Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i3.5000

COMPARATIVE CYTOTOXIC, ANTITUMOR AND ANTI-PROLIFERATIVE ACTIVITIES EVALUATION OF SEA BUCKTHORN (HIPPOPHAE RHAMNOIDES LINN) BERRIES EXTRACTS OF PAKISTAN

Javid Ali[1*], Muhammad Siddique[2], Arshad Hussain[3], Javed Abbas[4], Inayat Ur Rehman[5], Abdul WajidKhalil[6], Muhammad Tayyab Gul[7], Rehman Ullah[8], Anwar Zeb[9], Muhammad Akram[10], Muhammad Younas[11], Muhammad Saqib Khalil[12#]

[1*,2,3,4,5,6,8,9,10,11]Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex, Peshawar - Pakistan
[7]Departmnet of Chemical and Life Sciences, Qurtuba University of Science and Information Technology, Peshawar - Pakistan
[12#]Sarhad Institute of Allied Health Sciences, Sarhad University of Science and Information Technology, Peshawar - Pakistan

***Corresponding Author:** Javid Ali, Email: javedali_14@yahoo.com
[#]Co-corresponding Author: Muhammad Saqib Khalil**,** Email: saqib.biotech@suit.edu.pk

Abstract

The present study was carried out to investigate the antiproliferative, antitumor and cytotoxic potential of sea buckthorn berries from Pakistan. Folin-Ciocalteu reagent method was used to determine the Phenolic compounds, 2, 4 dinitro phenylhydrazine (DNPH) method was applied to quantified the ascorbic acid and cytotoxic activity determined by *Artemia salina* lethality bioassay method. The flavonoids, Lycopene and β-carotene were estimated spectrophotometrically. The antitumor potato disc assay using *Agrobacterium tumefaciens* was employ to evaluate the antitumor activities and Methyl thiazolyl tetrazolium (MTT) assay was carried out to determine the anti-proliferative properties. Maximum total phenolic content, flavenoids, ascorbic acid, lycopene and β-carotent were 13±01 mg of Gallic acid/gm, 5.2±0.5 mg of Rutin/gm, 410±4mg/100g, 25±2 mg/100g and 380±7 µg/g respectively in methanol extract. Highest brine shrimp mortality (73.33%) was observed in methanol extract at 1000 mg/L. Highest tumors inhibition activity (75.00%) was shown by methanol extracts at 1000 mg/L. The highest % cell death of human cervical (HeLa), colorectal adenocarcinoma (Caco-2), prostatic adenocarcinoma (PC-3), stomach adenocarcinoma (AGS) and mammary gland adenocarcinoma (MDA-MB-231) were 75%, 80.71%, 80.00%, 76% and 76.92% respectively in methanol extract. While in ethyl acetate maximum cell death 70.33% for mammary gland adenocarcinoma (MCF-7). The variation in cell inhibition, tumor and cytotoxic activities might be blame to the dissimilar quantification of bioactive compounds accountable for these activities. Every extracts have substantial intensity of antitumor, cytotoxic and cell inhibition with a dose dependant way. The present research outcomes proved that Sea Buckthorn berries extracts an effective natural resource of bioactive compounds with cytotoxic, antitumor and anticancer activities.

Keywords: Sea buckthorn berry, Bioactive compounds, Brine shrimp, Tumor inhibition, Cancer, Cellinhibition.

Introduction

Cancer is a composite of poly factors cell illness distinguished by irregular propagation cells. It inflicts a severe load on the community healthiness structure, and its management and therapy are technically difficult. Cancer is supposed to allege 09 million lives globally by 2015 (Zainal et al., 2014). Herbs have been utilized for long time as a vital medicinal foundation for cancer therapy as crude extracts, juice or teas of herbs, or standardize concentrated portions in medicinal formulation like capsules, tablets, powders, liquid extracts and tinctures (Dutt et al., 2014). Therefore, it is achievable that customary therapeutics herbs could be used as latent resource for formulation novel medicine and additional efficient therapy for cancer treatment (Caamal-Fuentes et al., 2011). In medical care centers, conventional medicines are generally recommended to cancer patients in medical care centers. However, because of less adverse effects and low toxicity of the plants bioactive compounds, the exploration on cancer and therapeutics plants has been strengthen (Suphachai, 2014). Common person due high cost does not afford the current advance cancer treatments. Alternative medicine such as treatment with phytochemicals have play a main role in the deterrence and treatment of cancer and medicinal plants are generally accessible and feasible (Asima et al., 2012). The occurrence of bioactive molecules in plant have possessed diverse properties like anticancerous, antioxidant, antibacterial, antifungal, immunostimulant, cytoprotective, anti-inflammatory, antiradiation, hepatoprotective, anti- artherosclerosis, anti-diabetic, anti-ulcer as well as wound healing actions (Harshit et al., 2013).

The botanical name of Sea Buckthorn is *Hippophae rhamnoides* (*H. rhamnoides*), is shrubs with orange or yellow fruits and leaves are fallen down seasonally. In various countries of Europe and Asiasuch small tress are being cultivated (Javid and Bashir (a), 2015). The fruit of Sea Buckthorn is berrytype with mean size 6-9 mm having soft heavy orange layer. The berries are locally utilized for the production of liquors, syrups and jams. Berries dried powders are utilized for numerous nutritional herbal teas (Otakar et al., 2014). The Sea Buckthorn fruits are recognized to be a wealthy resource of various therapeutic and nutrients compounds such as polyphenolic acids, β- sitosterol, minerals, lipids, flavonoids, proteins, vitamin E, vitamin A and vitamin C; therefore reported its anti-stress and antioxidant activities (Javid and Bashir (b), 2015). The berries were exercise in folk medication, mostly practices in Central Asia, Chinese, Mongolian and Tibetan system. Sea Buckthorn bark, leaves, juice and oils are documented regarding for its therapeutic purposes, and they are utilized to cure eye vision, dermal, skin, heart, gingivitis and elevated blood fat diseases (Mousmi and Handique, 2013). The current study were carried to determined the bioactive components of *H. rhamnoides* berries andits various extracts for cytotoxic, antitumor and antiprolifrative activities.

Materials and Methods

Sea Buckthorn berries were procured from Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratory Skardu, Gilgit Baltistan-Pakistan. These berries were brought to Food Technology Center of PCSIR Laboratories Complex Peshawar, Khyber Pakhtunkhwa-Pakistan. The berries were clean, remove unwanted materials, washed with tape water to remove dust particles, andthen rinsed with double distilled water. The clean berries were dried in shade dust free room, grindedto powder with the help of Wiley Mill Standard Model# 3 (USA). The berries powders kept in a sterilized glass bottles Duran (Germany) until used.

Extraction

Take fifty gram (50g) of berries powders and soaked individually in organic solvents (acetone, ethyl acetate, ethanol and methanol) and aqueous (water) extract for forty eight (48 hrs). The extracted materials of each solvent were filtered using Whatman® 40 filter paper 125mm Ø Whatman

International Ltd. Maidstone- England. The process was performing again with fresh solvents four times. The solvents mixtures were concentrated using Rotary Evaporator (R-200, Buchi Rotavapor-Switzerland) at 40°C. Theconcentrated semi solid extract were decanted from the round bottom flask of Rotary Evaporator into 100mL beaker and kept on Hot Plate (PCSIR-Pakistan) at 40°C until all the solvent traces were removed and received a constant weight. The final dried extract were preserve in a sterilize brown glass bottles at low temperature (10°C) in a cooled incubator (Gallenkamp-UK) for further experimental study.

Total phenolic contents

Take 0.1 mL of extract solution and mix with 3 mL of distilled water in a volumetric flask. Then add 0.5 mL Folin-Ciocalteu reagent. After waiting for three minutes, add 02 mL of twenty percent sodiumcarbonate. Then shake the blended solution and kept for one minute in boiling water. Cool the mixture and afterward take a concentration optical density using Shimadzu UV 160A Spectrophotometer at 650 nm. The total phenolic contents were extrapolated applying known prepared a gallic acid standardcurve (Mousmi and Handique, 2013).

Total flavonoids

Take extract of 1.0 mL having concentration of 01mg/mL and blended with five percent of sodium nitrite solution (0.3 mL) and4.0 mL distilled water. Waite for five minutes then added 1M of Sodiumhydroxide (NaOH) 2.0 mL and Aluminium chloride 0.3 mL to the mixture and add distilled water uptoprepared 10 mL of total volume. The mixture was carefully shacked and absorbance was calculated using blank with 510 nm by Shimadzu UV 160A Spectrophotometer. Reference standard compound was used Rutin (Shinvani and Chandresh, 2012).

Vitamin C

Measure 6.0 mL of 5% TCA and 2.0 mL of experimental extract. After thoroughly mix the solution was centrifuge (Hettich-Germany) at 2000 RPM for ten minutes. Take 2.0 mL of supernatant, pour it into sample tubes, and in another test tube take 2.0 mL of reference standard ascorbic acid. After that mix 01 drop of indophenols reagent and blended well. Then added five millimeter of DT reagent (DNPH 2gm + Thiourea (1gm) in 9N H_2SO_4). Incubation was carried out for all experimental tests tubes for one hour at60°C on water bath. After incubation, cool the tests tubes in ice water. Then added to tubes 2.5mL of 85% H_2SO_4 and well mixed. At 505 nm using Spectrophotometer (SP-3000 Plus, Optima Tokyo-Japan) the optical density (OD) was measured. The amount of Vitamin C in berries extracts was calculated from ascorbic acid standard curve (Shinvani and Chandresh, 2012).

Lycopene

The quantification of lycopene in Sea buckthorn berries extract was analyzed by the describes method of (Shinvani and Chandresh, 2012). The berries were extracted repetitively with acetone applying mortar andpestle unless the colorless residues were obtained. The acetone berries extracts were collected and shifted to a separating funnel holding petroleum ether twenty milliliter and afterward added twenty millimeter sodium sulphate (5%) solution and vibrate the mixture in the separating funnel. To the separating funnel added 20mL of petroleum ether unless two phases of clear separation were appeared. Two layers were disconnect and the inferior water layer was repeatedly extracted with extra twenty millimeter petroleum ether awaiting the colorless water extract layer was achieved. Little more distilled water was adjoining tothe petroleum ether berries extracts. The petroleum ether extract washed holding lycopene was shifted into a brown container holding approximately ten-gram sodium sulphate (anhydrous) and reserved to one side for thirty minutes. The extract of petroleum ether shifted into volumetric flask (100mL) in the courseof funnel holding cotton wool, the slurry of sodium sulphate was afterward rinsed with petroleum ether awaiting colorless. Make the volume upto 100mL with petroleum ether. The optical density was

calculatedat 503nm on UV-Vis Spectrophotometer applying petroleum ether as control.
Absorbance (1 unit) = 3.1206 µg lycopene/mL

$$\text{Lycopene mg/100g} = \frac{31.206 \times \text{Absorbance}}{\text{Weight of berries (g)}}$$

β-carotene

Take 1.0 mL aliquot of each extract and add with one-milliliter ethanol and three-milliliter petroleum ether. The mixture shaken vigorously and centrifugation was carried out for fifteen minutes at 2500rpm. The optical density was measured at 450nm (Shimadzu UV 160A Spectrophotometer) using run blank petroleum ether. β-carotene quantification in each samples of extracts were extrapolated from standard curve of β-carotene (Shinvani and Chandresh, 2012).

Cytotoxic activity

Fifty milligram of brine shrimp powder was applied to originate larva through incubation for forty-eight hours using artificial seawater. Each extract were prepared in a concentration of 10, 100 and 1000 mg/mL in dimethyl sulfoxide (DMSO). Waite overnight until the DMSO evaporation. Using three tubes for every concentration of individual berries extracts and to every concentration ten shrimp larva per tubes (thirty shrimp larva per dilution) shifted before added seawater 5mL/vial. During the experiment, the seawater and Etoposide used as negative and positive control respectively. After twenty-four hour the brine shrimps mortality were observed. The brine shrimp percent mortality measured as shown in the following formula (Javid and Bashir, (b) 2015).

$$\text{Mortality \%} = \frac{\text{No. of Immobile or Died Brine Shrimp} \times 100}{\text{Total Number of Brine shrimps}}$$

Potato Disk Tumor Induction Assay

The bacteria *Agrobacterium tumefaciens* (*A. tumefaciens*) were collected from PCSIR Laboratories Complex Peshawar-Pakistan. Prepare three different test samples with a concentration 10mg/ml, 100mg/ml and 100 mg/ml. Take bacterial culture 2 mL (1×10^8 CFU/mL) added distilled water 1.5 ml and test sample 0.5 ml dissolved in DMSO. The blank (control) was prepared for experiment by substituting the extract concentration with DMSO (0.5ml). Collected Red-skinned Potato Solanum tuberosum L., Solanaceae) and using sterilized cork borer prepared 5 mm×8 mm in size potato discs. These potato discs were placed on the agar surface and afterward 50 µL of inoculum was poured on each specified disc, sealed the Petri dishes with parafilm. The inoculated agar plates were paled in an incubator for three weeks at 27 °C. After incubation time completion, disc of potato were stained with Lugol's iodine (I_2 5% and Ki 10%) for thirty minutes and the using stereo microscope for tumors observation. In microscopic observation, the cells of tumors lack starch (appear like orange color). Tumor inhibition percentage were calculated using the following formula (Javid and Bashir, (a) 2015).

$$\text{Inhibition \%} = (1 - Ns/Nc) \times 100$$

Where, Ns=tumors (sample), Nc = tumors (negative control).

Antiproliferative activityCell culture

The cancer cell lines (Table 1) were incubated at 37°C in humidity environment possessing five percent carbon dioxide in media enriched with streptomycin (100µg/mL) and penicillin (100 units/mL).

Extracts Preparation Process

Prepared 1, 2, 3, 4 and 5 mg/mL of *H. rhamnoides* berries extract dilutions and inoculated into 96 well culture plates. To each well 500 µL culture of each cell line (10^5 cells/mL) was added. The control wellswere created using the suspension cell line culture exclusive of Sea buckthorn extracts. The incubation ofplates were carried out in Carbon dioxide incubator for 72 hrs at 37°C. The plate's examinations were carried using microscope for cells confluent monolayer, toxicity and turbidity (Javid and Bashir, (a) 2015).

MTT assay

After incubation, the mixture from each well aspirated and discarded carefully. Take fifty micro letter (50 µL) MTT reagents and supplemented into a specified well. The plates were kept in incubation for four hours at 37°C with five percent carbon dioxide to permit the soluble MTT yellow intracellular reduction to unsolvable crystals having purple color of formazan. The upper layer was detached; propanol fifty microliters mixed up and the plates was vertex cautiously to synthesize formazan soluble. The mixer shifted to a Spectrophotometer (Shimadzu-UV-160A) cuvette and at 570nm the optical density (OD) impression were recorded with DMSO applying as control. The death percentages of cell were computed applying the bellow formula (Javid and Bashir, (a) 2015).

$$\text{Cell Death \%} = 1 - (\text{Test OD}/\text{Control OD}) \times 100$$

Statistical analyses

The experimental parameters were analyzed in triplicates and average ± standard deviation outcomes werecommunicated. A value significant was considered as $p<0.05$.

Results

The bioactive compounds in *H. rhamnoides* berries were summarized as; total phenolic (mg of gallic acid/gm) content in methanol extract was 13±01, ethyl acetate was 11±01, aqueous was 10±01, ethanol was 08±0.5 and acetone was 05±0.5. The highest flavonoid (mg of rutin/gm) content was calculated in methanol extract 5.2±0.5, followed by ethyl acetate extract (4.5±0.3), aqueous extract (3.5±0.2), ethanol (3.2±0.2) and acetone (2.5±0.4). The lowest vitamin C (340 mg/100g) were examined in ethanol extract and in the extract of methanol maximum (410 mg/100g) was found, moderate values 395 mg/100g, 380 mg/100g and 370 mg/100g was calculated in the extract of ethyl acetate ($C_4H_8O_2$), aqueous and acetone (C_3H_6O) respectively. The Lycopene result showed 25 mg/100g, 21 mg/100g, 19 mg/100g, 17 mg/100g and 14 mg/100g for methanol, $C_4H_8O_2$, aqueous, ethanol (C_2H_5OH) and C_3H_6O respectively. The sea buckthorn berries extract of water, methanol (CH_3OH), C_2H_5OH, C_3H_6O and $C_4H_8O_2$ β-carotene (µg/g) contents were 350±5, 380±7, 280±9, 320±5 and 360±6 respectively.

The Sea buckthorn berries extract cytotoxic activities against *Artemia salina* results were summarized asfollows. The aqueous extract at 1000, 100 and 10 mg/L showed 66.66%, 50.00% and 36.66% mortality respectively. While methanol extract calculated highest mortality 73.33% @ 1000mg/L, 60.00% @ 100mg/L and 33.33% @10mg/L. The second highest mortality was observed for ethyl acetate extract were 70.00% @ 1000 mg/L, 56.66% @ 100 mg/L and 26.66% @ 10 mg/L. The ethanol extract was found

46.66% (1000 mg/L), 26.66% (100 mg/L) and 16.66% (10 mg/L) mortality. Comparative point of view the acetone extract at 1000 mg/L mortality was 40.00%, at 100 mg/L mortality was 20.00% and at 10mg/L mortality was 13.33%. The negative control (sea salt) was found no mortality. But the positive control (etoposide) was calculated the highest mortality i.e. 86.66% at 1000mg/L, 80.00% at 100 mg/L and 66.66% at 10mg/L. All results observed a dose dependent cytotoxic activity of each extracts against *Artemia salina*. The numbers of viable brine shrimp is shown in

Table 2.

The Sea buckthorn berries extracts antitumor activities were summing up as track. The aqueous extract showed 50.00%, 41.66% and 0% tumor inhibition at 1000, 100 and 10 mg/L respectively. The extract of methanol were found the maximum inhibition of tumor (75.00%) @ 1000 mg/L, 58.33 % at 100 mg/L and 50.00 mg/L at 10 mg/L. The ethyl acetate extract observed tumor inhibition 41.66% (1000 mg/L), 33.33% (100 mg/L) and 16.66% (10 mg/L). The tumor inhibition was 41.66%, 33.33% and 0% at 1000, 100 and 10 mg/L correspondingly in extract of ethanol. The extract of acetone at 1000 mg/L and 100 mg/L and 10 mg/L observed tumor inhibition percentage were 33.33, 8.33 and 0 respectively. All extract showeda dose dependant pattern for tumor inhibition. The numbers of tumors per disc (mean) is shown in **Fig1**.

To evaluate the inhibition activity of *H. rhamnoides* berries extract against six different cell lines. These cell lines were Hella cell, AGS cell, MCF Cell, PC-3, Caco-2, and MDA-MB (Table 1). Extracts were prepared in five solvents i.e. methanol, ethanol, water, acetone and ethyl Acetate. All these extract were prepared of five different concentrations 5, 4, 3, 2 and 1mg/mL. Cell death (%) of HeLa cells line by *H. rhamnoides* berries extracts are shown in **Figure 2**. Maximum cell death 75% was shown by methanolic extract against Hella cells at 5 mg/mL. While minimum 14% was exhibited by ethanolic extract at 1 mg/mL. Ethyl acetate showed 2nd maximum cell death i.e. 68 % at 5 mg/mL. Cell death (%) of AGS cellline by *H. rhamnoides* berries extracts are shown in **Figure 3**, in which the highest activity was exhibited by methanolic extract within the range of 76−34% (5−1mg/mL) and the lowest was by acetone i.e., 41−20% (5−1mg/mL). Cell death (%) of MCF-7 cell line by *H. rhamnoides* berries extracts are shown in**Figure 4**. In which the maximum inhibition was shown for ethyl acetate extract in the limits of 70−50 % (5−1mg/mL) and minimum for acetone extract in the range of 46.15−29.67% (5−1mg/mL). Cell death (%) of PC-3 cell line by *H. rhamnoides* berries extracts are shown in **Figure 5**. In this case the highest activity was shown by methanolic extract i.e. 80−65.33% (5−1mg/mL) and the lowest was 73.33−53.33%(5−1mg/mL) for acetone extract. Cell death (%) of Caco-2 cell line by *H. rhamnoides* berries extracts areshown in **Figure 6**. Maximum activity were shown by methanolic extract against Caco−2 cell, in range of 80.71−68.57% (5−1mg/mL).While minimum was exhibited by acetone extract which was 67.85−55.71%(5−1mg/mL). Cell death (%) of MDA-MB-231 cell line by *H. rhamnoides* berries extracts are shown in **Figure 7**. The maximum inhibition was noted for methanolic extract of 76.92−47.69 % (5−1mg/mL) andminimum 48.56−43.07% (5−1mg/mL) for acetone. It was observed in all extracts that cell line death takeplace in dose dependant manner. As a whole the methanol and ethyl acetate extract showed more potent cell death ability.

Discussion

Herbs and botanicals have served as medicines in every civilization throughout human history. Nowadays scientists are engaged to identify those herbs which were used for cancer treatment. The plants have possess biologically active constituents that have been identified as therapeutic agents.

The phenol (mg/g GAE) and flavenoids (mg/g QE) content in *Hippophae salicifolia* fruit juices were150.2±0.03 and 272.92±0.07 respectively (Arvind et al., 2013). Seabuckthorn fruit pulp total phenol (mg/100 g) was 560.00 ±0.91 (Selvamuthukumaran and Farhath, 2014). The maximum quantification of phenols substances of the leaves extracts, bark extracts, pulp extracts and seeds extracts were calculated in the subsequent order; extract of CH_3OH> C_3H_6O>chloroform>petroleum ether (Mousmi and Handique, 2013). The differences in phenolic substances quantification in the different extracts are because of different substances polarities possess in the berries and similar variation has been observed in the previous findings research for the seeds of other fruits (Mousmi and Handique, 2013).

The flavonoids have significant effects on cancer chemotherapy and chemoprevention by phenomena such as reversal of multidrug resistance, antioxidation, angiogenesis inhibition, induction of differentiation and apoptosis, cell cycle arrest, antiproliferation, inactivation of carcinogen and collection actions of these phenomena (Ren et al., 2003). Numerous significant

parameters of signal transduction pathways connected to cellular survival and growth are modulating by flavonoids. Therefore, the cell signaling pathways modulation might assist to inhibit cancer through; (i) DNA topoisomerase II inhibition (ii) Inducing and proliferation apoptosis inhibition (iii) changes in cell cycle such as prevention of cyclins and cdk or cdk-inhibitors of cip/kip family up-regulation (Kalyaniet al., 2010). The occurrences of flavonoids in all parts of Sea buckthorn are principally responsible for antioxidant and anticancer properties (Javid and Bashir, (a) 2015). It is observe that lycopene havebeen responsible to hold back cell division growth in breast, prostate and lung cell lines (Kalyani et al., 2010). The phenolic substances play a key role as antiproliferative mediators in cell cycle pathways and they have inhibited the tumor cells by using these mechanisms (Conforti et al., 2008). Pro- apoptotic activities have been reported by β-carotene in colon and leukemic cells because of augmenting GSSG/GSH and ROS ration connected with amplified NF-kB attachment ability, enhanced pro-apoptotic outcome in the cells of tumor and growth inhibition of cells. It is also has beenobserved that β-carotene have minimize the preparation of anti-apoptotic protein Bcl-2 in cancerous tissues and therefore stop the cancer cells growth (Kalyani et al., 2010).

Tumor is an ailment condition illustrated organized propagation and nonexistence of apoptosis. Programmed cell death or apoptosis is vital occurrences that participate in a significant responsibility in the growth of an organism and homeostasis (Kwon et al., 2006). The method of potato disks crowngall might be applied as normally which is statistically reliable, secure, cheap and quick comparativelypre analysis antitumor activities for 3PS (Fatma et al., 2012). The *Agrobacterium tumefaciens* inducted crown gall inhibition on the tissue of potato disk is the method which is originated on antimitotic activities that could be perceive a wide spectrum of novel and known antitumor properties (Mclaughlin *et al*., 1998; Coker *et al*., 2003). The legitimacy of this method is forecast on the examination that assured cancer phenomena are identical in animals and plants. It was observed that crown gall tumor inhibition on the disc of potato explained a similar identity with plant extracts and compounds acknowledged vigorous in the in-vivo murine leukemia anticancer activities (Fatma *et al*.,2012).

The cytotoxic method based on MTT reagents gives an easy assay for the measurement of survive cellquantity in order to judge the frequency cell growth and cytotoxic agents screening (Kalyani et al., 2010). The ascorbic acid levels of berries grown in Sweden and its relation with tumor cell growth inhibition was recognized (Dominique et al., 2007). The *Hippophae rhamnoides* berries these outcomes are owing to extremely wealthy phytochemicals of it like vitamin K, vitamin E, flavonoides,lycopene, β-carotene and sterol. Numerous population experiments showed that vitamin E intake minimize the occurrence of cancer (colorectal) by activator apoptosis of cancer cells by inducing a strong cell cycle inhibitor i.e. 21 wafi/cip1 (Kalyani et al., 2010).

It was recognized in recent times that plant sterols carried out process of programmed cell death in thehuman being large intestine cancer cell through targeting various phenomena signaling (Fatma et al.,2012). Various research reports demonstrated that there is a correlative connection between the anticancer activities of herbal phenolic compounds and herbal products. A relationships stay live between the shape oxidation nature and number, position and state of the group of atoms of the phenolssubstances and its anticancer activities (Yanez et al., 2004). The *Hippophae rhamnoides* is well recognized for its strong antitumor property (Jadhav et al., 2013). Inhibition of MDA-MB-231 and PC-3 cancer cell proliferation by *H. rhamnoides* berry juices with IC_{50} (µl/mL) were 35 and 22 respectively (Dominique et al., 2007). Aqua-alcoholic extract of Sea buckthorn seeds observed considerable and duration contingent on cell toxic activities against human leukemia cell lines, havinghalf-maximal inhibitory concentration (IC_{50}) records 50.0±13.3 µg/mL and 70.67±8.1 µg/mL after seventy two (72) and forty eight (48) hours respectively (Kalyani et al., 2010). Flavonoids have the capability to provide safeguard to the cells against oxidation stress and at the results, its stop genetic mutation and as a final point cancer (Shivani and Chandresh 2002). Aqua-alcoholic extract of *H. rhamnoides* seeds observed considerable and duration dependent anticancer activity against HL-60 cells, with IC_{50} value 50.0±13.3 and 70.67±8.1 µg/mL after 72 and 48 h and

respectively (Kalyani et al., 2010). The proliferation of cancer cells in liver were control by *H. rhamnoides* leaves (Zhao et al., 1987). The utilization of therapeutic plants exposed by customary culture has not been proving by systematic information. Bench top screening can give primary bioassay data. But, bioassays should be inexpensive, reliable, convenient, rapid, require little material and sensitive (Fatma et al., 2012). The utilization of plants extracts can offer a sustainable and cheap, means toward illness control, and can ultimately develop the value of life of the adjacent to city and rural poor people in the budding countries.

Conclusion

The *H. rhamnoides* berries extract were a rich source of bioactive compounds, with good cytotoxic and antitumor properties. Further selected berries extracts hold back the expression of six cell lines of cancer. Berries should be included in the meal, chosen for their significant antiproliferative abilities, which have given a remarkable input in the direction of cancer inhibition in the human being.

Additionally, the berries extract of Sea buckthorn should be formulated as nutraceutical products in first stage of cancer patients and prior to it might be methodically screen for separation, classification, characterization and assessment of anticancer drug and medicine development. Additional studies andexploration are required to separate the bioactive anti-tumor compounds from the Sea buckthorn berries extracts on industrial and commercial level. It will also concluded that state of the art animal and human model study is required in depth to finalize anticancer compounds drugs before any end could be ended on the effectiveness of every entity of extracts compounds.

Acknowledgments

The authors are thankful to the staff of Food Technology Center of PCSIR Laboratories Complex, Peshawar-Khyber Pakhtunkhwa-Pakistan for their help.

Statement Conflict of Interest

We announce that authors of this article have no conflict of interest.

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Table 1. Cell line used in the study.

Name of Cell lines	**Culture media used for growth**
Human cervical (He La)	Eagles minimum essential medium with 10% fetalbovine serum
Stomach adenocarcinoma (AGS)	F12-K medium containing 10% FBS
Mammary gland adenocarcinoma(MCF-7)	Minimum Essential Medium containing 0.01 mg/mL insulin and 10% FBS
Prostatic adenocarcinoma (PC-3)	Ham's F12 containing 10% calf serum
Colorectal adenocarcinoma(Caco-2)	Dulbecco's modified Eagle's medium (DMEM)containing 10% FBS, 10 µg/mL transferrin and 1 mM pyruvate
Mammary gland adenocarcinoma(MDA-MB-231)	a-MEM containing 10% FBS and 10 µg/mL insulin

Table 2. Cytotoxic activity of *H. rhamnoides* Berries extracts against *Artemia salina*.

Extract/Control	Dose (mg/L)	No. of Shrimp	No. of survivors
Aqueous	1000	30	10±0
	100	30	15±1
	10	30	19±1
Methanol	1000	30	8±0
	100	30	12±0
	10	30	20±1
Ethanol	1000	30	16±1
	100	30	22±1
	10	30	25±1
Ethyl acetate	1000	30	9±0
	100	30	13±0
	10	30	22±0
Acetone	1000	30	18±1
	100	30	24±1
	10	30	26±1
***Sea salt**	1000	30	30±1
	100	30	30±1
	10	30	30±1
***Etoposide**	1000	30	4±0
	100	30	6±0
	10	30	10±0

Values are mean ±SD, n=3, ***E**toposide = Positive control, ***S**ea salt = Negative control.

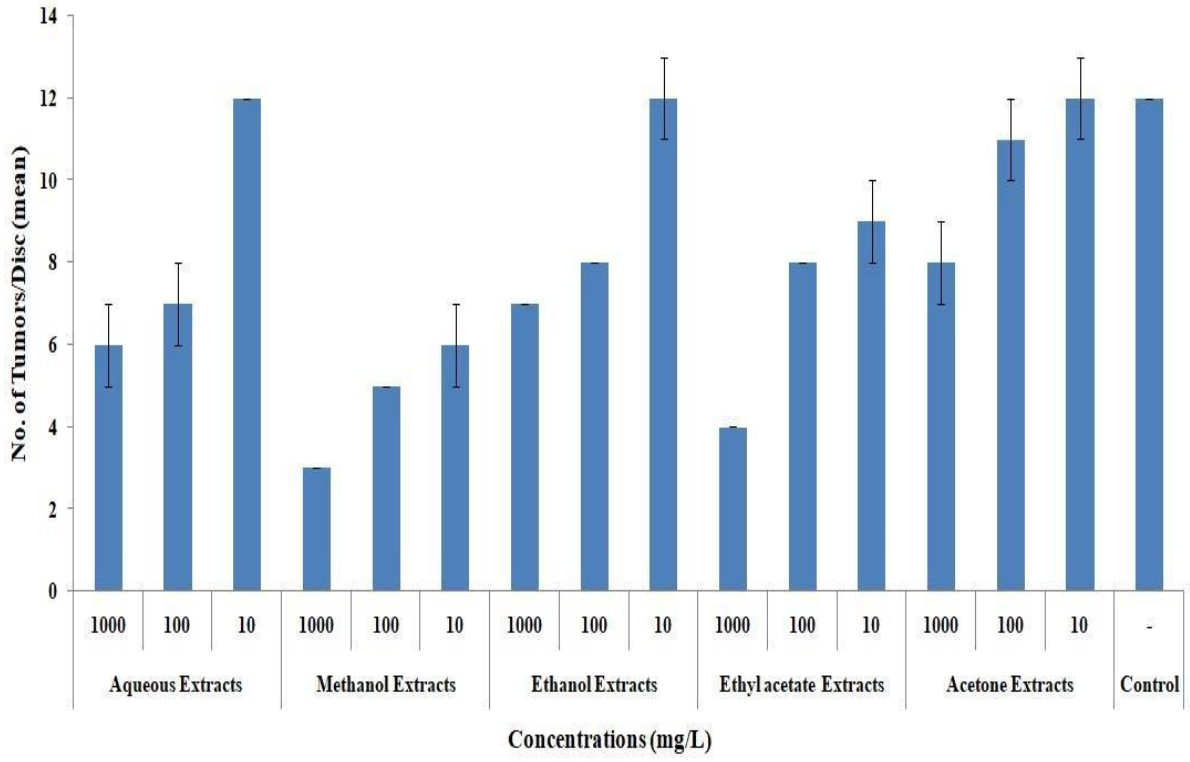

Fig. 1. Antitumor activity of *H. rhamnoides* Berries extracts.

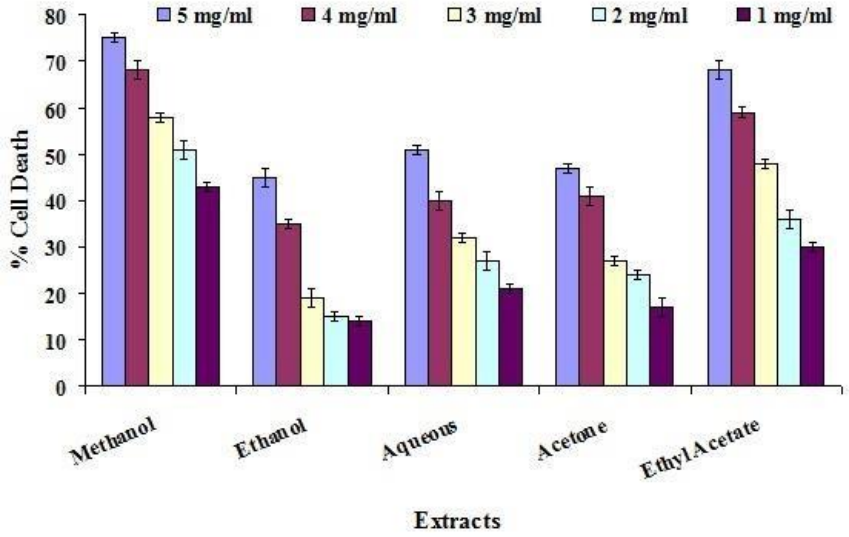

Fig. 2. % Cell death of HeLa cells line by *H. rhamnoides* berries extracts.

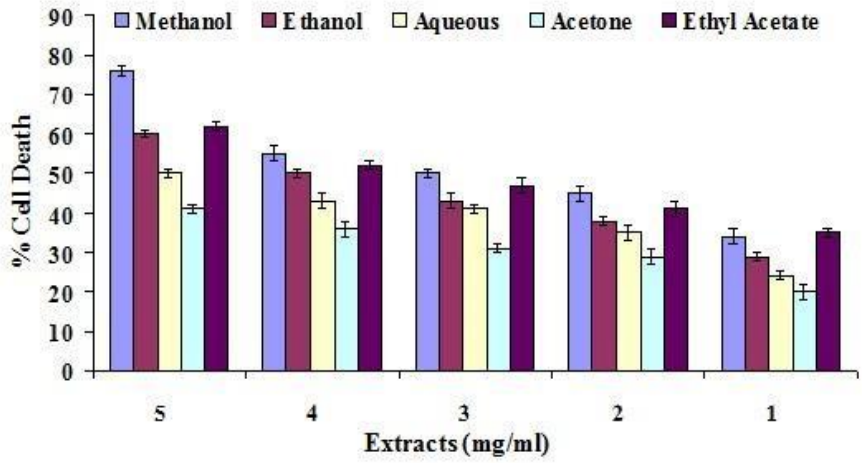

Fig. 3. % Cell death of AGS cell line by *H. rhamnoides* berries extracts.

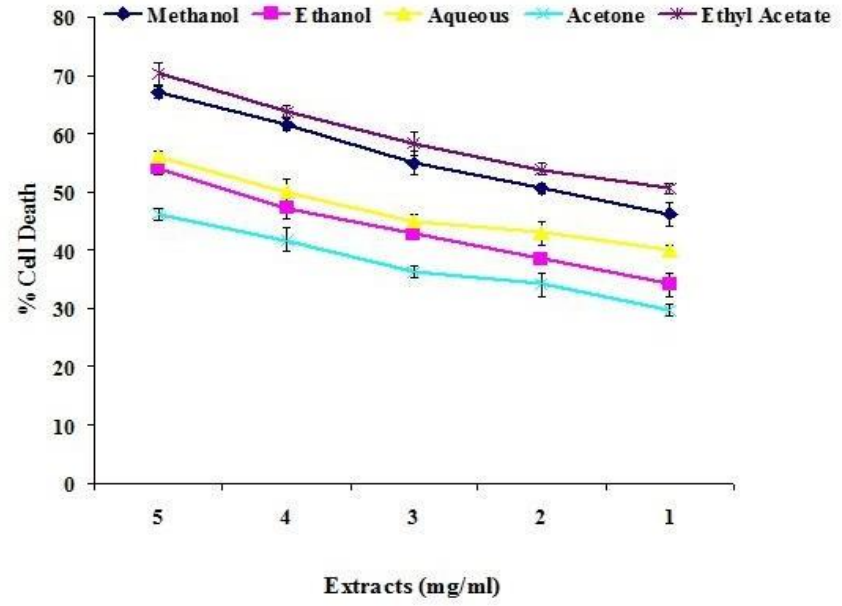

Fig. 4. % Cell death of MCF-7 cell line by *H. rhamnoides* berries extracts.

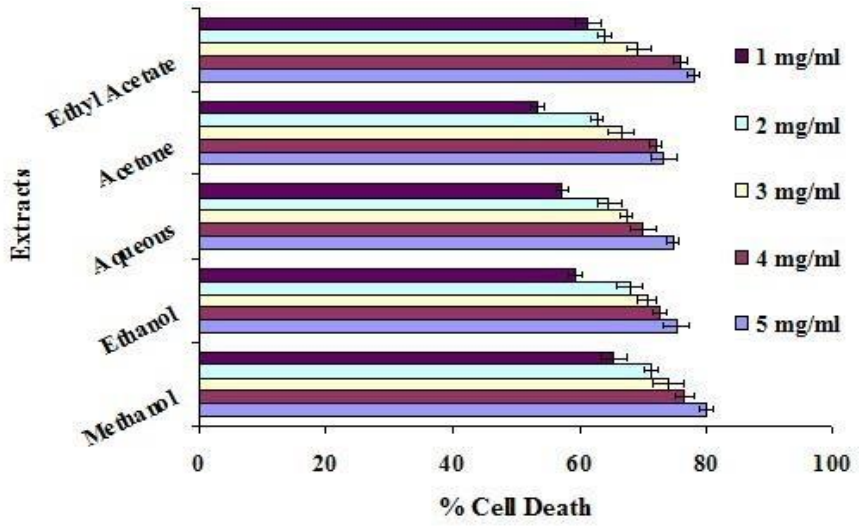

Fig. 5. % Cell death of PC-3 cell line by *H. rhamnoides* berries extracts.

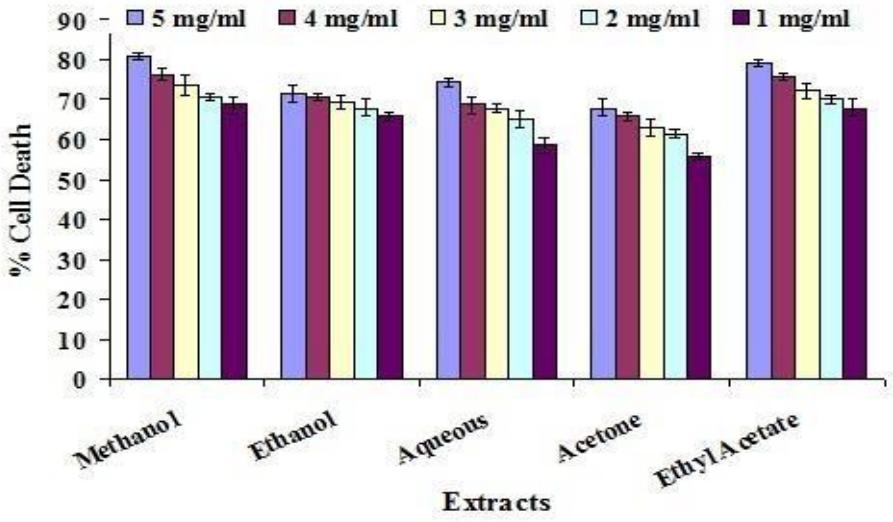

Fig. 6. % Cell death of Caco-2 cell line by *H. rhamnoides* berries extracts.

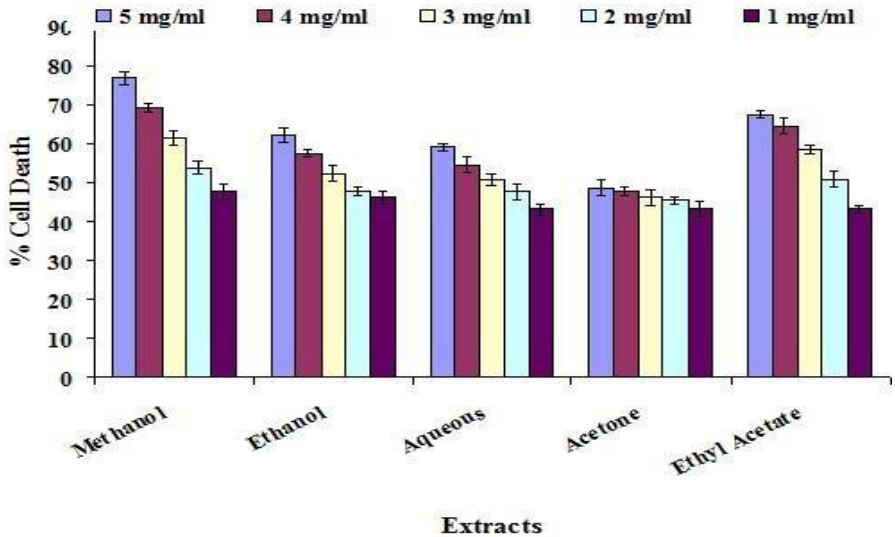

Fig. 7. % Cell death of MDA-MB-231 cell line by *H. rhamnoides* berries extracts.