JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2025/43942
RESEARCH ARTICLE

Chromatographic Analysis of Polyherbal Extract and Formulation by HPTLC and GC-MS Methods

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Abstract

Background: The World Health Organization (WHO) acknowledges that natural plants and their bioactive components have been recognized for their potential therapeutic benefits. The main issue facing the herbal industry is the lack of effective quality control for herbal extracts and formulations Establishment standards are essential to ensuring the efficacy and integrity of natural medicine development. Determining the precise compound responsible for therapeutic action poses an equally challenging task, as these compounds frequently work synergistically to produce medicinal benefits. **Aim:** In this exploration, GC-MS and high-performance thin-layer chromatography were used to analyze the formulation and polyherb extract. The HPTLC technique is often employed to analyze the sample and compare its fingerprint to reference standards to verify plant extracts' quality and formulations' quality. Using GC-MS, the phytochemical ingredients in the formulation and the extract have been determined. **Methods:** This research assessed a mixture of herb extract and formulation using analytical approaches. The combined hydroalcoholic extracts of the herbs were used to produce the polyherb extract of seeds of *Azadirachta indica*, *Carica papaya, Trigonella foenum-graecum, Gossipium herbaceum,* fruit of *Piper nigrum.* The required excipients were added before compressing the herbal mixture extract into tablets. **Results:** The extract and formulation's HPTLC chromatograms displayed Rf values of 0.14, 0.34, 0.48, 0.63, and 0.76.The results of the GC-MS, N-Hexadecanoic, 1,19-Eicosadiene, 9,12-Octadecadienoic Acid, Z, Z-4,16-Octadecadien-1-Ol Acetate, Piperine that were analyzed provide different peaks to determining the presence of phytochemicals found in the extract and formulation were confirmed. **Conclusion:** HPTLC and GC-MS aid in the identification of individual chemical constituents within herbal formulations by coupling chromatographic and mass spectral data of the sample with reference standards or databases, the identity of key compounds can be confirmed, ensuring authenticity, and has shown an intense correlation between the mixture of herb extract and formulation.

Major Findings: In this Research, GC-MS and high-performance thin-layer chromatography were used to analyze the formulation and polyherbal extract of key compounds can be confirmed, ensuring authenticity, and has shown an intense correlation between the mixture of herb extract and formulation.

Keywords: *Carica papaya,* GC-MS, HPTLC, Phytochemical Analysis, Polyherbal Formulation

Abbreviations: TLC: Thin layer Chromatography; HPTLC: High-Performance Thin layer Liquid chromatography; GC-MS: Gas Chromatograph-Mass Spectroscopy; µl: microlitre; Cm: Centimetre; Mg: milligram; NIST: National Institute of Standard and Technology

1. Introduction

The World Health Organization (WHO) acknowledges that natural shrub and its bioactive components have been recognised for their potential therapeutic benefits[1,2]. Since traditional times, herbal plants, their parts, and their functional biomolecules have been documented in prevention ailments, such as homeopathy, Chinese medicine, *Unani* medicine, and *Ayurvedic* medicine India needs to find out about the therapeutic valuable plants, and this can only be done if the herbal preparation has been validated through

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Article Received on: 09.05.2024 Revised on: 25.11.2024 Accepted on: 03.12.2024

modern standardisation procedures[3]. Standardisation of herbal formulations with suitable analytical instruments and production processes is needed to create a framework for overseeing the quality of medicinal products[4].

The scientific community faces a major obstacle in developing accurate analytical techniques to identify the phytochemical and quantitative assessments of markers. Establishment standards are essential to ensuring the efficacy and integrity of natural medicine development[5]. Quality control for herbal formulations presents greater challenges compared to synthetic drugs due to the intricate chemical complexity inherent in the ingredients. Characterising all the compounds in herbal preparations, which often encompass hundreds of mostly specific compounds, poses a significant challenge[6].

The evaluation of the quality of herbal formulations is crucial to support their acceptance in the current healthcare system. The main issue facing the herbal industry is the lack of effective quality control for herbal formulations. Significant attempts have been made since to control the quality of herbal materials and preparations using quantitative techniques and qualitative fingerprinting[7].

Determining the precise compound responsible for therapeutic action poses an equally challenging task, as these compounds frequently work synergistically to produce medicinal benefits[8,9]. The intricate interplay among these compounds complicates identifying the specific agent responsible for the therapeutic action. Thus, ensuring efficacy in herbal products from different batches is both difficult and essential, and it has recently received significant attention as a challenging analytical challenge.

HPTLC is frequently employed to develop reference fingerprints of herbs, which can be used to analyse raw materials and assay finished products. The fingerprint provides information about the presence of phytomarkers and botanical compound quality of the herbal formulation and plant extract. In GC-MS analysis, the mass spectra of unknown compounds are compared to those of known compounds stored in databases like NIST. Using GC-MS, the phytochemical ingredients in the formulation and the extract have been determined.

In this research, a mixture of herb extract and formulation has been assessed using analytical approaches. The extract has been produced from the combining hydroalcoholic extracts of seeds of *A. indica*, *C. papaya, T. foenum-graecum, G. herbaceum,* fruit of *P. nigrum*. After mixing the polyherbal extract with essential excipients, the tablets were compressed. HPTLC and GC-MS aid in the identification of individual chemical constituents within herbal formulations by coupling chromatographic and mass spectral data of the sample with reference standards or databases, the identity of key compounds can be confirmed, ensuring authenticity and has shown an intense correlation between the mixture of herb extract and formulation.

2. Materials and Methods

2.1 Procurement and Authentications of Plants

Fresh seed of neem (*A. indica*), papaya (*C. papaya)*, seeds of fresh papaya was procured from the surrounding market of Gujarat and dry part of the drug-like fruit of Piper, fenugreek (*Trigonella foenum-graecum),* cotton (*G. herbaceum)* was collected from local commercial market of the of Bharuch. After collection, fresh material was dried under the Shade. Herbarium sheets were prepared and Varified by Dr. P. S. Nagar, Department of Botany BARO herbarium M. S. University of Baroda, Gujarat with voucher specimen no BARO 123456725 (AN30725, AN30726, AN30723, AN30724, AN728) and submitted.

2.2 Preparation of Extract

For further use; the air-dried ingredients were ground into a powder, sieved through a 40 mesh, and placed in an airtight container[10,11]. Soxhlet apparatus employed for hot extraction methods using petroleum ether, chloroform, ethyl acetate, alcohol, and water solvents. Cooled the filtrate and filtered through filter paper. The solvent was removed utilizing a rotary evaporator (Buchi R-300). The resulting left an adhesive residue that was brownish-dark in appearance. A measured quantity of the concentrated extract was obtained. The resulting crude extract yield is referred to as the

polyherbal extract, which was preserved in an airtight desiccator for further analysis[12].

2.3 Preparation of Polyherbal Formulation[13-15]

An appropriate quantity of dry extracts was selected to formulate tablets, along with the necessary excipients. The wet granulation process was chosen for its suitability in small-scale preparations, Standardized extracts and other components for each formula were individually weighed, ground, sieved through sieve 80, and prepared the granules. Granules were dried at 35°C in the hot air oven. The granules, mixed with magnesium stearate, were punched into 600 mg tablets, with the die cavity adjusted for the required weight. The tablet granules were prepared using incorporating Hydroalcoholic extracts of each drug in different ratios, starch as a disintegrator, magnesium stearate as a lubricant, and lactose as a filler

2.4 Identification of Marker Compounds by HPTLC and GC-MS[16]

Planar chromatography is a highly suitable technique for identifying constituents from plant extracts. Advancements in chromatographic techniques, such as GC-MS, have simplified and made the analysis of small chemical quantities more cost-effective. Through GC-MS analysis, the identification of pure compounds can be achieved even when present in amounts less than 1 gram.

2.5 HPTLC Fingerprinting

High-performance thin-layer chromatographic generates a distinct pattern of spots after chromatographic separation, essentially creating a unique fingerprint for a given herbal extract or formulation, and comparing these fingerprints aids in quality control and authentication. HPTLC fingerprinting is a versatile and effective analytical technique for assessing the quality and composition of herbal materials and formulations[17,18].

2.6 Steps Involved in HPTLC Analysis[19,20]

2.6.1 Instrument

HPTLC CAMAG (Switzerland Muttenz) Linomat V automatic sample spotter; Syringe(Bonadug, Switzerland) 100µl or 500µl capacity, TLC Scanner III With three light sources-mercury vapor lamp, deuterium lamp, Densitometer: TLC Scanner with win CATS 1.2.3 software[21,22].

2.6.2 Preparation of Stock Solution

Using a 10 ml graduated flask, ten milligrams of piperine was dispersed in the methanol and the final amount was adjusted to 10 ml. This produced a standard solution for the marker analysis that included 1 mg/ml[23].

2.6.3 Preparation of Test Solution and Formulation

Using a mortar and pestle, around 5 gm of the extract and formulation were crushed. Following that, the precisely weighed 650 mg of powder was put inside a 25 mL measuring flask and allowed to be soluble in methanol. methanol was added to the volume until it was the right amount, and then the solution was sonicated for 10 min. Use the filtrate as a stroke solution after filtering the mixture[24-26].

2.6.4 Application of Sample

The sample was applied in the form of band lengths, on the prewashed TLC plates, based on the number of points placed.

2.6.5 Chromatography Conditions

Separation technique analysis applied of extracts and formulations was conducted using a planner chromatogram. The stationary phase used was Silica Gel 60 F254 with dimensions of 10 cm × 10 cm and a thickness of 250 mm[27].

Using a Camag Linomat V sample applicator and a 100 ml Hamilton syringe, chromatography was performed by placing extract and formulation on a precoated silica gel aluminum plate and Mobile phase: Toluene: ethyl acetate: Acetic acid (8:1.8:0.2) Scanning Wavelength: 254nm and 366 nm. HPTLC fingerprints of extracts were obtained along with the spot of respective standards The fingerprint provided an effective means to verify the final product's integrity. The formulation may be identified and its quality evaluated with efficiency using the HPTLC fingerprint.

2.7 GC-MS Studies

Gas Chromatograph with Mass Spectroscopy (GC-MS) Perkin Elmer, USA system was employed to identify an unknown compound structure. This method utilised to

ionisation energy of 70 eV with a scan interval of 0.5 s and parts ranging from 20 to 610 Daltans were utilised. In this instance, a silica column made entirely of 100% dimethyl polysiloxane, measuring 30 nm by 0.25 nm ID by 1 µm df, was utilised. Compounds were separated using helium as the mobile phase at a flow rate of 1 ml/minute. After injecting the 2 µl extracted sample into the device, the Turbo gold mass detector used the Turbo mass 5.1 software to detect it. The temperature in the oven was held at 110°C for two minutes during the GC extraction. Total GC running time was 48 min. The relative percentage amount of each component was calculated from the obtained peak area[28,29].

2.7.1 Sample Preparation

A screw-cap vial containing about 1 gm of sample was filled with 10 ml of methanol. After an hour of sonication, it was stored for 12 hours. The extract was employed in a 2µl for hybrid analysis[30].

3. Results and Discussion

In this research, a mixture of herb extract and formulation has been assessed using analytical approaches. The Polyherbal formulation has been produced from the combining hydroalcoholic extracts of seeds of *A. indica*, *C. papaya*, *T. foenum-graecum*, *G. herbaceum* fruit of *P. nigrum*. The formulation and extract were subjected to HPTLC analysis and the results revealed the presence of separated bands in plates A and B with good resolution in the solvent system Toluene: ethyl acetate: Acetic acid (8:1.8:0.2) depicted in Figures 1 and 2 indicated the presence of piperine in combined extract and formulation precisely matched with Rf values of 0.48 which illustrated in Figures 3,4 and 5. In GC-MS analysis, components of volatile oil from plant extract and polyherbal formulation were compared with mass spectra of known compounds of the NIST library database which contains more than 62,000 patterns to confirm identity. The GC-MS chromatogram presented in Figure6 shows the presence of phytocompounds like N-Hexadecanoic, 1, 19-Eicosadiene, 9, 12-Octadecadienoic Acid, Z,

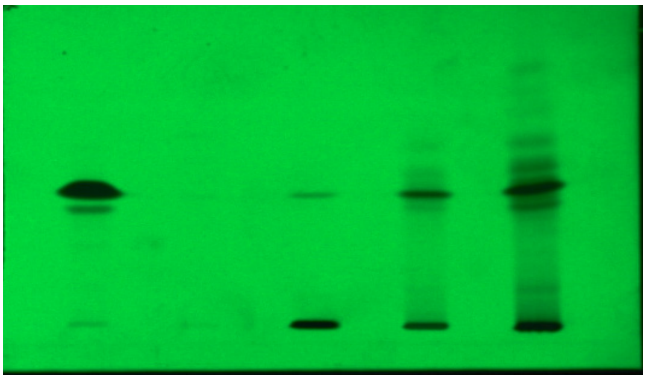


Figure 1. T1: piperine, T2: Diosgenin, T3: Trigonelline T4: combine extract, T5: Formulation Ultraviolet 254 nm.

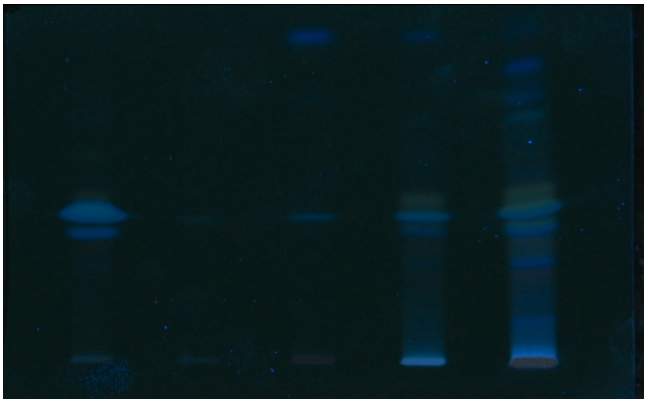


Figure 2. T1: piperine, T2: Diosgenin, T3: Trigonelline, T4: combine extract, T5: Formulation 366 nm.

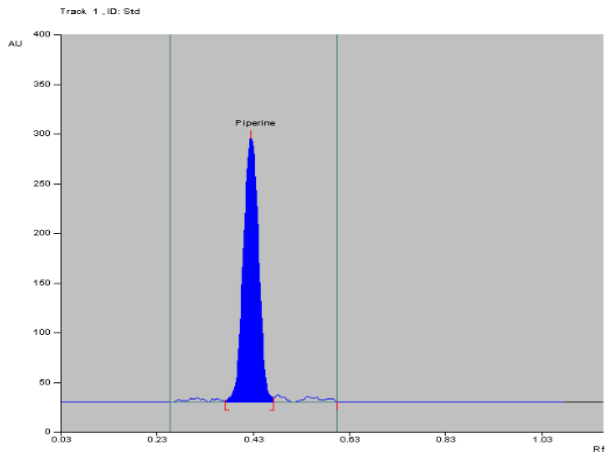


Figure 3. Chromatogram of A-Piper Std with rf value 0.43.

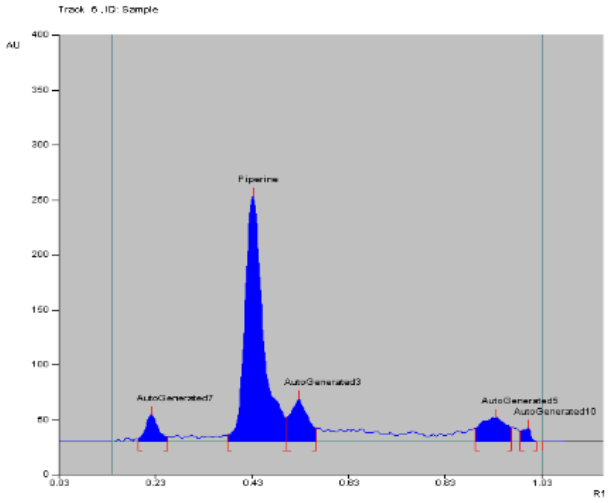


Figure 4. Chromatogram B: piper extract with rf value: 0.43.

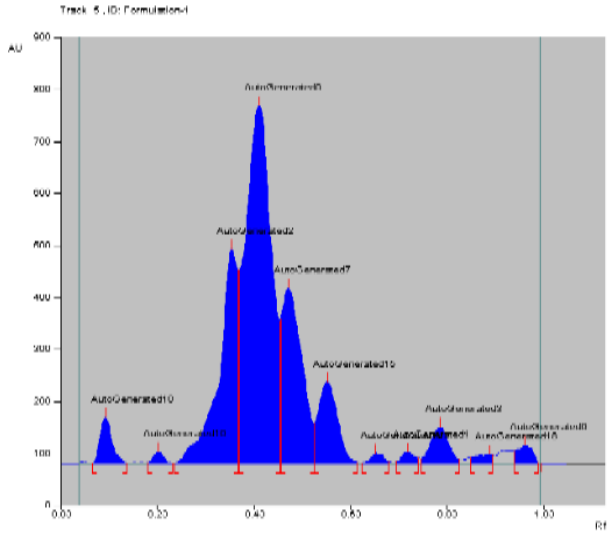


Figure 5. Chromatogram of formulation.

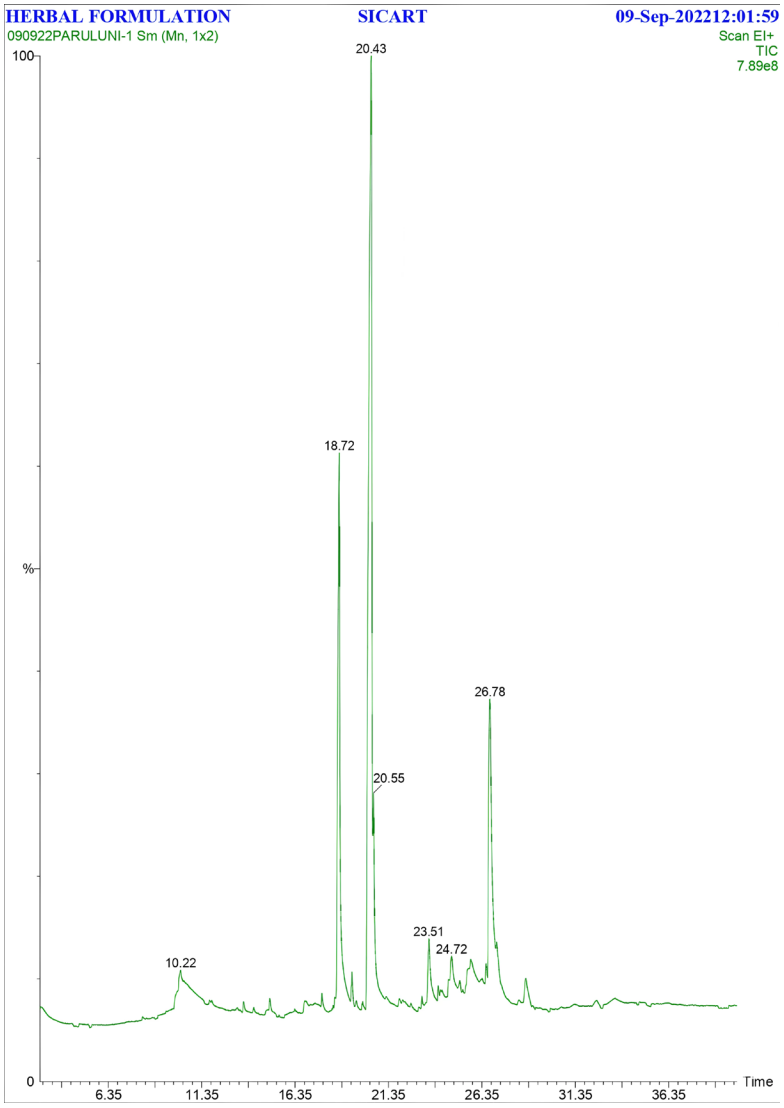


Figure 6. GC-MS chromatogram of the formulation.

Table 1. Compound identified by GC-MS analysis

S. No.	Compound Name Identified	RT value	Molecular Weight	Molecular Formula	% Peak Area
1	N-Hexadecanoic	18.72	256	$C_{16}H_{32}O_2$	17.17
2	1,19-Eicosadiene	20.43	278	$C_{20}H_{38}$	47.54
3	9,12-Octadecadienoic Acid (Z,Z)	20.55	280	$C_{18}H_{32}$	4.55
4	Z,Z-4,16-Octadecadien-1-Ol Acetate	23.51	308	$C_{20}H_{36}O_2$	2.09
5	Piperine	26.78	285	$C_{17}H_{19}O_3N$	16.06

Z-4,16-Octadecadien-1-Ol Acetate, Piperine along with molecular weight, formula, RT value and % peak area in Table 1**.** The presence of was verified Spectral matching using NIST library data is the basis for structural attribution of gas chromatography retention data of compounds.

4. Conclusion

The present study on selected plant extract and its formulation revealed the HPTLC fingerprinting and GC-MS profile. HPTLC fingerprinting indicated methanolic extract and formulations show well-separated bands with good resolution in the applied solvent system revealing the presence of phytoconstituents in combined extract and formulation precisely matched. In GC-MS analysis, components of volatile oil from plant extract and polyherbal formulation were investigated and compared with mass spectra of known compounds of the NIST library database.The combined hyphenated techniques are a valuable approach for assessing the quality and uniformity of herbal remedies. HPTLC and GC-MS aid in the identification of individual chemical constituents within herbal formulations by coupling chromatographic and mass spectral data of the sample with reference standards the identity of key compounds can be confirmed and ensure authenticity. This research provides a foundation for future studies on standardisation and quality control of polyherbal formulations. Further research could focus on developing more sensitive and specific analytical methods to identify and quantify a wider range of bioactive compounds in complex polyherbal mixtures.

5. Acknowledgment

The author would like to express our sincere gratitude to the principal and HOD of Pharmacognosy, PIPR, Waghodia, Vadodara for providing the necessary resources and support that made this research possible. The authors would also like to acknowledge, SICART, Anand, Gujarat, for doing the HPTLC and GC-MS analysis of the samples.

6. Author Contribution

Conceptualization by P,V. and G.S.V.; Methodology, and Formal analysis and investigation by P,V. and G.S.V. Writing—original draft, PV. Supervision by G.S.C. All authors have read and agreed to the published version of the manuscript.

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