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Research Article

STANDARDIZATION OF AN AYURVEDIC FORMULATION AJMODADI CHURNA

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ABSTRACT

With the increase in demand of herbal products, it is very important to develop standardization method for development of authentic formulations which are being used for many centuries. Keeping in mind this motivation this article is focussed on standardization of Ajmodadi churna, a polyherbal ayurvedic formulation used as an antispasmodic and carminative, and is a strong wormifuge and also restores normal digestive functions and also helps in all painful conditions like sciatica and stiffness in back. Ajmodadi churna was prepared according to process described in Ayurvedic Formulary of India. In house preparation and marketed formulation have been standardized on the basis of, physical characteristics, physicochemical properties, phytochemical screening and chemofinger printing methods were developed. The set parameters were found to be sufficient to evaluate the churna.

Keywords: *Ajmodadi churna, ayurvedic formulation, pharmacognosy.*

INTRODUCTION

Standardization of the herbal drugs is mandatory in perspective of its increasing worldwide acceptability. The main advantage of standardization is that it ensures consistently a good quality product. Additionally it also ensures positive identification that the herbal drug is what it is claimed to be according to its label (Andola et al., 2010).

India has rich wealth of traditional knowledge of Ayurveda, Siddha, Unani and other traditional medicine In order to market our products outside the country and also to maintain the quality of these medicines when shifted to industrial production requires validated analytical methods for maintaining the quality of these products. So in this direction we have chosen an ayurvedic formulation Ajmodadi churna.

MATERIALS AND METHODS

Plant material

Ajmodadi churna consists of 12 ingredients, v iz., *Apium graveolens* L, *Embelia ribes* Burm, Rock salt, *Cedrus deodar* (Roxb) Loud, *Plumbago zeylanica* Linn, *Pipper longum* Linn, *Foeniculum vulgare* Gaertn, *Pipper longum* Linn, *Pipper nigrum* Linn, *Terminalia chebula* Retz, *Argyreia speciosa* (L.f.) sweet, *Zingiber officinale* Roscoe. (Ayurvedic Formulary of India, 2003). The raw materials were procured from local market of Ranchi. The raw materials were separated from impurities and dried under

shade to remove excessive moisture content to aid in preservation during storage. Then each ingredient was made powder and packed in air tight containers until usage. According to Ayurvedic Pharmacopoeia, raw materials were authenticated microscopically.

Preparation of ajmodadi churna

The churna was manufactured following Ayurvedic Formulary of India.All the ingredi ents were
powdered separately, passed through 80 # sie ve and then mixed together in specified proportion to get uniformly blended churna.

Marketed samples

A marketed formulation (Sample B) of Vyas pharmaceuticals (Ajmodadi Churna) was procured and one in house formulation (Sample A) was prepared.Then they had been standardized on the basis of, physical characteristics, physicochemical properties, chemofinger printing and phytochemical screening methods were developed.

Physico-chemical determination

Physico-chemical investigations of formulatio ns were carried out, including the determinati on of extractive values, ash values, moisture content and fineness of particles (Mohapatra et al., 2008).

Determination of micromeritics properties of granules of churna

Physical characteristics like bulk density, tap density, angle of repose, Haussner ratio, and Carr's index were determined for different formulations (The British Pharmacopoeia, 2004).

Preliminary phytochemical screening of churna samples

The ethanolic extracts of samples were subjected to preliminary phytochemical screening for the detection of various plant constituents (Abbas et al., 2011).

Elemental analysis

Elements presentin the formulations were determined by using ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer) (Optical 2100dv, Perkin Elmer, USA).

Identification of pesticide residue in churna samples

Thin layer chromatography (TLC) has been used in pesticide residue analysis for gaining semi-quantitative information on the concentration of pesticide residues in the sample. This study was performed to investigate the possibilities of applying various TLC detection methods in combination with ethyl acetate extraction. Further, the purpose of the study was to establish the within-laboratory reproducibility of the Rf values of selected pesticides with the elution and detection methods, and to determine the limit of quantification of residues in samples. Two pesticides (Dichlorvos and Quinalphos) were selected for determination of Pesticides Residues in Powder Samples. Thin layer chromatographic method was used to detect the pesticide residues in the formulations (Ambrus, 2005).

Determination of anti-oxidant activity of ajmodadi churna

A great number of spices and aromatic herbs contain chemical compounds exhibiting

antioxidant properties. These properties are attributed to a variety of active phytochemical including vitamins, carotenoids, terpenoids, alkaloids, flavonoids, lignans, simple phenols and phenolic acids, etc. One of the main reasons behind the abdominal pain nowadays are the oxidative stress caused due to the generation of reactive oxygen species or the free radical. These free radical damage components of cell which can cause cell death, slow inflammation and destruction leading to pain in the abdomen. Since Ajmodadi churna is quite beneficial in abdominal pain therefore the antioxidant activity of various formulation needs to be compared (Pareessh, 2012).

Simultaneous estimation of piperine, eucalyptol, caryophyllene and eugenol in ajmodadi churna formulations

Piperine, Caryophyllene, Eucalyptol and Eugenol standards were procured from sigma Aldrich, India. Silica gel TLC plate 60F254 (20 cm × 20 cm, layer thickness 0.2 mm from E. Merck, Darmstadt, Germany) supplied by Anchrom Technologies, Mumbai, India were used as a stationary phase. All the chemicals and reagents were of analytical grade and obtained from Rankem, India. Ajmodadi Churna formulations as well as all the ingredients of the formulations were used for analysis.

RESULTS

Table 1: Total Ash values of various Ajmodadi churna Samples

Sl.No.	Samples	Total Ash Value ((% w/w)±SEM
1	A	10.35±0.016
2	B	10.33±0.016

Table 2: Water soluble Ash values of Ajmodadi churna samples

Sl. No.	Samples	Water soluble Ash Value (% w/w) ±SEM
1	A	6±0.02
2	B	7±0.02

Table 3: Acid insoluble ash of Ajmodadi churna samples

Sl. No.	Samples	Acid insoluble Ash Value (% w/w) ±SEM
1	A	3.5±0.04
2	B	3±0.06

Table 4: Methanol soluble extractive values of various Ajmodadi churna samples

Sl. No.	Samples	Extractive Value (Methanol %w/w) ±SEM
1	A	48±0.05
2	B	49±0.06

Table 5: Water soluble extractive values of various samples of Ajmodadi churna

Sl. No.	Samples	Extractive Value (Water % w/w) ±SEM
1	A	7.29±0.02
2	B	7.27±0.02

Table 6: Moisture content of various Ajmodadi churna samples

Sl. No.	Samples	Moisture Content (%w/w) ±SEM
1	A	4.6±0.03
2	B	4.2±0.04

Table 7: Fineness of Particles of Ajmodadi churna samples

Sl. No.	Samples	Description of powder.
1	A	Fine powder
2	B	Fine powder

Table 8: Preliminary Phytochemical Screening of Ajmodadi Churna

Compound	Experiment	Observation	Inference
Carbohydrates	Molish's test	A violet ring was obtained	presence of carbohydrates
	Fehling's test	A red precipitate was obtained	Presence of sugar.
	Barfoed's test	A red precipitate was obtained	Presence of sugar.
	Benedict's test	A characteristic coloured precipitate was obtained	Presence of sugar.
Alkaloids	Mayer's test	A white precipitate was obtained	presence of alkaloids
	Wagner's test	A reddish brown precipitate was obtained	presence of alkaloids
	Hager's Test	A prominent yellow precipitate was obtained	presence of alkaloids
	Dragendorff's Test	A prominent yellow precipitate was obtained	presence of alkaloids
Glycosides	Borntrager's Test	Pink colour was not obtained	absence of anthraquinone glycosides
	Legal Test	Pink colour was not obtained	absence of anthraquinone glycosides
Saponin	50 mg of extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for fifteen min	A stable layer of foam formed	indicates the presence of saponin
Proteins and Amino Acids	Millon's Test	A white precipitate was obtained	indicate the presence of proteins
	Biuret Test	Pink colour in the ethanolic layer was obtained.	indicate the presence of proteins
	Ninhydrin Test	A characteristic purple colour was not appeared	absence of amino acids

Steroids	Liebermann-Burchard Test	brown ring is formed at the layers junction	presence of steroids
	Salkowski Test	Red coloured lower layer; yellow coloured lower layer	presence of steroids ; presence of triterpenoids
Tannins	Ferric chloride Test	blue colour	presence of hydrolysable tannin
	Gelatin Test	White precipitate	presence of tannin
Flavonoids	Alkaline Reagent Test	Yellow fluorescence was obtained	presence of flavonoids
	Shinoda Test	pink scarlet, crimson red or occaissonally green to blue colour appear after few minutes	presence of flavonoids
	Zinc Hydrochloric Test	Appearance of red colour after few minutes.	presence of flavonoids

Table 9: Micromeritic properties of Ajmodadi Churna samples

S.No.	Parameters	Sample A Mean ± SEM	Sample B Mean ± SEM
1	Bulk density (g/ml)	0.42±0.06	0.34±0.04
2	Tapped density (g/ml)	0.63±0.16	0.47±0.05
3	Angle of repose	40.4°±1.17	37.6°±1.70
4	Hausner's ratio	1.5±0.06	1.38±0.06
5	Carr's index	33.3±0.71	27.6±0.7

Table 10: Micromeritic properties of Ajmodadi Churna granules

S.No.	Parameters	Sample A Mean±SEM	Sample B Mean±SEM
1.	Bulk density (g/ml)	0.45±0.02	0.57±0.02
2.	Tapped density (g/ml)	0.74±0.04	0.72±0.03
3.	Angle of repose°	39°±1.2	34.3°±1.6
4.	Hausner's Ratio	1.36±0.05	1.26±0.06
5.	Carr's index	26.7±0.06	20.83±0.07

Table 11: Elemental Analysis of Ajmodadi churna samples

S.No.	Name of Elements	Concentration of elements (mg/l) Sample A	Sample B
1	Al	1.462	2.622
2	Bi	0.011	0.012
3	Ca	15.9	32.32
4	Cd	0.001	0.001
5	Co	0.002	0.001
6	Cr	0.056	0.059
7	Cu	0.130	0.134
8	Fe	1.008	2.759
9	K	29.94	32.41
10	Mg	5.765	5.882
11	Mn	1.628	0.404
12	Na	35.99	33.38
13	Pb	0.035	0.037
14	Zn	0.373	0.324

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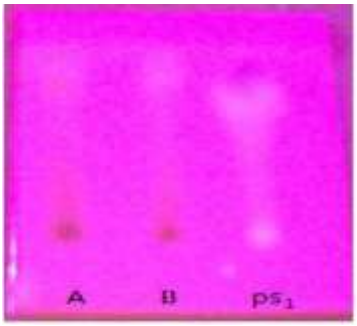
Identification of Pesticide residue in Ajmodadi churna samples

Two pesticides were selected; Dichlorvos (ps1) and Quinalphos (ps2) for TLC analysis. The plates were eluted in, ethyl acetate.The spots of the fluorescence obtained with the pesticides in the solvent systems, did not match with the spots of the Churna samples (Sample A, Sample B). The Rf value of Dichlorvos- 0.458, Quinalphos- 0.631 were found in the ethyl acetate solvent system.



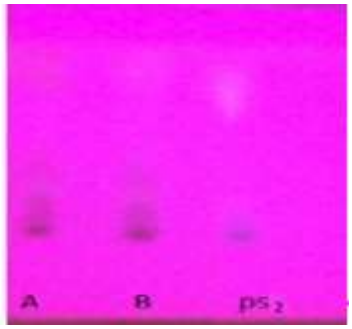


Table 12: DPPH free radical scavenging activity of samples of Ajmodadi churna

		% Inhibition		
S.No.	**Conc.(µg/ml)**	**ascorbic acid**	**Sample A**	**Sample B**
1	50	96.95	85.11	79.93
2	100	97.25	88.48	82.61
3	150	97.45	94.31	93.15
4	200	97.66	94.36	94.41

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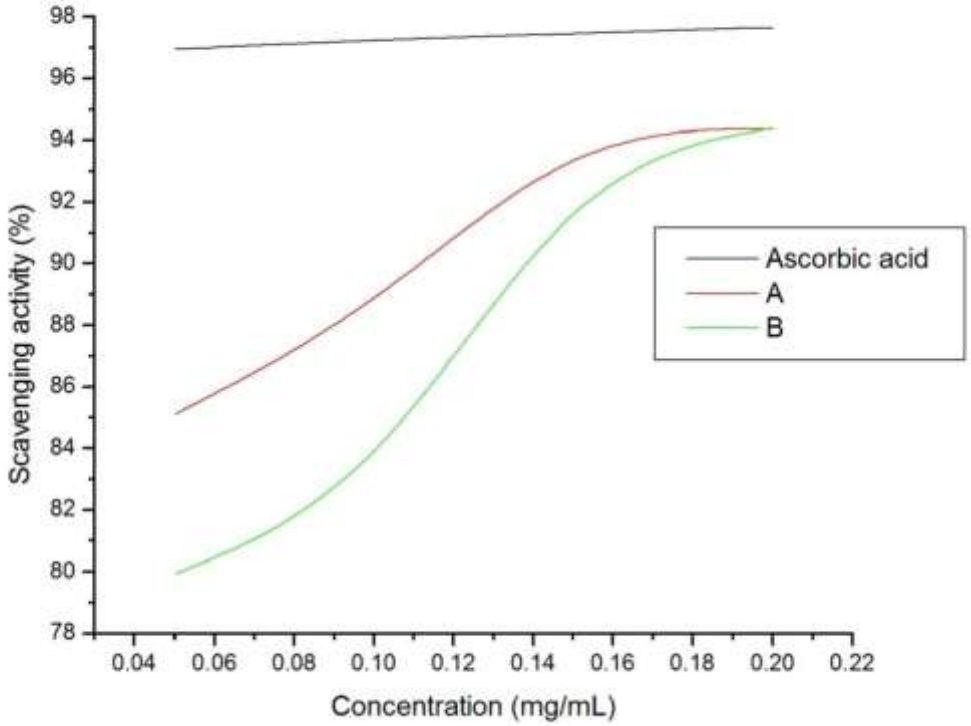

Fig. 1: Antioxidant activity of Ajmodadi churna formulation, Sample A, Sample B and Standard Ascorbic acid using DPPH method.

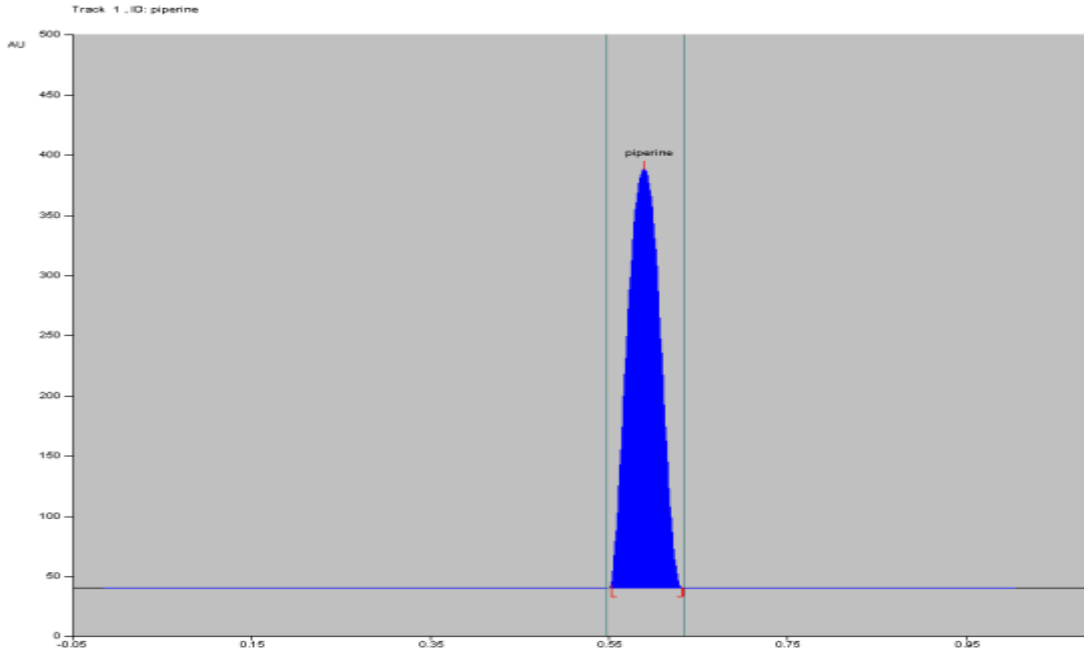

Fig. 2 (a) : HPTLC chromatogram of standard Piperine.

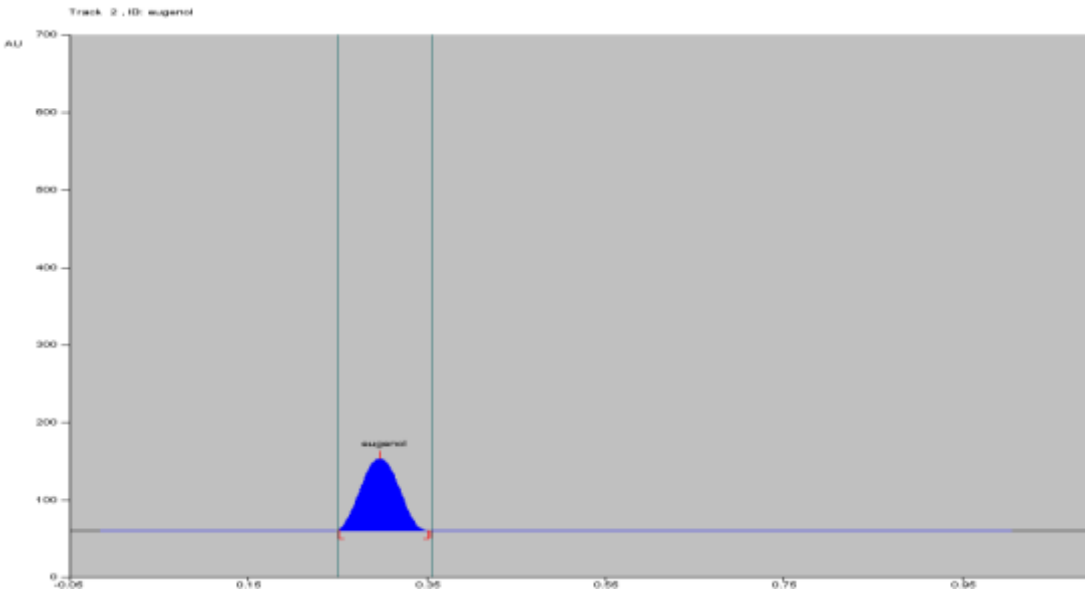

Fig. 2 (b): HPTLC chromatogram of standard Eugenol

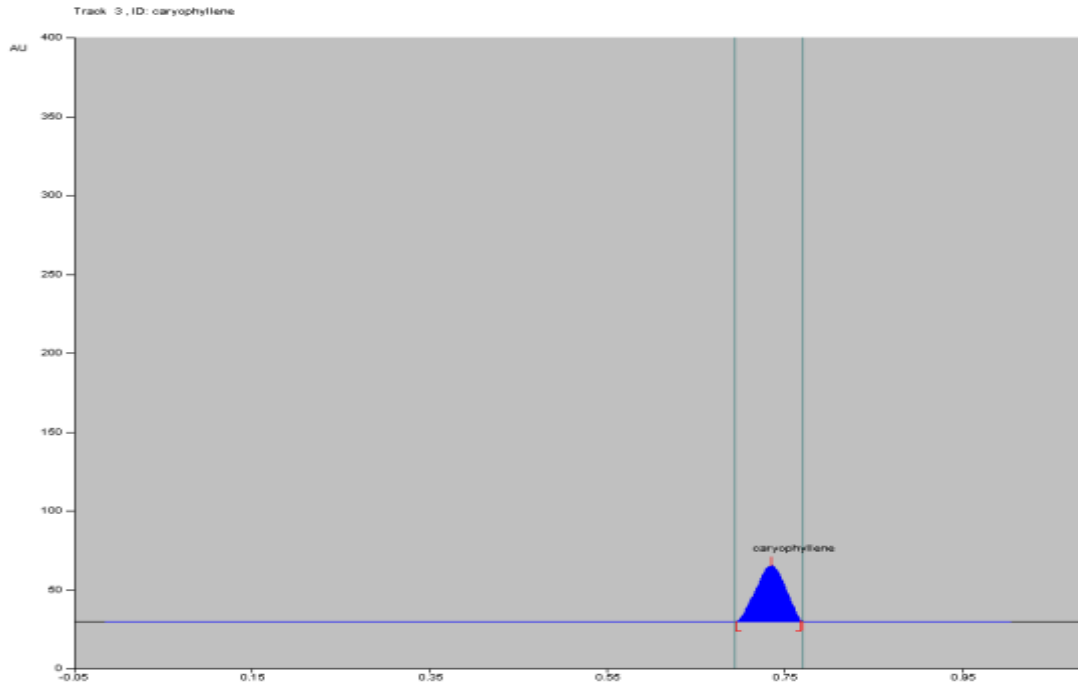

Fig. 3: HPTLC chromatogram of standard Caryophyllene.

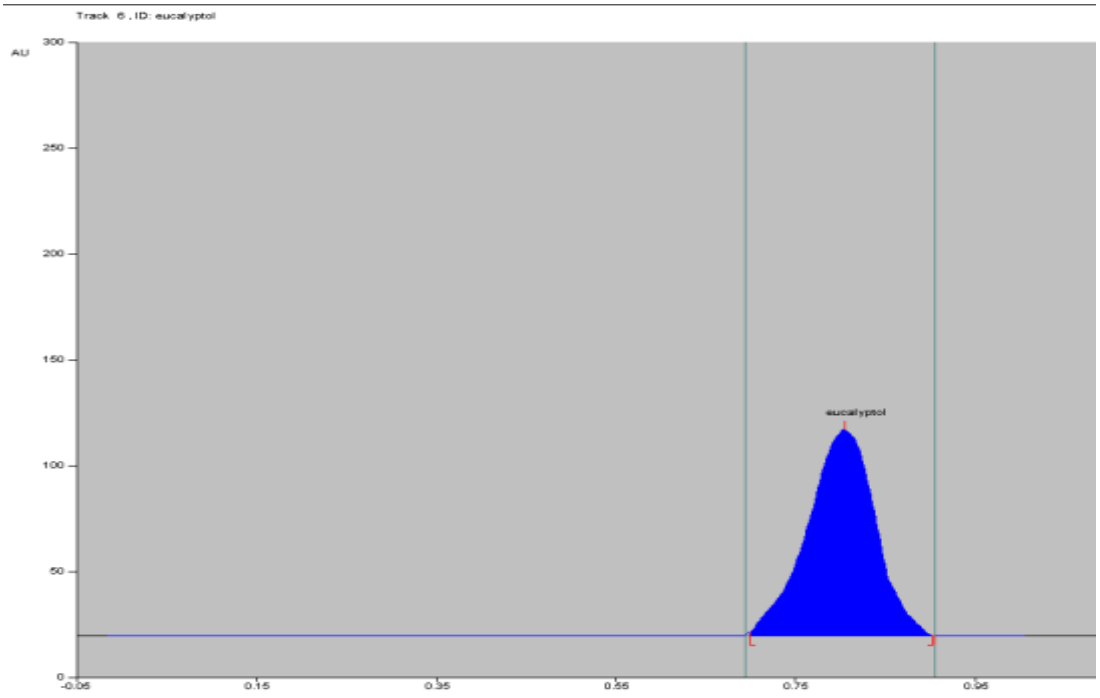

Fig. 4: HPTLC chromatogram of standard Eucalyptol.

Analysis of standard in formulation

Table 13: Estimation of piperine, eugenol, caryophyllene, eucalyptol in formulations of Ajmodadi churna.

Drug	R_f	Amount found in mg	Present in 100 mg
A	0.60	0.007062	0.7062
B	0.60	0.008679	0.8671
Piperine	0.59	0.00100	0.1
Drug	**R_f**	**Amount found in mg**	**Present in 100 mg**
A	0.30	0.0055375	0.55375
B	0.31	0.004000	0.4
Eugenol	0.30	0.002	0.2

Drug	R_f	Amount found in mg	Present in 100 mg
A	-	-	-
B	0.75	0.0345	3.45
Caryophyllene	0.74	0.002	0.2

Drug	R_f	Amount found in mg	Present in 100 mg
A	0.83	0.011795	1.1795
B	0.83	0.0065	0.65
Eucalyptol	0.81	0.002	0.2

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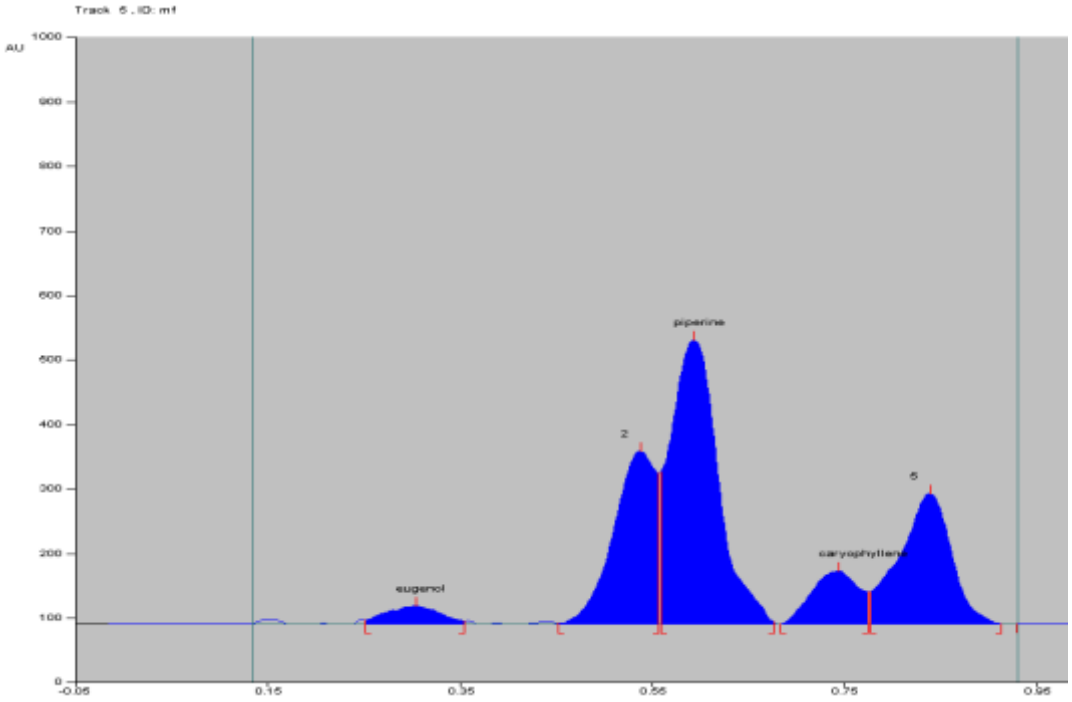

Fig. 5: HPTLC fingerprint of marketed formulation (scanned at 300nm).

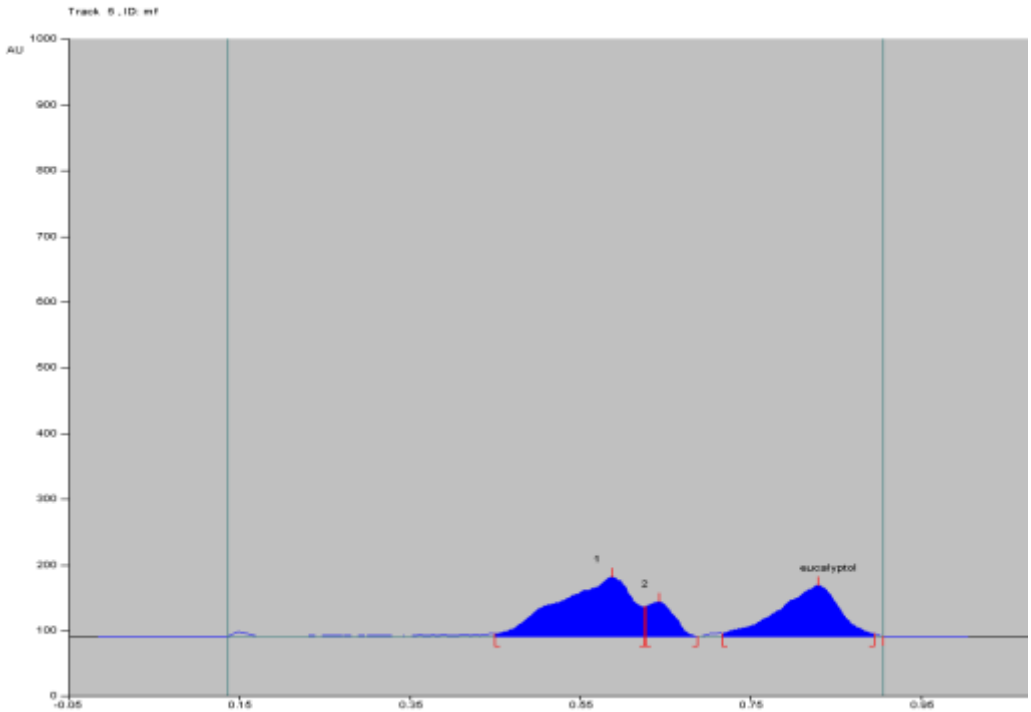

Fig. 6: HPTLC fingerprint of marketed formulation (scanned at 400nm).

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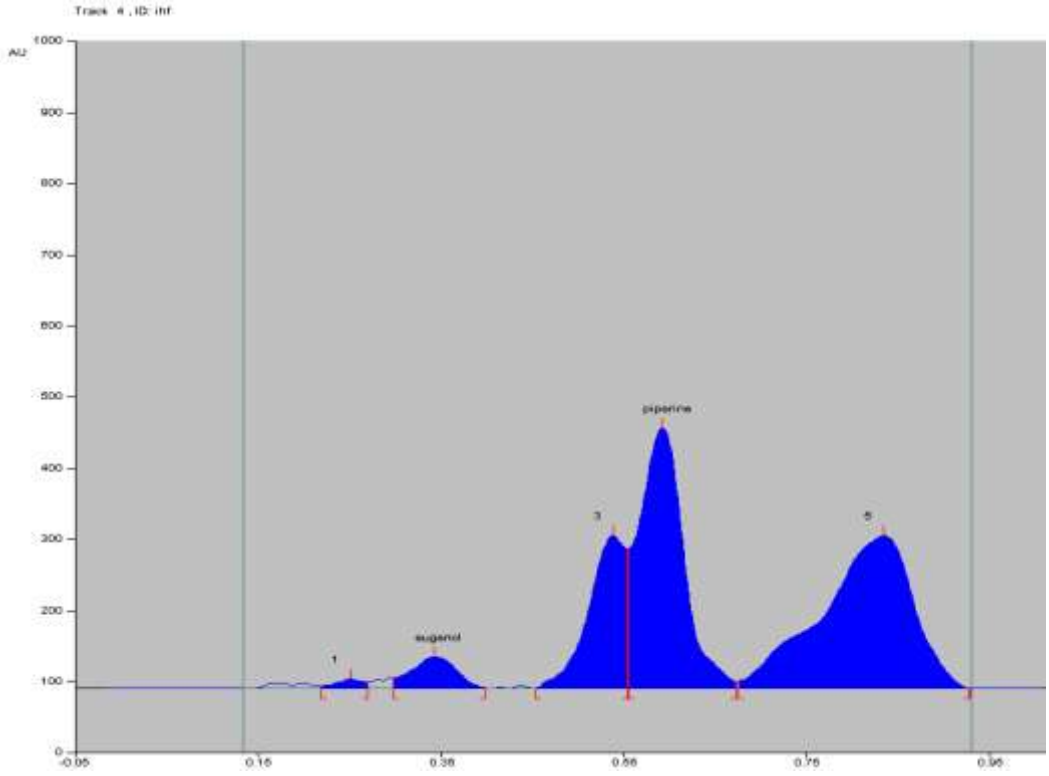

Fig. 7: HPTLC fingerprint of inhouse formulation (scanned at 300 nm).

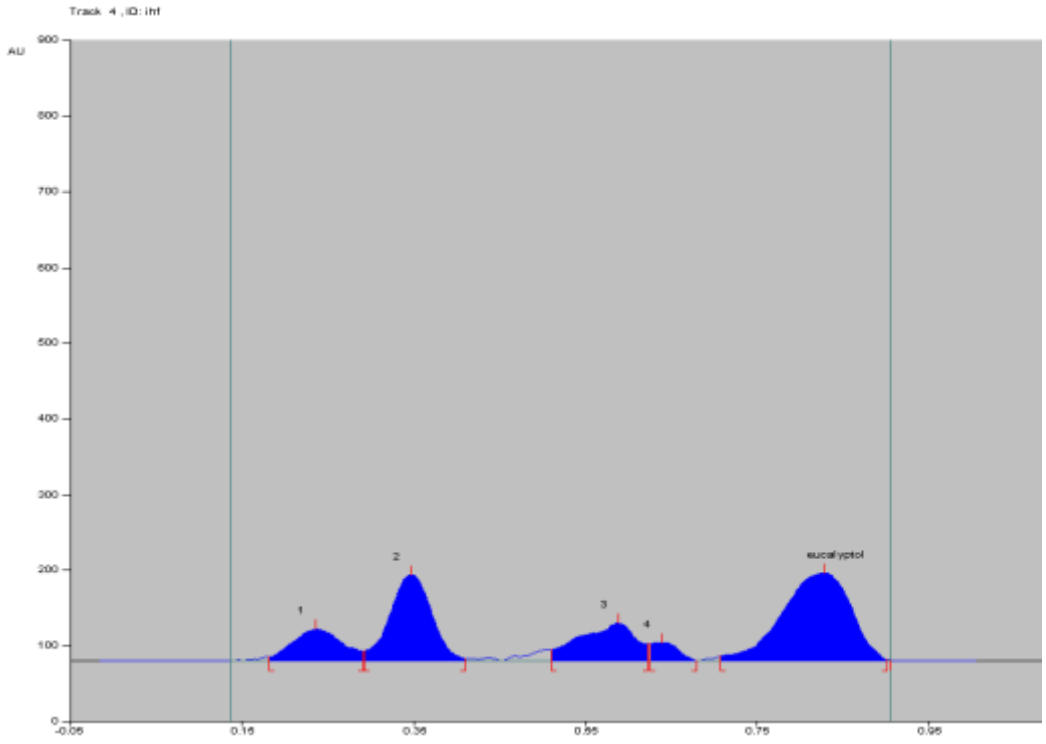

Fig. 8: HPTLC fingerprint of inhouse formulation (scanned at 400 nm).

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