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Pharmacological evaluation of *Ziziphus nummularia* leaves for phytotoxic and molluscicidal bioassays

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Medicinal plants have curative properties due to the presence of various complex chemical substances of different composition which contain secondary metabolites. In the present study, the phytochemical screening of *Ziziphus nummularia* leaves were carried out to investigate their phytoconstituents like alkaloids, carbohydrates, protein, saponin, tannins, fixed oil, fats, volatile oil, glycosides, phenol and flavonoids. The phytotoxic and molluscicidal potential of *Z. nummularia* leaves were evaluated which exhibit that both ethanolic and n-hexane extracts were highly molluscicidal and good phytopromotors. The results indicate that *Z. nummularia* have growth promoting capability and can be used in fertilizer industries.

Key words: Phytochemical screening test, phytotoxic, molluscicidal, *Ziziphus nummularia.*

INTRODUCTION

Pharmacognosy is the study of chemical, physical and biochemical properties of natural drugs, their potential, drug substances, origin and the discovery of new drugs from natural sources (Tyler, 1999). The study of multi-cellular and unicellular organism including fungi, bacteria, aquatic organisms, aquatic plants, terrestrial plants and animals, all comes under pharmacognosy preview.

Pharmacognosy helps us to know the past history, collection, cultivation, drying, preservation, storage, commerce and uses of drugs obtained from biological source (Evans, 2002). Morph-histological study is the preliminary step towards identification and standardization of plants and plants derived drugs (Younken, 1950).

Ziziphus nummularia (Family Rhamnaceae) commonly called Malla or Jher beri (Hindi) is used for its fruits. The fruit is cooling, astringent, appetizer, stomachic, cures mucous and increase biliousness effect (Oudhia, 2003). The bark has nematicidal, antihelminthic, antipyretic and and anti-inflammatory properties but showed no spermatotoxicity (Bachayaa et al., 2009). The leaf paste is used in boils and skin diseases like scabies etc. (Singh et al., 2002). Herbicides and weedicides are used for the effective control of weeds in order to increase crop yield (Kim, 1994; Santos, 2009). But sometimes the misuse and overuse of these synthetic weedicides may create some problems like resistance against the pesticides, water, soil and air pollutions (Judith et al., 2001). Due to these harmful effects, it is needed to reduce the use of synthetic weedicides or herbicides instead of using natural herbicides to overcome these problems.

Schistosomiasis and *Fascioliasis* are two prevalent diseases that infect about 207 million people worldwide (King and Cha, 2008). The best method for the control of these diseases is the parasites life cycle breakage(Mello-Silva et al., 2006; Jigyasu and Sing, 2010). Snails are the intermediate host for the transmission of these diseases.

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By the control of snails it is possible to reduce and eliminate these diseases (Giovanelli et al., 2001; Mello-Silva et al., 2006). In the present study, the phytotoxic or weedicidal and mulluscicidal potential of *Z. nummularia* was evaluated.

MATERIALS AND METHODS

Plant

Healthy and fresh leaves of *Z. nummularia* were collected from Peshawar University Campus, Pakistan. The collected leaves were washed with tap water to remove the dust particles. The leaves were shade dried for 10 to 15 days then powdered by electric grinder. The plant specimen with voucher number Bot. 20033 (PUP) was deposited in herbarium of Botany Department University of Peshawar, Pakistan.

Preparation of extracts

Two hundred grams of powder was soaked in absolute ethanol and n-hexane solvent for 72 h and then filtered through Whatman filter paper No. 1823. The process was repeated three times. The filtrate evaporated through rotary evaporator to get the extracts and preserved in refrigerator at 4°C for phytochemical screening analysis and pharmacological bioassays.

Phytochemical screening test

The ethanolic and n-hexane extracts were subjected to different qualitative chemical tests to find out the presence of different phytoconstituents that is, alkaloids, carbohydrate, phenolics and tannins, fats, fixed oils, proteins and amino acids, flavonoids, saponins by following detection methods.

Detection of carbohydrates, protein, alkaloids, phenol, tannins, saponins, fats and oil and flavonoids

Same volume of Fehling (copper sulphate in distilled water) and Fehling B (potassium tartarate and sodium hydroxide in distilled water) were added with few drops of sample solution of each extract and then boiled. The appearances of brick red precipitate of cuprous oxide confirm the presence of reducing sugar (Trease and Evens, 2002).

Extract solution of each sample was taken in test tube and 0.2% of Ninhyrin solution added in it and then boiled. The appearances of violet colour indicate the presence of protein otherwise not (Kumar and kiladi, 2009).

For the detection of alkaloids, few drops of Hager reagent were added in sample of each extract. The formation of yellow precipitate confirms the presence of alkaloids (Khandelwal, 2004).

2 ml of ferric chloride solution was taken in a test tube to which 2 ml of extract solution was added. The bluish green colour of the solution indicates the presence of phenol otherwise none (Dahiru et al., 2006).

To an extract solution, sodium hydroxide was added. Tannins were detected by the appearance of yellow to red precipitate (Kokate, 1994).

In a test tube, 5 ml of an extract solution was taken and then vigorously shaken. On surface of solution, the froth formation indicates the presence of saponins (Chaouche et al., 2011).

Powder drug of each sample were individually pressed between two filter papers. Existence of oily spots on filter paper shows the presence of fixed oil (Gomathi, 2010).

Few drops of sodium hydroxide were added with extract solution of each sample. The appearance of yellow to red precipitate confirms the presence of flavonoids (Kokate, 1994).

Phytotoxic activity

Phytotoxic activity of the extracts were carried out against *Lemna minor* following the standard procedure of Atta-ur-Rehman et al. (2001).

Materials used were *Lemna minor* fronds, extracts flask 250 ml, distilled water, micropipette (10 to 100 µl, 100 to 1000 µl), filter paper, glass vials, laminar flow hood, brush, oven etc.

The medium was prepared in distilled water and autoclaved at 1210°C for about 20 min, and by adding KOH pellets the pH was adjusted to 5.4 to 5.5. Stock solutions were prepared by dissolving 10 mg extracts in 40 ml ethanol and n-hexane. Then three different concentrations that is, 10, 100 and 1000 µ/ml were prepared from stock solutions by taking 5, 50 and 500 µl from stock solutions, respectively. The solvents were allowed to evaporate. In each Petri dish, 20 ml of medium were separately added and 10 plants of *L. minor* each with 2 or 3 fronds were added in each Petri dish. Methanol and paraquate were used as positive and negative controls, respectively. For seven days the Petri dishes were placed in growth chamber at 28°C. On day seven, the numbers of fronds in each Petri dish were counted. Percent growth promotion in both extracts was calculated by using the following formula:

$$\%\ \text{Regulation} = 100 - \frac{\text{Number of fronds in test samlpe}}{\text{Number of fronds in possitive control}} \times 100$$

Molluscicidal activity

The molluscicidal activity of ethanol and n-hexane leaves extracts of *Z. nummularia* was carried out by following the procedure of Atta Ur Rahman et al. (2001).

The materials used were *vLymnea acuminate,* extracts, distilled water, micropipettes (10, 100 and 1000 µl), Petri dishes, pinching needle, beakers (500 ml).

Aquatic snails (*L. acuminate*) were collected from different fresh water bodies of Peshawar, Pakistan. The snails were kept in de chlorinated tap water aquaria with 25±2°C. The same size snails were chosen for the activity. 10, 100 and 1000 ppm concentrations of crude extracts of leaves were prepared. Each concentration was replicated three times of both extract. snails were placed in each concentration and covered the flask with perforated aluminum foil to allow air circulation in flask. After 24 h all the snails were brought out from the test solution. The mortality of the snails were evaluated by punching needle and counted as dead when they did not show any movement or action. The control series contained only distilled water. With reference to positive control, the percent mortality of both extracts were calculated by the following formula:

$$\text{Percentage mortality} = 100 - \frac{\text{No of snails alive in test}}{\text{No of snails alive in control}} \times 100$$

Table 1. Phytochemical study of *Ziziphus nummularia.*

Constituent	Test name	Ethanolic extact	n-Hexan extract
Carbohydrates	Fehling's test	+	+
Protein	Ninhydrine	+	+
Alkaloids	Hagers test	+	+
Phenol	Ferric chloride test	+	+
Flavonoids	Alkali test	+	+
Tannins	Alkali test	+	+
Saponin	Frothing test	+	+
Glycosides	Killaer killini	-	-
Fixed oil and fats	Spot test	-	+
Volatile oil	Spot test	-	+

+ = present and - = absent.

Table 2. *Lemna* Phytotoxicity of *Ziziphus nummularia* leaves extracts.

Extracts used	Concentration (µg/ml)	No. of fronds		% growth promotion
		Initial reading	After 7 days	
Ethanol	1000	30	36	20
	100	30	34	13
	10	30	33	10
	0 (-ve control)	30	32	6
n-hexane	1000	30	39	30
	100	30	37	23
	10	30	34	13
	0 (-ve control)	30	33	10

RESULTS AND DISCUSSION

Phytochemical screening test

In the present study, the ethanol and n-hexane extracts of *Z. nummularia* leaves were screened for phytochemical analysis. Both the extracts showed the same result for carbohydrates, protein, alkaloids, phenol, flavonoids, tannins, saponins and glycosides, while fixed oil, fats and volatile oil were present in n-hexane and absent in ethanol extract. The results are presented in Table 1.

Phytotoxicity bioassay

Herbicides and weedicides are used for the effective control of weeds in order to increase crop yield (Kim, 1994; Santos, 2009). Due to their harmful effects, it is needed to reduce the use of synthetic weedicides or herbicides instead of using natural herbicides to overcome these problems. In the present study, the ethanolic and n-hexane extracts of *Z. nummularia* leaves were tested for their phytoxocicity against *L. minor*. The results indicated that both the extracts posses growth promotion potential. At 1000 µg/ml both ethanol and n-hexane extracts exhibited high growth promotion 20 and 30%, respectively, while at 100 µg/ml, both the extracts showed 13 and 23% growth regulation. Both the ethanol and n-hexane at concentration10 µg/ml revealed 10 and 13% growth regulation, respectively. Ali et al. (2009) *Euphorbia wallichii*; Khan et al. (2011) *Euphorbia prostrate* and Bashir et al. (2011) studied ethyl acetate, methanolic extracts and various other fractions of *Ziziphus jujuba* against *L. minor* and reported that *Z. Jujuba* has growth regulating potential. So our results are strongly supported by their research findings. Our results as shown in Table 2 indicate that as compared to control, the ethanolic and n-hexane extracts of *Z. nummularia* leaves are good growth promoter and can be used as a fertilizer for growth regulation.

Table 3. Molluscicidal activity of *Ziziphus nummularia* leaf.

Part	Extract conc. (mg/ml)	Total No. of individuals	No. of dead snails	% mortality	LD_{50}	95% CL		Least square line	X^2 (p)
						LCL	UCL		
				Ethanol					
Leaf	control	25	0	0	3.7674	6.136	13.67	Y= 3.19+1.12X	0
	5	25	13	52					
	10	25	15	60					
	15	25	19	76					
				n-Hexane					
	control	25	0	0	6.550	15.71	2.613	Y= 3.91+0.94X	0.037
	5	25	16	64					
	10	25	19	76					
	15	25	22	88					

Molluscicidal bioassay

Snails are the intermediate host for the transmission of *Fascioliasis* disease. Control of snails will lead to the reduction and elimination of this disease (Giovanelli et al., 2001). The use of plant derived compounds is safe, inexpensive and easily available for mollusk control (Singh and Singh, 2010).

In the present study, the ethanolic and n-hexane extracts of *Z. nummularia* leaves were evaluated for their molluscicidal potential. The ethanolic extract produced 52% mortality at a concentration of 5 mg/ml and 60% at 10 mg/ml while significant lethality of 88% at a concentration of 15 mg/ml with LD_{50} value of 3.7674 mg/ml. As compared to ethanolic extract, the n-hexane extracts also exhibited high lethality of 88% at 15 mg/ml and moderate lethality of 76% at 10 mg/ml while poor lethality of 64% at 5 mg/ml with LD_{50} value of 6.550 mg/ml. Many researchers studied different plants for molluscicidal potential like Sharma et al. (2009) who have studied the effect of ethanolic extracts of *Ricinus communis* and *Acalypha indica* against *L. acuminate* snails. Hassan et al. (2011) *Enterolobium contorisiliquum* and Jaiswal et al. (2009) studied the effect of nutmeg and mace of *Myristica fragrance* against vector snail *L. acuminate.* The results displayed in Table 3 indicate that both the extracts are highly molluscicidal but n-hexane extract of *Z. nummularia* showed good results for the control of snails and further can be used for the breaking of *Fascioliasis* disease cycle.

REFERENCES

Ali I, Naz R, Khan WN, Gul R, Choudhary MI (2009). Biological screening of different root Extracts of *euphorbia wallichii*. Pak. J. Bot. 41(4) :1737-1741.

Atta-ur-Rhman, Choudhary MI, Thomsen WJ (2001). Bioassay Technique for Drug Development Harwood Academic Publishers. The Netherlands.

Bachayaa HA, Zafar I, Nisar KM, Zia-ud-Din, Abdul J (2009). Anthelmintic activity of *Ziziphus nummularia* (bark) and *Acacia nilotica* (fruit) against Trichostrongylid nematodes of sheep. J. Ethnopharmacol. 123(2):325–329.

Bashir B, Khan I, Bashir S, Azam S, Hussain F (2011). Screening of *Zizyphus jujuba* for antibacterial, phytotoxic and haemagglutination activities. Afr. J. Biotechnol. 10(13):2514-2519.

Chaouche T, Haddouchi F, Bekkara FA (2011). Phytochemical study of root and leaves of the plant *Echium pycmanthum* Pomel. Der Pharm. Lett. 3(2):1-4.

Evans WC (2002). Pharmacognosy, 15th ed. English Language Book, Society Baillere Tindall: Oxford University Press.

Giovanelli A, Silva CL, Medeiros L, Vasconcellos MC (2001). The molluscicidal activity of the latex of *Euphorbia splendens* var. *hisloppii* on *Melanoides tuberculata* (Thiaridae), a snail associated with habitats of *Biomphalaria glabrata* (Planorbidae). Mem. Inst. Oswaldo Cruz. 96:123-125.

Gomathi V, Jayakar B, Kothai R, Ramakrishnan G (2010). Antidiabetic activity of leaves of *Spinacia oleracea* Linn. in Alloxan induced diabetic rats. J. Chem. Pharmaceut. Res. 2(4):266-274.

Hassan AA, Mahmoud AE, Hassan RA, Huseein AM (2011). Evaluation of *Euphorbia aphylla*, *Ziziphus spina-christi* and *Enterolobium contortisiliquum* as Molluscicidal Agents. J. Amer. Sci. 7(8):511-520.

Jaiswal P, Singh DK (2009). Molluscicidal Activity of Nutmeg and Mace (*Myristica fragrans* Houtt.) Against the Vector Snail *Lymnaea Acuminata.* J. Herbs Spices Med. Plants 15:177–186.

Jigyasu HV, Sing VK (2010). Effect of environmental factors on the fecundity, hatchability and survival of snail *Lymnaea* (Radix) *acuminate* (Lamarck): vector of fascioliasis. J. Water Health 8: 109-115.

Judith CS, Lemley AT, Hogan SI, Weismiller RA, Hornsby AG (2001). Health Effects of Drinking Water Contaminants. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Available online:http://edis.ifas.ufl.edu/SS299.

Kamboj A, Saluja A (2010). Microscopical and preliminary phytochemical studies on aerial part (leaves and stem) of *Bryophyllum pinnatum* Kurz. Pharmacogn. J. 2(9):254-259.

Khan RA, Shah AS, Ahmad M, Khan FU, Aslam N, Khan MR, Shah MS (2011). Phytotoxic activity of crude methanolic extract of *Euphorbia prostrata* collected from Bannu District (Pakistan). Afr. J.

Biotechnol. 11(10):2513-2517.
Khandelwal KR (2004). Practical Pharmacognosy, Techniques and experiments (12th Edn). Nirali Prakashan, Pune India. P 157.
Kim KU (1994). Integrated management of paddy weeds in Korea with an emphasis on allelopathy. In: Bay-Peterson J. (ed.), Integrated Management of Paddy and Aquatic Weeds in Asia. pp. 144–159.
King CH, Cha MD (2008). The unacknowledged impact of chronic schistosomiasis. Chronic Illn. 4:65–79.
Kokate CK (1994). Practical Pharmacognosy. 4th Edn. Vallabh Prakashan: New Delhi. pp. 120-124.
Kumar BJR, Kiladi CP (2009). Preliminary Phytochemical and Pharmacognostic Studies of *Holoptelea integrifolia* Roxb. Ethnobot. Leafl. 13:1222-1231.
Mello SCC, Vasconcellos MC, Pinheiro J, Rodrigus MLA (2006). Physiological changes in *Biomphalaria glabrata* (Say), (Pulonata: Planorbidae) caused by sublethal concentrations of the latex of *Euphorbia splendens var. hisloppii* N. E. B. (Euphorbiaceae). Mem. Inst. Oswaldo Cruz. 10(1):3-8.
Oudhia P (2003). Research note on medicinal herb of Chattisgarh, India having less known traditional uses, IX. Boir (*Ziziphus nummularia*, family: Rhamnaceae).
Santos BM (2009). Drip-applied metam potassium and herbicides as methyl bromide alternatives for *Cyperus* control in tomato. Crop Prot. 28: 68–71.
Sharma S, Singh T, Vijayvergia R (2009). Molluscicidal activity of some medicinal plants. J. Herbal Med. Toxicol. 3(2):155-157.
Sing SK, Sing A (2010). Metabolic changes in freshwater harmful snail *Lymnaea acuminata* due to aqueous extract of bark and leaf of *Euphorbia pulcherima* plant. Am. Eurasian J. Toxicol. Sci. 2(1):13-19.
Singh AK, Raghubanshi AS, Singh JS (2002). Medical ethnobotany of the tribals of Sonaghati of Sonbhadra district, Uttar Pradesh, India. J. Ethnopharmacol. 81(1):31–41.
Trease GE, Evans WC (2002). Pharmacognosy. 15th edition. English Language Book, Society Baillere Tindall, Oxford University Press. pp. 17, 417- 547.
Tyler VE (1999). Phytomedicines: back to the future. J. Nat. Prod. 62: 1589-1592.
Younken HW (1950). A Text Book of Pharmacognosy. The Blackiston Co Toronto, Philadelphia. pp. 1675 – 1676.