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Research Article

PRELIMINARY PHYTO–CHEMICAL STUDY ON THE EXTRACT OF VATAPATRADI LEPA - A POLY HERBAL FORMULATION

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Abstract

Vatapatradi lepa is a polyherbal formulation comprising six herbal drugs mentioned in Sharanghadhara Samhitha as a Varnya Lepa. As an attempt for the modification into ointment form, Vatapatradi Extract was prepared. Present invention provides a method of preparing aqueous extract of polyherbal formulation as per classics. In the present study Vatapatradi extract was evaluated for its physico-chemical properties, preliminary phytochemical analysis, Thin Layer Chromatography and High Performance Thin layer Chromatography. Preliminary phyto-chemical analysis revealed the presence of Carbohydrate, Flavanoids, Steroids, Saponins, Tannin, and Terpenoids. HPTLC analysis detected 11, 9 and 7 spots at different R_f values under 254nm, 366nm and 620 nm respectively. Reports highlight the therapeutic potential of the polyherbal formulation which can be incorporated in the preparation of topical dosage forms.

Key words: Vatapatradi Lepa; Extract; HPTLC; Phytochemical analysis; Polyherbal.

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INTRODUCTION

Man has been using the herbs and plant products for combating the diseases since time immemorial. Natural products such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity.[1] Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs, men turned to ethnopharmacognosy. They found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect.[2] Extraction pharmaceutically involves the separation of medicinally active portion of plant or animal tissue from inactive or inert components by using selective solvents in standard extraction procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or in dry powder form and are intended for oral or external use.[3] Pharmaceutical research across the world shows that natural products are potential sources of novel molecules for drug development.[4] Extracts are rich source of bio chemical constituents which can be incorporated in the preparation of different dosage forms. Keeping these points in consideration an attempt has been made to prepare the aqueous extract of a polyherbal topical formulation quoted in Ayurvedic classics. Vatapatradi Lepa is a polyherbal topical formulation mentioned in Sharanghadhara Samhita,[5] Vangasena.[6] Yogaratnakara[7] and Bhavaprakasha[8] as an external application in Vyanga (Melasma).[9] Present study focuses the preparation of polyherbal extract of Vatapatradi Lepa adopting the classical principle where water was used as a solvent. The main objective of the study was to analyze the presence of different phytochemicals and to develop the physico-chemical profile of the formulation. In addition to these, present study signifies the use of TLC and HPTLC fingerprint profiles for developing standards for the formulation.

MATERIAL AND METHODS

Procurement of raw materials

The dry herbs required for the preparation were collected from Bharath Pharmacy, a GMP Certified pharmacy, Moodbidri, Karnataka. Fresh drugs (Ripened leaves of Vata and fresh leaves of Jati) required were collected from local area of Moodbidri, Karnataka. All these raw drugs were identified as genuine samples by the Department of Dravya Guna, Alva's Ayurveda medical college, Moodbidri.

Pharmaceutical study was conducted in the P.G. Lab of Rasashasthra and Bhaishajya Kalpana Dept. of Alva's Ayurveda medical college. Analytical tests were carried out in SDM Center for Research in Ayurveda & Allied Sciences, Kutpadi, Udupi, Karnataka and P.G. Lab of Dravya Guna Dept. of Alva's Ayurveda medical college, Moodbidri, Karnataka.

Preparation of the Kwatha (Aqueous extract)

The ingredients of Vatapatradi extract are depicted in Table 1. Firstly the raw materials were cleaned properly and made into coarse powder and then soaked in 8 parts of water and kept overnight, next day the liquid was boiled over moderate flame until the reduction of 1/4th quantity.[10] Later the decoction was strained through cloth and collected liquid is further heated over moderate flame until it attains semisolid consistency. Later it was preserved in air tight container.

Analytical study

The physicochemical evaluation of the extract such as Loss on drying at 105^0 C,Total ash,

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Acid insoluble ash Water soluble ash, Ash analysis were carried out as per methods cited in the Ayurvedic Pharmacopoeia of India.[11]

Preliminary phyto-chemical tests

The aqueous extract was subjected for preliminary phyto-chemical analysis for determining the presence of secondary metabolites present in it. Presence of Alkaloids, Carbonates, Saponins, Flavanoids, Phenols, Terpenoids were determined following standard procedures.[12]

Chromatographic study

Aqueous extract was used for the study. The solvent system used was n-Butanol:Glacial acetic acid:Water in the ratio 4:1:1. The developed plates were visualized in UV 254 nm, 366 nm and then spraying with vanillin sulphuric acid reagent, scanned under UV 254 nm, 366 nm and 620 nm, R_f colour of the spots and densitometric scan were recorded.[13]

RESULTS AND DISCUSSION

The physico-chemical parameters of the extract are presented in Table 2. Ash analysis of the extract revealed the presence of Chloride, Sulphate, Phosphate, Potasssium, Sodium, Aluminium in the sample and results are depicted in Table 3. Aqueous extract was subjected for qualitative phytochemical screening for the identification of chemical constituents and for the detection of the functional groups various chemical tests were performed with aqueous extract. The tests showed the presence of Carbohydrate, Flavonoids, Steroid Saponins Tannin and Terpenoid in the sample. Previous research conducted on extract of leaf, stem bark and root bark of *Ficus bengalensis* showed the presence of Tannins, Flavonoids, Phenols, Saponins, Glycosides, Carbohydrates and Alkaloids.[14] Phytochemical analysis conducted on Jati patra (*Jasminum grandiflorum* Linn.) showed the presence of Carbohydrate, Tannins, Flavonoids, Saponins and Alkaloids.[15] Phytochemical analysis of Raktha Chandana (*Pterocarpus santalinus* Linn.) revealed the presence of Saponins, Tannins, Carbohydrates, Glycosides, Flavonoides, Alkaloids.[16] Phytochemical analysis of Daruharidra (*Berberis aristata*) extract revealed the presence of Alkaloid, Resin, Tannin, Saponin and Protein.[17] Same findings are reflected in the present analysis also. Results of Phytochemical analysis of Vatapatradi extract are summarized in Table 4, 5, 6 and 7.

Thin Layer Chromatography and High Performance Thin Layer Chromatography

TLC of the aqueous extract of Vatapatradi Lepa showed 1 spot under 254 nm, 8 spots under UV 366 nm, 5 spots after post-derivatisation vanillin sulphuric acid and 3 spots after post derivatisation at 366 nm. The colour of the R_f value were noted. (Table 8 and Figure 1)

HPTLC densitometric scan of aqueous extract of Vatapatradi Lepa was developed at 254 nm, 366 nm and 620 nm. The solvent system n-Butanol:Glacial acetic acid:Water in the ratio (4:1:1) efficiently resolved the components present in the extract. Totally 11, 9 and 7 spots were detected at 254 nm, 366 nm and 620 nm respectively in the chromatogram. Results of HPTLC profile are depicted in Figure 2 - 4 and Table 9, 10 and 11.

CONCLUSION

The different physico-chemical parameters of the aqueous extract of the Vatapatradi lepa formulation can be considered for the future references. The preliminary phytochemical screening revealed the presence of different functional groups like alkaloid, steroid tannin,triterpinoid and saponins. The developed TLC/HPTLC Chromatogram of the extract indicate the chemical profile of the extract.

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Table 1: Ingredients of Vatapatradi Lepa extract

Sanskrit name	Botanical name	Family	Part used	Quantity (g)
Vata	*Ficus bengalensis* Linn.	Moraceae	Leaf	150
Jati	*Jasminum grandiflorum* Linn.	Oleaceae	Leaf	150
Raktha chandana	*Pterocarpus santalinus* Linn.	Fabaceae	Heart wood	150
Kushta	*Saussurea lappa* C.B Clarke.	Asteraceae	Root	150
Daru haridra	*Berberis aristata* Dc.	Berberidaceae	Stem	150
Lodhra	*Symplocos racemosa* Roxb.	Symplocaceae	Bark	150

Table 2: Physico-chemical parameters of Vatapatradi Extract

Physicochemical Parameters	Result
Loss on Drying at 105^0 C (%w/w)	4.25
Total ash (%w/w)	7.01
Acid insoluble ash at 450^0 C (%w/w)	0.84
Water soluble ash at 450^0 C (%w/w)	4.81

Table 3: Ash analysis of the Vatapatradi extract

Test	Result
Carbonates	-ve
Fluorides	-ve
Chlorides	+ve
Sulphate	+ve
Chromate	-ve
Phosphate	+ve
Potassium	+ve
Sodium	+ve
Aluminium	+ve
Calcium	-ve

+ve – Present ; -ve Absent

Table 4: Results of Preliminary phytochemical analysis of Vatapatradi extract

Tests	Colour if positive	Vatapatra aqueous extract
	Alkaloids	
Dragendrof's test	Orange precipitate	Red color
Wagners test	Red precipitate	Red color
Mayers test	Dull white precipitate	White color
Hagers test	Yellow precipitate	Yellow color
	Steroids	
Liebermann- buchard test	Bluish green	Bluish green
Salkowski test	Bluish red to cherry red	Bluish red to cherry red
	Carbohydrate	
Molish test	Violet ring	Violet ring
Fehlings test	Brick red precipitate	Brick red precipitate
Benedicts test	Red precipitate	Red precipitate

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Table 5: Results of Preliminary phytochemical analysis of Vatapatradi extract

Tannin		
With $FeCl_3$	Dark blue or green or brown	Light brown color
Flavanoids		
Shinoda's test	Red to pink	Pink color
Saponins		
With $NaHCO_3$	Stable froth	Stable froth
Triterpenoids		
Tin and thionyl chloride test	Pink	Pink color
Coumarins		
With 2 N NaOH	Yellow	Yellow color

Table 6: Results of Preliminary phytochemical analysis of Vatapatradi extract

Phenols		
With alcoholic ferric chloride	Blue to blue black, Brown	Yellow color
Carboxylic acid		
With water and $NaHCO_3$	Brisk effervescence	No effervescence
Resin		
With aqueous acetone	Turbidity	No turbidity
Quinone		
5% NaOH	Pink/purple/red	Brown color
Amino acids		
Ninhydrine reagent	Purple color	Brown color

Table 7: Results of preliminary phytochemical tests

Test	Vatapatradi aq. Extract
Alkaloid	-
Carbohydrate	+
Carboxylic acid	-
Coumarins	-
Flavanoids	+
Phenol	-
Quinone	-
Resins	-
Steroid	+
Saponins	+
Tannin	+
Terpenoid	+
Amino acids	-

+ve – Present ; -ve Absent

Table 8: R_f values of Vatapatradi Aqueous extract

At 254nm	At 366nm	Post derivatisation	Post derivatisation at 366nm
-	0.14 (FL. blue)	-	-
-	0.27 (FL. blue)	-	-
-	-	0.30 (L. peach)	-
-	0.33 (FD. blue)	-	-
-	0.35 (FL. blue)	0.35 (L. peach)	-
-	-	0.42 (D. yellow)	0.42 (D. brown)
-	0.51 (FL. green)	-	0.51 (L. green)
0.58 (D. green)	0.58 (FL. green)	0.58 (L. yellow)	0.58 (L. green)
-	-	0.70 (L. peach)	-
-	0.88 (FL. blue)	-	-
-	0.93 (FL. blue)	-	-

F – Fluorescent; L – Light; D- Dark.

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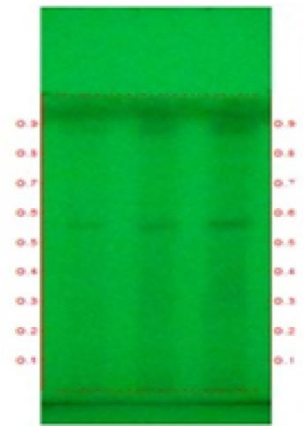
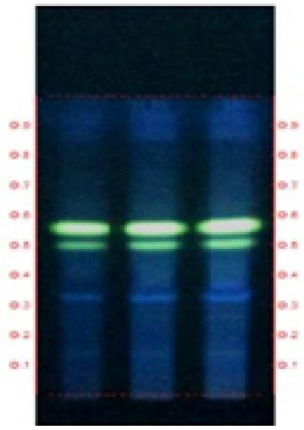
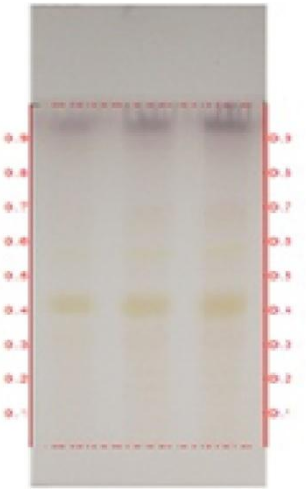
Figure 1: TLC Photo documentation of Vatapatradi aqueous extract



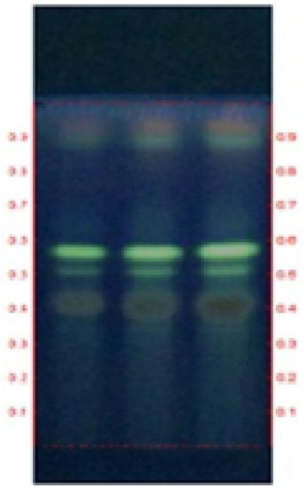
At 254nm



At 366nm



Post derivatisation



After dipping at 366nm

Track 1 –3 µl; Track 2 –6 µl; Track 3 – 9 µl

Solvent system: *n*-Butanol: Glacial acetic acid: Water (4: 1: 1)

Figure 2: HPTLC photo documentation at 254 nm - 9 µl

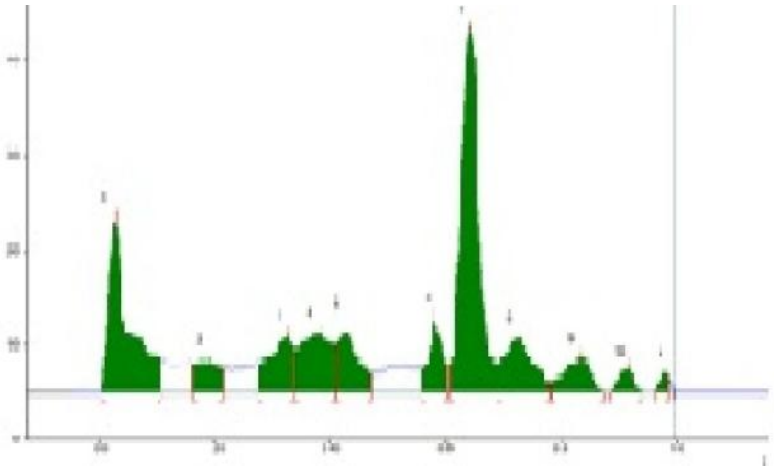


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Figure 3: HPTLC photo documentation at 366 nm - 9 µl

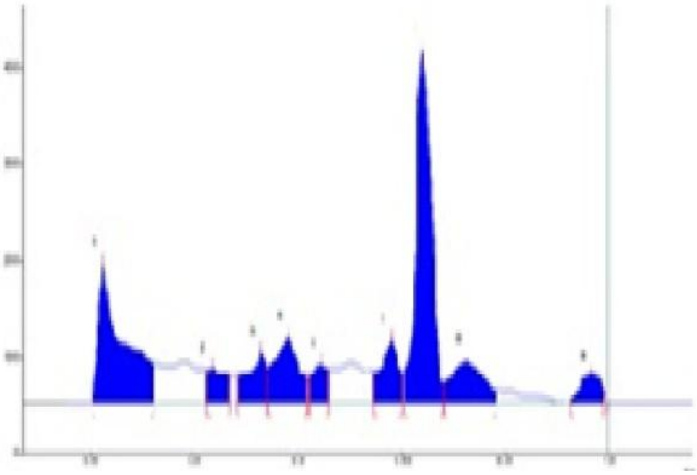

Figure 4: HPTLC photo documentation at 620 nm - 9 µl

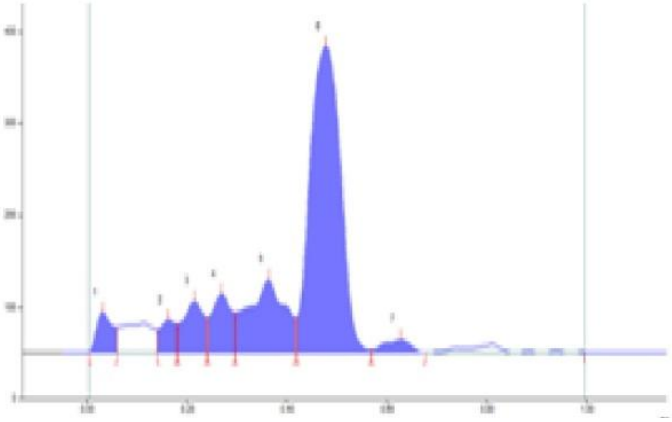

Table 9: Results of HPTLC profile at 254nm-9 µl

Peak	Start Position (Rf)	Start Height (AU)	Max Position (Rf)	Max Height (AU)	Max %	End Position (Rf)	End Height (AU)	Area (AU)	Area %
1	0.01	1.7	0.03	178.1	18.13	0.10	34.6	4274.3	17.34
2	0.16	28.0	0.19	32.3	3.29	0.21	22.4	856.0	3.47
3	0.28	29.3	0.33	58.5	5.95	0.34	45.4	1610.3	6.53
4	0.34	45.5	0.38	60.7	6.18	0.41	50.4	2433.6	9.87
5	0.41	50.6	0.43	67.1	6.83	0.47	17.9	1644.8	6.67
6	0.56	21.6	0.58	75.4	7.67	0.60	28.8	1404.5	5.70
7	0.60	28.9	0.64	380.3	38.71	0.69	28.1	8776.7	35.60
8	0.69	28.9	0.73	56.4	5.74	0.78	9.8	1963.3	7.96
9	0.79	9.9	0.84	34.2	3.48	0.88	0.1	1118.1	4.54
10	0.88	0.0	0.92	21.0	2.14	0.94	0.6	386.9	1.57
11	0.96	0.6	0.98	18.5	1.88	0.99	14.2	185.1	0.75

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Table 10: Results of HPTLC Profile at 366nm- 9µl

Peak	Start Position (Rf)	Start Height (AU)	Max Position (Rf)	Max Height (AU)	Max %	End Position (Rf)	End Height (AU)	Area (AU)	Area %
1	0.00	1.1	0.03	145.4	16.93	0.12	42.5	5046.8	21.61
2	0.23	36.1	0.23	37.6	4.37	0.27	27.9	975.2	4.18
3	0.28	27.1	0.33	53.8	6.27	0.35	39.4	1550.6	6.64
4	0.35	39.8	0.38	68.9	8.02	0.42	27.2	2189.1	9.37
5	0.42	27.3	0.45	42.4	4.94	0.46	36.9	906.4	3.88
6	0.55	34.3	0.58	66.5	7.75	0.60	33.5	1686.2	7.22
7	0.60	34.0	0.64	368.2	42.87	0.68	21.2	8254.1	35.34
8	0.68	21.4	0.73	46.5	5.42	0.79	11.7	1958.7	8.39
9	0.93	0.1	0.97	29.5	3.44	0.99	12.8	787.6	3.37

Table 11: Result of HPTLC profile at 620nm- 9µl

Peak	Start Position (Rf)	Start Height (AU)	Max Position (Rf)	Max Height (AU)	Max %	End Position (Rf)	End Height (AU)	Area (AU)	Area %
1	0.00	0.3	0.03	44.8	7.09	0.06	27.8	1028.5	4.12
2	0.14	25.5	0.16	36.4	5.76	0.18	31.7	829.6	3.33
3	0.18	31.8	0.22	56.7	8.98	0.24	38.7	1654.7	6.63
4	0.24	38.8	0.27	64.4	10.20	0.30	43.7	1830.2	7.34
5	0.30	43.8	0.36	80.1	12.67	0.42	38.4	4211.7	16.89
6	0.42	38.9	0.48	334.4	52.93	0.57	3.0	14812.8	59.39
7	0.57	3.1	0.63	15.0	2.37	0.68	0.0	574.6	2.30

The analysis carried out proves the therapeutic potential of the polyherbal formulation which can be incorporated to design various dosage forms.

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