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In-vitro and *in-vivo* antidiabetic activity of aerial parts of *Aitchisonia rosea* supported by phytochemical and GC-MS analysis

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Abstract: Medicinal plants contain a wide variety of bioactive phytoconstituents which can serve as new therapeutic agents for several diseases. This study examines the antidiabetic potential of *Aitchisonia rosea* in alloxan-induced diabetic rats and identifies its bioactive phytoconstituents using GC-MS. *In vitro,* antidiabetic potential was established using the α-amylase inhibition assay. *In vivo,* antidiabetic potential was investigated by employing the oral glucose tolerance test (OGTT). GC-MS analysis was used to identify the bioactive phytoconstituents. The *in vitro* and *in vivo* tests showed that the aqueous extract of *A. rosea* possesses better antidiabetic potential. The α-amylase inhibition assay highlighted an IC_{50} value of 134.87µg/ml. In an oral glucose tolerance test, rats given an aqueous *A. rosea* extract significantly lowered their blood sugar levels significant reduction in the blood glucose concentration was observed in the oral glucose tolerance test in rats treated with the aqueous *A. rosea* extract. GC-MS investigation revealed many phytoconstituents, with serverogenin acetate and cycloheptasiloxane tetradecamethyl being important antidiabetic agents. This study found anti-diabetic properties in *A. rosea* extract. The phytochemical and GC-MS investigation also found serverogenin acetate and cycloheptasiloxane tetradecamethyl, which could be used to develop new antidiabetic drugs.

Keywords: Antidiabetic activity, *Aitchisonia rosea*, OGTT, GC-MS, Serverogenin, α-amylase inhibitory activity.

INTRODUCTION

Diabetes is a chronic disease caused by insufficient secretion of insulin or the body's inability to utilize insulin, which eventually results in hyperglycemia (Keskes *et al*., 2014). Diabetes mellitus is usually associated with secondary complications such as cardiovascular complications, neuropathy, nephropathy and retinopathy (Bandawane *et al*., 2011). In 2019, globally, there were 463 million individuals with diabetes. This count is expected to rise to 578 million by 2030 and 700 million by 2045 (Iduye *et al*., 2022). Plant products are becoming increasingly popular for diabetes care due to their efficacy. Compared to the current medication for diabetes, it has fewer side effects and a lower cost (Ju *et al*., 2008). Because of the rising prevalence of diabetes around the world and associated side effects and the lack of efficacy of currently available antidiabetic medicines, there is an immediate need to investigate more effective and safer treatments derived from natural sources (Uzor and Osadebe, 2016).

The family Rubiaceae consists of 450 genera and over 6500 species. *Aitchisonia* is a monotypic genus belonging to the Rubiaceae family and consists of only one species, *Aitchisonia rosea*. The plant *Aitchisonia rosea* is a bushy shrub found in Pakistan, Iran and Afghanistan (Noor *et al*., 2009). In Pakistan, its aerial portions are used in traditional medicine to cure skin problems and the plant is most commonly found in the hilly regions of Baluchistan (Rasool *et al*., 2015).

Previously, it has been reported that *Aitchisonia rosea* possesses anticonvulsant, antimicrobial, analgesic and anti-inflammatory potential (Rasool *et al*., 2020). The literature survey of the plant revealed very limited data regarding the pharmacological studies conducted on it. Therefore, the present study aimed to investigate the undocumented antidiabetic potential of the plant, followed by characterization of the most active extract through GC-MS to identify the phytoconstituents responsible for exerting the antidiabetic effect.

MATERIALS AND METHODS

Drugs and reagents

Acarbose was obtained from Bayer Pvt. Ltd., Pakistan. Alloxan monohydrate was bought from Sigma-Aldrich in St. Louis, USA. Glibenclamide was obtained as gift from Sanofi-Aventis, Pakistan. All the chemicals and reagents utilized in the present study were procured from commercial sources and of analytical grade.

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Collection and extraction of plant material

The *Aitchisonia rosea* was collected from the mountain range of Quetta, Pakistan. Authentication of the plant was done by the Department of Botany, GCU, Lahore, Pakistan and voucher specimen number 1911 was deposited at the university herbarium for future citation purposes. The desiccated aerial components of plants were pulverised using an electric mill. Subsequently, a quantity of 1 kg of pulverised material was selected and treated to a series of extraction. Briefly, plant material was finely ground and immersed in 2.5 litres of n-hexane for a duration of seven days, after which it was separated using a filtration process. The marc was dried and subsequently immersed in 2.5 L of chloroform for seven days. The dried marc was then again filtered. After the marc was dried, it was subsequently immersed in 2.5 litres of distilled water for a week. Following this, the mixture was filtered and the residual marc was discarded. The three filtrates, consisting of n-hexane, chloroform and distilled water, were evaporated using a rotary evaporator (Eyela, Japan) at a temperature range of 38-40°C under reduced pressure (United States Pharmacopeia, 2018). The process of evaporation resulted in the production of three extracts: n-hexane, chloroform and aqueous, respectively.

Phytochemical analysis

All three extracts of *A. rosea* were analyzed after treatment with the chemical reagents to confirm the presence of a wide variety of phytoconstituents. Phytochemical analyses were conducted to ascertain the presence of primary metabolites, specifically carbohydrates and proteins as per standard protocols (Nawaz *et al*., 2020). The Molish, Fehling, Benedict (Al Jamal and Al Yousef, 2018), Seliwanoff (Besir *et al*., 2021) and iodine tests (Fleischer, 2019) were used to identify the presence of carbohydrates. The presence of proteins and amino acids was verified using Millon's and Ninhydrin tests (Pawar and Jadhav, 2015).

Whereas, standard procedures were used to evaluate secondary metabolites such as alkaloids, glycosides, terpenoids, tannins, phenolic compounds and flavonoids (Nawaz *et al*., 2020). The presence of alkaloids was verified through the utilization of Dragendorff's (Raal *et al*., 2020), Mayer's, Wagner's and Hager's tests (Pawar and Jadhav, 2015). The existence of tannins was confirmed using the ferric chloride and bromine water test (Asthana *et al*., 2019). The glycosides were detected through the application of the Legal test and the Keller-Killani test (Shaikh and Patil, 2020). The presence of flavonoids was assured using the lead acetate and alkaline reagent tests (Shaikh and Patil, 2020). The presence of terpenoids was identified using the Salkowski and Liberman Burchard tests (Malik, 2017).

Total phenolic and flavonoid content estimation

The total phenolic content was estimated using the Folin-Ciocalteu (FC) colorimetric technique (Chaovanalikit and Wrolstad, 2004). Gallic acid was utilized as the standard control. Concisely, test tubes were collected and filled with 500 µL FC reagent and 100 µL of each extract. These were then added to individual test tubes holding 7.5 mL of distilled water. The FC reagent and each extract were combined in distilled water. Subsequently, the mixture underwent incubation for a duration of 10 min at a temperature of 30^0C. Subsequently, 1.5 mL of a freshly produced solution of Na_2CO_3 with a concentration of 20% (w/v) was added to the mixture mentioned before. Next, a water bath set to a temperature of 40°C was utilized to heat the combination for a duration of 20 minutes. The heated mixture then cooled using an ice bath. The cooled solution underwent measurement using a UV spectrophotometer (Shimadzu UV-1700, Japan) and the absorbance at 725 nm was determined for each extract. The absorbance of gallic acid was measured at various concentrations (10, 20, 40, 80, 100 and 120 µg/mL) and a calibration curve was created by using UV-Probe V2.2 (Musdalipah *et al*., 2021). The total phenolic content (TPC) was expressed in GAE mg/g. The formula described below was used to calculate the TPC:

GAE mg/g = milligrams of gallic acid equivalents (GAE) per gram of samples.

Similarly, total flavonoid content (TFC) was determined using the same calorimetric method, as described by Dewanto and colleagues after some modifications (Dewanto *et al*., 2002). Catechin was used as standard. The TFC were calculated by the formula as described under:

CE mg/g = milligrams of catechin equivalent (CE) per gram of sample.

Assay for α-amylase inhibitory activity

All three extracts were tested for their ability to inhibit α-amylase using a standard protocol (Kwon *et al*., 2006). Stock solutions of all three extracts were prepared to carry out the assay. Each extract was tested at six different concentrations including 20, 40, 60, 80, 100 and 200 µg/ml. Test tubes with 0.5 mg/ml of α-amylase solution in 0.02 M sodium phosphate buffer are filled with extract (500 µl). The mixture was first incubated at 25 °C for 10 minutes, after which 1% starch solution (500 µl) was added to each test tube and the samples were incubated at 30 °C for another 10 minutes. To stop the process, 1000 µl of dinitro salicylic acid (DNSA) solution was added and the mixture was heated in a boiling water bath for 5 minutes before cooling at room temperature (Khadayat *et al*., 2020). The absorbance of each reaction mixture was measured at a wavelength of 540 nm by using UV spectrophotometer (Shimadzu UV-1700 Japan) after being diluted with distilled water (10 ml). Acarbose served as positive control.

The formula used to measure the percentage inhibition is as under:

$$\text{Inhibition activity \%} = \frac{(\text{Absorbance of control} - \text{Absorbance of extract})}{\text{Absorbance of control}} \times 100$$

Oral Glucose Tolerance Test (OGTT)

Animals

In the current study, male and female Wistar rats weighing 200-250 g were used. The animals were confined in propylene cages with a controlled temperature range of 22-25 °C and a 12-hour light and dark cycle Standard pellet food and water were provided ad libitum to the caged animals. All experimental procedures were approved by the Institutional Research and Ethics Committee (IREC), University of Lahore.

Induction of diabetes

A diabetic state was induced in the rats via intraperitoneal injection of alloxan monohydrate into their abdominal cavity at a dose of 150 mg/kg (Aamir *et al*., 2021). After 72 hours of alloxan monohydrate administration, animals were considered diabetic having blood glucose levels≥ 200 mg/dl ultimately selected to proceed with the study.

Experimental design

After induction of diabetes in all groups except normal control, the animals were split into 6 groups of 5 rats each (n=5). Groups 1 and 2 served as normal and diabetic control respectively and received 0.9% saline p.o. while group 3 served as positive control and received orally administered Glibenclamide (600 µg/kg). Nevertheless, diabetic rats in groups 4, 5 and 6 received a daily administration of a single dose of n-hexane, chloroform and aqueous extract of *Aitchisonia rosea* through oral gavage for a duration of 15 days (300 mg/kg).

Oral glucose tolerance test (OGTT)

The oral glucose tolerance test was performed according to a previously done method (Aamir *et al*., 2021). Before the glucose challenge, the fasting blood glucose levels of all rats were measured. After an oral dose of glucose (2000 mg/kg/bw), blood glucose levels were checked at 0, 30, 60, 90 and 120 minutes using a Accucheck instant S glucometer (924, Roche, Germany).

Gas chromatography-mass spectrometry (GC-MS) analysis

The aqueous extract of *Aitchisonia rosea* exhibiting maximum antidiabetic potential was selected to undergo GC-MS analysis. The GC-MS of the extract was performed at 70 eV via Agilent Technologies GC-MS system (California, USA) consisting of a 6850 Network GC system equipped with a 5973-mass selective detector. The sample was injected through the 7683B auto-injector into an HP-5MS capillary column (film thickness 0.25 µm, length 30 m, inner diameter 0.25 mm), which has 5% phenyl-methylpolysiloxane as its stationary phase. The helium carrier gas flow rate was adjusted at 1.2 ml/min. The programming of the oven's temperature is comprised of three phases. Initially, the temperature was raised from 45 °C to 150 °C through a constant 5 °C/min increase in temperature and the temperature of 150 °C was maintained for 5 minutes. In the second phase, the temperature was increased up to 280 °C through a constant 10 °C/min rise in temperature and maintained at 280 °C for 10 minutes. In the final phase, the temperature was further raised to 325 °C through a 20 °C/min increase in temperature and maintained at this temperature for 5 minutes. The identification of the constituents was done through comparison with standard alkane series (C_8- C_{24}) and with the National Institute of Standards and Technology (NIST 05) Mass Spectral Library and published spectra (Adams, 2001).

Ethical approval

The experimental protocols obtained approval from the Institutional Research Ethics Committee under the Ethics No. IREC-2016-125.

STATISTICAL ANALYSIS

Results were expressed as Mean ± SEM. The statistical differences between groups were evaluated by using one-way analysis of variance (ANOVA) followed by Tukey's range test. Differences were considered significant at $P<0.05$.

RESULTS

Evaluation of Phytoconstituents

The results of phytoconstituents present in *A. rosea* are depicted in table 1. In comparison to the n-hexane extract, the aqueous and chloroform extracts contained carbohydrates, glycosides, alkaloids, tannins, flavonoids and proteins.

Total Phenolic and Flavonoid contents

Total phenolic content (TPC) and total flavonoid content (TFC) of *A. rosea* extracts were determined and it was noticed that aqueous extract contains the highest quantity of both TPC and TFC when compared to chloroform and n-hexane extracts. The results are shown in figs. 1 and 2 and table 2.

Phenolic and flavonoids are important constituents of medicinal plants. The aqueous extract of *A. rosea* contains the maximum amount of total phenolic and flavonoid contents and the results of aqueous extract were found significant as compared to chloroform and n-hexane extracts which might be liable for antioxidant activity in *A. rosea*.

Effect of A. rosea extracts on α-amylase inhibition.

Among the *A. rosea* extracts, the aqueous extract showed maximum inhibition as reflected by an IC_{50} value of 134.87 µg/ml, following chloroform extract having an

IC_{50} value of 149.32 µg/ml and the IC_{50} value for the standard acarbose was 96.46 µg/ml. The results for the α-amylase inhibition assay are shown in table 3 and fig. 3.

Effect of A. rosea extracts on OGTT

When comparing the blood glucose levels of the normal control group with the group of diabetic rats that did not receive treatment (negative control) using the oral glucose tolerance test (OGTT), a statistically significant (*P<0.01*) increase in blood glucose levels was seen in the negative control group. However, the groups treated with Glibenclamide and the aqueous *A. rosea* extract showed a significant reduction (***P*<0.01) in the level of blood glucose. Moreover, results showed that chloroform extract also significantly reduced blood glucose levels (***P*<0.05). The results of OGTT are given in table 4 and fig. 4.

GC-MS characterization of the extract

Following the *in vitro* and *in vivo* antidiabetic assays, the aqueous *A. rosea* extract was selected to undergo GC-MS characterization to identify the bioactive phyto-constituents, as it exhibited the maximum antidiabetic activity. The chromatogram obtained is shown in fig. 5 the list of phytoconstituents identified by the compound library is given in table 5 and the structures of the phytoconstituents are shown in fig. 6.

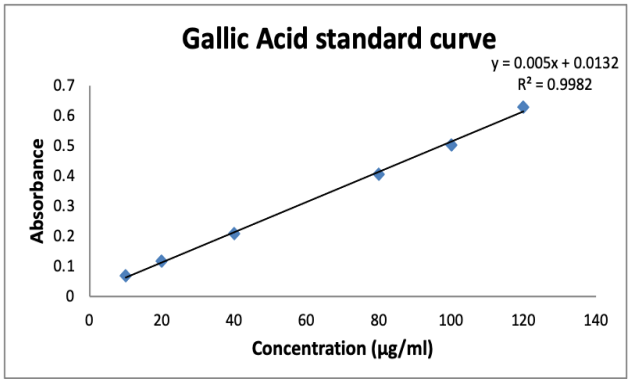


Fig. 1: Regression Line for Gallic Acid

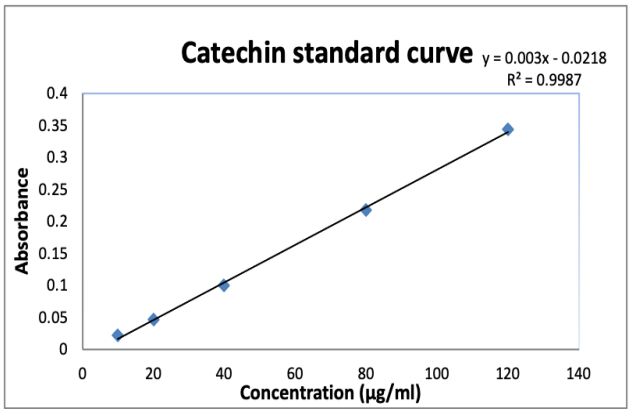


Fig. 2: Calibration Curve for Catechin Showing Linear Regression.

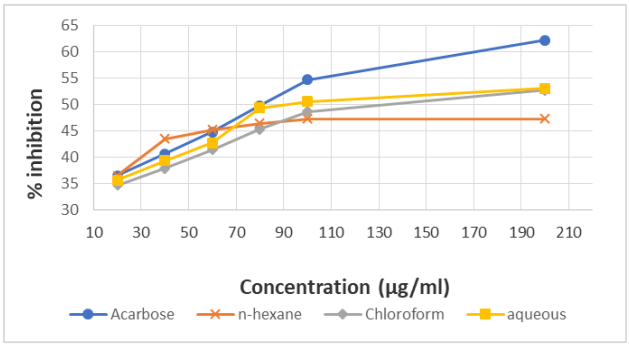


Fig. 3: α-amylase inhibition by *A. rosea* extracts and acarbose.

DISCUSSION

With the increasing prevalence of diabetes worldwide and the drawbacks associated with the available antidiabetic drugs, there has been an increased interest in developing antidiabetic agents from natural resources (Soni *et al*., 2018). Moreover, even with great developments in synthetic drugs, herbal medicine is usually preferred because of the decreased cost and the belief that it is safer to use as compared to synthetic drugs (Zaid *et al*., 2016). As a result, the search for potent natural antidiabetic agents having fewer side effects has become the main aim of scientists worldwide (Raafat and Omar, 2016).

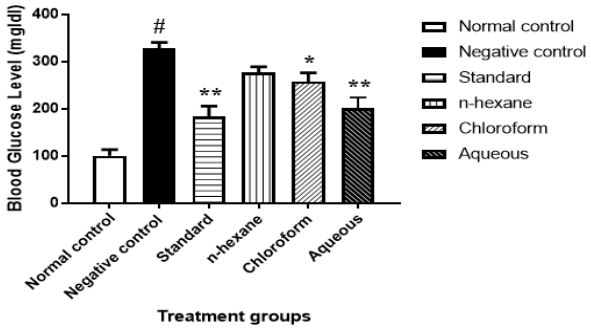


Fig. 4: Overall result of the effect of *A. rosea* extracts on OGTT. Values are expressed mean ± SEM, n=5. **P*<0.05, ***P*<0.01 as compared to negative control. #*P*<0.01, negative control as compared to normal control.

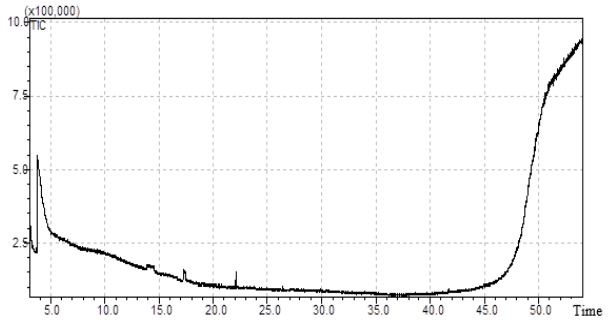


Fig. 5: GC-MS chromatogram of aqueous extract of *A. rosea*.

Table 1: Phytochemical analysis of extracts of *A. rosea*

Phytoconstituents	*n*-hexane extract	Chloroform extract	Aqueous extract
Carbohydrate	-	+	+
Proteins	-	-	+
Glycosides	-	+	+
Alkaloids	-	+	+
Saponins	-	-	+
Flavonoids	-	+	+
Tannins, Phenols	-	+	+
Terpenoids	-	-	+
Fats and Fixed oils	+	-	+

(+) means presence of phytoconstituents and (-) means absence of phytoconstituents

Table 2: Total phenolic and flavonoid contents

Extracts	TPC (GAE mg/g)	TFC (CE mg/g)
n-hexane	17.44±0.003	12.93±0.005
Chloroform	21.04±0.003	17.93±0.004
Aqueous	47.84**±0.002	40.60**±0.004

Data is expressed as Mean ± SEM, (n=3)

Table 3: IC_{50} values of acarbose and *A. rosea* extracts for α-amylase inhibition assay

Sample	IC_{50}[a] (µg/ml)
Acarbose (control)	96.46**
n-hexane extract	210.47
Chloroform extract	149.32*
Aqueous extract	134.87**

[a] Calculation of IC_{50} value done through linear regression equation by plotting graph of concentration vs. %inhibition. Values are expressed as mean ± SEM, *P<0.05, **P<0.01.

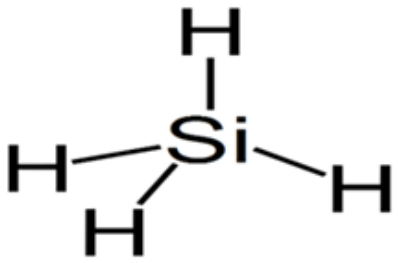
Table 4: Effect of *A.rosea* extracts on OGTT

Level of blood glucose in mg/dl at different time intervals	Treatment Groups with doses					
	Control 0.9% saline	Negative control 0.9% saline	Glibenclamide 600µg/kg	*n*-hexane extract 300mg/kg	Chloroform extract 300mg/kg	Aqueous extract 300mg/kg
0 min	77±1.07	282.2±2.80$^{\#}$	200±4.03**	278.6±3.97	260.0±3.08	206.2±3.02**
30 min	139.2±1.71	320±3.51$^{\#}$	236.8±3.56**	293.0±2.00	315.2±4.14	250.6±4.30**
60 min	121.4±1.08	333±4.76$^{\#}$	210.2±3.10**	308.4±2.32	273.2±2.82*	242.0±3.83**
90 min	95.2±1.20	344±3.49$^{\#}$	162.8±3.72**	263.2±3.89	234.2±3.95*	184.0±4.24**
120 min	69.6±1.16	358.2±4.35$^{\#}$	111.2±1.48**	244.0±3.21	205.8±4.15*	124.4±4.18**

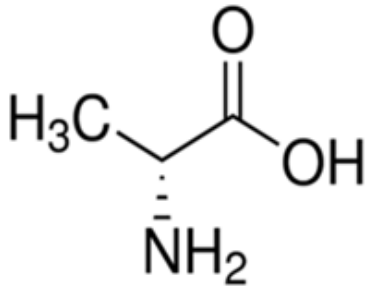
Values are expressed as mean ± SEM, n=5. *P<0.05, **P<0.01 as compared to negative control. #*P*<0.01, negative control as compared to normal control.

Table 5: List of phytoconstituents identified in GC-MS analysis of aqueous *A. rosea* extract

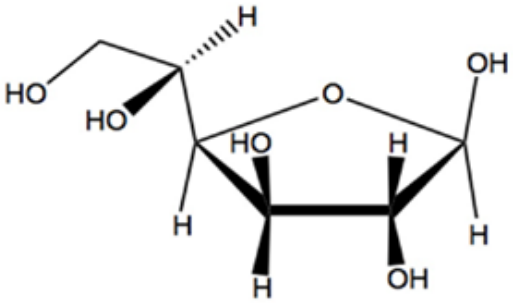
Chemical Compounds	Silane	d-alanine	Glucofuranose	Serverogenin acetate	Cycloheptasiloxane tetradecamethyl
Molecular Weight	458	89	518	544	519
Retention Time	22.1	26.425	19.15	3.908	17.35
Molecular Formula	$C_{20}H_{42}O_4Si_4$	$C_3H_7NO_2$	$C_{23}H_{34}O_{13}$	$C_{29}H_{36}O_{10}$	$C_{14}H_{42}O_7Si_7$



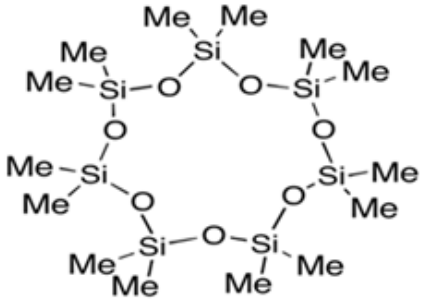
(a) **Silane**



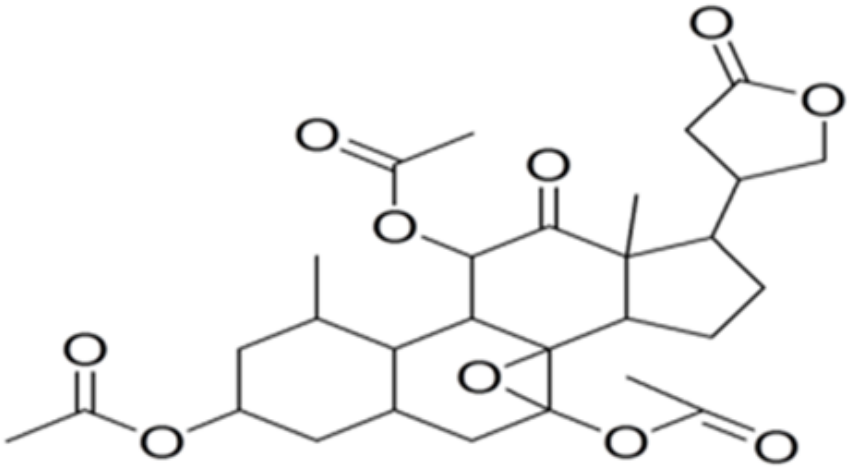
(b) **d-alanine**



(c) **Glucofuranose**



(d) **Cycloheptasiloxane tetradecamethyl**



(e) **Serverogenin acetate**

Fig. 6: Chemical structures of phytoconstituents detected through GC-MS in aqueous *A. rosea* extract.

The purpose of this research was to find out the antidiabetic potential of various *A. rosea* extracts by testing their effectiveness in *in-vitro* and *in-vivo* and then analysing the most effective extract using gas chromatography-mass spectrometry.

Glycaemic control refers to the average blood glucose levels in diabetic patients and attaining good glycaemic control is the overall goal of diabetes treatment (Tarnow *et al*., 2008). Loss of postprandial plasma glycaemic control is observed in diabetic patients which leads to elevated postprandial plasma glucose (PPG) levels known as postprandial hyperglycemia. In diabetic patients, there is an inadequate release of insulin following food consumption, because of which inhibition of glucagon secretion does not occur. Both these factors lead to the production of glucose from the liver and kidneys and also decreased uptake by the cells, ultimately leading to postprandial hyperglycemia (Gerich, 2013). Postprandial hyperglycemia causes hindrance in achieving ideal

glycaemic control and thus is of importance in managing not only diabetes mellitus but also the secondary complications associated with diabetes as well, such as cardiovascular diseases, stroke, retinopathy, nephropathy and neuropathy (Gerich, 2003).

Carbohydrate hydrolyzing enzyme inhibition reduces postprandial hyperglycemia. The enzymes pancreatic α-glucosidase and amylase present in the small intestine are involved in carbohydrate metabolism and convert polysaccharides into monosaccharides.

Increased glucose levels after a meal occur from the absorption of the glucose produced by these enzymes (Matsui *et al*., 2007). Drugs like acarbose, voglibose and miglitol that inhibit these enzymes help in controlling postprandial hyperglycemia and are used as effective treatments to control diabetes. Screening of potential antidiabetic drugs is done by studying their inhibitory action on these enzymes. Owing to their high cost and side effects associated with their long-term use, continuous efforts are being made to search for enzyme inhibitors from plants or microbial sources (Poovitha and Parani, 2016).

In the present research, the *in vitro* inhibition of the α-amylase enzyme was studied with starch being used as a substrate. Among the *A. rosea* extracts, the results revealed the aqueous extract to have the highest α-amylase inhibitory activity having an IC_{50} value of 134.87 µg/ml, followed by the chloroform extract which had an IC_{50} value of 149.32 µg/ml. The standard acarbose had an IC_{50} value of 96.46 µg/ml. Since the aqueous and chloroform extracts showed significant inhibition of the α-amylase enzyme, these can be utilized for developing a treatment for diabetes management that can decline the intestinal absorption of glucose. Similarly, the aqueous extract derived from the leaves of *Morinda lucida* demonstrated the ability to inhibit α-amylase in the context of metabolic syndrome (Kazeem *et al*., 2013).

The OGTT is commonly used to assess the body's ability to metabolize a glucose load after oral administration (Ramesh and Rani, 2019). The results of OGTT revealed that *A. rosea* extracts (aqueous and chloroform) controlled the blood glucose concentration after glucose administration in diabetic animals, with the aqueous extract exhibiting a stronger antidiabetic response as compared to chloroform extract. These results were also comparable with phytochemical analysis that confirmed the presence of maximum phytochemicals in aqueous extract as compared to chloroform and n-hexane extract. The results of our study are corroborated by Shewamene and colleagues, who showed that *Otostegia integrifolia* has an antidiabetic effect in rodents by enhancing glucose tolerance during oral glucose tolerance testing (OGTT) (Shewamene *et al*., 2015). Moreover, Aqueous extract also contains adequate amounts of total phenolic and flavonoid contents which may be responsible for the antidiabetic activity.

Based on the findings from *in vitro* and *in vivo* studies, the aqueous extract was selected to undergo GC-MS analysis as it showed maximum antihyperglycemic and α-amylase inhibition. Characterization of the *A. rosea* extract by GC-MS revealed the presence of diverse phytoconstituents, out of which serverogenin acetate and cycloheptasiloxane tetradecamethyl are of importance in exerting the antidiabetic effect.

Serverogenin acetate is the main bioactive phytoconstituent responsible for exerting the antidiabetic effect owing to its property in inhibiting the α-amylase, α-glucosidase and aldose reductase enzymes. A recent study conducted on the molecular docking interactions of serverogenin acetate with these enzymes revealed it to have a strong binding affinity with the binding sites of target proteins present in these enzymes (Konappa *et al*., 2020). The inhibition of the aldose reductase enzyme can block the progression of the polyol pathway, which is involved in diabetic complications such as diabetic retinopathy, nephropathy and neuropathy (Behl *et al*., 2016, Forbes *et al*., 2008).

Serverogenin acetate is also known to possess antioxidant properties (Konappa *et al*., 2020), similarly the aqueous extract of *A. rosea* possesses an adequate quantity of phenolic and flavonoid contents which can prove to be beneficial in oxidative stress conditions in hyperglycemic conditions. Both nicotinamide adenine dinucleotide hydrogen (NADH) and flavin adenine dinucleotide hydrogen (FADH) are formed in excess under hyperglycemic circumstances, this ultimately leads to the generation of surplus amounts of superoxide radical after the NADH and FADH enter the electron transport chain. This causes an imbalance in the homeostasis of the free radicals, thus leading to oxidative stress, which in turn is the main cause of diabetic complications including both microvascular as well as cardiovascular complications (Javed *et al*., 2015). Control of oxidative stress in type 2 diabetes is specifically important, since in addition to the insulin resistance already present, the oxidative stress can lead to the destruction of the pancreatic β cells (King and Loeken, 2004).

The other important bioactive phytoconstituent identified through GC-MS characterization is cycloheptasiloxane tetradecamethyl. This compound has been reported to possess immunomodulatory, antimicrobial and antitumor activity (Wahyuni *et al*., 2017). In type 1 diabetes, the destruction of pancreatic β cells takes place because of the autoimmune response of the body towards these cells. Many immunotherapies are currently being investigated to suppress the autoimmune response and preserve the

pancreatic β cells (Waldron-Lynch and Herold, 2011). The immunomodulatory action of the phytoconstituent cycloheptasiloxane tetradecamethyl, thus, can be manipulated in modulating the autoimmune response seen in type 1 diabetes.

The findings of the current research work are clear indications of the antioxidant as well as enzyme-inhibiting properties of the phytoconstituent in bringing about the antidiabetic effect of the aqueous *A. rosea* extract in alloxan-induced diabetic rats.

CONCLUSION

The current investigation was aimed at investigating the undocumented antidiabetic potential of *A. rosea* and then identifying the various bioactive phytoconstituents responsible for exerting the antidiabetic effect. The GC-MS analysis of the aqueous *A. rosea* extract revealed phytoconstituents of established diverse therapeutic potentials, which accordingly ameliorated the hyperglycemia in the alloxan-indued diabetic rats. The bioactive phytoconstituent serverogenin acetate can be utilized in developing an effective antidiabetic drug, owing to its α-amylase, α-glucosidase and aldose reductase enzyme inhibiting as well as antioxidant properties. Moreover, the immunomodulatory property of the phytoconstituent cycloheptasiloxane tetradecamethyl can also be utilized in developing an effective immunotherapeutic agent that works against the autoimmune response seen in type 1 diabetes.

REFERENCES

Aamir K, Khan HU, Hossain CF, Afrin MR, Jusuf PR, Waheed I, Sethi G and Arya A. (2021). Arjunolic acid downregulates elevated blood sugar and pro-inflammatory cytokines in streptozotocin (STZ)-nicotinamide induced type 2 diabetic rats. *Life Sci.*, **289**(5): 120232.

Adams RP (2001). Identification of essential oil components by gas chromatography, quadrupole mass spectroscopy. 3rd ed. Allured publishing corporation, Carol Stream, Ill. p.456.

Al Jamal A and Al Yousef M (2018). Phytochemical analysis of some herbal medicines. *Medbiotech. J.*, **2**(2): 82-84.

Asthana S, Maurya SP, Maurya P and Maurya A (2019). Qualitatve analysis of protein: egg albumin and milk. *Indian. J. Drugs.*, **7**(1): 30-33.

Bandawane D, Juvekar A and Juvekar M (2011). Antidiabetic and antihyperlipidemic effect of *Alstonia scholaris* Linn bark in streptozotocin induced diabetic rats. *Indian. J. Pharm.Educ. Res.*, **45**(2): 114-120.

Behl T, Kaur I and Kotwani A (2016). Implication of oxidative stress in progression of diabetic retinopathy. *Surv. Ophthalmol.*, **61**(2): 187-196.

Besir A, Yazici F, Mortas M and Gul O (2021). A novel spectrophotometric method based on Seliwanoff test to determine 5-(Hydroxymethyl) furfural (HMF) in honey: Development, in house validation and application. *Lwt.*, **139**(1): 110602.

Chaovanalikit A and Wrolstad R (2004). Total anthocyanins and total phenolics of fresh and processed cherries and their antioxidant properties. *J. Food Sci.*, **69**(1): FCT67-FCT72.

Dewanto V, Wu X, Adom KK and Liu RH. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J Agric. Food Chem.*, **50**(10): 3010-3014.

Fleischer H (2019). The iodine test for reducing Sugars-A safe, quick and easy alternative to copper (II) and silver (I) based reagents. *World J. Chem. Educ.*, **7**(2): 45-52.

Forbes JM, Coughlan MT and Cooper ME (2008). Oxidative stress as a major culprit in kidney disease in diabetes. *Diabetes.*, **57**(6): 1446-1454.

Gerich J (2013). Pathogenesis and management of postprandial hyperglycemia: role of incretin-based therapies. *Int. J. Gen Med.*, **6**: 877-895.

Gerich JE (2003). Clinical significance, pathogenesis and management of postprandial hyperglycemia. *Arch Intern Med.*, **163**(11): 1306-1316.

Iduye D, Wild S, Ostrishko K, Macdonald M, Helwig M, Iduye S and Jefferies K (2022). Lifestyle interventions for type 2 diabetes prevention in children and adolescents of African descent in OECD countries: A systematic review protocol. *JBI Evid. Synth.*, **20**(5): 1392-1403.

Javed S, Petropoulos IN, Alam U and Malik RA (2015). Treatment of painful diabetic neuropathy. *Ther. Adv. Chronic Dis.*, **6**(1): 15-28.

Ju JB, Kim JS, Choi CW, Lee HK, Oh TK and Kim SC (2008). Comparison between ethanolic and aqueous extracts from Chinese juniper berries for hypoglycaemic and hypolipidemic effects in alloxan-induced diabetic rats. *J. Ethnopharmacol.*, **115**(1): 110-115.

Kazeem M, Adamson J and Ogunwande I (2013). Modes of inhibition of α-amylase and α-glucosidase by aqueous extract of *Morinda lucida* Benth leaf. *BioMed Res. Int.*, **1**: 527570.

Keskes H, Mnafgui K, Hamden K, Damak M, El Feki A and Allouche N (2014). *In vitro* anti-diabetic, anti-obesity and antioxidant proprieties of *Juniperus phoenicea* L. leaves from Tunisia. *Asian Pac. J. Trop Biomed.*, **4**(2): S649-S655.

Khadayat K, Marasini BP, Gautam H, Ghaju S and Parajuli N (2020). Evaluation of the alpha-amylase inhibitory activity of Nepalese medicinal plants used in the treatment of diabetes mellitus. *Clin Phytosci.*, **6**(34): 1-8.

King GL and Loeken MR (2004). Hyperglycemia-induced oxidative stress in diabetic complications. *Histochem Cell Biol.*, **122**(4): 333-338.

Konappa N, Udayashankar A C, Krishnamurthy S, Pradeep CK, Chowdappa S and Jogaiah S (2020). GC-MS analysis of phytoconstituents from *Amomum nilgiricum* and molecular docking interactions of bioactive serverogenin acetate with target proteins. *Sci. Rep.,* **10**(1): 16438.

Kwon YI, Vattem DA and Shetty K (2006). Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension. *Asia Pac. J Clin. Nutr.*, **15**(1): 107-118.

Malik S (2017). Qualtitative and quantitative estimation of terpenoid contents in some important plants of Punjab, Pakistan. *Pak J. Sci.*, **69**(2): 150-154.

Matsui T, Tanaka T, Tamura S, Toshima A, Tamaya K, Miyata Y, Tanaka K and Matsumoto K (2007). α-Glucosidase inhibitory profile of catechins and theaflavins. *J. Agric. Food Chem.*, **55**(1): 99-105.

Musdalipah M, Tee SA, Karmilah K, Sahidin S, Fristiohady A and Yodha AW (2021). Total phenolic and flavonoid content, antioxidant and toxicity test with bslt of meistera chinensis fruit fraction from Southeast Sulawesi. *Borneo J. Pharm.*, **4**(1): 6-15.

Nawaz H, Shad MA, Rehman N andaleeb H and Ullah N (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean *Phaseolus vulgaris* seeds. *Braz J. Pharm. Sci.*, **56**(1): e17129.

Noor AT, Perveen S, Begum A, Fatima I, Malik A and Tareen RB (2009). Aitchisonides A and B, new iridoid glucosides from *Aitchisonia rosea. J. Asian Nat. Prod. Res.,* **11**(11): 985-989.

Pawar S, Jadhav M and Deokar TG (2016). Study of Phytochemical screening, physicochemical analysis and antimicrobial activity of *Bacopa monnieri* (L) extracts. *Int. J. Pharm. Clin. Res.,* **8**(8): 1222-1229.

Poovitha, S and Parani M (2016). *In vitro* and *in vivo* α-amylase and α-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd *Momordica charantia*. *BMC Complement Altern. Med.*, **16**(1): 185.

Raafat KM and Omar AG (2016). Phytotherapeutic activity of curcumol: Isolation, GC-MS identification and assessing potentials against acute and subchronic hyperglycemia, tactile allodynia and hyperalgesia. *Pharm Biol.,* **54**(8): 1334-1344.

Raal A, Meos A, Hinrikus T, Heinamaki J, Romane E, Gudienė V, Koshovyi O, Kovaleva A, Fursenco C and Chiru T (2020). Dragendorff's reagent: Historical perspectives and current status of a versatile reagent introduced over 150 years ago at the University of Dorpat, Tartu, Estonia. *Int. J. Pharm. Sci.,* **75**(7): 299-306.

Ramesh C and Rani AP (2019). *In vivo* and *in vitro* antidiabetic potentials of methanol extract of *Tephrosia pumila* against alloxan-induced diabetes in experimental animals. *Int. J. Health. Sci.,* **13**(3): 10-18.

Rasool S, Khan FZ and Ahmad M (2015). Evaluation of anticonvulsant, antimicrobial and hemolytic activity of *Aitchisonia rosea. Bangladesh J. Pharmacol.,* **10**(4): 966-971.

Rasool S, Uttra AM, Uttra MM, Ahmed F and Abbas K (2020). Evaluation of anti-inflammatory and analgesic activities of methanolic extract of *Aitchisonia rosea* in albino mice. *RADS J. Pharm. Pharm Sci.,* **8**(1): 31-36.

Shaikh JR and Patil M (2020). Qualitative tests for preliminary phytochemical screening: An overview. *Int. J. Chem. Stud.*, **8**(2): 603-608.

Shewamene Z, Abdelwuhab M and Birhanu Z (2015). Methanolic leaf exctract of *Otostegia integrifolia* Benth reduces blood glucose levels in diabetic, glucose loaded and normal rodents. *BMC Complement. Altern. Med.*, 15(1): 1-7.

Soni LK, Dobhal MP, Arya D, Bhagour K, Parasher P and Gupta R (2018). *In vitro* and *in vivo* antidiabetic activity of isolated fraction of *Prosopis cineraria* against streptozotocin-induced experimental diabetes: A mechanistic study. *Biomed. Pharmacother.,* **108**(1): 1015-1021.

Tarnow L, Groop PH, Hadjadj S, Kazeem G, Cambien F, Marre M, Forsblom C, Parving HH, Tregouet D and Thevard A (2008). European rational approach for the genetics of diabetic complications-EURAGEDIC: patient populations and strategy. *Nephrol. Dial. Transplant.*, **23**(1): 161-168.

United States Pharmacopeia (2018). The United States Pharmacopeial Convention. 2018. USP 41.

Uzor PF and Osadebe PO (2016). Antidiabetic activity of the chemical constituents of *Combretum dolichopetalum* root in mice. *EXCLI J.*, **15**(1): 290-296.

Wahyuni DK, Ansori AN and Vidiyanti F (2017). GC-MS analysis of phytocomponents in methanolic extracts of leaf-derived callus of *Justicia gendarussa* Burm. f. *Biosci. Res.*, **14**(3): 668-677.

Waldron-Lynch F and Herold KC (2011). Immuno-modulatory therapy to preserve pancreatic β-cell function in type 1 diabetes. *Nat. Rev. Drug Discov.*, **10**(6): 439-452.

Zaid H, Mahdi AA, Tamrakar AK, Saad B, Razzaque MS and Dasgupta A (2016). Natural active ingredients for diabetes and metabolism disorders treatment. *Evid. Based Complement. Altrn. Med.,* **2016**: 2965214.