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RESEARCH ARTICLE

Assessment of Antioxidant and Antibacterial Potential of Leaf Extracts from *Leucaena leucocephala*

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ABSTRACT:
Leucaena leucocephala traditionally utilized in folk treatment for its antimicrobial and anti-inflammatory properties. Despite its widespread availability, its phytochemical richness and associated biological activities remain underexplored. Qualitative & quantitative evaluation of phytochemical composition of chloroform extract of *Leucaena leucocephala* (CELL), ethyl acetate extract of *Leucaena leucocephala* (EAELL) and ethanolic extract of *Leucaena leucocephala* (EELL), and also assess the *in vitro* antioxidant and antibacterial activity of CELL, EAELL and EELL with a focus on identifying the most bioactive extract. The collected leaves were dried under shade, pulverized into a coarse powder, extracted serially by using different solvents according to polarity. Phytochemical constituents were identified through standard methods. Total phenolic and flavonoid contents were quantified using the Folin–Ciocalteu and aluminum chloride methods, respectively. Antioxidant activity was assessed via 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), Hydrogen Peroxide (H_2O_2) and Nitric Oxide (NO) assays. Antibacterial potential was assessed by the disk diffusion technique against selected Gram-positive strains (*Staphylococcus aureus* and *Streptococcus pneumoniae*) and Gram-negative strains (*Salmonella typhi* and *Escherichia coli*). The ethanolic extract exhibited the richest phytochemical profile, with the highest total phenolic (189.34 ± 0.982 µg GAE/mg) and flavonoid (46.50 ± 0.078 µg QE/mg) contents. It showed potent antioxidant activity in H_2O_2 (IC_{50} 55.59 µg/mL), medium activity in NO (IC_{50} 67.53 µg/mL) while lowest activity found in DPPH (IC_{50} 75.68 µg/mL) method. Furthermore, the ethanolic extract demonstrated the greatest antibacterial activity, with inhibition zones ranging from 11.23 to 18.54 mm .In contrast, the chloroform and ethyl acetate extracts showed comparatively lower phytochemical and biological activities. The study concluded that ethanolic extract of Leucaena leucocephala leaves as a promising source of antioxidants and antibacterial, credited may be to its high content of phenolic and flavonoid. Results support its potential application in the development of phototherapeutic products, warranting further investigation into its bioactive constituents and mechanisms of action.

KEYWORDS: Ethanolic extract of *Leucaena leucocephala*, Phytochemical screening, Phenolics, Flavonoids Antioxidant activity, Antibacterial activity.

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INTRODUCTION:
Herbal plants have elongated been familiar as a vital cradle of helpful agents in traditional medicine and modern pharmacology. They contain a diverse array of phytochemicals, such as alkaloids, saponins, flavonoids, tannins, terpenoids, and phenolic compounds, which are accountable for a wide range of biological activities, including antioxidant, antimicrobial, anticancer and anti-inflammatory effects[1]. The phytochemical composition

of plant extracts plays a crucial role in determining their therapeutic potential[2].

Due to a lack of modern medications and financial difficulties, the World Health Organization (WHO) estimates that 80% of the world's population depends on traditional medical treatments for basic well-being[3].

The development of new medications has increased the utilize of plants or organic materials in remedy for a variety of reasons all around the globe. In isolated areas, herbal treatments have been utilised often since they are safe and have fewer known side effects than modern generic medications[4,5].

Qualitative and quantitative phytochemical analyses are essential techniques to bioactive phytoconstituents identification. These methods help in standardizing plant extracts, ensuring quality, and providing insights into their pharmacological efficacy[6,7].

Quantitative estimation, particularly of total phenolic and flavonoid contents, is commonly employed to correlate phytoconstituent levels with antioxidant activity[8].

Oxidative stress arises when there is an imbalance between the production of free radicals and the body's antioxidant defenses, and it has been linked to the onset of various chronic diseases, including cancer, diabetes, heart conditions, and neurodegenerative disorders[9].

In vitro antioxidant assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, Hydrogen peroxide (H_2O_2) radical scavenging activity, and Nitric oxide (NO) radical scavenging activity are broadly used to estimate the antioxidant capacity of plant extracts[10,11].

These assays afford valued information about the aptitude of plant-derived compounds to neutralize free radicals.

Furthermore, the rise in multidrug-resistant microbial strains necessitates the search for novel antimicrobial agents from plant sources. Various studies have reported significant antibacterial effects of plant extracts, accredited to its phenolic, flavonoids, and other secondary metabolites[12,13]. Investigating the antibacterial efficacy through in vitro assays against both Gram-positive and Gram-negative pathogens can validate its therapeutic relevance.

Hence, the current study targets to estimate bioactive phytoconstituents, in-vitro antioxidant and antibacterial activity of leaf extracts of *Leucaena leucocephala.* This integrated approach not only provides insight into the phytochemical diversity of the plant but also supports its potential application in developing plant-based therapeutic agents. Image of leaves of *Leucaena leucocephala* are shown in figure 1.

Figure 1: Leaves of *Leucaena leucocephala.*

MATERIALS AND METHODS:

Drugs and Chemicals

Gallic acid, Quercetin, Ascorbic acid, Chloramphenicol, Ethyl acetate, Chloroform, Petroleum ether, Ethanol. Millon's, Molisch's, Ninhydrin, DPPH, Wagner's, Mayer's, Hager's, Folin- Ciocalteu, Dragendroff's, reagents. Sulphuric acid, copper sulphate, sodium hydroxide, Hydrochloric acid, Distilled water, Ferric Chloride, Lead acetate, Magnesium turnings, 2,6-Dichlorophenolindophenol (DCPIP), Aluminum chloride, Zinc dust, Methanol, Hydrogen peroxide, phosphate buffer, Sulfanilic acid, Phosphoric acid, Potassium acetate, Sodium nitroprusside, Naphthyl Ethylenediamine Dihydrochloride (NEDD) and Sodium carbonate.

Identification and Authentication

Leucaena leucocephala leaves were gathered from a garden situated in Hasanpur, within the Amroha district of Uttar Pradesh, India (PIN: 244241) during June 2021. NIScPR- New Delhi carried out the identification and authentication of the plant sample. The authentication number assigned NIScPR/RHMD/Consult/2021/3914-15-2. A voucher specimen was placed in the herbarium to serve as a reference for future.

Sample Preparation

Leucaena leucocephala leaves were gathered, cleaned with water, dried under shade and pulverized into a coarse powder. The coarse powder filled in container which is tightly closed and stored till further use.

Soxhlet Extraction

500 mg of coarse powder was poured into a 1000 mL Soxhlet and extracted using different solvents based on

polarity from low to high in following sequence petroleum ether (60-80°C) only for defatted purpose, chloroform, ethyl acetate and ethanol. Experimental Scheme for extraction of powdered drug is shown in figure 2. The confirmation of complete extraction was done by visual observation. The solvent in the extraction chamber remains clear, indicating no further extraction. Following the extraction process, excess solvent was eliminated using steam distillation. The concentrated extract was then further dried on a water bath maintained at 40°C. The percentage yield, color, and consistency of the extract were evaluated. Finally, the extracts were stored in airtight glass bottles and refrigerated at 4–8°C for future use[14].

Physical Properties of Extracts

Physical properties of obtained extracts were noted. % yield determined by formula 1.

$$\% \text{ Yield} = \frac{\text{(Amount of Extract)}}{\text{(Weight of coarse powder)}} \times 100 \quad \text{.. formula 1}$$

Qualitative Phytochemical Screening

Qualitative phytochemical screening test were performed to identify secondary metabolites (carbohydrates, amino acids, proteins, alkaloids, saponins, steroids, sterols, phenolics, vitamin c and flavonoids) and detailed procedure tabulated in table 1[15].

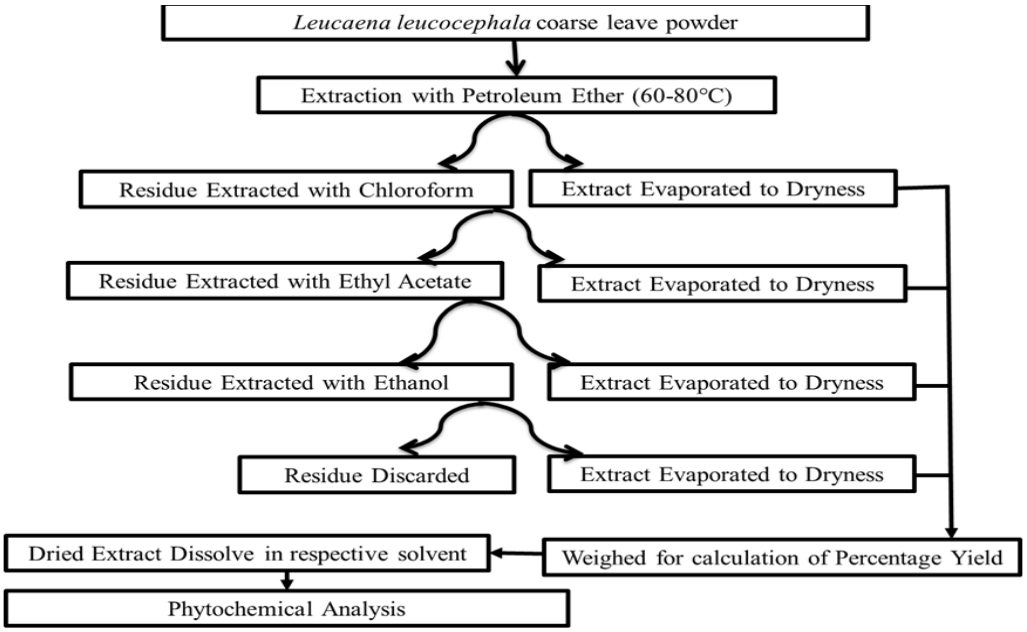

Figure 2: Flow chart of successive extraction of coarse powdered of *Leucaena leucocephala*.

Table 1: Qualitative Phytochemical Screening test

S. No	Phyto-constituent	Chemical Test	Procedure	Inference (Positive Result)
1	Carbohydrates	Molisch's Test	2 mL sample + Molisch's reagent (2 drops) +1 mL of conc. H_2SO_4.	Violet ring at the interface confirms the presence of carbohydrates.
2	Amino Acids	Ninhydrin Test	2 mL sample + Ninhydrin solution (Few drops) and boil for a few minutes.	Purple or blue color indicates amino acids.
3	Proteins	Biuret Test	2 mL sample + 2 mL of 10% NaOH solution, then add 2 drops of 1% $CuSO_4$ solution.	Violet or pink color indicates proteins.
		Millon's Test	2 mL sample + Millon's reagent (few drops) and heat the mixture gently.	When heated, white precipitate turns red, indicating the presence of proteins.
4	Alkaloids	Dragendorff's Test	2 mL sample + dilute HCl + Dragendorff's reagent (few drops).	Orange or reddish-brown precipitate indicates alkaloids.
		Mayer's Test	2 mL sample + Mayer's reagent (few drops) + dilute HCl.	Cream or white precipitate indicates alkaloids.
5	Saponins	Foam Test	2mL sample + 5 mL of distilled water shake vigorously for few minutes.	Persistent froth indicates saponins.
6	Steroids & Sterols	Salkowski Test	2 mL sample + 2 mL chloroform +2 mL conc. H_2SO_4.	The formation of a red or brown ring at the interface signifies the presence of steroids or sterols.

7	Phenolic Compounds	Ferric Chloride Test	2 mL sample + ferric chloride solution (few drops).	Phenols are indicated by being blue, green, or black in appearance.
8	Vitamin C	2,6-Dichlorophenolindophenol (DCPIP) Test	Add DCPIP solution dropwise to extract. Observe decolorization.	Decolorization of blue DCPIP indicates Vitamin C.
9	Flavonoids	Shinoda Test	2 mL sample + Magnesium turnings (few pieces) + conc. HCl.	Orange or red or pink color indicates flavonoids.
		Zinc-HCl Reduction Test	Sample + Zinc dust + Conc. HCl and then heat the mixture gently.	Red coloration indicates flavonoids.

Quantitative Phytochemical Screening

Total Phenolic Content (TPC)

TPC was measured as per Folic- Ciocalteu (FC) method. 2 millilitres of 7.5% Na_2CO_3, 2.5 millilitres of FC reagent, and 0.5 millilitres of a 1 mg/mL extract were put into a test tube. After fully mixing the mixture and letting it sit in the dark for half an hour, absorbance was noted at 760 nm by UV. Total phenolics were quantified by comparing absorbance values to a gallic acid calibration curve prepared in methanol at concentrations ranging from 10 to 100 µg/mL. Results were represented as µg GAE/mg of dry extract[16].

Total Flavonoid Content (TFC)

TFC was measured as per aluminium chloride method. A 0.5 mL aliquot of the extract (1 mg/mL) was put in a test tube together with 2.8 mL of distilled water, 1.5 mL of CH_3OH, 100 µL of 10% $AlCl_3$, and 100 µL of potassium acetate (CH_3COOK). After fully mixing, the mixture was left in the dark for half an hour. The UV spectrophotometer was used to measure the absorbance at 415 nm. The flavonoid content was quantified using a quercetin calibration curve (10–100 µg/mL in methanol), and results were expressed as µg of quercetin equivalents (QE) per mg of dry extract[17].

***In Vitro* Antioxidant Activity**

2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) Method

The DPPH assay was used to assess the antioxidant activity of CELL, EAELL, and EELL at different doses. Various conc. of CELL, EAELL, EELL, and the common antioxidant ascorbic acid were prepared (10, 20, 30, 40, 50, 60, 70, and 80 µg/mL). The assay was performed by adding 1 mL of each sample to a test tube and then 2 mL of a 0.1 mM DPPH solution, which was prepared by dissolving 4 mg of DPPH in 100 mL of ethanol. For half an hour, the combinations were kept in the dark after that. A UV-Visible spectrophotometer was used to measure absorbance at 517 nm. The percentage inhibition of DPPH radicals by CELL, EAELL, EELL, and ascorbic acid was calculated using Formula 2. The IC_{50} value, indicating the concentration at which 50% of the DPPH radicals were scavenged, was determined from a graph plotting percentage inhibition against concentration[18].

$$\text{Percentage Inhibition} = \frac{(\text{Absorbance control} - \text{Absorbance test})}{(\text{Absorbance control})} \times 100$$

…….formula 2

Hydrogen Peroxide (H_2O_2) Method

The technique of Ruch et al. was used to evaluate the hydrogen peroxide (H_2O_2) scavenging activity CELL, EAELL and EELL, with minor adjustments. A range of conc. of CELL, EALL, EELL, and the standard antioxidant ascorbic acid were prepared (10, 20, 30, 40, 50, 60, 70, and 80 µg/mL). By adding 8.8 mL of 30% H_2O_2 in 100 mL of phosphate buffer (pH 7.4), a 40 mM H_2O_2 solution was created. 0.4 mL of each conc. test solution and 0.6 mL of the 40 mM H_2O_2 solution were combined in separate test tubes for the experiment. For 10 minutes, the test tubes were kept in a dark room after the test. The absorbance at 230 nm was measured using a UV-Visible spectrophotometer. Formula 2 was used to determine the percentage inhibition of H_2O_2 by CELL, EALL, EELL, and the reference chemical. The IC_{50} values were determined from the plot of percentage inhibition versus concentration[19].

Nitric Oxide (NO) Scavenging Method

The Griess reagent technique was used to assess CELL, EALL and EELL nitric oxide scavenging capability. Different quantities of CELL, EALL, EELL and ascorbic acid were produced as solutions (10, 20, 30, 40, 50, 60, 70, and 80 µg/mL). In phosphate buffer, a new 10 mM sodium nitroprusside (SNP) solution was made. Equal parts of 0.1% naphthyl ethylenediamine dihydrochloride (NEDD) and 1% sulfanilic acid (dissolved in 5% phosphoric acid) were combined in distilled water to create the Griess reagent.

In separate test tubes, 0.5 mL of 10 mM SNP was combined with 1 mL of each test solution for the experiment. For three hours, the mixes were incubated at room temperature. Each tube received 1 mL of Griess reagent after incubation and 548 nm absorbance was noted. The % inhibition by CELL, EALL, EELL and the reference standard determined by above mentioned formula 2. The IC_{50} values were obtained from the plot of percentage inhibition versus concentration[20].

Antibacterial Activity:

Microorganisms:

Antibacterial action of extract was evaluated with both types of bacteria (gram positive and gram negative). Every bacterial culture came from the strain collection that was kept up to date by the IFTM University, Moradabad's School of Pharmaceutical Sciences.

The bacterial strains were grown and preserved on Mueller-Hinton agar at a temperature of 37 °C. For the duration of the experiment, the bacteria were cultured in Mueller-Hinton broth. The resulting bacterial suspensions were standardized to match the turbidity of a 0.5 McFarland standard whenever required [21].

Antibacterial Activity by Disk Diffusion Technique:

The antibacterial potential of CELL, EAELL, and EELL was assessed using the disk diffusion method. From its corresponding broth culture, A small inoculum of each test microorganism was transferred into a test tube containing 5 mL of distilled water. Adjusted turbidity to 0.5 McFarland standard. The standardized bacterial suspension was spread onto the Hinton agar plates using a sterile cotton swab, and the plates were left to air dry. Using sterile forceps, three discs of sterile Whatman filter paper were carefully positioned on the agar surface. 20 µL (100 mg/ mL conc.) of the corresponding plant extract (CELL, EAELL, or EELL) was applied to each disc. As a positive control, chloramphenicol (30 µg/mL) was applied following the same procedure. Every test was run in triplicate. Infected plates were incubated for 24 h at 37 °C. Effectiveness against bacteria was evaluated by measuring the size of the clear zones surrounding the discs post incubation. Experiments were carried out in triplicate. Data were analysed using GraphPad Prism software[22].

Data Analysis

Data showed in this research as mean ± SD. GraphPad Prism (version 8.2.4) was used to do result analysis. Tukey test was used after one way ANOVA to analysed the results. Significance difference was considered if $p< 0.05$.

RESULTS

Physical Properties of Extracts

Extraction was successfully done with Soxhlet apparatus. After the extraction physical properties of extracts of leaves of *Leucaena leucocephala* was noted and tabulated in table 2.

Table 2: Physical properties of extracts of *Leucaena leucocephala*.

S. No	Extract	% Yield W/W	Colour	Consistency
1	CELL	4.268	Dark Green	Sticky Mass
2	EAELL	7.684	Greenish Brown	Semisolid Mass
3	EELL	8.360	Greenish Black	Semisolid Mass

Qualitative Phytochemical Screening:

Extracts of *Leucaena leucocephala* were tested for the presence of phytoconstituents. CELL demonstrated the presence of alkaloids, steroids, phenolics, vitamin C, and flavonoids. Similarly, EAELL also contained alkaloids, steroids, phenolics, vitamin C, and flavonoids. EELL exhibited the widest range of phytoconstituents, showing the presence of all tested compounds except saponins. In comparison to other solvent extracts, it was discovered that the EELL had the maximum number of phytoconstituents. Presence of these phytoconstituents suggests the potential medicinal value of the *Leucaena leucocephala*.

Quantitative measurement of Total Phenolic and Flavonoid Content:

Total phenolic and flavonoid content of all the extract were measured and displayed in table 3. All the extract exhibited total phenolic and flavonoid content but EELL showed maximum content both phenolic and flavonoid.

Table 3: Results of total phenolic and flavonoid content of extracts.

S. No.	Extracts	Total Phenolic content (µg/mg of dry extract) Mean ± SD	Total Flavonoids Content (µg/mg of dry extract) Mean ± SD
1.	CELL	20.5 ± 0.924*	12.76 ± 0.954*
2.	EAELL	124.12 ± 0.613*	23.28 ± 0.625*
3.	EELL	189.34 ± 0.982*	46.50 ± 0.078*

Data represented as mean ± SD (n=3). One way ANOVA followed by Tukey's test was used to examined the data. Significance difference was considered if $p< 0.05$ vs among the groups.

***In-Vitro* Antioxidant Activity**

2, 2 Diphenyl 1 Picrylhydrazyl Method

Various concentrations of CELL, EAELL, EELL and ascorbic acid (10-80 µg/mL) were tested for their scavenging potential. The % inhibition of the samples increased proportionally with concentration, as shown in table 4 and figure 3. The IC_{50} values of DPPH method for the CELL, EAELL and EELL were 100.33 µg/mL, 88.96 µg/mL and 75.68 µg/mL in comparison to ascorbic acid, which demonstrated an IC_{50} of 49.24 µg/mL (table 4). These findings suggest that all the extracts possess notable antioxidant properties and may play a beneficial role in managing conditions associated with oxidative stress. Less IC_{50} values indicated high scavenging activity. Thus, EELL produced maximum antioxidant activity as compared to other extract because it had lowest IC_{50} value.

Table 4: Percentage scavenging activity and IC_{50} of Ascorbic acid, CELL, EAELL and EELL by DPPH method.

S. No.	Conc. (µg/mL)	% Inhibition			
		Ascorbic acid	CELL	EAELL	EELL
1	10	27.13 ± 1.23	4.66 ± 0.98	6.23 ± 1.09	8.81 ± 1.35
2	20	32.19 ± 0.87	9.02 ± 1.98	12.87± 0.76	15.50 ± 0.40
3	30	38.26 ± 0.67	14.08 ± 0.12	17.32 ± 0.23	21.65 ± 0.57
4	40	44.23 ± 0.54	20.00 ± 1.45	24.01 ± 0.12	29.16 ± 0.64
5	50	51.42 ± 0.45	25.87 ± 0.67	30.00 ± 0.32	35.86 ± 0.90
6	60	56.48 ± 0.98	29.09 ± 0.76	35.87 ± 0.67	41.10 ± 1.46
7	70	62.04 ± 0.56	34.54 ± 0.43	39.60 ± 0.34	46.16 ± 1.09
8	80	68.12 ± 0.45	40.82 ± 0.12	44.10 ± 0.85	51.22 ± 3.12
9	IC_{50}	**49.24 ± 1.23***	**100.33 ± 1.20***	**88.96 ± 1.54***	**75.68 ± 2.87***

Data represented as Mean ± SD (n=3). One way ANOVA followed by Tukey's test was used to examined the data. Significance difference was considered if $p< 0.05$ vs among the groups.

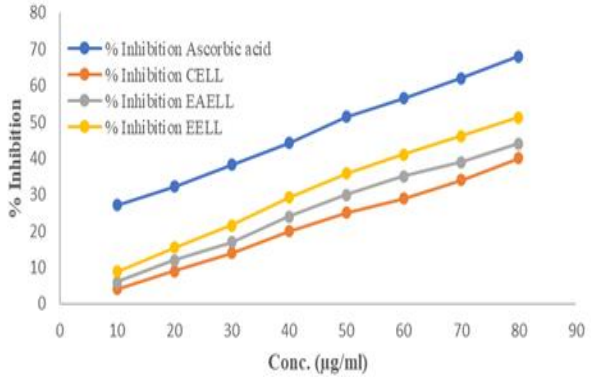

Figure 3: % Inhibition by CELL, EAELL, EELL and Ascorbic acid by DPPH method.

Hydrogen Peroxide (H_2O_2) Method

H_2O_2 is generated in the body through the activity of various oxidase enzymes, including superoxide dismutase. It is capable of diffusing across biological membranes and can slowly oxidize a range of biomolecules. When $H2O_2$ levels are high, they can cause oxidative stress, which is a dangerous situation that can injure cells. Many diseases are known to have oxidative stress as a major contributing factor in their development.

Various concentrations (10–80 µg/mL) of CELL, EAELL, EELL and ascorbic acid were tested for their antioxidant potential. As shown in table 5 and figure 4, the scavenging activity increased in a concentration-dependent manner. The IC_{50} values of H_2O_2 method for the CELL, EAELL and EELL were 88.75 µg/mL, 63.99 µg/mL and 55.59 µg/mL compared to ascorbic acid, which showed an IC_{50} of 44.16 µg/mL (table 5). These findings suggest that all the extracts possess notable antioxidant properties and may play a beneficial role in managing conditions associated with oxidative stress. Less IC_{50} values indicated high scavenging activity. Thus, EELL produced maximum antioxidant activity as compared to other extract because it had lowest IC_{50} value.

Table 5: % Inhibition and IC_{50} of Ascorbic acid, CELL, EAELL and EELL by H_2O_2 method.

S. No	Conc. (µg/mL)	% Inhibition			
		Ascorbic Acid	CELL	EALL	EELL
1	10	13.76 ± 0.89	4.09 ± 0.80	5.34 ± 1.87	5.56 ± 1.76
2	20	22.43 ± 0.34	9.11 ± 0.67	11.02 ± 1.34	14.39 ± 1.32
3	30	33.40 ± 0.54	14.99 ± 0.12	20.38 ± 0.45	24.82 ± 0.45
4	40	44.47 ± 0.67	20.23 ± 0.45	30.21 ± 0.86	36.03 ± 0.54
5	50	55.03 ± 0.65	26.12 ± 0.65	40.00 ± 0.76	45.38 ± 0.54
6	60	67.51 ± 0.23	32.21 ± 1.45	47.04 ± 0.54	54.53 ± 0.43
7	70	80.63 ± 1.74	39.42 ± 0.34	55.25 ± 0.12	63.97 ± 1.02
8	80	90.24 ± 1.09	46.71 ± 0.98	62.02 ± 0.34	72.85 ± 0.98
9	IC_{50}	**44.16 ± 1.45***	**88.75 ± 1.34***	**63.99 ± 0.98***	**55.59 ± 1.98***

Data represented as Mean ± SD (n=3). One way ANOVA followed by Tukey's test was used to examined the data. Significance difference was considered if $p< 0.05$ vs among the groups.

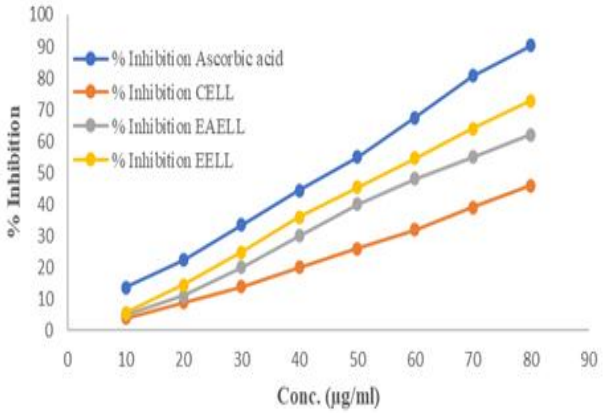

Figure 4: % Inhibition by CELL, EAELL and EELL, Ascorbic acid by H_2O_2 method.

Nitric Oxide (NO) Method

The Ruch et al. (1989) approach was used to assess the NO scavenging activity of CELL, EAELL, and EELL. Different concentrations (10–80 µg/mL) of CELL, EAELL, EELL and ascorbic acid were tested to assess their antioxidant potential. All samples showed an increase in scavenging activity that was concentration-dependent, as shown in table 6 and figure 5.

The IC_{50} values of NO method for the CELL, EAELL and EELL were 105.43 µg/mL, 87.79 µg/mL and 67.53 µg/mL related to ascorbic acid, which showed an IC_{50} of 43.25 µg/mL (table 6). These findings suggest that all the extracts possess notable antioxidant properties and may play a beneficial role in managing conditions associated with oxidative stress. Less IC_{50} values indicated high scavenging activity. Thus, EELL produced maximum antioxidant activity as compared to other extract because it had lowest IC_{50} value.

In-Vitro Antibacterial Activity

All the extracts (CELL, EAELL, and EELL) antibacterial activity was measured are presented in table 7 and figure 6. Overall, the standard antibiotic chloramphenicol produced inhibition zones ranging from 13.50 to 21.45 mm, which were generally larger than those observed for the plant extracts, which ranged from 7.67 to 18.54 mm. Among the tested extracts, EELL demonstrated the most pronounced antibacterial effect across all microbial strains, followed by EAELL and then CELL.

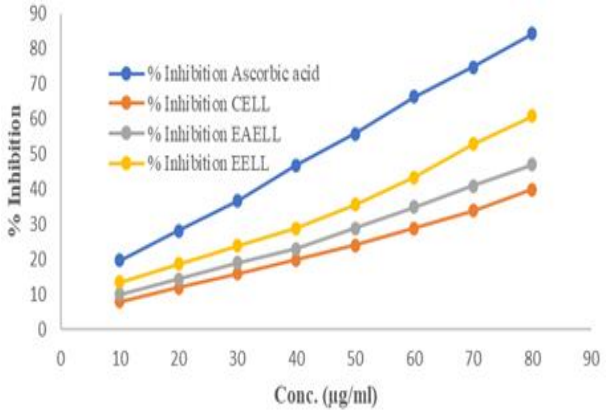

Figure 5: % Inhibition by CELL, EAELL and EELL as Compared to Ascorbic Acid in the NO method.

Table 6: % Inhibition and IC_{50} of Ascorbic acid, CELL, EAELL and EELL during NO method.

S. No.	Conc. (µg/mL)	% Inhibition			
		Ascorbic Acid	CELL	EAELL	EELL
1.	10	19.73 ± 0.80	8.67 ± 0.94	10.12 ± 0.82	13.52 ± 0.12
2.	20	28.18 ± 0.23	12.02 ± 1.43	14.40 ± 1.10	18.76 ± 1.23
3.	30	36.77 ± 0.98	16.00 ± 1.20	19.00 ± 1.00	23.85 ± 0.87
4.	40	46.96 ± 0.11	20.92 ± 0.78	23.21 ± 0.93	28.90 ± 0.17
5.	50	55.85 ± 1.22	24.87 ± 0.56	29.80 ± 0.43	35.60 ± 1.45
6.	60	66.92 ± 1.32	29.29 ± 0.65	35.51 ± 0.98	43.51 ± 2.12
7.	70	74.82 ± 0.43	34.70 ± 0.12	41.92 ± 0.87	52.91 ± 2.11
8.	80	84.38 ± 0.1.23	40.90 ± 1.90	47.47 ± 0.12	60.95 ± 0.20
9.	**IC_{50}**	**43.25 ± 1.76***	**105.43 ± 1.23***	**87.79 ± 1.20***	**67.53 ± 1.89***

Data represented as Mean ± SD (n=3). One way ANOVA followed by Tukey's test was used to examined the data. Significance difference was considered if $p < 0.05$ vs among the groups.

Table 7: Antibacterial activity (zone of inhibition) of CELL, EAELL and EELL and standard.

Microorganism	Zone of Inhibition (mm) Mean ± SD			
	CELL (100mg/ml)	EAELL (100mg/ml)	EELL (100mg/ml)	Chloramphenicol (30µg/ml)
***S. aureus* (Gram positive)**	11.66 ± 0.34	14.12 ± 0.65	18.54 ± 0.45	21.45 ± 0.67
***S. pneumoniae* (Gram positive)**	10.12 ± 0.23	12.40 ± 0.72	17.21 ± 0.28	20.23 ± 0.52
***S. typhi* (Gram negative)**	8.32 ± 0.22	10.22 ± 0.34	12.55 ± 0.70	15.76±0.23
***E. coli*(Gram negative)**	7.67 ± 0.45	9.10 ± 0.22	11.23 ± 0.98	13.50 ± 0.84

Data represented as Mean ± SD (n=3).

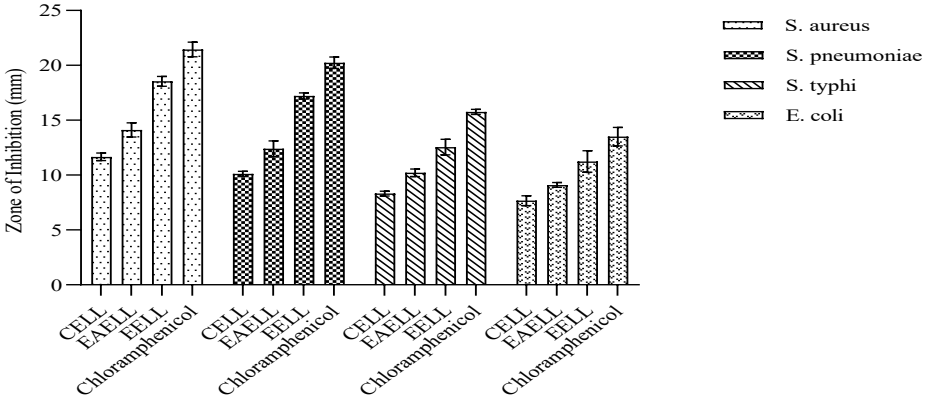

Figure 6: Antibacterial activity (zone of inhibition) of CELL, EAELL and EELL and standard.

All the extract exhibited antibacterial efficacy but EELL showed Maximum zone of inhibition. It is likely due to higher conc. of phenolic and flavonoids in the EELL as compared to CELL and EALEE.

DISCUSSION:

This study included preliminary phytochemical screening, quantitative estimation of total phenolics and flavonoids invitro antioxidant and antibacterial activities of different extracts of Leucaena leucocephala.

The findings unequivocally showed that the best solvent for removing bioactive substances from *Leucaena leucocephala* leaves is ethanol. The ethanolic extract showed the highest phytochemical richness, high content of phenolics and flavonoids, strong antioxidant activity, and broad-spectrum antibacterial effects, which are in line with previous studies[12,23]. The polar nature of ethanol likely enhances the extraction of phenolic and flavonoid compounds, which are known to possess strong free radical scavenging and antimicrobial properties[24].

The chloroform extract, on the other hand, showed the least amount of antibacterial activity. This could be because chloroform is non-polar and has a limited capacity to extract polar phytoconstituents. Ethyl acetate, being semi-polar, showed intermediate efficacy in all assays.

These results provide credence to the possibility of using *Leucaena leucocephala* ethanolic extract in the creation of natural antibacterial and antioxidant compounds.

CONCLUSION:

The results of the current study show that the leaves of *Leucaena leucocephala* are a rich source of bioactive phytochemicals with strong antibacterial and antioxidant properties. Among the different solvent extracts evaluated, the ethanolic extract shown the peak levels of phenolic and flavonoid content, which correlated strongly with its superior free radical scavenging activity and broad-spectrum antibacterial efficacy. The ethyl acetate extract showed moderate activity, while the chloroform extract was comparatively less effective in all assays.

These results confirm ethanol likely application in the production of natural antioxidant and antibacterial agents and imply that it is a solvent that can be used to extract pharmacologically significant chemicals from *Leucaena leucocephala*. Further isolation and characterization of individual active constituents, along with in vivo studies, are warranted to fully explore the therapeutic potential of this underutilized plant species.

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