

PHYTOCHEMICAL SCREENING AND ANTI-BACTERIAL ACTIVITY OF *ERYTHRINA VARIEGATA* LEAF, STEM AND ROOT EXTRACTS

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Abstract: *Erythrina variegata* is a potent medicinal plant belonging to the family Fabaceae. Present investigation was carried out the preliminary phytochemical screening of the *Erythrina variegata* to evaluate the presence of alkaloids, flavonoids, glycosides, phenols, tannins, steroids/triterpenoids, quinones, saponins by using different parts of the plant extracts such as leaf, stem and root in five different solvent systems (methanol, butanol, chloroform, ethanol, and distilled water) by cold maceration technique. According to our evaluation the high intensity of secondary metabolites like alkaloids and glycosides were strongly observed in leaf butanol extract and complete absence of saponins except in aqueous solvent was seen. In stem extracts butanol and chloroform were more efficient solvents for alkaloids, glycosides, tannins and moderate for phenols and steroids. The results of root extract revealed the strong presence of alkaloids, flavonoids, glycosides in butanol extract. Due to its efficiency in butanolic extract *Erythrina variegata* was used to test anti-bacterial activity. Which showed the highest zone of inhibition against *Bacillus subtilis* in leaf and root extract whereas in stem butanolic extract highest zone of inhibition was against *Proteus vulgaris*.

Keywords: anti-bacterial activity, *Bacillus subtilis*, *Erythrina variegata*, plant extracts, *Proteus vulgaris*.

Introduction

Medicinal plants have the ability to cure many diseases which can be used as a resource for many drugs [PAVANI & SHASTHREE, 2022]. The presence of different phytochemicals in the plant determines the medicinal value of the plant [SANTHIYA & al. 2016]. Most of the developed countries use the compounds obtained from medicinal plants for the production of traditional medicines [YADAV & AGARWALA, 2011]. However, these plants should be studied to better understand their properties, and the safety upon their usage. The phytoconstituents identified from the plant material helps to predict the possible pharmacological activity of that plant [SHAIKH & PATIL, 2020]. Therefore, understanding the phytochemical profile of *Erythrina variegata* can provide valuable insights into its therapeutic potential and lead to the development of novel natural remedies or pharmaceutical agents.

Phytochemical screening is a crucial process in the field of plant science and pharmacology. It involves the systematic analysis and identification of various biologically active compounds present in plants. These compounds, known as phytochemicals, are natural chemicals that contribute to the plant's characteristics and play essential roles in their growth, development, and defense mechanisms [DOUGHARI, 2012].

Phytochemicals are of great interest to scientists and researchers because of their potential health benefits and medicinal properties. Many of these compounds have been found to possess antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, among others, making them valuable for drug development and alternative medicine [RALTE & al. 2022].

The alkaloids extracted from the leaves of *E. variegata* are reported to have analgesic and anti-inflammatory activity whereas iso-flavonoids isolated have antibacterial activity [KUMAR & al. 2010], cytotoxic [NKENGFAK & al. 2001], anthelmintic [JESUPILLAI & PALANIVELU, 2009], antiulcer and diuretic properties [SACHIN & ARCHANA, 2009].

E. variegata holds immense potential as a natural source of antimicrobial agents. Its long-standing use in traditional medicine and recent scientific evidence of its efficacy against various pathogens highlight the importance of further exploration. The therapeutic properties of this plant may offer a sustainable and effective solution to combat infectious diseases and address the growing concern of antimicrobial resistance. The various parts of *E. variegata*, such as leaves, bark, flowers, and seeds, are used to treat a range of ailments, including microbial infections. The plant's therapeutic potential is attributed to its diverse phytochemical composition, which includes alkaloids, flavonoids, tannins, saponins, and other secondary metabolites [SANTHIYA & al. 2016].

Studies investigating the antimicrobial properties of *E. variegata* have shown promising results against a wide spectrum of pathogens. Some studies suggest that *E. variegata* bioactive compounds may interfere with crucial enzymatic processes within microbial cells, further compromising their viability [BASKAR & al. 2010].

As a result, this study is being conducted to give phytochemical analysis, employing various solvents and *Erythrina variegata* anti-bacterial characteristics.

Material and methods

Collection of plant material

E. variegata plant was collected from the department of biotechnology, Kakatiya University, Warangal where the plants were grown and maintained in proper condition. The collected plant materials were washed thoroughly under running tap to remove dirt. After that the parts were separated and shade dried for 10 days and were made in to powder form for further use (Figure 1).

Preparation of extract

The 3 grams of each plant part powder was taken along with 30 ml of each solvent in separate conical flask the solvents used here were aqueous, butanol ethanol, chloroform and methanol and were incubated in orbital shaker for about 48 and later the extracts were filtered using Whatman filter paper. The final extracts were kept in a rotating shaker for 48 hours at 28 °C. After 48 hours, the extract was filtered subsequently subjected for preliminary screening by using standard methods protocol.



Figure 1. Parts of *Erythrina variegata*. (a) – Leaves; (b) – Leaves powder; (c) – Leaves extract; (d) – Stem; (e) – Stem powder; (f) – Stem extract; (g) – Root; (h) – Root powder; (i) – Root extract.

Preliminary screening (qualitative) of phytochemicals in plant *Erythrina variegata* is determined by using the following tests (Table 1-6)

Table 1. Tests for alkaloids

Test	Procedure	Observation
Mayers test	Few drops of filtered plant extract and 1 to 2 drops of Mayer’s reagent (along the sides of test tube)	A creamy white/yellow ppt
Hager’s test	0.5 ml filtered plant extracts and mixed with 1-2 drops of Hagers reagent	A creamy white ppt
Tannic test	0.5 ml filtered plant extracts and mixed with 10% tannic acid solution	Formation of buff color ppt

PHYTOCHEMICAL SCREENING AND ANTI-BACTERIAL ACTIVITY OF ERYTHRINA VARIEGATA ...

Table 2. Tests for flavonoids

Test	Procedure	Observation
Alkaline reagent test	1 ml filtered plant extracts and mixed with 2 ml of 2% NaOH solution (and add few drops dil. HCl)	An intense yellow color, becomes color less on addition of diluted acid
Ferric chloride	Extract aqueous solution mixed with few drops 10% Ferric chloride solution	A green precipitate was appeared

Table 3. Tests for glycosides

Test	Procedure	Observation
Molisch test	2 ml filtered plant extracts and mixed with 2 drops of alcoholic α -naphthol and 1 ml conc. H ₂ SO ₄ (along the sides of test tube)	A violet ring was formed in the middle of the two layers of the liquids
Conc. H₂SO₄ test	5 ml filtered plant extracts mixed with 2 ml glacial acetic acid and few drop of 5% FeCl ₃ + conc. H ₂ SO ₄	A browning or red ppt was formed

Table 4. Tests for saponins

Test	Procedure	Observation
Foam test	0.5 gm plant extract mixed with 2ml distilled water and vigorously shaken for 10 to 15 mints	Persistent foam layer was formed

Table 5. Tests for phenols

Test	Procedure	Observation
FeCl₃ test	Plant extract mixed with few drops 5% ferric chloride sol.	Dark green/bluish black color
Ellagic acid test	Plant extract was mixed with few 5% glacial acetic acid and 5% sodium nitrite Solution	Solution turns muddy/Niger brown precipitate

Table 6. Tests for tannins

Test	Procedure	Observation
Alkali reagent test	1 ml plant extract was added to 2 ml of 2% NaOH solution (and add few drops dil. HCl)	An intense yellow color, becomes color less on addition of diluted acid
FeCl₃ test	1 ml of plant extract mixed with few drops 5% ferric chloride sol.	Dark green/bluish black color
Gelatin test	1.0 ml Plant extract is dissolved in 5 ml distilled water and add 1% gelatin Solution and 10% NaCl	A white precipitate

Anti-bacterial activity

Using the well diffusion method, the plant extracts anti-microbial activity was evaluated in aseptic condition. the process in the well diffusion method, includes a volume of the microbial inoculum which is distributed over the entire agar surface. Next, a volume (20-80 μ l) of the extract solution is put into the well by aseptically making a hole with a diameter of 6 to 8 mm using a sterile cork borer or tip. The test microorganism is then placed on an appropriate agar plate, and the incubation process is continued. The antibiotic ingredient spreads across the

agar media and stops the tested microbial strain from growing. All the plates were incubated at 37 °C for 24 hours. After incubation, the size (diameter) of the inhibition zones was measured.

Results and discussions

Phytochemical screening

Leaf extract

The phytoconstituents like alkaloids and glycosides in leaf extract of *E. variegata* were found to be in more concentration in butanol followed by chloroform extract and whereas the other phytoconstituents like flavonoids and phenols were found to be more in butanolic extract (Figure 1 and Table 7). Similarly phytochemical analysis of the ethanolic leaf extract of *E. senegalensis* showed the presence of alkaloids, saponins and flavonoids in moderate quantities [NNAMA & al. 2017].

Table 7. Phytochemical constituents of *Erythrina variegata*

Part of plant	Components	Methanol extract	Butanol extract	Chloroform extract	Ethanol extract	Aqueous extract
LEAF	Alkaloids	+	++	+	+	-
	Flavonoids	+	++	++	+	-
	Glycosides	+	++	+	+	+
	Phenols	+	++	+	+	+
	Tannins	+	+	+	+	+
	Steroids/triterpenoids	-	+	-	+	+
	Quinones	-	-	-	-	+
	Saponins	-	-	-	-	+
STEM	Alkaloids	+	++	+	+	++
	Flavonoids	+	+	+	+	+
	Glycosides	+	++	++	+	+
	Phenols	+	+	+	-	+
	Tannins	+	+	+	++	+
	Steroids/triterpenoids	+	+	+	+	-
	Quinones	-	-	+	-	-
	Saponins	-	-	-	-	++
ROOT	Alkaloids	+	++	++	+	+
	Flavonoids	++	++	+	++	-
	Glycosides	+	++	+	+	+
	Phenols	-	+	-	-	+
	Tannins	+	+	+	++	++
	Steroids/triterpenoids	+	-	+	+	+
	Quinones	++	+	++	++	-
	Saponins	-	-	-	-	+

Stem extract

The phytoconstituents such as alkaloids were more in concentration in butanol and aqueous extract whereas glycosides were found to be more in butanol extract along with them tannins were found more abundant in chloroform and ethanol extract. Similarly, results were observed in *Zingiber officinale* by AHMED & al. (2022).

Root extract

The phytoconstituents like alkaloids and glycosides were found to be more abundant in butanol extract whereas flavonoids were in more concentration in methanol and butanol extracts. Similar, results were reported in *Mutuntinia calabura* leaf methanolic extract shown maximum zone of inhibition against *Escherichia coli* [SUVARCHALA & al. 2022]. Among all the extracts used saponins were found only in aqueous extract of all three plant parts like leaf, stem, root.

Anti-bacterial activity for leaf butanolic extract

The anti-bacterial activity of leaf, stem and root butanolic extracts (20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml) and the control streptomycin (10 µg/ml) was evaluated. The butanolic leaf extract shown a maximum zone of inhibition at 80 µg/ml when compared to other concentrations. As the concentration of plant extract increased, the inhibition zone was also found to increase. The maximum antibacterial activity was shown towards *Bacillus subtilis* and *Escherichia coli* at 80 µg/ml concentration, followed by *P. vulgaris* and least zone of inhibition was shown by *B. Sphaericus* (Figure 2, 3). Similar work were reported in *M. cymbalaria* methanolic extract showed a high inhibition zone against *E. coli* [CHAITANYA & al. 2021] and the antibacterial activity of *Moringa oleifera* ethanolic leaf extracts shown towards *Staphylococcus aureus* and *Escherichia coli* [JAHAN & al. 2022].

Anti-bacterial activity for stem butanolic extract

The stem butanolic extract revealed considerable anti-microbial activity with large extent of inhibitory zones against *P. vulgaris*, followed by *E. coli* in contrast to the other bacterial strains tested (Figure 4, 5). However, it was shown that *B. sphaericus* have minimal antibacterial activity. The similar findings were reported in stem methanolic extract of *Sesbania grandiflora* [ANANTAWORASAKUL & al. 2011].

Anti-bacterial activity for root butanolic extract

The antibacterial properties of the various concentrations of root extract (20 µg/ml, 40 µg/ml, 60 µg/ml, and 80 µg/ml) were evaluated in comparison to the control (10 µg/ml). Compared to the other concentrations, the 80 µg/ml concentration displayed the largest zone of inhibition (Figure 6, 7). The root butanolic extract effectively inhibited *B. subtilis* in a zone. *E. coli*, *P. vulgaris* and *B. sphaericus*, however, demonstrated the least antibacterial action. Similar findings were reported in root extract of *Diploknema butyracea* [CHHETRY & al. 2022].

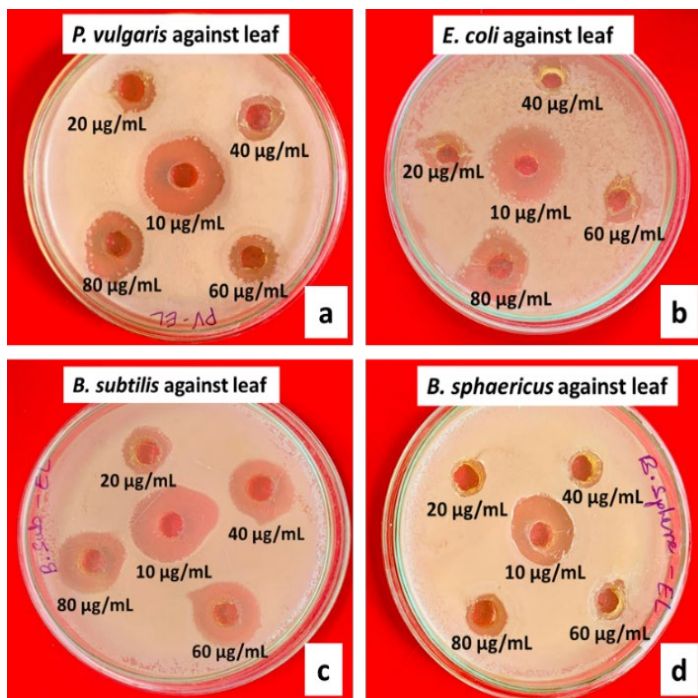


Figure 2. Anti-bacterial activity of butanolic leaf extracts of *E. variegata* against different bacterial strain
 a. Zone of inhibition against *P. vulgaris*; b. Zone of inhibition against *E. coli*;
 c. Zone of inhibition against *B. subtilis*; d. Zone of inhibition against *B. sphaericus*

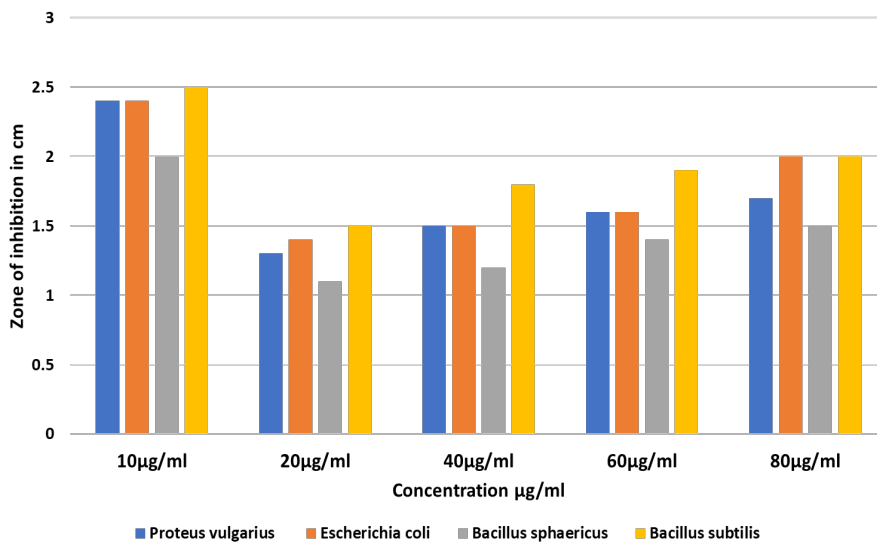


Figure 3. Zone of inhibition for butanolic leaf extract of *Erythrina variegata*

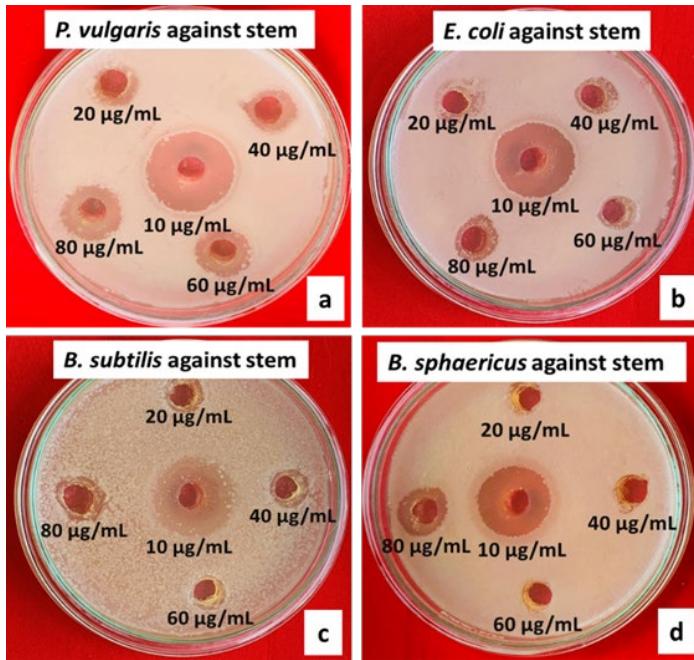


Figure 4. Anti-bacterial activity of butanolic stem extracts of *E. variegata* against different bacterial strain: a. Zone of inhibition against *P. vulgaris*; b. Zone of inhibition against *E. coli*; c. Zone of inhibition against *B. subtilis*; d. Zone of inhibition against *B. sphaericus*

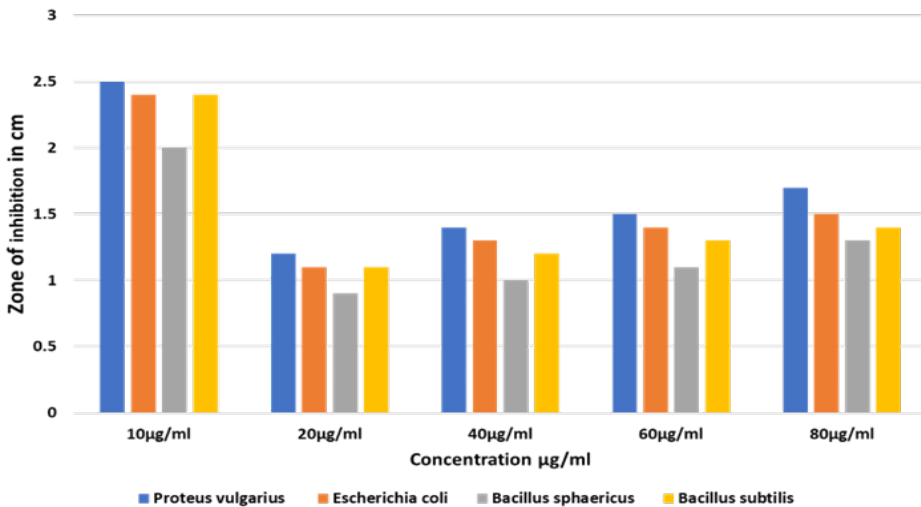


Figure 5. Zone of inhibition for butanolic stem extract of *Erythrina variegata*

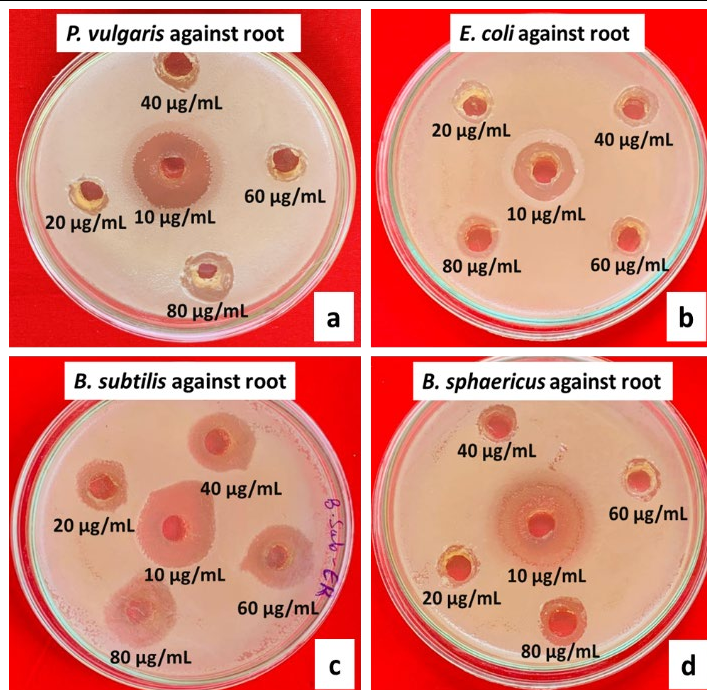


Figure 6. Anti-bacterial activity of butanolic root extracts of *E. variegata* against different bacterial strain. a. Zone of inhibition against *P. vulgaris*; b. Zone of inhibition against *E. coli*; c. Zone of inhibition against *B. subtilis*; d. Zone of inhibition against *B. sphaericus*

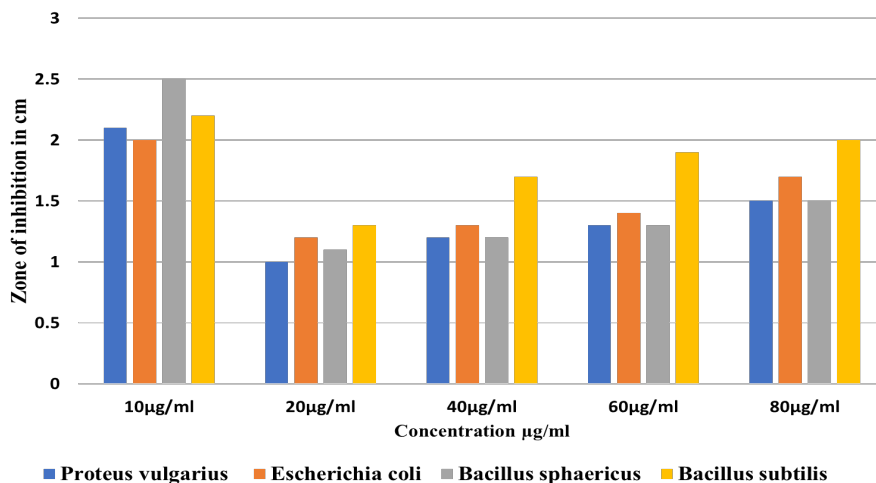


Figure 7. Zone of inhibition for butanolic root extract of *Erythrina variegata*

Conclusions

This study is concluded that the phytochemical screening of *E. variegata* showed positive results for the presence of phytochemicals like alkaloids, flavonoids glycosides, steroids and quinones in butanolic extracts of leaf stem and root. Anti-bacterial activity of butanolic extracts was carried out which showed a maximum zone of inhibition at 80 µg/ml compared to other concentrations. As the concentration of the plant extract increased, the inhibition zone was also found to increase, which concludes the anti-bacterial activity of *E. variegata* and secondary metabolites obtained from it have medicinal properties. It is useful for the production of medicine to treat various diseases.

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