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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING, TOTAL TANNIN, SAPONIN CONTENT AND ANTIBACTERIAL ACTIVITY OF LEAF EXTRACTS OF *ERYTHRINA VARIEGATA.* L

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ABSTRACT

In the present study aqueous leaf extract of *Erythrina variegata* were investigated the phytochemical screening, total tannin, total saponin content and antibacterial activity. Phytochemical screening of various extracts such as aqueous, ethanol, chloroform, acetone and petroleum ether, aqueous leaf extract revealed the presence of steroids, quinones, cardiac glycosides, saponins, tannins, phenols, flavonoids, terpenoids and alkaloids than other extracts. The leaf extract were quantitatively estimated for total tannin and saponin content. The optimum yield of tannins and saponin content was found in aqueous leaf extract (46.7mg TAE/ g) and (36.1mg/gm) of *Erythrina variegata*. Different concentrations of aqueous leaf extracts were tested for the anti-bacterial activity against *Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus subtilis* using the agar disc diffusion technique. The aqueous leaf extracts from *Erythrina variegata* had a superior level of antimicrobial activity. The powerful antibacterial effect is attributed to the greater amount of tannins and saponin compound in the aqueous leaf extract of *Erythrina variegata*.

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INTRODUCTION

Medicinal plants are the most exclusive source of life-saving drugs for majority of the world's population. For ages nature has gifted us plenty of herbs and plants which form the main source of traditional medicines used to help in relief from illness and are still widely used all over the world. Herbal treatment is still used for many health problems. Herbs are safe, less toxic, economical and a reliable key natural resource of drugs all over the world (Al-Essa *et al.,* 1998). The secondary metabolites are known to play a major role in the adaptation of plants to their environment and also represent an important source of pharmaceuticals (Makkar and Becker, 1998). Plant-derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. Dietary phytochemicals are considered as an effective tool to cure body disorder. They play important roles as therapeutic agents in the prevention of many diseases (Kareem *et al.,* 2010). Among the different plant derivatives, secondary metabolites have been proven to be the most important group of compounds that showed a wide range of antibacterial and antifungal activity (Ahmed *et al.,* 1999; Rahman *et al.,* 1999). Tannins are high polyphenolic compounds present in plants, foods, and beverages, soluble in water and polar organic solvents. These tannins are classified as hydrolysable and condensed tannins based on their chemical structure and biological activity (Feeny, 1970). Both types of tannins are capable of forming strong complexes with certain type of proteins depressing the rate of their digestion (Barry and Manley, 1984). Tannins may also bind to bacterial enzymes or form indigestible complexes with cell wall carbohydrates reducing the cell wall digestibility (Barry *et al.,* 1986). In recent years, tannins have been investigated to possess high antioxidants (Koleckar *et al.,* 2008), antimicrobial (Ho *et al.,* 2006), gastro protective, and anti-ulcerogenic activities (Ramirez *et al.,* 2003). Due to these therapeutic properties, tannins can be used in the treatment of various diseases to improve human health. Saponins are a group of secondary metabolites that are found a plenty in a variety of plants. They are amphiphilic high molecular weight glycosides with unique foaming characteristics, which can be attributed to a combination of hydrophobic or fat-soluble sapogenin and hydrophilic or water soluble sugar.

The saponins are mainly produced by plants, low marine animals, and a few species of bacteria (Riguera, 1997; Yoshiki *et al.,* 1998). The saponins are considered a part of plant's defense mechanism, however, the function of saponins in the plant cell has not been comprehensively studied and understood. In one of the studies the role of saponins was identified as a compound causing membrane permeability, which results in cell leak and cytotoxic and antibacterial effect (Wink, 2003). The genus *Erythrina* comprises of about 110 species of trees and shrubs. 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 *variegata*d leaves, as well as its seasonal showy red flowers **(**Muthukrishnan *et al.,* 2014). *Erythrina variegata* is belongs to the family Fabaceae or Indian coral tree, is in an average size and grows rapidly in the deciduous forests all over India. The studies on phytochemical of *Erythrina* variegata have demonstrated alkaloids and flavonoids as major constituents. *E. variegata* parts (leaves, flowers, barks and roots) have been used in the natural medicines as nervine sedative, febrifuge, anti-asthmatic and antiepileptic. Traditionally, it has potential effects to heal some of the diseases like convulsion, fever, alzheimer, inflammation, bacterial infection, cough, ulcer, cuts and wounds(Chu *et al.,* 2000). Therefore, the purpose of the present investigation was to evaluate the total tannin, saponin content and antibacterial activity of leaf extracts of *Erythrina variegata*.

MATERIAL AND METHODS

Collection of material: The healthy leaves of *Erythrina variegata* (Fig 1) were collected during the middle of February 2016 from Thiruvallur district, Tamil Nadu, India. The collected parts were brought to the laboratory and maintained at PG & Research Department of Botany, Presidency College, Chennai and healthy leaves used for further experimental studies.

Preparation of the plant extract: Preparation of the extracts was done according to the methods prescribed by Ramachandra Kumar *et al.,* 2017. The dried leaf powder of *Erythrina variegata* plant materials were extracted with acetone, ethanol (75%), chloroform, petroleum ether and aqueous extract for 1 minute using an ultra turax mixer (13,000 rpm) and soaked overnight at room temperature. The extracts were then filtered through what man No.1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-evator at 40°C to a constant weight and then dissolved in respective solvents. The concentrated extracts were stored in an airtight container in the refrigerator below 10°C.

Phytochemical screening of *Erythrina variegata*: The phytochemical screening of leaf extract of *Erythrina variegata* were assessed by standard methods Savithramma *et al.,* 2011. Phytochemical screening was carried out on the leaf extracts using different solvents to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides, coumarins and steroids. General reactions in these analyses revealed the presence or absence of these compounds in the leaf extracts tested.

Estimation of Tannins content in leaf and extracts of *Erythrina variegata:* Tannins content in leaf extract of *Erythrina variegata* was estimated by standard method (Fagbemi *et al.,* 2005). The aqueous leaf extracts (1 ml) were mixed with Folin-Ciocalteau's reagent (0.5 mL), followed by the addition of saturated sodium carbonate solution (1 mL) and distilled water (8 mL). The reaction mixture was allowed to stand for 30 min at room temperature. The supernatant was obtained by centrifugation and absorbance was recorded at 725 nm using UV-Visible Spectrophotometer. Different concentrations of standard tannic acid were prepared and the absorbance of various tannic acid concentrations was plotted for a standard graph. The tannin content was expressed as µg tannic acid equivalent (TAE) per gram of the sample.

Determination of Saponin in *Erythrina variegata*: Quantitative estimation of saponin was carried out following the method as described by Obadoni and Ochuko, (2001). 10 g of fine powdered of *Erythrina variegata* were weighed and dispersed in 100 ml of 20% ethanol. The extract was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The filtrate and the residue were re-extracted with another 100 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and about 30 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight and the saponin content was calculated.

Antibacterial activity of leaf extract of *Erythrina variegata*: The leaf extract of ***Erythrina variegata*** plant were used for antibacterial study (Ozkan *et al.,* 2004; Janarthanam and Sumathi 2010). Different concentrations (10, 20 and 30 mg/ml) of the concentrated aqueous leaf extract was tested for its antimicrobial activity against strains such as *Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis*. The bacterial cultures were grown in Mueller Hinton Agar and Mueller Hinton broth (Himedia) (Lopez *et al.,* 2001). Antibacterial activity was measured using the standard method of diffusion disc plates on agar (Erturk *et al.,* 2003). Then 0.1ml of each culture of bacteria was spread on agar plate surfaces. For antibacterial assay, all bacterial strains were grown in Mueller Hinton Broth Medium (Hi media) for 24 hours at 37°C and plated on Mueller Hinton Agar (Hi media) for agar diffusion experiments. Paper disc (6mm in diameter) were placed on the agar medium to load 20µl of different concentrations of aqueous leaf extract of *Erythrina variegata* were tested. Inhibition diameters were measured after incubation for 24 - 48 hours at 37°C. Blanks of solvent only (processed in the same way), were also tested for antibacterial activity.

RESULTS AND DISSCUSION

In the present study, the phytochemical analysis of five different solvent extracts such as ethanol, chloroform, petroleum ether, acetone and aqueous studied, showed that the aqueous leaf extract of *Erythrina variegata* were rich in secondary metabolites such as phenol, tannins, saponins, terpenoids, steroid, flavonoids, cardiac glycosides, coumarins and alkaloids followed by other solvent extracts (Table 1).

Table 1. Phytochemical screening from leaf extracts of *Erythrina variegata*

Phytochemicals Tested	Leaf Extracts of *Erythrina variegata*				
	Aqueous	Ethanol	Chloroform	Petroleum ether	Acetone
Tannins	++	+	-	-	+
Saponins	++	-	+	-	+
Quinones	++	++	+	+	++
Terpenoids	++	+	+	+	+
Steroids	++	+	+	+	++
Flavonoids	++	+	-	-	+
Phenol	++	++	+	+	+
Alkaloids	+	+	-	-	-
Glycosides	+	-	-	-	-
Cardiac glycosides	+	+	-	-	+
Coumarins	++	+	+	+	+
Antho cyanin	-	-	-	-	-
Beta cyanin	+	+	+	+	+

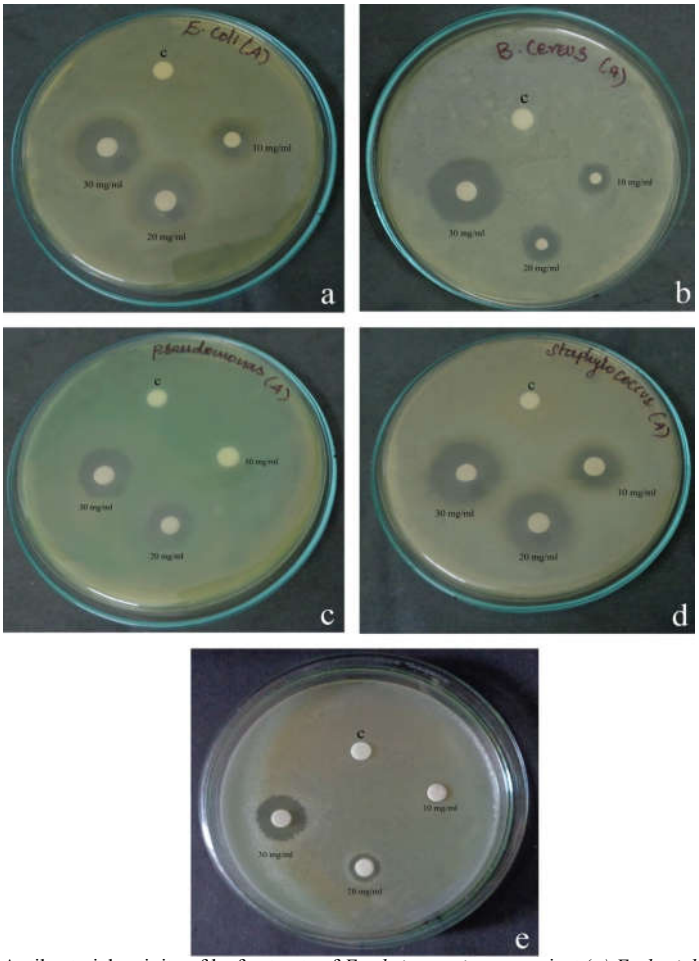
Key: + = positive, ++ = strong positive , - = negative

Table 2. Quantification of Phytochemicals from leaf extract of *Erythrina variegata*

Erythrina variegata	Total Tannin content (mg TAE/g)	Total Saponin content (mg/g)
Leaf	46.7	36.1

Table 3. Antibacterial activity from leaf extracts of *Erythrina variegata*

Micro-organisms Tested	Zone of inhibition (mm in diameter)		
	Concentrations of extract		
	10mg/ml	20mg/ml	30mg/ml
Erythrina variegata –Leaf extract			
Escherichia coli- MTCC No. 1563	8	10	16
Bacillus cereus- MTCC No. 10211	8	13	18
Pseudomonas aeruginosa- MTCC No. 14676	-	12	14
Staphylococcus aureus- MTCC No. 9542	9	10	16
Bacillus subtilis- MTCC No. 10224	-	11	16



Antibacterial activity of leaf extracts of *Erythrina variegata* against (*a) Escherichia coli, (b) Bacillus cereus, (c)Pseudomonas aeruginosa, (d) Staphylococcus aureus, (e) Bacillus subtilis*

Figure 2. Antibacterial activity from aqueous leaf extracts of *Erythrina variegata*

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, steroids, etc., (Britto and Sebastian, 2011) Thus, the preliminary screening test may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development (Doss *et al.,* 2009). The result of the present study recorded highest Tannins content in the leaf extract of *Erythrina variegata* and the tannins content was expressed as mg tannic acid equivalent (TAE) per gram of the sample. The optimum yield of tannins was found to be 46.7mg/gm TAE/ g dry weight from leaf of *Erythrina variegata* (Table 2). The effect of aqueous on extraction of tannins from *Erythrina variegata* leaf extracts was found to be good. The results corroborates with the findings of Vanimakhal and Ezhilarasi, (2016) who has reported the maximum yield of Tannins from aqueous extract of *Areca catechu.* Tannins are the natural polyphenolic compounds which can influence the nutritive value of different food stuffs utilized by human and other animals. Tannins also have large influence on the phytochemical and phytotherapeutic value of medicinal plants. Various methods have been used to increase the extraction efficiency of tannins from different medicinal plants for their use in pharmaceutical field (Cobzac *et al.,* 2005). The saponins content were estimated in the leaf extract of *Erythrina variegata*36.1 mg /g dry sample. The results support the findings of Rajat, (2015) that the maximum yield of saponin obtained from *Albizia chinensis.*

The data presented in Table 3, indicate that the leaf extracts of *Erythrina variegata* inhibit the growth of some microorganism in various concentration. The concentrations of 10 mg/ml -30 mg/ml aqueous leaf extract showed antimicrobial activity against *Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis* (Fig. 2). The maximum clear zone of inhibition was found at 30mg/ml of aqueous leaf extract of *Erythrina variegata*. In leaf extract, there is no zone of inhibition was found (*Bacillus subtilis* and *Pseudomonas aeruginosa*) in lower concentration 10mg/ml. Similar results were obtained on aqueous leaf extract of *Moringaoleifera***,** Crassocephalum crepidioides and *Chromolaenaodorata* which exhibited antibacterial activity. Kiran and Tafida, (2014); Omotayo *et al.,* 2015. Thus from our findings, it is concluded that the aqueous extracts from dry powdered leaf of *Erythrina variegata* had superior level of antimicrobial activity. The powerful antibacterial effect is attributed to the greater amount of tannins and saponins compound in the aqueous leaf extracts of *Erythrina variegata*.

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