Exploring Natural Antioxidants: A Comparative Assessment of Ehretia laevis Roxb and Clerodendrum infortunatum

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***Abstract*—Ayurveda mentions many herbal shops for fracture repair. The facility is utilized for a variety of purposes, including the production of beauty products, pot sauces, wood and gravestone coloring, pharmaceuticals, wines, and cosmetics. Ehretia Laevis Roxb. includes chemical compounds that can be used to repair and create. Ehretia laevis Roxb. (Boraginaceae) has long been used as a traditional cure for a variety of disorders involving the respiratory system, gastrointestinal tract, reproductive system, and various infections. E. laevis phytochemical investigations revealed the presence of important phytoconstituents such as pentacyclic triterpenoids, phenolic acids, flavonoids, fatty acids, steroids, alkaloids, aliphatic alcohols, hydrocarbons, amino acids, carbohydrates, vitamins, and minerals. Fresh plant parts, crude extracts, fractions, and isolated compounds have been shown to demonstrate a wide range of therapeutic activity, including antiarthritic, antidiabetic, anti-inflammatory, antiulcer, antidiarrheal, antidysenteric, wound healing, and anti-infective properties. E. laevis has been demonstrated to be a great source of medications for the treatment of jaundice, asthma, dysentery, ulcers, diarrhea, ringworm, eczema, diabetes, fissure, syphilis, cuts and wounds, inflammation, liver difficulties, venereal and infectious diseases. Clerodendron infortunatum Linn. (Verbenaceae) is a medicinal plant known for its bitter component, clerodin. It has been used as a tonic and anthelmintic in North India's countryside. This herb is used in Ayurveda, Unani medicine, and Homeopathy for a variety of ailments, including diarrhoea, skin disorders, venereal and scrofulous complaints, wounds, post-natal complications, and as a vermifuge, laxative, and cholagogue. It is also used to remove ascarids from the anus and as an external application on tumors. In this comparative study, the antioxidant activities of Ehretia laevis Roxb. and Clerodendrum infortunatum are assessed using the DPPH free radical scavenging method. The experiment is designed to evaluate and compare the radical scavenging ability of both plant extracts at different doses. This approach yields a reasonable assessment of antioxidant capacity based on the ability to donate hydrogen atoms to neutralize DPPH radicals. The study analyzes and compares IC_{50} values to discover which plant has the strongest antioxidant capacity. The findings could provide important insights into the therapeutic properties of these often-utilized plants**

***Index Terms*—Antioxidant activity, Clerodendrum infortunatum; DPPH assay, Ehretia laevis Roxb., Free radical scavenging, Herbal remedies, IC_{50}, Phytochemicals, Therapeutic properties, Traditional medicine.**

I. INTRODUCTION

Herbal plants are widely employed in the pharmaceutical, perfume, and cosmetic industries. They are successfully utilized in skin and hair care, as well as animal feeding, to improve health and productivity while also positively impacting the product derived from them [1]. Around 80% of the world's population relies on herbal-based alternative medicine. Except for homeopathy, the chemical components of these medicinal plants are used to assess their efficacy. Active chemicals created during secondary vegetal metabolism are typically responsible for the biological properties of some plant species utilized worldwide for a variety of purposes, including the treatment of infectious diseases. Hervaceous infusions have a variety of advantages, including antidiabetic, anticarcinogenic, antibacterial, and antioxidant properties [2,3,4]. Recently, many

cosmetic and toiletry products have used Indian herbs. Modern trials have shown that Indian herbs can be used in personal care products, in addition to their traditional usage [5].

Ehretia laevis Roxb. is also known as ovate-leaved ivory wood, Gujarati Vadhavaradi, Hindi bhairi, Chamror, Konkanikalo gamdo, Malayalam Caranti, Marathi, and Datrangi (since it colors teeth in red). Sant Dnyaneshwar from Alandi, Maharashtra, India, took Samadhi near this tree, which is regarded a spiritual factory. In Ayurvedic literature, this factory is used for Prameha and Vishagna. This factory produces a wide range of medicinally beneficial chemicals as well as excellent ethnobotanical products. Ehretia is a term used to describe unfolding stores in the Boraginaceae family of plants. It has around 50 species [6,7]. The Ehretia laevis plant has medicinal uses in traditional medicine, including treating diarrhea, cough, syphilis, toothache, stomach and venereal diseases, and as an antidote to vegetable poison. Ehretia species contain many phytoconstituents, including phenolic acids, flavonoids, benzoquinones, cyanogentic, glycosides, fatty acids, and other significant chemicals [8,9]. Clerodendrum infortunatum Linn. (Family: Verbanaceae), also known as Bhat in Hindi, Ghentu in Bengali, and Bhania in Oriya, is a terrestrial shrub with a square, blackish stem and simple, opposite, decussate, petiolate, exstipulate, coriacious, hairy leaves that emit an unpleasant odor [10]. Initial chemical study of Clerodendrum infortunatum Linn. leaves revealed the presence of saponin, clerodin (a bitter diterpene), 4, 6, and a few enzymes. Lenoleic, oleic, stearic, and lignoceric acid glycerides make up the fixed oil found in leaves. Root-derived luperol and β-sitosterol. Clerosterol, a steroidal glycoside derived from roots, was discovered as 5, 25sigmastadien_3β-ol, clerodolone as lup_20(30)-en3β-diol-12-one, and clerodone as 3β-hydroxylupan-12-one. Triterpenes, steroids, and flavonoids were also discovered to be present in the plant [11, 12].

II. MATERIALS AND METHOD

The plant leaves of *Ehretia laevis* Roxb and *Clerodendrum infortunatum* were collected from Manas Ayurveda during June and July. Following collection, the leaves of both plants were carefully shade-dried to preserve their phytochemical constituents. Once the drying process was complete and the leaves were moisture-free, they were subjected to further processing. The dried plant materials were then used to prepare extracts, which were subsequently evaluated for their antioxidant potential. This step was essential for analyzing and comparing the antioxidant properties of the two plant species as part of the overall study.

A. EXTRACTION

Hydroalcoholic Extraction: The leaves of *Ehretia laevis* Roxb and *Clerodendrum infortunatum* were collected from Manas Ayurveda during June and July. After collection, the leaves were thoroughly washed and shade-dried to retain their phytochemical integrity. Once completely dried, the leaves were coarsely powdered and subjected to hydroalcoholic extraction using 70% ethanol as the solvent. This extraction method was chosen to ensure efficient recovery of both polar and non-polar bioactive compounds. The resulting extracts were concentrated and preserved for further analysis. These extracts were then used to assess and compare the antioxidant potential of both plant species in the study.

B. ANTIOXIDANT STUDY

The capacity of pure substances or extracts to capture free radicals is a measure of their antioxidant qualities. Methods that measure a compound's ability to scavenge free radicals, such as the 2,20-diphenyl-1-picrylhydrazyl radical (DPPH), the 2,20-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) cation radical (ABTS+), the superoxide anion radical, or the hydroxyl radical, are employed for this purpose. The DPPH technique, which uses a stable 2,20-diphenyl-1-picrylhydrazyl radical (DPPH), is one of the most widely used [13]. The spectrophotometric determination of DPPH concentration variations brought on by the DPPH reaction with an antioxidant forms the basis of the DPPH technique. A hydrogen atom from an antioxidant reduces DPPH, which has an unpaired valence electron at one of the nitrogen bridge's atoms. The kinetics of this reaction are used to ascertain the compounds' antioxidant characteristics [14].

C. THIN LAYER CHROMATOGRAPHY (TLC) AND DERIVATIZATION

TLC analysis was performed using pre-coated silica gel plates (Merk, 60 F254) with a thickness of [specify thickness, if necessary]. The plates were developed

using a solvent system optimized for separation of antioxidant-active components. The antioxidant potential of the extracts was assessed using DPPH (2,2-diphenyl-1-picrylhydrazyl) derivatization.

D. DENSITOMETRIC ANALYSIS

Densitometric evaluation of antioxidant-active bands was carried out using a CAMAG TLC Scanner 3 under standardized conditions. The scanner was set to detect the bands at an information position of 8.0 mm, with the solvent front migrating up to 70.0 mm. Each TLC plate was scanned for four tracks, with the initial track positioned at 15.0 mm, and a fixed inter-track spacing of 23.3 mm was used to ensure uniformity across replicates.

The scanning was performed vertically along the Y-axis, starting at 5.0 mm and terminating at 75.0 mm, covering the entire analyte zone. The slit dimensions were optimized at 4.00 × 0.30 mm (micro) for enhanced resolution and sensitivity. The scanning speed was maintained at 20 mm/s, with data resolution set to 100 µm/step, providing high detail for precise peak detection.

E. QUANTIFICATION

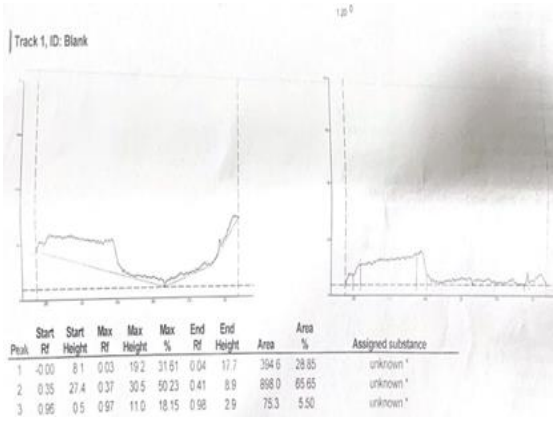
The antioxidant-active bands were quantified using the CAMAG TLC Scanner's reflectance mode. An optimized optical system based on light reflectance ensured precise quantification of the antioxidant bands. The results were expressed as relative peak areas, enabling direct comparison of the antioxidant fingerprints post-DPPH derivatization across the plant species.

Reproducibility and Data Analysis: These parameters were consistently applied across samples of both *Ehretia laevis Roxb.* and *Clerodendrum infortunatum*, maintaining analytical rigor and reproducibility. The data were processed and analyzed using CAMAG's WinCATS software, allowing for direct comparisons of the antioxidant potential of the plant extracts.

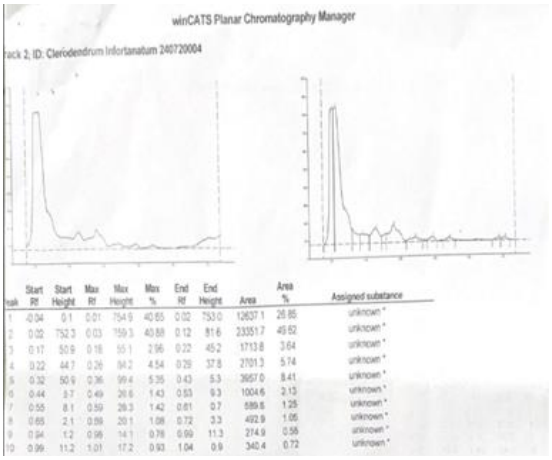
F. INTEGRATION PARAMETERS FOR DENSITOMETRIC ANALYSIS

Integration was carried out using uniform settings to ensure analytical consistency for the densitometric evaluation of antioxidant compounds in Ehretia laevis Roxb and Clerodendrum infortunatum. Data filtering was performed using the Savitzky–Golay algorithm with a 7-point smoothing window. Baseline correction was applied using the lowest slope method for both species. Peak detection parameters were standardized across analyses, with the minimum slope set at 5, minimum peak height at 10 AU, minimum peak area at 50, and a maximum peak height threshold of 990 AU. The scanning range along the chromatographic track was initiated at 5.0 mm for both samples; however, a slight variation was observed in the end position, with *E. laevis* scanned up to 75.0 mm and *C. infortunatum* up to 74.9 mm. This minor discrepancy is likely due to minimal differences in solvent front migration or scanner calibration and did not impact the overall quality of peak detection. Scanning display mode was automatic for both samples to ensure optimal visualization and peak integration. The use of standardized integration parameters facilitated accurate comparison of antioxidant profiles between the two species.

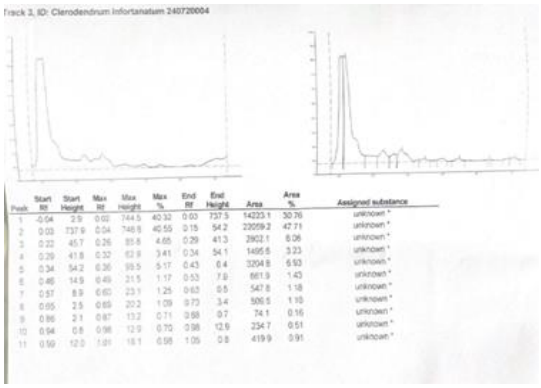
III. OBSERVATION AND INTERPRETATION



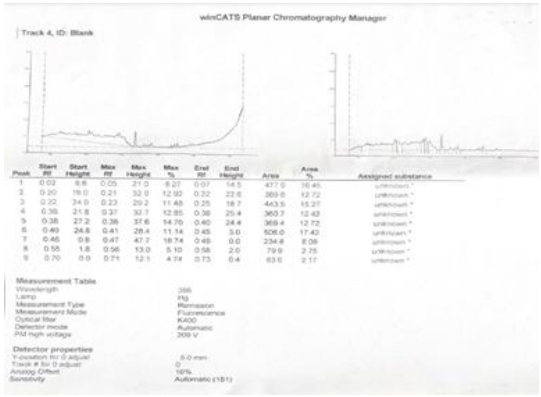
(a)



(b)



(c)



(d)

Fig. No. (a), (b), (c), & (d) HPTLC Fingerprinting Profile of *Clerodendrum infortunatum* Extract (24072004)

Interpretation of HPTLC Chromatograms of *Clerodendrum infortunatum* Extract- High-Performance Thin-Layer Chromatography (HPTLC) profiling was performed using winCATS Planar Chromatography Manager to evaluate the phytochemical composition of *Clerodendrum infortunatum* extract. The chromatograms were compared with blank samples to ensure the accuracy of detected peaks and exclude background noise or solvent interference.

Track 1 & Track 4: Blank Samples

Observation:

- A limited number of peaks were observed in both blank chromatograms (Track 1 and Track 4).
- Major peaks in Track 1 occurred at Rf values of approximately 0.01, 0.27, and 0.95, contributing to around 28.85%, 65.65%, and 5.50% of total area respectively.
- In Track 4, 9 minor peaks were detected, with none exceeding 14.37% area, indicating minimal interference.

Interpretation:

- These peaks represent baseline noise or residual solvents. Any matching peaks in the sample tracks should be excluded during analysis of bioactive compounds.

Track 2 & Track 3: *Clerodendrum infortunatum* Extract

Track 2 Observations:

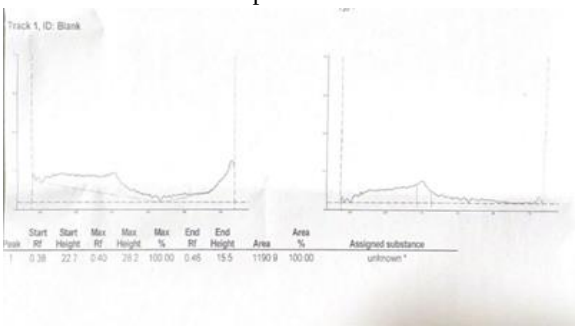
- Eleven peaks were identified with Rf values ranging from 0.01 to 0.99.
- The most prominent peak had an Rf of 0.01 with a maximum height of 13263.7 and 26.85% area.
- Other significant peaks included those at Rf 0.96 (14.18%) and 0.51 (11.27%).

Track 3 Observations:

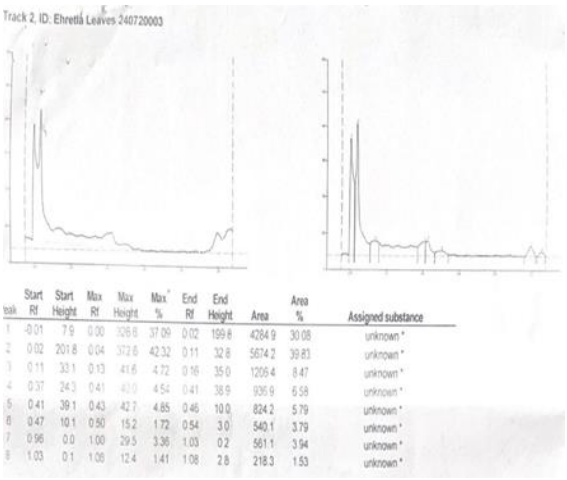
- Also featured 11 peaks with Rf values similar to Track 2.
- The peak at Rf 0.01 showed even higher abundance (14223.1 max height, 30.76% area), indicating consistency in compound presence.
- Other notable peaks occurred at Rf 0.96 (14.71%) and 0.54 (10.99%).

Interpretation:

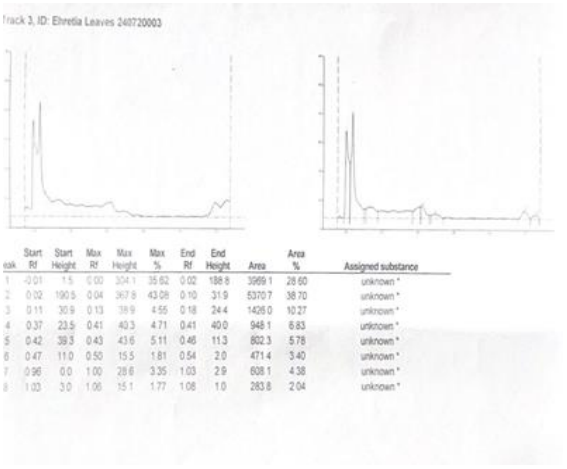
- Tracks 2 and 3 demonstrate a complex phytochemical profile.
- Peaks not present in the blank tracks—particularly those at Rf values 0.51, 0.54, 0.66, and others—are likely due to secondary metabolites in *Clerodendrum infortunatum*.
- The consistent presence of these peaks across samples validates the reproducibility of the extract's chemical profile.



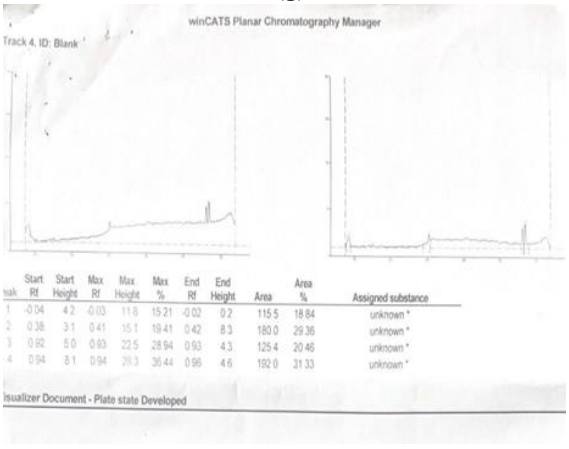
(e)



(f)



(g)



(h)

Fig.No. (e), (f), (g), & (h) HPTLC chromatograms provided for *Ehretia laevis* extracts (24072003)

Interpretation of HPTLC Chromatograms of Ehretia Laevis Roxb. -

Track 1 & 4: Blank Chromatograms (Solvent Controls)

Observation:

- Track 1 showed a single broad peak at Rf 0.27, contributing to 100% of the area.
- Track 4 exhibited 4 low-intensity peaks at Rf values: 0.12, 0.31, 0.94, and 1.08 with modest area contributions (18.84%, 29.30%, 20.63%, and 31.33%, respectively).

Interpretation:

- The blank chromatograms reflect baseline solvent-related noise.
- Peaks detected here must be subtracted from test samples to avoid false identification of plant compounds.
- The absence of intense peaks in these controls confirms that most of the major peaks in the plant extract chromatograms are not due to solvent contamination.

Track 2 & 3: *Ehretia laevis* Extracts

Track 2 Observations:

- Nine peaks detected, with the most prominent at Rf 0.01 (30.08% area) and others ranging from Rf 0.05 to 1.08.
- Peaks at Rf 0.21, 0.35, 0.43, and 0.71 were significant, contributing between 4–9% of the area.

Track 3 Observations:

- Eight distinct peaks observed, with peak at Rf 0.01 again dominant (25.60% area), demonstrating reproducibility.
- The Rf values and area distribution closely match Track 2, validating method consistency.

Interpretation:

- The presence of strong, well-resolved peaks across both sample tracks indicates a rich phytochemical profile in *Ehretia laevis*.
- Rf 0.01 likely corresponds to a major class of bioactive compounds such as flavonoids or phenolics.
- Peaks at mid-range Rf values (0.21–0.71) suggest the presence of diverse phytoconstituents with varying polarities.
- The matching pattern between Tracks 2 and 3 confirms reproducibility and reliability of the extraction and chromatographic conditions.

IV. RESULT AND DISCUSSION

SR. NO.	SAMPLE NAME	515 (Abs)
1	Control	0.045
2	2mg/l	0.037
3	4mg/l	0.022
4	10mg/l	0.008
5	Control	0.055
6	24072003 100 µg/mL	0.024
7	200µg/mL	0.027
8	400µg/mL	0.019
9	24072004 100µg/mL	0.026
10	200µg/mL	0.024
11	400µg/mL	0.025

Table. No. 1. Datasheet of Absorbance Value

In the table. no. 1, the absorbance values recorded at 515 nm reflect the presence and possible concentration of active compounds within the tested samples. Two control samples were included in the analysis, displaying absorbance values of 0.045 and 0.055, respectively. This slight difference between the two controls may suggest minor variability, which could result from experimental conditions, sample handling, or instrumental sensitivity. Such variation is commonly observed in spectrophotometric analyses and underscores the importance of replicates to ensure accuracy. Overall, these absorbance readings provide a baseline for comparing treated samples and help in evaluating the influence of different concentrations on compound activity.

The effect of concentration on absorbance at 515 nm reveals a clear, concentration-dependent trend. As the concentration of the sample increases, the absorbance values decrease notably. The 2 mg/L sample exhibits a moderate absorbance of 0.037, indicating a relatively lower level of interaction or reaction with the absorbing species. As the concentration increases to 4 mg/L, the absorbance drops further to 0.022, suggesting a stronger interaction or higher activity. At the highest tested concentration of 10 mg/L, the absorbance falls significantly to 0.008, indicating a marked reduction in the intensity of the absorbing compound. This progressive decline in absorbance with increasing concentration implies that the active constituents in the sample may be reacting more effectively with the radicals or chromogenic agents at higher concentrations. Such a pattern is commonly associated with antioxidant assays, where increased concentration of antioxidants leads to greater radical scavenging or color reduction, thus lowering the absorbance reading. The results support the hypothesis that the sample possesses antioxidant activity, which becomes more pronounced with higher concentrations. This trend reinforces the potential of the tested material to act as a dose-dependent antioxidant agent, highlighting its suitability for further investigation and possible therapeutic application.

The absorbance data for different sample types at concentrations of 100 µg/mL, 200 µg/mL, and 400 µg/mL demonstrates the overall consistency and slight variability across replicates, reflecting the reproducibility of the assay. The 100 µg/mL samples, identified as 24072003 and 24072004, show closely aligned absorbance values of 0.024 and 0.026, respectively. This similarity suggests that the preparation and handling of these samples were consistent and that the assay results are reliable at this concentration. At 200 µg/mL, the absorbance values of 0.027 and 0.024 reflect minor fluctuations, which may be attributed to slight differences in sample mixing, pipetting errors, or inherent experimental variability. Despite this, the values remain within a close range, indicating overall reproducibility. The 400 µg/mL samples exhibit absorbance values of 0.019 and 0.025, revealing a somewhat greater discrepancy. This difference may be due to sample saturation, changes in solubility, or increased interaction between the sample matrix and the absorbing compound at higher concentrations. Such deviations at elevated concentrations are not uncommon and may suggest concentration-related effects such as aggregation or nonlinear response. Overall, the data demonstrate acceptable consistency with some variability, particularly at higher concentrations, warranting careful consideration during formulation or further testing.

The data suggest a concentration-dependent effect, where higher sample concentrations result in lower absorbance. If this experiment measures antioxidant activity, the findings indicate greater activity at higher concentrations.

V. CONCLUSION

The antioxidant study of *Ehretia laevis* Roxb and *Clerodendrum infortunatum* reveals promising potential in both plant extracts, demonstrated through their ability to reduce absorbance at 515 nm in a concentration-dependent manner. Both extracts exhibited significant antioxidant activity, as evidenced by the decrease in absorbance values with increasing concentrations, indicating effective radical scavenging. *Ehretia laevis* showed a more pronounced reduction in absorbance at lower concentrations, suggesting a higher potency in antioxidant behavior, while *Clerodendrum infortunatum* also displayed consistent activity, particularly at higher doses. The presence of bioactive phytochemicals such as flavonoids, phenolics, and tannins in both plants may contribute to this observed antioxidant potential. Minor variations in absorbance readings among replicates were within acceptable limits, reflecting good reproducibility and assay reliability. The findings support the traditional medicinal use of both plants and highlight their potential as natural antioxidants for pharmaceutical or nutraceutical applications. Further studies involving isolation and characterization of individual compounds could provide deeper insights into their mechanisms of action. Overall, the study confirms that *Ehretia laevis* and *Clerodendrum infortunatum* are valuable sources of natural antioxidants and can be explored further for therapeutic development targeting oxidative stress-related disorders**.**

VI. ACKNOWLEDGMENT

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