Phytochemical Screening and Combined Invitro Anti-Microbial and Anti-Oxidant Studies of Various Extracts of Vinca Ixora Coccinea

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Abstract:

Ixora coccinea belonging to family Rubiaceous an evergreen shrub presents in many regions of India and throughout the world. It contains various therapeutic properties such as antitumour, wound healing, antioxidant activity, gastroprotective, hepatoprotective, antidiarrheal, antinociceptive, antimutagenic, antineoplastic and chemo preventive effects. Phytochemical investigation shows the presence of flavonoid, saponin, tannin, alkaloid, glycoside, phenol. Antioxidant is a chemical substance that prevents oxidation of other chemical substances by neutralising damaging effect of free radicals. Antioxidants are mainly present in phenolic compounds. Phenolic compounds show antioxidant capacity due to redox properties they act as a reducing agent, hydrogen donors, metal chelators singlet oxygen quenchers. Free radical is an atom or molecule processing unpaired electrons. The ROS oxygen derived free radicals such as superoxide anion[O2], Hydroxyl [OH], Hydroperoxyl [OOH], Peroxyl [ROO] and hypochlorous acid [HOCL].

Key Words: Ixora coccinea, Rubiaceous, antioxidant activity, antineoplastic, Phenolic compounds, Hydroperoxyl.

INTRODUCTION

Ixora coccinea belonging to family Rubiaceous an evergreen shrub presents in many regions of India and throughout the world. It contains various therapeutic properties such as antitumour, wound healing, antioxidantactivity, gastroprotective, hepatoprotective, antidiarrheal, antinociceptive, antimutagenic, antineoplastic and chemo preventive effects. Phytochemical investigation shows the presence of flavonoid, saponin, tannin, alkaloid, glycoside, phenol. Antioxidant is a chemical substance that prevents oxidation of other chemical substances by neutralising damaging effect of free radicals [1]. Antioxidants are mainly present in phenolic compounds. Phenolic compounds show antioxidant capacity due to redox properties they act as a reducing agent, hydrogen donors, metal chelators singlet oxygen quenchers [2]. Free radical is an atom or molecule processing unpaired electrons. The ROS oxygen derived free radicals such as superoxide anion[O2], Hydroxyl [OH], Hydroperoxyl [OOH], Peroxyl [ROO] and hypochlorous acid [HOCL][3]. Antioxidant compounds present in the food place an important role in health protecting factor. Antioxidant compounds like phenolic acids polyphenols and flavonoids have found in the plants to have multiple biological effects [4]. Synthetic antioxidants like Butylated Hydroxy Anisole [BHA], and Butylated Hydroxy Toluene [BHT] have very effective for industrial processes but have side effects toxic to humans [5]. Polyphenolic compounds are important for the human body they act as antioxidant and free radical scavengers [6]. Antioxidant basic drugs and formulations used for the prevention and treatment of complex diseases like Alzheimer's diseases and cancer during the last three decades [7].

PLANT PROFILE

Botanical study: -

Synonyms: *Ixora grandiflora Bot.* and *Ixora bandhuca Roxby*.

Common Names: Jungle of geranium or Flame of woods or Red *Ixora*

Kingdom: Plantae

Order: Gentianellas
Family: Rubiaceous
Subfamily: Ixoroideae
Tribe: Ixoreae

Genus: *Ixora*
Species: *coccinea*

Habit

An evergreen shrub.

Root

Branched tap root system

Stem

Aerial, erect, branched, cylindrical and woody.

Leaf

Simple, opposite decussate, oblong, stipulate with interpetiolar stipules and unicostate reticulate venation.

Inflorescence

Terminal or axillary dense corymbose cyme.

Flower

Bracteate, bracteolate, dichlamydeous, bisexual, complete, tetramerous, actinomorphic and epigynous.

Calyx

Sepals 4, green, gamosepalous showing valvate aestivation, regular and persistent.

Corolla

Petals 4, bright red or yellow or white, gamopetalous, showing twisted aestivation, hypo crateriform, corolla tube long and slender.

Androecium

Stamens 4, epipetalous, attached at the throat of the corolla tube, filaments short, alternate the petals. Anthers are dirhinous, basifixed, introse and dehiscing longitudinally.

Gynoecium

Ovary inferior, bicarpellary and syncarpous. Ovary bilocular with one ovule in each locule showing axile placentation. Style simple and filiform. Stigma bifid.

Fruit; -A berry.

Seed; -Endosperm

METHODOLOGY

1.Selection of the plant

1.Selection of the plant was based on through literature survey.

2.The plant was identified to have *invitro* antioxidant and *invitro* anti-microbial activity by various microorganisms.

3.The plant was identified and cultivated near Tirumala Hills.

2.Extraction of the Plant

Materials and Methods

The leaves of the plant *ixora coccinea* were collected from our college SreeVidyanikethan college of Pharmacy Medicinal Garden, Rangampet.A, Chittoor district, Andhra Pradesh, India. The fresh leaves were recognized and Authentified in the SreeVenkateswara University. The fresh leaves were dried for 10 days in shade. The powdered sample was stored in a bottle at room temperature before analysis.

MACERATION:

Maceration was a popular and inexpensive homemade technique for the preparation of tonic since a long time. Moreover, this technique was used for the extraction of essential oils and active compounds from plant materials. Generally, the maceration procedure consists of multiple steps in extraction. The whole or coarsely powdered crude drug undergoes grinding to increase the surface area for proper mixing of powdered materials with the solvent. This process is done in a closed vessel where an appropriate solvent (menstruum) is added. Next, the solvent is strained off followed by pressing the solid residue of the extraction process known as marc to recover an optimum amount of occluded solution. Both the obtained pressed out liquid and the strained solvent are mixed together and separated from unwanted materials by filtration. Frequent agitation during maceration facilitates extraction by two processes:

(1) Promotes diffusion,

(2) Separates concentrated solution from the sample surface by adding new solvent to the menstruum for increasing the extraction yield.

PREPARATION OF EXTRACT:

- ✓ The fresh leaves are collected from a healthy plant of *ixora coccinea.*
- ✓ Thereafter the leaves were air-dried in shade at room temperature for 10 days.
- ✓ Then the dried leaves were grinded with an electrical grinder to obtain fine powder.
- ✓ The obtain powder was stored in a sealed bottle at room temperature.

✓ 15gms of powdered extract was weighed and mixed with the solvent 150ml of ethanol.
✓ The mixture was allowed to macerate for a whole day. Whatsmann filter paper is used to filter the mixture after 24 hours.
✓ Finally, pressure was applied to the residue in order to extract the last bit of solvent.
✓ Stored it at room temperature in a tightly covered container to continue the phytochemical screening.

4.Phytochemical screening

1. Test for Alkaloids:
Dragendroffs test: To 1ml of extract, add 1ml of dragendroffs reagent (potassium bismuth iodide solution). An orange to red precipitate indicates the presence of alkaloids.

2. Test for Tannins:
Gelatin solution: To 2-3ml of extract, and add few drops of gelatin solution. Formation of white precipitate indicates presence of tannins.

3. Test for Flavonoid's:
1 ml of extract sample, few drops of dilute ammonium solution, and few drops of concentrated hydrochloric acid were added. The yellowish colour indicates the presence of flavonoids.

4. Test for Saponins:
1 ml of extract and 5 ml of distilled water were added and mixed strenuously. The formation of froth desired the presence of saponins.

5. Test for Phenolics:
1ml of extract sample and 1 ml of lead acetate solution were used. The formation of a precipitate shows the presence of phenols.

6. Test for Glycosides:
Legals test: A minimum quantity of the extract is hydrolysed with hydrochloric acid for 5 minutes on water bath. To the hydrolysate 1ml of pyridine and few drops of sodium nitroprusside solution is added, then it is made alkaline with sodium hydroxide solution. Colour change shows presence of glycosides.

7.Test for Phyto steroids:
Salkowski test: small quantity of extract is dissolved in 5ml of chloroform separately. To the 1ml of above prepared chloroform solution few drops of concentrated sulphuric acid is added. Formation of brown ring indicates the presence of Phyto steroids.

8.Test for Proteins:
Ninhydrin test :3ml test solution and 3drops 5% Ninhydrin solution were heated in boiling water bath for 10 mins observed for purple or bluish colour.

9.Test for Amino acids:
Biuret test: To 3ml of test solution added 4% NaOH and few drops of 1% CuSO4 solution observed for violet or pink colour

10.Test for Carbohydrates:
Fehling's test: 1ml Fehling's A and 1ml Fehling's B solutions was mixed and boiled for one minute. Added equal volume of solution. Heated in boiling water bath for 10 minutes was observed for a yellow, brick red precipitate.

11.Tests for Gums and Mucilage's:
Ruthenium red test: small quantity of extract is diluted with water and added with ruthenium red solution. A pink colour production shows the presence of gums and mucilages.

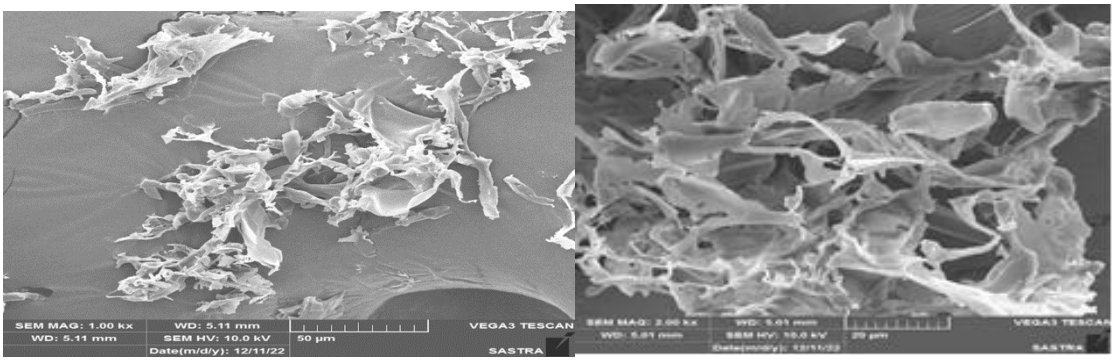
S.NO	Phytoconstuents	Ethanol extract of Ixora coccinea	Hot water extract of Ixora coccinea	Cold water extract of Ixora coccinea
01	Alkaloids	+	+	+
02	Glycosides	+	-	-
03	Phenols	_	_	_
04	Flavonoids	+	+	-

05	Saponins	+	+	+
06	Fixed oil/Fats	_	_	_
07	Gums/Mucilage	+	+	+
08	Carbohydrates	-	-	-
09	Amino acids	-	-	-
10	Proteins	-	-	-
11	Steroids	+	+	+
12	Tannins	+	+	+

RESULSTS

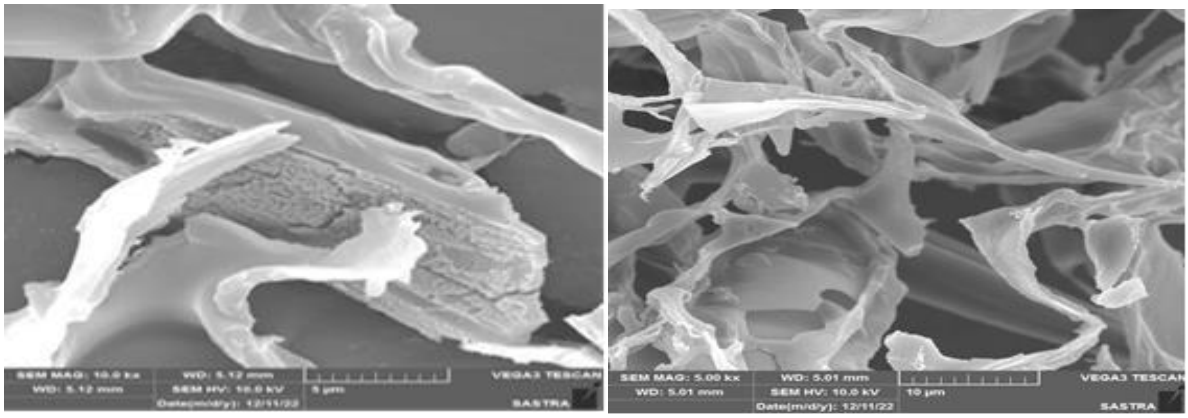
SEM Analysis-Ethanolic Extract of *Ixora coccinea*

The surface characteristics of Ethanolic Extract of *Ixora coccinea* with was studied by SEM (Vegan 3 Tuscan). The specimens were scanned with an electron beam of acceleration potential of 10 kV and the images were collected as secondary electron mode.



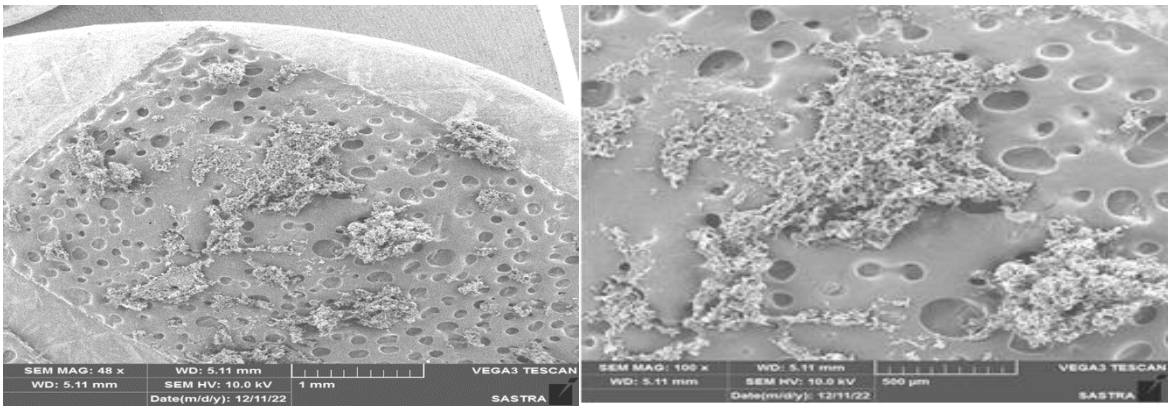
SEM Analysis-Hot water extract of *Ixora coccinea*

The surface characteristics of Hot water Extract of *Ixora coccinea* with was studied by SEM (Vegan 3 Tuscan). The specimens were scanned with an electron beam of acceleration potential of 10 kV and the images were collected as secondary electron mode.



SEM Analysis-cold water extract of *Ixora coccinea*

The surface characteristics of cold-water Extract of *Ixora coccinea* with was studied by SEM (Vegan 3 Tuscan). The specimens were scanned with an electron beam of acceleration potential of 10 kV and the images were collected as secondary electron mode.



Invitro antioxidant study

1.There is considerable epidemiological evidence indicating association between diets rich in fresh fruits and vegetables and a decreased risk of cardiovascular disease and certain forms of cancer. Free-radicals are generated continuously in the body due to metabolism and disease. In order to protect themselves against free radicals, organisms are endowed with endogenous (catalase, superoxide dismutase, glutathione peroxidase/ reductase) and exogenous (C and E vitamins, carotene, uric acid) defences; yet these defence systems are not sufficient in critical situations (oxidative stress, contamination, UV exposure, etc.) where the production of free radicals significantly increases. Free radicals can cause lipid peroxidation in foods, which leads to their deterioration. Antioxidants are important in the prevention of human diseases. Antioxidant compounds may function as free radical scavengers, complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation.

2.Antioxidants are often used in oils and fatty foods to retard their autoxidation. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxy anisole (BHA), have restricted use in foods as they are suspected to be carcinogenic. Therefore, the importance of search for natural antioxidants has greatly increased in the recent years. Ethnomedical literature contains a large number of plants that can be used against diseases, in which reactive oxygen species and free radical play important role. There is a plethora of plants that have been found to possess strong antioxidant activity. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases. So, many researchers have focused on natural antioxidants and in the plant kingdom numerous crude extracts and pure natural compounds were previously reported to have antioxidant properties.

The plants *Ixora Coccinea* are distributed in the warm regions of India and Southeast Asia. This plant used as antimicrobial activity. The bark decoction has been used in the traditional medicine for the treatment of asthma, intestinal worms and cough and leaves are used against colic's. Tannins were isolated from the bark. The fatty alcohol, $C_{22}H_{46}O$, named bridely alcohol besides fatty acids and a phlebotomine were isolated from the leaves of *Ixora Coccinea*. Traxanoxes was isolated from roots hexane extract. Based on the literature survey also revealed that lack of scientific report regarding antioxidant activity of the whole plant of *Ixora Coccinea.* However, no data are available in the literature on the antioxidant activity of whole plant of *Ixora Coccinea*. Therefore, we undertook the present investigation to examine the antioxidant activities of various extract of whole plant of *Ixora Coccinea* through different in vitro models.

MATERIAL AND METHODS

Collection and Identification of Plant materials

The plant *Ixora coccinea* were collected from Tirumala hills, India. The plants Ixora Coccinea Wild, were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40-mesh sieve.

Preparation of Extracts

The above powered materials were successively extracted with hydro alcohol (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus for 24 hrs then mark was subjected to Ethyl acetate (76-78^{0}C) for 24 hrs and then mark was subjected to Methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizes till dry powder was obtained.

Evaluation of Antioxidant activity by in vitro Techniques

IN-VITRO* ANTIOXIDANT POTENTIAL OF VARIOUS EXTRACTS *IXORA COCCINEA

Evaluation of Antioxidant activity by in vitro Techniques

Hydroxyl radical scavenging activity

This was assayed as described by Elizabeth and Rao (1990). The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+} -Ascorbate –EDTA –H_2O_2system (Fenton reaction). The reaction mixture contained 0.1 ml deoxyribose (2.8mM),0.1 ml EDTA (0.1 mM), 0.1 ml H_2O_2 (1mM), 0.1 ml Ascorbate (0.1mM), 0.1 ml KH_2PO_4-KOH buffer, P^H 7.4 (20mM) and various concentrations of plant extract in a final volume of 1 ml. The reaction mixture was incubated for 1 hour at 37^0 C. Deoxyribose degradation was measured as TBARS and the percentage inhibition was calculated. s

Nitric oxide radical scavenging activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garratt (1964) [13]. The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM), 0.5 ml of phosphate buffer saline (1M) were incubated at 25^0C for 150 mins. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphonic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization.

Then 1 ml of naphthyl ethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological P^H spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess Allostery reaction at 540 nm.

Total phenol

The measurement of total phenol is based on Mallick and Singh (1980) [14]. To 0.25g of sample, added 2.5 ml of ethanol and centrifuged at 2^oC for 10 mins. The supernatant was preserved. Then, the sample was re-extracted with 2.5 ml of 80% ethanol and centrifuged. The pooled supernatant was evaporated to dryness. Then, added 3 ml of water to the dried supernatant. To which added 0.5 ml of Folin's phenol reagent and 2 ml of sodium carbonate (20%). The reaction mixture was kept in boiling water bath for 1 min. the absorbance was measured at 650 nm in a spectrophotometer.

RESULTS AND DISCUSSION

Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases. They are also involved in autoimmune disorders like rheumatoid arthritis etc.

Hydroxyl radical scavenging activity

Table 1: Hydroxyl radical scavenging activity of Hot water extract of Ixora coccinea

The percentage of hydroxyl radical scavenging activity of Hot water extract of Ixora coccineapresented in Table 2. Hot water extract of Ixora coccinea was exhibited a maximum hydroxyl radical scavenging activity of 58.27 % at µg/ml whereas for ascorbate (standard) was found to be 75.23 % at 90 µg/ml. The IC_{50} values of Hot water extract of Ixora coccineaand ascorbate were found to be 600µg/ml and 90µg/ml respectively.

S. No	Concentration (µg/ml)	% of activity(±SEM) *	
		Sample (HWEIC)	Standard (Ascorbate)
1	25	20.11 ± 0.042	22.87 ± 0.076
2	50	22.11 ± 0.042	26.87 ± 0.076
3	75	37.21 ± 0.034	30.30 ± 0.054

4	100	43.36 ± 0.078	60.64 ± 0.022
5	200	48.27 ± 0.024	75.23 ± 0.014
6	400	53.36 ± 0.079	79.64 ± 0.022
7	600	58.27 ± 0.024	85.23 ± 0.014
		IC_{50} = 600 µg/ml	**IC_{50} = 90 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Nitric acid Scavenging activity

Nitric oxide is a diffusible free radical which is an important effector molecule in diverse biological systems. The animal studies suggested the role for NO in pathogenesis of inflammation and pain. So it is worthful to investigate the NO scavenging potential of the plant extract.

The reduction of nitric oxide radical by the Hot water extract of Ixora coccinea and ascorbate were illustrated in Table 2. The maximum nitric oxide scavenging activity of petroleum ether extract and ascorbate at 600 µg/ml were found to be 46.37 % and 75.23% respectively. The IC_{50} value of Hot water extract of Ixora coccinea and ascorbate were recorded as 600µg/ml and 210µg/ml respectively.

Table 2: Nitric oxide scavenging activity of Hot water extract of Ixora coccinea

S. No	**Concentration (µg/ml)**	**% of activity(±SEM) ***	
		Sample (HWEIC)	**Standard (Ascorbate)**
1	25	11.58 ±0 .015	26.87 ± 0.076
2	50	20.06 ± 0.049	30.30 ± 0.054
3	75	31.48 ± 0.030	60.64 ± 0.022
4	100	46.37 ± 0.027	75.23 ± 0.014
5	200	54.06 ± 0.059	90.30 ± 0.004
6	400	65.48 ± 0.038	105.64 ± 0.024
7	600	76.37 ± 0.017	121.23 ± 0.011
		IC_{50} = 600 µg/ml	**IC_{50} = 210 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Total Phenolic content

Table 3: The total Phenolic content of Hot water extract of Ixora coccinea

Phenolic compounds are known as powerful chain breaking antioxidants. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. The phenolic compounds may contribute directly to antioxidant action. The total phenolic content of Hydroalcoholic extract of Terminalia Billericaand ascorbate were illustrated in Table 3.

S. No	Extracts	Total phenol content (mg/g Gallic acid) (±SEM)*
1	Hot water extract of Ixora coccinea	1.50 ± 0.022

Based on the result the Hot water extract of Ixora coccinea was found higher content of phenolic components.

Hydroxyl radical scavenging activity

Table 4: Hydroxyl radical scavenging activity of Cold-water extract of Ixora coccinea

The percentage of hydroxyl radical scavenging activity of Cold-water extract of Ixora coccineapresented in Table 4. Cold water extract of Ixora coccinea was exhibited a maximum hydroxyl radical scavenging activity of 63.36 % at µg/ml whereas for ascorbate (standard) was found to be 74.64 % at 90 µg/ml. The IC_{50} values of Cold-water extract of Ixora coccinea and ascorbate were found to be 400µg/ml and 180µg/ml respectively.

S. No	Concentration (µg/ml)	% of activity(±SEM) *	
		Sample (CWEIC)	Standard (Ascorbate)
1	25	28.11 ± 0.032	32.87 ± 0.086
2	50	32.11 ± 0.041	36.87 ± 0.086
3	75	47.21 ± 0.024	42.30 ± 0.034
4	100	53.36 ± 0.088	58.64 ± 0.026
5	200	58.27 ± 0.034	65.23 ± 0.024
6	400	63.36 ± 0.069	74.64 ± 0.022
7	600	78.27 ± 0.014	82.23 ± 0.024
		IC_{50} = 600 µg/ml	**IC_{50} = 90 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Nitric acid Scavenging activity

Nitric oxide is a diffusible free radical which is an important effector molecule in diverse biological systems. The animal studies suggested the role for NO in pathogenesis of inflammation and pain. So, it is worthful to investigate the NO scavenging potential of the plant extract.

The reduction of nitric oxide radical by the Cold-water extract of Ixora coccinea and ascorbate were illustrated in Table 5. The maximum nitric oxide scavenging activity of Cold-water extract of Ixora coccinea and ascorbate at 600 µg/ml were found to be 86.17 % and 80.30% respectively. The IC_{50} value of Cold-water extract of Ixora coccinea and ascorbate were recorded as 600µg/ml and 200µg/ml respectively.

Table 5: Nitric oxide scavenging activity of Cold-water extract of Ixora coccinea

S. No	Concentration (µg/ml)	% of activity(±SEM) *	
		Sample (CWEIC)	Standard (Ascorbate)
1	25	21.58 ±0 .018	36.88 ± 0.016
2	50	30.09 ± 0.043	40.20 ± 0.059
3	75	41.68 ± 0.030	50.64 ± 0.022
4	100	56.47 ± 0.037	75.23 ± 0.018
5	200	64.09 ± 0.069	80.30 ± 0.024
6	400	75.18 ± 0.018	95.84 ± 0.054
7	600	86.17 ± 0.018	105.43 ± 0.081
		IC_{50} = 600 µg/ml	**IC_{50} = 200 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Total Phenolic content

Table 6: The total Phenolic content of Cold-water extract of Ixora coccinea

Phenolic compounds are known as powerful chain breaking antioxidants. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. The phenolic compounds may contribute directly to antioxidant action. The total phenolic content of Cold-water extract of Ixora coccinea and ascorbate were illustrated in Table 6.

S. No	Extracts	Total phenol content (mg/g Gallic acid) (±SEM)*
1	Cold water extract of Ixora coccinea	1.20 ± 0.080

Based on the result the Cold-water extract of Ixora coccinea was found higher content of phenolic components.

Hydroxyl radical scavenging activity

Table 7: Hydroxyl radical scavenging activity of Ethanolic extract of Ixora coccinea

The percentage of hydroxyl radical scavenging activity of Ethanolic extract of Ixora coccineapresented in Table 7. Ethanolic extract of Ixora coccinea was exhibited a maximum hydroxyl radical scavenging activity of 63.36 % at µg/ml whereas for ascorbate (standard) was found to be 74.64 % at 90 µg/ml. The IC_{50} values of Ethanolic extract of Ixora coccinea and ascorbate were found to be 200µg/ml and 100µg/ml respectively.

S. No	Concentration (µg/ml)	% of activity(±SEM) *	
		Sample (SEIC)	Standard (Ascorbate)
1	25	31.11 ± 0.032	35.87 ± 0.086
2	50	35.11 ± 0.041	38.87 ± 0.086
3	75	50.21 ± 0.024	45.30 ± 0.034
4	100	56.36 ± 0.088	61.64 ± 0.026
5	200	61.27 ± 0.034	68.23 ± 0.024
6	400	66.36 ± 0.069	73.64 ± 0.022
7	600	81.27 ± 0.014	85.23 ± 0.024
		IC_{50} = 200 µg/ml	**IC_{50} = 100 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Nitric acid Scavenging activity

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The reduction of nitric oxide radical by the Ethanolic extract of Ixora coccinea and ascorbate were illustrated in Table 7. The maximum nitric oxide scavenging activity of Ethanolic extract of Ixora coccinea and ascorbate at 600 µg/ml were found to be 86.17 % and 80.30% respectively. The IC_{50} value of Ethanolic extract of Ixora coccinea and ascorbate were recorded as 600µg/ml and 200µg/ml respectively.

Table 8: Nitric oxide scavenging activity of Ethanolic extract of Ixora coccinea

S. No	Concentration (µg/ml)	% of activity(±SEM) *	
		Sample (EEIC)	Standard (Ascorbate)
1	25	18.58 ±0 .018	26.88 ± 0.016
2	50	20.09 ± 0.043	30.20 ± 0.059
3	75	31.68 ± 0.030	40.64 ± 0.022
4	100	46.47 ± 0.037	65.23 ± 0.018
5	200	54.09 ± 0.069	74.30 ± 0.024
6	400	65.18 ± 0.018	85.84 ± 0.054

7	600	76.17 ± 0.018	95.43 ± 0.081
		IC_{50} = 600 µg/ml	**IC_{50} = 200 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Total Phenolic content

Table 9: The total Phenolic content of Ethanolic extract of Ixora coccinea

Phenolic compounds are known as powerful chain breaking antioxidants. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. The phenolic compounds may contribute directly to antioxidant action. The total phenolic content ofEthanolic extract of Ixora coccinea and ascorbate were illustrated in Table 6.

S. No	Extracts	Total phenol content (mg/g Gallic acid) (±SEM)*
1	Ethanolic extract of Ixora coccinea	1.80 ± 0.040

Based on the result the Ethanolic extract of Ixora coccinea was found higher content of phenolic components.

CONCLUSION

In the present study an attempt has been made to explore pharmacogenetic and phytochemical parameters besides evaluating antimicrobial activity against microorganisms causing skin infections, intestinal infections and urinary tract infections. The identification of the plant material taxonomically pharmacokinetically is important to provide standards and avoid of adulteration of drugs. The plant was identified by Dr. N. Savitramma. Assistant professor, head of Botany Department, Sree Venkateshwara University, Tirupati. The detailed botanical, pharmacogenetic studies with proper authentication of the plants help in m minimizing the adulteration and also for proper identification of the plant.

The preliminary phytochemical analysis of the extracts howed the presence of the alkaloids, flavonoids, paleobotanies, steroids, terpenoids, cardiacglycosides, anthraquinones and saponins constituents may be responsible for the healing potential of skin infections, intestinal, and urinary tract infections. Evolution of antimicrobial activity of ixoracoccinea 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 conform the antimicrobial activity of the prepared ixoracoccinea. From the above results it can be concluded that the ixora coccinea could effectively fight against microorganisms causing skin infection, intestinal and urinary tract infections. Antioxidant compound in food play an important role as a health protecting factor. Primary source of naturally occurring antioxidants are whole grains, fruits and vegetables. The main characteristics of an antioxidant are its ability to trap free radicals. Highly reactive free radicals and oxygen species can initiate degenerative diseases. Plant is one of the most sources of medicines. The medicinal are reach in secondary metabolites and essential oil of therapeutic importance. The Ethanolic extract of Ixora Coccinea has exhibited anti-oxidant properties. So it is used to inhibit free radicals and it ceases oxidation. Thus it can be used asantioxidant.

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