

Preliminary Phytochemical and Antimicrobial Investigations of the Aqueous Extract of *Ixora coccinea* Linn and *Commelina benghalensis* L. on Gram-Positive and Gram-Negative Microorganisms

¹Mukesh Chandra Sharma and ²Smita Sharma

¹School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore (M.P.) 452001, India

²Department of Chemistry Chodhary Dilip Singh Kanya Mahavidyalya Bhind (M.P.) - 477001, India

Abstract: The present work is aimed at exploring the antimicrobial activities of leaf extracts of *Ixora coccinea* Linn and *Commelina benghalensis*. The antimicrobial activity was studied using various organisms by means of agar diffusion method. Susceptibility of some Gram-negative organisms (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*) and Gram-positive organism (*Staphylococcus aureus*) were tested. Antibacterial activity was determined by measuring the diameter of zones of inhibition (mm) produced after incubation. The organisms were more sensitive to the hexane, chloroform extract of the leaves, where as extracts from other solvents like chloroform and hexane showed moderate to weak activity respectively. Similar results have been showed in MIC and MBC. The different extracts such as hexane, chloroform, exhibits comparable antimicrobial activity with the standard.

Key words: Anti-microbial activity • *Ixora coccinea* Linn and *Commelina benghalensis* • Disc diffusion method

INTRODUCTION

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug-resistant bacteria. It is essential to investigate newer drugs with lesser resistance [1]. The use of plant compounds to treat infections is an age-old practice in a large part of the world, especially in developing countries, where as there is dependence on traditional medicine for a variety of diseases [2, 3]. Many pharmacognostical and pharmacological investigations were carried out to identify new drugs or to find new lead structures for the development of novel therapeutic agents for the treatment of human diseases such as cancer and infectious diseases [4]. The use of herbs and medicinal plant as the first medicines is a universal phenomenon. Every culture on the earth, through written or oral tradition, has relied

on the vast variety of natural chemistries found in plants for their therapeutic properties. All drugs from the plant are substances with a particular therapeutic action extracted from plants [5]. The increased prevalence of antibiotic resistant bacteria due to the extensive use of antibiotics may render the current antimicrobial agents insufficient to control some bacterial diseases and hence research for identifying novel substances that are active against human pathogens is an urgent need [6]. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms [7]. The plant *Ixora coccinea* Linn (Rubiaceae) is found throughout India more common in west peninsula in scrub jungles widely cultivated to throughout the tropics [8]. The leaf and stem often used as an ablution for infantile, sedative. Root is useful in diarrhea, dysentery, gonorrhoea and fever. The flowers are used externally to sores, chronic ulcer, scabies and some type of dermatitis and also human internally for cholera, dysentery and gonorrhoea [9]. *Commelina benghalensis* L. is used for ophthalmia, sore throat and burns while in Zanzibar, the juice from the flower is used for eye treatment. In Lesotho it is used for the treatment

of infertility in women while in India it used as demulcent, refrigerant, laxative, bitter, emollient, depressant and for the treatment of leprosy in India [10, 11].

The present investigation was carried out to investigate the chemical and therapeutically potential by evaluating phytochemical and antibacterial activity of the fresh leaf extract of *Ixora coccinea* Linn (Rubiaceae) is being reported here.

MATERIALS AND METHODS

Plant Material: The fresh leaves of *Ixora coccinea* Linn and *Commelina benghalensis*, were collected from local area Indore [M.P], India. The sample was identified at the Department of Pharmaceutical Sciences Dr.Hari Singh Gaur University Sagar [M.P].

Extraction of Plant Material: The samples were carefully washed under running tap water followed by sterile distilled water. These were air dried at room temperature (40°C) for five days and pulverized to a fine powder using a sterilized mixer grinder and stored in air-tight bottles. Different solvents namely hexane, chloroform and methanol (LR grade, Merck, India) were used for extraction. A 100 g amount of pulverized fruits was separately soaked in 100ml of acetone, ethanol, methanol (100% each) and cold sterile distilled water for 24h. Also the same amount (i.e. 10g) of pulverized was immersed in 100ml of hot sterile distilled water and allowed to stand for 30min on a water bath with occasional shaking and kept undisturbed for 24h. Each preparation was filtered through a sterilized Whatman No.1 filter paper. The dried extract thus obtained was exposed to UV rays for 24hrs and checked for sterility on nutrient agar plates and stored in labelled sterile bottles in a freezer at 4°C until further use.

Experimental Method [Agar Well Diffusion]: Stock cultures of Gram-negative organisms (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*) and Gram-positive organism (*Staphylococcus aureus*) were obtained DAVV Indore [M.P]. They were maintained on Mueller-Hinton Agar (Himedia, Mumbai) slope at 4°C and subcultured into Mueller-Hinton broth by a picking off technique [12]. Twenty-four hour old pure cultures were prepared for use each time. *In vitro* antibacterial activity of the crude extracts was studied against Gram-negative and Gram positive bacteria by the agar well diffusion method [13]. The extracts were dissolved in dimethylsulfoxide (DMSO) to a final concentration of

50 mgml⁻¹. Pure DMSO was taken as the negative control and 0.05% Amoxicillin and Gatifloxacin as the positive control. Mueller- Hinton Agar (Himedia, Mumbai) was used as the bacteriological medium. It was prepared according to the manufacturer's instruction, autoclaved and dispensed at 20 ml per plate in 12 x 12 cm petri dishes. Set plates were incubated overnight to ensure sterility before use. Suspension of micro-organisms was made in sterile normal saline and adjusted to 3ml Macfarland standards (166 CfU/ml) [14]. Each labelled medium plate was uniformly inoculated with a test organism by using a sterile cotton swab rolled in the suspension to streak the plate surface in a form that lawn growth can be observed. A sterile cork borer of 5mm diameter was used to make wells on the medium. 100µl of the various extract concentration and control compound were dropped into each, appropriate labelled well. The inoculated plates were kept in the refrigerator for 1 hour to allow the extracts to diffuse into the agar. The Mueller Hinton Agar plates were incubated at 37°C for 24 hours.

Minimum Inhibitory Concentration (MIC): To measure the MIC values, micro-broth dilution method was used [15]. The reconstituted extracts was serially diluted 2-fold in Mueller- Hinton broth medium to obtain various concentrations of the stock, 100, 50, 25, 10 and 5 mgml⁻¹ and were assayed against the test organisms. The minimum inhibitory concentration was defined as the lowest concentration able to inhibit any visible bacterial growth [16].

Minimum Bactericidal Concentration (MBC): Equal volume of the various concentration of each extract and Mueller Hinton broth were mixed in micro-tubes to make up 1 ml of solution. 3ml of McFarland standard of the organism suspension was added to each tube [17]. The tubes were incubated aerobically at 37°C for 24 hours. Two control tubes were maintained for each test batch. These include tube-containing extract without inoculum and the tube containing the growth medium and inoculum.

RESULTS AND DISCUSSION

In vitro preliminary screening of the antimicrobial activity of the plant extracts from *Ixora coccinea* Linn was studied against some microorganisms using the filter paper disc diffusion method. The antimicrobial affect of plant extract against the different strains are illustrated in Table 1. The extract of *Ixora coccinea* Linn and *Commelina benghalensis* at the concentration

Table 1: Antibacterial activities profile of three extracts from the leaves of *Ixora coccinea* Linn and *Commelina benghalensis*

Test bacteria	Hexane	Chloroform	Methanol	Amoxicillin	Gatifloxacin
<i>S. aureus</i>	20.34	21.87	20.06	25.13	27.61
<i>P. aeruginosa</i>	27.19	22.76	18.22	15.11	23.91
<i>S. typhi</i>	21.37	19.07	24.61	22.53	20.28
<i>E. coli</i>	18.01	13.11	22.99	21.48	19.73

Table 2: Antimicrobial activity of *Ixora coccinea* Linn and *Commelina benghalensis*. Aqueous extract of different micro organisms

Sample Conc. in %	Zone of Inhibition in (mm)			
	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. typhi</i>
100	21	24	21	18
50	19	22	19	16
30	22	18	22	14
20	17	19	17	15
10	15	16	13	19

of 100% has antimicrobial activity on the tested microorganism from high to low respectively. *P. aeruginosa* (24mm), *E. coli* (21mm), *S. typhi* (18mm) and *S. aureus* (21mm), showed in Table 1. The data indicated that Gram negative *P. aeruginosa* was the most sensitive strain of those tested with the extract of *Ixora coccinea* Linn, with strongest inhibition zone of 19mm. The extract concentration of 100% also exhibit high antimicrobial activity against *E. coli* with modest activity against *S. typhi*, *S. aureus*. The antibacterial activities of the methanol extracts compared favourably with the standard antibiotic (Amoxicillin and Gatifloxacin) and have appeared to be broad spectrum as its activities were independent on Gram reaction. The minimum inhibitory concentration (MIC) of the *Ixora coccinea* Linn extract was measured which is depicted in the Table 1. It was observed that *S. Aureus* and *P. aeruginosa* have shown MIC value at 1% concentration of plant extract. Other bacteria have shown very small zone at 1% concentration of the extract. The minimum inhibitory concentration (MIC) of the methanol extract for different organisms ranged between 100 and 25.0 mgml⁻¹, while that of the chloroform extract ranged between 12.0 and 50.0 mgml⁻¹. The preliminary phytochemical analysis showed the presence of many constituents which would have played a role in the pharmacological activities studied. The result of antibacterial studies confirms the antibacterial activity of extracts which helps in the treatment of many infections.

In conclusion, results revealed that the crude extracts contain certain constituents like alkaloids which are known to be synthesized by plants in response to microbial infection. Hence, it is apparent that they have been found to be effective antibacterial

substances against a wide range of microorganisms. The plant can also be further explored for its activity against wide spectrum of microbes and can be developed into a powerful antibiotic. Further studies which aimed at the isolation and structure elucidation of antibacterial active constituents from the plant have been initiated.

ACKNOWLEDGEMENTS

The authors thank the referees for their valuable suggestions.

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