Current Research Journal of Pharmaceutical and Allied Sciences

Journal website: www.crjpasonline.com
Journal email: editor-crjpas@gips-guwahati.ac.in

ISSN: 2456-9674

Research Article

Antimicrobial Potential of *Coccinea cordifolia* and *Crateva magna*

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ABSTRACT

In the present study, *Coccinea cordifolia* root and *Crateva magna* bark were extracted with various solvents according to their increasing order of polarity and the extracts were subjected to preliminary phytochemical investigation. Depending on the phytochemicals present methanolic extract of *Coccinea cordifolia* and chloroform extract of *Crateva magna* were selected for the antimicrobial study. Both the extract has shown good antimicrobial potential against the microorganisms *S. aureus, E. coli, K. pneumoniae, S. typhi* sp., *V. cholera*, *P. aeruginosa, B. subtilis* and *S. faecalis* used in the study, when compared with the standard drug Lincomycin. Hence, from the present investigation it can be concluded that potential antimicrobial agents can be further isolated from both the plants for reproducible results.

ARTICLE INFORMATION

- ***Received*** : 11 November 2018
- ***Received in revised form*** : 29 December 2018
- ***Accepted*** : 30 December 2018
- ***Available online*** : 31 December 2018

Keywords:
Coccinea cordifolia
Crateva magna
Antimicrobial
Phytochemicals

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1. Introduction

The plant *Coccinea cordifolia* is said to be an annual creeper, found twilling around the trees and supports. Leaves are triangular or pentagonal in shape, dentate and have a length of 2 to 5 inch and breathe of 2 inch. Stems are pentagonal in shape, flowers are monocieus and white in colour. Fruits are pulpy, barrel shaped, 1-2 inch in length and green in color when unripe which turns scarlet red on ripening. This fruit posses numerous seeds. Roots are long and contain resins, few alkaloids, starch, gum, fatty acid and carbonic acid. Presence of minerals like calcium, iron and phosphorus are also reported. In Ayurveda the drug is said to be *Kapha, Pitta* suppressant. It is said to be a good wound healer, anti-inflammatory and also a good appetizer which helps improve digestion. It is a good laxative and stimulated liver for proper bile secration (Anonymous, 2011). Steroids, glycosides, saponins, tannins, alkaloids, phenols and carbohydrates are the reported chemical constituents present in the plant. The total phenolic content was found to be 38.25±0.86 (mg/gm) to 72.58±0.36 (mg/gm) whereas the alkaloidal content was in-between 12.52±0.48 (mg/gm) and 24.45±0.19 (mg/gm) for the methanolic extract (Ganga, 2010).

The plant *Crateva magna* is densely foliaceous, deciduous tree, grows up to 10 m in height, fairly smooth with horizontal wrinkles, wood is yellowish white, branches are grayish brown when dry (Pullaiah, 2007). Flower pedicles are up to 6.5 cm with ascending sepals, petals are white with stamens which are 5-5.5 cm ovary is oblong elliosoid, and the stigma are sessile. The berries are sub-spherical, 3.5 cm across, the seeds are dorsally crested and tuberculate (Karantha, 2013). The bark and leaves of the plant are astringent, acrid, diuretic, lithotriptic stimulant, expectorant, demulcent depurative, astiperiodic tonic and bitter. The leaves and barkare also useful in vitiated conditions of *Vata* and *Kapha* (Ayur.), dyspepsia, colic, flatulence, helminthiasis, strangury, renal and vesical calculi, cough, bronchitis, asthma, pruritus, skin diseases, intermittent fevers, visceramegaly, scrofula, inflammations and hepatopathy. The leaf paste isapplied on pilesexternally and leaf juice is used as a drink to get relieffrom bleeding piles (Benniamin, 2005; Warrier, 1994).

Antibiotic resistance has become of grave concern for public health that directly has speeded up the search for new antimicrobials from nature. Number of human pathogens has

evolved to become resistant to many of the currently available antibiotics, considerably increasing mortality and morbidity worldwide. Novel antibiotics are need of the hour to combat these life-threatening pathogens (Subramani, 2017; Kannan et al., 2017).

The increasing cases of drug-resistant pathogens are drawing the attention of the pharmaceutical and scientific researchers towards the potential antimicrobial activity of plant-derived substances, which are used in traditional medicine in different countries. And they are trying to identify and isolate the lead compounds responsible for the activity. While considering that many of these compounds have been used for centuries, they are also considered as source of new drugs. We can expect that, in the coming years some different molecules from ingenious screening programs will be discovered and thus different plant oils and extracts will become useful therapeutic tools (Savoia, 2012).

Of late, we have seen an increase in the number of studies to discover bioactive leads from plant origin to control antibiotic-resistant bacteria (Ruben, 2008). In this direction and based upon traditional claims we designed our study to evaluate the antimicrobial potential of extracts of bark of *Crateva magna* and root of *Coccinea cordifolia*.

2. Materials and Methods

2.1. Plant materials

Crateva magna bark was collected from Lakhimpur district of Assam near Assam-Arunachal Pradesh border of India. Whereas, *Coccinea cordifolia* roots and tubers were collected from Jalpaiguri District of West Bengal (India). Both the plant materials were washed and cleaned properly to remove adhered materials. Both the crude drugs were then sundried and powdered using mechanical grinder and were made to pass through sieve no. 10. The powdered materials were defatted using Petroleum ether followed by extraction with chloroform in a Soxhlet apparatus. Chloroform extract of *Crateva magna* bark and methanolic extract of *Coccinea cordifolia* root were used as test drug for the study.

2.2. Microorganisms

The microbial species were obtained from Microbiology Department of Jadavpur University, Kolkata (India). The collected species were *S. aureus, E.coli, K. pneumoniae, S. typhi* sp., *V. cholera, P. aeruginosa*, *B. subtilis* and *S. faecalis*. The cultures of bacteria were maintained on nutrient agar slants at 4°C and sub-cultures were transferred to nutrient broth, 24 hours before testing.

2.3. Preservation of bacterial strains

Strains of *S. aureus, E.coli, K. pneumoniae, S.typhi , V. cholera, P. aeruginosa, B. subtilis* and *S. faecalis* were preserved using slant cultures at 4ºC temperature. The routine sub culture of Gram positive strains were done using nutrient agar media and for Gram negative strains bromothymol blue lactose agar media was used (Peryasamy, 2010).

2.4. Standard antibiotic

Lincomycin (Lyka Labs, India) was used as standard drug for the study. The pure sample of the drug was received as gift sample from M/S Lyka Labs, India.

2.5. Preparation of impregnated discs of extract and standard antibiotics

Discs with 7.25 mm diameter were prepared by punching of Whatman no.1 filter paper and all the discs were sterilized by dry heat at 160ºC for an hour in batches in screw capped Bijou bottles. The Chloroform extract of *Crateva magna* bark and methanolic extract of *Coccinea cordifolia* root were weighed and dissolved in sterile distilled water to make the required solutions of concentration 128-2000 µg/mL. Similarly, the stock solution of the control antibiotic (lincomycin) within the range of 0-1000 µg/mL were prepared by dissolving the required amount of the drugs in sterile distilled water. All the prepared stock solutions were then kept at 4ºC. Now, for preparing antibiotic impregnated discs, 1.0 mL of the stock solutions of the antibiotic was added separately to sterile Bijou bottles containing 100 discs each. The same procedure was followed for preparing impregnated discs of the plant extracts. The discs were used while wet and can be stored for further use at 4ºC, as they can retain the moisture and potency for at least 3 months in the screw capped Bijou bottles (Jeya, 2012; Mahesh, 2008).

2.6. Antimicrobial assay of chloroform extract of *Crateva magna* bark and methanolic extracts of *Coccinea cordifolia* root

The antimicrobial assay was performed by disc diffusion method. Nutrient agar plates with inoculum size of 105-106 cfu/mL of the microorganisms were used. Previously prepared discs (Concentration 128-2000 µg/mL) and antibiotic and compound 1 (concentration 0-1000 µg/mL) were placed aseptically on petridishes. All the petridishes were then incubated at 37ºC±2ºC for 18 hour. After 18 hours, the activity was recorded by measuring the zone of inhibition on the petridishes around the discs. The zone of inhibitions thus observed, were measured using a transparent ruler and recorded with reference to the zone of inhibition of the standard drug. Lincomycin was taken as standard drug for this study (Estari, 2013; Abdullah, 2011).

2.7. Minimum Inhibitory Concentration (MIC)

MICs were determined using standard agar dilution method (Tanti, 2010). The extracts were dissolved in 0.5 ml of dimethyl sulphoxide and then further diluted using sterilized distilled water. The drug solution thus prepared was then added to the molten nutrient agar in different tubes to give a concentration in the range of 0–128 µg/mL and then subsequently increasing it two folds up to 2000 µg/mL. The pH of the tubes were adjusted to 7.2 to 7.4 and transferred to sterile petridishes. Bacterial cell suspensions (10 µL) were then inoculated on the petridishes using sterile bacterial planter. Number of cfu inoculated onto the petridishes was 105 for all the microbial strains. All the inoculated petridishes were then incubated for 18 hours at 37°C±2°C. The petridish with lowest concentration which did not show any visible growth of microorganism upon incubation was considered as MIC for that particular microbial strain. The petridishes containing sterile distilled water and Lincomycin solution, served as negative and positive control respectively.

3. Results

3.1. Antimicrobial assay and Minimum inhibitory concentration (MIC ± SD of three replicates) of methanolic extract of *Coccinea cordifolia* root.

The results of the antimicrobial assay for methanolic extract of *Coccinea cordifolia* root (Table 2) shows that, out of 54 bacterial strains, the growth of 34 strains were inhibited by the extract at concentrations within the range of 128 – 512 µg/mL, 12 strains were inhibited at a concentration of 1000 µg/mL, while the remaining 08 strains were inhibited at a concentration of >2000 µg/mL, which is the highest concentration for the methanolic extract. The MIC study shows that 10 out of 18 gram positive strains were sensitive between 128 and 256 µg/mL (Zone of inhibition 10-16 mm); whereas 14 out of 36 gram negative strains were sensitive at a concentration range between 128-256 µg/mL of the chloroform extract (Zone of inhibition 08-16 mm). So we can conclude that, the methanolic extract of *Coccinea cordifolia* root has shown antimicrobial activity against both gram positive and gram negative strains.

3.2. Antimicrobial assay and Minimum inhibitory concentration (MIC± SD of three replicates) of Chloroform extract of *Crateva magna* bark.

The results of the antimicrobial assay (Table 1) shows that out of 54 bacterial strains, the growth of 33 strains were inhibited by the extract at concentrations within the range of 128–512 µg/mL, 14 strains were inhibited at a concentration of 1000 µg/mL, while the remaining 07 strains were inhibited at concentration >2000 µg/mL, which was the highest concentration for extract. The MIC study shows that 5 out of 18 gram positive strains were sensitive between 128 and 256 µg/mL (zone of inhibition 12-16 mm); whereas 15 out of 36 gram negative strains were sensitive at a concentration range between 128-256 µg/mL of the chloroform extract (Zone of inhibition 08-12 mm). So we can conclude that, the Chloroform extract of *Crateva magna* has shown antimicrobial activity against both gram positive and gram negative isolates.

4. Discussion

The results shows antimicrobial potential for both the methanolic extract of *Coccinea cordifolia* root and the isolated compound 2, when compared with the standard drug Lincomycin, which has shown inhibitory effect against 16 out of 18 strain of gram positive and 32 out of 36 of gram negative strains at concentration range between 0.25-256 µg/mL with zone of inhibition between 10-18mm.

Whereas the results for *Crateva magna* has shows good antimicrobial potential for both the chloroform extract of Crateva magna, when compared with the standard drug Lincomycin, which has also shown inhibitory effect against 16 out of 18 strain of gram positive and 29 out of 36 of gram negative strains at concentration range between 0.25-256 µg/mL with Zone of inhibition between 12-20mm.

Conclusion

Hence, from the present investigation it can be concluded that potential antimicrobial agents can be further isolated from both the plants for reproducible results.

Table 1: *In vitro* antimicrobial assay and Minimum inhibitory concentration (MIC± SD of three replicates) at 600nm OD of methanolic extract of *Coccinea cordifolia* root.

Pathogens	Number of strain	MIC of leaf extracts (µg/mL)					Zone of inhibition (mm)	MIC of Lincomycin (µg/mL)						
		128	256	512	1000	>2000		0.25	0.5	8	64	128	256	>1000
S. aureus	07	02	03	01	-	01	++	-	03	-	01	02	-	01
K. pneumoniae	06	-	03	02	01	-	+	01	02	-	01	01	01	-
E. coli	06	01	-	02	02	01	++	01	-	02	-	01	01	01
Salmonella. sp	09	-	02	02	03	02	++	-	01	-	01	02	03	02
V. cholerae	07	01	02	01	02	01	++	01	02	-	01	03	-	-
B. subtilis	06	02	-	01	02	01	+	-	01	01	-	03	01	-
S. faecalis	05	-	03	01	-	01	++	-	02	01	-	01	-	01
P. aeruginosa	08	02	01	02	02	01	++	02	01	-	01	02	01	01
Total	54	08	14	12	12	08		05	12	04	05	15	07	06

Methanolic extract of *Coccinea cordifolia*; +: ≤ 10mm; ++: ≥12mm; the inoculum size used was 10^5cfu per spot for all the organisms except *S. aureus*, where the inoculum size per spot was 10^6cfu. The result represents the mean value of triplicate tests.

Table 1: *In vitro* antimicrobial assay and Minimum inhibitory concentration (MIC± SD of three replicates) at 600nm OD of chloroform extract of *Crateva magna* bark.

Pathogens	Number of strain	MIC of Chloroform extracts (µg/mL)					Zone of inhibition (mm)	MIC of Lincomycin (µg/mL)						
		128	256	512	1000	>2000		0.25	0.5	8	64	128	256	>1000
S. aureus	07	01	01	02	03	-	++	02	-	01	02	-	02	-
K. pneumoniae	06	03	01	01	-	01	+	-	01	-	01	01	01	02
E. coli	06	01	02	01	02	-	++	-	01	01	01	-	01	02
Salmonella. sp	09	-	03	02	02	02	++	-01	01	01	02	01	02	01
V. cholerae	07	02	-	02	03	-	+	-	01	01	02	02	-	01
B. subtilis	06	-	01	02	02	01	++	01	01	01	-	02	-	01
S. faecalis	05	-	02	01	01	01	++	-	01	01	-	01	01	01
P. aeruginosa	08	-	03	02	01	02	+	01	01	01	02	-	02	01
Total	54	07	13	13	14	07		05	07	07	10	07	09	09

Chloroform extract of *Crateva magna*; +: ≤ 10mm; ++: ≥12mm; the inoculum size used was 10^5cfu per spot for all the organisms except *S. aureus*, where the inoculum size per spot was 10^6cfu. The result represents the mean value of triplicate tests.

Conflict of Interest

No conflict of interest.

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How to cite this article:

Deb Roy S, Dash S, Das B. Antimicrobial Potential of *Coccinea cordifolia* and *Crateva magna*. Current Research Journal of Pharmaceutical and Allied Sciences. 2018; 2(4): 1-4.