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Biological Evaluation of the Plant *Mazus japonicus* Albiflorus (Scrophulariaceae).

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Research Article

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ABSTRACT

Mazus japonicus is an important plant of Scrophulariaceae family. In the present research work, the dichloromethane and methanol crude extracts of the plant *Mazus japonicus* were evaluated for phytotoxic, cytotoxic, antifungal and antibacterial activities. Both dichloromethane and methanol extracts of *Mazus japonicus* inhibited the growth of *Lemna minor* L. and showed significant phytotoxic activities. The dichloromethane extract showed cytotoxic activity at highest level of dose with LD_{50} 513.04 µg/ml, while methanol extract showed no cytotoxic activity. The dichloromethane extract of the plant showed 25% inhibition against *Fusarium solani* and methanol extract of the plant showed 30% inhibition against *Microsporum canis*. Both dichloromethane and methanol extract of the plant exhibited non-significant activity when tested against pathogenic gram positive and gram negative bacterial strains. The phytochemical tests indicated the presence of saponins in the plant.

INTRODUCTION

Trees and plants are of paramount importance for human life, not only in the present time but also in the remote past as well. The early man depended on them for his physical needs such as source of food, shelter, clothing, medicines, ornaments, tools and for spiritual needs such as magic.

Herbs have always been used since prehistoric time till to date for curative purposes. An organized study of natural products, started at the beginning of 19th century, has been a pivotal factor in the development of potent biologically active molecules from plants. Therefore plants not only continued to retain their historical significance as one of the important source of new medicine for the treatment of diseases like cancer, acquired immunodeficiency syndrome (AIDS), malaria and disorders of cardiovascular and central nervous system, and many more. For all these reasons, bioactive molecules of plants have been always available to serve as source of inspiration to advance the traditional medicine and to prepare it to accept new challenges in time to come [1].

Majority of pharmacologically active molecules separated from plants are secondary metabolites, which are sophisticated arsenal to protect plants from outside dangers. Secondary metabolites perform important ecological functions including defenses against herbivores, bacterial and fungal infections. Many compounds used by the plants for defensive purposes can also be used by humans for the same purpose. However, secondary metabolites, which have ecological significance for plants, have altogether different effects on humans. For example, L-Dopa isolated from many leguminous plants like *Vicia faba,* acts as an antifeedant, but in humans it is used for the treatment of various psychatory disorders [2].

Plants have been used as medicines since beginning of human civilization. There are written evidences of medicinal uses of plants in texts of the ancient Chinese, Indian and other civilizations. India has had a history of ancient traditional medicinal practice based mostly on Ayurveda, Siddha and Unani systems of medicine. Medicinal plants have always been the main constituents of the traditional medicine. Ayurveda is based on natural products of nearly 2,000 cultivated and wild plant species. The written records of Ayurveda like Charaka Samhita, Shushruta Samhita and others contain more than 8,000 herbal remedies. There are literally millions of plants, combinations, traditions and household remedies to treat varieties of diseases and to boost health [3,4].

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Scrophulariaceae is a large family with 220 genera and 3000 species distributed worldwide [5,6]. The genus Mazus of Scrophulariaceae family is eastern Asiatic, primarily of China. There are about 30 species in the genus, distributed in temperate and subtropical regions. In general the species of warmer regions and lower altitudes are widespread and of common occurrence. Some of the species of higher altitudes are restricted in their ranges. Among these species, the most widespread is *Mazus japonicus*, widely distributed in moist swampy places from India, China, Japan, south to the Philippines and Java, the only species that extends beyond the southern border of China [7,8,9].

MATERIALS AND METHODS

The present research work was carried out in natural product chemistry laboratory and phytochemistry laboratory, Department of Pharmacy, Bahauddin Zakariya University, Multan, from January, 2012 to December, 2012. Brief description of materials as well as methods adopted is described below.

Collection and identification of plant

The plant material was collected in March, 2012 from surroundings of Bahauddin Zakariya University, Multan. The plant was identified as *Mazus japonicus* by Dr. Altaf Hussain Dasti, Professor, Institute of pure and applied Biology, Bahauddin Zakariya University, Multan and assigned a catalog no. STW 653.

Preparation of plant extracts

To achieve efficient extraction, the whole plant was dried under shade for 30days, then grinded it to a uniform powder and weighed. Simple maceration was adopted for effective extraction. Extract was prepared by soaking 200g of the dry powdered plant material of *Mazus japonicus* in a measured volume of dichloromethane in a closed container along with vigorous shaking for 24hrs and then filtered. This procedure was adopted three times with dichloromethane. The extraction of marc was carried out with methanol using same procedure. Rotary evaporator was used to concentrate the dichloromethane and methanol extracts. The extracts of dichloromethane and methanol were taken in different vials and named as MJD and MJM respectively.

Determination of Phytochemical Constituents

Phytochemical studies were carried out to analyze the presence of secondary metabolites. Alkaloids were detected by using Dragendorff, Mayer, Wagner and Hager's Reagent. For detection of free and bound anthraquinone glycosides, Borntrager and modified Borntrager tests were performed. Keller Kiliani method was used for the confirmation of cardiac glycosides. Froth test was employed for the detection of saponins and for tannins Ferric chloride, Gelatin and Catechin tests were performed [6,10,11].

Phytotoxicity Assay

Lemna minor for Phytotoxicity Assay

Inorganic (E-medium) was prepared in which KOH pellets were added to attain pH 5.5-6.0. For testing, 10 vials per dose (500, 50, 5 ppm, control) were prepared. 15g of extract was dissolved in 15 ml of solvent. 1000, 100 and 10 µl solutions were dissolved to vials for 500, 40 and 5 ppm, after which solvents were allowed to evaporate overnight. 2 ml of E-medium and then a single plant containing healthy and green rosettes of three fronds were added to each vial. Vials were placed in a glass dish that is filled with 2cm of water. Then container was sealed. Dish with vials were placed in growth chamber for seven days at 26˚C along with other controlled conditions. After three and seven days number of fronds per vial were counted and recorded. In the end, data was analyzed as percent of control with ED_{50} computer program to determine FI_{50} values and 65% confidence interval [12,13].

Cytotoxicity Assay

Brine-Shrimp Lethality Assay

The dichloromethane and methanol extracts of *Mazus japonicus* were tested for their cytotoxic activity by performing Brine shrimp (*Artemia salina*) lethality bioassay. For this assay mature shrimp larvae were produced by placing them in artificial sea water. For testing, three vials each of concentration 1000, 100 and 10 µg/ml were prepared. Then, 20mg of extract was added in 2ml of solvent. From these solutions 500, 50, or 5 µl were transferred to vials having 1000,100, or 10 µg/ml respectively. Solvent was allowed to evaporate. Then 5ml of sea water and 10shrimp larvae were added in each vial with the aid of Pasteur pipette. These vials were placed under illumination. After 24 hours surviving shrimps were counted and recorded and finney computer program was used to analyze data and to determine LC_{50} values and 95% confidence intervals [12,14,15].

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Antifungal Assay

Agar Tube Dilution Assay

The dichlorometane and methanol extracts of *Mazus japonicus* were tested against *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glabrata*. A control experiment with test substance was performed to evaluate the antifungal activity of extracts. Stock solution was prepared by adding dichlorometane and methanolic extracts in DMSO. Sabouraud Dextrose Agar (SDA) was prepared and a known volume was transferred into screw capped test tubes which were autoclaved at 121˚C for 15 minutes and then test tubes were cooled to 50˚C. The test samples were transferred from stock solution to the non-solidified Sabouraud agar media. Tubes were then solidified at room temperature. 4mm diameter of inoculums removed from a seven day old culture of fungi was inoculated in each tube. Inhibition of fungal growth was observed after 7-10 days of incubation at 28±1˚C [12,15,16].

Antibacterial Assay

Agar diffusion method

The dichlorometane and methanol extracts of *Mazus japonicus* were tested against *Bacillus subtilis* (NCTC 8236)*, Staphylococcus aureus* (NCTC 6571)*, Salmonella typhi, Escherichia coli* (NCTC 10418)*, Shigella flexenari and Pseudomonas aeruginosa* (ATCC 10145) by using this method. To determine antibacterial activity by using this method already prepared plates of broth culture media were used. From these plates required number of holes was cut by using a sterile cork borer keeping in view the proper distribution in the periphery and one in the centre. By using 0.1ml sterile pipette, 100µl of the dichloromethane and methanolic extracts in a suitable solvent, standard antimicrobial agent and solvent were poured into their respective hole. These plates were placed at room temperature for 2 hrs to get proper diffusion of the sample and incubated at 37˚C for 24 hr. The diameter of the zones of inhibition was measured to the nearest mm [12,16,17].

RESULTS AND DISCUSSION

Preliminary Phytochemical Tests

For the detection of secondary metabolites preliminary phytochemical tests were performed. The results of phytochemical tests showed the presence of saponins as a major class of secondary metabolites in *Mazus japonicus.* Results also indicated the absence of alkaloids, cardiac glycosides, anthraquinone glycosides and tannins in *Mazus japonicus*.

Table 1: Secondary metabolites of *Mazus japonicus.*

Plant Name	Alkaloids	Anthraquinones	Cardiac glycosides	Saponins	Tannins
Mazus japonicus	-	-	-	+	-

+ : present, - : absent

Saponins have been reported earlier to protect acute and chronic heart disease by exerting strong antioxidant activities by neutralizing reactive oxygen species. Cardiotonic effect showed by saponins was due to calcium channel blocking. Saponins are responsible for significant decrease in platelet aggregation. Saponin also showed protective effect on CNS. Saponins have been reported earlier to inhibit cancer cell proliferation indirectly through cell cycle protein, apoptosis related proteins, growth factors and protein kinases [18]. Saponins also showed abortifacient, antizygotic and antiimplantation activity. Saponins were also found to be involved in enhancing immune system by cell mediated immune system and antibody production [19]. Saponins are identified as a major class of secondary metabolites in the plant *Mazus japonicus*. It is conceivable that saponins hold a wide range of therapeutic potential and could be a lead for isolation and purification of individual saponins and can be put to further evaluation for their therapeutic potential.

Phytotoxic activity

The *Lemna minor* assay is a quick measure of phytotoxicity of plants extracts [20]. The results of phytotoxic bioassay of crude dichloromethane and methanol extracts of *Mazus japonicus* are given in the Table No 2.

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Table 2: Results of *In vitro* phytotoxic bioassay of *Mazus japonicas*

Extracts	Plant Name	Conc. of Compound (µg/ml)	No. of Fronds		% Growth Regulation	Conc. of Standard Drug (Paraquat) (µg/ml)
			Sample	Control		
Dichloromethane extract	*Lemna minor*	1000	0	20	100	0.015
		100	6		70	
		10	9		55	
Methanol extract		1000	11	20	45	
		100	15		25	
		10	19		5	

The results of phytotoxic bioassay showed that the dichloromethane extract inhibited the growth of *Lemna minor* L. upto 100% at high dose, 70% at moderate dose and 55% at low dose. While methanol extract inhibited the growth of *Lemna minor* L. upto 45% at high dose, 25% at moderate dose and 5% at low dose. Several workers reported the phytotoxic activity of various medicinal like *Abroma augusta* seed oil inhibited the growth of *Lemna aequinoctialis* [21]. It has been reported that *Chamaesyce hyssopifolia, Melaleuca quinquenervia, Acacia farnesiana, Ageratum conyzoides* and *Alphitonia excelsa* showed phytotoxicity against *Lemna aequinoctialis* [22]. Hussain *et al.* reported that n-hexane, n-butanol, chloroform and water fractions of *Nepeta juncea* showed insignificant phytotoxic effect against *L. minor* [23]. The *Lemna* assay is a quick measure of phytotoxicity. The phytotoxicty bioassay is a useful primary screen for weedicide research. As weeds is one of the major factors of poor agricultural productivity in the developing countries. Synthetic weedicides are expensive, toxic and non specific. Weedicides from natural sources having improved characteristics could have a promising future [12, 24]. Phytotoxic natural products may be utilized either directly or as lead compounds for the development of herbicides. Furthermore, studies may be carried out to explore the phytotoxic components of the plant by isolation, purification and structure determination leading to the development of an effective herbicide.

Cytotoxic Activity

Brine shrimp (*Artemia salina*) lethality bioassay was performed to evaluate cytotoxic activity of dichloromethane and methanol extracts of *Mazus japonicus.* Results of Brine shrimp (*Artemia salina*) lethality bioassay of crude dichloromethane and methanol extracts of *Mazus japonicus* are given in the Table No 3.

Table 3: Results of Brine shrimp (*Artemia salina)* bioassay of *Mazus japonicas*

Extracts	Dose (µg/ml.)	No. of Shrimps	No. of Survivors	LD50 (µg/ml.)	STD. Drug	LD50 (µg/ml.)
Dichloromethane extract	1000	30	13	513.04	Etoposide	7.4625
	100	30	20			
	10	30	28			
Methanol extract	1000	30	18	2894.93	Etoposide	7.4625
	100	30	26			
	10	30	28			

The dichloromethane extract showed cytotoxicity at highest level of dose with LD_{50} 513.04 µg/ml, while methanol extract showed no cytotoxicity with LD_{50} 2894.93 µg/ml. Brine-shrimp lethality bioassay has been used extensively to monitor the cytotoxicity of the sample under study. This is a rapid, inexpensive, in house, general bioassay which has been developed for screening, fractionation and monitoring of physiologically active natural products [25].

Antifungal activity

In vitro antifungal bioassay was performed to evaluate the antifungal activity of *Mazus japonicus. In vitro* antifungal bioassay was performed by following Agar tube dilution protocol. Results of *In vitro* antifungal bioassay of crude dichloromethane and methanol extracts of *Mazus japonicus* are given in the table 4.

The dichloromethane and methanol extracts of *Mazus japonicus* showed non-significant activity against *Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani, Candida glabrata*. It has been noted that dichloromethane extract showed 25% inhibition with linear growth at 75mm, when compared with control; only against *Fusarium solani* and methanol extract showed 30% inhibition with linear growth at 70mm, when compared with control; only against *Microsporum canis*.

The results of antifungal activity of the plant is in consistent with other plants of family Boraginaceae like *Echium rauwolfii*, *Echium horridum* [26], *Cordia alliodora* [27], *Cordia linnaei* [28], *Cordia morelosana* [29], *Arnebia*

euchroma [30], *Arnebia hispidissima* [31], *Trichodesma amplexicaule* [32] and *Cynoglossum officinale* [33]. Moderate activity was reported by crude methanolic extract of *Onosma griffiithii* against *Aspergillus flavus* (55%) and *Fusarium solani* (40%) while its n-butanol and ethyl acetate extracts shows no activity [34]. No antifungal activity was recorded when aqueous and ethanolic extract of the plant, *Colendia procumbens* was tested against *Candida albicans* [35]. Aqueous and methanol extracts of *Anchusa italic* and *Trichodesma zeylanicum* also displayed no activity [36].

Table 4: Results of *In vitro* antifungal bioassay of *Mazus japonicas*

Extracts	Name of Fungus	Linear Growth (mm) Sample	Control	% Inhibition	Standard Drug	Mic (µg/ml)
Dichloromethane extract	*Candida albicans*	100	100	0	Miconazole	110.8
	Aspergillus flavus	100	100	0	Amphotericin B	20.20
	Microsporum canis	100	100	0	Miconazole	98.4
	Fusarium solani	75	100	25	Miconazole	73.25
	Candida glabrata	100	100	0	Miconazole	110.8
Methanol extract	*Candida albicans*	100	100	0	Miconazole	110.8
	Aspergillus flavus	100	100	0	Amphotericin B	20.20
	Microsporum canis	70	100	30	Miconazole	98.4
	Fusarium solani	100	100	0	Miconazole	73.25
	Candida glabrata	100	100	0	Miconazole	110.8

Antibacterial activity

In vitro antibacterial bioassay was performed to evaluate the antibacterial activity of *Mazus japonicus. In vitro* antibacterial bioassay was performed by using Agar tube diffusion method. Results of *In vitro* antibacterial bioassay of crude dichloromethane and methanol extracts of *Mazus japonicus* are given in the table 5.

Table 5: Results of *In vitro* antibacterial bioassay of *Mazus japonicas*

Extract	Name of bacteria	Zone of inhibition of sample (mm)	Zone of inhibition of standard drug (Imipenum) (mm)
Dichloromethane extract	*Eschericha coli*	-	25
	Bacillus subtilis	-	50
	Shigella flexinari	-	28
	Staphylococcus aureus	-	48
	Pseudomonas aeruginosa	-	23
	Salmonella typhi	-	28
Methanol extract	*Eschericha coli*	-	25
	Bacillus subtilis	-	50
	Shigella flexinari	-	28
	Staphylococcus aureus	-	48
	Pseudomonas aeruginosa	-	23
	Salmonella typhi	-	28

The dichloromethane and methanol extracts of *Mazus japonicus* were tested against *Eschericha coli, Bacillus subtilis, Shigella flexinari, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi* by using Agar tube diffusion method. Both the extracts showed no activity.

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CONCLUSION

The present findings have clearly demonstrated that the plant has excellent potential towards the phytotoxic and cytotoxic activity. The growth inhibition of the plant against *Microsporum canis* and *Fusarium* solani identified that the plant can be used as antifungal agent for the treatment of different fungal diseases. During preliminary phytochemical screening saponins are identified as a major class of secondary metabolites in this plant. So the further studies may be carried out to purify the active constituents of the plants that are responsible for its phytotoxic, cytotoxic and antifungal activity to get a valuable herbicidal, cytotoxic and antifungal agent. It is conceivable that saponins hold a wide range of therapeutic potentioal and could be a lead for isolation of therapeutically active novel saponins.

REFERENCES

1. Usmanghani K, Saeed A, Alam MT. 1997, Indusyunic medicine. Department of Pharmacognosy, University of Karachi, pp. 7.
2. Simmonds MSJ, Grayer RJ. 1999, Drug Discovery and Development. Chemicals from Plants: Perspectives on Plant Secondary Products. Ed. N. J. Walton & D. E. Brown, Imperial College Press, 215-249.
3. Pearce D, Moran D. 1994, Economic value of Biodiversity. In Association with the Biodiversity Programme of IUCN – The World Conservation Union, Earthscan Publications Ltd, London.
4. Subrat N, Iyer M, Prasad R. 2002, The Ayurvedic medicine industry: current status and sustainability. Sub-study of the India Country study of the international collaboration research project. Instruments for sustainable private sector forestry collaboration between Ecotech Services (India) pvt. Ltd. And International Institute for Environment and Development. ETS Publication.
5. Evans WC, Saunders WB, Trease and Evans Pharmacognosy, 15th edition, (2002).
6. Trease GE, Evans WC. Phamarcognosy, 13th edition, Bailliere Tindale Ltd, London, (1989).
7. Li H-L. The Genus Mazus (Scrophulariaceae). Brittonia. 1954;8:29-38.
8. Hsieh T. Revision of *Mazus Lour*. (Scrophulariaceae) in Taiwan. Taiwania.2000;45(2):131-146.
9. Barker WR. 1991. A taxonomic revision of *Mazus lour*. (Scrophulariaceae) in Australasia. Papers and Proceedings of the Royal Society of Tasmania, Aspects of Tasmanian Botany: A Tribute to Winifred Curtis: A Symposium arranged by the Royal Society of Tasmania March. pp. 85-94. ISSN 0080-4703
10. Brain KR, Turner TD. 1975, the practical evaluation of phytopharmaceuticals. Published by Wright Scientechnica, Bristol.
11. Ayoola G, Coker H, Adesegun S, Adepoju-Bello A, Obaweya K, Ezennia E, et al. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in southwestern Nigeria. Tropical J Pharm Res. 2008;7(3):1019-1024.
12. Atta-ur-rehman, Choudhary MI, Thomsen WJ. 2001, Bioassay technique for drug development. Harwood Academic publishers, Canada.
13. Dastagir G, Hussain F. Phytotoxic and insecticidal activity of plants of family Zygophyllaceae and Euphorbiaceae. Sarhad J Agric. 2013;29(1):83-91.
14. Hossain SF, Islam MS, Parvin S, Shams T, Kadir MF, et al. Antimicrobial Screening and Brine Shrimp Lethality Bioassay of *Calotropis gigantea* (Fam: Asclepiadaceae). Scholars Research Library. 2012;2(1):49-59.
15. Khan S, Khan GM, Mehsud S, Rahman A, Khan F. Antifungal activity of *Tamarix dioica*-an in vitro study. Gomal Journal of Medical Sciences. 2004;2:40-42.
16. Bano A, Ayub Z. Antibacterial and Antifungal Activity in Three Species of Siphonaria (Gastropoda: Pulmonata) Collected From Rocky Ledge of Mubarak Village, Karachi. Pakistan J Zool. 2012; 44(6):1493-1497.
17. Stepanovic S, Antic N, Dakic I, Svabicvlahovic M. Microbiological research. In vitro antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs. Microb. Res. 2003;158:353-357.
18. Yuan C, Wang C, Wicks SM, Qi L. Chemical and Pharmacological Studies of Saponins with a Focus on American Ginseng. J Ginseng Res. 2010;34:160-167.
19. Francis G, Kerem Z, Makkar HPS, Becker K. The biological action of saponins in animal systems: a review. British J Nutr. 2002;88:587-605.
20. Einhelling FA, Leather GR, Hobbs LL. J Chem Ecol. 1985;11:65.
21. Khan T, Zahid M, Asim M, Shahzad ul Hussan, Iqbal Z, Choudhary MI. et al. Pharmacological activities of crude acetone extract and purified constituents of *Salvia moorcraftiana* Wall. Phytomed. 2002;9(8):749-752.
22. Allan S, Adkins S. 2005, Searching for a natural herbicide: the role of medicinal plants. Proc. 4th World Cong. on Allelopath.
23. Hussain J, Jamila N, Gilani SA, Abbas G, Ahmed S. Platelet aggregation, antiglycation, cytotoxic, phytotoxic and antimicrobial activities of extracts of *Nepeta juncea*. Afric J Biotech. 2009;8(6):935-940.
24. Morimoto M, Cantrell CL, Bailey LL, Stephen O, Duke. Phytotoxicity of constituents of glandular trichomes and the leaf surface of camphorweed, *Heterotheca subaxillaris*. Phytochem. 2009;70:69-74.

25. Meyer BN, Ferrigni NR, Putnam JE, Jacobson LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plants constituents. Planta Med. 1982;45:31-34.
26. El-shazly A, Abdel-All M, Tie A, Wink M. Pyrrolizidine alkaloids from *Echium rauwolfii* and *Echium horridum* (Boraginaceae). Z Naturforsch. 1999;54;295-300.
27. Ionset JR, Marston A, Gupta M, Hostettmann K. Antifungal and larvicidal cordiaquinones from the roots of *Cordia curassavica*. Phytochem. 2000;53(5):613-617.
28. Ionset JR, Marston A, Gupta M, Hostettmann K. Antifungal and larvicidal meroterpenoid naphthoquinones and a naphthoxirene from the roots of *Cordia linnaei*. Phytochemistry. 1998;47(5):729-734.
29. Sanchez DOS, Najera GLA, Rivera IL, Ramirez OD, Cisneros MGV, Garcia VMN. Antimicrobial activity of Medicinal plants from the *Huautla sierra* biosphere reserve in Morelos (Mexico). Polibotanica. 2009;28: 213-225.
30. Antimicrobial and cytotoxic isohexenylnaphthazarins from *Arnebia euchroma* (Royle) Jonst. (Boraginaceae) callus and cell suspension culture. Molecules. 2012;17:14310-14322.
31. Shukla YN, Tandon JS, Bhakuni DS, Dhar MM. Chemical constituent of the Antibiotic fraction of *Arnebia nobilis*. Experentia. 1969;25:357.
32. Singh B, Singh S. Antimicrobial activity of Terpenoids from *Trichodesma amplexicaule* Roth. Phytother. 2003;17:814.
33. Plyta ZF, Li T, Papageorgion VP, Mellidis AS, Assimopoulo AN, Pitsinos AN, Couladouros EA. Inhibition of Topoisomerase I by naphthaquinone derivatives. Biorg Med Chem Lett. 1998;8:3385.
34. Ahmad B, Ali N, Bashir S, Choudhary MI, Azam S, Kan I. Parasiticidal, antifungal and antibacterial activities of *Onosma griffithii* Vatke. African J Biotechnol. 2009;8(19):5084-5087.
35. Ramakrishnan G, Kothai R, Jaykar B, Rathnakumar TV. In vitro Antibacterial activity of different extracts of leaves of *Colendia procumbens*. International Journal of PharmTech Research. 2011;3(2):1000-1004.
36. Bahraminejad S. In vitro and In vivo antifungal activities of Iranian plant species against *Pythium aphanidermatum*. Ann Biol Res. 2012;3(5):2134-2143.