ABSTRACT
In the present study various extracts of *Erythrina variegata* such as aqueous, methanol, ethanol, hexane and DMSO were evaluated for their phytochemicals and antimicrobial activity against selected five bacterial and two fungal strains by using cup diffusion method. The activities of the samples were compared with that of standard antibiotics. Phytochemical screening of *Erythrina variegata* leaf extract in five different solvents showed the presence of important phyto-constituents like phenols, alkaloids, flavonoids, tannins and saponins. In antimicrobial assay, all the organisms respond to the plant extract but inhibitory zone developed varied according to the concentration. Among the selected (aqueous, methanol ethanol, hexane and DMSO) extracts, the ethanolic extract showed a higher activity than other extracts. The ethanolic leaf extract of *E. variegata* showed higher activity against *Pseudomonas aeruginosa* followed by *Klebsiella pneumoniae, Escherichia coli* and *Bacillus cereus*. The methanolic leaf extract of *E.variegata* showed maximum zone of inhibition against *Staphylococcus aureus*. The ethanolic extract of *E.variegata* exhibited high antifungal activity against *Aspergillus niger* and *Aspergillus fumigatus* followed by methanol, DMSO, hexane and aqueous extracts. The results revealed that the antimicrobial activity exhibited by *E. variegata* ethanolic extract noted to be most effective than other solvents.

**Keywords:** Antimicrobial activity, Antibiotics, *Erythrina variegata*, Phytochemical screening.

INTRODUCTION
Nature has been a source of medicinal agent for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of these isolations were
based on the uses of the agents in traditional medicine. This plant – based traditional medicine system continues to play an essential role in health care, with about 80% of the world’s inhabitants relying mainly on traditional medicines for their primary health care.\cite{1,2} Many people still use traditional herbs to treat a variety of diseases including bacterial infections.\cite{3,4} In the past few years, the development of resistance by pathogens to many of the commonly used antibiotics provides sufficient impetus for further attempts to search for new antimicrobial agent.\cite{5} Recently, the acceptance of traditional medicine as an alternative form for health care and the development of microbial resistance to the available antibiotics has led authors to investigate the antimicrobial activity of medicinal plants.\cite{6-10} Secondary plant metabolites (phytochemicals) with antibacterial potency have been actively investigated as alternatives to and / or combination agents with antibiotics for the therapy of bacterial infections.\cite{11,12}

The genus *Erythrina* comprises of about 110 species of trees and shrubs. The name “coral tree” is used as a collective term for these plants. Coral tree is indigenous to the Old World tropics, possibly originally from India to Malaysia, but is native of ancient westward to Zanzibar and eastward to eastern Polynesia (the Marquesas). It is typically found on sandy soil in littoral forest, and sometimes in coastal forest up to 250m (800ft) in elevation. The most attractive type, var. *variegata*, is grown for its variegated leaves, as well as its seasonal showy red flowers.\cite{13-15} Different parts of *E. variegata* have used in traditional medicine as nervine sedative, febrifuge, anti-asthmatic and antiepileptic.\cite{16} In the some experiments, it has potential effects for treatment of some diseases like convulsion, fever, inflammation, bacterial infection, insomnia, helminthiasis, cough, cuts and wounds.\cite{17-20} We therefore undertook this study to evaluate the phytochemical constituents and antimicrobial potential of the various solvent extracts, from the leaves of *E. variegata*.

**MATERIALS AND METHODS**

**Collection of plant sample**

The fresh leaves of *E. variegata* were collected from Kolli Hills, Namakkal district, Tamilnadu, India.

**Preparation of leaf extracts**

The leaves were washed thoroughly with tap water and in distilled water and then dried the leaves at room temperature. The dried leaves were ground to a fine powder in a mechanic grinder. About 25gm of powdered plant material was uniformly packed into a thimble and
extracted with 250ml of different solvents separately. Solvents used were methanol, ethanol, hexane and DMSO. The process of extraction continues till the solvent in siphon tube of an extractor become colorless. Aqueous extract was prepared by the cold maceration method. The extracts were filtered through Whatman No.1 filter paper and the solvent was removed by evaporating in a water bath, which gave rise to a solid mass of the extract.

**Phytochemical Analysis**
The qualitative analysis of tannins, phenols, glycosides, alkaloids, steroids and flavonoids were analyzed by standard method. [21]

**Selected test microorganisms**
Extracts were tested against pathogenic microbes, including the bacteria *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa;* the fungi *Aspergillus niger and Aspergillus fumigatus.*

**Antibacterial Assay**
Antibacterial activity of plant extracts was carried using cup-plate agar diffusion method with some minor modifications. [22]

**Antifungal Assay**
The cup-plate agar diffusion method was adopted with some minor modifications to assess the antifungal activity of prepared extracts. [22]

**RESULTS AND DISCUSSION**

**Table 1: Preliminary phytochemical screening of selected solvent extracts of *E. variegata***

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the Phytochemicals</th>
<th>Aqueous</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>Hexane</th>
<th>DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Tannins</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Resins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

“+” indicates presence, “-” indicates absence.
With the increase in the incidence of resistance to antibiotics, alternative natural products of plants could be of interest. Some plant extracts and phytochemicals are known to have antimicrobial properties, which could be of great importance in the therapeutic treatments. The preliminary phytochemical results of selected solvent extracts of *E. variegata* were showed in the table - 1. Phytochemical screening of aqueous leaf extract of *E. variegata* contained Glycosides, phenols and tannins. Methanolic extract revealed the presence of flavonoids, glycosides, phenols, tannins and resins. The ethanolic extract showed the presence of alkaloids, flavonoids, phenols, tannins and resins. The qualitative phytochemical analysis indicates that hexane extract possess flavonoids, glycosides and saponins. DMSO extract were found to contain flavonoids, glycosides, phenols and tannins. From the phytochemical analysis it was noted that all the extracts of *E.variegata* leaf are rich in various secondary metabolites such as phenolics, alkaloids, flavonoids, tannins and saponins. Among the various solvents screened for phytochemicals, ethanolic extract is very effective followed by methanol, DMSO, hexane and aqueous extracts. It has been mentioned that antioxidant activity of plants might be due to their phenolic compounds. Flavonoids are most commonly known for their antioxidant activity. They are modifiers which modify the body’s reactions to allergens, viruses, and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anticancer activity. The presence of alkaloids explains its anti-bacterial activity, since this phytochemical is reported to have anti-bacterial activity. Tannins are reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects. Phytotherapeutically tannin-containing plants are used to treat nonspecific diarrhoea, inflammations of mouth and throat and slightly injured skins.

The antimicrobial activity have been screened because of its great medicinal relevance with the recent years, infections have increased to a great extent and resistance against antibiotics, become an ever increasing therapeutic problem. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobial compounds.

Table – 2 represents the antibacterial effect of selected solvent extracts of *E.variegata* by cup diffusion method against selected bacterial strains and the zone of inhibition was assessed in millimetre diameter. The aqueous extract of *E.variegata* showed moderate zone of inhibition against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia,*
Pseudomonas aeruginosa and minimal response against Bacillus cereus. Methanolic extract of E.variegata leaves showed significant activity against B. cereus (12 mm), E. coli (11.5 mm), Pseudomonas aeruginosa (11.5 mm) S.aureus (10 mm), and K. pneumoniae (9.5 mm) were documented. Methanolic extract exhibits significant antibacterial activity when compared with the standard antibacterial agents (Gentamicin and Ciprofloxacin). The ethanolic extract of E.variegata at 3000µg concentration shows maximum zone of inhibition (12mm) against Pseudomonas aeruginosa, it is also nearer to standard antibiotic ciprofloxacin. Ethanolic extract showed good antibacterial activity and the result also nearer to the zones produced by the (Gentamicin and Ciprofloxacin) standard anti-bacterial agents. Among the selected bacterial strains Pseudomonas aeruginosa was more sensitive to ethanolic extract of E.variegata. The antibacterial activity of hexane extract of E. variegata showed maximum zone of inhibition (11mm) at 3000µg against B. cereus compared to other bacterial strains.
### Table 2: Antibacterial activity of selected solvent extracts of *Erythrina variegata*

<table>
<thead>
<tr>
<th>Species</th>
<th>Aqueous extract</th>
<th>Methanol extract</th>
<th>Ethanol extract</th>
<th>Hexane extract</th>
<th>DMSO extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gm 10µg</td>
<td>1000 µg</td>
<td>2000 µg</td>
<td>3000 µg</td>
<td>Gm 10µg</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>8.5±0.5</td>
<td>2.5±0.6</td>
<td>5.0±0.4</td>
<td>6.5±0.3</td>
<td>13.5±0.1</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>8.5±0.2</td>
<td>3.0±0.5</td>
<td>5.0±0.5</td>
<td>4.0±0.3</td>
<td>14.5±0.5</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>10.5±0.4</td>
<td>3.5±0.3</td>
<td>5.0±0.5</td>
<td>7.0±0.5</td>
<td>14.0±0.3</td>
</tr>
<tr>
<td><em>Klebsiella Pneumonia</em></td>
<td>9.5±0.7</td>
<td>3.0±0.5</td>
<td>4.5±0.9</td>
<td>6.5±0.7</td>
<td>13.0±0.5</td>
</tr>
<tr>
<td><em>Pseudomonas Aeruginosa</em></td>
<td>11.0±0.5</td>
<td>3.0±0.5</td>
<td>4.0±0.7</td>
<td>6.0±0.5</td>
<td>13.5±1</td>
</tr>
</tbody>
</table>

Values are Mean ± S.D (n=3)
The DMSO extract of *E. variegata* at 3000µg concentration showed maximum zone of inhibition (11.0mm) against *B. cereus*, it is also nearly similar to standard antibiotic Gentamicin. The DMSO extract of *E. variegata* at 3000µg concentration against *Pseudomonas aeruginosa* showed minimum zone of inhibition. Among all the solvents, ethanol was proved as the most effective solvent for extracting broad spectrum of antibacterial compounds from plants. The antibacterial activity of the plants may be due to the presence of various active principles in them. In the present study the inhibitory action of the *Erythrina variegata* extract was found to increase with an increase in concentration against the selected bacterial and fungal strains. The zone of inhibition developed against the bacterial species seems to be interesting and notable. All the organisms responded to the plant extract but inhibitory zone developed varied according to the concentrations. Nearly the concentration (3000µg) seems to have a maximum inhibitory zone against the bacterial strains. All the selected extracts of *E. variegata* possess significant antibacterial activity against the gram positive and gram negative pathogens. Among the selected (ethanol, methanol, hexane, DMSO, aqueous) extracts of *E. variegata*, the ethanolic extract showed a higher activity than other extracts. This may be due to the solvent extract containing different constituents having antibacterial activity.

**Antifungal Screening**

Antifungal activity of various solvent extracts of *E. variegata* (Aqueous, Methanol Ethanol, Hexane and DMSO) were studied using Potato Dextrose Agar medium by Cup diffusion method using Ketoconazole as standard and the zone of inhibition was assessed in millimetre diameter. The fungal strains *A. niger* and *A. fumigatus* were used for this study.

The importance of fungicidal activity investigation cannot be over emphasized in view of the fact that, fungal infections of the skin, nails, and hair are a major source of morbidity throughout the world. \(^{[29]}\) From the table 3, the aqueous extract of *E. variegata* showed low zone of inhibition at 2000µg (2.5mm) and moderate zone of inhibition at 3000µg (4.5mm) against *A. niger*. Findings of cup diffusion method for methanolic extract of *E. variegata* against selected fungal strains showed good antibacterial activity and the methanolic extract of *E. variegata* showed maximum zone of inhibition (8mm) against *A. niger* and possess moderate zone of inhibition against *A. fumigatus*. 
Table 3: Antifungal activity of different solvent extracts of *Erythrina variegata*

<table>
<thead>
<tr>
<th>Fungal Species</th>
<th>Zone of Inhibition (mm)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ketoconazole</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10µg (std)</td>
<td>10.0±0.4</td>
<td>9.0±0.3</td>
<td>12.0±0.6</td>
<td>10.0±0.5</td>
<td>9.5±0.7</td>
<td></td>
</tr>
<tr>
<td><strong>Aspergillus niger</strong></td>
<td>2.5±0.7</td>
<td>4.5±0.5</td>
<td>5.0±0.9</td>
<td>8.0±0.5</td>
<td>5.0±0.6</td>
<td>8.5±0.5</td>
</tr>
<tr>
<td>10µg (std)</td>
<td>10.0±0.3</td>
<td>10.5±0.7</td>
<td>10.5±0.9</td>
<td>10.0±0.8</td>
<td>10.0±0.5</td>
<td></td>
</tr>
<tr>
<td><strong>Aspergillus fumigatus</strong></td>
<td>2.0±0.8</td>
<td>4.0±0.4</td>
<td>3.0±0.3</td>
<td>6.0±0.7</td>
<td>6.0±0.5</td>
<td>7.0±0.7</td>
</tr>
</tbody>
</table>

Values are Mean ± S.D (n=3)

The ethanolic extract of *E. variegata* possess notable zone of inhibition when compared to standard antibiotic (Ketoconazole) against two different fungal strains *A. niger* and *A. fumigatus*. The antifungal activity of hexane and DMSO extract of *E. variegata* against *A. niger* and *A. fumigatus* possess moderate zone of inhibition compared to standard antibiotic (Ketoconazole). Both the species (*A. niger* and *A. fumigatus*) showed responses against *E. variegata* at varied concentration from 2000 to 3000µg. From the selected extracts of *E. variegata*, ethanolic extract showed the maximum zone of inhibition against *A. fumigatus* and *A. niger*. The results of the present work indicate that the selected extracts of *E. variegata* possess antifungal properties.

**CONCLUSION**

The antimicrobial activity of the crude extract from the leaves of *E. variegata* may be due to the presence of various phytochemical constituents indulged in them. Therefore, the alcoholic extracts of *E. variegata* could be recommended as a source of pharmaceutical materials required for the preparation of new antimicrobial agents.

**ACKNOWLEDGEMENT**

We are thankful to Department of Biochemistry, Periyar University, Salem, Tamil Nadu, for providing the necessary infrastructural facilities.

**FUNDING**

We are thankful for the financial support provided by Periyar University (URF).
REFERENCES


