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Research Article

IN VITRO AND *IN VIVO* ASSESSMENT OF ANTIDIABETIC ACTIVITY OF *MALACHRA CAPITATA* WITH ENZYME INHIBITION AND ANTIOXIDANT EVIDENCE

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ABSTRACT

Objectives: *Malachra capitata* (family: Malvaceae) is known to contain various bioactive phytochemicals with potential therapeutic properties. The present study aimed to evaluate *M. capitata* as a potential source of hypoglycemic bioactive compounds using *in vitro* enzyme inhibition assays and *in vivo* experimental models.

Methods: Extracts of *M. capitata* were prepared using 70% ethanol, 100% ethanol, and water and subjected to phytochemical screening. *In vitro* antidiabetic potential was evaluated through α-amylase and α-glucosidase inhibitory assays. Acute oral toxicity of the 70% ethanol extract was assessed in Wistar rats according to standard guidelines. The hypoglycemic effect was further evaluated using an oral glucose tolerance test (OGTT) and a 28-day treatment study in Wistar rats. Antioxidant parameters, including superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and malondialdehyde (MDA), were also analyzed.

Results: Phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, phenolics, and tannins in all extracts. Among them, the 70% ethanol extract demonstrated the strongest enzyme inhibitory activity with α-amylase (IC_{50}=79.1 µg/mL) and α-glucosidase (IC_{50}=54.61 µg/mL) inhibition compared to other extracts. Acute oral toxicity testing showed no mortality up to 2000 mg/kg. *In vivo* studies revealed that the extract significantly improved glucose tolerance in OGTT and reduced fasting blood glucose levels from 245.0±15.82 mg/dL to 146.0±11.40 mg/dL after 28 days. Serum insulin levels increased to 627.5±142.78 pg/mL, while antioxidant defense improved through increased SOD, CAT, and GSH levels and decreased MDA levels.

Conclusion: The findings indicate that *M. capitata* possesses significant hypoglycemic and antioxidant activities, supporting its potential as a promising natural source for antidiabetic drug development. Further studies are required to isolate and characterize the active compounds responsible for these effects.

Keywords: *Malachra capitata*, Hypoglycemic, Bioactive compounds, Phytochemical screening, Antioxidant, Glucose tolerance.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic condition that induces sustained hyperglycemia owing to impaired insulin secretion, insulin action, or a combination of both. It is a major health concern worldwide and is defined by a rapidly rising incidence and associated morbidity and mortality. Diabetes is a primary contributor to cardiovascular disease, renal failure, visual impairment, and lower limb amputations worldwide [1]. Thus, a key element of type 2 diabetes treatment is effective management of postprandial hyperglycemia.

Inhibiting enzymes that hydrolyze carbohydrates, such as α-amylase and α-glucosidase, is a well-established therapeutic approach for managing postprandial blood glucose. When these enzymes are inhibited, glucose absorption is delayed, which lowers postprandial glucose spikes. These enzymes break down complex carbons into absorbable monosaccharides [2]. Although a number of synthetic enzyme inhibitors are clinically available, their long-term use is frequently linked to gastrointestinal adverse effects, which has led to the search for safer, plant-derived substitutes [3].

It has long been known that medicinal plants constitute a substantial reservoir of bioactive compounds substances as well as the potential to treat diabetes. A variety of methods, including enzyme inhibition, antioxidant action, enhanced insulin sensitivity, or modification of glucose metabolism, phytochemicals such as flavonoids, phenolic acids, alkaloids, and terpenoids have shown hypoglycemic benefits [4,5]. Therefore, by combining biological screening with sophisticated separation techniques, bioactivity-guided fractionation has become a logical and methodical method to discover and isolate such active ingredients.

In traditional medicine, *Malachra capitata* (L.), a member of the Malvaceae family, is used to treat pain, inflammation, and several metabolic conditions. According to phytochemical research, the genus *Malachra* contains phenolic compounds, flavonoids, and other secondary metabolites with antioxidant and enzyme-inhibiting properties [6,7]. Despite these observations, little research has been conducted on the hypoglycemic potential of *M. capitata* stems and the isolation of its active ingredients.

Phytoconstituent isolation from complicated plant matrices is now much more efficient and purer because of recent developments in chromatographic techniques, particularly preparative high-performance liquid chromatography (HPLC). Simultaneously, *in silico* methods such as molecular docking and interaction analysis have been developed into useful instruments for forecasting enzyme–ligand interactions, clarifying potential modes of action, and bolstering experimental results [8]. A thorough foundation for lead compound identification in natural product research is provided by the amalgamation of *in vitro* enzyme inhibition experiments as well as *in silico* investigations.

In this regard, the current study used a bioactivity-guided extraction and fractionation method to examine the hypoglycemic potential of *M. capitata* stems. To identify and validate the possible antidiabetic phytoconstituents, we used α-amylase inhibition as the main screening approach, followed by liquid–liquid partitioning, preparative HPLC-based isolation, chromatographic characterization, and supported *in silico* analysis.

METHODS

Drug chemicals and materials

Chemicals of AR grade used, such as ethanol (99%), starch, acarbose, α-amylase, α-glucosidase, (3,5-Dinitrosalicylic acid [DNSA]), (*p*-nitrophenyl-b-d-glucopyranoside), and 2,2-diphenyl-1-picrylhydrazine, were obtained from SRL PVT LTD Mumbai, and metformin was a gift from Sun Pharmaceutical Industries Ltd. Mumbai. All supplementary chemicals, as well as reagents required, were of AR quality from a local manufacturer.

Instruments and equipment

Ultrasonicator was used for the extraction of plant material. Absorbance for enzyme inhibition assays was measured using an Epoch Gen5 microplate reader. An analytical balance was used for weighing plant extracts. A water bath was used during the DNSA reaction step of the α-amylase assay. Blood glucose levels were measured using a digital glucometer. Serum separation was carried out using a laboratory centrifuge. Micropipettes were used for accurate measurement of reagents during *in vitro* assays. Experimental animals were housed in polypropylene cages, and oral dosing was performed using a gastric gavage needle.

Plant material

The *Malachra Capitata* (L.) area was selected for evaluation. Plant material was collected from a local area near the campus of B.P. Arts College, Dhule Road, Chalisgaon. The plant was Authenticated by Botany Department of Dr. Bababasaheb Aambedkar Marathwada University, Aurangabad (accession number 0791).

Experimental animals

Healthy Wistar rats, weighing 150–200 g and aged 6–8 weeks, were selected for this study. Animals were procured from LACSMI Biofarms, Alephata, and Pune. Before the initiation of the experimental procedures, the rats were adapted to the animal house conditions for 5–7 days to reduce stress related to environmental changes. The animals were housed in polypropylene cages in accordance with conventional laboratory setups and provided with a standard laboratory diet (VRK Nutritional Solutions, Sangli, India) and tap water ad libitum. All experimental protocols were performed in strict compliance with the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals and approved by the Institutional Animal Ethics Committee (Approval No.: 2166/PO/RcBI/S/22/CPCSEA).

Extraction of *M. capitata*

The Aerial material of *M. capitata* was extracted using several solvents and extraction methods, as outlined in conventional phytochemical protocols, the Aerial material of *M. capitata* was extracted [9,10]. Water, 70% ethanol, and 100% ethanol were used for extraction. The extraction procedures were reflux, maceration, and ultrasonication. According to previous research, ultrasonication-assisted extraction was used to improve the recovery of bioactive components [10]. To calculate the extractive yield, expressed as a percentage yield (% w/w), the extracted materials were concentrated and weighed. Compared to the maceration and reflux methods, the ultrasonication method produced the maximum yield, according to the extraction data. Thus, extracts obtained by ultrasonication that were aqueous (MCA), 70% ethanolic (MCHA), and 100% ethanolic (MCE) were chosen for additional bioactivity assessments.

Preliminary qualitative phytochemical analysis

Preliminary phytochemical evaluation was performed on the ultrasonicated extracts of *M. capetita* (L), namely the aqueous extract (MCA), 70% ethanolic extract (MCHA), and 100% ethanolic extract (MCE). Standard qualitative chemical tests were employed to ascertain the presence or absence of the principal categories of secondary metabolites. The extracts were screened for alkaloids, steroidal constituents, phenolic compounds, flavonoids, saponins, and tannins, using established phytochemical reagents and conventional test procedures [11,12].

In vitro antidiabetic activity

α-*Amylase inhibition assay*

The inhibitory effect against porcine pancreatic α-amylase was evaluated using the DNSA colorimetric method with slight modifications from established procedures [13,14]. The Porcine α-amylase was selected because it closely resembles human pancreatic α-amylase in catalytic properties and is widely used as a standard enzyme model for preliminary screening of carbohydrate-digesting enzyme inhibitors. Stock solutions of the acarbose, MCA, MCHA, and MCE extracts were prepared in distilled water. For the assay, 200 µL sodium phosphate buffer (20 mM, pH 6.9) and 100 µL α-amylase solution (1 U/mL) were mixed with varying concentrations of the test extracts or acarbose (10–100 µg/mL). The mixture was pre-incubated at 25°C for 10 min, followed by the addition of 200 µL of 1% soluble starch to initiate the reaction. After incubation, the reaction was terminated with DNSA reagent, and absorbance was measured at 540 nm. Acarbose served as the positive control, while a negative control containing buffer and solvent without inhibitor was included in each experimental run. All assays were performed in triplicate (three technical replicates) and repeated in three independent experiments, and IC_{50} values were calculated from dose–response curves using linear regression analysis.

α-*Glucosidase inhibition assay*

The inhibitory potential of several excerpts against α-glucosidase was ascertained using yeast α-glucosidase and p-nitrophenyl-α-D-glucopyranoside (pNPG) as the substrates, following a modified standard protocol [14,15]. Yeast α-glucosidase was selected because it is a commonly used model enzyme for preliminary screening of α-glucosidase inhibitors and provides reproducible results comparable to mammalian systems**.** The enzyme was prepared in 0.1 M phosphate buffer (pH 6.9). The reaction mixture contained buffer, enzyme solution, and different concentrations of plant extracts (10–100 µg/mL). After pre-incubation at 37°C, pNPG was added to initiate the reaction, and the mixture was incubated for 30 min. The reaction was terminated with sodium carbonate, and absorbance was recorded at 405 nm. Acarbose was used as the positive control, and a negative control containing buffer with solvent only was included in every experimental run. All experiments were conducted in triplicate with three independent repetitions, and IC_{50} values were determined from concentration–response curves.

Acute oral toxicity study

Acute oral poisoning by 70% ethanolic extract of *M. capitata* (MCHA) was assessed in female Wistar rats using OECD Guideline 423 (Acute Oral Toxicity - Acute Toxic Class Method) under Good Laboratory Practice settings [16,17]. Healthy, young, non-pregnant female Wistar rats weighing approximately 150–250 g were chosen for the investigation. The extract was dissolved in distilled water and delivered as a single oral dose using a gastric feeding needle at doses of 300, 1000, and 2000 mg/kg body weight (BW) (dose volume: 1 mL per animal). After treatment, each animal was individually watched during the first 30 min, frequently followed over the first 24 h, with a specific focus during the first 4 h, and then assessed daily for 14 days. Observations concentrated on detecting any indications of toxicity, including modifications in the skin and fur, ocular conditions, and mucous membrane alterations, along with variations in respiratory, circulatory, autonomic, and central nervous system functioning. Behavioral patterns and clinical symptoms were meticulously documented during the study period.

Oral glucose tolerance test (OGTT)

An OGTT was conducted to assess the glucose handling capacity. Before the experiment, the rats were starved overnight. Blood specimens were also collected via the tail vein at 0, 30, 60, 120, and 240 min after an oral glucose load of 2 g/kg BW was administered. A glucometer was used to measure the blood glucose levels [18,19].

***In vivo* hypoglycemic activity**

Grouping and dosing of animals

To evaluate the *in vivo* hypoglycemic activity, Wistar rats of both sexes were randomly allocated into four groups, each including six individuals. Group I: Normal control (non-diabetic rats receiving vehicle only) and Group II: Diabetic control (streptozotocin [STZ]-induced diabetic rats receiving vehicle). Group III received the Standard group (diabetic rats treated with metformin), whereas Group IV received the Test group (diabetic rats treated with *M. capitata* extract, MCHA). Experimental diabetes was induced in the designated groups by a single intraperitoneal injection of STZ at a dose of 40 mg/kg BW, freshly prepared in cold citrate buffer (0.1 M, pH 4.5), while the normal control group received only the vehicle. Before STZ administration, rats were fasted overnight (12 h) with free access to water to enhance the diabetogenic effect of STZ. After 72 h of STZ injection**,** fasting blood glucose levels were measured using a glucometer. Rats with fasting blood glucose levels ≥250 mg/dL were considered diabetic and included in the study, and only these confirmed diabetic animals were selected for the 28-day treatment protocol. The diabetic rats received oral treatment with either conventional medication or the test extract for 28 days. During the trial, BW and fasting blood glucose levels were recorded weekly. On completion of the treatment period, blood samples were obtained for the assessment of serum insulin and antioxidant metrics, including superoxide dismutase (SOD), catalase (CAT), reduced GSH, and malondialdehyde (MDA). After blood collection, the animals were euthanized, and the pancreas was removed, fixed in 10% formalin, and prepared for histological analysis to assess treatment-induced morphological alterations [20,21].

Dose administration

The dose of 100 mg/kg of the 70% ethanolic extract (MCHA) was selected based on the results of the acute toxicity study, where no mortality was observed up to 2000 mg/kg (OECD 423). Therefore, 100 mg/kg represents a safe pharmacological screening dose (approximately $1/20^{th}$ of the maximum tested dose). Metformin was administered orally at a dose of 125 mg/kg BW**,** which is a commonly used standard dose in experimental diabetic rat models. STZ was prepared at a dose of 10 mg in 0.2 mL of saline.

Blood withdrawal

On last day of treatment, blood samples were withdrawn in 1.5 mL Eppendorf tubes (0.4–0.6 mL), serum were separated and used for estimation of Bio-chemical Studies and Hematology.

Statistical analysis

All experimental data are expressed as mean±standard error of the mean (SEM)**.** Statistical analysis was performed using two-way repeated measures analysis of variance (ANOVA) followed by Bonferroni *post hoc* test for multiple comparisons to evaluate the effects of treatment and time on blood glucose levels and BW during the OGTT and 28-day study. For parameters measured at a single time point (such as serum insulin and antioxidant enzyme levels), one-way ANOVA followed by Bonferroni *post hoc* test was used. A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Extraction yield of *M. capitata* arial part

Extraction of *M. capitata* using different solvents and techniques showed that ultrasonication produced the highest extractive yield compared to the maceration and reflux methods (Table 1). Among the ultrasonication extracts, the 70% ethanolic extract (7.77%) yielded more than the aqueous extract **(**6.41%) and ethanolic extracts (3.45%) [22].

Preliminary phytochemical screening

The initial phytochemical analysis of water, 70% ethanol, and 100% ethanolic extracts of *M. capitata* revealed the existence of secondary metabolites (Table 2) [23].

***In vitro* hypoglycemic activity**

Alpha amylase inhibition

In this experiment, the extracts from *M. capitata* were tested against the positive control acarbose to determine their α-amylase inhibitory activity (Table 3 and Fig. 1). Among the extracts, MCHA showed the highest inhibition, followed by MCE, whereas MCA showed the lowest activity. At 100 µg/mL, MCHA showed inhibition comparable to that of the standard drug, acarbose.

MCHA had a lower IC_{50} value compared to MCA and MCE, which means a smaller amount of MCHA was needed to inhibit the enzyme. However, acarbose was slightly more effective than other extracts. Overall, the results suggest that MCHA has good α-amylase inhibitory activity and may help in controlling postprandial blood glucose levels by slowing down carbohydrate digestion [24,25].

Alpha glucosidase inhibition

All extracts showed a gradual increase in α-glucosidase inhibition with increasing concentrations (Table 4 and Fig. 2). Among these, MCHA showed the highest inhibitory activity, followed by MCE and MCA. At the highest concentration tested, inhibition by MCHA was close to that of acarbose. The IC_{50} values showed that MCHA required a lower concentration to inhibit α-glucosidase than MCA and MCE. This indicates that MCHA has a stronger inhibitory activity. The extracts displayed better inhibition of α-glucosidase than of α-amylase, which is beneficial because strong α-glucosidase inhibition helps control blood sugar with fewer digestive side effects [24,25].

Table 1: Extractive % yield of *Malachra capitata* extracts with different extraction technique

Extracting solvent	Raw material: Solvent	Method of extraction	Yield (g)	% Yield
Water	1:30	Ultrasonication	0.0641	6.41
70% Ethanol	1:30	Ultrasonication	0.077	7.77
100% Ethanol	1:30	Ultrasonication	0.0345	3.45
Water	1:30	Maceration	0.0549	5.49
70% Ethanol	1:30	Maceration	0.0597	5.97
100% Ethanol	1:30	Maceration	0.0330	3.30
Water	1:30	Reflux	0.0591	5.91
70% Ethanol	1:30	Reflux	0.070	7.0
100% Ethanol	1:30	Reflux	0.0435	4.35

Table 2: Phytochemical screening test for MCA, MCHA, and MCE extracts

Phytochemical active constituents test	MCA	MCHA	MCE
Carbohydrate Fehling's test	+	+	+
Alkaloids Wagner's test	+	+	+
Flavonoid Shinoda test	+	+	+
Phenol Lead acetate test	+	+	+
Steroid Liebermann-test	−	−	−
Terpenoid Burchard test	−	−	+
Glycosides Keller-Killani test	−	+	+
Fats and oil Spot test	−	−	+
Saponins Foam Test	+	+	+

Where (+) indicates the presence of phytochemicals and (−) indicates the absence of phytochemicals

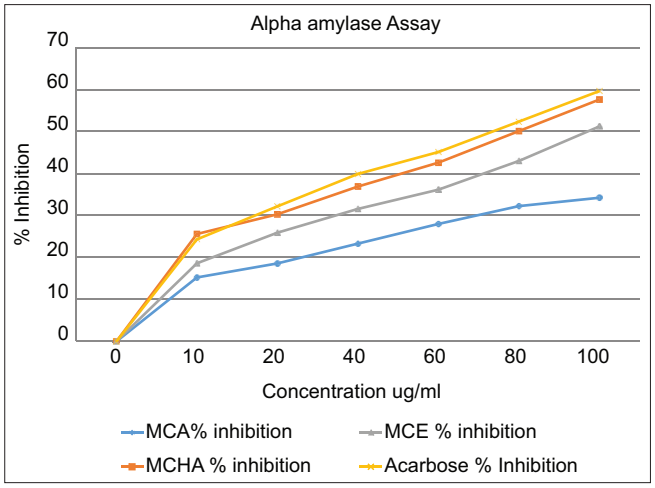


Fig. 1: α-Amylase inhibitory activity of *Malachra capitata* extracts (MCA, MCHA, and MCE) compared with the standard drug acarbose. Data are presented as mean±SD (n=3)

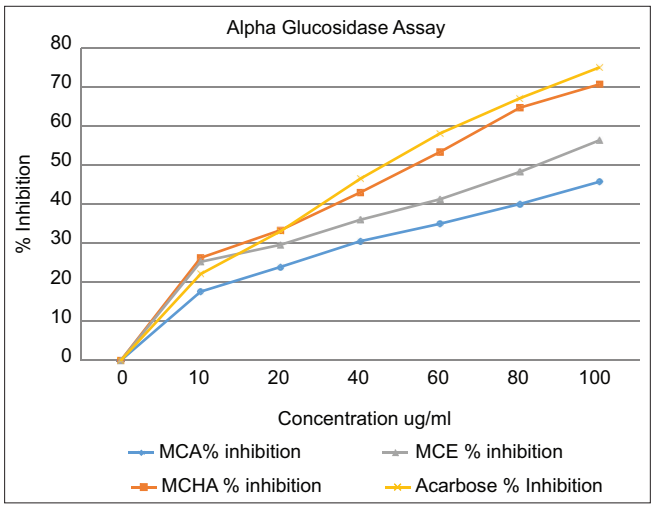


Fig. 2: α-Glucosidase inhibitory activity of *Malachra capitata* extracts (MCA, MCHA, and MCE) compared with the standard drug acarbose. Data are presented as mean±SD (n=3)

From the *in vitro* hypoglycemic assay, the MCHA extract showed promising inhibition compared to MCA and MCE.

Acute toxicity

No harmful effects were detected at 2000 mg/kg, categorizing *M. capitata*70% ethanolic extract (MCHA) as safe under GSH Category 5.

Although no mortality was observed across all dose groups, two animals (Animals 7 and 9) showed clinical signs of toxicity at the 2000 mg/kg dose level. BWs increased progressively in all animals, indicating that overall health was maintained [26].

The acute oral toxicity assessment of MCHA in female rats demonstrated a favorable safety profile, aligned with its categorization under GSH Category 5. As summarized in Table 5, no mortality was observed across any of the tested dose groups (up to 2000 mg/kg). Animals treated with 300 mg/kg and 1000 mg/kg showed no discernible signs of toxicity throughout the 14-day observation period. While two animals (Animal 7 and Animal 9) at the highest dose of 2000 mg/kg exhibited some clinical indicators of toxicity, these were not severe enough to cause mortality. Furthermore, the progressive increase in BW in all animals across all dose groups, from day 0 to day 14, indicated that the extract did not negatively impact the general health and growth of the rats. This collective evidence suggests that *M. capitata* extract has relatively low acute toxicity, confirming its safety for potential therapeutic applications within this dose range.

Table 3: Mean percentage inhibition of α-amylase by *Malachra capitata* extracts (MCA, MCHA, MCE) and acarbose (n=3)

Conc. µg/mL	MCA% inhibition	MCHA % inhibition	MCE % inhibition	Acarbose % inhibition
0	0	0	0	0
10	15.23	25.6	18.6	24.31
20	18.56	30.27	25.9	32.16
40	23.3	36.9	31.56	39.89
60	27.97	42.58	36.16	45.12
80	32.23	50.1	43.01	52.36
100	34.23	57.62	51.23	59.674
IC_{50}	166.88	79.1	98.32	72.64

Data are presented as mean±SD (n=3). IC_{50} values were calculated from the dose–response curve using non-linear regression analysis

Table 4: Mean percentage inhibition of α-glucosidase by *Malachra capitata* extracts (MCA, MCHA, MCE) and acarbose (n=3)

Conc. ug/mL	MCA% inhibition	MCHA % inhibition	MCE % inhibition	Acarbose % inhibition
0	0	0	0	0
10	17.6	26.24	25.26	22.12
20	23.9	33.31	29.58	33.04
40	30.5	42.96	35.98	46.46
60	34.96	53.31	41.23	58.06
80	40.01	64.66	48.23	67.06
100	45.73	70.66	56.36	74.99
IC_{50}	112.46	54.6195	83.89	49.6363

Data are presented as mean±SD (n=3). IC_{50} values were calculated using non-linear regression analysis from the dose–response curve.
SD: Standard deviation

Table 5: Summary of acute oral toxicity study of *Malachra capitata* 70% ethanolic extract in female rats

Animal No.	Dose (mg/kg)	BW (g) Day 0/ Day 7/Day 14	Clinical observations	Dose volume (mL)
Animal 1	300	179/185/191	No toxic signs	0.7
Animal 2	300	180/186/191	No toxic signs	0.7
Animal 3	300	184/190/195	No toxic signs	0.7
Animal 4	1000	237/248/256	No toxic signs	0.9
Animal 5	1000	235/240/248	No toxic signs	0.9
Animal 6	1000	230/245/256	No toxic signs	0.9
Animal 7	2000	230/238/245	Showed toxic signs	0.9
Animal 8	2000	250/263/274	No toxic signs	0.9
Animal 9	2000	237/250/264	Showed toxic signs	0.9

BW: Body weight. In the OGTT study, a higher dose (400 mg/kg) was used to evaluate the acute antihyperglycemic effect of the extract after glucose loading, while a lower dose (100 mg/kg) was selected for the 28-day chronic study based on safety findings from the acute toxicity study, and to evaluate the long-term antidiabetic efficacy.

Oral glucose tolerance test

A higher dose **(**400 mg/kg**)** was used in the OGTT experiment to evaluate the acute antihyperglycemic response**,** whereas a lower dose (100 mg/kg**)** was selected for the 28-day chronic study to assess sustained antidiabetic activity while ensuring safety. The extract

significantly reduced glucose levels after glucose loading at 60, 90, and 120 min, although it was slightly less effective than metformin [27].

Data are expressed as Mean±SEM (n=6). Statistical analysis was performed using two-way repeated measures ANOVA followed by Bonferroni's *post hoc* test. *p<0.001 and *p<0.05 compared to the glucose-loaded control group at the corresponding time point. Group1: Normal control (non-diabetic rats receiving vehicle). Group 2: Glucose-loaded group (2 g/kg glucose).Group3: Standard group (metformin 125 mg/kg+glucose). Group 4: Test group (MCHA 400 mg/kg+glucose).

The OGTT findings, as presented in Table 6 and Fig. 3, demonstrated the significant hypoglycemic potential of the *M. capitata*70% ethanolic extract of *M. capitata*. Following glucose loading, the extract notably decreased blood glucose levels at 60, 90, and 120 min, indicating its efficacy in improving glucose metabolism. While the extract showed a significant disparity in contrast with the glucose-loaded group (indicated by ***p<0.001 and *p<0.05), its effect was slightly less pronounced than that of the usual medication, metformin (500 mg/kg). Metformin consistently brought blood glucose levels closer to those of the standard test group at every post-load time point. Nonetheless, the ability of *M. capitata* 70% ethanolic extract to significantly mitigate the post-glucose surge suggests its potential as an antidiabetic agent, likely by enhancing glucose utilization or inhibiting glucose absorption, albeit with a slightly lower potency than the established drug. The data are expressed as mean±SEM and analyzed by One-Way ANOVA followed by Bonferroni *Post hoc* analysis, which robustly supports these findings.

***In vivo* hypoglycemic study**

Over 28 days, the extract-treated group showed a significant decrease in fasting blood glucose levels (from 245.0±15.82 mg/dL to 146.0±11.40 mg/dL). Serum insulin levels increased significantly (627.5±142.78 pg/mL) in comparison to the diabetes control [28,29].

The 28-day *in vivo* hypoglycemic study of *M. capitata* extract in Wistar rats provided compelling evidence of its antidiabetic potential, as detailed in Tables 7-9 and visually represented in Figs. 4-6. As shown in Table 9 and Fig. 6, the test extract markedly reduced the fasting blood sugar levels in diabetic rats over the 28-day period. The initial blood glucose concentration in the extract-treated group on Day 7 was 245.0±15.82 mg/dL, which progressively decreased to 146.0±11.40 mg/dL by Day 28. This substantial decrease (***p<0.0001 in comparison to prevention of illness) indicates a robust long-term hypoglycemic effect, effectively bringing glucose levels closer to the normal range, though the standard group (Metformin) demonstrated an even more pronounced reduction (109.7±3.83 mg/dL on Day 28). In contrast, the disease control group exhibited a consistent rise in blood glucose concentrations from 271.7±3.44 mg/dL on Day 7 to 310.5±13.10 mg/dL on Day 28 ($^{###}$p<0.0001 opposite to normal control group).

Furthermore, as seen in Table 9 and Fig. 7, *M. capitata* extract significantly increased serum insulin levels (627.5±142.78 pg/mL, *p<0.05) compared to that in the diabetes group (286.5±52.88 pg/mL). This indicates that the hypoglycemic effect of the substance might, at least in part, be mediated through enhanced insulin secretion from the pancreas. The general group showed a greater increase in insulin levels (879.83±218.85 pg/mL, ***p<0.0001), and the ability of the extract to boost insulin levels is a promising indicator [30].

Regarding BW, Table 6 and Fig. 4 illustrate that while the illness management group experienced a gradual reduction in BW over

Table 6: Effect of *Malachra capitata* extract (MCHA) on blood glucose levels during an oral glucose tolerance test in rats

Sr No.	Treatment and Dose	Blood Glucose Levels (mg/dL)					
		0 min Base Line	15 min	30 min	60 min	90 min	120 min
1	Normal control, Normal saline solution	87.00±3.61	96.67±11.59	90.67±4.73	87.33±1.15	84.33±1.53	82.33±1.53
2	Glucose loaded	90.00±2.65	163.00±12.77	171.00±15.72	157.33±16.50	142.00±14.53	124.33±6.81
3	Standard metformin (125 mg/kg)	89.67±4.04	145.33±12.50	125.67±7.77	104.00±8.89	97.33±5.51	90.33±1.53
4	Test MCHA (400 mg/kg)	89.33±5.86	164.67±5.51	145.67±6.11	128.33±6.66	112.67±6.03	95.33±1.53

Table 7: Effect of *Malachra capitata* extract on hypoglycemic activity and BW in Wistar rats

S. No	Treatment and Dose	Day 0	Day 7	Day 14	Day 21	Day 28
		BW g (Mean±SEM)				
1	Normal control	188.3±5.32	192.2±5.46	197.3±5.16	202.3±4.97	208.2±5.95
2	Disease control	190.3±4.63	179.2±4.40	172.0±4.69	166.5±3.27	160.8±5.98
3	Standard group	189.3±3.78	183.3±3.67	180.2±3.82	186.5±4.1	200.5±4.14
4	Test extract *Malachra Capetita*	191.8±5.64	186.7±6.02	181.8±5.98	184.0±3.69	188.2±4.36

Data are expressed as mean±SD (n=6). Statistical analysis was performed using two-way repeated measures ANOVA followed by Bonferroni's *post hoc* test. ###p<0.0001 compared to the normal control group.*p<0.0001 compared to the disease control group. ANOVA: Analysis of variance, SD: Standard deviation, BW: Body weight, SEM: Standard error of the mean

Table 8: Effect of *Malachra capitata* extracts on blood glucose levels in Wistar rats

S. No.	Treatment and dose	Day 0	Day 7	Day 14	Day 21	Day 28
		Blood Glucose Levels (mg/dL) (Mean±SEM)				
1	Normal control	89.5±1.87	91.3±2.07	89.5±1.87	90.2±1.33	89.8±1.47
2	Disease control	90.5±1.87	271.7±3.44$^{###}$	282.8±4.96$^{###}$	293.0±6.45$^{###}$	310.5±13.10$^{###}$
3	Standard group	89.8±1.72	227.3±9.58***	172.3±5.75***	133.8±6.24***	109.7±3.83***
4	Test extract *Malachra Capetita*	90.0±1.41	245.0±15.82***	216.8±9.41***	163.2±14.11***	146.0±11.40***

Data are expressed as mean±SEM (n=6), $^{###}$p<0.0001 versus normal control group, *p<0.001 versus disease control group, statistical analysis was performed using two-way repeated measures ANOVA followed by Bonferroni *post hoc* test. ANOVA: Analysis of variance, SD: Standard deviation, BW: Body weight, SEM: Standard error of the mean

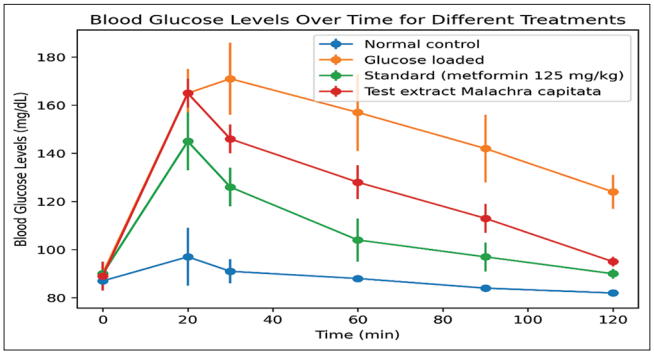


Fig. 3: Data are expressed as mean±standard error of the mean (n=6). Statistical analysis information and significance notations have been consistently formatted throughout the paper.

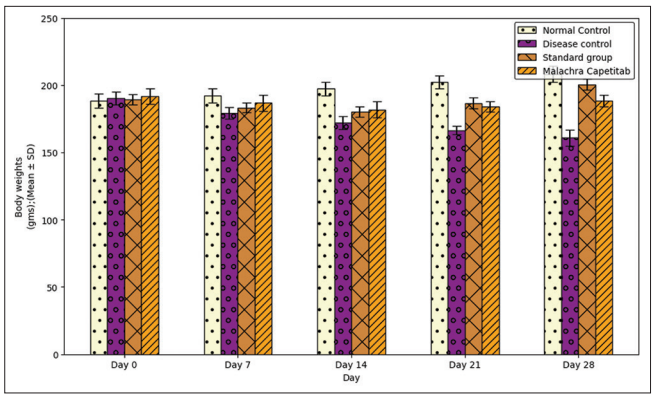


Fig. 4: Effect of *Malachra capitata* extract (MCHA) on body weight in streptozotocin-induced diabetic Wistar rats during the 28-day treatment period. Data are presented as mean±SD (n=6). Statistical symbols and formatting of captions are correct and compatible with the paper.

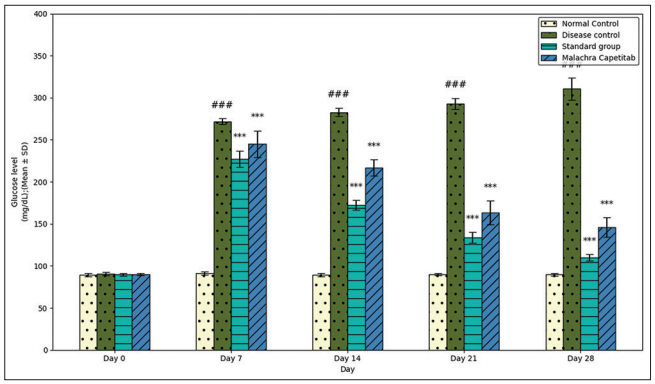


Fig. 5: Effect of *Malachra capitata* extract (MCHA) on fasting blood glucose levels in streptozotocin-induced diabetic Wistar rats over a 28-day treatment period. Data are presented as mean±SD (n=6). Statistical significance was determined using two-way repeated measures analysis of variance followed by Bonferroni's *post hoc* test. ###p<0.0001 compared to normal control group; *p<0.0001 compared to disease control group

the 28 days (from 190.3±4.63 g to 160.8±5.98 g), both the standard and test extract groups showed maintained or improved BWs. The *M. capitata* extract-treated group maintained a relatively stable BW, starting at 191.8±5.64 g and ending at 188.2±4.36 g. This suggests that

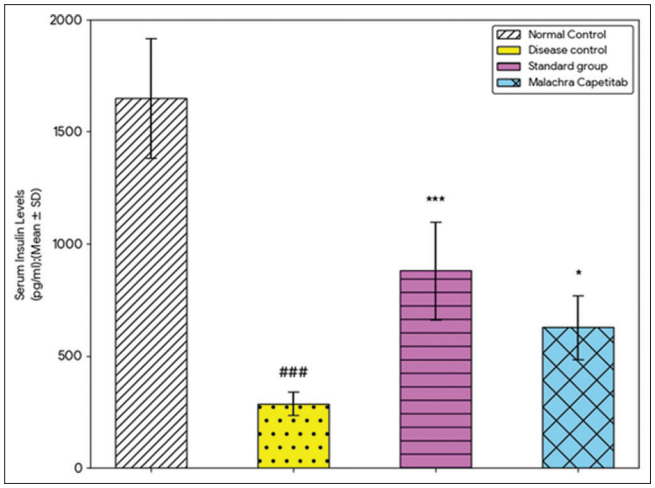


Fig. 6: Effect of *Malachra capitata* extract (MCHA) on serum insulin levels in streptozotocin-induced diabetic Wistar rats after 28 days of treatment. Data are presented as mean±SD (n=6). Statistical analysis was performed using One-way analysis of variance followed by Bonferroni's *post hoc* test. ###p<0.0001 compared to normal control group; *p<0.0001 and *p<0.05 compared to disease control group

Table 9: Effect of *Malachra capitata* extract on serum insulin levels in Wistar rats

Sr. No	Treatment and dose	Serum insulin levels (pg/mL) (Mean±SEM)
1	Normal control	1650.16±267.21
2	Disease control	286.5±52.88###
3	Standard group	879.83±218.85***
4	Test Extract 1 *Malachra Capetita*	627.5±142.78*

Data are expressed as mean±SD (n=6). Statistical analysis was performed using one-way ANOVA followed by Bonferroni's *post hoc* test. ###p<0.0001 compared to the normal control group. *p<0.0001 and *p<0.05 compared to disease control group. ANOVA: Analysis of variance, SD: Standard deviation, BW: Body weight, SEM: Standard error of the mean

the antidiabetic effect of the extract does not occur at the expense of general well-being or body mass, which is often a concern in diabetes management. The results, analyzed by Two-way ANOVA followed by Bonferroni *post hoc* analysis, highlight the significant beneficial effects of *M. capitata* extract on managing diabetes *in vivo*.

Antioxidant enzyme activity

SOD, CAT, and glutathione (GSH) levels improved significantly, while MDA levels decreased in the extract-treated groups, confirming antioxidant protection [31].

The evaluation of antioxidant enzyme activities demonstrated the protective effect of *M. capitata* extract against oxidative stress in STZ-induced diabetic rats. As shown in Table 10 and Fig. 7, the disease control group exhibited a significant decrease in endogenous antioxidant enzymes, including SOD, CAT, and reduced GSH, along with a marked increase in MDA, a biomarker of lipid peroxidation, compared with the normal control group. Specifically, SOD (4.17±1.04 U/mg protein), CAT (29.17±2.08 U/mg protein), and GSH (18.82±0.83 µmol/mg protein) levels were significantly reduced, while MDA levels (5.70±1.43 nmol/mg protein) were significantly elevated in the disease control group (###p<0.0001 vs. normal control).

Treatment with *M. capitata* extract (MCHA) partially restored the antioxidant defense system. The extract-treated group showed an increase in SOD, CAT, and GSH levels compared with the disease control group. However, the increase in SOD activity was moderate and did not

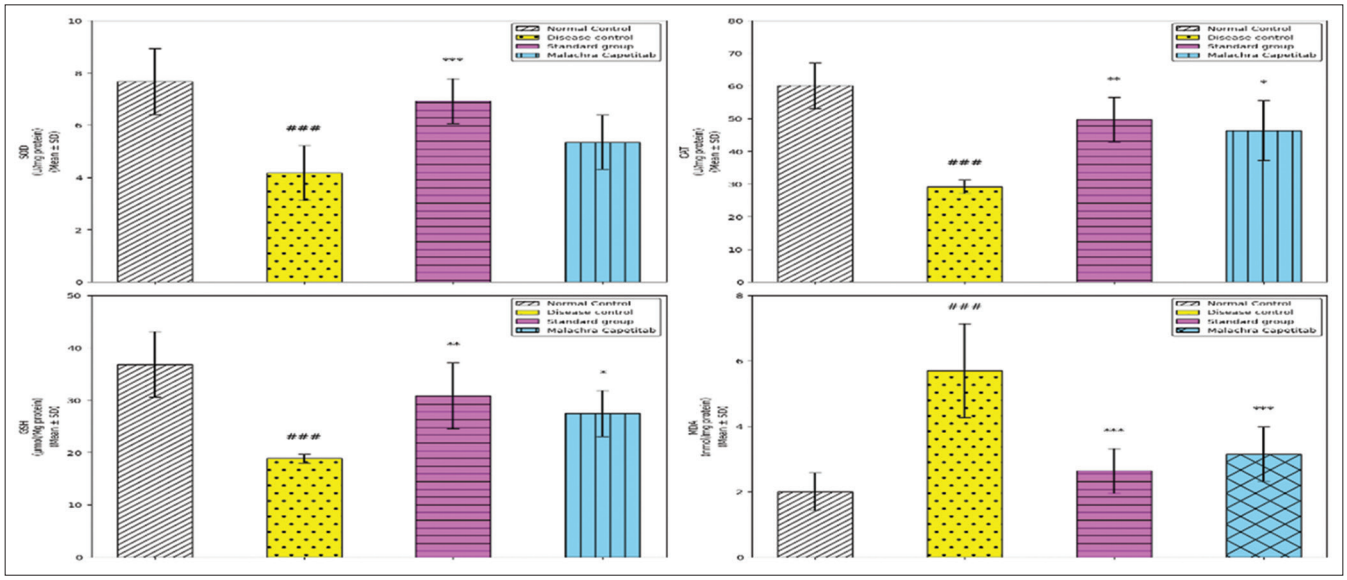


Fig. 7: Effect of *Malachra capitata* extract (MCHA) on antioxidant enzyme activities (superoxide dismutase, catalase, glutathione) and lipid peroxidation marker (malondialdehyde [MDA]) in streptozotocin-induced diabetic Wistar rats. Data are presented as mean±SD (n=6). Statistical significance was determined using one-way analysis of variance followed by Bonferroni's *post hoc* test. ###p<0.0001 compared to normal control group; ***p<0.0001, **p<0.01, *p<0.05 compared to disease control group

Table 10: Effect of *Malachra capitata* extracts on antioxidant enzyme activity SOD, CAT, GSH and MDA in Wistar rats

S. No.	Treatment and dose	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µmol/Mg protein)	MDA (nmol/mg protein)
		(Mean±SEM)			
1	Normal control	7.67±1.26	60.02±6.93	36.82±6.23	2.00±0.58
2	Disease control	4.17±1.04###	29.17±2.08###	18.82±0.83###	5.70±1.43###
3	Standard group	6.92±0.86***	49.73±6.80**	30.87±6.30**	2.63±0.68***
4	Test extract 1 *Malachra Capetita*	5.35±1.04*	46.30±9.16*	27.42±4.36	3.15±0.83***

Data are expressed as mean±SD (n=6). Statistical analysis was performed using one-way ANOVA followed by Bonferroni's *post hoc* test. ###p<0.0001 compared to normal control group. *p<0.0001 and *p<0.05 compared to disease control group. SOD: Superoxide dismutase, CAT: Catalase, GSH: Glutathione, MDA: Malondialdehyde

reach statistical significance, whereas CAT levels showed a statistically significant improvement (46.30±9.16 U/mg protein, *p<0.05 vs. disease control). In addition, MDA levels were significantly reduced (3.15±0.83 nmol/mg protein, ***p<0.0001 vs. disease control), indicating reduced lipid peroxidation and oxidative damage.

The standard treatment group (metformin) showed a more pronounced restoration of antioxidant enzyme activities, with significant increases in SOD, CAT, and GSH levels and a marked reduction in MDA levels compared with the disease control group. These findings suggest that the antidiabetic effect of *M. capitata* may be partly mediated through its antioxidant properties, which help mitigate oxidative stress associated with diabetes.

CONCLUSION

The present investigation clearly demonstrated that *M. capitata* possesses significant hypoglycemic and antioxidant potential, particularly when extracted with 70% ethanol. The bioactivity-guided approach revealed that this extract contains abundant pharmacologically important phytoconstituents, such as flavonoids, phenolics, alkaloids, and saponins, which are likely responsible for its biological effects. The strong inhibition of α-amylase and α-glucosidase indicates an effective mechanism for controlling postprandial hyperglycemia. *In vivo* studies further confirmed its antidiabetic efficacy, as evidenced by improved oral glucose tolerance, marked decrease in fasting blood glucose levels, and notable increase in serum insulin levels in diabetic rats. In addition, the extract mitigated oxidative stress by restoring endogenous antioxidant enzymes and reducing lipid peroxidation, thereby highlighting its protective role against diabetes-associated oxidative damage. An acute toxicity study established a favorable safety profile at therapeutically relevant doses. Collectively, these findings validate the traditional use of *M. capitata* and suggest that it is a promising natural source of anti-diabetic compounds. Further studies focusing on the isolation, structural characterization, and mechanistic evaluation of its active constituents are warranted to bolster its potential as a plant-based antidiabetic therapeutic agent.

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AUTHOR CONTRIBUTIONS

P. Mijgar: Concept, execution, writing. V. Thakare and V.K. Pandey: Toxicology and pathology supervision. U. Deokate: Supervision and review.

CONFLICTS OF INTEREST

None declared.

REFERENCES

1. Krentz AJ, Bailey CJ. Oral antidiabetic agents: Current role in type 2 diabetes mellitus. Drugs 2005;65:385-411.
2. Krentz AJ, Bailey CJ. Oral antidiabetic agents: Current role in type 2 diabetes mellitus. Drugs 2005;65:385-411. doi:10.2165/00003495-200565030-00005.
3. Tundis R, Loizzo MR, Menichini F. Natural products as alpha-amylase and alpha-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: An update. Mini Rev Med Chem. 2010;10(4):315-31. doi: 10.2174/138955710791331007,

PMID 20470247
4. Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TP. Indian herbs and herbal drugs used for the treatment of diabetes. J Clin Biochem Nutr. 2007;40(3):163-73. doi: 10.3164/jcbn.40.163, PMID 18398493
5. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac J Trop Biomed. 2012;2(4):320-30. doi: 10.1016/s2221-1691(12)60032-x, PMID 23569923
6. Patel A, Rahi DS. Phytochemical study of *Malachra capitata* medicinal plant. Int J Adv Acad Stud. 2023;5(9):13-5. doi: 10.33545/27068919.2023.v5.i9a.1045
7. Cervantes-Ceballos L, Sánchez-Hoyos J, Sanchez-Hoyos F, Torres-Niño E, Mercado-Camargo J, Echeverry-Gómez A. An overview of genus *Malachra* L.-ethnobotany, phytochemistry, and pharmacological activity. Plants (Basel). 2022;11(21):2808. doi: 10.3390/plants11212808, PMID 36365260
8. Meng XY, Zhang HX, Mezei M, Cui M. Molecular docking: A powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des. 2011;7(2):146-57. doi: 10.2174/157340911795677602, PMID 21534921
9. Kediso TE, Tolessa T, Getachew F, Makonnen E, Seifu D. Effect of 70% ethanol extract and its solvent fractions of *Artemisia afra* (Jacq. Ex Willd.) against pentylenetetrazole-induced seizure in mice. Evid Based Complement Alternat Med. 2021;2021:6690965. doi: 10.1155/2021/6690965, PMID 34239592
10. Mamoona, Nosheen S, Riaz S, Shah SI, Shahid S. Optimizing extraction methods: The role of solvent polarity in enhancing phenolic content and antioxidant activity in biowaste. Biomass Conv Bioref. 2024;15(11):16721-36. doi: 10.1007/s13399-024-06368-6
11. Kadali SR, Das MC, Bayya MG, Kumar MV. Preliminary phytochemical screening and evaluation of aqueous and ethanolic leaf extracts of *Chloroxylon swietenia* on blood glucose in streptozotocin-induced diabetic rats. Res J Pharm Technol. 2019;12(11):5295-300. doi: 10.5958/0974-360X.2019.00917.X
12. Saradhi Settaluri VS, Mohammed Al Anbari T, Khalifa Al Shukaili S, Mohammed Al Rawahi R, Najmul Hejaz Azmi S, Sah P. Phytochemical screening and *in vitro* evaluation of antioxidant and antimicrobial efficacies of *Pteropyrum scoparium* crude extracts. J King Saud Univ Sci. 2024;36(1):102995. doi: 10.1016/j.jksus.2023.102995
13. Khatri DK, Juvekar AR. α-glucosidase and α-amylase inhibitory activity of *Indigofera cordifolia* seeds and leaves extract. Int J Pharm Pharm Sci. 2014;6(11):152-5.
14. Wulandari WT, Tuslinah L, Aprillia AY, Anggraeni R, Budiani SA. Inhibitory effect of curcumin incorporated in chitosan nanoparticle on α-amylase and α-glucosidase activities. Int J App Pharm. 2022;14(4):45-8. doi: 10.22159/ijap.2022.v14s4.op06
15. Saepudin S, Susilawati Y. Alpha-glucosidase inhibitor activities and phytochemicals screening of the *Peperomia* genus cultivated in Indonesia. Int J App Pharm. 2022;14(5):117-22. doi: 10.22159/ijap.2022.v14s5.23
16. Organization for Economic Co-operation and Development. OECD Guideline for the Testing of Chemicals: Acute Oral Toxicity-Acute Toxic Class Method (Test No. 423). Paris: OECD Publishing; 2001.
17. Rao OU, Eswaraiah MC. Single dose oral toxicity study of ethanolic extract from inflorescence of *Casuarina equisetifolia* in Wistar rats. J Drug Deliv Ther. 2018;8(6-S):44-7.
18. Sato VH, Chewchinda S, Goli AS, Sato H, Nontakham J, Vongsak B. Oral glucose tolerance test (OGTT) evidence for the postprandial anti-hyperglycemic property of *Salacca zalacca* (Gaertn.) Voss seed extract. Molecules. 2023;28(19):6775. doi: 10.3390/molecules28196775, PMID 37836618
19. Goli AS, Sato VH, Sato H, Chewchinda S, Leanpolchareanchai J, Nontakham J. Antihyperglycemic effects of *Lysiphyllum strychnifolium* leaf extract *in vitro* and *in vivo*. Pharm Biol. 2023;61(1):189-200. doi: 10.1080/13880209.2022.2160771, PMID 36625086
20. Ayele AG, Kumar P, Engidawork E. Antihyperglycemic and hypoglycemic activities of the aqueous leaf extract of *Rubus Erlangeri Engl* (Rosacea) in mice. Metab Open. 2021;11:100118.
21. Ramachandran S, Rajasekaran A, Manisenthilkumar KT. Investigation of hypoglycemic, hypolipidemic and antioxidant activities of aqueous extract of *Terminalia paniculata* bark in diabetic rats. Asian Pac J Trop Biomed. 2012;2(4):262-68. doi: 10.1016/S2221-1691(12)60020-3, PMID 23569911
22. Saifullah M, McCullum R, McCluskey A, Vuong Q. Comparison of conventional extraction technique with ultrasound assisted extraction on recovery of phenolic compounds from lemon scented tea tree (*Leptospermum petersonii*) leaves. Heliyon. 2020;6(4):e03666. doi: 10.1016/j.heliyon.2020.e03666, PMID 32258513
23. Lohvina H, Sándor M, Wink M. Effect of ethanol solvents on total phenolic content and antioxidant properties of seed extracts of fenugreek (*Trigonella foenum-graecum* L.) Varieties and determination of phenolic composition by HPLC-ESI-MS. Diversity. 2022;14(1):7. doi: 10.3390/d14010007
24. Etsassala NG, Badmus JA, Marnewick JL, Iwuoha EI, Nchu F, Hussein AA. Alpha-glucosidase and alpha-amylase inhibitory activities, molecular docking, and antioxidant capacities of *Salvia aurita* constituents. Antioxidants (Basel). 2020;9(11):1149. doi: 10.3390/antiox9111149, PMID 33228164
25. Etsassala NG, Badmus JA, Marnewick JL, Egieyeh S, Iwuoha EI, Nchu F. Alpha-glucosidase and alpha-amylase inhibitory activities, molecular docking, and antioxidant capacities of *Plectranthus ecklonii* constituents. Antioxidants (Basel). 2022;11(2):378. doi: 10.3390/antiox11020378, PMID 35204260
26. Chiranthanut N, Lertprasertsuke N, Srisook E, Srisook K. Acute and subchronic oral toxicity assessment of extract from *Etlingera pavieana* rhizomes. Toxicol Rep. 2022;9:1472-83. doi: 10.1016/j.toxrep.2022.07.005, PMID 36518420
27. Chaimum-Aom N, Chomko S, Talubmook C. Toxicology and oral glucose tolerance test (OGTT) of Thai medicinal plant used for diabetes control, *Phyllanthus acidus* L. Pharmacogn J. 2017;9(1):58-61.
28. Yin P, Wang Y, Yang L, Sui J, Liu Y. Hypoglycemic effects in alloxan-induced diabetic rats of the phenolic extract from Mongolian oak cups enriched in ellagic acid, kaempferol and their derivatives. Molecules. 2018;23(5):1046. doi: 10.3390/molecules23051046, PMID 29710864
29. Zher LY, Zheng EK, Raymond RE, Jie YZ, Yap CG. Strategies for optimization of hypoglycemia rat models. Anim Models Exp Med. 2025;8(9):1590-610. doi: 10.1002/ame2.70045, PMID 40692212
30. Abdel-Haleem SA, Ibrahim AY, Ismail RF, Shaffie NM, Hendawy SF, Omer EA. *In-vivo* hypoglycemic and hypolipidemic properties of *Tagetes lucida* alcoholic extract in streptozotocin-induced hyperglycemic Wistar albino rats. Ann Agric Sci. 2017;62(2):169-81. doi: 10.1016/j.aoas.2017.11.005
31. Udi OA, Oyem JC, Ebeye OA, Chris-Ozoko LE, Igbigbi PS, Olannye DU. The effects of aqueous extract of *Ocimum gratissimum* on the cerebellum of male Wistar rats challenged by lead acetate. Clin Nutr Open Sci. 2022;44:28-41. doi: 10.1016/j.nutos.2022.06.001