

PHYTOCHEMICAL PROFILING PREDICTION OF In-vitro ANTIDIABETIC ACTIVITIES OF *Stephania glabra* L. TUBER AND *Clerodendrum phlomidis* L. LEAVES

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ABSTRACT

The current research investigates the antidiabetic potential of *Stephania glabra* L. tuber and *Clerodendrum phlomidis* L. leaves by targeting key enzymes involved in regulating hyperglycemia. It highlights how bioactive phytochemicals inhibit α -amylase and α -glucosidase and enhance glucose uptake mechanisms. These medicinal plants act by modulating enzymes responsible for hyperglycemia and stimulating glucose transporters. Ethanolic extracts of *Stephania glabra* tuber and *Clerodendrum phlomidis* leaves were prepared via maceration and stored at 25 °C for further analysis. Phytochemical detection bears the occurrence of phytochemicals such as alkaloids, flavonoids, carbohydrates, terpenoids, phytosterols, tannins, polyphenols, and saponins. Phytochemical analysis was conducted using liquid and gas chromatography coupled with mass spectrometry to identify non-evaporable and evaporable constituents. In total, 14 non-evaporable and 5 evaporable compounds were identified in *Stephania glabra* tuber, and 11 non-evaporable and 4 evaporable compounds in *Clerodendrum phlomidis* leaves, all associated with antidiabetic potential. The ethanolic extract of *Stephania glabra* inhibited α -amylase and glucosidase with IC₅₀ values of 41, 27, and 30 μ g/mL, while *Clerodendrum phlomidis* showed similar inhibition at 45, 25, and 26 μ g/mL, closely aligning with the standard drug acarbose (38, 22, and 23 μ g/mL). Glucose uptake in isolated rat hemidiaphragm tissue increased dose-dependently, reaching 222 mg/g for *Stephania glabra* and 214 mg/g for *Clerodendrum phlomidis* at 60 mg/kg. When combined with insulin, uptake rose to 286 mg/g and 270 mg/g, respectively, surpassing insulin alone (225 mg/g), indicating both insulin-dependent and independent mechanisms. This research highlights the therapeutic value of *Stephania glabra* and *Clerodendrum phlomidis* as effective plant-based agents for diabetes management. Their strong enzyme inhibition and glucose uptake enhancement support their development as safer, natural antidiabetic therapies. The dual mechanism of action holds promise for addressing insulin resistance in type 2 diabetes.

Keywords: Amylase, Acarbose, Diabetic, Diaphragm, and Glucosidase

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INTRODUCTION

According to the World Health Organisation (WHO), more than 500 million people are currently affected by diabetes worldwide, with new diagnoses occurring every few seconds. How many of these cases could be avoided through early detection and healthier daily habits? A relative's sudden health crisis, later linked to unnoticed type 2 diabetes, revealed just how quietly the disease can develop. It primarily affects individuals between the ages of 18 and 40 years of younger adults and is mainly considered a multifactorial condition, influenced by lifestyle factors, genetic predisposition, and ageing.¹ It is estimated by the World Health Organization (WHO) that diabetes cases may reach 643 million by 2030. Given its growing prevalence, managing type 2 diabetes has become a global diabetic emergency. The use of indigenous plants has shown promising effects in the management of type 2 diabetes. These plants possess metabolic glucose compounds that may help control blood glucose levels (BGL), offering a balancing method to standard treatment strategies. *Stephania glabra* L. is a medicinal climbing plant from the Menispermaceae family, native to Southeast Asia, and widely recognised for its rich phytochemical composition and broad therapeutic potential. This study aligns with the Sustainable Development Goals. (SDG) 3: Good Health and Well-being by investigating natural plant-based approaches for diabetes management. By targeting key enzymes and enhancing glucose uptake, the research explores safer alternatives to synthetic drugs. Additionally, the identification of bioactive compounds supports the

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development of accessible, affordable therapeutic options for chronic disease prevention.² Due to their rich content of bioactive compounds, medicinal plants have long been valued in traditional medicine for managing conditions such as diabetes, neurological disorders, pain, and digestive ailments. The active constituents include powerful alkaloids, flavonoids, and phytosterols, which together exert a wide range of pharmacological effects. These include antidiabetic, analgesic, antioxidant, antidepressant, and neuroprotective properties, with growing scientific interest in its potential role in managing Diabetes, Alzheimer's disease, and metabolic disorders.^{3,4} The presence of both volatile and non-volatile compounds enhances its bioactivity, making it potent for integrative therapeutic development, though its use must be carefully monitored due to the potency rich in phytochemicals. *Stephania glabra* L. *Clerodendrum phlomidis* L. is a potent medicinal shrub extensively employed in traditional Ayurvedic and folk medicine for its robust therapeutic profile. Abundant in functional compounds, namely flavonoids, phenolics, terpenoids, and sterols, it exhibits powerful antidiabetic, antidepressant, Antialzheimer, antioxidant, antimicrobial, and hepatoprotective activities.^{5,6} This study investigates the antidiabetic potential of selected plant extracts through α -amylase and α -glucosidase inhibition and enhancement of glucose uptake. Phytochemical profiling was conducted using LC-MS and GC-MS to identify bioactive constituents. The work addresses a significant research gap in the characterisation of natural compounds with potential therapeutic effects.⁷

EXPERIMENTAL

Authentication and Extraction Method

The tuber of *Stephania glabra* L. and the *Clerodendrum phlomidis* (L.) leaves were authenticated by a taxonomist (voucher numbers 1198 and 0964, respectively) and deposited in the Department of Botany, Sri Venkateswara University, Tirupati, India. The plant materials were shade-dried at 35 °C and coarsely powdered. Powdered samples (500 g) were subjected to maceration with ethanol in a 1:2 (w/v) ratio for 7 days at room temperature. The resulting ethanolic extracts were purified over cellulose filter paper (No. 1), and the filtrate was condensed using a Rota evaporator (40 °C) and stored in a vacuum desiccator at 25 °C. These extracts were later used for preliminary phytochemical profiling and *in-vitro* antidiabetic studies, following a universal standard protocol to detect the presence of phytochemicals.^{8,9}

Phytochemical Analysis

Preliminary phytochemical screening is carried out for bioactive constituents. The analysis shows the traced flavonoids, alkaloids, phytosterols, tannins, carbohydrates, saponins, and phenolics in the extract.¹⁰

LC and GC-MS Analysis of Non-Volatile and Volatile Compounds in the Tubers of *Stephania glabra* L. and *Clerodendrum phlomidis* L.

In liquid chromatography mass spectroscopy analysis, 1 mg/ml of ethanolic test extracts were dissolved in 1 ml of methanol (HPLC grade), sonicated for 15 minutes, and a 0.22 μ m syringe filter. Non-volatile constituents were profiled using a contrary-phase C₁₈ column under gradient elution of mobile phase (acidified water and acetonitrile). Detection employed electrospray ionization (ESI-positive and negative ions) over an m/z range of 100–1500. In parallel, gas chromatography mass spectroscopy (GC-MS) volatile constituents were analyzed. 1 mg/ml of ethanolic test extracts was injected in split mode into a capillary column, and separation was achieved by temperature-programmed elution, and detection used electron ionization (EI). Ingredients were recognized by likening retention times and mass spectra with valid standards and the NIST library.^{11,12}

Pharmacological *In-vitro* Antidiabetic Studies

For the α -amylase inhibition study, ethanolic test extracts (12.5 to 400 μ g/ml) were diluted in sodium phosphate solution (20 mM, pH 7.3) to assess α -amylase inhibition. α -Amylase (1 IU/ml) was added and incubated at 32 °C for 10 minutes, followed by 1 ml of (1%) starch solution and 30 minutes of incubation. The reaction was stopped with 3,5-Dinitrosalicylic acid (DNS) reagent, and Acarbose was used as a standard control. Absorbance was measured at 540 nm by the (ERBA diagnostic) semi-auto analyser.^{13,14} For the α -glucosidase inhibition study, ethanolic test extracts (12.5–400 μ g/ml) were diluted in 20 mM Sodium phosphate solution (pH 7.3) and transferred into labelled culture tubes. α -Glucosidase enzyme

(1 IU/ml of maltase and sucrase) was transferred, and the final mixture was gestated at 32 °C for 30 minutes. After stabilization, 1 ml of 4-Nitrophenyl α -D-glucopyranoside substrate was added to initiate the reaction.^{15,16} Glucose uptake was assessed using isolated hemidiaphragms. The tissues were transferred to labelled culture tubes and maintained at 25°C. Ethanolic extracts (20, 40, and 60 mg/kg) were prepared in (pH 7.3) 20mM Sodium phosphate solution. The experimental groups included vehicle control (buffer), insulin control (1 IU/ml), ethanolic test extract alone, and ethanolic test extract combined with insulin (1 IU/ml). Each group was incubated for 30 minutes, followed by the addition of (2%) glucose and a further 30-minute incubation. Glucose uptake was measured at 505 nm using an automated glucose analyser.¹⁷

Biostatistical Analysis

The present values as Mean $\pm$ SEM, and data were analyzed (one-way ANOVA). All experiments were conducted in triplicate to ensure reliability.

RESULTS AND DISCUSSION

Extraction and Phytochemical Screening

Ethanolic extraction yielded 25% from the tubers of *Stephania glabra* L. and 30% from *Clerodendrum phlomidis* L. The obtained extracts were subsequently utilized for preliminary phytochemical screening, analytical characterization of bioactive constituents, and *in-vitro* evaluation of antidiabetic activity. The categories of phytochemicals are shown below in Table-1.

Table-1: Phytochemical Screening of Ethanolic Crude Extracts

Category	Tuber of <i>Stephania glabra</i>	Leaves of <i>Clerodendrum phlomidis</i>
Carbohydrates	+	-
Alkaloids	+	+
Flavonoids	+	+
Proteins and amino acids	+	+
Terpenoids	+	+
Phenolic compounds	+	+
Tannis	+	-
Phytosterols	+	+

(Note: (+) indicates presence, whereas (-) indicates absence.)

Liquid and Gas Chromatography Linked Mass Spectroscopy Analysis

Liquid chromatography coupled mass spectrometry (LC-MS) phytochemical analysis of the ethanolic tuber extract of *Stephania glabra* L. identified 14 phytochemical constituents, while 11 compounds were detected in the ethanolic leaves extract of *Clerodendrum phlomidis* L., as detailed in Tables-2 and 4. Gas chromatography coupled mass spectroscopy (GC-MS) profiling bare 05 volatile compounds in the *Stephania glabra* tuber extract and 07 in the leaves extract of *Clerodendrum phlomidis* L. The identified compounds, including phenolic derivatives and long-chain alcohols, are recognized for their reported antidiabetic potential.^{18,19,20} Detailed compound profiles are presented in Tables 3 and 5, with corresponding chromatograms shown in Fig.-1, 2, 3, and 4.

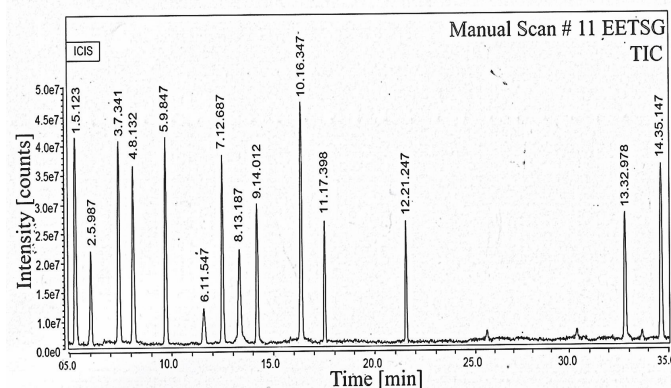
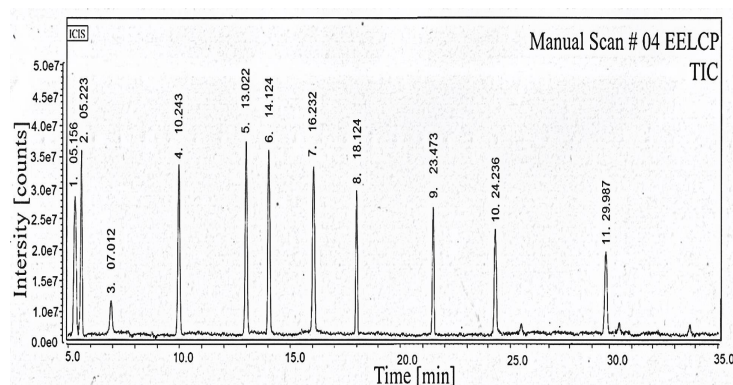
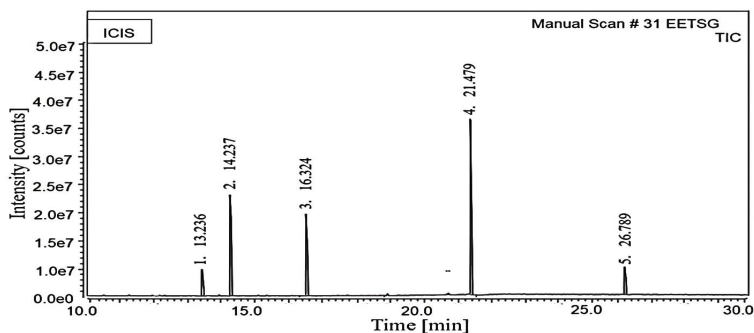
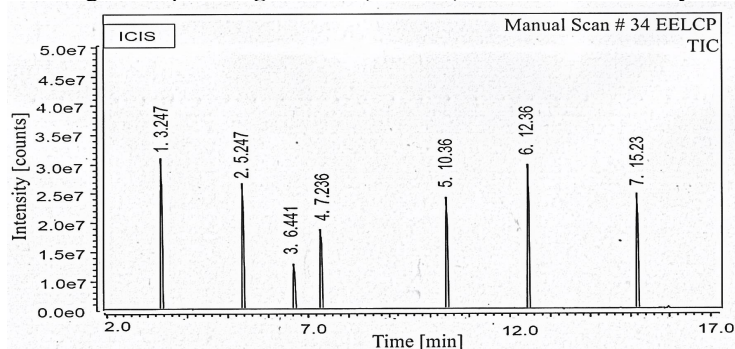


Fig.-1: LC-MS Study of Ethanolic Tuber Extract of *Stephania glabra* L. (EETSG)

Fig.-2: LC-MS Study of Leaf Extract of *Clerodendrum phlomidis* L. (EELCP)Fig.-3: *Stephania glabra* L. (EETSG) Tuber GC-MS StudyFig.-4: *Clerodendrum phlomidis* L. (EELCP) Leaves GC-MS Study

Pharmacological *In-vitro* Antidiabetic Studies

The ethanolic extracts of *Stephania glabra* tuber and *Clerodendrum phlomidis* leaves exhibited potent antidiabetic activity. At 60 mg/ml, α -amylase inhibition reached 89.85% and 86.35%, significantly, while α -glucosidase (sucrase and maltase) inhibition was 89.9%, 88.3%, 89.7%, and 90.8%, significantly. In glucose uptake by isolated rat hemidiaphragm, uptake increased from 187 mg/g to 222 mg/g, further rising to 286 mg/g with insulin. Insulin alone increased uptake from 172 mg/g to 214 mg/g, and up to 270 mg/g with insulin significantly. These findings suggest that both ethanolic extracts improve glucose utilization through insulin-independent mechanisms and synergize with insulin.^{21,22,23}

Table-2: Phytochemical Constituents of the Tuber of *Stephania glabra* L. by LC-MS

Peak No.	Compounds	Molecular Formula	Molecular weight (g/mol)	Retention Time (R _T)	Observed (m/z)	Rel. Area % TIC
01	Rutin	C ₂₇ H ₃₀ O ₁₆	610.52	05.123	609.23 [M-H] ⁻	08.19
02	Jatrorrhizine	C ₂₀ H ₂₀ NO ₄ ⁺	338.38	05.987	338.34 [M+H] ⁺	04.87
03	Palmatine	C ₂₁ H ₂₂ NO ₄ ⁺	352.40	07.341	352.29 [M+H] ⁺	08.38
04	Berberine	C ₂₀ H ₁₈ NO ₄ ⁺	336.36	08.132	336.28 [M+H] ⁺	07.43

05	Magnoflorine	C ₂₀ H ₂₄ NO ₄ ⁺	342.40	09.847	342.32 [M+H] ⁺	08.58
06	Cycleanine	C ₃₇ H ₄₂ N ₂ O ₆	610.74	11.547	611.24 [M+H] ⁺	02.92
07	Tetrandrine	C ₃₈ H ₄₂ N ₂ O ₆	622.75	12.687	623.32 [M+H] ⁺	07.60
08	Quercetin	C ₁₅ H ₁₀ O ₇	302.24	13.187	303.27 [M-H] ⁻	05.87
09	Kaempferol	C ₁₅ H ₁₀ O ₆	286.24	14.012	287.28 [M-H] ⁻	07.43
10	Apigenin	C ₁₅ H ₁₀ O ₅	270.24	16.347	271.32 [M-H] ⁻	12.36
11	Stigmasterol	C ₂₉ H ₄₈ O	412.69	17.398	413.31 [M+H] ⁺	05.65
12	β-Sitosterol	C ₂₉ H ₅₀ O	414.71	21.247	415.27 [M+H] ⁺	05.66
13	Lupeol	C ₃₀ H ₅₀ O	426.72	32.978	426.28 [M+H] ⁺	06.65
14	β-Amyrin	C ₃₀ H ₅₀ O	426.72	35.147	426.29 [M+H] ⁺	08.41

Table-3: Phytochemical Constituents of Tuber in *Stephania glabra* L. by GC-MS

Peak No.	Compounds	Chemical Formula	Molecular weight (g/mol)	Retention Time (R _T)	Observed (m/z)	Rel. Area % TIC
01	1-Chloroundecane	C ₁₁ H ₂₃ Cl	190.76	13.263	191.21 [M+H] ⁺	09.43
02	2,4-di-tert-butylphenol	C ₁₄ H ₂₂ O	206.32	14.237	207.23 [M-H] ⁻	23.58
03	3,5-Di-tert-butylphenol	C ₁₄ H ₂₂ O	206.32	16.324	207.27 [M-H] ⁻	20.75
04	4-[1-(1-Methyl-indol-3-yl) ethyl] phenol	C ₁₇ H ₁₉ NO	253.34	21.479	254.29 [M+H] ⁺	35.85
05	1-Hexadecanol	C ₁₆ H ₃₄ O	242.44	26.789	243.31 [M+H] ⁺	10.38

Table-4: Phytochemical Constituents of Leaves in *Clerodendrum phlomidis* L. by LC-MS

Peak No.	Compounds	Chemical Formula	Molecular mass (g/mol)	Retention Time (R _T)	Observed (m/z)	Rel. Area % TIC
01	Gallic acid	C ₇ H ₆ O ₅	170.12	05.156	171.22 [M-H] ⁻	08.48
02	Rutin	C ₂₇ H ₃₀ O ₁₆	610.52	05.223	609.23 [M-H] ⁻	10.91
03	N-Methyltryptamine	C ₁₁ H ₁₄ N ₂	174.24	07.012	175.31 [M+H] ⁺	03.33
04	Luteolin	C ₁₅ H ₁₀ O ₆	286.24	10.243	287.28 [M+H] ⁺	10.61
05	Quercetin	C ₁₅ H ₁₀ O ₇	302.24	13.022	303.34 [M-H] ⁻	11.52
06	Kaempferol	C ₁₅ H ₁₀ O ₆	286.24	14.012	287.28 [M-H] ⁻	11.21
07	Apigenin	C ₁₅ H ₁₀ O ₅	270.24	16.232	271.37 [M-H] ⁻	10.61
08	Clerodin	C ₂₄ H ₃₄ O ₆	418.52	18.124	373.32 [M+H] ⁺	10.02
09	Campesterol	C ₂₈ H ₄₈ O	400.68	23.473	401.14 [M+H] ⁺	09.07
10	Oleanolic acid	C ₃₀ H ₄₈ O ₃	456.70	24.236	457.26 [M+H] ⁺	07.27
11	Ursolic acid	C ₃₀ H ₄₈ O ₃	456.70	29.987	457.21 [M+H] ⁺	06.97

Table-5: Phytochemical Constituents of Leaves in *Clerodendrum phlomidis* L. by GC-MS

Peak No.	Compounds	Chemical Formula	Molecular weight (g/mol)	Retention Time (Min)	Observed (m/z)	Relative Area % TIC
01	p-Coumaric acid	C ₉ H ₈ O ₃	164.16	3.247	165.32 [M-H] ⁻	17.58
02	Ferulic acid	C ₁₀ H ₁₀ O ₄	194.19	5.247	195.21 [M-H] ⁻	14.29
03	Anabasine	C ₁₀ H ₁₄ N ₂	162.23	5.441	163.27 [M-H] ⁺	9.89
04	Terpinen-4-ol	C ₁₀ H ₁₈ O	154.25	7.236	155.26 [M-H] ⁺	10.99
05	Phytol	C ₂₀ H ₄₀ O	296.53	10.36	219.34 [M-H] ⁺	13.74
06	α-Bisabolol	C ₁₅ H ₂₆ O	222.37	12.36	205.23 [M-H] ⁺	18.13
07	Caryophyllene	C ₁₅ H ₂₄	204.36	15.23	297.24 [M-H] ⁺	15.38

Table-6: α-amylase Inhibition by the Tuber of *Stephania glabra* and *Clerodendrum phlomidis* Leaves

Groups	Concentration [μg/ml]	α-amylase		
		EETSG	EELCP	Acarbose
Group I	12.5	20.53 ± 0.01	22.53 ± 0.25	25.36 ± 0.10

Group II	25	37.53 ± 0.01	36.25±0.20	39.48 ± 0.01
Group III	50	56.53 ± 0.25	53.32±0.19	58.77 ± 0.02
Group IV	100	72.31 ± 0.66	69.76±0.21	76.77 ± 0.01
Group V	200	85.53 ± 0.46	80.4±0.32	93.76 ± 0.02
Group VI	400	89.85 ± 0.11	86.35±0.34	96.54 ± 0.01
	IC ₅₀ (µg/ml)	41	45	38

All values were presented as Mean ± SEM based on triplicate.

Table-7: α-glucosidase Levels of the Tuber of *Stephania glabra* and *Clerodendrum phlomidis* Leaves

Group	Dose (µg/ml)	Percentage Inhibition					
		EETSG		EELCP		Acarbose	
		Sucrase	Maltase	Sucrase	Maltase	Sucrase	Maltase
Group I	12.5	25.6±0.1	33.2±0.5	34.2 ± 0.1	37.1±0.6	39.6±0.4	20.3±0.3
Group II	25	40.7±0.1	48.7±0.4	49.6 ± 0.2	50.2 ±0.2	51.6±0.1	39.8±0.3
Group III	50	55.3±0.2	62.1±0.3	63.4 ± 0.2	65.7±0.2	68.4±0.2	60.5±0.8
Group IV	100	70.5±0.1	81.7±0.7	83.6 ± 0.2	80.4±0.4	86.6±0.2	75.2±0.1
Group V	200	80.3±0.1	86.4±0.7	87.6 ± 0.2	85.3±0.8	90.6±0.1	85.7±0.4
Group VI	400	89.9±0.2	88.3±0.	89.7 ± 0.1	90.8±0.2	93.1±0.1	95.4±0.8
	IC ₅₀ (µg/ml)	30	27	26	25	23	22

All values were presented as Mean ± SEM based on triplicate

Table-8: Glucose Uptake Levels by EETSG and EELCP Extracts

S. No	Treatments	Glucose uptake (mg/g tissue/30 min)
1.	Normal water	60
2.	Insulin 0.6 ml (1 IU/ml)	225
3.	EETSG 20 mg/kg	187
4.	EETSG 20 mg/kg + 0.6 ml Insulin (1 IU/ml)	249
5.	EETSG 40 mg/kg	204
6.	EETSG 40 mg/kg + 0.6 ml Insulin (1 IU/ml)	277
7.	EETSG 60 mg/kg	222
8.	EETSG 60 mg/kg +0.6 ml Insulin (1 IU/ml)	286
9.	EELCP 20 mg/kg	172
10.	EELCP 20 mg/kg + 0.6 ml Insulin (1 IU/ml)	235
11.	EELCP 40 mg/kg	192
12.	EELCP 40 mg/kg + 0.6 ml Insulin (1 IU/ml)	256
13.	EELCP 60 mg/kg	214
14.	EELCP 60 mg/kg + 0.6 ml Insulin (1 IU/ml)	270

All values were presented as mean ± sem based on tri-replicates

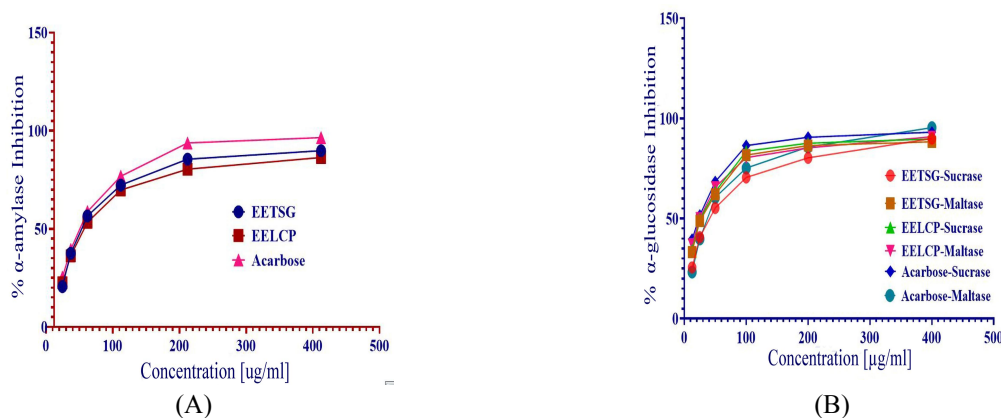


Fig.-5: (A.) α-amylase Inhibition of the Tuber of *Stephania glabra* L. and (B.) α-glucosidase Inhibition of Leaves of *Clerodendrum phlomidis*

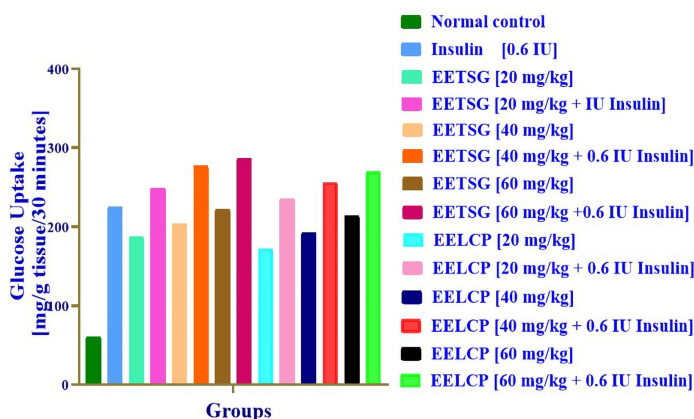


Fig.-6: Glucose Uptake by Rat Hemidiaphragm by Tuber of *Stephania glabra* L. and Leaves of *Clerodendrum phlomidis*

CONCLUSION

The ethanolic extracts of *Stephania glabra* tubers (EETSG) and *Clerodendrum phlomidis* leaves (EELCP) exhibited significant *in-vitro* antidiabetic activity, attributed to active phytochemicals that inhibit glucose-liberating enzymes, such as α -amylase and glucosidase, and modulate glucose transport mechanisms. These findings support their potential in developing antidiabetic therapies and contribute toward achieving SDG-3: Good Health and Well-being.

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CONFLICT OF INTERESTS

The authors report no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to SDG-3: Good Health and Well-being. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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