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Quality Characterisation and HPTLC fingerprinting of Vachadi syrup: A polyherbal formulation

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ABSTRACT

Polyherbal formulations (PHFs) are potential for their safety, cost effectiveness and better acceptability than allopathic drugs. PHFs help in amelioration of various serious disorders. Therapeutic efficacy of herbal medicine is exerted due to the bioactive constituents of plants. The present study was aimed to prepare and standardize a polyherbal formulation (Vachadi syrup) including traditional drugs such as *Acorus calamus* (rhizomes), *Trachyspermum ammi* (fruits), *Phyllanthus emblica* (fruits), *Terminalia bellirica* (fruits), *Terminalia chebula* (fruits), *Zingiber officinalis* (rhizomes). **Methods:** Prepared polyherbal formulation was subjected to determine the physical constants (pH determination, refractive index, specific gravity, total solids, reducing and non reducing sugar) and HPTLC fingerprinting. **Results:** The analysis revealed the physical constant such as refractive index 1.43418, specific gravity 1.256, pH 3.5, total solids 61.50, total sugar 17.64 and reducing sugar 4.46. HPTLC fingerprinting profile showed different band patterns at different wavelength under short UV, long UV and at 620nm after derivatisation with vanillin sulphuric acid spraying reagent. Unique Rf patterns were recorded. **Conclusion:** Vachadi syrup was authenticated according to pharmacopeial standards as its analysis was important to ensure the quality of drug.

Keywords: Vachadi syrup, Polyherbal formulation, Physical constants, HPTLC fingerprinting.

INTRODUCTION

Polyherbal formulations (PHFs) are now influential drug to traditional medicine practices in India. The Ayurvedic treatise '*Sarangdhara Samhita*' explored the polyherbalism concept to achieve greater therapeutic efficacy [1]. Quality assurance of Ayurvedic formulations is an important step to study chemical profile and safety of drug, as the standardisation of drug is emerging aspect in Ayurvedic drug preparation. Leading population of world is now relies on phytotherapy for health care as per World Health Organization (WHO) [2,3]. WHO consider phytotherapy is safe, cost effective and more significant without any side effect [4]. Phytotherapy is now emerging with issues regarding their quality, safety and efficacy. WHO has given specific guidelines, for the assessment of safety, effectiveness and quality of herbal drug [5]. Ayurvedic Pharmacopoeia of India and AYUSH also provides monograph on the preparation of Ayurvedic PHFs, that aided in standardisation of drug.

A polyherbal Ayurvedic formulation (Vachadi Syrup) was prepared using six different drugs including *Acorus calamus*, *Trachyspermum ammi*, *Phyllanthus emblica*, *Terminalia bellirica*, *Terminalia chebula*, *Zingiber officinalis*. The present study is an attempt to develop and standardize Vachadi syrup (PHFs). The syrup will be used in the treatment of Pratishyaya (Rhinitis). To fulfil the requirement of the emerging trend, the prepared polyherbal formulation was subjected for standardisation as per pharmacopeial procedures.

MATERIALS AND METHODS

Collection of raw drugs

Drugs as raw drugs were purchased from of SDM Ayurveda Pharmacy Udupi, Kuthpady, Karnataka for preparation of Vachadi syrup (PHFs). The syrup was standardized and authenticated at Pharmacognosy department of SDM Center for Research in Ayurveda and Allied Sciences, Udupi, Kuthpady, Karnataka and a specimen (740/16031601) is being maintained for future reference. The prepared syrup was subjected for characterization and HPTLC finger printing.

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Preparation of Vachadi syrup

Vachadi syrup was prepared by using *Acorus calamus*, *Trachyspermum ammi*, *Phyllanthus emblica*, *Terminalia bellirica*, *Terminalia chebula* and *Zingiber officinalis*. Different parts of plants were used for preparation (Table 1). The plant materials were identified morphologically with the description given in the Quality Standards of Indian Medicinal Plants. Formulation was prepared by taking all the drugs in equal proportion. Drugs were soaked in double volume of distilled water overnight at room temperature. After 24 hours of incubation the drugs were mixed using blender and then filtered. Filtered material was then subjected for boiling at 100°C by adding 40% sugar. Syrup was boiled till it was reduced to 1/4th of whole solution. The mixed formulation was packed in dark bottles^[6-9]. Prepared Vachadi syrup (PHFs) was subjected for authentication.

Table 1: Different parts of plants used in formulation preparation

S. No.	Name of Plants	Plant part used in formulation preparation
1.	<i>Acorus calamus</i>	Rhizome
2.	<i>Trachyspermum ammi</i>	Fruit
3.	<i>Phyllanthus emblica</i>	Fruit
4.	<i>Terminalia bellirica</i>	Fruit
5.	<i>Terminalia chebula</i>	Fruit
6.	<i>Zingiber officinalis</i>	Rhizome

Determination of physical constant

Determination of physical constants like pH determination, refractive index, specific gravity, total solids, reducing and non reducing sugar was done as per the standard procedure^[10].

HPTLC finger printing

10ml of syrup was partitioned in a separating funnel with 20ml of butanol. The butanol soluble portion was evaporated to dryness and was dissolved in 10.0ml of methanol. 4, 8 and 12µl of the above sample were applied on a pre-coated silica gel F254 on aluminum plates to a band width of 8 mm using Linomat 5 TLC applicator. Vachadi syrup plate was developed in Toluene: Ethyl acetate: Acetic acid: Methanol (2.0: 3.0: 0.8: 0.2 v/v). The developed plate was visualized under short UV, long UV and after derivatisation in vanillin-sulphuric acid spray reagent^[11]. The plate was scanned at 254nm, 366nm and 620nm after post derivatisation. R_f, color of the spots and densitometric scan were recorded using CAMAG Scanner 4^[12,13].

RESULTS

Physical constants

For standardisation of Vachadi syrup pH determination, refractive index, specific gravity, total solids, reducing and non reducing sugar were determined according to AYUSH protocol (Table 2).

Table 2: Standardisation of Vachadi syrup

Parameters	Vachadi syrup
Refractive index	1.43418
Specific gravity	1.256
pH	3.5
Total solids	61.50
Total sugar	17.64
Reducing sugar	4.46

HPTLC fingerprinting

R_f values and colour of the spots in chromatogram developed in Toluene: Ethyl acetate: Acetic acid: Methanol (2.0: 3.0: 0.8: 0.2 v/v) for Vachadi syrup were recorded (Table 3). TLC photo-documentation revealed presence of many phytoconstituents with different R_f values and HPTLC densitometric scan of the plates showed numerous bands under short UV, long UV and 620 nm (after derivatisation). On photo documentation 7 spots under 254nm, 7 spots under 366nm and 9 spots under 620 nm post-derivatisation with vanillin-sulphuric acid spray reagent (Fig: 1a-1c). Densitometric scan at 254 nm revealed 6 peaks corresponding to 6 different compounds in the vachadi syrup, compounds with R_f - 0.04 (19.30%), 0.18 (6.56%), 0.24 (14.21%), 0.34 (7.98%), 0.44 (4.65%) and 0.86 (47.31%) in Figure 2a Densitometric scan at 366 nm, (Figure 2b) showed 4 peaks, peak with R_f - 0.02 (57.09%), 0.45 (25.79%), 0.91 (3.22%) and 0.97 (13.90%). Figure 3c depicts 9 peaks- with R_f - 0.02 (2.57%), 0.06 (45.96%), 0.26 (11.12%), 0.32 (2.90%), 0.44 (6.33%), 0.70 (3.86%), 0.84 (19.78%), 0.91 (6.96%) and 0.96 (0.52%) (Table 4).

Table 3: R_f values of all the samples

At 254 nm	At 366 nm	After Derivatisation
0.11 (L. green)	0.11 (F. blue)	-
-	-	0.16 (L. purple)
0.20 (L. green)	-	0.20 (L. purple)
0.23 (L. green)	-	-
-	-	0.26 (L. purple)
0.29 (L. green)	-	-
-	-	0.36 (L. purple)
0.39 (L. green)	0.39 (F. blue)	-
-	0.48 (F. blue)	0.48 (L. purple)
-	0.53 (F. blue)	-
0.76 (D. green)	0.76 (F. blue)	0.76 (L. purple)
-	-	0.79 (L. purple)
-	0.86 (F. blue)	-
0.89 (D. green)	-	-
-	0.93 (F. blue)	0.93 (L. purple)
-	-	0.96 (L. purple)

Table 4: Densitometric scan of methanolic fraction of Vachadi syrup

254nm		366nm		620nm	
R _f	% Area	R _f	% Area	R _f	% Area
-	-	0.02	57.09	0.02	2.57
0.04	19.30	-	-	-	-
-	-	-	-	0.06	45.96
0.18	6.56	-	-	-	-
0.24	14.21	-	-	-	-
-	-	-	-	0.26	11.12
-	-	-	-	0.32	2.90
0.34	7.98	-	-	-	-
0.44	4.65	0.45	25.79	0.44	6.33
-	-	-	-	0.70	3.86
-	-	-	-	0.84	19.78
0.86	47.31	-	-	-	-
-	-	0.91	3.22	0.91	6.96
-	-	0.97	13.90	0.96	0.52

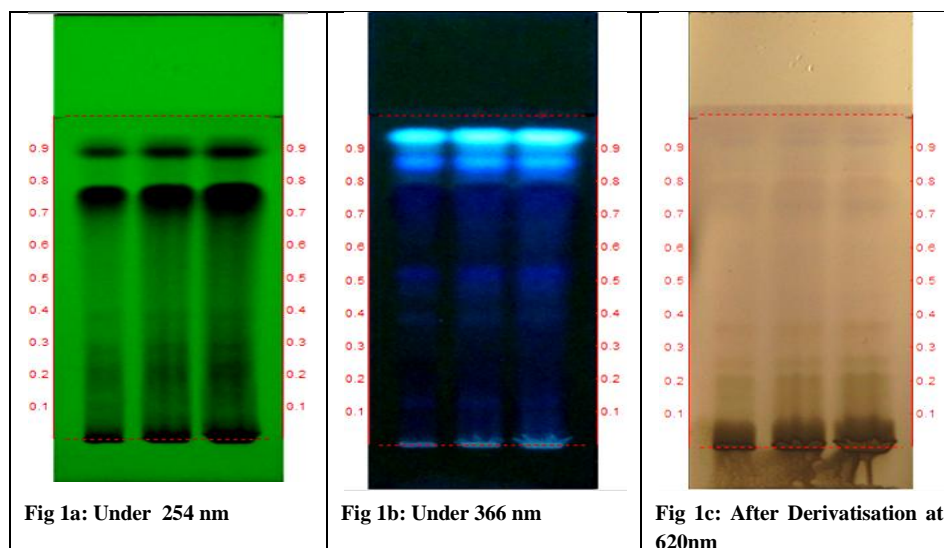


Figure: 1: HPTLC photodocumentation of methanolic fraction of Vachadi Syrup

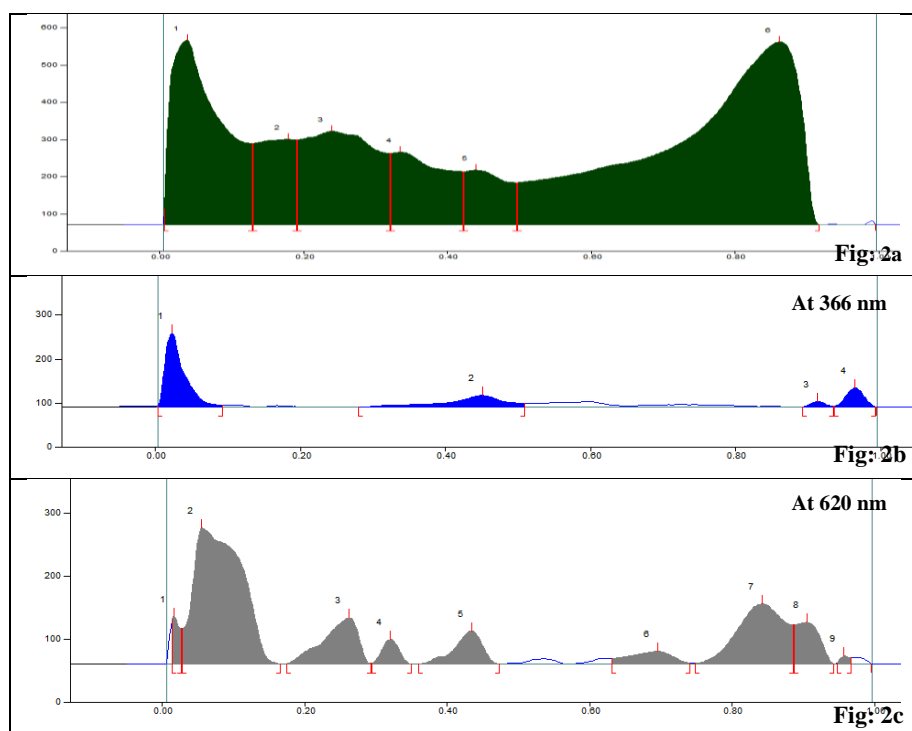


Figure: 2: HPTLC densitometric scan of methanolic fraction of Vachadi syrup

DISCUSSION

Standardisation of polyherbal formulation is specified level to assure the quality and efficacy of drug. The concept of standardisation provides a platform for scientific validation of drug. Physical constants such as refractive index (1.43), specific gravity (1.25), pH (3.5), total solids (61.50), Total sugar (17.64) and reducing sugar (4.46) for polyherbal formulation authentication are performed standard methods. HPTLC fingerprinting also revealed active constituents at different R_f values. Standardisation of Vachadi syrup reveals compliance with all the above discussed parameters and hence it can be concluded that Vachadi syrup is a well standardized product at essential pharmacopeial parameters. The results obtained from the study could be utilized as a reference for setting limits.

CONCLUSION

Characterization of the Vachadi syrup (PHFs) has been done as per pharmacopoeial methodology. The set of data obtained in the present investigation can serve as standard for the identification of syrup. The results will be helpful in sustaining and reproducibility of drug as well as to ensure the quality and safety of drug.

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