



PREPARATION AND STANDARDIZATION OF CHITRAKADI VATI

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ABSTRACT

The present work deals with the preparation and standardization of chitrakadi vati. All the parameters of both market formulation (MF1 & MF2) and Laboratory formulation (LF) were found to be within the limit prescribed in pharmacopoeia. Average weight and weigh variation of LF was 2057 mg and 0.2±0.1 %, disintegration time was 8±2 min, hardness was 4.6±0.2 Kg/cm², friability was 0.9722±0.2 %, ash values were 2.4 % (acid insoluble ash) and 2.15 % (water soluble ash), extractive values were 5.6 % (acid insoluble extractive) and 7.25 % (water soluble extractive). In order to ensure quality and stability of final product, some of the important chemical markers has been separated from the mixture and for analysis by high performance thin layer chromatography was selected for qualitative and quantitative purpose. The results of standardization parameters showed marked difference among three, which fingers to authenticity of market formulations. Even HPTLC fingerprinting showed some ingredients in market formulations were taken adulterated or inferior in quality. The HPTLC method for fingerprinting was found to be precise and accurate to quantify three chemical markers plumbagin (0.84%), piperine (0.92%), zingiberene (0.71%), and it further characterized by IR, NMR, Mass spectra.

Keywords: Chitrakadi vati, HPTLC, Piperine, Plumbagin, Zingiberene

INTRODUCTION

Ayurveda is an alternative medicine system with historical roots in the Indian subcontinent. The theory and practice of Ayurveda is pseudoscientific. Ayurveda is heavily practised in India and Nepal, where around 80% of the population report using it. Ayurvedic formulations are used to treat pathological conditions since thousands of years. The ayurvedic drugs are derived from vegetable sources from the various parts of the plant like root, stem, leaf, flower, fruit extract or plant as a whole. There are about 21 varieties of compound formulations¹⁻⁴.

Vati is one of the ayurvedic formulations which is prepared from the sugar or jaggery (guda) or guggulu is made like lehya on mild fire then the powders of the ingredients are added to the paka (lehya) which become soft mass paste like then vati is to be made by rolled into circular in shape⁵.

Chitrakadi vati is an ayurvedic formulation used for the improved digestion process. Chitraka is a Sanskrit word which means fire. Chitrakadi vati increases appetite and improves liver function. Chitrakadi vati shows a dual digestive effect. Its unique combination acts as an appetizer if taken before meal and as a digestive if taken after a meal. By stimulating fat burn it also helps in mobilizing over-deposited fat from the tissues. Chitrakadi vati increases the pitta and regulates vata in the stomach. It restores normal appetite. It promotes proper secretion of digestive Pitta. It influences fat metabolism thereby helping in mobilizing over deposited fat⁶.

Chitrakadi vati control aama dosha by increasing acid secretion in stomach. Aama leads to formation of endotoxins which is root cause of many auto-immune diseases like rheumatoid arthritis,

SLE, scleroderma, nephrotic syndrome, ankylosing spondylitis etc. Chitrakadi vati is useful in irritable bowel syndrome, anorexia, frequent loose stools, heaviness in the abdomen, gas formation. Chitrakadi vati maintains the peristaltic movements of the intestine and stops the flow of undigested food along with loose stool.

With the use of NDDS and phytochemistry aspects variety of novel ayurvedic formulation was successfully formulated and evaluated which have higher drug release capacity and more efficacy than traditional formulations.⁷⁻¹⁵

High-performance thin layer chromatography is a very popular method to separate plant constituents according to their polarity. It is a qualitative and quantitative method for identification, separation and quantification of plant chemicals. Separated constituents can be further characterised by IR, NMR and Mass spectra.

MATERIALS AND METHODS

Authentication of raw materials

Raw materials used for the preparation of chitrakadi vati were collected from the local source. Two market formulations of different manufacturers were procured from local chemist shop. Fresh leaf samples and dried powder of plant were studied macroscopically and microscopically¹⁶.

Procedure to prepare chitrakadi vati (Lab. Formulation)¹⁷

Raw materials were triturated to make fine powder with mortar pestle and with a mechanical grinder. Ingredients were mixed well in equal quantities except for Panchnamak. Panchnamak was added five times than the previous other ingredients' quantity.

Ingredients were mixed well and juice of dadim was added to make dough or compact bind form. The pills were rolled and tried to take equal quantities to roll. (0.20 gms/vati). The pills were dried in natural atmosphere for 24 Hrs and were packed in an airtight container.

Market Formulation

Two market formulations of different manufacturers were procured from local chemist shop. Both products were compared with laboratory formulation as prepared above.

Standardization of formulations

Following tests were done to standardize and compare the Laboratory Formulation with two different market formulations.

Test for average weight and weight variation

20 tablets were taken and weighed individually. Average weight and the individual tablet weight was calculated using digital weight balance and compared with the average.

Disintegration time

6 tablets are placed into disintegration assembly tubes. 2.5 liter water filled into jar. Temperature maintained $37 \pm 2^\circ\text{C}$. When tablets completely disintegrate record time and calculate average value.

Hardness test

Monsanto hardness tester was used to evaluate tablets hardness. 5 tablets from each batch were randomly selected and tested.

Friability Test

Roche friabilator was used to evaluate the friability of the tablets. 10 tablets from each batch were randomly selected and place into the friabilator. After completion of 100 rotation weight of tablets compare to before operation and calculate friability.

Determination of ash value

Total ash

About 2-4g of the ground air-dried material was taken and weight accurately. Material was transferred into crucible and heat up to 500-600°C. After completion of operation ash was collected and weight accurately and find out ash value.

$$\% \text{ASH} = \frac{(\text{ashes wt.}) - (\text{crucible wt.})}{(\text{crucible and sample wt.}) - (\text{crucible wt.})} \times 100$$

Acid-insoluble ash

1 gm of ash was taken in the crucible and 25 ml of 0.1N hydrochloric acid was added and heated on a hot plate for 5 min. Filter the insoluble matter and wash it 3 times with 25 ml water. Collect dried insoluble matter and weight accurately. Based on the readings calculate acid insoluble ash.

$$\text{Acid insoluble ash (gm)} = \frac{(\text{Mass of crucible plus ash} - \text{Mass of crucible})}{\text{Mass of sample}} \times 100$$

Water-soluble ash

The ash was boiled for 5 minutes with 25ml of distilled water. Residue collected on ashless filter paper, ignited and weight. Percentage of water-soluble ash was calculated with reference to the air-dried drug.

Determination of extractive value

Determination of alcohol soluble extractive

5 g of the air-dried drug was macerated with 100 ml of ethanol of the coarsely powdered in a closed flask for 24 hours, shaken frequently for 6 hours and allowed to stand for 18 hours. Filtered rapidly, taking precautions against loss of solvent, 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, and dried at 105° , to constant weight and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug.

Determination of water-soluble extractive

Proceeded as directed for the determination of alcohol soluble extractive, using chloroform water instead of ethanol.

HPTLC quantification and method development

Silica gel G60 F254 coated on aluminium sheet was used as mobile phase. Camag twin trough glass chamber (10 x 10 and 20 x 10) was used. Scanning speed was up to 100mm/s. Camag UV cabinet was used with dual wavelength UV lamp (254 and 366nm). Toluene: Ethyl acetate = 7:3 was used as mobile phase.

Sample Preparation

Lab. formulation and market formulation

50 g. of crushed vati was extracted exhaustively with 50 ml methanol for 1 hour and then filtered. The filtrate was concentrated. 1 gm of residue was mixed with 100 ml ethanol and from that mixture 1 ml of mixture taken and diluted up to 100 ml (100µg/ml). Both laboratory formulation and market formulation were prepared using same dilutions and used for further studies.

Standard drugs solutions

100µg/ml concentration solutions are used as standard solutions of piperine, plumbagin and Zingiberene. 0.5, 1, 1.5, 2, 2.5 and 3 µl of piperine, and plumbagin and 14, 16, 18, 20, 22, 24 µl of zingiberene were spotted on 10x10 cm precoated Silica gel G60 F254 plate was developed in Toluene: Ethyl acetate: 7:3 Mobile phase using twin trough chamber. All the chemical markers were detected under U.V. light. λ max of piperine 340 nm, plumbagin 420 nm and zingiberene 420 nm.

RESULTS AND DISCUSSIONS



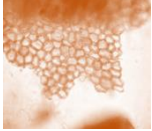
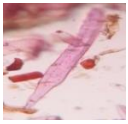



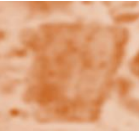

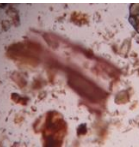
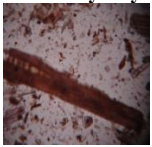
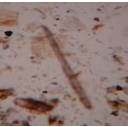
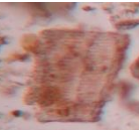
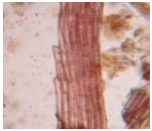
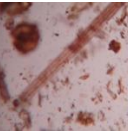
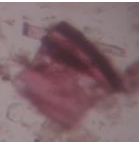
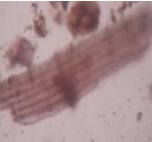








Sensory Characters of Powdered Drug

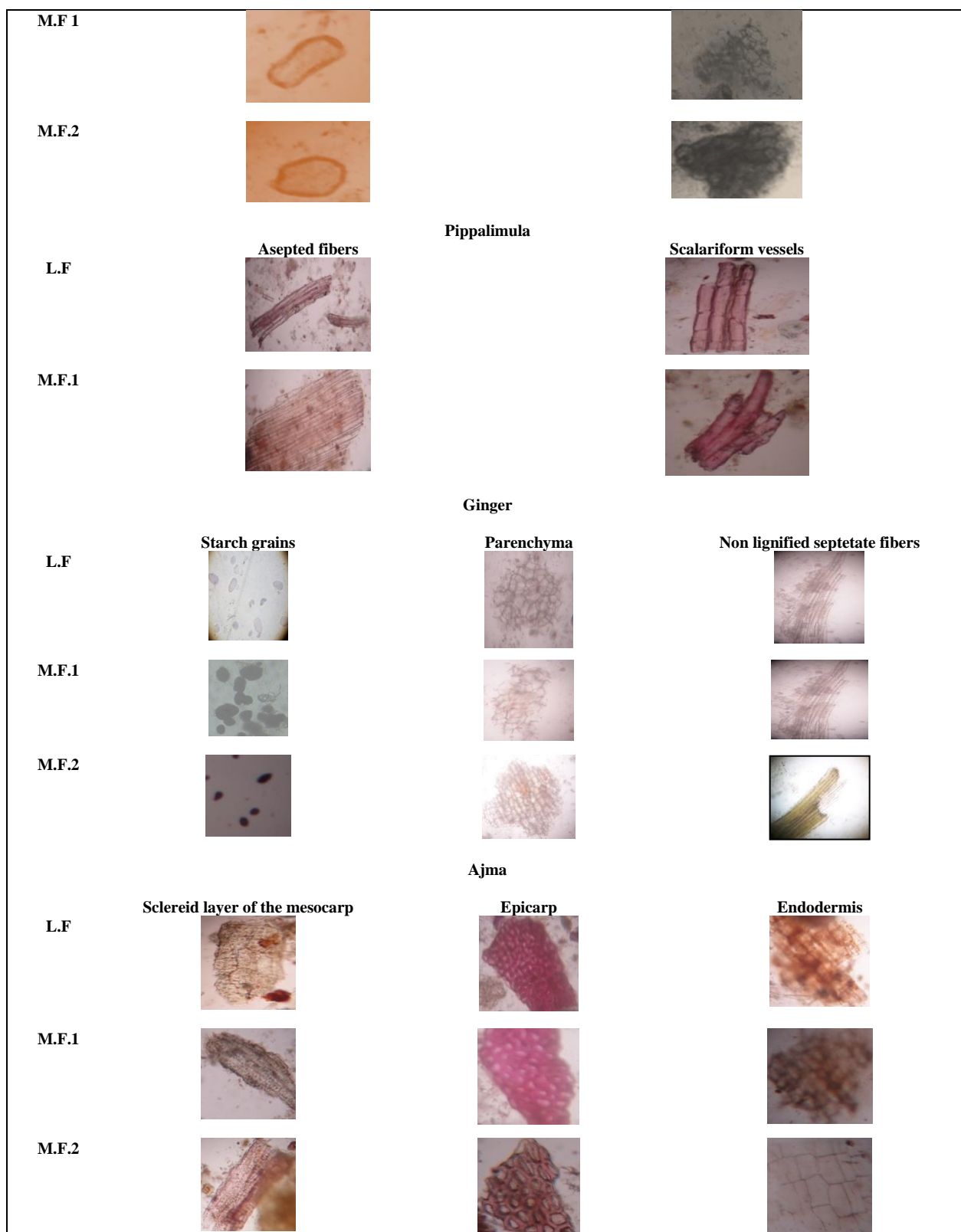
Table 1: Sensory characters of powdered drug

SN	Name of the ingredient	Colour	Odour	Taste	Texture
1	Chitrakmula	Deep yellow	Disagreeable	Acrid	Smooth
2	Pippalimula	Reddish brown to grey	Aromatic	Pungent	Smooth
3	Sunthi	Yellowish brown	Agreeable and aromatic	Agreeable and pungent	Smooth
4	Kalimirsch	Dark brownish black	Aromatic	Pungent	Smooth
5	Pippal	Greyish-black to black	Aromatic Strong	Pungent	Smooth
6	Hing	Dark yellow	Asafoetida like	Astringent	Smooth
7	Ajma	Light brown	Aromatic	Slightly bitter giving a sensation of warmth to tongue	Smooth
8	Chavya	Greenish black	Aromatic	Pungent	Smooth
9	Yavakshar	White	Agreeable	Salty	Smooth
10	Sajikshar	White to Yellow	Slight	Salty	Smooth
11	Panchnamak	Light buff white	Disagreeable	Salty	Smooth

Microscopic evaluation of vati

Table 2: Microscopic characters of raw material

Sample	Character		
	Chitrak		
	Phloem fiber	Reticulated vessels	Cork cell
L.F			
M.F1			
M.F2			
	Chavya		
	Fibers	Vessels	Medullary Rays
L.F			
M.F.1			
M.F2			
	Kalimirsch		
	Isodiametric stone cell	Starch grain	
L.F			
M.F1			
M.F.2			
	Pippal		
	Oval to elongated stone cell	Fragments of parenchyma	
L.F			



Description

Table 3: Result of descriptions of formulations

Formulation	Color	Odour	Taste
LF	Dark brown	Order like umbelliferae and piper	Strong Bitter
MF1	Creamish brown	Mild aromatic	Bitter
MF2	Light brown	No odour	Bitter

Sensory Characters of market formulations were found to be almost same as laboratory formulation.

Table 4: Results of evaluation parameters after formulation

Batch	Average Weight	% weight variation	Hardness Kg/cm ²	% Friability	Disintegration Time (min)
LF	2057 mg	0.2±0.1	4.6±0.2	0.9722±0.2	8±2
MF1	2190 mg	0.4±0.2	5.9±0.1	0.78±0.1	12±1
MF2	3156 mg	0.1±0.2	4.3±0.2	0.88±0.1	14±3

Determination of Ash Value

Table 5: Results of ash values

Formulation (5 gm)	Total Ash	Acid-insoluble ash	Water-soluble ash
LF	6.25 %	2.4 %	2.15 %
MF1	13.5 %	4.95 %	7.65 %
MF2	14 %	6 %	%

The water-soluble ash values of the individual marketed formulations were in range of 5-8%, while same of laboratory formulation were 2.15%. Acid-insoluble ash values of the market formulations were in range of 5-6% while the same of laboratory formulation was 2.4%. High total ash indicates improper removal

of sand (silica matter) from the crude drug. High acid insoluble ash indicates that the acid insoluble constituents like metals, silica, and certain minerals are present as contaminants in the crude drug used for preparation of market formulation.

Determination of extractive value

Table 6: Results for extractive values

Formulation	Alcohol soluble extractive	Water soluble Extractive
LF	5.6 %	7.25 %
MF1	7.72 %	9 %
MF2	9.12 %	11 %

The water-soluble extractive values of marketed formulations are in range of 9-11% while in house preparation shows 7.25%. The alcohol soluble extractive value of marketed formulations was found to be in range of 7-10% or beyond this while the same of Laboratory formulation was 5.6%. Above results shows that each

marketed preparation was developed with some adulteration, or some mistakes being done during mixing or drying process. Another attention was the % water-soluble extractive values were higher than alcohol soluble extractives. This indicates presence of more amounts of water-soluble contents in the formulations.

Separation and quantification of piperine, plumbagin and zingiberene in chitrakadi vati

Piperine

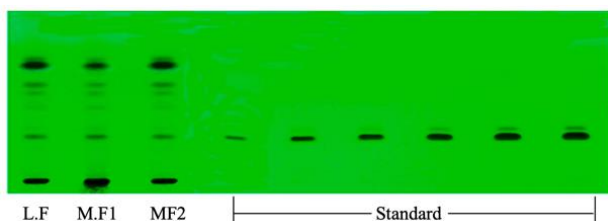


Fig. 1: HPTLC of standard and formulation piperine

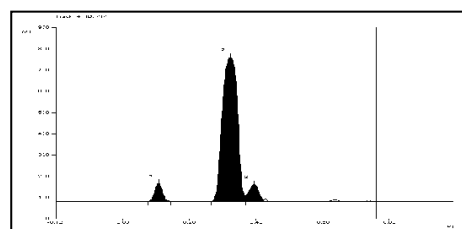


Fig. 2: HPTLC Chromatogram of std. piperine 50 µg/ml

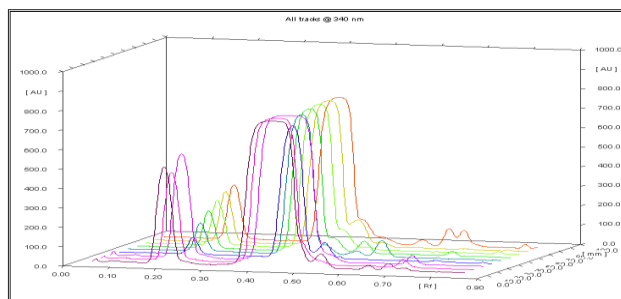
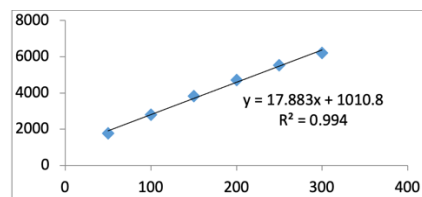


Fig. 3: Absorption spectra of piperine scanned at 340nm

Table 7: Calibration curve of piperine

Concentration of marker (mcg/ml)	Area
50	1766.3
100	2795.9
150	3829.9
200	4707.5
250	5536.7
300	6205.2



Plumbagin

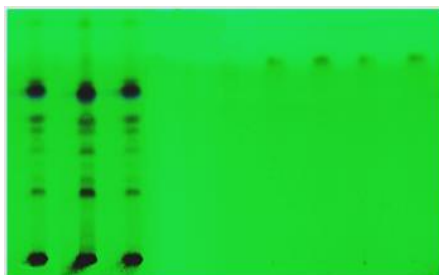


Fig. 4: HPTLC of standard and formulation plumbagin

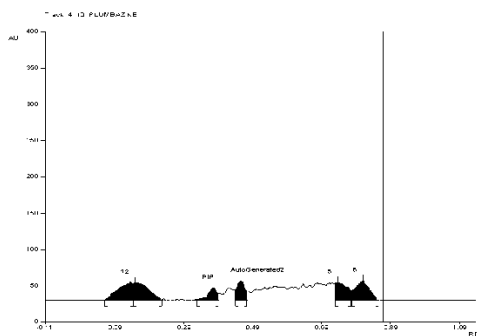


Fig. 5: HPTLC Chromatogram of std. plumbagin 50 µg/ml

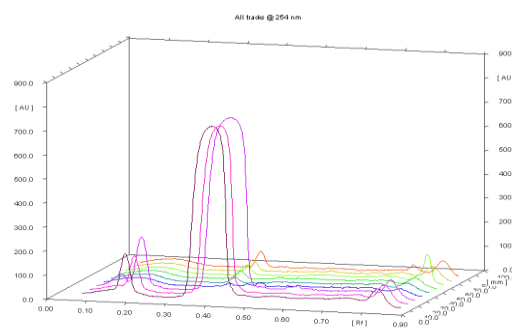
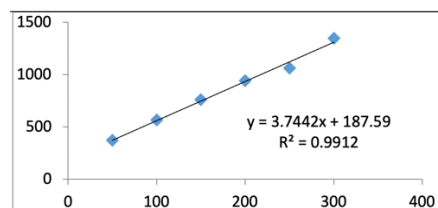


Fig. 6: Absorption Spectra of plumbagin scanned at 420nm

Table 8: Calibration curve of plumbagin

Concentration of marker (mcg/ml)	Area
50	372.6
100	567
150	762.3
200	942.8
250	1062.8
300	1349.5



Zingiberene

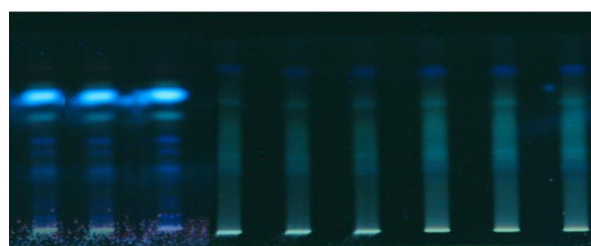
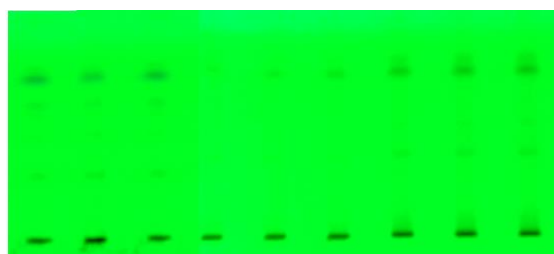


Fig. 7: HPTLC of standard and formulation zingiberene

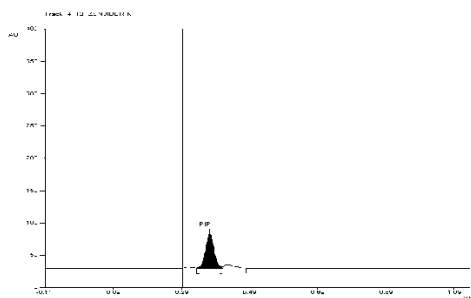


Fig. 8: HPTLC Chromatogram of std. zingiberene 140 µg/ml

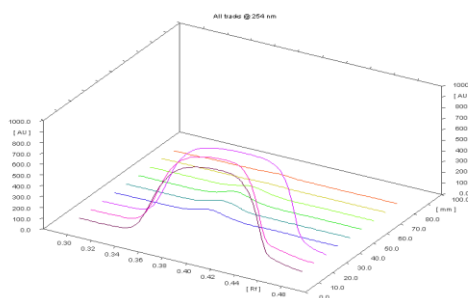
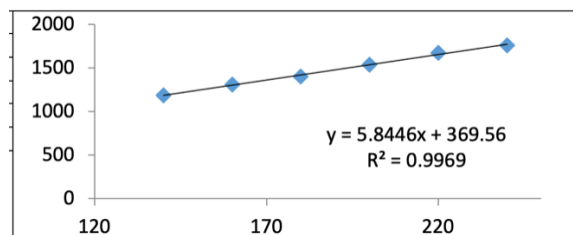


Fig. 9: Absorption Spectra of zingiberene scanned at 420nm

Table 8: Calibration curve of zingiberene

Concentration of marker (mcg/ml)	Area
140	1187.9
160	1310.8
180	1405.5
200	1541.3
220	1673.1
240	1761.6



Standardization of chitrakadi vati

Table 10: Results obtained from HPTLC quantification of chitrakadi vati

Formulation	Plumbagin	Piperine	Zingiberene
LF	0.84%	0.92%	0.71%
MF1	0.14%	0.59%	0.63%
MF2	0.61%	0.13%	0.89%

The amount of plumbagin in LF, MF1 and MF2 were found to be 0.84%, 0.14 and 0.61% respectively. The amount of piperine in LF, MF1 and MF2 were found to be 0.92%, 0.59% and 0.13% respectively. From the results it can be observed that the amount of piperine and plumbagin is more in LF as compared to MF1 and MF2. This indicates that the raw materials used in the MF1 and MF2 are not containing the specified amount of constituents or

are not standardized before formulating the market formulations. The amount of zingiberene in LF, MF1 and MF2 were found to be 0.71%, 0.63% and 0.89% respectively. The quantity of zingiberene in LF was found to be less as compared to MF2. This indicates that the MF2 contains more amount of ginger as compared to the quantity specified for its preparation.

Spectral Characterization

Table 11: Spectral Characterization of Plumabagin, Piperine and Zingiberene

Formulation	Structure	IR	¹ H NMR (δ ppm)	Mass (m/z)
Plumbagin		3065.32 (Aro.C-H) 2924.12, 2946.35, 2979.60 (Ali.C-H) 1640.38 (C=O) 1502.58 (C=C) 1169.57 (C-C)	1H, s, 12.49 (OH), 3H, s, 2.33 (CH ₃) 1H, s, 5.34, =CH 3H, m, 7.14-7.88 (Aromatic C-H)	188.09 M ⁺ MW:188.18
Piperine		3067.83(C-H Aro.) 2945.35 (C-H Ali.) 1640.27, 1658.38 (C=O) 1539.48 (C=C) 1248.38 (C-O) 1178.38 (C-N)	4H, t, 3.54-3.78, N-CH ₂ 4H, p, 2.03-2.19, CH ₂ 2H, p, 1.45-1.52, CH ₂ 1H, d, 5.34-5.41, CO=CH 3H, m, 4.98-5.39, =CH 3H, m, 7.18-8.03, Aromatic C-H 2H, s, 4.37, O-CH ₂ 2H, s, 6.07 (CH ₂),	285.30 (M ⁺) MW: 285.34
Zingiberene		2998.28, 2937.38 (Aliphatic C-H) 1670.38 (C=O) 1559.38 (C=C)	6H, s, 2.84 (CH ₃) ₂ 1H, t, 5.87-6.05 (=CH), 2H, q, 1.53-1.64 (CH ₂), 2H, q, 1.43 (CH ₂), 1H, h, 2.71-3.13 (CH), 3H, d, 1.34-1.37 CH ₃ 1H, p, 2.34-2.54, CH 2H, t, 1.43-1.49, CH ₂ 3H, m, 1.44-2.22 3H, s, 1.47, CH ₃	204.05 (M ⁺) MW :204.36

CONCLUSION

On the basis of above data, it was concluded that chitrakdi vati was successfully formulated and evaluated. Raw material was evaluated by microscopical characteristics. Formulated vati was evaluated by various pharmacopeial tests. Formulated vati passes in all evaluation tests. By the HPTLC biomarkers are separated and quantify. Biomarkers were further characterised by IR, NMR and Mass.

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