Keywords: Ayurvedic formulations; Churnas; Standardization; Markers; Fingerprint; HPTLC

Introduction
Ayurvedic formulations have numerous uses in Ayurveda. They effect or help to rectify the three doshas or humors in the body [1]. Churna is a fine powder of well dried drug or drugs described in ancient literature [2]. Quantitative estimation of chemical markers of each ingredient in the poly herbal preparation required ideal separation technique [3,4]. 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 [3,5]. HPTLC thus offers major advantages over other commonly available conventional chromatographic techniques [4]. 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 [3,6]. 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 [7].

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 may be applied to the powder prepared by a single drug or a combination of more drugs [8]. Ayurvedic formulary of India has given the specification for the composition of churnas [9,10].

Chaturjat churna: A polyherbal formulation consisting of 4 ingredients with specific morphological parts. The ingredients are Cinnamomum zeylanicum, Elettaria cardamomum, Cinnamomum tamala and Tribulus terrestris [11].

HPTLC profile: The crude drug sample extracted in Methanol (150 ml x 5) through Soxhlet apparatus was filtered and concentrated to 5-10 ml. 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 10 cm using Toluene: Ethyl acetate (9:1) as mobile phase, examined under Ultra Violet Light at 254 nm; and under 366 nm; after derivatization with 5% methanolic sulphuric acid solution different Rf 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) [12] (Figure 1).

Pancasama churna: Pancasama churna, a polyherbal formulation consists of rhizomes of Cyprus rotundus (Mustha), whole plant Terminalia chebula (haritaki), fruit of Piper longum (pippali), root of Operculina turpethum (Trivrat) and sandha lavana [7].

TLC/HPTLC Analysis: TLC and HPTLC finger printing profile of Pancasama Churna (ethanol extract) were developed in Toluene: Ethyl acetate: Formic acid (5:0.3:5:1.0 v/v) solvent system (Figure 2).

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 Triphala churna [9,10]. This well known formulation is made by combining Terminalia chebula, Terminalia belerica and Emblica officinalis, in equal proportions [13].

HPTLC: A HPTLC-densitometric method of analysis for markers

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### Table 1: HPTLC finger print of different churnas.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Churna/extract</th>
<th>Constituents</th>
<th>Solvent system and Scanning wavelength</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ajmodadi churna (Methanol) [3]</td>
<td>Piper species (Piper longum in both form root and fruit and Piper nigrum) [10]</td>
<td>Toluene: Ethyl acetate (07:03) at 336 nm.</td>
<td>Piperine</td>
</tr>
<tr>
<td>2.</td>
<td>Amukkara choornam (toluene) [30]</td>
<td>Piper nigrum, Piper longum, Zingiber officinale, Amukkara (Withania somnifera), Elettaria cardamomum, Cinnamomum wightii, Syzygium aromaticum</td>
<td>Toluene: Ethyl acetate (9:3 v/v) at 260 nm.</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>4.</td>
<td>Hingashak Churna (methanol) [31]</td>
<td>Piper longum, Piper nigrum, Cucumina longa, Thymus vulgaris</td>
<td>Toluene-ethyl acetate-methanol, 9:1:0.5 at 420, 333, and 277 nm</td>
<td>Curcumin, piperine, and thymol</td>
</tr>
<tr>
<td>7.</td>
<td>Nisamalaki churna (Methanol and Aqueous) [33]</td>
<td>Curcumina longa, Emblica officinalis</td>
<td>Chloroform-methanol (9:0.5:v/v), Ethanol: glacial acetic acid (9:1 v/v) at 500 nm</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>8.</td>
<td>Pancasama churna(Ethanol) [7]</td>
<td>Operculina turpethum; Terminalia chebula, Cypruss rotundus; Piper longum</td>
<td>Toluene: ethyl acetate: Formic acid (5:0:3.5:1.0 v/v) at 366 nm.</td>
<td>Piperine and gallic acid</td>
</tr>
<tr>
<td>9.</td>
<td>Panchshark churna (methanol) [34,35]</td>
<td>Cassia angustifolia, Foeniculum vulgare, Terminalia chebula, Zingiber officinale, Rock salt (Saindahva lavana).</td>
<td>Toluene: ethyl acetate at 260 nm</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>10.</td>
<td>Palas abijadi churna (methanol) [36]</td>
<td>Butea monosperma; Holarrhena antidysentrica,embelia ribes, Azadirachta indica, Swertia chirata</td>
<td>Toluene: Ethyl acetate (90:10 v/v) at 260 nm.</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>11.</td>
<td>Panichkol Churna [37]</td>
<td>Piper longum, Piper nigrum, Cuminum cyminum, Plumbago zeylanica, Embelia ribes, Zingiber officinale</td>
<td>Toluene: ethyl acetate (7.3) at 340, 420 nm.</td>
<td>Piperine, plumbagine, zingiberine</td>
</tr>
<tr>
<td>12.</td>
<td>Triphla Churna (aqueous) [13,14]</td>
<td>Terminalia chebula, Terminalia belerica and Embellia officialis</td>
<td>Ethanol: glacial acetic acid: tolenue (5.5:1:1.5) for ascorbic acid and Ethyl acetate: toluene: acetone (4.5:4:1) for gallic acid at 254 nm.</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>14.</td>
<td>Talishadi churna (methanol) [38]</td>
<td>Piper longum, Piper nigrum, Zingiber officinale, Elettaria cardamomum, Cinnamomum zeylanicum, Bambusa arundinacea,</td>
<td>Toluene: ethyl acetate (9:3 v/v) at 260 nm.</td>
<td>Not mentioned</td>
</tr>
</tbody>
</table>

i.e. Gallic acid [14] and ascorbic acid in Triphla churna (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 announcements 0.74 ± 0.1 on using ethanol: glacial acetic acid: toluene (5.5:1.5) and gallic acid showed R announcements 0.54 ± 0.1, using ethyl acetate: toluene: acetone (4.5:4:1) as mobile phase, scanned at 254 nm. Thus, a simple, precise and accurate method for quantitative estimation of ascorbic acid and gallic acid in herbal medicine (triphala churna) by HPTLC was developed. The gallic acid and ascorbic acid content in triphala churna was quantified (Figure 3).

### Trikatu churna:

Trikatu Churna is well known Ayurvedic 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) [15].

**HPTLC:** The fingerprint method for Trikatu churna by simple high performance thin layer chromatography (HPTLC) determination using piperine as a standard, which is as an important and major content in formulation. 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 Trikatu churna name TK-I, TK-II, TK-III, was 2.13% ± 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: ethylacetic acid: glacial acetic acid (8:2:0.1 v/v/v) at 550 nm gave Rf values of 0.42 ± 0.03 for piperine at 550 nm.

Better results were also obtained with mobile phase consisting of toluene: ethyl acetate (70:30 v/v), gave Rf values of 0.42 ± 0.03 for piperine [16] (Figure 4).

**Haritaki churna:** Haritaki churna mainly constitutes of dried fruit of Terminalia chebula [17].

**HPLC:** A high performance liquid chromatography method coupled with diode array detection was developed to simultaneously determine seven different marker compounds in Haritaki churna, an ayurvedic 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 Dionex Ultimate 3000 RSLC System equipped with a photodiode array detector.
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 (R2 > 0.998) between the peak area of each marker and concentration. In this study, an HPLC–DAD method for the qualification and quantification of phytoconstituents in Haritaki churna has been developed and successfully applied for comparison of three marketed samples (HC1, HC2, and HC3) (Table 2).
Terminalia chebula, Piper nigrum, Piper longum, Zingiber officinale, Elletaria cardamomum, Cinnamomum zeylanicum, Punica granatum seeds, Cuminum cyminum, Salts (sea salt, black salt, rock salt and vida salt) or decoctions. It acquires the consistency of a thick paste. The other ingredients of jaggery, sugar or sugar-candy and boiled with prescribed juices of the above ingredients. 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.

**Table 2: HPLC fingerprint of churnas.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Churna/extract</th>
<th>Constituents</th>
<th>Solvent system and Scanning wavelength Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Haritaki churna (methanol: water 70:30) [17]</td>
<td>Terminalia chebula</td>
<td>Tri ethyl amine eq. pH3 with Orthophosphoric acid and acetoneitrite at 272 nm and flow rate of 1 ml/min, an injection volume of 10 μl</td>
</tr>
<tr>
<td>2.</td>
<td>Lavanbhaskar churna (ethanol) [42]</td>
<td>Piper longum, Piper nigrum, Zingiber officinalis, Elletaria cardamomum, Cinnamomum zeylanicum, Punica granatum seeds, Cuminum cyminum, Salts (sea salt, black salt, rock salt and vida salt)</td>
<td>Methanol: water (69:31) at 343 nm and injection volume: 20 μl and flow rate were 1.5 ml/min</td>
</tr>
<tr>
<td>3.</td>
<td>Sitopaladi Churna (ethanol) [43]</td>
<td>Piper nigrum, Piper longum, Zingiber officinalis, Elletaria cardamomum, Cinnamomum wightii, sugar, Bombusa bombos.</td>
<td>Methanol at 342 nm and flow rate of 1.2 ml/min, an injection volume of 20 μg.</td>
</tr>
<tr>
<td>4.</td>
<td>Trikatu Churna (methanol) [44]</td>
<td>Piper longum, Piper nigrum, Zingiber officinalis</td>
<td>Methanol at 342 nm and flow rate of 1.2 ml/min, an injection volume of 20 μg.</td>
</tr>
<tr>
<td>5.</td>
<td>Triphla churna (Hexane and acetonitrile) [45]</td>
<td>Terminalia chebula, Terminalia belerica and Embellica officinalis</td>
<td>Acetonitrile: acetic acid 99.9: 0.10 at 212 nm. Flow rate 1 ml/min, Injection Volume10 μl</td>
</tr>
<tr>
<td>6.</td>
<td>Vidanga churna (Chlo-roform extract sonicated with methanol) [46]</td>
<td>Embelba ribes</td>
<td>Methanol: phosphate buffer pH 3 (90:10) at 291 nm and flow rate of 1.4 ml/min, an injection volume of 20 μl.</td>
</tr>
</tbody>
</table>

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 Haritaki churna.

**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. [8].

**Vyaghirhareetaki avaleha:** An Ayurvedic formulation, Vyaghirhareetaki avaleha (VHA) is a potent drug indicated for shwasa (Asthma), kasa (cough) etc. and used in the management of Tamaka Shwasa (Bronchial Asthma). Its consists of Solanum xanthocarpum, Terminalia chebula, Piper nigrum, Piper longum, Zingiber officinale, Cinnamomum zeylanicum, Cinnamomum tamala Elettaria cardamomum, Mesua ferrea, honey, jaggery [18].

**High Performance Thin layer chromatography (HPTLC):** Eight spots were observed in short wave UV 254 nm and five spots in long wave UV 366 nm. HPTLC fingerprinting for Vyaghirhareetaki Avaleha (methanol extract) with solvent system Toluene: Ethyl acetate: Glacial acetic acid: Formic acid (5:5:1:0.5) at 254 and 336 nm., reveals eight spots of rf values 0.01, 0.14, 0.30, 0.41, 0.50, 0.72, 0.82, 0.92 in short wave UV 254 nm. In long wave UV 366 nm five spots at 0.01, 0.12, 0.40, 0.62, 0.69 RI values were observed (Figure 5).

**Ikshvadi avaleha:** Ikshvadi Avaleha is very safe to be used for tuberculosis in children. Its consists of Phyllanthus urinaria, Saccharum officinarum, Bambusa arundinacea, Mucuna prurita, Piper nigrum, Cinnamomum zeylanicum, Elettaria cardamomum, and honey [19].

**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 RI 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 hRf values 43.90, 3.35, 32.69, 20.06. In short wave UV 254 nm and 3 spots corresponding to hRf 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 (Figure 6).**

**Vasavaleha:** Vasavaleha is a traditional Ayurvedic oral Herbal formulation consisting of five herbs, Vasaka (Adhatoda vasica Nees.), Pippali (Piper longum Linn.), Sugar, Ghee and Honey. It is available as a popular proprietary, from most manufacturers of ayurvedic drugs [20].

**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.
Piperine. Densiometric analysis was carried out in the absorbance gel 60 F254 as a stationary phase. The solvent system consists of (chloroform and methanol extract) as well as its bulk drug.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ayurvedic preparations/extract</th>
<th>Constituents</th>
<th>Solvent system and Scanning wavelength</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ashwagandha capsule (methanol) [47]</td>
<td>Withania somnifera</td>
<td>HPTLC: Chloroform: Methanol (9:1) at 254 and 366 nm.</td>
<td>Not mentioned</td>
</tr>
<tr>
<td></td>
<td>Ashwagandharishta (methanol) [41]</td>
<td>Withania somnifera</td>
<td>HPTLC:-Toluene:ethylacetate: formic acid at 540 nm.</td>
<td>Beta-sitosterol D glucoside</td>
</tr>
<tr>
<td></td>
<td>Ashwagandha vati (methanol) [41]</td>
<td>Withania somnifera</td>
<td>HPTLC:-Toluene:ethylacetate: formic acid at 540 nm.</td>
<td>Beta-sitosterol D glucoside</td>
</tr>
<tr>
<td>5.</td>
<td>Amritasprasa ghrita, brahmi ghrita, chagalyadi ghrita and phala ghrita (hexane) [48]</td>
<td>Not mentioned</td>
<td>HPLC:-Hexane-isopropanol at 220nm. injection volume: 20 μl and flow rate were 2 ml/min</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>6.</td>
<td>Arkavati, Kryyadras and Marichyadi taila (methanol) [49]</td>
<td>Mainly Piper nigrum</td>
<td>HPLC:-Methanol: water in the volume ratio of 70:30 at 342 nm. at a flow rate of 1.0 mL min-1</td>
<td>Piperine</td>
</tr>
<tr>
<td>7.</td>
<td>Polyherbal Acnovin capsule (aq., methanol, ethyl acetate) [50]</td>
<td>Mahamajishthadi kwath, Panch neem churna, Sariva, Sonamukhi, Khadir twak, Haridra, Amla, Bibhitaki, Haritaki and Gandhak rasayana</td>
<td>HPLC:-Water: acetonitrile: glacial acetic acid (9:1:0.2) at 540 nm.</td>
<td>Gallic acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HPLC:-Chloroform: Methanol (9:7) at 254 and 366 nm.</td>
<td>Gallic acid</td>
</tr>
<tr>
<td>12.</td>
<td>Punarnavashtak kwath (methanol) [53]</td>
<td>Boerhaavia diffusa, Picrorhiza Kurroa, Tinospora cordifolia, Zingiber officinalis, Berberis aristata, Terminalia chebula, Azadirachta indica. and Tricosanthes dioica</td>
<td>HPTLC:-Water: acetonitrile: methanol:formic acid (3:3:0:2:0:8) and scanned at 366 nm for berberine and at 280 nm for gallic acid</td>
<td>Gallic acid and berberine</td>
</tr>
<tr>
<td>13.</td>
<td>Sanjivani Vati (diethyl ether) [54]</td>
<td>Embelia ribes, zingiber officinalis, Terminalia chebula, Terminalia belerica, Tinospora cordifolia, Acoruntum heterophyllum, Emblica officinalis,</td>
<td>HPLC:-Acetonitrile: water (20:80), acetonitrile: water: acetic acid (48:52:1) at 254 nm at flow rate 1ml/min, injected vol. 10 μl</td>
<td>Embelin, Piperine</td>
</tr>
<tr>
<td>15.</td>
<td>Triphalaguduchyadhi Vati (methanol) [56]</td>
<td>Terminalia chebula, Terminalia belerica, Emblica officinalis, Cyperus rotundus, Tinospora cordifolia</td>
<td>HPLC:-Toluene:Ethylacetate: Acetic acid (7:2:1) at 254 and 366 nm.</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>16.</td>
<td>Triphala capsule (methanol) [57]</td>
<td>Terminalia chebula, Terminalia belerica, Phyllanthus emblica</td>
<td>HPLC:-Chloroform: Methanol (7:3) at 540 nm</td>
<td>Gallic acid</td>
</tr>
<tr>
<td>17.</td>
<td>Vasavaleha (chloroform and methanol) [20]</td>
<td>Vasaka (Adhatoda vasica Nees.), Pippali (Piper longum Linn.), Sugar, Ghee and Honey.</td>
<td>HPLC:-Dioxane:Toluene:Ethylacetate: Methanol: Ammonia (1.5:2:1:0.3% v/v) at 285 nm.</td>
<td>Vasicine and Piperine</td>
</tr>
<tr>
<td>18.</td>
<td>Vyaghrithareetaki avaleha (acid hydrolysed Methanol extract) [18]</td>
<td>Solarium xanthocarpum, Terminalia chebula, Piper nigrum, Piper longum, Zingiber officinalis, Cinnamomum zeylanicum, Cinnamomum tamala Elettaria cardamomum, Mesua ferrea, honey, jaggery</td>
<td>HPLC:-Toluene:Ethyl acetate :Glacial acetic acid: Formic acid (5:5:1:0:5) at 254 and 336 nm.</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>19.</td>
<td>Vatari guggulu (n-hexane) [58]</td>
<td>Commiphora wightii, Ricianus communis, Terminalia chebula, Terminalia belerica, Emblica officinalis,</td>
<td>HPLC:-Toluene:acetic (9:1) at 250 nm.</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>20.</td>
<td>Yogaraja guggulu (aq. and methanol) [59]</td>
<td>29 ingredients: Plumbago zeylanica, Piper longum, Hyoscyamus niger, Commiphora mukul, Ricianus communis, Terminalia chebula, Terminalia belerica, Emblica ribes etc.</td>
<td>HPLC:-Petroleum ether: Ethyl acetate</td>
<td>Not mentioned</td>
</tr>
</tbody>
</table>

Table 3: Fingerprint profile of other Ayurvedic preparations. (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 (1.5:2:1:0.3% v/v). This system was found to give compact spot for Vasicine and Piperine. Denisometric 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 (Figure 7).

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 [21].

High performance thin layer chromatography (HPTLC) study: In High performance thin layer chromatography (HPTLC) study of ashtaangaavaleha (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 Rf 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 Rf 0.02, 0.20, 0.49, 0.56, 0.65 in long wave UV 366 nm. Details are noted in the Table 1 and Figure 2. Then the plate was sprayed with Anisaldehyde sulphuric acid...
followed by heating and then visualized in day light shows 3 prominent spots at Rf 0.18, 0.36, 0.55 (Figure 8).

**Chyawanprash:** Chyawanprash is a traditional polyherbal formulation, which is widely used as rejuvenator, anabolic, immunomodulator and memory enhancer. Chyawanprash contains the pulp of *Emblica officinalis* as the prime ingredient, along with powder and extract of several other herbs [22].

**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 [23].

These are fermented preparations of medicinal plants. The fermentation procedure adopted to prepare these preparations is termed as ‘Sandhaana kalpanaa’ and the ferment used to stimulate fermentation is termed as ‘Sandhaana dravya’. 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. Asavas and Arishtas 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 [8].

**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 Woodfordia fruticosa [23].

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, gallic 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 (Figure 9).

**Chandanasaava:** 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), mutrakrcrehra (painful micturation), hdroga (heart diseases), agnimandya (loss of appetite) [24].

**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/v) respectively for extracts, visualised at 366 nm and chromatogram was scanned with spectrodensitometer.

**Tablet/Vati**

Vati and Gutiaka–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 [25]. In Ayurveda there are 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 *Emblica officinalis* 201 mg and 15 other ingredients. Amalant offers a multi-pronged approach in the treatment of hyperacidity and acid peptic disorders [26].

**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 Rf 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 Rf value (0.34) was coinciding with standard Rf value and peak area 5097.0. Amalant Tablet extract showed nine peaks, the fourth peak Rf value (0.34) was coinciding with standard Rf value and peak area 5097.0. Amalant Tablet extract showed nine peaks.

**Sulaharan yoga:** Sulaharan Yoga (SY), an Ayurvedic polyherbal

**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 (Figure 11).

From superimposition study a band (Rf 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 [28].

**Nisha amalaki vati:** Nisha Amalaki Vatti is a polyherbal formulation containing Curcuma longa and Phyllanthus emblica used as anti-diabetic agents marketed by Ayush, India [29].

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 (Figure 12).

**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 Ayurvedic churna/preparations.

References


