ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

DETERMINATION OF POLYPHENOLIC COMPOUNDS PRESENT IN *GUDUCHYADI KASHAYA* USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS)

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Article info

Article History:
Received: 12-12-2023
Accepted: 15-01-2024
Published: 04-02-2024

KEYWORDS:
Guduchyadi kashaya, LC-MS/MS, Polyphenolic compounds, Polyphenolic profiling, Phytochemical analysis.

ABSTRACT

The utilization of various contemporary analytical methods has become indispensable when it comes to assessing polyherbal Ayurvedic formulations, aiming to ensure their quality, safety, and effectiveness. Quality control of herbal medicines involve analytical evaluation using instrumental techniques such as TLC, HPTLC, HPLC, GC-MS, LC-MS and spectrophotometer etc. Among them Liquid Chromatography with tandem Mass Spectrometry (LC-MS/MS) is a powerful hyphenated analytical technique that combines the separating power of liquid chromatography with the mass analysis capabilities of mass spectrometry. Generally, its application is oriented towards the specific detection and potential identification of chemicals in a complex mixture. Polyphenolic compounds are usually referred to as a diverse group of naturally occurring compounds containing multiple phenolic functionalities. The anti-inflammatory, anti-aging, antiproliferative, and antioxidant like biological properties of phenolic compounds have been described in several studies. This research study was taken to analyse polyphenolic compounds in *Guduchyadi kashaya* by LC-MS/MS. Phenolic compounds are abundant in the ingredients of *Guduchyadi kashaya*, as evidenced by previous studies. So, to determine the polyphenolic contents of *Guduchyadi kashaya*, this study was conducted. The quantification of the polyphenols was carried out on Shimadzu Shim Pack GISS C18 column with 0.1% formic acid as mobile phase. A total of 28 polyphenolic compounds were analysed, and 24 of these compounds were found to be present in *Guduchyadi kashaya*. The results showed that the phenolic compounds present in the ingredients were also present in the formulation in high abundance.

INTRODUCTION

The therapeutic effects of a polyherbal formulation are due to the diverse array of phytoconstituents present in it. Polyphenols, which are the largest group of plant-specialized metabolites, are generally recognized as chemicals involved in plants for the purpose of defence against stress. Polyphenolic compounds are usually referred to as a group of naturally occurring compounds containing multiple phenolic functionalities. There are more than 8,000 different types of polyphenols that have been identified so far. Although phenolic compounds differ greatly in structure, their common feature is the presence of one or more hydroxyl substituents, either one or more simple phenolics or polyphenols, that are directly connected to one or more aromatic or benzene rings. They can be divided into phenolic acids, flavonoids, stilbenoids, and lignans based on their structures.[1] The biological properties of phenolic compounds are diverse depending on their chemical structure, concentration, and interaction with other molecules in the body. The anti-inflammatory, anti-aging, antiproliferative, and antioxidant properties of phenolic compounds have been described in several studies. As powerful natural antioxidants that may scavenge free radicals, inactivate other pro-oxidants, and interact with a variety of biologically significant molecules, polyphenols are becoming increasingly important. Evidence suggests that additive and synergistic interactions of natural antioxidants significantly strengthen the protective effects against

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https://doi.org/10.47070/ijapr.v12i1.3087

oxidative damage in the body. [2] Polyphenols may offer protection against development of certain cancers, cardiovascular diseases, diabetes, and neurodegenerative diseases.[3] Previous studies reveal phenolic compounds are abundant in the ingredients of *Guduchyadi kashaya*.

The utilization of various contemporary analytical methods has become indispensable when it comes to assessing polyherbal Ayurvedic formulations, aiming to ensure their quality, safety, and effectiveness. Quality control of herbal medicines involve analytical evaluation using instrumental techniques such as thin layer chromatography (TLC), High-performance thin-layer chromatography (HPTLC), High-performance liquid chromatography (HPLC), Gas chromatography-mass spectrometry (GC-MS), Liquid Chromatography Mass Spectrometry (LC-MS), near infrared (NIR), and spectrophotometer etc. [4] Among them Liquid Chromatography with tandem Mass Spectrometry (LC-MS/MS) is a powerful hyphenated analytical technique that combines the separating power of liquid chromatography with the mass analysis capabilities of mass spectrometry. [5] This research study was taken to analyse 28 polyphenolic compounds in *Guduchyadi kashaya* by LC-MS/MS. Preparation of *Guduchyadi kashayas* were done as per the reference of Sarnghadhara Samhita by adding 16 times water boiled and reduced to 1/8th part.[6] Most of the Ayurvedic manufacturers use heartwood of *Patranga* instead of heartwood of *Padmaka*. So, two types of *Guduchyadi kashaya*, one containing *Padmaka* and the other containing *Patranga*, are available in the market. Therefore, two samples of *Guduchyadi kashaya* were prepared in this study.

AIM AND OBJECTIVES

To determine the polyphenolic compounds of *Guduchyadi kashaya* using liquid chromatography tandem mass spectrometry (LC-MS/MS)

MATERIALS AND METHODS

Collection of Raw Materials

Good quality raw materials of *Guduchyadi kashaya* after proper identification were procured from genuine sources. All the herbal ingredients were authenticated at the drug testing lab, Government Ayurveda College, Thiruvananthapuram.

The ingredients of *Guduchyadi kashaya* are *Guduchi* (*Tinospora cordifolia*), *Padmaka* (*Prunus cerasoides*), *Nimba* (*Azadirachta indica*), *Dhanyaka* (*Coriandrum sativum*), *Rakthachandana* (*Pterocarpus santalinus*).[7] In Kerala most of the herbal drug manufacturers are using *Patranga* (*Caesalpinia sappan*) instead of *Padmaka* (*Prunus cerasoides*), so two samples (GKP and GKC) were prepared using both *Padmaka* and *Patranga.*

GKP - *Guduchyadi kashaya* prepared using *Guduchi (Tinospora cordifolia), Padmaka (Prunus cerasoides), Arista (Azadirachta indica), Dhanyaka (Coriandrum sativum),* and *Rakthachandana (Pterocarpus santalinus).*

GKC - *Guduchyadi kashaya* prepared using *Guduchi (Tinospora cordifolia), Patranga (Caesalpinia sappan), Arista (Azadirachta indica), Dhanyaka (Coriandrum sativum),* and *Rakthachandana (Pterocarpus santalinus).*

Table 1: Ingredients of *Guduchyadi kashaya*

S.No	Drug	Botanical name	Family	Part used
1	*Guduchi*	*Tinospora cordifolia* Miers	Menispermaceae	Fresh Stem
2	*Padmaka (Patranga)*	*Prunus cerasoides* D. Don (*Caesalpinia sappan* L)	Rosaceae (Caesalpiniaceae)	Heart wood
3	*Nimba*	*Azadirachta indica* A. Juss	Meliaceae	Stem bark
4	*Dhanyaka*	*Coriandrum sativum* L	Umbelliferae	Dried ripe fruits
5	*Rakthachandana*	*Pterocarpus santalinus* Linn.	Fabaceae	Heartwood

Preprocessing of raw drugs

The raw drugs, *Padmaka, Patranga, Nimba, Dhanyaka, Rakthachandana* were made free from physical impurities and foreign matter by thorough washing with water, dried in sun and stored in airtight containers and out of which the required quantity was taken for preparation. *Guduchi* was taken in fresh form after peeling off the external skin.

Preparation of *Guduchyadi kashayas*

125g of each ingredient was taken and cut it into *Yavakuta churna*. Two samples of *Guduchyadi kashaya* were prepared, first *Kashaya* (GKP) containing *Guduchi, Padmaka, Nimba, Dhanyaka, Rakthachandana* as ingredients and 16 times of water was added into the *Yavakuta churna* of the ingredients taken in a mud vessel, boiled in mild fire and reduced to 1/8th part. Second sample of *Kashaya* (GKC) prepared using *Patranga* instead of *Padmaka* in the same method.

Lyophilisation of *Kashayas* into dry mass

1000ml from above prepared *Kashayas* were lyophilized into powder form. The freeze dryer Alpha 3-4 LS basic of ice condenser capacity 4kg and ice condenser temperature- 105°C was used for this

purpose. Amount of lyophilized *Kashaya* obtained per 1 L *Kashaya* were 24.6gm and 28.3gm for GKP and GKC *Kashaya* sample respectively. [8]

Preparation of Methanolic Extract of Dried form of *Kashaya*

To render the raw drugs and lyophilized *Kashaya* soluble in methanol, a hot reflex extraction process was carried out. 3g lyophilised *Kashaya* sample was added in 50ml methanol and reflexed for 5 hours and this methanolic extract of *Kashaya* was taken for further phytochemical analysis. [9]

Organoleptic Evaluation

Organoleptic evaluation of the two samples of *Guduchyadi kashaya* were done. Colour, odour, taste, consistency of both samples of *Kashaya* were analysed.[10]

Physicochemical Analysis

Physicochemical analysis was done to ascertain the quality of *Guduchyadi kashaya*, which includes total solid, pH and specific gravity. Procedures were done as per standard methods. [11]

Total solid

Total solids are the weight of total dissolved solids (TDS), total suspended solids (TSS) and settleable solids per unit volume of water. Total solid includes both organic and inorganic material.

$$\text{Total solid} = \frac{\text{(Weight of beaker with residue-Weight of empty beaker) x100}}{\text{Weight of sample}}$$

Specific gravity

Specific gravity is the ratio of weight of a given volume of substance to the weight of an equal volume of water at the same temperature.

$$\text{Specific gravity} = \frac{\text{Weight of bottle with } \textit{Kashaya} - \text{Weight of empty bottle}}{\text{Weight of bottle with distilled water}}$$

pH

pH value specifies the H+ ion concentration of the solution. It is determined by means of a glass electrode and p^H meter.

Preliminary Phytochemical Analysis of Methanolic extract of *Kashayas*

Preliminary phytochemical screening of methanolic extract of *Guduchyadi kashaya* was done for the qualitative detection of major class of phytochemicals using standard procedures. Qualitative analysis was done to analyze the presence of steroids, flavonoids, phenol, alkaloids, tannins, triterpenoids and saponins. [12]

Polyphenolic Profiling and Quantification using LCMS/ MS of *Kashayas*

The quantitative analysis of 28 polyphenols catechol, catechin, quinine, naringenin, tocopherol, gallic acid, chlorogenic acid, epicatechin, syringic acid, vanillic acid, caffeic acid, epigallocatechin, ferulic acid, myricetin, quercetin, para-coumaric acid, luteolin, apigenin, kaempherol, rutin, daidzein, hesperetin, shikimic acid, ellagic acid, morin, genistein, cinnamic acid, chrysin were performed by LC-MS/MS system (Nexera with LC-MS- 8045 Shimadzu corporation, Kyoto, Japan)- HPLC (Nexera LC-30AD) equipped with an autosampler (SIL-30AC), temperature controlled column oven (CTO-20AC) and prominence diode array detector (SPD-M20A) coupled to triple quadrupole mass spectrometer (Nexera with LC-MS- 8045 Shimadzu corporation, Kyoto, Japan). Working standards were prepared by diluting the stock solution with water concentration ranging from 0.01 to 1µg/ml. The quantification of the polyphenols in two samples of *Guduchyadi kashaya* was carried out on Shimadzu Shim- Pack GISS C18 column (150* 2.1mm I.D, 1.9µm) that used 0.1% formic acid as mobile phase A and 100% methanol as mobile phase B. Polyphenols were eluted with a linear gradient system as follows. 0.5 to 1.9 min – 5% of solvent B, 2 to 10 min- 98% of solvent B, 10.1 to 15 min - 98% of solvent B and 15.1 to 17 min -5% of solvent B with a flow rate 0.3ml/min, injection volume was 10µL and oven temperature 40^0 C. Positive and negative modes of multiple reaction monitoring (MRM) mode were operated during LC-MS/MS with Electron Spray ionization (ESI). LC- MS/MS data were collected and processed by LabSolutions software Shimadzu corporation, Kyoto, Japan and interface temperature of 400°C was conditioned for ionization, desolvation line temperature of 300°C, heat block temperature of 400°C, nebulizing gas flow (Nitrogen) at 3L/min and drying gas (Nitrogen) 10L/min. Each calibration solution was analysed in triplicate and the average value of the result was used as the representative for each point. [13]

RESULTS

Evaluation of Organoleptic Characters of *Guduchyadi kashaya*

Table 2: Organoleptic characters of two samples of *Guduchyadi kashaya*

Organoleptic characters	GKP	GKC
Colour	Dark brown colour	Brownish orange colour
Odour	Aromatic	Characteristic
Taste	Bitter	Bitter
Consistency	Liquid	Liquid

Table 3: Physicochemical parameters of two samples of *Guduchyadi kashaya*

Physicochemical parameters	GKP	GKC
Specific gravity	1.011	1.013
Total solid	2.31	2.82
pH	5.48	5.47

Table 4: Preliminary phytochemical analysis of two samples of *Guduchyadi kashaya*

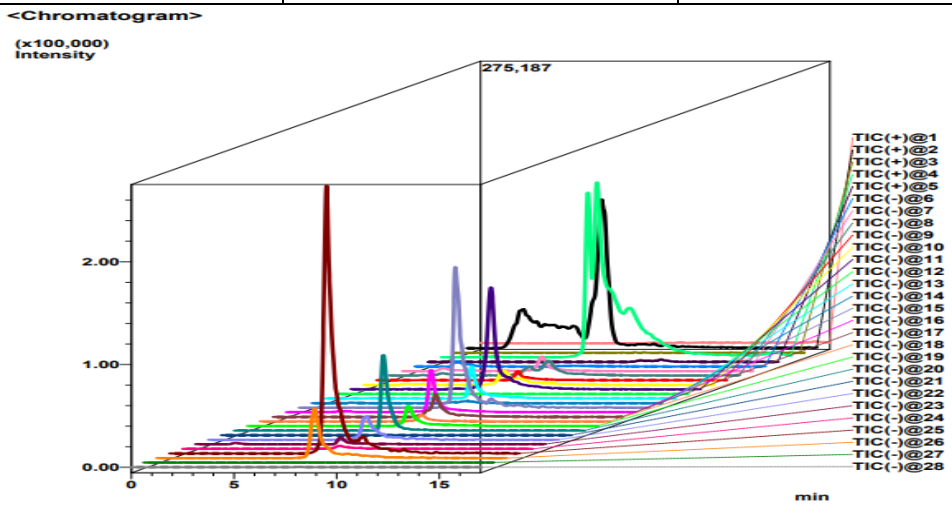
Phytoconstituents	Test	GKP	GKC
Steroids	Liebermann-Burchard's Test	+	+
Alkaloids	Wagner's reagent test	+	+
Phenol	Ferric chloride test	+	+
Flavonoids	Magnesium ribbon and concentrated HCl	+	+
Tannins	Ferric chloride test	+	+
Triterpenoids	Con. H_2SO_4 and chloroform	-	+
Saponins	Frothing *Test*	+	+

Polyphenolic quantification of *Guduchyadi kashaya* using LCMS/MS

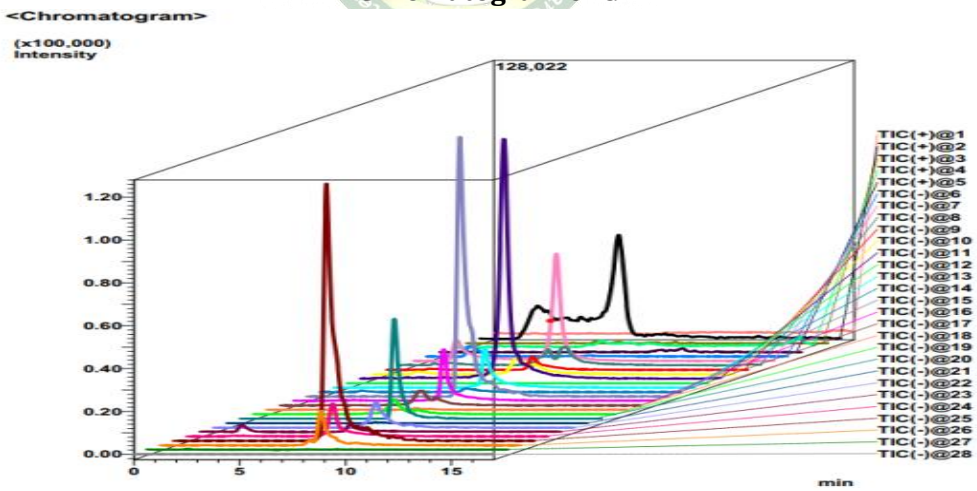
Table 5: Quantification of polyphenolic compounds in two samples of *Kashaya*

S.No	Name	GKP (ppb in 1000ppm)	GKC (ppb in 1000ppm)
1	Catechol	0	0
2	Catechin	30.27	81.96
3	Quinine	0.095	0.063
4	Naringenin	99.34	11.46
5	Tocopherol	2.67	2.54
6	Gallic acid	65.28	28.015
7	Chlorogenic acid	31.56	119.37
8	Epicatechin	7.055	55.91
9	Syringic acid	152	155.8
10	Vannilic acid	233.2	155.4
11	Caffeic acid	62.68	71.63
12	Epigallocatechin	0	0
13	Ferulic acid	48.95	35.83
14	Myricetin	4.48	2.69
15	Quercetin	47.46	28.22
16	P-coumaric acid	62.85	46.41
17	Luteolin	17.52	0.96

18	Apigenin	3.81	0.067
19	Kaempferol	282.8	19.67
20	Rutin	48.48	5.8
21	Diadzein	0	0
22	Hesperetin	5.2	1.88
23	Shikimic acid	0	0
24	Elagic acid	86.16	389.4
25	Morin	6.07	0
26	Genistein	90.59667	33.63
27	Cinnamic acid	2.45	2.58
28	Chrysin	0.971667	0



Chromatogram of GKP



Chromatogram of GKC

Figure 1: Chromatogram of GKP and GKC

DISCUSSION

Organoleptic analysis aids in the initial assessment of a product's quality by evaluating by the senses. As a preliminary quality check, the organoleptic parameters of two samples of *Guduchyadi kashaya* were tested, and the results showed that GKP *Kashaya* sample had an aromatic odour, dark brown colour and bitter taste whereas GKC sample had brownish orange colour and bitter taste. The specific aromatic odour of GKP sample may be due to the aromatic components present in the *Padmaka*.

Physicochemical analysis was employed as part of routine evaluation to determine quality of the *Kashaya*. The specific gravity, pH, total solids of the *Kashaya* were determined, which will serve as

reference for future analysis. The pH of two samples of *Guduchyadi kashaya* were 5.48 and 5.47, that means they were slightly acidic.

Qualitative phytochemical tests were carried out to identify the major phytochemical groups present in the *Guduchyadi kashaya*. Preliminary phytochemical analysis of methanolic extract of two samples of *Kashayas* were analysed. Triterpenoids were not detected in GKP sample using Salkowski test, while they were present in GKC sample. Steroids, alkaloids, phenol, flavonoids, tannins, saponins were present in both samples.

Among 28 polyphenolic compounds analysed in LC-MS/MS, 24 compounds were found in *Guduchyadi kashaya* containing *Padmaka* and 22 compounds present in *Guduchyadi kashasya* containing *Patranga.* Raw drugs of *Guduchyadi kashaya* contain abundant polyphenolic compounds which are extracted into the *Kashaya* also. The phytocompounds chrysin and morin were found in *Guduchyadi kashaya* containing *Padmaka* only. Chrysin and morin are abundantly present in the ingredient *Padmaka* (*Prunus cerasoides*). Based on a comprehensive analysis of scientific literature, it appears identified compounds possess a multitude of therapeutic effects with the potential to improve human health.

Epidemiological studies revealed an inverse relationship between the intake of polyphenolic rich food and medicines and threat of chronic human ailments. Phenolic components have been reported on their effective antioxidant, anti-cancerous, antibacterial, cardioprotective, anti-inflammatory, immunomodulatory like therapeutic properties that exhibit preventive and therapeutic effects for cardiovascular diseases, neurodegenerative diseases, cancer, diabetes, obesity etc. [14] Hence polyphenolic compounds may play a pivotal role in the therapeutic potential of *Guduchyadi kashaya*.

CONCLUSION

Abundant polyphenolic compounds present in the raw drugs of *Guduchyadi kashaya* were extracted in to the *kashaya* also. The therapeutic potential of *Guduchyadi kashaya* is significantly influenced by the actions of polyphenolic compounds. *Guduchyadi kashaya* may exhibit antioxidant, anticancerous, antibacterial, cardioprotective, anti-inflammatory, and immunomodulatory like therapeutic properties. Further research studies should be conducted to know the biological activities of the polyphenols in *Guduchyadi kashaya* to learn more about their potential health benefits. Organoleptic characters, physicochemical analysis, phytochemical analysis through LCMS/MS carried out in this analytical work can be used as reliable methods for standardization of *Guduchyadi kashaya.*

ACKNOWLEDGEMENT

We extend our gratitude to all the faculties of Department of Rasasastra and Bhaishajya Kalpana, Government Ayurveda College, Thiruvananthapuram, for their valuable support and help. We also thank faculties of the CSIR NIIST, Pappanamcode, Thiruvananthapuram, Drug Testing Lab, Government Ayurveda College, Thiruvananthapuram, for providing the facilities for the analytical works.

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Cite this article as:
Sruthi S S, S Anand. Determination of Polyphenolic Compounds Present in Guduchyadi Kashaya Using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). International Journal of Ayurveda and Pharma Research. 2024;12(1):11-17. https://doi.org/10.47070/ijapr.v12i1.3087
Source of support: Nil, Conflict of interest: None Declared

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