BJP

Bangladesh Journal of Pharmacology

Research Article

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A Journal of the Bangladesh Pharmacological Society (BDPS) **Bangladesh J Pharmacol 2014; 9: 161-166**
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index
ISSN: 1991-0088

In vitro antimicrobial screening of methanolic extracts of *Cleome chelidonii* and *Cleome gynandra*

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Article Info

Received: 25 January 2014
Accepted: 1 March 2014
Available Online: 30 March 2014

DOI: 10.3329/bjp.v9i2.17759

Cite this article:
Sridhar N, Kiran BVVSS, Sasidhar DT, Kanthal LK. *In vitro* antimicrobial screening of methanolic extracts of *Cleome chelidonii* and *Cleome gynandra.* Bangladesh J Pharmacol. 2014; 9: 161-66.

Abstract

Antimicrobial activity of methanolic extracts of plants was screened by disc diffusion assay against four bacteria and four fungal cultures. Streptomycin (10 µg/disc) and nystatin (10 µg/disc) are used as standards for bacteria and fungi respectively. Minimum inhibitory concentration (MIC) of the extracts was evaluated through micro broth dilution method. The antimicrobial potency of plant extracts was assessed by their zone of inhibition and activity index values. Total activity of extracts was evaluated to quantitatively compare the activity of two plants. Methanolic extract of *Cleome gynandra* showed maximum antibacterial activity against *Staphylococcus aureus* (IZ- 22 ± 0.22 mm, AI- 0.917, MIC- 0.039 mg/mL, MBC- 0.039 mg/mL). Maximum antifungal potential was shown by *C. chelidonii* against *Candida albicans* (IZ- 25 ± 0.92 mm, AI-1.000, MIC- 0.039 mg/mL, MFC- 0.039 mg/mL). Both the extracts exhibited good antimicrobial activity with low range of MIC.

Introduction

Many antibacterial agents are available in the market, which are developed by the great scientists. But still the microbes are challenging the scientists by developing the resistance to the presently available drugs. Plants are known to produce a variety of compounds to protect themselves against a variety of their pathogens and therefore considered as potential source for different classes of antimicrobial substances (Sridhar et al., 2012).

Fungal diseases are classified at the fourth range of nosocomial infections. Victims of fungi infections can be found elsewhere in the world, with the predomination in a third of the world's countries (43%). The development of resistance to drug by pathogens and toxic side effects of available antifungal therapies have emphasized the search for new efficient and none or less-toxic antifungal drugs (Bouguerra et al., 2004; Barchiesi et al., 2006; WHO, 1996). Recently, *Amberboa divaricata* (Iqbal et al., 2014), *Artemisia annua* (Zhang et al., 2012), *Asparagus laricinus* (Ntsoelinyane and Mashele, 2014), *Asparagus racemosus* (Shah et al., 2014), *Berberis jaeschkeana* (Alamzeb et al., 2013), *Buddleja polystachya* (Fawzy et al., 2013), *Leucas clarkei* (Das et al., 2012), *Phyllostachys edulis* (Shen et al., 2012), *Pithecellobium jiringa* (Bakar et al., 2012), *Rhododendron arboretum* (Nisar et al., 2013) and *Sarcochlamys pulcherrima* (Mazumder et al., 2014) showed antimicrobial activities.

Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial and antifungal agents, a systematic investigation was undertaken to screen the antibacterial and antifungal activity of *Cleome chelidonii* and *Cleome gynandra.*

Cleome genus represents the species of herbaceous

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annual or perennial plants and shrubs widely distributed in tropical and subtropical regions. *C. chelidonii* Linn (Latin name is *Polanisia chelidonii*) is a perennial plant belonging to the family Capparaceae. It grows as perennials throughout dry seasons. *C. chelidonii* is generally known to be used for the treatment of colic, dysentery, headache, otitis, and rheumatism (Kirtikar and Basu, 1991). It has also been found to possess multiple therapeutic properties such as its use a vermifuge, the treatment of skin diseases and its anti-inflammatory, antinociceptive, antipyretic properties (Parimalakrishnan et al., 2007) and hepatoprotective activities (Ethadi et al., 2013). *C. chelidonii* contains gluco -capparin and glucocleomin (Songsak et al., 2004).

C. gynandra L. Syn *Gynandropsis pentaphylla* DC (Capparidacea) is a common weed, which grows in most tropical countries. In India the common names include hurhur and karaila. The medicinal application of *C. gynandra* is also described in Ayurvedic pharmacopoeia of India and also in other ancient medical texts. In Ayurvedic medicine it is a chief constituent in Narayana Churna. In Ayurveda it is used as an anthelmentic, in ear diseases, pruritis and several other diseases like gastro intestinal disorders and gastrointestinal infections etc (The Ayurvedic Pharmacopoeia of India, 2007). The leaves and seeds of the plant have long been in use as indigenous medicine for treatment of headaches and stomach aches. Sap from leaves has been used as an analgesic particularly for head ache, epileptic fits and ear ache. A decoction or infusion of boiled leaves and/or roots has been administered to facilitate childbirth. Bruised leaves, which are rubefacent and vesicant, are also used to treat neuralgia, rheumatism and other localized pains (Rastogi et al., 2009). It has also been found to possess anti-inflammatory (Mule et al., 2008), Immunomodu-latory (Rastogi et al., 2009), hypoglycemic (Shaik et al., 2013) properties. Plant contains Cleogynol, 5, 7- dihydroxy-chromone, 5-hydroxy-3,7,4-trimethoxyflavone, luteolin, hexacosanol, sitosterol and kaem-pferol (Das et al., 1999; Gupta et al., 1968; Jain et al., 1999).

Materials and Methods

Plant materials and extraction

Fresh leaves of *C. chelidonii* and *C. gynandra* which are free from disease were collected from local areas of Kakinada, Andhra Pradesh, India. The plants are authenticated by Botanical Survey of India, Hyderabad (BSI/DRC/2013-14/Tech./522). The plant leaves were shade dried at room temperature of (32 ± 2)°C and the dried leaves were ground into fine powder using pulverizer. The powdered part was sieved and stored in cellophane bags. The powdered leaves are extracted successively in soxhlet apparatus, using methanol. The extracts were evaporated to dryness and stored at 4°C until used.

Preparation of nutrient agar

The weighed amount of NaCl (5 g), peptone (10 g), Beef extract (10 g) are dissolved in 1, 000 mL of the water, then agar (20 g) is added slowly on heating with continues stirring until agar is completely dissolved and pH is adjusted to 7.2 to 7.4. This nutrient agar medium is then sterilized by moist heat sterilization method using autoclave at temperature of 120°C at 15 lb pressure maintained for 15 min.

Preparation of potato dextrose agar (PDA)

The potato was peeled and 100 g was measured, finely chopped and boiled to a mash in distilled water. The dextrose was measured (12.5 g) and placed in a 1L measuring cylinder. Agar was measured (12.5 g) and added to the measuring cylinder (with the dextrose). The potato mash was stirred and strained into the cylinder. Hot distilled water was added to make up 500 mL. The contents was continuously poured and stirred until consistency was achieved. The content was then poured into a conical flask, plugged with cotton wool, over which aluminum foil was tightly wrapped. The flask was then autoclaved at 121°C for 24 hours (Murray et al., 1995).

Microbial cultures

Bacterial cultures of Gram positive bacteria *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 3160) and Gram–negative bacteria *Escherichia coli* (MTCC 46), *Pseudomonas aeruginosa* (MTCC 1688) and fungal cultures of *Aspergillus flavus* (MTCC 277), *Aspergillus niger* (MTCC 2723), *Candida albicans* (MTCC 183), *Fusarium axisporum* (MTCC 1755) were procured from Institute of Microbial Technology, Chandigarh, Punjab, India.

Antimicrobial activity

Plant extracts were investigated by the disc diffusion method using 6 mm filter discs (Andrews et al., 2001). Whatmann filter paper (No. 1) discs of 6 mm diameter were made by punching the paper and the blank discs were sterilized in hot air oven at 160°C for one hour. Then the sterile filter paper discs were impregnated with 50 µL and 100 µL of 10 mg/mL solution of each plant extract to give a final concentration of 500 and 1,000 µg/disc. The test discs are left to dry in vacuum so as to remove residual solvent, which might interfere with the experimental result. Nutrient agar and potato dextrose agar base plates were seeded with the bacterial and fungal inoculums, respectively with inoculums size 1×10^8 CFU/mL for bacteria and 1×10^7 cell/mL for fungi (Baker et al., 1983). The impregnated discs with extracts were then planted at equidistant points on top of the inoculated agar medium by sterile forceps. Each extract was tested in triplicate with streptomycin (10

μg/disc) and nystatin (10 μg/disc) as standards for bacteria and fungi, respectively. The plates were kept at 4°C for 1 hour for diffusion of extract, thereafter were incubated at 37°C for bacteria (24 hours) and 28°C for fungi (48 hours). At the end of the incubation period the antimicrobial activity was evaluated by measuring the average of inhibition zones using digital vernier caliper (including disc diameter of 6 mm). Activity index for each extract was calculated by using following formula.

Activity index (AI) = Inhibition zone of the sample/ Inhibition zone of the standard

Determination of Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

MIC is the concentration required to inhibit growth of a specific isolate in vitro under standardized conditions. The MIC, MBC and MFC were performed by a serial dilution technique using 96-well microtiter plates (Barsi et al., 2005). Plant extracts were re-suspended in acetone (which has no activity against test microorganisms) to make 10 mg/mL final concentration and then two fold serially diluted. To measure the MIC values, various concentrations of the stock, 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039, 0.019 mg/mL were assayed against the test pathogens. 1 mL of each extract was added to test tubes containing 1 mL of sterile Nutrient agar media (for bacteria) Potato dextrose agar media (for fungi). The tubes were then inoculated with standard size of microbial suspension (for bacteria 1×10^8 CFU/mL and 1×10^7 cell/mL for fungi) and the tubes were incubated at 37°C for 24 hours for bacteria and 28°C for 48 hours for fungi in a BOD incubator and observed for change in turbidity after 24 hours and compared with the growth in controls. A tube containing nutrient broth and inoculums but no extract was taken as control. Bacterial and fungal suspensions were used as negative control, while broth containing standard drug was used as positive control. Each extract was assayed in duplicate and each time two sets of tubes were prepared, one was kept for incubation while another set was kept at 4°C for comparing the turbidity in the test tubes. The lowest concentration which completely inhibited the microbial growth (no turbidity) was recorded as MIC (mg/mL). The turbidity was measured by ELISA micro plate reader (Bio-tech USA) at 540 nm and the growth was indicated if OD value that increased twice its initial value. The MBC/MFC was determined by subculturing 50 μL from each well. Least concentration of extract showing no visible growth on subculturing was taken as MBC/MFC (Singh et al., 2013).

Total activity determination

Total activity is the volume at which the test extract can be diluted with the ability to kill microorganisms. It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract or compound isolated and is expressed in mL/g (Eloff, 2004).

Total activity = Extract per gram dried plant part/MIC of extract.

Results and Discussion

The antimicrobial potency of methanolic extracts of the two plants *C. chelidonii* and *C. gynandra* were assessed by their zone of inhibition (IZ) and activity index (AI) values which reveals that these two plant shows significant antimicrobial effects compared to standards (Figure 1).

Methanolic extract of *C. gynandra* showed maximum antibacterial activity on *Staphylococcus aureus* (IZ- 22 ± 0.22 mm, AI-0.917, MIC- 0.039 mg/mL, MBC- 0.039

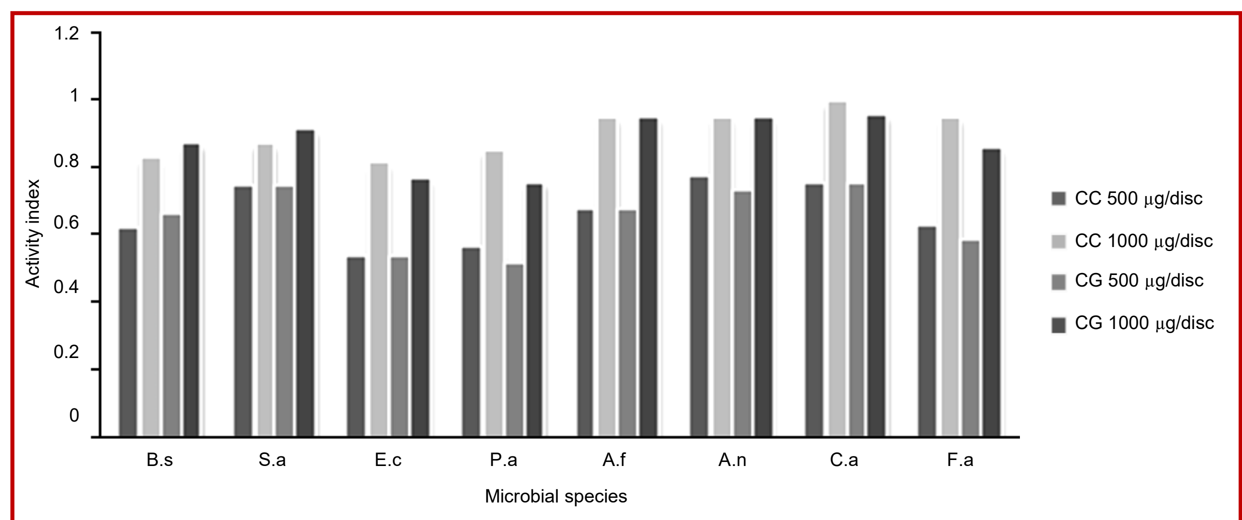

Figure 1: Comparative antimicrobial activity of plant extracts

mg/mL) followed by *Bacillus subtilis* (IZ- 21± 0.52 mm, AI-0.875, MIC- 0.078 mg/mL, MBC- 0.078 mg/mL). Methanolic extract of *C. chelidonii* showed maximum antibacterial activity on *Staphylococcus aureus* (IZ- 21 ± 0.16 mm, AI-0.875, MIC- 0.078 mg/mL, MBC- 0.078 mg/mL) followed by *Pseudomonas aeruginosa* (IZ- 18 ± 0.26 mm, AI-0.857, MIC- 0.156 mg/mL, MBC- 0.312 mg/mL). Maximum antifungal potential of *C. chelidonii was* observed against *Candida albicans* (IZ- 25 ± 0.92 mm, AI-1.000, MIC- 0.039 mg/mL, MFC- 0.039 mg/mL) followed by *Aspergillus niger* (IZ- 22± 0.66 mm, AI-0.956, MIC- 0.078 mg/mL, MFC- 0.078 mg/mL). *C. gynandra* shows maximum antifungal potential against *Candida albicans* (IZ- 24 ± 0.18 mm, AI- 0.960, MIC- 0.039 mg/

Table I

Antibacterial activity of *C. chelidonii* and *C. gynandra*

	Extract concentration (µg/disc)	Test microorganism							
		Pseudomonas aeruginosa							
		IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI
Streptomycin	10	24 ± 0.2	-	24 ± 0.2	-	22 ± 0.3	-	21 ± 0.2	-
C. chelidonii	500	15 ± 0.7	0.6	18 ± 0.4	0.8	12 ± 0.4	0.5	12 ± 0.2	0.6
C. chelidonii	1000	20 ± 0.2	0.8	21 ± 0.2	0.9	18 ± 0.7	0.8	18 ± 0.3	0.9
C. gynandra	500	16 ± 0.2	0.7	18 ± 0.8	0.8	12 ± 0.3	0.5	11 ± 0.5	0.5
C. gynandra	1000	21 ± 0.5	0.9	22 ± 0.2	0.9	17 ± 0.2	0.8	16 ± 0.2	0.8

IZ of negative control for each bacteria is 0.0 mm. Values are mean ± SEM; n = 3, (p>0.05)

Table II

Antifungal activity of *C. chelidonii* and *C. gynandra*

	Extract concentration (µg/disc)	Test microorganism							
		Aspergillus flavus							
		IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI
Nystatin	10	22 ± 0.6	-	23 ± 0.1	-	25 ± 0.1	-	22 ± 0.4	-
C. chelidonii	500	15 ± 0.3	0.7	18 ± 0.4	0.8	19 ± 0.5	0.8	14 ± 0.2	0.6
C. chelidonii	1000	21 ± 0.2	1.0	22 ± 0.7	1.0	25 ± 0.9	1.0	21 ± 0.3	1.0
C. gynandra	500	15 ± 0.3	0.7	17 ± 0.4	0.7	19 ± 0.3	0.8	13 ± 0.3	0.6
C. gynandra	1000	21 ± 0.3	1.0	22 ± 0.2	1.0	24 ± 0.2	1.0	19 ± 0.2	0.9

IZ of negative control for each fungus is 0.0 mm. Values are Mean ± SEM; n = 3, (p>0.05)

Table III

MIC and MBC values (mg/mL) of plant *C. chelidonii* and *C. gynandra*

Plant	Test microorganism							
	Bacillus subtilis		*Staphylococcus aureus*		*Escherichia coli*		*Pseudomonas aeruginosa*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
C. chelidonii	0.078	0.156	0.078	0.078	0.156	0.312	0.156	0.312
C. gynandra	0.078	0.078	0.039	0.039	0.156	0.625	0.625	1.250

Table IV

MIC and MFC values (mg/mL) of *C. chelidonii* and *C. gynandra*

Plant	Test microorganism							
	Aspergillus flavus		*Aspergillus niger*		*Candida albicans*		*Fusarium axisporum*	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
C. chelidonii	0.078	0.156	0.078	0.078	0.039	0.039	0.156	0.312
C. gynandra	0.078	0.156	0.078	0.156	0.039	0.078	0.312	0.312

Table V

Total activity of *C. chelidonii* and *C. gynandra* extracts

	Total activity of microorganisms (mL/g)								
	Amount (mg/g)	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aspergillus flavus*	*Aspergillus niger*	*Candida albicans*	*Fusarium axisporum*
C. chelidonii	81.6	1046.2	1046.2	523.1	523.1	1046.2	1046.2	2092.3	523.1
C. gynandra	93.4	1197.4	2394.9	598.7	149.4	1197.4	1197.4	2394.9	299.4

mL, MFC- 0.078 mg/mL) followed by *Aspergillus niger* (IZ- 22 ± 0.24 mm, AI-0.956, MIC- 0.078 mg/mL, MFC- 0.156 mg/mL). Both the plants showed maximum effects on fungi than that of bacteria (Table I and II). Fungi and Gram-positive bacteria are found to be more susceptible to the extracts and Gram-negative bacteria are intermediately susceptible. Gram-positive bacteria was found to be the most susceptible organism than Gram-negative bacteria to the plant extracts which may be due to difference in cell wall structural between these classes of bacteria. *C. gynandra* showed more antibacterial activity compared to that of *C. chelidonii* and *C. chelidonii* showed more antifungal activity compared to that of *C. gynandra.*

MIC and MBC/MFC values (Table III and IV) were recorded for the plant extracts. The lowest MIC values were recorded for *C. chelidonii* against *Candida albicans* (0.039 mg/mL) and for *C. gynandra* against *Staphylococcus aureus* (0.039 mg/mL) and *Candida albicans* (0.039 mg/mL). These low MIC values suggesting the more susceptible nature of these organisms towards the plant extracts and supporting the AI values obtained in the assay. Higher values of MBC/MFC than that of MIC indicated that bacteriostatic/fungistatic nature of the extracts, which were observed for the active extracts.

Total activity and quantity of extracts from each plant was calculated and recorded (Table V). Total activity indicates the volume at which extract can be diluted which still having ability to kill microorganism. Both the extracts showed high total activity values against *Candida albicans* (2394.9 mL/g). *C. gynandra* showed high total activity values against *Staphylococcus aureus* (2394.9 mL/g) compared to other bacterial culture. These total activity values indicating the potential of plant extracts to inhibit growth of the test microorganisms even at low concentration. The two plant extracts inhibited the growth of selected bacteria and fungi, indicating broad spectrum bioactive nature of plants.

Plants are reservoirs of blockbuster molecule which can be used as antimicrobial agents. Plant based new antimicrobials have enormous therapeutic potential as the microbes are developing resistance towards the currently available antibiotics. Keeping this as research envisage the present screening was done and we got significant results to prove the traditional medicinal plants *C. chelidonii* and *C. gynandra* are having good antimicrobial properties.

Conclusion

The methanol leaf extract of *C. chelidonii* and *C. gynandra* possess significant antibacterial and antifungal activity, hence it can be further exploited for developing safe and potent antimicrobials.

Financial Support

Self-funded

Conflict of Interest

Authors declare no conflict of interest

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