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Research Article

Comprehensive GC-MS and Phytochemical Profiling of Momordica charantia Extracts: Therapeutic Insights from Acetonic and Alcoholic Solvent Systems

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ABSTRACT

Momordica charantia, a perennial member of the Cucurbitaceae family, is widely cultivated across the globe due to its extensive therapeutic properties and ability to thrive in all seasons. Despite its bitter taste, the plant holds significant medicinal value, offering antidiabetic benefits and aiding in the treatment of heart diseases, cancer, neurological disorders, colds, and coughs. This study focuses on the phytochemical and GC-MS analysis of *M. charantia* extracts from its leaves, seeds, and fruit pulp prepared using acetone and alcohol as solvents. Extraction was performed via Soxhlet and natural evaporation methods. Key bioactive compounds identified include pentanoic acid, neophytadiene, n-hexadecanoic acid, methyl citrate, octadecanoic acid, and pentyl ester. Additionally, secondary metabolites such as steroids, triterpenoids, alkaloids, flavonoids, phenols, tannins, proteins, and saponins were detected. These compounds exhibit diverse industrial and pharmaceutical applications, including antioxidant, anti-inflammatory, antimicrobial, insecticidal, and cancer-preventive properties. The findings underscore the potential of *M. charantia* as a source of bioactive agents for therapeutic and industrial use.

INTRODUCTION

M.charantia (commonly known as bitter melon, bitter gourd, or karela in Hindi) is a medicinally significant plant from the Cucurbitaceae family. It thrives in tropical and subtropical regions, including Asia, East Africa, and the Caribbean. Its cultivation has also extended to South America, where it is valued as both a food source and a medicinal plant. Despite its distinctly bitter taste, *M. charantia* is highly regarded for its nutritional, economic, and therapeutic properties. As noted by the Botanical Survey of India, more than 46,000 plant species have been identified, with over 7,000 species acknowledged for their medicinal value (Bisoi and Panda, 2015). Among these, *M. charantia* is particularly prominent due to its phytochemical richness, which includes alkaloids, flavonoids, phenols, tannins, and glycosides.

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These compounds are primarily responsible for its diverse pharmacological activities, such as antidiabetic, anti-inflammatory, and anticancer effects (Singh et al., 2007). Research emphasizes *M. charantia*'s potential to lower blood glucose levels and enhance insulin sensitivity. Key bioactive compounds like charantin, vicine, and polypeptide-p have demonstrated their efficacy in glucose metabolism regulation, positioning the plant as a promising agent for type 2 diabetes management (Dwijajant et al., 2020). Flavonoids and phenols in *M. charantia* exhibit anti-inflammatory effects, helping to manage chronic inflammatory conditions effectively. Bioactive components such as charantin and momordicosides have shown notable anticancer activities by inducing apoptosis and inhibiting the proliferation of tumor cells (Singh et al., 2017). Different parts of *M. charantia* have been traditionally utilized for specific ailments. Leaves are employed to treat helminthiasis, intermittent fever, and burning sensations, while the fruits are commonly used for managing diabetes, asthma, and skin disorders. Additionally, seeds are known for their effectiveness in treating ulcers and other gastrointestinal issues. Gas Chromatography-Mass Spectrometry (GC-MS) is an essential technique for identifying and isolating bioactive compounds in plants. It is particularly effective in detecting long-chain hydrocarbons, esters, steroids, amino acids, and nitro compounds, making it a valuable tool for pharmaceutical research. Studies using GC-MS on *M. charantia* have identified significant secondary metabolites, including n-hexadecanoic acid, octadecanoic acid, neophytadiene, and phytol, which contribute to its therapeutic potential (Bortolotti et al., 2019). This study aims to analyze the chemical composition and bioactive compounds present in acetonic and alcoholic extracts of *M. charantia* leaves, seeds and fruit pulp. By employing Soxhlet extraction followed by GC-MS analysis, the research seeks to evaluate the pharmaceutical potential of these extracts for various therapeutic applications.

MATERIALS AND METHODS

Collection of Plant Material

Plant material from *M. charantia* was collected from a local area in Satara city during the summer season, as this period is known to influence optimal phytochemical composition. Leaves, seeds, and fruit pulp were gathered and shade-dried. Identification of *M. charantia* was conducted using relevant literature.

Preparation of Powder and Extract

The dried material was crushed using a mortar and pestle to obtain a fine powder for the extraction process. Soxhlet extraction, a widely used method for efficient extraction, was employed. For this process, 20 g of powdered material was extracted using 200 ml of solvent (acetone or alcohol). The extraction continued until the solvent's color changed, which typically required 6–8 hours at a temperature of approximately 60–70°C, indicating completion. The resultant material was subjected to natural evaporation, leaving the extract in a Petri dish. The extract was stored in a refrigerator for further analysis.

GC-MS Analysis

The acetonic and alcoholic extracts were subjected to Gas Chromatography-Mass Spectrometry (GC-MS) to identify bioactive compounds, as these solvents effectively dissolve a wide range of polar and non-polar bioactive constituents, facilitating a comprehensive analysis. GC-MS analysis was conducted using a Shimadzu QP2010 system equipped with a non-polar 60m RTX-5ms column. Initial oven temperature was set at 50°C (held for 4 minutes), increasing to a final temperature of 250°C. The injection temperature was maintained at 250°C in split mode, with a total run time of 45 minutes. Compounds were identified by comparing chromatographic retention times with those in the NIST Library using a Quadrupole detector. Quantitative determination was achieved by correlating peak areas to TLC areas obtained from the GC-MS. Internal standards were utilized during the process to ensure accuracy and reproducibility of the measurements.

Identification of Phytochemical Compounds

1.Tests for Glycosides

i). Keller-Killani Test (Cardiac Glycosides): Add a few drops of glacial acetic acid and 2 ml of ferric chloride solution to the test solution. Add concentrated sulfuric acid along the test tube sides. A reddish-brown lower layer and a bluish-green upper layer indicate glycosides, as these color changes result from complexation reactions between glycosides and the reagents used. The formation of these layers highlights the distinct chemical interactions and serves as a specific indicator for the presence of cardiac glycosides.

ii). Raymond's Test: Treat the test solution with dinitrobenzene in hot methanolic alkali. A violet color indicates glycosides.

iii). Legal's Test: Treat the test solution with 1 ml pyridine and 1 ml sodium nitroprusside. A pink to red color confirms glycosides.

2.Tests for Alkaloids

i). Mayer's Test: Treat the test solution with Mayer's reagent (potassium mercuric iodide). A cream-colored precipitate indicates alkaloids.

ii). Wagner's Test: Treat the acidic test solution with Wagner's reagent (iodine in potassium iodide). A brown precipitate confirms alkaloids.

iii). Hager's Test: Treat the acidic test solution with Hager's reagent (saturated picric acid solution). A yellow precipitate confirms alkaloids.

3.Tests for Flavonoids

i). Ferric Chloride Test: Add a few drops of ferric chloride solution to the test solution. An intense green color confirms flavonoids.

ii). Shinoda Test: Add magnesium ribbon fragments and concentrated hydrochloric acid to the test solution. A pink to magenta red color indicates flavonoids.

iii). Zinc-Hydrochloric Acid Reduction Test: Treat the test solution with zinc dust and a few drops of hydrochloric acid. A magenta red color confirms flavonoids.

iv). Alkaline Reagent Test: Treat the test solution with sodium hydroxide. An increase in yellow intensity, turning colorless upon adding dilute acid, confirms flavonoids.

v). Lead Acetate Test: Add lead acetate solution (10% w/v) to the test solution. A yellow precipitate confirms flavonoids.

4.Tests for Steroids

i). Chloroform Test: Dissolve 1 mg of crude plant extract in 1 ml chloroform, then add concentrated sulfuric acid along the sides. A red upper layer and yellow with green fluorescence in the sulfuric acid layer indicate steroids.

ii). Salkowski's Test: Mix the extract with concentrated sulfuric acid carefully. A reddish-brown interface confirms steroids.

5.Tests for Phenols

i). Ferric Chloride Test: Add 1-2 drops of $FeCl_3$ to a small amount of ethanolic extract in water. The appearance of blue, green, red, or purple colors during this test is significant as it indicates the presence of phenols. These color variations arise due to the complexation of phenols with ferric ions, which produce characteristic chromophores, helping in their identification and differentiation from other compounds.

6.Tests for Terpenoids

i). Salkowski's Test: Add concentrated sulfuric acid to the test solution, shake, and allow it to stand. A red lower layer indicates sterols.

ii). Liebermann-Burchard Test: Treat the test solution with acetic anhydride, mix well, and add concentrated sulfuric acid along the sides. A brown ring at the junction and a green upper layer confirm terpenoids.

7.Tests for Saponins

i). Foam Test: Shake saponin with water. Persistent foam for at least 15 minutes indicates saponins.

ii). Hemolysis Test: Add 2 ml of filtrate to 2 ml of 18% sodium chloride and a few drops of blood. Observing hemolysis under a microscope confirms saponins.

8.Tests for Carbohydrates

i). Molisch's Test: Add Molisch's reagent to the test solution, then slowly add concentrated sulfuric acid. A purple ring at the junction confirms carbohydrates.

ii). Barfoed's Test: Treat the test solution with Barfoed's reagent and boil. A brick-red precipitate indicates carbohydrates.

iii). Benedict's Test: Treat the test solution with Benedict's reagent and boil. A reddish-brown precipitate confirms carbohydrates.

9.Tests for Proteins

i). Millon's Test: Heat the test solution with Millon's reagent. A yellow stain confirms proteins.

ii). Xanthoproteic Test: Boil the test solution with concentrated nitric acid. A yellow precipitate indicates proteins.

iii). Biuret Test: Treat the test solution with 40% sodium hydroxide and dilute copper sulfate solution. A blue color confirms proteins.

iv). Ninhydrin Test: Treat the test solution with ninhydrin reagent. A blue color indicates proteins.

10.Tests for Starch

i). Starch Test: Heat 1 ml of extract with 10 ml NaCl solution, then add starch reagent. A blue-purple color indicates starch.

11.Tests for Tannins

i). Gelatin Test: Dissolve the plant extract in distilled water and add 1% gelatin solution and 10% NaCl. A white precipitate confirms tannins.

ii). NaOH Test: Add 4 ml of 10% NaOH to 0.4 ml of extract. Shake well; an emulsion indicates tannins.

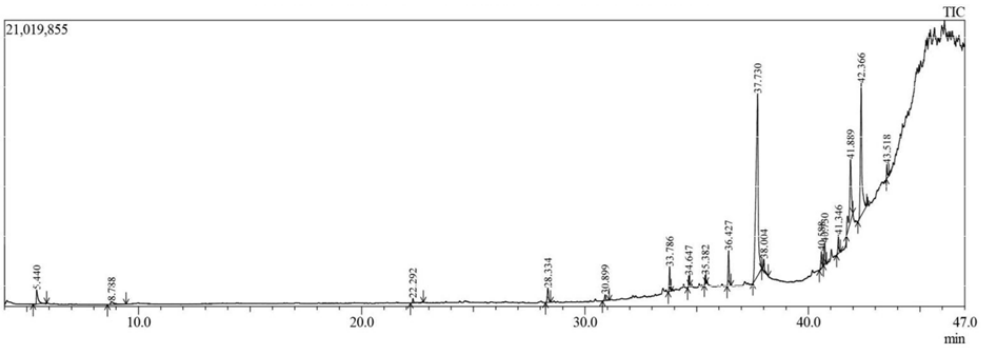
12. Test for Anthocyanins

i). HCl Test: Mix 2 ml of plant extract with 2 ml of 2N HCl. Adding ammonia changes the pink-red solution to blue-violet, confirming anthocyanins.

RESULTS:

GC-MS Analysis of *M. charantia* Fruit Acetone Extract

Gas chromatography-mass spectrometry (GC-MS) analysis of the *M. charantia* fruit acetone extract identified seventeen compounds (Fig. 1). The analysis revealed a complex mixture of compounds, with varying abundances. The most abundant compound identified was n-hexadecanoic acid (44.31% area), a saturated fatty acid with known biological activities. Other significant compounds included its methyl ester (hexadecanoic acid, methyl ester, 3.81% area) and octadecanoic acid (20.95% area), another saturated fatty acid. Several unsaturated fatty acids and their esters were also detected, including 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (1.85% area), 11,14,17-Eicosatrienoic acid, methyl ester (2.67% area), and Z-5,17-Octadecadien-1-ol acetate (12.32% area). The presence of neophytadiene (0.82% area), a diterpene known for its biological activity, is also noteworthy. Other compounds identified in lower abundance include various ketones, alkanes, and alcohols.



GC-MS Analysis of *M. charantia* Seed Alcohol Extract

Gas chromatography-mass spectrometry (GC-MS) analysis of the *M. charantia* seed alcohol extract identified twelve compounds (Fig. 2). The analysis revealed a complex mixture dominated by saturated fatty acids and their esters. The most abundant compound was octadecanoic acid (34.99% area), a saturated fatty acid, followed closely by n-hexadecanoic acid (17.40% area). The methyl ester of hexadecanoic acid was also present (1.00% area). Pentanoic acid (15.63% area) and its pentyl ester (1.31% area) were also significant components. The presence of

neophytadiene (1.26% area), a diterpene with established biological activities, was detected. Methyl stearate (3.06% area) and 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (1.16% area), both fatty acid derivatives, were also identified, amongst other minor components. One compound remains unidentified (40.860 min).

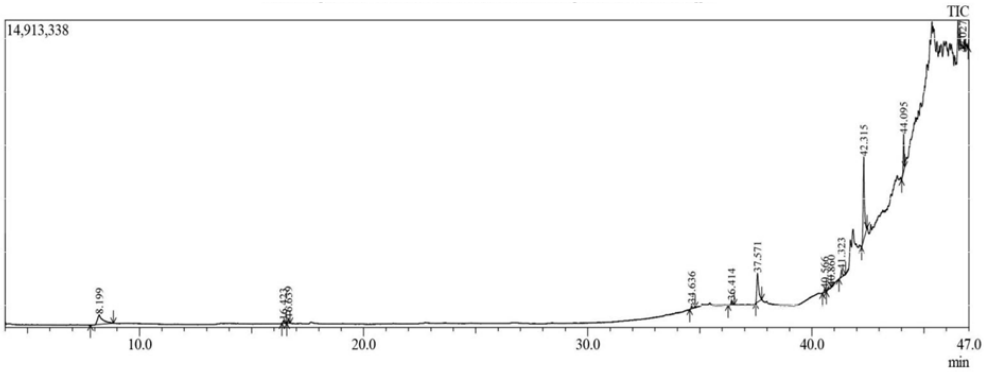


Fig 2: M. Charantia seed alcohol Chromatograph

GC-MS Analysis of *M. charantia* Leaf Alcohol Extract

Gas chromatography-mass spectrometry (GC-MS) analysis of the *M. charantia* leaf alcohol extract identified nine compounds (Fig. 3). The chromatogram shows a less complex profile compared to the seed and fruit extracts, although still showing several compounds with significant relative abundances. The most abundant compound was n-hexadecanoic acid (77.07% area), a saturated fatty acid. Phytol (12.63% area), a diterpenoid alcohol, was also present in a considerable amount. Neophytadiene (9.84% area), a diterpene, was another major component. Other compounds identified in much lower abundance include dodecane, tetradecane, phosphoric acid diethyl nonyl ester, and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol. One compound remains unidentified (42.285 min).

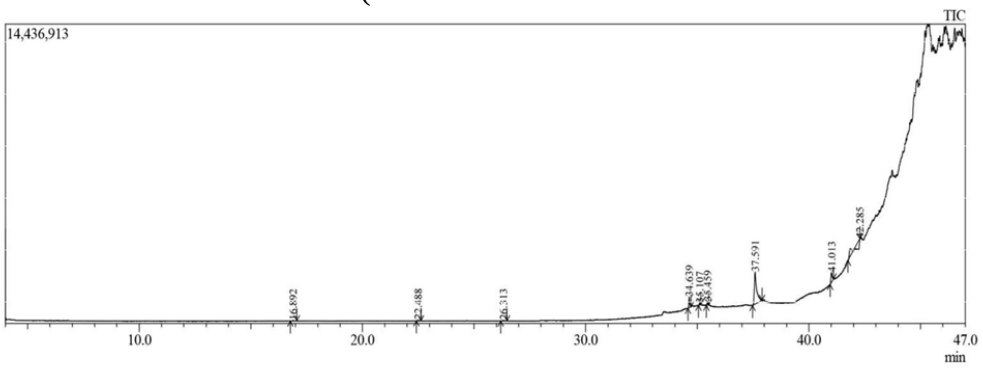


Fig 3: Momordia charantia leaves alcohol chromatograph

GC-MS Analysis of *M. charantia* fruit Alcohol Extract

The GC-MS analysis reveals a sample predominantly composed of fatty acids, with n-Hexadecanoic acid and Oleic Acid being the most abundant, comprising 41.07% and 38.47% of the total area, respectively (Fig. 4). Octadecanoic acid is also significant at 14.15%. These main components suggest that the sample could be an oil or lipid-based substance. Additionally, trace elements such as Tetradecanoic acid, 4H-Pyran-4-one, and various esters and hydrocarbons add to the complexity of the mixture. Such a profile is typical for applications in food, cosmetics, or as a component in biodiesel, where understanding the composition is crucial for quality and efficacy.

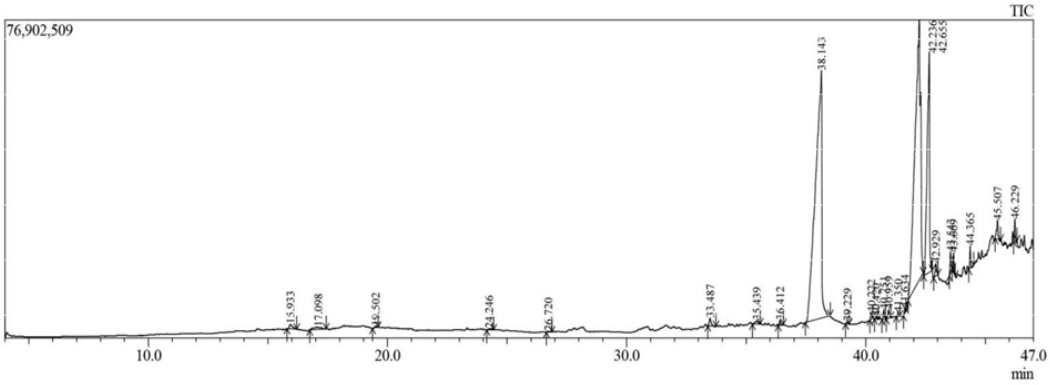


Fig 4*: M. charantia* fruit alcohol chromatograph

GC-MS Analysis of *M. charantia* Leaf Acetone Extract

Gas chromatography-mass spectrometry (GC-MS) analysis of the *M. charantia* leaf acetone extract identified twenty-four compounds (Fig. 5). The chromatogram reveals a complex mixture of compounds with a wide range of abundances. The most abundant compound was n-hexadecanoic acid (41.07% area), a saturated fatty acid. Oleic acid (38.47% area), an unsaturated fatty acid, was also highly abundant. Other significant components include several esters of fatty acids, such as octadecanoic acid (14.15% area), hexadecanoic acid, 2-hydroxyethyl ester (0.71% area), and glycidyl palmitate (0.48% area). Several other compounds were identified in trace amounts. The presence of 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (0.36% area) is noteworthy, along with other compounds like 2,4-dodecadienal (E,E)- (0.45% and 0.07% area) and various other ketones, alkanes, and substituted benzene derivatives.

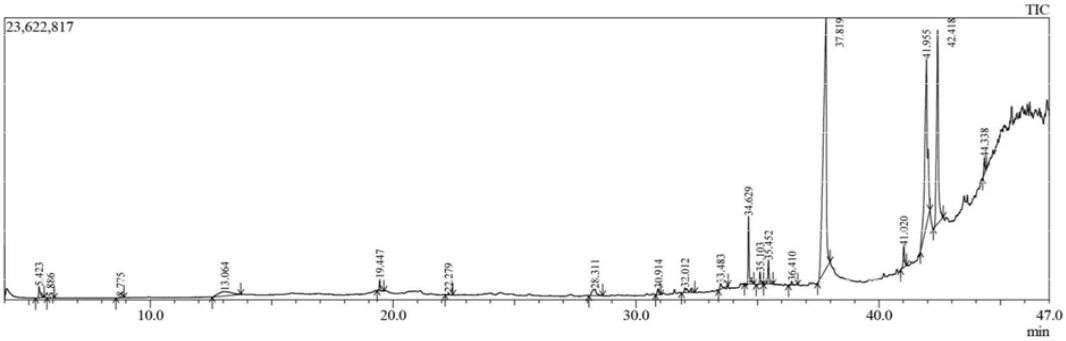


Fig 5: M. charantia leaves acetone Chromatograph

GC-MS Analysis of *M. charantia* Seed Acetone Extract

M. charantia seed compounds using acetone as the solvent in a gas chromatograph, a total of 18 compounds were identified based on their retention times and peak areas (Fig. 6). The chromatographic profile indicates a diverse composition of chemical compounds, with significant variability in their relative abundances. The compound identified as Octadecanoic acid exhibited the highest peak area, accounting for 30.64% of the total chromatographic area, indicating it as the predominant compound present in the extract. This was followed by n-Hexadecanoic acid, which represented 23.26% of the total area, and cis-9-Hexadecenal at 9.82%. Several esters were detected, including Hexadecanoic acid, pentyl ester (7.57%) and Pentanoic acid derivatives such as Pentanoic acid, pentyl ester, contributing 2.95% and 1.84% respectively. Notably, the compound 2-Pentanone, 4-hydroxy-4-methyl-, had a significant early elution at 5.420 minutes, accounting for 9.22% of the peak area. Minor components such as Ethylbenzene, p-Xylene, and various nonanoic and esters compounds were present in lower

quantities (ranging from 0.15% to 2.5%). An unidentified compound was noted at a retention time of 40.731 minutes, which constituted a negative peak area (-0.13%), possibly due to baseline noise or interference. The identification of these compounds suggests various biochemical roles and potential pharmacological activities inherent in *M. charantia* seeds.

The presence of significant fatty acids such as hexadecanoic and octadecanoic acids might implicate these seeds in lipid metabolic pathways and antioxidant activities. Further studies are recommended to characterize the unidentified compound and to explore the biological activities of these components, contributing to existing literature on the utilization of *M. charantia* seeds in nutraceutical and medicinal applications.

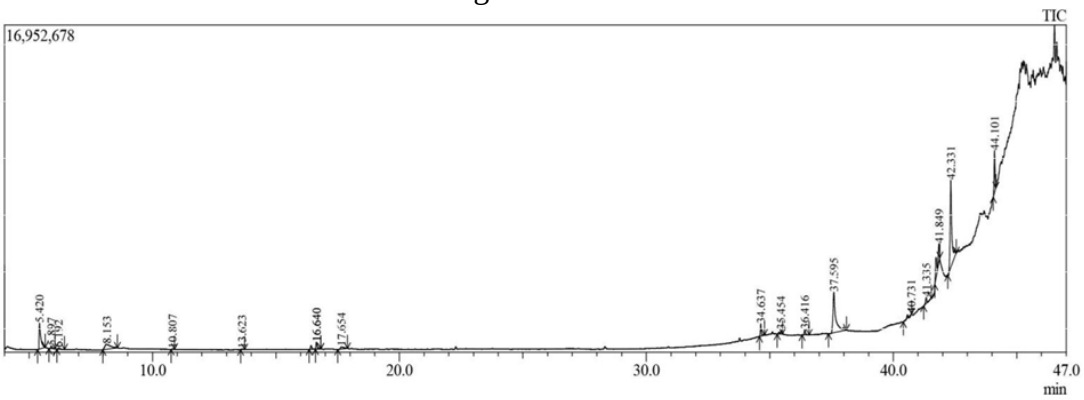


Fig 6: M. charantia seed acetone chromatograph

Qualitative phytochemical Analysis of *M. charantia* from Leaf, Seed and Fruit pulp

The qualitative phytochemical analysis revealed a diverse profile of secondary metabolites in *M. charantia*, with variations observed across different plant parts (leaf, seed, fruit) and extraction solvents of acetone and alcohol (Tab. 1). Steroids and triterpenoids were consistently detected across most samples, suggesting their significant presence in the plant. The presence of proteins and some glycosides varied across samples, indicating a potential influence of plant part and extraction method on their yield and solubility. Saponins were found predominantly in the leaf acetone and fruit alcohol extracts. Carbohydrates were detected in all samples, reflecting the presence of various sugars and polysaccharides. The detection of alkaloids, flavonoids, phenols, and tannins showed considerable variation among different samples, implying that their distribution is not uniform within the plant. The absence of starch in all samples indicates a lack of starch accumulation in the analyzed plant material. The inconsistent results for some phytochemicals could reflect variations in their solubility or stability across different solvents. Finally, the presence of an unidentified compound highlights the complexity of the plant's chemical profile and suggests a need for further exploration to fully elucidate its composition.

Table 1: Qualitative phytochemical Analysis of *M. charantia* from Leaf, Seed and Fruit pulp

Sr. No.	Phytochemical constituent	Test	Result					
			Leaf		Seed		Fruit	
			Ac	Al	Ac	Al	Ac	Al
1	Steroid	Chloroform test	+	-	+	-	+	+
		Salkowaski test	+	+	+	+	+	+

		Sulphur test						
2	**Triterpenoid**	Salkowaski test	+	+	+	+	+	+
		Liebermann Burchard test	+	+	+	+	+	+
		Biuret test	+	-	+	+	+	+
		Ninhydrin tes	-	-	-	-	-	-
3	**Glycosides**	Raymond's test	-	-	-	-	-	-
		Keller killiani test	+	-	-	+	+	-
		Legal's test	-	-	-	+	-	-
4	**Saponins**	Foam test	+	-	-	-	-	+
		Raymond's test	+	-	-	-	-	+
5	**Carbohydrates**	Molish test	+	+	+	+	+	+
		Benedict's test	-	-	-	-	-	-
		Leagal's test		-		+	-	-
6	**Alkaloids**	Wagner's test	-		+		-	+
		Hager's test	+	+	+		+	
		Mayer's test	+	+	+	-	+	+
7	**Flavonoids**	Lead acetate solution test	-	+	-	-	+	+
		Shinoda test	-	-	-	-	+	-
		Ferric chloride test	+	-	-	-	-	-
		Zinc-hydrocloric acid reduction test	+	-	-	-	-	-
		Alkaline reagent test	+	+	+	+	+	+
8	**Phenol**	Fecl3 test	-	+	-	-	-	+
9	**Protein**	Xanthoproteic test	+	-	+	+	+	+
		Milion's Test	-	-	+	-	+	+
		Ninhydrin test			-	-	-	
10	**Tannis**	Gelatin test	-	-	+	-	-	+
		NAOH test	-	-	+	+	+	-
11	**Starch**	Starch reagent	-	-	-	-	-	-
12	**Anthocynin**	HCL Test	-	-	-		-	-

The results of the GC-MS analysis and the qualitative phytochemical analysis of *M. charantia* demonstrate a rich profile of biologically active compounds that underline its potential applications in nutraceuticals and medicine. The high abundance of fatty acids, particularly n-hexadecanoic and octadecanoic acids, across various extracts signals their importance in lipid metabolism and possibly antioxidant activities, making *M. charantia* an attractive candidate for studies focused on lipid-based health benefits.

The comparative data further emphasize the variations in compound profiles depending on the part of the plant and the extraction solvent used, which suggests that the choice of extraction method can significantly influence the yield and

nature of bioactive compounds. The presence of triterpenoids, steroids, and other phytochemicals such as saponins and alkaloids reinforces the therapeutic potential of *M. charantia*, especially considering their known pharmacological activities. However, the variability in specific compounds among different extracts raises questions about optimizing extraction processes to maximize beneficial components. These findings provide a strong basis for further research aimed at isolating and characterizing these compounds for their health benefits and potential industrial applications.

DISCUSSION:

This study provides a comprehensive analysis of the bioactive compounds present in the leaf, seed, and fruit pulp extracts of *M. charantia*, highlighting its potential in medicinal applications. Utilizing Gas Chromatography-Mass Spectrometry (GC-MS), we identified sixty bioactive constituents, underscoring the plant's significance as a medicinal resource. Among the most prevalent compounds identified, n-hexadecanoic acid stands out for its widespread occurrence and known pharmacological benefits, such as anti-inflammatory and antimicrobial properties (Aparna V et al., 2012). Its presence in both *M. charantia* and other species like *Moringa peregrina* suggests a conserved biochemical role across plant species (Kumar et al., 2021).

Similarly, oleic acid and 9-octadecanoic acid were detected, both recognized for their beneficial cardiovascular and anticancer properties, aligning with previous research (Carrillo et al., 2012). Oleic acid, particularly, is known to inhibit cancer cell proliferation, further emphasizing the therapeutic potential of *M. charantia*. Neuophytadine, predominantly found in this study, has demonstrated anxiolytic and anticonvulsant activities, making it a compound of interest for neurological disorder treatment (Gonzalez et al., 2023). Furthermore, compounds such as hexadecanoic acid ethyl ester and 9,12-octadecadienoic acid, detected in this study, have known anti-inflammatory properties, corroborating findings from research in other plant extracts (Muzahid et al., 2022). This supports the potential for *M. charantia* to be used in developing new anti-inflammatory therapies. Recent comparisons with other medicinal plants such as *Holigarna grahamii*, which share some bioactive compounds, suggest a promising avenue for multi-plant utilization in pharmaceuticals. Additionally, the discovery of hexadecanoic acid methyl ester in both *Bacopa monnieri* and *M. charantia* underscores its phytopharmaceutical relevance, emphasizing its necessity in further drug development research (Jiménez-Nevárez et al., 2023). Its role as a biomarker for therapeutic potential in lipid metabolism and inflammatory conditions could be invaluable (Jeyasri et al., 2020).

CONCLUSION:

The current study successfully identified a total of sixty bioactive compounds and several phytochemicals from the alcoholic and acetonic extracts of the leaf, seed and fruit pulp of *M. charantia* using gas chromatography-mass spectrometry and qualitative phytochemical analysis. The diverse array of bioactive compounds and phytochemicals underscores the pharmaceutical potential of *M. charantia*. However, to fully harness the therapeutic benefits of this plant, further research is necessary to evaluate its potential toxicity, specifically on the chorioallantoic membrane (CAM) of *Gallus gallus domesticus*. These investigations are crucial to ensure the safe application of *M. charantia* in pharmaceutical formulations.

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