Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Document heading doi: 10.12980/JCLM.3.201514J100

Brine shrimp cytotoxic activities of *Hippophae rhamnoides* Linn leaves extracts

Javid Ali[1,2*], Bashir Ahmad[1]

[1]*Center of Biotechnology and Microbiology, University of Peshawar, KPK- Pakistan*

[2]*PCSIR Laboratories, Complex Jamrud Road, Peshawar, KPK- Pakistan*

PEER REVIEW

Peer reviewer

Dr. Abdul Latif Khan, Research Assistant Professor, UoN Chair of Oman's Medicinal Plants and Marine Natural Products, University of Nizwa, Sultanate of Oman.

Comments

This is a valuable research work in which authors have demonstrated the antitumor and antiproliferative activities of *H. rhamnoides* leaves extracts by brine shrimp cytotoxicity.

Details on Page 324

ABSTRACT

Objective: To evaluate brine shrimp lethality assay of solvent extracts (aqueous, methanol, ethanol, acetone, ethyl acetate, chloroform and *n*-hexane) of *Hippophae rhamnoides* (*H. rhamnoides*) leaves.

Methods: Brine shrimp cytotoxicity assay was used to assess the cytotoxic potential of *H. rhamnoides* leaves extracts. Three vials for concentration of each extract were made and 10 shrimps per vial (30 shrimps per dilution) were transferred to specific concentration of each extract.

Results: The mortality of aqueous extract was 46.7%, methanol extract was 46.7%, ethanolic extract was 50.0%, ethyl acetate was 26.7%, acetone extract was 33.3%, chloroform extract was 40.0% and *n*-hexane extract was 33.3%. The lowest LD_{50} was found in methanol extracts (1 199.97 μg/mL). Brine shrimp cytotoxicity of tested extracts of *H. rhamnoides* showed that mortality rate was concentration dependent.

Conclusions: It is concluded that bioactive components are present in all leaves extracts of *H. rhamnoides*, which could be accounted for its pharmacological effects. Thus, the results support the uses of this plant species in traditional medicine.

KEYWORDS

Sea buckthorn leaves, Solvent extracts, Brine shrimp, Diseases, Mortality

1. Introduction

According to World Health Organization, more than eighty percent of the world's inhabitants rely on traditional medicines for numerous kinds of disorders. The plants' remedial properties are potent in various components that give a precise biological function in the individual body. The main vital components of these biochemical plants are tannins, flavonoids, alkaloids and phenolic compounds[1].

Hippophae rhamnoides Linn (*H. rhamnoides*) commonly known as sea buckthorn is a medicinal plant, grown in large areas of Asia and Europe. Its different components have been

*Corresponding author: Javid Ali, Center of Biotechnology and Microbiology, University of Peshawar, KPK- Pakistan.

Tel: +92-091-9216244

Fax: +92-091-9216232

E-mail: Javedali_14@yahoo.com

Article history:

Received 24 Dec 2014

Received in revised form 27 Dec, 2nd revised form 30 Dec 2014

Accepted 12 Jan 2015

Available online 6 Mar 2015

long utilized for the cure of many diseases including pain, rheumatoid arthritis and colds[2]. *Hippophae* leaves contain folic acid, catechins, esterified sterols, triterpenols and isoprenols[3]. The sea buckthorn berries are rich source of flavonoids, vitamins, carotenoids and sterols, which have great therapeutic values[4]. Generally, the plant bioactive compounds have potent antifungal, antibacterial and antioxidant activities[5]. The study documented that glycoside, terpenoids, steroids, flavonoids, reducing sugars and tannins were present in *H. rhamnoides* (twigs)[6]. The methanol extract of leaves, seed and pomace of sea buckthorn were reported to have total phenolic content (mg GAE/g extract) (278.80, 162.56 and 107.01 respectively)[7]. Similarly, aqueous extract exhibited phenolic content (mg GAE/g extract) (184.89, 109.57 and 87.35 respectively). Seed oils, fresh juice, syrup and berry are used for fever, colds, cancer, stomach ulcers and metabolic disorders treatments[8].

Many health problems especially heart attack, cancer, AIDS, hepatitis, skin and gastrointestinal diseases are proliferated rapidly in the whole world particularly in undeveloped countries and their treatment by medicine (synthetic) is unaffordable by poor people. To utilize the active secondary metabolites from plants, *Hippophae* is a natural therapy for curing these health problems. The current research work was designed to evaluate the cytotoxic activities of *H. rhamnoides* leaves extracts.

2. Materials and methods

2.1. Collection of H. rhamnoides leaves

The fully matured healthy leaves of *H. rhamnoides* were collected from Pakistan Council of Scientific and Industrial Research, 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).

2.2. H. rhamnoides leaves extraction

Fifty grams of *H. rhamnoides* leaves powder was extracted in 250 mL of water, ethanol, acetone, methanol, ethyl acetate, chloroform and *n*-hexane for 48 h. These extracts were then filtered under vacuum in the course of 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.

2.3. Brine shrimp cytotoxicity assay of H. rhamnoides leaves extracts

Cytotoxicity of *H. rhamnoides* leaves extracts was determined through brine shrimp (*Artemia salina*) assay[9]. In this method, eggs of 50 mg were used to hatch larvae during incubation in artificial seawater for 48-72 h. A total of 20 mg of each extract was dissolve in 2 mL dimethyl sulfoxide to make stock solution. From this stock solution, 500, 50 and 5 μL were transferred to vials corresponding to 1 000, 100 and 10 μg/mL respectively and vials were to evaporate dimethyl sulfoxide overnight. Three vials for each concentration of each extract were made and 10 shrimps per vial (30 shrimps per dilution) were transferred to each concentration before 5 mL/vial addition of sea water. Cytotoxic drug (etoposide) served as positive control and sea water was regarded as negative control. The brine shrimps were observed for its mortality after 24 h. The % mortality of brine shrimp was calculated as shown below.

$$\%\text{Mortality} = \frac{\text{Number of died or immobile brine shrimp}}{\text{Total number of brine shrimps}} \times 100$$

2.4. Statistical analysis

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

3. Results

Cytotoxic effects (% mortality) of the *H. rhamnoides* extracts were summarized as follows. Brine shrimp cytotoxicity of tested extracts of *H. rhamnoides* showed that mortality rate was concentration dependent. The mortality was 46.7%, 16.7% and 6.7% at 1 000, 100 and 10 μg/mL respectively in aqueous extract. The methanol extract percent mortality was 46.7, 26.7 and 6.7 at 1 000, 100 and 10 μg/mL. The ethanolic extract at 1 000, 100 and 10 μg/mL showed % mortality of 50.0, 26.7 and 16.7 respectively. No mortality was found at 10 μg/mL in ethyl acetate and acetone extract, but these extracts showed 6.7 percent mortality at 100 μg/mL. While 26.7 and 33.3 mortality (%) were calculated at 1 000 μg/mL for ethyl acetate and acetone respectively. Chloroform and *n*-hexane extracts exhibited 6.7% mortality at 10 μg/mL. At 1 000 and 100 μg/mL, chloroform extract mortality were 40.0%

and 23.3% and *n*-hexane extract mortality were 33.3% and 16.7% respectively. No mortality was recorded for negative control and 100% mortality was noted in positive control.

The LD_{50} values of *H. rhamnoides* leaves extracts were shown below. The LD_{50} of methanol extracts was 1 199.97 μg/mL, while ethanolic extract's LD_{50} was 1 206.91 μg/mL. The *n*-hexane extract had the highest LD_{50} value of 6 146.53 μg/mL. The other LD_{50} values for aqueous, ethyl acetate, chloroform and acetone were 1 515.09 μg/mL, 4 050.53 μg/mL, 2 198.80 μg/mL and 2 282.31 μg/mL respectively.

4. Discussion

Cytotoxic activity was the initial footstep in exploration for development of anti-cancer drug. The brine shrimp cytotoxic assay has been widely used in the key screening of the isolated compounds as well as crude extracts to assess the toxicity towards brine shrimps, which could also give a sign of possible cytotoxic properties of the test substances[10]. Because this bioassay is an easy and simple protocol. The procedure allows the use of smaller amount of the extracts and allows larger number of dilutions and samples within shorter time than using the original test vials[11]. Furthermore, it has been established that the cytotoxic materials commonly show significant activity in the brine shrimp cytotoxicity assay and this assay can be suggested as a channel for the discovery of pesticidal and anti-tumor compounds because of its low cost and simplicity[12]. Alkaloids are commonly considered as antibacterial, cytotoxic, antimalarial and anticancerous agents[13]. While saponins contain antimicrobial and larvicidal characteristics[14]. Anthraquinones exhibit cytotoxicity and antimicrobial activities, whereas terpenoids have larvicidal and antibacterial activities[15]. Flavonoids have been exposed to cover antioxidant, antiviral, anti-thrombotic, anti-inflammatory, vasodilatory, antibacterial, antiallergic and antineoplastic activities[16]. Tannins have shown potential antioxidant, antibacterial, anticancer and antiviral activities[17]. The preliminary phytochemical analysis of *H. rhamnoides* berries claims the existence of saponins, glycosides, alkaloids, flavonoids, tannins and anthraquinones[18]. Plants containing flavonoids, tannins and sterols may be used for antiviral activity[19]. The LC_{50} of methanolic extract of *H. rhamnoides* (twigs) was 1 584.89 μg/mL and the difference in brine shrimp cytotoxicity results may be due to the variation in the type and quantity of cytotoxic phytochemicals (*e.g.* flavonoids, triterpenoids, tannins, or coumarins) found in the plants crude extracts[7]. *H. rhamnoides* leaves have been found free from heavy metal contamination and no side effect when taken orally[20,21].

The present study documented the potent cytotoxic activity of the *H. rhamnoides* leaves extracts especially ethanol and methanol. The phytochemical constituents such as phenols and flavonoids are the major components which are responsible for the potential cytotoxic activity. As the *H. rhamnoides* leaves are still in use as a traditional herbal medicine, containing a number of useful phytochemicals can provide a scientific base for further primary health care system.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgements

The authors wish to thank Center of Biotechnology and Microbiology (COBAM), University of Peshawar-Pakistan and Higher Education Commission Islamabad-Pakistan for their supports.

Comments

Background

Using medicinal plants against one of the most major health issues, cancer, has been always an area of interest for researchers. Because of the presence of diverse array of chemical constituents, medicinal plants offer a good alternative to search cure for cancer. *H. rhamnoides* (sea buckthorn) is a multipurpose plant holds many bioactive compounds like phenols, flavenoids, carotenoids and proteins *etc*, which have possessed numerous pharmacological activities. The present work describes the potential of this plant for brine shrimp cytotoxicity.

Research frontiers

The present research work described cytotoxic activities of *H. rhamnoides* leaves extracts by brine shrimp cytotoxicity inhibition percentage.

Related reports

Screening of natural products has received the attention of researchers around the world. The folklore medicine has evidence for effectiveness of herb extract in treating various disorders. The study showed cytotoxic activity of methanolic extracts of *H. rhamnoides*. The sea buckthorn juice also revealed the inhibition of prostatic adenocarcinoma (PC-3) and mammary gland

adenocarcinoma (MDA-MB-231) cancer cell proliferation.

Innovations and breakthroughs

H. rhamnoides commonly known as sea buckthorn is a medicinal shrub used in different folk medicines to treat many diseases including pain, rheumatoid arthritis and colds. *H. rhamnoides* leaves extracts could be a strong accepted source of antitumor and has been used in health foods for therapeutic and additive purposes. Further, extracts were found to be used as nutritional supplements.

Applications

From the literature survey, it has been found that *H. rhamnoides* is safe to humans. This scientific study supports and suggests that this plant is commonly used as antitumor and antiproliferative agent. The leaves are utilized to isolate one or more anticancer and antitumor compounds from crude extract and the careful utilization of such compounds can prevent the progression of cancer.

Peer review

This is a valuable research work in which authors have demonstrated the antitumor and antiproliferative activities of *H. rhamnoides* leaves extracts by brine shrimp cytotoxicity.

References

[1] Ahmad B, Ali J. Physiochemical, minerals, phytochemical contents, antimicrobial activities evaluation and fourier transform infrared (FTIR) analysis of *Hippophae rhamnoides* L. leaves extracts. *Afr J Pharm Pharmacol* 2013; **7**(7): 375-388.

[2] Jeong JH, Lee JW, Kim KS, Kim JS, Han SN, Yu CY, et al. Antioxidant and antimicrobial activities of extracts from a medicinal plant, sea buckthorn. *J Korean Soc Appl Biol Chem* 2010; **53**(1): 33-38.

[3] Efterpi C. *Hippophae rhamnoides* L. (sea buckthorn): a potential source of nutraceuticals. *Food Public Health* 2012; **2**(3): 69-72.

[4] Ahmad B, Ali J. Evaluation of larvicidal activity of *Hippophae rhamnoides* L. leaves extracts on *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). *Middle East J Sci Res* 2013; **13**(5): 703-709.

[5] Ali J, Ahmad B. Evaluation of phytotoxic activity of sea buckthorn (*Hippophae rhamnoides* Linn) leaves extracts. *Am-Euras J Toxicol Sci* 2014; **6**(4): 103-106.

[6] Bhattarai K, Shrestha TM, Bajracharya R, Jain SC, Lamichhane J. Biological activities of three different medicinal plants from Himalayan Region of Nepal. *Nepal J Sci Technol* 2010; **11**: 139-146.

[7] Arora R, Mundra S, Yadav A, Srivastava RB, Stobdan T. Antimicrobial activity of seed, pomace and leaf extracts of sea buckthorn (*Hippophae rhamnoides* L.) against food borne and food spoilage pathogens. *Afr J Biotechnol* 2012; **11**(45): 10424-10430.

[8] Bal LM, Meda V, Naik SN, Satya S. Sea buckthorn berries: a potential source of valuable nutrients for nutraceuticals and cosmoceuticals. *Food Res Int* 2011; **44**: 1718-1727.

[9] McLaughlin JL, Rogers LL, Anderson JE. The use of biological assays to evaluate botanicals. *Drug Inf J* 1998; **32**: 513-524.

[10] Meyer BN, Ferrigni NR, Putnam JE, Jacobsen JB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. *Plant Med* 1982; **45**: 31-34.

[11] Wah ST. Toxicity testing using the brine shrimp: *Artemia salina*. In: Colegate SM, Molyneux RJ, editors. *Bioactive aatural products detection, isolation, and structural determination*. London: CRC; 1993, p. 441-456.

[12] Mazid MA, Datta BK, Nahar L, Sarker SD. Assessment of anti-bacterial activity and brine shrimp toxicity of two *Polygonum* species. *Ars Pharm* 2008; **49**(2): 127-134.

[13] Wirasathien L, Boonarkart C, Pengsuparp T, Suttisri R. Biological activities of alkaloids from *Pseuduvaria setosa*. *Pharm Biol* 2006; **44**: 274-278.

[14] Sparg SG, Light ME, van Staden J. Biological activities and distribution of plant saponins. *J Ethnopharmacol* 2004; **94**(2-3): 219-243.

[15] Kanokmedhakul K, Kanokmedhakul S, Phatchana R. Biological activity of anthraquinones and triterpenoids from *Prismatomeris fragrans*. *J Ethnopharmacol* 2005; **100**: 284-288.

[16] Miller AL. Antioxidant flavonoids: structure, function and clinical usage. *Alt Med Rev* 1996; **1**: 103-111.

[17] Jamil M, Mirza B, Yasmeen A, Khan MA. Pharmacological activities of selected plant species and their phytochemical analysis. *J Med Plants Res* 2012; **6**(37): 5013-5022.

[18] Khan BA, Akhtar N. Phytochemical analysis and acute toxicity tests of two medicinal plant extracts. *J Med Plants Res* 2012; **6**(23): 3545-3548.

[19] Mothana RA, Mentel R, Reiss C, Lindequist U. Phytochemical screening and antiviral activity of some medicinal plants from the island Soqotra. *Phytother Res* 2006; **20**: 298-302.

[20] Saggu S, Divekar HM, Gupta V, Sawhney RC, Banerjee PK, Kumar R. Adaptogenic and safety evaluation of sea buckthorn (*Hippophae rhamnoides*) leaf extract: a dose dependent study. *Food Chem Toxicol* 2007; **45**: 609-617.

[21] Upadhyay NK, Kumar R, Mandotra SK, Meena RN, Siddiqui MS, Sawhney RC, et al. Safety and wound healing efficacy of sea buckthorn (*Hippophae rhamnoides* L.) seed oil in experimental rats. *Food Chem Toxicol* 2009; **47**: 1146-1153.