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Gas chromatography-mass spectrometric profiling of hexane fraction of whole plant *Tragia involucrata* L. and methanol fraction of *Psidium guajava* L. leaves

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ABSTRACT

Background: Medicinal plants are an important source of bioactive compounds with significant therapeutic potential, forming the basis of many traditional and modern pharmacological preparations. *Tragia involucrata* and *Psidium guajava* are widely used in traditional medicine for treating gastrointestinal, respiratory, metabolic, and infectious conditions. Despite their traditional relevance, a detailed chemical characterization of specific solvent fractions, particularly using advanced analytical methods, remains limited. Gas chromatography–mass spectrometry (GC-MS) offers a powerful tool for the identification of volatile and semi-volatile phytochemicals that may contribute to the pharmacological properties of these plants. **Objective:** This study aimed to identify and characterize the bioactive phytoconstituents present in the hexane fraction of *Tragia involucrata* whole plant and the methanol fraction of *Psidium guajava* leaves using GC-MS analysis, and to highlight their potential pharmacological relevance. **Materials and Methods:** Whole plant material of *T. involucrata* and leaves of *P. guajava* were collected from Kollam, Kerala, authenticated through DNA barcoding, shade-dried, powdered, and extracted using methanol. The crude extracts were successively fractionated with solvents of increasing polarity, and the hexane fraction of *T. involucrata* and methanol fraction of *P. guajava* were selected for GC-MS profiling. Analyses were carried out using a Shimadzu Nexus GC-2030 system under optimized temperature and carrier gas conditions. Compounds were identified based on retention time, mass spectral data, and comparison with the NIST 20 library. **Results:** GC-MS analysis of the *T. involucrata* hexane fraction identified fourteen phytoconstituents representing diverse chemical classes. These included alkylated phenols (2,4-di-tert-butylphenol; benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy, methyl ester), terpenoids (neophytadiene), diterpene alcohols (3,7,11,15-tetramethyl-2-hexadecen-1-ol), and fatty acid derivatives such as hexadecanoic acid methyl ester, methyl stearate, 9,12-octadecadienoic acid (Z,Z) methyl ester, 9,12,15-octadecatrienoic acid (Z,Z,Z) methyl ester, and n-hexadecanoic acid. Higher-chain esters, including hexadecanoic acid bis(2-ethylhexyl) ester, were also present. Additionally, phytol, squalene, and the antioxidant dl-α-tocopherol were detected, reflecting the chemical richness and potential bioactivity of the extract. The methanol fraction of P. guajava identified 10 bioactive compounds, predominantly saturated fatty acids (tetradecanoic, pentadecanoic, octadecanoic acids, oleic acid), long-chain hydrocarbons (dotriacontane, pentacosane), triterpene (squalene) and saturated fatty acids namely 13-docosenamide, and tetradecyl palmitate. The presence of these structurally diverse metabolites in both the *T. involucrata* hexane fraction and the *P. guajava* methanolic fraction highlights their notable phytochemical abundance and potential relevance for broad pharmacological applications. **Conclusion:** GC-MS profiling of *T. involucrata* and *P. guajava* demonstrates a diverse array of pharmacologically important metabolites, including diterpenes, terpenoids, phenolic derivatives, fatty acid esters, squalene, and α-tocopherol. These findings support their traditional medicinal relevance and indicate their promise as sources of bioactive lead molecules for drug development. Further biological evaluation is recommended to validate their therapeutic potential.

Keywords: Medicinal plants, *Tragia involucrate*, *Psidium guajava*, GC-MS analysis, Phytochemical profiling, Bioactive constituents.

INTRODUCTION

The rising use of complementary and alternative medicine underscores the limitations of conventional pharmacological strategies in managing chronic diseases, particularly with respect to efficacy and safety [1]. Medicinal plants, integral to traditional medical systems worldwide, represent a critical therapeutic resource owing to their diverse bioactive phytochemicals. The World Health Organization (1977) defines these plants as natural sources of compounds with either inherent pharmacological activity or potential as serve as molecular templates for drug development. Importantly, plant-derived extracts and metabolites

continue to contribute substantially to modern therapeutics, supporting the development of drugs for cardiovascular, hepatic, hypertensive, analgesic, and other pathological conditions [2].

Psidium guajava, a perennial evergreen shrub, belongs to the family *myrtaceae*, and genus *Psidium*. It is a widely cultivated tropical fruit species, commonly known as guava [3]. The leaves of *P. guajava* have an extensive history of use in traditional medicine. Primary applications include the oral consumption of leaf decoctions and infusions to treat gastrointestinal ailments such as diarrhea, dysentery, and gastroenteritis, as well as cough, diabetes mellitus, and rheumatism. Topically, leaf preparations are applied as poultices for wound care, skin ulcers, and to alleviate rheumatic pain. Other common uses include chewing the leaves for toothache and oral hygiene, using decoctions as a gargle for mouth ulcers, and employing preparations for their anti-bactericidal properties and as a febrifuge. A decoction of leaves and bark is also traditionally used to expel the placenta after childbirth [4,5]

Tragia involucrata is a perennial herb with a well-documented history in traditional medicine. Its ethnopharmacological applications are extensive, with roots in classical Ayurvedic texts for managing epilepsy, fever, and respiratory disorders, urinary disorders [6]. Traditional uses include the treatment of gastrointestinal complaints like constipation, respiratory illnesses such as whooping cough, and metabolic conditions including diabetes [7,8]. Topical applications of root and seed paste are employed for dermatological purposes, including treating scorpion stents and preventing hair loss [9]. These diverse uses are supported by modern research indicating its antidiabetic and therapeutic potential [10].

MATERIALS AND METHODS

Instruments and Chemicals

Rotary vacuum evaporator (M/s Buchi, Switzerland), HPLC-grade solvents (Hexane, chloroform, ethyl acetate, methanol were purchased from M/s Merck Life Science Pvt. Ltd, Mumbai, India, Gas chromatography- mass spectrometer used was Biomate 3S (M/s Shimadzu GC-MS).

Plant Material Collection and Authentication

The whole plant of *T. involucrata* L. (Kodithoova) and leaves of *P. guajava* L. (Pera) were collected from Thrikkovilvattom, Kollam, Kerala (8.896066° N, 76.667228° E) in July 2024. Samples were cleaned, shade-dried, and stored under dry, dark conditions. Species identity was confirmed by DNA barcoding at the Regional Facility for DNA Fingerprinting, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala, India.

Preparation of Crude Extracts

Powdered samples of *T. involucrata* L. and *P. guajava* L. were reflux-extracted with methanol (99% v/v) at a solvent-to-solid ratio of 25:1 (v/w) for 24 h at 67 °C. The extracts were concentrated under reduced pressure at 40 °C using a rotary evaporator and dried.

Fractionation of Extracts

Methanolic extracts of *T. involucrata* (MTI) and *P. guajava* (MPG) (500 mg each) were suspended in 50 mL distilled water and successively partitioned with solvents of increasing polarity (hexane → chloroform → ethyl acetate → methanol) using a separatory funnel. Each solvent extraction was performed thrice (100 mL each), and the combined organic phases were filtered (Whatman No. 1) and concentrated by solvent evaporation.

GC–MS Analysis

The phytochemicals present in the hexane fraction of *T. involucrata* and methanol fraction of *P. guajava* were identified using a Gas Chromatography–Mass Spectrometry (GC–MS) system at the Centre for Analytical Instrumentation, Kerala Forest Research Institute (KFRI), Peechi, Kerala. The analysis was performed with a Shimadzu Nexus GC-2030 instrument operating within a mass range of 50–500 m/z. Helium was employed as the carrier gas at a constant flow rate of 1 mL/min. For the hexane fraction, the oven temperature was initially set at 70 °C, increased at a rate of 8 °C/min, and held for 2 minutes, after which it was raised to 280 °C at a rate of 4 °C/min and maintained isothermal for 5 minutes. For the methanol fraction, the carrier gas flow rate was 1 mL/min. The oven temperature was set at 80 °C and held isothermal for 4 minutes, then increased to 280 °C at a rate of 5 °C/min and maintained at that temperature for 6 minutes. Aliquots of the extracts were injected into the chromatographic column after establishing a clear baseline. Major constituents were identified by comparing the obtained mass spectra with the NIST 20 library.

RESULTS

GC-MS analysis of hexane fraction of *T. involucrata* (HFT)

Gas chromatography–mass spectrometry (GC–MS) was employed to characterize and quantify the chemical constituents present in the plant sample. The analysis of the HFT revealed 14 hexane soluble phytocompounds. Compound identification was established through evaluation of molecular formulae, retention times, and peak areas. The comprehensive results of the GC–MS profiling, including the chromatogram, are presented in Figure 1, Table 1 and Table 2.

GC-MS analysis of methanol fraction of *P. guajava* (MFP)

In the present investigation, the MFP was subjected to GC–MS analysis, which resulted in the detection of 10 methanol-soluble phytocompounds. The identification of these metabolites was accomplished through careful evaluation of their molecular formulas, retention times, and peak areas, which ensured reliability and accuracy of the findings. The overall chromatographic and mass spectral data, along with the corresponding chromatogram, are systematically illustrated in Figure 2, Table 3 and Table 4, providing a comprehensive representation of the chemical profile of the extract.

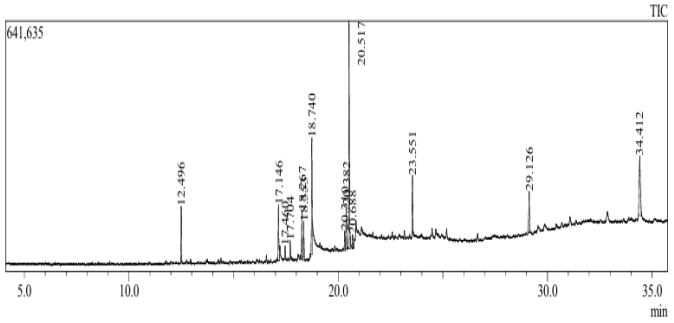

Figure 1: Chromatogram of HFT analysed using GC-MS

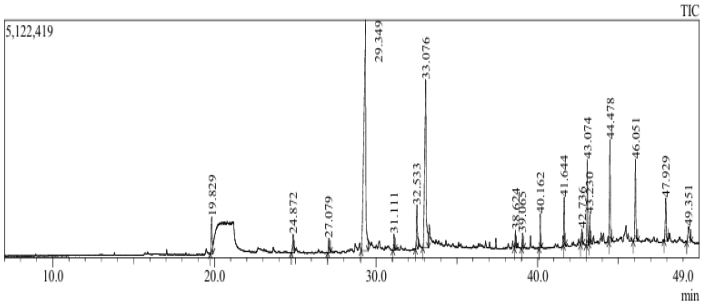

Figure 2: Chromatogram of MFP analysed using GC-MS

Table 1: GC-MS analysis of HFT

Peak#	R. Time	Area	Area%	Height	Height%	A/H	Mark	Identified compound
1	12.496	252940	5.36	142629	6.88	1.77	MI	2,4-Di-tert-butylphenol
2	17.146	323417	6.85	142020	6.85	2.28	MI	Neophytadiene
3	17.46	77062	1.63	34991	1.69	2.2	MI	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
4	17.704	91084	1.93	46401	2.22	1.98	MI	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
5	18.267	224811	4.76	123418	5.96	1.82	MI	Hexadecanoic acid, methyl ester
6	18.353	168135	3.94	98403	4.75	1.89	MI	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester
7	18.74	719010	15.23	297493	14.38	2.41	MI	n-Hexadnedioic acid
8	20.31	96830	2.05	49935	2.41	1.94	MI	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
9	20.382	219884	4.66	111262	5.37	1.98	MI	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
10	20.517	1250693	26.5	387593	18.12	2.14	MI	Phytol
11	20.688	68984	1.46	34994	1.69	1.97	MI	Methyl stearate
12	23.551	297701	6.18	151081	7.29	1.93	MI	Hexadecanoic acid, bis(2-ethylhexyl) ester
13	29.126	303361	6.43	103880	5.01	2.92	MI	Squalene
14	34.412	614012	13.01	151474	7.31	4.05	MI	dl-alpha-Tocopherol

Table 2: Phytochemical Profiling of Identified Compounds from HFT

Identified compound	Class of compound	Molecular formula
2,4-Di-tert-butylphenol	Alkylated phenol	$C_{14}H_{22}O$
Neophytadiene	Terpenoids	$C_{20}H_{38}$
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Diterpene	$C_{20}H_{40}O$
Hexadecanoic acid, methyl ester	Saturated fatty acid	$C_{17}H_{34}O_2$
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Polyunsaturated fatty acid (PUFA)	$C_{19}H_{34}O_2$
n-Hexadecanoic acid	Saturated fatty acid	$C_{16}H_{32}O_2$
Phytol	Diterpene	$C_{20}H_{40}O$
Methyl stearate	saturated fatty acid ester	$C_{19}H_{38}O_2$
Hexadecanoic acid, bis(2-ethylhexyl) ester	Saturated fatty acid	$C_{38}H_{76}O_2$
Squalene	Triterpene	$C_{30}H_{50}$
dl-alpha-Tocopherol	Fat soluble vitamin	$C_{29}H_{50}O_2$
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Polyunsaturated fatty acid (PUFA)	$C_{19}H_{32}O_2$
Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	Alkylated phenol	$C_{18}H_{28}O_3$

Table 3: GC-MS analysis of MFP

Peak #	R. Time	Area	Area %	Height	Height %	A/H	Mark	Name
2	24.872	1918760	1.70	399318	1.80	4.87	MI	Tetradecanoic acid
3	27.079	1285357	1.14	308528	1.40	4.20	MI	Pentadecanoic acid
4	29.349	41649997	36.95	4970027	22.76	8.38	MI	n-Hexadecanoic acid
5	31.111	1084317	0.96	445200	1.58	3.14	MI	Octadecanoic acid
6	32.533	3991027	3.53	945280	4.33	4.21	MI	Oleic Acid
7	33.076	23975858	21.27	3642970	16.60	6.61	MI	Octadecanoic acid
8	36.824	1028472	0.91	486199	1.75	2.11	MI	Pentacosane
10	40.162	2060929	1.83	728190	3.33	2.83	MI	Pentacosane
11	42.044	2831501	2.51	1029010	4.71	2.75	MI	Pentacosane
12	42.736	1148000	1.05	328041	1.50	3.61	MI	13-Docosenamide, (Z)-
13	43.070	5435849	4.83	1864627	8.45	2.91	MI	Dotriacontane
14	43.306	15624905	13.95	611871	2.80	25.54	MI	Squalene

15	44.478	7141766	6.34	2199812	10.07	3.25	MI	Dotriacontane
16	45.610	6751939	6.09	1765843	8.08	3.82	MI	Dotriacontane
17	47.929	4183688	3.71	922444	4.22	4.54	MI	Dotriacontane
18	49.351	2439464	2.16	324100	1.49	7.52	MI	Tetradecyl palmitate

Table 4: Phytochemical Profiling of Identified Compounds from MFP

Isolated compound	Class of compound	Molecular formula
Tetradecanoic acid	saturated fatty acid	$C_{14}H_{28}O_2$
Pentadecanoic acid	saturated fatty acid	$C_{15}H_{30}O_2$
n-Hexanedioic acid	saturated fatty acid	$C_6H_{10}O_4$
Octadecanoic acid	saturated fatty acid	$C_{18}H_{36}O_2$
Pentacosane	saturated hydrocarbon	$C_{25}H_{52}$
13-Docosenamide, (Z)-	unsaturated fatty acid	$C_{22}H_{43}NO$
Dotriacontane	saturated hydrocarbons (alkanes)	$C_{32}H_{66}$
Squalene	Triterpene	$C_{30}H_{50}$
Tetradecyl palmitate	saturated fatty acid	$C_{30}H_{62}O_2$
Oleic acid	Saturated fatty acid	$C_{18}H_{34}O_2$

DISCUSSION

Gas chromatography (GC) separates volatile and non-polar compounds based on their physical and chemical properties and their interaction with the stationary phase of the column. After separation, the compounds are analyzed by tandem mass spectrometry (MS/MS), where ionization and fragmentation of ions occur, enabling detection of both precursor and product ions. In MS/MS, two mass analyzers are arranged around a collision cell; ions selected in the first analyzer undergo collision-induced fragmentation with an inert gas in the cell, which enhances analytical specificity and precision [11]. Phytochemical profiling and chemotaxonomic research of medicinal plants rich in bioactive compounds heavily rely on the analytical capabilities of GC-MS techniques, [12] with the present study specifically focusing on the identification of biologically active phytochemicals through this technique. In a previous investigation, GC–MS analysis of ethanol extracts prepared from air-dried powdered leaves of *T. involucrata* revealed the presence of phytoconstituents closely comparable to those identified in the current study. The reported compounds included 3,7,11,15-tetramethyl-2-hexadecen-1-ol, (Z)-13-docosenamide, octadecanoic acid, neophytadiene, hexanedioic acid, phytol, and α-tocopherol, thereby demonstrating a strong concordance with the present findings [13].

Neophytadiene is classified within the sesquiterpenoid group of organic compounds and has been reported to exhibit diverse pharmacological properties, including analgesic, antipyretic, anti-inflammatory, antioxidant and antimicrobial activities [14]. Hexanedioic acid also known as adipic acid and represented by the molecular formula $(CH_2)_4(COOH)_2$, has been reported to possess antibacterial activity. It demonstrates inhibitory effects against the growth and proliferation of several pathogenic microorganisms, including *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Shigella dysenteriae* [15]. Phytol has been recognized as a safe and economical adjunct in the treatment of schistosomiasis, a parasitic infection caused by helminths of the genus *Schistosoma* [16]. Its antimicrobial effectiveness is well established, showing activity against *Escherichia coli*, *Candida albicans*, and *Aspergillus niger* [17]. The compound is further distinguished by strong antioxidant potential and notable antinociceptive effects. Importantly, phytol also counteracts the teratogenic impact of retinol and restricts the growth of *Staphylococcus aureus* [18,19]. α-Tocopherol contributes to cardiovascular protection through its ability to suppress inflammatory processes and regulate proteins responsible for lipid uptake, transport, and breakdown. Its combined antioxidant, anti-inflammatory, and anti-atherogenic functions make it an important compound in reducing the risk and progression of atherosclerosis and related cardiovascular diseases [20].

Gas chromatography-mass spectrometry analysis of the methanolic extract of *Olea dioica* revealed the presence of tetradecanoic acid, pentadecanoic acid, oleic acid, and squalene, compounds that were also detected in the methanol fraction of *P. guajava*. These phytoconstituents exhibited notable antibacterial activity across multiple test concentrations, with the most pronounced inhibition observed against *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Xanthomonas campestris*, and *Salmonella typhimurium* [21]. Oleic acid, a long-chain unsaturated fatty acid, has been shown to inhibit the proliferation of *Staphylococcus aureus* by interfering with enoyl-acyl carrier protein reductase (FabI), an essential enzyme required for bacterial fatty acid biosynthesis.long chain unsaturated fatty acids [22]. Octadecanoic acid (stearic acid) found in the methanolic fraction of guava leaf, has been reported to disrupt the cell cycle and trigger apoptotic cell death in Hep-G2 cells [23]. Squalene, a bioactive triterpene, is recognized for its cardioprotective, antioxidant, antibacterial, antifungal, anticancer, and detoxifying properties. Numerous investigations have highlighted its significance across nutritional, medicinal, and pharmaceutical applications. It is regarded as a promising chemopreventive and chemotherapeutic compound, capable of suppressing tumor development in organs such as the colon, skin, lungs, and breast. Additionally, squalene has been shown to enhance immune responses, supporting its potential therapeutic use in managing conditions including HIV, H1N1 influenza, leukemia, papilloma, and herpes [24-27].

CONCLUSION

In conclusion, the comprehensive phytochemical profiling of HFT and MFP using GC-MS highlights the significance of advanced analytical methods in medicinal plant research. GC-MS enables precise identification and characterization of volatile and semi-volatile bioactive constituents, providing a detailed chemical fingerprint essential for chemotaxonomic studies and therapeutic assessment. The detection of pharmacologically important compounds such as neophytadiene, phytol, α-tocopherol, squalene, and diverse fatty acids, not only validates earlier findings but also expands our understanding of these plants' medicinal potential. Compounds identified in this study are associated with a broad spectrum of biological activities, including anti-inflammatory, antimicrobial, antioxidant, and cardioprotective effects, underscoring the utility of GC-MS in discovering molecules of pharmaceutical interest. By elucidating the phytochemical diversity of

T. involucrata and *P. guajava*, this investigation supports their continued exploration as valuable sources of drug leads and bioactive natural products for the development of novel therapeutic agents.

Conflict of interest

The authors declared no conflict of interest.

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