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HPTLC PROFILING OF AYURVEDIC NEBULIZING FLUID

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ABSTRACT

This study is a prime attempt of High Performance Thin Layer Chromatography (HPTLC) profiling of a novel Ayurvedic nebulizing fluid (ANF) prepared with the herbal compositions of Shirisharishta, mentioned in Bhaishajya Ratnavali, a text of classical treasures in Ayurveda. Shirisharishta has significant therapeutic effect in treatment of Tamaka Swasa i.e. bronchial asthma. In contemporary medical science, bronchial asthma requires long term administration of oral medicaments along with local inhalation therapy. By considering this pharmaceutico-clinical need, the compositions of the formulation Shirisharishta have been converted in to ANF. However, new dosage form always requires specific fingerprinting for authenticity and for reproducibility. Thus, HPTLC profiling of ANFs, which were prepared in two batches with aqueous and alcoholic extracts of Twak (bark) and Sara (heartwood) and Patra (leaves) of Shirisha (*Albizzia lebbeck* Benth) along with rest of the nine herbs present in the formulation of Shirisharishta was done. Four marker components viz. curcumin, piperin, catechin and epicatechin are used in HPTLC profiling. The result showed that these marker components are present in ANF but below quantification level. The Rf value were found for curcumin, piperine, catechin and epicatechin at 0.92, 0.90,0.32 and 0.36 respectively. It is concluded that ANF is a novel pharmaceutical development of Shirisharishta. Marker components are present but below quantification level. It is suggested that further detail studies and clinical trial are required to verify the results.

Keywords: Ayurvedic Nebulizing Fluid, HPTLC, Marker compound, Shirisharista, Bronchial Asthma, Tamak Swasa

INTRODUCTION

Respiratory problems such as asthma are on the rise in many cities due to the dangerous levels of pollution. Although, contemporary medication does have a treatment for acute asthma attacks but it does not provide a long-term cure for this disease. This lack of a long-term treatment for asthma forced scientists and researchers to look toward alternative forms of medication for the treatment of asthma[1]. Medicinal plants used for the treatment of asthma should have anti-inflammatory, immunomodulatory, antihistaminic, smooth-muscle relaxants, and allergic activity. According to Ayurveda antiasthmatic drug should have properties such as Kapha- Vata Shamak (subside the aggravated Kapaha and Vata)[2]. Shirisha (*Albizia lebbeck* Benth) is a drug with multidimensional activities mentioned in Ayurvedic classics for different disease conditions including bronchial asthma[3]. The therapeutic attributes explained for the drug are Swasahara (antiasthmatic), Vishahara (antipoisonous), etc. Shirisharista, this compound formulation is the combination of 12 ingredients with Shirisha as main ingredient and jaggery as base. The plant extract has been proven to be efficacious in cases of allergic rhinitis. Other ingredients of the formulation such as Pippali (*Piper longum* Linn), Haridra (*Curcuma longa* Linn), Shunthi (*Zingiber officinalis* Linn) have been evaluated individually for their antitussive activity. Studies conducted in recent past reveals anti-asthmatic[4], anti-tussive[5], anti-allergic[5], of Shirisha (*Albezzia lebbeck*) and other ingredients of the formulation like Pippali[5] (*Piper longum* Linn), Haridra[6] (*Curcuma longa* Linn), have been evaluated individually for their anti-tussive activity.

Ayurvedic medicines are undergoing drastic changes in the present era, with advances in pharmaceutics. In contemporary medical science, bronchial asthma requires long term administration of oral medicaments along with local inhalation therapy. By considering this pharmaceutico-clinical need, the compositions of the formulation Shirisharishta have been converted in to ANF. Now a day's people accept more elegant and easy intake forms of Ayurvedic products.

Here, an effort has been made to provide safer and faster relief by using Ayurvedic drugs in newer form i.e. nebulizer to meet the need of the hour. Inhalation therapy is not new to the Ayurveda. In Ayurveda classics also, the drug is administered through nose, which is called Nasya in Ayurveda.Nasya play important role in the treatment of diseases related to Shira and Pranavaha Srotasa. The vitiation of Pranavaha Srotasa cause Shwasa (dyspnea)[7]. This procedure alleviates the vitiated doshas situated in head shira is considred as uttama aanga and is the prime controller of the all activities of the body[8].

Marker components are those chemical compounds in herbs which are used to authenticate the medicinal plant. For therapeutic effect of any herbal product, presence of active constituent of herbs is must in those herbal products. Then only any herbal product will show it's efficacy. To know the presence of active constituents in plant product, or extract a chromatography technique is required. These active components act as active markers in chromatographic profiling. Active markers are the constituents or groups of constituents with known pharmacological activity that contribute to efficacy viz.

curcumin in Haridra, piperine in Pippali, catechin in shirisha etc. HPTLC may be considered as one of the standardization parameter for plant extracts. It is the advance form of TLC because it provides better resolution and separation of spots. HPTLC fingerprinting authenticates the constituents of plant material. In addition, it also provides some information regarding active constituent of plant extracts i.e. identity and purity profile of a plant extract. The quantity of active component may also be known through densitometery. Thus, HPTLC technique had been used here to identify the marker compounds present in Ayurvedic nebulizing fluid. The four marker components which are used here are curcumin, piperine, catechin and epicatechin. The objective of this work was to describe and develop HPTLC analysis i.e. fingerprint and densitometry of ANF, with the help of these marker components.

MATERIAL AND METHODS

Preparation of test formulation: The raw materials of Ayurvedic Nebulizer (Table 1) were authenticated by the Pharmacognosy laboratory of IPGT and RA, Gujarat *Ayurved* University, Jamnagar, followed by size reduction in a mixer and sieving through #72. the raw material, three samples were made; taking leaf, bark and heartwood of Shirisha as main drug and rest nine drugs were taken in all three samples in the same ratio as mentioned in classics in the preparation of Shirisharista. Aqueous extracts and Alcoholic extracts of all three samples were made from reflux method and soxhlet method respectively then whole solvent was evaporated and dried solid extracts were obtained[9]. Final nebulizing fluid were made from dissolving solid extracts into solvent of distilled water and absolute alcohol (7:3). The respective samples were coded according to their nature (Table 2).

HPTLC profile[10]: Different solvent systems were selected through trial and error method for all three samples. The developed plate was visualized under visible day light, short UV (254 nm), long UV (366 nm). The Rf values were recorded. For HPTLC analysis the prepared extracts were dissolved in methanol and out of this 10µL solution was injected in HPTLC system.

Concentrations of the extract samples were prepared in Methanol

- *Shirisha Twaka* (bark): 158.5 mg / 10 ml Methanol
- *Shirisha Sara* (heartwood): 154.1 mg / 10 ml Methanol
- *Shirisha Patra* (leaf): 155.8 mg / 10 ml Methanol

Standard solutions

- The standard solution of Epicatechin, Catechin were procured from - Natural Remedies Pvt. Ltd. Bangalore.
- Piperine and Curcumin were procured from Sigma Aldrich Pvt. Ltd. Mumbai.

Concentration of the samples prepared

- **Epicatechin and Catechin**: 1mg/ml
- **Curcumin and Piperine**: 1mg/ml

HPTLC was performed on silica gel 60, 20X10 cm HPTLC plates. The solvent system was decided by trial & error method. Toluene: Ethyl acetate (8: 1.5 v/v) was selected as mobile phase because maximum number of constituents showed in this solvent system. The standards (Catechin, Epicatechin, Pipperin and Curcumin) solutions (5.0 µL of each concentration 1 mg/mL) were applied to the plates as 10 mm bands, sample application with CAMAG-Linomat IV automated spray on band applicator equipped with a 100 µL syringe. CAMAG TLC Scanner 3 was used to densitometrically to quantify the bands using WIN CATS software. The whole scanning process was done at an optimized wavelength 254, 366 nm and in visible range.

RESULT AND DISCUSSION

HPTLC Profiling of Marker Compounds in Different Extracts

All the prepared samples were analyzed for presence of marker components. Rf values for Catechin and Epicatechin is 0.32 and 0.36 respectively. No spot was present at 366 nm for Catechin. Table 4 shows Rf values of Curcumin and Piperin in which is present at 0.90 and 0.92 respectively at both wavelengths.

HPTLC profiling shows the Rf values in aqueous extracts of leaf, bark and heartwood extracts. The HPTLC profiling of extracts (Figure 1-7) and related Rf values (Table 3-8) are given.

Table 3-8 shows the details of HPTLC profiles of all tracks at 254 nm and 366 nm. The above data shows that in all aqueous extracts and alcoholic extracts, all four marker compounds were present at the detection level but these compounds were present below quantification level therefore quantification was not done.

Numbers of spots present in alcoholic samples were more in comparison to aqueous samples. It shows more components were present in alcoholic samples, among them heartwood sample contain even more spots, which reveal that number of components present more in alcoholic heartwood sample. This densitometric HPTLC fingerprint profile may be used as marker for quality evaluation and standardization of the drug. Thus, HPTLC fingerprint profile along with their Rf values were recorded, which would serve as a reference standard for the scientist engaged in research on the medicinal properties of plant.

In an experimental study, aqueous extract of leaves, bark and heartwood were tested for their anti-tussive and anti-histamanic activities[11]. It was revealed in the study that aqueous bark extract showed more significant activity in comparison to heartwood. In spite of the fact that HPTLC fingerprinting analysis showed more phytoconstituents in aqueous heartwood extract. It may be because, therapeutic active phytoconstituents were present in bark extract.

Table 1: Formulation composition of aqueous extract

Sr. No.	Ingredients	Botanical name	Part used	Quantity
1	***Shirisha***	*Albizzia lebeck* Benth.	Leaf/bark/heartwood	50 parts
2	***Pippali***	*Piper longum* Linn.	Fruit	1 part
3	***Priyangu***	*Callicarpa macrophylla* Vahl.	Flower	1 part
4	***Kustha***	*Saussurea lappa* C.B.Clarke	Root	1 part
5	***Ela***	*Elettaria cardamomum* Maton	Seed	1 part
6	***Nilini***	*Indigofera tinctoria* Linn.	Root	1 part
7	***Haridra***	*Curcuma longa* Linn.	Rhizome	1 part
8	***Daruharidra***	*Berberis aristata* DC	Stem	1 part
9	***Naagkeasar***	*Mesua ferrea* Linn.	Stamen	1 part
10	***Shunthi***	*Zingiber Officinalis* Linn.	Rhizome	1 part

Table 2: Coding of Different Formulations

S.No	Sample Name	Sample Code
1	Shirisha bark & nine herbs aqueous extract	**Aqbr**
2	Shirisha bark & nine herbs alcoholic extract	**Alcbr**
3	Shirisha heartwood & nine herbs aqueous extract	**Aqht**
4	Shirisha heartwood & nine herbs alcoholic extract	**Alcht**
5	Shirisha leaf & nine herbs aqueous extract	**Aqlf**
6	Shirisha leaf & nine herbs alcoholic extract	**Alclf**

Table 3: HPTLC results of methanolic extract of Catechin and Epicatechin

Mobile phase: Toluene: Ethyl Acetate: Acetic Acid- 5:3:2v/v				
Sample	**254 nm**		**366 nm**	
	No. of spots	**Rf value**	**No. of spots**	**Rf value**
Catechin	3	0.02,0.**32,**0.86	-	-
Epicatechin	15	0.13,0.19,0.21,0.24,0.28,0.30,0.32,**0.36,**0.39,0.43,0.46,0.50,0.55,0.64,0.92	24	0.00,0.04,0.10,0.15,0.19,0.21,0.24,0.28,0.30,0.32,**0.36**,0.40,0.43,0.46,0.50,0.55,0.62,0.64,0.71,0.73,0.76,0.81,0.86,0.90

Table 4: HPTLC results of methanolic extract of Curcumin and Piperin

Mobile phase: Toluene: Ethyl Acetate: Acetic Acid- 7:2:1v/v				
Sample	**254 nm**		**366 nm**	
	No. of spots	**Rf value**	**No. of spots**	**Rf value**
Curcumin 1(2.5µg/ml)	3	0.00,0.69,0.89,**0.90**	3	0.00,0.68,**0.90**
Curcumin 2 (5 µg/ml)	4	0.00,0.68,0.84,0.88,**0.90**	3	0.00,0.68,**0.90**
Curcumin 3 (7.5 µg/ml	4	.0.00,0.70,0.84,0.87,**0.9**	4	0.00,0.08,0.69,**0.9**
Piperin 1(2.5 µg/ml)	3	0.67,0.84,0.87,**0.92**	2	0.67,0.89,**0.92**
Piperin 2 (5 µg/ml)	3	0.69,0.84,**0.92**	2	0.69,**0.92**
Piperin 3 (7.5 µg/ml)	9	0.27,0.36,0.45,0.49,0.52,0.60,0.69,0.68,**0.92**	4	0.52,0.63,0.78,**0.92**

Table 5: HPTLC results of methanolic extract of three Extracts for Epicatechin and Catechin

Mobile phase: Mobile phase: Toluene: Ethyl Acetate: Acetic Acid- 5:3:2v/v				
Sample	**254 nm**		**366 nm**	
	Rf value	**No. of spots**		
Aqht	6	0.00,0.02,**0.32,0.36**,0.49,0.87	-	-
Aqbr	6	0.00,0.02,**0.32,0.36**,0.51, ,0.90	-	-
Aqlf	4	0.02,0.**32,0.36**,0.92	-	--

Table 6: HPTLC results of methanolic extract of three extracts for Curcumin and Piperin

Mobile phase: Toluene: Ethyl Acetate: Acetic Acid- 7:2:1v/v				
Sample	**254 nm**		**366 nm**	
	No. of Spots	**Rf Value**	**No. of Spots**	**Rf Value**
Aqht	4	0.30,0.86,**0.90,0.92**	5	0.36,0.86,**0.90,0.92**,0.93
Aqbr	3	0.62,**0.90,0.92**	3	0.55,**0.90,0.92**
Aqlf	4	0.59,**0.90,0.92**,0.93	5	0.00,0.32,0.59,**0.90,0.92**

Table 7: HPTLC results of methanolic extract of three extracts for Epicatechin and Catechin

Mobile phase: Mobile phase: Toluene: Ethyl Acetate: Acetic Acid- 5:3:2v/v				
Sample	**254 nm**		**366 nm**	
	Rf value	**No. of spots**	**Rf value**	
Alcht	7	0.00,0.05,**0.32,0.36**,0.56,0.60,0.73,0.83	8	0.05,0.21,0.34,**0.36**,0.62,0.70,0.88,0.92
Alcbr	6	0.01,0.08,**0.32**,0.35,**0.36,**0.73,0.81,0.90	8	0.01,0.13,0.31,0.34,**0.36**,0.37,0.39,0.69,0.83,0.93
Alclf	1	0.01,**0.32**,0.34,**0.36**	1	0.01,0.31,0.35,**0.36**

Table 8: HPTLC results of methanolic extract of three extracts for Curcumin and Piperin

Mobile phase: Toluene: Ethyl Acetate: Acetic Acid- 7:2:1v/v				
Sample	**254 nm**		**366 nm**	
	No. of spots	**Rf value**	**No. of spots**	**Rf value**
Alcht	4	0.01,0.05,**0.90,0.92**	7	0.21,0.33,0.47,0.51,**0.90,0.92**,0.93
Alcbr	5	0.01,0.30,**0.90,0.92**,0.93	5	0.01,0.30,0.83,**0.90,0.92**
Alclf	5	0.01,0.32,0.89,**0.90,0.92**	5	0.01,0.08,0.30,**0.90,0.92**

Figure 1

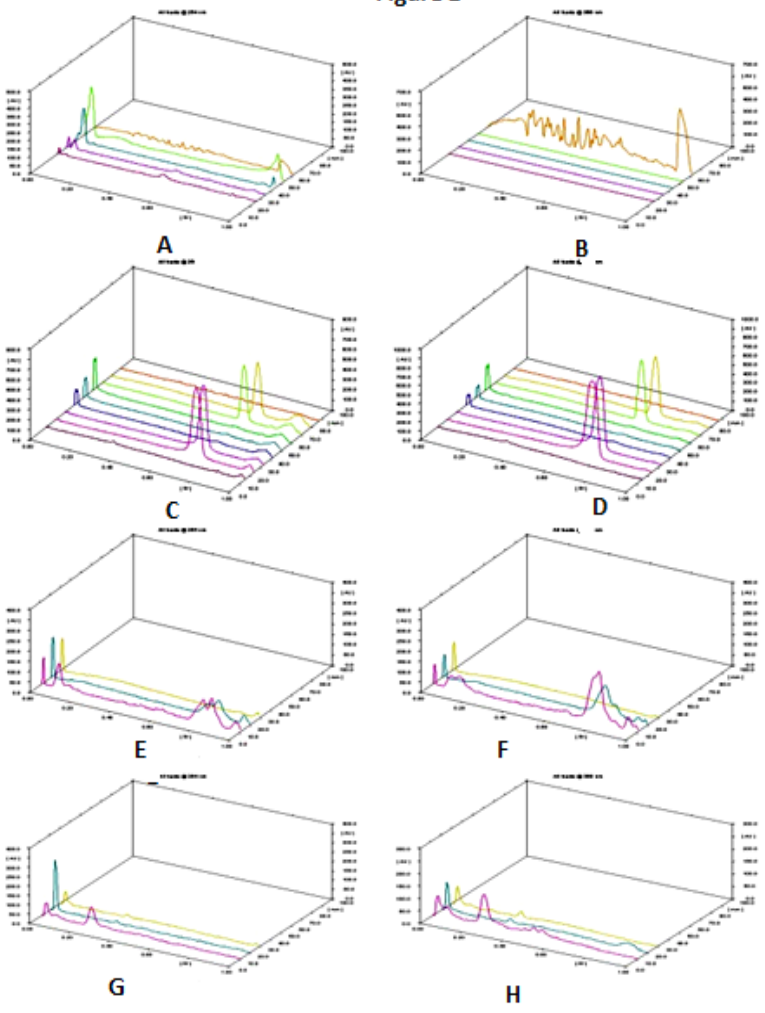

Figure 1: Three-dimensional view of tracks at both wavelengths of all samples with standards
A.Aqueous extracts with Epicatechin and Catechin at 254 nm**, B.**Aqueous extracts with Epicatechin and Catechinat 366 nm**, C.**Aqueous extracts with curcumin and piperine at 254 nm**, D.**Aqueous extracts with curcumin and piperine at 366 nm**, E.**Alcoholic extracts, for presence of Epicatechin and Catechinat 254 nm**, F.**Alcoholic extracts,for presence of Epicatechin and Catechinat 366 nm**, G.**Alcoholic extracts, for presence of curcumin and piperin at 254 nm**, H.**Alcoholic extracts for presence of curcumin and piperin at 366 nm

Figure 2

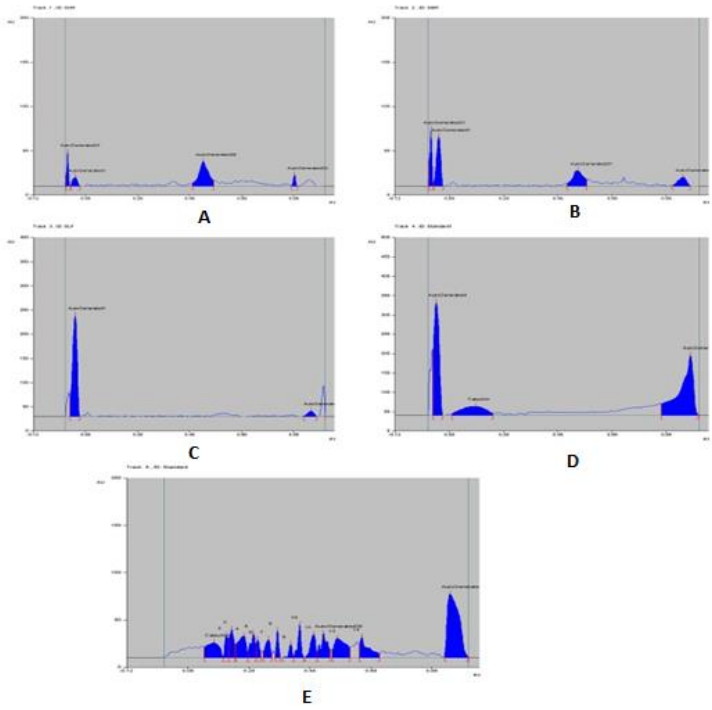

Figure 2: Peak display of Aqueous extracts with standards Epicatechin and Catechin at 254 nm
A.Heartwood extract**, B.**Bark extract, **C.**Leaf extract**, D.S**tandard Catechin, **E**.Standard Epicatechin

Figure 3

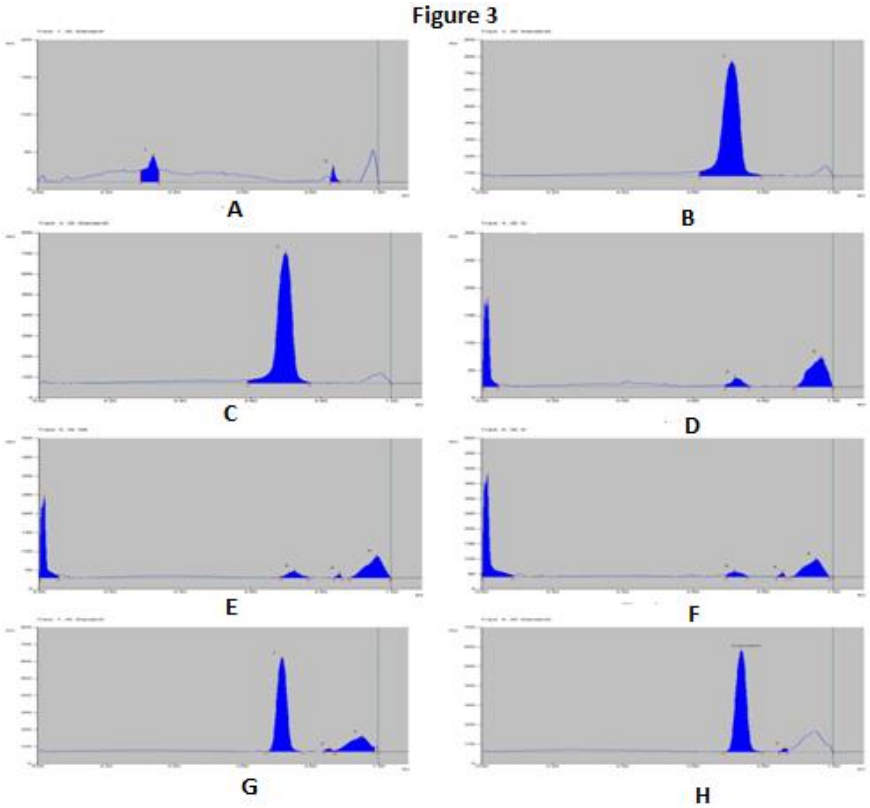

Figure 3: Peak display of Aqueous extracts with Standards Curcumin and Piperin at 254 nm
A.Standard curcumin at 2.5 µg/ml, **B.**Standard curcumin at 5 µg/ml**, C.**Standard curcumin at 7.5 µg/ml**, D.**Heartwood extract**, E.**Bark extract**, F.**Leaf extract**, G.**Standard piperin at 2.5 µg/ml**, H.S**tandard piperin at 5 µg/ml

Figure 4

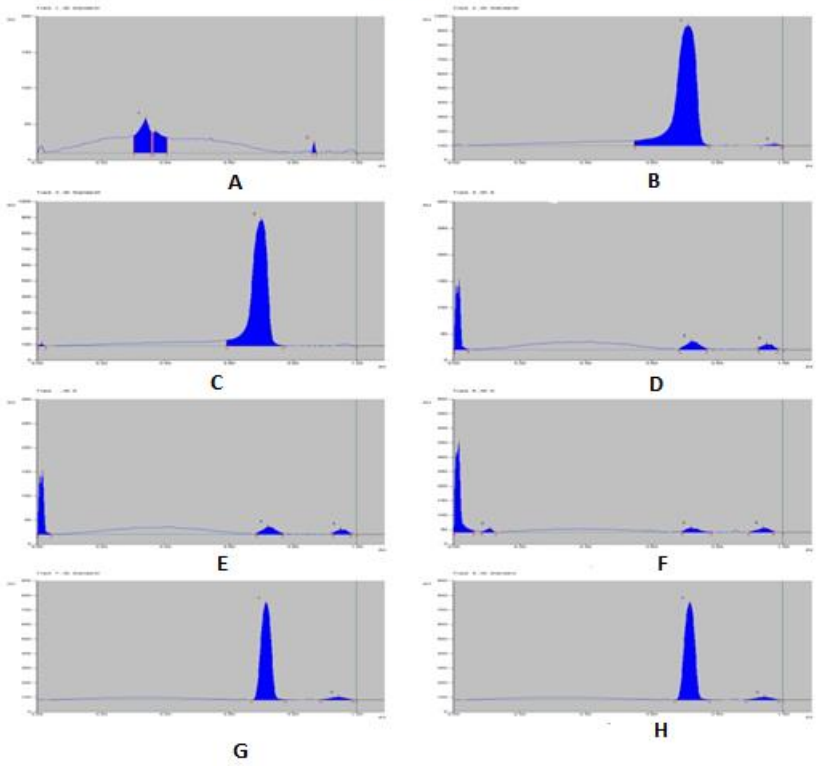

Figure 4: Peak display of Aqueous extracts with Standards Curcumin and Piperin at 366 nm
A.Standard curcumin at 2.5 µg/ml, **B.**Standard curcumin at 5 µg/ml**, C.**Standard curcumin at 7.5 µg/ml, **D.**Heartwood extract**, E.**Bark extract**, F.**Leaf extract**, G.**Standard piperin at 2.5 µg/ml**, H.**Standard piperin at 5 µg/ml

Figure 5

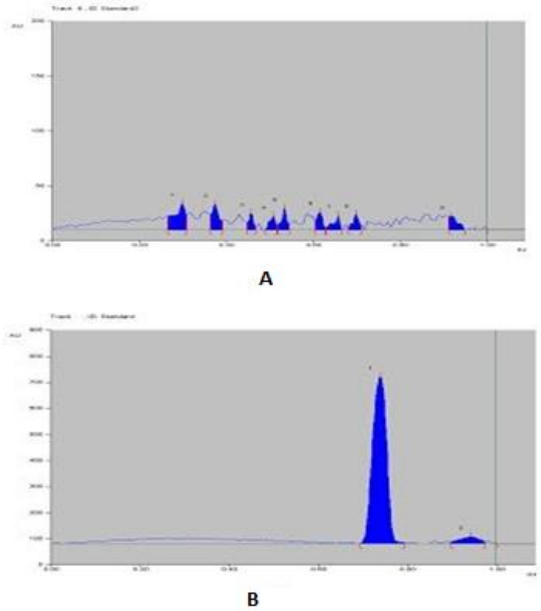

Figure 5: Peak display of 9th track of standard Piperin at both wavelength
A.Standard piperin 7.5 µg/ml at 254 nm, **B.**Standard piperin 7.5 µg/ml at 366 nm

Figure 6

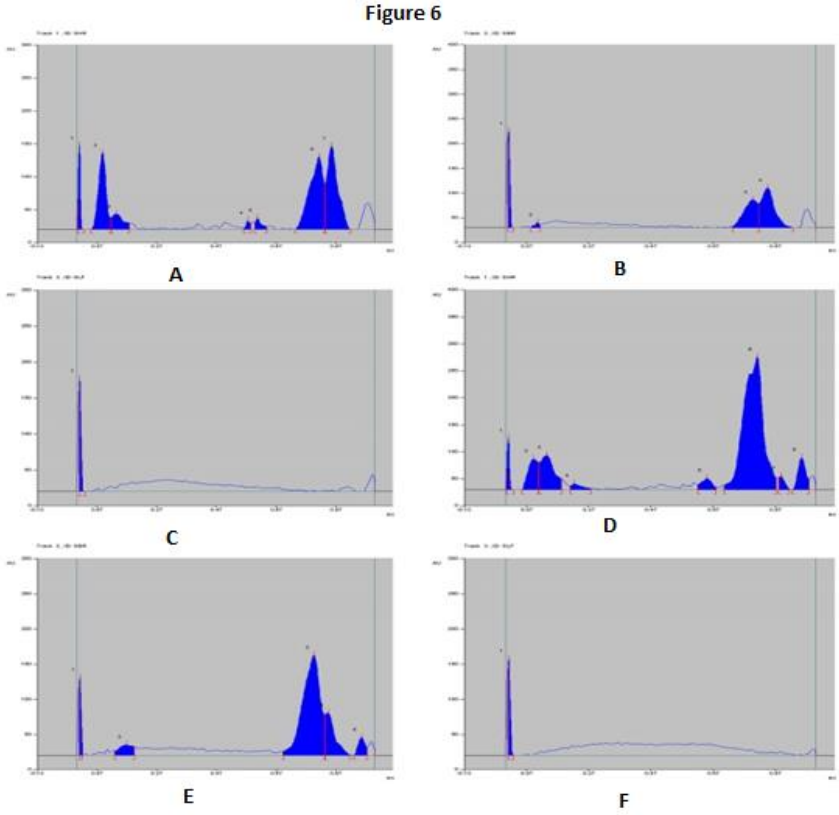

Figure 6: Peak display of Alcoholic extracts for presence of Standards Epicatechin and Catechin
A.Heartwood extract at 254 nm**, B.**Bark extract at 254 nm**, C.**Leaf extract at 254 nm**, D.**Heartwood extract at 366 nm**, E.**Bark extract at 366 nm**, F.**Leaf extract at 366 nm

Figure 7

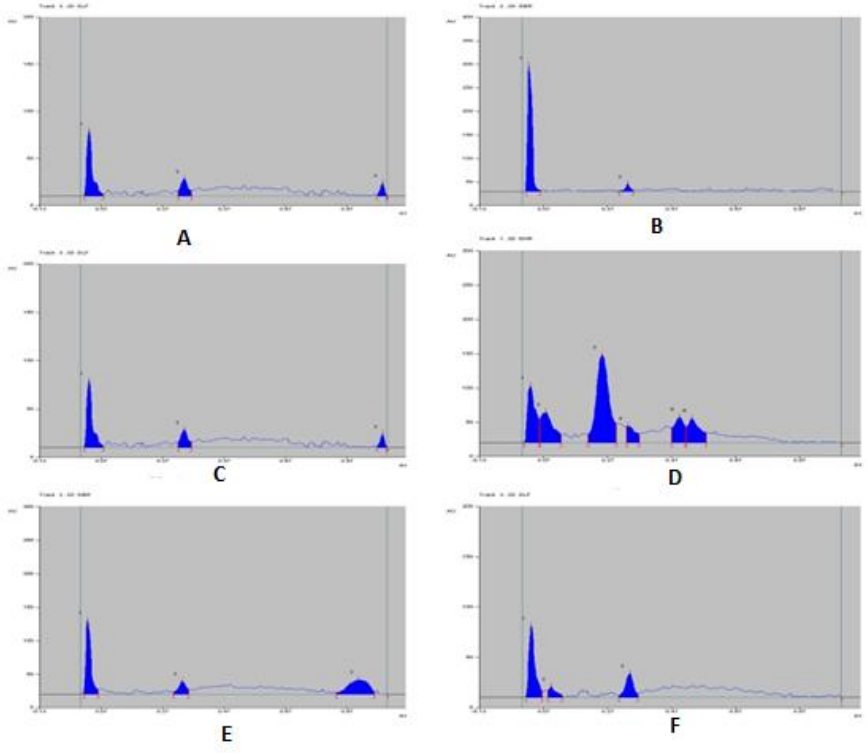

Figure 7: Peak display of Alcoholic extracts for presence of Standards Curcumin and Piperin
A.Heartwood extract at 254 nm**, B.**Bark extract at 254 nm**, C.**Leaf extract at 254 nm**, D.**Heartwood extract at 366 nm**, E.**Bark extract at 366 nm**, F.**Leaf extract at 366 nm

CONCLUSION

HPTLC is an advanced technique of TLC which has been widely used for the determination of phyto-constituents in different extracts and to authenticate the medicinal plants by providing fingerprinting of phyto-constituents. As marker components are important tool for authentication of plant and herbal product. In present study fingerprinting analysis of different extracts of prepared from the herbal ingredients of Shirisharista has been carried out. In the result, it is revealed that all four-marker component present in extracts but below quantification level and heartwood extract had showed maximum no. of spots in comparison to leaves and bark. It clearly indicates the presence of more phytoconstituents in heartwood extract. As aqueous bark extract showed more therapeutic effect in spite of the fact that heartwood has more number of phytoconstituent. It is suggested to validate the alcoholic extracts for their therapeutic valve through analytical and experimental studies.

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