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SAFETY EVALUATION, IN VITRO ANTIDIABETIC STUDY AND GC-MS PROFILING OF A FORMULATED POLYHERBAL SYRUP

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ABSTRACT

Diabetes mellitus remains a major metabolic disorder requiring safer and affordable therapeutic alternatives. This study evaluated the safety profile, in vitro antidiabetic activity, and phytochemical composition of a formulated polyherbal syrup (PHS) prepared from *Vernonia amygdalina*, *Moringa oleifera*, *Zingiber officinale*, *Allium cepa*, and *Allium sativum*. Aqueous extracts of onion, garlic, and ginger were obtained by decoction of 20 g powdered samples in 100 mL distilled water at 40 °C for 3 h, while fresh leaves of *Moringa oleifera* and *Vernonia amygdalina* were extracted by manual juice expression and filtration. The extracts were combined with honey to produce the polyherbal syrup. Acute toxicity evaluation in albino rats using oral doses of 1000–16000 mg/kg body weight revealed no mortality or visible signs of toxicity, indicating an LD_{50} greater than 16000 mg/kg. Sub-acute toxicity studies conducted at doses of 25–400 mg/kg also showed no adverse behavioural or physiological changes. In vitro antidiabetic assays revealed that PHS is concentration-dependent. The PHS exhibited IC_{50} values (µg/mL) of 8.88 for glucose uptake, 2.55 for α-amylase inhibition, and 3.50 for α-glucosidase inhibition, compared with the standard drug acarbose which showed IC_{50} values of 3.55, 1.89, and 3.39 µg/mL respectively. Although the standard drug showed slightly higher potency, the PHS demonstrated comparable α-glucosidase inhibitory activity. GC–MS profiling revealed numerous bioactive phytochemicals including 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, 5-hydroxymethylfurfural, thymine, n-hexadecanoic acid, and octadec-9-enoic acid. The combined presence of these compounds suggests possible synergistic pharmacological interactions responsible for the observed antidiabetic effects. These findings indicate that the formulated polyherbal syrup is safe and possesses promising antidiabetic potential.

Keywords: Polyherbal Formulation, Antidiabetic, Syrup, LD50, GC-MS, Diabetes Mellitus

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. The condition disrupts the metabolism of carbohydrates, lipids, and proteins and is associated with long-term complications affecting the cardiovascular system, kidneys, nerves, and eyes (Farzaei *et al*., 2017). Despite the availability of several synthetic antidiabetic drugs, their use is often limited by adverse effects, high cost, and reduced long-term efficacy. Consequently, there is growing interest in the development of safer, cost-effective therapeutic alternatives derived from natural products. Medicinal plants have long served as important sources of therapeutic agents due to their rich diversity of bioactive phytochemicals. Numerous plant-derived compounds such as flavonoids, phenolic acids, alkaloids, terpenoids, and glycosides have demonstrated significant pharmacological activities, including antihyperglycemic, antioxidant, and anti-inflammatory effects (Tran *et al*., 2020). These phytochemicals can modulate multiple pathways involved in glucose metabolism, including enhancement of insulin sensitivity, stimulation of glucose uptake, inhibition of carbohydrate-digesting enzymes, and reduction of oxidative stress. As a result, phytotherapy has become an increasingly attractive approach in the management of diabetes and other metabolic disorders (Tran *et al*., 2020). In recent years, attention has shifted toward polyherbal formulations, which involve the combination of two or more medicinal plants to achieve enhanced therapeutic efficacy. The concept of polyherbalism is based on the principle of synergism, where different phytoconstituents interact to produce additive or complementary pharmacological effects. Such combinations may improve therapeutic outcomes by targeting multiple biochemical pathways simultaneously, increasing bioavailability of active compounds, and reducing potential toxicity compared to high doses of single plant extracts (Ramaiah *et al*., 2013; Dauda *et al*., 2020; 2025). Furthermore, polyherbal formulations may overcome the limitations associated with single-plant therapies, in which bioactive phytochemicals often occur in low concentrations that may be insufficient to elicit significant therapeutic effects when administered individually (Lakshmi *et al*., 2025). The present study focuses on a polyherbal syrup formulated from *Vernonia amygdalina, Moringa oleifera, Zingiber officinale, Allium cepa,* and *Allium sativum*, plants that are widely consumed as food or traditional medicine and are recognized for their diverse pharmacological properties. *Vernonia amygdalina* (bitter leaf) is rich in sesquiterpene lactones, flavonoids, and phenolic compounds, which have been reported to exhibit antihyperglycemic, antioxidant, and anti-inflammatory activities. These compounds can enhance glucose utilization and improve insulin sensitivity (Great *et al*., 2023). *Moringa oleifera* leaves contain abundant polyphenols, vitamins, and minerals and have been widely documented for their hypoglycemic, lipid-lowering, and antioxidant effects, making them valuable in metabolic disease management (Vergara-Jimenez *et al*., 2017).

Zingiber officinale (ginger) contains bioactive constituents such as gingerols, shogaols, and paradols, which have been shown to improve glucose metabolism, inhibit carbohydrate-digesting enzymes, and enhance insulin sensitivity (Li *et al*., 2012). Similarly, *Allium cepa* (onion) possesses flavonoids such as quercetin that have demonstrated significant antidiabetic effects through antioxidant activity, inhibition of glucose absorption, and improvement of pancreatic β-cell function (Kianian *et al*., 2021). *Allium sativum* (garlic) is rich in organosulfur compounds such as allicin and diallyl sulfides, which are known to reduce blood glucose levels, improve lipid metabolism, and protect against oxidative stress (Shang *et al*., 2019).

The selection of these plants for the formulation was therefore based on their documented antidiabetic potential, complementary mechanisms of action, nutritional value, and widespread availability in traditional medicine. Combining these plant extracts into a single polyherbal formulation may provide synergistic therapeutic benefits by simultaneously targeting multiple pathways involved in glucose regulation, including enzyme inhibition, glucose adsorption, enhancement of cellular glucose uptake, and antioxidant protection. Such a multi-target approach is particularly beneficial in diabetes management, where complex metabolic dysregulation is involved.

Therefore, the present study aimed to evaluate the safety, in vitro antidiabetic activity, and phytochemical profile of a formulated polyherbal syrup prepared from *Vernonia amygdalina*, *Moringa oleifera*, *Zingiber officinale*, *Allium cepa*, and *Allium sativum* using acute toxicity studies, enzyme inhibition assays, glucose uptake and adsorption analyses, and GC–MS profiling.

MATERIALS AND METHODS

Sample Collection

While the leaves of *Moringa oleifera* (drum stick) and *Vernonia amygdalina* (bitter leaf) were gathered from Anyigba in the Dekina Local Government Area of Kogi State, Nigeria, the bulb of *Allium cepa* (onion), the clove of *Allium sativum* (garlic), and the rhizome of *Zingiber officinale* (ginger) were bought from a nearby market. After that, the samples were brought in a polythene bag to the lab. Mr. Ayegba, O. Sule of the Botany Unit's Herbarium division at Kogi State University in Anyigba, Nigeria, identified the samples before they were analyzed. *Allium cepa*, *Allium sativum*, *Zingiber officinale*, *Moringa oleifera*, *Vernonia amygdalina*, and *Zingiber officinale* have specimen voucher numbers PT-017, PT-018, PT-0318, and PT-0307 respectively.

Preparation of Onion (Allium cepa) Sample

The onion bulbs were cleaned and all unwanted dirt washed away by running a faucet. Using a knife, the clean onions were peeled by hand and then cut into 5 mm-thick slices. Before being used, the sliced onions were kept fresh and dry in food-grade plastic containers that were sealed tightly.

Preparation of Garlic (Allium sativum) Sample

To get rid of dirt, the garlic cloves were cleaned. Using a knife, the cleaned garlic was chopped into smaller pieces. After being air-dried, the sliced garlic was kept until further examination in an airtight polythene bag.

Preparation of Ginger (Zingiber officinale) Sample

Ginger rhizomes were washed to remove dirt. With a knife, the clean gingers were cut into smaller pieces. The sliced gingers were air-dried before being stored in an airtight polythene bag until further examination.

Preparation of Drumstick (Moringa oleifera) and Bitter Leaf (Vernonia amygdalina) Samples

Leaves of Moringa and Bitter Leaf leaves were washed to remove dirt. The cleaned leaves were kept fresh in a polythene bag until they were analyzed.

Preparation of Extracts

i. Twenty grams (20 g) of each pulverized sample (onion, garlic and ginger) was extracted with 100 mL of distilled water by decoction method for a period of 3 hours at 40 °C. Each resulting mixture was filtered, and the filtrate collected and stored in separate containers
ii. Fifty gram each of the fresh leaves of Moringa and bitter leaf were juiced by hand squeezing. Each resulting juice mixture was collected separately by filtration and stored in the refrigerator for further analysis.

Formulations of Polyherbal Syrup (PHS)

The polyherbal syrup was prepared and formulated using extracts of *Vernonia amygdalina* (bitter leaf), *Moringa oleifera* (drumstick), *Allium cepa* (onion), *Allium sativum* (garlic), and *Zingiber officinale* (ginger), as presented in Table 1.

Table 1: Formulation of Polyherbal Syrup (PHS)

Ingredients	Formulation (mL) PHS
Allium cepa (Onion bulbs)	10
Allium sativum (Garlic cloves)	10
Zingiber officinale (Ginger rhizome)	10
Moringa oleifera (Moringa leaves)	10
Vernonia amygdalina (Bitter leaves)	10
Honey	50

Lethal dose (LD_{50}) Analysis

Animals

Matured and healthy Albino rats of both sexes weighing between 134 and 175 g were purchased. The rats were housed in a well-ventilated wooden cage with a stainless-steel top grill and pelleted food facilities. For two weeks prior to the experiment, the animals were kept in a well-ventilated animal house on a 12-hour light and dark cycle at room temperature. The animals were fed a standard growers' mash diet and had access to clean drinking water.

Toxicity Range Finding Test

Prior to determining the LD_{50}, ten albino rats (134-175 g) were used in a pilot study to select dose ranges for subsequent toxicity testing. The syrup was administered orally to five pairs of rats in doses ranging from 1000, 2000, 4000, 8000, and 16000 mg/kg body weight. Over the course of 24 hours, the treated rats were monitored for signs of toxicity and death. The highest dose that killed no rats was recorded, as was the lowest dose that killed 50 % of the animals.

Sub-Acute Toxicity Screening

The purpose of this study was to determine the median lethal dose (LD_{50}) of polyherbal syrup (PHS). For this study, twenty Albino rats of both sexes were used. The syrup was given orally to four pairs of rats at doses ranging from 25, 50, 100, 200, and 400 mg/kg body weight. Over the course of 24 hours, the treated rats were monitored for signs of toxicity and death. Karber's arithmetic method was used to calculate the lethal dose (LD_{50}) (Shetty and Alwar, 2007). The LD_{50} was calculated using the formula:

$LD_{50} = DH - \sum(Dd\ x\ Md)/N$

DH = Highest dose (LD_{100})

N = Number of animals per group

DD = Dose difference

MD = Mean death

Gas Chromatography Mass Spectroscopic (GC-MS) Analysis of the PHS

GC-MS analysis was carried out in a combined gas chromatograph system (Agilent 6890N) and mass spectrophotometer (5973 MSD), fitted with DB-5MS capillary column (30.0 m x 0.25 mm; film thickness 0.25 µm). 2 mL of the sample extract (PHS) was injected into the GC column for analysis. The initial temperature was set at 40 °C which increased to 150 °C at the rate of 10 °C/min. The temperature was again increased to 230 °C at the rate of 5 °C/min. The process continued till the temperature reached 310 °C at the rate of 20 °C/min which was held for 8 minutes. The injector port temperature remained constant at 280 °C and the detector temperature was 250 °C then. Helium was used as the carrier gas with a flow rate of 1mL/min. Split ratio and ionization voltage were 110:1 and 70 eV respectively.

The unknown components in the sample (PHS) were identified based on their individual mass spectra peak value, and the mass spectrum was interpreted using the National Institute of Science and Technology's (NIST) 2014 database. The molecular weight, retention duration, and percentage content of the samples were then determined.

In vitro Antidiabetic Study

Glucose Adsorption Assay

The syrup's glucose adsorption capability was evaluated using the Ou et al. (2001) technique. One gram of the syrup was mixed into 100 mL of glucose solution at five different concentrations (5, 10, 15, 20, 30, and 60 mM). These combinations were well mixed, agitated, and incubated in a shaker water bath at 37 ℃ for 6 hours each. After incubation, the mixture was centrifuged at 4800 rpm for 20 minutes, and the glucose concentration in the supernatant was measured with a glucose oxidase peroxidase testing kit. The quantity of bound glucose was calculated using the following formula:

$$Glucose\ bound = \frac{G1 - G6}{weight\ of\ sample}\ x\ volume\ of\ sample$$

Glucose Uptake Assay

This assay used Cirillo's well-defined technique (Cirillo *et al*., 1962). One percent (1%) suspension of commercial baker's yeast was prepared by dissolving it in distilled water. The suspension was kept overnight at ambient temperature (25 ℃). In the following days, the yeast cell culture was centrifuged for 5 minutes at 4200 rpm (Microfuge16 Centrifuge, FX241.5P Rotor, 50/60Hz and 220-240V). The operation was repeated by adding distilled water to the pallet until a clear supernatant was formed. Exactly 10 parts of the clear supernatant fluids were mixed with 90 parts of distilled water to make a 10%v/v yeast cell solution.

5 mg of syrup was combined with dimethyl sulfoxide (DMSO) until dissolved. The mixture was then combined with varying amounts of 1 mL glucose solution and incubated for 10 minutes at 37 ℃. To begin the process, 100 μL of yeast suspension was mixed with glucose and syrup, vortexed, and incubated for an additional 60 minutes at 37 ℃. Following incubation, the tubes were spun at 3800 rpm for 5 minutes before glucose was measured at 520 nm with a spectrophotometer (UV 5100B). Absorbance for the appropriate control was also measured at the same wavelength. The percentage increase in uptake was estimated using the following formula:

$$\%\ glucose\ uptake = \frac{(Abs.of\ control - Abs.of\ sample)}{Abs.\ of\ control}\ x\ 100$$

In Vitro A-Amylase Inhibitory Studies

This experiment was carried out utilizing McCue and Shetty's modified approach (Mccue and Shetty, 2004). In a tube, 250 µL of extract was mixed with 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase. After pre-incubating at 25 ℃ for 10 minutes, 250 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at regular intervals and incubated at 25 °C for another 10 minutes. Following incubation, the reaction was halted using 500 µL of dinitrosalicylic acid (DNS) reagent. After 5 minutes of incubation in boiling water, the tubes were cooled to room temperature. The reaction mixture was diluted with 5 mL of distilled water, and the absorbance was measured at 540 nm with a spectrophotometer.

A control was made by using the same approach but replacing the extract with distilled water. The α-amylase inhibitory activity was calculated as a percentage inhibition. The syrup concentrations that inhibit enzyme activity by 50 % (IC_{50}) were graphically determined.

$$\%\ inhibition = \frac{Abs.of\ control - Abs.of\ sample}{Abs.of\ control}\ x\ 100$$

In Vitro A-Glucosidase Inhibitory Studies

The syrup's influence on α-glucosidase activity was tested using α-glucosidase from Saccharomyces, as described by Kim et al. (2005). The substrate solution, p-nitropheynyl glucopyranoside (pNPG), was produced in 20 mM phosphate buffer at pH 6.9. 100 µL of α-glucosidase (E.C. 3.2.1.20) was pre-incubated with 50 µL of extract concentrations (acetone, ethanol, and water) for 10 minutes. The reaction was initiated by adding 50 µL of 3.0 mM (pNPG) substrate diluted in 20 mM phosphate buffer (pH 6.9). The reaction mixture was incubated at 37 ℃ for 20 minutes before stopping with 2 mL of 0.1 M Na_2CO_3. The activity of α-glucosidase was evaluated by measuring the yellow para-nitrophenol produced from pNPG at 405 nm. The data were presented as a percentage of the blank control. The syrup concentrations that inhibit enzyme activity by 50 % (IC_{50}) were graphically calculated.

$$\%\ inhibition = \frac{Abs.of\ control - Abs.of\ sample}{Abs.of\ control}\ x\ 100$$

RESULTS AND DISCUSSION

The results of the analyses of the polyherbal syrup (PHS), including acute toxicity (LD_{50}), in vitro antidiabetic activity, and GC–MS profiling, are presented in Tables 2–5 and Figures 1–9. Tables 2-4 show the LD_{50} results and clinical behavioural signs from the toxicity study. Figures 1–8 present the in vitro antidiabetic activity, while Figure 9 and Table 5 show the GC–MS profile of the polyherbal syrup and the identified phytochemical constituents.

Table 2: Acute Toxicity of the Polyherbal Syrup in Albino Rats

Group	No. per group	No. of Death	Weight (g)	Dose (mg/kg)
1	2	0	147	1000
2	2	0	167	2000
3	2	0	160	4000
4	2	0	175	8000
5	2	0	134	16000

Table 3: Sub-acute Toxicity of the Polyherbal Syrup in Albino Rats

Dose (mg/kg)	No. of Death	Mean Death (Md)	Dose Difference (Dd)	Md x Dd
25	0	0	0	0
50	0	0	25	0
100	0	0	50	0
200	0	0	100	0
400	0	0	200	0

Table 4: Effect of Test doses of PHS on Clinical Signs of Behaviour and Physical Parameters in Sub-acute Toxicity Study Rats

Clinical parameters	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Itching	N	N	N	N	N
Eye discharge	N	N	N	N	N
Nasal discharge	N	N	N	N	N
Skin lesson	N	N	N	N	N
Abnormal movement	N	N	N	N	N
Urination	Normal	Normal	Normal	Normal	Normal
Food intake	Normal	Normal	Normal	Normal	Normal
Water intake	Normal	Normal	Normal	Normal	Normal

N = no clinical sign, (M) = moderate clinical sign, (S) = severe clinical sign

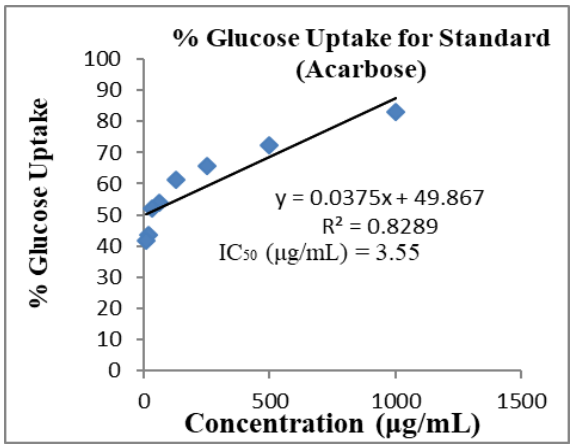


Figure 1: Percentage Glucose Uptake of Standard Drug

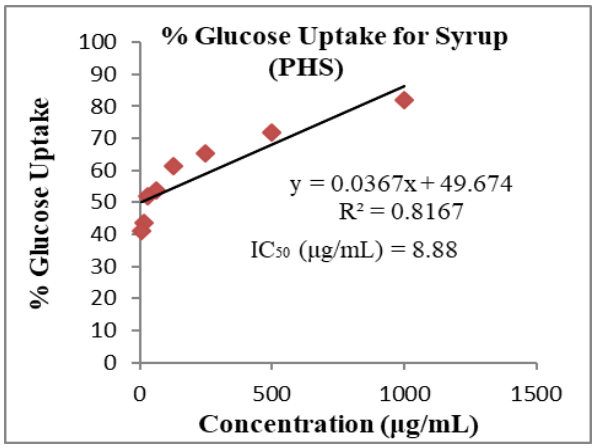


Figure 2: Percentage Glucose Uptake of Polyherbal Syrup

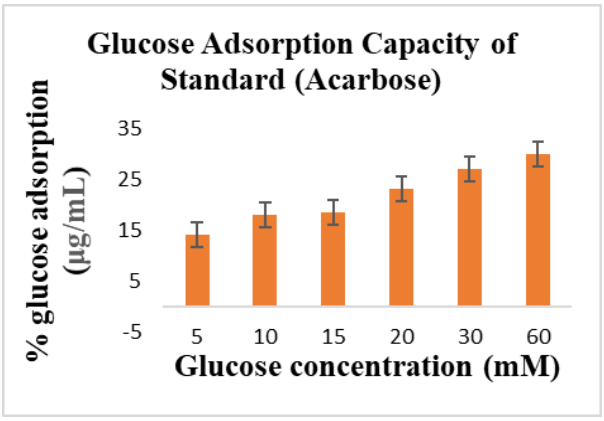


Figure 3: Percentage Glucose Adsorption of Standard Drug

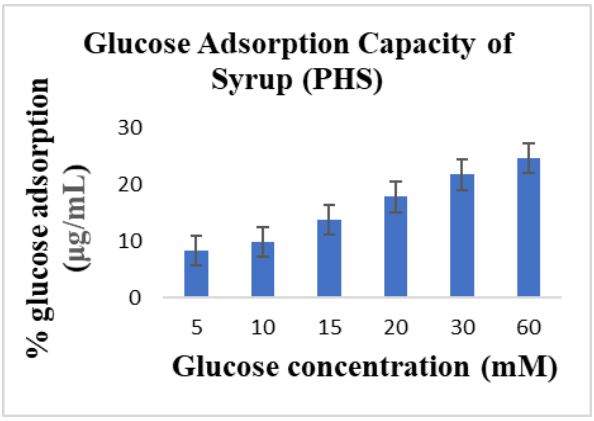


Figure 4: Percentage Glucose Adsorption of Polyherbal Syrup

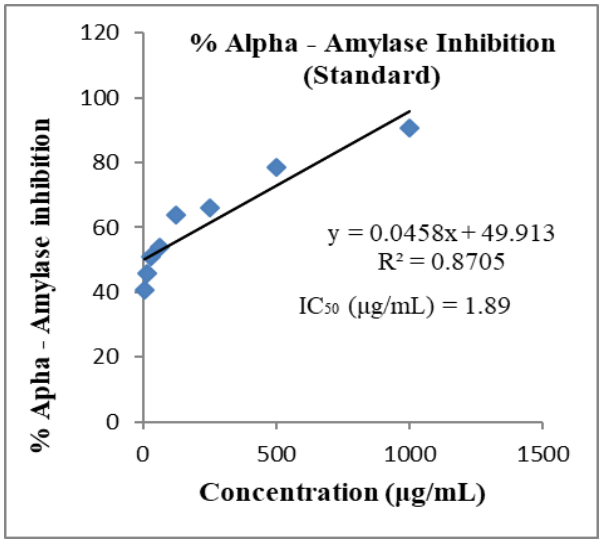


Figure 5: Percentage Inhibition of α-amylase on Standard Drug

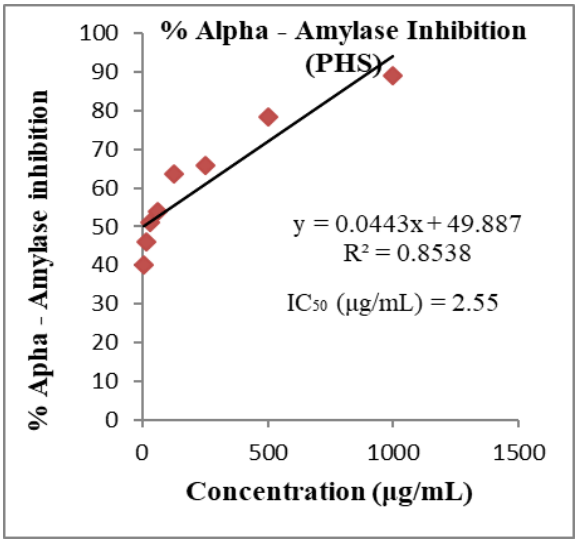


Figure 6: Percentage Inhibition of α-amylase on Polyherbal Syrup

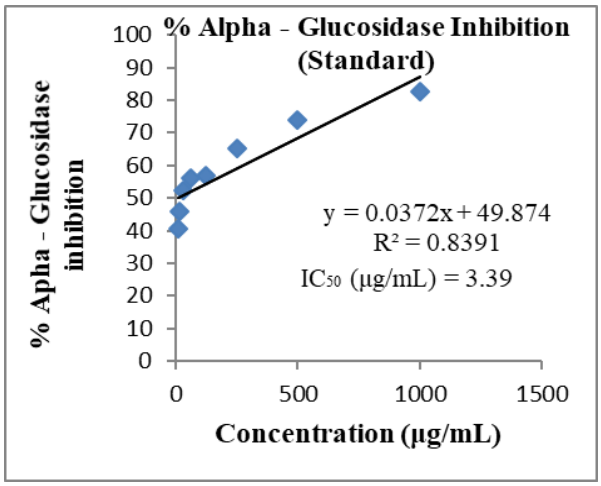


Figure 7: Percentage Inhibition of α-Glucosidase on Standard Drug

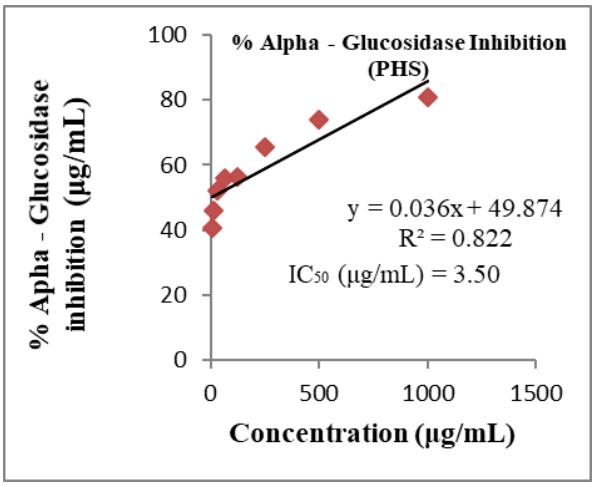


Figure 8: Percentage Inhibition of α-glucosidase on Polyherbal Syrup

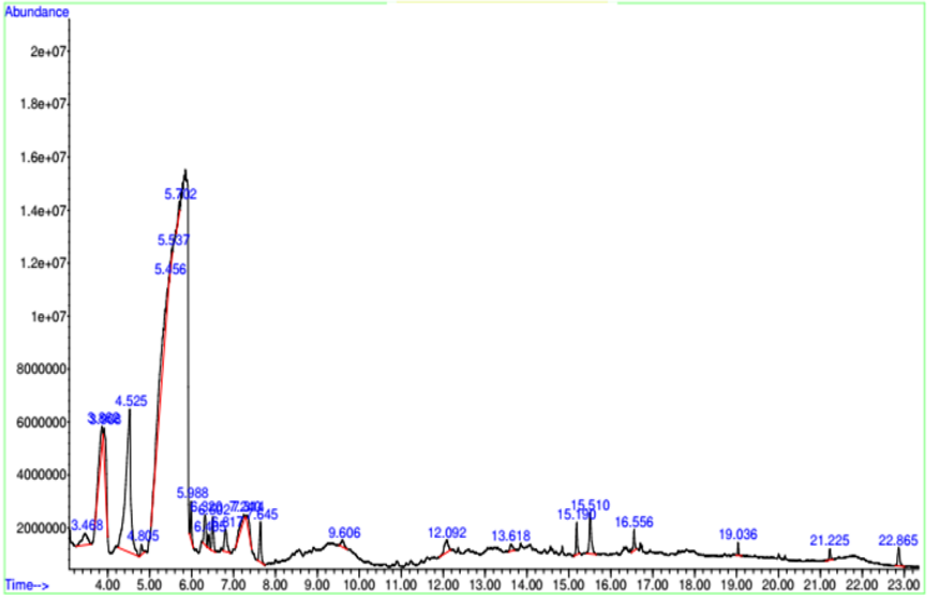
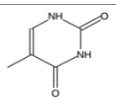
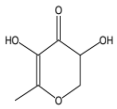
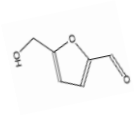
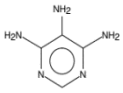
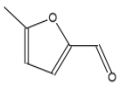


Figure 9: GC-MS Chromatogram of the Polyherbal Syrup (PHS)

Table 5: Phytochemical Profile of the Polyherbal Syrup (PHS) by GC-MS

S/N	RT	Peak Name	MF	MW	% Peak Area	Structure	Class of Compound	Activity
2	3.862	Thymine	$C_5H_6N_2O_2$	126	5.67		Pyrimidine	Stabilizes nucleic acid structures, conversion of carbohydrate into energy (Yazan and Kimberly, 2023)
4	4.525	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	$C_6H_8O_4$	144	34.08		Pyran ketone	Antitumor, antibiotic, antibacterial, antiallergic, hypolipidemic (Sarita *et al*., 2020)
6	5.456	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	19.12		Furan Aldehyde	Antioxidant, anti-allergic, anti-inflammatory, anti-sickling, anti-hypoxic (Shapla *et al*., 2018)
13	7.645	4,5,6-Pyrimidinetriamine	$C_4H_7N_5$	125	3.47		Aromatic heterocycle (amino pyrimidine)	Anticancer, anti-inflammatory, antioxidant (BenchChem, 2023)
17	15.190	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	2.01		Fatty acid (palmitic acid)	Antioxidant, antimicrobial, hypocholesterolemic, nematicide, pesticide, hemolytic (Starlin *et al*., 2019a)
18	15.510	5-Methyl-2-furancarboxaldehyde	$C_6H_6O_2$	110	4.11		Furan aldehyde	Flavouring agent, human metabolite, acetolactate synthase inhibitor (Kumar *et al*., 2019)
19	16.556	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282	1.33		Fatty acid (Oleic acid)	Anticancer, anti-inflammatory, reduces cardiovascular diseases, antioxidant, hypocholesterolemic, anticoronary, anti-arthritic (Starlin *et al*., 2019b)

RT = Retention Time MF = Molecular Formula MW = Molecular Weight

Discussion

Safety Evaluation

The safety evaluation of the polyherbal syrup (PHS) demonstrated a high margin of toxicological safety based on both acute and sub-acute toxicity assessments in albino rats. Acute toxicity assessment showed that oral administration of the formulation at doses up to 16000 mg/kg body weight produced no mortality or observable toxic symptoms, indicating that the median lethal dose (LD_{50}) is greater than this value (Tables 2 and 3). Based on the Hodge and Sterner toxicity scale, substances with LD_{50} values above 5000 mg/kg are considered practically non-toxic, suggesting that the polyherbal syrup is relatively safe for oral use. In addition to the absence of mortality, no clinical signs of toxicity such as itching, nasal discharge, abnormal movement, or changes in feeding and drinking behaviour were observed during the sub-acute toxicity study (Table 4). The animals maintained normal physiological activities including food intake, water consumption, and urination, indicating that the formulation did not adversely affect metabolic or neurological functions. These findings are consistent with recent studies on polyherbal formulations, which have reported high LD_{50} values and minimal toxic effects following oral administration (Sholikhah et al., 2020; Singh and Ilango, 2024). Such safety profiles are commonly attributed to the synergistic effects of phytochemicals such as flavonoids, phenolics, and alkaloids present in medicinal plants. Overall, the results indicate that the polyherbal syrup is a promising natural therapeutic candidate.

In vitro Antidiabetic Study

Effect on Glucose Uptake

The effect of the standard drug, acarbose, and the polyherbal syrup on glucose uptake is presented in Figures 1 and 2. The results demonstrated a concentration-dependent increase in glucose uptake for both samples, indicating enhanced glucose utilization with increasing concentration. However, acarbose exhibited significantly higher glucose uptake activity with a

lower IC_{50} value (3.55) compared with the polyherbal syrup (IC_{50} = 8.88). The lower IC_{50} value of acarbose indicates greater potency, as a smaller concentration is required to achieve 50 % glucose uptake activity. This observation is consistent with the pharmacological properties of acarbose, which improves glycemic regulation by inhibiting carbohydrate-digesting enzymes and reducing glucose availability in the intestinal lumen. Despite its lower activity relative to the standard drug, the polyherbal syrup demonstrated appreciable glucose uptake across the tested concentrations. This suggests that the herbal formulation contains bioactive phytochemicals capable of stimulating glucose transport and cellular utilization. Plant-derived compounds such as flavonoids, phenolic acids, and alkaloids have been reported to enhance glucose uptake through mechanisms including stimulation of insulin signaling pathways and activation of glucose transporter proteins such as GLUT4 (Hanhineva et al., 2010). Therefore, the observed activity of the polyherbal formulation may be attributed to the synergistic actions of multiple phytochemical constituents present in the medicinal plants used in the formulation.

Effect on Glucose Adsorption

The glucose adsorption capacities of acarbose and the polyherbal syrup are illustrated in Figures 3 and 4. Both samples exhibited increasing glucose adsorption with increasing concentration, indicating a dose-dependent interaction between the test samples and glucose molecules. Across the tested concentration range, acarbose showed higher glucose adsorption capacity (14.06 – 29.92 %) compared with the polyherbal syrup (8.23 – 24.58 %). The higher adsorption capacity observed for acarbose suggests stronger glucose-binding ability and greater efficiency in limiting glucose availability for absorption. Glucose adsorption is considered an important mechanism in the control of postprandial hyperglycemia because it reduces the amount of free glucose available in the gastrointestinal tract for absorption. Compounds capable of binding glucose molecules may delay glucose diffusion and subsequently reduce the rate at which glucose enters the bloodstream (Ou et al., 2001). Although the polyherbal syrup demonstrated comparatively lower adsorption capacity, its moderate glucose binding ability suggests that it may contribute to glycemic regulation by delaying intestinal glucose absorption. The adsorption activity observed may be associated with dietary fiber and polyphenolic compounds present in the herbal components, which are known to interact with glucose molecules through hydrogen bonding and physical adsorption mechanisms.

α-Amylase Inhibitory Activity

Inhibiting the α-amylase and α-glucosidase enzymes involved in carbohydrate metabolism is a known approach in diabetes care for lowering postprandial hyperglycemia (Adefegha and Oboh, 2012). To successfully control the glycemic index in diabetes, moderate doses of α-amylase inhibitors and powerful levels of α-glucosidase inhibitors are required. These inhibitors help regulate dietary sugar levels for absorption in the small intestine (Ojo et al., 2022). The inhibitory activities of the samples against Alpha-amylase are presented in Figures 5 and 6. Both the standard drug and the polyherbal syrup exhibited concentration-dependent inhibition of α-amylase activity. Acarbose demonstrated stronger inhibition with a lower IC_{50} value (1.89) compared with the polyherbal syrup (IC_{50} = 2.55), indicating greater enzyme inhibitory potency. This observation aligns with the established mechanism of acarbose, which competitively inhibits carbohydrate-hydrolyzing enzymes in the digestive tract and prevents the rapid conversion of starch into glucose. Inhibition of α-amylase is beneficial in the management of Diabetes mellitus because it slows the digestion of complex carbohydrates, thereby reducing the rate of glucose release into the bloodstream and preventing rapid postprandial spikes in blood glucose levels (Chau et al., 2003; Kwon et al., 2007). This impact would delay the breakdown of starch and oligosaccharides, resulting in decreased glucose absorption and, as a result, inhibiting the increase in postprandial blood glucose (Lee et al., 2011).

α-Glucosidase Inhibitory Activity

The inhibitory activities of the samples against Alpha-glucosidase are presented in Figures 7 and 8. Both acarbose and the polyherbal syrup exhibited dose-dependent inhibition of α-glucosidase activity. Acarbose showed strong inhibitory activity with an IC_{50} value of 3.39, confirming its effectiveness as a standard inhibitor of carbohydrate digestion. Interestingly, the polyherbal syrup demonstrated comparable inhibitory activity with an IC_{50} value of 3.50, indicating a relatively similar potency in inhibiting this enzyme. α-Glucosidase catalyzes the final step in the hydrolysis of oligosaccharides and disaccharides into glucose in the small intestine. Inhibition of this enzyme delays carbohydrate digestion and reduces the rate of glucose absorption, thereby helping to maintain stable blood glucose levels after meals (Kim et al., 2005). The relatively close IC_{50} values observed for the polyherbal syrup and acarbose suggest that the herbal formulation possesses promising α-glucosidase inhibitory potential. This inhibitory activity may result from synergistic interactions among multiple phytochemical constituents present in the polyherbal formulation. Several plant-derived secondary metabolites, including flavonoids, tannins, and phenolic compounds, have been reported to inhibit digestive enzymes through interactions with catalytic residues or conformational modification of enzyme structures (Tadera et al., 2006). The presence of such phytochemicals in the herbal formulation may therefore account for the observed inhibitory activity. Collectively, the results of this study demonstrate that the polyherbal syrup possesses significant glucose-modulating properties through multiple mechanisms, including enhanced glucose uptake, glucose adsorption, and inhibition of key carbohydrate-digesting enzymes such as α-amylase and α-glucosidase. Although the activity of the polyherbal formulation was generally lower than that of acarbose in most assays, its comparable α-glucosidase inhibitory activity and moderate effects on glucose uptake and adsorption suggest promising antidiabetic potential.

The antidiabetic activities observed in the present study are consistent with findings from recent investigations on polyherbal formulations that target multiple pathways involved in glucose metabolism. Polyherbal therapies are increasingly recognized for their multi-target pharmacological effects in the management of diabetes mellitus due to synergistic interactions among diverse phytochemical constituents. For example, a study by Kitphati et al. (2024) reported significant antihyperglycemic effects in a polyherbal formulation composed of several medicinal plant extracts through mechanisms such as enhanced glucose uptake, inhibition of intestinal glucose absorption, and suppression of carbohydrate-digesting enzymes. These findings are comparable with the results of the present study, where the polyherbal syrup demonstrated concentration-dependent glucose uptake and inhibitory effects on α-amylase and α-glucosidase enzymes. Although the standard drug acarbose exhibited stronger activity, the polyherbal syrup

showed considerable inhibitory potential, particularly against α-glucosidase. Similarly, Sridevi and Thirumal (2025) reported that a polyherbal combination containing Curcuma longa, Emblica officinalis, and Trigonella foenum-graecum significantly inhibited digestive enzymes and enhanced glucose uptake. These activities were attributed to synergistic phytochemical interactions. The observed enzyme inhibition in the present study may also be associated with flavonoids and phenolic compounds, which are known to interact with enzyme active sites, thereby slowing carbohydrate hydrolysis and reducing postprandial glucose levels (Lam et al., 2024). Therefore, the findings of this study support the potential use of the polyherbal syrup as a natural therapeutic agent for the management of diabetes mellitus. Nevertheless, further in vivo studies, pharmacokinetic investigations, and clinical trials are required to validate its efficacy, safety, and mechanism of action.

GC-MS Profiling of the Polyherbal Syrup

The GC–MS analysis of the polyherbal syrup (PHS) revealed a diverse range of bioactive phytochemical compounds (Table 5 and Figure 9), indicating that the formulation contains multiple secondary metabolites that may contribute to its pharmacological activities. The compound with the highest abundance was 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (34.08%), a pyran ketone known for its antibacterial, antitumor, antiallergic, and hypolipidemic properties (Sato et al., 2025), suggesting a possible contribution to the therapeutic activity of the syrup. Another major compound detected was 5-hydroxymethylfurfural (19.12%), which possesses antioxidant, anti-inflammatory, anti-allergic, and anti-hypoxic properties (Kong et al., 2019). Antioxidant compounds are particularly important in diabetes management because they help reduce oxidative stress associated with hyperglycemia and protect pancreatic β-cells (Lowell et al., 2024). Other compounds identified include thymine, 5-methyl-2-furancarboxaldehyde, and 4,5,6-pyrimidinetriamine, which are heterocyclic compounds involved in metabolic and antioxidant activities (Banerjee, 2012). The presence of fatty acids such as n-hexadecanoic acid (palmitic acid) and octadec-9-enoic acid (oleic acid) suggests additional pharmacological benefits, including anti-inflammatory, antioxidant, and hypocholesterolemic effects that are beneficial in diabetes management (Kaur et al., 2014).

CONCLUSION

The present study demonstrated that the formulated polyherbal syrup containing Vernonia amygdalina, Moringa oleifera, Zingiber officinale, Allium cepa, and Allium sativum possesses a high safety margin and promising antidiabetic potential. Acute toxicity assessment showed no mortality or observable toxic effects in albino rats at doses up to 16,000 mg/kg body weight, indicating that the formulation is practically non-toxic. In vitro antidiabetic assays revealed concentration-dependent glucose adsorption, enhanced glucose uptake, and significant inhibition of key carbohydrate-digesting enzymes, α-amylase and α-glucosidase, which are important in the regulation of postprandial hyperglycemia. GC–MS analysis further identified several bioactive phytochemicals with known antioxidant, anti-inflammatory, and metabolic regulatory properties that may contribute synergistically to the observed biological effects. Overall, the findings suggest that the polyherbal syrup represents a promising natural candidate for diabetes management; however, further in vivo studies and clinical investigations are required to confirm its therapeutic efficacy and long-term safety.

REFERENCES

Adefegha, S. A. and Oboh, G. (2012). Inhibition of key enzymes linked to type 2 diabetes and sodium nitroprusside-induced lipid peroxidation in rat pancreas by water extractable phytochemicals from some tropical spices. Pharmaceutical Biology, 50, 857–865

Banerjee, R. (2012). Medicinal significance of furan derivatives: A Review. International Journal of Review Life Science, 2(1), 7-16

Chau, C., Huang, Y. and Lee, M. (2003). In Vitro Hypoglycemic Effects of Different Insoluble Fiber-Rich Fractions Prepared from the Peel of Citrus Sinensis L. Journal of Agricultural and Food Chemistry, 51, 6623–6626

Cirillo, V. P. (1962). Mechanism of glucose transport across the yeast cell membrane. Journal of Bacteriology, 84, 485–491

Dauda, J. A., Ameh, E. M., Adaji, M. U., Ocheme, G. W. and Nayo, R. O. (2020). Nutritional Composition and Antioxidant Analyses of Ageratum conyzoides Whole Plant. International Journal of Scientific research Publication, 10(8), 922-928

Dauda, J. A., Khan, M. E., Abdullahi, S., Jacob, A. D., Idowu, O. L., Owoyemi, W. I., Akor, J. A., Olupinyo, O. and Oteno. F. (2025). Physicochemical Properties, Phytochemical Contents, Antibacterial and Antioxidant Potentials of a Formulated Polyherbal Syrup. ChemClass Journal, 9(4), 261-276.

Farzaei, F., Morovati, M. R., Farjadmand, F. and Farzaei, M. H. (2017). A mechanistic review on medicinal plants used for diabetes mellitus in traditional persian medicine. Journal of Evidence-Based Complementary and Alternative Medicine, 22, 944–955.

Great, I. E., Princess, O. S., Agatha, N. J., Favour, O. O., Laura, I. I., Promise, O., Ajiri, R. O., Joy, E, Victor, O. I., Chinenye, F. O., Eunice, U., Jonathan, O., Miracle, I., Ifechukwude, D. N., Sheyi, R. A. and Joy, E. O. (2023). Biological and bioactive components of bitter leaf (Vernonia amygdalina leaf): Insight on health and nutritional benefits. A review. Food Chemistry Advances, 3, 100488

Hanhineva, K., Törrönen, R. and Bondia-Pons, I. (2010). Impact of dietary polyphenols on carbohydrate metabolism. International Journal of Molecular Sciences, 11, 1365–1402

Kaur, N., Chugh, V. and Gupta, A. K. (2014). Essential fatty acids as functional components of foods- a review. Journal of food science and technology, 51(10), 2289–2303. https://doi.org/10.1007/s13197-012-0677-0

Kianian, F., Marefati, N., Boskabady, M., Ghasemi, S. Z. and Boskabady, M. H. (2021). Pharmacological Properties of Allium cepa, Preclinical and Clinical Evidences; A Review. Iranian journal of pharmaceutical research (IJPR), 20(2), 107–134

Kim, Y. M., Jeong, Y. K., Wang, M. H., Lee, W. Y. and Rhee, H. I. (2005). Inhibitory effects of pine bark extract on alpha-glucosidase activity and postprandial hyperglycemia. Nutrition, 21, 756-761

Kim, Y. M., Jeong, Y. K., Wang, M. H., Lee, W. Y. and Rhee, H. I. (2005). Inhibitory effect of pine extract on α-glucosidase activity. Nutrition, 21, 756–761

Kitphati, W., Sato, V. H., Peungvicha, P., Saengklub, N., Chewchinda, S., Kongkiatpaiboon, S., Goli, A. S., Sangfuang, M., Nontakham, J., Chatsumpun, N., Mangmool, S. and Sato, H. (2024). Antihyperglycemic activity of a novel polyherbal formula (HF344), a mixture of fifteen herb extracts, for the management of type 2 diabetes: Evidence from in vitro, ex vivo, and in vivo studies. Heliyon, 10(19), e38703. https://doi.org/10.1016/j.heliyon.2024.e38703

Kong, F., Lee, B. H. and Wei, K. (2019). 5-Hydroxymethylfurfural Mitigates Lipopolysaccharide-Stimulated Inflammation via Suppression of MAPK, NF-κB and mTOR Activation in RAW 264.7 Cells. Molecules (Basel, Switzerland), 24(2), 275. https://doi.org/10.3390/molecules24020275

Kumar, M., Myagmardoloonjin, B., Keshari, S., Negari, I. P. and Huang, C. M. (2019). 5-methyl Furfural reduces the production of Malodors by inhibiting sodium I-lactate Fermentation of Staphylococcus epidermidis Implication for Deodorants Targetting the Fermentation skin Microbiome. Microorganism, 7(8), E239

Kwon, O., Eck, P., Chen, S., Corpe, C. P., Lee, J-H., Kruhlak, M. and Levine, M. (2007). Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. FASEB Journal, 21, 366–377.

Lakshmi S. R., Anand, T., Saravanan, K. M., Prabhu, K., Mohamed Yasar, A., Vivek, S., Chetan, A. and Srikanth, J. (2025). Polyherbal Formulation as a Therapeutic Strategy for Lung Cancer: Phytochemical Profiling, Molecular Docking, and Pharmacological Evaluation. Asian Pacific journal of cancer prevention (APJCP), 26(5), 1841–1852

Lam, T. P., Tran, N. N., Pham, L. D., Lai, N. V., Dang, B. N., Truong, N. N., Nguyen-Vo, S. K., Hoang, T. L., Mai, T. T. and Tran, T. D. (2024). Flavonoids as dual-target inhibitors against α-glucosidase and α-amylase: a systematic review of in vitro studies. Natural products and bioprospecting, 14(1), 4. https://doi.org/10.1007/s13659-023-00424-w

Lee, S. H., Lee, B. W., Won, H. K., Moon, J. H., Kim, K. J., Kang, E. S. and Lee, H. C. (2011). Postprandial triglyceride is associated with fasting triglyceride and HOMA-IR in Korean subjects with type 2 diabetes. Diabetes and Metabolic Journal, 35(4), 404-410.

Li, Y., Tran, V. H., Duke, C. C. and Roufogalis, B. D. (2012). Preventive and Protective Properties of Zingiber officinale (Ginger) in Diabetes Mellitus, Diabetic Complications, and Associated Lipid and Other Metabolic Disorders: A Brief Review. Evidence-based complementary and alternative medicine: eCAM, 2012, 516870

Lowell, D., Dewayne, S., Aldeam, F., Felix, O., Shada, M. and Felix, O. O. (2024). Diabetes and the associated complications: The role of antioxidants in diabetes therapy and care. Biomedicine and Pharmacotherapy, 181, 117641

Mccue, P. and Shetty, K. (2004). Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase in vitro. Asia Pacific Journal of Clinical Nutrition, 13(1), 101–106

Ngo, L. T., Okogun, J. I. and Folk, W. R. (2014). 21st century natural product research and drug development and traditional medicines. Natural Product Reports, 30, 584–592

Ojo, O. A., Amanze, J., Oni, A. P., Grant, S., Iyobhebhe, M., Elebiyo, T. C., Rotimi, D., Asogwa, N. T., Oyinloye, B. E., Ajiboye, B. E. and Ojo, A. B. (2022). Antidiabetic activity of avocado seeds (Persea americana Mill.) in diabetic rats via activation of PI3K/AKT signaling pathway. Scientific Reports, 12, 2919. https://doi.org/10.1038/s41598-022-07015-8

Ou, S. K., Kwok, C., Li, Y. and Fu, L. (2001). In vitro study of possible role of dietary fiber in lowering postprandial serum glucose. Journal of Agricultural and Food Chemistry, 49, 1026–1029

Ramaiah, M., Chakravath, I. G. and Yasaswini, K. (2013). In vitro biological standardization, formulation and evaluation of directly compressed polyherbal anthelmintic tablets. Pharmacognosy Journal, 5, 130-134

Sarita, K., Yogesh, K. T., Esha, R. and Mahendra, K. (2020). Use of Sustainable Organic Transformations in the Construction of heterocyclic Scaffolds. Advances in Green and Sustanable Chemistry, 265, 245-352

Sato, T., Tomizawa, M., Segawa, S., Matsuoka, M. and Aurues, T. (2025). Effect of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one-containing lactic acid bacterial beverages on skin moisture: a randomized, double-blind, placebo-controlled, parallel study. Journal of clinical biochemistry and nutrition, 76(2), 195–201. https://doi.org/10.3164/jcbn.24-178

Shang, A., Cao, S. Y., Xu, X. Y., Gan, R. Y., Tang, G. Y., Corke, H., Mavumengwana, V. and Li, H. B. (2019). Bioactive Compounds and Biological Functions of Garlic (Allium sativum L.). Foods (Basel, Switzerland), 8(7), 246

Shapla, U. M., Solayman, M., Alam, N., Khalil, M. I. and Gan, S. H. (2018). 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: effects on bees and human health. Chemistry Central Journal, 12(1), 35

Shetty, A. J. and Alwar, M. (2007). Acute toxicity studies and determination of median lethal dose. Current Science, 93, 917-920

Sholikhah, E. N., Mustofa, M., Nugrahaningsih, D. A. A., Yuliani, F. S., Purwono, S., Sugiyono, S., Widyarini, S., Ngatidjan, N., Jumina, J., Santosa, D. and Koketsu, M. (2020). Acute and Subchronic Oral Toxicity Study of Polyherbal Formulation Containing Allium sativum L., Terminalia bellirica (Gaertn.) Roxb., Curcuma aeruginosa Roxb., and Amomum compactum Sol. ex. Maton in Rats. BioMed research international, 2020, 8609364. https://doi.org/10.1155/2020/8609364

Singh, A. and Ilango, K. (2024). Acute and sub-chronic toxicity study of novel polyherbal formulation in non-alcoholic fatty liver using Wistar rats. Future science OA, 10(1), FSO910. https://doi.org/10.2144/fsoa-2023-0118

Sridevi, N. and Thirumal, M. (2025). Evaluation of polyherbal synergy against diabetes: in-vitro analysis. Future science OA, 11(1), 2468128

Starlin, T., Nishanthini, A., Mohan, V. R. and Jeeva, S. (2019b). Phytochemical, FT-IR and GC-MS analysis of stem and leaf of Tiliacora acuminate (Ian). International Journal of Pharmaceutical Sciences and Research, 5(9), 3977-3986

Starlin, T., Prabha, P. S., Thayakumar, B. K.A. and Gopalakrishnan, V. K. (2019a) Phytochemical Screening and GC-MS profiling of ethanolic extract of Tylophora pauciflora. Biomedical Informatics, 15(6), 425-429

Tadera, K., Minami, Y., Takamatsu, K. and Matsuoka, T. (2006). Inhibition of α-glucosidase and α-amylase by flavonoids. Journal of Nutritional Science and Vitaminology, 52, 149–153

Tayade, J. A. and Patil, A. V. (2015). Polyherbal formulation; 3rd International conference and exhibition on pharmacognosy, phytochemistry and Natural Products. Natural Products Chemistry and Research, 3, 6

Tran, N., Pham, B. and Le, L. (2020). Bioactive Compounds in Anti-Diabetic Plants: From Herbal Medicine to Modern Drug Discovery. Biology, 9(9), 252. https://doi.org/10.3390/biology9090252

Vergara-Jimenez, M., Almatrafi, M. M. and Fernandez, M. L. (2017). Bioactive Components in Moringa Oleifera Leaves Protect against Chronic Disease. Antioxidants (Basel, Switzerland), 6(4), 91. https://doi.org/10.3390/antiox6040091

Yazan, H. and Kimberly, C. (2023). Thymine Structure and function. Retrieved from https://study.com/learn/lesson/thymine.html#