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Research Article

PHYSICOCHEMICAL AND HPTLC ANALYSIS OF *PANCHAVALKAL* AND ITS SUBSTITUTES MENTIONED IN *HIMVAN AGADA*

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A B S T R A C T

Ayurveda has grouped the stem bark of five plants *viz. Nyagrodha* (*Ficus bengalensis* Linn.), *Udumbara* (*Ficus glomerata* Linn.), *Ashwatha* (*Ficus religiosa* Linn.), *Parisha* (*Thespesia populnea* Soland Ex Correa) and *Plaksha (Ficus infectoria* Roxb.) as *Panchavalkal* (Sample A). Interestingly it has been found that there is a mention of the use of *Shirish* (*Albizzia lebbeck* Benth.) and *Vetasa* (*Salix caprea* Linn.) in place of *Udumbara* and *Parisha* in the *Panchavalkal* (B) identified by *Arundatta* the commentator of *Ashtanga Hrudaya*) which belong to different families, genera & species and with different active chemical. Therefore the present study has been planned to comparatively explore and analyze the poly herbal combination *Panchavalkal* (A) and (B) with different set of herbs by using HPTLC fingerprinting and to further assess their antimicrobial properties. HPTLC finger printing revealed that Few Rf values and their colour (visualization) same which confirms that some ingredients are common in both samples. Antimicrobial activity found to be significant when compared between sample A & B at each concentration with related standard drug ZOIs.

INTRODUCTION

Ayurveda has grouped the stem bark of five plants *viz. Nyagrodha* (*Ficus bengalensis* Linn.), *Udumbara* (*Ficus glomerata* Linn.), *Ashwatha* (*Ficus religiosa* Linn.), *Parisha* (*Thespesia populnea* Soland Ex Correa) and *Plaksha (Ficus infectoria* Roxb.) as *Panchavalkal* (the stem bark of five trees).[1]

These *Panchavalkal* (A) (used together) are indicated as *Varnya (improve complexion)*, *Stanyashodhana* (purifies breast milk), *Vranaghna* (wound healing), *Visarpa* (for treating Herpes zooster) and *Shophaghna* (anti-inflammatory). Many references with different herbs as components of *Panchavalkal* have been found[2- 3]. Interestingly it has been found that there is a mention of the use of *Shirish* (*Albizzia lebbeck* Benth.) and *Vetasa* (*Salix caprea* Linn.) in place of *Udumbara* (*Ficus glomerata* Linn.) and *Parisha* (*Thespesia populnea* Soland Ex Correa) in the *Panchavalkal* (B) identified by *Arundatta* the commentator of *Ashtanga Hrudaya* [4]. In the *Himavan Agada* (anti-poisonous formulation) used to treat the *Mandali Sarpa Visha*, there is this difference in the opinion of *Panchavalkal*. The *Panchavalkal* (B) combination of different herbs however shares similar pharmacological property as that of *Panchavalkal* (A) *viz. Shophaghna* (anti-inflammatory).

First and foremost the rationale for the combination of these five medicinal plants as *Panchavalkal* is very peculiar. Further the substitution of plants which belong to different families, genera & species and with different active chemical constituents are more interesting. Therefore the present study has been planned to explore the scientific rationale for the substitution of the medicinal plants. In addition we wish to validate the traditional *Ayurvedic* principles.

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MATERIALS AND METHODOLOGY

Preparation of Panchavalkal sample A and B

Procurement and Authentication of raw drugs for preparation of Samples

Raw drugs required for preparation were procured form authenticated market dealer. Procured drugs were authenticated in Dravyaguna Department, Uttaranchal Ayurvedic College, Dehradun. Drugs were pounded individually in *Khalwa Yantra* to get *Yawakuta* form. After pounding drugs are mixed (I part each) to prepare

Panchavalkala A and B. Ingredient of *Panchvalkala* are mentioned in table (1and 2).

Table no 1 Ingredients of *Panchavalkal* Sample A

Sl no	Name of Ingredient of *Panchvalkala* sample A	Quantity
1.	*Nyagrodha (Ficus bengalensis* Linn.)	1 part
2.	*Udumbara (Ficus glomerata* Roxb.)	1 part
3.	*Asvattha (Ficus religiosa* Linn.)	1 part
4.	*Parisha (Thespesia populnea* Soland ex.Correa)	1 part
5.	*Plaksha (Ficus infectoria)*	1 part

Table no. 2 Ingredients of *Panchavalkal* Sample B

Sl no	Name of Ingredient of *Panchvalkala* sample B	Quantity
1.	*Nyagrodha (Ficus bengalensis* Linn.)	1 part
2.	*Shirish (Albizzia lebbeck Benth.)*	1 part
3.	*Asvattha (Ficus religiosa* Linn.)	1 part
4.	*Vetasa (Salix caprea Linn.)*	1 part
5.	*Plaksha (Ficus infectoria)*	1 part

The sample of *Panchavalkal* (A) and (B) were later subjected to Physico-chemical analysis and preliminary phytochemical analysis. The following material and methods were used for analyzing both the sample i.e. *Panchavalkal* (A) and (B).

Physico-Chemical Analysis

Physico- chemical Analysis like total ash, acid insoluble ash, water-soluble ash, alcohol soluble extractive and, water-soluble extractives were carried out at S.R Labs, Haldighati Marg, Rajasthan.

Thin layer Chromatography (TLC) Test[5]

TLC is the technique in which a solute undergoes distribution between two phases, stationary phase and a mobile phase. The stationary phase acts as an adsorbent in a relatively thin uniform layer of a dry finely powdered material applied to a glass, plastic or metal sheet. Separation may be achieved on the basis of partition a or a combination of partition and adsorption, depending on the particular type of stationary phase, its preparation and uses of different solvents.

High Performance Thin layer Chromatography (TLC) Test [6]

HPTLC is the technique in which a solute undergoes distribution between two phases, stationary phase and a mobile phase. The stationary phase acts as an adsorbent in a relatively thin uniform layer of a dry finely powdered material applied to a glass, plastic or metal sheet. Separation may be achieved on the basis of partition a or a combination of partition and adsorption, depending on the particular type of stationary phase, its preparation and uses of different solvents. Instrument contains different parts to applicate, scan and visualize the elution of different spots after development of TLC plate. So, we can easily develop a fingerprint profile for the same.

Phytochemical Analysis[7]

Aqueous extract of *Panchavalkal* was subjected for Qualitative analysis, which has shown presence of Carbohydrates, Reducing sugar, Alkaloids, Proteins, Amino acids, Fats and Oils, Steroids, Flavonoids, Saponins was present given in table- 5 and 6 respectively.

Antimicrobial Activity[182, 183,184]

To lay down the procedure for Antimicrobial activity to be performed in formulation with reference of using standard culture.

Test organisms used

Use cultures of the following microorganisms *Candida* MTCC no 227, *Escherichia coli* MTCC No. 1687 and *Staphylococcus aureus* MTCC No. 737. The viable microorganisms used in the test must not be more than five passages removed from the original MTCC culture or any other equivalent cultures. In vitro antibacterial activity of formulations was carried out by using the Agar well diffusion method.

OBSERVATIONS AND RESULTS

Table no. 3 Results of Preliminary Physicochemical and Phytochemical Analysis

Sr. No.	Parameters	Sample A	Sample B
1.	Alcohol Soluble Extractive	8.33%	7.62%
2.	Water Soluble Extractive	11.75%	15.26%
3.	LOD	10.15%	10.29%
4.	Total Ash	12.67%	8.16%
5.	Water Soluble Ash	4.05%	2.55%
6.	Acid Insoluble Ash	1.04%	0.91%

Organoleptic characters

Table no. 4 Organoleptic characters of *Panchavalkal A and B*

Sr. No.	Parameters	*Panchavalkal*
1	Appearance	Crude raw drug
2	Odour	Characteristic
3	Taste	-
4	Colour	Brownish

Qualitative Parameters

Table no. 5 Results of Phytochemical profile of *Panchavalkal A*

Sr. No	Qualitative chemical Test	Results	Method
1.	Alkeloids	Negative	
2.	Glycosides	Positive	
3.	Tannins	Positive	
4.	Flavonides	Positive	API
5.	Saponins	Negative	2008
6.	Steroids	Negative	

Table no.6 Results of Phytochemical profile of *Panchavalkal B*

Sr. No	Qualitative chemical Test	Results	Method
1.	Alkeloids	Negative	
2.	Glycosides	Positive	
3.	Tannins	Positive	
4.	Flavonides	Positive	API
5.	Saponins	Negative	2008
6.	Steroids	Negative	

Table no.7 Results of TLC analysis– Sample A

Test Parameter	Limits	Result	Test Method
TLC Profile Sample B	254nm	0.68	API
	366nm	0.93,0.82,0.62,0.4,0.2	2008
	Derivatized	0.93, 0.82, 0.75, 0.70, 0.5, 0.31	

Table no.8 Results of TLC analysis– Sample B

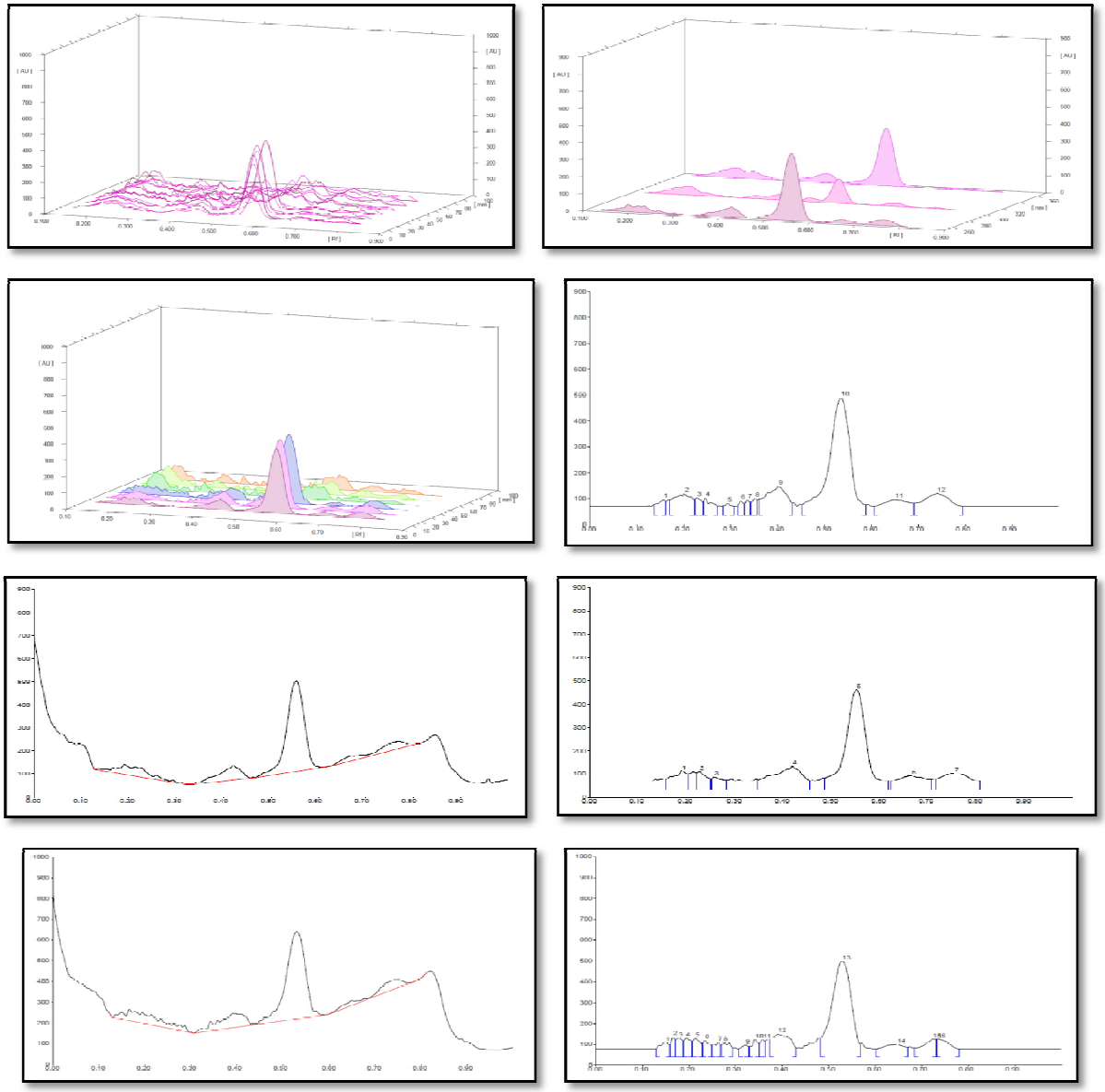
Test Parameter	Limits	Result	Test Method
TLC Profile Sample A	254nm	0.87.0.66	API 2008
	366nm	0.93,0.82,0.62, 0.2	
	Derivatized	0.93, 0.82, 0.5, 0.16	

Table no.9 Results of HPTLC fingerprinting analysis

Test Parameter	Limits	Result
HPTLC Fingerprinting Profile sample A	254 nm	0.19, 0.2, 0.26, 0.4, 0.53, 0.65, 0.73, 0.74
	310nm	0.19, 0.23, 0.42, 0.55, 0.68
	366 nm	0.2, 0.22, 0.23, 0.42, 0.53, 0.78
HPTLC Fingerprinting Profile sample B	254 nm	0.18, 0.29, 0.54, 0.57, 0.64
	310 nm	0.18, 0.54, 0.58, 0.66, 0.68
	366 nm	0.18, 0.34, 0.54, 0.58, 0.65, 0.66, 0.7, 0.72, 0.79

indicates the replacing of one drug either in whole or in part by another drug. It is usually done with fraudulent intent but under certain condition a substitution may be justified. In case of drugs, substitutes should have proven efficacy as near as the original drug[9]. Considering above said present study has been initiated to evaluate and explore the scientific rationale for the substitution of the *Panchavalkal* A with *Panchavalkal* B. to achive the same HPTLC fingerprinting.

Images of HPTLC fingerprinting at various nm, For Sample A and Sample B



DISCUSSION

In *Ayurveda* there is mentioning of substitution of different herbs explained as *Pratinidhi Dravya* which are defined as; "when there is unavailability of any particular drug during preparation of a compound, one should try to get another drug having similar potency in terms of *Rasa*, *Guna*, *Veerya*, and *Vipaka*[8]."

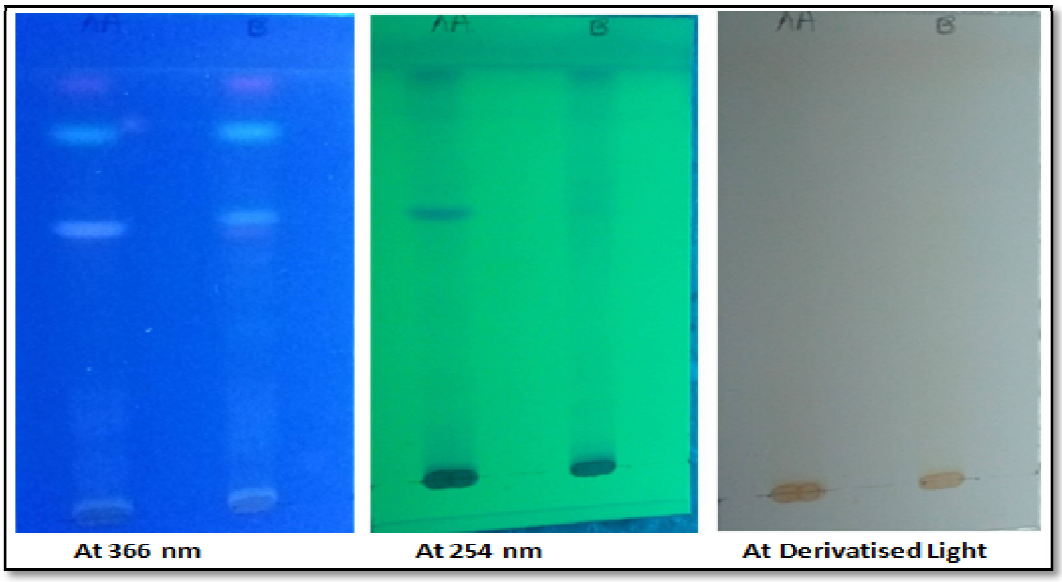
In terms of pharmacognosy, substitution is generally done when original material is not available or if available is in insufficient quantity. In words of Youngken, substitution

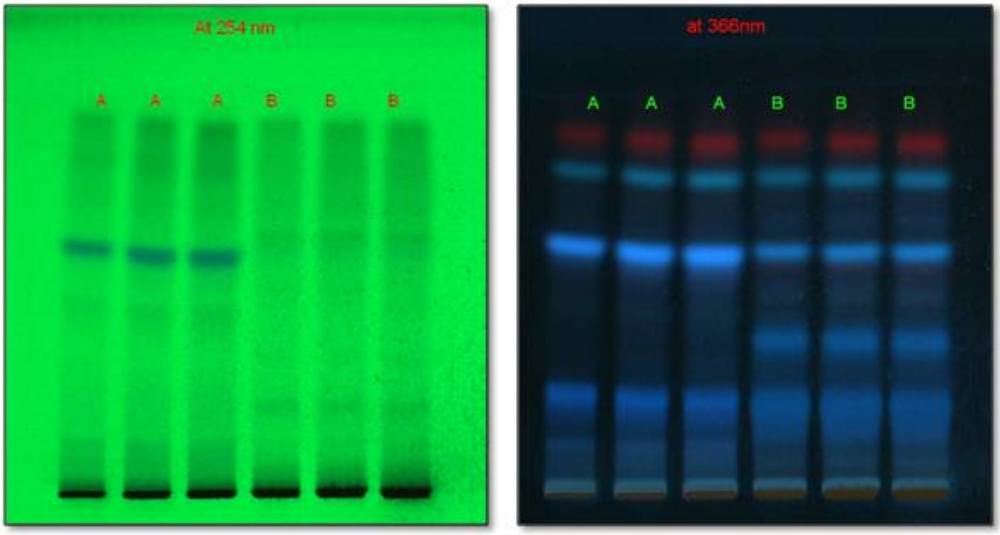
Discussion on Physico-Phytochemical analysis

The quantitative tests assays are the methods upon which the standards of Pharmacopoeia depend. So it is necessary to analyze *Ayurvedic* formulation on these parameters.

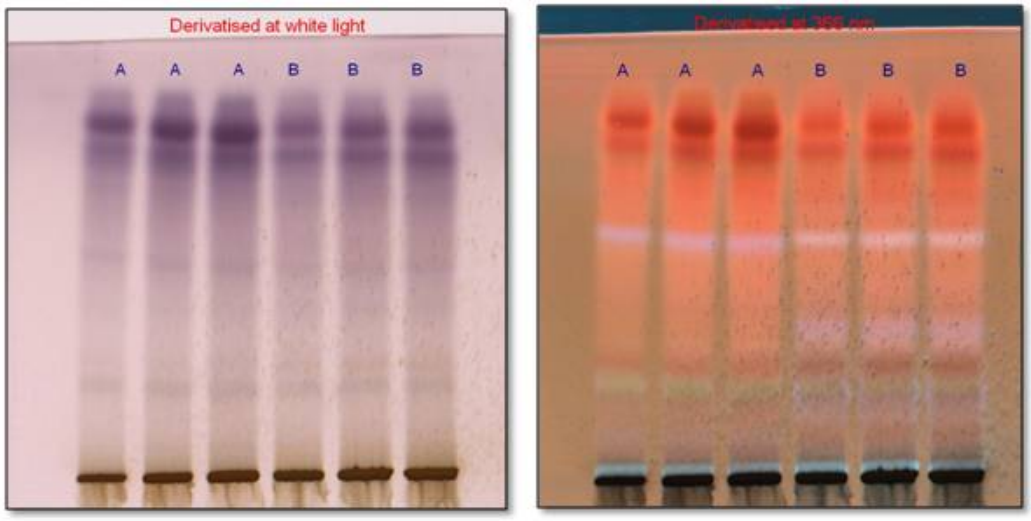
Phytochemical identification has been performed in order to know the phytochemical similarities between sample A and B and its found that same of phytochemical such as Glycosides, Tannins, Flavonides are common in both sample.

Images of Thin Layer Chromatography and High Performance Thin Layer Chromatography





HPTLC fingerprinting at various nm



HPTLC fingerprinting at various nm

Contents of Sample A and B

Sample A

Sample B

Discussion on HPTLC fingerprinting profile

Comparative analysis has performed between sample A and B. The different concentration of Methanolic extract of samples was applied on HPTLC plates in 6, 8 and 10 µl. Plate was developed and elution of different spots visualized under 254, 366nm and white light after derivatization with Anisaldehyde sulphuric acid reagent.

Sample A was shown more and intense spot in 254nm and profile was entirely different compared to sample B and Sample B shown intense spots at lower Rf which are absent in sample A.

Related Rf values are stated in below table that confirm about elution of different spots in both the samples.

Few same Rf values and their colour (visualization) confirm that some ingredients are common in both samples.

CONCLUSION

The substitution of ingredients in a formulation is of great economic importance to the country and efforts should be made for the systematic identification and evaluation by pharmacognostical and phytochemical chemical analytical studies. In present study HPTLC fingerprinting revealed that Few Rf values and their colour (visualization) same which confirms that some ingredients are common in both samples.

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