

Evaluation of Phytochemicals, Total Flavonoids Content and Antioxidant Activity of *Leucaena Leucocephala* (Lam.) De Wit.

Jyoti Prajapati*, Anusha Maitreya, Himanshu Pandya, Hitesh Solanki

Department of Botany, Bioinformatics and Climate Change Impacts Management, Gujarat University, Ahmedabad-380009, Gujarat India

ABSTRACT

The plant species *Leucaena leucocephala* (Lam.) De Wit. belongs to the Fabaceae family of Angiospermae, has been found in the Navrangpura area of Ahmedabad district of Gujarat state. The purpose of this study is to prepare the extracts of the leaves and stem of the plant using a polar solvent (Methanol) and a non-polar solvent (Chloroform) and conduct preliminary phytochemical screening and quantitative analysis of flavonoids using colorimetric method, respectively, as well as an antioxidant activity using the DPPH (2,2 diphenyl-1-picrylhydrazyl) method. The methanolic extract of leaf and stem revealed a higher number of phytochemicals compared to chloroform extract. The methanolic extract of leaf showing maximum total flavonoids content as compared to stem. The methanol extract of leaf and stem showed less antioxidant activity as compared to standard ascorbic acid.

Keywords: *Leucaena leucocephala*, Phytochemical screening, Total Flavonoids Content, Antioxidant activity

INTRODUCTION

Leucaena leucocephala (Lam.) De Wit. originates from Central America and Mexico and belongs to Fabaceae family. It is also known as *Acacia leucocephala* (Lam.), *Mimosa leucocephala* (Lam.) and *Leucaena glabrata* (Rose). It is widely distributed and cultivated in more than 150 countries especially in tropical and Sub-tropical zones. *Leucaena leucocephala* is a perennial evergreen, flowering, non-climbing, thornless, fast-growing, small to moderate size tree with a long life. In the genus *Leucaena*, about 23 well-defined species of small trees are present (KHALID et al., 2024). *Leucaena* is derived from Greek words in which “leuc” means “white” and “cean” means “new” mentioned to the whitish flowers. The flowers are also mentioned by species name: *leucocephala* in which “leu” means “White” and “cephala” means “Head” (Zayed et al., 2018). *Leucaena leucocephala* is a medium-wood plant that can be recognized by its physical characteristics, such as its thornless growth and height of 7 to 18 meters. This plant typically has bipinnate leaves with 6-8 pairs of pinnae bearing 11-23 pairs of leaflets that its usual size is between 8-16 mm long.

Flowers are round, light yellow that quickly develops into a fruit with flat brown pods that are 13-18 mm long and contain 15-30 flat oval seeds (Zarina et al., 2017). *Leucaena leucocephala* are medium sized fast – growing plant. It is also derived as “Miracle Tree” for its excellent multipurpose uses. The trees has many uses like controls soil erosion, fuel wood, timber, fodder, green manure etc. It also has medicinal properties like Antimicrobial, Anthelmintic, Antibacterial, Anti- proliferative, Anti- diabetic, Anticancer, Cancer preventive, Diuretic, Anti-inflammatory, Antioxidant, Antitumor, Antihistaminic, Nematicide, Pesticide, Anti-androgenic, Hypo-cholesterole (Deivasigamani, 2018). *Leucaena leucocephala* is a multipurpose tree, valued for its wood, which is used to create paper pulp, tiny furniture, and high-quality charcoal. Its seeds, young leaves, and young shoots can be eaten as vegetables. Additionally, seeds can be used as jewellery or as an alternative to coffee. It is planted to promote soil fertility, control erosion, prepare sites for reforestation, and provides shades for commercial crops like coffee and cacao. Cattles, water buffalo and goats are frequently fed the protein-rich leaves and legumes (Verma, 2016). *Leucaena leucocephala* are

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



medicinal plants which is used to control stomach ache, like contraception and abortifacient (Zayed et al., 2016). It is used to cure intestinal worms, measles, scurf, diabetes. The roasted seeds are used to increase the flow of menstruation (Chaurasia et al., 2015). *Leucaena leucocephala* (Chinese petai) leaves and seeds are used as medicinally for Indonesians. It leaves contain active flavonoids that reduce pain. It seeds have anthelmintic properties, insomnia, swelling, kidney inflammation, and diabetes. The plant used by people as wound medicine. Because of it has no side effects towards the skin irritation it uses as traditionally (Septina et al., 2020). It's bark and roots are used as household remedies. The roots contains emmenagogic and abortive qualities (De Angelis et al., 2021).

MATERIALS AND METHODS

2.1 Plant Collection

The leaves and stem of the plant were collected from Navrangpura area of Ahmedabad district of Gujarat state. The plant parts were washed with water and dried at a room temperature for 2-3 days. After drying, the plant parts were made into a coarse powder using a grinder, and stored in air-tight plastic box at room temperature.

2.2 Plant Extracts Preparation

The extracts were prepared by using cold extraction method. The dried powder of the plant was dissolved in the solvent (methanol and chloroform) using the ratio of 1:10 g/ml to their respective conical flasks and which was then covered it with parafilm and aluminium foil and then incubate for 48 hours at a room temperature. All the samples were separately filtered by using the Whatman filter paper 1. The filtrate are transferred into petri-plates for solvent evaporation for 2-3 days at a room temperature. The petri-plates were stored in laboratory refrigerator for the further process. The percentage yield was calculated by the standard formula-

Yield (%) = (Weight of Dry Extract ÷ Weight of Plant Powder Taken) × 100

2.3 Qualitative Analysis (Phytochemicals Screening)

30 mg extract was dissolved in 30 ml methanol and chloroform solvents separately to prepare 1mg/ml concentration of the stock solution. Both the methanolic and chloroform extracts were further subjected for phytochemical analysis to determine the presence of various secondary metabolites like alkaloids, phenols, quinones, steroids, flavonoids, saponins, carbohydrates, proteins and glycosides. Preliminary phytochemical screening was done by following methods (Monisha et al., 2017) (Sahrawat et al., 2018) (Shah et al., 2021).

• Test for Alkaloids

Mayer's Test: Take 1ml extract and add 2ml Mayer's reagent, the formation of white creamy ppt. or yellow colour indicates the presence of alkaloids.

Wagner's Test: Take 1ml extract and add 1ml Wagner's reagent, the formation of red brown ppt. indicates the presence of alkaloids.

• Test for Carbohydrates

Fehling's Test: Take 1ml extract and add 1ml Fehling's A solution then add 1ml Fehling's B solution and incubate in water bath for 2-4 minutes, the formation of red colour settled in bottom of test tube indicates the presence of carbohydrates.

• Test for Glycosides

Ferric Chloride Test: Take 1ml extract and add 0.5ml Glacial acetic acid then add few drops of 1% Ferric chloride solution then add conc. H₂SO₄, the formation of brownish or red-green colour indicates the presence of glycosides.

Ammonia Test: Take 1ml extract and add 3ml Chloroform then add 1ml 10% Ammonia solution, the formation of pink colour indicates the presence of glycosides.

• Test for Terpenoids

Salkowski's Test: Take 1ml extract and add 1ml Conc. H₂SO₄ then incubate in water bath for 2-4 minutes, the formation of greyish colour indicates the presence of terpenoids.

• Test for Proteins



Nitric acid Test: Take 1ml extract and add Conc. Nitric acid, the formation of yellow colour indicates the presence of proteins.

- **Test for Phenols**

Ferric chloride Test: Take 1ml filtrate and add 5% Ferric chloride solution, the formation of dark green or blue or black colour indicates the presence of phenols.

Folin Ciocalteu Test: Take 1ml filtrate and add 1ml Folin Ciocalteu reagent, the formation of blue-green colour indicates the presence of phenols.

Lead acetate Test: Take 1ml extract and add 0.5ml Lead acetate solution, the formation of white precipitates indicates the presence of phenols.

- **Test for Tannins**

Ferric chloride Test: Take 1ml filtrate and add 5% Ferric chloride solution, the formation of dark green or blue or black colour indicates the presence of tannins.

- **Test for Quinones**

Sodium hydroxide Test: Take 1ml extract and add 1ml of 2% NaOH, the formation of blue green or red colour indicates the presence of quinones.

- **Test for Flavonoids**

Shinoda's Test: Take 1ml extract and add few drops of Conc. HCl, the formation of pink scarlet colour indicates the presence of flavonoids.

Lead acetate Test: Take 1ml extract and add few drops of 10% lead acetate solution, the formation of yellow precipitates indicates the presence of flavonoids.

Sodium Hydroxide Test: Take 1ml extract and add 3ml of 2% NaOH, the formation of yellow colour seen then add dil. H₂SO₄ the yellow colour will disappears which indicates the presence of flavonoids.

- **Test for Saponins**

Foam Test: Take 1ml extract and add 3ml distilled water and shake it vigorously, the formation of foam layers indicates the presence of saponins.

- **Test for Steroids:**

Salkowski's Test: Take 2ml extract and shake with chloroform then add Conc. H₂SO₄ side by side, the formation of red colour at the lower chloroform layer indicates the presence of steroids.

2.4 Quantitative Analysis

The stock solution was prepared with the concentration of 1mg/ml in the methanol solvent. In case of sample, 10 mg of dry extract was dissolved in 10 ml of solvent (methanol) to make stock solution. Similarly, the standard solutions (Quercetin) were prepared with a concentration of 1mg/ml.

2.4.1 Quantification of Total Flavonoids Content

The total flavonoids content was determined by using colorimetric method with slight modifications. The standard solution used in the concentration gradient series of 10mg/ml to 100mg/ml. To the series, 0.3ml of 5% Sodium nitrite, 0.3ml of 10% Aluminium chloride, 2ml 1M Sodium hydroxide are added. Similarly for plant extract, 1ml plant extract, 0.3ml of 5% Sodium nitrite, 0.3ml of 10% Aluminium chloride, 2ml 1M Sodium hydroxide are added. Final volume made up to 5ml in both standard and extract and incubate for 5 minutes. The absorbance was determined at 510nm. The flavonoid content was calculated from standard curve of Quercetin and expressed as mg QEg⁻¹ (Quercetin equivalent) using the formula,

$$TFC = (C \times V) / M$$

Where, C = concentration of quercetin obtained from the standard calibration curve in mg/ml

V = volume of the extract in ml

M = weight of the extract in g

2.5 Antioxidant Activity

2.5.1 DPPH Radical Scavenging Assay

Antioxidant activity was done by using the DPPH radical scavenging assay. The DPPH is a 2,2-diphenyl-1-picrylhydrazyl reagent. The DPPH was freshly prepared by dissolving 4mg DPPH in 100ml of methanol to make 0.004% DPPH solution. Ascorbic acid was used as standard on the concentration of 0.2mg/ml. The concentration gradient series ranging from 40µg/ml to 200µg/ml, and it is replaced with 0.2mg/ml sample extract when the sample is under process. 2ml of prepared DPPH was added in standard and sample extract and incubate in complete darkness for 15 minutes. The absorbance was measured by using a spectrophotometer at $\lambda = 517\text{nm}$, a gradual decrease was determined. The test was repeated in triplicates to avoid any errors. The scavenging activity is calculated based on the percentage of DPPH radical scavenged (I%) with the help of the following formula,

$$I\% = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where, A_{control} is the absorbance of control (containing all reagents except the sample), and A_{sample} is the absorbance of sample.

RESULT AND DISCUSSION

3.1 Yield (%) value

The yield (%) of plant extracts is calculated for the purpose of quantification of the phytochemical constituents with respect to the dry plant extract. The

methanolic leaf extract 10.49% yield and in stem 5.26% yield observed. While in chloroform leaf extract 3.33% and in stem 1.37% yield was observed. The standard formula used was,

$$\text{Yield (\%)} = (\text{Weight of Dry Extract} \div \text{Weight of Plant Powder Taken}) \times 100$$

Calculations:

1. Yield (%) for Methanolic Leaf Extract = $(1.049 \div 10) \times 100 = 10.49\%$
2. Yield (%) for Methanolic Stem Extract = $(0.526 \div 10) \times 100 = 5.26\%$
3. Yield (%) for Chloroform Leaf Extract = $(0.333 \div 10) \times 100 = 3.33\%$
4. Yield (%) for Chloroform Stem Extract = $(0.137 \div 10) \times 100 = 1.37\%$

3.2 Qualitative Analysis

Methanolic extract of leaf and stem of *Leucaena leucocephala* (Lam.) De Wit. evinced the presence of alkaloids, glycosides, proteins, flavonoids, saponins, and steroids. Whereas, the chloroform extract of leaf revealed only the occurrence of alkaloids and glycosides and in stem alkaloids, terpenoids, proteins and steroids are occurred. This result proves that the polar methanol solvent tends to extract more phytochemicals from the plant sample as compared to the non-polar chloroform solvent.

Table 1: Preliminary Phytochemical Tests

Sr. No	Phytochemicals	Test Name	Methanol		Chloroform	
			Leaves	Stem	Leaves	Stem
1	Alkaloids	Mayer's Test	+	+	-	-
		Wagner's Test	+	+	+	+
2	Carbohydrates	Fehling's Test	-	-	-	-
3	Glycosides	Ferric Chloride Test	+	+	+	-
		Ammonia Test	-	-	-	-
4	Terpenoids	Salkowaski's Test	-	-	-	+
5	Proteins	Nitric acid Test	+	+	-	+
6	Phenols	Ferric Chloride Test	-	-	-	-
		Folin Ciocalteu Test	-	-	-	-
		Lead acetate Test	-	-	-	-
7	Tannins	Ferric Chloride Test	-	-	-	-
8	Quinones	Sodium Hydroxide Test	-	-	-	-
9	Flavanoids	Shinoda's Test	-	-	-	-
		Lead acetate Test	-	+	-	-
		Sodium Hydroxide Test	+	+	-	-
10	Saponins	Foam Test	-	+	-	-
11	Steroids	Salkowaski's Test	+	+	-	+

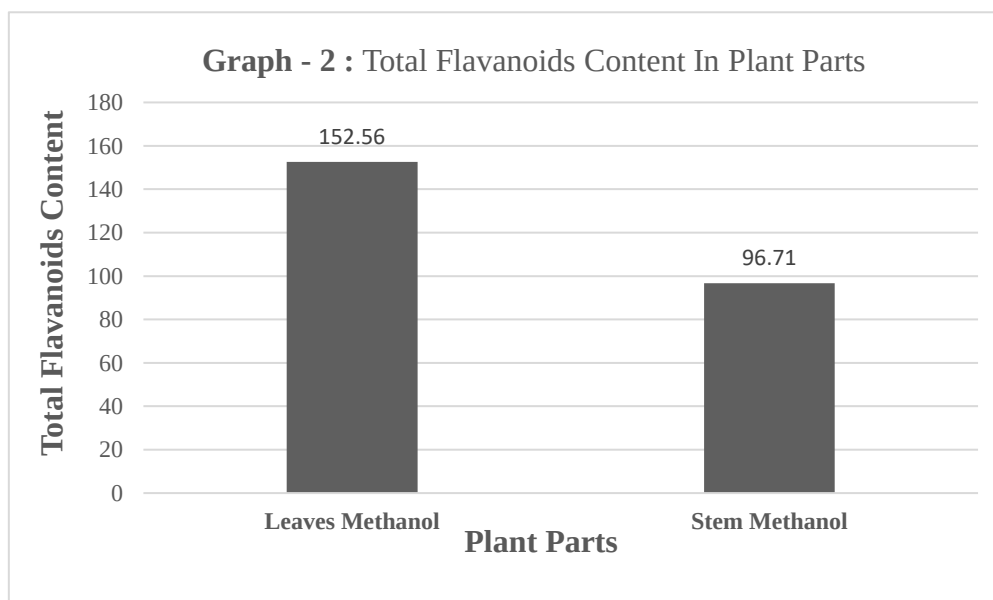
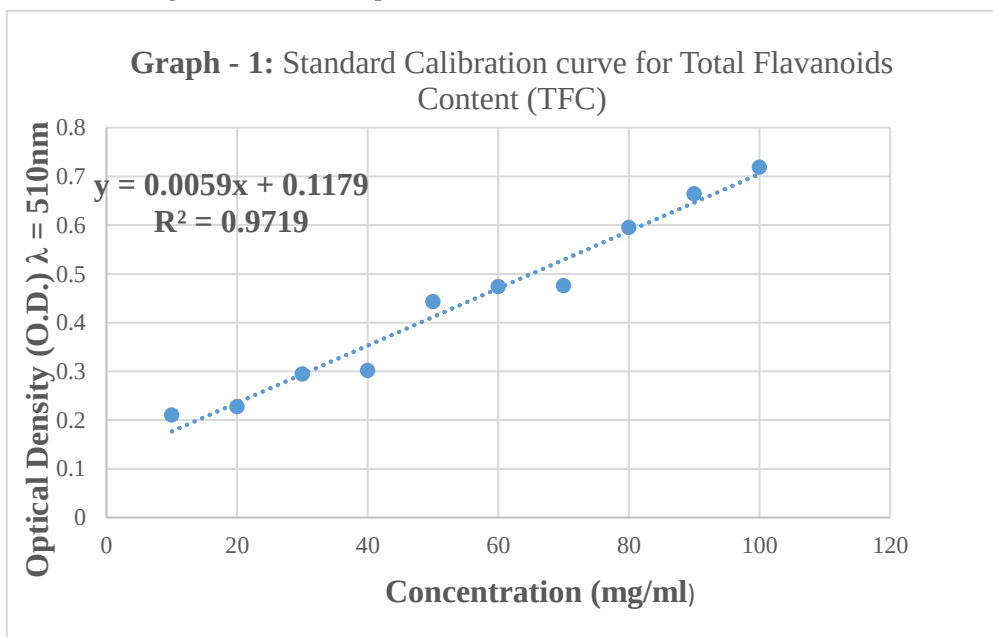
The table represents the results of the phytochemical tests performed. '+' sign indicates the presence and '-' sign indicates the absence of the respective phytochemicals.

3.3 Quantitative Analysis

3.3.1 Total Flavonoids Content

Aluminium chloride colorimetric method is run by the principle of $AlCl_3$ forming acid stable complex with

C4 keto group or C3/C5 hydroxyl group of flavonoids or flavones thus it is used to evaluate the total flavonoid content. The calibration curve was obtained by running the standard, Quercetin. The curve provides the following equation $y = 0.0059x + 0.1179$ $R^2 = 0.9719$. From the above equation, the total flavonoid content of the plant samples was calculated. The leaves consists 152.56 mg QE/g and stem 96.71 mg QE/g of total flavonoid content of samples.



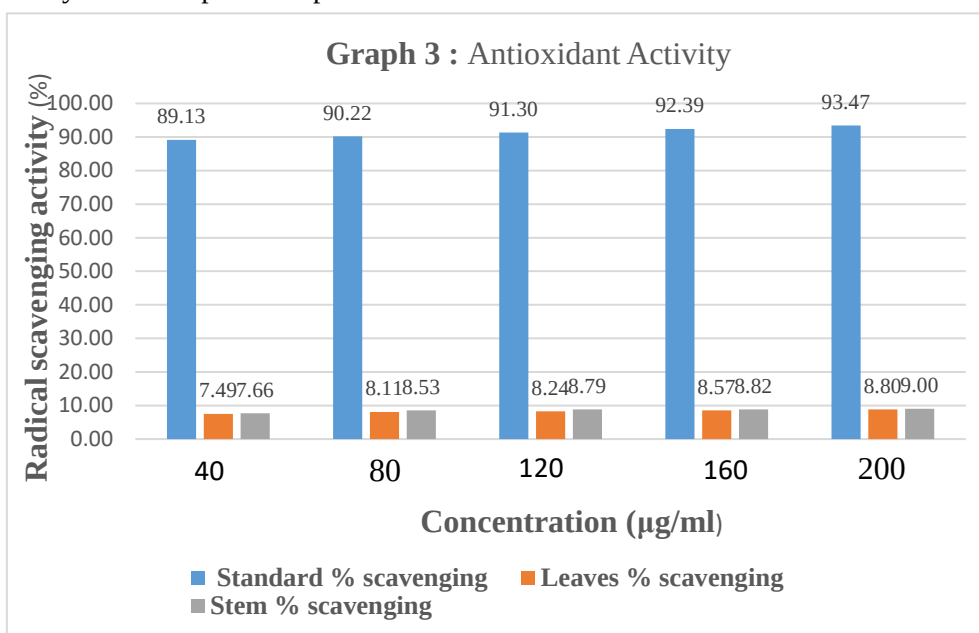
3.4 Antioxidant activity

3.4.1 DPPH Radical Scavenging Assay

The antioxidant activity of the plant was assessed by DPPH radical scavenging assay. DPPH is the most commonly used chemical for testing the antioxidant activity of plant samples. It is a rapid effective method for colorimetric method. The antioxidant agent

reduces DPPH which is signified by the colour change from purple to yellow with a sequential decline in the absorbance at 517 nm which is measured using a spectrophotometer. To study the antioxidant activity of *Leucaena leucocephala* leaves and stem, ascorbic acid was used as the standard antioxidant agent for the comparison. From the comparison of standard and plant samples, the standard shows the high scavenging activity instead of plant samples. From the

shows result the value of DPPH scavenging activity of standard ascorbic acid highest (93.47) at concentration 200 µg/ml and lowest (89.13) at concentration 40 µg/ml. For leaves extract highest (8.80) at concentration 200 µg/ml and lowest (7.49) at concentration 40 µg/ml. For stem extract highest (9.00) at concentration 200 µg/ml and lowest (7.66) at concentration 40 µg/ml.



In the present study we performed the phytochemical screening of successive methanol and chloroform extracts obtained from cold extraction. The methanol extracts obtained from cold extraction of leaves and stem were utilized in quantitative analysis (total flavonoid content) and antioxidant activity test. The methanolic leaf extract shows the highest yield value about 10.49% of dry extracts. The phytochemical screening revealed the presence of alkaloids, glycosides, proteins, flavonoids and steroids in leaves methanolic extract and in stem alkaloids, glycosides, proteins, flavonoids, saponins and steroids are present. In chloroform extract, the leaves contains only alkaloids and glycosides and in stem alkaloids, terpenoids, proteins and steroids are present. Carbohydrates, phenols, tannins and quinones were undetected in any of the extracts. From quantitative analysis (total flavonoid content), the methanolic extract of leaves showed the maximum amount of total flavonoid content with 152.56 mg QE/g as compared to methanolic extract of stem shows 96.71 mg QE/g of total flavonoid content. However, flavonoids and phenolic acids are identified as typical

phenolics with antioxidant properties. Fruits, vegetables and other plants have been shown to naturally contain antioxidants called phenolic acids (Shilpakar et al., 2009). In the present study the antioxidant activity test was found to be positive in methanolic extract which can be attributed to the presence of only flavonoids as shown in the phytochemical screening test. From the comparison of standard and plant samples, the standard shows the high scavenging activity about 93.47% and plant samples (leaves – 8.80% and stem – 9.00%) shows least scavenging activity. Additionally, this study shows that the polar solvents are preferred for isolating any phytoconstituents and that methanol, a polar solvent, is more effective than a non-polar solvents at retaining or extracting phytoconstituents (Shah et al., 2021). Phytoconstituents such as tannins, phenolics, and flavonoids play a vital role in antioxidant and is frequently used in the pharmaceutical sector due to their anti-inflammatory, anti-tumor, anti-cancer, anti-ageing, anti-diabetic, and anti-obesity properties. They are employed in the management of a range of conditions, including

various cardiovascular diseases, diabetes, age-related eye disorders, neurodegenerative diseases such as Alzheimer's disease, hypertension, and in providing protective effects against Inflammatory Bowel Disease (IBD), which encompasses, ulcerative colitis (UC) and Crohn's disease (Zhang et al., 2015).

CONCLUSION

Qualitative analysis, quantitative analysis and antioxidant activity test of *Leucaena leucocephala* (Lam.) de Wit. were performed. From our study it can be concluded that various phytochemicals, including flavonoids are present in the leaves and stem of this plant. Quantitative analysis of total flavonoid content, the leaves shows the high flavonoid content as compared to stem in methanolic extract. Antioxidant activity of methanolic extract of *Leucaena leucocephala* was done by using DPPH radical scavenging method. Antioxidant efficacy of methanolic extracts were found to be higher in standard ascorbic acid compared to plant parts at concentration of 200µg/ml. From this study we can conclude that this plant consists several phytoconstituents. Also, this plant consists antioxidant property though they were comparatively lower than that of the standard used in the study.

REFERENCE

1. Chaurasia, S., & Sharma, P. (2015). Evaluation of antibacterial and antimutagenic potential of *Acokanthera oppositifolia* and *Leucaena leucocephala*. *Am J Pharm Health Res*, 3(1), 246-58.
2. De Angelis, A., Gasco, L., Parisi, G., & Danieli, P. P. (2021). A multipurpose leguminous plant for the mediterranean countries: *Leucaena leucocephala* as an alternative protein source: A review. *Animals*, 11(8), 2230.
3. Deivasigamani, R. (2018). Phytochemical analysis of *Leucaena leucocephala* on various extracts. *J Phytopharm*, 7(6), 480-482.
4. KHALID, A. M., MAHMOD, A. I., TALIB, W. H., & AFIFI, F. U. (2024). LC-MS Analysis And Antioxidant, Antimicrobial And Antiproliferative Investigations Of *Leucaena Leucocephala* Flowers Growing In Jordan Extracts. *Farmacia*, 72(2), 337-345.
5. Monisha, M., Sowmiya, M., Ragunathan, R., & Johny, J. (2017). Extraction of bio active compounds from *Cassia Auriculata* pods and leaves and its medicinal uses. *Int J Curr Microbiol App Sci*, 6(8), 425-434.
6. Sahrawat, A., Sharma, J., Rahul, S., Tiwari, S., Joshi, M. D., & Pundhir, A. (2018). Phytochemical analysis and Antibacterial properties of *Azadirachta indica* (Neem) leaves extract against *E. coli*. *Journal of Pharmacognosy and Phytochemistry*, 7(4), 1368-1371.
7. Septina, E., Yetti, R. D., & Rivai, H. (2020). Overview of traditional use, phytochemical, and pharmacological activities of chinese petai (*leucaena leucocephala*). *Int. J. Pharm. Sci. Med*, 5(12), 1-10.
8. Shah, R., Sharma, P., & Modi, N. (2021). Preliminary phytochemical analysis and assessment of total phenol and total flavonoid content of *Haworthiopsis limifolia* Marloth. and its antioxidant potential. *International Journal of Botany Studies*, 6(3), 902-909.
9. Sharma, P., & Chaurasia, S. (2015). Evaluation of total phenolic, flavonoid contents and antioxidant activity of *Acokanthera oppositifolia* and *Leucaena leucocephala*. *Int J Pharmacogn Phytochem Res*, 7, 175-80.
10. Shilpakar, A., Gaire, B. P., Bahadur, S. C., Lamichhane, R., & Neupane, S. (2009). Phytochemical screening and analysis of antibacterial and antioxidant activity of *Ficus auriculata*, Lour. Stem bark. Pokhara University, Pokhara, Nepal.
11. Srivastava, S., Panchani, D., & Modi, N. (2022). Phytochemical Analysis and Antioxidant Activity of *Dalechampia scandens* var. *Cordofona* (Hochst. ex A. Rich) Muell. Arg. *Indian Journal of Natural Sciences*, 13(73), 46737-46746.
12. Verma, S. (2016). A review study on *Leucaena leucocephala*: A multipurpose tree. *International Journal of Scientific Research in Science, Engineering and Technology*, 2(2), 103-105.
13. Zarina, Z., Ghazali, C. M. R., & Sam, S. T. (2017, September). Characterization analysis for leaves of *Leucaena leucocephala* by using phytochemical screening assay. In *AIP Conference Proceedings* (Vol. 1885, No. 1). AIP Publishing.

14. Zayed, M. Z., & Samling, B. (2016). Phytochemical constituents of the leaves of *Leucaena leucocephala* from Malaysia. *Int J Pharm Pharm Sci*, 8(12), 174-179.
15. Zayed, M. Z., Sallam, S. M. A., & Shetta, N. D. (2018). Review article on *Leucaena leucocephala* as one of the miracle timber trees. *International Journal of Pharmacy and Pharmaceutical Sciences*, 10(1), 1-7.
16. Zhang, Y. J., Gan, R. Y., Li, S., Zhou, Y., Li, A. N., Xu, D. P., & Li, H. B. (2015). Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Molecules*, 20(12), 21138-21156.

HOW TO CITE: Jyoti Prajapati*, Anusha Maitreya, Himanshu Pandya, Hitesh Solanki, Evaluation of Phytochemicals, Total Flavonoids Content and Antioxidant Activity of *Leucaena Leucocephala* (Lam.) De Wit., *Int. J. Sci. R. Tech.*, 2025, 2 (8), 227-234. <https://doi.org/10.5281/zenodo.16847693>