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Original Article

Phytochemical Screening, Heavy Metal Content and HPLC Estimation of Bioactive Compound from Leaves and Flower Extracts of *Abutilon indicum* for *in vitro* Antidiabetic Activity

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Abstract

This research focused on the phytochemical screening, estimation of heavy metal content, and HPLC analysis of bioactive compounds exhibiting *in vitro* hypoglycemic activity in the ethanol extracts derived from the leaves and flowers of *Abutilon indicum*. The assessment of heavy metal content was conducted using atomic absorption spectrophotometry, while the estimation of phytochemicals was performed through HPLC. The *in vitro* antidiabetic activity was evaluated using methods that inhibit the enzymes alpha amylase and alpha glucosidase. Cytotoxicity was assessed *via* an MTT assay on the L6 muscle cell line. The efficiency of the extracts was determined by testing the translocation of the glucose transporter-4 (GLUT4) in L6-GLUT4myc cells. The ethanol extract from the leaves was found to contain alkaloids, flavonoids, phenolics, sterols, saponins, and tannins, whereas the flower extract comprised glycosides, flavonoids, phenolics, and saponins. Both extracts were found to contain Zn, Cu, Fe, Mn, and Co, but were devoid of Hg, Cd, Ni, Cr, and Pd. The leaves exhibited a higher concentration of steroidal compounds, particularly beta-sitosterol (5.34 mg), while the flower extract revealed a greater amount of the polyphenolic compound gallic acid (6.28 mg) as identified by HPLC. The *in vitro* antidiabetic activity was notably more significant in the flower extract, which also led to a substantial increase in GLUT4 levels when compared to the leaf extract. In conclusion, the findings indicated that both extracts demonstrated strong antidiabetic activity; however, the flower extract exhibited superior efficacy, likely attributable to its higher gallic acid content.

Keywords: *Antidiabetic Activity; Extracts; in vitro Study; Phytochemicals*

Introduction

Diabetes Mellitus (DM) is a severe metabolic syndrome (Thomas & Philipson, 2015). Globally, one billion people are pre-diabetic, eventually developing diabetes, costing approximately 1,200 billion dollars for diagnosis, treatment, and care (Ong et. al., 2023). It is primarily linked to sedentary lifestyles, poor diets, obesity, and various genetic and environmental factors. Untreated diabetes leads to serious complications and death (Singh *et al*., 2021). Current therapies do not prevent beta cell destruction and insulin resistance. Herbal agents, due to fewer side effects, high availability, and lower costs, are being explored for their antioxidant, antidiabetic, and antihyperlipidemic properties (Rubin *et al*., 2008). The herbal agents that have pharmacological activities like antioxidants,

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antidiabetic, and antihyperlipidemic can be utilized for diabetic nephropathy. Hence, herbal medicines are being focused in the present study.

Abutilon indicum, from the Malvaceae family, is a small shrub native to tropical regions, widely found in India. The roots and bark are used for their nervine tonic, aphrodisiac, and diuretic properties (Sharma, 2013), while the seeds are used as laxatives and the leaves for anti-inflammatory, antidiabetic, and wound healing activities (Bolleddu *et al*., 2021; Kaushik *et al*., 2009). The aerial parts exhibit anti-inflammatory activity for asthma treatment (Paranjape & Mehta, 2008), and the ethanolic leaf extract has antibacterial (Poonkothai, 2006), antifungal (Saini *et al*., 2014), antidiarrheal (Chandrashekhar *et al*., 2004), and immunomodulatory properties (Dashputre & Naikwade, 2010). Flower extract traditionally increases semen in men (Rajeshwari & Sevarkodiyone, 2018). The plant contains amino acids, carbohydrates, alkaloids, flavonoids, saponins, phenolics, and terpenoids (Sunil *et al*., 2023), responsible for various therapeutic activities. Despite its many applications, research on flower extract is limited. Although the antidiabetic activity of leaves is known, further studies on leaves and flower extracts are needed, including heavy metal analysis, which might affect nutrient content and enzymatic metabolic processes. The first time was investigated with the focus on comparative *in vitro* antidiabetic activity for both leaves and flower extracts with the presence of heavy metals and constituents, estimated through HPLC analysis.

Materials and Methods

Materials

Gallic acid and 98% pure β-sitosterol were procured from Sigma Aldrich, Mumbai. HPLC-grade solvents were supplied by Fine Chemicals, Navi Mumbai, while the MTT reagent was obtained from Thermo Fisher Scientific, Bangalore.

Plant profile

Leaves and flowers of the *Abutilon indicum* plant were collected from rural Bangalore. The plant material was authenticated by Dr. P. E. Rajasekharan, Principal Scientist at the Indian Institute of Horticultural Research, Bangalore. A voucher specimen (AI-0932) was deposited in the Department of Pharmacology, Bharat Institute of Higher Education and Research, Chennai, Tamil Nadu.

Determination of heavy metals

To assess heavy metal content, 100 g of dried leaves and flowers (dried at 105 °C for 20 minutes) were digested using a mixture of sulfuric and perchloric acids (2:1) at 200 °C until white fumes appeared. The digested residue was diluted to 50 ml with deionized water. Atomic Absorption Spectrophotometry was used for analysis, with air-acetylene as the oxidant gas. Wavelengths were selected based on concentration ranges, and blank samples were used for correction. All measurements were performed in triplicate to ensure accuracy, following the method of Das *et al*. (2019).

Preparation of Extracts

A total of 500 g each of shade-dried leaves and flowers (dried for 21 days) were ground into coarse powder. Ethanol extraction was carried out using a Soxhlet apparatus for 9 hours at 40 °C. The extracts were filtered and concentrated using a rotary evaporator at 35 °C for 40 minutes. The final extracts were stored in glass bottles at 6 °C for future use.

Phytochemical Analysis

Preliminary phytochemical screening was conducted using standard protocols (Das *et al*., 2022). Thin-layer chromatography (TLC) was employed to detect alkaloids, steroids, and flavonoids. Steroids were identified using a solvent system of toluene: ethyl acetate: formic acid (4.5:4.5:1), alkaloids with toluene: ethyl acetate (8:2), and flavonoids with ethyl acetate: methanol: water (10:1:1). Pre-coated silica gel plates were used for all TLC analyses.

HPLC analysis

Reverse-phase HPLC (RP-HPLC) was used to quantify gallic acid and β-sitosterol in the extracts. A Phenomenex LC column (150 × 4.6 mm) was operated in isocratic mode. For β-sitosterol, the mobile phase consisted of methanol and acetonitrile (90:10 v/v) at a flow rate of 1.5 ml/min, with UV detection at 202 nm (Khonsa *et al*., 2022). For gallic acid, methanol and water (60:40 v/v) served as the mobile phase, with a flow rate of 1.0 ml/min and UV detection at 272 nm (Das *et al.,* 2019).

Preparation of stock solution and calibration curve

Standard stock solutions of gallic acid and β-sitosterol were prepared by dissolving 10 mg of each compound in 10 ml of their respective mobile phases, yielding 1000 µg/ml solutions. Calibration curves were constructed using six dilutions (10–100 µg/ml) prepared by serial dilution. Each concentration was analyzed in triplicate. Method validation included assessment of retention time precision, and calculation of the limit of detection (LOD) and limit of quantification (LOQ) using the formulas LOD = 3.3 × σ/slope and LOQ = 10 × σ/slope, where σ is the standard deviation. Calibration curves were plotted based on concentration versus area under the curve (AUC).

In vitro antidiabetic activity

Alpha amylase inhibition: Alpha-amylase inhibition was assessed by mixing 0.5 mg/ml of the enzyme with extract concentrations ranging from 100 to 500 µg/ml. A 1% starch solution and 100 µl of phosphate buffer (pH 6.9) were added, and the mixture was incubated at 37 °C for 5 minutes. The reaction was stopped by adding 2 ml of 3,5-dinitrosalicylic acid reagent and heating at 100 °C for 15 minutes. After cooling in an ice bath, absorbance was measured at 540 nm. Acarbose was used as the standard (Alqahtani *et al*., 2019).

Alpha-glucosidase inhibitory activity: Alpha-glucosidase inhibition was evaluated by incubating starch in Tris buffer with the extracts and enzyme at 35 °C for 40 minutes. The reaction was terminated, and absorbance was measured. IC_{50} values were calculated for each extract, with acarbose serving as the reference standard (Alqahtani *et al.,* 2019).

MTT assay

Cytotoxicity was assessed using the MTT assay in a 96-well plate format. Cells were seeded at a density of 100,000 cells/ml in 10% FBS and incubated for 24 hours. After washing, various concentrations of extracts were added and incubated for 72 hours. MTT solution was then added and incubated for 3 hours. Formazan crystals were solubilized with DMSO, and absorbance was measured at 540 nm. Growth inhibition percentages and IC_{50} values were calculated from dose-response curves (Tolosa *et al*., 2014).

Glucose uptake by the L6 cell line:

Glucose uptake was measured in L6 rat skeletal muscle cells treated with *Abutilon indicum* plant extracts. Cells were cultured and refreshed with DMEM containing BSA, then incubated with insulin, plant extracts, and glucose. Glucose levels in the supernatant and cell lysate were quantified using a reagent-based colorimetric method, with absorbance measured at 530 nm (Gupta *et al*., 2009).

Statistical Data Analysis

For the correlation studies conducted in this research, Pearson's correlation coefficient (r) was used to assess the linear relationship between heavy metal concentrations and phytochemical levels (β-sitosterol and gallic acid) in leaf and flower extracts. The strength and direction of these correlations were quantified, and statistical significance was determined using a two-tailed test.

To evaluate the significance of these correlations, a p-value threshold of 0.05 was applied. Correlations with $p < 0.05$ were considered statistically significant, indicating a meaningful association between the variables. Additionally, linear regression analysis was performed to examine the relationship between bioactive compound concentrations and in vitro α-amylase inhibitory activity. The

coefficient of determination (R^2) and regression equations were reported to describe the predictive strength of gallic acid and β-sitosterol on enzyme inhibition.

Results

Determination of Heavy Metals

Heavy metals in *Abutilon indicum* dried leaves and flower powders showed higher Cu and Zn levels in the flower powder (4.23 mg/kg and 1.14 mg/kg, respectively) and higher Fe and Mn levels in the leaves (5.41 mg/kg and 1.07 mg/kg, respectively) (Table-1). Co was present in leaves, but Ni, Pd, Hg, Cd, and Cr were absent in both samples.

Table 1: *Elemental Content in Leaves and Flowers of Abutilon indicum*

Elements	Content (mg/kg)	
	Dried Leaves powder	Dried flowers powder
Fe	5.41 ± 0.12	0.15 ± 0.31
Mn	1.07 ± 0.20	0.62 ± 0.02
Cu	0.92 ± 1.04	4.23 ± 0.13
Zn	0.37 ± 0.54	1.14 ± 1.01
Co	0.03 ± 0.22	0.01 ± 0.11
Ni	ND	ND
Cd	ND	ND
Pb	ND	ND
Cr	ND	ND
Hg	ND	ND

Values were mean ± SEm (n=3); ND = Not detected

Extraction of Plant Materials

500 g of powdered leaves and flower samples of *Abutilon indicum* were extracted by ethanol, resulting in a yield of 43.7 g and 23.4 g, respectively (Figure 1). The percent yields were 8.74 and 4.68 percent, respectively.

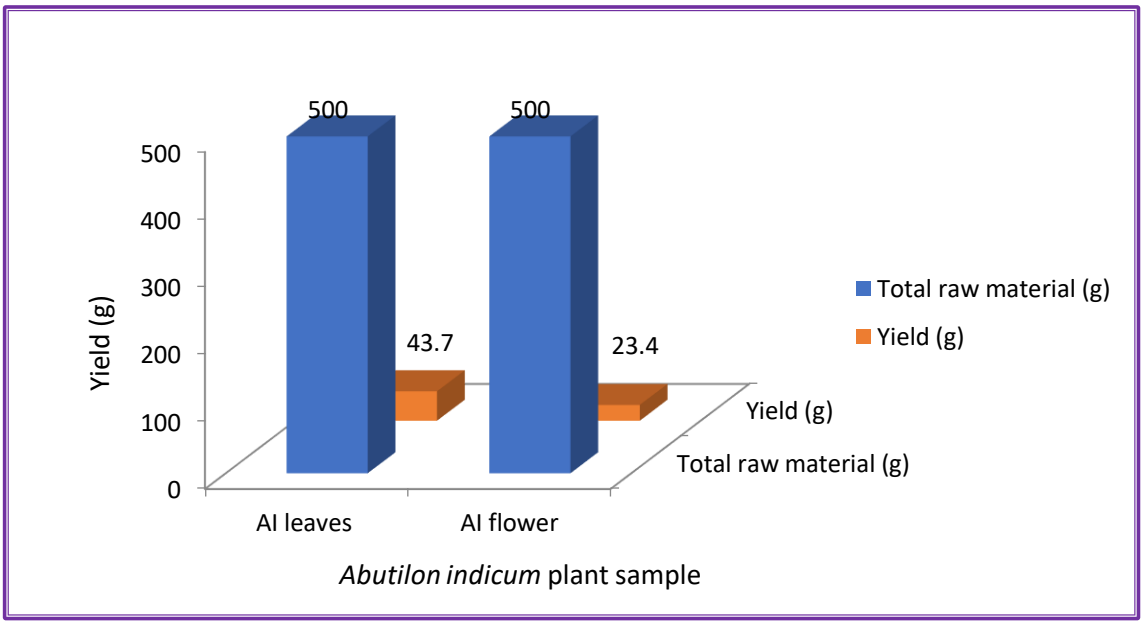


Figure 1: *Yield of the Extracts*

Phytochemical Screening of Extracts

Different chemical tests were performed to detect the groups of bioactive compounds present in the extracts. Carbohydrates, proteins, alkaloids, steroids, flavonoids, and phenolic compounds were present in the leaf extract of the *Abutilon indicum* plant. In contrast, glycosides, alkaloids, flavonoids,

proteins, saponins, and phenolics were detected in the flower extract of the *Abutilon indicum* plant (Table 2).

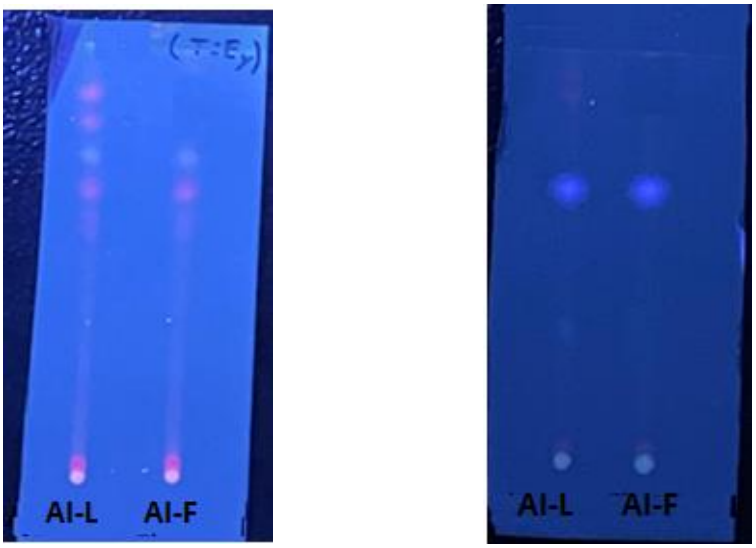
Table 2: *Chemical Tests for Abutilon indicum Plant Extracts*

Chemical Tests	*Abutilon indicum* **Leaves Extract**	*Abutilon indicum* **Flower Extract**
Protein	+	--
Alkaloids	++	++
Glycosides	+	++
Carbohydrate	+	+
Flavonoids	++	++
Tannins	+	+
Phytosterol	++	++
Saponin	--	+
Phenolics	++	++
Terpenoids	+	++
Resins	--	--
Oils and gums	--	--

(++) = Prominent; (+) = Present; (--) = Absent

Detection and separation of phytochemicals by TLC

Based on the availability of the constituents common to both extracts, further TLC was run with various solvent systems, and steroids and polyphenolics were identified and separated (Figure 2).



Detection of steroidal compounds Detection of phenolic compounds

Figure 2*: TLC of leaves (Abutilon indicum -L) and flower (Abutilon indicum -F) extracts of Abutilon indicum.*

TLC of steroidal compounds was detected in the first plate, where the leaf extract contained more compounds than the flower extract, but both extracts contained steroidal compounds. Similarly, in 2nd plate, polyphenolic compounds were identified in the second plate.

HPLC Analysis

Based on the TLC separation, further HPLC analysis was carried out and identified beta-sitosterol as a steroidal compound and gallic acid as a polyphenolic compound from both the leaf and flower extracts of *Abutilon indicum*, which were authenticated by run with standard beta-sitosterol and gallic acid sample (Figure-3 to 8).

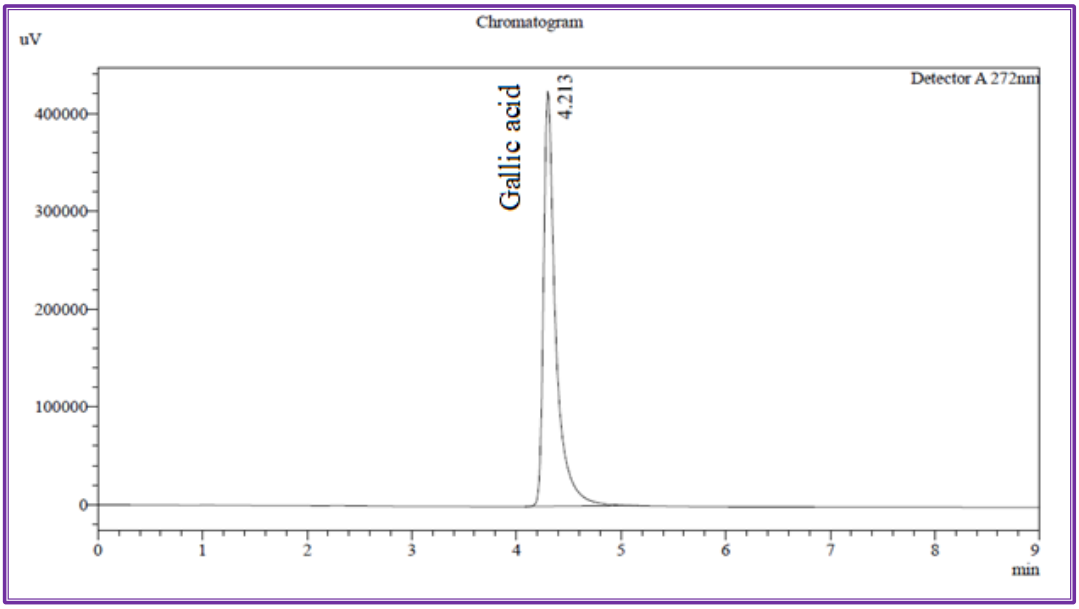


***Figure 3**: HPLC of Standard Gallic Acid*

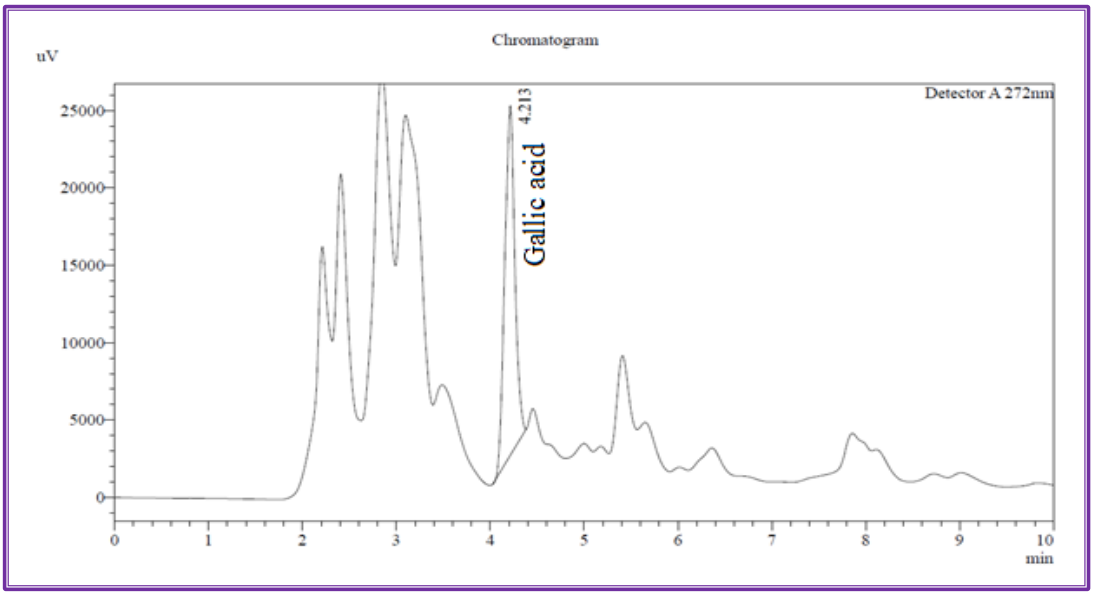


***Figure 4**: Gallic Acid in Flower Extract of Abutilon indicum*

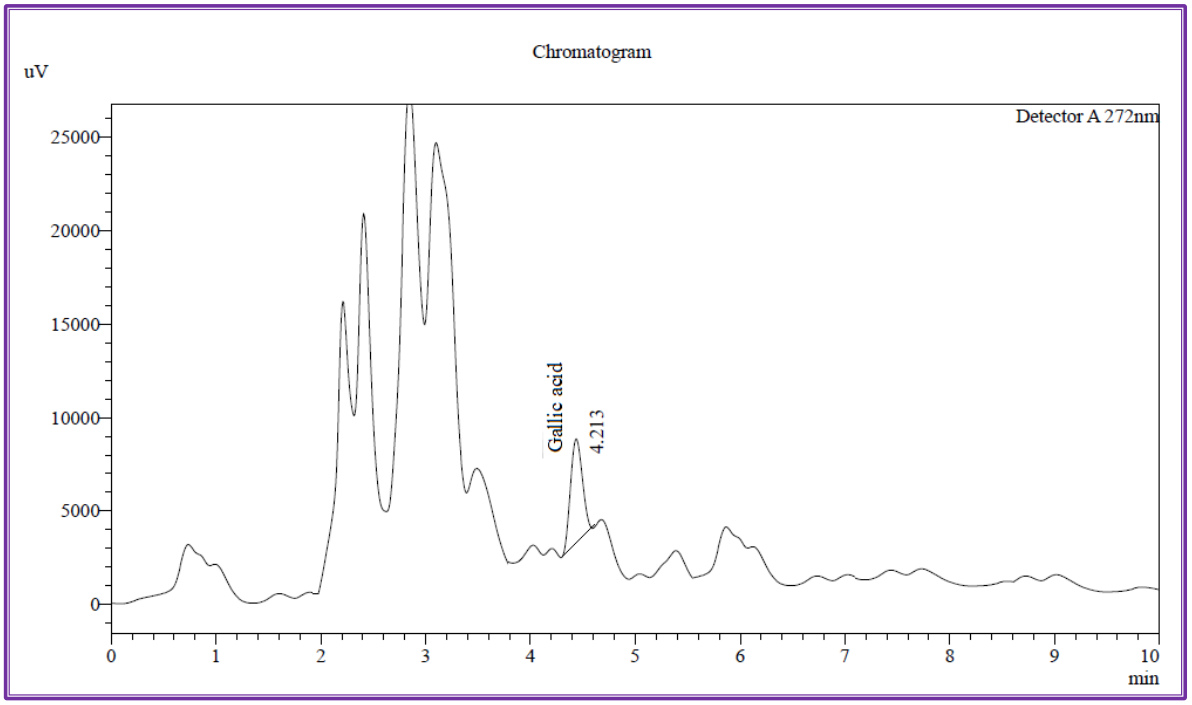


Figure-5: *Gallic Acid in Leaves Extract of Abutilon indicum*

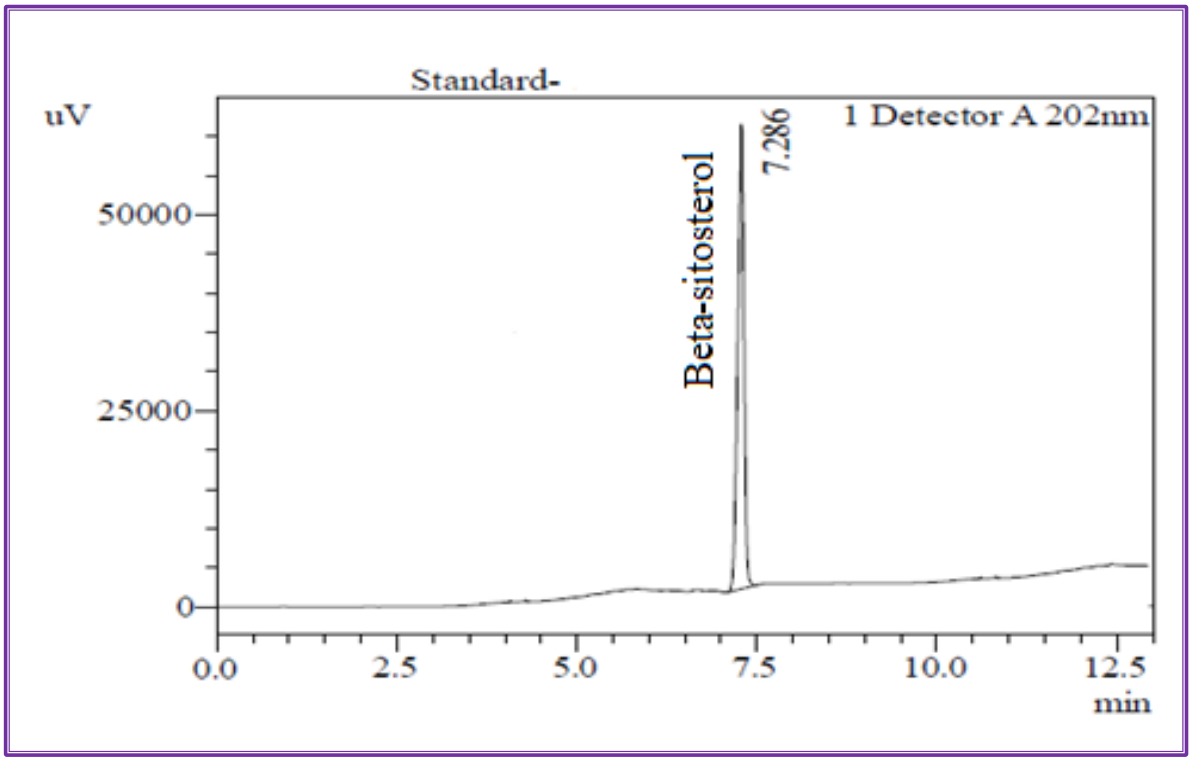


Figure-6: *HPLC of Standard Beta-sitosterol*

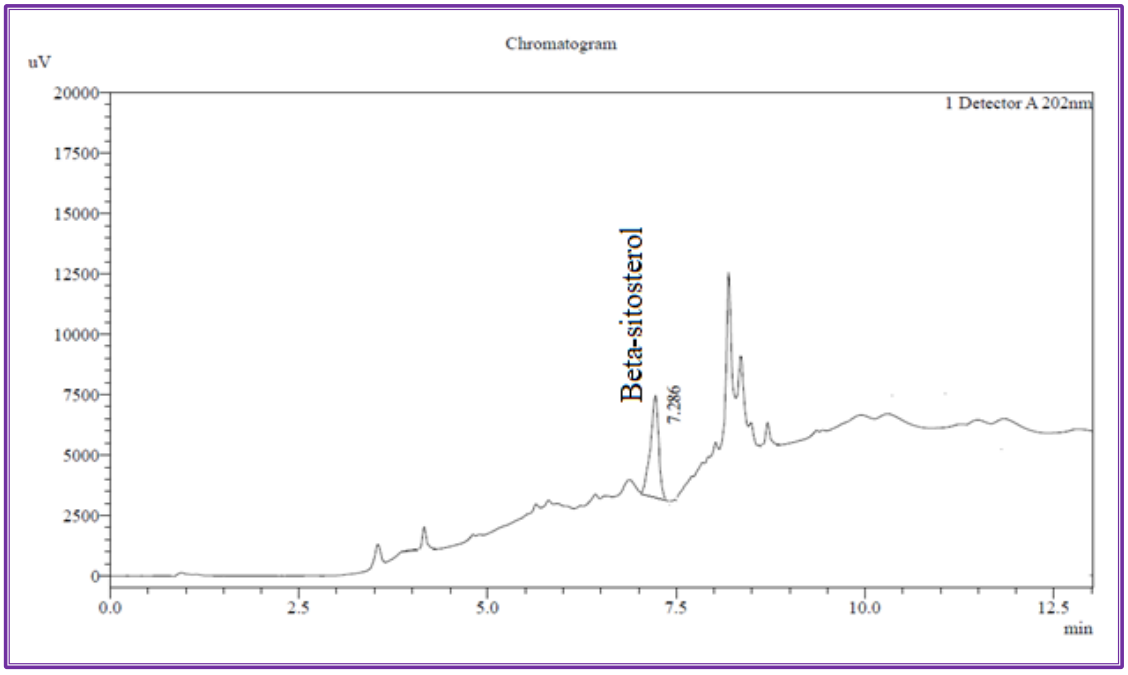


***Figure-7**: HPLC of Beta-sitosterol Present in Leaves Extract of Abutilon indicum*

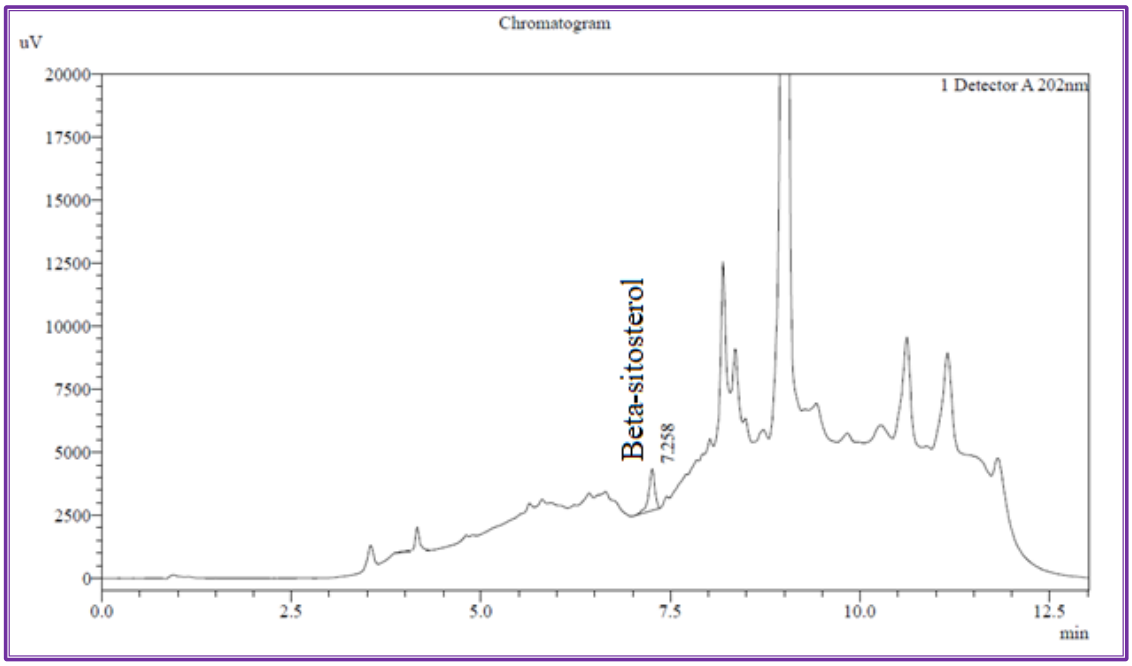


***Figure-8**: HPLC of beta-sitosterol present in flower extract of Abutilon indicum.*

Standard Gallic acid showed a sharp peak at the retention time (Rt) of 4.213 min, whereas at the same Rt, flower and leaf extracts showed the same pear, gallic acid, and beta-sitosterol. Furthermore, the contents of beta-sitosterol and gallic acid were estimated concerning the standard, and the amounts are tabulated in Table 3.

***Table 3:** Estimation of Beta-Sitosterol and Gallic Acid in Abutilon indicum Leaves and Flower Extracts*

Components	**Leaves Extract of *Abutilon indicum***	**Flower Extract of *Abutilon indicum***
Beta sitosterol	5.34 mg	0.63 mg
Gallic acid	1.42 mg	6.28 mg

Specificity

The applied methods were found to be specific, as there was no other interference from any constituents, as shown in figures 3 and 6 when HPLC eluted standard gallic acid and beta-sitosterol.

Linearity

The selected standard drugs, gallic acid, and beta-sitosterol, showed a linear response at concentrations ranging from 10 to 100 µg/ml (Figure-9 and 10).

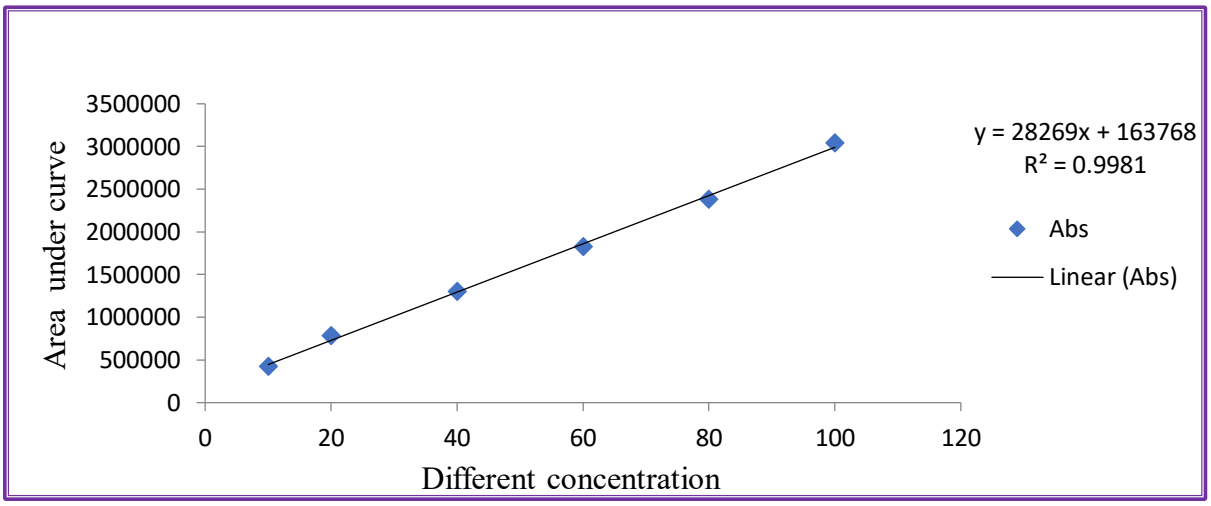


***Figure-9**: HPLC calibration curve of Gallic acid.*

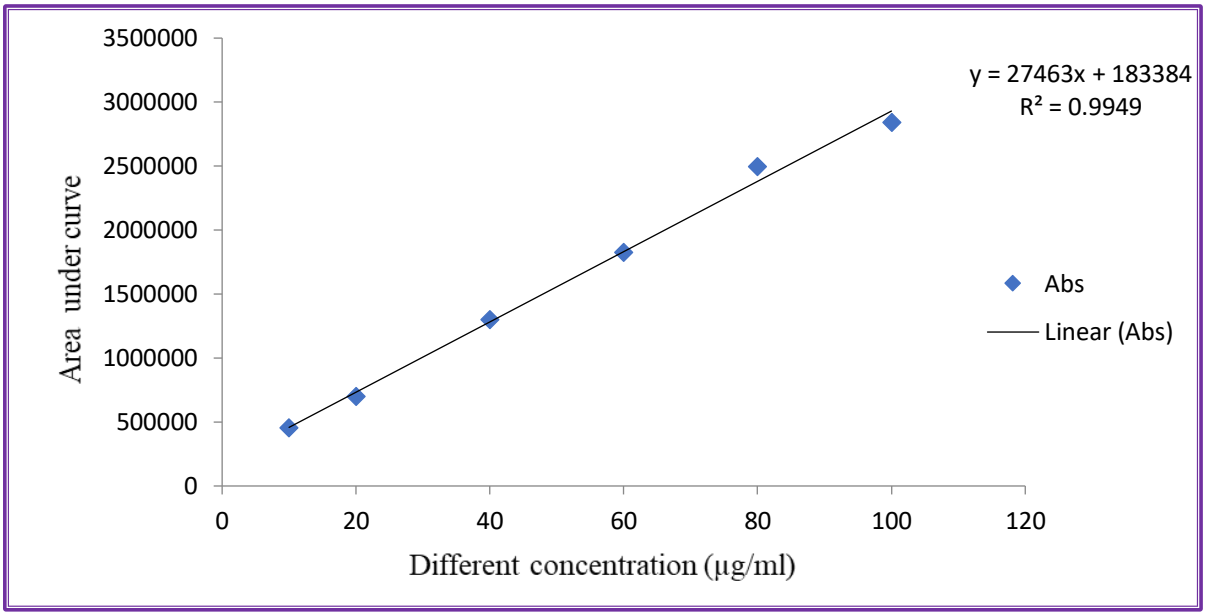


***Figure-10:** HPLC Calibration Curveo of Beta-Sitosterol*

The Linearity was validated for gallic acid and beta-sitosterol by the high value of the correlated coefficients (R^2) of 0.9981 and 0.9949, respectively, which were the acceptable criteria for the method. Further, LOD and LOQ were determined by applying the above formula; LOD and LOQ for gallic acid were 0.78 and 3.23, respectively, whereas the same for beta-sitosterol were 0.64 and 2.89, respectively.

In vitro antidiabetic activity

Alpha-amylase inhibition activity

The leaf and flower extracts of *Abutilon indicum* were used for alpha amylase inhibition activity, which resulted in dose-dependent activity. Table 4 shows that at different concentrations ranging from 100 to 500 µg/ml, the leaf and flower extracts of *Abutilon indicum* inhibited 93.75 and 91.14%, respectively, at 500 µg/ml. The IC_{50} value was determined for flower extract (164.15 µg/ml and leaf extract (276.67 µg/ml when compared with the standard IC_{50} value of acarbose (20. 89 µg/ml).

Table 4: *Alpha Amylase Inhibition Activity of Leaves and Flower Extracts of AI*

Samples	Concentration (µg/ml)	Absorbance at 540 nm	% inhibition	IC_{50} (µg/ml)
Control	0	0.384	0.00	
Flower extract of *Abutilon indicum*	500	0.024	93.75 ± 0.07	164.15
	400	0.043	88.80 ± 0.11	
	300	0.120	68.75 ± 0.21	
	200	0.191	50.26 ± 0.34	
	100	0.218	43.23 ± 0.28	
Leaves extract of *Abutilon indicum*	500	0.034	91.14 ± 0.22	276.67
	400	0.118	69.27 ± 0.21	
	300	0.132	65.63 ± 0.22	
	200	0.196	48.96 ± 0.43	
	100	0.223	41.93 ± 0.30	
Acarbose standard	500	0.020	94.93 ± 0.52	20.89
	400	0.031	92.15 ± 0.01	
	300	0.071	82.02 ± 0.11	
	200	0.143	63.79 ± 0.02	
	100	0.164	58.47 ± 0.40	

Values are presented as mean ± SEm (n =3).

Alpha-glucosidase inhibition activity

Leaf and flower extracts of *Abutilon indicum* were further tested in vitro for α-glucosidase enzyme inhibition activity, and interestingly, the same trend was observed. The flower extract showed a concentration-dependent potent inhibition of alpha-glucosidase enzyme (91.93 %) compared to the leaf extract (89.78 %), and the values were close to the standard (92.50 %) at 500 µg/ml (Table-5).

Table 5: *Alpha-Glucosidase Inhibition Activity of Leaves and Flower Extracts of Abutilon indicum Plant*

Tests	Concentration (µg/ml)	Abs	Percent Inhibition	IC_{50} value (µg/ml)
Control	0	0.372	0.00	
Abutilon indicum **Flower Extract**	500	0.030	91.93± 0.43	127.76
	400	0.063	83.06 ± 0.16	
	300	0.123	66.94 ± 0.38	
	200	0.145	61.02 ± 0.32	
	100	0.202	45.69 ± 0.20	
Abutilon indicum **Leaves Extract**	500	0.038	89.78 ± 0.11	143.59
	400	0.068	81.72 ± 0.08	
	300	0.112	69.89 ± 0.21	
	200	0.149	59.94 ± 0.02	
	100	0.218	41.40 ± 0.32	
Acarbose Standard	500	0.028	92.50 ± 0.18	122.33
	400	0.046	87.70 ± 0.31	
	300	0.082	78.75 ± 0.44	
	200	0.196	47.59 ± 0.11	
	100	0.221	40.91 ± 0.20	

Values were expressed as the mean ± SEM (n =3)

Cytotoxicity Study

The MTT assay was performed to determine the cytotoxicity of both plant extracts (leaves and flowers) at 31.25 1000 µg/ml concentrations. The study was conducted against the L6 cell line, and the percentage of cell viability after treatment decreased with increasing concentration (Table 6). Table 6 showed at 1000 µg/ml, the cell viability was 82.21 percent for the flower extract and 80.63% for the leaf extract.

Table 6: *In Vitro Cell Viability of Ethanol Leaves and Flower Extracts of Abutilon indicum Plant on Rat Skeletal Muscle (L6) Cell Line by MTT Assay*

Samples	Concentration (µg/ml)	Cytotoxicity effect
Abutilon indicum flower extract	1000	82.21 ± 0.20
	500	86.11 ± 0.42
	250	93.87 ± 0.48
	125	94.92 ± 1.23
	62.5	97.27 ± 0.32
	31.25	98.18 ± 0.26
Abutilon indicum leaves extract	1000	80.63 ± 0.33
	500	84.12 ± 1.10
	250	89.28 ± 0.74
	125	93.23 ± 1.34
	62.5	95.10 ± 0.48
	31.25	98.20 ± 1.42

Further, the percentage of glucose uptake by the cells was investigated, and a dose-dependent increase in glucose uptake was revealed compared with the standard metformin. *Abutilon indicum* flower extract showed a higher inhibition of 68.38 percent, near standard drug (71.23 percent), whereas leaf extract inhibited 57.20 percent at 500 µg /ml concentration (Table 7).

Table 7: *Percent Glucose Uptake of All the Extracts Over Cell Control In In Vitro L6 Cells*

Sl.no	Treated sample	Percent glucose uptake over cell control
1	Standard- Metformin (250 µg/ml)	71.23 ± 0.11
2	Flower extract @ 500 µg/ml	68.38 ± 0.24
3	Flower extract @ 250 µg/ml	53.21 ± 0.31
4	Leaves extract @ 500 µg/ml	57.20 ± 0.40
5	Leaves extract @ 250 µg/ml	49.27± 0.33

Values are mean ± SEM; (n =3)

Correlation Study

Heavy metal content with phytochemicals

The heavy metal content was correlated with the phytochemicals in the leaf and flower extracts of the *Abutilon indicum* plant. Table 8a showed a positive correlation of beta-sitosterol and gallic acid with Fe and Mn for the leaf extract of *Abutilon indicum*. Still, a significant ($p<0.05$) correlation was observed with beta-sitosterol. Furthermore, the values showed maximum negative and non-significant values for Cu and Zn. On the other side (table-8b), Cu and Zn content showed a positive correlation with beta-sitosterol and gallic acid. Still, it was highly significant with the amount of bioactive compound gallic acid.

Table-8a: *Correlation Coefficient Study of Heavy Metal Contents with Bioactive Compounds in Leaves Extract of Abutilon indicum*

Metals	Leaves extract of ***Abutilon indicum***						
	Fe	**Mn**	**Cu**	**Zn**	**Co**	**BS**	**GA**
Fe	1						
Mn	0.135	1					
Cu	0.447	0.312	1				
Zn	0.809	0.497	-0.139	1			
Co	-0.632	0.426	-0.943	-0.056	1		
BS	0.986*	0.968*	-0.169	0.669	0.239	1	
GA	0.861	0.785	-0.136	-0.454	0.289	0.986*	1

Significant at p<0.05, BS= Beta-sitosterol; GA = Gallic Acid

Table-8b: *Correlation Coefficient Study of Heavy Metal Contents with Bioactive Compounds in Flower Extract of Abutilon indicum*

Metals	Flower Extract of ***Abutilon indicum***						
	Fe	**Mn**	**Cu**	**Zn**	**Co**	**BS**	**GA**
Fe	1						
Mn	0.214	1					
Cu	-0.016	0.571	1				
Zn	0.308	-0.328	0.672	1			
Co	0.867	0.043	0.508	-0.023	1		
BS	0.421	0.302	0.871	0.108	0.452	1	
GA	-0.306	-0.089	0.982*	0.971*	0.677	0.980*	1

Significant at p<0.05, BS= Beta-sitosterol; GA = Gallic Acid

Correlation study of bioactive compounds with in vitro antidiabetic activity (in alpha-amylase inhibition)

In vitro, antidiabetic activity was correlated with higher estimated bioactive compounds, *viz.* gallic acid, and beta-sitosterol from flower and leaf extracts, respectively, and a positive correlation was depicted in figure-11 and 12. Figure 11 showed that the R^2 value of gallic acid with α-amylase inhibitory activity was 0.9918 with y=0.7308x + 89.174, whereas Figure 12 shows that the R^2 value of β-sitosterol with α-amylase inhibitory activity was 0.9643 with y= 0.06429x+ 87.691.

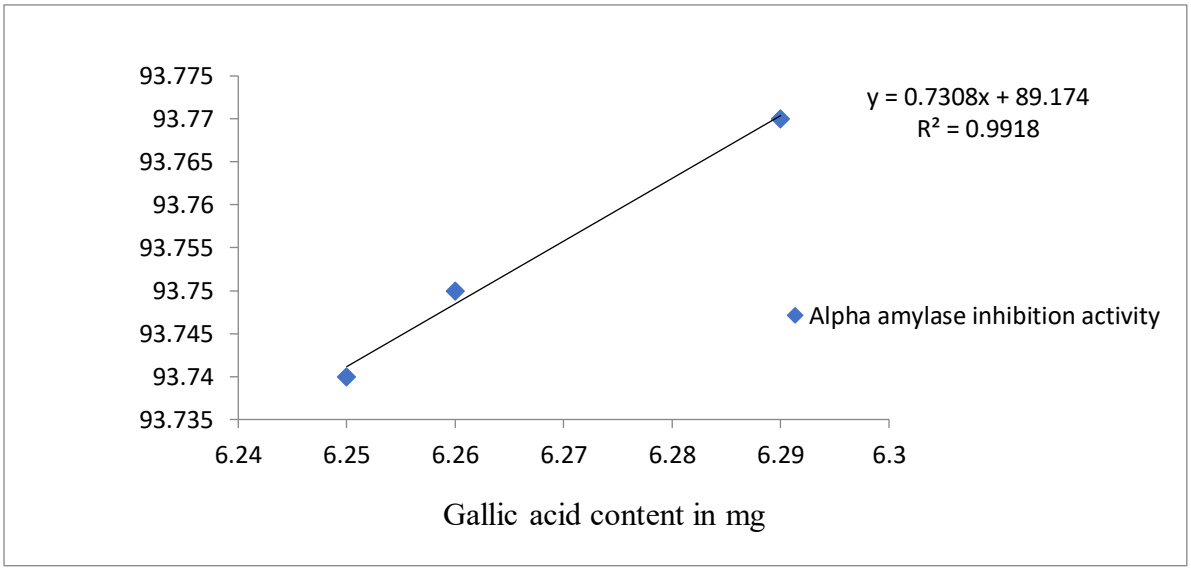


Figure-11: *Correlation Coefficient between Gallic Acid and In Vitro Alpha Amylase Inhibitory Activity at Dose of 500 µm/ml (Flower extract of Abutilon indicum).*

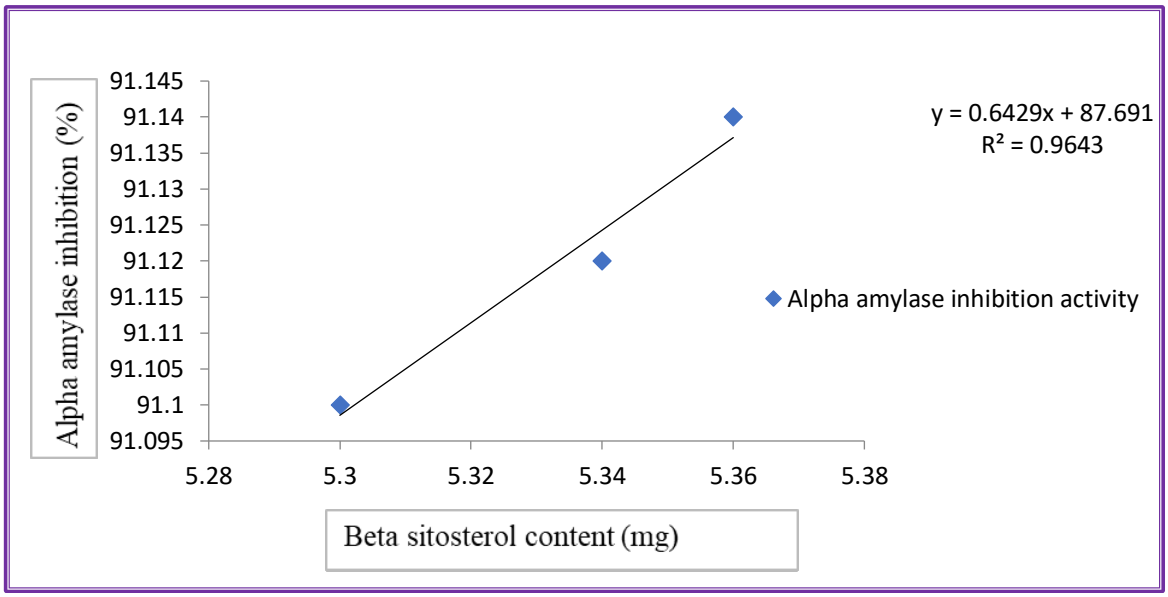


***Figure 12**: Correlation Coefficient between Beta-Sitosterol and in Vitro Alpha Amylase Inhibitory Activity at A Dose of 500 µm/ml (Leaves extract of Abutilon indicum).*

Discussion

Ethanol was used to extract the leaves and flowers of *Abutilon indicum* plants. Thus, ethanol is safe and readily available. Moreover, it has a high dielectric constant and can extract bioactive compounds. Earlier studies also revealed a similar effect of ethanol solvent in extracting maximum plant secondary metabolites (Das *et al*., 2023). Due to the use of ethanol solvent, the present study showed a higher yield in leaf extract than in flower extract in *Abutilon indicum* plants because maximum compounds were identified in both leaves and flower extracts when various chemical tests were performed.

The analysis of heavy metal content in *Abutilon indicum* plant extracts revealed a distinct distribution pattern between leaves and flowers, which appears to influence their phytochemical profiles. Specifically, higher concentrations of Iron (Fe) and Manganese (Mn) were detected in the leaf extracts. These metals are known to play pivotal roles as enzymatic cofactors in the biosynthesis of phytosterols, such as β-sitosterol. This observation aligns with previous findings by Li *et al*. (2023), who reported that elevated Fe and Mn levels can upregulate sterol biosynthetic pathways in plant tissues, thereby enhancing phytosterol accumulation. Conversely, the flower extracts exhibited higher levels of Copper (Cu) and Zinc (Zn), both of which are essential trace elements involved in the regulation of polyphenol biosynthesis. These metals are known to activate key enzymes such as Phenylalanine Ammonia-Lyase (PAL) and Polyphenol Oxidase (PPO), which are critical in the phenylpropanoid pathway responsible for polyphenol production. The elevated gallic acid content observed in the flower extracts through HPLC analysis is consistent with this mechanism. Similar correlations between Cu/Zn enrichment and increased polyphenolic content have been documented by Adamczyk-Szabela and Wolf (2024), further supporting the notion that micronutrient availability can modulate secondary metabolite synthesis in plants. Importantly, non-essential heavy metals such as lead (Pb), Cadmium (Cd), and Mercury (Hg) were either absent or below detectable limits in both extracts. This is a favorable outcome, as the accumulation of such toxic elements can not only compromise the safety of plant-based therapeutics but also interfere with metabolic pathways, leading to the formation of harmful byproducts. According to standard phytopharmacological guidelines, the absence of non-essential heavy metals is crucial for ensuring the therapeutic efficacy and safety of herbal formulations (Das *et al.,* 2011). Overall, these findings underscore the nuanced role of essential heavy metals in enhancing the biosynthesis of bioactive compounds like phytosterols and polyphenols, which contribute to the antidiabetic potential of *Abutilon indicum* plant extracts. This metal–metabolite relationship offers valuable insights for optimizing cultivation and extraction strategies aimed at maximizing phytochemical efficacy in medicinal plants.

In vitro antidiabetic activity was assessed using alpha-amylase and α-glucosidase inhibition methods. Inhibitors of alpha-amylase slow down the breakdown of carbohydrates in the small intestine and lessen the postprandial blood glucose surge in diabetes. One method for treating diabetes is to reduce postprandial glucose levels by blocking carbohydrate-digesting enzymes such as alpha-amylase and alpha-glucosidase from absorbing glucose in the intestine (Kwon *et al*., 2007; Das *et al.,* 2020). The present study showed that the flower extract of *Abutilon indicum* is more than the leaf extract. This finding was consistent with an earlier study that suggested modest pancreatic alpha-amylase inhibitory activity is preferable since severe inhibition could cause aberrant bacterial fermentation of the colon of partially digested carbs (Thilagam *et al.,* 2013). The present study followed similar results as stated earlier.

Furthermore, skeletal tissue maintains normal blood glucose levels. Skeletal muscle glucose absorption anomalies in response to insulin stimulation are common in non-insulin-dependent diabetic mellitus (NIDDM). Glucose transporter protein type-4 (GLUT4) is the principal glucose transporter expressed in insulin-responsive tissues, including skeletal muscle and adipose tissue. Acute insulin stress causes these tissues to swiftly transfer GLUT4 from an intracellular storage site to the plasma membrane (Kwon *et al*., 2007). The present study showed concentration-dependent activity, where cell viability was higher with a decrease in the concentration of the combined extracts. The same trend was observed in a previous report (Thilagam *et al*., 2013). In the digestive tract, alpha-amylase and alpha-glucosidase are key enzymes that break down carbohydrates. Alpha-amylase breaks down complex carbohydrates, such as starch, into smaller polysaccharides called oligosaccharides.

In contrast, alpha-glucosidase breaks down disaccharides, such as maltose and sucrose, into individual glucose molecules (Gupta *et al*., 2009) and results in postprandial hyperglycemia. Therefore, inhibiting these two enzymes is essential for managing hyperglycemia by delaying the breakdown of carbohydrates and lowering postprandial plasma glucose levels. Hence, this study used acarbose, a synthetic drug, as a standard for competitive inhibition of both enzymes. The inhibitory activity was dose-dependent, as determined by measuring the IC_{50} value (Prasanna *et al.*, 2019). In our study, the flower extract showed a similar result to that mentioned in the previous literature.

MTT assay is a reliable method for determining cell cytotoxicity. The number of live cells and the degree of cytotoxicity were inversely correlated with cell formation. Cell viability increased with decreasing concentrations, similar to that reported in an earlier study (Kadan *et al*., 2013). According to previous reports, the ethanol extract of a combined sample can help strengthen a host's defenses against diabetes by restoring balance and conditioning bodily tissues. Similarly, combined ethanol extract-tagged GLUT4 (L6-GLUT4myc) was steadily expressed in cells from the rat L6 muscle cell line. Skeletal muscle glucose absorption anomalies in response to insulin stimulation are common in Non-Insulin-Dependent Diabetic Mellitus (NIDDM). The glucose transporter protein type-4 (GLUT4) is the principal glucose transporter expressed in insulin-responsive tissues, including skeletal muscle and adipose tissue (Zhao *et al*., 2018). In the present study, cell-ELISA tests were used to examine the effects of PA methanol extract on the translocation of glucose transporter-4 (GLUT4) to the plasma membrane in L6 muscle cells that stably expressed myc-tagged GLUT4 (L6-GLUT4myc). The facilitative hexose transporter GLUT4 is a membrane protein that alternates between intracellular reserves and the plasma membrane to facilitate glucose uptake into skeletal muscle. Insulin mainly increases the rate at which GLUT4 is exocytosed and fused with the plasma membrane. This process is known as GLUT4 translocation, which increases the amount of GLUT4 on the cell surface (Zaid *et al*., 2008). In the present study, the flower ethanol extract of AI showed a high percentage of cell viability. It decreased cytotoxicity with decreased and increased concentrations compared to the leaf extract.

Conclusion

Recent research revealed that ethanol extracts from the leaves and flowers of *Abutilon indicum* plants possess promising antidiabetic properties. Flower extracts, rich in gallic acid, showed stronger enzyme inhibition, lower cytotoxicity, and enhanced glucose uptake compared to leaf extracts, which

contain beta-sitosterol. Heavy metal analysis suggested a positive link between metal content and the synthesis of these bioactive compounds, indicating their role in boosting the plant's antidiabetic potential.

Conflict of Interest

The authors declare that they have no competing interests.

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