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EVALUATION OF ANTIMICROBIAL ACTIVITY OF *ERYTHRINA MYSORENSIS* Gamb.

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Abstract

In the present investigation the Petroleum ether (60-80), chloroform, alcohol and aqueous extracts of *Erythrina mysorensis* Gamb, stem bark were investigated for antibacterial and antifungal activities. Chloroform and ethanol extracts possessed antibacterial activity in a concentration dependent manner against the gram +ve and gram –ve organisms. The effects of these extracts are compared to standard drugs, benzyl penicillin and streptomycin. Both the ethanol and aqueous extracts possessed antifungal activity in a concentration dependent manner against the fungus. The effects of these extracts are compared to standard drug gentamycin. The phytochemical screening of the extracts revealed the presence of secondary metabolites like glycosides, alkaloids, flavonoids, tannins, triterpenoids and saponins etc.

Key words:
Erythrina mysorensis Gamb, antibacterial, antifungal.

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INTRODUCTION

Despite of tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses are still a major threat to public health. Their impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance[1]. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases[2]. Therefore, researchers are increasingly diverting their attention to folk medicine, looking for new leads to develop better drugs against microbial infections [3]. Current research on natural molecule and products primarily focuses on plants since they can be sourced more

easily and be selected based on their ethno-medicinal uses [4].

Erythrina (Leguminosae), a genus of trees or shrubs, rarely herbs, is widely distributed in tropical and subtropical regions. About eight indigenous species and ten introduced ones occur in India [5]. *Erythrina mysorensis* is a small tree with a few or no prickles [6]. Many of the plants belonging to *Erythrina* genus possess secondary metabolites which are usually produced under stress conditions and often in response to infections. These secondary metabolites contain profound antimicrobial activities. The root extracts of *Erythrina variegata* possess antimicrobial activity against *Staphylococcus aureus* and *Mycobacterium smegmatis*[7]. Hence, the present work was undertaken to evaluate the antimicrobial activity in *Erythrina mysorensis* Gamb.

MATERIAL AND METHODS

Plant material:

The stem bark of *Erythrina mysorensis* Gamb., was collected from the regions of Shimoga District, Karnataka, India and authenticated by Prof. V. Krishna (one of the authors). The collected stem barks were washed, cut into small pieces and dried in the shade for about a week. Later the shade dried material was kept in an oven at 40°C to ensure complete drying. The dried material was finally grounded into coarse powder and preserved in an airtight container.

Preparation of Extracts

Coarse powder was subjected for successive extraction with petroleum ether 60-80, chloroform and alcohol in a Soxhlet apparatus and finally with chloroform and water by maceration. All the extracts were concentrated in vacuum using Rotary Flash Evaporator. They were further concentrated and dried in desicator. All the extracts were distilled, dried and used in the present study.

Drugs Used :

Streptomycin and Benzyl penicillin were used as standard for gram negative and gram positive antibacterial activity respectively, and Gentamycin was used as standard for antifungal activity.

Cultures:

Bacillus subtilis (NCIM 2920), *Staphylococcus aureus* (NCIM 5022), *Escherichia coli* (NCIM 2065), *Pseudomonas aeruginosa* (NCIM 2945), *Candida albicans*, and *Aspergillus niger*. These cultures were procured from National Collection of Industrial Micro organism (NCIM), Pune, India.

Phytochemical investigation:

Phytochemical tests were carried out to find out the presence of phytoconstituents viz., alkaloids, glycosides, carbohydrates, proteins, flavonoids, tannins, triterpenoids, steroids and saponins etc.

Antibacterial activity:

The extracts and the standard drugs were dissolved in minimum quantity of DMSO and adjusted to make up the volume with sterile distilled water to get 50, 100 and 200 µg/ml. concentrations. The benzyl penicillin was used against Gram positive and streptomycin was used against Gram negative bacteria as standard drugs. The antibacterial activity tests were performed by cup plate method, [8-9]. The fresh cultures of bacteria, *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* were cultivated by inoculating into peptone broth and incubated at 37±2°C for 18-24 hours. This culture was mixed with nutrient agar media and poured into petridishes by following aseptic techniques. After solidification of the media, five bores were made at equal distance by using sterile steel cork borer. Different concentrations 50, 100 and 200µg/ml of test extracts and standard drugs were introduced into these cups. DMSO was used as a control. The plates were placed in a refrigerator at 8-10°C for proper diffusion of drugs into the media. After two hours of

cold incubation, the petriplates were maintained in an incubator at 37°C for 24hrs.The plates were observed for clear zone formation around the well and the experiment was carried out in triplicate. Antibacterial activities were expressed in millimeter (Table 2).

Anti-fungal activity:

The antifungal activity was studied by cup plate method as described above. The fresh cultures of *Candida albicans* (NCIM 3103) and *Aspergillus niger* (NCIM 798) were introduced into Potato-Dextrose Agar media and poured into petriplates. After solidification five bores were made with the help of sterile cork borer. Standard drug Gentamycin (100 µg/ml) and extract solutions (50µg 100 and 200 µg/ml) were prepared in DMSO separately and introduced into the wells. Only DMSO was introduced into a well, which served as control. The test plates were incubated at 25°C for 24 hrs and zone of inhibition were measured, and the results are tabulated in Table 3.

RESULTS AND DISCUSSION

Phytochemical Screening:

Investigations on the phytochemical screening of *Erythrina mysorensis* stem bark extracts revealed the presence of alkaloids, glycosides, steroids, saponins, tannins, proteins, phenolic compounds and flavonoids. These compounds are known to be biologically active and therefore aid in the antimicrobial activities of *Erythrina mysorensis*. These secondary metabolites exert antimicrobial activity through different mechanisms. Tannins have been found to form irreversible complexes with prolinerich protein, resulting in the inhibition of cell protein synthesis. It is reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues.[10] The flavonoids and phenolic compounds in particular of the plant are important for the plant growth and defense against infection and injury. These compounds while exhibiting antioxidant property are usually also act as good antimicrobial agents. [11]

Antibacterial activity:

Petroleum ether, chloroform, ethanolic and aqueous extracts of *Erythrina mysorensis* bark were subjected to antibacterial activity. The results revealed that only chloroform and ethanolic extracts possessed antibacterial activity and are presented in table-1.Where petroleum ether and aqueous extracts failed to show prominent antibacterial effects. Both chloroform and ethanolic extract showed antibacterial activity at 50, 100 and 200 µg/ml in a concentration dependent manner and are comparable with the standard drug streptomycin and benzyl penicillin. The effect of the extract was however found to be lower than the reference drugs at concentrations studied Benzyl penicillin 100µg/ml(22,22mm), Streptomycin 100µg/ml (16,24 mm). In the chloroform extract, maximum zone of inhibition was recorded by *E. coli, Gram –ve bacteria* at the concentrations of 50,100 and 200µg/ml,10, 12 and 22 mm. respectively, and more sensitive followed by *Pseudomonas aeruginosa* 10, 14 and 20 mm. respectively. In ethanolic extract maximum zone of inhibition was recorded by *Staph. Aureus, Gram +ve bacteria at the concentrations of* 50,100 and 200µg/ml,10, 14 and 16 mm. respectively, followed by *Bacillus subtilis, Gram +ve bacteria* 10, 12 and 16 mm. respectively. Further, among the two extracts chloroform extract is found to be more effective in case of gram negative bacteria. We can justify the above results in the light of earlier reports of Erythrina Burttii, Erythrina poeppigiana and Erythrina latissima of antibacterial activities.[12,13,14]

Antifungal activity:

Among the four extracts studied for antifungal activity the aqueous and ethanolic extracts found to possess antifungal activity for all the three doses studied, the results are shown in table 2.Petroleum ether and chloroform extracts did not show the antifungal activity. Among the two extracts, ethanolic extract seems to be more effective in terms of antifungal activity. The zone of inhibition for *Candida albicans* exhibited 12, 14 and 16 mm at 50, 100 and 200 µg/ml respectively and *Aspergillus niger* has shown 11, 13 and 15 mm zone of inhibition indicating *Candida albicans* more sensitive than *Aspergillus niger***.** However, the zone of inhibition was less compared to reference standard at 100µg/ml, 18 and 20 mm. for Candida albicans and Aspergillus niger respectively .We can justify the above results in the light of the earlier antifungal activity reports of Erythrina burttii and Erythrina latissima.[12,14]

CONCLUSION:

The results obtained in this study justify the antimicrobial effects of the crude ethanol extract of *Erythrina mysorensis,* is an indication of its broad spectrum antimicrobial potential which may be helpful in the management of microbial infections. However, further studies are necessary to isolate and reveal the active compound(s) contained in the crude extract *Erythrina mysorensis* and to establish the mechanism(s) of action.

Table 1: Antibacterial activity of Erythrina mysorensis Gamb.

Extract	Conc. µg/ml	Bachillus subtilis (in mm)	Staph. Aurens (in mm)	e. coli (in mm)	Pseudomonas aeruginosa (in mm)
Chloroform	50	10	8	10	10
	100	16	12	12	14
	200	18	16	22	20
Ethanol	50	10	10	8	10
	100	12	14	10	14
	200	16	16	12	16
Benzyl penicillin	100	22	22	--	--
Streptomycin	100	--	--	16	24

Table 2: Antifungal activity of Erythrina mysorensis

Extract	Conc. µg/ml	C. albicans (in mm)	A. niger (in mm)
Aqueous Ext	50	8	6
	100	10	8
	200	12	10
Ethanol Ext	50	12	11
	100	14	13
	200	16	15
Gentamycin	100	18	20

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