Research Article

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EFFICACY OF *IXORA COCCINEA* AGAINST COMMON FISH PATHOGENS

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ABSTRACT

Ixora coccinea (Rubiaceae family) has been used traditionally for a variety aliments and also cultivated for ornamental purposes. The present study was conducted to evaluate the antibacterial activity of methanol and aqueous extracts of flower of *Ixora coccinea* against six fish virulence strains of bacterial isolates *viz*., *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Vibrio cholerae, Aeromonas hydrophila* and *Pseudomonas aeruginosa*. The antibacterial potential of *Ixora coccinea* methanol extract was tested by using Agar well diffusion method. The methanol extract of *Ixora coccinea* showed maximum zone of inhibition when compared to aqueous extract. Phytochemical tests were performed from the both extracts and showed that the antibacterial activity of *Ixora coccinea was* due to the presence of Phytochemical compound like alkaloids, tripenoids, saponins, flavonoids, phenols, tannins, protein, carbohydrates and glycosides. *Ixora coccinea* methanol flower extracts was analyzed by using the HPTLC.

Keywords: *Ixora coccinea* flower extract , phytochemical test, HPTLC, antibacterial activity.

INTRODUCTION

Ixora coccinea is a shrub growing throughout India as an ornamental flower. Flowers are numerous and found to grow in clusters. They are bright scarlet color in dense, odorous, senssile corymbiform cymes. It is extensively used in traditional medicine. It is a common flowering shrub native to Asia [1,2]. Apart from their use as antiseptic, flowers of the plant are reported to possess medicinal values such as antihelmintic[3], antiasthmatic[4], astringent, sedative, stomachic, antidiarrhoeal, anti-inflammatory[5], antibacterial and wound healing activities[6]. A large number of bacteria cause disease to plants, animals and humans. Pathogenic bacteria harm the host either by direct attacking on a host cells or by releasing in toxins, which are of two types exotoxins and endotoxins[7]. The flowers are used to cure dysentery, leucorrhoea and bronchitis. It is a remedy for fever, gonorrhea, anorexia, dysentery, sores, and skin diseases[8]. Plants are considerably useful and economically essential. Flowers of *Ixora coccinea* (Rubiaceae) are commonly used as an antiseptic throughout India especially in southern parts. A decoction of the leaves is employed as a lotion for eye troubles and can cure sores and ulcers. The roots possess astringent and antiseptic properties.

Since plants are not associated with side effects and have enormous therapeutic potential to heal many infectious diseases. This is due to the presence of secondary metabolites. Therefore, researchers are increasingly turning their attention to folk medicine to develop better drugs with no side effects, especially against microbial infections. The methanol and aqueous flower extract of this plant was reported to have alkaloids, phenols, flavonoids, tannins, saponins, carbohydrates, glycosides, sterols and proteins. Flower extract also contains triterpenoid and ursolic acid. The flowers afforded two new cycloartenol esters, lupeol fatty ester, lupeol, oleanolic acid and sitosterol. Flowers are reported to contain rutin, leucocyanadin glycoside, cyanadin-3-rutinoside and delphinidin monoglycoside [9]. The chloroform extract of *I.parviflora* was column chromatographed over silica gel and the compounds eluted and identified as β-sitosterol, kaempferol, β-sitosterol- β -D-glycoside, kaempferol-7-Omethyl ether.[10] Antimicrobial activity was performed on 50% ethanolic extract of *I. Coccinea.* The effective inhibitory concentration of extract for both bacteria and fungus was found to be 125µg/ml beyond which the inhibitory activity declined and organism started reviving from antimicrobial principle [11]. In recent years, drug resistance to human pathogenic bacteria has been commonly and widely reported in literature. Plants have been an essential part of human society since the start of civilization. Around 250 drugs have been identified from plants during Rig Veda and Atharva Veda descriptions of the Veda period. The rural population in different parts of the world is more disposed to traditional ways of treatment because of easy availability and cheaper cost. It is estimated that 80% of the African population is consulting with traditional healers[12,13].

MATERIALS AND METHODS

Collection of flowers

The red flowers of *Ixora coccinea* were collected from VELS University campus, Chennai. It was authenticated by Prof. P. Jayaraman, Ph.D. and Reg. No:Parc|2015|3030. Institute of Herbal Botany, Plant Anatomy Research centre, Tambaram, Chennai, India. The red flowers of *Ixora coccinea* were washed thoroughly four times with running tap water and once with distilled water. The plant materials were shade dried and powdered. The powdered samples were aseptically sealed in separate polythene bags.

Preparation of Plant Extract

40 g of powdered red flowers of *Ixora coccinea were* extracted successively with 200 ml of methanol at 56-60°C and aqueous extract at 90-100°C in Soxhlet extractor until the extract was clear. The extracts were evaporated to dryness and the resulting extracts were stored in a refrigerator at 4°C for future use.

Collection of fish

Catla fish was collected aseptically from the Tamilnadu fish farm, Thiruvallur district. These fishes were kept in laboratory conditions up to one week for acclimatization. After acclimatized these fish were used for the experimental purpose.

Preparation of Inoculums

E.coli, Klebsiella pneumoniae, Staphylococcus aureus, Vibrio cholerae, Pseudomonas aeruginosa and *Aeromonas hydrophila* was used as an infectious agent in this experiment. These strains were collected from microbiology lab and subculture was maintained. The strains were grown for 12 hours at 28 ^{0}C in nutrient broth. These strains were injected to group of fishes aseptically, then the virulent strain was isolated from those fishes. This isolated virulent strain was injected to next group of fishes. This process was repeated for three times.

Antimicrobial activity

Screening of antibacterial activity was performed by well diffusion method[14]. The Mueller Hinton agar plates were seeded with 0.1 ml of the inoculum for each test organism. The inoculums was spread evenly over plate with sterile glass spreader. The seeded plates were allowed to dry for 30 minutes. A standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the MHA and 75 and 100 µl of each flower extract was introduced in the well. Tetracycline was used as a positive control. The inoculated plates were incubated at 37°C for 24 hours and zone of inhibition was measured to the nearest millimeter (mm).

Phytochemical screening

Phytochemical test were done in both extracts in order to find the presence of active chemical constituents in the *Ixora coccinea* such as alkaloids, flavonoids and phenols. These were identified by characteristic of color change using standard procedure[15,16].

HPTLC

Methanol extract of flower *Ixora coccinea* was standardized by HPTLC using CAMAG LINOMAT V and TLC scanner III. Rutin was used as a standard compound. Methanol extract and rutin was dissolved in an ethyl acetate. 5- 20µl of sample was taken from each extract in different concentrations and loaded in a pre-coated silica gel with help of syringe. The solvent system was developed. Chloroform and methanol (90:10) was used as a solvent system. Developed plates were dried and scanned at 254nm.

RESULT AND DISCUSSION

The preliminary phytochemical screening for methanol and aqueous extracts of *Ixora coccinea* flowers revealed the presence of alkaloids, glycosides, tannins, flavonoids, steroids, phenols, proteins, terpenoids and carbohydrates. The methanol extracts of red flowers showed the presence of alkaloids, flavonoids, and phenols. The aqueous extracts of *I. coccinea* flowers showed the presence of alkaloids and flavonoids. The results were reported in the Table 1.

Table 1: Phytochemical analysis in methanol and aqueous extract of *Ixora coccinea* flowers

S.No	Name of the compound	Methanol Extract	Water Extract	Name of the test
1	Alkaloids	+++	+ ++	Dragendorff's
2	Phenols	+++	-	5% of ferric chloride
3	Flavonoids	+++	++	Shinoda test

Note: +++ shows strong positive, ++ shows weak positive, - shows negative.

HPTLC fingerprinting (Figure 1) had revealed the presence of five peaks in methanol extract and rutin used as standard gave a sharp and well-defined single peak with less diffusion and spreading with RF value of 0.15 and the content was found to be 100%. This peak was observed at 254 nm. In the phenolic compound rutin was present. Antioxidant and antibacterial activity are found in this compound. So, rutin was used as a standard compound in this experiment.

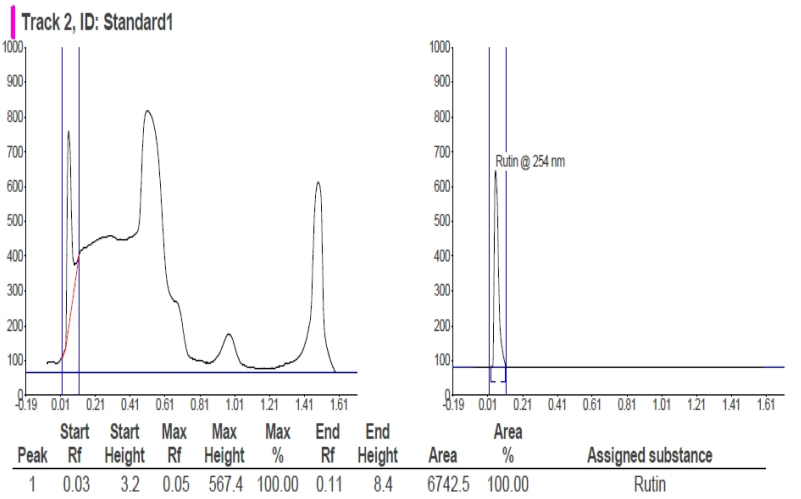
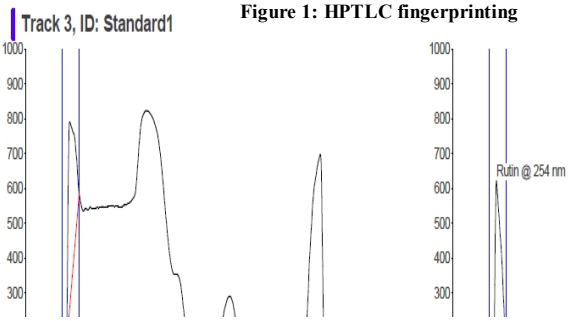


Figure 1: HPTLC fingerprinting



The antibacterial activities of medicinal plants have been reported by many researchers[17]. Most antibacterial of medicinal plants are active against microorganisms[18]. In the present study, methanol and aqueous extract of *Ixor*a *coccinea* was assayed at two different concentrations (75µl and100µl) for the antibacterial activity against various fish bacterial pathogens and the results were showed in (Table 2). It shows antibacterial activity against the common fish pathogens such *as Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Vibrio cholera, Aeromonas hydrophila* and *Pseudomonas aeruginosa.* The zone of inhibition of 75µl and 100µl of methanol extract of *Ixor*a *coccinea* against bacteria was, *Aeromonas hydrophila* (10mm & 15mm), *Escherichia coli* (12 & 19mm*), Klebsiella pneumonia* (18 & 24mm*), Pseudomonas aeruginosa* (15 & 20mm*), Staphylococcus aureus* (17 & 21mm), *Vibrio cholera* (14 & 11mm*).* The zone of inhibition of 75µl and 100µl of aqueous extract of *Ixor*a *coccinea* against bacteria was, *Aeromonas hydrophila* (NZ), *Escherichia coli* (10 & 13 mm), *Klebsiella pneumonia* (11 & 15mm*), Pseudomonas aeruginosa* (4mm), *Staphylococcus aureus* (8 & 11mm), *vibrio cholera* (NZ). The standard antibiotic of tetracycline used as a positive control. In comparison, the zone of inhibition of methanol extract of *Ixor*a *coccinea* against bacteria was more when compared to aqueous extract.

Table 2: Antibacterial activity of *Ixor*a *coccinea* of methanol and aqueous extract against bacterial pathogens

Name of the organism	Methanol extract		Aqueous extract	
	75µl (mm)	100µl (mm)	75µl (mm)	100µl (mm)
Aeromonas hydrophila	10	15	NZ	NZ
Escherichia coli,	12	19	10	13
Klebsiella pneumoniae	18	24	11	15
Pseudomonas aeruginosa	15	20	NZ	4
Staphylococcus aureus	17	21	8	11
Vibrio cholerae	14	11	NZ	NZ
Tetracycline(antibiotic)	25	27	23	25

Note: NZ=No zone.

CONCLUSION

The study of antibacterial activity of herbal plant extract of *Ixora coccinea* flower showed that the methanol extract shows strong potential of antibacterial activity against bacterial fish pathogens when compared to aqueous extract. Phytochemical analysis showed that the antibacterial activity of *Ixora coccinea* flower was due to the presence of detected Phyto-constituents. These plants act as a useful source of new antimicrobial agents.

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