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SCREENING OF SEEDS AND LEAVES OF *LEUCAENA LEUCOCEPHALA* FOR TREATMENT OF DIABETES

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ABSTRACT

The diabetes is increasing at alarming rate. *Leucaena Leucocephala* (Family: Fabaceae) has often been used as traditional medicine, especially the leaves and seeds. It is observed that plant parts were able to show antidiabetic potency.

KEYWORDS: *Leucaena Leucocephala,* antidiabetic, leaves, seeds.

INTRODUCTION

The diabetes is increasing at alarming rate. *Leucaena Leucocephala* (Family: Fabaceae) has often been used as traditional medicine, especially the leaves and seeds. These have also traditional importance among the tribal people of Tripura and Manipur, India, to use sometime as anthelmintic, antidiabetic, anticancer agent. Considering its traditional use, present study was attempted to know the antidiabetic potency (in-vitro) of seeds and leaves of *Leucaena Leucocephala* grown in state Tripura, India, along with the phytoconstituents. Humans in Thailand, Indonesia, and Central America eat leucaena. Both processed and untreated varieties are edible. The fermented seeds are consumed in Java as bean cakes or sprouts. The immature pods are prepared like a vegetable in the Philippine Islands, and the young, dry seeds are then popped like popcorn. Young leaves, blossoms, and pods are often consumed in soups and salads in Indonesia, Thailand, Mexico, and Central America. Along with other leguminous, seeds are being regarded as unconventional sources of protein. *Leucaena leucocephala* leaves and seeds have many

medical uses, including as an abortifacient, a contraceptive, and a way to relieve stomach aches. Leaves particularly showed the presence of 30 compounds including mimosine.[1-7]

MATERIALS AND METHODS

Chemicals and reagents were used of analytical grade. Instruments were utilized : Electronic balance (Model No: M300 DR, Ahmed Instruments(p) Ltd), Magnetic stirrer, Heating mantle, Water bath, Micropipette, Digital pH meter (Model No: Digital pH Meter MINI 2 POINT CALIBRATION LJ-141), Incubator, Hot air oven, Flash chromatography (Model No: Combi Flash Next Gen 100).

Pharmacognostical study

The medicinal plants can be identified on the basis of their morphological and physiochemical parameters studies. Thus the macroscopical character (size, shape, colour, odour, texture etc.) of the leaves were observed. Physicochemical observation[7] were also carried out for leaves by determining moisture content, total ash value, water soluble ash value and acid insoluble ash value.[8-10]

Extract preparation and Preliminary Phytochemical Screening[9-12]

Fresh Leucaena leaves and seeds were collected from forests during July-August, 2024, in Tripura, India. The plant was identified as *Leucaena leucocephala* (lam.) de Wit; by the Department of Botany, Tripura University, under Accession No. 4962.

5Kg of healthy leaves and seeds of *Leucaena Leucocephala* were washed separately with plenty of fresh water to remove the dust adhered on the leaves and then allowed to dry at room temperature for 12 days. The dried leaves then crushed by hand and bark are separated through the screen of size 20 mesh. Then it was grinded with pulveriser in powder form.

100 Ml of methanol was taken in the 1000 ml beaker to carry out the methanolic extraction. 10 Gm of seeds and leaves powder were separately taken and dissolved into 100 ml methanol in two separate beakers, stirred and tightly covered with a aluminium foil for 3 days, so that there will be no evaporation of methanol. After 3 days the sample mixture was filtered with the help of filter paper to separate out the marc and filtrate.

The solid residue was pressed & left over after straining to recover more of the solution. The liquid extract was concentrated using water bath. The concentrated methanolic extract of leaves and seeds were covered & kept in room temperature for further use.

The extract of seeds and leaves of *Leucaena Leucocephala* were subjected to the preliminary phytochemical screening for the identification of active constituents, such as alkaloids, tannins, carbohydrates, glycosides, saponins, phytosterols, proteins, amino acids, flavonoids, terpenoids, anthraquinones.

Flash Chromatography[13,14]

One purification method designed especially for quick separation is flash chromatography. In contrast to gravity-fed chromatography, which is slow and ineffective, flash chromatography uses air pressure to accomplish separation more quickly and effectively. This approach differs from the traditional column technique in that it uses somewhat smaller silica gel particles and applies pressured gas at pressures between 50 and 200 pounds per square inch (psi). Commonly employed in chemical separations, flash chromatography columns are made of prepackaged plastic cartridges that contain 40–60 mm silica gel particles.

In flash column chromatography, a chemical compound is applied on top of a bed of silica gel or another material (stationary phase) loaded in a glass column, as opposed to thin layer chromatography, which uses a piece of chromatography paper or glass slide coated with silica gel as the stationary phase. The sample is typically covered with a solvent combination (mobile phase), which is then forced through the vertical silica gel column to elute the sample and separate it into its constituent parts. 150 Mg of a crude extract of *Leucaena Leucocephala* leaves / seeds were used for the flash chromatography, and for 25 minutes; n-hexane : ethyl acetate was applied with a solvent (1:1). Material separated/collected in 24 test tubes the value of collected material is placed in Table No. 1 and 2.

Thin Layer Chromatography[15,16]

TLC has been carried out for the separated samples obtained from Flash chromatography, by taking mobile phase n-hexane : ethyl acetate and identifying the spot in the iodine chamber. The R_f values are recorded accordingly.

Table 1: Report of Flash chromatography of leave extracts and R_f values.

Sl. No	Test tube Number	Sample collected(ml)	Peak obtained	Peak not obtained	R_f value in TLC by using hexane:ethyl acetate as the mobile phase

1	1	12	+		0.8
2	2	14		_	
3	3	12	+		0.545
4	4	14		_	
5	5	12	+		0.969
6	6	14		_	
7	7	12	+		No movement of solute
8	8	14		_	
9	9	12	+		0.25
10	10	14		_	
11	11	12	+		0.33
12	12	14		_	
13	13	12	+		No movement of solute
14	14	14		_	
15	15	12	+		No movement of solute
16	16	14		_	
17	17	12	+		0.909
18	18	14		_	
19	19	12	+		0.267
20	20	14		_	
21	21	12	+		No movement of solute
22	22	14		_	
23	23	12	+		No movement of solute
24	24	14		_	

+: Indicates the presence of peak; - : Indicates that there is no peak.

Table 2: Report of Flash chromatography of seeds extract and R_f values.

Sl. No	Test tube number	Sample collected	Peak obtained	Peak not obtained	R_f value in TLC by using hexane:ethyl acetate as the mobile phase
1	1	3		_	
2	2	12	+		0.272
3	3	12	+		No movement of solute
4	4	12	+		0.571
5	5	14		_	
6	6	14		_	
7	7	12	+		0.4
8	8	14		_	
9	9	12	+		No movement of solute
10	10	14		_	
11	11	14		_	
12	12	14		_	
13	13	14		_	
14	14	14		_	
15	15	14		_	
16	16	14		_	
17	17	12	+		0.653

18	18	14		_	
19	19	12	+		0.288
20	20	14		_	

+ : Indicates the presence of peak; - : Indicates that there is no peak.

In-vitro Antidiabetic test

Alpha-Amylase Inhibition Assay[17]

It is necessary to examine the dosage dependency of test samples on alpha-amylase at varying concentrations (between 15 and 1000 mg/mL) in order to assess their potency. Inhibition of porcine pancreatic alpha-amylase was based on the Laishram et al technique. Alpha-amylase solution (0.5 mg/mL, 5.0 MU/mL) must be incubated at 25 °C for 10 minutes with sample solution (200 µL) and 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride, 500 µL). It is necessary to add 500 µL of a 1% starch solution in 0.02 M sodium phosphate buffer following pre-incubation. The next step is to incubate the reaction mixture for 10 minutes at 25°C. Dinitro salicylic acid (DNS) in 1.0 mL is required to halt the process.

A boiling water bath must then be used to incubate the reaction mixture for five minutes before it is allowed to cool to room temperature. Distilled water must then be added to the reaction mixture to dilute it, and an ELISA microplate reader or UV spectrometer must be used to measure absorbance at 540 nm. Alpha amylase is the primary enzyme responsible for hydrolyzing the α-1,4-glucan bonds found in starch, maltodextrins, and other similar carbohydrates, which breaks down the polymeric substrate into shorter oligomers. As a result, higher inhibition indicates greater activity. The results are given in Table No. 3.

Table 3: Maximum % of alpha amylase inhibition by plant extract.

SAMPLE : SEED EXTRACT				
Conc. (µg/m)	**Absorbance of test sample (T)**	**Absorbance of Blank (C)**	**(C-T)/C**	**% Inhibition**
15.62	1.132	1.152	0.017361	1.736111
31.25	1.102	1.152	0.043403	4.340278
62.5	1.054	1.152	0.085069	8.506944
125	1.005	1.152	0.127604	12.76042
250	0.948	1.152	0.177083	17.70833
500	0.872	1.152	0.243056	24.30556
1000	0.831	1.152	0.278646	27.86458
SAMPLE : LEAF EXTRACT				
15.62	0.982	1.152	0.147569	14.75694
31.25	0.928	1.152	0.194444	19.44444
62.5	0.804	1.152	0.302083	30.20833
125	0.712	1.152	0.381944	38.19444

250	0.605	1.152	0.474826	47.48264
500	0.512	1.152	0.555556	55.55556
1000	0.402	1.152	0.651042	65.10417
15.62	0.982	1.152	0.147569	14.75694

Alpha-Glucosidase Inhibition Assay[17]

To evaluate the potency of test extracts, the dose dependency of alpha-glucosidase has to be measured using different concentrations (between 15 and 1000 µg/mL) by referring the method of Laishram et al.

a) Add 25 µL of sample solution in 0.1M Phosphate buffer (pH 6.8).
b) Add 25 µL of Enzyme solution (2U/mL) in 0.1M Phosphate buffer, pH 6.8
c) Mix well and incubate at 37±1°C for 10 minutes.
d) Add 25 µL of the substrate i.e. P-nitro-phenyl α D glucopyranoside (0.5 mM in 0.1 M phosphate buffer, pH 6.8).
e) Incubated at 37°C for 30 minutes.
f) Terminate the reaction by adding 100 µL of 0.2 M sodium carbonate solution.
g) Measure the absorbance of the solution produced at 405 nm

Alpha glucosidase is responsible for cleaving a single glucose from a starch chain or maltose to create more glucose in the fermentable sugar profile. Complex nonabsorbable carbohydrates are changed into simple absorbable carbs by enzymes that they competitively block. These enzymes consist of isomaltose, maltase, sucrase, and glucoamylase. The results are given in Table No.-4.

Table 4: Effect of plant extracts on alpha-glucosidase inhibition.

SAMPLE : SEED EXTRACT				
Conc.(µg/m)	**Absorbance of test sample (T)**	**Absorbance of Blank (C)**	**(C-T/)/C**	**%Inhibition**
15.62	1.072	1.231	0.129163	12.91633
31.25	0.968	1.231	0.213647	21.36474
62.5	0.844	1.231	0.314379	31.43786
125	0.752	1.231	0.389115	38.91145
250	0.635	1.231	0.484159	48.41592
500	0.542	1.231	0.559708	55.97076
1000	0.412	1.231	0.665313	66.53128
SAMPLE : LEAF EXTRACT				
15.62	1.182	1.231	0.039805	3.980504
31.25	1.162	1.231	0.056052	5.605199
62.5	1.134	1.231	0.078798	7.8797737
125	1.095	1.231	0.110479	11.05793

250	0.998	1.231	0.189277	18.9277
500	0.922	1.231	0.251015	25.10154
1000	0.881	1.231	0.284322	28.43217

RESULTS AND DISCUSSION

Macroscopical Characteristics of *Leucaena leucocephala*

The macroscopic examination of *Leucaena leucocephala* revealed distinguishing features valuable for identification and pharmacognostic evaluation. The crushed leaves emitted a characteristic pungent odour. The leaves were bipinnate, composed of small grey-green leaflets that responded to environmental stress by folding. The flowers were observed as white or pale cream in dense, globular heads. Fruits were identified as flat, thin, and explosive pods measuring 9–19 cm in length and 13–21 mm in width. Seeds were shiny brown, ranging from 6.7–9.6 mm in length and 4–6.3 mm in width, with an average weight of approximately 0.070–0.080 gm.

Physical and Physico-Chemical Properties

Physical analysis showed that the melting point of the dried plant extract was around 80°C and the pH of the extract was slightly acidic at pH 5.44. Solubility tests revealed that the extract was immiscible in chloroform and only slightly miscible in water. The density was determined to be 1.1127 gm/ml.

The physico-chemical parameters, including moisture content, total ash, water-soluble ash, and acid-insoluble ash were studied for both leaf and seed powders. Leaves showed a moisture content of 9.8%, while seeds contained 8.4%. Total ash content in leaves and seeds were 17.33% and 16.56%, respectively. Water-soluble ash was 7.73% (leaves) and 7.75% (seeds), whereas acid-insoluble ash was found to be 6.8% and 6.9% in leaves and seeds, respectively.

Preliminary Phytochemical Screening

Phytochemical analysis revealed the presence of several bioactive constituents. Both the leaves and seeds tested positive for alkaloids (Hager's, Dragendorff's, Mayer's tests), glycosides (Molisch's, Legal test), flavonoids (conc. H_2SO_4, aqueous NaOH test), saponins (foam test), carbohydrates (Fehling's test), tannins (bromine water test), proteins (ninhydrin and xanthoproteic test), and triterpenoids (Salkowski test). Anthraquinones and phytosterols were not detected in the plant parts tested.

Flash Chromatography Analysis

Flash chromatography was used to fractionate the methanolic extracts of both leaf and seed samples. Out of 24 test tubes in case of leaf extract, 12 demonstrated the presence of peaks, indicating presence of chemical constituents. The R_f values ranged from 0.25 to 0.969. Similarly, seed extract analysis showed 11 positive test tubes out of 20, with R_f values ranging between 0.272 and 0.653. R_f values were determined by using hexane:ethyl acetate as the mobile phase in Thin Layer Chromatography (TLC). The diversity of R_f values suggests the presence of various phytochemicals, potentially including phytosterols, terpenoids, and benzoquinones.[18,19]

Enzyme Inhibition Assays

The antidiabetic potentiality of *Leucaena leucocephala* was evaluated through alpha-amylase and alpha-glucosidase inhibition assays, which are tabulated in Table – 3&4. The leaf extract showed a dose-dependent inhibition of alpha-amylase activity, with 65.10% inhibition at 1000 µg/ml. The seed extract demonstrated relatively lower inhibition (27.86%) at the same concentration. In contrast in regard to alpha gucosidase inhibition assay, seed extract exhibited higher inhibitory activity (66.53%) at 1000 µg/ml, compared to leaf extract which achieved 28.43% inhibition. These results indicate differential enzyme inhibition potentiality between the two plant parts, where with seed extracts showing stronger activity against alpha-glucosidase and leaf extracts showing more potent against alpha-amylase. The results further were compared with standard (Acrabose) inhibitor data.[17]

CONCLUSION

The study confirms that *Leucaena leucocephala* possesses significant antidiabetic potential, which was observed through dual enzyme inhibition. The leaf extract showed strong α-amylase inhibition (65.10% at 1000 µg/ml), while the seed extract was more effective against α-glucosidase (66.53% at 1000 µg/ml). When compared to standard inhibitor (Acrabose), the crude methanolic extracts showed promising activity, suggesting antidiabetic action may be obtained upon regular intake at edible concentrations, the plant parts may gradually match the therapeutic effect of standard drugs. These findings support the traditional use of *Leucaena leucocephala* and encourage further studies for dosage standardization and clinical application.

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Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this research article.

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