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FINGERPRINTING PROFILING OF AYURVEDIC PREPARATION: AN OVERVIEW

B. S. Khurana*, S. Jain and K.Shukla
College of Pharmacy, Dr. A. P. J. Abdul Kalam University
Indore-Dewas Bypass Rd, Arandia, Jhalariya, Indore, Madhya Pradesh 452016. India
Corresponding Author : Baljeet Singh Khurana
College of Pharmacy, Dr. A. P. J. Abdul Kalam University, Indore-Dewas Bypass Rd, Arandia, Jhalariya, Indore, Madhya Pradesh 452016.
Email: jeet.khurana@gmail.com

ABSTRACT

Ayurvedic formulations such as solid dosage (vati, churna), semisolid (avaleha, ghritas), liquid (asava, arishta) have numerous uses in Ayurveda. They effect or help to rectify the three doshas in the body, and restore homeostatic balance that builds up in the body's digestive system and spreads to the tissues. Standardization and analysis of the chemical marker of the Ayurvedic and other poly herbal formulation has always been a concern. For Researchers, Standardization is the need of hour for the present era to set standards for maintaining the quality and efficacy ofthe herbal products. HPTLC offers major advantages over other conventional chromatographic techniques such as unsurpassed flexibility (stationary and mobile phase), choice of detection wavelength, user friendly, rapid and cost effective/economical. The present study compiles the progress made on the development of optimized and validated HPTLC/HPLC method for the simultaneous estimation of markers in different Ayurvedic Churnas/preparations. HPTLC/HPLC profile is quite helpful in setting up of standards. The present study is an attempt tocompile the major studies carried out on Ayurvedic preparations likeChurnas, avaleha, asava, arishta, vati, rasa, taila, ghritas and herbal capsules etc. which may be of use to develop/compile the fingerprint profile for evaluating the purity & quality of Ayurvedic formulations, thus helpful as a reference in developing pharmacopoeial standards.

Keywords: Ayurvedic formulations, Churnas, Standardization, Markers, Fingerprint, HPTLC.

Introduction

Ayurvedic formulations have numerous uses in Ayurveda. They effect or help to rectify the three dos has or humors in the body (Rathi *et al.,* 2010).Churna is a fine powder of well dried drug or drugs described in ancient literature (Waghmare & Kochar, 2011). Quantitative estimation of chemical markers of each ingredient in the poly herbal preparation required ideal separation technique (Gupta & Jain 2011; Mukherjee, 2005). For herbal preparations (including polyherbal), there is an urgent need for scientific proof/validation with chemical standardization protocols/procedures, biological assays, animal models and clinical trials (Ong, 2004). HPTLC thus offers major advantages over other commonly available conventional chromatographic techniques. The proposed method was validated on the basis of its selectivity, linearity, limit of detection (LOD) and limit of quantification (LOQ) according to ICH requirements (Gupta & Jain 2011). HPTLC profile is quite helpful in setting up of standards for evaluating the purity and quality of Ayurvedic preparations. This will be helpful to overcome batch to batch variations in different Ayurvedic churna/preparations (Meena *et al.,* 2010).

Churna

Churna is a fine powder of a drug or drugs which is prepared by mixing clean, finely powdered and sieved drugs. The term churna maybe applied to the powder prepared by a single drug or a combination of more drugs (Ayurvedic Pharmacopoeia of India 2007). Ayurvedic formulary of India has given the specification for the composition of churnas (Ayurvedic Pharmacopoeia of India Part-I 2007; Ayurvedic Pharmacopoeia of India 2001).

Chaturjatchurna: A polyherbal formulation consisting of 4ingredients with specific morphological parts. The ingredients are *Cinnamomum zeylanicum*, *Elettaria cardamomum*, *Cinnamomum tamala* and *Tribulus terrestris* (Gautam *et al.,* 2011).

HPTLC profile: The crude drug sample extracted in Methanol (150ml×5) through Soxhlet apparatus was filtered and concentrated to 5-10ml. High Performance Thin Layer Chromatography was carried out by applying 6 µl of the sample on TLC Silica gel plate 60 F 254 (from Merck India Ltd, Germany) and developed the plate to a distance of 10cm using Toluene: Ethyl acetate (9:1) as mobile phase, examined underUltra Violet Light at 254 nm; and under 366 nm; after derivatization with 5% methanolic sulphuric acid solution different R_f value in TLC finger print was found to be 0.24, 0.47, 0.54, 0.76, 0.80, 0.84, and 0.92.

HPTLC finger printing profile of Caturjata churna was also developed in Toluene: Ethyl acetate (93:7) solvent system (Table 1) (Sitapara *et al.,* 2011).

Pancasamachurna: Pancasamachurna, a polyherbal formulation consists of rhizomes of Cyprus rotandus (Mustha), whole plant *Termenalia chebula* (haritaki), fruit of *Piper longum* (pippali), root of *Operculina turpethum* (Trivrat) and sandhalavana.

TLC/HPTLC Analysis: TLC and HPTLC finger printing profile of Pancasama Churna (ethanol extract) were developed in Toluene: Ethylacetate: Formic acid (5.0:3.5:1.0 v/v) solvent system.

Triphala churna: It is an age old commonly used Ayurvedic powdered preparation in Indian systems of medicine. Ayurvedic formulary of India has given the specification for the composition of Triphlachurna (Ayurvedic Pharmacopoeia of India Part-I 2007; Ayurvedic Pharmacopoeia of India 2001).This well-known formulation is made by combining *Terminalia chebula*, *Terminalia belerica* and *Embellica officinalis*, in equal proportions (Mali *et al.,* 2011).

HPTLC: A HPTLC-densitometric method of analysis for markers *i.e.* Gallic acid (Jain *et al*., 2011) and ascorbic acid in Triphlachurna (methanol extract) was developed. Water was selected as a solvent for preparing standard solutions.

Quantitative estimation of Gallic acid and ascorbic acid was performed separately on aluminum backed silica gel 60 F254 TLC plates(10 cm×10 cm plate size, layer thickness 0.2 mm, E-Merck, Darmstadt, Germany). Ascorbic acid shows R_f value of 0.74 ± 0.1 using ethanol: glacialacetic acid: toluene (5.5:1:1.5) and Gallic acid showed R_f valueof 0.54 ± 0.1, using ethyl acetate: toluene: acetone (4.5:4:1) as mobilephase, scanned at 254 nm. Thus a simple, precise and accurate method for quantitative estimation of ascorbic acid and Gallic acid in herbalmedicine (triphala churna) by HPTLC was developed. The Gallic acid and ascorbic acid content in triphalachurna was quantified.

Trikatuchurna: Trikatu Churna is well known Ayurveda Formulation, comprised of the fruits of two medicinal important plants of *Piper longum* (Pipali) along with *Piper nigrum* (Marica) and rhizomes of *Zingiber officinalis* (Saunth) (Shailajan *et al.,* 2011).

HPTLC: The fingerprint method for Trikatuchurna by simple high performance thin layer chromatography (HPTLC) determination using piperine as a standard, which is as an important and major content informulation. The concentration of piperine present in raw materials was found to be 4.2% ± 0.43 w/w in *Piper nigrum* (Maricha), and 2.15%± 0.68 w/w in *Piper longum* (Pipali) respectively and in three identical laboratory batch of Trikatuchurna name TK-I, TK-II, TK-III, was2.13% ± 0.62, 2.42% ± 0.67, 2.18% ± 0.41 w/w respectively with mean value 2.24% ± 0.48 w/w. The piperine content of all the three batches is found to be in close proximities with each other. Obtained results were compared with marketed formulations. Better results were obtained with mobile phase consisting of Toulene: ethylacetate: glacial acetic acid(8:2:0.1 v/v/v) at 550 nm gave R_f values of 0.42 ± 0.03 for piperine at550 nm.

Better results were also obtained with mobile phase consisting of toluene: ethyl acetate (70:30 v/v), gave R_f values of 0.42 ± 0.03 for piperine (Vyas *et al.,* 2011).

Haritakichurna: Haritakichurna mainly constitutes of dried fruit of *Terminali achebula* (Mahajan & Pai 2011).

HPLC: A high performance liquid chromatography method coupled with diode array detection was developed to simultaneously determine seven different marker compounds in Haritakichurna, anayurvedic formulation. These markers are gallic acid, methyl gallate, ethyl gallate, ellagic acid, chebulagic acid, chebulinic acid and penta-O-galloyl-β-D-glucose. HPLC analysis was carried out at wavelength 272 nm. The chromatographic separation was performed on Thermo Scientific BDS HYPERSIL Phenyl reversed-phase column (100 mm×4.6 mm, 3 μm). The mobile phase was consisted of 0.02% triethyl amine aqueous pH 3.0 with ortho-phosphoric acid (A) and acetonitrile (B) at a flow rate of 1.0 ml/min gradient mode. The flow rate was 1.0 ml/ min and aliquots of 10 μl were injected. Regression equations showed good linear relationships (R_2>0.998) between the peak area of each marker and concentration. In this study, an HPLC–DAD method for the qualification and quantification of phtyoconstituents in Haritakichurna has been developed and successfully applied for comparison of three marketed samples (HC_1, HC_2, and HC_3) (Table 2).

This method is validated for good accuracy, repeatability and precision, and can be used to evaluate the quality of the drug. This multi-phytoconstituents assay method will be helpful to quality control and stability studies of Haritakichurna.

Avaleha

It is a semi-solid preparation of the drugs, prepared with addition of jaggery, sugar or sugar-candy and boiled with prescribed juices or decoctions. It acquires the consistency of a thick paste. The other similar forms are known as Modaka, Guda, Khanda, Lehya, Praasa etc. e.g: Vasa Avaleha, Chyavanprasha Avaleha, Kushmanda Avaleha etc.

Vyaghrihareetaki Avaleha: An Ayurvedic formulation, Vyaghrihareetakiavaleha (VHA) is a potent drug indicated for shwasa (Asthma), kasa (cough) etc. and used in the management of Tamakashwasa (Bronchial Asthma). It consists of *Solanum xanthocarpum*, *Terminalia chebula*, *Piper nigrum*, *Piper longum*, *Zingiber officinale*, *Cinnamomum zeylanicum*, *Cinnamomum tamala, Elettaria cardamomum*, *Mesuaferrea*, honey, jaggery (Roshy *et al.,* 2011).

Ikshvadi Avaleha: Ikshvadi Avaleha is very safe to be used for tuberculosis in children. Its consists of *Phyllanthusurinaria*, *Saccharum officinarum*, *Bambusa arundinacea*, *Mucunaprurit*a, *Piper nigrum*, *Cinnamomum zeylanicum*, *Elettaria cardamomum*, and honey (Gohel *et al.,* 2011).

HPTLC: TLC and HPTLC were carried out after organizing appropriate solvent system in which maximum 4 spots were distinguished in TLC and 3 spots in HPTLC and most of the R_f values were identical when done with different sample extractive methods. It is inferred that the formulation meets the minimum qualitative standards as reported in the API at a preliminary level.

HPTLC study of the Unsaponifiable fraction of the Ikshvadi Avaleha (methanol extract) was also carried out by using the same solvent system of Toluene: Ethyl acetate (7:3 v/v). After completion of HPTLC post chromatographic deprivation was done with Methanol extract.

Densitometry scanning of the HPTLC pattern showed 4 spots corresponding to hR_f values 43.90, 3.35, 32.69, 20.06. In short wave UV 254 nm and 3 spots corresponding to hR_f values 36.01, 5.34, 58.66, obtained in long wave UV 366 nm. Though it may not be able to identify particular chemical constituent from the spots obtained, the pattern may be used as a reference standard for further quality control researches.

Vasavaleha: Vasavaleha is a traditional Ayurvedic oral Herbal formulation consisting of five herbs, Vasaka (*Adhatodavasica* Nees.), Pippali (*Piper longum* Linn.), Sugar, Ghee and Honey. It is available as a popular proprietary, from most manufacturers of ayurvedic drugs (Patel *et al.,* 2010).

HPTLC: A selective, precise and accurate High Performance Thin Layer Chromatography (HPTLC) method has been developed for the simultaneous quantification of Vasicine and Piperine in Vasavaleha (chloroform and methanol extract) as well as its bulk drug.

The method employed TLC aluminum plate precoated with silica gel 60 F254 as a stationary phase. The solvent system consists of Dioxane: Toluene: Ethyl acetate: Methanol: Ammonia (1.5:2:1:1:0.3 % v/v). This system was found to give compact spot for Vasicine and Piperine. Densiometric analysis was carried out in the absorbance mode at 285 nm. The linear regression analysis data for the calibration plot showed good linear relation with r^2=0.992 and 0.993 with respect to peak area for Vasicine and Piperine respectively, in concentration range 2-10 µg/spot.

The data generated indicate that Vasavaleha contains a number of markers that may have a prominent role to play, for the therapeutic activity. The proposed HPTLC methods for simultaneous estimation of Vasicine and Piperine from Vasavaleha, seems to be accurate, precise, reproducible and repeatable. It is the first attempts, when both the markers in Vasavaleha were simultaneously estimated and compared for the respective raw materials.

AshtaangaAvaleha: Ashtaangaavaleha is indicated for the management of Jwara (Fever), Kaasa (Cough), Swaasa (Dyspnoea/ Asthma), Aruci (Tastelessness) and Chardi (Emesis). There has been an increase in demand for the Phyto-pharmaceutical products of Ayurveda so a new pharmaceutical preparation in the form of Ashtaangaavaleha was tried to standardize which is economical in terms of time and machinery usage. The phytochemical analysis and High Performance Thin Layer Chromatography has been performed to confirm its identity, quality and purity (Dubey *et al.,* 2011).

High performance thin layer chromatography (HPTLC) study: In High performance thin layer chromatography (HPTLC) study of ashta angavaleha (methanol extract) using Toluene: Ethyl acetate (9:1v/v), visual observation under UV light showed few spots, but on analyzing under densitometer much more was observed. Chromatogram shows 8 prominent spots at R_f 0.02, 0.13, 0.22, 0.32, 0.49, 0.56, 0.77, 0.94 in short wave UV254 nm and 5 prominent spots at R_f0.02, 0.20, 0.49, 0.56, 0.65 in long wave UV 366 nm. Details are noted in the Table 1. Then the plate was sprayed with Anisaldehyde sulphuric acid

Chyawanprash: Chyawanprash is a traditional polyherbal formulation, which is widely used as rejuvenator, anabolic, immunomodulator and memory enhancer. Chyawanprash contains the pulp of *Embelica officinalis* as the prime ingredient, along with powder and extract of several other herbs (Kasar *et al.,* 2006).

HPTLC: HPTLC analysis of aqueous and methanol extract was performed using toluene: ethyl acetate: formic acid: ethanol (6:4:0.3:0.4) for developing finger print profile of piperine, catechin, epicatechin and gallic acid at 254 and 366 nm.

Asava and arishta

Asavas and arishtas are very popular in India, probably due to their taste and alcoholic content in addition to their medicinal uses and physiological importance (Lal *et al.,* 2009).

These are fermented preparations of medicinal plants. The fermentation procedure adopted to prepare these preparations is termed as 'Sandhaanakalpanaa' and the ferment used to stimulate fermentation is termed as 'Sandhaanadravya'. Arishtas are usually prepared by fermenting expressed juice ('swarasa'), whereas 'Asavas' are prepared from fermentation of decoction (Kwaatha). Sugar or Jaggery and powders (Choorna) of other medicinal plants as required along with a natural ferment are added to these two liquids and they are left in a closed container till the fermentation is completed. Aasava and Aristaas can be prepared from 'swarasa' or 'kwaatha' (as the case may be) of single plant or from a mixture of 'swarasa' or 'kwaatha' from multiple plants. This facilitates the extraction of the active principles contained in the drugs.

Arjunarishta: Arjunarishta (Parthadyarishta) is an important Ayurvedic formulation used for cardiovascular disorders and is prepared by fermenting the decoction of specified plant materials using flowers of Wood for diafruticosa (Lal*et al.,* 2009).

An HPLC-PDA method was developed for the standardization of Arjunarishta by quantitative estimation of major antioxidant compounds, ellagic acid, gallic acid, ethyl gallate, quercetin and kaempferol as markers.

HPLC: HPLC method was developed for the formulation after several trials for separation of phenolic acids and flavonoids. The flavonoids showed very high retention time (>75 minutes) with the reported method. In the present study, a shorter run time (45 min) was achieved with gradual increase of organic phase (acetonitrile). Five phenolic compounds were identified in Arjunarishta; these were gallic acid, ethyl gallate, ellagic acid, quercetin and kaempferol. The chromatogram also showed several other unidentified peaks. A binary gradient system consisting of water–acetonitrile–acetic acid as mobile phase was able to separate these compounds.

Chandanasava: Chandanasava is one of ancient, commonly used Ayurvedic formulations. The herbal formulation is made up of Santalum album and other 24 plant ingredients. Chandanasava is prescribed for treatment of karsya (malnutrition), sukramehe (presence of semen in urine), mutrakrcchra (painful micturation), hrdroga (heart diseases), agnimandya (loss of appetite) (Katekhaye& Singh 2012).

HPTLC: Fingerprinting of different extracts (petroleum ether, dichloromethane, ethyl acetate) was done by using selected solvent system pet. Ether: ethyl acetate (9.5:0.5), pet. Ether: ethyl acetate (9:1), toluene: ethyl acetate: acetic

acid: water (3:3:0.8:0.2 v/v) respectively for extracts, visualised at 366 nm and chromatogram was scanned with spectrodensitometer.

Tablet/Vati

Vati and Gutika–These are in the herbal preparation in the form of tablets or pills made of one or more drugs of plant or mineral origin and these too comprise other several items (Lather *et al.,*2010). In Ayurveda there is several other different type of formulation like Vatis-Gutika (Pills), Rasa yoga (mineral based herbal formulation), Tailas (oil based herbal formulation), Guggulu etc.

Amalant tablet: Each tablet contains *Embelica officinalis*201 mg and 15 other ingredients. Amalant offers a multi-pronged approach in the treatment of hyperacidity and acid peptic disorders (Patel & Telange 2011).

HPTLC of gallic acid

Mobile phase for Gallic Acid is Toluene: Ethyl Acetate: Formic Acid (6:3:1 v/v/v), and Scanning wavelength: 254 nm, Mode of scanning: Absorption [Deuterium], Standard: Gallic acid 0.1 mg/ml [10 µl].

The R_f value of Standard Gallic Acid was found to be 0.34 and peak area 5097.0. Amalant Tablet extract showed nine peaks, the fourth peak R_f value (0.34) was coinciding with standard R_f value and its area calculated was 249.71 at 100 µg/ml of standard and sample concentration, The amount of gallic acid was found to be 4.89%.

Sulaharan yoga: Sulaharan Yoga (SY), an Ayurvedicpolyherbal formulation, consists of *Strychnosnux vomica* and other seven ingredients in Vati (Tablet) form, 1. *Terminalia chebula* (Family: Combretaceae, part: dried fruit), 2. *Zingiber officinale* (Family: Zinglberaceae, part: dried rhizome), 3. *Piper nigrum* (Family: Piperaceae, part: dried fruit), 4. *Piper longum* (Family: Piperaceae, part: dried fruit), 5. *Strychnosnux vomica* (Family: Fabaceae, part: dried seed), 6. *Ferula foetida* (Family: Umbelliferae, part: oleo-gum-resin), 7.Sulphur and 8.Rock salt (Saindhavalavana) (Pattanaya *et al.,* 2010).

HPTLC: HPTLC study of extracts (methanolic) of the separate ingredients, formulation (laboratory scale) and formulation (commercial scale) were carried out using the different biomarker compounds corresponding to the therapeutically active ingredients to ensure the presence of active ingredients in all the formulations. HPTLC fingerprint profile of an ayurvedic Sulaharan Yoga formulations are depicted in figure represents the presence of all major ingredients in proportional quantity in the formulations, in absence of any impurities. This confirms the consistency in the batches of the laboratory scale preparation and commercial scale.

From superimposition study a band (R_f 0.44) corresponding to Gallic acid is visible in both *Terminalia chebula* and Sulaharana yoga formulations, indicate the presence of *Terminalia chebula* in the formulations.

It is generally believed that for monitoring quality control parameters, HPTLC fingerprinting is an ideal option which involves comparative parameter between a standard and a test sample. The use of biomarkers ensures that the concentration and ratio of components in the herbal mixture are present as per claims and also in in reproducible levels in raw materials batches and in the final dosage form batches. In this way use of markers and chromatographic fingerprinting technique can give useful information assisting manufacturing control, minimising variations in production batches and assuring batch to batch consistency, with reproducible results (Pattanayak *et al.,* 2011).

Nishaamalakivati: Nisha Amalaki Vatti is a polyherbal formulation containing *Curcuma longa* and *Phyllanthus emblica* used as anti-diabetic agents marketed by Ayush, India (Rubesh *et al.,* 2010). Standardisation by UV, HPLC and HPTLC method has been studied for the simultaneous analysis of curcuminoids and gallic acid in combined polyherbal formulation. The proposed method was found to be simple, sensitive, accurate, precise, economical and rapid for the routine simultaneous estimation of these two phytoconstituents in a combined dosage form. The value of the standard deviation and coefficient of variation were satisfactory. In the simultaneous equation method wavelength of respective absorbance maxima i.e. 227 nm for gallic acid and 427 nm for curcuminoids were used for the analysis of the phytoconstitution in the standard and tablet.

Conclusion

Fingerprint profile is quite helpful in setting up of standards and thus to keep a check on intentional/ unintentional adulteration. The present review is an attempt to compile the major studies carried out on Ayurvedic Churnas/preparations, which may be of use to develop/ compile the fingerprint profile for evaluating the purity and quality of churnas/preparations, thus helpful as a reference in developing pharmacopoeial standards, present compilation would also be helpful to overcome batch to batch variations in traditional preparation of different AyurvedicChurna/ preparations.

Table 1 : HPTLC finger print of different churnas.

S.No.	Churna/extract	Constituents	Solvent system and Scanning wavelength	Standard
1.	AjmodadiChurna (Methanol)	Piper species (*Piper longum* in both form root and fruit and *Piper nigrum*)	Toluene: Ethyl acetate (07:03) at 336 nm.	Piperine
2.	Amukkarachoornam (toluene) (Patra & Suresh 2009)	*Piper nigrum, Piper longum, Zingiber officinale, Amukkara (Withania somnifera), Elletariacardamomum, Cinnamomum wightii, Syzygiuma romaticum*	Toluene: Ethyl acetate (9:3 v/v) at 260 nm.	Not mentioned
3.	ChaturjatChurna (Methanol)	*Cinnamomum zeylanicum, Elettariacar damomum, Cinnamomum tamala and Tribulus terrestris*	Toluene: Ethyl acetate (9:1) at 254 nm.	Not mentioned

4.	HingashtakChurna (methanol) (Verma& Joshi 2006)	*Piper longum, Piper nigrum, Curcuma longa, Thymus vulgaris*	Toluene-ethyl acetate-methanol, 9:1:0.5 at 420, 333, and 277 nm	Curcumin, piperine, and thymol
5.	Kuberaksha/Kantakikaranjapatra Churna (methanol) (Prasad *et al.,* 2010)	*Caesalpinia bonduc*	Ethyl acetate: methanol: water (100:13.5:10) at 254 nm.	Not mentioned
6.	LaghugangadharChurna (methanol)	*Cyperus rotundus, Symplocos racemosa, Woodfordia fruticosa, Aegle marmelos,*	Toluene: Ethyl acetate (90:10) at 254 and 366 nm.	Not mentioned
7.	NisamalakiChurna (Methanol and Aqueous) (Kumar & Kumar 2011)	*Curcuma longa; Emblica officinalis*	Chloroform-methanol (9.5:0.5 v/v), Ethanol-glacial acetic acid (9:1 v/v) at 500 nm	Curcumin; Ascorbic acid
8.	PancasmaChurna (Ethanol)	*Operculina turpethum; Terminalia chebula, Cyprus rotundus; Piper longum*	Toluene: ethyl acetate: Formic acid (5.0:3.5:1.0 v/v) at 366 nm.	Piperine and gallic acid
9.	PanchaskarChurna (methanol) (Priyanka *et al.,* 2010)	*Cassia angustifolia, Foeniculum vulgare, Terminalia chebula, Zingiber officinale, Anethum sowa , Rock salt* (Saindhavalavana).	Toluene: ethyl acetate at 260 nm	Not mentioned
10.	PalasabijadiChurna (methanol) (Rastogi *et al.,* 2008)	*Buteamonos perma; Holarrhena antidysentrica, embeliaribes, Azadirachta indica, Swertia chirata*	Toluene: Ethyl acetate (90:10 v/v) at 260 nm.	Not mentioned
11.	PanchkolChurna (Mistry *et al.,* 2010)	*Piper longum, Piper nigrum, Cuminum cyminum, Plumbago zeylanica, Embelia ribes, Zingiber officinale*	Toluene: ethyl acetate (7:3) at 340, 420 nm.	Piperine, plumbagine, zingiberine
12.	TriphlaChurna (aqueous)	*Terminalia chebula, Terminalia belerica and Embellica officinalis*	Ethanol: glacial acetic acid: toluene (5.5:1:1.5) for ascorbic acid and Ethyl acetate: toluene: acetone (4.5:4:1) for gallic acid at 254 nm.	Ascorbic acid and gallic acid [13]
13.	TrikatuChurna (methanol)	*Piper longum, Piper nigrum, Zingiber officinale*	Toulene: ethylacetate: glacial acetic acid (8:2:0.1 v/v/v) at 550 nm.	Piperine
14.	TalishadiChurna (methanol) (Patra *et al.,* 2011)	*Piper longum, Piper nigrum, Zingiber officinale, Elletaria cardamomum, Cinnamomum zeylanicum, Bambusa arundinacea,*	Toluene: ethyl acetate (9:3 v/v) at 260 nm.	Not mentioned
15.	Vidangachurna (methanol) (Sudani *et al.,* 2011)	*Embelia ribes*	Chloroform: ethyl acetate: formic acid (5:4:1 v/v/v) at 291 nm.	Embelin
16.	AshwagandhaChurna (methanol) (Kaur *et al.,* 2010; Tatke *et al*., 2010)	*Withania somnifera*	Toluene: ethyl acetate: formic acid at 540 nm.	Beta-sitosterol D glucoside

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