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Standardization of Ayurvedic Formulation (Marichyadi Vati) Using HPLC and HPTLC Methods

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***Abstract*—**The present investigation was aimed to develop methodology for the standardization of Marichyadi Vati and its raw materials. Standardization was carried using systematic Pharmacognostical and physicochemical parameters as per WHO guidelines. The detailed standardization of Marichyadi Vati, it is concluded that there are no major differences prevailed in the quality of marketed products and laboratory samples of Marichyadi Vati. However, market samples showed slightly better amount of Piperine than the laboratory sample by both methods. This is the first attempt to generate complete set of standards required for the Marichyadi Vati.

***Keywords*—**Marichyadi Vati, Standardization, Piperine.

I. Introduction

THE flora and fauna of India is popular throughout the world. The Indian herbal formulation contains compounds which have proposed therapeutic activity but are not properly identified have no uniform process of manufacturing and no SOP's is available for production, analysis, validation and lack of analytical procedures. The present investigation was aimed to develop methodology for the Standardization of Marichyadi Vati. Medicines prepared in the form of tablet or pills are known as Vati. These are made of one or more drugs of plant, animal or mineral origin. Marichyadi Vati is famous Ayurvedic formulation, which is given in the Ayurvedic formulary and used in cough and asthma. Marichyadi Vati contains two drugs *Piper longum* and *Piper nigrum*. According to ayurveda the fruits powder of these drugs are used for preparation which is described under general method of preparation of Vati. [1]

Formula

1) Marica	-12part
2) Pippali	-12part
3) Yavaksara (yava)	-6 gm
4) Dadima	-24 gm
5) Guda	-96 gm.

II. Materials and Methods

A. Materials

The Raw Material for the preparation of Marichyadi Vati, powdered *Piper nigrum* and *Piper longum* was procured from the two different drug suppliers (sample 1 of LVG and sample 2 of (Akhand Ayurveda) from the local market in Ahmedabad. Marketed sample of Marichyadi Vati was procured from Baidyanath pharmacy. Sample 2 (Akhand Ayurveda) was used to preparation as it shows better result than sample 1 (LVG).

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B. Methods

Piper nigrum and *Piper longum* are dried and made into fine powders passing through sieve no. 120, separately. Yavaksara and fruit rind of Dadima was added to the prepared powdered drug after passing to sieve no. 120, one by one. All the ingredients were introduced to Guda and ground to a soft paste with the prescribed fluids. Then it was kept on mild fire and removed from the oven. The criterion to determine the final stage of the formulation before making pills is that it should not stick to the fingers when rolled. Pills were dried in shade as specified. [1]

C. Pharmacognostical and Physicochemical Evaluation of Raw Material [2]-[9]

1. Microscopical characteristics of *Piper nigrum* and *Piper longum* are shown in Fig. 1.

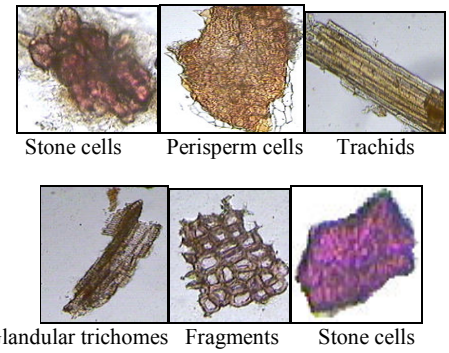


Fig. 1 Microscopical characteristic of thin walled cells of *Piper nigrum* and *Piper longum*

2. Determination of foreign matter results are shown in Table I.

TABLE I
Determination of Foreign Matter for Raw Materials

Sample		Foreign Matter (%W/W)	As Per Indian Herbal Pharmacopoeia.
Piper nigrum	Sample 1	1.2 %	Not More Than 2.0%
	Sample 2	1.1%	
Piper longum	Sample 1	1.6 %	
	Sample 2	1.4 %	

3. Determination of ash value results are shown in Table II.

TABLE II
DETERMINATION OF ASH VALUE FOR RAW MATERIALS

Piper nigrum			
---	Total Ash	Acid In-Soluble Ash	Water Soluble Ash
As Per Indian Herbal Pharmacopoeia.	(Not More Than 6 % W/W)	(Not More Than 1 % W/W)	-
Sample 1	5 %	0.33 %	4.33 %
Sample 2	4.5 %	0.25 %	4.0 %
Piper longum			
---	Total Ash	Acid In-Soluble Ash	Water Soluble Ash
As Per Indian Herbal Pharmacopoeia.	(Not More Than 6 % W/W)	(Not More Than 1 % W/W)	-
Sample 1	5.5 %	0.66 %	4.50 %
Sample 2	5 %	0.55 %	4.17 %

4. Determination of loss on drying results are shown in Table III.

TABLE III
DETERMINATION OF LOSS ON DRYING FOR RAW MATERIALS

Sample		Loss On Drying (%W/W)	As Per Indian Herbal Pharmacopoeia.
Sample 1	*Piper nigrum*	10.50 %	Not More Than 13 %
	Piper longum	7.20 %	
Sample 2	*Piper nigrum*	11.60 %	
	Piper longum	7.80 %	

5. Determination of extractive values results are shown in Table IV.

TABLE IV
DETERMINATION OF EXTRACTIVE FOR RAW MATERIALS

Piper nigrum				
---	Water	Ethanol	Solvent Ether	N-Hexane
As Per Indian Herbal Pharmacopoeia.	(Not Less Than 9 % W/W)	(Not Less Than 5 % W/W)	-	-
Sample 1	9.60 %	5.60 %	6.40 %	2.40 %
Sample 2	10 %	6 %	6.60 %	2.40 %
Piper longum				
---	Water	Ethanol	Solvent Ether	N-Hexane
As Per Indian Herbal Pharmacopoeia.	(Not Less Than 35 % W/W)	(Not Less Than 9 % W/W)	-	-
Sample 1	35 %	9 %	3.20 %	2.20 %
Sample 2	36.80 %	9.50 %	3.50 %	2.40 %

D.Pharmacognostical and Physicochemical Evaluation of Market & Laboratory

1. Microscopical Characteristic of Marichyadi Vati results are shown in Fig. 2.

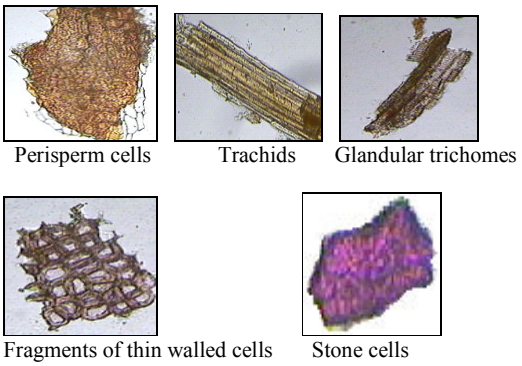


Fig. 2 Pharmacognostical and physicochemical evaluation of Market & Laboratory Marichyadi Vati

2. Determination of Ash Value of Market & Laboratory Vati results are shown in Table V.

TABLE V
DETERMINATION OF ASH VALUE OF MARKET & LABORATORY MARICHYADI VATI

Sample	Total Ash (% W/W)	Acid In-Soluble Ash (% W/W)	Water Soluble Ash (% W/W)
Laboratory	7 %	0.55 %	6.2 %
Market	6.17 %	0.33 %	5.0 %

3. Determination of Loss on Drying of Market & Laboratory Marichyadi Vati results are shown in Table VI.

TABLE VI
DETERMINATION OF LOSS ON DRYING OF MARKET & LABORATORY MARICHYADI VATI

Sample	Loss On Drying (%W/W)
Laboratory	7.50 %
Market	6.75 %

4. Determination of Extractive Values of Market & Laboratory Marichyadi Vat. results are shown in Table VII.

TABLE VII
DETERMINATION OF EXTRACTIVE VALUES OF MARKET & LABORATORY MARICHYADI VATI

Sample	Water (%W/W)	Ethanol (%W/W)	Solvent Ether (%W/W)	N-Hexane (%W/W)
Laboratory	55 %	26 %	5.5 %	3 %
Market	51 %	27 %	5 %	2 %

E. Standardization of Raw Material, Market and Laboratory Marichyadi Vati by HPTLC [10]-[13]

1. Chromatographic Condition

Stationary Phase : 10 x 10 cm^2 aluminum backed silica gel 60 F_{254} plates.
Mobile Phase : Toluene: Diethyl ether:

	Dioxane: 6.25: 2.15: 1.6
Spraying reagent	: Dragandroff reagent
Chamber saturation	: 30 minutes
Band width	: 5 mm
Distance between bands	: 5 mm
Rate of spotting	: 10 sec/µl
Distance run	: 80 mm
Scanning wavelength	: 366 nm
Scanning speed	: 5 mm/sec
Temperature	: 25 ^{o}C

2. Preparation of Standard and Sample Solutions

Standard stock solution of Piperine was prepared by diluting 10 mg in 10 ml methanol (1000 µg/ ml). And different concentration was spotted in aliquots of 2 µg, 4 µg, 6 µg, 8 µg and 10µg numbered as spot 1,2,3,4 and 5 respectively. The Standard curve is shown in Fig. 3. The HPTLC Fingerprinting was shown in Fig. 4 and 3D view in Fig. 5.

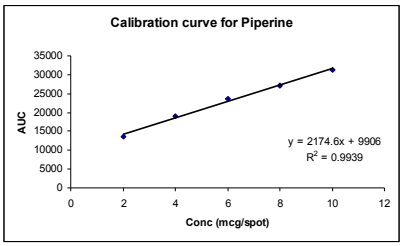


Fig. 3 Standard curve of Piperine

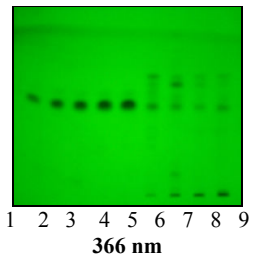


Fig. 4 HPTLC Fingerprinting

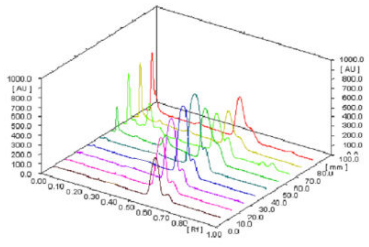
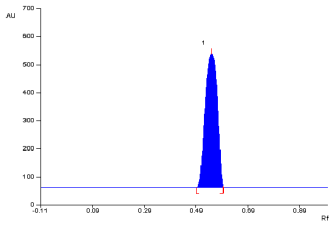


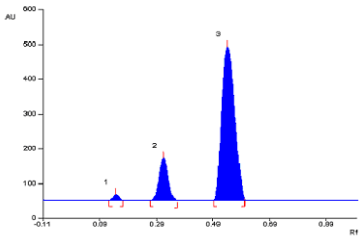
Fig. 5 **3 D View**

3. HPTLC Chromatograms

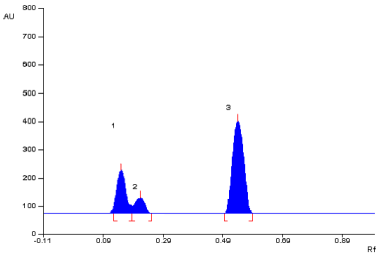
The chromatogram for standard Piperine, Piperine in *Piper Nigrum*, *Piper Longum,* and in Laboratory Vati and in market Vati was shown in Figs. 6 (a)-(e), respectively. The total amount of Piperine content was shown in Table VIII.



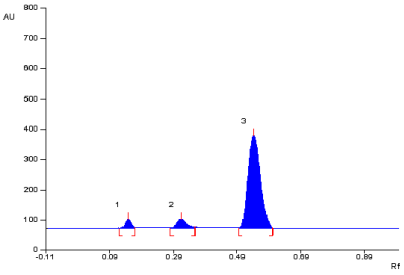
(a) Standard Piperine



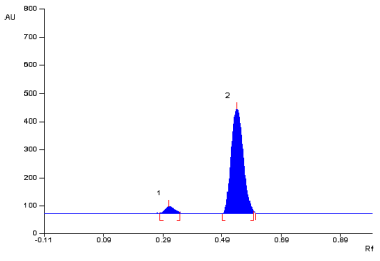
(b) Piperine in *Piper nigrum*



(c) Piperine in *Piper longum*



(d) Piperine in laboratory prepared Marichyadi Vati



(e) Piperine in Market Marichyadi Vati

Fig. 6 (a)-(e) HPTLC chromatograms

TABLE VIII
DETERMINATION OF PIPERINE RAW MATERIAL, MARKET AND LABORATORY MARICHYADI VATI BY HPTLC

Sample	Concentration (µg/Spot)	Auc	R_f	Piperine (%W/W)
Piper nigrum	3	36580	0.54	4.09
Piper longum	3	37487	0.54	4.23
Laboratory Preparation	3	12440	0.54	0.39
Market Preparation	3	12831	0.54	0.45

F. Standardization of Raw Material, Market and Laboratory Marichyadi Vati by HPLC [10]-[13]

1. Chromatographic Condition

Model : Shimadzu DGU-20A5
Column : Luna 5uC_{18} (2) 100A
Detector : Photo Diode Array (PDA)
Detection : 343 nm
Mobile Phase : Methanol: Water: 70: 30
Flow Rate : 1.5 ml/minute
Temperature : 25 ^oC
Injection : 20 µl

2. Preparation of Standard and Sample Solutions

Standard stock solution of Piperine was prepared by diluting 1 mg in 10 ml methanol (100 µg/ ml) and different concentration was prepared and injected in aliquots of 2.5 µg/ml, 5 µg/ml, 5 µg/ml, 7.5 µg/ml, 10 µg/ml, 12.5 µg/ml, 15 µg/ml and 17.5 µg/ml . For the stock solution of *Piper longum* and *Piper nigrum* was prepared by 0.5 gm, extracted separately in 50 ml methanol on water bath for 30 minutes repeatedly twice and filtered. From this solution 1 ml was taken and volume was adjusted to 10 ml by methanol. The stock solution of lab and market Vati was prepared by extracting 0.5 gm separately in 50 ml methanol on water bath for 30 minutes repeatedly twice and filtered. From this solution 0.5 ml was taken and volume was adjusted to 10 ml by methanol (500 µg per ml). The Standard curve is shown in Fig. 7.

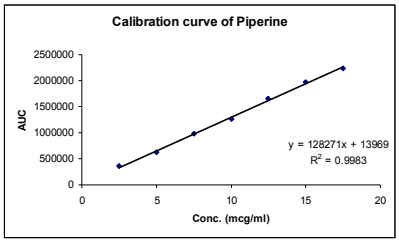
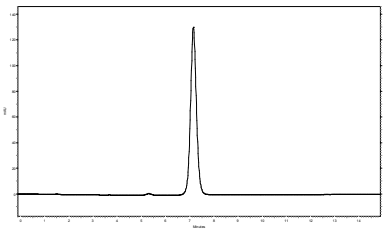


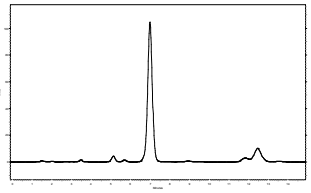
Fig. 7 Standard curve of Piperine

3. HPLC Chromatograms

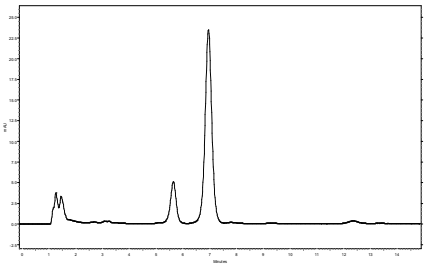
The chromatogram for standard Piperine, Piperine in *Piper Nigrum*, *Piper Longum,* In Laboratory Vati and in market Vati were shown in Fig. 8 (a to e) respectively. The total amount of Piperine content was shown in Table IX.



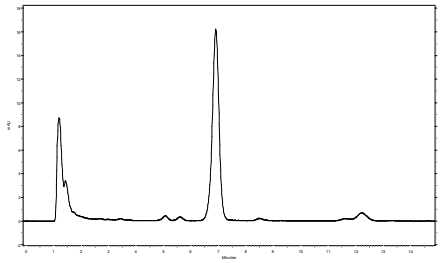
(a) Standard Piperine



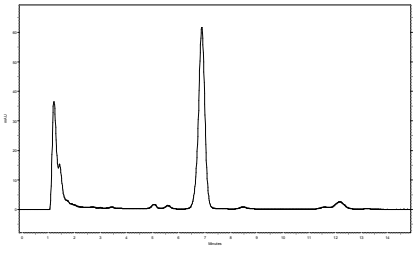
(b) Piperine in *Piper nigrum*



(c) Piperine in *Piper longum*



(d) Piperine in laboratory prepared Marichyadi Vati



(e) Piperine in Market Marichyadi Vati

Fig. 8 (a)-(e) HPLC chromatograms

TABLE IX
DETERMINATION OF PIPERINE RAW MATERIAL, MARKET AND LABORATORY MARICHYADI VATI BY HPLC

Sample	Concentration (µg/Ml)	Auc	Rt	Piperine (%W/W)
Piper nigrum	500	2715220	7.102	4.20 %
Piper longum	500	2718235	7.104	4.22 %
Laboratory Preparation	2000	292963	7.205	0.44
Market Preparation	2000	315867	7.208	0.48

III. RESULTS AND DISCUSSION

The major objective of the present study was standardization of Marichyadi Vati and to develop set of standardization parameters for further research. In this study, the raw material for the preparation of Marichyadi Vati, powdered *Piper nigrum* and *Piper longum* was procured from the different drug suppliers, from local market in Ahmedabad. It is concluded from the pharmacognostical and physiochemical studies that both samples are genuine and no adulteration is reported. Marketed sample of Marichyadi Vati was procured from Baidyanath Pharmacy. The laboratory sample of Vati was prepared from material procured from local market in Ahmedabad. Pharmacognostical and physiochemical studies was performed on both lab and market sample.

The HPTLC method for the analysis of raw material for the marker compound Piperine in *Piper nigrum* and *Piper longum* was performed. Market sample 1 and 2 of *Piper nigrum* procured from local market showed Piperine content 4.23 and 4.09% w/w respectively whereas, market sample 1 and 2 of *Piper longum* contained 2.36 and 2.29% w/w of Piperine respectively. The HPLC method for the analysis of raw material for the marker compound Piperine in *Piper nigrum* and *Piper longum* was performed. Market sample 1 and 2 of *Piper nigrum* procured from local market showed Piperine content 4.22 and 4.20% w/w respectively whereas, market sample 1 and 2 of *Piper longum* contained 2.22 and 2.21% w/w of Piperine respectively. Hence, we can conclude that both HPTLC and HPLC analytical method have very good reliability in the analysis of Piperine in the drug as they gave almost similar results. The HPTLC and HPLC methods for the analysis of Marichyadi Vati for the Piperine content were performed. Market sample procured from Baidyanath Pharmacy showed Piperine content of 0.45% w/w in HPTLC and 0.48% w/w in HPLC. Laboratory sample showed Piperine content of 0.39% w/w in HPTLC and 0.44% w/w in HPLC. Hence, it is clear from the study that market formulation shows little higher amounting of Piperine than laboratory sample of Vati. However, it is very important to note that laboratory sample shows Piperine content in accordance with the theoretical amount of Piperine present in the ingredient drugs of *Piper nigrum* and *Piper longum*.

IV. CONCLUSION

In this present work, it is concluded from the detailed standardization of Marichyadi Vati, the uniform manufacturing process is followed as per the Ayurvedic Herbal Pharmacopoeia. No considerable differences in the result of Pharmacognostic parameter are found for both the sample, laboratory prepared and market preparation. But from the result it is concluded that market preparation showed slight better result in comparison with laboratory prepared Marichyadi Vati. The standardization process and the value concluded in the analysis and standardization of the laboratory sample of Marichyadi Vati can be used as a set of standard for others who involved in the manufacturing and processing of this formulation. This is the first attempt made in the complete standardization of Marichyadi Vati.

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