ORIGINAL ARTICLE

Antioxidant, antimicrobial and cytotoxic bioassay of *Mollugo oppositifolius* L

Md. Torequl Islam, Md. Ahad Ali Khan, Jasmin Akther Hossain, Joy Barua
Department of Pharmacy, Faculty of Science and Engineering, Southern University Bangladesh, Mehedibag, Chittagong, Bangladesh

The present study was conducted according to the traditional uses of *Mollugo oppositifolius* L. by the kabiraj (traditional practioner) for the treatment of infectious diseases of the ill fated and poor people of Bangladesh. For this antioxidant, antimicrobial and biolethality potentials were conducted by methanol (MOME), ethanol (MOEE) and petroleum ether (MOPE) extractives of the suspected species. To determine the antioxidant activity the DPPH inhibition method was used. For antimicrobial test, antibacterial and antifungal sensitivities were performed by disc diffusion method and serial tube dilution method was carried out to determine the minimum inhibitory concentrations on some human pathogenic bacteria and fungi. For cytotoxicity test, brine shrimp lethality bioassay was conducted. Among the three crude extracts, MOEE produced more significant inhibition of DPPH (IC_{50}; 27 µg/ml); MOPE produced highest zone of inhibition against *Bacillus subtilis* (16.67 mm) and *Microsporum spp.* (16.0 mm). On the other hand, MOME produced mild cytotoxicity as 50% and 90% mortality (LC_{50} and LC_{90}) 8.0 µg/ml and 85.12 µg/ml.

Key words: Antioxidant, antimicrobial, cytotoxicity, *Mollugo oppositifolius*

INTRODUCTION

Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world.[1] Many of the plant materials used in traditional medicine are readily available in rural areas and this has made traditional medicine relatively cheaper than modern medicine.[2] Medicinal plants contain pharmacologically active principles which over the years have been exploited in traditional medical practice for the treatment of various ailments.[3] Bangladesh is a developing country; a large number of people having inability to access medical supports. Most of the ill-fated people are usually dependent upon kabiraj (traditional medicine practitioners) for their health troubles.

Mollugo oppositifolius (L) (Family: *Molluginaceae*) is an annual, creeping herb that can form open mats, found mostly in tropical/warm regions. It has little economic value.[4] In Bangladesh, the plant has been used medicinally by the traditional practioners for the treatment of abdominal pain and burn [Table 1].

Access this article online

Quick Response Code:

Website: www.greenpharmacy.info

DOI: 10.4103/0973-8258.85179

In Bangladesh, it is frequently used as a popular vegetable. Previously the important bioactive principles L-arginine[5] and pectic polysaccharides[6] were isolated from the plant, *Glinus oppositifolius* L. (synonyoumous plant of *M. oppositifolius*). The aim of the present study is to evaluate the antioxidant, antimicrobial activity as well as toxic responses of crude methanol, ethanol and petroleum ether extracts of the targeted plant and to search logical evidence for its folk use and further exploitation.

MATERIALS AND METHODS

Plant Material

The plant *M. oppositifolius* L. was collected from the Chittagong hill in the month of March, 2011 and identified by Forest Research Institute (FRI); Chittagong, Bangladesh.

Extraction

The plant was subjected for shade dry.[7] The dried plant was then ground into coarse powder and subjected for hot extraction[8] with methanol, ethanol and petroleum ether by Soxhlet Apparatus (Quickfit, England).[9] Each extraction was carried out about 18 h and the extracts

Table 1: Traditional uses of *M. oppositifolius* L. in Bangladesh

Used parts	Indications	Practitioners
Leaf (aqueous extract)	Burn, wound	Local
Whole body without root (aqueous extract)	Abdominal pain, eye infection	Local

Address for correspondence: Mr. Md. Torequl Islam, Department of Pharmacy, Southern University Bangladesh, 739/A, Academic Building-III, 22-Shaheed Mirza Lane (East), Mehedibag Road, Chittagong - 4000, Chittagong, Bangladesh. E-mail: mti031124@yahoo.com
Received: 01-06-2011; **Accepted:** 10-06-2011

were filtered through cotton plug followed by Whatman filter paper, number 1. The extracts were then concentrated by using rotary evaporator (Heidolph, 560 91110 00 0, Germany).

Antioxidant Activity

The antioxidant (free radical scavenging) activity[10] of the crude extracts was determined by the inhibitory action of stable radical 1, 1 diphenyl 2 picrylhydrazyl (DPPH). A series of concentration (1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 µg/ml) were made with 0.004% DPPH solution. Then the reaction mixtures were allowed to stand for 20 min. The absorbance was determined at 517 nm (colorimeter, *AE11M*, Abs_{max}; logT; 0-2.0, India) and from these values the corresponding percentages of inhibitions were calculated by using the following equation:

$$\% \text{ Inhibition of DPPH action} = [1 - (ABS_{sample} \div ABS_{control}) \times 100]$$

Then % inhibitions were plotted against respective concentrations used and from the graph IC_{50} were calculated using ascorbic acid (ASCOSON Inj., 500 mg/5ml ampoule, Jayson Pharmaceuticals Ltd.), a potential antioxidant, as standard.

Antimicrobial Screening

The disc diffusion method[11,12] was used for antibacterial and antifungal sensitivity tests of the crude extracts against 11 pathogenic bacteria: *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, *Vibrio cholerae*, *Shigella sonnei* and *Salmonella paratyphi* and 7 pathogenic fungi: *Aspergillus niger*, *Blastomyces dermatitidis*, *Candida albicans*, *Pityrosporum ovale*, *Trichophyton spp.*, *Microsporum spp.* and *Crytococcus neoformans*. They were collected as pure culture from the Bangladesh Council of Scientific and Industrial Research (BCSIR), Chittagong, Bangladesh. Antimicrobial sensitivity was determined by measuring the diameter of zone of inhibition in mm. The experiment was carried out in triplicate and the mean values were taken. Standard, ciprofloxacin (CIPROCIN, 500 mg/Tab. Square Pharmaceuticals Ltd.) and fluconazole (FLUGAL, 50 mg/Tab. Square Pharmaceuticals Ltd.) were used for antibacterial and antifungal sensitivity tests respectively. Again, the minimum inhibitory concentration test[13] by serial tube dilution method was also carried out against the above 18 pathogens.

Brine Shrimp Lethality Bioassay

Brine shrimp lethality bioassay[11] technique was applied for determination of general toxic property of the plant crude extract. Di-methyl sulfo-oxide (DMSO) solutions of the sample were applied against *Artemia salina* in one day *ex-vivo* assay. For the experiment, ten successive concentrations (0.5, 1, 2, 4, 8, 16, 32, 64, 128 and 256 µg/ml) the crude extracts of *M. oppositifolius* were selected. Vincristin sulfate (VINCRISTIN RICHTER Inj., powder for reconstitution, 1 mg vial, Gedeon Richter/City Overseas Ltd.) was used as positive control. With the help of a Pasteur's pipette 10 living shrimps were kept to each of the test tubes. After 24 h the number of survived nauplii in all tubes was counted.

Statistical Analysis

Experimentally obtained primary data were manipulated as the source of responses. All experiments were performed in duplicate and replicated at least three times. All statistical

Table 2: Antioxidant activity of the extractives of *M. oppositifolius*

Sample	IC_{50} (µg/ml)
ASC	14.0
MOME	40.0
MOEE	27.0
MOPE	28.0

ASC – Ascorbic acid; MOME – Crude methanol extract of *M. oppositifolius*; MOEE – Crude ethanol extract of *M. oppositifolius*; MOPE – Crude petroleum ether extract of *M. oppositifolius*

Table 3: Antibacterial potential of the crude extracts of *M. oppositifolius*

Test bacteria	Zone of inhibition (mm)		
	MOME	MOEE	MOPE
Bacillus subtilis	10.0 ± 1.0^{d}	8.0 ± 1.0^{c}	16.67 ± 1.53^{f}
Bacillus megaterium	Nd	11.33 ± 1.53^{d}	7.33 ± 0.58^{b}
Bacillus cereus	Nd	Nd	Nd
Staphylococcus aureus	Nd	Nd	10.0 ± 1.0^{d}
Escherichia coli	14.0 ± 1.0^{e}	Nd	Nd
Pseudomonas aeruginosa	8.0 ± 1.0^{d}	11.0 ± 1.0^{e}	Nd
Salmonella typhi	Nd	8.33 ± 0.58^{b}	Nd
Shigella dysenteriae	11.0 ± 1.0^{b}	Nd	9.33 ± 1.53^{b}
Vibrio cholerae	Nd	6.67 ± 0.58^{b}	8.0 ± 1.0^{b}
Shigella sonnei	Nd	Nd	7.67 ± 1.53^{d}
Salmonella paratyphi	7.0±1.0b	Nd	Nd

$^{a}P< 0.001$; $^{b}P< 0.01$; $^{c}P< 0.02$; $^{d}P< 0.05$; $^{e}P< 0.10$; $^{f}P< 0.50$; Nd – Not detected; A diameter less than 7 mm was considered as inactive; MOME – Crude methanol extract of *M. oppositifolius*; MOEE – Crude ethanol extract of *M. oppositifolius*; MOPE – Crude petroleum ether extract of *M. oppositifolius*

Table 4: Antifungal sensitivity of the crude extracts of *M. oppositifolius*

Test bacteria	Zone of inhibition (mm)		
	MOME	MOEE	MOPE
Aspergillus niger	11.67 ± 1.53^{a}	Nd	8.0 ± 1.0^{b}
Blastomyces dermatitidis	8.33 ± 1.53^{c}	Nd	Nd
Candida albicans	12.0±1.0b	8.0 ± 1.0^{b}	Nd
Pityrosporum ovale	Nd	Nd	7.33 ± 1.53^{e}
Trichophyton spp.	Nd	16.0 ± 2.65^{f}	Nd
Microsporum spp.	Nd	Nd	16.0 ± 1.0^{d}
Crytococcus neoformans	8.0 ± 1.0^{b}	10.33 ± 1.15^{c}	Nd

$^{a}P< 0.001$; $^{b}P< 0.01$; $^{c}P< 0.02$; $^{d}P< 0.05$; $^{e}P< 0.10$; $^{f}P< 0.50$; Nd – Not detected; A diameter less than 7 mm was considered as inactive; MOME – Crude methanol extract of *M. oppositifolius*; MOEE – Crude ethanol extract of *M. oppositifolius*; MOPE – Crude petroleum ether extract of *M. oppositifolius*

Table 5: Minimum inhibitory concentrations of the extracts against the test bacteria

Extracts	Minimum inhibitory concentration (µg/ml)										
	1	2	3	4	5	6	7	8	9	10	11
MOME	62.50	nd	nd	nd	31.25	125	nd	62.50	nd	nd	500
MOEE	125	62.50	nd	nd	nd	62.50	125	nd	500	nd	nd
MOPE	31.25	250	nd	125	nd	nd	nd	125	250	250	nd

Nd – Not detected; MOME – Crude methanol extract of *M. oppositifolius*; MOEE – Crude ethanol extract of *M. oppositifolius*; MOPE – Crude petroleum ether extract of *M. oppositifolius*; 1 – *Bacillus subtilis*; 2 – *Bacillus megaterium*; 3 – *Bacillus cereus*; 4 – *Staphylococcus aureus*; 5 – *Escherichia coli*; 6 – *Pseudomonas aeruginosa*; 7 – *Salmonella typhi*; 8 – *Shigella dysenteriae*; 9 – *Vibrio cholerae*; 10 – *Shigella sonnei* and 11 – *Salmonella paratyphi*

Table 6: Minimum inhibitory concentrations of the extracts against the test fungi

Extracts	Minimum inhibitory concentration (µg/ml)						
	1	2	3	4	5	6	7
MOME	62.50	250	62.50	nd	nd	nd	250
MOEE	nd	nd	250	nd	31.25	nd	125
MOPE	250	nd	nd	500	nd	31.25	nd

Nd – Not detected; MOME – Crude methanol extract of *M. oppositifolius*; MOEE – Crude ethanol extract of *M. oppositifolius*; MOPE – Crude petroleum ether extract of *M. oppositifolius*; 1 – *Aspergillus niger*; 2 – *Blastomyces dermatitidis*; 3 – *Candida albicans*; 4 – *Pityrosporum ovale*; 5 – *Trichophyton spp.*; 6 – *Microsporum spp.* and 7 – *Crytococcus neoformans*

Table 7: Brine shrimp lethality bioassay of crude extracts of *M. oppositifolius*

Sample	LC_{50} (µg/ml)	LC_{90} (µg/ml)
VS	0.47	1.23
MOME	8.0	85.12
MOEE	8.0	96.0
MOPE	12.0	160

VS – Vincristine sulphate; LC – Lethal concentration; MOME – Crude methanol extract of *M. oppositifolius*; MOEE – Crude ethanol extract of *M. oppositifolius*; MOPE – Crude petroleum ether extract of *M. oppositifolius*

analyses were carried out using STATGRAPHICS Centurion XV. Statistical differences between extract activities were determined using ANOVA followed by Least Significant Difference (LSD) testing. Differences were considered statistically significant when $P<0.50$

RESULTS

In case of screening for antioxidant activity [Table 2] ethanol extract of the *M. oppositifolius* (MOEE) exhibited the highest free radical scavenging activity as IC_{50} 27.0 µg/ml. On the other hand, the crude extractives MOPE and MOME produced IC_{50} 28.0 µg/ml and 40.0 µg/ml while the standard, ascorbic acid (14.0 µg/ml).

In the antimicrobial screening, the extracts of *M. oppositifolius* showed mild to moderate growth inhibitory activity as well as minimum inhibitory concentrations (MICs) against the tested microorganisms. MOME produced zone of inhibition as 10.0±1.0, 14.0±1.0, 8.0±1.0, 11.0±1.0, 7.0±1.0 11.67±1.53, 8.33±1.53, 12.0±1.0 and 8.0±1.0 mm against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Salmonella paratyphi*, *Aspergillus niger*, *Blastomyces dermatitidis*, *Candida albicans* and *Crytococcus neoformans* respectively. But it was found to be inactive against the other pathogens [Tables 3 and 4]. Moderate MIC (31.25 µg/ml) of MOME was found against *Escherichia coli* [Tables 5 and 6].

For MOEE, the highest zone of inhibition was found to be 16.0±2.65 mm against *Trichophyton spp*. Then followed by 11.33±1.53, 11.0±1.0, 10.33±1.15, 8.33±0.58, 8.0±1.0, 8.0±1.0 and 6.67±0.58 mm against *Bacillus megaterium*, *Pseudomonas aeruginosa*, *Crytococcus neoformans*, *Salmonella typhi*, *Bacillus subtilis*, *Candida albicans* and *Vibrio cholerae* respectively. But it was found to be inactive against the other pathogens [Tables 3 and 4]. Moderate MIC (31.25 µg/ml) of MOEE was found against *Trichophyton spp.*

Again, for MOPE, the maximum growth inhibition (16.67±1.53) was observed against *Bacillus subtilis*. Then followed by 16.0±1.0, 10.0±1.0, 9.33±1.53, 8.0±1.0, 8.0±1.0, 7.67±1.53, 7.33±0.58 and 7.33±0.58 mm against *Microsporum spp.*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Aspergillus niger*, *Vibrio cholerae*, *Shigella sonnei*, *Bacillus megaterium and Pityrosporum ovale* respectively. But it was found to be inactive against the other pathogens [Tables 3 and 4]. Significant MIC (31.25 µg/ml) of MOEE was found against *Bacillus subtilis*.

Table 7 shows the results of the *in-vitro* brine shrimp lethality bioassay of the extracts. Among them, the methanol extract of the plant, after 24 h of exposure; 50% and 90% mortality (LC_{50} and LC_{90}) were found to be 8.0 and 85.12 µg/ml in comparison to standard, vincristine sulphate.

DISCUSSION

The present study indicated that the petroleum ether and methanol extracts of the whole part of *M. oppositifolius* have got profound antimicrobial and moderate antioxidant effect and may have potential use in medicine. In comparison with the positive control (vincristine sulphate), the cytotoxicity exhibited by the methanolic crude extract of the plant showed mild activity. It may be concluded from this study that *M. oppositifolius* is active against the tested pathogenic microorganisms and also have antioxidant effect. In addition, the results confirm the use of the plant in traditional medicine. The results of the investigation do not reveal that which chemical compound is responsible

for aforementioned activity. Now our next aim is to explore the lead compound liable for aforementioned activity from this plant.

ACKNOWLEDGMENT

We want to thank Mr. Mohiuddin, Director, Forest Research Institute, Chittagong, Bangladesh to identify plant. We also owe to the authority of the Southern University Bangladesh for providing laboratory facilities to conduct the study.

REFERENCES

1. WHO. Traditional Medicine: Growing Needs and Potential. WHO Policy Perspectives on Medicines 2002;1-6.
2. Apulu N, Dada JO, Odama LE, Galadima M. Antibacterial activities of aqueous extracts of some Nigerian medicinal plants. Niger J Bot 1994;45-8.
3. Adebanjo AO, Adewumi CO, Essien EE. Anti-infective agents of higher plants. Niger J Biotechnol 1983;8:15-7.
4. Soerjani M, Kostermans AJ, Tjitrosoepomo G. Weeds of Rice in Indonesia. Balai Pustaka 1987.
5. Sahakitpichan P, Disadee W, Ruchirawat S, Kanchanapoom T. L (-) (N transcinnamoyl)-arginine, an acylamino acid from *Glinus oppositifolius* (L.) Aug. DC. Molecules 2010;6186-92.
6. Inngjerdingen KT, Debes SC, Inngjerdingen M, Hokputsa S, Harding SE, Rolstad B, *et al*. Bioactive pectic polysaccharides from *Glinus oppositifolius* (L.) Aug. DC., a Malian medicinal plant, isolation and partial characterization. J Ethnopharmacol 2005;204-14.
7. Trease GE and Evans WC. The Text Book of Pharmacognosy. The Williams & Wilkins Company, Baltimore 2, Maryland, 15th ed. 1966; p. 64.
8. AOAC. Official methods of analysis. Association of Analytical Chemists, 13th ed. Washington DC 1980.
9. Bhal BS, Bhal A. A Text Book of Organic Chemistry. 13th ed New Delhi, S. Chand and Company Ltd 1992;5,6,11,12,112-4.
10. Sadhu SK, Okuyama E, Fujimoto H, Ishibashi M. Separation of *Leucas aspera*, a medicinal plant of Bangladesh, guided by prostaglandin inhibitory and antioxidant activities. Chem Pharm Bull 2003;595-8.
11. Bauer AW, Kirby WM, Sherries JC, Tuck M. Antibiotic susceptibility testing by a standard disc diffusion method. J Am Clin Pathol 1966;493-6.
12. Rahman MS, Rashid MA. Antimicrobial activity and cytotoxicity of *eclipta prostrate*. Orient Pharm Exp Med 2008;47-52.
13. Aboaba OO, Smith SI, Olude FO. Antibacterial effect of edible plant extracts on *Escherichia coli* 0157:H7. Pak J Nutr 2006;325-7.

How to cite this article: Islam M, Khan MA, Hossain JA, Barua J. Antioxidant, antimicrobial and cytotoxic bioassay of *Mollugo oppositifolius* L. Int J Green Pharm 2011;5:141-4.

Source of Support: Nil, **Conflict of Interest:** None declared.