

Comparative Chemical Profiling of a Polyherbal Formulation with Respect to its Ingredient Plants Using Spectrophotometric and High-performance Thin-layer Chromatographic Techniques

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Abstract

Background: Guggulutiktam Kashayam (GTK) is a water decoction used in Ayurveda for the treatment of various diseases like skin diseases, ulcers, anemia, thyroid problems, stress, liver disease, and leucorrhoea. It is a polyherbal formulation prepared from specific parts of 28 medicinal plants.

Objectives: The study aimed at a comparative phytochemical evaluation of an important polyherbal formulation with its ingredient plants.

Materials and Methods: Chemical profiling of GTK has been carried out in comparison with its ingredient plants. Spectrophotometric estimation of major class compounds and high-performance thin-layer chromatography (HPTLC) profiling were done using a standardized protocol.

Results: The results of the study showed that the phytochemical contents of the finished formulation are different from those of the ingredient plants, which might be due to the numerous synergistic and antagonistic interactions of numerous chemical components extracted from different ingredient drugs. HPTLC-based comparative chromatograms showed the differences and similarities in certain compounds that are specific to some of the plant ingredients.

Conclusion: The study established the cumulative chemical interactions of various phytochemicals during the preparation of an important polyherbal formula used in Ayurveda.

Keywords

Chemical profiling, polyherbal formulation, phytochemistry, HPTLC

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Introduction

The majority of Ayurvedic formulations are polyherbal in nature and contain several ingredients in specific proportions, as prescribed in the ancient Ayurvedic classics. Polyherbal formulations (PHFs) are considered to be more active due to the presence of multiple ingredients, which is commonly known as polyherbalism (Sulaiman et al., 2021). Guggulutiktam Kashayam (GTK) is one of the well-known classical PHFs used to treat skin diseases, ulcers, anemia, thyroid problems, stress, liver disease, and leucorrhoea. Many phytochemicals, including phenolics, flavonoids, coumarins, and catechins, have been reported from this

formulation (Sulaiman et al., 2020). The phytochemicals present in the finished PHFs are cumulative chemical entities after many synergistic and antagonistic chemical

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interactions. The chemical pattern of any finished formulation is different from that of its individual ingredient plants due to the herb–herb interaction that normally leads to the synergistic chemical transformation of many of the phytochemicals extracted from the various ingredient raw drugs. The range of such interactions depends on the nature of the raw drugs used and the method of preparation. In most cases, Ayurvedic formulations are prepared by prolonged heating of the different raw drugs in boiling water. Another phenomenon, antagonism, in which the effect of some compounds is nullified by other constituents, is also happening during the PHF preparation (Ministry of Health and Family Welfare, 2003).

The therapeutic efficacy of any herbal preparation depends on the various chemical constituents present in it that are capable of possessing the desired biological action. Chemical profiling of finished PHFs is one of the most reliable methods to correlate their active constituents with their pharmacological activities. Comparative phytochemical profiling with raw ingredient drugs may provide a preliminary understanding of the synergism and antagonism occurring during the preparation of the formulation. High-performance thin-layer chromatography (HPTLC) is a strong analytical tool for the comparative profiling of PHFs with their raw drugs. Chromatograms with good resolution can be used to distinguish the presence or absence of individual chemical constituents in PHF with respect to the individual ingredient plants. HPTLC techniques have been widely used for the comparative chemical profiling of plants and herbal products (Akhtar et al., 2022; Parasuraman et al., 2014; Singh et al., 2014). In this study, a comparative chemical evaluation of an Ayurvedic PHF has been carried out by various spectrophotometric and HPTLC analyses. The study has been conducted to ensure the quality of the selected finished formulation with respect to its ingredients. A comparative phytochemical evaluation of this formulation with its ingredient drugs has not yet been reported.

Materials and Methods

GTK was obtained from the Product Development Department of the institute. Aluminium-backed precoated HPTLC plates Si 60F₂₅₄, and all the analytical-grade solvents were procured from Merck India Pvt. Ltd. Gallic acid was procured from Sigma Aldrich Chemicals.

Ingredient plant materials for GTK were obtained from the Materials Department of the institute, and all the materials were authenticated by the Pharmacognosy Division of the institute. Voucher specimens were deposited in the Raw Drug Repository (Table 1).

Preparation of Extracts

Ten grams of each of the shade-dried materials were ground into coarse powder and refluxed with 100 mL of distilled water for 6 h. It was then filtered and kept in the refrigerator until various phytochemical analyses.

Physicochemical Screening

Physicochemical tests such as total ash, acid-insoluble ash, water-insoluble ash, sulfated ash, alcohol-soluble extractive, and water-soluble extractive of all the ingredient plants were done as per the standard procedures of the Ayurvedic Pharmacopoeia of India (The Ayurvedic Pharmacopoeia of India, 2001).

Estimation of Phenolics

Major phytochemicals were estimated using an ultraviolet (UV)-visible spectrophotometer (Shimadzu, Japan). Total phenolic content was estimated for GTK and its ingredient plants. The Folin-Ciocalteu method was used for the total phenol estimation (Singleton & Rossi, 1965; Sulaiman & Balachandran, 2012; Sulaiman et al., 2014; Sulaiman et al., 2020). The values were calculated with respect to standard gallic acid.

HPTLC Profiling

HPTLC analysis was performed using a CAMAG HPTLC system (Switzerland) equipped with an automatic sample applicator (CAMAG ATS-IV) on an aluminium-backed, precoated silica gel 60F₂₅₄ HPTLC plate (Merck India). The stock solution of GTK was prepared by sonicating 2 g in 10 mL of distilled water using an ultrasound bath. The mobile phase was optimized as per the previous method with slight modifications (Sulaiman et al., 2020; Sulaiman et al., 2022). Ideal separation was obtained with the mobile phase containing toluene, ethyl acetate, and methanol in the ratio 7:3:1. Comparative HPTLC profiling of aqueous extracts of ingredient plants along with GTK was done in triplicate with the same mobile phase. The chromatogram was developed in a twin-trough glass chamber presaturated for 5 min at room temperature with the optimized mobile phase. After development, the plates were dried and scanned using CAMAG HPTLC Scanner-III at UV-254, UV-366, and in visible light after derivatizing with anisaldehydesulphuric acid reagent. All the developed plates were documented by the CAMAG HPTLC photo-documentation system.

Table 1. List of Ingredient Plants of GTK.

Ingredient Plants	Sample Code	Raw Drug Repository Number
<i>Aconitum heterophyllum</i> Wall. ex. Royle	AH	RDR/AYUSHCOE/26
<i>Aconitum palmatum</i> D. Don	AP	RDR/AYUSHCOE/27
<i>Acorus calamus</i> L.	AC	RDR/AYUSHCOE/22
<i>Azadirachta indica</i> A. Juss.	AI	RDR/AYUSHCOE/01
<i>Cedrus deodara</i> (Roxb. ex Lamb.) G. Don	CD	RDR/AYUSHCOE/08
<i>Cissampelos pareira</i> L.	CP	RDR/AYUSHCOE/06
<i>Commiphora wightii</i> (Arn.) Bhandari	CW	RDR/AYUSHCOE/29
<i>Curcuma longa</i> L.	CL	RDR/AYUSHCOE/11
<i>Dolomiaea costus</i> (Falc.) Kasana & A.K. Pandey	DC	RDR/AYUSHCOE/14
<i>Embelia tsjeriam-cottam</i> (Roem. & Schult.) A. DC.	ET	RDR/AYUSHCOE/07
<i>Foeniculum vulgare</i> Mill.	FV	RDR/AYUSHCOE/12
<i>Holarrhena pubescens</i> Wall. ex G. Don	HP	RDR/AYUSHCOE/17
<i>Justicia adhatoda</i> L.	JA	RDR/AYUSHCOE/03
<i>Picrorhiza kurroa</i> Royle ex Benth.	PK	RDR/AYUSHCOE/20
<i>Piper longum</i> L.	PL	RDR/AYUSHCOE/23
<i>Piper nigrum</i> L.	PN	RDR/AYUSHCOE/16
<i>Piper retrofractum</i> Vahl	PR	RDR/AYUSHCOE/13
<i>Pluchea lanceolata</i> (DC.) C.B. Clarke	PLT	RDR/AYUSHCOE/24
<i>Plumbago zeylanica</i> L.	PZ	RDR/AYUSHCOE/19
<i>Rubia cordifolia</i> L.	RC	RDR/AYUSHCOE/25
<i>Scindapsus officinalis</i> (Roxb.) Schott	SO	RDR/AYUSHCOE/09
<i>Semecarpus anacardium</i> L.f.	SA	RDR/AYUSHCOE/21
<i>Solanum virginanum</i> L.	SV	RDR/AYUSHCOE/05
<i>Tinospora cordifolia</i> (Willd.) Hook.f. & Thomson	TC	RDR/AYUSHCOE/02
<i>Trachyspermum ammi</i> (L.) Sprague	TA	RDR/AYUSHCOE/28
<i>Trichosanthes dioica</i> Roxb.	TD	RDR/AYUSHCOE/04
<i>Zanthoxylum armatum</i> DC.	ZA	RDR/AYUSHCOE/15
<i>Zingiber officinale</i> Roscoe	ZO	RDR/AYUSHCOE/10

Abbreviations: GTK, guggulutiktam kashayam.

Results

This study was carried out to identify the major class of chemical compounds in the extracts and distinguish the presence or absence of individual chemical constituents in PHF with respect to the individual ingredient plants.

Physicochemical Screening

Physicochemical properties such as total ash, acid-insoluble ash, water-insoluble ash, sulfated ash, water-soluble extractive, and alcohol-soluble extractive were evaluated in all the ingredient drugs of GTK, and the results are presented in Table 2. The physicochemical parameters of raw herbal drugs are an important set of quality markers as they ensure

the required quality of raw materials (Jeevitha et al., 2021). The Ayurvedic Pharmacopoeia of India (API) is the regulatory document that fixes the quality parameters of raw drugs. All the samples were analyzed to confirm their authenticity and quality.

Estimation of Major Phytochemicals

Medicinal plants are rich sources of polyphenols with diverse biological properties. Quantitative estimation of total polyphenols provides the preliminary chemical pattern of the plant extracts. Spectrophotometric methods have been employed for the estimation of total polyphenols in herbal drugs and finished formulations (Dinakaran et al., 2018; Sulaiman et al., 2021). The total phenolics (TPC) of all the

Table 2. Physicochemical Parameters of Ingredient Plants of GTK.

Sample	Total Ash (%)	Water-insoluble Ash (%)	Acid-insoluble Ash(%)	Sulfated Ash (%)	Water Extractive Value (%)	Alcohol Extractive Value (%)
AH	0.94	1.42	0.51	3.65	33.32	10.90
AP	7.89	5.58	2.5	12.04	31.17	8.75
AC	6.59	2.42	0.59	8.6	36.09	18.16
AI	4.82	4.2	0.579	6.79	9.57	10.2
CD	0.33	0.1914	0.1097	0.54	5.77	7.61
CP	7.5	6.38	3.66	9.43	14.61	10.28
CW	7.57	6.27	2.81	10.93	93.53	3.22
CL	6.19	3.47	1.1	7.7	15.76	6.6
DC	4.2	2.9	1.9	6.07	32.2	25.47
ET	4.1392	1.1	0.152	5.75	9.28	10.1
FV	7.74	4.2	0.316	12.51	18.86	7.94
HP	6.24	3.68	0.2	8.69	15.15	9.73
JA	2.09	5.54	0.92	3.08	5.86	3.17
PK	4.66	3.17	1.34	6.01	38.94	31.84
PL	8.56	4.766	0.2076	10.81	24.98	16.68
PN	5.15	1.73	0.18	7.49	12.31	9.92
PR	6.55	3.75	0.89	9	11.41	6.609
PLT	16.75	5.27	2.25	19.77	30.47	10.04
PZ	3.17	1.9	0.89	4.76	13.37	12.08
RC	6.89	3.04	0.22	8.87	21.33	14.44
SO	11.509	4.1	0.08	17.01	20.53	4.29
SA	7.20	6.48	3.43	11.26	1.40	6.43
SV	10	7.37	0.245	13.88	11.89	5.22
TC	9.39	7.21	0.568	11.68	11.52	5.86
TA	7.57	4.163	0.243	9.57	31.22	6.27
TD	19.46	10.60	0.49	21.38	28.76	7.07
ZA	9.94	3.18	0.38	9.58	12.28	10.66
ZO	6.01	2.89	1.3	8.86	19.99	5.64

Abbreviation: GTK, guggulutiktam kashayam.

ingredient plants, along with GTK, were estimated. The TPC was calculated from the calibration curve of gallic acid ($R^2 = 0.999$, Figure 1). The TPC contents of GTK and its ingredient plants are given in Table 3.

The highest TPC was shown by *Justicia adhatoda* (JA) (39.42 ± 0.468), followed by *Scindapsus officinalis* (SO) (37.29 ± 1.248), *Zingiber officinale* (ZO) (23.50 ± 0.316), and *Pluchea lanceolata* (PLT) (17.821 ± 0.1010). The lowest value was observed for *Piper longum* (PL) (2.133 ± 0.008), followed by *Rubia cordifolia* (RC) (2.20 ± 0.010), and *Azadirachta indica* (AI) (2.23 ± 0.104). The TPC of GTK was found to be 2.46 ± 0.028 , and the value is much lower

compared to that of other ingredient plants. This might be due to the chemical transformations that occurred due to the synergistic and antagonistic interactions of various polyphenols extracted from the ingredient plants. Variations in phenolics with respect to the herbal drug samples have been reported earlier (Mehmood et al., 2022; Siddiqui et al., 2017; Tungmunthum et al., 2018); however, no previous reports are available on the comparative estimation of the polyphenols with respect to the finished formulation. Synergistic interactions of polyphenols with respect to the ratio of plant materials have been reported earlier (Aslam et al., 2017). More studies are required to establish the effect of

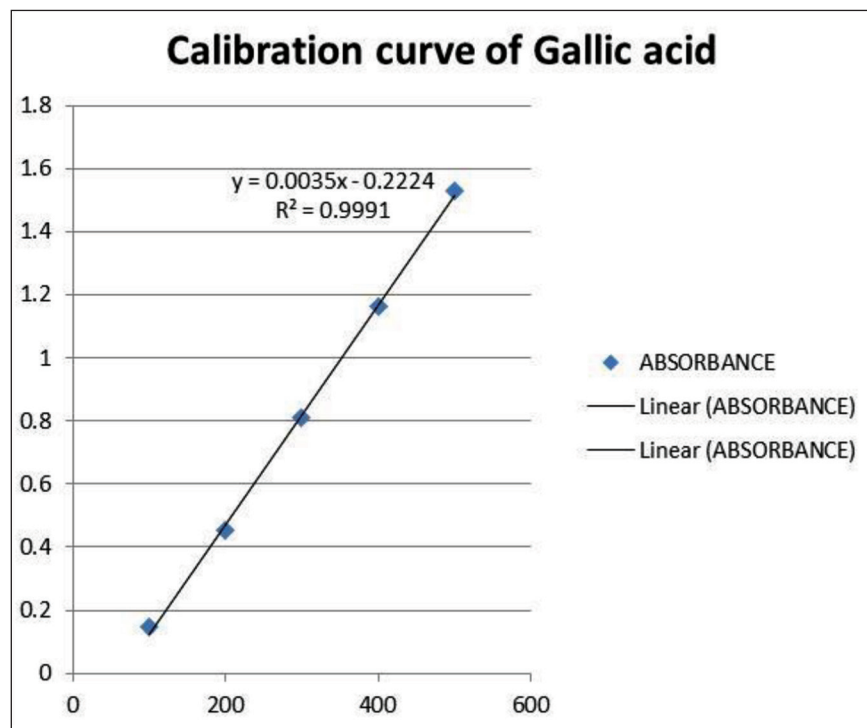


Figure 1. Calibration of Curve of Gallic Acid Standard.

various raw drugs on the total phenolic composition of finished PHFs.

HPTLC Profiling

HPTLC has emerged as an important analytical tool for the qualitative and quantitative phytochemical analysis of herbal raw drugs and PHFs (Lalhriatpuii, 2020). Comparative HPTLC profiling has been carried out for GTK with all the ingredient plants. The chromatogram documented under various UV visualizations was compared with respect to the R_f values. Chemical quenching at UV-254 nm and fluorescence at UV-366 nm have complied, and the data is presented in Table 4. On evaluating under UV-254, bands with R_f 0.03, 0.09, 0.11, 0.17, 0.22, 0.29, 0.47, 0.57, 0.70, and 0.77 have been observed in GTK, and out of these compounds with R_f 0.03 (AP, AC, PN, RC), 0.09 (PK, SA, ZA, SO, TC, TD), 0.11 (AH, PK, JA, ZO), 0.17 (PN, RC), 0.29 (SA, CL), 0.70 (SA, ZA), and 0.77 (CD) are identified. On evaluating under UV-366 nm, bands with R_f 0.06, 0.12, 0.20, 0.21, 0.28, 0.31, 0.37, 0.40, 0.47, 0.50, 0.53, 0.57, 0.64, 0.69, 0.77, and 0.82 have been observed in GTK. Out of these compounds with R_f 0.06 (PN, CD, SA), 0.12 (PTA, TA), 0.28 (CL), 0.31 (CW), 0.37 (SA), 0.50 (SA), 0.64 (CD, PN, PR), 0.69 (PZ), and 0.82 (SA) are identified (Figure 2).

Comparative chemical profiling showed that there are differences in the chemical pattern of the finished formulation

with respect to the chemical constituents of all the ingredient plants. The changes might be due to the chemical interactions of various phytochemicals extracted from the different raw drugs. Evidence is reported to support the occurrence of synergy within a total extract of a single herb as well as between different herbs in a formulation. Positive and negative aspects of interactions, including antagonistic reactions, have also been reported (Hemaiswarya et al., 2022; Williamson, 2001; Zhou et al., 2016). The present study also supports the previous reports on the herb-herb interaction and synergistic and antagonistic chemical reactions possibly happening during the preparation of PHFs.

Discussion

The majority of the herbal formulations used in Ayurveda are polyherbal in nature and contain multiple ingredient plants. Quality standardization of each ingredient drug is very important as it directly affects the quality and safety of the finished formulation. The phytochemical standardization achieved by multiple chemical profiling, along with preliminary screening and quantification of marker compounds, can assure the reproducibility of the activity of the PHFs. The clinically pertinent scientific data to support the asserted synergistic therapeutic effects of Ayurvedic PHFs needs to be established (Tungmunthum et al., 2018).

Table 3. Spectrophotometric Estimation of TPC in GTK and its Ingredient Drugs.

Plant/Formulation	Phenolic Content
GTK	2.46 ± 0.02
AH	3.25 ± 0.10
AP	6.36 ± 0.13
AC	3.46 ± 0.02
AI	2.23 ± 0.10
CD	2.89 ± 0.01
CP	7.64 ± 0.07
CW	6.69 ± 0.36
CL	2.93 ± 0.03
DC	4.13 ± 0.10
ET	7.995 ± 0.02
FV	5.277 ± 0.11
HP	9.177 ± 0.05
JA	39.42 ± 0.46
PK	16.05 ± 0.10
PL	2.133 ± 0.12
PN	9.355 ± 0.07
PR	5.0072 ± 0.05
PLT	17.821 ± 0.10
PZ	13.531 ± 0.06
RC	2.20 ± 0.01
SO	37.29 ± 1.24
SA	2.609 ± 0.07
SV	2.74 ± 0.01
TC	4.75 ± 0.09
TA	5.176 ± 0.05
TD	3.28 ± 0.04
ZA	7.93 ± 0.05
ZO	23.50 ± 0.31

Abbreviations: TPC, total phenolics; GTK, guggulutiktam Kashayam.

Phytochemical studies have been reported in some other finished formulations like Pathyashadangam Kwath, Trikatu Churna, and so on (Govindarajan et al., 2016; Abraham et al., 2020; Karigar, 2021; Kurian et al., 2021). In this study, a comparative phytochemical evaluation of the ingredient plants of an important PHF has been carried out using various chromatographic and spectrophotometric techniques. Quality standardization has been ensured with the API (Dinakaran et al., 2019). Major classes of compounds, such as TPC and flavonoids, were estimated by photometric assays. Chemical fingerprints have been compared by HPTLC analysis. Comparative evaluation of PHFs with respect to their

ingredient plants is a novel analytical method to ensure their quality and to understand chemical transformations happening during the preparation of the product. The chemical constituents of the finished formulations are the cumulative compounds extracted after several interactions among the various phytoconstituents from different ingredient plants (Sulaiman et al., 2021). The present study also confirms the synergistic and antagonistic reactions occurring in polyherbal preparations. In the case of TPC and flavonoids, the value of GTK is much lower compared to that of individual drugs. The banding pattern of HPTLC also confirms the possibility of such interactions in the formulation. Certain specific

Table 4. HPTLC R_f Values of Various Compounds Separated from GTK and its Ingredient Plants.

Plant/Formulation	254 nm	366 nm
GTK	0.03, 0.09, 0.11, 0.17, 0.22, 0.29, 0.47, 0.57, 0.70, 0.77	0.06, 0.12, 0.20, 0.21, 0.28, 0.31, 0.37, 0.40, 0.47, 0.50, 0.53, 0.57, 0.64, 0.69, 0.77, 0.82
AH	0.04, 0.06, 0.08, 0.11, 0.16, 0.24, 0.27, 0.81	0.02, 0.09, 0.11, 0.26, 0.31, 0.49
AP	0.03, 0.06, 0.23, 0.26	0.03, 0.05, 0.08, 0.13, 0.25, 0.48
AC	0.03, 0.05	0.04, 0.11, 0.17, 0.20, 0.31, 0.36, 0.72
AI	0.10, 0.16, 0.26	0.05, 0.08, 0.16, 0.26
CD	0.12, 0.14, 0.18, 0.24, 0.27, 0.3, 0.35, 0.38, 0.45, 0.52, 0.65, 0.67, 0.77	0.06, 0.17, 0.22, 0.33, 0.64
CP		0.09, 0.13, 0.15
CW	0.65, 0.72, 0.76	0.05, 0.07, 0.12, 0.18, 0.2, 0.24, 0.31, 0.50, 0.56, 0.61, 0.74, 0.8, 0.89
CL	0.04, 0.10, 0.23, 0.29, 0.36, 0.42, 0.60, 0.65	0.04, 0.24, 0.28, 0.42, 0.60, 0.68
DC		0.07, 0.09, 0.10, 0.28, 0.40
ET	0.07	0.07, 0.10, 0.35, 0.89
FV	0.23, 0.27, 0.39	0.05, 0.10, 0.18, 0.21, 0.46, 0.58
HP	0.04, 0.10, 0.24	0.10, 0.19, 0.22, 0.31, 0.44
JA	0.04, 0.11	0.04, 0.11, 0.31, 0.41, 0.65
PK	0.09, 0.11, 0.27, 0.61	0.07, 0.11, 0.15, 0.24, 0.30, 0.33, 0.43, 0.52, 0.67, 0.9
PL	0.08, 0.15, 0.18	0.04, 0.06, 0.08, 0.14, 0.19
PN	0.03, 0.04, 0.17, 0.32, 0.44, 0.64	0.03, 0.06, 0.09, 0.17, 0.19, 0.25, 0.44, 0.64, 0.74
PR	0.04, 0.16	0.06, 0.64
PLT	0.12, 0.21	0.12, 0.15, 0.17, 0.21, 0.44, 0.89
PZ	0.15, 0.25, 0.69	0.04, 0.09, 0.16, 0.18, 0.25, 0.31, 0.69
RC	0.03, 0.13, 0.21, 0.76	0.03, 0.04, 0.06, 0.09, 0.20, 0.23, 0.26, 0.35, 0.45, 0.67, 0.76
SO	0.09, 0.16,	
SA	0.05, 0.09, 0.13, 0.20, 0.25, 0.29, 0.39, 0.49, 0.63, 0.70, 0.80, 0.89	0.06, 0.14, 0.29, 0.63, 0.82, 0.88
SV	0.12	0.02, 0.14
TC	0.01, 0.09, 0.13, 0.16, 0.25	0.26, 0.9
TA	0.12, 0.19, 0.26	0.05, 0.12, 0.24, 0.45, 0.52, 0.66
TD	0.09, 0.14, 0.23	
ZA	0.07, 0.09, 0.11, 0.14, 0.18, 0.24, 0.27, 0.34, 0.41	0.04, 0.14, 0.21, 0.25, 0.30, 0.35, 0.39, 0.45, 0.55, 0.67, 0.74
ZO	0.11, 0.14, 0.19	

Abbreviations: HPTLC, high-performance thin-layer chromatography; GTK, guggulutiktam kashayam.

compounds from individual plants were also identified in the formulation, and some other bands were found to be absent in the formulation. This might be due to the chemical transformations and interactions that happened during the preparation.

Conclusion

The phytochemical evaluation of PHF is the very first step in the correlation of their biological properties with the active

compounds identified. In the case of Ayurvedic formulations, all the compounds are important, irrespective of their abundance. Hence, comparative chromatographic fingerprinting is a useful tool to understand the chemical pattern of formulations along with ingredient drugs. In this study, comparative phytochemical studies have been carried out in an important Ayurvedic formulation with respect to all of the ingredient drugs. Quality standardization of all the raw materials was done as per the pharmacopoeial standards. Major groups of chemicals, such as total phenols and total flavonoids, have been estimated, and

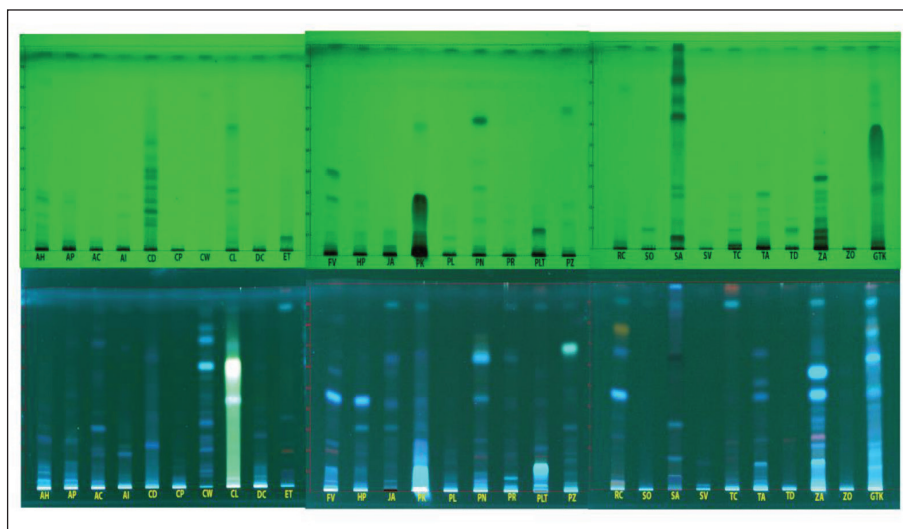


Figure 2. Comparative HPTLC Profiling of GTK with its Ingredient Drugs. Tracks: AH, AP, AC, AI, CD, CP, CW, CL, DC, ET, FV, HP, JA, PK, PL, PN, PR, PLT, PZ, RC, SO, SA, SV, TC, TA, TD, ZA, ZO, GTK.

Abbreviations: HPTLC, high-performance thin-layer chromatography; JA, *justicia adhatoda*; SO, *scindapsus officinalis*; ZO, *zingiber officinale*; PLT, *pluchea lanceolata*; PL, *piper longum*; RC, *rubia cordifolia*; AI, *azadirachta indica*; CD, *cedrus deodara*; CP, *cissampelos pareira*; CW, *commiphora wightii*; CL, *curcuma longa*; DC, *dolomiaea costus*; ET, *embelia tsjeriam-cottam*; FV, *foeniculum vulgare*; HP, *holarrhena pubescens*; PK, *picrorhiza kurroa*; PN, *piper nigrum*; PR, *piper retrofractum*; PZ, *plumbago zeylanica*; SA, *semecarpus anacardium*; SV, *solanum virginianum*; TC, *tinospora cordifolia*; TA, *trachyspermum ammi*; TD, *trichosanthes dioica*; ZA, *zanthoxylum armatum*; AH, *aconitum heterophyllum*; AP, *aconitum palmatum*; AC, *acorus calamus*; GTK, *guggulutiktam kashayam*.

chromatographic comparison was done by HPTLC profiling. The result of the study showed the differences in the chemical constituents of the finished formulation when compared to its ingredient drugs, which provide a lead for further studies to confirm the synergy and antagonism in the selected formulation.

Summary

GTK is an important Ayurvedic PHF that contains a variety of phytoconstituents with numerous biological properties.

Abbreviations

GTK: *guggulutiktam kashayam*; HPTLC: high-performance thin-layer chromatography; API: ayurvedic pharmacopoeia of India; TPC: total phenolic content; GaE: gallic acid equivalent; PHF: poly herbal formulation; ATS: automatic TLC sampler; AH: *aconitum heterophyllum*; AP: *aconitum palmatum*; AC: *acorus calamus*; AI: *azadirachta indica*; CD: *cedrus deodara*; CP: *cissampelos pareira*; CW: *commiphora wightii*; CL: *curcuma longa*; DC: *dolomiaea costus*; ET: *embelia tsjeriam-cottam*; FV: *foeniculum vulgare*; HP: *holarrhena pubescens*; JA: *justicia adhatoda*; PK: *picrorhiza kurroa*; PL: *piper longum*; PN: *piper nigrum*; PR: *piper retrofractum*; PLT: *pluchea lanceolata*; PZ: *plumbago zeylanica*; RC: *rubia cordifolia*; SO: *scindapsus officinalis*; SA: *semecarpus anacardium*; SV: *solanum virginianum*; TC: *tinospora cordifolia*; TA: *trachyspermum ammi*; TD: *trichosanthes dioica*; ZA: *zanthoxylum armatum*; ZO: *zingiber officinale*.

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