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Multitargeted Polyherbal Formulation for Diabetes-Associated Depression: Physicochemical Characterization and In-Vitro Safety Assessment

Amrut Arun Swami[1], S. Shruti[2], Gayathridevi S[3], Perbhat Kansal[4], Anand Bhide[5*]

[1]Department of Community Medicine, Dr S. S. Tantia Medical College, Hospital and Research Centre, Sri Ganganagar, Rajasthan, India

[2]Rasa Shastra and Bhaishajya Kalpana Department, Sri Ganganagar College of Ayurvedic Science and Hospital, Sri Ganganagar, Rajasthan, India

[3]Senior scientific officer, Chemistry section, Regional Forensic Science Laboratory, Mysuru, Karnataka, India

[4]Department of Pharmacology, Dr S. S. Tantia Medical College, Hospital and Research Centre, Sri Ganganagar, Rajasthan, India

[5*]Department of Community Medicine, MIMER Medical College, Talegaon, Pune, Maharashtra, India

***Corresponding Author E-mail: dranandrbhide@gmail.com**

Abstract

Diabetes mellitus and depression are closely associated chronic disorders that together impose a substantial burden on individual health and healthcare systems, particularly in developing countries. The bidirectional relationship between metabolic dysregulation and neuropsychological stress indicates that there are therapeutic strategies that can address both conditions simultaneously. Polyherbal formulations are increasingly explored as alternative interventions due to their multi-targeted mechanisms, synergistic pharmacological effects, and comparatively lower risk of adverse reactions than synthetic drugs. In the present study, a polyherbal extract (PHE) was formulated using *Aegle marmelos*, *Prosopis cineraria*, and *Linum usitatissimum*, plants traditionally recognised for their antidiabetic, antioxidant, and neuroprotective properties. Extraction was carried out using Soxhlet extraction with ethanol at 50°C, along with mucilage preparation techniques. The formulated extract was evaluated for physicochemical parameters, including moisture content, ash values, and foreign matter, followed by qualitative phytochemical screening to identify major bioactive constituents. Cytotoxicity and preliminary safety were assessed using the MTT assay on cultured cells. Phytochemical analysis confirmed the presence of flavonoids, alkaloids, terpenoids, tannins, steroids, carbohydrates, and glycosides, indicating a diverse bioactive profile. Physicochemical evaluation demonstrated acceptable stability, with moisture content ranging from 7.49 to 8.67%, ash values between 10.53 and 12.23%, and foreign matter below 1.1%. The MTT assay revealed an IC_{50} value of approximately 212 µg/ml, with more than 80% cell viability observed at 100 µg/ml, suggesting low cytotoxicity at therapeutically relevant concentrations. Overall, the findings indicate that the formulated polyherbal extract possesses favourable stability, safety, and phytochemical characteristics, supporting its potential application in the management of diabetes-associated depression. Further in vivo studies and bioanalytical investigations are necessary to validate its therapeutic efficacy and elucidate underlying mechanisms of action.

Keywords: Diabetes, Depression, Polyherbal Extract, *Aegle marmelos*, *Prosopis cineraria*, *Linum usitatissimum*.

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1. Introduction

Diabetes mellitus (DM) is a chronic, irreversible disorder characterised by impaired insulin production or utilisation, which results in elevated blood glucose levels and excessive urinary excretion. According to the International Diabetes Federation (IDF), the global prevalence of DM reached approximately 537 million in 2021, with projections indicating an alarming increase to 783 million by 2045 [1–2].

Depression, often referred to as major depressive disorder (MDD), is a leading cause of global morbidity, affecting an estimated 264 million individuals worldwide. It is marked by persistent sadness, a lack of interest in activities, and cognitive and behavioural disturbances. The World Health Organization (WHO) reports approximately 800,000 suicide deaths annually, underscoring the significant public health burden of MDD [3-4].

The frequent co-occurrence of DM and depression worsens disease outcomes, making their integrated management a pressing clinical challenge. Nature has long been recognised as a source of pharmacologically active compounds, with phytotherapy offering promising alternatives to synthetic medications. Numerous plants exhibit antidiabetic and antidepressant properties, but single-herb treatments often provide limited efficacy [5-6].

Polyherbalism, combining multiple herbs in a single formulation, enhances therapeutic potential through pharmacodynamic and pharmacokinetic synergism. Such formulations can simultaneously target multiple biological pathways, improving efficacy, minimising adverse effects, and providing holistic disease management [7-8].

In this context, a polyherbal extract (PHE) comprising *Aegle marmelos*, *Prosopis cineraria*, and *Linum usitatissimum* was developed for managing diabetes-associated depression. These herbs were selected for their well-documented pharmacological profiles: *Aegle marmelos* and *Prosopis cineraria* are rich in phenolic compounds like protocatechuic acid, chlorogenic acid, caffeic acid, ferulic acid, β-sitosterol, rutin, and gallic acid, which exhibit antidiabetic, antioxidant, and

neuroprotective effects. *Linum usitatissimum* contributes omega-3 fatty acids, including alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), which regulate glucose and lipid metabolism, reduce insulin resistance, and alleviate depressive symptoms [9-10].

This combination is hypothesised to provide multitargeted therapeutic benefits, addressing the complex pathophysiology of diabetes and depression concurrently. The present study focuses on the formulation, physicochemical evaluation, phytochemical profiling, and in vitro cytotoxicity assessment of this PHE.

2. Materials and Methods

2.1. Selection and Collection of Raw Materials

Polyherbal extract (PHE) was prepared using leaves of *Aegle marmelos* and *Prosopis cineraria* and mucilage of *Linum usitatissimum* (flaxseed), chosen for their reported antidiabetic and antidepressant properties. while *Linum usitatissimum* seeds were purchased from the local market. The leaves were shade-dried at room temperature for 15 days, pulverised, and stored in airtight containers. All plant materials were authenticated by the Department of Botany.

2.2.Preparation of Polyherbal Extract

Soxhlet Extraction (*Aegle marmelos* and *Prosopis cineraria*)

Powdered leaves of *Aegle marmelos* and *Prosopis cineraria* (80 g each) were placed in a cellulose thimble and extracted with 450–500 ml of ethanol using a Soxhlet apparatus at 50°C. The extraction was continued for approximately 6 hours (four complete cycles) or until the syphon solvent turned colourless. Solvent flow was maintained at ~2–3 ml/min to ensure optimal extraction. The resulting extract was concentrated under reduced pressure to one-fourth of its original volume using a rotary evaporator at 60°C.

2.3.Preparation of *Linum usitatissimum* Mucilage

Flaxseed mucilage was prepared by soaking *Linum usitatissimum* seeds in distilled water at a ratio of 7:1 (ml/g) at 40–45°C for 90 minutes. The mucilage was filtered through a 200-mesh cheesecloth and stored at 4°C for use in the formulation.

2.4.Physicochemical Evaluation

Foreign Matter

A 250 g sample was visually inspected (with 6X–10X magnification) for extraneous matter, including insect parts or non-plant materials. The percentage of foreign matter was calculated as:

$$\% \text{ Foreign Matter} = (\text{Weight of foreign matter} / \text{Total sample weight}) \times 100$$

2.5.Moisture Content (Loss on Drying)

Moisture content was determined using a gravimetric method. Two grams (W) of PHE were placed in a pre-weighed porcelain dish (W_1) and dried in a hot-air oven at 105°C until a constant weight (W_2) was obtained. Moisture percentage was calculated as:

$$\% \text{ Loss on Drying} = ((W_1 - W_2) / W) \times 100$$

2.6.Ash Content

Ash values were determined to assess inorganic impurities.

Total Ash: Three grams of air-dried extract were incinerated in a silica crucible at increasing temperatures in a muffle furnace until a carbon-free white residue was obtained. Total ash was calculated as

$$\% \text{ Total Ash} = ((W_3 - W_1) / (W_2 - W_1)) \times 100$$

Acid-insoluble Ash: The total ash was boiled with 25 ml dilute HCl for 5 minutes, filtered, rinsed with hot water, and the residue incinerated to constant weight (W_4).

$$\% \text{ Acid-insoluble Ash} = ((W_4 - W_1) / (W_2 - W_1)) \times 100$$

Water-soluble Ash: Total ash was boiled with 25 ml distilled water, filtered, dried, and ignited to constant weight.

$$\% \text{ Water-soluble Ash} = ((W_7 - W_6) / \text{Sample weight}) \times 100$$

Extractive Values

Water-Soluble Extractive: Five grams of powdered sample were macerated with 100 ml of chloroform water for 18 hours, filtered, and 25 ml of the filtrate evaporated in a petri dish at 105°C to constant weight.

Alcohol-Soluble Extractive: Two grams of sample were macerated in 100 ml ethanol for 6 hours (with intermittent shaking) and left to stand for 18 hours. Ten millilitres of filtrate were evaporated and dried at 105°C to constant weight. Extractive values were expressed as a percentage w/w.

2.7.Phytochemical Evaluation

Qualitative phytochemical screening was conducted using standard tests:

- **Alkaloids:** Mayer's, Hager's, and Dragendorff's tests
- **Glycosides:** Legal's, Bontrager's, Foam test
- **Flavonoids:** Sulphuric acid test
- **Tannins:** Nitric acid test
- **Proteins & Amino Acids:** Millon's and Ninhydrin Tests
- **Steroids:** Salkowski test
- **Carbohydrates:** Molisch's, Fehling's, and Benedict's tests

2.8.Cytotoxicity Evaluation (MTT Assay)

Cell viability was assessed using the MTT assay. Cultured cells were seeded in 96-well plates (1×10^4 cells/well) and incubated for 24 hours. Cells were treated with different PHE concentrations and incubated for another 24 hours. Subsequently, 20 µl of MTT solution (5 mg/ml) was added to each well and incubated for 4 hours. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell viability was expressed as a percentage relative to the untreated control.

All experiments were conducted in triplicate ($n = 3$). Statistical analysis was performed using one-way ANOVA in GraphPad Prism 8.0, with $p < 0.05$ considered significant.

3. RESULT AND DISCUSSION

3.1. Physicochemical Analysis of the Polyherbal Extract (PHE)

The physicochemical parameters for PHE are summarised in Table 1. The total ash content of the extract ranged from 10.53 ± 0.21 % to 12.23 ± 0.29 %, reflecting a low level of inorganic matter such as silicates, carbonates, and phosphates (Figure 1). Water-soluble ash and acid-insoluble ash values were within the pharmacopeial limits for the individual plants, consistent with previous reports for *Aegle marmelos*, *Prosopis cineraria*, and *Linum usitatissimum* [11-12].

Moisture content was measured between 7.49± 0.28% and 8.67± 0.31%, remaining well below the pharmacopeial threshold of 15%, thereby confirming the satisfactory stability of the extract (Figure 2). Foreign matter content was low (< 1.1%), indicating minimal contamination and high sample purity (Figure 3).

Extractive values varied significantly between water-soluble and alcohol-soluble fractions ($p < 0.05$), with higher yields in the water-soluble fraction, which may reflect a greater abundance of polar phytoconstituents (Figure 4). Such values are consistent with extracts rich in flavonoids, phenolic acids, and glycosides, corroborating the results of the qualitative phytochemical screening.

3.2. Cytotoxicity (MTT Assay)

The cytotoxic potential of the PHE was assessed using the MTT assay. The extract exhibited > 80% cell viability at concentrations up to 200 µg/ml, with an IC_{50} of ~212 µg/ml (Figures 5 and 8). These results indicate low cytotoxicity at therapeutic levels**,** aligning with previously reported cytotoxicity data for *Prosopis cineraria* and *Aegle marmelos* extracts [13-14]. This safety profile supports the suitability of the PHE for further in vitro and in vivo investigations.

3.3. Mechanistic Insights

The therapeutic potential of the PHE can be attributed to the synergistic pharmacodynamics of its bioactive compounds. Flavonoids and phenolic acids improve glucose metabolism, attenuate oxidative stress, and modulate serotonergic/dopaminergic neurotransmission, while omega-3 fatty acids regulate lipid/glucose metabolism and reduce insulin resistance. These combined actions are hypothesized to mitigate the complex pathophysiology of diabetes-associated depression.

3.4. Limitations and Future Directions

Although the study provides valuable insights into the in vitro stability, safety, and phytochemical profiles of PHE, it lacks in vivo behavioural and biochemical validation**.** Future research should focus on preclinical diabetic-depression models**,** bioanalytical profiling of active compounds**,** and evaluation of pharmacokinetics to confirm its translational potential.

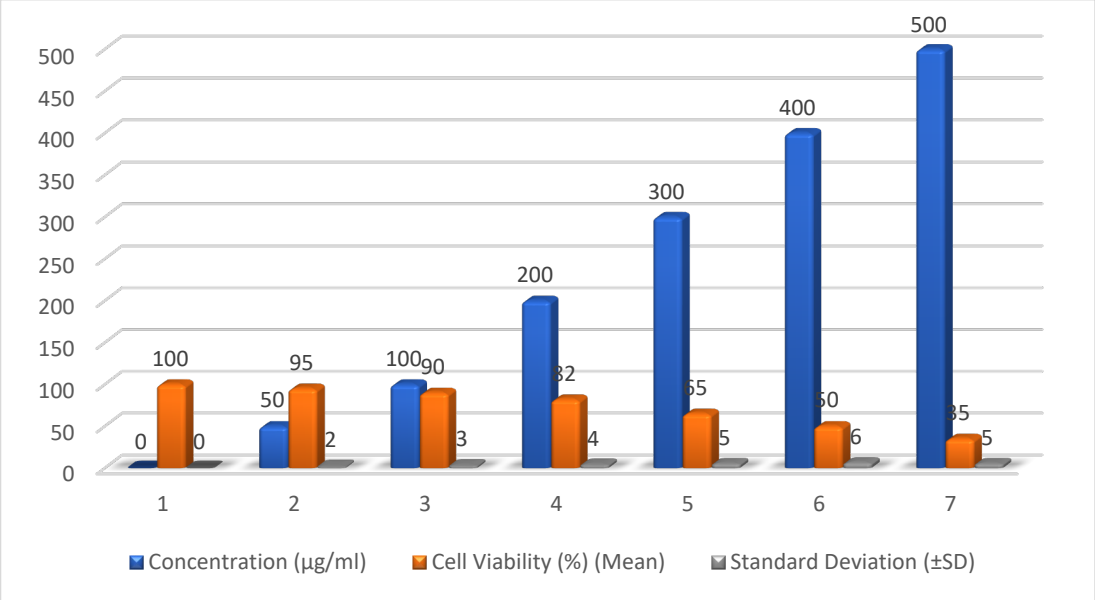

Figure 5: MTT assay results showing the percentage of viable cells after treatment with different concentrations of the polyherbal extract. Values are expressed as mean ± SD (n = 3).

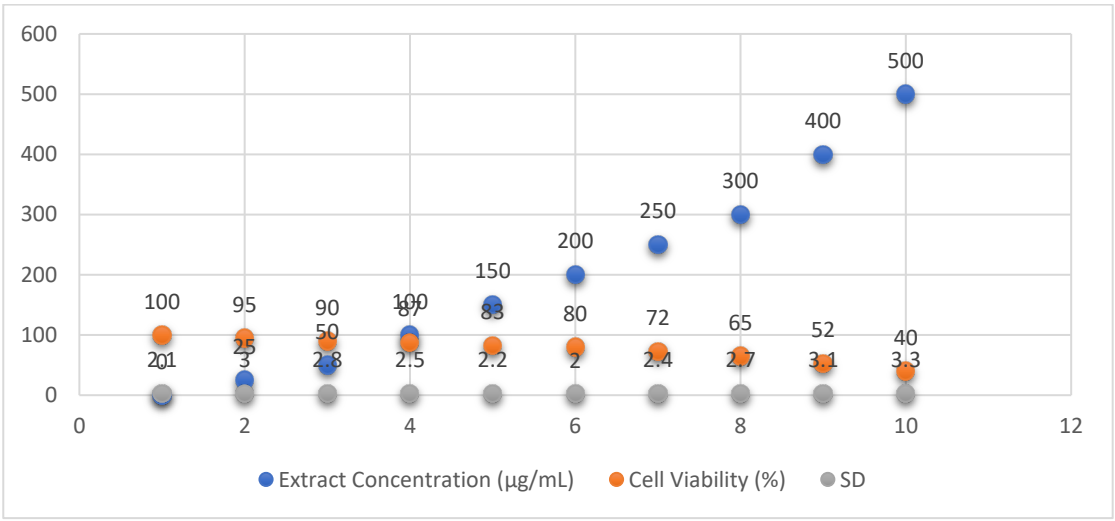

Figure 6. Dose-response curve of the polyherbal extract in the MTT assay. Cell viability (%) is plotted against extract concentrations (µg/mL). Data are mean ± SD (n = 3). The IC_{50} (~212 µg/mL) is highlighted.

Table 1. Physicochemical Parameters of Polyherbal Extract (PHE) (Mean ± SD, n = 3)

Parameter	*Aegle marmelos*	*Prosopis cineraria*	*Linum usitatissimum*	p-value (ANOVA)
Ash value (% (w/w)				
Total ash	10.53 ± 0.21	11.70 ± 0.34	12.23 ± 0.29	0.031

Water-soluble ash	2.34 ± 0.24	2.11 ± 0.05	2.23 ± 0.06	0.089
Acid-insoluble ash	3.34 ± 0.11	2.34 ± 0.08	3.43 ± 0.10	0.027
Moisture content (%)	7.49 ± 0.28	8.67 ± 0.31	7.87 ± 0.22	0.045
Foreign matter (% (w/w)	1.09 ± 0.12	1.10 ± 0.03	0.97 ± 0.02	0.064
Extractive value (% (w/w)				
Water-soluble extractive	25.57 ± 0.60	24.57 ± 0.93	14.55 ± 0.44	0.014
Alcohol-soluble extractive	12.23 ± 0.19	11.02 ± 0.07	8.02 ± 0.03	0.018

*Values are expressed as mean ± SD (n = 3). Statistical significance was assessed by one-way ANOVA; *p < 0.05 was considered significant.

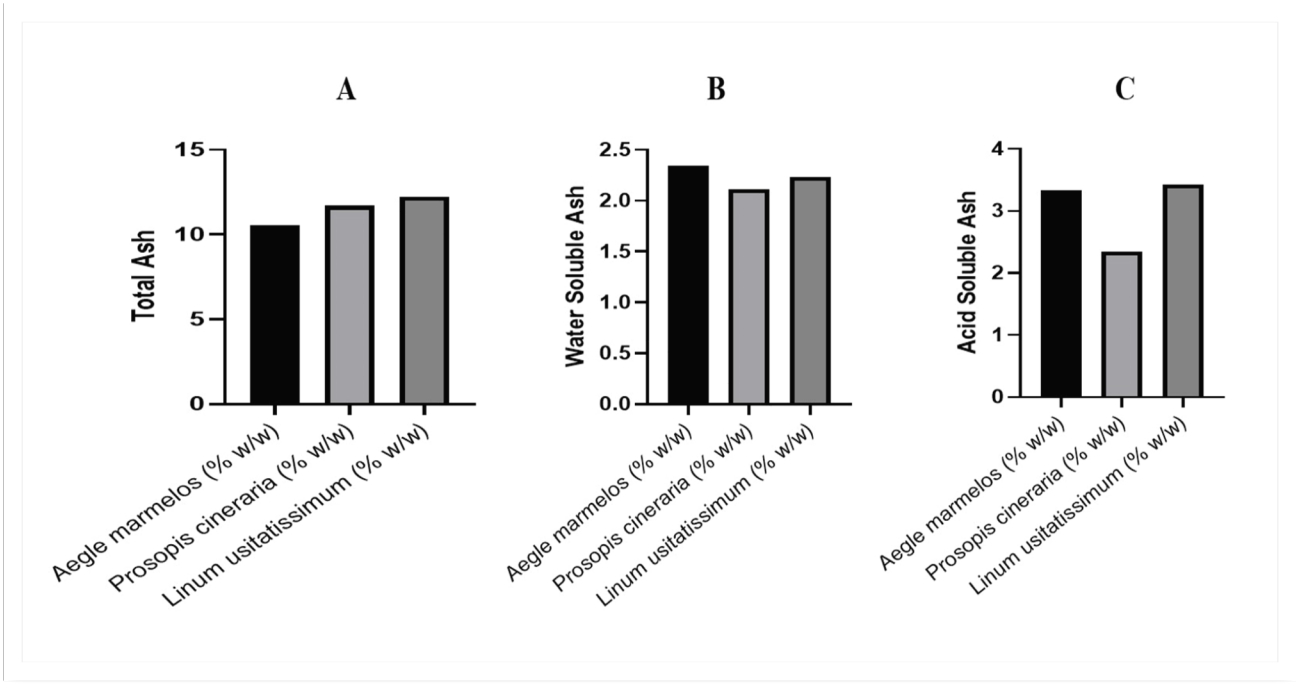

Figure 1: Ash value of individual herbs is expressed as % w/w; A-Total Ash; B- Water Soluble Ash; C- Acid Soluble Ash

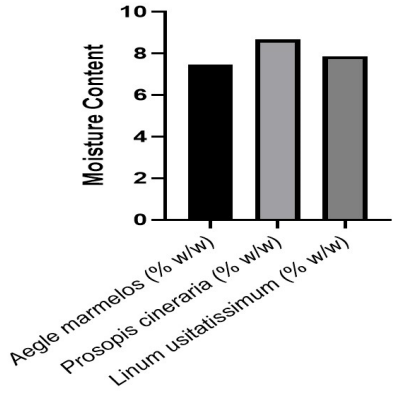

Figure 2: Moisture content of individual herbs is expressed as % w/w.

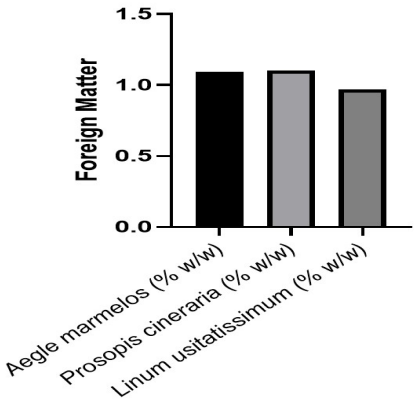

Figure 3: Foreign Matter Content of individual herbs is expressed as % w/w.

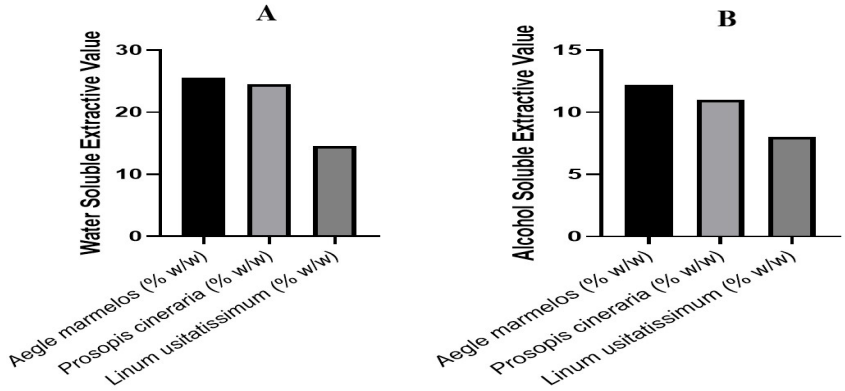

Figure 4: Extractive value of individual herbs is expressed as % w/w; A- Water-Soluble Extractive Value; B- Alcohol-Soluble Extractive Value

4. Phytochemical Screening

The PHE demonstrated a diverse phytoconstituent profile, including alkaloids, glycosides, flavonoids, tannins, proteins, amino acids, steroids, and carbohydrates (Table 2). The high abundance of flavonoids (+++), phenolic acids, and omega-3 fatty acids (from *Linum usitatissimum*) supports its antidiabetic, antioxidant, and neuroprotective properties. These findings are consistent with existing evidence that polyphenols enhance insulin sensitivity and modulate neurotransmission pathways involved in depression [15-16].

Table 2. Semi-Quantitative Phytochemical Profile of Polyherbal Extract (PHE)

Phytochemical Group	Test Name	*Aegle marmelos*	*Prosopis cineraria*	*Linum usitatissimum*
Alkaloids	Mayer's / Hager's / Dragendorff's	++	+++	+
Cardiac Glycosides	Legal's Test	++	+	++
Saponin Glycosides	Foam Test	++	+	++
Anthraquinone Glycosides	Bontrager's Test	–	–	–
Flavonoids	Sulphuric Acid Test	+++	++	+++
Tannins	Nitric Acid Test	++	++	+
Proteins	Millon's Test	+	+	+
Amino Acids	Ninhydrin Test	++	++	+
Steroids	Salkowski Test	+	++	+
Carbohydrates	Molisch's / Fehling's / Benedict's	+++	++	+++

Legend:
– = Not detected;

- = Low abundance;
  ++ = Moderate abundance;
  +++ = High abundance.

5. CONCLUSION

This study successfully formulated and characterised a polyherbal extract (PHE) comprising *Aegle marmelos*, *Prosopis cineraria*, and *Linum usitatissimum* for potential application in managing diabetes-associated depression. The extract exhibited acceptable stability, with moisture and ash values within pharmacopeial limits, and contained diverse bioactive constituents including flavonoids, alkaloids, tannins, steroids, and glycosides, which are known for their antidiabetic, antioxidant, and neuroprotective properties. Cytotoxicity (MTT assay) confirmed >80 % cell viability at concentrations up to 200 µg/ml and an IC_{50} of ~212 µg/ml, suggesting a favorable safety profile at therapeutic doses.

Collectively, these findings support the PHE as a promising candidate for integrated management of diabetes and depression. Future work should include in vivo validation using diabetic-depression models, advanced bioanalytical profiling of active compounds, and mechanistic studies to elucidate its multitargeted pharmacological actions.

Ethics Approval
Not applicable.
Competing Interest
There is no conflict of interest.
Funding

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Availability of data and materials

Data and supportive information are available within the article.

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