IJBPAS, September, 2022, 11(9): 4199-4205

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PRELIMINARY PHYTOCHEMICAL AND HPTLC ANALYSIS OF SUKUMARAM KASAYAM: A CLASSICAL AYURVEDIC FORMULATION

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Received 16th Nov. 2021; Revised 20th Dec. 2021; Accepted 12th Feb. 2022; Available online 1st Sept. 2022

https://doi.org/10.31032/IJBPAS/2022/11.9.6369

ABSTRACT

The objective of the present work is to investigate the phytochemicals present in of Sukumaram kasayam. Phytoconstituents investigations were carried by applying standard preliminary phytochemical tests and HPTLC. These phytochemical screenings revealed or showed that the Sukumaram kasayam contained important phytoconstituents and gave positive tests for the presence of carbohydrate, protein, phytosterol, glycoside, saponin, flavonoids, polyphenols and alkaloids. The HPTLC fingerprinting of marketed Sukumaram Kasayam showed 05 peaks having maximum R_f value 0.13, 0.46, 0.62, 0.75, 0.94 at 254 nm. Similarly the marketed Sukumaram Kasayam showed 06 peaks having maximum R_f values 0.21, 0.32, 0.47, 0.51, 0.72, 0.85 at 366nm. The information gathered from the phytochemical and HPTLC study of Sukumaram Kasayam which will serve to determine the quality and standardization of the ayurvedic formulation in the future.

Keywords: Sukumaram kasayam, phytoconstituents. phytochemical screening, HPTLC

INTRODUCTION

Sukumaram Kasayam is a classical ayurvedic medicine available in either decoction liquid form or concentrated extract tablet form. In Ayurveda, Sukumaram Kasayam are being used many ailments. It helps with indigestion, bowel spasms, upset stomach, menstruation irregularities, and female sterility, that according Ashtanga Hridayam **[1]**. Sukumaram Kashayam is also beneficial in the treatment of polycystic illness, ovarian

cysts, and uterine fibroids, among other things. Among the different ayurvedic medicines, Sukumaram Kasayam was selected to study its phytochemical constituents. The goal of this study is to explore the phytochemicals contained in Sukumaram Kasayam by using preliminary phytochemical screening and HPTLC analysis.

MATERIALS AND METHODS

Procurement of samples

The Sukumaram Kasayam (Batch No. 18B034) used in the present study was procured from the local market from the registered Ayurvedic Pharmacy

Preliminary phytochemical analysis

Therapeutic activity of ayurvedic drugs depends upon the type of constituents present in them. The decoction of ayurvedic formulation was screened for the presence of phytoconstituent(s) using different chemical tests **[2, 3, 4]**.

High performance thin layer chromatography

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve or to attain precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable and helpful quality assessment tool for the evaluation of botanical materials efficiently, accurately, and cost effectively. HPTLC method offers high degree of selectivity, sensitivity, and rapidity combined with single-step sample preparation **[5, 6]**. In addition it is a reliable, decisive method for the quantization of nanogram levels of samples. Thus this method can be conveniently, easily adopted for routine quality control analysis and investigation. It provides a chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of medicinal plant raw materials **[7, 8, 9, 10]**.

Basic steps involved in HPTLC

Extracts used : Methanol

Application mode : CAMAG Linomat 5

Development mode : CAMAG Twin Trough chamber.

Sample application

The samples were dissolved in same solvent and 10 µl quantity of sample was applied on the HPTLC silica merk 60F 254 graded plate sized 6cm x 10 cm as narrow bands using CAMAG Linomat 5 injector.

Chromatogram development

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out

according to the adsorption capability of the component to be analyzed. After elution, plates were taken out of the chamber and dried.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis

The Ayurvedic formulation was subjected to different phytochemical tests and the results obtained by all the tests procedures reveal the genuineness of the formulation. The market formulation was screened for phytochemical investigation by different phytochemical tests to check the presence or absence of a group of phytochemical constituents. These phytochemical screening shown or gave positive tests for the presence of Carbohydrate, protein, phytosterol, glycoside, saponin, flavonoids, polyphenols and alkaloids as mentioned in **(Table 1)**.

HPTLC analysis

Standardization of drugs measure confirmation of its identity, quality determination and purity. Standardisation which incorporate the important step for the establishment of a persistent biological activity and chemical profile, or commonly a quality assurance program for production and manufacturing of herbal drugs. However, it is an important condition for providing and estimating the quality and safety of polyherbal formulations since these are combinations of more than one herb to obtain the desire therapeutic effect.

High Performance Thin Layer Chromatography (HPTLC) technique is most simple, elementary and fastest separation technique or approach available today which gives better, improved, precision and accuracy with extreme flexibility and resilience for various steps. HPTLC profile generated and achieved in this study provides a standard tool for the authenticity of Ayurvedic polyherbal formulation and genuineness of the final product.

The HPTLC fingerprinting of marketed Sukumaram Kasayam showed 05 peaks having maximum R_f value 0.13, 0.46, 0.62, 0.75, 0.94 at 254 nm shown in **Figure 1 and Table 2**. Similarly the marketed Sukumaram Kasayam showed 06 peaks having maximum R_f values 0.21, 0.32, 0.47, 0.51, 0.72, 0.85 at 366nm shown in **Figure 2 and Table 3**.

Depending on the number of finger prints we can depicts the number of active principles. This HPTLC technique may be useful for the identification and the quality evaluation of ayurvedic preparations.

Table 1: Preliminary phytochemical analysis of Sukumaram Kasayam	
Phytochemical tests	**Marketed formulation**
Test for Cabohydrates	
1. Molisch's Test	+
2. Fehling's Test	+
3. Benedict's Test	+
4. Barfoed's Test	+
5. Aniline Acetate Test	-
6.Bial's test	-
Test for Proteins and Amino Acid	
1. Ninhydrin Test	+
2. Xanthoprotein Test	+
Test for Fixed Oils and Fats	
1. Spot Test	-
Test for Phytosterols	
1. SalkowSKi's Test	-
2. Libermann-Burchard's Test	+
Test for Glycosides	
1. Baljet's Test	+
2. Legal's Test	+
3. Borntrager Test	-
Test for Saponins	
1. Foam Test	+
2. Froth Test	++
Test for Flavonoids	
1. Ferric Chloride Test	++
2. Lead Acetate Test	++
Test for Tannins and Phenolic Compounds	
1.Goldbeater SKin Test	++
2.Gelatin Test	+
Test for Alkaloids	
1. Mayer's Test	+
2. Dragendorff's Test	-
3. Wagner's Test	-
4. Hager's Test	+

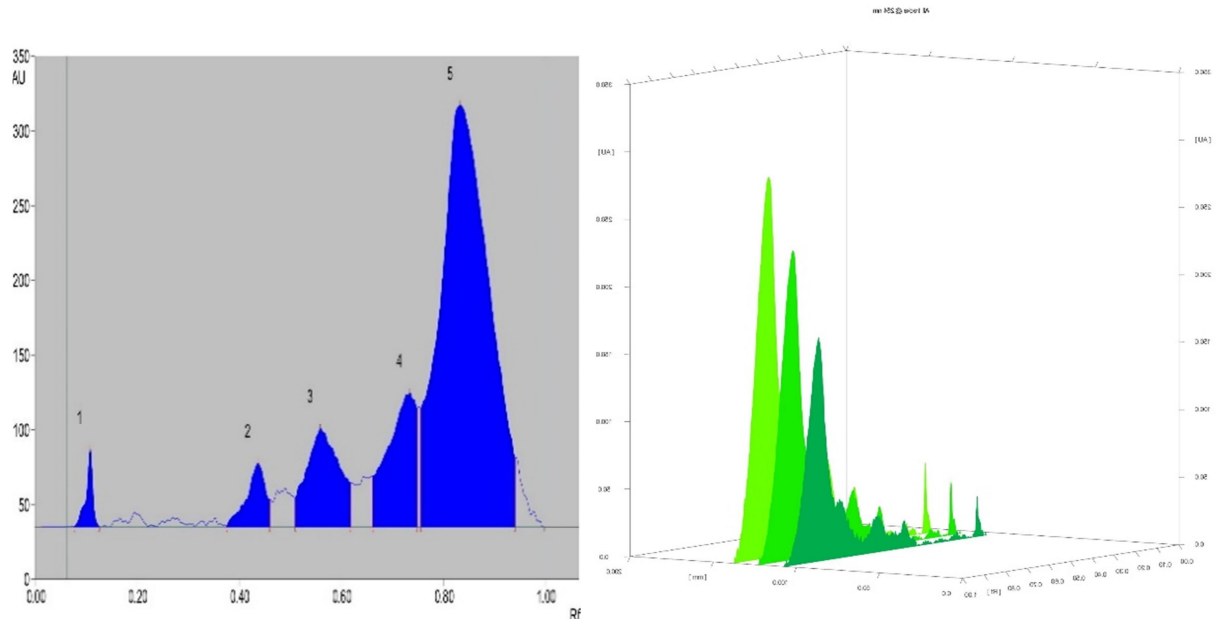

Figure 1: HPTLC chromatogram of Sukumaram Kasayam extract at 254 nm

Table 2: HPTLC spectral analysis of in-house Sukumaram Kasayam at 254 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.08 Rf	0.1 AU	0.11 Rf	52.1 AU	9.77 %	0.13 Rf	0.1 AU	584.4 AU	1.74 %	unknown *
2	0.38 Rf	0.6 AU	0.44 Rf	42.8 AU	8.04 %	0.46 Rf	3.2 AU	1355.5 AU	4.04 %	unknown *
3	0.51 Rf	19.2 AU	0.56 Rf	66.0 AU	12.39 %	0.62 Rf	9.6 AU	3697.6 AU	11.02 %	unknown *
4	0.66 Rf	34.1 AU	0.74 Rf	89.7 AU	16.84 %	0.75 Rf	9.2 AU	4296.7 AU	12.81 %	unknown *
5	0.76 Rf	79.3 AU	0.83 Rf	282.1 AU	52.97 %	0.94 Rf	3.1 AU	3614.2 AU	10.39 %	unknown *

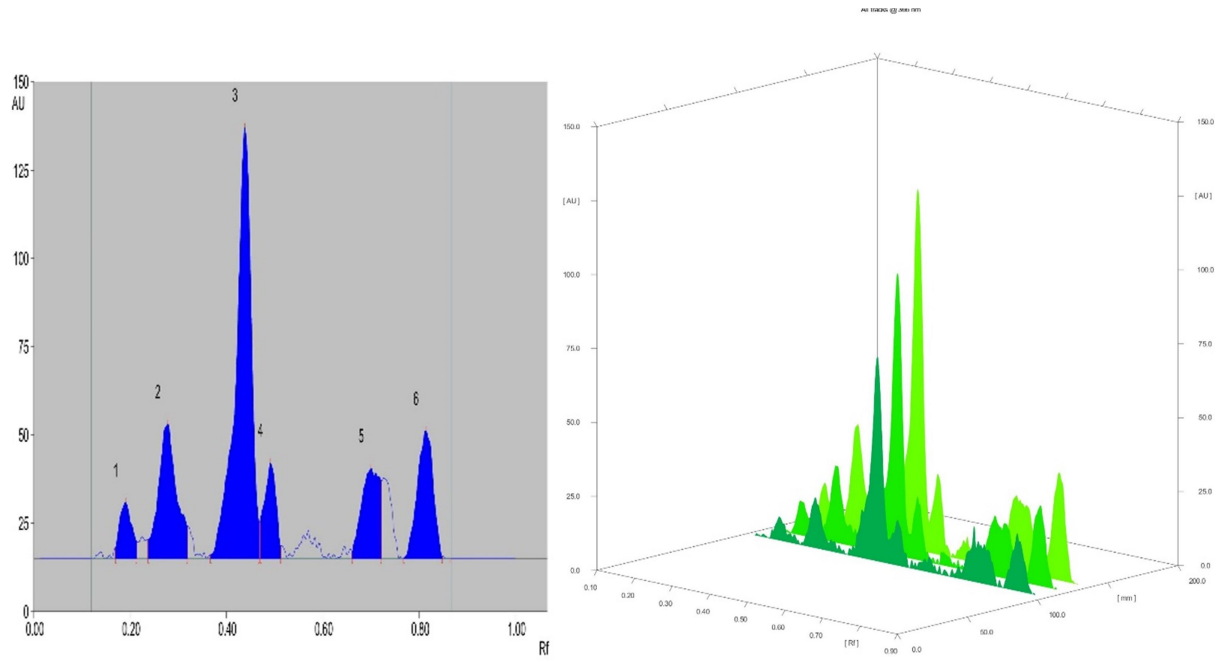

Figure 2: HPTLC chromatogram of SukumaramKasayam extract at366 nm

Table 3: HPTLC spectral analysis of in-house Sukumaram Kasayam at 366 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.17 Rf	3.1 AU	0.19 Rf	15.9 AU	6.00 %	0.21 Rf	4.7 AU	356.8 AU	4.77 %	unknown *
2	0.24 Rf	5.3 AU	0.28 Rf	38.1 AU	14.38 %	0.32 Rf	9.1 AU	248.6 AU	16.69 %	unknown *
3	0.37 Rf	1.0 AU	0.44 Rf	22.0 AU	16.07 %	0.47 Rf	0.2 AU	1415.0 AU	15.66 %	unknown *
4	0.47 Rf	0.8 AU	0.49 Rf	27.1 AU	10.25 %	0.51 Rf	3.5 AU	573.7 AU	7.67 %	unknown *
5	0.66 Rf	1.9 AU	0.70 Rf	25.5 AU	9.62 %	0.72 Rf	2.0 AU	840.6 AU	11.24 %	unknown *
6	0.77 Rf	0.0 AU	0.81 Rf	36.2 AU	13.68 %	0.85 Rf	0.5 AU	1045.3 AU	13.97 %	unknown *

CONCLUSION

The present work focuses on the phytochemical screening of Sukumaram Kasayam. As there was no phytochemical work in this classical ayurvedic drugs, present work is taken up in the view to lay down the presence of phytochemicals, which could be used in deciding the genuineness and effectiveness of the selected ayurvedic drug. The phytochemical screening provides the evidence of saponin glycoside, flavonoids and tannin in marketed formulation which may contributes in curing the diseases mentioned in literatures. The phytochemical and HPTLC data obtained from the results might be useful in the determining the authenticity of the drugs.

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