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Phytochemical Profiling and Assessment of the Hypolipidemic Properties of *Melissa parviflora* Whole Plant

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Abstract

Medicinal plants represent a rich source of bioactive compounds with potential therapeutic applications. *Melissa parviflora* (*M. parviflora*) is traditionally used for various ailments, but its antioxidant and antihyperlipidemic properties remain underexplored. This study aimed to evaluate the phytochemical composition, antioxidant capacity, and enzyme inhibitory activities of extracts and fractions from *M. parviflora* against key metabolic enzymes involved in hyperlipidemia. The whole plant was collected, cleaned, and crude extracts were prepared using solvents of varying polarity, and fractions were obtained through sequential partitioning. Phytochemical screening was performed to identify major compound classes. Total phenolic and flavonoid contents were quantified spectrophotometrically. Antioxidant activity was assessed using DPPH, ABTS, and FRAP assays. Inhibitory effects on α-amylase, αglucosidase, pancreatic lipase, and HMG-CoA reductase were determined *in-vitro*. Phytochemical screening revealed the presence of diverse bioactive compounds, including flavonoids, phenols, alkaloids, glycosides, and terpenoids. The ethyl acetate fraction (EFMP) of *M. parviflora* exhibited the highest antioxidant activity across all assays, correlating with its rich phenolic and flavonoid content. In enzyme inhibition studies, the methanolic fraction

(MFMP) showed strong α-amylase inhibition, while EFMP was most effective against αglucosidase, pancreatic lipase, and HMG-CoA reductase. Solvent polarity significantly influenced extraction efficiency and bioactivity, with intermediate-polarity fractions demonstrating superior efficacy. The findings highlight the therapeutic potential of *M. parviflora* fractions, particularly EFMP and MFMP, as natural sources of antioxidant and antihyperlipidemic agents. Further studies are warranted to isolate active compounds and validate efficacy *in-vivo*.

Keywords: *Melissa parviflora*, Antioxidant, Enzyme inhibition, Hyperlipidemia, Flavonoid.

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Introduction

For millennia, natural products have served as the foundation of medicinal systems across diverse cultures, offering a rich and largely untapped reservoir of therapeutic compounds (Sanjai, Gaonkar, & Hakkimane, 2024). Plants, in particular, synthesize a vast array of secondary metabolites—such as alkaloids, flavonoids, terpenoids, and phenolic compounds— as part of their defense mechanisms and ecological interactions (Anjali et al., 2023). These compounds exhibit a broad spectrum of biological activities, including antioxidant, antiinflammatory, antimicrobial, anticancer, and metabolic-regulatory properties. In modern pharmacology, natural products continue to play a pivotal role, inspiring the development of novel drugs and therapeutic agents. Approximately 40% of modern pharmaceuticals are derived from natural compounds or inspired by their structures. The appeal of plant-based medicines lies not only in their historical efficacy and cultural acceptance but also in their multi-target mechanisms, which often result in synergistic effects and reduced side effects compared to synthetic single-target drugs (Narayanankutty, Famurewa, & Oprea, 2024). This is particularly relevant in the management of chronic conditions such as hyperlipidemia, diabetes, and cardiovascular diseases, where complex pathophysiology demands a multifaceted therapeutic approach. The World Health Organization (WHO) estimates that over 80% of the world's population relies on traditional plant-based medicines for primary healthcare, underscoring the global importance and relevance of phytomedicine research (Rauf et al., 2022).

Melissa parviflora Benth. (*M. parviflora*) is an aromatic perennial herb belonging to the Lamiaceae (mint) family. Renowned for its profound therapeutic influence, it has been a cornerstone of the Unani system of medicine for centuries. The constituents of the essential oil of the plant in various climates are different, but citral (geranial and neral), citronellal, and geraniol are the main components (Bhatt et al., 2022). Its medicinal reputation is further elevated by the endorsement of the renowned Persian physician Avicenna (Ibn Sina), who specifically advocated for its use in managing heart conditions. The plant is documented to possess a broad range of pharmacological properties, such as antitubercular, antipyretic, analgesic, and stomachic effects. It is also commonly used as a remedy to eliminate bad breath and strengthen the gums. However, its most used and primary action is as a potent tranquillizer and nervine relaxant. *M. parviflora* is greatly esteemed for its pronounced calming properties, which underpin its historical use in alleviating conditions of the central nervous system and stress-related ailments (A. Khan, Siddiqui, & Jamal, 2019).

Phytochemical profiling is essential for identifying and quantifying the bioactive constituents in plant extracts. Techniques, including solvent-based extraction, chromatographic separation, and spectroscopic analysis, enable the characterization of compounds responsible for observed biological activities (Parani, Vijay, & Chitra, 2024). For instance, phenolic acids, flavonoids, and terpenoids are frequently linked to lipid-lowering and antioxidant effects, providing a chemical basis for the ethnopharmacological use of medicinal plants. Establishing a clear link between the phytochemical profile and observed biological activities provides a scientific basis for the ethnopharmacological use of plants and guides the isolation of novel bioactive compounds for drug development (A. Roy et al., 2022).

Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses, is a key pathological mechanism underlying hyperlipidemia and its cardiovascular complications (Dash et al., 2025). Antioxidant assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline6-sulfonic acid)), and FRAP (Ferric Reducing Antioxidant Power) are widely employed to evaluate the free radical scavenging and reducing capacities of plant extracts. These assays help correlate phytochemical composition with the ability to mitigate oxidative damage, a key mechanism in preventing lipid peroxidation and endothelial dysfunction. Plants rich in polyphenols, flavonoids, and vitamins often demonstrate strong antioxidant activity in these assays, which correlates with their ability to mitigate oxidative damage and support metabolic health (Munteanu & Apetrei, 2021).

Hyperlipidemia, a condition marked by elevated levels of triglycerides, cholesterol, and lipoproteins in the bloodstream, is a major modifiable risk factor for cardiovascular diseases (CVDs), including coronary artery disease, stroke, and myocardial infarction (Soppert, Lehrke, Marx, Jankowski, & Noels, 2020). Antihyperlipidemic agents work through various mechanisms to normalize lipid profiles by reducing intestinal cholesterol absorption, inhibiting endogenous cholesterol and triglyceride synthesis, enhancing lipoprotein clearance, and promoting bile acid excretion. Pancreatic Lipase is secreted by the pancreas, hydrolyzes dietary triglycerides into absorbable free fatty acids and monoglycerides. Inhibiting pancreatic lipase reduces fat absorption, thereby lowering postprandial hyperlipidemia and overall caloric intake (Subramaniyan & Hanim, 2025). α-Amylase and α-Glucosidase are involved in carbohydrate digestion. α-Amylase breaks down starch into oligosaccharides, while α-glucosidase further hydrolyzes disaccharides into glucose. Inhibiting these enzymes delays glucose absorption, flattening postprandial blood glucose

and insulin spikes(Alqahtani et al., 2019). HMG-CoA Reductase is the rate-limiting enzyme in the mevalonate pathway, which catalyzes the conversion of HMG-CoA to mevalonate, a crucial step in cholesterol biosynthesis. Inhibitors of HMG-CoA reductase (statins) are first-line drugs for hypercholesterolemia, effectively reducing LDL-cholesterol and cardiovascular risk (Gesto, Pereira, Cerqueira, & Sousa, 2020). The existing studies on *M. parviflora* have primarily focused on its nutritional value, mucilaginous properties, or preliminary antimicrobial effects. There is a notable lack of comprehensive phytochemical profiling coupled with systematic evaluation of its antioxidant and antihyperlipidemic activities. Specifically, the effects of *M. parviflora* extracts on key metabolic enzymes—such as pancreatic lipase, α-amylase, α-glucosidase, and HMG-CoA reductase—have not been investigated. This gap hinders the validation of its traditional use and the exploration of its potential as a source of novel lipid-lowering agents. This study addresses the existing research gap by comprehensively evaluating the antihyperlipidemic potential of *M. parviflora* through phytochemical profiling and *in vitro* bioactivity assays. By integrating chemical analysis with bioactivity assessments, this work not only identifies the key metabolites responsible for the observed effects but also provides mechanistic insights into the plant's hypolipidemic potential. Furthermore, this research highlights the most effective fractions for future isolation of active compounds and in vivo studies, contributing to the development of evidence-based natural therapies for hyperlipidemia.

Materials and Methods Reagent

All chemicals have been of analytical grade.

Plant materials

Whole plant *M. parviflora* (WPMP) was purchased from the local market of Buraydah, Saudi Arabia

Preparation of *M. parviflora* leaf powder

The collected whole plant of *M. parviflora* was washed, air-dried in shade, and crushed with the help of a grinder. The resulting *M. parviflora* was stored and labelled.

Extraction

The crushed part of *M. parviflora* (500 g) was extracted using 95% methanol, 99% ethanol, hydroalcoholic extract (75% methanol+25% water), and water. The filtrate was then concentrated under reduced pressure using a rotary evaporator, maintaining controlled temperature and pressure

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conditions. The obtained powder was weighed, and the % yield was obtained (Ali, Khan, Sajid, & Zahra, 2018).

$$\% \text{ yield} = \frac{\text{Weight of the powder obtained}}{\text{Weight of the crude drug}} \text{ X } 100$$

Fractionation

The whole plant material of *M. parviflora* (500 g) was subjected to exhaustive extraction with 99% ethanol. The resulting solution was filtered, and the filtrate was concentrated under reduced pressure using a rotary evaporator, with careful regulation of temperature and pressure to prevent thermal degradation. The obtained crude ethanolic extract was then subjected to a sequential liquid-liquid fractionation process using solvents of increasing polarity. This process began with hexane, followed by chloroform, ethyl acetate, and methanol. Each fraction was thoroughly concentrated to complete dryness under high vacuum conditions using a rotary evaporator. The dried fractions were subsequently accurately labeled and stored in a desiccator under anhydrous conditions to prevent moisture absorption until further phytochemical and biological analysis (Hossain, Al-Hdhrami, Weli, Al-Riyami, & Al-Sabahi, 2014).

Phytochemical screening

A preliminary qualitative phytochemical analysis was conducted on the crude ethanolic extract of *M. parviflora* to identify the presence of major classes of secondary metabolites. The investigation was carried out employing established protocols and standard procedures as described in contemporary phytochemical methodology (Dubale, Kebebe, Zeynudin, Abdissa, & Suleman, 2023).

Total polyphenolic content

The total phenolic content of the root extract was quantified spectrophotometrically utilizing the Folin-Ciocalteu method, with gallic acid serving as the reference standard. For the assay, a reaction mixture was prepared by combining 0.5 mL of the root extract with 3 mL of distilled water and 0.25 mL of Folin-Ciocalteu reagent, followed by vigorous shaking. This mixture was kept in darkness for 5 minutes, after which 1 mL of a 7.5% sodium carbonate (Na_2CO_3) solution was introduced. The resulting solution was then incubated for 90 minutes at ambient temperature, protected from light. A reagent blank was prepared concurrently using distilled water in place of the extract. Absorbance readings for all samples were taken at 760 nm against this blank using a double-beam UV/Vis spectrophotometer. The total phenolic content was calculated from a gallic

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acid calibration curve and expressed as milligram gallic acid equivalents per 100 grams of sample (mg GAE/100 g). All measurements were performed in triplicate to ensure analytical precision (Seifu, Mehariffi, Atlabachew, & Chandravanshi, 2017).

Determination of flavonoid content

The quantification of total flavonoid content was conducted employing the aluminum chloride colorimetric method, as adapted from established protocols. In this procedure, a 0.5 mL aliquot of the plant extract was transferred to a 10 mL test tube and combined with 2 mL of distilled water. Subsequently, 0.15 mL of a 5% sodium nitrite ($NaNO_2$) solution was introduced to the mixture. Following a 5-minute incubation period, 0.15 mL of a 10% aluminum chloride ($AlCl_3$) solution was added. After an additional minute, 1 mL of 1 M sodium hydroxide (NaOH) was incorporated, and the total volume was brought to 5 mL using distilled water. The solution was allowed to stand for 10 minutes, after which the absorbance was measured at 510 nm using a spectrophotometer. A standard curve was prepared using catechin, and the total flavonoid content was calculated and expressed as milligrams of catechin equivalent per 100 grams of sample (mg CE/100 g). All analyses were performed in triplicate to ensure reproducibility (Ayele, Akele, & Melese, 2022).

Antioxidant activity

ABTS radical scavenging assay:

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity was assessed following the method. The $ABTS^+$ radical cation solution was prepared by mixing equal volumes (v/v) of 7 mM ABTS stock solution and 4.95 mM potassium persulfate. This mixture was allowed to incubate overnight at room temperature in the dark to facilitate the formation of the stable radical cation. For the assay, 1.0 ml of the prepared extract was combined with 3.9 ml of the $ABTS^+$ solution and 0.1 ml of phosphate buffer (pH 7.4). The decrease in absorbance of this reaction mixture was measured at 745 nm. A blank was prepared using an identical protocol, but replacing the extract with phosphate buffer (Al Jaafreh, 2024). The radical scavenging capacity was calculated as a percentage of inhibition using the formula:

$$\% \text{ Inhibition} = \frac{(A_{control} - A_{sample})}{A_{control}} \text{ X } 100$$

where A_control is the absorbance of the control reaction and A_sample is the absorbance in the presence of the extract.

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The results were standardized against gallic acid and are expressed in mg of gallic acid equivalents (GAE) per ml. The efficacy of the extract was further quantified by determining its IC_{50} value, which represents the concentration required to scavenge 50% of the ABTS radicals. This value was derived by plotting the percentage inhibition against a series of sample concentrations.

Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power (FRAP) was evaluated. For the assay, 1.0 ml of this extract was combined with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of a 1% potassium ferricyanide solution. The mixture was incubated at 50°C for 20 minutes. Following incubation, 2.5 ml of a 10% trichloroacetic acid solution was added. The solution was then centrifuged at 3,000 rpm for 10 minutes. From the resulting supernatant, 2.5 ml was aliquoted and mixed with 2.5 ml of distilled water and 0.5 ml of a 0.1% ferric chloride solution. The absorbance of the final solution was measured at 700 nm. An increase in the absorbance of the reaction mixture corresponds to a higher reducing power (Walasek-Janusz, Papliński, Mysiak,
& Nurzyńska-Wierdak, 2025).

The reducing power was quantified relative to a standard of butylated hydroxytoluene (BHT) and expressed as mg BHT equivalents per ml. The antioxidant activity was also characterized by an IC_{50} value, which denotes the concentration of the extract required to achieve 50% scavenging activity. This value was determined by plotting the percentage of inhibition against the sample concentration. The percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \frac{(A_{control} - A_{sample})}{A_{control}} \text{ X } 100$$

where A_control is the absorbance of the control reaction and A_sample is the absorbance in the presence of the extract.

Assay of DPPH Scavenging Activity

The DPPH radical scavenging activity was determined according to a protocol. Briefly, 0.5 g of the sample was homogenized using a mortar and pestle with 50% ethanol while kept on ice. The homogenate was then centrifuged at 5000 rpm for 15 minutes. The resulting supernatant was collected, and its final volume was adjusted to 4.0 mL with 50% ethanol. Subsequently, a 1.0 mL aliquot of this extract was mixed with 1.0 mL of a 0.1 mM DPPH solution. A control was prepared using an equivalent volume of ethanol in place of the extract. After vigorous mixing, the reaction mixture was incubated in the dark for 30 minutes, and its absorbance was measured at 517 nm.

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The radical scavenging activity was quantified as a percentage of inhibition, calculated using the formula:

$$\% \text{ Inhibition} = \frac{(A_{control} - A_{sample})}{A_{control}} \text{ X } 100$$

where A control is the absorbance of the control reaction and A sample is the absorbance in the presence of the extract.

The results were standardized against a reference antioxidant, ascorbic acid, and are expressed as milligrams of ascorbic acid equivalent per gram of fresh sample weight (mg AAE/g FW). Furthermore, the extract's scavenging efficiency was expressed as an IC_{50} value, which denotes the concentration required to achieve 50% radical scavenging. This value was derived by plotting the percentage inhibition against a range of sample concentrations (Kiran, Kumar, Singh, & Jain, 2024).

Antihyperlipidemic Activity

The antihyperlipidemic potential of various fractions of *M. parviflora* whole plant parts was evaluated through in vitro assays, including α-amylase inhibition, α-glucosidase inhibition, pancreatic lipase activity, and HMG-CoA reductase inhibition.

HMG CoA reductase inhibition assay

The HMG-CoA reductase inhibitory activity was evaluated using a spectrophotometric assay kit (Sigma-Aldrich Co., St. Louis, MO, USA). The reaction mixture was prepared in a final volume of 200 µL of potassium phosphate buffer (100 mM, pH 7.4), containing 400 µM nicotinamide adenine dinucleotide phosphate (NADPH) and 400 µM HMG-CoA. The reaction was initiated by adding 2 µL of HMG-CoA reductase enzyme (0.5–0.75 mg/mL) to the mixture. To assess inhibition, 2 µL of the test sample (HAPF; 20–100 µg/mL) was included in the experimental group, while the control reaction was performed without the inhibitor. Atorvastatin was employed as a reference standard inhibitor.

The reaction was incubated at 37°C for 10 minutes and performed in triplicate. Enzyme activity was determined by monitoring the decrease in absorbance at 340 nm, which corresponds to the oxidation of NADPH during the reduction of HMG-CoA to mevalonate. The inhibitory activity was calculated based on the reduction in absorbance relative to the control reaction (Chaudhary et al., 2023).

The percentage of inhibition was determined using the provided formula:

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$$\% \text{ Inhibition} = \frac{(A_{control} - A_{sample})}{A_{control}} X\ 100$$

where Abs control represents the absorbance of the control reaction (containing all reagents except the plant extract or standard inhibitor), and Abs sample is the absorbance in the presence of the plant extract or Atorvastatin.

Alpha-glucosidase inhibitory assay

The α-glucosidase inhibitory activity of the plant extracts was assessed using an adapted protocol based on the method of (Pistia-Brueggeman & Hollingsworth, 2001). Plant extract aliquots (50 µL), at concentrations ranging from 12.5 to 400 µg/mL, were pre-incubated with 10 µL of α-glucosidase enzyme solution (1 U/mL, from yeast; Sisco Research Laboratories

Pvt. Ltd.) and 125 µL of 0.1 M phosphate buffer (pH 6.8) for 20 minutes at 37 °C. Following this incubation, the reaction was initiated by adding 20 µL of 1 mM p-nitrophenyl-α-Dglucopyranoside (pNPG) as the substrate. The resulting mixture was incubated for an additional 30 minutes at 37°C. The reaction was terminated by adding 50 µL of 0.1 M sodium carbonate (Na_2CO_3). The absorbance of the released p-nitrophenol was measured at 405 nm using a Biotek multi-well plate reader. The inhibitory activity was calculated using the formula:

$$\% \text{ Inhibition} = \frac{(OD_{control} - OD_{sample})}{OD_{control}} X\ 100$$

where OD_control represents the absorbance of the control reaction (containing all reagents except the test extract) and OD_sample is the absorbance in the presence of the plant extract or standard inhibitor. One unit of enzyme activity was defined as the amount of enzyme required to release 1 µmol of p-nitrophenol from pNPG per minute under the assay conditions.

The half-maximal inhibitory concentration (IC_{50}) was determined by plotting the percentage inhibition against the logarithm of the inhibitor concentration and performing non-linear regression analysis. Acarbose, used as the standard reference inhibitor across the same concentration range, served as the positive control. All analyses were performed in triplicate.

Alpha-amylase inhibitory assay

The α-amylase inhibitory potential of the plant extracts was evaluated using an iodine-starch test, based on the assay that relies on the principle that starch forms a blue-violet complex upon reaction

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with iodine; a reduction in color intensity indicates starch hydrolysis by α-amylase, which is inhibited in the presence of an active compound.

Briefly, 0.2 mL of each plant extract was mixed with 0.4 mL of 1% starch solution and incubated at 37 °C. A defined quantity of α-amylase enzyme was then introduced to the mixture, followed by an additional 15-minute incubation period. The reaction was halted by adding 0.8 mL of 0.1 M HCl. Subsequently, 200 µL of 1 mM iodine solution was added (Mogole, Omwoyo, & Mtunzi, 2020).

The percentage inhibition of α-amylase activity was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{(A_{control} - A_{sample})}{A_{control}} \text{ X } 100$$

where Abs_control represents the absorbance of the control reaction (containing all reagents except the plant extract or standard inhibitor), and Abs_sample is the absorbance in the presence of the plant extract or acarbose. Acarbose was used as the standard reference inhibitor.

All assays were conducted in triplicate to ensure reproducibility.

Pancreatic lipase activity

The lipase inhibitory activity of the pollen extracts was evaluated using a previously described protocol with slight modifications. An enzyme buffer was prepared by combining 30 µL (10 units) of porcine pancreatic lipase (Sigma, St. Louis, MO) solution with 850 µL of Tris-buffer (100 mM Tris-HCl and 5 mM $CaCl_2$, pH 7.0). The lipase solution was prepared in 10 mM MOPS buffer containing 1 mM EDTA (pH 6.8).

Subsequently, 100 µL of the pollen extract or the standard inhibitor Orlistat (Roche, Switzerland) was added to 880 µL of the enzyme buffer, and the mixture was incubated at 37 °C for 15 minutes. Following this, 20 µL of substrate solution, consisting of 10 mM pnitrophenyl butyrate (p-NPB) in dimethyl sulfoxide (DMSO), was introduced to initiate the reaction. The enzymatic hydrolysis was allowed to proceed for 15 minutes at 37 °C.

The activity of pancreatic lipase was quantified by monitoring the release of p-nitrophenol from p-NPB at a wavelength of 405 nm using a microplate reader (OPTIMA, Germany). The inhibitory activity was calculated as the percentage reduction in optical density (OD) compared to the control reaction, which contained no inhibitor. Orlistat was used as a positive control.

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The results are presented as percent inhibition, and the IC_{50} value, which denotes the concentration of extract required to achieve 50% enzyme inhibition, was determined from a dose-response curve (Yang et al., 2022).

Statistical analysis

The statistical analysis was conducted using GraphPad Prism version 7.0. Data are presented as the mean ± SD, and group differences were evaluated using one-way analysis of variance (ANOVA). Significance levels were denoted as *P < 0.05, **P < 0.01, and ***P < 0.001.

Result Extraction

A comparison of different crude extraction solvents on *M. parviflora* whole plant showed significant variation in extraction efficiency. The 99% ethanolic extract (ETMP) yielded the highest amount of extractable material at 24.92%. The 95% methanolic extract (MEMP) also performed well with a yield of 21.09%. The hydroalcoholic extract (HAMP) and the aqueous extract (AQMP) were less effective, yielding 15.63% and 11.47%, respectively. The aqueous extract (AQMP) had the lowest yield, demonstrating that water alone was the least efficient solvent for extracting components from the plant material (Table 1). **Table 1:** Plant material and its percentage yield

Plant materials	**Crude extracts**	**% Yield**
***M. parviflora* whole plant (MPWP) (500 g)**	95% methanol (MEMP)	21.09
	99% ethanol (ETMP)	24.92
	Hydroalcoholic extract (75% methanol+25% water) (HAMP)	15.63
	Water (AQMP)	11.47

Fractionation

The fractionation of the crude ethanolic extract (ETMP) from *M. parviflora* whole plant, which itself had a yield of 24.92%, resulted in four distinct fractions with varying yields. The fractionation of the crude ethanolic extract (ETMP, 24.92% yield) using solvents of increasing polarity successfully partitioned the plant material into distinct components. The ethyl acetate fraction (EAMP) yielded the highest amount at 36.71%, suggesting that the majority of the compounds in *M. parviflora* are of intermediate polarity, as ethyl acetate is a mid-polar solvent. The moderately polar methanol fraction (MFMP) also constituted a significant portion at 29.44%. The lower yield of the chloroform fraction (CFMP, 16.27%), a solvent of low-tointermediate polarity, and the lowest yield from the non-polar hexane fraction (HFMP, 11.38%) indicate that highly non-polar compounds are present in a smaller proportion within this plant.

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This polarity-based separation reveals that *M. parviflora* is richest in mid-polarity phytochemicals (Table 2).

Table 2: Percentage yield of plant extract and its different fractions

Plant materials	**Crude extracts**	**% Yield**	**Fractions**		**% Yield**
M. parviflora Whole Plant (MPWP) (500 g)	Ethanolic (ETMP)	24.92	Hexane Chloroform Ethyl acetate Methanol	(HFMP) (CFMP) (EAMP) (MFMP)	11.38 16.27 36.71 29.44

Phytochemical screening

Based on the phytochemical analysis, the methanolic extract of *M. parviflora* was found to contain a diverse range of bioactive compounds. The tests confirmed the presence of diterpenes, carbohydrates, flavonoids, anthocyanins, steroids, coumarins, phenols, alkaloids, proteins, and glycosides.

Specifically, the extract tested positive for alkaloids across all three standard tests (Mayer's, Dragendroff's, and Wagner's tests). Both Legal's and Baljet tests indicated the presence of glycosides. The presence of steroids was confirmed by the Salkowski and LiebermannBurchard tests. Flavonoids were detected using the zinc hydrochloride reduction and alkaline reagent tests. Carbohydrates were present, as shown by positive results in Molisch's, Fehling's, and Benedict's tests. Positive Biuret and Ninhydrin tests identified proteins. Phenols were detected with the ferric chloride and lead acetate tests. Tannins were obtained by (lead acetate and vanillin hydrochloride tests). Furthermore, the extract tested positive for the presence of diterpenes, anthocyanins, and coumarins. The extract tested negative for saponins (foam and froth tests), fixed oils (spot test), volatile oils (fluorescence test), and quinones (alcoholic KOH test) (Table 3).

Table 3: Phytochemical constituents found in *M. perviflora*

Phytochemicals	**Name of the test**	***M. perviflora***
Diterpenes	Copper acetate test	+
Carbohydrate	Molisch’s test	+
	Fehling’s test	+
	Benedict’s test	+
Flavonoids	Zinc hydrochloride reduction test	+

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	Alkaline reagent test	+
Anthocyanins	HCl test	+
Steroids	Salkowski test	+
	Liebermann- Burchard test	+
Coumarins	NaOH+Chloroform	+
Tannins	Lead acetate test	+
	Vanillin hydrochloride test	+
Phenols	Ferric chloride test	+
	Lead acetate test	+
Alkaloid	Mayer's test	+
	Dragendroff's test	+
	Wagner's test	+
Proteins	Biuret test	+
	Ninhydrin test	+
Glycoside	Legal's test	+
	Baljet test	+
Saponins	Foam test	-
	Froth test	-
Fixed Oils	Spot test	-
Volatile Oils	Fluorescence test	-
Quinones	Alcoholic KOH test	-

Total Phenols & Flavonoid Content

The ethanolic extract of *M. parviflora* was found to contain significant levels of phenolic and flavonoid compounds. The total phenol content was determined to be 36.83 ± 3.71 mg of Gallic Acid Equivalent (GAE) per gram of dry weight. The total flavonoid content was quantified as 47.19 ± 5.04 mg of Catechin Equivalent (CE) per gram of dry weight. These results indicate that the plant is a rich source of these potent antioxidant phytochemicals (Table 4).

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Table 4: Total phenol and flavonoid contents

Total Phenol Content (mg of Gallic acid equivalent/g of dry weight)	**Total Flavonoid (mg of Catechin equivalent/g of dry weight)**
36.83±3.71	47.19±5.04

Antioxidant Activity

ABTS Assay

Based on the ABTS radical cation decolorization assay graph, the results demonstrate a clear dose-dependent increase in antioxidant activity for all tested fractions of *M. parviflora* and the standard. The ethyl acetate fraction (EFMP) consistently exhibits the highest percentage of inhibition across the concentration range, indicating it is the most potent scavenger of $ABTS^{+}$ radicals among the extracts. This is followed by the methanolic fraction (MFMP), which shows strong but lesser activity. The chloroform (CFMP) and hexane (HFMP) Fractions demonstrate progressively lower scavenging abilities.

The standard antioxidant displays the steepest curve, achieving near-complete inhibition at the lowest concentrations, confirming its superior potency as a positive control. The results from this assay are consistent with the DPPH and FRAP findings, conclusively identifying the ethyl acetate fraction (EFMP) as the most antioxidant-rich extract. Based on the ABTS radical scavenging assay, the antioxidant activity of various fractions of *M. parviflora* was quantified and expressed as the half-maximal inhibitory concentration (IC_{50}), where a lower value indicates higher potency in neutralizing the $ABTS^{+}$ radical.

The results demonstrate that the Ethyl Acetate Fraction (EFMP) possessed the strongest antioxidant activity among the tested extracts, with the lowest IC_{50} value of 54.87 µg/ml. The methanolic fraction (MFMP) and chloroform fraction (CFMP) showed moderate and comparable activity, with IC_{50} values of 89.11 µg/ml and 90.31 µg/ml, respectively. The hexane fraction (HFMP) exhibited the weakest scavenging ability, with an IC50 of 111.30 µg/ml. As expected, the standard antioxidant, Trolox, demonstrated the most potent activity with a significantly lower IC_{50} value of 3.25 µg/ml, serving as a benchmark for the assay. The ABTS assay results corroborate the findings from the DPPH and FRAP assays, consistently identifying the ethyl acetate fraction (EFMP) as the most effective antioxidant extract, which aligns with its high content of phenolic and flavonoid compounds.

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FRAP Assay

The reducing power, which indicates the ability of the extract to donate electrons, was strongest in the ethyl acetate fraction (EFMP), as it achieved the highest absorbance (or percentage reduction) across the tested concentration range. This was followed by the methanolic fraction (MFMP). The chloroform (CFMP) and hexane (HFMP) Fractions demonstrated progressively lower reducing power, respectively.

The results from this assay confirm the trend observed in the DPPH analysis, reinforcing that the ethyl acetate fraction possesses the most significant antioxidant activity among the extracts. Based on the ferric reducing antioxidant power (FRAP) assay, the antioxidant activity of various fractions of *M. parviflora* was quantified and expressed as the half-maximal effective concentration (IC_{50}), with a lower value indicating a stronger ability to reduce ferric ions. The results indicate that the ethyl acetate fraction (EFMP) exhibited the most potent reducing power among the extracts, with the lowest IC_{50} value of 27.08 µg/ml**.** This was followed by the chloroform fraction (CFMP)**,** which showed moderate activity with an IC_{50} of 65.91 µg/ml**.** The methanolic (MFMP) and hexane (HFMP) fractions demonstrated considerably weaker reducing power, with IC_{50} values of 93.45 µg/ml and 96.42 µg/ml, respectively.

As anticipated, the standard antioxidant, Ascorbic acid, displayed the strongest reducing activity with a significantly lower IC_{50} value of 7.63 µg/ml, serving as a positive control for the assay. The FRAP assay results confirm the superior antioxidant potential of the ethyl acetate fraction (EFMP), which is consistent with the findings from the DPPH assay, highlighting its significant electron-donating capacity.

DPPH Assay

Based on the DPPH radical scavenging assay graph, the results demonstrate a clear dosedependent increase in antioxidant activity for all tested fractions of *M. parviflora* and the standard ascorbic acid. Among the plant extracts, the ethyl acetate fraction (EFMP) shows the steepest curve, achieving the highest percentage of inhibition at each concentration, which indicates it is the most potent antioxidant fraction. This is followed by the methanolic fraction (MFMP), then the chloroform (CFMP) and hexane (HFMP) fractions, which show progressively lower activity across the tested concentration range. Based on the DPPH radical scavenging assay, the antioxidant activity of various fractions of *M. parviflora* and a standard ascorbic acid was evaluated and expressed as the half-maximal inhibitory concentration (IC_{50}), where a lower value indicates higher potency.

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The results demonstrated a clear dose-dependent increase in antioxidant activity for all tested fractions. Among the plant extracts, the ethyl acetate fraction (EFMP) exhibited the strongest antioxidant potential with the lowest IC_{50} value of 35.66 µg/ml. This was followed by the methanolic fraction (MFMP) with an IC_{50} of 54.24 µg/ml. The chloroform (CFMP) and hexane (HFMP) fractions showed comparatively weaker antioxidant activity, with IC_{50} values of 79.56 µg/ml and 88.91 µg/ml, respectively.

The standard antioxidant, ascorbic acid, demonstrated the most potent activity with a significantly lower IC_{50} value of 4.88 µg/ml, serving as a benchmark for the assay. The data from both the graph and the table indicate that the ethyl acetate fraction (EFMP) possesses the most significant free radical scavenging activity among the tested extracts, which correlates with the high phenolic and flavonoid content previously identified, suggesting these compounds are major contributors to its antioxidant effect (Table 5; Figure 6).

Table 5: IC_{50} value of diverse fractions of *M. parviflora* whole plant and standard

Fractions	**IC_{50} (µg/ml)**		
	DPPH	**ABTS**	**FRAP**
HFMP	88.91	111.30	96.42
CFMP	79.56	90.31	65.91
EFMP	35.66	54.87	27.08
MFMP	54.24	89.11	93.45
Ascorbic acid (Standard)	4.88	-	7.63
Trolex Solution	-	3.25	-

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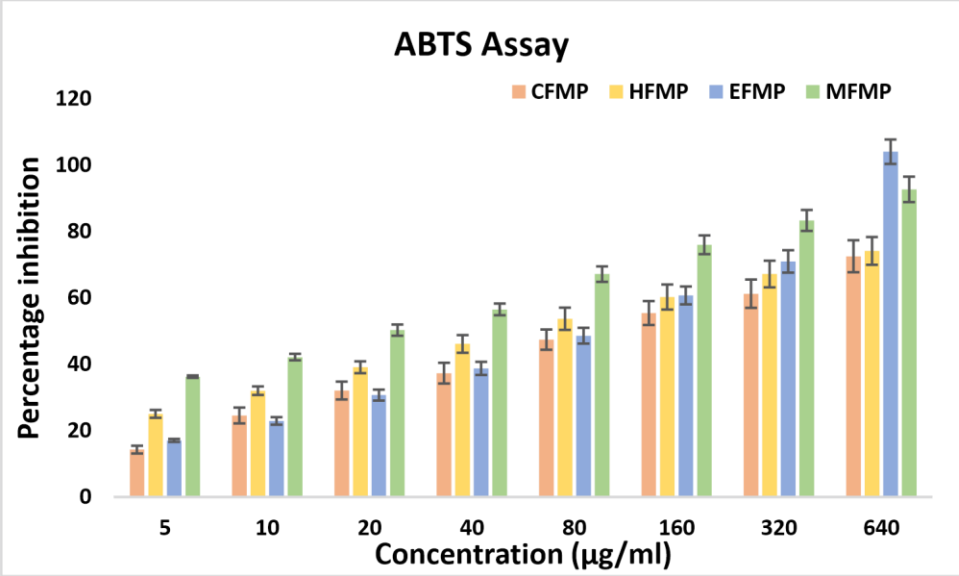

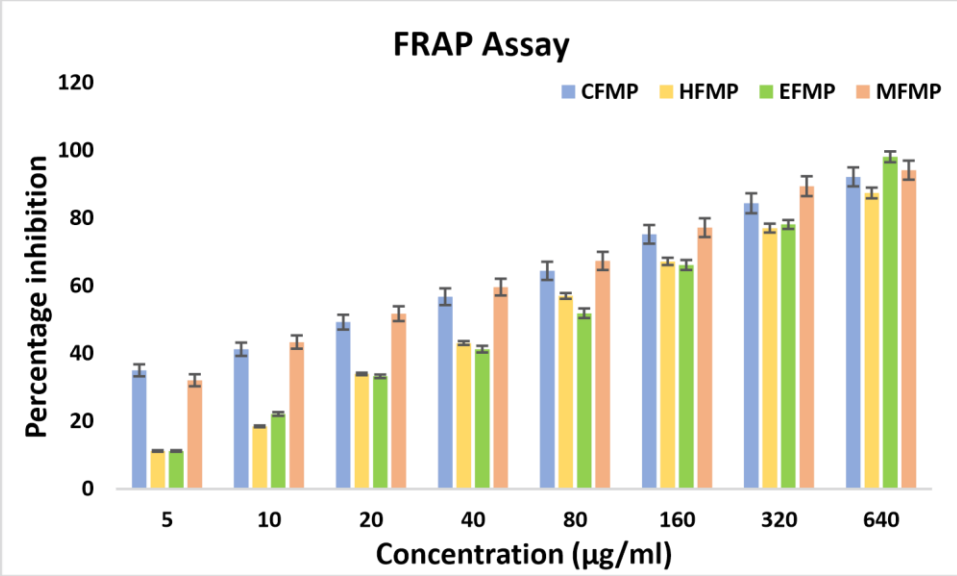

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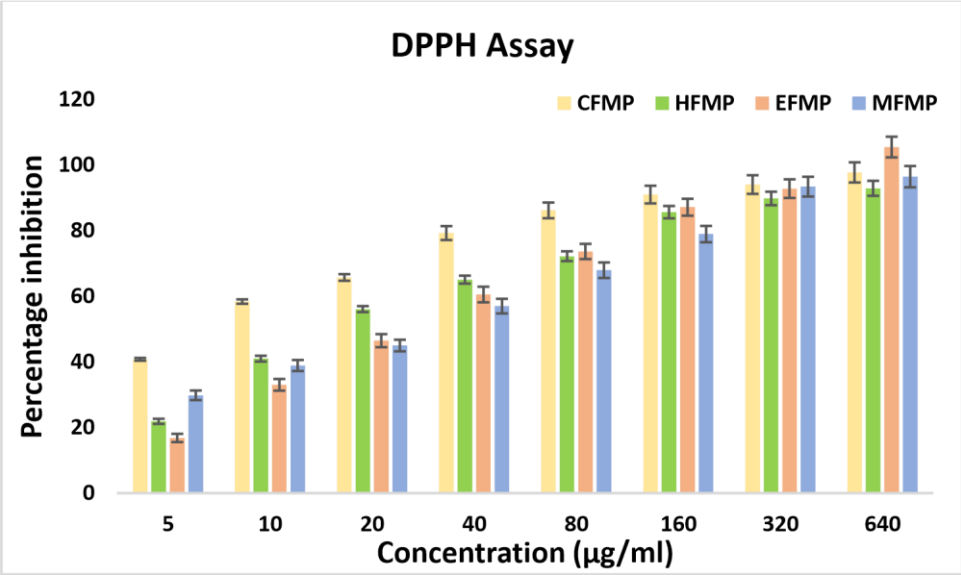

Figure 1: The antioxidant estimation of different fractions of M. Parviflora against FRAP, ABTS, and DPPH assay

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Antihyperlipidemic Activity α-Amylase Inhibitory Effect

At the lowest tested concentration of 75 µg/ml, the methanolic fraction (MFMP) and ethyl acetate (EFMP) exhibited very significant inhibitory activity (**p<0.01), while the chloroform (CFMP), and hexane (HFMP) fractions showed significant inhibition (*p<0.05).

At 150 µg/ml, the methanolic (MFMP), chloroform (CFMP), and hexane (HFMP) fractions demonstrated significant inhibition (*p<0.05), while the ethyl acetate (EFMP) showed very significant inhibitory activity (**p<0.01).

At 300 µg/ml, the methanolic (MFMP) and chloroform (CFMP) fraction showed very significant inhibition (**p<0.01), while the ethyl acetate (EFMP) displayed highly significant (***p<0.001) and hexane (HFMP) fraction showed significant (*p<0.05) inhibition.

At the highest concentration of 600 µg/ml, the methanolic (MFMP) fraction showed a very significant (**p<0.01) reduction, the chloroform (CFMP) and hexane (HFMP) fractions showed significant inhibition (*p<0.05), while the ethyl acetate (EFMP) displayed highly significant (***p<0.001) inhibition when all these fractions were compared with acarbose.

The standard inhibitor, acarbose, consistently exhibited the most potent inhibitory effect across all concentrations tested. These results indicate that the methanolic fraction possesses the most substantial alpha-amylase inhibitory potential among the tested extracts. The MFMP fraction exhibited the most potent α-amylase inhibitory activity among all tested plant fractions, with an IC_{50} value of 89.16 µg/mL. This was followed by the HFMP fraction (IC_{50} = 116.48 µg/mL) and the EFMP fraction (IC_{50} = 154.39 µg/mL). The CFMP fraction was the least effective against this enzyme, with an IC_{50} of 189.81 µg/mL. As expected, the standard drug Acarbose demonstrated significantly stronger inhibition (IC_{50} = 17.09 µg/mL) than all plant fractions.

***α*-Glucosidase Inhibitory Effect**

At 75 µg/ml, the hexane (HFMP) and chloroform (CFMP) fractions exhibited significant inhibition (*p<0.05) while ethyl acetate (EFMP), and methanolic (MFMP) fractions showed very significant inhibition (**p<0.01). At 150 µg/ml, the hexane (HFMP) and chloroform (CFMP) fractions exhibited significant inhibition (*p<0.05) while ethyl acetate (EFMP), and methanolic (MFMP) fractions showed very significant inhibition (**p<0.01). At 300 µg/ml, the hexane (HFMP) and methanolic (MFMP) fractions exhibited very significant (**p<0.01) inhibition, chloroform (CFMP) fractions exhibited significant inhibition (*p<0.05), while ethyl acetate (EFMP) showed highly significant inhibition (***p<0.001).

At 600 µg/ml, HFMP, CFMP, and MFMP fractions showed very significant inhibition (**p< 0.01), EFMP showed highly significant inhibition (***p<0.001). All these fractions were compared with acarbose.

The EFMP fraction was the most effective α-glucosidase inhibitor, showing a markedly low IC_{50} value of 34.73 µg/mL. The MFMP fraction also showed considerable activity (IC_{50} = 59.49 µg/mL), followed by the CFMP fraction (IC_{50} = 123.51 µg/mL). The HFMP fraction was the least active against this enzyme (IC_{50} = 177.86 µg/mL). Again, the standard Acarbose was the most potent inhibitor overall (IC_{50} = 12.26 µg/mL) (Figure 2).

Pancreatic Lipase Inhibitory Activity

At 75 µg/ml, the hexane (HFMP), chloroform (CFMP), ethyl acetate (EFMP), and methanolic (MFMP) fractions exhibited significant inhibition (*p<0.05). At 150 µg/ml, the hexane (HFMP) and methanolic (MFMP) fractions exhibited significant inhibition (*p<0.05), while chloroform (CFMP), and ethyl acetate (EFMP) fractions showed very significant inhibition (**p<0.01). At 300 µg/ml, the hexane (HFMP) and methanolic (MFMP) fractions exhibited significant inhibition (*p<0.05), while chloroform (CFMP) and ethyl acetate (EFMP) fractions showed very significant inhibition (**p<0.01). At 600 µg/ml, the hexane (HFMP), chloroform (CFMP), and methanolic (MFMP) fractions exhibited very significant inhibition (**p<0.01) while ethyl acetate (EFMP) fractions showed highly significant inhibition (***p<0.01) when all four fractions were compared to Orlistat.

The calculated IC_{50} showed that MFBM and HFBM inhibition potential is significant with respect to other fractions when compared with orlistat, with an IC_{50} of 42.34 (EFBM), 99.53 (CFBM), 158.7 (HFBM) and 72.53 (MFBM) µg/ml against 18.18µg/ml noted for orlistat (Figure 2).

HMG-CoA Reductase Inhibitory Activity

At 75 µg/ml, the chloroform (CFMP) fraction showed non-significant (p>0.05) inhibition of this enzyme, while hexane (HFMP) and methanolic (MFMP) fractions showed significant (*p<0.05) inhibition, and ethyl acetate (EFMP) exhibited very significant inhibition (**p<0.01). At 150 µg/ml, 300 µg/ml, and 600 µg/ml, the chloroform (CFMP) and hexane (HFMP) fractions exhibited significant (*p<0.05) inhibition, while ethyl acetate (EFMP) and methanolic (MFMP) fractions showed very significant (**p<0.01) inhibition when all four fractions were compared to Atorvastatin.

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The calculated IC_{50} showed that MFBM and HFBM inhibition potential is significant with respect to other fraction when compared with atorvastatin, with an IC_{50} of 57.03 (EFBM), 211.5 (CFBM),
88.58 (HFBM), and 31.88 (MFBM) µg/ml against 13.99 µg/ml noted for atorvastatin (Figure 2).

Inhibitory effects of the fraction

The inhibitory effects (expressed as IC_{50} values) of the various *M. parviflora* (HFMP, CFMP, EFMP, MFMP) and standard drugs on the activities of key metabolic enzymes are presented in Table 9.

The MFMP fraction exhibited the most potent α-amylase inhibitory activity among all tested plant fractions, with an IC_{50} value of 89.16 µg/mL. This was followed by the HFMP fraction (IC_{50} = 116.48 µg/mL) and the EFMP fraction (IC_{50} = 154.39 µg/mL). The CFMP fraction was the least effective against this enzyme, with an IC_{50} of 189.81 µg/mL. As expected, the standard drug Acarbose demonstrated significantly stronger inhibition (IC_{50} = 17.09 µg/mL) than all plant fractions. The EFMP fraction was the most effective α-glucosidase inhibitor, showing a markedly low IC_{50} value of 34.73 µg/mL. The MFMP fraction also showed considerable activity (IC_{50} = 59.49 µg/mL), followed by the CFMP fraction (IC_{50} = 123.51 µg/mL). The HFMP fraction was the least active against this enzyme (IC_{50} = 177.86 µg/mL). Again, the standard Acarbose was the most potent inhibitor overall (IC_{50} = 12.26 µg/mL). For pancreatic lipase inhibition, the EFMP fraction was the most effective plant-derived inhibitor with an IC_{50} of 61.67 µg/mL. The CFMP and HFMP fractions showed moderate activity, with IC_{50} values of 111.04 µg/mL and 93.29 µg/mL, respectively. The MFMP fraction was the least effective at inhibiting lipase (IC_{50} = 149.88 µg/mL). The inhibitory activity of all fractions was considerably weaker than that of the standard drug Orlistat (IC_{50} = 7.42 µg/mL). The EFMP fraction demonstrated the strongest inhibition of HMG-CoA reductase among the fractions, with an IC_{50} value of 47.71 µg/mL. The MFMP fraction also showed good activity (IC_{50} = 84.05 µg/mL), while the HFMP and CFMP fractions were less effective, with IC_{50} values of 149.62 µg/mL and 187.35 µg/mL, respectively. The standard drug Atorvastatin was the most potent inhibitor by a significant margin (IC_{50} = 16.37 µg/mL) (Table 6).

Table 6: IC_{50} of *M. parviflora* whole plant fractions & standard drugs on HMG-CoA reductase activities, α-glucosidase, pancreatic lipase & α-amylase.

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***M. parviflora* fraction & standard drugs**	**Inhibitory Concentration (IC_{50})**			
	HMG-CoA reductase	**α - amylase**	**Pancreatic lipase**	**α glucosidase**
HFMP	149.62	116.48	93.29	177.86
CFMP	187.35	189.81	111.04	123.51
EFMP	47.71	154.39	61.67	34.73
MFMP	84.05	89.16	149.88	59.49
Acarbose	-	17.09	-	12.26
Orlistat	-	-	7.42	-
Atorvastatin	16.37	-	-	-

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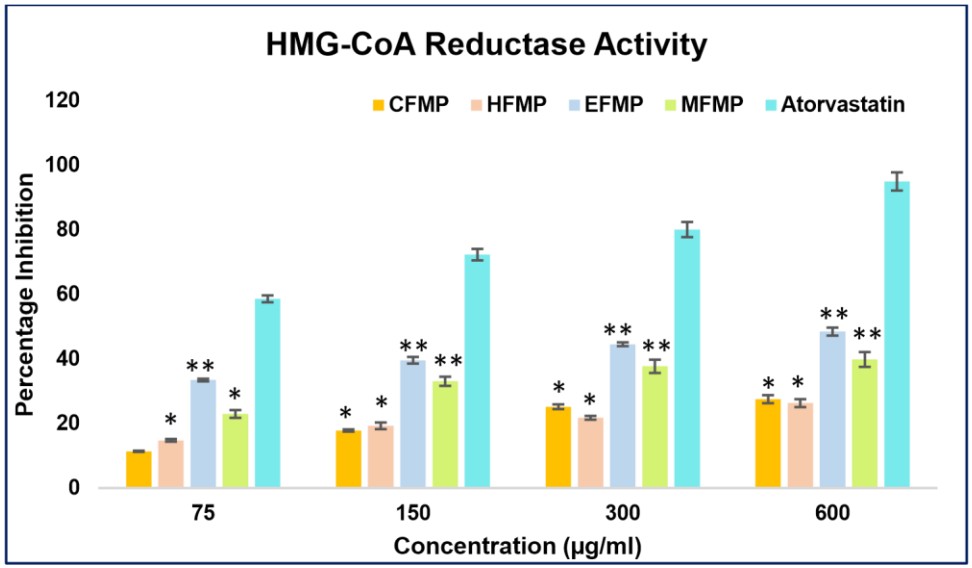

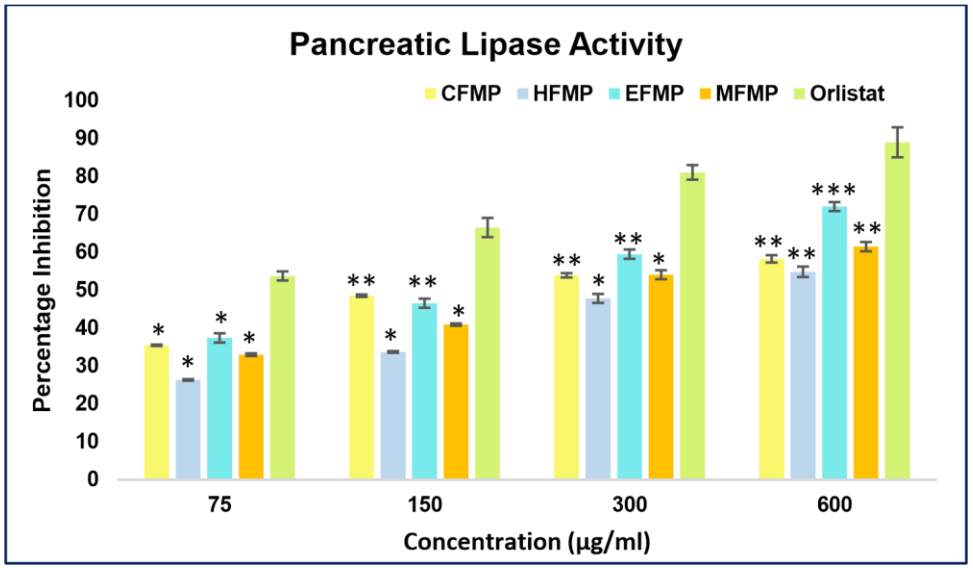

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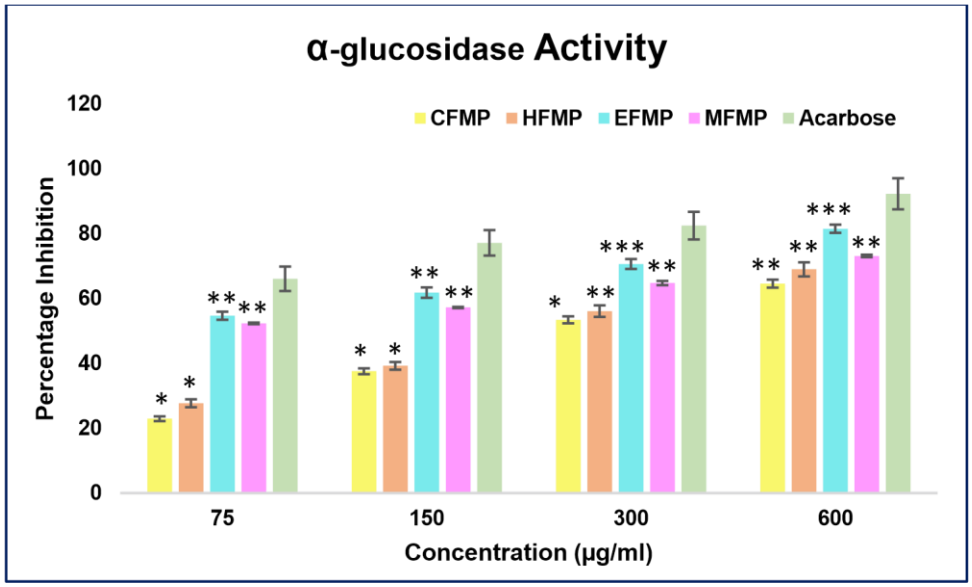

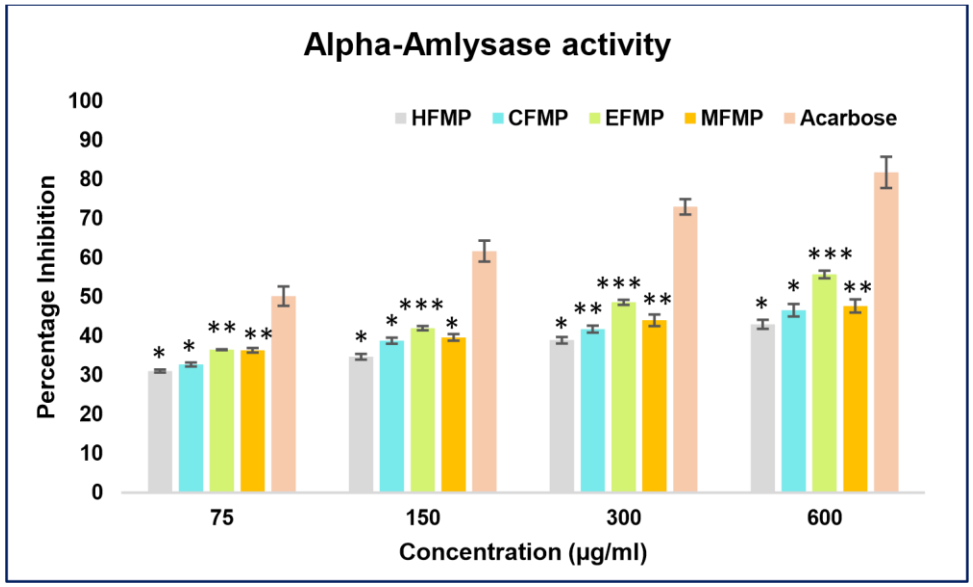

Figure 2: Antihyperlipidemic activity of *M. parviflora* through different lipid controlling enzymes

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Discussion

The plant kingdom represents an immense and largely untapped reservoir of biodiversity, offering a vast array of species endowed with significant therapeutic potential. For millennia, diverse human civilizations have relied on botanical sources to develop traditional medicines for treating a wide spectrum of ailments (Siddiquie et al., 2024). In the contemporary era, scientific inquiry has turned towards these natural repositories, seeking to validate their historical uses and elucidate the precise mechanisms of action behind their healing properties. This rigorous investigation aims to isolate novel bioactive compounds that can serve as the foundation for new pharmaceuticals or functional foods aimed at promoting human health and combating chronic diseases (S. Khan, Ahsan, Mahmood, & Bano, 2024).

Among this rich diversity, *M. parviflora* stands out as a plant of considerable pharmacological interest. It is renowned for its complex chemical profile, which includes a potent mixture of secondary metabolites such as flavonoids, phenolic acids, tannins, terpenoids, and saponins. These compounds are not merely incidental; they are the active agents responsible for the plant's documented biological activities. Extensive research has correlated the presence of these phytochemicals with robust antioxidant, antimicrobial, and anti-inflammatory effects, highlighting the plant's role in natural defense mechanisms (A. Khan et al., 2019).

A particularly promising application of these bioactive compounds lies in the management of metabolic disorders, including hyperlipidemia and related cardiovascular conditions. Hyperlipidemia, characterized by elevated levels of lipids in the bloodstream, is a major modifiable risk factor for atherosclerosis and coronary artery disease (Nelson, 2013). Modern therapeutic strategies increasingly look to natural products that can support and modulate lipid metabolism safely and effectively. Plant-derived substances offer a multi-faceted approach by interacting with various physiological pathways. They can inhibit key enzymes involved in fat and carbohydrate digestion, modulate the synthesis of cholesterol and fatty acids in the liver, enhance antioxidant defenses to reduce oxidative stress, and suppress inflammatory pathways that exacerbate metabolic syndrome (D. Roy, Kaur, Ghosh, Choudhary, & Rangra, 2024).

A critical mechanism in managing postprandial blood glucose levels—a key factor influencing lipid metabolism—involves the inhibition of carbohydrate-digesting enzymes in the digestive tract. By targeting enzymes like α-amylase and α-glucosidase, bioactive compounds can delay the breakdown of complex carbohydrates into absorbable sugars. This action prevents the sharp spikes in blood glucose after a meal, thereby reducing the subsequent insulin demand and the conversion

of excess sugars into stored fats (Tucci, Boyland, & Halford, 2010). The investigation into *M. parviflora's* capacity to inhibit these specific enzymes is therefore central to understanding its antihyperlipidemic potential.

Furthermore, the antioxidant prowess of plant extracts is intrinsically linked to their richness in polyphenolic compounds. Phenols and flavonoids, in particular, are powerful scavengers of free radicals, such as the stable DPPH radical commonly used in assays. The ability to donate electrons and neutralize these reactive oxygen species helps protect cellular structures from oxidative damage, which is an underlying contributor to chronic diseases like hyperlipidemia. The synergistic action of various secondary metabolites—including alkaloids, phytosterols, diterpenes, and glycosides— further amplifies these antioxidant effects (Rauf, Ahmad, Formanowicz, Ribaudo, & Alomar, 2024). The initial extraction phase is critical for evaluating the solubility and preliminary composition of bioactive compounds within *M. parviflora*. The use of solvents with varying polarities—from pure organic solvents to water—provides a fundamental understanding of the plant's chemical landscape (Hlatshwayo, Thembane, Krishna, Gqaleni, & Ngcobo, 2025).

The markedly higher yield observed with the pure ethanolic extract, compared to the other solvents, offers a significant initial insight. This result strongly suggests that the majority of the extractable secondary metabolites in *M. parviflora* are of intermediate polarity. Compounds such as flavonoids, polyphenols, and terpenoids, which are frequently associated with pronounced biological activities, are typically efficiently solubilized by solvents like ethanol and methanol. The comparatively lower yield from the aqueous extract implies that while highly polar compounds like saponins or polar glycosides are present, they constitute a less dominant portion of the plant's extractable chemical profile (Lim et al., 2024). Consequently, the ethanolic extract was selected for further fractionation, as it appears to most effectively capture the broad spectrum of the plant's medium-polarity bioactive constituents.

The subsequent fractionation of the chosen crude extract provides a more refined dissection of the plant's phytochemical composition. This step, which employs a series of solvents with systematically increasing polarity, allows for the segregation of compounds based on their solubility, effectively creating extracts enriched with specific classes of molecules (Jiménez et al., 2022).

The results from this fractionation are highly informative. The exceptionally high yield obtained in the ethyl acetate fraction is particularly notable. Ethyl acetate, a solvent of intermediate polarity, is renowned for its efficacy in extracting a valuable range of secondary metabolites, most notably

polyphenols, flavonoids, and tannins. This outcome powerfully indicates that *M. parviflora* is abundant in these particular classes of compounds, which are often linked to strong antioxidant and enzyme-inhibitory properties.

The substantial yield of the methanol fraction confirms the successful extraction of the remaining polar constituents from the initial crude extract (Ponphaiboon et al., 2023). The yields from the hexane and chloroform fractions, which target non-polar and moderately non-polar compounds, respectively, align with expectations for the extraction of substances like lipids, waxes, and chlorophyll. This structured separation successfully creates a series of fractions with increasingly polar character.

The sequential extraction and fractionation process has effectively mapped the solubility profile of *M. parviflora*'s constituents. The high recovery of material in the intermediate-polarity fractions strongly suggests they are the most promising repositories of therapeutically relevant compounds. This finding provides a compelling rationale for focusing subsequent phytochemical analysis and pharmacological assays—such as antioxidant, anti-inflammatory, or metabolic enzyme inhibition studies—primarily on these fractions to identify the active principles responsible for the plant's traditional medicinal uses.

The total phenolic content observed in the extract indicates a considerable presence of polyphenolic compounds. Phenolics compounds are known for their potent redox properties, which enable them to act as reducing agents, hydrogen donors, and metal chelators. These compounds contribute significantly to the antioxidant defense mechanisms of plant extracts, helping to neutralize free radicals and mitigate oxidative stress—a key factor in the progression of chronic and degenerative diseases (Guneidy, Zaki, Gad, Saleh, & Shokeer, 2022). The abundance of these compounds in *M. parviflora* suggests a strong capacity for radical scavenging, which may underlie some of its traditional uses in alleviating inflammation and supporting metabolic health.

Similarly, the flavonoid content detected underscores the plant's potential as a source of bioactive molecules. Flavonoids, with their diverse subclasses such as flavones, flavonols, and flavanones, can modulate enzyme activity and signal transduction pathways, further enhancing their therapeutic value (Belew, Hanan, Meshesha, & Akele, 2025). The significant flavonoid levels detected in *M. parviflora* align with its purported medicinal applications and highlight its suitability for further exploration in nutraceutical and pharmaceutical research.

The concurrent presence of substantial phenolic and flavonoid compounds suggests the possibility of synergistic interactions, which may enhance the overall bioactivity of the extract. It is well

documented that combinations of polyphenols can produce additive or synergistic effects, improve antioxidant efficacy, and expand the scope of biological actions compared to individual compounds. These results not only validate the traditional use of *M. parviflora* but also provide a foundation for its future application in evidence-based natural product development.

Antioxidant activity is a critical indicator of the potential health benefits of plant extracts, as oxidative stress is implicated in the pathogenesis of numerous chronic diseases (Selby-Pham, Wise, & Selby-Pham, 2025). The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely used method to evaluate the free radical scavenging capacity of plant extracts. It measures the ability of antioxidants to donate hydrogen atoms or electrons to stabilize the DPPH radical, resulting in a color change from purple to yellow (Baliyan et al., 2022). Lower IC_{50} values in this assay indicate higher antioxidant potency, as less extract is required to neutralize the radicals (Oraibi et al., 2025). The results revealed significant differences in DPPH radical scavenging activity among the fractions. The EFMP fraction demonstrated the most potent activity, suggesting a high concentration of hydrogen-donating compounds, such as phenolics and flavonoids, which effectively neutralize free radicals. The MFMP fraction also exhibited strong activity, though to a lesser extent than EFMP, indicating a substantial presence of antioxidants. The CFMP and HFMP fractions showed moderate to lower activities, aligning with their expected lower polarity and reduced abundance of polar antioxidant compounds. These findings highlight the EFMP fraction as the most promising for further investigation into radical scavenging mechanisms.

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay measures the ability of antioxidants to scavenge the $ABTS^{+}$ radical cation, which is generated through oxidation. This assay is effective for assessing antioxidant activity in both hydrophilic and lipophilic systems, providing a broader understanding of radical neutralization capacity (Cano, Maestre, Hernández-Ruiz, & Arnao, 2023).

Similar to the DPPH results, the EFMP fraction displayed the highest ABTS radical scavenging activity, indicating its superior ability to quench radicals across diverse environments. The MFMP and CFMP fractions showed intermediate activity, suggesting a balanced profile of antioxidants capable of acting in both aqueous and lipid phases. The HFMP fraction, with the lowest activity, likely contains fewer compounds effective against the $ABTS^{+}$ radical. The consistency between DPPH and ABTS results reinforces the robustness of EFMP's antioxidant properties and its potential as a source of broad-spectrum radical scavengers.

The FRAP (Ferric Reducing Antioxidant Power) assay evaluates the reducing capacity of antioxidants by measuring their ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). This electron-donating ability is a key mechanism of antioxidant action, particularly in mitigating metalinduced oxidative stress (Gulcin & Alwasel, 2025).

The EFMP fraction again exhibited the strongest reducing power, underscoring its high electrondonating capacity, likely due to its rich content of reducing agents like polyphenols and flavonoids. The MFMP and CFMP fractions showed moderate reducing power, while the HFMP fraction demonstrated the weakest activity. The pronounced reducing ability of EFMP aligns with its performance in radical scavenging assays, suggesting a complementary role in combating oxidative stress through both radical neutralization and metal ion reduction.

The collective results from the DPPH, ABTS, and FRAP assays provide a comprehensive profile of the antioxidant potential of *M. parviflora* fractions. The EFMP fraction consistently emerged as the most active across all assays, highlighting its enrichment with potent antioxidants capable of acting through multiple mechanisms.

α-Amylase is a key enzyme in carbohydrate digestion, catalyzing the hydrolysis of starch into smaller oligosaccharides and disaccharides in the small intestine. Inhibiting α-amylase slows the breakdown of complex carbohydrates, reducing postprandial blood glucose levels. This mechanism is clinically relevant for managing hyperglycemia and type 2 diabetes(Ćorković, Gašo-Sokač, Pichler, Šimunović, & Kopjar, 2022). Among the fractions, MFMP demonstrated the most potent αamylase inhibitory activity, suggesting a high affinity for the enzyme's active site. HFMP also exhibited notable inhibition, while EFMP and CFMP showed moderate effects. The superior performance of MFMP may be attributed to its enrichment with polar compounds, such as polyphenols or flavonoids, known to interact with digestive enzymes. Although none of the fractions matched the efficacy of the standard drug acarbose, the significant inhibition by MFMP highlights its potential as a natural adjunct for glycemic control.

α-Glucosidase, located on the brush border of the small intestine, further breaks down disaccharides into absorbable monosaccharides. Inhibiting this enzyme delays glucose absorption, flattening postprandial blood glucose spikes(Ćorković et al., 2022). The EFMP fraction emerged as the most effective α-glucosidase inhibitor, displaying remarkable activity. MFMP also showed strong inhibition, while CFMP and HFMP were less potent. The exceptional performance of EFMP suggests a high concentration of bioactive compounds, possibly flavonoid glycosides or alkaloids,

that specifically target α-glucosidase. This fraction's efficacy underscores its potential for developing natural therapies to complement existing antidiabetic drugs like acarbose.

Pancreatic lipase is the primary enzyme responsible for hydrolyzing dietary triglycerides into free fatty acids and monoglycerides. Inhibiting lipase reduces fat absorption, making it a strategic target for managing obesity and hyperlipidemia(Rajan, Palaniswamy, & Mohankumar, 2020). EFMP again demonstrated the strongest inhibitory effect against pancreatic lipase, indicating its broad-spectrum bioactivity. HFMP and CFMP showed moderate inhibition, while MFMP was the least effective. The potency of EFMP may stem from its content of terpenoids, saponins, or other nonpolar compounds that interfere with lipase function. Although orlistat, the standard inhibitor, was far more potent, the significant lipase inhibition by EFMP suggests its utility in weight management strategies.

HMG-CoA reductase is the rate-limiting enzyme in the mevalonate pathway, responsible for cholesterol biosynthesis. Inhibiting this enzyme reduces endogenous cholesterol production, a cornerstone of hyperlipidemia management(Friesen & Rodwell, 2004). EFMP exhibited the highest inhibition of HMG-CoA reductase, followed by MFMP. HFMP and CFMP showed weaker effects. The activity of EFMP may be linked to its content of steroidal compounds, polyphenols, or flavonoids that structurally mimic HMG-CoA, competing with the substrate. While atorvastatin, a synthetic statin, remained vastly more potent, the notable inhibition by EFMP highlights its potential as a natural alternative for cholesterol-lowering interventions. While this study provides valuable insights into the antioxidant and enzyme-inhibitory potential of *M. parviflora* fractions, certain limitations should be acknowledged. The findings from in-silico docking and in-vitro assays, though promising, lack validation through in-vivo studies. Such studies are critical to confirm the bioavailability, pharmacokinetics, and physiological efficacy of the identified bioactive compounds. Additionally, the research focused on previously documented compounds, and the isolation or characterization of novel phytoconstituents was beyond its scope. The study also restricted its focus to a limited number of enzymatic targets (e.g., HMG-CoA reductase, lipase, α-amylase, and αglucosidase), leaving other potential mechanisms in lipid metabolism and oxidative stress pathways unexplored. To build upon these findings, future work should prioritize *in-vivo* validation of the observed *in-vitro* and *in-silico* results. Well-designed animal models or clinical trials would help elucidate the real-world therapeutic efficacy, optimal dosing, and safety profile of the most active fractions, such as EFMP. Moreover, advanced phytochemical studies—including bioassay-guided fractionation, NMR spectroscopy, and mass spectrometry—should be

employed to isolate and characterize novel compounds unique to *M. parviflora*. Expanding the scope of molecular targets to include other key regulators of lipid metabolism, inflammation, and oxidative stress could provide a more holistic understanding of the plant's multipotential benefits. Finally, long-term clinical studies would be essential to translate these preclinical results into safe and effective natural-based therapies for human use.

Conclusion

This study comprehensively demonstrates the significant phytochemical and pharmacological potential of *M. parviflora*. The extraction and fractionation processes revealed distinct polaritybased compound distributions, with ethyl acetate and methanol fractions exhibiting the highest yields and bioactivity. Phytochemical screening confirmed the presence of diverse bioactive compounds, including phenols, flavonoids, alkaloids, and glycosides, which correlated with strong antioxidant activities. Notably, the EFMP showed the strongest free radical scavenging and reducing power, correlating with its high phenolic and flavonoid content, indicating potent antihyperlipidemic and antidiabetic properties. These findings scientifically validate the traditional uses of this plant and highlight its potential as a source of natural therapeutic agents for managing metabolic disorders.

Conflict of Interest

There is no conflict of interest

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Authors Contribution

MMUK: Performed and written the manuscript

Data Availability Statement

All data mentioned in the manuscript

Ethics Approval

Not applicable

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