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Original Research Article

Antidiabetic Medicinal Plants *Cinnamomum zeylanicum* and *Lupinus albus* having Insulin Mimetic Properties and Investigation if Its Metabolites Profile Using GC-MS Technique

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Abstract: Multiple countries include cinnamon bark and essential oil in their pharmacopoeias whereas the substances also function as food additives alongside condiments and flavoring agents supporting their role as carminatives and antioxidants and preservatives. The "white lupin" Lupinus albus grows across a wide range of the Mediterranean area. The Mediterranean region constitutes the entire planting area of this species. Lupin seeds just like legume seeds possess significant amounts of protein and minerals and dietary fibre content. Around the world diabetes mellitus exists as one of the major health implications that produces a severe long-term complex metabolic condition. The medical condition of diabetes mellitus identifies hyperglycemia through its effects on metabolic disturbances that affect carbohydrates and proteins and fats. We will use GC-MS technique to analyze the metabolites present in the plant. Evaluation of Cinnamomum zeylanicum and Lupinus albus volatiles used GC-MS for separation and identification of their contents. The results showed that twenty individual volatile components were present trans-Cinnamaldehyde, Cyclohexane methylamine, Caryophyllene, 4-(1-Hydroxyethyl) benzaldehyde, naphthalene, cis, trans-1,5-Cyclodecadiene, Octahydrodimethyl-4,7-methano-1H-indenol, 2-Methoxycinnamaldehyde, coumarin, 3-Phenylpropan-1-ol, Terbenthene, alpha-Bisabolol for Cinnamomum zeylanicum. 2-Ethylhexan-1-ol, 2-hydroxy-5-propanamidobenzoic acid, 2-Methyloctan-3-one, beta-Myrcene, 2-(hydroxymethyl)-6-[2-(4-methylcyclohex-3-en-1-yl) propan-2-yloxy] oxane-3,4,5-triol, Heptane, 2,3-dimethyl-, 2,3-Dimethylheptane, cis-beta-Ocimene in Lupinus albus. Research suggests that Cinnamomum zeylanicum along with Lupinus albus display quality as potential hypoglycaemic agents.

Keywords: Cinnamomum zeylanicum, Lupinus albus, Metabolites Profile, Antidiabetic, GC-MS.

INTRODUCTION

Cinnamon functions as traditional medicine because it possesses distinctive medicinal functions and aromatic properties for treating patients with anorexia and heart disease alongside intestinal disorders and worm infections. Cinnamaldehyde functions as the main volatile component that occurs in all cinnamon species since it demonstrates effective inhibition against various food spoilage microorganisms. In addition, in international trade, the higher the content of total cinnamic aldehydes in CEO. The extraction procedure should maximize the concentration of cinnamic aldehyde because it functions as the dominant volatile compound [1; 2]. The ultrasound-assisted and microwave-assisted extraction techniques demonstrate their value through brief extraction periods and elevated yield amounts while maintaining excellent quality extraction results. The essential oil production industry uses microwave-assisted steam distillation (MASD) as its leading technology because it merges benefits from conventional methods and state-of-the-art techniques. [3-5]. White lupin seed maintains a protein composition of between 33-47% which exceeds other legumes' protein values and approaches those of soybeans. Lupins have proven themselves as useful plants throughout history for decorative needs in home gardens along with their status as an agricultural crop. Food manufacturers incorporate lupin seeds together with lupin flour during the production of numerous cereal-based products which include pasta, crisp, bread, cookie, cake and

breakfast cereal. Scientists across the world view white lupin seeds as potential human food materials because their high-quality proteins and dietary fiber content makes them an attractive alternative food source [6, 7]. Breeding and crossing investigations depend strongly on seed content definitions. The method of solid phase microextraction (SPME) lets analytes stick onto fused silica fibers with appropriate stationary phases before direct extraction to run chromatographic tests. Target analytes absorb onto the fibre through direct sample immersion or by HS-SPME using sample headspace. This method produces high reductions of sample matrix interferences. The analytical method reduces time needed for analysis and enhances detection limits through two primary operational modes including direct extraction and headspace configurations. Medical Diarrhea remains among the main factors which cause infant mortality throughout developing countries specifically during periods of malnutrition and poor sanitary conditions. The identification and examination of easily accessible medicinal plants for infectious diarrhea treatment stands important because they serve as alternatives to existing antimicrobial drugs. The antimicrobial properties of traditional medicines have been studied through various investigations for treating infectious diarrhea by researchers. People commonly exploit cinnamon barks as a seasoning ingredient. The Cinnamon bark tea infusion addressed conditions associated with stomach distension as well as flatulence and mild cramp feelings caused by decreased gastric juice production. Traditional medicine uses Cinnamon bark treatment for digestive disorders and functional asthenias as well as weight gain promotion therapy. Multiple scientific studies show that Cinnamon displays antimicrobial properties against wide ranges of microbial agents [8, 9]. Cinnamon bark consists of volatile oils which represent 14% of cinnamaldehyde (60%), eugenol (up to 10%) and trans-cinnamic acid (51%) along with phenolic compounds (41%), condensed tannins, catechins, and proanthocyanidins and contains monoterpenes and sesquiterpenes (pinene) and calcium-monoterpenes oxalate and gum and mucilage and resin starch sugars and traces of coumarin. Diabetes mellitus stands as the most widespread endocrine disorder which consists of multiple medical disorders within a single group. Medical signs such as hyperglycemia, polyphagia, polydipsia and reduced body weight commonly occur when a person has diabetes. The diabetes treatment using alloxan caused weight reduction in subjects during the experiment. Rats given cinnamon experienced greater body weight increases than rats enrolled in the diabetic control group. The high food consumption in diabetic rats compared to controls did not prevent protein and fat utilization that led to decreased body weights. The lack of insulin created a reduction of protein content through muscular tissue proteolysis. Scientific evidence from this research shows that cinnamon functions effectively as a compound to decrease serum glucose levels. The concentration of serum glucose that rose from alloxan decreased by 50.02% during week four with cinnamon powder supplementation ($p < 0.001$). The diabetic rats developed high blood glucose levels through alloxan exposure but the consumption of cinnamon extract led to a decrease in blood glucose levels. The treatment with cinnamon extract resulted in decreased plasma glucose levels in diabetic rats exposed to streptozotocin. The research shows flavonoids exhibit two important effects: they help repair β-cells that alloxan has injured in rats and they stimulate insulin production [10, 11]. Research shows that bioactive anti-diabetic compounds consist of steroidal, phenolic acid, flavonoid and terpenoid molecules.

MATERIAL AND METHODS

Plant Materials

The dried barks of cinnamon zeylanicum together with lupinus albus seeds were obtained from Al-Jabawi Medical Herbals Office in Babil governorate in Iraq before their identification by the Botany department.

Extraction of plant materials

Maceration extraction with methanol included shade drying and powdering the plant materials followed by shaking the solution for 3 days. The residue was observed under 50 to 60^{o} drying conditions before weighing it for yield determination. Subsequently, the extracted substance underwent preliminary phytochemical tests. Protection from air resulted in drying the extract which was then put into a sealed container.

GC/MS analysis of plant materials

The obtained materials proceeded to GC MS testing. The Gas chromatography– Mass spectroscopy (Agilant 6890/Hewlettpackard 5975) operated with electron impact mode for analysis. The instrument utilized Helium gas as its carrier medium at a speed of 1mL/min. The program set the initial temperature at 80o C for 5 minutes before raising it to 300^{o} C while following a 15^{o} C/min increase. The temperature values for the injector and EI detector (70eV) operated at 280^{o} C and 300^{o} C. A 29µL solution of each plant extract received injection through a Hamilton syringe into the GC/MS instrument.

A-amylase inhibitory assay

The standard procedure for measuring α-amylase inhibitory activities required minor modifications to evaluate both extract samples from Cinnamomum zeylanicum and Lupinus albus along with their fractions. The α-amylase solution containing two international units per millilitre was combined with 200 millilitres of extract or fractions that had varying 0.5 milligramme per millilitre concentrations and 500 millilograms of 6.8 phosphate buffer with one hundred millimolar phosphate. Researchers placed the mixture into a 96-well plate before keeping it at 37 °C for 20 minutes. The temperature selection for incubation amounted to 37°C throughout the preincubation period. Afterward, the mixture was moved into

another 37 degrees Celsius incubator for 30 more minutes. Simultaneously, 20 litres of 1% soluble starch in 100 mM phosphate buffer pH 6.8 were added as substrate. An hour of boiling at constant pressure followed the mixture of 100 liters DNS colour reagent with the liquid solution. The measurement served to establish values for the final mixture absorbance. The standard acarbose concentration values extended from 0.1 to 0.5 mg/ml served as baseline references. A simultaneous synthesis of the extract (Cinnamomum zeylanicum and Lupinus albus) with fractions which underwent no experimental manipulation led to the productive material. The results appeared as inhibition percentage through the use of the applied formula. The enzyme inhibition data points enabled researchers to obtain the IC50 value through graphical analysis of the different fraction concentrations.

The percentage of inhibition could be determined by applying the following formula:

$$\% \text{ Inhibition} = (Abs_{control} - Abs_{extract}) / Abs_{control} \times 100$$

A-Glucosidase Inhibitory Assay

An analysis was performed to determine the α-glucosidase inhibitory potential of the extract from Cinnamomum zeylanicum and Lupinus albus combined with its fractions. The standard protocol with modified steps served to execute the analysis when applied alone. The 96 well plate consisted of pre-cooled serum samples which received three-dimension centigrade incubation for fifteen minutes. The liquid solution contained nine extracts and fractions of extracts at 0.500 mg/mL, ten units of purified alpha-glucosidase, fifty litres of 100 mM 6.8 phosphate buffer solution. Thirty-seven degree centigrade served as the temperature for pre incubation. After thirty-seven degrees centigrade, the mixture required twenty additional minutes of incubation. An addition of twenty liters P-NPG solution with five millimolar concentration served as the substrate. The scientists terminated the reaction with a 50-litre addition of a 0.1 M sodium carbonate solution. The standard measurement employed acarbose while the examined sample contained 0.5 mg/mL of acarbose. The experiment included an immediate control test which omitted the use of the investigated chemical compound. The research evaluated α-glucosidase inhibitory activity by determining the percentage of inhibition through this expression:

$$\% \text{ Inhibition} = (Abs_{control} - Abs_{extract}) / Abs_{control} \times 100$$

The analysis features A control as absorbance measure for control solution while A extract shows the absorbance readings of each fraction. The data analysis allowed researchers to determine IC50 values by reading the graphic representations which showed the necessary fraction amounts that caused 50% enzyme inhibition.

RESULTS AND DISCUSSION

A gas chromatography–mass spectrometry (GC-MS) analysis separated then identified the volatiles present in both Cinnamomum zeylanicum and Lupinus albus. A total of twenty volatile components existed in the analyzed samples according to results trans-Cinnamaldehyde, Cyclohexane methylamine, Caryophyllene, 4-(1-Hydroxyethyl) benzaldehyde, naphthalene, cis, trans-1,5-Cyclodecadiene, Octahydrodimethyl-4,7-methano-1H-indenol, 2-Methoxycinnamaldehyde, coumarin, 3-Phenylpropan-1-ol, Terbenthene, alpha-Bisabolol for Cinnamomum zeylanicum. 2-Ethylhexan-1-ol, 2-hydroxy-5-propanamidobenzoic acid, 2-Methyloctan-3-one, beta-Myrcene, 2-(hydroxymethyl)-6-[2-(4-methylcyclohex-3-en-1-yl) propan-2-yloxy]oxane-3,4,5-triol, Heptane, 2,3-dimethyl-, 2,3-Dimethylheptane, cis-beta-Ocimene in Lupinus albus. Many scientists think that terpenes serve as chemical defense elements in plant latex and resins against pathogens that cause disease in human beings and animals [12]. The activity of terpenes depends on their lipid-friendliness as well as the strength of functional groups and their ability to dissolve in water.

Table 1: Activity of phytocomponents identified in the *Cinnamon zeylanicum*

No.	Compounds	Molecular Formula	Molecular Weight
1	trans-Cinnamaldehyde	C_9H_8O	132.16 g/mol
2	Cyclohexane methylamine	$C_7H_{15}N$	113.20 g/mol
3	Caryophyllene	$C_{15}H_{24}$	204.35 g/mol
4	4-(1-Hydroxyethyl) benzaldehyde	$C_9H_{10}O_2$	150.17 g/mol
5	naphthalene	$C_{10}H_8$	128.17 g/mol
6	cis, trans-1,5-Cyclodecadiene	$C_{10}H_{16}$	136.23 g/mol
7.	Octahydrodimethyl-4,7-methano-1H-indenol	$C_{12}H_{20}O$	180.29 g/mol
8.	2-Methoxycinnamaldehyde	$C_{10}H_{10}O_2$	162.18 g/mol
9.	coumarin	$C_9H_6O_2$	146.14 g/mol
10.	3-Phenylpropan-1-ol	$C_9H_{12}O$	136.19 g/mol
11.	Terbenthene	$C_{10}H_{16}$	136.23 g/mol
12.	alpha-Bisabolol	$C_{15}H_{26}O$	222.37 g/mol

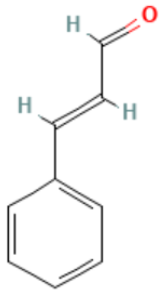
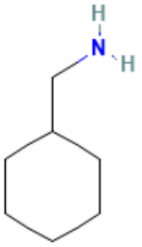
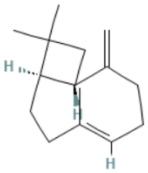
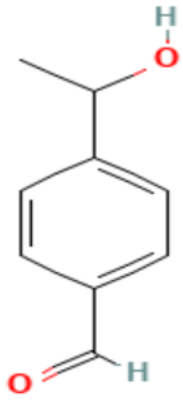
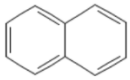
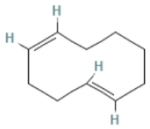
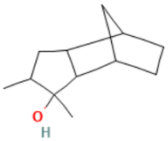
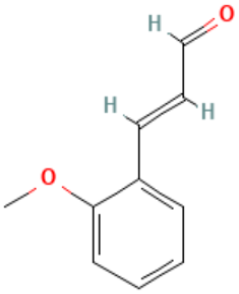
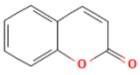
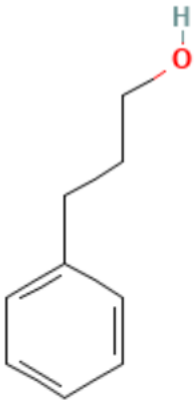
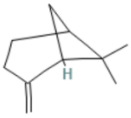
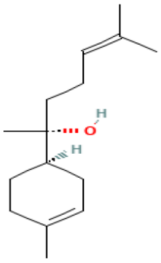
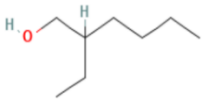
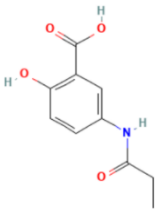
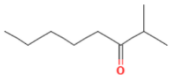
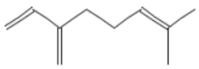
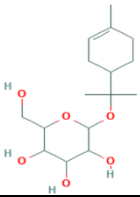
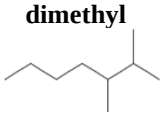
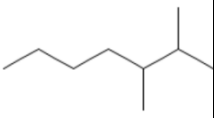
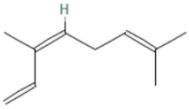
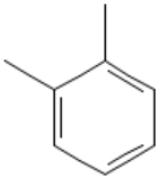
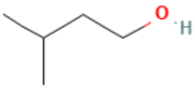
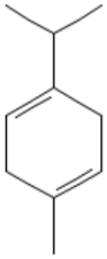
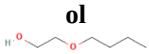
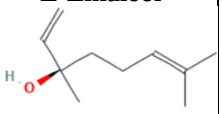
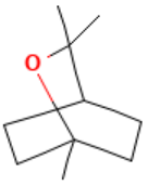
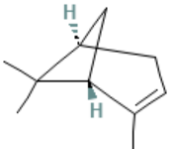
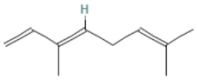
trans-Cinnamaldehyde 	**Cyclohexanemethylamine** 	**Caryophyllene** 	**4-(1-Hydroxyethyl)benzaldehyde** 	**Naphthalene** 	**cis,trans-1,5-Cyclodecadiene** 
Octahydrodimethyl-4,7-methano-1H-indenol 	**2-Methoxycinnamaldehyde** 	**Coumarin** 	**3-Phenylpropan-1-ol** 	**Terbenthene** 	**alpha-Bisabolol** 

Table 2. Activity of phytocomponents identified in the *Lupinus albus*

No.	Compounds	Molecular Formula	Molecular Weight
1	2-Ethylhexan-1-ol	$C_8H_{18}O$	C8H18O
2	2-hydroxy-5-propanamidobenzoic acid	$C_{10}H_{11}NO_4$	209.20 g/mol
3	2-Methyloctan-3-one	$C_9H_{18}O$	142.24 g/mol
4	beta-Myrcene	$C_{10}H_{16}$	136.23 g/mol
5	2-(hydroxymethyl)-6-[2-(4-methylcyclohex-3-en-1-yl)propan-2-yloxy]oxane-3,4,5-triol	$C_{16}H_{28}O_6$	316.39 g/mol
6	Heptane, 2,3-dimethyl-	C_9H_{20}	128.25 g/mol
7.	2,3-Dimethylheptane	C_9H_{20}	128.25 g/mol
8.	cis-beta-Ocimene	$C_{10}H_{16}$	136.23 g/mol
9.	1,2-Xylene	C_8H_{10}	106.16 g/mol
10.	3-Methylbutan-1-ol	$C_5H_{12}O$	88.15 g/mol
11.	p-Mentha-1,4-diene	$C_{10}H_{16}$	136.23 g/mol
12.	2-Butoxyethanol	$C_6H_{14}O_2$	118.17 g/mol
13.	L-Linalool	$C_{10}H_{18}O$	154.25 g/mol
14.	1,8-Cineole	$C_{10}H_{18}O$	154.25 g/mol
15.	alpha-Pinene	$C_{10}H_{16}$	136.23 g/mol
16.	(E)-3,7-Dimethylocta-1,3,6-triene	$C_{10}H_{16}$	136.23 g/mol
17.	4-(2-Methoxy-phenyl)-thiazol-2-ylamine	$C_{10}H_{10}N_2OS$	206.27 g/mol

Table 2: Activity of Phytocomponents identified in the plant *Lupinus albus* (GC-MS)

2-Ethylhexan-1-ol 	**2-hydroxy-5-propanamidobenzoic acid** 	**2-Methyloctan-3-one** 	**beta-Myrcene** 	**2-(hydroxymethyl)-6-[2-(4-methylcyclohex-3-en-1-yl)propan-2-yloxy]oxane-3,4,5-triol** 	**Heptane, 2,3-dimethyl** 
2,3-Dimethylheptane 	**cis-beta-Ocimene** 	**1,2-Xylene** 	**3-Methylbutan-1-ol** 	**p-Mentha-1,4-diene** 	**2-Butoxyethanol** 
L-Linalool 	**1,8-Cineole** 	**alpha-Pinene** 	**(E)-3,7-Dimethylocta-1,3,6-triene** 		

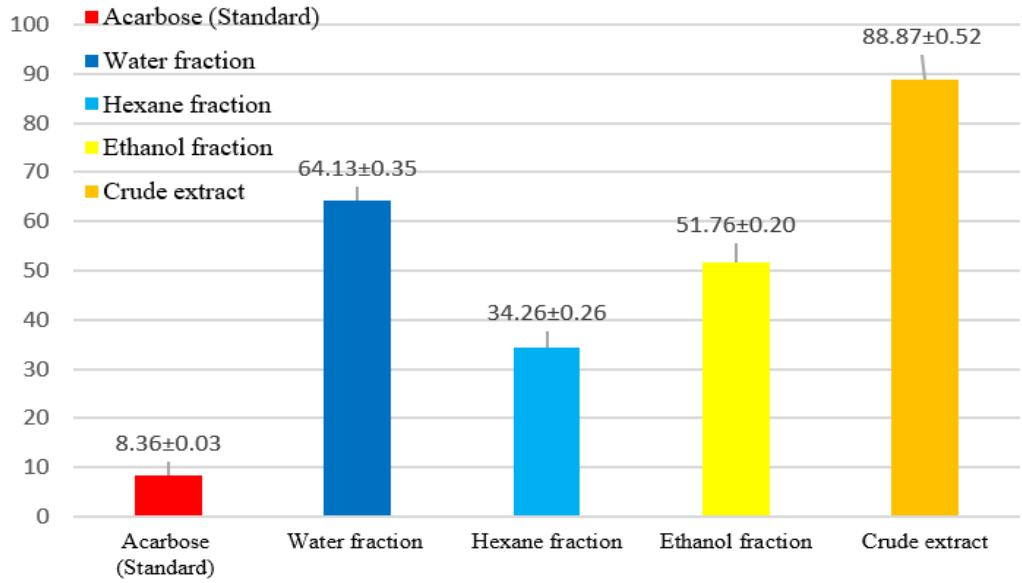


Figure 1. Antidiabetic medicinal plant *Cinnamomum zeylanicum* Crude, Ethanol fraction, Hexane fraction, Water fraction and acarbose as standard against α-amylase activity

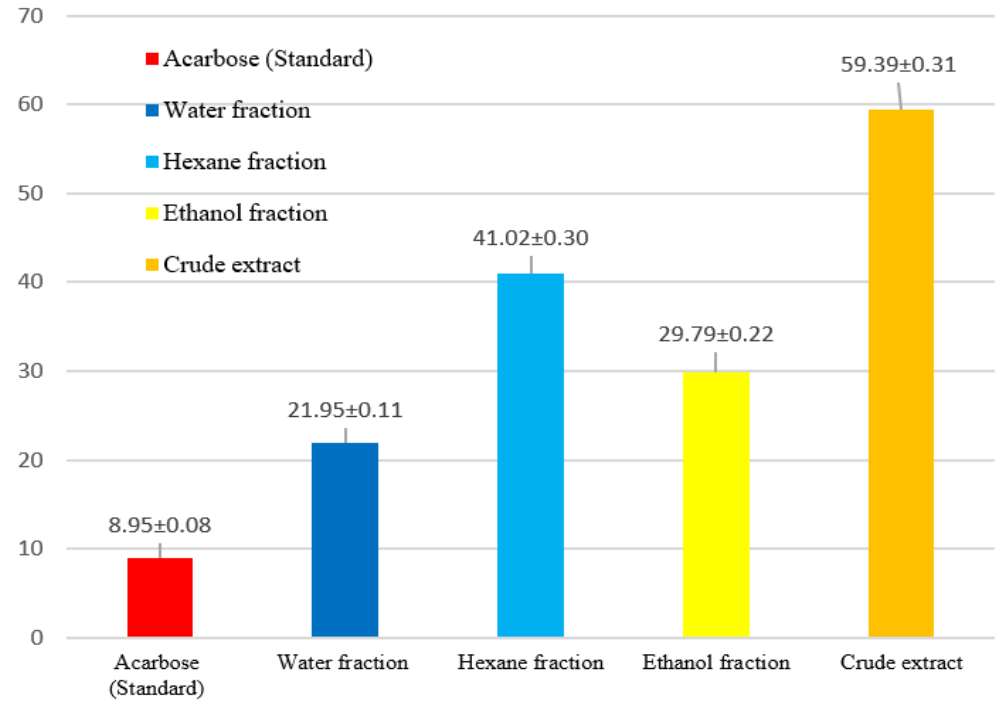


Figure 2. Antidiabetic medicinal plant *Cinnamomum zeylanicum* Crude, Ethanol fraction, Hexane fraction, Water fraction and acarbose as standard against α- glucosidase activity

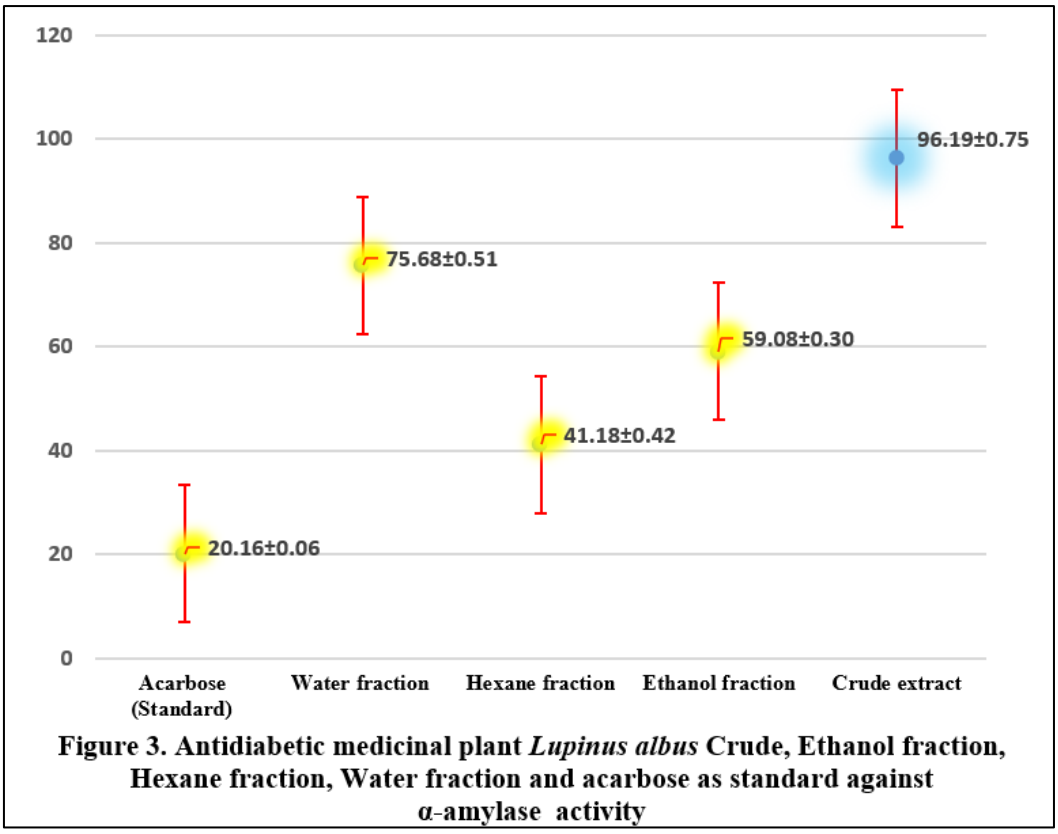


Figure 3. Antidiabetic medicinal plant *Lupinus albus* Crude, Ethanol fraction, Hexane fraction, Water fraction and acarbose as standard against α-amylase activity

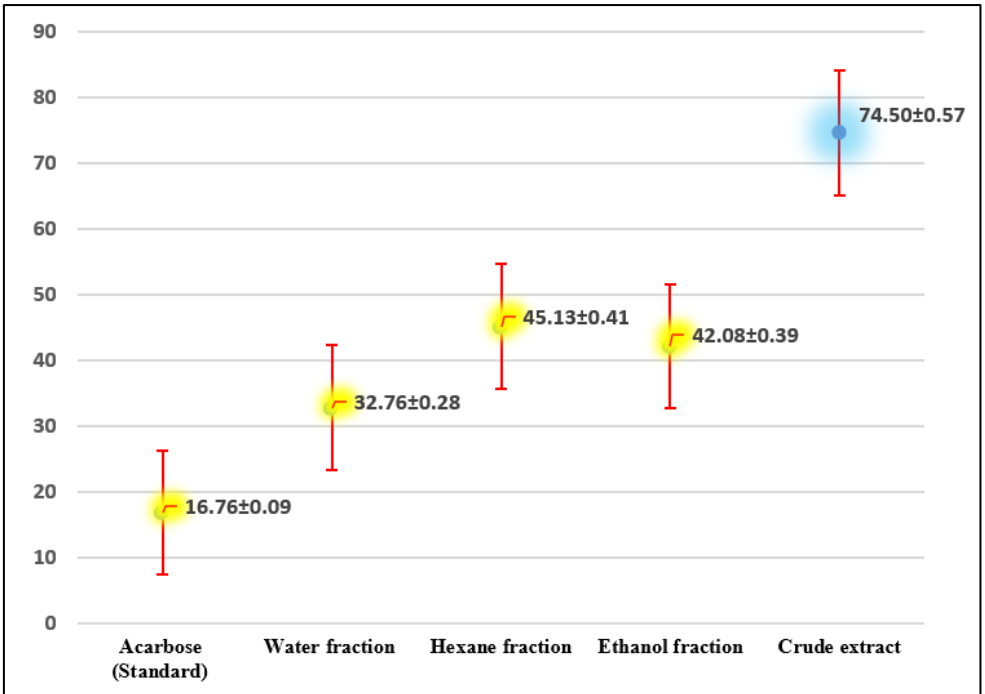


Figure 4. Antidiabetic medicinal plant *Lupinus albus* Crude, Ethanol fraction, Hexane fraction, Water fraction and acarbose as standard against α- glucosidase activity

According to the type of extract Cinnamomum zeylanicum (Crude extract, Ethanol fraction, Hexane fraction, Water fraction and acarbose (Standard) recorded (88.87±0.52, 51.76±0.20, 34.26±0.26, 64.13±0.35 and 8.36±0.03) respectively inhibitory potency against α-amylase. While recorded (59.39±0.31, 29.79±0.22, 41.02±0.30, 21.95±0.11, and 8.95±0.08) respectively inhibitory potency against α- glucosidase activity. The percent inhibition of α-glucosidase showed that methanol and ethanol fraction produced significant ($P < 0.05$) more potent effects when compared to acarbose. According to the type of extract Lupinus albus (Crude extract, Ethanol fraction, Hexane fraction, Water fraction and acarbose (Standard) recorded (96.19±0.75, 59.08±0.30, 41.18±0.42, 75.68±0.51 and 20.16±0.06) respectively inhibitory potency against α-amylase. While recorded (74.50±0.57, 42.08±0.39, 45.13±0.41, 32.76±0.28, and 16.76±0.09) respectively inhibitory potency against α- glucosidase activity. The metabolic disease impacts 4% of worldwide population and experts predict its numbers will grow by 5.4% during 2025. The combination of higher oxidative stress and weaker antioxidant protection occurs in diabetes patients and scientists currently believe these alterations lead to the initiation and worsening of complications linked to diabetes. Excessive glucose levels cause both cell harm and promote lipid peroxidation damage. Medical research has revealed that diabetic patients show changes in their antioxidant defense system enzymes catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD). Scientific researches implement β-cell destroying chemicals to produce experimental diabetes in rat models because the method is easy to execute. Alloxan along with streptozotocin serve as the main chemicals used to create diabetes in rat models. The research community needs to understand both β-cell changes in the pancreas and organism-level modifications after exposing animals to alloxan or streptozotocin for using these compounds as diabetogenic agents [13-15]. A combination of proper diet with exercise activity along with insulin replacement therapy serves as the main control method for managing diabetes. Unpleasant side effects emerge from hypoglycemic drug usage with insulin and biguanides and sulfoylureas and α-glucosidase inhibitors which cause severe hypoglycemia along with lactic acidosis and peripheral edema and abdominal discomfort. Synthetic hypoglycemic agents generate major adverse effects whereas natural-derived bioactive compounds show secured performance as well as affordable pricing. Traditional medicine relies on multiple plant treatments for diabetes while many researchers have identified these plants' capabilities in diabetes management through studies [16, 17]. Scientific literature shows that more than 400 species of plants contain hypoglycemic properties yet the ongoing search for novel antidiabetic and antihyperlipidemic and antioxidant natural plant medicines remains attractive. Plasma lipids in diabetics typically become elevated because elevated lipids function as strong risk factors for coronary heart disease. Research by scientists documented elevated plasma cholesterol together with phospholipids and free fatty acids and triglycerides in alloxan diabetic patients [18-21]. Insulin normally blocks hormone sensitive lipase but diabetic patients have abnormally high plasma lipid levels because free fatty acids increase in peripheral depots. The research demonstrated that cinnamon treatment caused a reduction of triglyceride and cholesterol levels in alloxan diabetic rats. Body weight administration of 20 mg/Kg cinnamon reduced cholesterol and triglyceride concentrations in serum blood together with a substantial insulin boost in blood plasma. The postprandial intestinal overproduction ofapoB48-containing lipoproteins might benefit from raising intestinal insulin sensitivity through cinnamon extract treatment therefore leading to improved lipid metabolic

control. Application of cinnamon essential oil reduced diabetes symptoms while simultaneously protecting DNA from harm and lowering cholesterol [22-25]. The polyphenols, polymers and anthocynins existing in cinnamon demonstrate antioxidant properties and enhance insulin effects which lead to beneficial diabetes control mechanisms [26, 27]. The consumption of cinnamon extract with polyphenols lead to better cholesterol levels in individuals who had type 2 diabetes. Insulin release in pancreatic islets increases due to sparteine sulphate (alkaloids from Lupinus) which contributes to its effect on lowering blood sugar levels in lupin. Subjects with type-2 diabetes who received intravenous sparteine [28, 29] sulphate treatment had reduced glucose levels and higher insulin results.

CONCLUSION

The current research demonstrated for the initial time that Lupinus albus and Cinnamomum zeylanicum supplementation has promising capabilities to enhance both fasting and post-meal plasma glucose measurements. These findings indicate Lupinus albus could achieve its hypoglycaemic effect through mechanisms beyond insulin stimulation from β-cells which include stimulation of glucose uptake and a potential correction of insulin resistance and blocking endogenous glucose production and activation of glycogenogenesis in liver and muscles. The combination of Cinnamomum zeylanicum and Lupinus albus shows potential to serve as hypoglycaemic medications.

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