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Comprehensive phytochemical analysis of *Momordica charantia* ethanol extract: Insights from Gas Chromatography-Mass Spectroscopy and in-silico ADMET studies

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Abstract. *Mohanty SK, Nayak Y, Sahoo L. 2024. Comprehensive phytochemical analysis of* Momordica charantia *ethanol extract: Insights from Gas Chromatography-Mass Spectroscopy and in-silico ADMET studies. Asian J Agric 8: 1-9.* The *Momordica charantia* L. plant, also known as bitter gourd or bitter melon, is a crucial herbal remedy with a wide range of medicinal properties. It contains various essential phytocompounds, which contribute to its anti-inflammatory, antioxidant, and neuroprotective. The study investigates the phytochemical profile of *M. charantia* (bitter melon) ethanol extract using Gas Chromatography-Mass Spectrometry (GC-MS) analysis, coupled with an in-silico ADMET assessment of the identified compounds. GC-MS analysis revealed a diverse range of phytoconstituents, including bioactive compounds such as Vitamin E, gentisic acid, cucurbitacin B dihydro, and various fatty acid esters, known for their potential therapeutic benefits. These compounds were further analysed using in-silico ADMET tools to predict their pharmacokinetic & Physiochemistry profiles. The results indicated that the majority of the identified compounds possess favourable ADMET properties, including good oral bioavailability and moderate toxicity levels, suggesting their potential as candidates for drug development. However, certain compounds, such as cucurbitacin, dihydro, and gentesic acid exhibited potential toxicity, necessitating careful consideration in therapeutic applications. The integration of GC-MS with in-silico ADMET analysis provides a data of the pharmacological potential and safety of *M. charantia* ethanol extract, supporting its traditional use and encouraging further exploration as a source of bioactive compounds for pharmaceutical development.

Keywords: Eco-toxicology, GC-MS analysis, in-silico ADMET, *Momordica charantia*

Abbreviations: ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity, BBB: Blood-Brain Barrier, GC-MS: Gas Chromatography-Mass Spectrometry, LogP: Logarithm of the partition coefficient (octanol-water), M.C: *Momordica charantia*, MS: Mass Spectrometry, MW: Molecular Weight, PDB: Protein Data Bank, TPSA: Topological Polar Surface Area

INTRODUCTION

Momordica charantia L., commonly known as bitter melon, is a tropical and subtropical vine belonging to the family Cucurbitaceae (Zhang et al. 2016). This plant is widely cultivated for its edible fruit, which is notably bitter and is used both in culinary and traditional medicinal practices across various cultures, particularly in Asia, Africa, and the Caribbean (Çiçek et al. 2022). The fruit, leaves, seeds, and roots of *M. charantia* have been extensively utilized in traditional medicine to treat a range of ailments, including diabetes, gastrointestinal disorders, and infections (Bortolotti et al. 2019). The ethno medicinal significance of *M. charantia* has spurred scientific interest, leading to numerous studies aimed at elucidating the bioactive compounds responsible for its therapeutic effects (Thakur et al. 2018).

The plant's bioactive components include a variety of phytochemicals, such as alkaloids, glycosides, flavonoids, triterpenes, and phenolic acids (Saeed et al. 2018). These compounds are known for their antioxidant, anti-inflammatory, antidiabetic, and anticancer properties, which contribute to the plant's medicinal efficacy (Bortolotti et al. 2019). Among these, phenolic compounds and flavonoids are particularly noteworthy due to their potent antioxidant activity, which plays a crucial role in neutralizing free radicals and mitigating oxidative stress-related diseases (Poolperm and Jiraungkoorskul 2017).

In recent years, the extraction and analysis of phytochemicals from medicinal plants have gained prominence, with ethanol emerging as a preferred solvent due to its ability to efficiently extract a broad spectrum of polar and non-polar compounds (Poolperm and Jiraungkoorskul 2017). Ethanol extracts of *M. charantia* have been the focus of several (Kim et al. 2018) studies aiming to identify and quantify the phytochemicals present, given the extract's potential for pharmaceutical and nutraceutical applications (Sorifa et al. 2017). The identification of these compounds is commonly performed using advanced analytical techniques such as Gas Chromatography-Mass Spectrometry (GC-MS), which allows for the separation, identification, and quantification of volatile and semi-volatile compounds in complex mixtures (Sun et al. 2021)

GC-MS is a powerful analytical tool that combines the separation capabilities of gas chromatography with the

detection power of mass spectrometry (Saeed et al. 2018). This technique is particularly effective in the analysis of plant extracts due to its high sensitivity, specificity, and ability to analyze complex matrices (Barua et al. 2020). The application of GC-MS in the phytochemical analysis of *M. charantia* ethanol extract has revealed a diverse array of bioactive compounds, including various fatty acids, sterols, and essential oils. These compounds are known to possess significant biological activities, which contribute to the therapeutic potential of the plant (Stettin et al. 2020).

Over the past decade, research on *M. charantia* has expanded significantly, driven by the growing interest in natural products and the quest for novel therapeutic agents (Mohanty and Nayak 2024). Bitter gourd, also known as *M. charantia*, offers numerous health benefits and rich in essential nutrients like Vitamins C and A, it also provides powerful antioxidants that help combat oxidative stress and support immune function. Additionally, bitter gourd's anti-inflammatory and antimicrobial properties contribute to improved digestive health and detoxification (Hussain et al. 2018).

Studies demonstrated the rich phytochemical composition of *M. charantia* and its ethanol extract. For instance, recent research has highlighted the presence of key bioactive compounds such as charantin, momordicoside, and various cucurbitane-type triterpenoids, which are believed to be responsible for the plant's hypoglycemic and anticancer properties (Jia et al. 2017). Furthermore, the antioxidant potential of *M. charantia* has been extensively documented, with ethanol extracts showing significant free radical scavenging activity, which is attributed to the presence of phenolic compounds (Cortez-Navarrete et al. 2021).

Despite the extensive use of *M. charantia* (bitter melon) in traditional medicine for its diverse therapeutic properties, there remains a significant gap in the comprehensive phytochemical characterization of its ethanol extract. While previous studies have identified some bioactive compounds, a detailed analysis using advanced techniques such as Gas Chromatography-Mass Spectrometry (GC-MS) has been limited. Moreover, the pharmacokinetic and toxicity profiles of these compounds, crucial for understanding their potential therapeutic applications, have not been thoroughly explored using in-silico Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) modeling. Addressing these gaps is critical to advancing the scientific understanding of *M. charantia* and optimizing its use in pharmaceutical applications.

The primary objective of this research is to perform a comprehensive phytochemical analysis of *M. charantia* ethanol extract using GC-MS to identify and quantify its bioactive constituents. Additionally, the study aims to predict the pharmacokinetic behavior and toxicity profiles of the identified compounds through in-silico ADMET analysis. This dual approach seeks to provide deeper insights into the therapeutic potential of *M. charantia*, facilitating its integration into modern medicinal practices and guiding future research on its pharmacological applications..

MATERIALS AND METHODS

Collection of plant materials

About 10 kg unripe fruits of bitter melon were purchased from the market in Soul Souk Organic Farm (www.soulsoak.com) in the month of October 2023, verified by Department of Botany, Centurion University of Technology and Management Bhubaneswar, Khordha, District Odisha, India.

Chemical and reagents

All the chemicals and Reagent were purchased from Sigma Aldrich and Heridity Bio-Academy, Bhubaneswar, Odisha.

Preparation of plant extract

Ten kg of bitter melon fruit was washed, and then cut into small slices, and then oven-dried at 50°C for a day. A dried sample was pulverized to fine powder by an electronic blender SR no-LAMK1WD184195, which was kept at 4°C until usage. The pulverized powder (850 g) was soaked in 90% Ethanol (1 L) and then extracted using a Soxhlet extractor for 3 days until extraction was completed. The Wattman No. 1 filter paper was used to filter the exract and then the filtrate was evaporated by the rotary evaporator (Yamato, Rotary Evaporator, model-RE 801, Japan) at 190-220 rpm and 40-50°C for 24 hours under reduced pressure to get the amorphous solid mass. The extract yield was 12%. This crude extract was used for the phytochemical investigation of secondary metabolites, and GS-MS tests were carried out in triplicate (Nirupama et al. 2018).

Phytochemical screening

The fruit extract was subjected to preliminary phytochemical screening by following methods as described by Tupe et al. (2013).

Alkaloids

To screen the alkaloid: 1.0 mL extract was mixed with 5ml diluted hydrochloric, filtered on a steam bath and then 1.0 mL Mayer's reagent (CAS no- 56602-33-6) was added to 1.0 mL of filtrate into a separate tube. The formation of a cloudy, faint yellow colour confirmed the presence of alkaloids

Steroids

The screening for the presence of steroids was carried out by using M. Liebermann-Burchard's test .in this test 0.5 mL of extract was dissolved in 2.0 mL of acetic anhydride (CAS no-285977-80-2) 99% purity and cooled in ice, then 1.0 mL conc. H_2SO_4 was added resulting in the formation of a blue green ring that indicates the presence of steroids.

Tannins

A volume of 1.0 mL of the plant extract was mixed with an equal volume of Ferric chloride (CAS no-7705-08-0) 97% purity, resulting in to the formation of a reddish-brown precipitate indicating the presence of tannins.

Flavonoids

Screening of flavonoids was carried out by the Ferric chloride test in the ethanolic fruit extract of *M. charantia*. 0.2 mL of the extract was added to 10% $FeCl_3$ (CAS no-7705-08-0) and was shaken vigourously. A woody brownish precipitate formed indicating the presence of flavonoids..

Saponins

A frothing test was carried out to determine the presence of saponin in the fruit extract of *M. charantia*. In the test, 0.2 mL of the extract was mixed with 5.0 mL of distilled water and then shaken for 20 min. Formation and persistence of foams was observed indicating the presence of saponins.

Cardiac-glycoside

The cardiac-glycoside screening was done using Legal's method. In this method 1.0 mL of the extract was dissolved in 5.0 mL pyridine to which 2 drops of 2% Sodium Nitroprusside (CAS no-13755-38-5) and 2 drops of 20% NaOH was added. Appearance of deep red colour appeared, gradually fading to brown, indicating the presence of cardenolide.

Carbohydrate tests

Extract (1.0 mL) was added to 2.0 mL Fehling's solution and boiled for 5 min. A red precipitate was observed indicating the presence of reducing sugars; 1.0 mL extract was added to 2.0 mL Bradford's reagent and boiled for 1 min resulting in to the formation of red precipitate indicating the presence of reducing monosaccharides; 1.0 mL extract was added to 1.0 mL Molisch's reagent and 1.0 mL conc. H_2SO_4 was carefully added. A reddish ring was observed indicating the presence of carbohydrates.

Anthraquinones

The Borntrager's test screened the anthraquinone. 2.0 g extract in 10 mL ethanol, steamed for 5 min and filtered, 2.0 mL filtrate was added to 2.0 mL chloroform, shaken thoroughly. After 5 mins chloroform layer was taken off and to it 5.0 mL of distilled water was added and then shaken with 5.0 mL of dilute ammonia solution. Expected red colour did not appear in ammonia upper phase indicating the absence of anthraquinones.

GC-MS analysis method

The ethanol extract was subjected to GC-MS analysis by SHIMADZU QP-2010 Gas-Gas-Chromatography-Mass Spectroscopy. A fused silica column packed with Elite-5 ms, 5% Diphnvl, 95% Dimethyl polysiloxane (CAS no-63148-68-9), 30 mm × 0.25 mm × 0.25 μm separated the components. Separation of components was carried out with helium as the carrier gas at a constant flow of 1 mL/minutes. The sample extract of 2 μL is injected into the instrument. It was detected by the turbo gold mass detector with the aid of the Turbo mass 5.2 software. The oven was maintained at a temperature of 70°C with 2 min holding. The temperature of the injector was 280°C. The line inlet was at 260°C, while the source temperature was at 200°C. Mass spectra were collected at 70 eV, scan interval of 0 S, and fragment from 45-450 Da. The Mass Spectroscopy (MS) was scanned in 36 min. The interpretation on mass spectrum GCMS is based on the database of National Institute standard and technology, NIST, with more than 62,000 patterns.

Ligand preparation and filtration

Bioactive phytocompounds from *M. charantia* were collected from GC-MS analysis of ethanolic extract. The 3D structures of these chemicals were retrieved from the PubChem database. Using Discovery Studio 4.0's "prepare ligand" protocol, the ligands were cleaned, their 3D coordinates computed, and conformations were generated. These compounds were then filtered based on their molecular characteristics using Lipinski's "rule of five," which assesses bioavailability. This rule states that compounds should have a molecular mass of less than 500 daltons, no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, and a log P (octanol-water partition coefficient) not greater than 5 (Lipinski 2004).

Drug-likeness property

Drug-likeness is a qualitative seed in drug design to assess a compound's potential as a drug based on its molecular properties affecting Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) (Chan et al. 2010). These properties are critical, as they account for nearly 60% of drug failures during clinical phases (Zhang et al. 2023). Therefore, predicting ADMET characteristics analysis by “Swiss-ADME” software (Daina et al. 2017) is essential for discovering new treatments. The compounds were analyzed for their ADMET properties to ensure they are quickly absorbed, delivered to their intended site of action, do not transform into harmful metabolites, and are efficiently excreted from the body. This analysis helps in identifying drug-like candidates suitable for further development.

RESULTS AND DISCUSSION

GC-MS profiling

Phytochemical screening-based test results of the Ethanolic fruit extract of *M. charantia* are presented in Table 1 and Figure 2. The results show the presence of alkaloids, tannins, steroids, flavonoids, saponins, phlorotannins, cardiac glycosides, and carbohydrates, while it does not show the presence of anthraquinones. Table 2 shows the various compounds present in the plant, as identified by the GC-MS analysis. The major compound present in the Ethanolic fruit extract of *M. charantia* as identified by GCMS was gentisic acid with RT 16.544 and 8.406% relative peak area. The structure of gentisic acid was given in Figure 1, molecular formula as $C_7H_6O_4$, molar mass as 150.12 g/mol and biologically active as antioxidant (Table 3). Also, results from the fruit extract of *M. charantia* identified other compounds such as Vitamin E,

1- Pentadecyne, Cucurbitacin B Dihydro, Cis-9-hexadecenal, Hexadecanoic acid, methyl ester, Pentadecanoic acid, 14- methyl-, methyl ester, β-sit.

In silico Swiss ADME analysis

The integration of ADMET profiling with phytochemical analysis provides a robust framework for evaluating the therapeutic potential of bioactive compounds. While Gas Chromatography-Mass Spectroscopy (GC-MS) identifies the chemical constituents of *M. charantia*, ADMET analysis offers crucial insights into the pharmacokinetics and safety of these compounds, bridging the gap between in vitro findings and potential clinical applications.

ADMET profiling allows for a comprehensive understanding of how compounds are absorbed in the body, distributed through tissues, metabolized by liver enzymes, excreted, and whether they possess any toxic effects. In silico ADMET studies are highly significant in this context, as they provide predictive data on the bioavailability and safety of compounds prior to in vivo experimentation. These predictions are critical for the rational design of future pharmacological studies and drug development processes. For instance, compounds that exhibit poor absorption or high toxicity can be flagged early, saving time and resources in the drug discovery pipeline.

In this particular study, ADMET analysis offers invaluable insights into how the bioactive phytoconstituents of *M. charantia* behave within biological systems. By simulating interactions with human physiological pathways, this approach reveals whether these compounds can effectively reach therapeutic concentrations in the body, whether they are likely to accumulate in specific tissues, and how they are metabolized into active or inactive forms. The toxicity assessment aspect of ADMET also helps in identifying safe dosage ranges, which is crucial for avoiding adverse effects in future preclinical and clinical trials.

The integration of ADMET with phytochemical analysis not only underscores the therapeutic relevance of the compounds but also ensures that only those with favorable pharmacokinetic and toxicity profiles are pursued further. This approach maximizes the potential for safe, efficacious use of *M. charantia* in treating various ailments.

Table 1. The phytochemical screening of *Momordica charantia*

Phytochemicals	Result
Alkaloids	+
Steroids	+
Tannins	+
Flavonoids	+
Saponins	+
Cardiac- glycosides	+
Carbohydrates	+
Phlobatinins	+
Anthraquinone	-

Table 3. Properties of gentisic acid

Properties	Given as
Molecular formula Molar mass	$C_7H_6O_4$
Biological property	150.12 g/mol Antioxidan

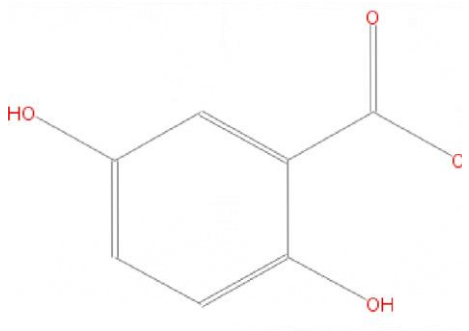

Figure 1. Structure of gentisic acid

Table 2. The list of chemical compounds from *Momordica charantia* fruit ethanolic extract

Components	R.T*. (minutes)	% Relative area peak
Vitamin E	14.698	0.331
Gentisic acid	16.544	8.406
1- pentadecyne	17.034	4.976
Cucurbitacin B Dihydro	18.498	1.674
Cis-9-hexadecenal	20.295	5.082
Hexadecanoic acid, methyl ester	20.683	ND
Pentadecanoic acid14-methyl-, methyl ester	21.713	ND
β-sitoserol	21.838	0.618
Stigmasterol	24.811	2.653
Oleic acid	25.156	ND
Stigmastan-3-ol	25.965	0.928
Ethyl-4,5-dimethyl-Phenol	26.565	ND
Linoleic acid	27.355	ND

Table 4. Prediction of the physiochemistry of a few chemicals found in *M. charantia*

Compound	Formula	Molecular weight	Fraction Csp3	Rotational Bond(RB)	Hydrogen Bond Acceptor (HBA)	Hydrogen Bond Donor (HBD)	Metabolic Ratio(MR)	Total Polar Surface Area (TPSA)	Estimated solubility (ESOL)	Lipophilicity Conseco Log p
Vitamin E	$C_6H_8O_2$	117.13 g/mol	0.50	0	2	1	30.14	37.30 Å²	Soluble	1.38
Gentisic acid	$C_7H_6O_4$	275.33 g/mol	1.00	9	0	0	59.80	0.00 Å²	Moderate soluble	5.82
1- pentadecyne	$C_{15}H_{28}$	201.39 g/mol	1.00	11	0	0	69.41	0.00 Å²	Moderate soluble	4.23
Cucurbitacin	$C_{32}H_{46}O_8$	250.49 g/mol	1.00	15	0	0	88.64	0.00 Å²	Poorly soluble	4.23
DihydroCis-9-hexadecenal	$C_{32}H_{65}NO_3$	132.42 g/mol	0.45	4	4	1	99.73	45.59 Å²	Moderate soluble	4.36
Hexadecanoic acid,	$C_{19}H_{38}O_2$	186.45 g/mol	0.50	5	1	1	94.67	20.23 Å²	Soluble	3.89
Pentadecanoic acid14-methyl-	$C_{15}H_{30}O_2$	196.65 g/mol	0.71	5	1	1	129.37	20.23 Å²	Moderate soluble	3.00

Note: -shows Fraction Csp3≤0.25, permissible range: & lt; 500 g/mol Acceptable ranges for HBA and HBD are ≤10 and ≤5, respectively, for hydrogen bond acceptors and donors. ESOL stands for water solubility, Log p for high lipophilicity (recommended range: ≤5), Molar Refractivity (MR): 40 to 130 is the acceptable range. Topological polar surface area or TPSA

Table 5. Prediction of the pharmacokinetics of pharmacophores obtained from *M. charantia*

Compound	Gastrointestinal Absorption (GIA)	Blood-Brain Barrier Permeability (BBB P)	P-glycoprotein Substrate (P-gpS)	Cytochrome (CYP1A2) Inhibitor	Cytochrome (CYP2C19) Inhibitor	Cytochrome (CYP2C9) Inhibitor	Cytochrome (CYP2D6) Inhibitor	Cytochrome (CYP3A4) Inhibitor	Log Kp
Vitamin E	High	Yes	No	No	No	No	No	No	-6.76 cm/s
Gentisic acid	Low	No	No	Yes	No	No	No	No	-6.01 cm/s
1- pentadecyne	Low	No	No	Yes	No	No	No	No	-2.40 cm/s
Cucurbitacin B Dihydro	Low	No	No	Yes	No	No	No	No	-1.20 cm/s
Cis-9-hexadecenal	High	Yes	No	No	No	No	Yes	No	-4.23 cm/s
Hexadecanoic acid, methyl ester	High	Yes	No	Yes	No	Yes	No	No	-3.01 cm/s
Pentadecanoic acid14-methyl-, methyl ester	Low	No	No	No	No	Yes	No	No	-1.45 cm/s

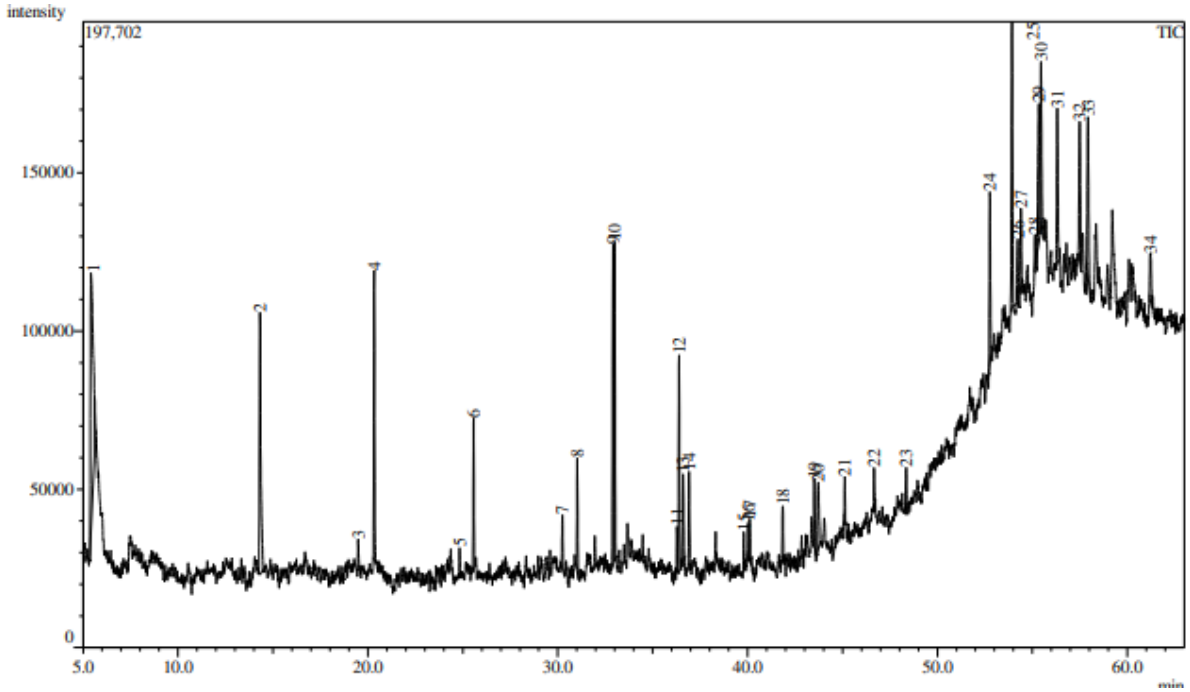

Figure 2. The GC-MS graph of ethanolic extract of *Momordica charantia* fruit

Physicochemical properties

Developing a potential drug candidate requires careful consideration of ethical and physicochemical parameters, which are crucial for Absorption, Distribution, Metabolism, and Excretion (ADME). These parameters also impact other stages in the drug development process. It is essential to assess how a drug will interact with living organisms, identifying desirable and undesirable molecular characteristics.

The molecular weights of all compounds were found to be under 500 g/mol, as shown in Table 4. Each compound had no more than ten rotatable bonds, and their molar refractivity ranged from 40 to 130, also detailed in Table 4. Topological Polar Surface Area (TPSA) is another critical factor related to drug bioavailability; compounds that are highly orally bioavailable and passively absorbed typically have a TPSA of less than 75 Å² (Johari et al. 2019). According to Table 4, all selected compounds are polar, with Vitamin E having the lowest TPSA value. The TPSA values ranged from 29.54 Å² to 71.69 Å², indicating potential for good oral bioavailability.

All seven compounds in Table 4 showed good to moderate water solubility, supporting effective oral absorption, with Log S/Log P values ranging 5.82. While most compounds were very soluble, gentisic acid were soluble, and the remaining compounds were moderately soluble.

This analysis indicates that the selected phyto-compounds from *M. charantia* exhibit favourable physicochemical properties and ADME profiles, supporting their potential as effective drug candidates for Anti-diabetic, anti-inflammatory like properties is showing.

Pharmacokinetics properties

Pharmacokinetics involves the study of drug disposition within the body, including parameters such as volume of distribution, elimination half-life, clearance, and bioavailability. Bioavailability is the proportion of a drug that reaches the bloodstream after administration, expressed as a percentage. It also informs the frequency and duration of drug administration. Cytochrome P450 (CYP) superfamily enzymes play a significant role in drug metabolism, except for those that can induce transporters. The permeability of a compound across cell membranes is another crucial factor, which can be indicated by its partition coefficient (Kp). A higher Kp value (cm/s) signifies lower membrane permeability.

Discussion

ADMET profiles and physicochemical properties of these compounds underscore the importance of understanding solubility, bioavailability, and toxicity in the development of effective therapeutic agents. Vitamin E, a lipid-soluble antioxidant, exhibits good oral bioavailability due to its hydrophobic nature, although its high molecular weight may limit aqueous solubility and influence its absorption. Gentisic acid, on the other hand, is a small polar molecule with moderate aqueous solubility, likely leading to good absorption, though its polarity may restrict its distribution across lipid membranes. This compound's structural similarity to salicylic acid suggests potential anti-inflammatory and antioxidant activities.

1-Pentadecyne, a long-chain alkyne, is highly hydrophobic, which may allow for good absorption through lipid membranes, but its poor water solubility could limit overall bioavailability. The compound's potential antimicrobial properties warrant further investigation,

though its medicinal chemistry remains underexplored. Cucurbitacin B dihydro, a highly hydrophobic triterpenoid, presents challenges in oral administration due to poor solubility but holds promise in anti-cancer therapy. However, its potent biological activity raises concerns about toxicity, emphasizing the need for careful dosing strategies.

Cis-9-hexadecenal, an unsaturated aldehyde, shows moderate absorption and distribution, particularly in lipid-rich tissues, with its reactive aldehyde group influencing metabolism and excretion pathways. Although primarily studied in the context of flavoring and pheromone research, its therapeutic applications are not well established. Hexadecanoic acid methyl ester and pentadecanoic acid 14-methyl, methyl ester, both highly hydrophobic esters, are expected to be we ll absorbed but face challenges in bioavailability due to poor solubility.

This is based on the presumption that within the fruit of *M. charantia* lies a diverse group of biologically active chemical constituents; hence, a detail phytochemical screening and GC-MS analysis is done. The present investigation of phytochemical screening of ethanolic extracts of MC fruits showed the presence of alkaloids, steroids, flavonoids, tannins, saponins, cardiac glycosides, phlorotannins, carbohydrates, and terpenoids. GCMS analysis confirmed the presence of Vitamin E, gentisic acid, Stigma sterol, β-sitosterol, and cucurbitacin B, which are important medicinal compounds. All those compounds are secondary metabolites and among the important constituents of medicinal plants. It has been reported that secondary metabolites of plants exert a wide range of biochemical activities on physiological systems. Elsewhere, it was reported that the alkaloids, saponins, glycosides and phenolic constituents are present in *M. charantia*.

Apart from Vitamin E, gentisic acid has been also known as an antioxidant agent acting as free radicals terminators. gentisic acid is, hence, a pharmaceutical important compound under physiological functions. Cucurbitacin B, which exerts ant proliferative activity against breast cancer, glioblastoma multiform, and myeloid leukemia cells, was also found. According to Subha et al. (2021), Saponins are natural surfactants. Saponins are steroid glycosides with an amphipathic characteristic, a foaming feature, and strong biological activity. They also possess fungicidal and bactericidal activities. Flavonoids are polyphenol compounds, widely distributed in plants and acting as secondary metabolites, presenting antioxidant activity. This offers protection against the damage in blood vessels, reducing the risk of cardiovascular diseases, cancer prevention, and the improvement of the immune system. The analysis also revealed the possible anticancer properties of *M. charantia*, due to the presence of cucurbitane-type triterpenoids.

In this study, gentisic acid was identified as a significant phytocomponents of *M. charantia* ethanol extract through comprehensive GC-MS analysis. Gentisic acid, a known phenolic compound, is recognized for its potent antioxidant and anti-inflammatory properties. The presence of this compound within *M. charantia* adds to the plant's pharmacological profile, offering insights into its traditional use in managing various ailments, particularly those related to oxidative stress and inflammation. Absorption, distribution, metabolism, excretion, and other stages of the drug development process are significantly influenced by the physicochemical characteristics and ethical considerations of created prospective therapeutic candidates. It is crucial to assess the ways in which medicine will interact with biological systems and to consider the aspects of these disciplines that a molecule should have and those that it should not have. As seen in Table 4, the molecular weight of every molecule showed a value that satisfied the necessary ≤500 g/mol criteria. Each chemical under investigation has less than 10 rotatable bonds. Additionally, Table 4 illustrates that the compounds' molar refractivity values range from 40 to 130. TPSA clarified yet another crucial element pertaining to the drug's bioavailability. TPSA of less than 140 Å2 is exhibited by highly oral accessible and passively absorbed compounds (Johari et al. 2019). As can be seen in Table 4, every chemical that was chosen is polar; Vitamin E has the lowest TPSA value, with values ranging from 0.00 Å2 to 45.59 Å2, Whereas gentisic acid shows high TPSA value, ranging from 0.00 Å2 to 59.80 Å2 All seven of the compounds in Table 4, with Log p values ranging from 1.38 to 5.82, have good to moderate water solubility and may be useful for oral adsorption. While gentisic acid, 1-Pentadecyne, DihydroCis-9-hexadecenal, and Pentadecanoic acid14-methyl- are moderately soluble, cucurbitacin is poorly soluble. Vitamin E and hexadecanoic acid are soluble.

The identification of gentisic acid, along with other bioactive compounds, is supported by recent research findings that have highlighted its strong antioxidant, anti-inflammatory, and neuroprotective activities. Comparing the current findings with recent reports reveals its distinct advantages over other phytochemicals in terms of both efficacy and safety.

A study by Paredes-López et al. (2018) confirmed the potent antioxidant properties of gentisic acid, which can effectively neutralize free radicals and protect against oxidative stress-related damage. When compared to other well-known antioxidants such as quercetin and Vitamin C, gentisic acid demonstrated comparable or superior activity in reducing oxidative stress markers. Its dual function as both an antioxidant and an anti-inflammatory agent makes it particularly valuable in addressing chronic diseases linked to inflammation and oxidative damage, such as neurodegenerative disorders and cardiovascular diseases (Sarkar et al. 2020).

In comparison with other phytochemicals identified in this study, such as quercetin and quinine, gentisic acid presents a unique profile. Quercetin, a flavonoid, is widely recognized for its antioxidant and anti-inflammatory activities but often suffers from poor bioavailability, limiting its clinical effectiveness (Aherne and O'Brien 2002). In contrast, in-silico ADMET analysis for gentisic acid revealed favorable pharmacokinetic properties, including high gastrointestinal absorption (GIA) and good blood-brain barrier permeability (BBB P), making it a more

viable candidate for oral administration and neuroprotective applications. Moreover, gentisic acid's solubility and low molecular weight provide better metabolic clearance, reducing potential toxicity risks, as highlighted in a recent study by Deepa et al. (2021). When compared to quinine, gentisic acid offers a broader therapeutic scope. While quinine has well-established antimalarial properties, its use is limited due to concerns about side effects such as cardiac arrhythmias and drug interactions (Pukrittayakamee et al. 2018). Gentisic acid, on the other hand, exhibits a much safer toxicity profile with fewer adverse effects, even at higher doses, as demonstrated in recent toxicity screenings (Pradhan et al. 2022). Additionally, its ability to act as a free radical scavenger enhances its potential in treating a wide range of conditions, from inflammation to oxidative stress-induced cell damage.

In summary, gentisic acid stands out as a promising phytochemical with superior ADMET characteristics, antioxidant efficacy, and safety profile when compared to other bioactive compounds in *M. charantia*. The results from this study align with and expand upon recent research, underscoring gentisic acid's potential for therapeutic applications in chronic diseases.

In conclusion, the study investigates the physicochemical properties of *M. charantia* fruit, focusing on its solubility, bioavailability, and toxicity. It reveals that Vitamin E, gentisic acid, Stigma sterol, β-sitosterol, and cucurbitacin B are important medicinal compounds. The phytochemical screening and GC-MS analysis of the ethanolic fruit extract of *M. charantia* revealed various compounds. Gentisic acid, an antioxidant, has physiological functions, while Cucurbitacin B has ant proliferative activity against breast cancer, glioblastoma multiform, and myeloid leukemia cells. Saponins are natural surfactants with strong biological activity. The comprehensive phytochemical analysis of *M. charantia* ethanol extract using Gas Chromatography-Mass Spectroscopy (GC-MS) and subsequent in-silico ADMET studies highlighted gentisic acid as a significant phytocomponents. The identification of gentisic acid within the extract underscores its potential therapeutic value, given its well-documented anti-inflammatory, antioxidant, and analgesic properties. The in-silico ADMET profiling further corroborated the suitability of gentisic acid for drug development, demonstrating favourable ADMET characteristics. These findings suggest that gentisic acid contributes substantially to the pharmacological effects of *M. charantia* and warrants further investigation for its role in therapeutic applications, particularly in the context of chronic inflammatory and oxidative stress-related diseases.

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