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Chemical profiling of Mandak - A novel polyherbal combination

Research Article

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Abstract

Introduction: In Ayurveda, drug formulation adheres to two principles: single-drug use and compound drug use, the latter known as polyherbalism. *Mandak*, a Novel Polyherbal combination of six drugs have shown better efficacy individually in the management of *twak vikaras*. Methods: This study aimed to evaluate raw and end product's *(Mandak)* organoleptic, physicochemical, and phytochemical properties, along with chromatographic screenings via Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC), and Fourier Transform Infrared Spectroscopy (FTIR) to assess the synergistic effects of this novel combination. Results and Discussion: The microbial load of both the raw drugs and the compound mixture was within permissible limits. Physicochemical and phytochemical analyses confirmed the presence of compounds such as flavonoids, alkaloids, and tannins, recognised for their antioxidant, anti-inflammatory, anti-allergic, and antimicrobial properties. HPTLC profiling identified berberine (Rf ~0.36) and gallic acid (Rf ~0.59), with colour bands indicating various phytoconstituents such as red and yellow for alkaloids and blue for flavonoids and tannins etc. FTIR analysis detected hydroxyl groups linked to alcohols and phenols, which exhibit antioxidant properties, and carboxyl groups associated with lipids and proteins synthesis. The phytoconstituent composition may vary due to geographical distribution and time of collection. The compound drug's components might work synergistically, enhancing antioxidant, anti-inflammatory, and wound healing activities. Conclusion: The diverse pharmacological activities of *Mandak* stem from its phytoconstituents, which could help create standard monographs for this novel combination. This study emphasises the importance of *Mandak* by evaluating its phytochemical properties and potential benefits in Ayurvedic practice.

Keywords: *Mandak, Polyherbal combination, HPTLC, FTIR, Phytoconstituents, Twak vikaras.*

Introduction

Herbal medications are largely derived from the kingdom of plants. Even in recent times, people have become more aware of the significance of medicinal herbs. (1) A lot of research is being done on medicinal flora in an effort to create novel medications that are less expensive and more safely able to cure microbial infections than allopathic medications, which are sometimes linked to undesirable side effects. (2) *Mandak*- a polyherbal combination comprising of novel combination of drugs, viz., *Meshashringi* (*Gymnema sylvestre* R.Br.), *Arjuna* (*Terminalia arjuna* (Roxb)W. &A), *Nimba* (*Azadirachta indica* A. Juss.), *Daruharidra* (*Berberis aristata* DC) *Asana* (*Pterocarpus marsupium* Roxb.), and *Khadira* (*Acacia catechu* (Linn) Willd) are combined together in equal ratio and studied for their physico-chemical and phytochemical analysis.

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Traditionally, all the individual drugs been mentioned in the treatment of *twak vikaras* (skin ailments). Maximum of the drugs have been taken from *asanadi gana* (a group of 23 drugs) mentioned in *Ashtanga hridya* and *Ashtang sangraha* under *vividhaganasamgraha* adhyaya (3) and *shodhanadiganasamgraha adhyaya* (4) except *Nimba*. This *gana* is mainly indicated for the management of *shwitra, kushtha, prameha, krimi, pandu, kaphaja vikara and medodosha*. *Nimba* traditionally have been indicated in the treatment of *kushtha, krimi, prameha, vrana, visa roga, arsha, kandu* etc. (5) It has been proven for its activities such as anti-inflammatory, anti-diabetic, anti-microbial, immunostimulant and for skin disorders. (6) Considering the utility of the *nimba* in ayurvedic as well as contemporary science, this drug was combined together with the other 5 drugs in order to examine their effect all together.

The existence of numerous active components in herbs, which when combined can produce a potentiating effect that may not be possible with a single ingredient, is a significant potential benefit above traditional single-component medications. Plant-based pharmacological compounds found in polyherbal formulations have the potential to function in synergistic, potentiative, agonistic, or antagonistic ways due to their varied active principles. (7) Bioactive chemicals found in medicinal

plants include tannins, alkaloids, carbohydrates, terpenoids, steroids, flavonoids, and phenols. These compounds have specific physiological effects on the human body.

The quality of herbal medications can be influenced by various external factors, including the region of cultivation, growth period, and manufacturing technique. (8) Different drugs exhibit varying activities in different solvent systems, and in ayurveda many are used in the form of *kashaya* preparations (decoction) due to their phytochemical affinity and solubility in water. The study focuses on evaluating the quality of the drugs used in this combination. Separation by HPTLC and FTIR are some other famous methods used to control the quality of raw materials and final herbal formulations. As the drugs incorporated are of different physical natures and because there is no previous data available on this combination, the present study was carried out to analyse the qualitative and quantitative parameters of *Mandak*- A polyherbal combination.

Aims and Objectives

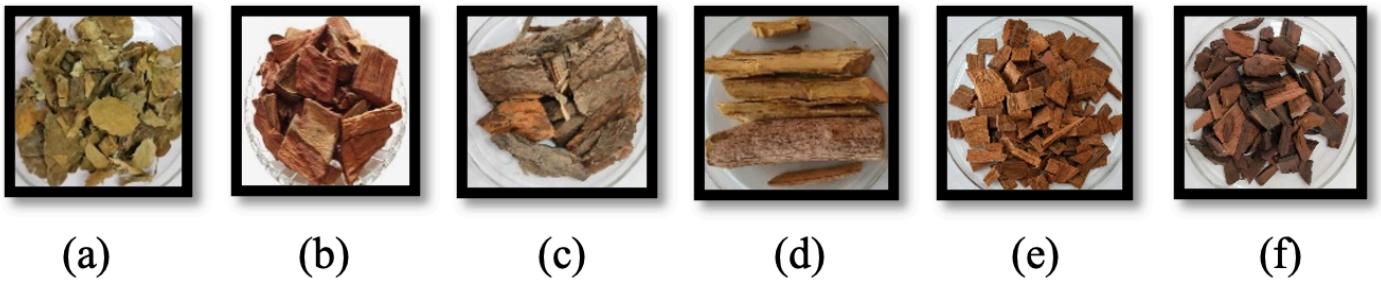
To evaluate the qualitative and quantitative analysis of individual drugs and novel compound drug mixture- *Mandak* for chemical profiling.

Materials and methods

Collection of raw drugs

The raw drugs required for the combination, viz., leaves of *Gymnema sylvestre* R.Br., stem bark of *Terminalia arjuna* (Roxb)W. &A., stem bark of *Azadirachta indica* A. Juss., stem of *Berberis aristata* DC., heartwood of *Pterocarpus marsupium* Roxb., and heartwood of *Acacia catech*u (Linn) Willd. were collected from the various parts of Belgaum, Karnataka. All raw drugs were authenticated by experts at AYUSH approved Drug Testing Laboratory for ASU drugs at KAHER's Shri B. M. Kankanawadi Ayurveda Mahavidyalaya, Belagavi, Karnataka, India. Microbial limit test (MLT) of all the individual raw drugs was performed.

Figure 1: Raw drugs



(a) Leaves of *Gymnema sylvestre* R.Br. (b) Stem bark of *Terminalia arjuna* (Roxb)W. &A (c) Stem bark of *Azadirachta indica* A. Juss. (d) Stem of *Berberis aristata* DC (e) Heartwood of *Pterocarpus marsupium* Roxb (f) Heartwood of *Acacia catech*u (Linn) Willd.

Preparation of *Mandak* compound

The individual drugs were cleaned; dried and standard methodology was used to prepare the *Kwathachurna* (coarse powder of the formulation). Prepared drug mixture was subjected to microbial limit test (MLT).

Fluorescence analysis of raw drugs as well as *Mandak*

All individual raw drugs and compound drug was converted into fine powders. Small quantity of fine powdered drugs was taken and observed for visual colour change in daylight, in short UV- light (254 nm) and long UV-light (366 nm). Fig 2. Represents the colour of powders in various wavelengths.

Physico- and Phyto-chemical analysis of individual raw drugs and *Mandak* compound

All the individual drugs and *Mandak* (compound drug) was subjected to standard methods to determine the physicochemical parameters and preliminary phytochemical analysis. Different solvent extracts, such as aqueous and alcohol, were used to conduct tests for the phytoconstituents.

TLC analysis was carried by using suitable solvent media for both individual drugs as well as compound drug *viz*., *Meshashringi* – n-hexane: Toluene: Ethyl acetate (5:10:2), *Arjuna* – Ethyl acetate: Formic acid: Methanol (10:3:1:2), *Nimba* – Toluene: Ethyl acetate (9:1), *Daruharidra* – n-butanol: Glacial acetic acid: water (6.5:1.5:2), *Asana* – Ethyl acetate: n-hexane (5.5:4.5), *Khadira* – Toluene: Ethyl acetate: Formic acid (5:4:1), and *Mandak* - Toluene: Ethyl acetate (7:3)

HPTLC analysis of *Mandak*

HPTLC analysis was carried out for alcoholic as well as aqueous extract using silica gel 60 F 254 plates for spotting. Toluene: chloroform: methanol (8:3:1) was used as the mobile phase. After development, densitometric scan was performed with a CAMAG TLC Scanner "Scanner_171019" S/N 171019 (2.01.02) in reflectance absorbance mode at UV detection as 254 nm and 366 nm and visualised by CAMAG Visualiser: 171217 (Visualizer_171217). HPTLC analysis was carried out at CARe KERALAM Ltd., KINFRA Small Industries Park, Koratty, Thrissur.

FTIR analysis of *Mandak*

The *Mandak* was analysed on potassium bromide (KBr) discs to obtain an infrared spectrum (FTIR Spectrum) using Shimadzu IR Affinity-1, Japan. Samples were scanned and characteristic peaks were detected. The peak values of the FT-IR were recorded.

The analysis was carried out at KLE College of Pharmacy, Nehru Nagar, Belagavi, Karnataka, India.

Results

MLT of all the individual raw drugs as well as of *Mandak* (combination of 6 drugs) were within limits and has been depicted in the Table 1. The organoleptic characteristics and results of the physico-chemical and phytochemical analyses of individual raw drugs and *Mandak* were noted. After the data was evaluated, its findings were interpreted. Results for various analysis are depicted in the following tables:

Table 1: Microbial limit test

Test for specified micro-organisms (qualitative)								
S. No.	Organisms	***Meshashringi***	***Arjuna***	***Nimba***	***Daruharidra***	***Asana***	***Khadira***	***Mandak***
1	E. coli	A	A	A	A	A	A	A
2	S. aureus	A	A	A	A	A	A	A
3	P. aeruginosa	A	A	A	A	A	A	A
4	S. abony	A	A	A	A	A	A	A
Microbial limit test (quantitative)								
5	Total bacterial	NG	NG	NG	NG	NG	NG	NG
6	Total fungal	NG	NG	4cfu/ml	2cfu/ml	4cfu/ml	NG	NG

A - Absent, NG – No Growth

Table 2: Organoleptic characters

S. No.	Parameters	*Meshashringi*	*Arjuna*	*Nimba*	*Daruharidra*	*Asana*	*Khadira*	*Mandak*
1	Appearance/ Part	Leaves	Stem bark	Stem bark	Stem	Heartwood	Heartwood	Coarse powder
2	Colour	Light green	Greyish pink	Rusty grey	Pale yellowish brown	Golden yellowish brown	Brownish red	Reddish brown
3	Odour	Unpleasant	Charact-eristic	Charact-eristic	Characteristic	Characteristic	Charact-eristic	Characteristic
4	Taste	Bitter and acrid	Bitter & astringent	Bitter	Bitter	Astringent	Astringent	Bitter

Table 3: Fluorescence analysis of powders of individual and compound drug

S.No.	Drugs	Visible	Short wavelength	Long wavelength
1	*Meshashringi*	Light green	Light green	Dark yellow
2	*Arjuna*	Light brown	Light brown	Light brown
3	*Nimba*	Brown	Brown	Dark blue
4	*Daruharidra*	Yellow	Fluorescent green	Fluorescent green
5	*Asana*	Reddish brown	Green	Brown
6	*Khadira*	Reddish brown	Brown	Brown
7	*Mandak*	Brown	Yellow	Fluorescent yellow

Figure 2: Fluorescence study of individual and compound drug

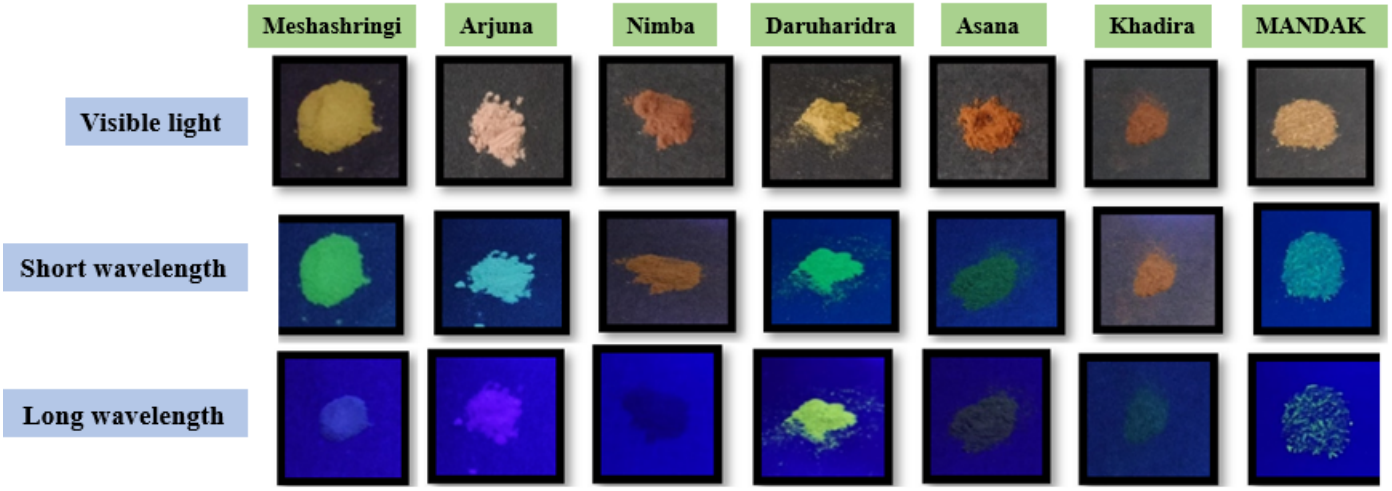


Table 4: Physico-chemical parameters

S. No.	Parameters	*Meshashringi*	*Arjuna*	*Nimba*	*Daruharidra*	*Asana*	*Khadira*	*Mandak*
1	Loss on Drying	6.287%	4.588%	5.591%	2.876%	3.888%	4.110%	6.281%
2	Ash value	8.605%	23.088%	4.187%	1.677%	1.666%	1.979%	6.679%
3	Acid insoluble ash	1.298%	0.977%	0.394%	0.148%	0.245%	0.494%	1.080%
4	Water soluble ash	5.048%	5.102%	0.923%	1.174%	0.249%	0.291%	3.439%
5	Alcohol extractive value	11.811%	26.259%	7.481%	7.244%	9.898%	4.497%	20.157%
6	Water extractive value	30.025%	24.644%	6.963%	9.748%	13.470%	4.086%	22.345%

Table 5: Phyto-chemical analysis of raw drugs

S.No.	Tests	*Meshashringi*		*Arjuna*		*Nimba*		*Daruharidra*		*Asana*		*Khadira*		*Mandak*	
		Aq.	A	Aq.	A	Aq.	A	Aq.	A	Aq.	A	Aq.	A	Aq.	A
1	Carbohydrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	Reducing sugar	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	Monosaccharide	+	+	+	+	+	+	+	-	+	+	+	+	+	+
4	Pentose sugar	-	-	+	+	+	+	+	-	-	-	+	-	+	+
5	Hexose	-	-	+	+	+	+	-	-	-	-	-	-	+	-
6	Non reducing sugar	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	Protein	+	+	+	+	-	-	-	-	+	+	-	+	+	+
8	Amino acid	+	-	+	-	-	-	-	-	+	-	-	-	-	-
9	Steroids	+	-	-	-	-	-	+	+	+	-	+	-	+	+
10	Saponin	+	-	+	-	+	-	+	-	+	-	+	-	+	-
11	Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	Alkaloids	+	-	+	-	-	-	+	+	+	-	-	-	+	-
13	Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	Cardiac glycosides	+	+	+	+	+	-	-	-	+	+	-	+	+	+
15	Anthraquinone glycosides	+	-	-	-	-	-	-	+	+	-	-	-	-	-

Aq. - Aqueous extract, A- Alcoholic extract, '+': Detected, '- ': Not detected

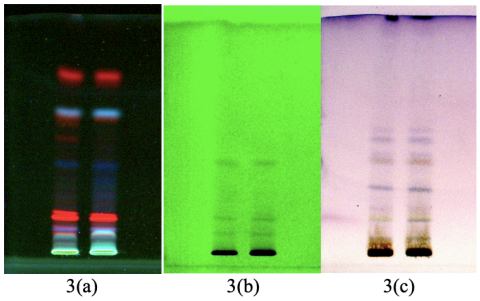
Table 6: Rf values of raw drugs and *Mandak* (alcoholic extract)

S. No	Samples	Short wave	Long wave
1	*Meshashringi*	**0.11**, 0.24, 0.29, 0.37, 0.44, 0.52	**0.13**, 0.19, 0.24, 0.26, **0.32**, 0.42, 0.44**, 0.49**
2	*Arjuna*	**0.38**, 0.48, 0.50, 0.51, 0.63, 0.93	0.40, 0.51, 0.55, **0.60**, 0.90
3	*Nimba*	0.17, 0.23, 0.29, 0.58, 0.77	0.23, 0.32, 0.45, **0.50**, 0.52, **0.80**
4	*Daruharidra*	**0.13**, **0.40**, 0.48, 0.59, 0.90	**0.04, 0.13**, 0.24, 0.33, **0.39**, **0.50**, **0.57,** 0.65, **0.87**, 0.96
5	*Asana*	0.02, 0.50, 0.54, 0.60, 0.66, 0.72, 0.83,	0.04, 0.20, 0.33, **0.40, 0.50**, 0.63, 0.83
6	*Khadira*	0.51, 0.58, 0.62, 0.69, 0.77, 0.95	**0.35, 0.46,** 0.54, **0.59,** 0.64, 0.72, **0.87**
7	*Mandak*	**0.04**, **0.08**, 0.18, 0.24, **0.35,** 0.78, 0.86, 0.91, 0.95, 0.98	**0.06, 0.10**, 0.29, **0.47, 0.60, 0.79,** 0.81, **0.84**, 0.91, 0.96, 0.98

HPTLC

High Performance Thin layer chromatography of *Mandak* (alcoholic and aqueous extract). The developed plates and separation of bands have been illustrated in fig 3. Both the alcoholic and aqueous extracts, which were made at the same concentration, are represented by the first and second tracts, respectively. Fig 4 and 5 represents the peaks in graph of both alcoholic and aqueous extract at 254nm and 366 nm respectively.

Figure 3: HPTLC fingerprinting of *Mandak*



3(a) 3(b) 3(c)

3(a) at 366nm., 3(b) at 254nm., 3(c) visible light

Figure 4: HPTLC graphs with peaks (a) alcoholic extract (b) water extract at 254nm

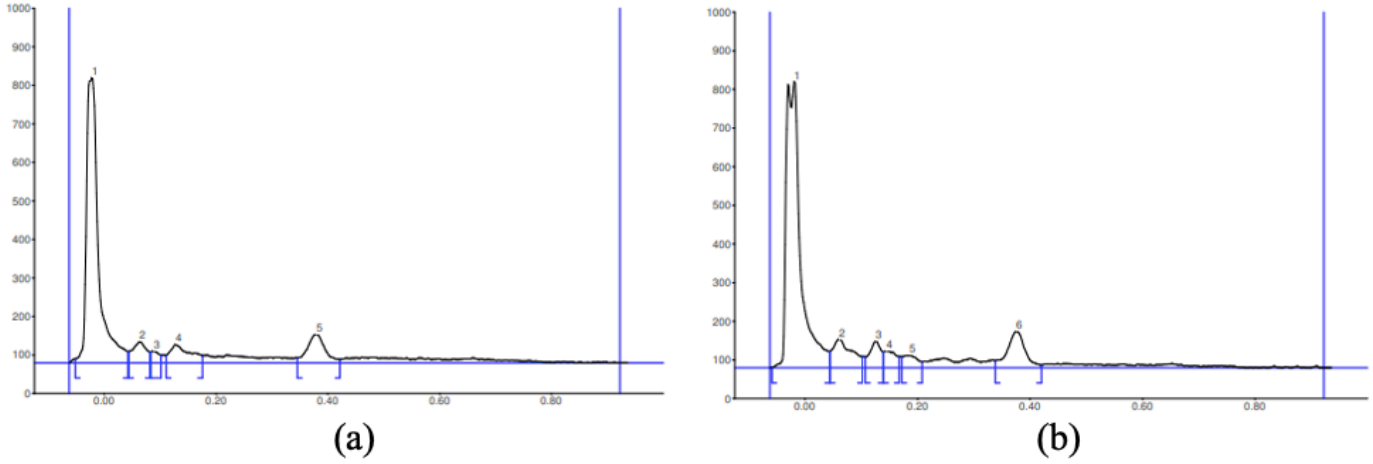


Figure 5: HPTLC graphs with peaks (a) alcoholic extract (b) water extract at 366nm

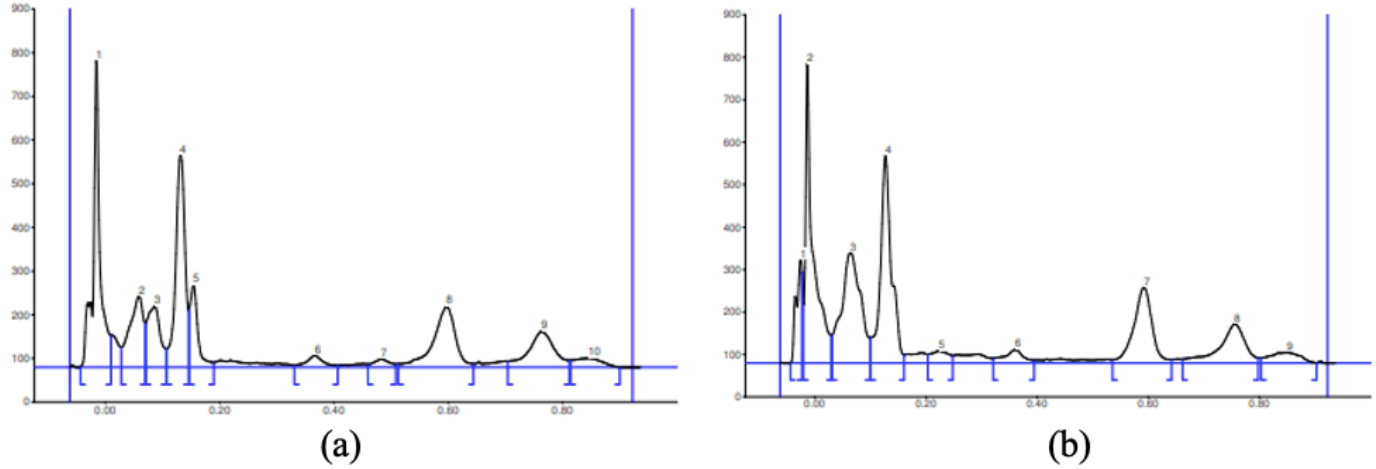


Table 7 (A): HPTLC (High Performance Thin layer chromatography) of Mandak (alcoholic extract).

a) Short wavelength (254nm)

Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %
1	-0.05	11.5	-0.02	742.2	77.99	0.04	29.6	14827.9	72.86
2	0.04	29.8	0.06	55.1	5.79	0.08	29.1	1292.1	6.35
3	0.08	29.2	0.09	30.9	3.24	0.10	20.1	428.9	2.11
4	0.11	20.0	0.13	48.2	5.07	0.18	19.6	1605.8	7.89
5	0.35	13.8	0.38	75.3	7.91	0.42	9.6	2197.0	10.80

b) Long wavelength (366nm)

Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %
1	-0.04	0.9	-0.02	702.3	35.7	0.01	73.5	7846.6	22.40
2	0.03	46.5	0.06	163.2	8.30	0.07	100.5	3719.0	10.62
3	0.07	101.7	0.09	139.8	7.11	0.11	43.1	2915.1	8.32
4	0.11	43.2	0.13	486.1	24.72	0.15	129.4	7433.9	21.22
5	0.15	133.5	0.15	188.2	9.57	0.19	11.5	2404.3	6.86
6	0.33	5.8	0.37	26.7	1.36	0.41	4.8	776.6	2.22
7	0.46	7.2	0.48	18.5	0.94	0.51	7.6	499.1	1.42
8	0.51	7.9	0.60	139.3	7.08	0.64	8.0	5028.4	14.35
9	0.70	14.4	0.76	81.8	4.16	0.81	16.1	3448.7	9.85
10	0.81	16.3	0.85	20.9	1.06	0.90	0.5	957.9	2.73

Table 7 (B): HPTLC (High Performance Thin layer chromatography) of *Mandak* (aqueous extract).

c) Short wavelength (254nm)

Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %
1	-0.06	0.9	-0.02	740.9	70.13	0.04	42.8	17649.0	68.52

2	0.05	43.7	0.06	74.8	7.08	0.10	29.1	2344.6	9.10
3	0.11	28.7	0.13	69.4	6.56	0.14	41.1	1282.1	4.98
4	0.14	41.2	0.14	45.0	4.26	0.17	29.4	892.1	3.46
5	0.17	29.1	0.19	32.1	3.04	0.21	16.3	802.6	3.12
6	0.34	19.1	0.38	94.4	8.93	0.42	7.6	2786.8	10.82

d) Long wavelength (366nm)

Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %
1	-0.04	0.4	-0.03	243.9	11.87	-0.02	214.6	2357.5	5.64
2	-0.02	136.9	-0.01	704.6	34.30	0.03	65.7	9191.2	2,199
3	0.03	66.0	0.06	260.2	12.67	0.10	60.3	8419.9	2015
4	0.10	60.6	0.13	488.3	23.77	0.16	20.1	9061.2	21.68
5	0.20	21.4	0.22	28.8	1.40	0.25	18.5	897.9	2.15
6	0.32	11.8	0.36	31.9	1.55	0.40	7.3	1052.6	2.52
7	0.54	8.6	0.59	178.9	8.71	0.64	9.0	5492.4	13.14
8	0.66	10.2	0.76	92.5	4.50	0.80	12.8	3989.8	9.55
9	0.80	13.2	0.85	25.2	1.23	0.90	0.8	1328.8	3.18

FTIR

Figure 5 illustrates the various functional groups present in the compound (*Mandak*) based on the dips (in transmittance mode) and spikes (in absorbance mode) of the graph.

Figure 5: FTIR Spectrum of *Mandak*

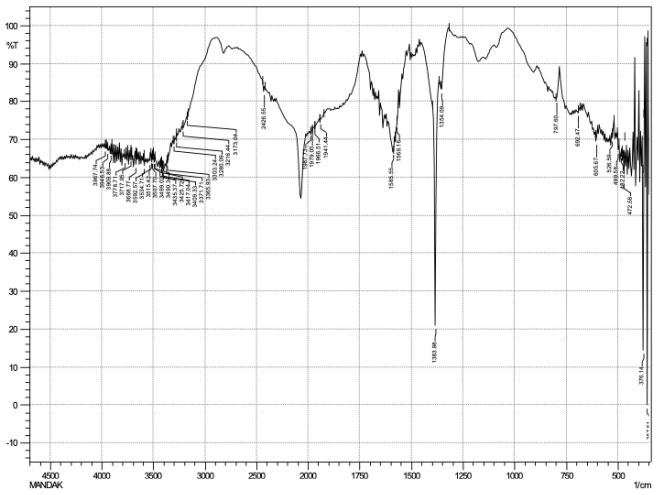


Table 8: FTIR analysis of *Mandak*

S.No.	Peak Range	Bond	Functional group
1	3668.77-3534.71	O-H Stretching	Alcohol
2	3507.70- 3365.93	N-H Stretching	Primary amine
3	3280.92	O-H Stretching	Carboxylic acid
4	3173.04	O-H Stretching	Alcohol
5	1987.72	C=C=C Stretching	Allene
6	1585.55	N-H Bending	Amine
7	1383.98	C-H Bending	Aldehyde
8	1354.09	O-H Bending	Alcohol
9	1354.09	O-H Stretching	Phenol
10	797.60	C-C Bending	Alkene
11	692.47	C-Br Stretching	Halo compound
12	605.67	C-I Bending	Halo compound

Discussion

In the fields of research and the healthcare system, proper drug authentication and identification plays a very crucial role (9). The quality of the herbal drugs is crucial for maintaining the standards of research, as it is essential for the reproducible efficacy and safety of these remedies. Herbal drugs tainted with microbiological loads may not exhibit the necessary potency and may shorten the formulation's shelf life (10). The microbial load of all the raw drugs and the end product, as shown in table no. 1, was either absent or within acceptable bounds, indicating that the drugs were free of microbial contamination and ensuring the quality of the drugs used.

Organoleptic characters can be referred to as observing the characteristics of a drug with help of sense organs. This ascertains some distinctive characteristics of the material, serving as the initial stage in assessing the material's nature and level of purity (11). Another important diagnostic characteristic of the plants are the organoleptic and physico-chemical parameters of the plant components, which are listed in table 2 & 4. All the parameters were within the standard limits. When assessing the drug's purity, the total ash value is especially crucial. It is the presence or absence of exogenous matter, such as silica or metallic salts like carbonates and oxalates etc. The determination of extractive values facilitates the identification of chemical constituents in specific solvents involved in the herbs and also ensures that the drug is adequately soluble in both polar and non-polar solvents (12). Loss on drying of *Mandak* was 6.67%, indicating a lower moisture content or the least amount of hydroscopic activity in the compound drug. Although there is no discernible difference between the water and alcohol soluble extractive values (22.34% and 20.15%, respectively), of *Mandak* it can nevertheless be inferred that the drug may be more extracted and soluble in water than in alcohol.

Many chemical components in plant materials exhibit fluorescence. For instance, alkaloids like berberine do not glow in daylight but emit fluorescence

under UV light. Non-fluorescent chemicals can be converted into fluorescent derivatives using reagents. Consequently, fluorescence is often used to qualitatively evaluate crude drugs, making it a crucial pharmacognostic indicator (13). Table 3 and fig. 2 displays the colours of individual drugs and the combined drug (*Mandak*) under visible, short, and long wavelengths. Various reagents cause distinct colour changes, but the emphasis is on the colour variations of the drug powders alone, without any reagent mixture. *Daruharidra* stem powder exhibits a brilliant fluorescent green colour under both short and long wavelengths. Similarly, the end product shows the fluorescent yellow colour. *Meshashringi* and *Nimba* appear dark yellow and dark blue, respectively, under 366 nm, while *Asana* shows a green colour under 254 nm, reflecting the phytoconstituents present in these plants.

Phyto-chemical results revealed in table 5 shows the presence of phytoconstituents which are crucial primary and secondary metabolites that gives the compound drug their potent therapeutic properties. For instance, flavonoids have anti-allergic and anti-thrombotic properties. Likewise, the antimicrobial and antioxidant properties can be attributed to alkaloids (14). Tannins possess antioxidant, free-radical scavenging and anti-inflammatory properties (15). These plant characteristics could be considered a blessing for humans because they can help with healing and eradicate harmful bacteria when consumed.

Table no 6 shows the various Rf values of obtained during TLC profiling of individual drugs. The combination of toluene and ethyl acetate has the ability to dissolve both polar and nonpolar liquids. Thus, it can be considered universal solvent with a wide range of applications. Even at very low concentrations, the TLC fingerprint of the extracts could be utilised to verify the drug's quality and purity (16).

One of the advanced instrumental approaches based on TLC, that raises the benchmark for drug quality control is HPTLC. A specific plant/compound drug can be identified and separated from adulterants and substitutes using its HPTLC fingerprint. Figures 3,4 and 5 display the HPTLC photo-documentation profiles and densitogram profiles of the alcohol as well as aqueous extracts of the plant materials at UV long (366 nm), UV short (254 nm), and white light following derivatization (17).

The Rf values of aqueous and alcoholic extract have been mentioned in Table no 7. These peaks can be ascribed to the several phytoconstituents that are significant components of the plants under analysis. In the fig 3(a), 3(b) and 3(c), the first spot corresponds to the alcoholic extract and the second spot corresponds to the aqueous extract. On the plate, the separated compounds show up as discrete coloured bands at various heights. The colour, intensity, and position of these bands can vary based on the chemical nature of the compounds.

The peak observed at 0.36 indicates the presence of berberine, as it aligns closely with the standard values, confirming its identification (18). Similarly, the peak at 0.59 corresponds well with the standard values for gallic acid, thus confirming its presence (19). Similarly, other rf values can be compared with other standard biomarkers. For instance, the presence of specific anthocyanin types may be indicated by the red band; similarly, the presence of specific flavonoids, phenols, and alkaloids is indicated by the blue colour; and the presence of chlorophyll compounds and specific phenols, such as catechins, is indicated by the shades of green (20). To aid in precise identification, each compound has distinct colour responses and Rf values based on the solvent system used.

There are many peaks of individual drugs whose presence can be seen in HPTLC profile of *Mandak* confirming their presence in the same. Variation in Rf values is influenced by geographical regions and collection times. Plants like *Asana* and *Khadira*, from arid regions (*jangala desha*), (21) and *Daruharidra*, from tropical region (*anupa* and *sadharan desha*), (22,23) may have different chemical compositions due to the same. Harvest times also impact the chemical constituents of these plants.

FTIR provides information about molecular bonding, chemical structure and identifies organic functional groups. The formation of the infrared spectrum, which is related to the vibration of particular sets of chemical bonds established within a molecule, results from the absorption of electromagnetic radiation at various frequencies (24). Based on the peak values in the region of IR radiation, the FTIR spectrum of *Mandak* showed and confirmed the presence of halo compounds, alcohols, primary amines, and carboxylic acids as active components. The region from 600-1500 cm^{1} is known as the fingerprint region as the absorption pattern in this range is highly specific to each individual compound, much like a human fingerprint (25).

The presence of alcohols, phenols, and carbohydrates may be linked to the hydroxyl group (-OH), which has antioxidant properties; on the other hand, the presence of fatty acids and amino acids, which are the building blocks of proteins and lipid membranes, may be represented by the carboxyl group (26,27). Alcohol-containing molecules have the ability to scavenge free radicals and operate as cofactors as well as coenzymes (28). Comprehending the functional groups discerned in an FTIR spectrum facilitates understanding of the molecular roles and activities.

Certain biochemical attributes and processes necessary for life, such as energy production, structural integrity, signalling, and catalysis, are facilitated by the contributions of each functional group. Determining these groups aids in clarifying the molecular makeup and possible biological effects of the substances being investigated.

Conclusion

The present study provides various resourceful information regarding the qualitative and quantitative analysis of *Mandak*- A novel polyherbal formulation. The identification and isolation of significant phytoconstituents that are responsible for their

therapeutic properties is facilitated by TLC, HPTLC and FTIR analysis. These results may prove useful in the identification and validation of *Mandak*. Scientists might additionally benefit from using this important information on the identity and characteristics of this unique polyherbal combination, which can be further evaluated for in-vitro and in-vivo research.

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