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LC-MS profiling of Kanchnar Guggulu - A polyherbal Ayurvedic formulation using two solvent systems

Research Article

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Abstract

Kanchnar Guggulu is an Ayurvedic Polyherbal Formulation made up of 12 ingredients. Liquid chromatography coupled with mass spectrometric detection is a powerful analytical technique for separating, identifying, and quantifying various analytes within a mixture. Objective: This study aims to characterise the phytochemical composition of *Kanchnar Guggulu* using two different solvent systems: (100% Water + 0.1 % Formic acid) and (100 % Acetonitrile + 0.1 % Formic acid). Materials and Methods: Samples obtained from a GMP-certified pharmacy underwent solid-liquid extraction employing two distinct solvents, followed by LC-MS analysis. Results: Analysis revealed the presence of 1976 & 859 phytochemical compounds in the (100% Water + 0.1 % Formic acid) and (100 % Acetonitrile + 0.1 % Formic acid) extracts, respectively. Notably, 696 phytochemicals were common in both extracts, likely due to slight differences in solvent polarity. Among Total Compounds from polar solvent, 17.68% Steroids,15.24 % Terpenoids, 15.8 % Alkaloids, 9.75 % Phenolic compounds, 6.7 % Hormones, 4.8 % Flavonoids, 3.65 % Lactones, 3.65 % Carboxyllic acid & Derivatives,3.4 % Esters & Lactones, 2.43 % Organic acid,2.43 % Nitrogen-Containing Compounds,1.82 % Carbohydrates & Sugar derivatives, 0.6 % Saponins were found. And among Total compounds from Nonpolar solvent 14.81% Alkaloids, 14.81 % Terpenoids, 11.11% Esters,12.96 % Fatty acid & derivative,7.4 % Steroidal compounds,7.4 % Hydrazones, 5.55 % Cinnamic acid derivative, 3.7 % Lactones, 3.7 % Quinones, 3.7 % Piperazine derivatives and 1.85 % Acridines were found. Conclusion: 2291 unique phytochemical constituents were identified in the two solvent extracts of the same sample, highlighting the significance of solvent selection in phytochemical profiling studies.

Keywords: *LC-MS, Kanchnar Guggulu,* Metabolites, Acetonitrile, Formic acid, Ayurvedic.

Introduction

Kanchanar Guggulu is a renowned Ayurvedic formulation primarily used to support thyroid health and manage various glandular disorders. (2) It combines several potent herbs, each contributing unique chemical components and secondary metabolites.

LC-MS

Liquid chromatography-mass spectrometry (LC-MS) stands as a versatile technique highly suitable for the metabolomic profiling of individual or blended plant samples (3). This method enables the comprehensive exploration of a wide array of phytochemicals within a sample in a single analytical run, leveraging their inherent properties. Identification of metabolites is facilitated by mass spectrometry, which determines their mass-to-charge ratio.

As LC-MS (Liquid Chromatography-Mass Spectrometry) allows for complex combinations of phytochemicals present in the polyherbal formulation, it was chosen for the analysis of *Kanchnar Guggulu*. It is well known for having a high sensitivity, which enables the identification and measurement of substances in extremely minute quantities.

Although HPLC is capable of separating substances, it is not as accurate in identifying them as mass spectrometry.

The inability of GC-MS to analyse non-volatile substances, including the polar and bigger molecules found in herbal compositions like *Kanchnar Guggulu*, is due to its requirement that the compounds be volatile. Since LC-MS doesn't require volatile substances, it is better suited to the variety of metabolites that are anticipated in an Ayurvedic formulation.

Solvent system

The solvent systems chosen in this study—(100% Water + 0.1% Formic Acid) and (100% Acetonitrile + 0.1% Formic Acid)—were selected to extract both polar and non-polar compounds:

(100% Water + 0.1% Formic Acid): This polar solvent system is ideal for extracting hydrophilic (water-soluble) compounds, such as flavonoids, tannins, and other polar metabolites.

(100% Acetonitrile + 0.1% Formic Acid): Acetonitrile, being less polar, is effective in extracting lipophilic (fat-soluble) compounds, such as certain alkaloids, essential oils, and lipophilic phytochemicals.

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Table 1: Ingredients of *Kanchanara Guggulu*

S/No.	Ingredients	Latin/ English name	Family	Part used	Ratio
1	*Kanchanar*	*Bauhinia variegata* Blume	Fabaceae	Bark	*10 parts*
2	*Shunthi*	*Zingiber officinale* Roxb.	Zingiberaceae	Rhizome	1 *part*
3	*Kana*	*Piper Longum*	Piperaceae	Fruit	1 *part*
4	*Maricha*	*Piper Nigrum*	Piperaceae	Fruit	1 *part*
5	*Haritaki*	*Terminalia chebula* Retz	Combretaceae	Fruit	2 *part*
6	*Bibhitaki*	*Terminalia belerica*. Roxb	Combretaceae	Fruit	2*part*
7	*Amalaki*	*Emblica officinalis*. Gaertn	Phyllanthaceae	Fruit	2 *part*
8	*Varuna*	*Crataeva nurvala*	Capparaceae	Bark	1 *part*
9	*Patraka*	*Cinnamomum tamala*	Lauraceae	Leaves	1 *part*
10	*Ela*	*Elettarai cardamomum*	Zingiberaceae	Seeds	1 *part*
11	*Twaka*	*Cinnamomum zeylanicum* Blume	Lauraceae	Bark	1 *part*
12	*Guggulu*	*Commiphora wightii* (Arn.)	Burseraceae	Resin	Equal amount of all the ingredients in total.

To optimise the variety of compounds extracted, the two solvents of different polarities were taken . The phytochemicals in the formulation can be thoroughly profiled since acetonitrile targets less polar or non-polar molecules, whereas polar solvents like water extract more polar compounds.

Acetonitrile with formic acid and water with formic acid are two solvent systems that are frequently utilised in LC-MS analysis since they are: Volatile: Only the chemicals of interest for mass spectrometry remain after they readily evaporate. Ion-pairing agents: Formic acid increases the sensitivity of a variety of chemicals detection by assisting in ionisation during mass spectrometric analysis.(4)

Formic acid (0.1%) was added to both solvents to create a slightly acidic environment. This helps stabilise compounds during extraction and facilitates better ionisation during LC-MS analysis, which improves the detection of certain compounds, particularly those that ionise more efficiently in acidic conditions (5).

Finding a greater variety of phytochemicals in the same formulation is more likely when these two complementing solvent systems are used.The study provides a detailed chemical profile of *Kanchnar Guggulu* by employing two distinct solvent systems: (100% Water + 0.1 % Formic acid) and (100% Acetonitrile + 0.1 % Formic acid) via liquid chromatography-mass spectrometry. This information is essential for comprehending the bioactive ingredients in the formulation, which are connected to its therapeutic effects. For its traditional usage to be validated by evidence, such thorough profiling is necessary.

The study illustrates the significance of solvent selection in phytochemical research by showing how various solvents (water vs. acetonitrile) remove distinct types of chemicals. This data is essential for maximising the formulation's efficacy through the optimisation of extraction techniques in both industrial production and research. Deeper investigation of mechanisms of action is made possible by the identification of particular bioactive substances, such as guggulsterones, flavonoids, tannins, and saponins. This opens the door for preclinical and clinical research to determine how these substances contribute to the anti-inflammatory, lipid-lowering, and antioxidant properties that have been seen in therapeutic settings.This study provides critical insights into the chemical makeup of *Kanchnar Guggulu*, enhancing its scientific validation, standardisation, and potential for broader therapeutic application.

It also helps in identifying potentially **toxic** or **undesirable compounds** that may be present in the formulation. This is crucial for establishing **safe dosage limits** and preventing adverse effects.

By characterising the metabolites, researchers can investigate the **synergistic interactions** between compounds, leading to a deeper understanding of how the formulation works at a **molecular level.**

It can aid in the isolation and development of **novel therapeutic agents** from the formulation, promoting the integration of Ayurvedic knowledge into modern pharmacology.

Materials and Methods (Experimental)

Kanchnar Guggulu sourced from a GMP-certified Ayurvedic Pharmacy was utilised for the experiment. A 10 g of the sample was mixed with 50 ml of (100% Water + 0.1% Formic acid) in a conical flask and subjected to agitation using an orbital flask shaker at 21°C and 60 rpm for a duration of four hours. A similar procedure was conducted with (100% Acetonitrile + 0.1% Formic acid).

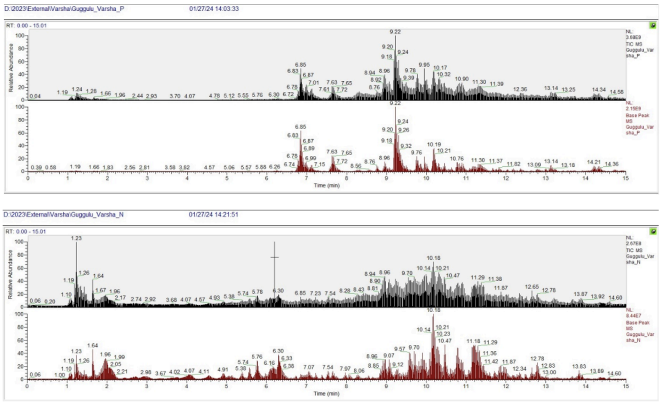
Subsequently, the mixture was allowed to settle in separating funnels, and the solvent layer was carefully collected. The solvent layer was then evaporated on Water bath, and the resulting residue was sent to IISC, Bangalore for LC-MS analysis.

LC-MS analysis was conducted utilising an MS Instrument - Orbitrap Fusion (Tribrid Series) with a Hypersil Gold Column of dimensions 150 mm length and 2.1 mm internal diameter. A one-microliter sample was injected through a 250°C injection port . The electron ionisation (EI) energy utilised was 70 eV, and the mass analyser temperature was maintained at 150°C.

Results

Kanchnar Guggulu exhibited a diverse range of phytochemicals including acids, Tannins, and flavonoids, Alkaloids etc.

Figure 1: LC-MS profile of *Kanchanara Guggulu*



Result of Analysis revealed 1976 & 859 phytochemical compounds in the (100% Water + 0.1 % Formic acid) and (100 % Acetonitrile + 0.1 % Formic acid) extracts, respectively. Notably, 696 phytochemicals were common in both extracts, likely due to slight differences in solvent polarity.

Classification of Phytochemicals

Non-Polar Compounds

859 phytochemicals were found; all were arranged alphabetically, and repetitions were removed. 753 unique compounds were found. Each metabolite is checked for its chemical properties using databases such as PubChem, Chemspider, ChEBL, Drug Bank and different Indexed Journal.

All the phytochemicals having similar chemical properties are grouped.

Polar Compounds

A total of 1976 phytochemicals were found; all were arranged in alphabetical order, and repetitions were removed. 1538 unique compounds were found. Each metabolite is checked for its chemical properties using databases such as PubChem, Chemspider, ChEBL, Drug Bank and Different Indexed Journal.

All the phytochemicals having similar chemical properties grouped together.

Table 2: Analysis Data set

S/N0.	Group	Polar Solvent		Non- Polar Solvent	
1	Alkalloids	204 compounds	17.6%	105 compounds	14.81%
2	Steroids	228 compounds	15.24%	53 compounds	7.4%
3	Terpenoids	197 compounds	15.8%	105 compounds	14.81%
4	Phenolic Compounds	126 compounds	9.75%	-	
5	Hormones	86 compounds	6.70%	-	
6	Flavinoids	62 compounds	4.8%	-	
7	Lactones/Furano-coumarins	47 compounds	3.65%	26 compounds	3.7%
8	Carboxylliic acids & Derivatives	47 compounds	3.65%	39 compounds	5.55%
9	Esters & Lactones	39 compounds	3.04%	105 compounds	14.81%
10	Organic acids	31 compounds	2.43%	-	
11	Nitrogen containing compounds	31 compounds	2.43%	-	
12	Carbohydrates & Sugar Derivatives	23 compounds	1.82%	-	
13	Saponins	8 compounds	0.60%	-	
14	Fatty acids & derivatives	-		92 compounds	12.96%
15	Hydrazones	-		53 compounds	7.4%
16	Spiro compounds	-		39 compounds	5.55%
17	Cinnamic acid Derivatives.	-		39 compounds	5.55%
18	Quinones	-		26 compounds	3.7%
19	Piperazine derivatives	-		26 compounds	3.7%
20	Acridines	-		13 compounds	1.85%
21	Others	66 compounds	4.87%	104 compounds	14.6%

The dataset obtained from this analysis is available as Supplementary Material S1 and S2.For detailed data, see Supplementary Table S1 and S2 in the accompanying Excel file.

Discussion

The choice of solvents for extraction plays a crucial role in isolating compounds from *Kanchnar Guggulu*. Hydrophilic compounds were effectively extracted using (100% Water + 0.1% Formic acid), while lipophilic components were extracted using (100% Acetonitrile + 0.1% Formic acid). The polarity of the solvent used significantly impacts the types of constituents extracted. Polar solvents tend to extract more polar compounds, whereas non-polar solvents favour the extraction of compounds with similar characteristics.

(-)-Pinellic acid is a conjugated fatty acid with potential anti-inflammatory proerties. (6) Brefeldin A is a macrocyclic lactone with antiviral and antifungal activity. It also disrupts protein transport within cells, making it valuable in cell biology studies. (+/-)12(13)-DiHOME is a dihydroxy-octadecenoic acid that is involved in lipid signalling. It is a metabolite that plays a role in inflammatory processes in the body. (7) (12E)-9,10-Dihydroxy-12-octadecenoic acid is part of the hydroxylated fatty acids with possible bioactive properties, often involved in metabolic and inflammatory pathways. (8) (13E,15E)-12-Oxo-13,15-henicosadienoic acid a long-chain unsaturated fatty acid, potentially involved in metabolic and signaling pathways related to lipid metabolism.(9) (15Z)-9,12,13-Trihydroxy-15-octadecenoic acid a hydroxy derivative of octadecenoic acid, this compound is involved in metabolic pathways, especially in lipid oxidation and inflammatory responses. (10) (1E)-7-Chloro-3-(2,4-dichlorophenyl)-10-hydroxy-1-[(4-methyl-1-piperazinyl)imino]-1,3,4,10-tetrahydro-9(2H)-acridinone is a derivative of acridinone with potential pharmacological properties, often explored in medicinal chemistry due to its complex ring structure and potential as an antibacterial or anticancer agent. (11) (20S)-Protopanaxadiol is a metabolite of ginsenosides and has shown various pharmacological activities, including antioxidant, anti-inflammatory, and potential anticancer effects.(12) (2E)-3-(3,4-Dimethoxyphenyl) acrylic acid is related to phenylpropanoids, a class of plant secondary metabolites. It has potential antioxidant, antimicrobial, and anti-inflammatory properties.(13) (2E,4Z)-2-Hydroxy-6-(2-hydroxyphenoxy)-6-oxo-2,4-hexadienoic acid is a complex organic acid with both phenoxy and hydroxy groups, potentially having antioxidant or anti-inflammatory properties due to its structure.(14) (2S,4R,9a'S)-1'-Hydroxy-4-{2-[(1S)-1-hydroxyethyl]-4-oxo-3(4H)-quinazolinyl}-2',2'-dimethyl-1',9a'-dihydro-3H-spiro[furan-2,9'-imidazo[1,2-a]indole]-3',5(2'H,4H)-dione A complex quinazolinone derivative with potential biological activity, such as anti-cancer or antibacterial, due to its fused-ring structure. (15).(3E,3'E)-3,3'-[1,4-Piperazinediylbis(2,1-ethanediylnitrilo)]bis(1-phenyl-1-butanone) is a piperazine-based compound, often found in bioactive molecules, with potential pharmacological properties such as anti-inflammatory or anticancer activities.(16) (3Z)-2-([(6-Methoxy-8-quinolinyl) amino]methyl)-1-azabicyclo[2.2.2]octan-3-one oxime is a quinoline-based compound with oxime functionality, potentially bioactive as an antibacterial or antiviral agent.(17) (5E)-2-Anilino-1-phenyl-5-(phenylimino)-1,5-dihydro-4H-imidazol-4-one is a substituted imidazole with potential antitumor or antimicrobial properties, reflecting the importance of imidazole rings in medicinal chemistry.(18) (7Z)-3-Hydroxy-7-octadecenoic acid is a fatty acid known for its potential role in lipid metabolism and cardiovascular health.(19) (E)-p-coumaric acid is phenolic compound that exhibits antioxidant and anti-inflammatory properties, widely present in food sources.(20)

Alkaloids are nitrogen-containing organic compounds that offer analgesic, antimicrobial, and anticancer effects (e.g., morphine, quinine).(21) Steroids are lipid-based compounds that play key roles in hormonal and structural functions in the body such as anti-inflammatory, hormone modulators, and support cardiovascular health .(22) Terpenoids also known as isoprenoids, are a large and diverse group of naturally occurring organic chemicals derived from terpenes which provide antimicrobial, anticancer, antioxidant, and neuroprotective benefits .(23) Phenolic Compounds Known for their antioxidant properties, they help reduce oxidative stress, exhibit anti-inflammatory effects, and protect against cardiovascular diseases.(24) Flavonoids are polyphenolic compounds have strong antioxidant, anti-inflammatory, and antimicrobial effects. They also show anticancer and neuroprotective properties.(25) Lactones Often found in medicinal plants, lactones display antimicrobial, anti-inflammatory, and anticancer activities.(26) Carboxylic Acid Derivatives show anti-inflammatory and antimicrobial effects.(27) Esters are known for their anti-inflammatory and analgesic effects, esters are also used in fragrance and flavour industries for their pleasant aromas.(28)

These diverse chemical components and secondary metabolites collectively contribute to the therapeutic properties of *Kanchanar Guggulu* in Ayurvedic practice.

Conclusion

2291 distinct phytochemicals were successfully identified through LC-MS Analysis. Among which 753 compounds were identified using Non- Polar solvent and 1538 phytochemicals were found using Polar Solvent . These findings provide valuable insights into the medicinal properties of Kanchnar Guggulu, such as its Anticancer action, Anti-inflammatory, Antimicrobial, Antioxidant and Lipolytic action, contributing to a deeper understanding of its therapeutic potential and paving the way for further research in this domain.

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