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Phytochemical profiling of methanolic leaf extract of *Erythrina variegata* L[#]

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Abstract

This study investigates the phytochemical composition of the methanolic leaf extract of Erythrina variegata (MLEV) using qualitative phytochemical screening, Fourier Transform Infrared (FTIR) spectroscopy and Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS). The extract exhibited a broad spectrum of bioactive compounds including alkaloids, steroids, tannins, flavonoids, glycosides, diterpenes, triterpenes, fatty acids and phenolic constituents. The FTIR spectroscopy revealed various chemical bonds or functional groups present in phytochemicals. The GC-MS/MS analysis identified 62 phytochemicals belonging to diverse classes such as terpenoids, fatty acid esters, alkaloids and phenolics, supporting the plant's traditional therapeutic value.

Keywords: *Erythrina variegata, qualitative phytochemical screening, FTIR, GC-MS/MS.*

Biologically active plant-derived compounds have gained increasing importance as therapeutic agents due to the growing acceptance of herbal medicine (Afolayan et al., 2009). Phytochemicals such as alkaloids, flavonoids, tannins, steroids, terpenoids, carbohydrates, and phenolic compounds constitute the major bioactive classes (Sowmya et al., 2015). *Erythrina variegata* (Fabaceae), traditionally used in Ayurveda and other Asian medicinal systems, is known for its nervine sedative, anti-asthmatic, antiepileptic, antiseptic, and anti-rheumatic applications, largely attributed to its rich content of alkaloids, flavonoids, and triterpenoids. Its hepatoprotective, antioxidant, anti-inflammatory, and CNS depressant activities have also been scientifically validated (Whistler and Elevitch, 2006). Extracts of *E. variegata* exhibit significant antioxidant and anti-inflammatory properties and have been reported to restore antioxidant balance, suppress inflammation, and modulate CYP2E1 activity key mechanisms involved in acetaminophen (APAP) induced hepatotoxicity (Mohapatra et al., 2023). However, comprehensive mechanistic evidence remains limited. Therefore, the present study aimed to evaluate the hepatoprotective potential of *E. variegata* through phytochemical screening, FTIR, and GC-MS/MS analyses to characterise its bioactive constituents.

Fresh leaves of *Erythrina variegata* were collected in January 2025 from Kanchiyar, Idukki district, Kerala, India (9.741736° N, 77.071453° E). The samples were washed with distilled water, shade-dried, and powdered using an

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electric pulveriser. A total of 51 g of powdered material was extracted with 900 mL methanol using a Soxhlet apparatus at 67–70 °C for three days (15–20 cycles). The solvent was removed under reduced pressure using a rotary vacuum evaporator, and the concentrated extract was stored at four degree Celsius until further analysis.

Extraction yield was calculated as: Yield (%) = (Weight of extract / Weight of sample) × 100

The methanolic extract was subjected to qualitative screening to detect steroids, alkaloids, glycosides, tannins, phenolics, flavonoids, diterpenes, triterpenes and saponins following (Table 1) standard procedures (Harborne, 1998).

Table 1. Qualitative phytochemical screening tests

Sl. No.	Phytochemical screened	Screening test
1	Steroids	Salkowski's test
2	Alkaloids	Dragendroff's test
		Mayer' test
		Wagner's test
		Hagner's test
3	Glycoside	Barford's test
4	Phenolic compounds	Ferric chloride test
5	Tannins	Ferric chloride test
		Gelatin test
6	Flavanoids	Ferric chloride test
		Lead acetate test
7	Diterpenes	Copper acetate test
8	Triterpenes	Salkowski's test
9	Saponins	Froth test

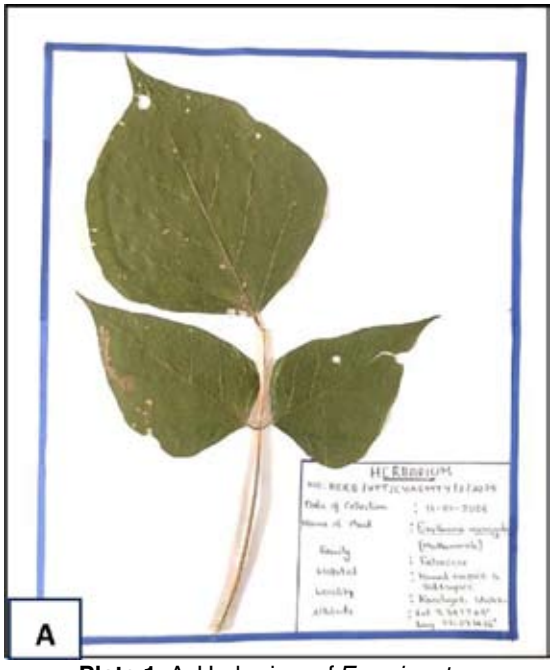

Plate.1. A. Herbarium of *E. variegata*

The ATR–FTIR spectroscopy was performed to identify functional groups and structurally related compounds in the methanolic leaf extract of *Erythrina variegata* (MLEV) using a PerkinElmer Spectrum Two™ FTIR spectrometer. Spectral interpretation was carried out using an FTIR functional group database with the Insta NANO search tool (Patil, 2025).

The phytochemical constituents of MLEV were further analysed using GC–MS/MS at the Central Instruments Laboratory, College of Veterinary and Animal Sciences, Mannuthy, Kerala and compounds identification was performed by comparison with the NIST MS Search 2.0 library (Gopalakrishnan et al., 2019).

The herbarium prepared (Plate1.A) from the leaves of *Erythrina variegata* was authenticated from Research and Postgraduate Department of Botany, St. Thomas College, Thrissur, Kerala.

The extraction yield of the methanolic extract from the leaves of *E. variegata* (MLEV) was determined to be 12.7 per cent. Mujahid et al. (2016) reported a comparable extraction yield of 12.5 per cent (w/w) for *E. variegata* leaves using methanol as the solvent.

The qualitative phytochemical screening of the methanolic leaf extract of *Erythrina variegata* (MLEV) revealed the presence of a wide range of secondary metabolites with potential biological significance. The extract tested positive for alkaloids, steroids, tannins, flavonoids, glycosides, diterpenes and triterpenes, while saponins were found to be absent. The results of the phytochemical tests are summarised in Table 2. These findings are consistent with earlier reports on *E. variegata*. Previous studies have documented the presence of

Table. 2. Qualitative phytochemical screening of MLEV

Sl. No.	Phytochemical screened	Screening test	MLEV
1	Steroids	Salkowski's test	+
2	Alkaloids	Dragendroff's test	+
		Mayer' test	+
		Wagner's test	+
		Hagner's test	+
3	Glycoside	Barford's test	+
4	Phenolic compounds	Ferric chloride test	+
5	Tannins	Ferric chloride test	+
		Gelatin test	+
6	Flavanoids	Ferric chloride test	+
		Lead acetate test	+
7	Diterpenes	Copper acetate test	+
8	Triterpenes	Salkowski's test	+
9	Saponins	Froth test	-
+ indicates presence; - indicates absence			

alkaloids, flavonoids, phenols, tannins, glycosides, steroids, and terpenoids in methanolic leaf extracts, reinforcing the phytochemical richness of the plant and supporting its traditional medicinal use.

The FTIR analysis of MLEV (Figure 1) revealed characteristic functional groups indicative of biochemical diversity. A broad absorption band at 3340 cm^{-1} corresponds to O–H stretching of alcohols and phenolic compounds, while medium-intensity peaks at 2918 and 2850 cm^{-1} represent C–H stretching vibrations of alkanes, suggesting the presence of long-chain hydrocarbon structures.

The present findings are consistent with the FTIR observations reported by Hemmalakshmi et al. (2017), who documented the presence of hydroxyl groups, amines, carboxylic acids, nitriles, terminal alkynes, carbonyl compounds, aromatic structures and alcohols in the ethanolic leaf extract of *E. variegata*. The agreement between the current study and previous literature reinforces the chemical complexity of *E. variegata* leaves and highlights the abundance of polyphenolic and nitrogenous compounds, which may contribute to its therapeutic potential.

The GC–MS/MS analysis of the MLEV revealed the presence of 62 bioactive phytocompounds. The chromatogram obtained is presented in Figure 2.

The GC-MS/MS analysis of the methanolic leaf extract of *Erythrina variegata* (MLEV) revealed a diverse phytochemical profile dominated by fatty acid esters, terpenoids, phenolics, and alkaloids. The major constituents identified included hexadecanoic acid methyl ester, methyl pentadecanoate derivatives, 2,6,10,10-tetramethyl-1-oxaspiro[4.5]decan-6-ol, tricycloundecan-5-ol, and α-amyrin. Among these, fatty acid esters were the most abundant, while monoterpenoid, sesquiterpenoid, and triterpenoid compounds were also prominently represented, with α-amyrin identified as a major triterpenoid constituent.

A wide range of terpenoids was detected, including diterpenes (gibberellic acid, ingenol derivatives, columbin, methyl abietate), monoterpenes (ethyl geranyl acetate), and sesquiterpenes such as bisabolene epoxide, widdrol hydroxyether, azulene derivatives, and 6-epi-shyobunol. The extract also contained significant levels of polyunsaturated fatty acid derivatives, free fatty acids, phenolic acids, and bioactive alkaloids.

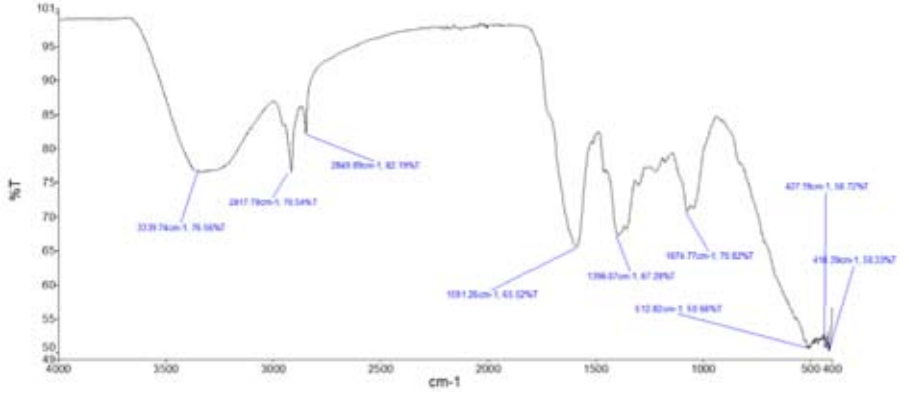

Fig. 1. FTIR spectra of MLEV depicting the absorption peaks corresponding to chemical groups present, with wave number along X axis and absorbance along Y axis.

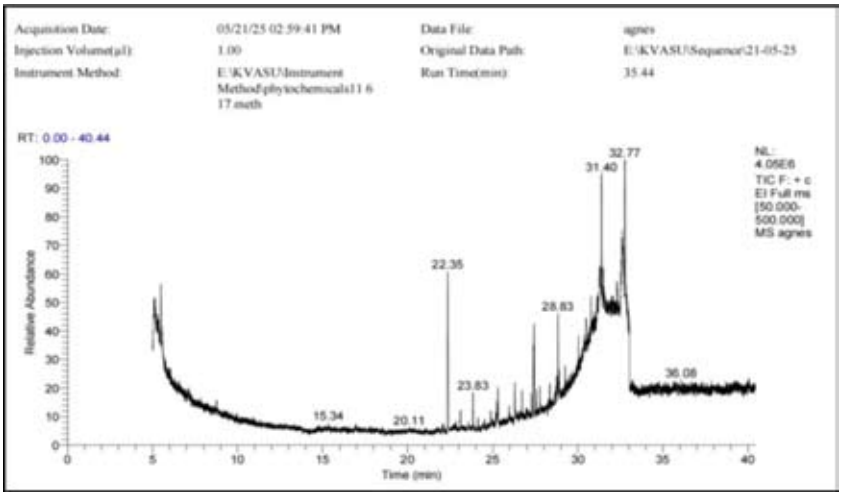

Fig. 2. Gas chromatography-tandem mass spectrometry chromatogram of MLEV with time (min) along X axis and relative abundance along Y axis

These findings are consistent with previous GC–MS reports on *E. variegata* leaf extracts, which documented similar classes of fatty acids, terpenoids, sterols, and phenolic compounds. Kavitha et al. (2023) reported several phytochemical constituents in the ethanolic leaf extract of *E. variegata*, including 1,2,3-butanetriol, diethyl phthalate, 6-hydroxy-4,4,7a-trimethyl-5,6,7, 7a-tetrahydrobenzofuran-2(4H)-one, n-hexadecanoic acid, 9,12,15-octadecatrienoic acid (Z,Z,Z), 9,12-octadecadienoic acid (Z,Z) 2-hydroxy-1-(hydroxymethyl) ethyl ester, 9-octadecenoic acid (Z) 2,3-dihydroxypropyl ester and eicosanoic acid. Similarly, Halimatussakdiah et al. (2025) identified neophytadiene, methyl hexadecanoate, methyl (9Z,12Z)-octadecadienoate, phytol, 3,7,11,15-tetramethyl-2-hexadecen-1-ol and stigmasterol through GC-MS analysis of MLEV.

The presence of phenolics, flavonoids, and terpenes, the well-established contributors to antioxidant and hepatoprotective activities along with structurally related hepatoprotective molecules, supports the therapeutic relevance of *E. variegata* leaves in mitigating drug-induced liver injury.

Summary

The present study provided a comprehensive phytochemical characterisation of the MLEV using qualitative screening, FTIR spectroscopy and GC–MS/MS analysis. The extract was found to contain a wide spectrum of secondary metabolites, including alkaloids, flavonoids, phenolics, tannins, glycosides, steroids, diterpenes, triterpenes, fatty acids and terpenoid derivatives. The FTIR analysis confirmed the presence of key functional groups such as hydroxyl, alkane, amine, carboxylic acid and metal-oxygen bonds, reflecting the biochemical diversity of the extract. The GC–MS/MS revealed 62 phytoconstituents belonging to multiple bioactive classes, with notable representation of fatty acid esters, terpenoids, phenolic compounds and alkaloids. The study highlights the therapeutic potential of *E. variegata* leaves and provides a strong foundation for future biological, pharmacological and *in-silico* investigations aimed at validating and harnessing its medicinal properties.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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