

## Antibacterial Activity of the Leaves of *Coccinia indica* (W. and A) Wof India

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**Abstract:** The aim of the present research was focused on investigating the antibacterial and preliminary phytochemical properties of *Coccinia indica* (W.A.) via in vitro approach. The aqueous and organic solvent (Petroleum ether, chloroform and ethanol) extracts from the leaves of *Coccinia indica* (Cucurbitaceae) were tested against *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *Salmonella typhimurium* by agar well diffusion method and broth dilution method. Results showed promising antibacterial activity against the bacteria tested. Among these, ethanol and aqueous extracts were found to have a more potent inhibitory effect comparing with the other extracts. Which prove the potentiality of the plant extracts for the treatment of various skin and gastrointestinal infections in humans.

**Key words:** *Coccinia indica* • Phytochemical • Antibacterial activity • Pathogens • HPTLC

### INTRODUCTION

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country [1]. Herbal medicine is still the mainstay of about 75-80% of the whole population and the major part of traditional therapy involves the use of plant extract and their active constituents. Following the advent of modern medicine, herbal medicine suffered a set back, but during last two or three decades, advances in phytochemistry and in identification of plant compounds, effective against certain diseases have renewed the interest in herbal medicines [2]. Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions [3]. This situation forced to search for new antimicrobial substances. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants. Antimicrobials of plant origin have enormous therapeutic potential [4]. They are effective in the treatment of infectious diseases while simultaneously mitigating many

of the side effects that are often associated with synthetic antimicrobials [5]. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins and phenol compounds, flavonoids, steroids, resins fatty acids gums which are capable of producing definite physiological action on body [6].

*Coccinia indica* belongs to the family Cucurbitaceae. It is growing wild throughout India and also cultivated in various parts of India. It is commonly known as kundru [7]. The whole plant is traditionally used for various medicinal purposes. Leaves of this plant are used in Indian folk medicine for treatment of number of ailments including diabetes, wounds, ulcers, inflammation, in eruptions of skin, fever, asthma and cough. Earlier scientific investigation of *Coccinia indica* showed that the crude extract has hepatoprotective [8 - 10] antioxidant [11, 12] anti-inflammatory and anti-nociceptive [13, 14] anti-diabetic [15 - 20] hypolipidemic [18] anti-bacterial [21] and antitussive activities [22]. Though the plant has been reported for many biological activities, no scientific data available to identify the genuine sample.

The present study aimed to screen and evaluate antibacterial activity of crude extracts of *Coccinia indica* leaf and to find out minimum inhibitory concentration (MIC) of these extracts against both Gram positive as well as Gram negative bacteria.

## MATERIALS AND METHODS

**Collection and Authentication:** The fresh leaves of wildy growing plant *Coccinia indica* were collected from the field areas of eastern Uttar Pradesh region during the month of February, 2009. For identification and taxonomic authentication, sample of plant material was given to National Botanical Research Institute (NBRI) Lucknow, India. The text report from National botanical research institute, Lucknow, India and confirmed the authenticity of plant material sample was *Coccinia indica* with voucher specimen no. NBRI -SOP-202 Receipt no. and date 19/72, 24-02-09. The fresh leaves were used for the study of macroscopic and microscopical characters. Whereas collected plants were shade-dried and coarsely powdered. This coarse powder was used for the determination of ash values, extractive values and preliminary phytochemical investigation was studied as per standard methods.

**Extraction of Plant Materials:** 100 g coarse powder of air dried leaves of *Coccinia indica* were packed in muslin cloth and subjected to soxhlet extractor for continuous hot extraction with distilled water, ethanol, petroleum ether and chloroform for 8 hrs separately. Then the each extracts were filtered and filtrate was evaporated to dryness. The percentage yield of the water, ethanol, petroleum ether and chloroform extracts was 23.8, 7.03, 2.45% and 4.15%, respectively.

**Preliminary Phytochemical Screening:** Preliminary phytochemical screening for the detection of various was carried out by using standard procedures described by Harborne [23] and Khandelwal [24].

**Thin Layer Chromatography and High Performance Thin Layer Chromatography (HPTLC):** Thin layer chromatography studies of the ethanol and chloroform extracts were carried out in various solvents at 30°C using Silica gel G as adsorbent and the  $R_f$  values were determined [25]. The same mobile phase was used for the HPTLC profiles of these extracts.

### Screening of Antibacterial Activity *Coccinia indica* (W. and A)

**Bacterial Strains Used:** About five human pathogenic bacterial strains were used. Both the Gram-negative (*Enterobacter aerogenes*, *Pseudomonas aeruginosa*,

*Salmonella typhimurium*) and Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus epidermidis*) were included. Axenic cultures of bacterial strains were obtained from the Department of Biotechnology, Integral University, Lucknow.

**Agar Well Diffusion Method:** Antibacterial activity was screened by agar well diffusion method [26, 27]. Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with eight-hours-old broth culture of respective bacteria. Using the sterile cork borer, the well (6mm) was made into the each Petri-plate. Various concentrations of petroleum ether, chloroform, ethanol and aqueous extracts (25, 50 and 75mg/ well) were used to assess the dose dependent activity of the extracts. The extracts were prepared in DMSO (Dimethyl sulphoxide) which showed no zone of inhibition and acts as a negative control and were added into the wells by using sterile micropipettes. Simultaneously the standard antibiotics (as positive control) were tested against the pathogens. Discs of Doxycycline (30 µg), Gentamicin (10 µg), Penicillin (10 units/disc), Streptomycin (300µg), Ampicillin (10 µg) and Tetracycline (10µg) were used as positive antibacterial controls. All product of Himedia Laboratories Mumbai (India) were used in this study. Then the plates were incubated at 37°C for 24 - 48 hours. After the incubation period, the diameter of the inhibition zones of each well was measured. And the values were noted. Triplicates were maintained in each extract and the average values were calculated for the eventual antibacterial activity.

**Broth Dilution Test:** Broth dilution test is used to determine the Minimum Inhibitory Concentration (MIC) of the antimicrobial drugs. Freshly prepared nutrient broth was used as diluents. Overnight cultures of the test bacteria grown in nutrient broth cultures were diluted 100 folds in nutrient broth. (100 µl bacterial cultures in 10 ml NB). Increasing concentrations of the extract were added to the test tubes containing the bacterial cultures to know the inhibitory concentration in a particular tube inhibiting the bacterial growth. All tubes were incubated at 37°C for 24 hours. The tubes were examined for visible turbidity and optical density of cultures were determined at 620 nm using NB as a control. Control tubes without the tested extracts were assayed simultaneously. The lowest concentration that inhibited visible growth of the tested organisms was recorded as MIC [28, 29].

## RESULTS

**Preliminary Phytochemical Screening:** The preliminary phytochemical investigation of the aqueous, ethanol, petroleum ether and chloroform extracts of *Coccinia indica* showed the presence of phytosterols, flavonoids, terpenoid saponins, carbohydrates, tannins, glycosides alkaloids proteins organic acids Table 1.

**Thin Layer Chromatography and High Performance Thin Layer Chromatography (HPTLC):** Thin layer chromatography of the ethanol and chloroform extracts was carried out using Toluene: Ethyl acetate (8.5: 1.5) as mobile phase, respectively and the R<sub>f</sub> values were recorded (Table 2). The visualizing reagent employed was anisaldehyde-sulphuric acid reagent to effect visualization of the resolved spots (Fig. 4). TLC and HPTLC finger printing studies on ethanol extract showed presence of various phytoconstituents with their respective R<sub>f</sub> values. The ethanolic extract was developed on chromatographic plates with many ratios of different solvents and the best eluent mixture was used further for HPTLC profile to minimize errors in TLC pattern.

Table 1: Qualitative analysis of phytochemicals in *Coccinia indica* Leaf Extracts

Extracts	Petether	Chloroform	Ethanolic	Aqueous
Sterols	+	+	+	-
Tannins	-	+	+	+
Flavonoids	-	-	+	+
Proteins and amino acids	-	+	+	+
Glycosides	-	+	+	+
Phenols	-	-	+	+
Acidic compounds	-	-	-	-
Carbohydrates	-	-	+	+
Saponins	-	+	+	-
Alkaloids	-	+	+	+

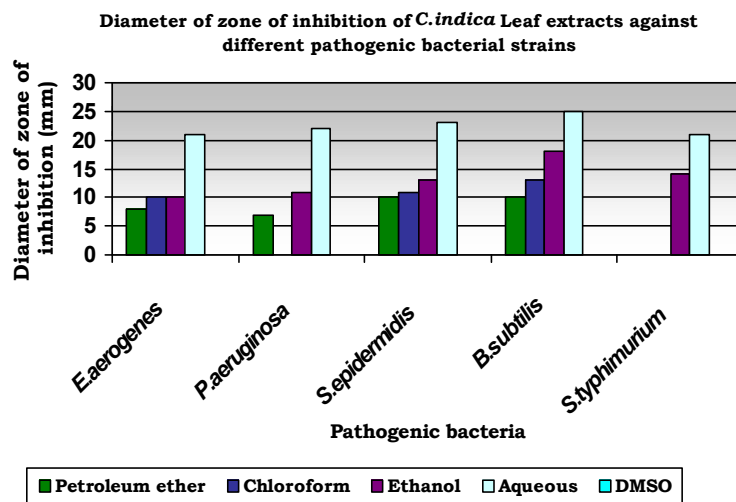
+ Present - Absent

Table 2: Thin layer chromatography of leaf extracts of *Coccinia indica*

S.No.	Test extract	Solvent system	Number of spots	R <sub>f</sub> value
1	Chloroform extract	Toluene: Ethylacetate (8.5: 1.5)	6	0.07
				0.15
				0.31
				0.69
				0.76
2	Ethanol extract	Toluene: Ethylacetate (8.5: 1.5)	5	0.07
				0.15
				0.72
				0.79
				0.99

The preliminary HPTLC studies revealed that the solvent system Toluene: Ethyl acetate (8.5: 1.5) was ideal and gave well resolved sample peaks. The spots of the chromatogram were visualized at 366 nm (Fig. 5).

**Antibacterial Activity:** Results of antibacterial activities by agar well diffusion method were presented in Fig. 1 and minimum inhibitory concentration (MIC) values were tabulated in Fig. 3. The antibacterial activity was tested on the basis of the magnitude of zones of inhibition (in mm) and minimum inhibitory concentration (in mg/ml). The activity of *Coccinia indica* has also been compared with the broad spectrum commercially available antibiotics. Some bacteria were found to be resistant towards commercially used antibiotics while others were found to be sensitive (Fig. 3). The detailed analysis of the antibacterial activity of the petroleum ether extract showed dose dependent activity and the activity was shown at an amount of 75 mg/ml well. (*E. aerogenes* and *P. aeruginosa*). While less activity was shown when 50 mg/well amount of extract was used (*S. epidermidis* and *B. subtilis*). *S. epidermidis* and *B. subtilis* showed maximum susceptibility followed by *Enterobacter aerogenes* and *Pseudomonas aeruginosa*. While *Salmonella typhimurium* was resistant to the extract. Gram positive bacteria were more susceptible towards this extract than tested Gram negative ones. The MIC of extract for *E. aerogenes* was 7.5 mg/ml, for *P. aeruginosa* was 7.5 mg/ml, for *S. epidermidis* was 5.0 mg/ml, for *B. subtilis* was 5.0 mg/ml. while no activity was shown by this extract against *S. typhimurium*. When compared to the standard antibiotics, it was seen that petroleum ether extract was effective than ampicillin, doxycycline and penicillin against *E. aerogenes* and *P. aeruginosa* while these antibiotics were resistant to this bacteria. Extract was found to more effective than tetracycline only in case of *P.aeruginosa* and more effective than streptomycin only in case *S. epidermidis*. It was found less active than gentamicin. The Chloroform extract of *Coccinia indica* was found to be effective at an amount of 50mg/ well (*E. aerogenes* and *S. epidermidis*). While activity was observed when 25 mg/well amount of extract was used (*B. subtilis*). *B. subtilis* showed maximum susceptibility followed by *S. epidermidis* and *Enterobacter aerogenes*. While *Pseudomonas aeruginosa* and *Salmonella typhimurium* were resistant to the extract. Gram positive bacteria were more susceptible towards this extract than tested Gram negative ones. The MIC of extract for *E. aerogenes* was 5.0 mg/ml, for *S. epidermidis* was 5.0 mg/ml, for *B. subtilis* was



Data is a mean of two replications

"-" No inhibition observed

Antibiotics were used as positive control

10% DMSO were used as negative control (No inhibition observed)

Fig. 1: Antibacterial activity of leaf extracts of *Coccinia indica* against different pathogenic bacterial strains by Agar well diffusion method

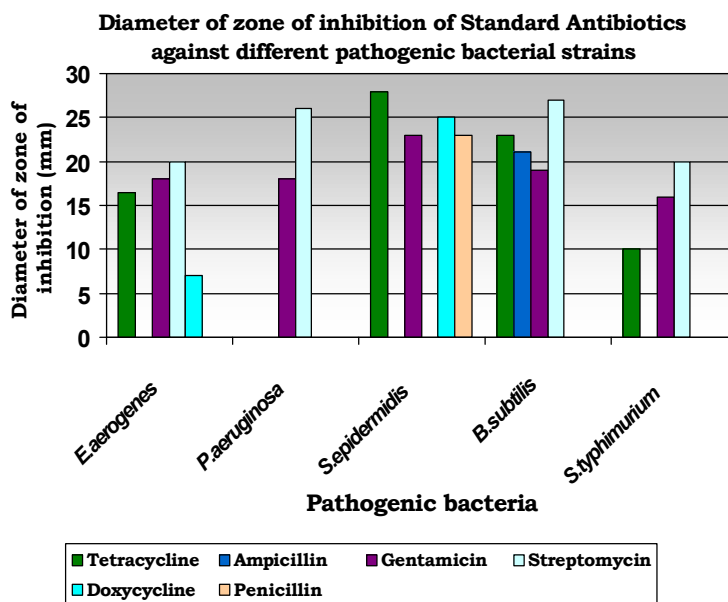


Fig. 2: Diameter of Inhibition zones of antibiotics against pathogenic bacterial strains

2.5 mg/ml, while no activity was shown by this extract against *S. typhimurium* and *P. aeruginosa*. When compared to the standard antibiotics, it was seen that chloroform extract showed more activity than doxycycline and penicillin in case of *E. aerogenes* and *B.subtilis*. Chloroform extract was also found to more effective than

streptomycin only in the case of *S. epidermidis* and better than ampicillin in case of *S. epidermidis* and *E. aerogenes* both. It was found less active than tetracycline and gentamicin. Ethanol extract was found to be effective at a dose of 50 mg/well (*E. aerogenes*, *P. aeruginosa* and *S. epidermidis*). While, activity was

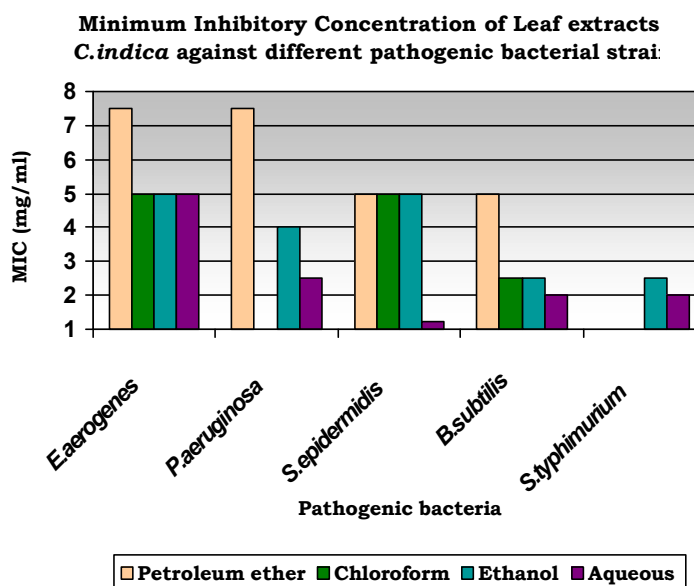


Fig. 3: Minimum inhibitory concentration (MIC) of leaf extracts of the plant *Coccinia indica* against different pathogenic bacterial strains



Fig. 4: TLC finger printing of ethanolic and chloroform extract of leaf of *Coccinia indica*.

shown when 25 mg/well amount of extract was used (*B. subtilis* and *S. typhimurium*). Gram positive and Gram negative both bacteria were found to be susceptible towards this extract. The MIC of extract for *E. aerogenes* was 5.0 mg/ml, for *P. aeruginosa* was 4.0 mg/ml, for *S. epidermidis* was 5.0 mg/ml, for *B. subtilis* was 2.5 mg/ml. and for *S. typhimurium* was 2.5 mg/ml. When compared to the antibiotics it was found that ethanol extract was more effective than tetracycline in the case

of only *P. aeruginosa* because this bacterium is resistant to the tetracycline. Also found to be more effective than ampicillin against all the microorganisms except *B. subtilis*. Better than streptomycin against *S. epidermidis*, better effective than doxycycline and penicillin against all tested organisms except *S. epidermidis*. Ethanol extract was less effective than gentamicin.

Aqueous extract also showed dose dependent activity and the activity was shown at an amount of 25 mg/well against all the tested pathogens except *E. aerogenes*. *E. aerogenes* was sensitive when 50 mg/well amount of extract was used. Gram positive and Gram negative both bacteria were found to be susceptible towards this extract, but extract was found to be most effective against Gram positive ones. The MIC of extract for *E. aerogenes* was 5.0 mg/ml, for *P. aeruginosa* was 2.5 mg/ml, for *S. epidermidis* was 1.25 mg/ml, for *B. subtilis* was 2.0 mg/ml and for *S. typhimurium* was 2.0 mg/ml. When compared to the standard antibiotics, it was shown that aqueous extract was more effective than tetracycline and doxycycline against all tested pathogens except *S. epidermidis*. When compared to the ampicillin it was found to be more effective in case of all the tested microorganisms. When activity of aqueous extract was compared to the gentamicin it was seen similar effective (23mm) in case of *S. epidermidis* but more effective against rest of bacteria. When compared to the streptomycin extract was seen less effective in case of

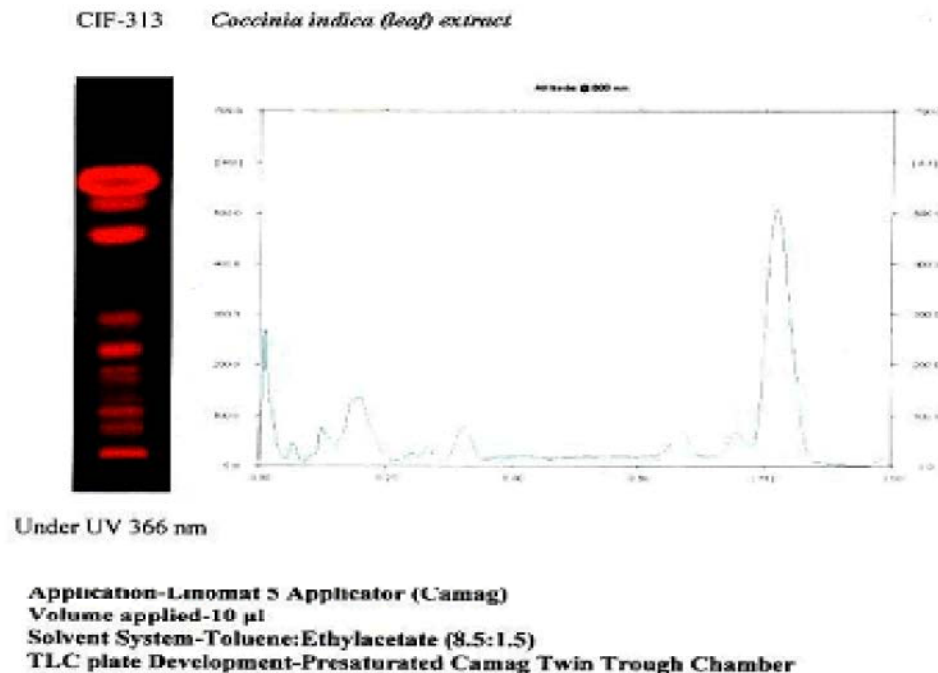


Fig. 5: HPTLC Finger printing of ethanolic extract of leaf of *Coccinia indica* scanned at wavelength 366 nm

*P. aeruginosa* and *B. subtilis* while more effective against rest of three bacteria. Aqueous extract was similar effective like penicillin (23 mm) in case of *S. epidermidis* while found to be more effective against rest of bacteria.

The result showed that *Salmonella typhimurium* was the most resistant strain and *Bacillus subtilis* was the most sensitive strain toward leaf extracts of the plant. This result was interesting because this bacteria form resting spores and are more resistant to environmental conditions than any other tested bacteria.

By analyzing the overall data it was observed that the gram positive pathogens were more susceptible towards the different leaf extracts tested. While Gram negative bacteria showed little resistance towards some extracts. It is not known exactly why Gram negative bacteria should be less susceptible, but it may be related to its outer membrane which endows the bacterial surface with strong hydrophilicity and acts as a strong permeability barrier [28].

Some of the phytochemical compounds e.g. glycoside, tannin, flavonoids, alkaloids, have variously been reported to have antimicrobial activity [30]. It might be possible that the antibacterial activity of *Coccinia indica* leaf extracts was due to the inhibition of bacterial cell wall synthesis or because of leakage from cell membranes of bacteria or it might be

possible that the effect of extract was shown due to the inhibition of protein synthesis of bacterial cell or due to the possibility to interfere with DNA function of the bacteria.

## DISCUSSION

In the present work, *in vitro* studies concluded that the plant extract inhibited bacterial growth but their effectiveness varied. The antimicrobial activity has been attributed to the presence of some active constituents in the extracts. This antibacterial study of the plant extracts demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases [31]. These findings support the traditional knowledge of local users and it is a preliminary, scientific, validation for the use of these plants for antibacterial activity to promote proper conservation and sustainable use of such plant resources [32].

It is interesting to note that the aqueous extract of leaf of *Coccinia indica* could be used against salmonella. Since Salmonella is a frequent candidate causing food-borne illnesses in addition to the typhoid and paratyphoid infection, the therapeutic value of this

plant could be an effective remedy. Further, it was revealed that all the extract has the ability to inhibit the growth of *Bacillus subtilis*, another organism responsible for food borne gastroenteritis. Hence it would be interesting to investigate the potentiality of this plant for possible application in foods to increase shelf life or promote safety [33]. It can also be suggested to promote the use of this plant against various gastrointestinal disorders as well as in skin infections.

This study is a substantial step and it further required a long term study to evaluate therapeutic efficacy and toxicity of leaf. This result may provide a basis for the isolation of compounds of from *Coccinia indica*. Further studies are needed to identify the pure component and establish the mechanism of action for antibacterial action of the plant extract.

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