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Investigating the Therapeutic Effects of *Cassia absus* Leaf Extract on Hyperglycemia and Oxidative Stress in Alloxan-Induced Diabetic Rats

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Abstract

In this study, rats with diabetes induced by alloxan were used to test the anti-diabetic effects of the ethyl acetate extract of *Cassia (C.) absus* leaves. The bioactive constituents of *C. absus* leaf showed a significant amount of phenol and flavonoid, had a hypoglycemic effect by ameliorating insulin secretion and preventing the alterations induced by alloxan. Thirty-six rats were split into six groups: the first group was given a normal diet, the second group was diabetic control, the third group was treated with metformin, fourth, fifth, and sixth groups were treated with *C. absus* ethyl acetate extract at 200, 400, 600 mg/kg dose rate for 28 days. On the 29th day, blood samples were taken. The phytochemical analysis and antioxidant activity of leaf extract were determined. Glycemic markers, hepatic and renal function, oxidative stress markers and histopathology of pancreatic tissue were determined. A significant alteration was observed in the diabetic control group. It was observed that *C. absus* ethyl acetate extract significantly improved the levels of serum glucose, insulin, ALT, AST, creatinine, urea, SOD, and CAT in treatment groups. Pancreatic tissue histology revealed a significant increase in the number of beta cells, with a normal architecture of the islets of Langerhans. Therefore, it was concluded that *C. absus* leaf extract has antioxidant and anti-diabetic qualities. The data was analyzed using both one-way and two-way analysis of variance (ANOVA).

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by elevated blood sugar levels, which can result from abnormalities in insulin secretion, insulin action, or both (Blagov et al., 2024). Diabetes mellitus, considered persistent hyperglycemia, is a heterogeneous group of disorders having multiple etiologies. About 537 million of adults between 20-79 years of age are alive with diabetes. The total number of persons with diabetes is predicted to increase to 643 million by 2030 (Abbas et al., 2025). Insulin resistance and beta cell dysfunction are hallmarks of type 2 diabetes mellitus. Insulin resistance results from the disruption of many cellular pathways and results in a diminished reaction to insulin, especially in muscle, liver, and adipose tissue

(Berbudi et al., 2025). T2DM risk factors comprise a blend of genetic, metabolic, and environmental factors that interrelate with one another contribute to its prevalence (Yang et al., 2024).

With time, numerous efforts have done for the diabetes treatment, but exact achievement has not been accomplished. Medications presently accessible for managing diabetes mellitus are problematic and have some limits. So, research for novel methods is desirable to distribute and target anti-diabetic securely to the site of action (Aloke et al., 2022). Plants have been traditionally utilized to treat a wide range of medical conditions since ancient times. Since herbal treatments typically have harmful side effects, there has been a surge in interest in the need for plant-based medications to treat a variety of illnesses in recent decades (Najmi et al., 2022). In both industrialized and developing nations, herbal remedies are utilized in primary healthcare. Numerous herbal medications have anti-diabetic properties and are frequently utilized because of their better bioavailability, low cost, and few adverse effects (Sukhikh et al., 2023). There are many potential ways that medicinal herbs can result in hypoglycemia. These include improving insulin synthesis and secretion from the β-cells, preventing additional damage, boosting the regeneration or renewal of damaged pancreatic β-cells, and lowering the GI system's absorption of glucose (Kamiloglu et al., 2024). It has been noted that natural products or their derivatives account for about half of all medications in contemporary medicine. Approximately 35% of medications on the market worldwide are derived from natural sources (Xie et al., 2025).

The annual erect plant *C. absus* is a member of the Fabaceae family. Chaksu is the local name for it. In medicine, *C. absus* leaves and seeds are useful. Throughout history, it has been used to treat a number of ailments, such as inflammation, bronchitis, conjunctivitis, liver problems, and hypertension. Antioxidant, anti-inflammatory, antibacterial, antifungal and

antihypertensive properties are among the many biological activities of *C. absus* that have been documented. Because of their comparable structures and numerous apparent impacts on the human body, plant metabolites are significant substances (Sajid et al., 2025). In this work, the anti-diabetic activity of ethyl acetate extract of *C. absus* leaves was determined against alloxan-induced diabetes in the rat model.

MATERIALS AND METHODS

CHEMICALS

Metformin and alloxan monohydrate used in this investigation were purchased from CCL Pharmaceuticals, Pakistan and Appli Chem, Ottoweg-4 Germany, respectively. All the chemicals used in the current research were of analytical grade.

PREPARATION OF *C. ABSUS* LEAF EXTRACT

The leaves of *C. absus* were purchased from a herbal store in Faisalabad. The leaves were washed thoroughly with tap water and cleaned followed by distilled water and then subjected to shade drying. The leaves were grinded into coarse powder by mechanical grinder and sieved. For extraction, 100 g powdered plant material was weighed and soaked in 70% ethyl acetate in a beaker. The beaker was tightly enclosed with an aluminum foil and placed for three days at room temperature and stirred at 150 revolutions per min for 24 hrs. The filtrate was concentrated under a rotary vacuum evaporator (Hussein et al., 2025).

QUALITATIVE PHYTOCHEMICAL ANALYSIS

With a few minor adjustments, phytochemical analysis was done to determine the presence of various bioactive phytochemicals such as alkaloids, flavonoids, phenols, glycosides, tannins and saponins of ethyl acetate leaves extract of *C. absus* (Subhan et al., 2021).

In vitro assessment of the antioxidant property of *C. absus*

DPPH ASSAY (2,2-DIPHENYL-1-PICRYLHYDRAZYL)

The DPPH free radicals scavenging action of ethyl acetate leaves extract of *C. absus* was evaluated using a previously described method (Abbas et al. 2025). Ascorbic was used as standard and absorbance was measured at a 515nm using spectrophotometer and the following formula was used to calculate the scavenging activity:

DPPH assay %= [(Abs blank– Abs sample)/Abs blank]/100

INDUCTION OF DIABETES MELLITUS

Alloxan monohydrate mixed in normal saline (0.9% saline) was intraperitoneally injected to thirty rats except healthy control group for the induction of diabetes. All animals' blood glucose levels were measured using a glucometer after three days, and those whose fasting blood glucose levels were higher than 250 mg/dL were added to the study.

EXPERIMENTAL DESIGN

Thirty-six rats were divided into six groups (n=6) on the basis of treatments received by oral gavage on daily basis for 21 days as follows:

Group I: Healthy control rats received normal diet

Group II: Untreated diabetic rats received normal diet

Group III: Diabetic rats treated with standard drug (metformin 150mg/kg/daily)

Group IV: 200 mg/kg of CAEAE was administered to diabetic rats.

Group V: Rats with diabetes receiving 400 mg/kg of CAEAE

Group VI: Rats with diabetes receiving 600 mg/kg of CAEAE

COLLECTION OF BLOOD SAMPLES AND ORGANS

On 29th day of study, overnight fasted rats were sacrificed under anesthesia withchloroform. To separate the serum, the blood specimens were taken, cent rifuged for 10 to 15 min at 4000 rpm, and then stored at -20°C for additional biochemical analysis. Pancreatic tissue of rats was excised, and preserved in 10% neutral buffered formalin for histopathology studies.

ESTIMATION OF DIABETIC MARKERS

The serum glucose and serum insulin were measured with the use of commercially available kit with compliance of manufacturer's instructions.

BIOCHEMICAL ANALYSES

A commercially accessible QCA®, Spain kit was used to spectrophotometrically quantify the levels of creatinine, urea, ALT, and AST in the serum of every animal.

ESTIMATION OF SERUM OXIDATIVE STRESS MARKERS

The level of superoxide dismutase and catalase was measured commercially available kit of QCA®, Spain in accordance to instructions of manufacturer.

HISTOPATHOLOGICAL EXAMINATION

The preserved tissue samples of the pancreas in 10% formalin solution were used. Hematoxylin and eosin stained was done for the examination of histopathology under light microscope.

STATISTICAL ANALYSIS: The statistical analysis was performed by one way ANOVA by using Tukey's multiple comparison test.

RESULTS

QUALITATIVE PHYTOCHEMICAL ANALYSIS

The table displayed the presence of the qualitative phytochemicals in CAEAE.

TABLE 1. PHYTOCONSTITUENTS PRESENT IN ETHYL ACETATE EXTRACT OF *C. ABSUS*

Phytochemical	Result
Alkaloids	+
Flavonoids	+
Phenols	+
Glycosides	-

Tannins	+
Saponins	-

DPPH assay

Cassia absus leaf extract's antioxidant capacity was assessed using the DPPH test technique. Different concentrations of *C. absus* leaf extract and AA (ascorbic acid) possess significant DPPH free radical scavenging action depending upon their concentrations (Table 2).

TABLE 2. ANTIOXIDANT ACTIVITY OF CAEAE USING DPPH ASSAY

Samples	Percentage inhibition of DPPH					IC_{50}
	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	100 (µg/ml)	(µg/ml)
CAEAE	16.46	26.2	33.4	44.8	66.1	72.2
AA	14.9	34.9	49.02	55.9	70.2	38.8

INFLUENCE OF CAEAE ON GLYCEMIC MARKERS

The diabetic control group's mean serum glucose level was significantly higher than that of both treatment as well as normal control groups. Serum glucose levels were significantly lower in the CAEAE group than in the disease group. However, when treated with CAEAE in a dose-dependent way, blood insulin levels significantly increased in the treatment groups while they significantly decreased in the untreated diabetes group.

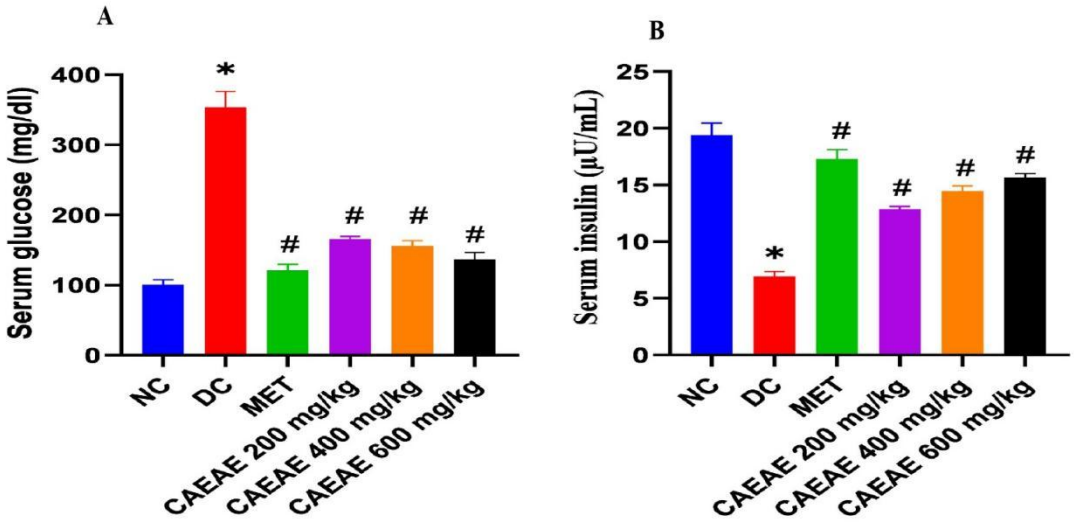

FIGURE 1. Effect of *C. absus* ethyl acetate extract on glycemic markers A) serum glucose, B) serum insulin. Values represent means ± standard deviation (n=6). Key: NC; normal control, DC; disease control, MET; metformin (150 mg/kg), CAEAE; *C. absus* ethyl acetate extract. $^{*}P$ <0.05 compared to NC and $^{\#}P$ < 0.05 compared to DC.

INFLUENCE OF CAEAE ON BIOMARKERS

Serum levels of AST, ALT, creatinine, and urea were significantly higher in diabetic control rats compared to the normal control, according to statistical analysis of LFTs and RFTs. Following treatment with metformin (150 mg/kg) and CAEAE at varying dose rates in treatment groups, AST, ALT, creatinine, and urea levels decreased and then returned to normal.

TABLE 3. IMPACT OF *C. ABSUS* ETHYL ACETATE EXTRACT ON BIOMARKERS

Groups	Parameters			
	AST	ALT	Urea	Creatinine
NC	119.16 ± 3.53	69.12 ± 3.18	12.12 ± 0.09	31.87 ± 3.06
DC	257.04 ± 1.52*	463.75 ± 7.42*	37.29 ± 0.92*	75.86 ± 1.41*
MET	146.70 ± 3.22#	113.75 ± 1.31#	13.53 ± 0.12#	36.95 ± 0.65#

CAEAE 200 mg/kg	178.33 ± 7.43#	151.22 ± 5.93#	19.22 ± 1.61#	46.89 ± 2.14#
CAEAE 400 mg/kg	167.93 ± 3.95#	138.61 ± 0.84#	17.10 ± 0.14#	42.21 ± 1.47#
CAEAE 600 mg/kg	158.82 ± 7.49#	126.08 ± 0.55#	15.52 ± 0.62#	39.03 ± 0.91#

Values represent means ± standard deviation (n=6). Key: NC; normal control, DC; disease control, MET; metformin (150 mg/kg), CAEAE; *C. absus* ethyl acetate extract. $^{*}P < 0.05$ compared to NC and $^{\#}P < 0.05$ compared to DC.

INFLUENCE OF CAEAE ON OXIDATIVE STRESS MARKERS

The current study's findings showed that alloxan-induced diabetes significantly increased serum oxidative stress markers, which led to the pancreatic beta-cells in the diabetic control group being eradicated more quickly than in the normal control group. On the other hand, hyperglycemic rats treated with metformin and CAEAE (200, 400, and 600 mg/kg) showed dose-dependent improvements in blood SOD and catalase levels. Therefore, the administration of CAEAE to diabetic rats increased their antioxidant system, allowing them to better withstand oxidative damage and, consequently, the advanced death of β-cells.

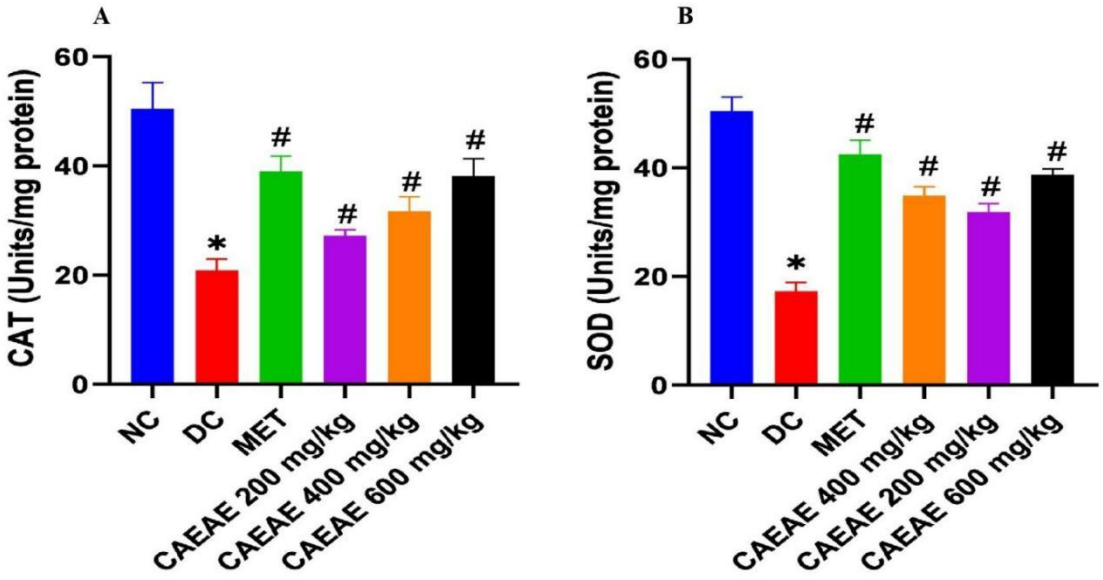

FIGURE 2. Effect of *C. absus* ethyl acetate extract on oxidative stress markers A) Catalase, B) Superoxide dismutase. Values represent means ± standard deviation (n=6). Key: NC; normal control, DC; disease control, MET; metformin (150 mg/kg), CAEAE; *C. absus* ethyl acetate extract. $^{*}P < 0.05$ compared to NC and $^{\#}P < 0.05$ compared to DC.

HISTOPATHOLOGICAL EXAMINATION

The pancreatic histomorphology showed that the diabetic control group had more beta-cells eliminated than the normal control group due to alloxan-induced diabetes. Alloxan-induced diabetes causes the destruction of the beta cells of the pancreas when treated with metformin, and CAEAE at different doses increased the number of beta-cells and restored the normal structure of islets of Langerhans.

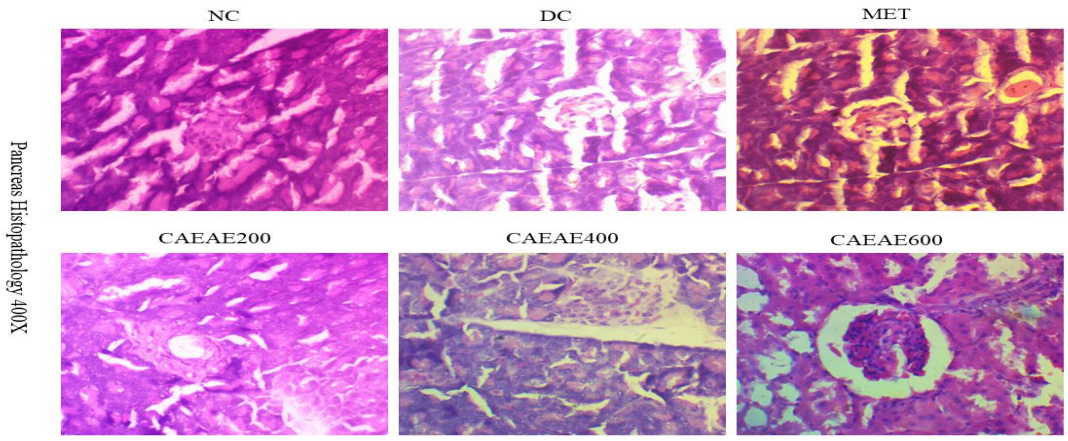

FIGURE 3: Photomicrographs of beta-cells of the pancreas of Normal Control, Diabetic control, Standard drug, Low, Medium, and High dose of *C. absus* treatment groups (H&E staining).

DISCUSSION

Globally, diabetes mellitus is one of the leading causes of morbidity and mortality due to rising rates of obesity and sedentarism. Many patients employ traditional plant-based therapies to treat their illnesses, even though there are many excellent medications available (Al-attar et al., 2019). Natural products have been the subject of extensive research in an effort to combat infectious diseases, epidemics, and pandemics. However, a lot of work has been done to identify and isolate bioactive chemicals from natural items that are widely accessible, reasonably priced, have few adverse effects, and serve a variety of purposes (Pye et al., 2017).

The qualitative phytochemical analysis of *C. absus* ethyl acetate leaves extract showed a signficant presence of flavonoids, alkaloids, tannins, saponins, phenols while absence of glycosides. All these detected components have reported anti-diabetic and antioxidant property. Aditionally, the extract of *C. absus* demonstrated antioxidant activity (IC_{50} = 72.2 µg/mL) in contrast to ascorbic acid. The presence of active biochemical constituents in *C. absus* has been associated with its beneficial effects. Results have proved that CAEAE has significant potential to quest free radicals. Our result was according to the previous study (Al-omar et al., 2020).

In current research, we used alloxan for the induction of diabetes which causes destruction of pancreatic β-cells destruction. The alteration of glucose homeostasis resulted in considerable serum glucose, and insulin. Additionally, after treatment with metformin and CAEAE at 200, 400, and 600 mg/kg dose ameliorated serum glucose and serum insulin level which might be attributed to the hypoglycemic action of identified phenolics (Motto et al., 2022).

The liver is essential for gluconeogenesis, the breakdown of proteins, lipids, and carbs, as well as for the storage of glucose as glycogen. Acute liver disease, elevated liver enzymes, non-alcoholic fatty liver, and acute liver disease are all associated with diabetes (Kumar et al., 2025). Serum levels of liver enzymes were tested as a marker for liver functioning and might be used to diagnose the degree of liver damage. Serum levels of these kidney and liver function measures decreased in diabetic rats treated with metformin and various dosages of *C. absus* extract; this observation may corroborate the renal injury.

According to Unuofin and Sogolo (2020), oxidative stress is a physiological state in which the system's antioxidant capacity causes the generation of ROS levels to change, either by reducing removal or increasing

synthesis. Reactive oxygen species are extremely potent free radicals that cause major alterations in the structure and functionality of cells. Although diabetic rats had lower levels of SOD and CAT, *C. absus* treatment dramatically raised these levels in a dose-dependent manner and demonstrated antioxidant capacity.

In present work, histomorphological findings of pancreas of diabetic rats treated with CAEAE indicated improved pancreatic architecture with increased number of β-cells as well as reduction in pancreatic lesions in comparison to diabetic control rats. These results supported previous findings that regeneration of islets lead to increase the production of insulin. Therefore, in the present study, *C. absus* ethyl acetate extract may protect β-cells function as observed from improvement of glycemic control, and antioxidant levels. This study concludes that *C. absus* leaf extract can be employed as an anti-diabetic medication and may soon form the basis for new formulations for broad use.

AUTHORS CONTRIBUTION

Muhammad Rehan Sajid, Rimsha Ikram, and Nawal Altaf; Conceptualization and Experimental research. Muhammad Adeel Razzaq, Huma, and Sadia Shafique; Data analysis and writing. Warda Saleem, Natasha Iqbal and Hifza Tariq; Critical review and draft preparation.

REFERENCES

Abbas, I., Majeed, W., Aslam, B., & Faisal, M. N. (2025). Salsola imbricata attenuates type 2 diabetes mellitus in rats by regulating oxidative stress and insulin signaling. *Asian Pacific Journal of Tropical Biomedicine*, *15*(5), 210-218.

Al-Attar AM, Alsalmi FA. Effect of Olea europaea leaves extract on streptozotocin induced diabetes in male albino rats. Saudi J Biol Sci 2019; 26: 118-128.

Aloke, C., Egwu, C. O., Aja, P. M., Obasi, N. A., Chukwu, J., Akumadu, B. O., ... & Achilonu, I. (2022). Current advances in the management of diabetes mellitus. *Biomedicines*, *10*(10), 2436.

Al-Omar, M. S., Mohammed, H. A., Mohammed, S. A., Abd-Elmoniem, E., Kandil, Y. I., Eldeeb, H. M., ... & Khan, R. A. (2020). Anti-microbial, anti-oxidant, and α-amylase inhibitory activity of traditionally-used medicinal herbs: a comparative analyses of pharmacology, and phytoconstituents of regional halophytic plants' diaspora. *Molecules*, *25*(22), 5457.

Berbudi, A., Khairani, S., & Tjahjadi, A. I. (2025). Interplay between insulin resistance and immune dysregulation in type 2 diabetes mellitus: implications for therapeutic interventions. *ImmunoTargets and Therapy*, 359-382.

Blagov, A., Nedosugova, L., Kirichenko, T., Sukhorukov, V., Melnichenko, A., & Orekhov, A. (2024). Mitochondrial dysfunction as a factor of energy metabolism disorders in type 2 diabetes mellitus. *Frontiers in Bioscience-Scholar*, *16*(1), 5.

Hussein, I., Ahmad, F. U. D., Asif, A., Khan, S., Chohan, M. S., & Sewell, R. D. (2025). The potential mitigating activity of Cassia absus seed extract on carbon tetrachloride-induced liver injury. *Journal of Herbmed Pharmacology*, *14*(1), 90-96.

Kamiloglu, S., Günal-Köroğlu, D., Ozdal, T., Tomas, M., & Capanoglu, E. (2024). Recent advances on anti-diabetic potential of pigmented phytochemicals in foods and medicinal plants. *Phytochemistry Reviews*, 1-31.

Kumar, A., Arora, A., Choudhury, A., Arora, V., Rela, M., Jothimani, D. K., ... & APASL ACLF Research Consortium. (2025). Impact of diabetes, drug-induced liver injury, and sepsis on outcomes in metabolic dysfunction associated fatty liver disease–related acute-on-chronic liver failure. *Official*

journal of the American College of Gastroenterology| ACG, *120*(4), 816-826.

Motto AE, Lawson-Evi P, Eklu-Gadegbeku K. Antidiabetic and antioxidant potential of total extract and supernatant fraction of the roots of Anogeissus leiocarpus in HFD-fed and Streptozocin-induced diabetic rats. Biomed Pharmacother 2022; 154: 113578.

Najmi, A., Javed, S. A., Al Bratty, M., & Alhazmi, H. A. (2022). Modern approaches in the discovery and development of plant-based natural products and their analogues as potential therapeutic agents. *Molecules*, *27*(2), 349.

Pye CR, Bertin MJ, Lokey RS, Gerwick WH, Linington RG. Retrospective analysis of natural products provides insights for future discovery trends. Proc Natl Acad Sci 2017; 114: 5601-5606.

Sajid, M.R., B. Aslam, F. Hassan and Z.U.D. Sindhu. 2025. Determination of Phytochemical Constituents and Anti-Dyslipidemic Potential of Cassia absus Seed Extract to Combat High-Fat Diet-Induced Dyslipidemia in Rats Model. Pakistan Journal of Agricultural Sciences. 62: 261-267.

Subhan, F., Aslam, B., Muhammad, F., Faisal, M. N., Hussain, A., & Bari, M. U. (2021). The efficiency of Cressa cretica (Linn.) extract in attenuating vancomycin-induced nephrotoxicity and oxidative stress in rabbits. *Comparative Clinical Pathology*, *30*(5), 829-839.

Sukhikh, S., Babich, O., Prosekov, A., Kalashnikova, O., Noskova, S., Bakhtiyarova, A., ... & Ivanova, S. (2023). Antidiabetic properties of plant secondary metabolites. *Metabolites*, *13*(4), 513.

Unuofin, J. O., and S. L. Lebelo. 2020. Antioxidant effects and mechanisms of medicinal plants and their bioactive compounds for the prevention and treatment of type 2 diabetes: an updated review. Oxid. Med. Cell. Longev. 2020.

Xie, S., Zhan, F., Zhu, J., Xu, S., & Xu, J. (2025). The latest advances with natural products in drug discovery and opportunities for the future: a 2025 update. *Expert Opinion on Drug Discovery*, *20*(7), 827-843.

Yang, X., Ding, W., Chen, Z., Lai, K., & Liu, Y. (2024). The role of autophagy in insulin resistance and glucolipid metabolism and potential use of autophagy modulating natural products in the treatment of type 2 diabetes mellitus. *Diabetes/Metabolism Research and Reviews*, *40*(1), e3762.