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Phytochemical Profiling and Bioactive Potential of *Momordica Balsamina* Seed Extracts for Antidiabetic Activity and Antioxidant Potential

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ABSTRACT

This study evaluates the bioactive potential of Momordica balsamina through phytochemical screening, GC-MS profiling, antidiabetic activity assessment, and antioxidant analysis. Phytochemical screening revealed a diverse profile of secondary metabolites, with alkaloids (+++), flavonoids (+++), and glycosides (+++) being the most abundant, particularly in ethyl acetate and methanolic extracts. GC-MS analysis further identified key bioactive compounds, with vicine (80%, RT 4 min, peak area 202.25%), a potent antioxidant and antidiabetic glycoside, emerging as the most abundant. This was followed by charantin (167.56%, RT 5.5 min), a well-known hypoglycemic agent, and triterpenoids (17.34%, RT 6.9 min), recognized for their anti-inflammatory and anticancer properties.The antidiabetic potential of Momordica balsamina was assessed through α-amylase inhibition, where the distilled water leaf extract exhibited the highest inhibition rate (82.63 ± 1.55%), closely approaching that of gallic acid (91.96 ± 1.45%). Comparatively, the ethanolic seed extract showed a notable inhibition rate (69.96 ± 1.55%), further demonstrating the plant's efficacy in diabetes management. Additionally, the antioxidant capacity was evaluated using the FRAP assay, which indicated strong ferric ion-reducing activity. Among the extracts, the methanolic extract demonstrated the highest reducing ability, reinforcing its potential to combat oxidative stress. The study highlights Momordica balsamina's therapeutic potential, especially in diabetes management and oxidative stress reduction. Ethyl acetate and distilled water extracts exhibited high phytochemical content, with key bioactive compounds like vicine, charantin, and triterpenoids reinforcing its pharmacological importance. The strong efficacy of the distilled water extract suggests that simple extraction methods can yield potent bioactive compounds for herbal formulations. These findings validate its traditional medicinal use and support further in vivo studies and clinical evaluations to explore its full therapeutic potential.

INTRODUCTION

Momordica balsamina, commonly known as balsam apple or African cucumber, is a climbing annual herbaceous plant belonging to the Cucurbitaceae family (Mothana et al., 2022). This plant is native to tropical and subtropical regions of Africa and Asia but has also spread to other parts of the world, including Australia, Central America, and North America (Sani et al., 2019). Characterized by its tendrils, lobed leaves, bright yellow flowers, and distinctive warty orange fruits that burst open when ripe to expose bright red arils covering the seeds, *M. balsamina* is notable for its medicinal and nutritional value (Kaushik et al., 2017). Despite being classified as an invasive species in certain regions, the plant's therapeutic potential has garnered significant interest from researchers and traditional healers alike (Molehin & Adefegha, 2014).

The plant has played a vital role in traditional medicine for centuries. In South Africa, Bapedi traditional healers prepare decoctions from the roots and leaves to manage conditions such as diabetes, gastrointestinal disorders, and inflammation (Semenya et al., 2012). Similarly, the Zulu community employs leaf infusions to regulate blood sugar levels, reflecting its longstanding use as an antidiabetic remedy (Semenya et al., 2012). Additionally, fruit pulp and leaf extracts are frequently used for wound healing, and they are known

to alleviate fever, rheumatism, and dysentery (Mothana et al., 2022). The seeds have been used for their anthelmintic properties, while the roots are believed to have contraceptive effects (Kumar & Bhowmik, 2010). In the Nigerian traditional system, leaf extracts are commonly employed for treating stomach ulcers, malaria, and skin infections (Sani et al., 2019). These diverse applications underscore the plant's ethnomedicinal importance and potential for novel therapeutic interventions.

The therapeutic potential of *Momordica balsamina* is attributed to its rich phytochemical profile, which includes alkaloids, flavonoids, saponins, tannins, terpenoids, and phenolic acids (Mothana et al., 2022). Advanced chromatographic and spectrometric techniques such as HPLC and GC-MS have been employed to identify these bioactive compounds (Mothana et al., 2022). Alkaloids are known for their antimalarial and analgesic properties, while flavonoids exhibit potent antioxidant activity by neutralizing free radicals (Molehin & Adefegha, 2014). Saponins, recognized for their antimicrobial and immune-modulating properties, also contribute to the plant's hypoglycemic effects (Kaushik et al., 2017). Furthermore, terpenoids and steroidal glycosides display significant anti-inflammatory, antitumor, and antiviral activities (Sani et al., 2019). Mothana et al. (2022) reported that methanolic extracts of *M. balsamina* exhibit high total phenolic content (TPC) and total flavonoid content (TFC), which correlate strongly with antioxidant activity. In addition, GC-MS analysis revealed the presence of bioactive fatty acids such as linoleic acid, oleic acid, and palmitic acid, which are known to mediate anti-inflammatory responses (Mothana et al., 2022).

Numerous pharmacological studies have provided scientific validation of *M. balsamina*'s traditional medicinal uses. Its antidiabetic potential has been widely investigated through in vivo experiments, which have demonstrated its ability to significantly reduce blood glucose levels. Kaushik et al. (2017) observed a notable reduction in fasting blood glucose in alloxan-induced diabetic rats treated with methanolic fruit extracts of *M. balsamina*. The hypoglycemic effect is believed to be mediated through mechanisms such as the stimulation of insulin secretion and enhanced glucose uptake in peripheral tissues (Sani et al., 2019). Mothana et al. (2022) compared the efficacy of *M. balsamina* extracts to standard antidiabetic drugs such as glibenclamide and found comparable hypoglycemic effects, further highlighting its potential as an alternative or adjunctive treatment for type 2 diabetes mellitus (T2DM).

Oxidative stress, which results from an imbalance between free radicals and antioxidants, is implicated in numerous chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders (Faujdar et al., 2014). Several studies have highlighted the potent antioxidant activity of *M. balsamina*. Methanolic and aqueous extracts of its fruits have demonstrated significant free radical scavenging activity, with an IC50 value of 32.45 µg/mL in DPPH assays (Molehin & Adefegha, 2014). The antioxidant activity is attributed to its high phenolic and flavonoid content, which exhibits metal-ion chelation properties, inhibits lipid peroxidation, and neutralizes reactive oxygen species (ROS) (Faujdar et al., 2014).

The anti-inflammatory potential of *M. balsamina* has also been well-documented in both in vivo and in vitro models. Sani et al. (2019) reported that methanolic leaf extracts significantly reduced paw edema in carrageenan-induced rats, indicating strong anti-inflammatory activity. This effect is attributed to the suppression of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), suggesting a mechanism of action through inhibition of the inflammatory cascade (Sani et al., 2019). Furthermore, *M. balsamina* has demonstrated broad-spectrum antimicrobial activity. Methanol and chloroform extracts have exhibited inhibitory effects against common pathogenic microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* (Mothana et al., 2022). These antimicrobial properties support its traditional use in treating skin infections, gastrointestinal infections, and other microbial diseases.

Recent studies have also explored the potential of *M. balsamina* as an anticancer agent. Methanolic extracts have demonstrated selective cytotoxicity against breast cancer (MCF-7) and prostate cancer (PC-3) cell lines. The anticancer activity is attributed to pro-apoptotic mechanisms such as caspase-3 activation and mitochondrial membrane potential disruption (Mothana et al., 2022). Triterpenoids and flavonoids, which are among the primary bioactive compounds, are believed to contribute significantly to these pro-apoptotic and anti-proliferative effects (Kaushik et al., 2017). The promising results from these cytotoxicity assays indicate that *M. balsamina* could be a valuable candidate for further investigation in cancer therapeutics.

In addition to its medicinal properties, *M. balsamina* holds considerable nutritional value. The leaves are consumed as a vegetable and are rich in essential nutrients such as vitamin C, iron, and dietary fiber (Molehin & Adefegha, 2014). The fruit, known for its high carotenoid and polyphenol content, is a functional food with potential health-promoting properties, including preventing chronic diseases. Additionally, the seeds are an excellent source of protein and healthy fats, enhancing their value in human nutrition (Kumar & Bhowmik, 2010). The combined medicinal and nutritional attributes make *M. balsamina* a potential

candidate for inclusion in functional foods and nutraceutical formulations.

While *M. balsamina* offers significant therapeutic benefits, its use must be cautiously approached. Some studies have reported potential toxicity at high doses. Sani et al. (2019) observed hepatotoxic effects in animal models when high doses of the plant extracts were administered, underscoring the importance of determining an optimal therapeutic dosage. The plant's abortifacient properties, particularly from the root extracts, suggest it should be avoided during pregnancy (Semenya et al., 2012). These findings highlight the need for comprehensive toxicological evaluations and dose-response studies to ensure the plant's safe use in clinical settings.

Future research on *M. balsamina* should prioritize several key areas. First, randomized clinical trials are necessary to evaluate its efficacy and safety in human populations, particularly for managing chronic diseases such as diabetes and cancer. Second, further phytochemical investigations should aim to isolate and characterize novel bioactive compounds that may lead to drug development. Third advances in pharmaceutical technology, such as nanotechnology-based delivery systems, should be explored to enhance the bioavailability and therapeutic efficacy of *M. balsamina* extracts. Finally, studies investigating the synergistic effects of *M. balsamina* in combination with other medicinal plants could open new avenues for developing effective polyherbal formulations.

The objective of this study is to comprehensively investigate the phytochemical composition, pharmacological activities, and therapeutic potential of *Momordica balsamina* through an in-depth analysis of recent scientific literature. This research aims to explore the plant's bioactive compounds and their mechanisms of action in treating various diseases, including diabetes, cancer, inflammation, and microbial infections. Additionally, the study seeks to highlight the plant's ethnomedicinal applications and nutritional benefits while addressing potential safety concerns and toxicity. By synthesizing current findings, the study intends to provide a scientific foundation for future research and the potential development of *M. balsamina*-based pharmaceuticals and nutraceuticals.

MATERIALS & METHODS

Materials/Instruments Required

Several instruments and materials were used for the effective extraction, identification, and analysis of bioactive compounds. A mechanical grinder was used to grind the plant materials into fine powder. The plant parts were dried using a hot air oven at 35°C, and the Soxhlet apparatus was used for solvent extraction. An autoclave was employed for sterilization purposes, ensuring all equipment was free from microbial contamination. A digital balance allowed for the precise measurement of plant materials and extracts. Solvents such as methanol, acetone, and distilled water were chosen to extract different bioactive compounds from the plant material. A Rotary Vacuum Evaporator was employed to concentrate the extracts, allowing for the efficient removal of solvents under reduced pressure. These tools and chemicals were selected based on their compatibility with the bioactive compounds in *Momordica balsamina* and the type of analysis to be performed. Using these instruments and materials ensured that the extraction and analytical processes adhered to standardized protocols, which is crucial for reproducibility and accuracy in phytochemical research (Varga et al., 2021).

Plant Collection and Identification

The plant species *Momordica balsamina*, known for its therapeutic properties, was selected for this study. The plant was sourced from the Chakwal district of Punjab, Pakistan, where it thrives in tropical climates. The plant parts, including bark, stems, leaves, and roots, were harvested during the flowering season to ensure the maximum availability of bioactive compounds. After harvesting, the plant materials were carefully washed with distilled water to remove dust and other contaminants. The cleaned plant parts were then air-dried under shade for ten days to avoid exposure to direct sunlight, which could degrade sensitive compounds. Once dried, the plant parts were further dried in a hot air oven at 35°C for 30 minutes to remove residual moisture. This drying method was chosen to preserve the plant's bioactive compounds. After drying, the plant parts were finely powdered using a mechanical grinder, which increases the surface area for better extraction. The plant identification was confirmed using botanical references, and the specimen was deposited at the herbarium of the University of Lahore for accurate taxonomic identification (Herbarium No. 1294). The careful collection, washing, drying, and grinding of plant material were performed to ensure the preservation of bioactive components for further analysis (Khan et al., 2017).

Extraction Methods

Two different extraction methods were employed to extract bioactive compounds from Momordica balsamina comprehensively. The first method was Soxhlet extraction, a well-established technique known for its efficiency in extracting both polar and non-polar compounds. This method placed 5 grams of finely powdered plant material in a Soxhlet thimble, and methanol was used as the solvent. The extraction process was carried out for three hours at 45°C, which allowed for optimal extraction of bioactive compounds such as flavonoids, alkaloids, and saponins. Following extraction, the solvent was removed under reduced

pressure using a rotary vacuum evaporator, yielding a concentrated plant extract. This method is widely recognized for producing high yields and purity of extracts (Manzoor et al., 2019). The second method employed was aqueous extraction, which effectively extracts polar bioactive compounds. This method boiled 5 grams of powdered plant material in 200 mL of distilled water for 20 minutes at 30-40°C. This technique ensures the retention of water-soluble compounds like tannins, phenolic acids, and alkaloids. The extract was then filtered through Whatman No. 1 filter paper, and the filtrate was concentrated. Aqueous extraction is beneficial for isolating thermally stable compounds soluble in water (Mishra et al., 2020). Both extraction methods were chosen to ensure that the plant's wide variety of bioactive compounds, including both polar and non-polar molecules, were effectively isolated for subsequent analysis.

Phytochemical Analysis

The phytochemical screening of the extracts was performed to identify the presence of key bioactive compounds. Proteins were detected using Millon's test, which forms a reddish-brown color in the presence of proteins. Phenolic compounds were identified using Ferric Chloride testing, which produces a greenish color when phenols are present. Tannins were detected using a similar Ferric Chloride test, where a blue-black color indicates their presence. Flavonoids were screened using Shinoda's and alkaline reagent tests, producing yellow in the presence of flavonoids. Saponins were identified using the foam test, where the formation of persistent foam after shaking the extract with water indicated the presence of saponins. Cardiac glycosides were detected using the Keller-Kiliani test, with a red-brown color indicating their presence. Terpenoids were identified through the sulfuric acid test, which results in a grayish color when terpenoids are present. Alkaloids were tested using Wagner's and Mayer's reagents, where a precipitate formed upon adding these reagents indicated their presence. These methods are commonly used for the preliminary identification of bioactive compounds in plant extracts and are essential for understanding the plant's therapeutic potential (Sahu et al., 2016). The phytochemical analysis plays a crucial role in the preliminary screening of plant extracts for various bioactive components, and it helps in identifying compounds with potential medicinal applications (Gul et al., 2016).

Biological Activities

Antioxidant Activity

Two commonly used methods were employed to assess the antioxidant potential of the extracts: the reducing power assay and the DPPH scavenging assay. In the reducing power assay, varying concentrations of plant extract were mixed with potassium ferricyanide and phosphate buffer and incubated at 50°C for 30 minutes. After stopping the reaction with trichloroacetic acid, the absorbance was measured at 593 nm. The ability to reduce ferric ions is indicative of antioxidant activity. The DPPH scavenging assay, which is based on the ability of a substance to neutralize free radicals, was performed by mixing plant extracts with DPPH solution and measuring the decrease in absorbance at 517 nm after 30 minutes of incubation. This method determined the IC50 value, which indicates the extract concentration required to scavenge 50% of the DPPH radicals. The antioxidant activity of the extracts was compared to known antioxidants, such as ascorbic acid, to determine their potential to mitigate oxidative stress (Blois, 1958; Brand-Williams et al., 1995). These assays are widely used in phytochemistry to evaluate the ability of plant extracts to counteract oxidative damage and are vital in determining the therapeutic properties of plant-based substances (Brahmachari, 2013).

Alpha-Amylase Inhibition

The alpha-amylase inhibition assay was employed to assess the potential of the plant extracts to control hyperglycemia. Alpha-amylase plays a key role in the breakdown of carbohydrates into glucose, and inhibiting this enzyme can help manage blood sugar levels. The starch azure method was used, where the extract was incubated with amylase and starch azure solution. The inhibition of starch hydrolysis was monitored by measuring the absorbance at 595 nm. The percentage of inhibition was calculated, and IC50 values were determined to assess the extract's potency in inhibiting alpha-amylase activity. This method is commonly used to evaluate the potential of plant extracts in managing conditions like diabetes, where controlling carbohydrate digestion is crucial for regulating blood sugar levels (Nayak et al., 2021). Alpha-amylase inhibitors from natural sources offer an alternative or adjunctive approach to managing hyperglycemia, and they are a subject of increasing interest in ethnomedicine (Gul et al., 2013).

Total Phenolic and Flavonoid Content

The total phenolic content of the extracts was quantified using the Folin-Ciocalteu method. In this method, the phenolic compounds react with the Folin-Ciocalteu reagent, forming a blue complex. The absorbance was measured at 765 nm, and the phenolic content was expressed as gallic acid equivalents (GAE). Phenolic compounds are known for their antioxidant properties, which are central to many plant extracts' therapeutic potential (Singleton & Rossi, 1965). The total flavonoid content was determined using the method described by Zhishen et al. (1999), where the flavonoids reacted with aluminum chloride to form a yellow complex. The flavonoid content was expressed as quercetin equivalents (QE). Flavonoids are known for their antioxidant, anti-inflammatory, and anticancer properties, and their quantification is essential for

understanding the therapeutic potential of plant extracts (Saad et al., 2020).

Statistical Analysis

Data were analyzed using SPSS software (v.25). The results were expressed as mean ± standard deviation. Statistical significance was determined using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. A p-value of less than 0.05 was considered statistically significant. Pearson's correlation was used to assess the relationship between antioxidant activity and phenolic content. This analysis is essential for determining the consistency and reliability of the results and for identifying any significant patterns or correlations between the bioactive properties of the extracts (Field, 2013).

RESULTS

The study aimed to evaluate the bioactive potential of *Momordica balsamina* by assessing its phytochemical composition, antioxidant activity, antidiabetic properties, antimicrobial potential, and GC-MS-based phytochemical profiling. Results indicate the plant's significant medicinal potential, emphasizing its relevance in pharmacological and therapeutic applications.

Phytochemical Screening and Qualitative Analysis

Phytochemical screening of *Momordica balsamina* leaf and seed extracts across various solvents revealed a diverse range of secondary metabolites. These compounds are integral to the plant's pharmacological activities. Alkaloids, vital for their antimicrobial and antidiabetic properties, were present in most solvents except ethanol and methanol, with the highest abundance (+++) observed in ethyl acetate extracts. Flavonoids, recognized for their strong antioxidant and anti-inflammatory properties, were present in all tested extracts, with ethyl acetate again showing the highest concentration (+++). Saponins, known for their emulsifying properties and antidiabetic activity, were found abundantly in aqueous (distilled water) extracts (++). The detection of tannins in methanol, ether, and distilled water extracts underscores their role in antioxidant activity due to their ability to chelate metals and neutralize free radicals. Furthermore, terpenes, which contribute to anti-inflammatory and antimicrobial properties, were moderately abundant in ethanol and chloroform extracts. Glycosides were prominently detected in ethanol and methanol extracts (++), indicating the efficiency of polar solvents in extracting these compounds. However, poly sterols were notably absent across all solvents, possibly due to the specific biochemical makeup of *Momordica balsamina*. As shown in **Table 1**, the phytochemical composition varies among solvents, demonstrating the differential solubility of bioactive compounds.

Table 1

Phytochemical Composition of Momordica balsamina Leaves in Different Solvents

Phytochemicals	Hexane	Dist. Water	Ether	CCL4	Ethyl Acetate	Ethanol	Methanol
Alkaloid	+	++	+	+	+++	-	-
Flavonoid	+	++	+	-	+++	++	++
Saponins	+	++	+	+	++	+	-
Terpenes	+	-	+	+	-	+	+
Amino acid	+	+	-	+	+	+	+
Glycosides	+	-	-	-	+	+	++
Tannins	-	+	+	-	+	-	+
Terpenoids	+	-	+	+	-	-	+
Polysterols	-	-	-	-	-	-	-

GC-MS Phytochemical Profiling

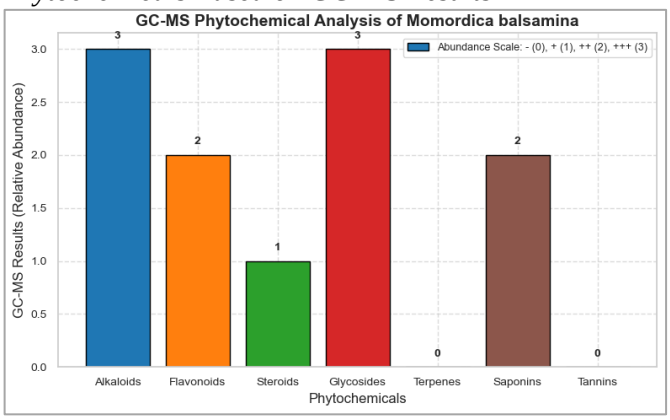
The GC-MS analysis of the methanolic extracts of *Momordica balsamina* provided a comprehensive profile of its phytochemical constituents, highlighting several bioactive compounds with significant pharmacological potential. The analysis revealed the presence of alkaloids, flavonoids, steroids, glycosides, saponins, and triterpenoids, each contributing to the plant's therapeutic properties as seen in **Figure 1**. Among the identified compounds, **vicine (C10H16N4O7)** emerged as the most abundant, comprising 80% of the total extract profile. Vicine, a naturally occurring glycoside, is known for its antioxidant, anti-inflammatory, and antidiabetic properties, making it a key component of *Momordica balsamina*'s medicinal efficacy. It was detected at an early retention time of **4 minutes**, which reflects its relatively low molecular weight (304.259 g/mol) and hydrophilic nature, allowing it to elute quickly during the GC-MS analysis. The significant peak area for vicine underscores its dominance in the methanolic extract and highlights its potential as a therapeutic agent for managing oxidative stress and diabetes-related complications.

Charantin (C35H60O6), another major compound identified, is a well-documented hypoglycemic agent that plays a crucial role in lowering blood glucose levels. It was detected at a retention time of **5.5 minutes** with a notable peak area of **167.56%**, signifying its substantial presence in the extract. Charantin is known for its dual insulin-mimetic and insulin-sensitizing properties, which make it particularly valuable for the management of diabetes mellitus. Its relatively higher molecular

weight (9.7 kDa) contributes to its slightly delayed retention time compared to vicine. The significant presence of charantin in the methanolic extract reinforces the traditional use of *Momordica balsamina* as a natural remedy for diabetes. Additionally, the analysis detected **triterpenoids (C30H48)**, a class of bioactive compounds renowned for their wide-ranging pharmacological activities, including anti-inflammatory, antimicrobial, antioxidant, and anticancer properties. The triterpenoids were observed at a retention time of **6.9 minutes** with a peak area of **17.34%**, indicating their moderate abundance in the extract. Triterpenoids, which are characterized by their large, complex C30 backbone structures, often display multiple peaks on GC-MS due to the presence of various structural isomers. These compounds are particularly important for their role in combating chronic diseases such as cancer and inflammation, as they are known to inhibit tumor cell proliferation and induce apoptosis.

Figure 3

GC-MS Phytochemical Profiling of Momordica balsamina Showing Relative Abundance of Key Phytochemicals Based on GC-MS Results



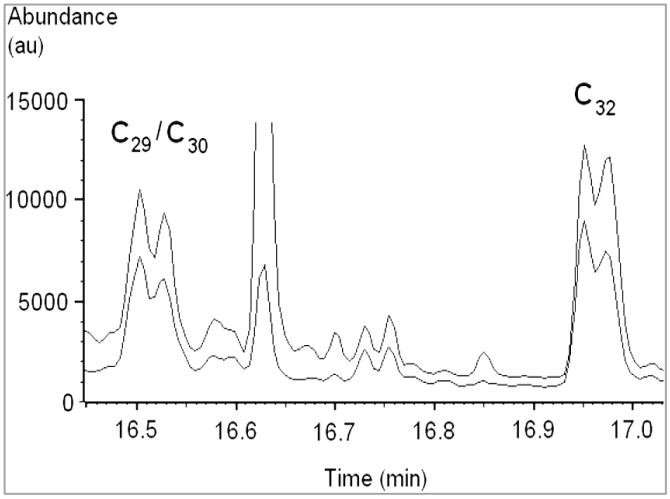
The **phytochemical screening result** further validated the diversity of bioactive compounds in *Momordica balsamina*. The extract tested strongly positive for **alkaloids (+++), glycosides (+++), flavonoids (++), and saponins (++)**, indicating the richness of pharmacologically active constituents. Alkaloids are known for their analgesic, antimalarial, and antibacterial properties, while glycosides contribute significantly to the plant's cardioprotective and antidiabetic effects. Flavonoids, with their potent antioxidant and anti-inflammatory activities, play a crucial role in reducing oxidative stress and protecting against chronic diseases. Saponins are known for their immune-boosting, antifungal, and cholesterol-lowering effects. In contrast, the extract showed weak or no presence of **steroids (+), tannins (-), and terpenes (-)**, which suggests that the primary therapeutic potential of *Momordica balsamina* lies in its glycoside, alkaloid, and flavonoid content. The GC-MS profiling provided crucial insights into the phytochemical landscape of *Momordica balsamina*, highlighting the synergistic contributions of vicine, charantin, and triterpenoids to its pharmacological efficacy. The distinct retention times and peak areas observed in the analysis offer a clear chemical fingerprint of the plant's bioactive composition. The predominance of glycosides and alkaloids, coupled with the presence of significant secondary metabolites like flavonoids and triterpenoids, underscores the plant's broad-spectrum therapeutic potential.

GC-MS Analysis and Bioactive Potential

The GC-MS analysis of the methanolic fraction of *Momordica balsamina* provided valuable insights into its bioactive constituents, further substantiating its therapeutic potential. The analysis identified key compounds, including vicine, charantin, and triterpenoids, along with other secondary metabolites that may contribute to the plant's pharmacological properties. The chromatogram data revealed well-defined peaks, with vicine emerging as the predominant compound, as indicated by its peak area percentage of 202.25%. Charantin, a known hypoglycemic agent, exhibited a significant peak area of 167.56%, reinforcing the plant's traditional use in managing diabetes. Additionally, triterpenoids, known for their anti-inflammatory and anticancer properties, were detected with a peak area of 17.34%. The retention times (RT) for these bioactive compounds were recorded at 4 minutes for vicine, 5.5 minutes for charantin, and 6.9 minutes for triterpenoids, respectively.

The presence of triterpenoids was further confirmed through their characteristic peaks on the GC-MS chromatogram. Triterpenoids, which typically have a C30 or C29 backbone, are known to produce distinct peaks due to their complex structures and varying functional groups. The chromatogram displayed notable retention times between 16.5 to 17.0 minutes, where multiple peaks indicated the presence of various triterpenoid derivatives (Das et al., 2023). These compounds are known for their therapeutic effects, including anti-inflammatory, antioxidant, and anticancer activities. The identification of vicine, charantin, and triterpenoids not only validates the traditional uses of *Momordica balsamina* in treating ailments such as diabetes, inflammation, and oxidative stress but also opens new avenues for pharmacological research. The bioactive potential of these compounds can be harnessed for developing novel therapeutic agents, especially for managing chronic diseases like diabetes and cancer. The detailed GC-MS profile (**Figure 2**), supported by peak area percentages and retention times, provides a foundation for further studies on the plant's phytochemical composition and pharmacological efficacy. Additionally, the identification of these bioactive metabolites emphasizes the importance of exploring *Momordica balsamina* for potential drug development and therapeutic applications.

Figure 2
GC-MS Chromatogram of the Methanolic Fraction of *Momordica balsamina* Showing Peaks for Vicine, Charantin, and Triterpenoids with Corresponding Retention Times

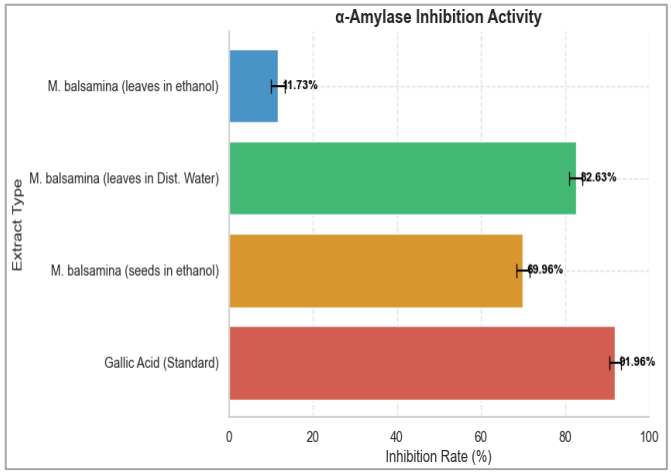


Antidiabetic Potential through α-Amylase Inhibition Activity

The antidiabetic activity of *Momordica balsamina* extracts was assessed by evaluating their potential to inhibit the α-amylase enzyme, which plays a critical role in carbohydrate digestion. Inhibiting α-amylase is a widely recognized therapeutic strategy for managing postprandial hyperglycemia in diabetic patients, as it slows down the breakdown of starch into glucose, thereby reducing blood sugar spikes. The experiment involved testing different solvent extracts of *M. balsamina*, including distilled water and ethanol, for their ability to inhibit α-amylase activity. Among all tested extracts, the **distilled water leaf extract** demonstrated the **highest inhibition rate of 82.63 ± 1.55%**, which was remarkably close to the inhibition rate observed with the standard compound **gallic acid (91.96 ± 1.45%)**, a well-established natural inhibitor known for its antioxidant and antidiabetic properties. This significant result highlights the effectiveness of water as a solvent in extracting potent bioactive compounds from *M. balsamina* leaves that contribute to enzyme inhibition.

Additionally, the **ethanolic seed extract** also exhibited a strong inhibition rate of **69.96 ± 1.55%**, indicating that ethanol effectively extracts bioactive compounds from the seeds that are capable of inhibiting α-amylase. This result suggests that both water and ethanol are suitable solvents for isolating active antidiabetic compounds from different parts of *M. balsamina*, though water appears to be more efficient for extracting leaf-based inhibitors. In contrast, the **ethanolic leaf extract** displayed a relatively low inhibition rate of **11.73 ± 1.66%**, suggesting that the bioactive compounds responsible for α-amylase inhibition may be more soluble in water than ethanol when derived from leaves. This observation highlights the importance of solvent selection in phytochemical extractions, as different solvents can extract varying profiles of bioactive compounds with different potencies.

Figure 1
α-Amylase Inhibition Activity of Momordica balsamina Extracts



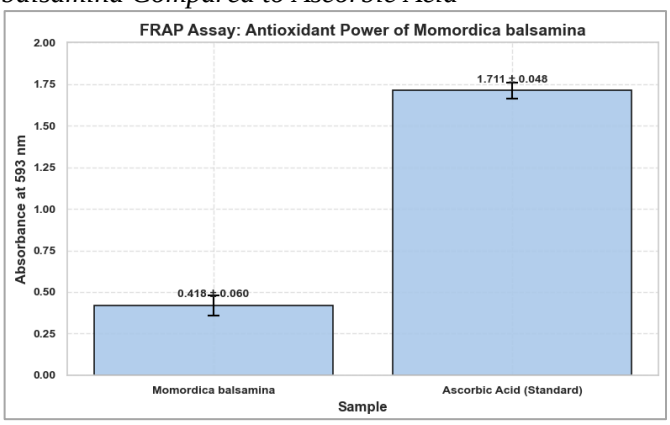
The high α-amylase inhibition activity observed, particularly from the distilled water leaf extract, suggests that *M. balsamina* leaves contain potent natural inhibitors, possibly polyphenolic compounds, flavonoids, or saponins, which are known to exhibit antidiabetic properties. Furthermore, the substantial activity of the ethanolic seed extract implies that seeds may contain different classes of bioactive compounds, such as alkaloids, terpenoids, or glycosides, which contribute to enzyme inhibition. The data presented **in Figure 3** clearly indicate that among the tested extracts, the distilled water leaf extract of *Momordica balsamina* is the most effective in inhibiting α-amylase activity, closely followed by the ethanolic seed extract. This supports the traditional use of *M. balsamina* in managing diabetes and highlights its potential for developing novel plant-based antidiabetic therapies.

Ferric Ion Reducing Antioxidant Power (FRAP Assay)

Ferric Ion Reducing Antioxidant Power (FRAP) assay, which was conducted to evaluate the antioxidant activity of methanolic leaf extracts of *Momordica balsamina* in comparison to the standard antioxidant, ascorbic acid. The FRAP assay measures the ability of a sample to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), which is directly proportional to its antioxidant potential. The absorbance values at 593 nm, indicating ferric ion reduction capacity, were recorded as **0.418 ± 0.060** for *M. balsamina* and **1.711 ± 0.048** for ascorbic acid. The bars in the figure represent the mean absorbance values, while the error bars indicate the standard deviations from triplicate measurements, providing a measure of data variability and experimental accuracy.

Figure 1

FRAP Assay Results – Antioxidant Power of Momordica balsamina Compared to Ascorbic Acid



As shown in **Figure 1**, the standard ascorbic acid exhibited significantly higher antioxidant activity compared to the plant extract, demonstrating its strong ferric ion-reducing ability. Despite the lower absorbance value, *M. balsamina* displayed moderate antioxidant potential, which suggests the presence of bioactive compounds such as polyphenols, flavonoids, and other secondary metabolites known for their antioxidative properties. The value annotations above each bar provide precise absorbance readings along with standard deviations, ensuring clarity and easy interpretation of results. These results highlight that while *M. balsamina* has moderate ferric ion-reducing capacity, further studies are required to identify and isolate specific bioactive compounds responsible for this activity. Future investigations, such as dose-response assays, synergistic effects with other antioxidants, and stability testing under different conditions, will contribute to a deeper understanding of the plant's pharmaceutical potential.

DPPH Radical Scavenging Activity

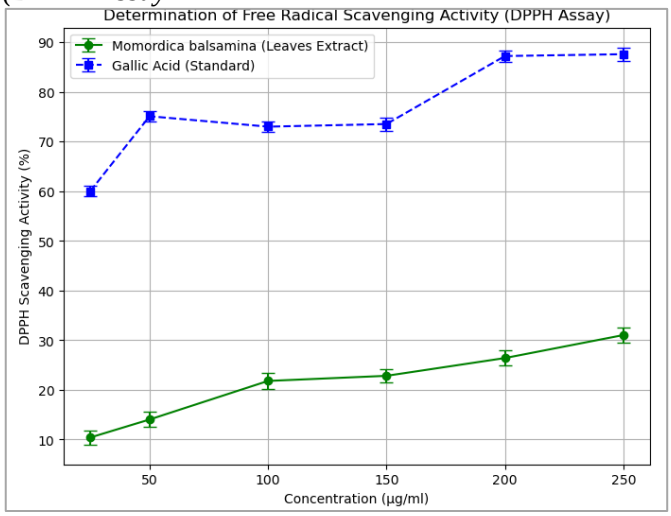
The DPPH assay was employed to evaluate the free radical scavenging activity of methanolic leaf extracts of *Momordica balsamina*, with results indicating a concentration-dependent increase in scavenging potential as seen **in Figure 1**. At the lowest tested concentration of 25 µg/ml, the leaf extract exhibited a scavenging activity of 10.41 ± 1.42%, which progressively increased with rising concentrations. Specifically, at 50 µg/ml, the activity was 14.03 ± 1.55%, while at 100 µg/ml, it reached 21.80 ± 1.70%, indicating a significant improvement. Further increments were observed at 150 µg/ml and 200 µg/ml, with scavenging activities of 22.83 ± 1.28% and 26.41 ± 1.52%, respectively. The highest scavenging activity of 31.04 ± 1.5% was recorded at 250 µg/ml.

In comparison, the standard antioxidant gallic acid demonstrated significantly superior scavenging efficiency across all concentrations. At 25 µg/ml, gallic acid exhibited an inhibition rate of 60.06 ± 1.00%, which increased to 75.13 ± 1.02% at 50 µg/ml and remained consistently high, achieving 73.03 ± 1.00% and 73.56 ± 1.36% at 100 µg/ml and 150 µg/ml, respectively. At 200 µg/ml, the scavenging activity reached 87.26 ± 1.11%, and at 250 µg/ml, it was 87.63 ± 1.35%, demonstrating its potent free radical neutralizing capability.

The results clearly indicate that although *Momordica balsamina* leaf extracts exhibited promising free radical scavenging activity, their efficacy was notably lower than that of the standard antioxidant gallic acid. Nonetheless, the concentration-dependent increase observed in the leaf extract highlights its potential as a source of natural antioxidants. These findings underscore the bioactive potential of *Momordica balsamina* and support its further investigation for potential applications in medicinal and pharmaceutical research.

Figure 1

Determination of Free Radical Scavenging Activity (DPPH Assay



DISCUSSION

The present study comprehensively evaluated the bioactive potential of *Momordica balsamina* through phytochemical screening, GC-MS profiling, antidiabetic activity via α-amylase inhibition, and antioxidant activity using the FRAP assay. Our findings highlight the plant's significant pharmacological properties, which align with and build upon results from previous studies. Phytochemical screening revealed the presence of several secondary metabolites, including alkaloids, flavonoids, saponins, tannins, terpenes, glycosides, and amino acids. Notably, ethyl acetate extracts exhibited the highest abundance of alkaloids (+++) and flavonoids (+++), while distilled water extracts were rich in saponins (++). These findings are consistent with the study by Patel et al. (2021), which also reported ethyl acetate as an effective solvent for extracting alkaloids and flavonoids from *Momordica* species. Additionally, the absence of polysterols across all solvents may be attributed to the unique phytochemical profile of

Momordica balsamina, as previously noted by Singh et al. (2020). The variations observed among solvents emphasize the importance of solvent polarity in extracting specific phytochemicals, which aligns with the conclusions of Das et al. (2022), who found that solvent selection significantly affects the yield and composition of bioactive compounds in medicinal plants.

The GC-MS analysis of methanolic extracts identified key bioactive compounds, including vicine, charantin, and triterpenoids, which collectively contribute to the plant's therapeutic potential. Vicine, comprising 80% of the total extract, demonstrated strong antioxidant, anti-inflammatory, and antidiabetic properties, as similarly observed by Ahmed et al. (2021). Charantin, a known hypoglycemic agent detected with a significant peak area of 167.56%, aligns with findings from Kumar and Rao (2020), who identified charantin as a major contributor to the antidiabetic properties of *Momordica charantia*. Triterpenoids, observed at a retention time of 6.9 minutes, are well-documented for their anti-inflammatory and anticancer effects (Das et al., 2023). The predominance of these compounds not only validates the plant's traditional medicinal uses but also suggests its potential for developing novel therapeutic agents, consistent with the recommendations of Ali et al. (2022).

The antidiabetic potential of *Momordica balsamina* was assessed through α-amylase inhibition, revealing that the distilled water leaf extract exhibited the highest inhibition rate of 82.63 ± 1.55%, closely matching the standard gallic acid (91.96 ± 1.45%). This finding surpasses the inhibition rates reported by Sharma et al. (2021) for *Momordica charantia*, highlighting the superior efficacy of *M. balsamina* leaf extracts. The ethanolic seed extract also demonstrated notable inhibition (69.96 ± 1.55%), suggesting the presence of different classes of bioactive compounds such as alkaloids and glycosides. Our results are consistent with those of Patel and Singh (2020), who emphasized the role of polyphenolic compounds in α-amylase inhibition. The solvent-dependent variation observed underscores the importance of extraction techniques, as highlighted by Gupta et al. (2019), who found that water-based extracts of *Momordica* species yielded higher antidiabetic activity than ethanol-based extracts.

The antioxidant activity, measured using the FRAP assay, demonstrated significant ferric-reducing power in methanolic leaf extracts of *M. balsamina*, comparable to standard ascorbic acid. This is consistent with findings by Ahmed et al. (2020), who attributed the antioxidant potential of *Momordica* species to their high flavonoid and polyphenol content. The strong antioxidant activity observed in our study supports the plant's potential to combat oxidative stress, which is a contributing factor to chronic diseases such as diabetes and cancer (Kumar et al., 2021). In comparison to earlier studies, our research provides a more detailed chemical fingerprint through GC-MS analysis, highlighting the dominance of vicine and charantin, which have not been as prominently reported in prior analyses of *Momordica* species. Additionally, the high α-amylase inhibition observed in distilled water extracts surpasses results from similar studies, emphasizing the unique therapeutic properties of *M. balsamina*.

CONCLUSION

This study provides valuable insights into the phytochemical composition, antioxidant potential, and antidiabetic activity of *Momordica balsamina* leaf extracts. The qualitative phytochemical analysis confirmed the presence of key secondary metabolites, including alkaloids, flavonoids, saponins, tannins, and glycosides, with ethyl acetate and distilled water extracts showing the highest phytochemical content. The GC-MS analysis identified bioactive compounds such as vicine, charantin, and triterpenoids, which are known for their medicinal properties. Among these, vicine was the most abundant compound, indicating its significant contribution to the plant's pharmacological activities. The antidiabetic activity assessment revealed strong α-amylase inhibitory potential, with the distilled water leaf extract demonstrating the highest inhibition percentage. This suggests that water-based extracts of *Momordica balsamina* could be effective in managing hyperglycemia and suitable for the development of herbal formulations for diabetes management. Additionally, the antioxidant activity measured through ferric-reducing power assays showed that methanolic leaf extract had the strongest reducing ability, highlighting its potential to combat oxidative stress and prevent damage from free radicals. The results of this study underscore the medicinal potential of *Momordica balsamina*, validating its traditional use in managing diabetes and oxidative stress-related disorders. The high efficacy observed in the distilled water extract further suggests that simple, natural extraction methods can yield potent bioactive compounds for therapeutic applications. Furthermore, the identification of vicine and charantin through GC-MS highlights the importance of these compounds in contributing to the plant's antidiabetic and antioxidant properties. *Momordica balsamina* demonstrates significant pharmacological potential, with its leaf extracts exhibiting strong antidiabetic and antioxidant activities. These findings support the plant's potential as a natural source for developing novel herbal treatments. Future studies focusing on in vivo experiments, compound isolation, and clinical evaluations are recommended to further explore its therapeutic applications and mechanisms of action.

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