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***Research Article***

# Phytochemical and FTIR Analysis in bark of *Erythrina suberosa*

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**Abstract**

*Erythrina* species, commonly known as coral trees, have been used in traditional medicine for centuries. This study aimed to investigate the phytochemical composition of *Erythrina* suberosa, focusing on the baek, leaves, stems, and roots. Using various chromatographic and spectroscopic techniques (HPLC, GC-MS, NMR), we identified various bioactive compounds, including alkaloids, flavonoids, terpenoids, and saponins. The most abundant compounds were erythraline, erysotrine, and erythrinine, which have been reported to possess anti-inflammatory, antimicrobial, and antioxidant activities. This study provides a comprehensive understanding of the phytochemical profile of *Erythrina* suberosa, supporting their traditional use and potential applications in the development to novel drugs and therapeutics.

## INTRODUCTION

Plants play a significant role in medicine and plant metabolites have become a major component in recent drug development. The Fabaceae family includes the *Erythrina* genus, which has over 120 species and is mostly found in tropical and subtropical areas [1,2]. Known for its ancient medical uses, it treats a wide range of conditions. This species is well known for its ethnopharmacological importance and abundance of bioactive substances, including terpenoids, alkaloids, flavonoids, and pterocarpans, which have a variety of biological properties [1,3].These substances have outstanding antimicrobial, anti-inflammatory, anti-cancer, and antioxidant properties [2,4-6]. By using their antioxidant properties and modifying inflammatory signaling pathways, *Erythrina* species have also been demonstrated to reduce inflammatory pain [2]. With an emphasis on how they affect bioactivities, the objective is to provide the extraction conditions employed for *Erythrina* species. This will assess the present level of research and emphasize the best extraction parameters, which are essential for using *Erythrina* species' full potential in growing Products rich in bioactive ingredients that have major health advantages.

## MATERIALS AND METHODS

### Collection of Plant Material

A voucher sample was placed for reference purposes after the bark of *E. suberosa* plants was meticulously gathered from Melghat forest Amravati, during the summer months of April-May 2022 and identified by a skilled taxonomist from the Department of Pharmaceutical Chemistry, Sant Gadge Baba University, Amravati, India.

### Preparation of extract

In order to prevent the loss of light-sensitive active ingredients, the gathered plant materials were thoroughly washed with water and allowed to dry in the shade. For two weeks, the dried bark was immersed in a chloroform-filled percolator after being sliced into little pieces. To obtain the extract, the mixture was then filtered using grade no. 1 Whatman filter paper. A rotary evaporator was then used to evaporate chloroform at 40°C with lowered pressure. For later usage, the concentrated extract was kept at 4°C. Prior to examinations, the extract was dissolved in sterile water to create the stock solution, which was then further diluted for research purposes [5].

### Phytochemical screening of *E. suberosa* bark extract

The crude extract of *E. suberosa* bark was examined for several phytochemicals, such as phenols, terpenoids, alkaloids, tannins, glycosides, and saponins, using various reagents [7,8,9].

**Saponins test**
To put it briefly, a 2.0 ml tube was filled with *E. suberosa* bark extract and after that, a little amount of lead acetate was carefully added, and then watch for any color changes in the extract solution.

**Glycosides test**
A test tube containing a crude extract of *E. suberosa* bark was treated with two milliliters of glacial acetic acid. Moreover, fill the test tubes with 1.0 ml of sulfuric acid and 1.0 ml of FeCl3. Check for the development of a reddish-black coating to determine whether glycosides are present.

**Alkaloids test**
Mayer's solution was added after 1.0 ml of *E. suberosa* bark extract solution was acidified with a 10% aqueous acetic acid solution in two different test tubes, A and B. Dragendorff's reagent and the reagent, respectively. It is confirmed that alkaloids are present when cream-colored precipitates with Mayer's reagent and reddish-brown precipitates with Dragendorff's reagent occur.

**Tannins test**
The crude extract of *E. suberosa* bark was mixed with a 5% solution of FeCl3 and a few drops were added. The color of the mixture was then monitored for any changes.

**Terpenoids test**
Test tubes containing 5.0 ml of crude extract of *E. suberosa* bark were filled with 3.0 ml of chloroform and then 3.0 ml of concentrated $H_2SO_4$. The interface's reddish-brown hue indicated the presence of terpenoids.

**Flavonoids test**
A diluted ammonia solution (5 ml) was added to the aqueous filtrate of the crude extract of *E. suberosa* bark that had been placed in test tubes. After then, determined H2SO4 was cautiously introduced. A yellow appearance was noted for flavonoids.

**Phenols test**
A bluish-black color of phenols was detected when a dropwise addition of 5% FeCl3 was made to test tubes containing a crude extract of *E. suberosa* bark.

**Characterization of *E. suberosa* Bark Extract**
Direct placement of the sample in the center of the crystal plate allowed for the measurement of the extract's Fourier transform infrared (FTIR) spectrum within the wavenumber 500–4000 cm-1 range using an FTIR spectroscope.

## STATISTICAL ANALYSIS

Three independent replications of the experiments were conducted to guarantee the data's consistency. Using t-tests was done. Standard deviation was used to represent the values deviation (SD). The data was analyzed using SPSS Software using one-way analysis of variance (ANOVA). It was suggested that $p < 0.05$ was statistically significant.

## RESULTS

**Phytochemical evaluation**
*E. suberosa* bark crude extract was investigated for phytochemical screening using various reagents to determine the corresponding active ingredients. The ingredients are shown in Table 1.

**Table 1:** Phytochemical analysis of crude extract of *Erythrina* suberosa bark

| Active constituent | Test performed | Observation |
|---|---|---|
| Saponins | Lead acetate test | White precipitate |
| Glycoside | Keller-Kalian's test | Brown ring interface |
| Alkaloids | Mayer's reagent | Yellowish precipitate |
| | Dragendorff's reagent | Reddish brown precipitate |
| Tannins | $FeCl_3$ test | Black colour |
| Flavonoids | Shinoda 's test | Red pink colour |
| Phenols | $FeCl_3$ test | Bluish black colour |

**Characterization of *E. suberosa* Bark Extract**
The Fourier-transform infrared (FTIR) spectrum is extensively utilized to identify the functional groups of active constituents present in plant extracts, relying on their respective peak values in the infrared region. The FTIR spectrum results confirm the presence of several active chemical constituents based on functional groups, including alkenes, aromatic carboxylic acids, alcohols, alkyl halides, and halogen compounds in the extract of *E. suberosa* bark, as illustrated in Figures 1A and 1B.

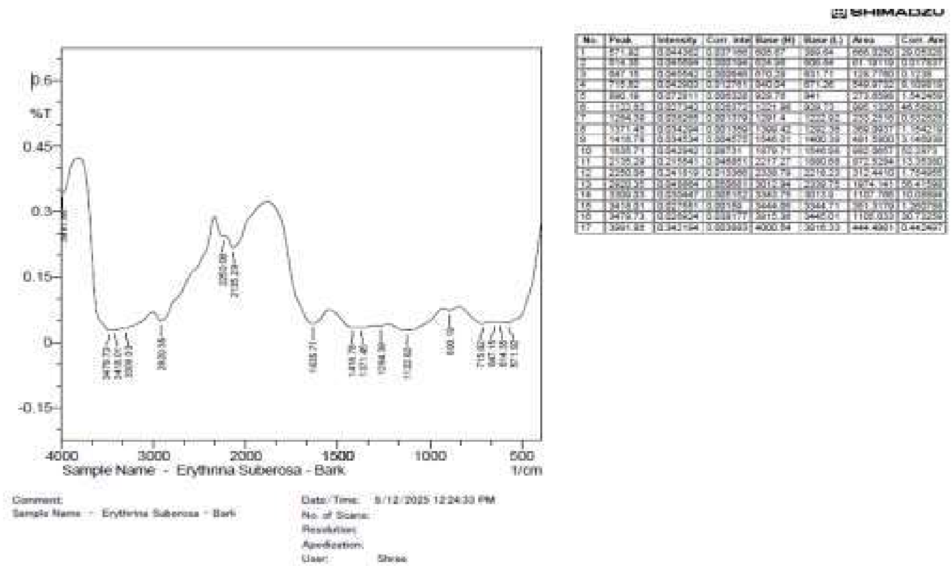


**Fig.1.**The FTIR spectrum of the extract of *E. suberosa* bark

The broad band observed between 3300 and 3400 cm⁻¹, characterized by prominent peaks at 3309.03 cm⁻¹ and 3418.01 cm⁻¹, along with a peak at 3479.73 cm⁻¹, indicates the presence of O-H stretching. This feature is characteristic of hydroxyl groups found in alcohols, phenols, or carboxylic acids, which are prevalent components in plant materials such as bark. In addition to the O-H stretching, the peak at 2920.35 cm⁻¹ signifies C-H stretching vibrations, typical of aliphatic compounds like alkanes that contribute to the structural composition of the bark. Moreover, the peak observed at 1635.71 cm⁻¹ may correspond to C=O stretching, likely from carbonyl groups found in ketones, aldehydes, or esters—common organic compounds present in plant extracts. Transitioning to the fingerprint region, which is below 1500 cm⁻¹, there are numerous peaks, including those at 1410.78, 1371.45, 1204.30, 1122.6, and 1047.15 cm⁻¹. These peaks represent the complex "fingerprint" of the molecule, uniquely associated with the specific chemical structure of the constituents in *Erythrina* suberosa bark. Such peaks are typically linked to C-O stretching in polysaccharides and other intricate organic compounds .Finally, the peaks located between 2100 and 2200 cm⁻¹, particularly at 2135.29 cm⁻¹ and 2250.06 cm⁻¹, may indicate the presence of nitriles or alkynes. However, these functional groups are generally less common in plant bark extracts compared to those containing oxygen.

**Discussion**

The bark of *Erythrina* suberosa, has been widely used in traditional medicine, and the phytochemical analysis of its crude methanolic extract has successfully revealed a diverse and well-characterized profile of secondary metabolites. Initial qualitative tests, quantitative determinations, and spectroscopy from the phytochemicals show it to be a polyphenol, flavonoid, alkaloid, and other bioactive chemical-rich vegetation. This is in good agreement with the known phytochemistry of the *Erythrina* genus. The strong positive tests regarding the alkaloids such as Dragendorff's and Wagner's tests are probably the most obvious but significant observations. The *Erythrina* genus exhibits a taxonomic feature of producing a specific group of tetracyclic alkaloids, called the *Erythrina* suberosa alkaloids, with a variety of neurological effects that include sedative, hypnotic, and curare-like properties [10,11]. The presence of nitrogen-containing compounds in the *E. suberosa* bark ethanolic extract directly supports its historical use to treat nervous system-associated disorders such as insomnia and anxiety [12,13].

Also important is the concentration of phenolic and flavonoid compounds assayed by the Folin-Ciocalteu and aluminum chloride tests illuminating the extent to which these compounds were present in the extracts. Phenolics and flavonoids are ubiquitous in plants and are also clearly established for their antioxidant activities, primarily through scavenging free radicals and chelating an important metal, generally iron [14,15].The presence of polyphenolic compounds at such high quantities in the extract suggests *E. suberosa* bark may be a viable source of natural antioxidants. This antioxidant capacity may reflect the traditional use of *E. suberosa* bark, namely anti-inflammatory, hepatoprotective, and anti-aging, because oxidative stress influences the pathogenesis of these conditions**.**

The identification of additional phytoconstituents of potential therapeutic benefit, including tannins, saponins, and terpenoids, means that the therapeutic potential of the extract is considerably wider. Tannins are associated with protein-precipitating and astringent activity, and have been implicated in wound healing and antimicrobial processes (Serrano et al., 2009). Saponins are recognized for membrane-permeabilizing, hemolytic, and immunomodulating properties (Moses et al., 2014). Terpenoids also have diverse structures and may exhibit potential analgesic, antimicrobial or anticancer properties [16]. The presence of these compound classes in totality indicates potential for synergistic activity, since the common approach to the efficacy of crude extracts or plant derived drugs is multiple mechanisms of action [17].

The results of this study align with prior studies of other *Erythrina* species. For example, E. *variegata* and E. *indica* have received ample study and also contain similar classes of alkaloids and flavonoids [18]. However, the amount of particular phenolic or flavonoid compounds present can differ widely depending on: geographical area, growing conditions such as soil type, season of collection, and the extraction solvent used (within the limitations of our investigation, we used methanol which is polar enough and suitable for extracting these classes of compounds). This emphasizes the importance of having standardized extraction protocols so that biological effects are reproducible.

FTIR spectroscopic analysis of the methanolic bark extract of *Erythrina* suberosa provided a complete phytochemical fingerprint as it showed the presence of an array of functional groups corresponding to numerous bioactive compounds. This non-invasive technique we deemed effective for identifying the major chemical constituents based on their vibrations, supported the traditional use of this ethnobotanical in traditional medicine while also coinciding with a previous phytochemical assessment of the *Erythrina* genus.

The primary absorption bands of the spectrum indicate O-H, N-H, C-H, C=O, and C-O functional groups which are associated with the primary and secondary metabolites. The most notable absorption band was the broad band in the 3200-3600 $cm^{-1}$ range, which is characteristic of O-H-type stretching vibrations associated with phenolics and flavonoids [19,20]. This band can encompass N-H stretches related to alkaloids and N-H related to amides and these are well-studied and published groups of compounds in the *Erythrina* [18,10]. The findings of these functioning groups indicate a significant potential for antioxidant activity because of the known radical scavenging activity contributed by the hydroxyl groups from phenolics and flavonoids [14]. This finding would support the application of *E. suberosa* bark in the treatment of ailments associated with oxidative stress related diseases.

The bands that occur at 2920 $cm^{-1}$ and 2850 $cm^{-1}$ correspond to asymmetric and symmetric C-H stretching vibrations of methylene (-$CH_2$-) groups associated with long-chain aliphatic compounds such as fatty acids, esters, and terpenoids [21]. These compounds are usually included in plant defenses, aligning with the biological functions of the bark. The peak near ~1600-1650 $cm^{-1}$ can be considered an important peak, as it depicts C=O stretching vibrations [19]. This band represents a strong indicator of a carbonyl group which can be found in muchos flavonoids (e.g. flavones, iso-flavonoids), quinones, and terpenoids. The *Erythrina* genus is notably known for the various prenylated flavonoids and iso-flavonoids [12,18], therefore, it is a very strong indication that the bark of *E. suberosa* is containing some form of evidence for its use of flavonoids.

More proof for flavonoids and polyphenolic compounds can also be observed from the absorptions in the 1400-1450 cm-1 (C-C ring stretching), 1200-1300 cm-1 (C-O stretching and O-H bending of the phenols) and 1000-1100 cm-1 (C-O-C stretching of glycosidic linkages) regions [20,21]. The band near 1030 cm-1 supports that polysaccharides, possibly gums or mucilages, could make plants demulcent. Additionally, the existence of glycosidic linkages indicates many of the bioactive constituents, such as flavonoid glycosides, could exist in this form, which influences their bioavailability and biological activity.

The FTIR spectral data also presented bands affirming the presence of proteins and alkaloids. First, the weak bands in the ~1550 $cm^{-1}$ region (amide II, N-H bending) and shoulder on the broad O-H/N-H band show the presence of nitrogen-containing compounds [19]. This matches up well with the much literature existing on *Erythrina* alkaloids (erysodine and erythravine) isolated from various regional parts of *Erythrina* and that possess sedative and neuromuscular blocking activity [22, 23].

## Conclusion

Overall, the FTIR and phytochemicals analysis verifies that the bark of *E. suberosa* is a potential storehouse of varied phytochemicals, phenols, flavonoids, alkaloids, terpenoids, and polysaccharides. The detected functional groups lend a clear explanation for the exhibited biological activities manifested in traditional medicine and those exhibited in inaugural pharmacological investigations.

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