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PHYTOCHEMICAL SCREENING AND INVITRO ANTIMICROBIAL ACTIVITY OF WHOLE PLANT OF IXORA COCCINEA

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ABSTRACT

Over one-third of the population in developing countries lack access to essential medicines. The provision of safe and effective TM/CAM therapies could become a critical tool to increase access to health care. While China, the democratic people's republic of Korea, the republic of Korea and Vietnam have fully integrated traditional medicine into their health care systems, many countries are yet to collect and integrate standardized evidence on this type of health care. 70 countries have a national regulation on herbal medicines model. This is because medicinal products or herbs are defined differently in different countries and diverse approaches have been adopted with regard to License. The limited scientific evidence about TM/CAM's safety and efficacy as well as other considerations. .

Keywords: *Ixora coccinea*, Traditional Medicine, Phlobatonins, Antibacterial activity, Ciprofloxacin.

INTRODUCTION

Medicinal plants play a key role in the human health care. About 80% of the world populations rely on the use of traditional medicine which is predominately based on plant materials. The traditional medicine refers to a board range of ancient, natural health care practices including folk/tribal practices as well as Ayurveda, Siddha and Unani. These medicinal practices originated from time immemorial and developed gradually, to a large extent, by relying or based on practical experiences without significant references to modern scientific principles [1]. These practices incorporated ancient beliefs and were passed on from one generation to another by oral tradition and/or guarded literature. Although herbal medicines are effective in the treatment of various ailments very often these drugs are unscientifically exploited and/or improperly used. Therefore, these plants drugs deserve detailed studies in the light of modern science. It is estimated that about 7,500 plants are used in local health traditions mostly in, rural and tribal villages of India [2]. Out of these, the real medicinal value of over 4,000 plants is either little known or hitherto unknown to mainstream population. The classical systems of medicines such as Ayurveda, Siddha, Unani and Tibetan use about 1,200 plants. A detailed investigation and documentation of plants used in local health traditions and pharmacological evaluation of these plants and their taxonomical relatives can lead to the development of invaluable plant drugs for many dreaded diseases. Random screening of plants has not proved economically effective.

Priorities for the use of Traditional Medicines

Over one-third of the population in developing countries lack access to essential medicines. The provision of safe and effective TM/CAM therapies could become a critical tool to increase access to health care. While China, the democratic people's republic of Korea, the republic of Korea and Vietnam have fully integrated traditional medicine into their health care systems, many countries are yet to collect and integrate standardized evidence on this type of health care. 70 countries have a national regulation on herbal medicines model. This is because medicinal products or herbs are defined differently in different countries and diverse approaches have been adopted with regard to License. The limited scientific evidence about TM/CAM's safety and efficacy as well as other considerations [3].

PLANT PROFILE

- **Common Name:** Flame of Woods
- **Kingdom:** Plantae
- **Botanical Name:** Ixora coccinea
- **Natural Order:** Asteriadae
- **Family:** Rubiacea
- **Genera:** Ixora
- **Species:** Coccinea

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Selection, Authentication and Collection of Ayurvedic Plants

The many ayurvedic plants traditionally used for treatment of Infectious diseases. But there is no scientific report for proving the effects. Through literature survey, we will select three ayurvedic plants such as *Ixora coccinea* (Family: Rubiaceae) which will be traditionally used for the treatment of Bacterial and funal infections was shown in figure 2. This plant will be selected and collected from Tirumala Hills, Tirupati, Andhra Pradesh for the present study. The plant materials will be identified and authenticated by Botanist.

Extraction and Purification

Plant material will be dried, powdered and extracted with suitable solvent like Cold water,Hot water and Ethanol by maceration in which all the phytoconstituents get extracted. Phytoconstituents present in the extract identified by chemical tests [4]. The extract subjected to study antibacterial and antifungal activity.

Preliminary Phytochemical Analysis

Chemical Tests

A preliminary phytochemical investigation will be carried out for all the extracts obtained from the medicinal plants.

Detection of Alkaloids

Small portions of solvent-free chloroform, alcohol and aqueous extracts will be stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate will be tested with various alkaloid reagents.

Mayer's test

Acidic test solution will be treated with potassium mercuric iodide (Mayer's reagent) and the formation of cream coloured precipitate indicates the presence of alkaloids.

Dragendroff's test

Acidic test solution will be treated with potassium bismuth iodide (Dragendroff's reagent) and formation of reddish brown precipitate indicates the presence of alkaloids [5].

Detection of Glycosides

Preparation of test solution

The test solution will be prepared by dissolving extract in the alcohol or hydro-alcoholic solution.

Test for Cardiac Glycosides

Baljet's test

The test solution will be treated with sodium picrate. Formation of yellow to orange colour indicates presence of cardiac glycosides.

Bromine water test

Test solution will be dissolved in bromine water.Formation of yellow precipitate indicates presence of cardiac glycosides.

Borntrager's tesT

Powdered drug will be boiled with 5 ml of 10% sulphuric acid for 5 mins. Filtered while hot, cooled and the filtrate will be shaken gently with equal volume of benzene. Benzene layer will be separated and then treated with half of its volume solution of ammonia (10%). The ammonical layer with rose pink colour indicates the presence of anthraquinones.

Cyanogenetic glycosides

Grignard's test:

Strips of sodium picrate filter paper will be inserted between split cork stoppers which will be fitted in to the neck of the test tube containing a small amount of powdered drug in water. Care will be exercised that the paper didn't touch the inner side of the test tube. The content will be warmed for half an hour. The red colour of the strips indicates the presence of cyanogenetic glycosides [6].

Detection of Carbohydrates

Small quantity of alcohol and aqueous extracts will be dissolved separately in distilled water and filtered. The filtrate will be subjected to various tests to detect the presence of different carbohydrates.

Molisch's test

Filtrates will be treated with alcoholic solution of α -Naphthol and a few drops of conc. Sulphuric acid will be added through the sides of the test tube. The formation of violet ring at the junction of the liquids indicates the presence of carbohydrates.

Fehling's test

Filtrates will be treated with few ml of dilute hydrochloric acid and heated on a water bath for 30 minutes. After hydrolysis the solutions will be neutralized with sodium hydroxide solution. To the neutralized solutions, equal quantities of Fehling's A & Fehling's B solutions will be added and heated on a water bath for a few minutes. Formation of red-orange precipitate indicates the presence of reducing sugars [7].

Detection of Phytosterols

Petroleum ether, chloroform, ethanol and aqueous extracts will be refluxed separately with solution of alcoholic potassium hydroxide till complete saponification took place. Saponified mixtures will be diluted with distilled water and extracted with solvent ether. Ethereal extract will be evaporated to dryness and the residue subjected to Liebermann-Burchard's test.

Triterpenoids

Preparation of test extracts solution

The test extract solution will be prepared by dissolving extract in the chloroform.

Salkowski test:

Few drops of concentrated sulphuric acid will be added to the test solution, shaken and on standing lower

layer turns golden yellow which indicates the presence of triterpenoids.

Detection of Saponins

Foam test:

About 1 ml of alcohol and aqueous extracts will be diluted separately with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes.
Formation of any froth above the surface indicates the presence of saponins.

Detection of Phenolic compounds and Tannins

Small quantities of alcohol and aqueous extracts will be diluted separately in water and will be tested for the presence of phenolic compounds and tannins.

Ferric chloride test:

To the test solutions, a few drops of 5% ferric chloride solution will be added. Formation of a bluish-black or greenish-black colour indicates the presence of phenolic compounds and tannins.

Gelatin test:

To the test solutions a few drops of 1% gelatine solution in 10% sodium chloride will be added. Formation of white precipitate indicates the presence of tannins.

Detection of Proteins and Free Amino Acids

Biuret test:

To the test solutions, a few drops of 0.7% copper sulphate solution will be added. Formation of a purplish violet colour indicates the presence of amino acids.

Ninhydrin test:

To the test solutions, a few drops of Ninhydrin solution will be added in a water bath. Formation of a bluish colour indicates the presence of amino acids [8].

METHODOLOGY

In-Vitro Anti Microbial Activity

Microbial strains tested

In this study, Microorganisms were selected to cover Gram-positive bacteria and Gram-negative bacteria namely , *Escherichia coli*, *Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas aureginosa*, *Malassezia furfur*. The tested strains were obtained from Microbiology Laboratory, SVCP, A.Rangampet, Tirupati. The Microorganisms were allowed to grown over night at 37^0 C in 2% nutrient agar at pH 7. The sensitivity of Microorganisms to the reference antibiotic was checked. For this purpose Ciprofloxacine was used as reference antibiotic [9, 10]

Preparation of inocula

The inocula were prepared by inoculating a loop of each bacterial strain from a 24 hours of old culture into a sterile nutrient broth aseptically in the laminar air flow unit. The culture growth was allowed for 24 hours in incubator at 37^0 C.

Determination of antimicrobial activity

The screening of Anti-microbial efficacy of the various extract of *Solanum erianthum* was performed on various microorganisms by using agar well diffusion method [11]. The agar plates were prepared by pouring 20 ml of sterile molten Mueller-Hinton (MH) agar (Himedia Lab Pvt. Ltd, Mumbai, India). The bacterial cultures were prepared by adding the seed culture in the autoclaved agar medium followed by pouring into Petri plates. The solid agar medium was gently punctured with the aid of 8mm sterile cork borer to make a proper well. 50µl of *Ixora coccinea* extract (50mg/ml) was added in the pre labelled wells together with reference antibiotic i.e. Ciprofloxacine [12]. Here various extract of *Ixora coccinea* served as test and Ciprofloxacine served as standard. The reference Antibiotic was used in the concentration range of 100µg/ml. It was taken care that the sample should be placed at the level of cavity. The diffusion of extract was allowed for 1hr at room temperature on a sterile bench. Then the Petri plates were incubated for 48 hrs at 37^0 C. After 48 hrs the plates were observed for the presence of inhibition of bacterial growth and that was indicated by clear zone of inhibition of bacterial growth around the wells. The size of Inhibitory zone was measured in mille meters (mm). Minimum Inhibitory Concentration (MIC) was determined [13]

RESULTS AND DISCUSSION

The results of modified agar well diffusion method (Table 1) showed that prepared CWEIC, HWEIC, EEIC, having Inhibitory effect on the microorganisms which are responsible for the intestinal infections skin infections and urinary tract infection. The Anti-microbial activity of the herbal extract has been comparable to that of market antibiotic (Ciprofloxacine) [14, 15]. The diameter of Zones of inhibitions was also given in the table 2, 3, 4, 5 respectively.

Table 1: Phytochemical analysis of various extracts of Ixora coccinea

S.NO	TEST	CWEIC	HWEIC	EEIC
1.	Alkaloids	+	+	+
2.	Flavonoids	+	-	+
3.	Phlobatannins	+	-	-
4.	Steroids	+	-	-
5.	Terpenoids	+	+	+
6.	Cardiac	+	-	-
7.	Saponins	+	-	+
8.	Glycosides	+	+	+

Table2: Antimicrobial activity of Cold water extract of Ixora coccinea

Microorganisms	Zone of inhibition
	CWEIC
Escherichia coli	29±1.0
Bacillus subtilis	31±1.0
Proteus mirabilis	27±2.0
Pseudomonas aureginosa	30±1.0
Malassezia furfur	30±2.0

Table3: Antimicrobial activity of Hot water extract of Ixora cocinea

Microorganisms	Zone of inhibition(mm)
	HWEIC
Escherichia coli	**26±1.0**
Bacillus subtilis	**24±1.0**
Proteus mirabilis	**25±2.0**
Pseudomonas aureginosa	**25±1.0**
Malassezia furfur	**22±2.0**

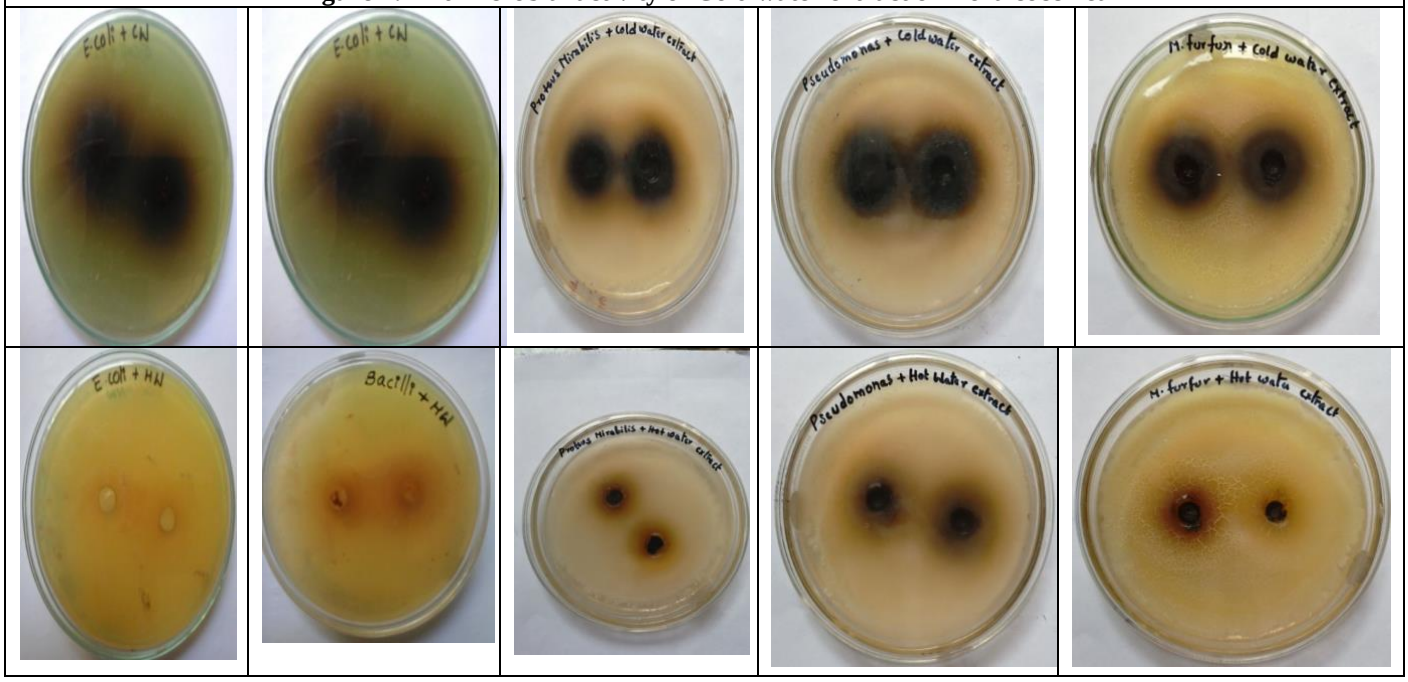
Table 4: Anti-microbial activity of Ethanolic extract of Ixora coccinea

Microorganisms	Zone of inhibition(mm)
	EEIC
Escherichia coli	**23±2.0**
Bacillus subtilis	**23±1.0**
Proteus mirabilis	**23±1.0**
Pseudomonas aureginosa	**25±2.0**
Malassezia furfur	**25±1.0**

Table 5: Antimicrobial activity of standard Ciprofloxacine

Microorganisms	Zone of inhibition
	Standarad (Ciprofloxacine)
Escherichia coli	**27±1.0**
Bacillus subtilis	**25±1.0**
Proteus mirabilis	**28±2.0**
Pseudomonas aureginosa	**22±2.0**
Malassezia furfur	**23±1.0**

Figure 1: Antimicrobial activity of Cold water extract of Ixora coccinea



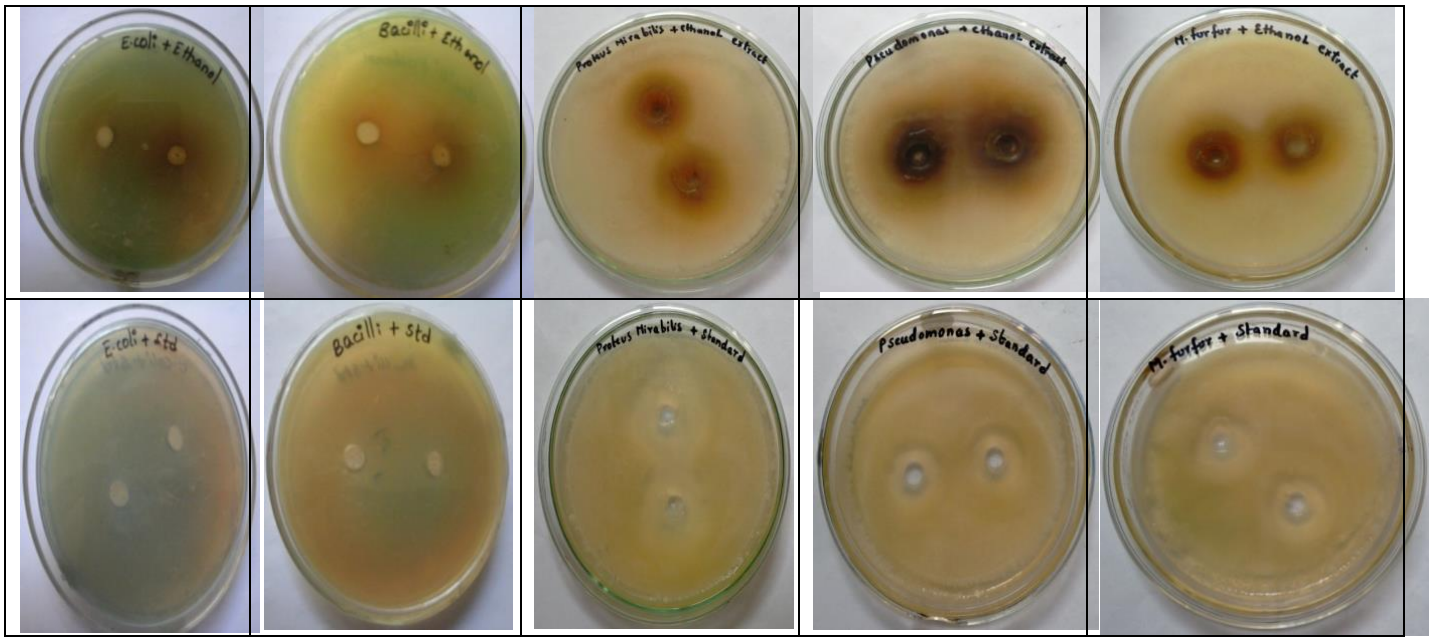


Figure 2: Ixora coccinea

SUMMARY AND CONCLUSION

In the present study an attempt has been made to explore Pharmacognostical and phytochemical parameters besides evaluating antimicrobial activity against microorganisms causing skin infection, intestinal infections and urinary tract infection. The identification of plant material taxonomically and Pharmacognostically is important to provide standards and avoid adulteration of drugs. The plants were identified and authenticated by Prof.N.Yasodamma, Head of Botany Department, Sree Venkateswara Univerity. Tirupathi. The detailed botanical, Pharmacognostical studies with proper authentication of the plants helps in minimizing the adulteration and also for proper identification of the plant.

Preliminary phytochemical analysis of the extract showed the presence of the Alkaloids, Flavonoids, Phlobatannins, Steroids, Terpenoids, Cardiac Glycosides, Anthraquinones and Saponins constituents may be responsible for the healing potential of Skin infections ,Intestinal and Urinary tract infections

Evaluation of antimicrobial activity of Ixora coccinea against microorganisms causing skin infections intestinal and urinary tract infections was done by using agar well diffusion method. After 24 hrs we measured the zone of inhibition to confirm the antimicrobial activity of the prepared Ixora coccinea. From the above results it can be concluded that the Ixora coccinea could effectively fight

against microorganisms causing Skin infections, Intestinal and Urinary tract infections.

CONFLICT OF INTEREST
No Interest

ACKNOWLEDGEMENT
Nil

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