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SCREENING OF ANTIMICROBIAL, CYTOTOXIC AND PESTICIDAL ACTIVITIES OF *COCCINIA GRANDIS* (L.) VOIGT

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ABSTRACT

This study was undertaken to assess the antibacterial, antifungal, cytotoxic and pesticidal activities of *Coccinia grandis* roots extract. The antimicrobial activity was evaluated using disc diffusion method against some pathogenic microorganisms. Cytotoxicity was determined using brine shrimp lethality bioassay method. The plant extract was screened for pesticidal activity towards *Sitophilus oryzae* adults. Minimum inhibitory concentration (MIC) was also studied against test organisms, using serial dilution technique to determine the antibacterial potency. In antibacterial screening, large inhibition zones were observed against the tested gram-positive (*Bacillus subtilis*, *Sarcina lutea* and *Stapphylococcus aureus*) and gram-negative (*Salmonella typhi* and *Shigella dysenteriae*) bacteria. In antifungal screening, the extract showed moderate antifungal activities against the tested fungi (*Candida albicans* and *Colletotrichum falcatum*). In cytotoxicity activity test, LC_{50} (lethal concentration, 50%) of the extract against brine shrimp nauplii was 15.00µg/ml. The plant extract also showed moderate pesticidal activities towards *S. oryzae* adults. These results suggested that the plant extract has significant antibacterial activity against the tested bacteria, moderate antifungal, cytotoxic and pesticidal activity towards the tested fungi, brine shrimp nauplii and *S. oryzae* adults, respectively.

Keywords: *Coccinia grandis*, methanol extract, antimicrobial activity, cytotoxicity, pesticidal activity

INTRODUCTION

Coccinia grandis (L.) Voigt (family of Cucurbitaceae), locally known as Telakucha, is an important medicinal plant, distributed in Indian sub-continent, Eastern Africa, and Central America. Different parts of the plant are used by humans mostly as a food crop in several countries in Australia, Asia, Caribbean, the southern United States, and Pacific Islands (**Pekamwar *et al.*, 2013**). Fruits may be eaten immature and green, or mature and deep red **(Hasanuzzaman *et al.*, 2013)**. The young shoots and leaves may also be eaten as greens. The fruits, stems, roots and leaves of the plant are popularly used in the treatment of edema, eye diseases, carminative, hypertension, fever, anti-inflammatory, headache, typhoid, sunstroke, hypnotic, jaundice, stomach pain, anti-pyretic, mental disease, leucorrhea, alopecia, dermatitis, eczema, emetic, dysentery, scabies and blood purifier (**Sivaraj *et al.*, 2011; Abbasi *et al.*, 2009**). Phytochemical screening of *Coccinia grandis* revealed the presence of saponins, cardenolides, flavonoids and polyphenols that may be attributed to antibacterial activity (**Sivaraj *et al.*, 2011**). The root of this plant contains resin, alkaloids, starch, fatty acids, carbonic acid, triterpenoid, saponin coccinoside, flavonoid glycoside, lupeol, β-amyrin, β-sitosterol, taraxerol (**Deokate and Khadabadi, 2011**).
The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immunocompromised patients in developing countries. To overcome this problem, there is a constant need for new and effective infection fighting strategies. Therefore, there is a need to develop alternative therapeutic agents for the treatment of infectious diseases from medicinal plants (**Tumpa *et al.*, 2015**). Medicinal plants continue to be used world-wide for the treatment of various diseases and have a great potential for providing novel drug leads with novel mechanism of action (**Singh *et al.*, 2012**). There are many reports on *Coccinia grandis* plant including, antibacterial and antifungal activity of leaves extract (**Bhattacharya *et al.*, 2010**), antiplasmodial activity against the *Plasmodium falciparum* (**Ravikumar *et al.*, 2012**), anti-inflammatory activity of leaves and stem extracts (**Deshpande *et al.*, 2011**), anthelmintic activity (**Tamilselvana *et al.*, 2011**), antioxidant activity (**Ashwini *et al.*, 2012**), antipyretic activity (**Aggarwal *et al.*, 2011**), anticancer activity of leaves extract (**Bhattacharya *et al.*, 2011**), antitussive activity of fruit extract (**Pattanayak and Sunita, 2009**), antiulcer activity of leaves extract (**Santharam *et al.*, 2013**) and others (**Kumar, 2012; Ravikumar *et al.*, 2012**). But there is no sufficient report on antimicrobial, cytotoxic and pesticidal activity on this valuable plant root extract.
The present study was designed to determine the role of methanolic extract of *Coccinia grandis* roots for potential antibacterial and antifungal activities against some pathogenic bacteria and fungi. The cytotoxicity and pesticidal activities of the plant extract were also determined using brine shrimp nauplii and *S. oryzae* adults, respectively.

MATERIAL AND METHODS

Plant material

Coccinia grandis plants were collected from Rajshahi University Campus, Rajshahi, Bangladesh and were identified by Md. Shahed Alam, Senior Technical Officer, Herbarium Museum, Department of Botany, University of Rajshahi, Bangladesh, where its voucher specimen was deposited for reference. Roots of these plants were used as plant material for this present investigation.

Chemicals and reagents

Methanol and DMSO (dimethyl sulfooxide) were purchased from Merck, Germany. Kanamycin was purchased from Square Pharmaceuticals Ltd., Bangladesh. Gallic acid and vincristine sulfate were purchased from Cipla Ltd., Goa, India. All the chemicals and reagents used throughout the investigation were of reagent grade.

Organisms

Antibacterial activity and MIC values were determined against six gram-positive bacteria (*Bacillus cereus, Bacillus subtilis, Bacillus megaterium, Sarcina lutea, Staphylococcus aureus*, and *Stapphylococcus-β-haemolyticus*) and seven gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Shigella boydii, Shigella dysenteriae, Shigella flexneri* and *Shigella sonnei*). Antifungal screening was carried out against seven fungi (*Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Candida albicans, Colletotrichum falcatum,*

Rhizopus oryzae and *Tricophyton rubrum*). Cytotoxicity was determined against brine shrimp nauplii (*Artima salina*). Brine shrimp nauplii were obtained by hatching brine shrimp eggs (Carolina Biological Supply Company, Burlington, NC, USA) in artificial sea water (3.8% sodium chloride solution) for 48h. These organisms were collected from the Microbiology Laboratory, Department of Microbiology and Institute of Nutrition and Food Sciences (INFS), University of Dhaka, International Centre for Diarrhoea Diseases Research Bangladesh (ICDDRB), Dhaka, Bangladesh. Pesticidal activity was tested against *Sitophilus oryzae* L. adult insect. The insects were collected from the "Integrated Pest Management Laboratory" Institute of Biological Sciences, University of Rajshahi, Bangladesh.

Media

Nutrient agar media (Difco laboratories) pH 7.2, nutrient broth media (Difco Laboratories) pH 6.8, Sabouraud dextrose agar media (Biolife Vole Monza) pH 5.6 and artificial seawater (3.8% sodium chloride solution) pH 8.4 were used for antibacterial screening, MIC determination, antifungal screening and cytotoxicity determination, respectively. A standard mixture of rice and powdered brewer's yeast in the ratio of 19:1 was used as food medium to culture *S. oryzae*.

Plant material extraction and fractionation

Collected roots of the plants were cut, air-dried powdered in a grinding machine and stored in an airtight polybag. Powdered dried roots (400g) of the plant were extracted (cold) with methanol (1.25 Liter) in flat bottom conical flask, through occasional shaking and stirring for 10 days (**Jeffery *et al*., 2000**). The content was pressed through the markin cloth to get maximum amount of extract. The whole mixture was then filtered by Whatman filter paper No. 41 and the remaining filtrate was dried (**Hussain *et al*., 2010**) in vacuo to afford a blackish mass. The output extract and fraction were collected to glass vials and preserved in a refrigerator at 4°C.

Antibacterial screening

Antibacterial screening was performed by disc diffusion method (**Hussain *et al*., 2010**) against six gram-positive and seven gram-negative bacteria at the concentration of 300µg/disc, which is a qualitative to semi quantitative test. Briefly, 20 ml quantities of nutrient agar were plated in petri dish with 0.1 ml of a 10^{-2} dilution of each bacterial culture. Filter paper discs (6 mm in diameter) impregnated with the concentration of plant extract was placed on test organism-seeded plates. Methanol was used to dissolve the extract and was completely evaporated before application on test organisms seeded plates. Blank disc impregnated with solvent methanol followed by during off was used as negative control. The activity was determined after 16 h of incubation at 37°C. The diameter of zone of inhibition produced by the plant extract were then compared with the zones produced by standard antibiotic (kanamycin 30 µg/disc).

Determination of minimum inhibitory concentration (MIC)

Serial tube dilution technique (**Hussain *et al*., 2010**) was used to determine MIC of the extract against six gram-positive and seven gram-negative bacteria. The plant extract (1.0 mg) was dissolved in 2 ml distilled water (2 drops tween-80 was added to facilitate dissolution) to obtain stock solution. After preparing the suspensions of test organisms (10^7 organisms per ml), 1 drop of suspension (20µl) was added to each broth dilution. After 16 h incubation at 37°C, the tubes were then examined for the growth. The MIC values of the extract were taken as the lowest concentration that showed no growth. Growth was observed in those tubes where the concentration of the extract was below the inhibitory level and the broth medium was observed to be turbid (cloudy). Distilled water with 2 drops of tween-80 and kanamycin were used as negative and positive control, respectively.

Antifungal screening

The antifungal activity of the extract was tested by disc diffusion method (**Hussain *et al*., 2010**) against the five pathogenic fungi at the concentrations of 300µg/disc for each. Here, 20 ml quantities of nutrient agar were plated in petri dish with 0.1 ml of a 10^{-2} dilution of each bacterial culture. Filter paper discs (6 mm in diameter) impregnated with the concentration of plant extract was placed on test organism-seeded plates. Methanol was used to dissolve the extract and was completely evaporated before application on test organisms seeded plates. Blank disc impregnated with solvent methanol followed by during off was used as negative control. The activity was determined after 72 h of incubation at room temperature (32°C). The diameter of zone of inhibition produced by the extract were then compared with the zones produced by standard antibiotic (kanamycin 30 µg/disc).

Cytotoxicity bioassay

Cytotoxicity of *C. grandis* roots was screened against *Artemia salina* in a one day *in vivo* according to published protocols (**Rahman *et al*., 2010**). Brine shrimp nauplii were obtained by hatching brine shrimp eggs in artificial sea water (3.8% sodium chloride solution) for 48 h in 25°C. Dissolution for extract was performed in artificial sea water using DMSO. Serially diluted test solutions (0.5, 1, 2, 5, 10, 20 and 40µg/ml) were added to the sea water (5 ml) containing 10 nauplii. After incubation for 24 h at 25°C, the numbers of survivors was counted. From this data, lethal concentration (LC_{50}) and 95% confidence intervals of the test samples were calculated using probit analysis method described by Finney (**Finney, 1971**). Each sample was used in triplicate for the determination of the LC_{50} (50% lethal concentrations, µg/ml). Gallic acid and vincristine sulfate were used as standards in this bioassay.

Assessment of pesticidal activity

Pesticidal activity of *C. grandis* root extract was assessed against *S. oryzae* adults as previously described (**Roy *et al*., 2005**). The mortality test was performed at the concentrations of 100, 50, 25 and 12 mg/ml. 1ml solution of each doses were dropped onto the petri dishes (6 cm diameter), spread and then air dried for five minutes. Ten adult insects (10-12 days old) were release into each petri dish, kept in room temperature and mortality (%) were recorded at 24, 48 and 72 h after treatment. For determining repellency test, required amount of extract was dissolved in methanol to obtain the concentrations as 100, 50, 25 and 12mg/ml. Filter papers (Whatman No. 40, diameter 9 cm) were cut into two half, and 1 ml solution of each dose was applied to each half uniformly with a dropper. The treated half of the papers were then air-dried at attached with the untreated half with a cello-tape at middle and paper was then placed on the petri dish. Ten adult insects were release in treated papers and petri dishes were placed in the laboratory at room temperature. The insects present on each half of the paper strip were counted at 24, 48 and 72 h after treatment.

Statistical analysis

The experimental results are presented as mean for three triplicates for studied parameters. The median lethal concentration (LC_{50}) and 95% confidence intervals of the test samples were calculated using probit analysis method described by Finney (**Finney, 1971**).

RESULTS AND DISCUSSION

Antibacterial activity

The results representing antibacterial activity of methanol extract of roots are presented in **Table 1**. The highest inhibition zones of root extract were 26.0 mm diameter found against *B. subtilis*, *S. aureus* and *S. lutea* (gram-positive) followed by 25.0 mm diameter against *S. typhi* and *S. dysenteriae* (gram-negative) at the concentration of 300 µg/disc. The lowest activity of plant extract was 16.0 mm diameter of zone inhibition observed against *Bacillus cereus* at the concentration of 300µg/disc. But, the extract found no inhibition zone against *S. sonnei* and *S. flexneri* at the concentration of 300 µg/disc. Negative control exhibited no zone of inhibition against all the organisms. In comparison to reference standard antibiotic (kanamycin, 30 µg/disc), the methanol extract of root exhibited significant antibacterial activities at the concentration of 300 µg/disc against almost all of the organisms tested (**Table 1**). **Bhattacharya *et al.* (2010)** evaluated the aqueous extract of leaves of *Coccinia grandis* for antibacterial activity against *Shigella flexneri* NICED, *Bacillus subtilis*, *Escherichia coli*, *Salmonella choleraesuis*, *Shigella dysenteries*, and *Shigella flexneri*. Ethanolic extract of *Coccinia grandis* leaf showed high antibacterial activity against *S. pigeons*, *E. coli*, *B. ceres*, *K. pneumonia* and *S. aureus* (**Sivaraj *et al.*, 2011**). **Hasanuzzaman *et al*., (2013)** reported 12 mm of zone of inhibition of *Coccinia grandis* root extract against *Staphylococcus aureus*. Previous studies on antibacterial activity of fruits, leaves and stem of *Coccinia grandis* (**Aggarwal *et al.*, 2011; Hussain *et al*., 2010**) have also detected the significant activity of methanol extract against different pathogenic bacteria providing support to the fact that methanol is a better solvent for extraction and screening of phytochemicals having antimicrobial activity.

Table 1 Antibacterial activities of methanol extract of *Coccinia grandis* roots.

Test organisms	Diameter of zone of inhibition (in mm)	
	Methanol extract (300µg/ disc)	Kanamycin (30µg/disc)
Gram-positive		
Bacillus cereus	16	24
Bacillus subtilis	26	31
Bacillus megaterium	23	32
Sarcina lutea	26	26
Stapphylococcus aureus	26	33
Stapphylococcus-β-haemolyticus	18	28
Gram-negative		
Escherichia coli	22	29
Pseudomonas aeruginosa	19	27
Salmonella typhi	25	31
Shigella boydii	23	31
Shigella dysenteriae	25	32
Shigella flexneri	0.0	21
Shigella sonnei	0.0	19

Note: Data are represented in the form of mean of three tested of the standard groups.

Minimum inhibitory concentration (MIC) measurement

The Minimum inhibitory concentration (MIC) values of the extract against tested bacteria were shown in **Table 2**. The MIC values were 64, 16, 32, 16, 16, 64, 32, 64, 32, 64, 32, 64 and 128 µg/ml respectively, against the tested organisms (six gram positive and seven gram negative). The MIC values against the tested gram positive bacteria ranged from 16 to 64 µg/ml and against gram negative bacteria from 32 to128 µg/ml. Negative controls exhibited no inhibition against all the organisms. The standard antibiotic (kanamycin) had MIC values varying 4 to16 µg/ml against the tested organisms. Antibacterial potency of the plant extract against these bacteria expressed in MIC values indicated that the plant extract is more effective against gram-positive bacteria than gram negative bacteria. **Sivaraj *et al*., (2011)** reported with 31.25 µg/ml MIC values of *Coccinia grandis* leave extract against *Stapphylococcus aureus*. **Bhattacharya *et al*. (2010)** showed 1000-1750 µg/ml of MIC values of *Coccinia grandis* leaves extract against some gram positive and gram negative bacteria. **Hasan and Rahman, 2011; Saikot *et al*., (2012); Khan *et al*., (2013)** reported similar MIC values for different plant extracts which supported our present findings.

Table 2 Minimum inhibitory concentration (MIC) of methanol extract of *Coccinia grandis* roots.

Test organisms	Methanol extract (µg/ml)	Kanamycin (µg/ml)
Gram-positive		
Bacillus cereus	64	16
Bacillus subtilis	16	4
Bacillus megaterium	32	4
Sarcina lutea	16	4
Stapphylococcus aureus	16	8
Stapphylococcus-β-haemolyticus	64	8
Gram-negative		
Escherichia coli	32	8
Pseudomonas aeruginosa	64	4
Salmonella typhi	32	4
Shigella boydii	64	8
Shigella dysenteriae	32	8
Shigella flexneri	64	8
Shigella sonnei	128	16

Note: Data are represented in the form of mean of three tested of the standard groups.

Antifungal activity

The antifungal activities of methanol extract of the plant root (300µg/disc) and standard kanamycin (30µg/disc) were determined against seven pathogenic fungi (**Table 3**). The highest activity was 19.0 mm diameter of zone inhibition observed against *C. albicans* and *C. falcatum* followed by 17.0 mm diameter of zone inhibition against *A. niger* at the concentration of 300 µg/disc. The lowest activity was 7.0 mm diameter of zone inhibition found against *Aspergillus fumigatus* at the concentration of 300µg/disc. The lowest activity was 6 mm diameter of zone inhibition found against *A. fumigatus* at the concentration of 300µg/disc. The plant extract showed no inhibition zone against *A. flavus* and *T. rubrum.* Negative control exhibited no zone of inhibition against all the organisms. In comparison to reference standard antibiotic (kanamycin, 30 µg/disc), the methanol extract of root exhibited significant antifungal activities at the concentration of 300 µg/disc against almost all of the organisms tested (**Table 3**). **Bhattacharya *et al*., (2010)** evaluated the antifungal activity of the *Coccinia grandis* leaves extract against the *Candida albicans*-II, *Candida tropicalis*, *Aspergillus niger*, *Saccharomyces cerevisiae*, *Candida tropicalis* II, *Cryptococcus neoformans* and *Candida albicans* ATCC. **Satheesh and Murugan (2011)** reported that *C. grandis* leaves have a strong inhibition activity against pathogenic fungus *C. albicans*, *M. indicus*, *P. notatum*, *A. flanus* and *C. neoformans.* Previous studies on antifungal activity of different plants crude extracts **(Hasanuzzaman *et al.*, 2013; Khan *et al.*, 2013; Hasan *et al*., 2009)** have also detected the significant activity against some pathogenic fungi which support our present findings.

Table 3 Antifungal activities of methanol extract of *C. grandis* roots.

Test organisms	Diameter of zone of inhibition (in mm)	
	Methanol extract (300µg/ disc)	Kanamycin (30µg/disc)
Aspergillus fumigatus	7	19
Aspergillus flavus	0.0	17
Aspergillus niger	17	23
Candida albicans	19	24
Colletotrichum falcatum	19	22
Rizopus oryzae	15	21
Tricophyton rubrum	0.0	19

Note: Data are represented in the form of mean of three tested of the standard groups.

Cytotoxicity bioassay

The LC_{50} values of the brine shrimp lethality bioassay obtained for root extract of the plant and that of the positive controls gallic acid and vincristine sulfate, has been presented in **Table 4**. The extract showed significant cytotoxicity against brine shrimp nauplii. The LC_{50} value of the plant extract was 15.00 µg/ml, whereas the cytotoxicity of standard gallic acid and vincristine sulfate LC_{50} values were 7.50 and 2.90 µg/ml respectively. No mortality was found in the control group. **Hasanuzzaman *et al*., (2013)** showed significant cytotoxicity with LC_{50} of 2.49 µg/ml in ethanolic extract of *C. grandis* roots. **Saikot *et al*., (2012)** showed cytotoxicity against brine shrimp nauplii with LC_{50} of 7.06 µg/ml in acetone extract of *Abroma augusta* leaves. **Hasan and Rahman, (2011)** reported cytotoxicity with LC_{50} of 35.45 µg/ml in ethanol extract of *Polygonum hydropipper* stem. These cytotoxicity results support our present findings.

Table 4 Cytotoxic activity of methanol extract of *Coccinia grandis* roots on brine shrimp nauplii.

Samples	LC_{50} (µg/ml)	95% confidence limits (µg/ml)	Regeneration equation	X^2 value
Plant extract	15.00	10.5-25.00	Y=2.65+2.01X	1.71
Gallic acid	7.50	5.10-13.50	Y=3.83+1.52X	1.25
Vincristine sulfate	2.90	1.34-5.55	Y=2.16+1.98X	0.61

Note: LC_{50} values, confidence limits, regeneration equations and X^2 values were calculated by probit analysis.

Assessment of pesticidal activity

In the present investigation, pesticidal activity of methanol extract of the plant root against *Sitophilus oryzae* has been determined. The mortality (%) and repellency (%) of *S. oryzae* adults in different concentrations, at different exposure periods has been given in **Fig. 1 and 2**. The highest percentage of mortality was 73.3% found at the concentration of 100mg/ml after 72 hours of treatment, followed by 66.6% at the same concentrations after 48 hours. On the other hand, no mortality (0.0%) was observed in the concentration of 12mg/ml after 24 hours (**Fig. 1**). Similarly, the highest percentage of repellency was 80.0% observed at the concentration of 100mg/ml after 72 hours followed by 73.3% at the same concentration after 48 hours**.** On the left hand, the lowest percentage of repellency was 6.6% found at the concentration of 12mg/ml after 24 hours **(Fig. 2)**. The control group had no mortality and repellency; therefore, it has not been presented. **Mobki *et al*., (2014)** reported with 83.3% mortality and 95% repellency of *Tribolium castaneum* larvae using garlic extract. **Roy *et al*., (2005)** reported with 56.71% mortality and 55.34% repellency of *Sitiphilus oryzae* using *Blumea lacera* plant extract. **Rahman *et al*., (2007)** reported with highest 34.0% mortality and 22.43% repellency in *Sapindus mukorossi* fruits extract against *Sitiphilus oryzae* adult insect. These results was similar to our present findings.

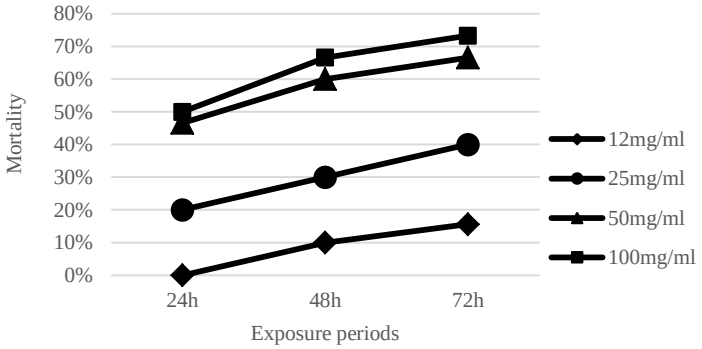

Figure 1 Regression line of mortality (%) on different doses (mg/ml) of root extract of methanol on *S. oryzae* at different exposure periods.

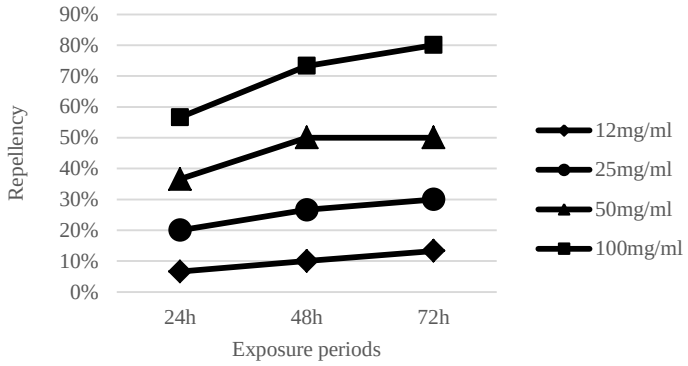

Figure 2 Regression line of repellency (%) on different doses (mg/ml) of root extract of methanol on S. oryzae at different exposure periods

CONCLUSION

The results observed in the present study demonstrated that *Coccinia grandis* roots have promising antimicrobial (300µg/disc), cytotoxic (15.00 µg/ml) and pesticidal (100mg/ml) activities. These antimicrobial, cytotoxic and pesticidal activities are probably first reported for the methanol extract of roots of *Coccinia grandis.* Further, remarkable antimicrobial, cytotoxic and pesticidal activities found by the experiments support the claims of traditional medicine. The present findings can be source of antibiotic substances for possible treatment of microbial infections. The cytotoxicity and pesticidal results revealed that the *Coccinia grandis* roots extract might be considered as a moderate toxic. However, to isolate these active phytochemicals and determine their activities are in progress.

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