0.69 R_f	27.3 AU	0.73 R_f	53.9 AU	20.88%	0.80 R_f	15.9 AU	2546.2 AU	35.38%
Results of HPTLC of Pet- Ether Extract at 366nm									
Peak	Start position	Start Height	Max position	Max Height	Max %	End position	End Height	Area	Area %
1	0.17 R_f	0.1 AU	0.21 R_f	14.5 AU	16.61%	0.24 R_f	0.8 AU	315.8 AU	20.80%
2	0.63 R_f	4.1 AU	0.65 R_f	28.5 AU	32.62%	0.66 R_f	18.0 AU	333.9 AU	22.00%
3	0.66 R_f	18.4 AU	0.67 R_f	21.5 AU	24.63%	0.69 R_f	9.7 AU	368.1 AU	24.24%
4	0.70 R_f	9.7 AU	0.72 R_f	22.8 AU	26.14%	0.75 R_f	2.2 AU	500.4 AU	32.96%

Table 7: **Results of HPTLC - Methanolic extract at different UV-visualisation.**

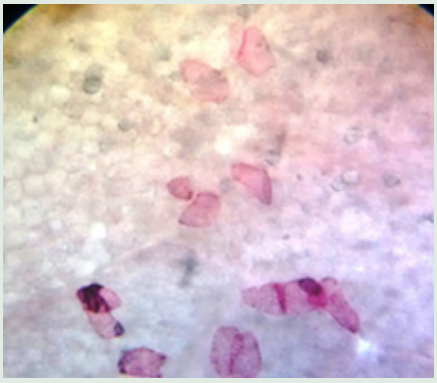
Results of HPTLC of Methanolic Extract at 254 nm									
Peak	Start position	Start Height	Max position	Max Height	Max %	End position	End Height	Area	Area %
1	0.23 R_f	5.3 AU	0.29 R_f	157.8 AU	34.84%	0.33 R_f	25.2 AU	6653.4 AU	40.25%
2	0.33 R_f	27.2 AU	0.35 R_f	35.1 AU	7.75%	0.38 R_f	19.3 AU	926.3 AU	5.60%
3	0.38 R_f	19.6 AU	0.41 R_f	106.6 AU	23.53%	0.45 R_f	10.6 AU	2815.3 AU	17.03%
4	0.48 R_f	10.0 AU	0.54 R_f	34.0 AU	7.50%	0.59 R_f	3.9 AU	1719.7 AU	10.40%
5	0.62 R_f	0.4 AU	0.67 R_f	119.4 AU	26.37%	0.73 R_f	6.6 AU	4417.2 AU	26.72%
Results of HPTLC of Methanolic Extract at 366 nm									
Peak	Start position	Start Height	Max position	Max Height	Max %	End position	End Height	Area	Area %
1	0.21 R_f	0.3 AU	0.25 R_f	44.8 AU	19.67%	0.29 R_f	2.8 AU	1366.3 AU	16.14%
2	0.35 R_f	7.3 AU	0.41 R_f	97.5 AU	42.80%	0.45 R_f	25.5 AU	3162.6 AU	37.35%
3	0.48 R_f	31.9 AU	0.53 R_f	62.0 AU	27.20%	0.60 R_f	2.3 AU	3232.1 AU	38.17%
4	0.65 R_f	6.8 AU	0.68 R_f	23.5 AU	10.33%	0.72 R_f	0.1 AU	705.8 AU	8.34%



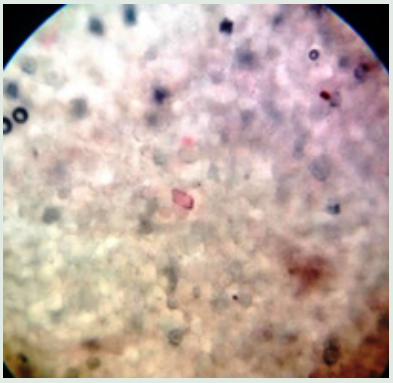
2.a



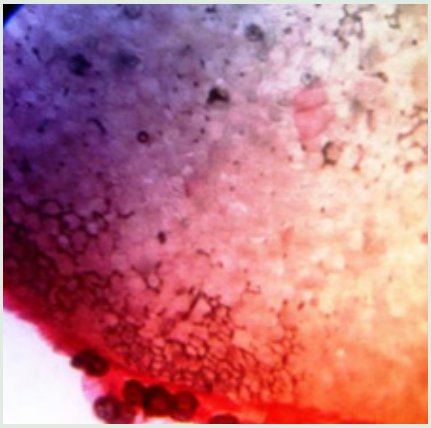
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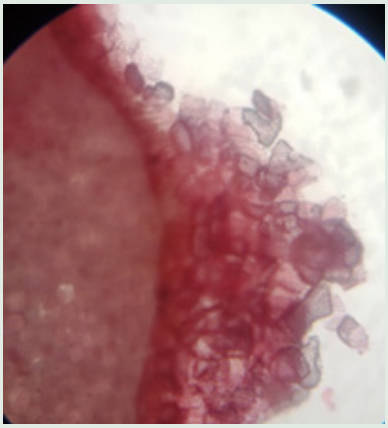
2.c



2.d



2.e



2.f

Figure 2: From top row, 2.a. *Terminalia bellarica* fruit, 2.b. Epidermal cells elongate to form hair like protuberance with swollen base. Middle row, 2.c. Horizontal and Vertical strands, 2.d. Starch grains and Stone cells. In the bottom row, 2.e. Starch grains and 2.f. Vascular Strands.

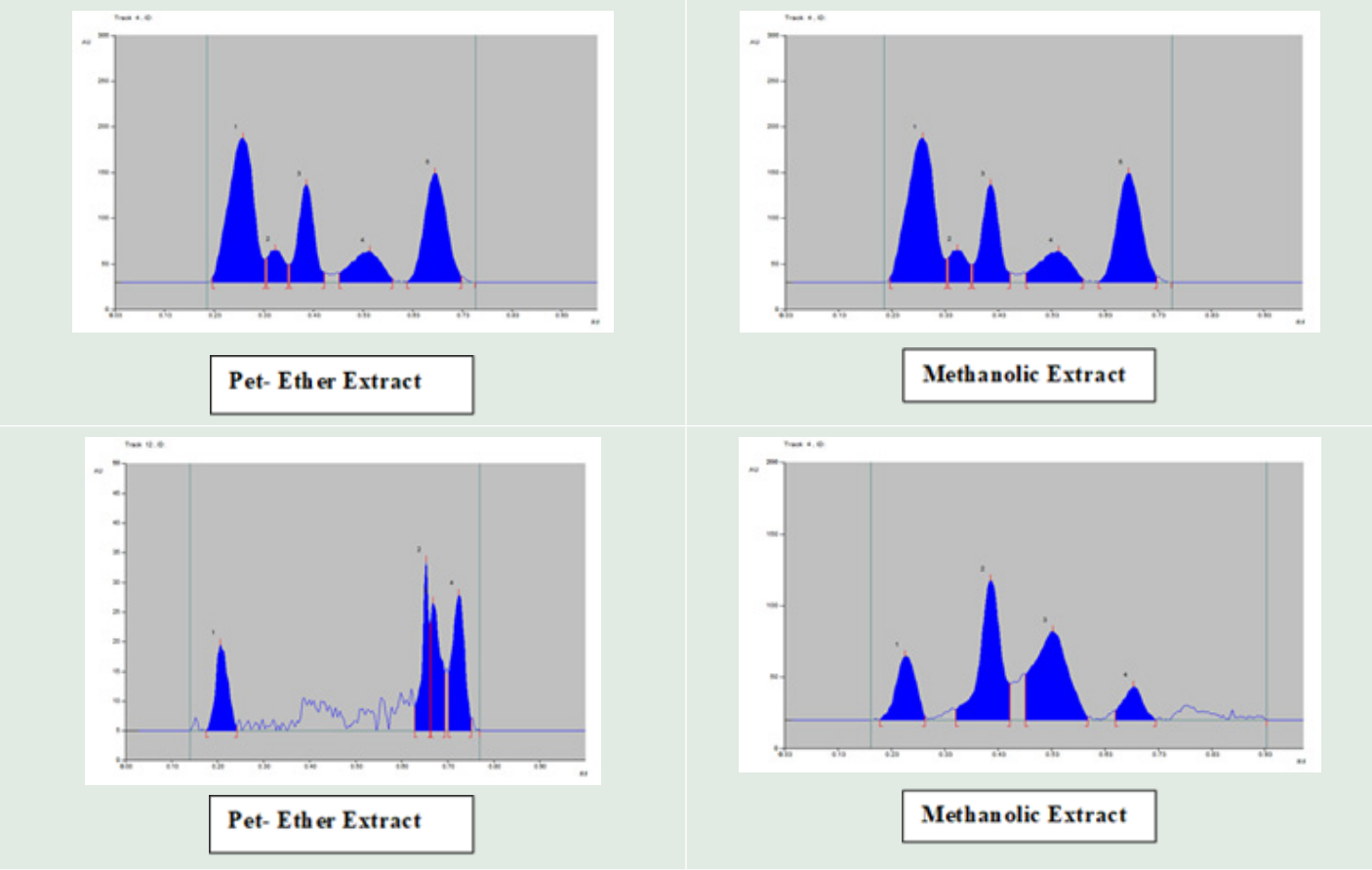


Figure 3: 2D graph images of HPTLC imaging, the top row depicts the HPTLC images at 254 nm and the bottom row shows the HPTLC image at 366nm.

CONCLUSION

According to *Charaka*, all the Dravya should undergo *Bheshaja pariksha* before usage of the medicine in the clinics. So as to prevent adulteration we need to perform all the basic Pharmacognostic tests. Thus, the study had given the evidence regarding the macroscopy, microscopy of the seeds and has described the various components of the part. It has also given the presence of various phytochemicals that has various pharmacological actions. Furthermore, the TLC and HPTLC has also given the presence of various non-polar and polar components validating the basic phytochemical study.

Thus, with the evidences and the proof the study not only validates the pharmacognosy of the seed but also phytochemical of *vibitaki*. Hence, the main objective of the study i.e. to standardize the seed of *Terminalia bellarica* is accomplished, but further *in vitro* study on its individual quantification of phytochemical, GCMS, LCMS and other cell-line based activity will further more confirm its numerous pharmacological actions.

ABBREVIATIONS

UV: Ultraviolet; **TLC:** Thin-Layer Chromatography; **HPTLC:** High-Performance Thin-Layer Chromatography; **GC-MS:** Gas Chromatography-Mass Spectrometry; **LC-MS:** Liquid Chromatography-Mass Spectrometry.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This study provides a comprehensive pharmacognostic and phytochemical profile of the *Terminalia bellerica* fruit kernel. Microscopic analysis identified key diagnostic features like starch grains and elongated epidermal hairs, while HPTLC fingerprints established specific R_f values (e.g., 0.22, 0.73 for petroleum ether) to ensure quality control and prevent adulteration of this traditional Ayurvedic drug.

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