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LC–MS BASED PHYTOCHEMICAL PROFILING AND EVALUATION OF DIABETES INDUCED BIOCHEMICAL PARAMETERS AND ANTIOXIDATIVE ACTIVITY OF *Stephania glabra* TUBER AND *Clerodendrum phlomidis* LEAVES

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ABSTRACT

Stephania glabra L. and *Clerodendrum phlomidis* L. are traditionally used to manage various endocrine complications. Considering the oxidative damage in the progression and trigger the diabetes, the current study aimed to evaluate the phytochemical profile and glucose regulation of their ethanolic substances. The plant materials were extracted by maceration using (95%) ethanol, and preliminary phytochemical screening along with liquid chromatography linked mass spectroscopy (LC–MS) analysis revealed the occurrence of phytochemical compounds. Antidiabetic activity was assessed in streptozotocin-produced diabetic rats (55 mg/kg), glucose levels above 250 mg/dl were pondered diabetic. Oral administration of the extracts (100, 200, and 400 mg/kg) significantly reduced glycosylated haemoglobin levels (6.12% and 4.6%), improved haemoglobin levels (14.6 and 13.9 g/dL), and decreased creatinine (0.66 mg/dL) and urea (28 mg/dL). In addition, the ethanolic extract of *Stephania glabra* tuber (400 mg/kg) markedly enhanced antioxidant status by increasing superoxide dismutase (SOD) 0.43 to 1.13 IU/mg, catalase 0.36 to 2.12 IU/mg, reduced glutathione (GSH) 3.10 to 6.21 IU/mg, and reducing malondialdehyde (MDA) 10.66 to 5.83 IU/mg. Similarly, *Clerodendrum phlomidis* leaves extract (400 mg/kg), increased SOD (1.05 IU/mg), catalase (2.02 IU/mg), and GSH (5.89 IU/mg), while decreasing MDA (6.26 IU/mg), indicating attenuation of oxidative stress. These findings suggest that flavonoids, alkaloids, and phenolic compounds may exert antidiabetic consequences by foraging responsive oxygen species and enhancing endogenous antioxidant defenses, thereby improving diabetes-associated biochemical parameters.

Keywords: Antidiabetic, Creatinine, Glycated Haemoglobin, Haemoglobin, Renal, SDG-3 and Urea.
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INTRODUCTION

Diabetes mellitus is rising globally and has become a major public health challenge. Recent estimates show that almost 537 million adults were active with diabetes in 2021, increasing to nearly 589 million by 2024–2025, and projections suggest that this number may reach about 643 million by 2030 if current trends persist.[1] This rapid escalation highlights an urgent need for stronger preventive approaches, earlier detection, and improved long-term clinical management to reduce future health burdens. Diabetes emerges as sustained hyperglycemia elevates fasting blood glucose above 126 mg/dl. The glycosylated haemoglobin is a key indicator of long-term blood glucose regulation in Diabetes mellitus, with levels ≥ 6.5% reflecting impaired glycemic control. In Diabetes mellitus, impaired insulin responsiveness reduces cellular glucose utilisation, resulting in persistent hyperglycemia.[2] This metabolic disturbance alters several systemic biochemical parameters, including increased Glycosylated Haemoglobin, along with changes in haemoglobin, total protein, creatinine, and urea, reflecting disrupted protein metabolism and renal function. Sustained hyperglycemia also enhances the liberation of reactive oxygen radicals, leading to oxidative damage.[3] Therefore, endogenous antiradical defenses like catalase, superoxide dismutase (SOD), and reduced glutathione (GSH) are compromised, while lipid peroxidation increases, indicated by elevated malondialdehyde (MDA). These biochemical alterations collectively illustrate the interplay between metabolic imbalance and oxidative stress in diabetes progression. Phytochemicals exert potent antidiabetic effects by reducing elevated glycosylated haemoglobin and improving overall systemic biochemical parameters.[4,5] Several plant-derived compounds also improve insulin sensitivity of metabolic

functions, further promoting glucose uptake. Their inherent antiradical effects additionally shield metabolic tissues from oxidative stress–induced damage. Through these coordinated mechanisms, phytochemicals contribute to effective and sustained management of diabetes. *Stephania glabra* L. tuber and *Clerodendrum phlomidis L*. leaves demonstrated notable antidiabetic effects by lowering elevated glycosylated haemoglobin and improving overall systemic biochemical parameters.[6] These plants also augment the endogenous antioxidant system by elevating antioxidative enzymes. Through these integrated metabolic and antioxidant effects, both species contribute effectively to the management of diabetes. Despite reports on the antidiabetic potential of medicinal plants, the biochemical mechanisms underlying their activity remain insufficiently characterised.[7] LC–MS-based identification of bioactive metabolites in *Stephania glabra* L. tuber and *Clerodendrum phlomidis* L. leaves was limited. Therefore, systematic evaluation of their phytochemical profile and effects on glucose-metabolising enzymes is required. The current study was to analyses the ethanolic extracts through phytochemical screening and liquid chromatograph linked mass spectroscopy (LC–MS) to identify their active constituents. The diabetic associated systemic biochemical parameters and antioxidant properties of ethanolic extracts of *Stephania glabra* L. tuber and *Clerodendrum phlomidis* L. leaves on Glycosylated haemoglobin levels, haemoglobin, creatinine and urea levels and Antioxidant activity were also evaluated by measuring catalase and superoxide dismutase to determine their potential in diabetes management.[8] The work contributes to SDG-3: Good Health and Well-being through its focus on natural, plant-based methods for diabetes management.

EXPERIMENTAL

Authentication and Extraction Method

The tubers of *Stephania glabra* L. and leaves of *Clerodendrum phlomidis* L. were collected and authenticated by a botanist at the Botany Department, Sri Venkateswara University, Tirupati. Carnet (1198 and 0964) was a plank in the departmental herbarium. The plant materials were shade-dried at 35 °C and ground into coarse powder. Approximately 500 g of each crushed sample was macerated with a compatible solvent (ethanol 1:2 w/v) for seven days at 35 °C. The extracts were filtered through filter paper (Whatman No. 01), concentrated at 40 °C using a rotary evaporator, and kept in a vacuum desiccator at 25 °C until further analysis.[9,10]

Animals

Albino Rats (150-180 g) were acclimatised for 48 hours before experimentation. The study of approval was permitted by the IAEC of Hindu College of Pharmacy, Guntur, approval No. IAEC-HOCP/2022/06.

Phytochemical Analysis

Preliminary phytochemical evaluation of several bioactive groups in ethanolic extracts. The analysis indicated detectable levels of flavonoids, alkaloids, phytosterols, tannins, carbohydrates, saponins, and phenolic compounds.[11,12]

Determination of Active Compounds by Liquid Chromatography Linked Mass Spectroscopy

Phytochemical profiling of the ethanolic extracts was accomplished using liquid chromatography linked mass spectrometry (LC–MS). The extracts concentration 1 mg/ml by HPLC-grade methanol-99%, sonicated (15 minutes), and sorted through a 0.22 µm needle filter prior to analysis. C_{18} column-based separation was performed on a reverse-phase column using a gradient eluent consisting of acidified water and acetonitrile. The mass spectrometer was operated in electrospray ionisation (ESI) mode, acquiring spectra in both (+ ve and -ve ion) modes scan range of m/z 100 to 1500. Compounds were hesitantly identified by assessing their retention time values and MS data with authenticated standards and the NIST spectral library.[13,14]

Pharmacological *In-Vivo* Diabetic Induced Biochemical Parameters and Antioxidative Study

Albino rats (male, 150–180 g) were randomly split into nine groups (I–IX), each with six animals. Diabetes was induced by streptozotocin (55 mg/kg) after fasting, and rats with blood glucose above normal (>250 mg/dl) were considered diabetic. Normal and diabetic control groups received plain water, while treatment groups received ethanolic extracts of *Stephania glabra* tuber and *Clerodendrum phlomidis* leaves at 100, 200, and 400 mg/kg. The positive control received glibenclamide (2.5 mg/kg).

Treatments were given orally once daily. Antidiabetic activity was assessed by glycosylated haemoglobin (HbA1c), haematological status by haemoglobin (Hb), and renal function by total protein, creatinine, and urea. Antioxidant status was determined by using superoxide dismutase, catalase, glutathione, and malondialdehyde.[15,16]

Biostatistical Analysis

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

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The ethanolic extracts of *Stephania glabra L.* tuber and *Clerodendrum phlomidis L.* leaves yielded 25% and 30%, respectively. Preliminary phytochemical screening revealed the presence of active compounds. The detailed classes of identified are presented in Table-1.

Table-1: Phytochemical Screening of Ethanolic Test Extracts

Category	*Stephania glabra L.* Tuber	*Clerodendrum phlomidis L.* Leaves
Carbohydrates	+	-
Alkaloids	+	+
Flavonoids	+	+
Proteins and amino acids	+	+
Terpenoids	+	+
Phenolic compounds	+	+
Tannis	+	-
Phytosterols	+	+
Volatile oils	+	+
Saponins	+	+
Phytosterols	+	+
Resins	-	-

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

LC-MS Identification of Active Constituents of Ethanolic Extracts

Liquid chromatography–mass spectrometry (LC–MS) analysis of *Stephania glabra* tubers revealed several bioactive constituents, including alkaloids such as berberine, palmatine, jatrorrhizine, magnoflorine, cycleanine, and tetrandrine, along with flavonoids (quercetin, rutin, kaempferol, and apigenin) and phytosterols (β-sitosterol, stigmasterol, lupeol, and β-amyrin). In a similar manner, LC–MS analysis of *Clerodendrum phlomidis* leaves identified phenolic compounds including gallic acid and rutin, flavonoids such as luteolin, quercetin, kaempferol, and apigenin, the alkaloid N-methyltryptamine, and terpenoids including clerodin, campesterol, oleanolic acid, and ursolic acid. Collectively, these constituents possess antidiabetic and antioxidant properties and may contribute to reduced glycosylated haemoglobin, increased haemoglobin levels, decreased creatinine and urea, improved total protein, and enhanced antioxidant defense in treated diabetic rats. these phytoconstituents which may contribute to the therapeutic effects observed in the present study.[17,18]

Effects of Ethanolic Extracts on Diabetic Induced Systemic Biochemical Parameters

Ethanolic extracts produced statistically significant effects, dose-dependent improvements in systemic biochemical parameters of groups. After 28 days, ethanolic extract of *Stephania glabra* tuber treatment reduced glycosylated haemoglobin to 6.1%, increased haemoglobin to 16.6 g/dl, lowered creatinine to 0.56 mg/dl and urea to 28.47 mg/dl, while total protein increased to 6.9 g/dl. Similarly, treatment with ethanolic extract of *Clerodendrum phlomidis* leaves reduced glycosylated haemoglobin to 6.5%, increased haemoglobin to 15.9 g/dl, decreased creatinine to 0.58 mg/dl and urea to 27.54 mg/dl, and elevated total protein to 6.8 g/dl. These changes indicate improved glycemic control along with restoration of protein metabolism and renal function in diabetic animals.[19,20]

Effects of Ethanolic Extracts on Antioxidant Study

Ethanolic extracts of *Stephania glabra* tubers and *Clerodendrum phlomidis* leaves significantly improved antioxidant enzyme activity in streptozotocin-induced diabetic rats. After 28 days, *Stephania glabra* increased superoxide dismutase to 1.13 IU/mg, catalase to 2.12 IU/mg, and glutathione to 6.21 IU/mg, while malondialdehyde decreased to 5.83 IU/mg, with the strongest effect observed at 400 mg/kg.

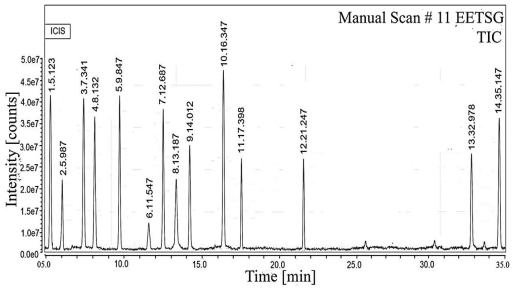

Fig.-1: LC chromatogram of the tuber of *Stephania glabra L*

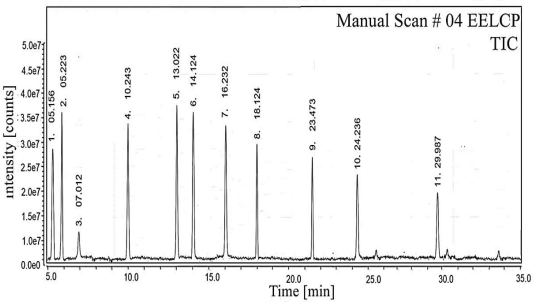

Fig.-2: LC chromatogram of *Clerodendrum phlomidis L.* Leaves

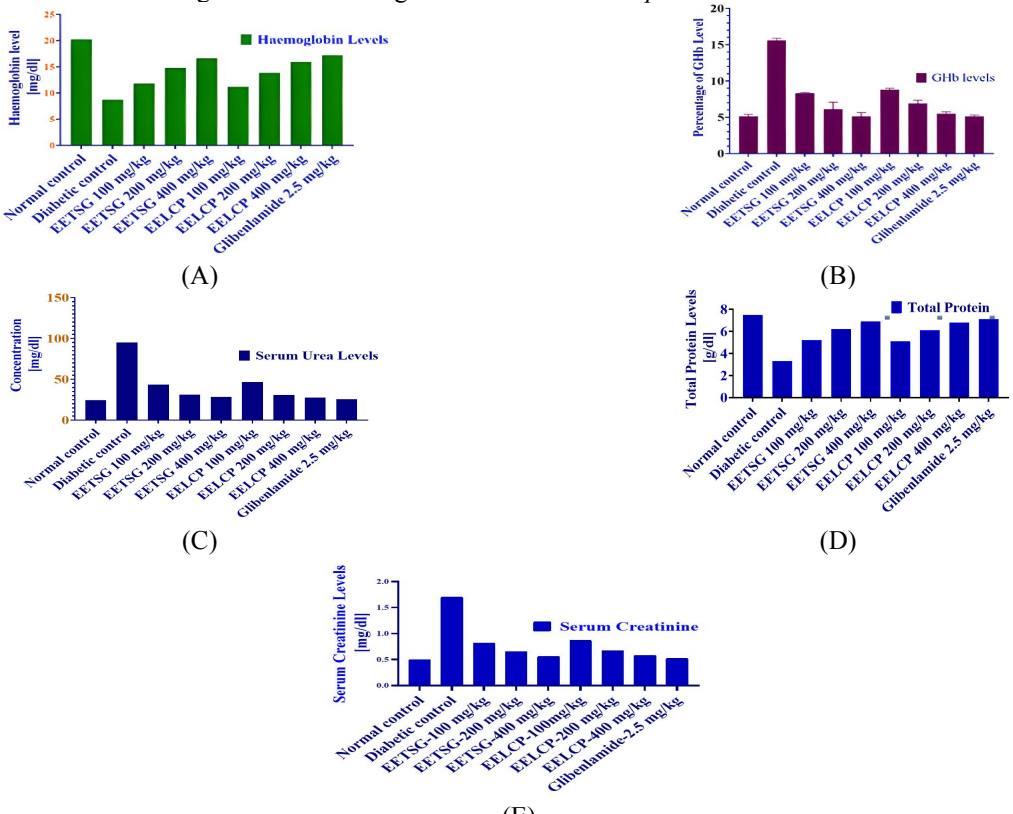

Fig.-3: Ethanolic Extracts on Systemic Biochemical Parameters (A) Total Haemoglobin Levels, (B) Glycosylated Haemoglobin Levels, (C) Urea Levels, (D) Creatinine Levels, (E) Total Protein Levels. Data Wererepresented as Mean ± SEM of (n=6) Measurements

Similarly, *Clerodendrum phlomidis* increased superoxide dismutase to 1.05 IU/mg, catalase to 2.02 IU/mg, and glutathione to 5.89 IU/mg, while malondialdehyde declined to 6.26 IU/mg. All values flashed statistically substantial variations contrasted with diabetic control, indicating improved antioxidant defence and reduced lipid peroxidation.[21,22]

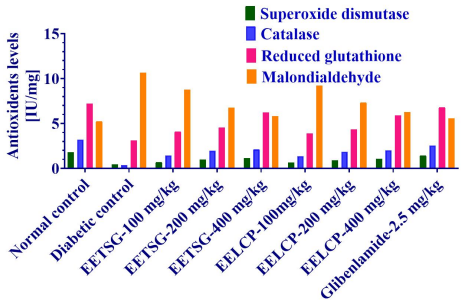

Fig.-4: Effect of Ethanolic Extracts on Antioxidant Enzymes. Data wererepresented as Mean ± SEM of (n=6) Measurements

CONCLUSION

This study demonstrates that ethanolic extracts of *Stephania glabra* L. tubers and *Clerodendrum phlomidis* L. leaves contain bioactive phytochemicals with antidiabetic potential. Treatment improved key diabetic biochemical parameters and enhanced antioxidant enzyme activities in diabetic rats. The extracts also supported metabolic regulation associated with glucose homeostasis. These discoveries suggest their capability as promising phytochemicals for diabetes management.

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CONFLICT OF INTERESTS

The authors declare that there is 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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