International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 8 (5): 422-427, 2012
ISSN 1811-7775 / DOI: 10.3923/ijp.2012.422.427

Phytochemical Investigation and Chromatographic Evaluation with Antimicrobial and Cytotoxic Potentials of *Cuscuta epithymum*

[1]S.K. Biswas, [1]A. Chowdhury, [1]J. Das, [2]U.K. Karmakar, [3]S.Z. Raihan, [4]A.C. Das, [1]M.A. Hannan, [1]M.A. Monsur Dinar, [1]M.J. Hassan, [1]M.I. Hossain and [1]M.R. Farhad
[1]Department of Pharmacy, BGC Trust University, Bangladesh
[2]Pharmacy Discipline, Khulna University, Bangladesh
[3]Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Bangladesh
[4]Coles Express, Hallet Cove, SA, Australia

Abstract: *Cuscuta epithymum* Murr. (Convolvulaceae) is found in Bangladesh and has been used in traditional medicine. The present study was designed to investigate the preliminary phytochemical screening, cytotoxicity and antibacterial activities of the methanol extract of the plant stem. The preliminary phytochemical analysis was performed on the basis of the standard procedures and thin layer chromatography to confirm the presence of chemical compounds in the methanol extract of the plant. The minimum inhibitory concentration and cytotoxic activities of the extract were carried out according to broth dilution assay and brine shrimp lethality bioassay, respectively. The results of preliminary phytochemical analysis showed the presence of flavonoid, glycoside, alkaloids, carbohydrates, saponins and steroids in the plant extract. In this study, the methanol extract was subjected to Thin Layer Chromatography (TLC) in order to detect the phytoconstituents present in the extract and the best result of TLC was obtained from the solvent system comprising of toluene and ethyl acetate at the ratio of 5.5:4.5 which identified the maximum number of spots. Moreover, the extract showed moderate antibacterial activities against *Bacillus megaterium, Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi* with MIC values of 4.96±0.20, 3.03±0.16, 3.47±0.20 and 4.07±0.08 mg mL^{-1}, respectively. The methanol extract showed lethality against brine shrimp nauplii (LC_{50}: 36.31 μg mL^{-1} and LC_{90}: 83.18 μg mL^{-1}). The results indicated that the methanol extract of the stem of the plant possessed antibacterial and cytotoxic properties with several numbers of chemical compounds.

Key words: *Cuscuta epithymum*, phytochemical, cytotoxicity, brine shrimp lethality, bioassay

INTRODUCTION

The Convolvulaceae is a family of twining, prostate herbs that has about 1000 species in 40 genera, of which only one genus, *Cuscuta*, is parasitic. The herb is most widely distributed in man's crops. The flowering and seed production of *Cuscuta epithymum* continues for several months but it does not require a host stimulant for seed germination. The Cuscuta plant does not grow equally on all plants that it parasitizes. It may grow exceptionally well, may survive, produce seed quickly and die or may simply retain attached and wait for a better host. The herb is known as 'Swarnalata' in India and Bangladesh and as 'Dodder' in Australia (Holm *et al*., 1997). *Cuscuta epithymum* Murr. belongs to the family of Convolvulaceae (Pagani and Ciarallo, 1974). The herb is a weed parasitizing and it is very difficult to kill it (Dimitrova, 2004). Moreover, they are not photosynthetically active and the leaves are very little (Holm *et al*., 1997).

The phytochemical compounds present in the traditional medicinal plants can be used in the treatment of different types of diseases (Pareta *et al*., 2011). *Cuscuta epithymum* possesses mild laxative and diuretic properties. The plant can also be employed for the treatment of sciatica, scurvy and scrofula derma (Holm *et al*., 1997). It was reported that the onset of action of seizure was delayed by the methanol extract of *Cuscuta epithymum* stem in pentylenetetrazol induced mice at the dose of 100 mg kg^{-1}. The plant also possessed effective anticonvulsant components (Mehrabani *et al*., 2007) and some flavonoids (Pagani and Ciarallo, 1974).

Corresponding Author: S.K. Biswas, Department of Pharmacy, BGC Trust University Bangladesh, Chittagong, Bangladesh

From the literature survey, it was observed that no extensive research was done on the plant. Due to the lack of scientific data of this medicinal plant, the present study was carried out to investigate the presence of phytochemicals, antibacterial activity and brine shrimp lethality bioassay for cytotoxic activities of the methanol extract of *Cuscuta epithymum* stem.

MATERIALS AND METHODS

Collection of plant materials and preparation of the extract: The stems of *Cuscuta epithymum* were collected from Chittagong, Bangladesh and were taxonomically identified by the experts at Bangladesh Forest Research Institute. The voucher specimen was deposited at the Department of Pharmacy of BGC Trust University Bangladesh. About 200 g of the dried powdered material of the plant was weighed and soaked in 600 mL of methanol. The mixture was then filtered and the excess solvent was completely evaporated to make a yellowish residue (yield 19.2%) which was known as crude methanol extract. Finally, the methanol extract was stored in a desiccator before use.

Culture media and test microorganisms: In this study, nutrient agar was used as a media for bacterial growth. Both gram positive bacteria such as *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus cereus*, *Staphylococcus aureus* and gram negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella dysenteriae*, *Shigella sonnei*, *Salmonella typhi* and *Vibrio cholerae* were used in this study. All of the strains were collected from International Centre for Diarrhoeal Disease and Research, Bangladesh.

Standard drug: The standard drug, amoxycillin was collected from Incepta Pharmaceuticals Limited, Dhaka, Bangladesh.

Preliminary phytochemical screening: Qualitative phytochemical tests were performed for the methanol extract of *Cuscuta epithymum* according to the standard procedures described by Trease and Evans (1989).

Thin layer chromatography (TLC): Thin layer chromatography is a common and most convenient tool for phytochemical analysis of plant constituents, particularly for their initial separation, detection and identification. The methanol extract of *Cuscuta epithymum* was subjected to thin layer chromatographic analysis to observe the presence of number of chemical constituents to support the phytochemical tests. The thin layer chromatographic analysis was done according to the procedure described by Mendham *et al.* (2002). Silica gel slurry was used to prepare the TLC plates and the solvent system selected for the TLC of methanol extract of *Cuscuta epithymum* was toluene and ethyl acetate as a mobile phase with different ratio of 9.5:0.5, 8.5:1.5, 7.5:2.5, 6.5:3.5, 5.5:4.5, 4.5:5.5, 3.5:6.5, 2.5:7.5, 1.5:8.5 and 0.5:9.5.

Cytotoxic activity: The cytotoxic activity of the crude methanol extract was performed on brine shrimp nauplii according to brine shrimp lethality bioassay (Meyer *et al.*, 1982). From this study, LC_{50} ($\mu g\ mL^{-1}$) and LC_{90} ($\mu g\ mL^{-1}$) of the crude methanol extract of the plant were also determined.

***In vitro* antimicrobial assay and minimum inhibitory concentration (MIC):** The antimicrobial activity of the extract was carried out using the agar disc diffusion method (Bauer *et al.*, 1966). The concentration of the plant extract was 500 $\mu g\ disc^{-1}$ while the standard drug, amoxycillin was used at 10 $\mu g\ disc^{-1}$. The minimum inhibitory concentration was determined by broth dilution assay with minor modifications (Kubo *et al.*, 1994; Sahin *et al.*, 2003).

Statistical analysis: Three replicates of each sample were used for statistical analysis and the results of the experiment were expressed as Mean±standard deviation (SD).

RESULTS

Phytochemical screening: The results of various chemical tests for the detection and identification of chemical constituents are summarized in Table 1. The phytochemical analysis showed the presence of

Table 1: Results of preliminary screening of methanol extract of *Cuscuta epithymum*

Phytochemicals	Crude extract of *Cuscuta epithymum*
Flavonoids	+
Tannins	−
Glycoside	+
Alkaloids	+
Anthraquinones	−
Carbohydrates	+
Resins	−
Proteins	−
Saponins	+
Steroids	+

+: Present, −: Absent

flavonoid, alkaloids, glycoside, carbohydrates, saponins and steroids while tannins, anthraquinones, resin and protein were not found in the extract.

TLC analysis: The solvent system selected for the TLC of methanol extract of *Cuscuta epithymum* was toluene and ethyl acetate as a mobile phase with the ratio of 9.5:0.5, 8.5:1.5, 7.5:2.5, 6.5:3.5, 5.5:4.5, 4.5:5.5, 3.5:6.5, 2.5:7.5, 1.5:8.5 and 0.5:9.5 resulted in identification of spots with different R_f values which are shown in Table 2. The study results revealed that a good number of chemical compounds were present in the crude extract. The solvent system having toluene and ethyl acetate of the ratio 5.5:4.5 produced maximum four spots at 365 nm with R_f values of 0.34, 0.671, 0.7968 and 1.00. The TLC plates of the extract of *Cuscuta epithymum* are shown in Fig. 1.

Fig. 1: TLC plate of methanol extract of *Cuscuta epithymum*

***In-vitro* antibacterial activities and minimum inhibitory concentration:** The results of antibacterial activities are summarized in Table 3. The antibacterial potentials of the methanol extract of *Cuscuta epithymum* were carried out against ten bacterial strains and the results were compared with a standard disc of amoxycillin (10 μg $disc^{-1}$). At 500 μg $disc^{-1}$, the methanol extract of the plant showed moderate antibacterial activity against four bacterial strains such as *Bacillus megaterium* (11.63±0.10 mm), *Pseudomonas aeruginosa* (6.13±0.10 mm),

Table 2: Solvent system with R_f values of the compounds present in methanol extract of *Cuscuta epithymum*

	No. of spot(s) under UV light at		R_f values	
Solvent system (toluene:ethyl acetate)	254 nm	365 nm	254 nm	365 nm
9.5:0.5	1	2	0.123	0.230 and 0.33
8.5:1.5	2	2	0.333 and 0.70	0.333 and 0.70
7.5:2.5	0	1	-	0.40
6.5:3.5	0	1	-	1.00
5.5:4.5	2	4	0.109 and 0.25	0.34, 0.671, 0.7968 and 1.00
4.5:5.5	1	3	0.1846	0.2165, 0.3692 and 0.9538
3.5:6.5	2	2	0.317 and 0.9523	0.317 and 0.9523
2.5:7.5	0	2	-	0.5454 and 0.6363
1.5:8.5	0	1	-	0.0833
0.5:9.5	2	1	0.34 and 0.56	1.00

Table 3: Antibacterial activities of methanolic extract of *Cuscuta epithymum*

		Diameter of zone of inhibition (mm)	
Bacterial strains	Negative control (blank)	Crude extract of *Cuscuta epithymum* (500 μg $disc^{-1}$)	Standard drug, amoxycillin (10 μg $disc^{-1}$)
Gram positive			
Bacillus subtilis	-	-	25.45±0.41
Bacillus megaterium	-	11.63±0.10	21.05±0.82
Bacillus cereus	-	-	-
Staphylococcus aureus	-	-	20.22±0.69
Gram negative			
Pseudomonas aeruginosa	-	6.13±0.10	16.92±0.12
Escherichia coli	-	9.72±0.21	23.67±0.94
Shigella dysenteriae	-	-	23.33±0.94
Shigella sonnei	-	-	16.00±0.82
Salmonella typhi	-	9.95±0.07	15.22±0.31
Vibrio cholerae	-	-	18.58±0.42

Data were represented as Mean±SD of triplicate determination, -: No inhibition

Table 4: The MIC values of methanol extract of *Cuscuta epithymum* against the tested microorganisms

		MIC values (mg mL^{-1})	
Bacterial strains	Control (blank)	Crude extract of *Cuscuta epithymum*	Standard drug, amoxycillin*
Gram positive			
Bacillus subtilis	-	-	74.83±0.76
Bacillus megaterium	-	4.96±0.20	80.00±0.15
Bacillus cereus	-	-	77.61±0.54
Staphylococcus aureus	-	-	82.23±0.83
Gram negative			
Pseudomonas aeruginosa	-	3.03±0.16	67.43±0.55
Escherichia coli	-	3.47±0.20	78.33±0.52
Shigella dysenteriae	-	-	55.10±0.80
Shigella sonnei	-	-	48.33±0.71
Salmonella typhi	-	4.07±0.08	75.88±0.78
Vibrio cholerae	-	-	64.46±0.68

Data were represented as Mean±SD of triplicate determination, -: No inhibition, *Amoxycillin all values were in μg mL^{-1}

Table 5: Brine shrimp lethality bioassay of methanolic extract of *Cuscuta epithymum*

		No. of alive shrimp						
Conc. of sample (μg mL^{-1})	Log Conc.	Test-1	Test-2	Test-3	Avg.	Mortality (%)	LC_{50} (μg mL^{-1})	LC_{90} (μg mL^{-1})
10	1.00	10	9	10	9.67	3.33	36.31	83.18
20	1.30	8	8	7	7.67	30.30		
30	1.48	7	6	7	6.67	44.44		
40	1.60	6	7	5	6.00	53.85		
50	1.70	6	4	5	5.00	64.29		
60	1.78	5	3	3	3.67	75.56		
70	1.85	4	4	2	3.33	79.17		
80	1.90	3	2	2	2.33	86.27		
90	1.95	2	1	1	1.33	92.59		
100	2.00	2	0	1	1.00	94.74		
150	2.18	1	0	1	0.67	96.67		
200	2.30	0	0	0	0.00	100.00		
250	2.40	0	0	0	0.00	100.00		
300	2.48	0	0	0	0.00	100.00		
350	2.54	0	0	0	0.00	100.00		
400	2.60	0	0	0	0.00	100.00		

Escherichia coli (9.72±0.21 mm) and *Salmonella typhi* (9.95±0.07 mm) while *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Shigella sonnei* and *Vibrio cholerae* were not susceptible to the tested crude extract. The maximum zone of inhibition obtained against gram positive bacterial strain such as *Bacillus megaterium* (11.63±0.10 mm). Subsequent tests were also carried out to determine the inhibitory concentrations of the methanol extract of Cuscuta epithymum. Table 4 summarized MIC values against *Bacillus megaterium*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi* were 4.96±0.20, 3.03±0.16, 3.47±0.20 and 4.07±0.08 mg mL^{-1}, respectively. The obtained MIC values were also compared with the standard drug, amoxycillin.

Cytotoxic activities: The results of cytotoxic activities are presented in Table 5. The extract showed positive result having LC_{50} and LC_{90} values of 36.31 μg mL^{-1} and 83.18 μg mL^{-1} by brine shrimp lethality bioassay testing which indicated significant cytotoxic activity of the plant extract. The values were calculated from the best-fit line slope (Fig. 2).

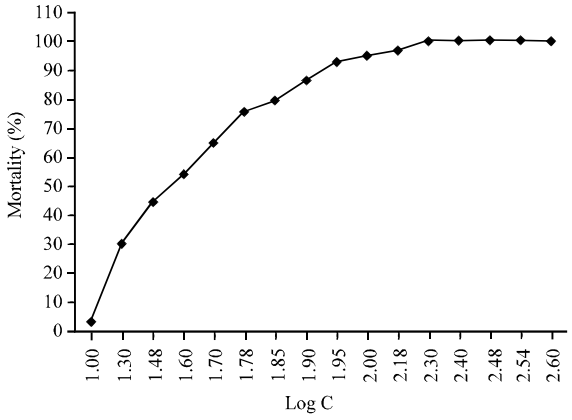

Fig. 2: Graphical presentation of LC_{50} (μg mL^{-1}) and LC_{90} (μg mL^{-1}) of methanol extract of *Cuscuta epithymum*

DISCUSSION

No extensive research was done on the antibacterial and cytotoxic activities of the methanol extract of

Cuscuta epithymum. The present study results showed the presence of flavonoid, glycoside, carbohydrates, saponins, steroids. Thin layer chromatography also confirmed the presence of phytochemicals. The presence of natural flavonoids in the medicinal plants could show the antioxidant and free radical scavenging properties (Middleton and Kandaswami, 1992; Okwu and Orji, 2007). Moreover, tannins are useful in urinary tract infections (Agbafor *et al*., 2011).

It has also been reported that flavonoids and steroids possess anti-inflammatory (Hossinzadeh *et al*., 2002; Zakaria *et al*., 2006; Okokon *et al*., 2008; Garg and Paliwal, 2011) and analgesic activities (Ramaswamy *et al*., 1985). Alkaloids also show the inhibition of pain perception (Rahman *et al*., 2011). Amabeoku and Kabatende (2011) also reported that saponins present in the medicinal plant show the analgesic activity. Moreover, the presence of glycosides in the plant may contribute hypoglycemic effect (Okontak and Aguwa, 2007). Thus, the plant might possess the promising hypoglycemic, analgesic and anti-inflammatory effects which should be investigated. The present study also revealed the antibacterial and cytotoxic activities of the methanol extract of the plant stems. Out of ten bacterial strains, *Bacillus megaterium* (11.63±0.10 mm), *Pseudomonas aeruginosa* (6.13±0.10 mm), *Escherichia coli* (9.72±0.21 mm) and *Salmonella typhi* (9.95±0.07) were susceptible to the crude methanol extract of the stems. The lowest MIC value was obtained against *Pseudomonas aeruginosa* (3.03±0.16 mg mL^{-1}). In addition to this, the study also confirmed the cytotoxic activities of the plant having LC_{50} and LC_{90} values of 36.31 μg mL^{-1} and 83.18 μg mL^{-1}, respectively. Thus, the plant may be a potential source of new antibacterial and anticancer drug.

CONCLUSION

The results of this study indicate that the plant is potentially a good source of antibacterial and anticancer drugs. The broad spectra of activity of both plants extract is promising and the isolation of active constituents present in the plant can be the subject for the future scientists.

ACKNOWLEDGMENTS

The authors are thankful to Professor Dr Samir Kumar Sadhu, Pharmacy Discipline of Khulna University, Bangladesh to provide all supports in progress of the research works. We also express our gratitude to the authority of International Centre for Diarrhoeal Disease and Research, Bangladesh and Incepta Pharmaceuticals Ltd., Bangladesh.

REFERENCES

Agbafor, K.N., E.I. Akubugwo, M.E. Ogbashi, P.M. Ajah and C.C. Ukwandu, 2011. Chemical and antimicrobial properties of leaf extracts of *Zapoteca portoricensis*. Res. J. Med. Plant, 5: 605-612.

Amabeoku, G.J. and J. Kabatende, 2011. Antinociceptive and anti-inflammatory activities of leaf methanol extract of *Cotyledon orbiculata* L. (Crassulaceae). Adv. Pharmacol. Sci., 10.1155/2012/862625.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by standardised single disc method. Am. J. Clin. Pathol., 44: 493-496.

Dimitrova, T., 2004. Check of *Amaranthus blitoides* W. var. *Reverchoni* Th.-An element of the control of *Cuscuta epithymum* Murr. in lucerne (*Medicago sativa* L.). Bulg. J. Agric. Sci., 10: 579-582.

Garg, V.K. and S.K. Paliwal, 2011. Anti-inflammatory activity of aqueous extract of *Cynodon dactylon*. Int. J. Pharmacol., 7: 370-375.

Holm, L., J. Doll, E. Holm, J. Pancho and J. Herbenger, 1997. World Weeds: Natural Histories and Distribution. John Wiley and Sons, New York, USA., pp: 249-265.

Hossinzadeh, H, M. Ramezani, M. Fedishei and M. Mahmoudi 2002. Antinociceptive, anti-inflammatory and acute toxicity effects of *Zhumeria majdae* extracts in mice and rats. Phytomedicine, 9: 135-141.

Kubo, I., H. Muroi and A. Kubo, 1994. Naturally occurring antiacne agents. J. Nat. Prod., 57: 9-17.

Mehrabani, M., E. Modirian, A.R. Ebrahimabadi, J. Vafazadeh, S. Shahnavaz and M.R. Heidari, 2007. Study of the effects of hydro-methanol extracts of *Lavandula vera* DC and *Cuscuta epithymum* Murr on the seizure induced by pentylentetranzol in mice. J. Kerman Univ. Med. Sci., 14: 25-32.

Mendham, J., R.C. Denney, J.D. Barnes and M. Thomas, 2002. Vogel's Textbook of Quantitative Chemical Analysis. Pearson Education (Singapore) Ltd., Delhi, India, pp: 251-258.

Meyer, B.N., N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols and J.L. Mclaughlin, 1982. Brine shrimp: A convenient bioassay for active plant constituents. Planta Med., 45: 31-34.

Middleton, Jr. E. and C. Kandaswami, 1992. Effects of flavonoids on immune and inflammatory cell functions. Biochem. Pharmacol., 43: 1167-1179.

Okokon, J.E., B.S. Antia and E. Umoh, 2008. Analgesic and anti-inflammatory effects of ethanolic root extract of *Hippocratea africana*. Int. J. Pharmacol., 4: 51-55.

Okontak, J.M. and C.N. Aguwa, 2007. Evaluation of hypoglycemic activity of glycosides and alkaloids extracts of *Picralima nitida* Stapf (Apocynaceae) seed. Int. J. Pharmacol., 3: 505-509.

Okwu, D.E. and B.O. Orji, 2007. Phytochemical composition and nutritional quality of *Glycine max* and *Vigna unguiculata* (L.) Walp. Am. J. Food Technol., 2: 512-520.

Pagani, F. and G. Ciarallo, 1974. New flavonoids of the *Cuscuta epithymum* murr (Convolvulaceae). Boll. Chim. Farm., 113: 30-35.

Pareta, S.K., K.C. Patra, P.M. Mazumder and D. Sasmal, 2011. Establishing the principle of herbal therapy for antiurolithiatic activity: A review. J. Pharmacol. Toxicol., 6: 321-332.

Rahman, M.A., E. Haque, M. Hasanuzzaman and I.Z. Shahid, 2011. Antinociceptive, antiinflammatory and antibacterial properties of *Tamarix indica* roots. Int. J. Pharmacol., 7: 527-531.

Ramaswamy, S., N.P. Pillai, V. Gopalkrishnan, N.S. Parmar and M.N. Ghosh, 1985. Analgesic effect of O-(β-hydroxyethyl) rutoside in mice. Indian J. Exp. Biol., 23: 219-220.

Sahin, F., I. Karaman, M. Gulluce, H. Ogutcu, M. Sengul, A. Adiguzel, S Ozturk and R. Kotan, 2003. Evaluation of antimicrobial activities *Satureja hortensis* L. J. Ethnopharmacol., 87: 61-65.

Trease, G.E. and W.C. Evans, 1989. Trease and Evan's Textbook of Pharmacognosy. 13th Edn., Cambridge University Press, London, Pages: 546.

Zakaria, Z.A., R.N.S.R.M. Nor, M.R. Sulaiman, Z.D.F.A. Ghani, G.H. Kumar and C.A. Fatimah, 2006. Antinociceptive and anti-inflammatory properties of *Melastoma malabathricum* leaves chloroform extract in experimental animals. J. Pharmacol. Toxicol., 1: 337-345.