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Phytochemical Characterization, Hypoglycemic Effect, and Preliminary Toxicological Evaluation of Methanolic and Aqueous Bark Extracts of *Lavandula stoechas* L.

Pratiksha Pandurang Tamundkar*, Kinza Khan and Pratyush Jain

RKDF College of Pharmacy, Bhopal-462046, M.P. India

Abstract

Background: Diabetes mellitus involves chronic hyperglycaemia; plants rich in phenolics, flavonoids, tannins, and terpenoids are explored for glucose-lowering potential. *Lavandula stoechas* (Ustukhuddus) contains diverse bioactives.
Objective: To prepare methanolic (LSME) and aqueous (LSAE) bark extracts, assess phytochemical profiles, glucose-lowering activity, and preliminary oral toxicity.
Methods: Bark powder was defatted and extracted with methanol/water. Extractive yields and phytochemicals were identified. Acute toxicity was tested in mice (500–2000 mg/kg). A 14-day study compared control vs. LSME (200, 400 mg/kg) and LSAE (250, 500 mg/kg). Biochemical and haematological parameters were measured.
Results: Yields: LSME 8.3%, LSAE 11.1%. LSME contained alkaloids, saponins, steroids, phenolics, tannins, flavonoids, terpenoids, glycosides, proteins, carbohydrates; LSAE had saponins, phenolics, tannins, flavonoids, terpenoids, proteins, carbohydrates. Acute toxicity showed dose-dependent behavioural toxicity and mortality (especially LSME). In the 14-day study, no mortality occurred. Serum glucose fell significantly (control 91.58 mg/dL → 60.30, 47.18, 48.28, 25.66 mg/dL in treated groups). Hepatic enzymes decreased at higher doses; protein, albumin, haemoglobin remained stable.
Conclusion: Both extracts showed strong glucose-lowering activity, likely due to phenolics, flavonoids, tannins, saponins, and terpenoids. However, acute toxicity indicates a narrow safety margin. Further studies in diabetic models with OECD-compliant toxicology and mechanistic evaluation are needed before therapeutic application.
Keywords: *Lavandula stoechas*; Ustukhuddus; diabetes mellitus; hypoglycaemic activity; phytochemical screening; methanolic extract; aqueous extract; oral toxicity.

***Correspondence Info:**
Ms. Pratiksha Pandurang Tamundkar
RKDF College of Pharmacy,
Bhopal-462046, M.P. India

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1. Introduction

Diabetes mellitus is a heterogeneous metabolic disorder characterized by chronic elevation of blood glucose caused by deficient insulin secretion, reduced tissue sensitivity to insulin, or a combination of these abnormalities. Persistent hyperglycaemia is associated with progressive damage to the cardiovascular system, kidneys, liver, eyes, and peripheral nerves [1–3]. The increasing prevalence of diabetes and its long-term complications has created a substantial burden on healthcare systems.

Although several synthetic oral antidiabetic agents are available, prolonged pharmacotherapy may be associated with adverse effects, drug interactions, secondary failure, treatment cost, and poor patient adherence. These limitations have encouraged the systematic investigation of medicinal plants traditionally used for the management of diabetes [4,5]. Plant-derived compounds may reduce blood glucose by stimulating insulin release, enhancing peripheral glucose utilization, protecting pancreatic β-cells, inhibiting intestinal glucose absorption, or reducing oxidative stress.

Lavandula stoechas L. belongs to the family Lamiaceae and is commonly known as French lavender or Ustukhuddus. The plant is traditionally used in Unani medicine and is reported to possess sedative, anticonvulsant, antispasmodic, antimicrobial, antioxidant, and other pharmacological properties. Its reported constituents include carbohydrates, glycosides, phenols, steroids, terpenes, resins, β-sitosterol, ursolic acid, apigenin, luteolin, rosmarinic acid, fenchone, camphor, eucalyptol, and myrtenol.

Phenolic compounds, flavonoids, tannins, triterpenoids, and saponins have frequently been associated with antioxidant and glucose-regulating activities. These compounds may protect tissues from oxidative injury and influence enzymes and receptors involved in carbohydrate metabolism [6–10]. Nevertheless, the biological activity and safety of a plant extract depend on the plant part, extraction solvent, dose, chemical composition, and duration of administration.

Therefore, the present investigation was undertaken to prepare methanolic and aqueous bark extracts of *L. stoechas*, characterize their preliminary phytochemical composition, evaluate their serum glucose-lowering activity, and assess their acute and 14-day oral toxicological profiles in experimental animals.

2. Materials and Methods

2.1 Drugs, chemicals, and instruments

Alloxan monohydrate, streptozotocin, glibenclamide, petroleum ether, methanol, gum acacia, and analytical-grade reagents were used during the study. Commercial diagnostic kits were used for the estimation of glucose, triglycerides, total cholesterol, total protein, albumin, bilirubin, creatinine, urea, alkaline phosphatase, SGOT, SGPT, HDL, and LDL. Major instruments included a UV-visible spectrophotometer, haemoglobinometer, haemocytometer, centrifuge, and blood glucose monitoring system.

2.2 Collection and authentication of plant material

The bark of *L. stoechas* was collected during March from Bhopal, Madhya Pradesh, India. The plant material was identified and authenticated by a qualified botanist, and a voucher specimen was deposited in the institutional herbarium. The bark was washed, shade-dried, pulverized into moderately coarse powder, and stored in an airtight container until extraction. The uploaded experimental chapter describes the collection, authentication, Soxhlet extraction, animal conditions, and ethical approval.

2.3 Preparation of extracts

Approximately 1000 g of powdered bark was packed in a Soxhlet apparatus and defatted with petroleum ether at 40–60°C for approximately nine hours. Complete defatting was confirmed by placing a drop of the solvent from the thimble on filter paper and observing the absence of an oily spot.

The defatted material was air-dried to remove residual petroleum ether and subsequently extracted separately using methanol and water. The liquid extracts were concentrated by distillation, and the remaining solvent was removed under reduced pressure. Each extract was dried to constant weight, and percentage yield was calculated as:

$$\text{Percentage yield} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100$$

2.4 Preliminary phytochemical screening

The methanolic and aqueous extracts were screened for major classes of secondary metabolites using standard qualitative procedures:

- Alkaloids: Dragendorff's, Mayer's, Hager's, and Wagner's tests
- Flavonoids: Shinoda, alkaline reagent, lead acetate, and ferric chloride tests
- Tannins and phenolics: ferric chloride, gelatin, and lead acetate tests
- Steroids and triterpenoids: Salkowski and Liebermann–Burchard reactions
- Saponins: foam and haemolysis tests
- Glycosides: general glycoside, Keller–Killiani, Legal's, and Baljet's tests
- Carbohydrates: Molisch's, Fehling's, Benedict's, and Barfoed's tests
- Proteins and amino acids: Biuret, Millon's, xanthoproteic, and ninhydrin tests

2.5 Experimental animals and ethical considerations

Swiss albino mice of either sex were maintained in clean polypropylene cages under controlled environmental conditions, with a 12-hour light–dark cycle and free access to standard feed and water. Male and female animals were housed separately. Experimental procedures were conducted according to CPCSEA guidelines after approval from the Institutional Animal Ethics Committee under approval number **279/TALP/2025**.

2.6 Acute oral toxicity study

The acute toxicity of LSME and LSAE was evaluated separately. For each extract, mice were divided into five groups containing two animals per group:

- Group I: 2% gum acacia, normal control
- Group II: extract 500 mg/kg
- Group III: extract 1000 mg/kg
- Group IV: extract 1500 mg/kg
- Group V: extract 2000 mg/kg

The extracts were administered orally as a single dose. Animals were observed continuously during the initial four hours, intermittently for the following six hours, and subsequently at 24, 48, and 72 hours. Grooming, locomotor activity, sedation, convulsions, respiratory abnormalities, motor activity, and mortality were recorded.

2.7 Fourteen-day oral study

Thirty Swiss albino mice were divided into five groups of six animals:

Table 1: Animal Groups

Group	Treatment
I	Normal control, 2% gum acacia
II	LSME, 200 mg/kg orally
III	LSME, 400 mg/kg orally
IV	LSAE, 250 mg/kg orally
V	LSAE, 500 mg/kg orally

Treatments were administered orally once daily for 14 days. Animals were monitored for mortality and general behavioural changes. On day 15, overnight-fasted animals were subjected to blood collection for biochemical and haematological investigations.

2.8 Biochemical and haematological parameters

Serum was separated by centrifugation and used for the determination of:

- Serum glucose
- Urea and creatinine
- Bilirubin
- SGOT and SGPT
- Total protein and albumin
- Alkaline phosphatase
- Haemoglobin
- Total white blood cell count

2.9 Statistical analysis

Results were expressed as mean ± standard error of mean. Differences between treatment groups and the control group were analysed using analysis of variance with SPSS version 20. Values of $p<0.05$ were considered significant, while $p<0.01$ indicated high statistical significance.

3. Results and Discussion

3.1 Extractive yield and physical characteristics

Both extracts were dark brown and possessed a characteristic odour. The aqueous extract produced a higher yield than the methanolic extract.

Table 2: Percentage yield of extract

Extract	Weight of bark	Weight of extract	Percentage yield	Appearance
LSME	1000 g	83 g	8.3%	Dark brown, characteristic odour
LSAE	1000 g	111 g	11.1%	Dark brown, characteristic odour

The higher aqueous extractive yield suggests that the bark contained a comparatively greater proportion of water-soluble polar constituents. The difference in yield may also reflect variations in solvent polarity and the solubility of carbohydrates, tannins, saponins, phenolics, and other hydrophilic components.

3.2 Phytochemical profile

The methanolic extract contained a wider variety of phytochemical classes than the aqueous extract.

Table 3: Phytochemical profile

Phytoconstituent	LSME	LSAE
Alkaloids	++	–
Saponins	+	++
Steroids	+	–
Phenolic compounds	+	+
Tannins	+++	++
Flavonoids	+	++
Terpenoids	++	+
Glycosides	+	–
Proteins and amino acids	+	+
Carbohydrates	+	+

+++ highly present; ++ moderately present; + faintly present; – absent.

The strong occurrence of tannins in LSME and the moderate presence of flavonoids and saponins in LSAE may be pharmacologically important. Phenolics and flavonoids can donate hydrogen atoms or electrons and may reduce oxidative stress associated with disturbances in glucose metabolism. Saponins and terpenoids have also been reported to influence glucose uptake, intestinal carbohydrate digestion, and insulin signalling. Nevertheless, the qualitative tests indicate only the presence or absence of constituent classes and do not establish their concentrations or specific identities. The reported yields and phytochemical findings are taken directly from the results chapter.

3.3 Acute oral toxicity

LSME produced marked dose-related toxicity. At 500 mg/kg, sedation, respiratory abnormalities, convulsions, and reduced motor activity were reported in approximately 50% of animals, followed by 50% mortality at 72 hours. At 1000–2000 mg/kg, severe behavioural toxicity and 100% mortality were reported.

LSAE appeared comparatively less acutely toxic at the lower doses. No sedation was reported at 500 mg/kg during the initial observation period. At 1000 mg/kg, delayed sedation, respiratory abnormalities, convulsions, reduced motor activity, and mortality were observed in some animals. Severe toxicity and mortality occurred at 1500 and 2000 mg/kg.

These findings indicate that the methanolic extract had greater acute toxicity than the aqueous extract. Methanol may have extracted comparatively higher concentrations of alkaloids, steroids, glycosides, terpenoids, or other constituents capable of producing central nervous system, respiratory, or cardiovascular toxicity. However, the acute study included only two animals in each group; therefore, an accurate median lethal dose or no-observed-adverse-effect level cannot be established. The reported mortality and behavioural observations require confirmation through an OECD-compliant toxicity protocol.

3.4 Effect of repeated administration on biochemical and haematological parameters

No mortality was reported during daily administration of LSME at 200 and 400 mg/kg or LSAE at 250 and 500 mg/kg for 14 days.

Table 4: Effect of repeated administration on biochemical and haematological parameters

Parameter	Control	LSME 200 mg/kg	LSME 400 mg/kg	LSAE 250 mg/kg	LSAE 500 mg/kg
Glucose, mg/dL	91.58 ± 2.76	60.30 ± 3.99*	47.18 ± 2.36**	48.28 ± 1.43*	25.66 ± 2.08**
Urea, mg/dL	16.74 ± 1.28	21.17 ± 1.02*	22.64 ± 1.44**	15.78 ± 1.03 NS	16.02 ± 0.76 NS
Creatinine, mg/dL	0.83 ± 0.25	0.52 ± 0.05 NS	0.65 ± 0.08 NS	1.04 ± 0.18**	0.79 ± 0.17 NS
Bilirubin, mg/dL	0.58 ± 0.14	0.52 ± 0.04 NS	0.42 ± 0.06 NS	0.58 ± 0.05 NS	0.36 ± 0.03*
SGOT, IU/L	60.03 ± 1.43	57.88 ± 2.60 NS	52.67 ± 2.15*	60.91 ± 3.17 NS	52.67 ± 2.15*
SGPT, IU/L	46.24 ± 5.03	30.78 ± 2.16*	20.47 ± 1.69**	45.38 ± 3.82 NS	26.54 ± 2.11*
Total protein, g/dL	6.81 ± 0.08	6.49 ± 0.45 NS	6.93 ± 0.31 NS	6.85 ± 0.18 NS	6.97 ± 0.22 NS
Albumin, g/dL	3.45 ± 0.26	3.64 ± 0.34 NS	4.00 ± 0.27 NS	3.61 ± 0.33 NS	3.74 ± 0.21 NS
ALP, U/L	72.16 ± 4.53	70.38 ± 5.48 NS	60.47 ± 8.37**	82.86 ± 7.40*	60.47 ± 8.37**
Haemoglobin, g/dL	7.40 ± 0.21	7.36 ± 0.23 NS	7.53 ± 0.32 NS	8.03 ± 0.32 NS	8.16 ± 0.22 NS
WBC count, cells/mm³	1900 ± 355.90	1866.66 ± 251.21*	1500 ± 61.72**	1700 ± 85.63*	1566.66 ± 140.63**

Values are mean ± SEM, n=6.

* p<0.05; ** p<0.01 compared with the normal control; NS: not significant.

3.4.1 Serum glucose

Both extracts produced marked dose-dependent reductions in serum glucose. Compared with the control group, glucose was reduced by approximately:

- 34.2% with LSME 200 mg/kg
- 48.5% with LSME 400 mg/kg
- 47.3% with LSAE 250 mg/kg
- 72.0% with LSAE 500 mg/kg

The greatest effect was observed with LSAE at 500 mg/kg. The activity may be related to the combined presence of saponins, flavonoids, phenolics, tannins, and terpenoids. Possible mechanisms include enhanced peripheral glucose utilization, reduced intestinal glucose uptake, inhibition of carbohydrate-hydrolysing enzymes, increased insulin secretion, or protection against oxidative injury.

However, glucose was measured in animals not described as diabetic in the reported 14-day results. Therefore, the findings demonstrate a hypoglycaemic effect, but do not independently prove antidiabetic efficacy. Excessive glucose lowering, particularly at 500 mg/kg LSAE, could also represent a safety concern.

3.4.2 Renal parameters

LSME produced significant increases in urea at both tested doses, suggesting that the methanolic extract may affect nitrogen metabolism, renal handling of urea, protein catabolism, or hydration status. Serum creatinine was not significantly altered by LSME.

LSAE at 250 mg/kg produced a significant elevation in creatinine, whereas the value at 500 mg/kg was close to the control. This non-linear response requires confirmation. Renal histopathology, electrolyte estimation, urine analysis, and longer-duration studies would be necessary to determine whether the observed changes represent renal toxicity.

3.4.3 Hepatic parameters

Higher doses of both extracts reduced SGOT, SGPT, and alkaline phosphatase. Total protein and albumin were not significantly altered, indicating that hepatic synthetic function was not appreciably affected during the 14-day period. The reduction in transaminases could be related to antioxidant or membrane-stabilizing effects of phenolics and flavonoids.

Nevertheless, enzyme reductions alone cannot establish hepatoprotection. Liver weight, histopathology, oxidative-stress markers, and comparison with a hepatotoxic control would be required.

3.4.4 Haematological findings

Haemoglobin values remained statistically unchanged, suggesting that neither extract produced overt anaemia during the study. In contrast, total WBC counts decreased in all treated groups, with larger reductions at

higher doses. This finding may indicate an immunomodulatory action, altered leukocyte production, or experimental variability. A complete differential leukocyte count and bone-marrow evaluation should be included in future safety studies.

The complete biochemical findings and graphical results were reported in the uploaded results chapter and table index.

4. Conclusion

The present study demonstrated that the bark of *Lavandula stoechas* contains several potentially bioactive phytochemical classes. The aqueous extract produced a higher percentage yield, whereas the methanolic extract contained a broader spectrum of phytoconstituents.

Both extracts significantly reduced serum glucose after repeated administration, with the most pronounced response observed with LSAE at 500 mg/kg. Higher doses also influenced hepatic enzymes, while serum proteins, albumin, and haemoglobin remained comparatively stable.

Despite these potentially beneficial effects, severe acute toxicity and mortality were reported, particularly with the methanolic extract. Changes in urea, creatinine, alkaline phosphatase, and WBC counts further indicate that safety cannot be assumed. Therefore, *L. stoechas* bark extracts should not be considered therapeutically established solely on the basis of the present findings.

Future studies should include chemical standardization, quantitative estimation of phenolics and flavonoids, identification of marker compounds, OECD-guided acute and repeated-dose toxicity, histopathological examination, and properly reported alloxan- or streptozotocin-induced diabetic models. Isolation of the active constituents and investigation of their molecular mechanisms are also required. The uploaded conclusion similarly recommends isolation of the responsible compounds and clarification of their antidiabetic mechanism.

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