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Research Paper

Phytochemical Profiling, *In vitro* α-Amylase Inhibition, Antioxidant Potential, and GC–MS Analysis of *Millingtonia hortensis* L.f. flower

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ABSTRACT

Diabetes mellitus has emerged as a modern era epidemic affecting millions of people worldwide. Oxidative stress is one of the major culprits behind occurrence of diabetes. Millingtonia hortensis L.f. (Bignoniaceae); commonly known as Indian cork tree, is an ethnomedicinal plant used to treat various diseases such as asthma, fever, sinusitis, indigestion, cough, vomiting and diarrhoea as well as diabetes. The present study aimed to investigate the phytochemical composition and in vitro α-amylase inhibitory and antioxidant activities of methanolic extract of M. hortensis flower. Preliminary phytochemical analysis confirmed the presence of flavonoid, phenol, phytosterol, tannins, phlobatannin, cardiac glycoside, terpenoid, alkaloid and protein. Quantitative estimation revealed Total Phenolic Content (TPC) of 81.14 ± 5.15 mg GAE/g and Total Flavonoid Content (TFC) of 29.27 ± 2.53 mg QE/g. The α-amylase inhibitory activity was assessed through in vitro 3,5-dinitrosalicylic acid (DNSA) and starch-iodine assays and found to be concentration dependent in both the assays. The IC_{50} values of 28.87 ± 0.09 mg/mL (acarbose 8.38 ± 0.03 mg/mL) and 128.83 ± 3.29 mg/mL (acarbose 8.09 ± 0.01 mg/mL) were found for DNSA and Starch-iodine assays, respectively. Antioxidant activity was evaluated using in vitro DPPH radical scavenging assay with an IC_{50} value of 15.95 ± 2.04 mg/mL as compared to ascorbic acid having an IC_{50} value of 11.57 ± 7.61 mg/mL. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of methanol extract revealed 56 peaks corresponding to 48 phytocompounds. The abundant compounds were 2-D,2-Pentadecyl-1,3-dioxepane (30.82%), 1-Methyl-3-vinylcyclopentane, monodeuterated (21.68%), Cyclobutanecarboxylic acid, dodecyl ester (6.92%), Ethanimidic acid, ethyl ester (3.53 %), Heneicosane (3.28%), 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (2.72%) and 5-Hydroxy-9-oxabicyclo [3.3.1] nonan-2-one (2.50 %). These results warrant further in vivo and clinical studies to validate the anti-diabetic efficacy of M. hortensis flower and corresponding mechanism of action.

KEYWORDS: *Akas nim,* Diabetes, Bignoniaceae, DPPH, β-sitosterol, Indian cork tree

INTRODUCTION

Diabetes mellitus (DM), a metabolic disease associated with chronic hyperglycaemia, is one of the most severe global health concerns and its mortality rate and prevalence have been rising[1]. The quality of life is considerably affected by diabetes in terms of physical health, along with social and psychological well-being[2]. Type 1 Diabetes is insulin dependent due to the non-functionality of β-cells which is utterly reliant on exogenous insulin whereas Type 2 Diabetes (DM2) is insulin-independent, which can be managed with medicine, exercise, and dietary modifications[3].

The majority of people consume a significant amount of starch. In humans, the pancreas and salivary glands release α-amylase enzyme to digest the ingested starch[4]. α-Amylase is a calcium-dependent metalloenzyme that breaks down larger polysaccharide molecules into smaller ones, such as maltose which results in postprandial hyperglycemia and Type 2 Diabetes[5]. Acarbose, voglibose and miglitol are synthetic enzymatic inhibitors which act against α-amylase[6-7]. However, these drugs have been associated with some undesirable gastrointestinal side effects, including abdominal distention, cramps, flatulence, and diarrhoea[8-9]. Therefore, one possible strategy to inhibit the absorption of carbohydrates from food is to employ natural resources as carbohydrate digestive enzyme inhibitors, which have fewer adverse effects and are cost-effective than synthetic medications[10-11].

In this context, ethnomedicinal plants are being researched to validate their therapeutic potential scientifically and to identify their phytocompounds using advanced techniques such as GC-MS. Among these, several bioactive compounds act as α-amylase inhibitors by blocking the active site of the enzyme and reducing the elevated blood glucose level after meals. Preliminary phytochemical screening and GC-MS provide a detailed phyto-constituent profile of the plant extract, which provides the foundation for further *in silico* studies for designing rational drugs and development of precision medicines[12].

Millingtonia hortensis L.f., [Figure 1] belonging to family Bignoniaceae, is a tall, deciduous, perennial tree. It can grow up to 24 meters in height. It has rich green foliage and a straight trunk with few branches. Corymbose or terminal panicles are long, tubular having pinkish or silvery-white flowers with a pleasant aroma. The flowers open at night and close before daybreak. It is commonly known as *Mini Chambeli, Neem Chameli, Akas Nim*, Indian cork tree, and Tree Jasmine[13].

The plant is reputed for its medicinal values in Southern Asia including Cambodia, Myanmar, Laos, Burma, Vietnam, Thailand, Southern China and usually cultivated in India, Malaysia and Indonesia[14-15]. Various parts of *M. hortensis* are utilized to treat different ailments by ethnic communities. According to Sastri[13], it is used as an antipyretic in Indonesia and dried bloom is considered good for the lung associated problems. In Myanmar, leaves are used to treat menstrual troubles and high blood pressure. Either eating shoots or a soup made with flowers can help cure hypertension and heart palpitations. Dizziness as well as heart palpitations are also treated with a root paste mixed with sugar or salt[15]. Indian ethnic communities utilise the plant to treat a variety of ailments, including liver illnesses, sinusitis, asthma, indigestion, vomiting, fever, diarrhoea, cough, and stomach discomfort. Its flowers are used for treatment of diabetes by local communities of Arunachal Himalaya in North-East India[16].

Several pharmacological properties, including antioxidant[17], antimicrobial[18], anti-inflammatory[19], anthelmintic[20], hepatoprotective[21], antimutagenic[22], cytotoxic[18], larvicidal[23], and anti-aging[24], have been reported from different parts of *M. hortensis* along with several phytocompounds.

An extensive literature survey on *M. hortensis* revealed that it has various pharmacological activities due to the presence of different bioactive compounds. However, scientific validation of its flowers for anti-diabetic potential has not yet been carried out. Thus, the present investigation was carried out to know *in vitro* α-amylase inhibition and antioxidant activity along with GC-MS analysis of *M. hortensis* flower.

MATERIALS AND METHODS

Plant Collection and Authentication

After proper identification of *M. hortensis* plant, the flowers were collected from Gulab Bagh, Brahmpuri, Udaipur, Rajasthan. The voucher specimen (Field no. 07) was prepared and the plant material was authenticated at Arid Zone Regional Centre of BSI at Jodhpur (No. BSI/AZRC/I.12012/2023-24/Tech. (Pl.Id.)/317 dated 03.08.2023).

Figure 1: *Millingtonia hortensis* L.f.

Preparation of Plant Extract

The flowers were shade dried, finely powdered and stored for further use. For 72 hours, 10 g of the dried flower powder was extracted using 100 mL of methanol in a shaker at 30 °C and 90 rpm. After filtration, the filtrate was left to evaporate in a pre-weighed glass petri dish without any disturbance at room temperature until a consistent weight was achieved. Petri dish was weighed once again after complete dryness. After proper labelling, the extract; thereafter called as *M. hortensis* flower's methanolic extract (MFM) was stored in glass vials at 4°C for further use, and the yield percentage was computed[25] as:

Extraction percentage yield =

$$\frac{\text{Weight of crude extract}}{\text{Weight of powdered sample}} \text{ X } 100$$

Preliminary Phytochemical Screening

The qualitative preliminary screening of methanolic flower extract for presence or absence of various phytoconstituents such as protein, carbohydrate, flavonoid, alkaloid, terpenoid, phytosterol, tannin, phlobatannin, phenol, cardiac glycoside, and saponin was performed using the standard methodology with minor modifications[26].

Assessment of Total Phenolic Content (TPC)

Folin-Ciocalteu method was used to determine the Total Phenolic Content in which 1 mL of flower extract was diluted with nine mL of distilled water, followed by addition of one mL of Folin-Ciocalteu reagent with thorough mixing. After five minutes, ten mL of 7% Sodium Carbonate was mixed and diluted to 25 mL. A set of reference solutions (positive control) of gallic acid (GA) in concentrations of 20, 40, 60, 80, 100, 150, 200, 300 and 400 µg/mL were prepared similarly as explained earlier. The standard and sample were incubated at room temperature for 90 minutes, and absorbance was measured at 550 nm using a UV/Visible spectrophotometer against the blank. TPC was demonstrated as milligrams of gallic acid equivalents (GAE) per gram of extract[27].

Assessment of Total Flavonoid Content (TFC)

Aluminium chloride colorimetry method was used to estimate Total Flavonoid Content. The reaction mixture was prepared by adding one mL of test sample to four mL of distilled water, followed by 0.3 mL of 5% Sodium Nitrite. After five minutes of incubation, 0.3 mL aluminium chloride (10%) was added and kept for five minutes. Thereafter, two mL of Sodium Hydroxide (1M) was added, and final volume was adjusted to ten mL using distilled water. A set of reference solutions (positive control) containing 20, 40, 60, 80, 100, 150, and 200 µg/mL concentration of quercetin was prepared. The absorbance of the standard and sample was recorded against the blank at 510 nm and TFC was quantified as milligrams of quercetin equivalent (QE) per gram of extract[27].

Assessment of *in vitro* α-Amylase Inhibitory Activity

3,5-Dinitrosalicylic Acid (DNSA) Assay

The chromogenic DNSA technique was used for the inhibition assay following the method of Miller[28] with slight modifications. The assay combination comprised 500 µL of 0.02 M sodium phosphate buffer (pH 6.9, containing 6 mM sodium chloride), 1 ml amylase extract, and 400 µL of methanolic flower extract with concentrations between 2 and 10 mg/mL, incubated for 10 minutes at 37°C. After pre-incubation, each tube was supplemented with 580 µL of a 1% (w/v) starch solution, which was then incubated for 15 minutes at 37°C. After adding 1 mL of DNSA reagent to terminate the reaction, it was permitted to cool at room temperature for 5 minutes before measuring absorbance at 540 nm. The control exhibited 100% enzyme activity, devoid of plant sample. A sample of the reaction mixture devoid of the enzyme was incorporated as a suitable negative control to negate the absorbance attributed to the plant extract. The α-amylase inhibition % was calculated as follows:

Percentage inhibition of *α*-amylase = $(Ab_{control} - Ab_{sample}/Ab_{control})$ x100

Starch-Iodine Assay

Methanolic flower extract was also assessed for *in vitro* α-amylase inhibitory potential utilising the methodology of Xiao *et al.*[29], with some modifications. The complete assay mixture, comprising 120 µL of 0.02M sodium phosphate buffer (pH 6.9 with 6 mM NaCl), 1.5 mL amylase extract, and varying concentrations of methanol extract (2-10 mg/mL), was incubated for 10 minutes at 37°C. Subsequently, each reaction mixture was combined with 1% w/v soluble starch and incubated for 15 minutes at 37°C. Following the termination of the enzymatic reaction with 60 µL of 1 M hydrochloric acid, 300 µL of iodine reagent (5 mM I_2 and 5 mM KI) was introduced and absorbance was measured at 620 nm. Complete enzyme activity was shown by reaction tubes lacking any plant sample. Extract controls devoid of the enzyme were also examined to eliminate the absorbance produced by the plant extract. A dark blue colour of reaction mixture signifies the presence of starch, yellow colour denotes complete starch degradation, and a brownish tint shows partial hydrolysis of starch. In the presence of the extracts, the starch in the enzyme assay mixture remained intact, resulting in a dark-blue colour complex; in the absence of the inhibitor, no colour complex developed, indicating that α-amylase had completely hydrolysed the starch.

Percent relative enzyme activity = 100- $(Ab_{control} - Ab_{sample}/Ab_{control})$ x 100

Assessment of *in vitro* Antioxidant Activity

1,1-Diphenyl 2-picryl-hydrazyl (DPPH) Assay

The 1,1-diphenyl 2-picryl-hydrazyl (DPPH) approach was used to determine the potential of *M. hortensis* flower extract to scavenge DPPH radicals. A 100 µl plant sample was mixed with 3.9 ml of a 0.1 mM methanolic DPPH solution. After then, the mixture was kept on vortex mixer and incubated for 30 minutes in the dark. Methanol served as a blank, and at 515 nm, absorbance was determined. The percent radical scavenging activity was computed as follows:

$(Ab_{control} - Ab_{sample}/Ab_{control}) \times 100$

Where $Ab_{control}$ is the absorbance of the DPPH solution and Ab_{sample} is the absorbance of sample.

The IC_{50} values were calculated using a linear plot of concentration against percentage inhibition.

Gas Chromatography-Mass Spectrometry (GC-MS) Study

The GC-MS profiling of MFM was conducted at the Advanced Instrumentation Research Facility of Jawaharlal Nehru University, New Delhi, using a GC-MS-QP-2010 Plus Ultra (Shimadzu company). An Rxi-5 SIL MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness) made of 5% diphenyl and 95% dimethyl polysiloxane was used to achieve separation. At an energy of 70 eV, the instrument was run in electron impact ionisation mode. With a split ratio of 10:1 and a sample injection volume of 1 μL, helium was used as the carrier gas at a steady flow rate of 1.21 mL/min. After starting the oven temperature program at 60 °C and keeping it there for two minutes, the temperature was gradually raised to 300 °C at a rate of 10 °C per minute, where it remained for 19 minutes. An interface temperature of 270 °C, an ion source temperature of 220 °C, a solvent delay of 2.50 minutes, a scan speed of 3333, and a mass detection range of m/z 40–600 with a threshold value of 1000 were among the analytical parameters. Compound identification was achieved by comparing retention time and mass spectrum fragmentation patterns with entries in the NIST and Wiley spectral libraries using data obtained in total ion chromatogram (TIC) mode. The relative concentration of each chemical was determined by calculating the percentage of the peak area to the total peak area. The chemical structures of phytocompounds were taken from PubChem database.

Statistical Analysis

All the assays were conducted in triplicate. The data were expressed as Mean ± Standard Deviation. Microsoft Office Excel 2019 was utilized to calculate the data and plot the graphs.

RESULTS

Extraction Yield

The percentage yield of *M. hortensis* flowers after extraction was found to be 29.52%. The extract showed a light brown colour.

Preliminary Phytochemical Qualitative Analysis

Preliminary phytochemical screening of MFM determined the presence of many phytoconstituents, including alkaloid, tannin, protein, phytosterol, cardiac glycoside, phlobatannin, flavonoid, phenol and terpenoid. However, carbohydrate and saponin were not detected in the plant sample [Table 1].

Total Phenolic Content (TPC)

Total Phenolic Content was ascertained using the Folin–Ciocalteu method. The result was derived from a calibration curve ($y = 0.0007x + 0.1072$, $R^2 = 0.9893$) of the standard compound gallic acid containing the concentration of 50 to 450 μg/mL. The TPC in MFM is quantified as 81.14 ± 5.15 mg GAE/g [Table 2].

Table 1: Preliminary phytochemical screening of methanolic extract of *M. hortensis* flower

Phytochemical	Result
Carbohydrate	-
Protein	+
Tannin	+
Alkaloid	+
Saponin	-
Cardiac glycoside	+
Phytosterol	+
Phlobatannin	+
Terpenoid	+
Phenol	+
Flavonoid	+

+ = Present **-** = Absent

Total Flavonoid Content (TFC)

Total Flavonoid Content is expressed as milligrams of quercetin equivalent (QE) per gram of extract. A calibration curve (y = 0.0016x + 0.0955, R^2 = 0.9982) of the standard compound quercetin (50–250 µg/mL) was used to determine the flavonoid content. The concentration of flavonoids in the methanol extract was found to be 29.27 ± 2.53 mg QE/g [Table 2].

Table 2: Quantitative determination of total flavonoid and total phenolic contents in the methanolic extract of *M. hortensis* flower

Total Flavonoid Content	29.27 ± 2.53 mg of QE/g
Total Phenolic Content	81.14 ± 5.15 mg of GAE/g

Results for TFC and TPC are given as mean ± standard deviation in terms of quercetin equivalents (mg of QE/g) and gallic acid equivalents (mg of GAE/g) respectively.

***In vitro* α-amylase Inhibitory Activity**

3,5-Dinitrosalicylic Acid (DNSA) Assay

In vitro α-amylase inhibition by methanol extract of *M. hortensis* flowers (MFM) was observed to be concentration dependant. The concentration of extract and acarbose varied from 2 to 10 mg/mL. IC_{50} refers to the concentration of plant sample, which retain 50% inhibition of α-amylase. An IC_{50} value of 28.87 ± 0.09 mg/mL was recorded for MFM and 8.38 ± 0.03 mg/mL for acarbose [Table 3].

Table 3: α-Amylase inhibitory activity and IC_{50} values of methanol extract of *M. hortensis* flowers (MFM) and standard acarbose for Glucose-DNSA

S. No.	Extract/ Standard	Percentage α-amylase inhibitory activity at different concentrations (mg/mL)					IC_{50} (mg/mL)
		2	4	6	8	10	
1.	MFM	0.68 ± 0.06	1.34 ± 0.24	2.24 ± 0.03	4.67 ± 0.04	18.48 ± 0.07	28.87 ± 0.09
2.	Acarbose	28.16 ± 0.04	37.22 ± 0.13	44.87 ± 0.09	49.51 ± 0.05	53.18 ± 0.11	8.38 ± 0.03

Starch-Iodine assay

The MFM was also assessed for its *in vitro* α-amylase inhibitory potential using starch-iodine assay, as shown in Fig. 2. An IC_{50} value of 128.83±3.29 mg/mL was recorded for methanolic flower extract. However, acarbose had an IC_{50} value of 8.09 ± 0.01 mg/mL [Table 4].

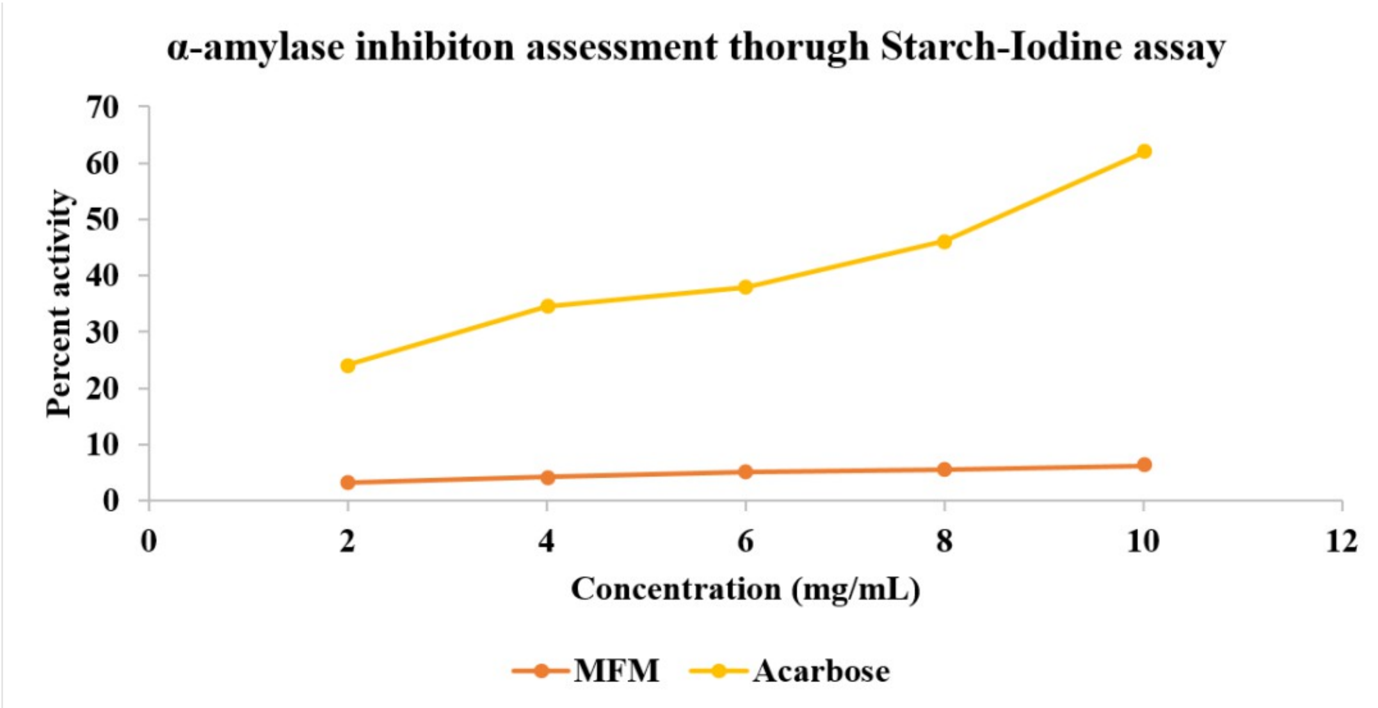


Figure 2: Inhibitory Activity of Methanolic Flower Extract of *M. hortensis* (MFM) Against α-amylase in Comparison with Acarbose in Starch-iodine Assay

Table 4: IC_{50} values for methanolic extract of *M. hortensis* flowers (MFM) and standard acarbose for DNSA and Starch-Iodine assays

S. No.	Extract/ Standard	IC_{50} value (mg/mL)	
		DNSA assay	Starch-Iodine assay
1.	MFM	28.87 ± 0.09	128.83 ± 3.29
2.	Acarbose	8.38 ± 0.03	8.09 ± 0.01

Assessment of *in vitro* antioxidant activity

1,1-Diphenyl 2-picryl-hydrazyl (DPPH) assay

In vitro DPPH radical scavenging activity of MFM was observed in a concentration dependent manner in DPPH assay (Fig.3). An IC_{50} value was found to be 15.95 ± 2.04 mg/mL for methanol extract. However, the standard ascorbic acid had an IC_{50} value of 11.57 ± 7.61 mg/mL.

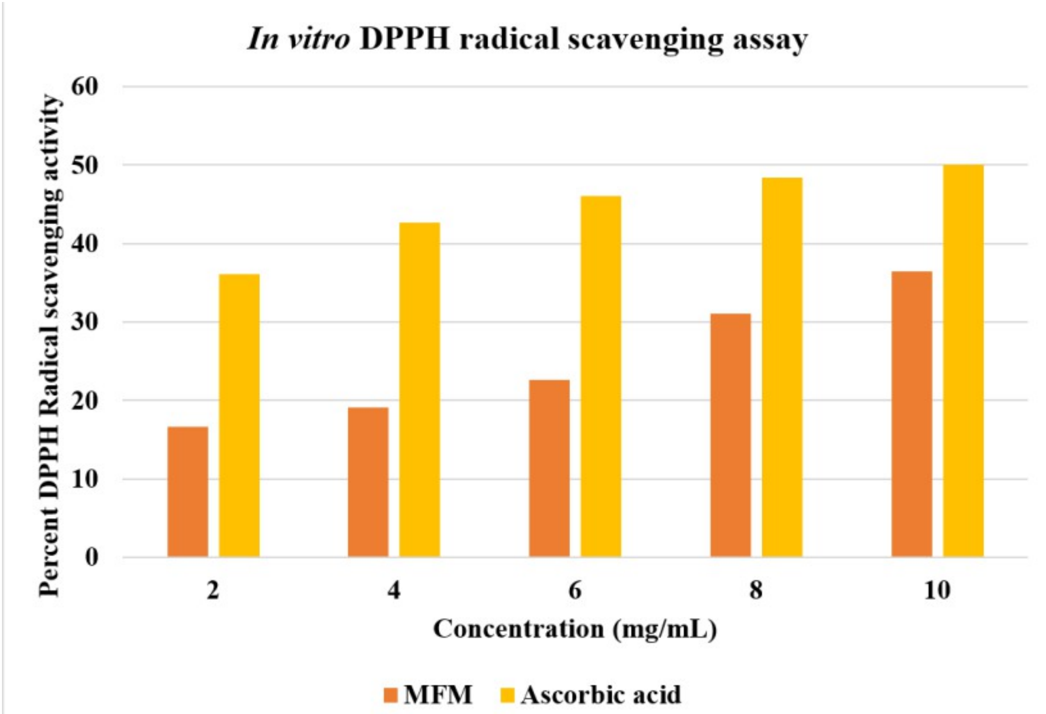


Figure 3: Percent DPPH Radical Scavenging Activity by Methanolic Extract of *M. hortensis* Flower (MFM) and Standard Ascorbic Acid at Different Concentrations

GC-MS investigation

Methanolic extract of flowers of *M. hortensis* was investigated through GC-MS and fifty six peaks were examined in chromatogram (Fig. 4) corresponding to forty eight compounds as 2-trans-6-trans-Farnesyl stearate, Tetratetracontane, n-Tetracosanol-1, Heneicosane and 5-Isopropyl-6-methyl-3-heptyne-2,5-diol were obtained more than one time (Table 5). The most abundant compounds were 2-D,2-Pentadecyl-1,3-dioxepane (30.82%), 1-Methyl-3-vinylcyclopentane, monodeuterated (21.68%), Cyclobutanecarboxylic acid, dodecyl ester (6.92%), Ethanimidic acid, ethyl ester (3.53 %), Heneicosane (3.28%), 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (2.72%), and 5-Hydroxy-9-oxabicyclo [3.3.1] nonan-2-one (2.50 %).

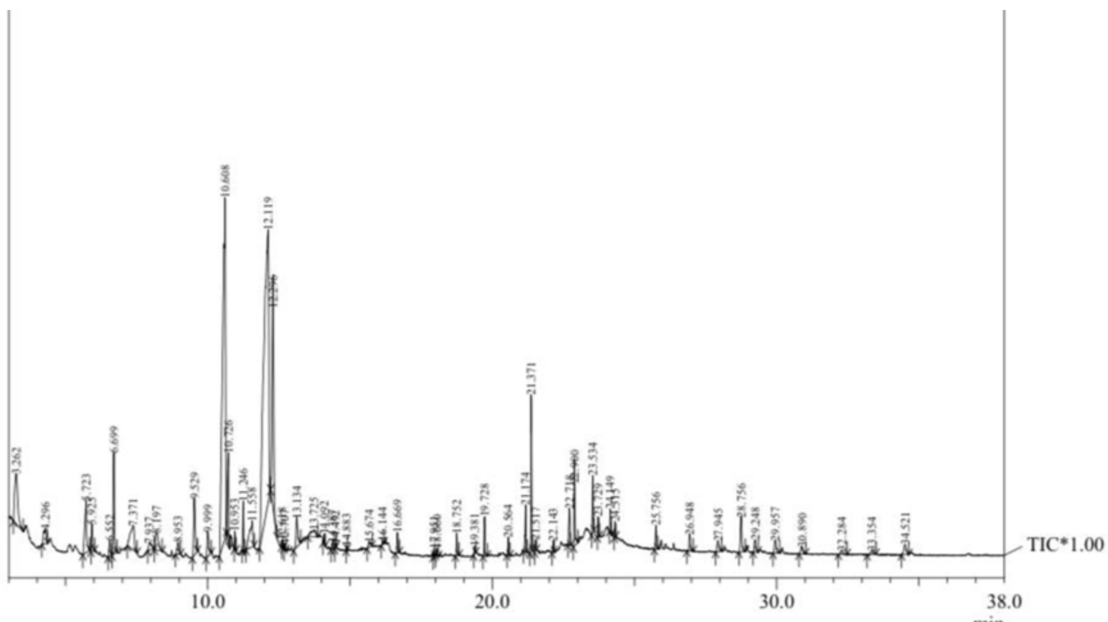
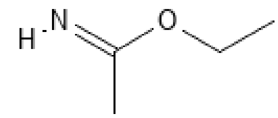
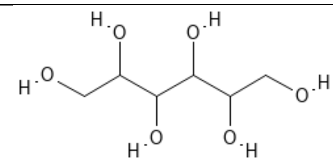
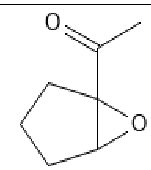
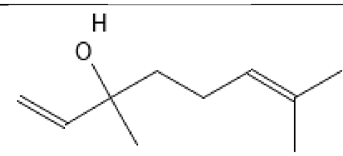
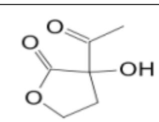
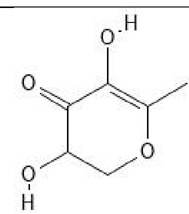
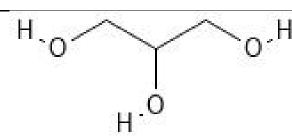
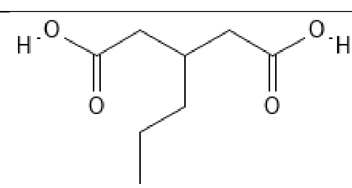
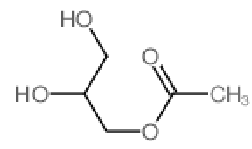
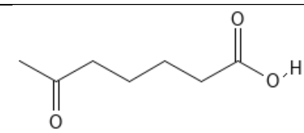
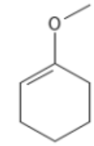
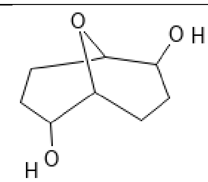
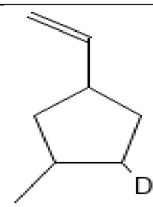
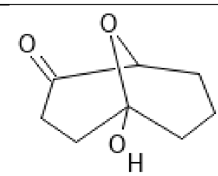
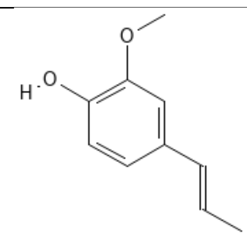
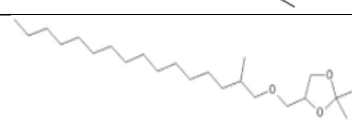
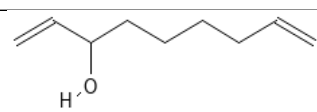
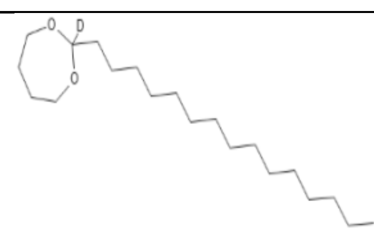
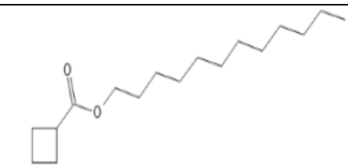
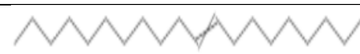
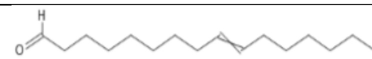
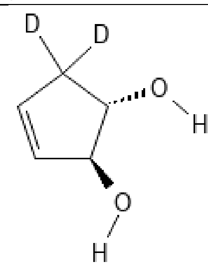
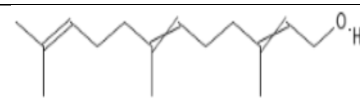
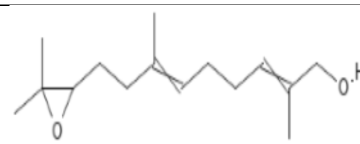
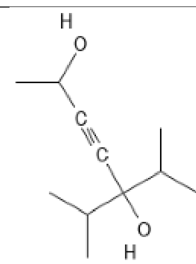
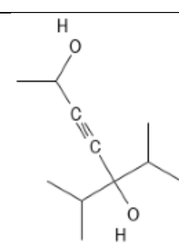
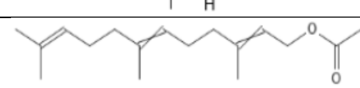


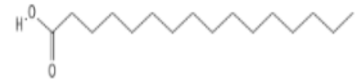
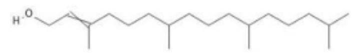
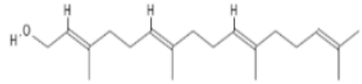
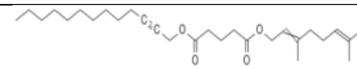
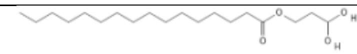
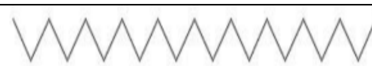
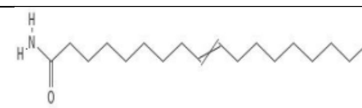
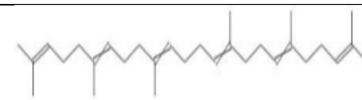
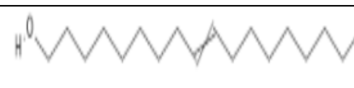
Figure 4: GC-MS Chromatogram of Methanolic Extract of *M. hortensis* Flower

Table 5: Peak Report of GC-MS analysis of methanolic flower extract of *M. hortensis* showing Retention Time (RT), % area, name, molecular formula, structure and molecular weight of analysed phytocompounds

Peak	RT	Area%	Name	Molecular Formula	Structure	Mol. Weight (g/mol)
1.	3.262	3.53	Ethanimidic acid, ethyl ester	C_4H_9NO		87
2.	4.296	0.86	D-mannitol	$C_6H_{14}O_6$		182
3.	5.723	2.22	1-acetyl-1,2-epoxy-cyclopentane	$C_7H_{10}O_2$		126
4.	5.925	0.58	3,7-Dimethyl-1,6-octadien-3-ol (Linalool)	$C_{10}H_{18}O$		154
5.	6.552	0.45	2-acetyl-2-hydroxy-. gamma. -butyrolactone	$C_6H_8O_4$		144
6.	6.699	2.72	3,5- Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	$C_6H_8O_4$		144
7.	7.371	2.18	1,2,3-propanetriol (Glycerol)	$C_3H_8O_3$		92
8.	7.937	0.46	3-Propylglutaric acid (3-Propylpentanedioic acid)	$C_8H_{14}O_4$		174

9.	8.197	1.17	1,2,3-Propanetriol, 1-acetate (1-Monoacetin)	$C_5H_{10}O_4$		134
10.	8.953	0.50	6-Oxoheptanoic acid / 5-Acetylvaleric Acid	$C_7H_{12}O_3$		144
11.	9.529	1.99	1-methoxy-cyclohexene	$C_7H_{12}O$		112
12.	9.999	0.92	9- Oxabicyclo[3.3.1] nonane-2,6-diol	$C_8H_{14}O_3$		158
13.	10.608	21.68	1-methyl-3-vinylcyclopentane, monodeuterated	$C_8H_{13}D$		111
14.	10.726	2.50	5-hydroxy-9-oxabicyclo [3.3.1] nonan-2-one	$C_8H_{12}O_3$		156
15.	10.953	0.30	Isoeugenol	$C_{10}H_{12}O_2$		164
16.	11.246	0.78	2,2-Dimethyl-4-([(2-methylhexadecyl)oxy]methyl)-1,3-dioxolane	$C_{23}H_{46}O_3$		370
17.	11.558	1.95	1,8-Nonadien-3-ol	$C_9H_{16}O$		140
18.	12.119	30.82	2-D,2-Pentadecyl-1,3-dioxepane	$C_{20}H_{39}DO_2$		313

19.	12.296	6.92	Cyclobutanecarboxylic acid, dodecyl ester	$C_{17}H_{32}O_2$		268
20.	12.618	0.10	9-eicosene/ 9-Eicosene, (E)-	$C_{20}H_{40}$		280
21.	12.707	0.08	(Z)-7-Hexadecenal	$C_{16}H_{30}O$		238
22.	13.134	1.22	N-[2-(diethylamino) ethyl]-2-methylacrylamide	$C_{10}H_{20}N_2O$		184
23.	13.725	0.69	5,5-d2-trans-3,4-dihydroxy-cyclopentene	$C_5H_6D_2O_2$		102
24.	14.092	0.13	Farnesyl alcohol	$C_{15}H_{26}O$		222
25.	14.387	0.15	9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol	$C_{15}H_{26}O_2$		238
26.	14.492	0.09	5-Isopropyl-6-methyl-3-heptyne-2,5-diol	$C_{11}H_{20}O_2$		184
27.	14.883	0.05	1-Heptadecene	$C_{17}H_{34}$		238
28.	15.674	0.26	5-Isopropyl-6-methyl-3-heptyne-2,5-diol	$C_{11}H_{20}O_2$		184
29.	16.144	0.05	Farnesyl acetate 3	$C_{17}H_{28}O_2$		264

30.	16.669	0.39	Hexadecanoic acid / Palmitic acid/ Cetylic acid	$C_{16}H_{32}O_2$		256
31.	17.951	0.12	7-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$		268
32.	18.060	0.12	Phytol	$C_{20}H_{40}O$		296
33.	18.752	0.37	trans-Geranylgeraniol	$C_{20}H_{34}O$		290
34.	19.381	0.15	Glutaric acid, tridec-2-yn-1-yl geranyl ester	$C_{28}H_{46}O_4$		446
35.	19.728	0.62	Heneicosane	$C_{21}H_{44}$		296
36.	20.564	0.25	Heneicosane	$C_{21}H_{44}$		296
37.	21.174	0.86	n-Tetracosanol-1	$C_{24}H_{50}O$		354
38.	21.371	2.24	Heneicosane	$C_{21}H_{44}$		296
39.	21.517	0.19	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$		330
40.	22.143	0.17	Heneicosane	$C_{21}H_{44}$		296
41.	22.718	0.83	n-Tetracosanol-1	$C_{24}H_{50}O$		354
42.	22.900	1.49	Tetratetracontane	$C_{44}H_{90}$		618
43.	23.534	0.95	9-Octadecenamide	$C_{18}H_{35}NO$		281
44.	23.729	0.30	Squalene	$C_{30}H_{50}$		410
45.	24.149	0.42	cis-9-Octadecen-1-ol / oleyl alcohol	$C_{18}H_{36}O$		268
46.	24.315	0.23	Tetratetracontane	$C_{44}H_{90}$		618

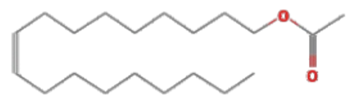
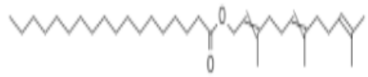
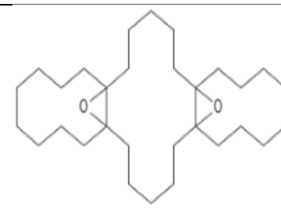
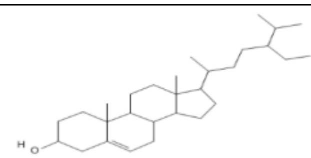
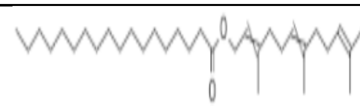
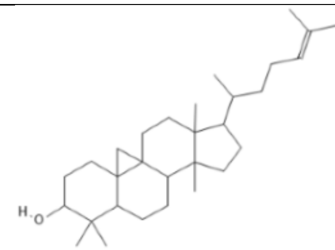
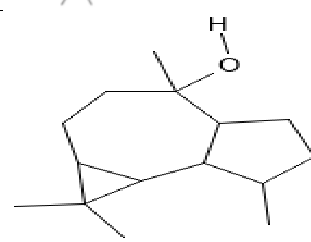
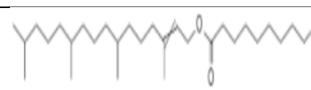
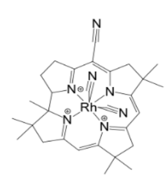
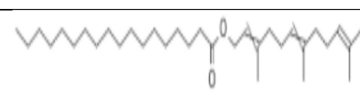
46.	24.315	0.23	Tetratetracontane	$C_{44}H_{90}$		618
47.	25.756	0.51	Oleyl alcohol , acetate	$C_{20}H_{38}O_2$		310
48.	26.948	0.48	2-trans-6-trans-Farnesyl stearate	$C_{33}H_{60}O_2$		488
49.	27.945	0.48	Tricyclo[20.8.0.0(7,16)]tri acontane, 1(22),7(16)-diepoxy-	$C_{30}H_{52}O_2$		444
50.	28.756	1.23	β-sitosterol	$C_{29}H_{50}O$		414
51.	29.248	0.45	2-trans-6-trans-Farnesyl stearate	$C_{33}H_{60}O_2$		488
52.	29.957	0.52	Handianol / Cycloartenol / 9,19-Cyclo-9.beta.-lanost-24-en-3.beta.-ol	$C_{30}H_{50}O$		426
53.	30.890	0.34	(1aR,4R,4aR,7R,7aS,7bS)-1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulen-4-ol	$C_{15}H_{26}O$		222
54.	32.284	0.17	Phytyl decanoate/ caprate phytyl ester	$C_{30}H_{58}O_2$		450
55.	33.354	0.22	Rhodium dinitrile heptamethylnitrile porphine complex	$C_{29}H_{34}N_7Rh$		583
56.	34.521	0.56	2-trans-6-trans-Farnesyl stearate	$C_{33}H_{60}O_2$		488
		100.00				

Table 6: Biological activities of GC-MS identified phytocompounds from methanolic flower extract of *M. hortensis* as reported in scientific literature

S. No.	Name	Biological activities	References
1.	Ethanimidic acid, ethyl ester	antioxidant, anti-cancer	Rassem *et al.*[30]
2.	Mannitol	antioxidant	Liu *et al.*[31]
3.	3,7-Dimethyl-1,6-octadien-3-ol (Linalool)	Anti-inflammatory and anti-cancer properties anti-cancer, anti-inflammatory	Al-Marzoqi *et al.*[32]
4.	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	anti-inflammatory, antimicrobial	Mujeeb *et al.*[33]
		anti-diabetic	Siddique *et al.*[34]
		antioxidant	Yu *et al.*[35]
5.	1,2,3-propanetriol (Glycerol)	antibacterial	Nalawade *et al.*[36]
6.	Isoeugenol	antioxidant, antibacterial	Zhang *et al.*[37]
7.	Farnesyl alcohol	anticancer, anti-inflammatory	Jung *et al.*[38]
8.	Hexadecanoic acid / Palmitic acid/ Cetylic acid	antimicrobial	Rahman *et al.*[39]
		antioxidant, antidiabetic, hemolytic, nematicide, hypocholesterolemic, antiandrogenic	Fagbemi *et al.*[40]
9.	Phytol	antioxidant	Costa *et al.*[41]
		anti-diabetic	Abdel-Hady *et al.*[42]
		antimicrobial, anti -inflammatory, anti-diuretic	Kavitha[43]
10.	Trans-Geranylgeraniol	anticancer, antimicrobial	Nguyen *et al.*[44]
11.	Heneicosane	antibacterial	Rautela *et al.*[45]
12.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester /	anti-inflammatory and anthelmintic activities antioxidant, anthelmintic, anti-inflammatory	Al-Marzoqi *et al.*[32]
13.	9-Octadecenamide	antibacterial, anti-inflammatory	Idan *et al.*[46]

13.	9-Octadecenamide	antibacterial, anti-inflammatory	Idan *et al.*[46]
14.	Squalene	antioxidant, hypocholesterolemic, anti-diabetic, hepatoprotective, anti-ageing, anti-inflammatory, analgesic, anticancer, antileukemic	Sudha *et al.*[47]
		antimicrobial	Rahman *et al.*[39]
15.	β-Sitosterol	anticancer, antifertility	Gopu *et al.*[48]
		anti-diabetic	Bharanishankar *et al.*[49]
		anti-diabetic, antioxidant, antimicrobial	Gopu *et al.*[48]; Fagbemi *et al.*[40]
		anti-inflammatory	Fagbemi *et al.*[40]
16.	(1aR,4R,4aR,7R,7aS,7bS)-1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulen-4-ol	antimicrobial	Tan *et al.*[50]
		antioxidant, antimutagenic	Rodriguez *et al.*[51]

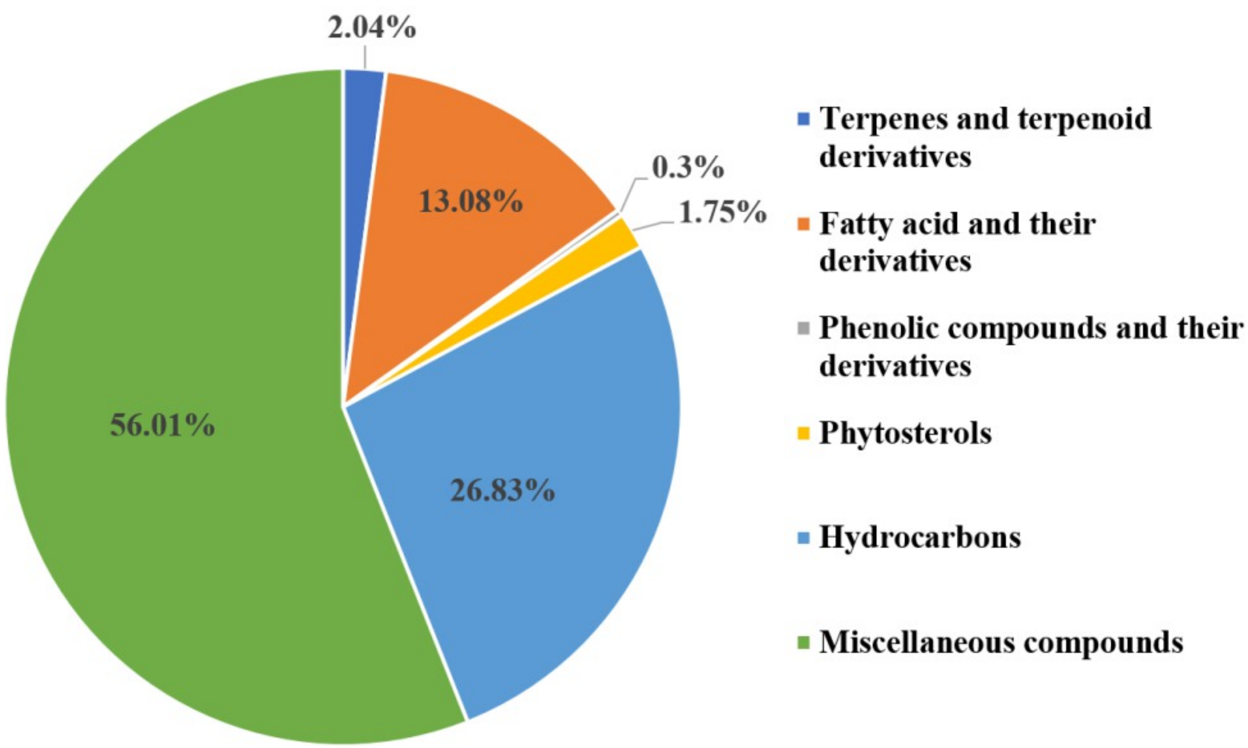


Figure 5: Phytochemical Composition of Methanolic Flower Extract of *M. hortensis* (MFM) Determined by GC-MS

DISCUSSION

Diabetes mellitus is a worldwide epidemic of several metabolic impairments[52] and over 800 million people are predicted to be affected by diabetes in 2045[53]. The oxidative stress caused by free radicals produced during glucose oxidation and the oxidative destruction of glycated proteins is thought to be the cause of diabetes complications. To prevent such issues, it is therefore commonly advised to take antioxidants in addition to anti-diabetic medications[54]. The use of *M. hortensis* flowers in treating diabetes is still devoid of scientific exploration. Therefore, this study was conducted to assess *in vitro* α-amylase inhibitory and antioxidant activities of methanolic extract of *M. hortensis* flower and determine the phytochemical composition through preliminary phytochemical screening and GC-MS analysis.

Phytocompounds, found in plants such as polyphenols, flavonoids, alkaloids, tannins etc. have been utilised to manage various ailments due to their diverse pharmacological potential[55]. These are natural resources for producing new medications that are more biocompatible and effective. GC-MS is an effective method to find out bioactive molecules in plant extracts[56]. Moreover, methanol is the most effective extraction solvent as efficient to extract a greater number of phytocompounds[57] including phenol, flavonoid, alkaloid and terpenoids[58]. Methanol was used to determine the phytochemical profiling of *M. hortensis* flowers in this study and the extraction yield was obtained as 29.52%.

Preliminary phytochemical analysis of the methanolic flower extract of *M. hortensis* confirmed the presence of proteins, tannins, flavonoids, phenolic compounds, phlobatannins, alkaloids, phytosterols, terpenoids, and cardiac glycosides. The presence of these various metabolites suggests that *M. hortensis* flowers are a rich reservoir of biologically active compounds that may synergistically provide the therapeutic potential of the plant. The results of the present study align with previous reports of different parts of *M. hortensis*. Chumbhale *et al*.[59] demonstrated the presence of carbohydrates, proteins, phenolic compounds, flavonoids, tannins, glycosides, and phytosterols in different stem extracts of *M. hortensis*, indicating that these phytocompounds are widely distributed across different plant parts. Janani and Ananthi[60] reported the presence of reducing sugars, carbohydrates, along with glycosides, flavonoids, and phenolic compounds in the ethanolic extract of *M. hortensis* flowers. However, reducing sugars and carbohydrates were not detected in present study. These variations may be attributed to differences in plant part, solvent polarity, extraction techniques, and environmental or geographical factors influencing the biosynthesis of metabolites.

In this study, 81.14 ± 5.15 mg GAE/g and 29.27 ± 2.52 mg QE/g values were found for TPC and TFC, respectively, suggesting a considerable presence of antioxidant phytocompounds in flowers. Babitha *et al*.[21] reported 241.2 ± 3.0 mg TPC equivalent to per gram of tannic acid and 58.5 ± 2.06 mg TFC equivalent to catechin per gram of extract for ethanol extract of *M. hortensis* flower. Sivaraj *et al*.[17] reported TPC and TFC for methanolic leaves extract of *M. hortensis* as 190.51 ± 0.38 µg/mg of GAE and 7.71±0.14 µg/mg of QE, respectively, indicating that the presence of phytocompounds not only depends on solvent polarity but also on plant part and metabolic activity. According to Rawal *et al*.[61], phenol and flavonoid contents also exhibit antioxidant and hypoglycaemic activities. Overall, the significant flavonoid and phenolic contents reported in the current study support the antioxidant potential and recommend its applicability for pharmacological research.

For the management of postprandial hyperglycemia in diabetic patients, the inhibition of α-amylase is a key therapeutic strategy[62]. Several plants have shown promising α-amylase inhibition potential for example, *Aegle marmelos, Zingiber officinale*, *Allium sativum*, *Murraya koenigii, Curcuma longa, Moringa oleifera, Citrus limon*, *Punica granatum*, *Momordica charantia, Eugenia cumini, Syzigium aromaticum Phyllanthus emblica* etc.[53]. In the present study, α-amylase inhibitory activity was found in a concentration dependant manner and IC_{50} values were found to be 28.87 mg/mL and 128.83 mg/mL for DNSA and starch-iodine assays, respectively. In contrast, the standard inhibitor, acarbose showed significantly lower IC_{50} values of 8.38 mg/mL and 8.09 mg/mL for DNSA and starch-iodine assays, respectively. Jayaprakasam *et al*.[63] have also demonstrated concentration-dependent α-amylase inhibition with an IC_{50} value of 6.67±0.14 µg/mL comparable to acarbose 5.87±0.19 µg/mL in methanolic extract of *M. hortensis* leaves. Similarly, methanolic plant extracts have shown strong α-amylase inhibitory activity in other medicinal plants. For example, Muthukumar *et al*.[2] reported that methanolic extract of *Cassia auriculata* flower possess α-amylase inhibitory activity with an IC_{50} value of 5µg/mL using DNSA method. Nisar *et al*.[64] demonstrated that the methanolic root extract of *Picrorhiza kurroa* exhibited the *in vitro* α-amylase inhibitory activity with an IC_{50} value of 0.39 ± 0.41mg/mL in DNSA method. Overall, the methanolic flower extract of *M. hortensis* demonstrated moderate α-amylase inhibitory activity in comparison to acarbose. However, its concentration-dependent inhibition supports the presence of bioactive constituents that can modulate α-amylase.

Oxidative stress is generated in the body due to an imbalance between free radicals and antioxidant system[65]. These free radicals damage important biomolecules such as proteins, lipids and nucleic acids and play a significant role in the development of *Diabetes mellitus* and its complications, as well as several other chronic diseases such as neurodegenerative disorders, cardiovascular and respiratory diseases, cancer, rheumatoid arthritis etc. Therefore, controlling oxidative stress through antioxidants is crucial for disease prevention and management. Antioxidants derived from plants reduce oxidative stress, neutralise dangerous free radicals, and significantly decrease the risk of diabetes and its complications. Synthetic antioxidants may have unfavourable side effects and frequently have low efficacy. Therefore, there is a rising need to discover safer and more effective antioxidants. In the present study, methanolic flower extract of *M. hortensis* revealed notable *in vitro* antioxidant activity with an IC_{50} value of 15.95 mg/mL, comparable with the standard antioxidant, ascorbic acid (11.75 mg/mL). Similar antioxidant potential of *M. hortensis* has been reported by Sivaraj *et al.*[17] in methanolic leaf extract of *M. hortensis* where significant DPPH radical scavenging activity was observed with an IC_{50} value of 140.97 µg/mL, compared with the standard ascorbic acid (IC_{50} = 11.98 µg/mL). The variation in IC_{50} values between studies may be due to differences in plant parts used, extraction methods, and geographical area. Overall, these results support the antioxidant potential of *M. hortensis*.

GC-MS profiling provides comprehensive information on the nature of bioactive compounds present in plants (Fig. 5). In this study, antioxidant and α-amylase inhibitory activities of methanolic flower extract of *M. hortensis* are possibly owing to the presence of bioactive compounds reported in its GC-MS analysis. GC-MS screening identified forty eight compounds, some of which have been documented in prior studies for various pharmacological activities including antioxidant and anti-diabetic properties (Table 5 & 6). It has been reported that ethanimidic acid ethyl ester, identified in methanolic extract of *Hibiscus* flower, exhibited antioxidant activity[30]. A pyranone derivative, 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, was confirmed for antioxidant activity[35] as well as α-amylase inhibitory activity in *Malus* species fruit extracts[34]. Phytol is an alcoholic diterpenoid, which has shown antioxidant potential in DPPH and ABTS assay as well as decreased lipid peroxidation and nitrate contents and improved reduced glutathione, superoxide dismutase and catalase activities in Swiss mouse hippocampus[41]. Phytol is also reported for its anti-diabetic activity isolated from *Ocimum canum* leaves[42]. Squalene was identified by Rajeswari *et al.*[66] in GC-MS analysis of ethanolic extracts of *Hugonia mystax* bark and reported its antioxidant activity whereas Sudha *et al.*[47] observed it in the ethanolic extract of *Fluggea leucopyrus* and mentioned its anti-diabetic activity. Likewise, Isoeugenol, has been shown to possess significant antioxidant activity[37]. β-sitosterol is a ubiquitous natural phytosterol which is well-known for its beneficial role in management of diabetes through its antioxidant, antidiabetic, anticancer, antimicrobial, and immunomodulatory activities[67].

The GC-MS study suggests that the methanolic extract of *M. hortensis* flowers is abundant in pharmacologically active compounds with various therapeutic properties (Table 6). These results corroborate its traditional medical practices and highlight its potential for further exploration of antioxidant and anti-diabetic activities. Additional research, including *in vivo* validation and isolation of compounds, is necessary to ascertain the mechanistic foundation of its bioactivity.

CONCLUSION

The rising prevalence of *Diabetes mellitus* worldwide necessitates the exploration of novel natural therapies that can effectively manage blood glucose levels[68]. For the first time, methanolic flower extract of *M. hortensis* has demonstrated *in vitro* α-amylase inhibitory property, which can be ascribed to the presence of tannins, phenolics, flavonoids, and phytosterols as observed in preliminary qualitative phytochemical analysis. The results showed moderate *in vitro* inhibition of α-amylase in DNSA and Starch-Iodine assays. Moreover, TPC and TFC values were also found satisfactory in describing its antioxidant activity revealed through DPPH assay. The results support methanol as a suitable solvent for extracting bioactive compounds and signify the potential of *M. hortensis* flowers in managing diabetes and oxidative stress-related ailments. However, *in vivo* and large-scale clinical studies are required to establish its anti-diabetic potential. Moreover, the corresponding hypoglycemic bioactive molecule present in the flowers needs to be identified.

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