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Phenolic extract of *Ixora coccinea* and *Hydro-alcoholic* extract of *Coccinia grandis fruit* evaluated for antibacterial & antioxidant activity in combined formulation

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Abstract
Present research work was to develop novel formulation methanolic extract of *Ixora coccinea* leaves with combination of alcoholic extract of coccinia grandis, which is reported to possess Anti-oxidant activity anti-bacterial activities and antimicrobial activity. Ixora *coccinea and alcoholic extract of coccinia grandis* combined *formulation* showed significant activities antibacterial & antioxidant assays compared to the standard antioxidant in a dose dependent manner. It is evident that formulation F-1 containing both alcoholic extract of coccinia grandis (3%) and *Ixora coccinea* (7%) showed larger zone of inhibition in comparison to other formulations.

Keywords: formulation, antioxidant activity, *Ixora coccinea*, *Alcoholic extract of Coccinia grandis* antibacterial

Introduction
Ixora coccinea Linn (Rubiaceae) plant used number of pharmacological activity such as hepatoprotective, Chemoprotective, antimicrobial, anti-oxidant, anti-nociceptive, anti–mitotic and anti-inflammatory activities [1]. Free radicals or oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders [2]. In literature is observed that the hydro alcoholic extract of coccinia grandis contain lupeol & taraxerone as active ingredients for particularly wound healing as well as anti-inflammatory activity [8]. In view of the above, we designed the formulation & evaluate the antioxidant potential and antibacterial effect of *Ixora coccinea and alcoholic extract of coccinia grandis combined* formulation.

Materials and methods
Materials
Clove oil was extracted at own Laboratories. Hydro-alcoholic extract of *Coccinia grandis* fruit kindly supplied by Green Chwem Herbal Banglore.Emulsifying wax, white soft paraffin, liquid paraffin and all other chemicals were of analytical grade and used without further purification. Bacterial culture of Staphylococcus aureus and Escherichia coli were obtained from Department of Pharmacognosy R.G. Sapkal College of Pharmacy.

Preparation of crude extract
The dried leaves were exhaustively extracted with 250ml of methanol in a Soxhlet apparatus for 6 Hours. The extract was then concentrated by evaporation methanol and air-dried [7].

Method for preparation of ointment [3, 7]
Mixed *Ixora cocconiea* extract, Hydro-alcoholic extract of *Coccinia grandis* fruit and clove oil. Add this mixture in ointment base with continuous stirring & heated to 70-75°C to melt it completely. Then clove oil and / or *Ixora coccinea* and Hydro-alcoholic extract of *Coccinia grandis* fruit were dissolved in it under stirring and then cooled at room temperature. Disperse acacia and tragacanth in distilled water in a beaker. Add the dispersed phase in the mixture by Doubling Method. Add Preservative.

Table 1: Composition of emulsifying ointment base

Sr. No.	Ingredients	Quantity
1	Ixora Coccinea Extract	7%
2	Hydro-alcoholic extract of *Coccinia grandis* fruit	3%
3	Clove oil	0.7%
4	Accacia	1gm
5	Tragacanth	2gm
6	Benzoic Acid	0.5gm
7	Ointment Base	20gm

Table 2: Composition of different ointment formulations

Item	Material name	Quantity (%)						
		F1	F2	F3	F4	F5	F6	F7
1	Clove oil	0.7	0.7	0.7	0.7	--	--	--
2	Hydro-alcoholic extract of *Coccinia grandis* fruit	3	2	1	1	3	2	1
3	*Ixora coccinea*	7	2	4	1	3	5	6
4	Benzoic acid, Accacia& Tragacanth	q.	q.	q.	q.	q.	q.	q.
5	ointment base	s.	s.	s.	s.	s.	s.	s.

Spreadability [7]
Spreadability was determined using the following formula:

$S = M/T$

Where S is the spreadability in g/s, M is the mass in grams and T is the time in seconds.

Extrudability
A closed collapsible tube containing ointment was pressed firmly at the crimped end. When the cap was removed, ointment extruded until pressure dissipated. Weight in

grams required to extrude 0.7 cm ribbon of ointment in 10 seconds was determined.

Viscosity

Brookfield digital viscometer (model/make-LU-DVE/Brookfield Eng lab.) was used to measure the viscosity (in cps) of the prepared ointment formulations as such that is in semisolid state.

Pharmacological evaluation

a. Antibacterial activity

The compounds were tested in-vitro for their antibacterial activity against two microorganisms *viz*. *Escherichia coli* (NCTC 10418), and *Staphylococcus aureus* (NCTC 6571) which are pathogenic in human beings.

a) Method: Cup-plate agar diffusion method using Nutrient agar.

b. Preparation of test solutions

Each test compound (5 mg) was dissolved in dimethylformamide (5 mL) to give stock solution of concentration 100 mcg/mL. Then 0.1 mL of this solution was used for testing.

c. Preparation of standard solution

Standard drug Norfloxacin was used. The concentration was 100 mcg/mL.

d. Method of testing

Nutrient agar plates were prepared as per research paper reference no [7].

Table 4: Inhibition zone diameters of different formulations

Sr.No.	Compd.	Zone of inhibition	
		E.coli	***S. aureus***
1	F-1	32.3	34.9
2	F-2	24.0	24.2
3	F-3	28.5	30.4
4	F-4	21.3	25.8
5	F-5	15.2	16.6
6	F-6	17.7	18.1
7	F-7	18.4	19.0
Standard	Norfloxacin	31.1	32.4

* ZoI- Zone of inhibition. Values are average of three determinations.

Ferric reducing power assay

The reducing antioxidant power of the plant extracts were determined by the method of Oyaizu [6]. Different concentrations of plant extracts as per research paper [7] then above solutions used for measured absorbance at 700 nm against a blank using UV-Vis spectrophotometer.

Table 5: Ferric reducing power assay

Plant parts	Solvents	Amount (mg of gallic acid equivalents/g of plant material)
Coccinia grandis fruit	*Hydro-alcoholic*	4.1
Ixora coccinea Leaves	Methanol	11.9

Hydrogen peroxide radical scavenging activity

In this method, when a scavenger is incubated with hydrogen peroxide, the decay or loss of hydrogen peroxide can be measured spectrophotometrically at 230 nm. Different concentrations were prepared as per research paper [7].

The data was expressed as % inhibition.

Hydrogen peroxide scavenging activity (%) = [A0– A1/ A1] × 100

Table 6: Hydrogen peroxide radical scavenging activity

Sr.No.	***Formulation***	***Standard (Ascorbic acid)***	***IC***
1	F-1	89.27 ± 0.12	60.31 ± 0.11
2	F-2	48.64 ± 0.13	41.22 ± 0.24
3	F-3	36.21 ± 0.23	24.12 ± 0.52
4	F-4	19.90 ± 0.13	15.32 ± 0.13
5	F-5	58.64 ± 0.13	51.22 ± 0.24
6	F-6	70.44 ± 0.15	45.41 ± 0.32
7	F-7	46.21 ± 0.23	24.12 ± 0.52

Conclusion

The present study concludes that formulation of *Ixora coccinea and alcoholic extract of coccinia grandis combined formulation.* contain high antioxidant and antibacterial property.

The formulations were evaluated for anti-bacterial activity & antioxidant activity, spreadability, viscosity, extrudability. From the results, it is clearly evident that all formulations showed good extrudability, viscosity and spreadability. From the data, it is evident that formulation F-1 containing *alcoholic extract of coccinia* (3%), clove oil (0.7%) and *Ixora coccinea* (7%) showed larger zone of inhibition in comparison to other formulations. So antibacterial & antioxidant activity of formulation F-1 found to be superior to other formulations. Results of all other evaluation parameters of F-1 were also satisfactory among all the formulations.

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