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Evaluation of Phytotoxic Activity of Sea Buckthorn (*Hipphophae rhamnoides* Linn) Leaves Extracts

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Abstracts: The present study describes the phytotoxic activity of the leaves crude extracts of *Hipphophae rhamnoides*. The crude extracts were aqueous, methanol, ethanol, chloroform, acetone, ethyl acetate and*n*-hexane, which were subjected to *lemna minor* inhibition assays to explore the phytotoxic potential of this medicinally important herb. The results indicated that the crude extracts leaves showed varied degree of phytotoxic activity. It was observed that *Hipphophae rhamnoides* exhibited dose dependent toxicity towards *Lemna minor*, with no toxicity at 05µg/ml, low toxicity at 100µg/ml and moderate activity at 1000µg/ml. The LD_{50} (µg/ml) values of aqueous (2751.26), ethanol (1356.12), acetone (1619.55), methanol (1344.79), chloroform (15069.88) and ethyl acetate (2025.26) were recorded.

Key words: Leman Minor · Allelopathy · Solvent Extracts · Sea buckthorn Leaves · LD_{50}

INTRODUCTION

Hippophae rhamnoides Linn (*H. rhamnoides* L.) commonly known as Sea buckthorn is a medicinal plant, grown on in large areas of Asia and Europe. Its different components have been long utilized for the cure of many diseases including pain, rheumatoid arthritis and colds. It has possessed many types of active compounds, which have latent applications in human health. The seeds, leaves and fruits hold high amounts of useful substances such as flavonoids, carotenoids, phenolic compounds and vitamins with antibacterial, antifungal and free radical scavenging activities [1]. Iron, magnesium, calcium, sodium, potassium, phosphorus and silver were found in Sea buckthorn seeds [2]. The twigs of *H. rhamnoides* methanol and chloroform: methanol extracts have reported [3] the presence of glycoside, terpenoids, steroids, flavonoids, reducing sugar and tannins, while alkaloids, coumarins and saponins were absent. It has been investigated scientifically that *H. rhamnoides* extracts from berries, oil and leaf have been found various pharmacological activities such as anti-inflammation, immune-modulation, radio protective and tissue regeneration [4].

The *H. rhamnoides* pomace extracts reducing power were rises as the concentration of the extracts increases and maximum was 70% in methanolic extract [5]. In plant kingdom allelopathy is an essential ecological factor as well as phytotoxic in medicinal plants. It has been reported that flavonoids work as allelochemicals [6]. The current research work was designed to evaluate the phytotoxic activities of *Hippophae rhamnoides L.* leaves extracts.

MATERIAL AND METHODS

Collection of *H. Rhamnoides* L. Leaves: The fully matured healthy leaves of *H. rhamnoides* L. were collected from Pakistan Council of Scientific and Industrial Research (PCSIR) Skardu Gilgit Baltistan, Pakistan. The leaves were slightly washed to remove any dust, shade dried and powdered with a laboratory mill. The crushed leaves were kept in an air-tight plastic bag till used.

The voucher specimen was deposited in Department of Botany, University of Peshawar, Khyber Pakhtunkhwa- Pakistan, with herbarium number Bot.20006 (PUP).

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H. Rhamnoides L. Leaves Extraction: Fifty grams powder of *H. rhamnoides* L. leaves was extracted in 250 ml of water, ethanol, acetone, methanol, ethyl acetate, chloroform and *n*-hexane for 48 hrs. These extracts were afterward filtered under vacuum in the course of No.1 Whatman filter paper into a Buchner flask. The extracts were concentrated in rotary evaporator and transferred in a sterilized beaker for heating on water bath at 50°C to obtain dried residue. The resultant crude extract was transferred into airtight sample bottles and kept at 4°C until used.

Phytotoxic Assay of *H. Rhamnoides* L. Leaves Extracts: *H. rhamnoides* L. leaves extracts were subjected for its phytotoxic activity against the *Lemna minor* [7]. Each extract (15 mg) was dissolve in 15 ml DMSO, 10, 100 and 1000 µl solution were added to vials to make 5, 50 and 500 ppm solution. Evaporate all solvent overnight. Then add 2 ml E- medium (PCSIR Peshawar) and a single plant to each vial (10 vials/dose), sterile E medium was used as a negative and Paraquat (PCSIR Peshawar) as positive control. Then vials were placed in the growth chamber (27-29ºC) for 7 days and were checked on daily basis. After incubation fronds per vials were counted and the growth regulation (in percent) was measured with reference to negative controls.

$$\%\text{Growth Regulation} = 100 - \frac{\text{Number of fronds in sample (test)}}{\text{Number of fronds in control (negative)}} - \text{X } 100$$

Statistical Analysis: All experiments were carried out in triplicate. Values are presented as mean ± SD (n = 3). The LD_{50} values were determined by using computer program SPSS.

RESULTS

Phytotoxic Activity of *H. Rhamnoides* L. Leaves Extracts: The phytotoxicity results (Figs. 1 and 2) indicated that all extracts obtained from *H. rhamnoides* L. leaves did not cause any phytotoxicity at the concentration of 5 µg/ml. The methanol extract showed growth regulation of 33.33% at 500 µg/ml and 20.00% at 50 µg/ml, while LD_{50} was 1344.79 µg/ml. The percent growth regulation of ethanol extract was 33.33 for 500 µg/ml concentration and 16.67 at 50 µg/ml and aqueous extract was 26.76% and 13.33% at 500 and 50 µg/ml respectively. The acetone extract %GR at 500 µg/ml was 30.00 and at 50 µg/ml was 10.00. The chloroform and ethyl acetate percent GR at 500 µg/ml

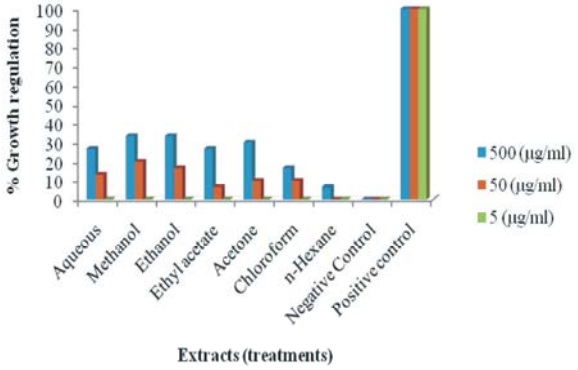

Fig. 1: Phytotoxic Activity of *H. rhamnoides* L. Leaves Extracts against *L. minor*

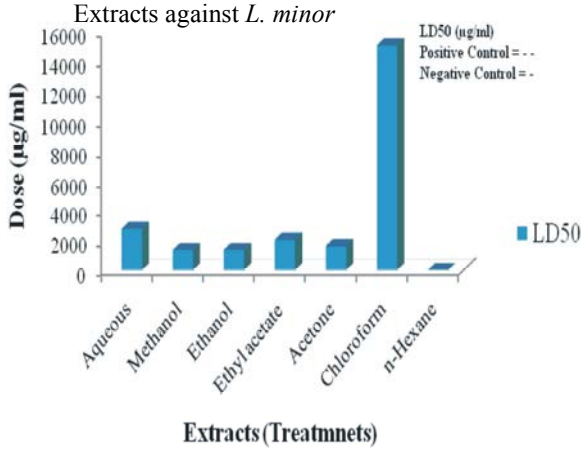

Fig. 2: LD_{50} of *H. rhamnoides* L. Leaves Extracts against *L. minor*

was 16.67 and 26.76 and at 50 µg/ml was 10.00 and 6.67 respectively. The LD_{50} (µg/ml) values of aqueous, ethanol and acetone were 2751.26, 1356.12 and 1619.55 respectively. The LD_{50} values 15069.88 µg/ml and 2025.26 µg/ml were calculated for chloroform and ethyl acetate respectively.

DISCUSSION

The variations in the inhibition of *Lemna* by plants might be due to solvent and plant material differences. Allelopathic effects are related to plants, their parts and extraction procedures [8]. Glycosides and terpenoids have exhibit phytotoxicity [9]. Several researchers reported that amino acids have allelopathic effect against plants [10, 11]. Allelochemicals inhibit electron transport chain in mitochondria [12]. Phenolic compounds and its derivatives were latent inhibitors of seedling growth and germination and have allelopathic applications in forestry

and agriculture as herbicides [13]. The active constituents detected in the *H. rhamnoides* L. leaves extracts could be accountable for the inhibition of *Lemna minor* in the current study. Phytotoxins [14] affect enzymatic activity, ion uptake, cell division, membrane permeability, electron transport in respiratory chains and photosynthesis. The presence of polyphenolic compounds and tannins are lethal and simply enter into the host cells leading to fatality of host tissues. The phenolic compounds can arbitrate hazardous connections indirectly or directly by connecting autotrophs toward each other and to herbivores [15]. Phytochemicals investigation showed that tannins, phenols and flavonoids were present in large quantity in *Hipphophae rhamnoides* Linn Leaves Extracts [16]. The leaves extracts of Sea buckthorn were possessed larvacidal activity against Aedes *aegypti* and *Anopheles stephensi* [17]. The Phytochemicals present in the aqueous extracts of Sea buckthorn leaves were the ability to synthesized silver nanoparticles [18].

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REFERENCES

1. Jong, H.J., W.L. Ji, S.K. Kyoung, K. Ju-Sung, N.H. Sang, Y.Y. Chang, K.L. Ju, S.K. Yong and J.K. Myong, 2010. Antioxidant and antimicrobial activities of extracts from a medicinal plant, Sea Buckthorn. Journal of Korean Society for Applied Biological Chemistry, 53(1): 33-38.
2. Alam, Z. and M. Ijaz, 2009. Biochemical characterization of sea buckthorn (*Hippophae rhamnoides* L. *spp. turkestanica*) seed. African Journal of Biotechnology, 8(8): 1625-1629.
3. Keshab, B., M.S. Tirtha, B. Ramita, S.C. Jain and L. Janardan, 2010. Biological activities of three different medicinal plants from Himalayan Region of Nepal. Nepal Journal of Science and Technology, 11: 139-146.
4. Gill, N.S., R. Sharma, R. Arora and M. Bali, 2012. Antioxidant and antibacterial activity of *Hippophae rhamnoides* methanolic leaf extracts from dry temperate agro-climate region of Himachal Pradesh. Journal of Plant Sciences, DOI: 10.3923.
5. Geetha, S. and G. Asheesh, 2011. Medicinal and therapeutic potential of Sea buckthorn (*Hippophae rhamnoides L.*). Journal of Ethnopharmacology, 138: 268-278.
6. Samanta, A., G. Das and S.K. Das, 2011. Roles of flavonoids in plants. International Journal of Pharmaceutical Science and Technology, 6(1): 12-35.
7. McLaughlin, J.L. and L.L. Rogers, 1998. The use of biological assays to evaluate botanicals. Drug Information Journal, 32: 513-524.
8. Begum and F. Hussain, 1980. Allelopathic effects of *Panicum antidotale.* Pakistan Journal of Scientific and Industrial Research, 23(5): 182-188.
9. Ghulam, D. and H. Farrukh, 2013. Phytotoxic and Insecticidal Activity of Plants of Family Zygophyllaceae and Euphorbiaceae. Sarhad Journal of Agriculture, 29(1): 83-91.
10. Uma, B., K. Prabhakar, S. Rajendran and Y.L. Sarayu, 2009. Antimicrobial activity of *Albizzia lebbeck* Benth against infectious diarrhoea. The Internet Journal of Microbiology, 7(1): 23-26.
11. Williams, R.D. and R.E. Hoagland, 2007. Phytotoxicity of mimosine and albizziine on seed germination and seedling growth of crops and weeds. Allelopathy Journal, 19(2): 423-430.
12. Kapoor, R.T., 2011. Bioherbicidal potential of leaf-residue of *Hyptis suaveolens* on the growth and physiological parameters of *Parthenium hysterophorus* L. Current Research Journal of Biological Sciences, 3(4): 341-350.
13. Li, Z.H., Q. Wang, X. Ruan, C.D. Pan and D.A. Jiang, 2010. Phenolics and plant allelopathy. Molecule, 15: 8933-8952.
14. Romero-Romero, T., A.L. Anaya and R.C. Ortega, 2002. Screening for effects of phytochemical variability on cytoplasmic protein synthesis pattern of crop plants. Journal of Chemical Ecology, 28(3): 617-629.
15. Waterman, P.G. and S. Mole, 1994. Analysis of Phenolic Plant Metabolites. Oxford University Publications, Oxford, pp: 251.
16. Bashir, A. and A. Javid, 2013. Physiochemical, minerals, phytochemical contents, antimicrobial activities evaluation and fourier transform infrared (FTIR) analysis of *Hippophae rhamnoides L.* leaves extracts. African Journal of Pharmacy and Pharmacology, 7(7): 75-388.

17. Bashir, A and A. Javid, 2013. Evaluation of Larvicidal Activity of *Hippophae rhamnoides* L. Leaves Extracts on *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). Middle-East Journal of Scientific Research, 13(5): 703-709.

18. Bashir, A., A. Javid and B. Shumaila, 2013. Optimization and Effects of Different Reaction Conditions for the Bioinspired Synthesis of Silver Nanoparticles Using *Hippophae rhamnoides* Linn. Leaves Aqueous Extract. World Applied Sciences Journal, 22(6): 836-843.