RESEARCH PAPER

Evaluation of Anti-Diabetic Activity of Bark and Leaf Extract of Thespesia Populnea: HR-LCMS Based Approach

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ABSTRACT

Globally, one among the major health problems is Diabetes Mellitus (DM). Currently, DM's incidence along with associated mortality is increasing. DM's current epidemic in the world indicates the urgent need to develop new therapeutic drugs that are cheaper, more safe, and available to face this health challenge. Conventional Anti-Diabetic (AD) drugs are effectual; yet, they also have inevitable side effects. Yet, medicinal plants may act as AD agents' alternative source to treat diabetes patients worldwide and are popular as nutraceuticals. Therefore, the presented study is presented to examine the assessment of the AD activity of bark along with Leaf Extract (LE) of Thespesia Populnea (TP) grounded on a High-Resolution Liquid Chromatography-Mass Spectrometry (HR-LCMS) approach. Therefore, by using HR-LCMS, bioactive compound characterization in TP bark and leaf is extracted. Fresh leaves and barks of TP are collected and shade-dried; further, aqueous extracts are prepared. In vivo studies are conducted using streptozotocin (STZ)-induced diabetic Wistar rats to appraise the extracts' AD activity. As per the outcome, TP plants' leaf and bark extracts show AD activities. When weighed against other drugs, the standard drug of glibenclamide achieves the most substantial reduction of 51%, lowering glucose levels as of 241 mg/dL to 123 mg/dL.

Keywords: Medicinal plant, Thespesia populnea, Diabetes, Anti-diabetic activity, Herbal formulations

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1. INTRODUCTION

Over the past few decades, in herbal medicine, there has been exponential growth. Owing to their natural origin, along with fewer side effects, those drugs are gaining popularity globally. Globally, the World Health Organization (WHO) has recorded 21,000 plants that are wielded for medicinal commitments. Plant research is focused worldwide. Also, to depict plants' boundless potential wielded in several traditional systems, data have been collected. One among the precious drugs wielded in herbal medicine, as well as recognized as Paras-Pipal, is TP Linn (Family-Malvaceae) [1, 2]. In India's coastal forests along with south-eastern areas, TP, which is termed "Portia Tree" (Malvaceae), is extensively distributed. For treating cutaneous infections along with brain and liver disorders, the plant is medicinally employed. Therefore, the different parts of TP in herbal medicine treat various diseases, including roots, leaves, flowers, fruits, and bark. Modern research has confirmed TP's therapeutic potential, which highlights the significance of discovering natural remedies along with plants' potential to offer novel treatments for various illnesses [3, 4]. Due to potential AD effects in the plant's various parts, TP is studied. The ethanolic extracts as of plant bark, along with leaf, have AD effects in rats induced by streptozotocin [5].

A serious, chronic, along with intricate metabolic disorder of numerous aetiologies with profound concerns, acute as well as chronic, is termed DM. DM, along with its complications, affects people, resulting in a major socioeconomic challenge. DM has severe impacts on patients as well as society. DM incidence is estimated to be 382 million people, which is predicted to rise to 439 million by 2030 [6]. DM treatment is grounded on parenteral insulin along with oral AD drugs. Presently, Oral hypoglycemic agents have austere side effects; therefore, there is a necessity to search for a fresher AD agent, which has high therapeutic efficacy with minimal side effects. This might be satisfied by treating DM with traditional medicine by employing AD agents as of medicinal plants [7]. Diabetes's improper management might cause human body organ deterioration. In the present Westernized medicine world, the treatment, along with the whole cure for diabetes, is an inexplicable question. To date, the only ethical drug approved for non-insulin-dependent DM patients' treatment is metformin; also, it is also derived from a medicinal plant.

Likewise, diabetes and its complications are allied with oxidative stress, which results in cellular damage owing to reactive oxygen species or ineffective Anti-Oxidant (AO) system generation in the human body[8, 9,10]. DM's current management could be attained with synthetic hypoglycemic medications' assistance; yet, these medications' longer-term usage might result in numerous side effects. Thus, there is a requirement for natural compounds with AD potential devoid of any side effects. Also, no studies have been conducted to identify the preliminary photochemical and HR-LCMS analysis in TP, particularly bark and LE. Thus, to fulfill this aforementioned gap, the presented study aims to analyze the AD potential of TP bark and LEs through HR-LCMS analysis, phytochemical screening, and in vivo evaluation with a focus on understanding their mechanism of action and safety for therapeutic use.

Objective of the study

The research objectives are specific, along with measurable goals that outline the researcher's accomplishments within a study. Hence, the research study's objectives are described below,

1. To identify and characterize the bioactive compound in TP bark and LEs using HR-LCMS.
2. To evaluate phytochemical presence like flavonoids, tannins, along with phenols, in the extracts through qualitative and quantitative screening.
3. To appraise the extracts' AD activity in STZ-induced diabetic rats, along with appraise their efficacy with a standard drug (glibenclamide).
4. To appraise the extracts' effects on oxidative stress makers, including SOD, catalase, and MDA levels in diabetic rats.
5. To perform acute toxicity studies to appraise the safety profile of the bark and LEs.

The pattern of this paper is structured as: Section 2 reviews the relevant literature of the study. Next, the study materials and methods are explained in Section 3. Later, Section 4 presents complete results and analyses of the study, as well as the analysis of the collected data and findings of the research. Finally, the study conclusion, limitations, future scope, and implications are summarized in section 5.

2. LITERATURE REVIEW

S. N. Belhekar *et al.* [11] intended to appraise the antihyperlipidemic and AD effects of TP fruit pulp in rats on alloxan-induced experimental diabetes. Afterward, diabetes was also established through alloxan treatment given to the rats. Further, the rats were subdivided into five groups only after the establishment of successful experimentally induced diabetes. By employing analysis of variance, Statistical analysis for testing the significance was done, after Dunnett's various comparison tests, along with a paired t-test. The therapeutic dose of extract was 200 mg/kg based on an acute toxicity study. Blood Glucose Levels (BGL) were minimized by the aqueous and alcoholic extract (treatment (28 days)). Finally, the research indicated that $P \leq 0.05$ was the minimum statistical significance level. But, these plants' mechanism was not defined clearly.

Digambar Subhashrao Pawar and Sahera Nasreen [12] evaluated the significant chemical constituents in leaves' methanol extract by using the method of HR-LCMS. The fresh and healthy leaves of Annona squamosa and Casuarina equisetifolia plants were collected from the Government Institute of Science, Aurangabad campus. Next, the food poisoning technique was employed to check the extracts' antifungal activity. The study claimed that HR-LCMS separated and identified the antimicrobial components from the leaves of Annona squamosa and Casuarina equisetifolia. Further, it also revealed that the activities against Fusarium oxysporum and Colletotrichum capsici exhibited antifungal activities. The HR-LCMS analysis specifies that the methanolic extract of leaves from Casuarina equisetifolia and Annona squamosa contained numerous secondary compounds of value to treat many diseases.

S.N. Belhekar *et al.* [13] considered the anti-hyperglycemic activity of TP seed extracts in normal, along with alloxan-induced diabetic rats. In acute along with chronic studies, the ethanolic and aqueous extracts were administered to normal along with alloxan-induced rats. After drug treatment, a sole-dose study of extracts was conducted on normal and diabetic rats' blood samples, which were withdrawn at 2, 4, 6, 8, and 24 hrs. At '2' dose levels-200 along with 400 mg/kg, the single-dose study of EETP and AETP caused a key ($p<0.01$) lowering in BGLs in the normal along with diabetic rats at 2, 4, 6, 8 h. Moreover, the acute study reported a maximum reduction at 6h. EETP, along with AETP at '2' dose levels depicted ($p<0.01$) reduction of BGLs at the study (21st, 28th days).

R. Kavitha *et al.* [14] examined the in vitro analysis of Anti-Inflammatory (AI) along with AD activities of Polyalthia longifolia Benth. and Hook., Solanum torvum Swartz. and TP (L.) Soland. Ex Correa. Medicinal plants. Therefore, all three plants' fresh leaves and barks were collected and shade-dried; also, aqueous extracts were prepared. The AD properties and inhibitory effect on α-amylase activity were carried out by applying the DNSA method. A study demonstrated that all plants' leaf and bark extracts showed AD along with AI activities. These extracts resulted in a major decrease in releasing maltose. Solanum torvum leaves depicted 53.58% Inhibition over control; also, TP value was 46.60% at 200 µg concentration. Nevertheless, the phytochemical constituents and their exploration in the pharmaceutical industries were not the focus of this study.

Indeewari K. S. Lindamulage and Preethi Soysa [15] focused on anticancer property evaluation of decoction

with TP L. along with Adenanthera pavonina L. Therefore, the study of the cytotoxicity along with anti-proliferative motion beside the Hep-2 cells, the Sulforhodamine B (SRB) assays and Lactate Dehydrogenase (LDH) release, (3-(4, 5-Dimethylthiazol-2-yl)-2, together with 5-diphenyltetrazolium bromide) MTT were considered. EC50 mean (± SD) values were 195.50 (±40.68), 120.02 (±29.82), together with 77.06 (±8.80) µg/ml for LDH, MTT, along with SRB. The outcomes were strongly correlated with the morphological variations in cells canned with the decoction. Also, the brine shrimp lethality depicted an EC50 value at a greater concentration (1.96 mg/mL).

Sangeetha L. A. Rajbanshi *et al.* [16] demonstrated the ethanol-prompted alterations in TP LE's cardiac enzymes-ameliorative effect. Furthermore, change estimation in cardiac enzymes, like antioxidants, along with cardiac enzymes in rats, was examined. Male adult Wistar rats were allocated into 10 clusters of '6' rats each. Ethanol (20%, 2g/kg) treatment resulted in a reduction in each of the appraised enzymes' activities, with a slight drop in Mg2+ ATPase (29.26%) along with the greatest drop in CAT (71.05%). Recovery with 200 mg Thespesia LE was fewer, reaching as of 24.1% for Mg2+ATPase to 190.91% for CAT, when analogized with ethanol-canned rats as controls. With 48.19% (minimum) for Mg2+ ATPase, along with 222.73% (maximum) for CAT, 400 mg Thespesia extort affected a more significant recovery.

Konda Jeevitha *et al*. [17] aimed to assess the phytochemical analysis along with in vitro anticancer activity of TP's fruit extract beside Hela (cervical cancer), along with k562 (leukaemia cancer) cancer cell lines. Using the extraction method, the fruit sample of TP with amassed polarity from methanol, ethyl acetate, mintroleum, along with hydro-alcohol solvents was analyzed. Using different solvents based on polarity and the phytochemical constituents of the plant, TP was calculated. Qualitative extract analysis revealed flavonoids, phenols, alkaloids, sterols, tannins, along with reducing sugars were present. A high percentage of extractives were seen in hydro-alcohol using Soxhlet extraction. The hydro-alcoholic fruit extract was cytotoxic against the K562 and HeLa in-vitro cell lines with IC50 values of 2886 and 2284, respectively.

Prawesty Diah Utami *et al*. [18] aimed to study the anti-malarial activity of TP solanad ex correa extract by employing a malaria mouse model infested with P. berghei. In total, 27 mice were selected and then randomly allocated into 3 groups: aquades, received chloroquine, along with 200mg/kg bw of TP (L.) Soland ex Correa extract together with Chloroquine. Parasitemia levels followed from the first up to the fourth day by statistical analysis depicted that the LE TP's administration in a dose 200 mg/kg bw with chloroquine had the effects lowered degree of parasitemia along with increased hemoglobin signification than the G1 groups. Nevertheless, there was no key difference betwixt the groups regarding G2. It also demonstrated that an effect on anemia prevention was produced by the extract of TP, given no significant differences in the control of 200 mg/kg bw.

Gowtham R *et al*. [19] elucidated the antimicrobial activity impact of TP along with Abutilon indicum against clinical microbes. From Gopal Nagar, Thanjavur, Tamilnadu, India, TP L. and Abutilon indicum L. healthy plants were gathered. Afterward, the selected leaf materials were cleaned along with shade-dried, free as of dirt particles. To detect antimicrobial activity, the agar well diffusion was followed. Compared to other aqueous extract solvents, TP and A.indicum leaf's antimicrobial properties with methanolic, along with diethyl ether extract of maximum zone inhibition, had brilliant performance. Also, the study indicated that T. populnea flowers' ethanolic crude extract activity showed key antibacterial as well as antifungal activities, along with the possession of antimicrobial activities against many microorganisms.

Sayyeda Farha *et al*. [20] reported the phytochemical along with anti-psoriatic activity of the TP leaves' ethanolic extract. From Sims Park, Coonoor, TP (L.) Webb & Berth (Malvaceae) fresh leaves were collected. For the presence of numerous phytoconstituents tests, the extract's preliminary phytochemical analysis was screened. Appraising anti-psoriatic activity was the goal. The plants' dried leaves were subjected to a solution with 95% ethanol, along with phytochemical studies were done. The anti-psoriatic activity was appraised by employing the Mouse-Tail. The reproducible morphometric allowed quantitative evaluation of anti-psychiatics effects via epidermal differentiation. TP (92.68±8.8) ethanolic extract depicted a key difference ($P < 0.05$) when weighed against control (100±10.7) in relative epidermal thickness.

Menna B. Abdel Halim *et al*. [21] presented the evaluation for TP L. bark's wound healing activity, an approach to accelerate healing via nanoparticles, along with isolation of key active constituents. TP (L.) Sol. Ex M.P. Correa bark was gathered as of Al Qanatir Al Khayriyyah Nurseries, Al-Qalyubiyya Governorate, Egypt, in the budding stage in September 2020. The utmost erasure wound remedial action was witnessed in petroleum ether (PetB), after ethyl acetate (EtacB) fractions at the higher dose (2%). In addition, bioactive fractions' phytochemical studies caused isolation of diverse chemical classes' several compounds viz; beta-sitosterol along with lupeol acetate inaccessible as of the PetB, with cyanidin as well as delphinidin from the EtacB.

Mohini Ashok Phanse *et al*. [22] identified the isolation, characterization, along with preclinical studies of TP's metal complex meant for the latent peroxisome proliferator-triggered receptors-γ agonist action. TP's dry bark was attained as of the Hilly area of Dehu from Pune,

Maharashtra, India. The sesquiterpene isolated as of TP bark's hexane fraction was synthesized synthetically through the analytical technique of electron paramagnetic resonance spectra. Next, grounded on anti-hyperglycemic along with hypoglycemic potential, the AD activity was evaluated. Findings of a study revealed that vanadium complex's nti-hyperglycemic and hypoglycemic activity in glucose-loaded along with normal animals, minimized plasma BGLs. The treatment with Vanadium complex depicted a key reduction in plasma glucose level while weighed against the Control Group (CG).

S. Solomon *et al*. [23] studied the AI activity along with the antioxidant activity of the flowers of TP. TP's Fresh flowers were gathered as of O. Koothur Village, Ariyalur district, Tamil Nadu, India. By employing the HRBC and albumin denaturation method, Ethanolic extract was appraised, by which the AI activity was detected. Likewise, by using the DPPH assay along with ABTS, the ethanolic extract's antioxidant activity was measured. The DPPH assay, total antioxidant, along with ABTS, depicted robust antioxidant activity, as well as the stabilization of albumin denaturation, HRBC membrane, and inhibition indicated AI activity. The study confirmed a strong link betwixt the TP flowers' antioxidant and AI activities.

Jancy Varghese *et al*. [24] aimed to analyze TP L. flower's the potential crude extracts on numerous drug-resistant opportunistic pathogens that existed in HIV/AIDS patients. T. populnea L. antifungal and antibacterial potential was tested on several drug-resistant opportunistic pathogens, Pseudomonas aeruginosa, along with Candida albicans in HIV/AIDS patients. In tube and plate methods, it is done through the major factors affecting resistance testing on the formation of the biofilm. Using mass spectroscopy and gas chromatography on the withered flower powder gave sauxlaut hot extraction, along with the crude extort was analyzed. As per the tested extracts along with the selected compounds, a key outcome was revealed in inhibiting human opportunistic pathogens. Also, the increased level of the extracts was better for inhibiting the microbes.

Vasundaramma. J and Kamakshamma [25] anti-hyperglycemic activity of TP seeds methanol extract on STZ-induced albino rats. The matured fruits of TP were collected from Tirupati's surrounding areas, Chittoor District of Andhra Pradesh, India, in November 2014. Using the ELISA method, the insulin level was measured in the plasma of normal along with STZ-induced diabetic rats. Thus, T. populea's Methanol seed extract depicted a remarkable decrease in BGLs and varied lipid profile parameters in Diabetic treated rats. Also, it demonstrated that the T. populnea seeds' methanol extract possessed significant anti-hyperglycemic activity.

3. MATERIALS AND METHODS

The study aims to investigate the AD potential of TP bark along with LEs through a systematic research process. The plant materials are collected, authenticated, and subjected to ethanolic extraction using the cold maceration method. Phytochemical screening is performed to detect the presence of bioactive compounds, followed by HR-LCMS analysis to identify and characterize specific phytochemicals, such as quercetin, kaempferol, and gossypol. In vivo studies are conducted using STZ-induced diabetic Wi star rats to appraise the extracts' AD activity. BGLs are monitored, along with oxidative stress markers like SOD, catalase, and MDA, are analyzed to assess the AO effects. Additionally, acute toxicity studies are performed to determine the extracts' safety profile. The findings are statistically analyzed to create the therapeutic potential of the extracts in managing diabetes and associated oxidative stress. The schematic diagram in Figure 2 illustrates the following research process.

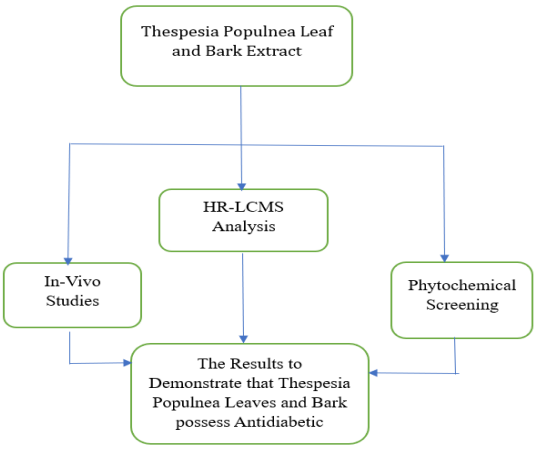

Figure 2: Schematic research diagram

Plant Material Collection and Authentication

The bark and leaves of TP are collected, washed carefully with distilled water, along with air-dried under shade to preserve phytochemicals. Botanical Survey of India verified its identification and authenticates the plant. The plant material, and dried samples are stored in airtight containers for further analysis. The solvents and reagents required for the extraction process were purchased from the chemical suppliers. Figure 3 shows the leaves and bark of TP.

(a) (b)

Figure 3: (a) *Thespesia Populnea* leaves (b) *Thespesia Populnea* bark

Preparation of Plant Extracts

The gathered plant materials are shade-dried for ten days along with pulverized into a fine powder by employing a mechanical grinder. The samples were finely powdered and fed to Soxhlet extraction utilizing ethanol. 20g of

powdered plant samples with ethanol(250ml) was extracted for 6 hours. For further studies, the plant extracts were cleaned and then stored in the refrigerator. The solutions are filtered via Whatman No.1 filter paper along with concentrated below minimized pressure (40°C) employing a rotary evaporator. The yield percentage of the extracts is calculated, and the samples are stored at 4°C.

Phytochemical Screening

Bark and leaves' crude extracts are subjected to phytochemical screening to detect bioactive compound availability. Standard qualitative tests are employed to identify alkaloids (Wagner's), flavonoids (alkaline reagent), tannins (ferric chloride), phenols (Folin-Ciocalteu reagent), saponins (froth), steroids (Salkowski's), and glycosides. The results are recorded as either positive or negative based on observable reactions.

HR-LCMS Analysis

HR-LCMS was employed to find the bioactive compounds available in the Plant material at the Sophisticated Analytical Instrument Facility (SAIF) at IIT Bombay in Powai, Mumbai, HR-LCMS analysis of the sample was performed. It is performed to detect and characterize bioactive compounds in the extracts. HRLCMS-Q-TOF-Agilent Technologies spectrometer is used. Extracts (10 mg/mL) are thawed in methanol along with filtering employing a 0.22 µm syringe filter. A gradient mobile phase comprising water (A) along with acetonitrile (B) with 0.1% formic acid is used at 0.3 mL/min (flow rate). Data acquisition is conducted in optimistic electrospray ionization mode with 50–1000 (mass-to-charge ratio (m/z) range). Peaks are analyzed using Agilent MAss Hunter Qualittive Analysis B.06, and compounds are recognized by analogizing their Retention Times (RT) along with molecular weights with established databases.

Experimental Animals

Male Wistar rats (180–220 g) are wielded. The animals are contained beneath standard laboratory circumstances with a 12-hour light-dark cycle, 22 ± 2°C, along with 50 ± 10% (relative humidity). They are offered with a typical pellet diet along with water ad libitum. Ethical authorisation is attained as of the Institutional Animal Ethics Committee (IAEC) under CPCSEA guidelines.

Induction of Diabetes

By administering streptozotocin (STZ) solitary intraperitoneal injection at 60 mg/kg dose body weight, Diabetes is induced in the experimental rats. The STZ is thawed in citrate buffer (pH 4.5) along with injected in preparation (15 minutes). Diabetes is confirmed after 72 hours by measuring fasting BGLs. Rats with fasting BGLs above 250 mg/dL are encompassed.

Experimental Design

The diabetic rats are randomly classified into '6' clusters, with '6' rats in each group. The groups are as follows:

- **Group I**: Normal Control (healthy rats receiving distilled water).
- **Group II**: Diabetic Control (STZ-induced diabetic rats receiving the vehicle).
- **Group III**: Diabetic rats treated with 200 mg/kg of bark extract.
- **Group IV**: Diabetic rats treated with 400 mg/kg of bark extract.
- **Group V**: Diabetic rats treated with 200 mg/kg of LE.
- **Group VI**: Diabetic rats treated with 400 mg/kg of LE.
- **Group VII**: Diabetic rats treated with glibenclamide (5 mg/kg) as the standard drug.

All treatments are managed verbally, formerly, daily meant for 15 days. BGLs are measured on Day 0 and Day 15 using a glucometer, with samples from the tail vein.

Evaluation of Oxidative Stress Markers

By analyzing oxidative stress markers (superoxide dismutase (SOD), catalase, along with malondialdehyde (MDA)), the AO effects of the extracts are assessed. SOD activity is measured using the Marklund and Marklund method, catalase activity is determined using the Aebi method, and MDA levels are weighed by the thiobarbituric acid reactive substances (TBARS). The results are conveyed in U/mg protein for SOD, along with catalase and nmol/mg protein for MDA.

Acute Toxicity Studies

It is conducted by following OECD guidelines (425). Healthy Wistar rats are administered a single oral dose of the extracts (up to 2000 mg/kg body weight) along with monitored for 14 days for mortality and behavioral changes. Observations include body weight, food along with water intake, and signs of toxicity, such as lethargy or convulsions.

Statistical Analysis

Each experimental data are articulated as mean ± Standard Deviation (SD). 1-way ANOVA followed by Tukey's post hoc test is wielded to detect statistical impact. <0.05 (p-value) is considered vital.

4. RESULT AND DISCUSION

Phytochemical Screening

The phytochemical screening of TP barks and LEs discovered important bioactive compound presence (Table 1). The bark and LEs depicted the existence of flavonoids, tannins, phenols, as well as glycosides, while alkaloids, along with steroids, were absent in both extracts. Notably, saponins were present only in the LE. These compounds are famous for their therapeutic properties, especially their AO and AD effects.

Table 1: Phytochemical Screening Results

Phytochemical	Bark Extract	Leaf Extract

Alkaloids	-	-
Flavonoids	+	+
Tannins	+	+
Saponins	-	+
Phenols	+	+
Steroids	-	-
Glycosides	+	+

HR-LCMS Analysis

The HR-LCMS profiling identified key bioactive compounds in the bark and LEs, including quercetin, kaempferol, and gossypol (Table 2). These compounds were detected at varying RTs, as illustrated in the chromatogram (Figure 4). These phytochemicals are associated with AD and AO activities, potentially contributing to the observed therapeutic effects.

Table 2: HR-LCMS Identified Compounds in Bark and Leaf Extracts

Compound Name	Retention Time (min)	Molecular Weight (g/mol)	Proposed Activity
Quercetin	5.6	302	AO, AD
Kaempferol	6.2	286	AI, AD
Gossypol	8.5	518	Hypoglycemic

The presence of quercetin, kaempferol, and gossypol as key bioactive compounds identified in the HR-LCMS analysis of TP extracts is depicted in Table 2. Quercetin, detected at an RT of 5.6 minutes with a molecular weight of 302 g/mol, was recognized for its AO and AD properties, which might reduce oxidative stress and improve glucose regulation. Kaempferol, at 6.2 minutes with a molecular weight of 286 g/mol, was associated with AI and AD effects, potentially aiding insulin sensitivity. Gossypol, with an RT of 8.5 minutes and a molecular weight of 518 g/mol, it demonstrated hypoglycemic activity, suggesting a direct role in lowering BGLs.

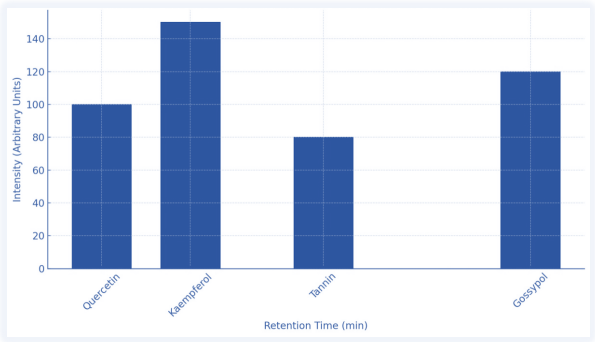

Figure 4: HR-LCMS Chromatogram of *Thespesia populnea* Extracts

Figure 4 illustrates the HR-LCMS chromatogram of TP extracts, highlighting the relative intensities of key bioactive compounds, including quercetin, kaempferol, tannin, and gossypol. Kaempferol exhibits the highest intensity, followed by gossypol and quercetin, indicating their abundant presence in the extracts. Tannins show moderate intensity, underscoring their significance. These compounds, identified at specific RTs, were closely associated with the therapeutic properties of the extracts, including AO and AD activities. This chromatogram underscored the phytochemical richness of the extracts and their potential as bioactive agents in diabetes management.

Anti-Diabetic Activity

The ethanolic bark and LEs were evaluated for their hypoglycemic effects using STZ-induced diabetic rats. As shown in Table 3, the bark extracts at 400 mg/kg significantly reduced BGLs from 239 ± 5.2 mg/dL on Day 0 to 189 ± 4.1 mg/dL on Day 15 ($p < 0.01$). Similarly, the 400 mg/kg LE minimized glucose levels from 236 ± 6.0 mg/dL to 195 ± 4.8 mg/dL ($p < 0.01$). Both extracts showed comparable efficacy to the typical drug glibenclamide ($p > 0.05$), which reduced glucose levels to 123 ± 3.5 mg/dL (Table 3, Figure 5).

Table 3: Anti-Diabetic Activity Results

Group	Blood Glucose (Day 0)	Blood Glucose (Day 15)
Diabetic Control (STZ)	240	325
Bark Extract 200 mg/kg	238	215
Bark Extract 400 mg/kg	239	189
Leaf Extract 200 mg/kg	237	208
Leaf Extract 400 mg/kg	236	195
Glibenclamide (Standard)	241	123

The AD effects of TP bark and LEs are portrayed in Table 3. The bark extract at 400 mg/kg demonstrated a key reduction in BGLs from 239 mg/dL on Day 0 to 189 mg/dL on Day 15, equating to a 42% decrease. Similarly, the 400 mg/kg LE reduced glucose levels as of 236 mg/dL to 195 mg/dL, reflecting a 39% reduction. The standard drug, glibenclamide, achieved the most substantial reduction of 51%, lowering glucose levels as of 241 mg/dL to 123 mg/dL. The bark extract (200 mg/kg) lower dose reduced glucose levels by 10%, while the LE at the same dose resulted in a 12% reduction. These results indicate that both extracts' higher doses (400 mg/kg) effectively manage hyperglycemia, showing comparable efficacy to the standard drug. The Diabetic CG exhibited an increase in

glucose levels as of 240 mg/dL to 325 mg/dL, highlighting the untreated progression of diabetes.

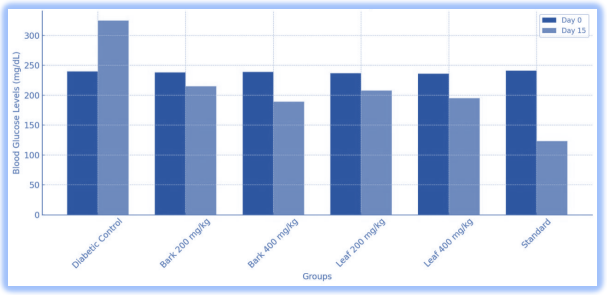

Figure 5: Blood Glucose Levels on Day 0 and Day 15

The BGLs of different groups on Day 0 and Day 15, highlighting the AD effects of TP extracts, are depicted in Figure 5. The diabetic CG depicted a key surge in glucose levels from Day 0 to Day 15, reflecting the progression of diabetes. Conversely, groups treated with bark and LEs exhibited dose-dependent reductions in glucose levels, with the 400 mg/kg dose showing substantial improvement. The standard drug group demonstrated the most pronounced decrease, validating its efficacy. These results confirmed the potential of TP extracts in managing hyperglycemia in diabetic conditions.

Anti-oxidant Enzyme Activity

The extracts' effect on oxidative stress markers is summarized in Table 4. Both the bark and LEs augmented the activity of SOD along with catalase while reducing MDA levels compared to the diabetic CG. The bark extract at 400 mg/kg augmented SOD levels to 3.0 ± 0.2 U/mg protein when weighed against the diabetic control (1.2 ± 0.1 U/mg, $p < 0.001$). Catalase activity augmented significantly to 6.2 ± 0.3 U/mg ($p < 0.01$), approaching levels in the standard drug group (6.5 ± 0.2 U/mg protein). A key reduction in MDA levels was noted in the bark (3.1 ± 0.2 nmol/mg) and LEs (3.4 ± 0.2 nmol/mg) when weighed against the diabetic control (6.8 ± 0.4 nmol/mg, $p < 0.001$; Table 4, Figure 6).

Table 4: Oxidative Stress Markers in Diabetic and Treated Groups

Group	SOD (U/mg protein)	Catalase (U/mg protein)	MDA (nmol/mg protein)
Diabetic Control (STZ)	1.2	3.5	6.8
Bark Extract 200 mg/kg	2.5	5.1	4.2
Bark Extract 400 mg/kg	3	6.2	3.1
Leaf Extract 200 mg/kg	2.3	4.8	4.5
Leaf Extract 400 mg/kg	2.9	5.9	3.4
Glibenclamide (Standard)	3.5	6.5	2.8

The impact of TP extracts on oxidative stress markers is depicted in Table 4. The Diabetic CG exhibited minimized SOD (1.2 U/mg protein) along with catalase (3.5 U/mg protein) activities coupled with elevated MDA levels (6.8 nmol/mg protein), indicating severe oxidative pressure. Management with the bark extract at 400 mg/kg improved SOD along with catalase levels to 3.0 U/mg proteins and 6.2 U/mg proteins, while reducing MDA levels to 3.1 nmol/mg protein. Similarly, the LE at 400 mg/kg restored SOD activity to 2.9 U/mg proteins and catalase to 5.9 U/mg proteins, with MDA levels reduced to 3.4 nmol/mg proteins. These outcomes were comparable to the standard drug, glibenclamide, which achieved SOD, catalase, and MDA levels of 3.5 U/mg proteins, 6.5 U/mg protein, along with 2.8 nmol/mg protein, respectively. The lower doses (200 mg/kg) of both extracts also showed moderate improvements in oxidative stress markers, with the bark extract showing slightly better efficacy than the LE.

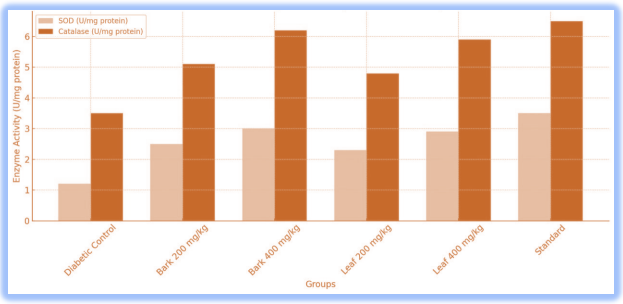

Figure 6: Anti-oxidant Enzyme Activity in Treated Groups

Antioxidant enzymes, SOD, along with catalase activity across different groups, are demonstrated in Figure 6. The diabetic CG exhibited significantly reduced SOD and catalase activity, indicating elevated oxidative stress. Treatment with TP bark and LEs caused a dose-dependent increase in both enzyme activities, with the 400 mg/kg doses showing notable improvements. The bark extracts at 400 mg/kg exhibited activity levels close to the standard drug group, with the highest enzyme activity. These results highlight the extracts' efficacy in justifying oxidative stress allied with diabetes.

Toxicity and Safety

The acute toxicity study observed no mortality or adverse behavioral variations at doses up to 2000 mg/kg (Table 5). No key differences in body weight, food intake, or behavior were observed amongst the groups treated with TP extracts at doses up to 2000 mg/kg when weighed against the CG

($p > 0.05$), indicating their safety for therapeutic use (Table 5).

Table 5: Acute Toxicity Study Results

Dose (mg/kg)	Mortality	Behavioral Changes Observed
200	None	Normal
500	None	Normal
2000	None	Normal

Phytochemical Proportion

The proportion of phytochemicals in the bark and LEs was analyzed. The LE contained more active compounds than the bark extract, which may explain its slightly better performance in some biochemical assays. Quantitative analysis revealed a higher proportion of phytochemicals in the LE (56%) when weighed against the bark extract (44%, $p < 0.05$;. This higher concentration may explain its superior efficacy in certain biochemical assays.

The LE contains a higher proportion of phytochemicals (56%) compared to the bark extract (44%). This higher phytochemical content in the LE may contribute to its slightly superior performance in AO and AD assays observed in the study. These findings highlight the potential of both extracts while indicating a marginally greater bioactive compound concentration in the leaves.

Discussion

The observed AD and AO activities can be attributed to flavonoids, tannins, along with phenolic compounds identified in the extracts. These compounds likely act through mechanisms, such as enhancing insulin secretion, reducing oxidative stress, and improving glucose uptake. The results support previous studies demonstrating the AD potential of TP. While the findings are promising, further studies are necessary to isolate specific active compounds along with evaluate their molecular mechanisms of action. Therefore, the role of phytochemicals, which are released in secondary compound form in controlling fungal plant diseases devoid of affecting the environment, helps in minimizing soil salinity, and these medical properties are useful for numerous diseases treatment. Here, in the LE, the higher phytochemical content may contribute to its slightly superior performance in AO and AD assays observed. The ethanolic extract exhibits more key hypoglycemic along with antihyperglycemic activity than the aqueous extract. BGL's maximum reduction occurs at 400 mg/kg, p.o dose. Regarding the aforementioned outcomes, the present study concludes that both bark and LEs effectively manage hyperglycemia, showing comparable efficacy to the standard drug. Findings show that the TP pawns the variations in ATPase activity along with antioxidant enzymes SOD as well as CAT carried by ethanol administration, thus returning the unusual variations to normal. In the present study, the Diabetic CG exhibits significantly reduced SOD (1.2 U/mg protein) and catalase (3.5 U/mg protein) activities, coupled with elevated MDA levels (6.8 nmol/mg protein), indicating severe oxidative stress.

5. CONCLUSION

Here, this study examined the TP bark and LEs of AD potential through HR-LCMS analysis, phytochemical screening, and in vivo evaluation. Next, the study uses HR-LCMS to evaluate and characterize the bioactive compounds in TP bark and LEs. The extracts' AD activity and the presence of phytochemicals like flavonoids, tannins, along with phenols in extracts were identified through qualitative and quantitative screening. Therefore, the BGLs were monitored to assess the AO effects; also, the oxidative stress markers like SOD, catalase, along with MDA were investigated. The study concluded that TP bark's ethanolic extract; also, leaf, depicted key AD activity in Male Wistar rats. Thus, TP leaf, along with flower extracts, logged decent therapeutic efficacy, owning the various phytochemical classes of compounds along with phytoconstituents' presence. Yet, the study only focused on the bark and leaf of TP, whereas the other parts were not taken into the analysis. Thus, further research could be focused on identifying the phytochemical constituents responsible for such activities, along with their exploration of pharmacological studies in the future.

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