Biological Activities of the Methanolic Extracts of *Coccinia indica* and *Mikania scandens* Leaves Available in Bangladesh

Md. Akhtar-E-Ekram, Md. Enamul Haq Sarker, Afrin Priya Talukder, Badhan Sarker, Md. Salah Uddin and Shahriar Zaman

Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh

(Received: March 25, 2017; Accepted: May 26, 2017; Published (web): June 21, 2017)

ABSTRACT: Biological activities of the methanolic leaf extracts of *Coccinia indica* and *Mikania scandens* were observed through antimicrobial assay, cytotoxic assay and antioxidant activity through DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay with a comparison of IC_{50} values of extracts with standard antioxidant BHT (butylatedhydroxytoluene). In case of crude extract of *Coccinia indica*, antimicrobial assay showed that *Pseudomonas* sp., *Escherichia coli* were susceptible at a dose of 150µg/disc out of five tested bacteria. Again, out of five fungi strains, *Phytopthora* sp., *Penicillium notatum, Aspergillus niger* were sensitive against 150 µg/disc containing crude extract. In the case of *Mikania scandens,* antimicrobial assay showed that *Pseudomonas* sp., *Rhizobium* for *Vigna mongu (*RVM), *Rhizobium* for *Cicer arietinum* (RCA)were susceptible at a dose of 150 µg/disc, while *Escherichia coli* was susceptible at only 75 µg/disc. Furthermore, out of five fungi strains, *Phytopthora* sp., *Penicillium notatum* were sensitive against 150µg/disc while *Aspergillus niger* was sensitive against 100 µg/disc and 150 µg/disc of *M. scandens* crude extract. The DPPH free radical scavenging activity of *C. indica* leaf extract displayed that it was capable of scavenging the 50% DPPH at the dose of 130 µg/ml and itindicated that the plant extract had moderate to high antioxidant activity. However, *Mikania scandens*, the IC_{50} value was 125µg/ml which indicated that *M. scandens* leaf extract had strong antioxidant potentialities than the leaf extract of *C. indica.* Cytotoxic assay showed that the methanolic leaf extracts of *C. indica* and *M. scandens* were highly toxic for the aquatic organisms at the concentrations of above 104.60 and 89 µg/ml, respectively.

Key words: *Coccinia indica, Mikania scandens*, antimicrobial activity, brine shrimp assay, DPPH (1, 1-diphenyl-2-picrylhydrazyl)radical scavenging assay

INTRODUCTION

Medicinal plant extracts are important reservoirs for modern antimicrobial compound due to the existence of natural bioactive compounds. *Coccinia indica*, commonly known as 'Telakucha' belongs to the family Cucurbitaceae.[1] The Cucurbitaceae family is commonly known as gourd, melon and pumpkin family which comprises 125 genera and 960 species.[2] *C. indica* is a useful climber tree that is traditionally used for various medicinal purposes in Bangladesh. [3]Leaves of this plant are also used in Bangladesh as well as India as folk medicine in the form of ailments herbal for treatment of number of diseases including diabetes, wounds, ulcers, inflammation, fever, asthma, cough and interruptions of skin.[4] In Bangladesh, the roots are used to treat osteoarthritis and joint pain.[5]

Mikania scandens commonly known as 'Ashamlota'is a species of flowering plant in the Aster family commonly known as Asteraceae or Compositae. *M. scandens* plants have been used in traditional herbal medicine in Bangladesh to treat various ailments including pain, inflammation and some other infectious diseases.[6] Aqueous leaf extracts of this plant have been used in folk medicine to treat stomach ulcers. Its leaf juice is applied to the affected area of the body to treat wounds and bruises. The

Correspondence to: Md. Akhtar-E-Ekram
E-mail: ekram_2012@ru.ac.bd

plant is considered as a rich source of vitamin A and C. The plant also contains vitamin B, mikanin, friedelin, efifriedinol, sesquiterpene, lactone scandenolide, aflavonol and some sesquiterpene dilactones including mikanolide, dihydromikanolide, deoxymikanolide and scandenolide.[7] Considering their medicinal properties we want to explore the bioactive properties of these two plants to obtain naturally available antimicrobial agents.

MATERIALS AND METHODS

Plant materials. The leaves of *C. indica* and *M. scandens* were collected from the gardens of Rajshahi University campus during March, 2014. These plants were identified by the taxonomist, Dept. of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh. The voucher specimen number of *Coccinia indica* and *Mikania scandens* were 37 and 72, respectively.

Solvents and chemicals. Methanol, DPPH (1, 1-diphenyl-2-picrylhydrazyl), BHT (butylated-hydroxytoluene), standard medium (Luria broth liquid, Luria broth agar, potato dextrose agar).

Preparation of extracts. The collected leaves of the plants were washed, unwanted materials were discarded and were dried in room temperature for 30 days. The dried leaves were grinded into small fine particles by a grinder machine, the powder was transferred to an air tight container and kept in a cool and dark place until the screening of their bioactive potentialities. For extraction, about 100 g powder of each plant material (leaves) was taken in 1 L conical flask. Each sample was soaked in (500-600) ml of methanol. The conical flasks with its contents were then sealed and kept on orbital shaker for continuous shaking at 150 rpm for 2 days. The mixtures were then filtered through Whatman No.1 filter paper. Using rotary evaporator, the methanolic extract of each plant was evaporated at (55-60)ºC temperature and at a rotation speed of (160-180) rpm. After 30 minutes of drying process, a slurry concentration was obtained, which was kept in small vial for further drying. After (20-30) days, the solvents were completely evaporated and the methanol extract of each plant became ready for experiment.

Antimicrobial activity. Antimicrobial screening was conducted according to Clinical and Laboratory Standards Institute (CLSI), USA as a standard of antimicrobial susceptibility test.[8] It was done against five gram negative bacteria (*Rhizobium* for *Vignamongu*-RVM*, Rhizobium* for *Cicerarietinum* -RCA, *Pseudomonas* sp., *Escherichiacoli* and *Acetobacter* sp.) and six fungal strains (*Phytopthora* sp., *Penicillium notatum, Aspergillus niger, Rhizopus* sp., *Colletotrichum* sp. and *Fusarium* sp.) at the doses of 10, 25, 50, 75, 100 and 150 µg/disc of extracted sample in triplicate. In case of antibacterial activity, 5 mg of each plant leaf extracts (*C. indica*and *M. scandens*) were taken in two separate vials and 5ml of solvent (methanol) were carefully added in each vial. The extracts were then dissolved well in the solvent by inverting the tube. Each of the stock solution (Conc. 1 µg/µl) was then labeled and was ready to use for sensitivity test. In this study, Luria broth (LB) liquid and Luria broth (LB) agar medium were used as culture medium for the growth of bacteria. Antibacterial screening was undertaken with disc diffusion method.[9,10] Kanamycin (10 µg/disc) was used as a standard antibiotic disc.

The extract samples were prepared for antifungal activity test in the same way of antibacterial activity test described before. In this study, potato dextrose agar (PDA) and liquid medium was used to perform the antifungal activity test and for subculture of the test organisms. Antifungal screening was also carried out by the disc diffusion method. Nystatin (10 µg/disc) was used as a standard antibiotic disc.

Cytotoxic activity. Cytotoxic activities of two experimental plant samples were conducted at the concentrations of 10, 25, 50, 75, 100 and150 µg/ml of extracted sample through brine shrimp (*A. salina*) lethality assay.[11] Brine shrimp eggs were hatched in simulated seawater to get nauplii. Sample solutions are prepared by dissolving the test materials in pre-calculated amount of methanol. Ten nauplii were taken in each vial containing 10 ml of simulated seawater. The samples of different concentrations were added to the pre-marked each vial with a micropipette. The assays were performed using three

replicates. Survivors were counted after 24 hours. These data were processed in a simple program for probit analysis to estimate LC_{50} values with 95% confidence intervals for statistically significant comparisons of potencies.

Antioxidant activity. Antioxidant activities of two experimental plant samples were conducted at the concentrations of 50, 100 and 150µg/ml of extracted sample through DPPH free radical scavenging assay.[12]

To prepare the experiment of DPPH scavenging activity test of BHT at different concentrations, at first we took 4 autoclaved test tubes and numbered them as 1, 2, 3 and control. From previously prepared BHT stock solution we took 10, 20 and 30 µl solution with the help of micropipette and added to first, second and third test tube respectively, except control test tube. Then, we also added 990, 980, 970 and 1000 µl solvent (methanol) in first, second, third and control test tube, respectively. Therefore, the concentrations of the BHT in first three test tubes were 50, 100 and 150 µg/ml, respectively. Finally, 1.5 ml of DPPH solution was added to each of the test tube. The test tubes were then incubated at room temperature for 30 minutes in dark to complete the reaction.

After 30 minutes of incubation, absorbance of each solution was measured at 519 nm using a spectrophotometer against blank. The percentage (%) of inhibition activity was calculated from the following equation:

$$\% I = \{(A_o - A_1)/A_o\} X 100$$

where, % I is percentage of inhibition activity, A_0 is the absorbance of the control, and A_1 is the absorbance of the BHT.

Procedure for DPPH radical scavenging assay, activity and IC_{50} value of the extracts were conducted almost same way as the experimental procedure of BHT. The only difference was that we added extract solution from stock solution of *C. indica* and *M. scandens* leaf extracts instead of BHT solution.

RESULTS AND DISCUSSION

In antibacterial activity test, *Pseudomonas* sp., *E. coli* and *Acetobacter* sp. were susceptible at the dose of 150µg/disc to *C. indica* leaf extract with (18.33 ± 1.53) mm, (18.67 ± 1.00) mm and (16.33 ± 0.58) inhibition zones, respectively. However, RVM and RCA were strongly resistant to *C. indica* crude extract (Table1). Shaheen *et al.*[13] investigated about the bioactive compounds in fruits of *C. indica* against some pathogenic bacteria. According to Farrukh *et al.*[14] water extract of leaves and ethanolic extract of stem showed significant activity against the gram negative bacteria *Shigella boydii* and *Pseudomonas aeruginosa* at the dose of 200µg/disc. In our experiment, we also found antibacterial activity of methanolic extract of *C. indica* against pathogenic bacteria. Again, in case of *M. scandens*, RVM, RCA, *Pseudomonas* sp. and *E. coli* were susceptible at the dose of 150 µg/disc with inhibition zones (22.00 ± 1.00) mm, (20.00 ± 1.00) mm, (19.00 ± 1.00) mm, (23.33 ± 1.00) mm, respectively. It was previously reported by Ghosh *et al.*[15] that *M. scandens* leaf extract has antibacterial effect against *Bacillus subtilis* MTCC 441, *Escherichia coli* MTCC 739 at the dose of 100µg/disc. But, *Acetobacter* sp. was resistant in case of *M. scandens*. Overall, *M. scandens* leaf extract possesses stronger antimicrobial activity than that of *C. indica.* All the bacterial strains were strongly susceptible to antibiotic Kanamycin (10 µg/disc) and the leaf extracts of both the plants showed nearable effect like this antibiotic.

In case of antifungal assay, we clearly demonstrated that *Phytopthora* sp., *Penicillium notatum* and *Aspergillus niger*were susceptible to *C. indica* leaf extract at the dose of 150 µg/disc with (18.33 ± 1.53) mm, (19.67 ± 2.08) mm, (18.67 ± 1.15) mm inhibition zones, respectively. It was reported that aqueous and ethanol extract of *C. indica* leaves showed potent antifungal activity against *Aspergillus niger* ATCC 1204 and *Candida albicans* at the dose of 100µg/disc.[16] Another investigation reported that *Aspergillus flavus, Candida albicans, Mucor indicus* were more sensitive to *C. indica* leaf extract at the dose of 100 and 150 µg/disc.[17] In case of both *C. indica* and *M. scandens* plant extracts, *Colletotrichum* sp. was intermediately resistant and remaining other two fungal species *Rhizopus* sp. and *Fusarium* sp. were strongly resistant at the dose of

150 μg/disc. In our current study, we also obtained that *Phytopthora* sp., *Penicillium notatum* and *Aspergillus niger* were susceptible to *M. scandens* crude extract at the dose of 150 μg/disc with (18.33 ± 0.58) mm, (18.67 ± 1.53) mm, (21.00 ± 1.00) mm inhibition zones, respectively. It was also reported by Siddiqui *et al*.[18] that essential oil of *M. scandens* leaf has antifungal effect against *Pythium graminicola*, *Fusarium oxysporum* at the dose of 125 μg/disc and *Tricoderma harzianum* was susceptible at the dose of 200 μg/disc. All the fungal strains were strongly susceptible to standard antibiotic Nystatin (10 μg/disc) except *Rhizopus* sp. and *Fusarium* sp. So, this current experiment showed similar result with previous records of the antifungal activity and also indicates promising antifungal effect for both plant extracts against four pathogenic fungal strains.

Table 1. Antimicrobial activity of *C. indica* and *M. scandens* methanolic extracts.

Name of bacteria	Diameter of Zone of inhibition (mm)		
	C. indica extract (150µg/disc)	*M. scandens* (150µg/disc)	Kanamycin (10µg/disc)
RVM	8.67 ± 0.58	22.00 ± 1.00	30.67 ± 1.15
RCA	8.33 ± 0.58	20.00 ± 1.00	28.00 ± 1.00
Pseudomonas sp.	18.33 ± 1.53	19.00 ± 1.00	31.33 ± 1.15
Escherichia coli	18.67 ± 1.00	23.33 ± 1.00	30.33 ± 2.52
Acetobacter sp.	16.33 ± 0.58	6.00 ± 0	19 ± 1.00
Fungi			**Nystatin (10µg/disc)**
Phytopthora sp.	18.33± 1.53	18.33 ± 0.58	30.67 ± 1.15
Penicillium notatum	19.67 ± 2.08	18.67 ± 1.53	24.33 ± 0.58
Aspergillus niger	18.67 ± 1.15	21.00 ± 1.00	29.67 ± 0.58
Rhizopus sp.	0 ± 0	0 ± 0	0 ± 0
Colletotrichum sp.	14 ± 1.00	11.67 ± 0.58	22.67 ± 0.58
Fusarium sp.	0 ± 0	0 ± 0	0 ± 0

The present study investigated the cytotoxic effect of *C. indica* leaf extracts at six concentrations of 10, 25, 50, 75, 100 and 150 μg/ml. For *C. indica* leaf extract, it was found that LC_{50} was 104.60 μg/ml and the regression equation was Y=1.957371 + 1.5066X, while the 95% confidence limits were 71.90 to 152.17 μg/ml for the of exposure (Table 2). *C. indica* leaf extract showed ability to kill the 50% of brine shrimp at the concentration 104.60 μg/ml. So, it was clear that *C. indica* leaf extract showed 50% toxicity against aquatic organism at the concentration of 104.60 μg/ml.

The present study revealed the cytotoxic effect of *M. scandens* leaf extract at six different concentrations of 10, 25, 50, 75, 100 and 150 μg/ml. For *M. Scandens* leaf extract, we found that the LC_{50} is 89μg/ml and the regression equation was Y = 2.235752 + 1.417984X, while the 95% confidence limits were 62.03 to 127.71μg/ml for the 24 hours of exposure (Table 2).

Therefore, we concluded that *M. scandens* leaf extract was responsible of 50% mortality of *A. salina* at the concentration of 89 μg/ml.

The cytotoxicity of plant material would indicate the presence of bioactive compounds in plant extract and whether the plant extract has toxicity at cell level of aquatic organism.[19] According to ample investigation by various researchers the ethanolic root extract of *C. indica* showed 50% mortality (LC_{50}) of aquatic organism (*A. salina*) at the concentration of 125 μg/ml.[20] It was also reported that ethanolic leaf extract of *C. indica* showed 50% mortality (LC_{50}) of *A. salina* at the concentration of 95μg/ml. In case of *M. scandens,* it was depicted that its same genera, the ethanolic extract of *M. cordata* showed 50% mortality of aquatic organism (*A.

salina) at the concentration of 90 µg/ml.[21] Dewi *et al.*[22] confirmed that the ethanolic root extract of *M. cordata* showed toxicity with LC_{50} at 40.43 µg/ml.

Table 2. LC_{50}, 95% confidence limits, regression equation and Chi-square value of cytotoxicity of *C. indica and M. scandens* leaf extracts against *A. salina* with 24 hours of exposure.

Plant sample	LC_{50} value (µg/ml)	95% confidence limits		Regression equation	Chi-square value (df)
		Upper	Lower		
C. indica	104.60	152.17	71.90	Y = 1.957371+1.5066X	0.3309 (4)
M. scandens	89	127.71	62.03	Y = 2.235752 +1.417984X	0.3522 (4)

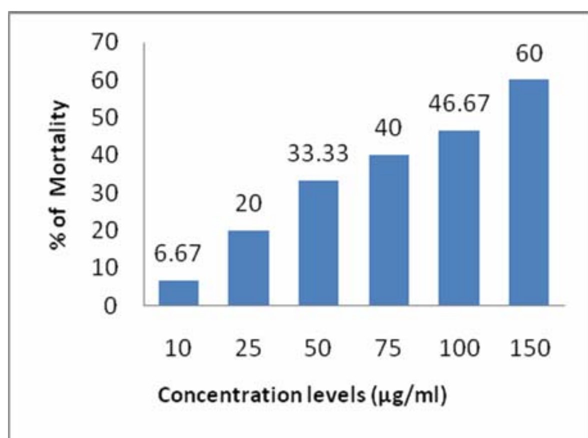

Figure 1.Percent of mortality of brine shrimp by *C. indica.*

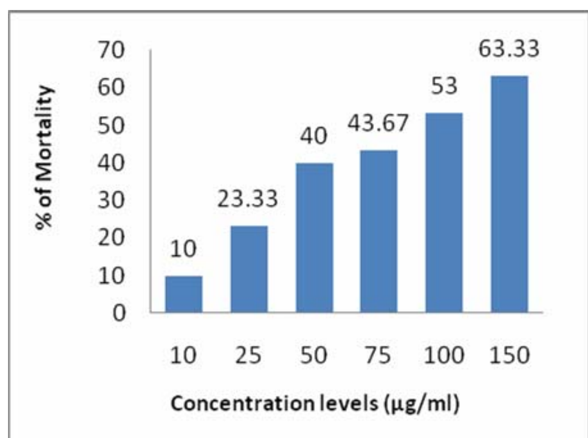

Figure 2. Percent of mortality of brine shrimp by *M. scandens.*

In our present study we revealed that the leaf extract of *C. indica* and *M. scandens* showed toxicity to *A. salina* with LC_{50} at 104.60 and 89 µg/ml, respectively. So, our findings showed similarity with previous researcher and indicated that the methanolic leaf extract of *C. indica* and *M. scandens* are promising for the presence of bioactive compounds and are highly toxic for the aquatic organism at the concentration of above 104.60 µg/ml and 89 µg/ml concentrations, respectively.

The present study explored the antioxidant effect (DPPH free radical scavenging activity) of BHT standard at three concentrations 50 and 100 and 150 µg/ml. The DPPH free radical scavenging activities of BHT were found as 63.68%, 88.50% and 95.83% at the concentrations of 50 and 100 and 150 µg/ml, respectively with IC_{50} value of 36 µg/ml (Table 3). So, BHT exhibited high antioxidant activity with IC_{50} value of 36 µg/ml.

The present study also revealed the antioxidant effect (DPPH free radical scavenging activity) of *C. indica* leaf extract among the three doses (50, 100 and 150µg/ml). The DPPH free radical scavenging activity of *C. indica* leaf extract were found as 26.84, 43.42 and 54.36% at the doses of 50, 100 and 150 µg/ml, respectively, with IC_{50} value of 130µg/ml (Table 3). So, we can say that this plant extract had moderate to high antioxidant effect although this effect was not nearable to the synthetic antioxidant (BHT). On the otherhand, we recorded the antioxidant effect of *M. scandens* leaf extract among three concentrations (50, 100 and 150 µg/ml). The antioxidant activities of *M. scandens* leaf extract were found as 39.96, 45.38 and 54.82% at the concentrations of 50, 100 and 150 µg/ml, respectively, with IC_{50} value of 125 µg/ml (Table 3). So, it was proved that *M. scandens* leaf extract had strong antioxidant potentialities than the leaf extract of *C. indica*.

Ashwini *et al.*[23] demonstrated that methanolic fruit extract of *C. indica* has potent antioxidant

activity with IC_{50} value 140 µg/ml. Another scientific research demonstrated that *C. indica* root extract has high antioxidant activity with IC_{50} value 135 µg/ml.[24] It was also assessed that the hydromethanolic leaf extract of *M. scandens* has effective antioxidant activity with IC_{50} value 130 µg/ml.[25] We also found similar results in case of *C. indica* and *M. scandens* leaf extracts with the IC_{50} value 130µg/ml and 125µg/ml, respectively. Our findings indicated that the leaf extracts of *C. indica* and *M. scandens* had enormous potentiality as a natural source of antioxidant. The positive responses obtained in the assays suggest that the extracts may contain important biologically active compounds. Therefore, we confirmed that if both the plant leaf extracts are used as alternative of agrochemical, these will not pose cancer or oxidative damage on human body because of their potent antioxidant efficiency.

Table 3. Percentage of scavenging of DPPH with IC_{50} value of standard antioxidant (BHT), *C. indica* and *M. scandens* leaf extracts.

Name of sample	Conc. (µg/ml)	Absorbance			Absorbance Mean ± STD	% of DPPH Scavenging	IC_{50} (µg/ml)
		a	b	C			
	50	0.288	0.274	0.281	0.281±0.007	63.97	
BHT	100	0.094	0.092	0.090	0.092±0.002	88.50	36
	150	0.033	0.034	0.030	0.356±0.001	95.83	
	50	0.542	0.599	0.571	0.570±0.028	26.84	
C. indica	100	0.425	0.458	0.441	0.441±0.016	43.42	130
	150	0.363	0.349	0.356	0.356±0.007	54.36	
	50	0.475	0.462	0.468	0.468±0.006	39.96	
M. scandens	100	0.433	0.419	0.426	0.426±0.007	45.38	125
	150	0.319	0.370	0.320	0.319±0.001	59.10	

ACKNOWLEDGEMENT

Authors are highly grateful to the Authority of Microbiology Lab, Department of Genetic Engineering and Biotechnology, Rajshahi University for purveying all the necessities to fulfill the research work.

REFERENCES

1. Wasantwisut, E. and Viriyapanich, T. 2003. Ivy gourd (*Cocciniagrandis Voigt, Cocciniacordifolia, Cocciniaindica*) in human nutrition and traditional applications. *World Rev. Nutr. and Diete*. **91,** 60-66.
2. Rao, G. M., Rao, C. and Mehrotra, S. 2003. Hepatoprotective effect of *Cocciniaindica* against CCI4 induced hepatotoxicity. *Nat. Prod. Sci.* **9,** 13-17.
3. Azad Khan, A. K., Akhtar, S. andMahtab, H. 1979. *Coccinia indica*in the treatment of patients with diabetes mellitus. *Bangladesh Med. Res. Coun. Bull*. **5,** 60-6.
4. Zakaria, D. M., Islam, M., Anisuzzaman, S. M., Kundu, S. K., Khan, M. S. and Begum, A. A. 2011. Ethnomedicinal survey of medicinal plants used by folk medical practitioners in four different villages of Gazipur district, Bangladesh. *Adv. Nat. and Appl. Sci.* **5,** 458-465.
5. Hussain, A., Wahab, S., Zarin, I. and Hussain, M. S. 2010. Antibacterial activity of the Leaves of *Coccinia indica* (W. and A) wof India. *Adv. Biol. Res.* **4**, 241-248.
6. Uddin N. S. 2006. Traditional Uses of Ethnomedicinal Plants of the Chittagong Hill Tracts. first edition, Bangladesh National Herbarium, Dhaka, Bangladesh, pp. 679.
7. Bohlmann, F., Adler, A., Jakupovic, J., King, R. M. and Robinson, H. 1982. A dimeric germacranolide and other sesquiterpene lactones from *Mikania* species. *Phytochemistry*. **21**, 1349-1355.
8. Cockerill, F. R. 2011. Performance standards for antimicrobial susceptibility testing: twenty-first informational supplement. Clinical and Laboratory Standards Institute (CLSI), USA.
9. Bauer, A. W., Kirby, W. M., Sherris, J. C. and Turek, M. 1966. Antibiotic Susceptibility Testing by a standardized single Disk method. *Am. J. Clin. Pathol*. **45,** 493-496.
10. Barry, A. L. 1976. Principle and Practice of Microbiology. Lea and Fabager. Philadelphia, pp. 36.
11. Ramesh, P. and Okigbo, R. N. 2008. Effects of plants and medicinal plant combinations as anti-infectives. *Afirican J. Pharm. and Pharmacol*. **2,** 130-135.
12. Brand-williams, W., Cuvelier, M. E. and Berset, C. 1995. Use of free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft und Technol.* **28**, 25-30.

13. Shaheen, S. Z., Bolla, K., Vasu, K. and Charya, M. S. 2009. Antimicrobial activity of the fruit extracts of *Cocciniaindica*. *African J. Biotechnol.* **8,** 7073-7076.

14. Farrukh, U., Shareef, H., Mahmud, S., Ali, S. A. and Rizwani, G. H. 2008. Antibacterial activities of *Cocciniagrandis* L. *Pakistan J. Bot.* **40,** 1259-1262.

15. Ghosh, A., Das, B. K., Roy, A., Mandal, B. and Chandra, G. 2008. Antibacterial activity of some medicinal plant extracts. *J. Nat. Med.* **62,** 259-262.

16. Pal, P., Chakraborty, S., Samanta, A., Bhattacharya, B. and Samanta, M. 2010. Evaluation of Antifungal and Antibacterial Activities of the Plant *Cocciniagrandis* (L.) Voigt. (Family- Cucurbitaceae). *J. Phytology.* **2,** 52-57.

17. Satheesh, L. S. and Murugan, K. 2011. Antimicrobial activity of protease inhibitor from leaves of *Cocciniagrandis*(*L.*) Voigt. *Indian J. Expt. Biol.* **49,** 366-374.

18. Siddiqui, S. A., Islam, R., Jamal, A. H. M., Parvin, T. and Rahman, A. 2013. Chemical composition and antifungal properties of the essential oil and various extracts of *Mikania scandens* (L.) Willd. *Arabian J. Chem.* **17,** 106.

19. Martin-Cordero, C., Saenz, M. T., Ayuso, M. J. and Caviedes, M. A. 1995. Cytotoxic activity of Retamasphaerocarpa. *Fitoterapia*. **66,** 495-498.

20. Hasanuzzaman, M., Sayeed, M. S. B., Islam, M. S., Sarwar, M. S., Mizanur, M., Moghal, R. and Islam, M. S. 2013. Preliminary antimicrobial activity and cytotoxicity of plant extract (roots) of *Cocciniagrandis* (Family: cucurbitaceae). *Intl. J. Pharma. Sci. and Res.* **4,** 1466.

21. Al Nayeem, A., Khatun, A., Rahman, M. S. and Rahman, M. 2011. Evaluation of phytochemical and pharmacological properties of *Mikaniacordata* (Asteraceae) leaves. *J. Pharmacognosy and Phytotherapy*. **3,** 20-20.

22. Dewi, K. S. and Ridwanuloh, J. 2011. Phytochemical screening, antibacterial and cytotoxic activity of *Mikaniacordata* extracts. *J. Nat. Med.* **56,** 232-234.

23. Ashwini, M., Lather, N., Bole, S., Vedamutrhy, A. B. and Balu, S. 2012. *In vitro* antioxidant and anti-inflammatory activity of *Cocciniagrandis*. *Intl. Pharm. and Pharma. Sci.* **4,** 239-242.

24. Bhadauria, P., Arora, B., Vimal, B. and Kulshrestha, A. 2012. *In vitro* antioxidant activity of *Cocciniagrandis* root extracts. *Indo Global J. Pharma. Sci.* **2,** 230-238.

25. Hasan, S. R., Jamila, M., Majumder, M. M., Akter, R., Hossain, M. M., Mazumder, M. E. and Rahman, S. 2009. Analgesic and antioxidant activity of the hydromethanolic extract of *Mikaniascandens* (L.) Willd. leaves. *Amer. J. Pharmacol. and Toxicol*. **4,** 1-7.