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Full Length Research Paper

Physiochemical, minerals, phytochemical contents, antimicrobial activities evaluation and fourier transform infrared (FTIR) analysis of *Hippophae rhamnoides L.* leaves extracts

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The present research study was designed to characterize the physiochemical, minerals, phytochemicals, antimicrobial activities and FTIR spectra analysis of various extracts of *Hippophae rhamnoides L.* leaves. The physiochemical and mineral content of *H. rhamnoides L.* leaves revealed that fiber was 18.0 ± 2.64%, protein was 10.45 ± 0.88% and carbohydrate value calculated was found to be 68.75 ± 1%. Sodium was 3,000 ± 1 ppm, calcium was 7,800 ± 1 ppm and potassium was 6,200 ± 2 ppm, referred to as a high concentration while qualitative phytochemicals investigation showed that tannins, phenols and flavonoids were present in large quantity. The highest antibacterial zone of inhibition was observed in aqueous and methanolic extracts against *Staphylococcus aureus* and *Vibrio cholerae* (21 ± 1 mm), and the lowest zone of inhibition measured 07 ± 0 mm against *V. cholerae* (ethyl acetate extracts). The antibacterial minimum inhibitory concentration values of extracts were determined, ranging between 40 to 120 mg/ml, and minimum bactericidal concentration values of the extracts ranged between 50 and 135 mg/ml. The highest antifungal zone of inhibition was calculated against *Alternaria alternata* (18 ± 0 mm) followed by *Aspergillus parasiticus* (17 ± 1 mm) of methanolic extract, while *A. alternata* (07 ± 1 mm) and *Penicillium digitatum* (07 ± 0 mm) were the least, found in chloroform and aqueous extract, respectively. Further assessment of antifungal minimum inhibitory concentration and minimum fungicidal concentration ranged between 40 to 135 and 50 to 180 mg/ml, respectively. The Fourier transform infrared (FTIR) spectra of all extracts revealed the presence of different functional groups ranging from hydroxyl (OH) stretching for hydroxyl group, alkanes (C-H), alkenes (C=C), aromatic rings (C=O), carboxylic (C=O) and amides (aromatic).

Key words: Seabuckthorn leaves, antibacterial, antifungal, secondary metabolites, *Hippophae rhamnoides L.* leaves, functional groups.

INTRODUCTION

Medicinal plants are abundantly available all over the world and are more focused than ever because they have the ability to produce many benefits to human society, especially for the treatment of various types of diseases.

According to World Health Organization (WHO), more than 80% of the world's population relies on conventional medicines for various types of ailment. The therapeutic properties of plants are present in some chemical compounds that cause a specific physiological action in the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (Duraipandiyan et al., 2006). The alkaloids have antimicrobial, anticancer, cytotoxic

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and antimalarial properties while flavonoids possess high antibacterial activity and are more effective in the treatment of inflammation, allergy, cancer, viral infection and hypertension. Tannin has shown high activities against viral and bacterial infection as well as act as strong antioxidant (Maryam et al., 2012).

The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants (Duraipandiyan et al., 2006). Worldwide generally and in the third world countries particularly, the infectious diseases (bacterial) are responsible for many human deaths (Nathan, 2004). Bacterial microorganism such as *Pseudomonas*, *Bacillus*, *Staphylococcus* and *Salmonella* are the major pathogens which are responsible for many live threatening diseases. These human bacterial pathogens have the capacity and ability to live in unsuitable environment due to their poly habitats in the earth (Ahameethunisa and Hoper, 2010). Pathogenic fungi are responsible for many different types of diseases due to subcutaneous, deep inside and superficial infections. Humans infected with pathogenic fungi have increased drastically in all parts of the world. Human systemic infections is caused by *Candida albicans, Aspergillus flavus and A. niger,* which are found throughout the world and caused different types of diseases such as candidiasis, aspergillosis etc. (Gutal, 2011). So there is a need to develop new antibiotics from natural sources, especially from plants, which do not exhibit many of the side effects always associated with the synthetic antibiotics. A normal human diet consists of water, vitamins, fats, proteins, carbohydrates and minerals. Human health always depends on balanced mineral contents in the body. If the balance is disturbed (below or above the limits), it results in the abnormalities of human health, so balanced mineral diet play important role in relation to human health (Archa et al., 2010).

Hippophae rhamnoides L. commonly known as sea-buckthorn is a shrub belonging to the family Elaeagnaceae in which male and female plants are different (unisexual). The leaves of seabuckthorn are commonly green on the upper side while green silver ash color on lower side, with a rough and thick bark (Raj et al., 2011). The whole plant, particularly leaves and berry, are considered a good source of vitamins (C, E, A and K) and other important vital compounds (flavonoids, sterols and carotenoids), which possess highest medicinal and nutritional properties (Chen et al., 1991; Yang and Kallio, 2001). The seabuckthorn plant has been used tradition-ally as nutritional supplement, medicine, fuel and as fence. The plant has been extensively used for treatment of gastrointestinal tract (GIT) disorder, ulcer, hepatic disorder, thrombosis, cancer, tendon and ligament injuries (Tsering et al., 2010).

Sea-buckthorn spp *turkestanica* seed elemental analy-sis revealed that it has Na, K, P, Mg, Ca, Zn, Fe and Ag (Alam and Ijaz, 2009). The stem and root extracts of *H. rhamnoides* showed strong antibacterial and antifungal activities (Jong et al., 2010). Sanjay et al. (2011) studied the antimicrobial activity of seed extract and leaf crude extract and seed oil of sea-buckthorn (*H. salicifolia* D. Don) and found that fresh leaves [supernatant concentrated and supernatant dried extract dissolved in Dimethyl sulfoxide (DMSO)] and seed extract have antibacterial activity against gram +ve bacteria. While gram -ve bacteria showed resistance against these extracts except *Agrobacterium tumefaciens.* Only seed extract exhibited antifungal activity against *Tilletia and Mucor*, whereas no activity was observed in case of *Rhizopus.*

Keeping in view the importance of sea-buckthorn, the present study was aimed to evaluate sea-buckthorn leaves extracts for its physiochemical, minerals, phytochemicals content, antimicrobial activities and Fourier transform infrared (FTIR) spectra profiling analysis.

MATERIALS AND METHODS

Sample collection

The fully grown healthy sea-buckthorn leaves (SBL) were collected from Pakistan Council of Scientific and Industrial Research (PCSIR) Skardu, Pakistan. The leaves were slightly washed to remove any dust, and dried in shade. The dried leaves were then converted into powder and transferred into air tight plastic bags until they were used.

Leaves extraction

Fifty grams powder of sea-buckthorn leaves were taken and extracted in 250 ml of aqueous ethanol, acetone, methanol, ethyl acetate, chloroform and n-hexane for 48 h. These extracts were then filtered under vacuum through Whatman filter paper (No.1) into a Buchner flask. The extracts were concentrated in a rotary evaporator and then transferred into a sterilized beaker for heating in a water bath at 50°C to obtain a dry residue. The resultant crude extracts were transferred into airtight sample bottles and kept at 4°C until they were used.

Physiochemical analysis of leaves

The SBL powder used for physiochemical analysis, which included parameters like moisture, ash, fat, pH, total suspended solids (TSS), acidity, crude fiber, nitrogen, protein, total sugar, reducing sugar and non-reducing sugar. They were determined in accordance with standard procedures (Association of Official Analytical Chemists (AOAC), 2003). Percentage carbohydrate value was calculated by the given method (Indrayan et al., 2005).

Mineral analysis of leaves

Elemental contents were determined by wet digestion procedure. Minerals like sodium and potassium were determined with the help of Flame Photometer (Jenway PFP7). Heavy and alkaline metals like Ca, Mg, Fe, Al, Mn, Zn, Si, Ba, Cd, Pb, Cr and Ni, were determined by Atomic absorption spectrophotometer (Hitachi Zeeman Japan Z-8000) according to the standard methods (AOAC, 2003).

Phytochemical qualitative screening

Phytochemicals were analyzed qualitatively using the method as described by Harborne (1998).

Antimicrobial activities

Tested microorganism

Bacterial cultures of *Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Vibrio cholerae, Salmonella typhi, Bacillus cereus, B. subtilis, Citrobacter freundii* and pure cultures of *A. niger, A. parasiticus, A. flavus, A. fumigatus, A. oryzae, Fusarium oxysporum, Rhizopus arrhizus, Alternaria alternata, C. albicans and Penicillium digitatum* of fungi were obtained from Food Microbiology Laboratory of PCSIR Laboratories Complex, Jamrude Road, Peshawar Khyber Pakhtunkhwa (KPK), Pakistan, and used in the present study. These cultures were maintained on slants of nutrient agar (bacteria) and potato dextrose agar (fungi) and kept in a refrigerator to subculture after every week.

Antibacterial activity determination

Antibcterial activity was determined using well agar diffusion method (El-mahmood, 2009) with minor modifications. One milliliter (1 ml) of tested bacterial strains adjusted to 0.5 Mac Farland units (1.0×10^8 cfu/ml) was inoculated into plate. The molten nutrient agar at 45°C was added into each plate and gently shaken to ensure uniform mixing of the media and culture. Wells were punched in the solidified agar with the help of a sterile 6 mm cork borer. The dried SBL extracts, 1000 mg, were dissolved in 5 ml of DMSO. Fifty microliter (50 µl) of the 200 mg/ml of each extracts was pipetted into each well. Fifty microliter of each of the dimethyl sulfoxide (negative control) and Ciproxin (0.5 mg/ml) solution as positive control was used. The plates were incubated at 37°C for 18 h. The antibacterial activity was determined by measuring the diameters of zones of inhibition.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The broth dilution method was carried out to determine the minimum inhibitory concentration. The sea-buckthorn leaves extracts were diluted to various concentrations ranging from 20 to 200 mg/ml in nutrient broth. Five hundred microliter (500 µl) of each concentration was added to sterile nutrient broth (2 ml) in test tubes placed on a test tube rack. Then, 1 ml (1×10^8 cfu/ml) of bacterial culture of the respective strain was added to the content of the test tubes and incubated at 37°C for 18 h, Ciproxin solution with different concentration range from 0.10 to 0.5 mg/ml was used as positive control. The lowest concentration of the testing material that did not allow any visible growth against experimental bacteria was taken as MIC. The MBC was determined by pipetting 100 µl of culture from each of the broth tubes having no growth, and introduced into fresh agar plates. The plates were incubated for 48 h and then observed for growth. The concentration of the extracts/control without visible growth was calculated as the MBC (EL-mahmood, 2009).

Antifungal activity

Antifungal activities were assessed according to the method of Ogu et al. (2011) with minor modifications. One ml of each standardized spore suspension (10^5 spores/ml) was spread on the surface of the *Sabouraud dextrose agar* (SDA) plates. Then, sterile cork borer (6 mm in diameter) was used to make a well at the centre of each inoculated/cultured plates. SBL extracts of 1,000 mg were dissolved in 5 ml of DMSO (200 mg/ml), and 50 µl of each extract was applied into each respective well. 50 µl each of pure dimethyl sulfoxide and fluconazole (0.5 mg/ml) were used as negative and positive control, respectively. The plates were kept in the incubator for 1 to 7 days at ambient temperature and then observed for anti-fungal activities.

Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

The MIC of the extracts was carried out using broth dilution method of Ogu et al. (2011), with minor modification. Serial dilutions of each extracts in the broth were made to obtain the concentration in the range of 20 to 200 mg/ml (extracts) and 0.1 to 0.5 mg/ml (fluconazole). One milliliter (1 ml) standardized inoculums (10^5 spores/ml) were inoculated into each broth having different extract concentrations and then incubated for 1 to 7 days at 30°C to observe turbidity. MIC was calculated on the basis of no turbidity observation of lowest concentration in the test tubes. For MFC determination, the contents of the MIC in the serial dilution were then sub-cultured on the media and incubated at 30°C for one to seven days and observed for colony growth. The MFC was the plate with the lowest concentration of extracts and without colony growth.

Fourier transforms infrared spectroscopy (FTIR) analysis of SBL extracts

Fourier transform infrared (FTIR) technique was used for the identification of different functional groups in each extract. The infrared spectroscopy (IR) spectrum was obtained using FTIR Prestige -21 Shimadzu Japan. The sample was scanned from 3,900 to 500 cm^{-1}, operating at a resolution of 4 cm^{-1}, with 10 number of scans.

Statistical analysis

Statistical package for social sciences (SPSS) version 14 was used to analyze the data. The data were presented in the form of mean ± standard deviation (SD).

RESULTS

Extractive values, physiochemical and mineral analysis

The physiochemical analyses of SBL are shown in Figure 1. The moisture (%) content was 8.0 ± 1, ash (%) content was 7.0 ± 0.3, fat (%) value was 5.8 ± 0.4, pH value was 4.0 ± 0.2, TSS was 1.0 ± 0.25%, acidity was 0.60 ± 0.17%, fiber was 18.0 ± 2.64%, total sugar was 0.8 ± 0.01%, reducing sugar was 0.7 ± 0.01%, non-reducing sugar was 0.1 ± 0.01%, nitrogen was 1.82 ± 0.24% and protein was 10.45 ± 0.88%. The carbohydrate value calculated in sea-buckthorn leaves was found to be 68.75 ± 1%. The mineral content of seabuckthorn leaves are shown in Figure 2. Sodium was found in highest concen-

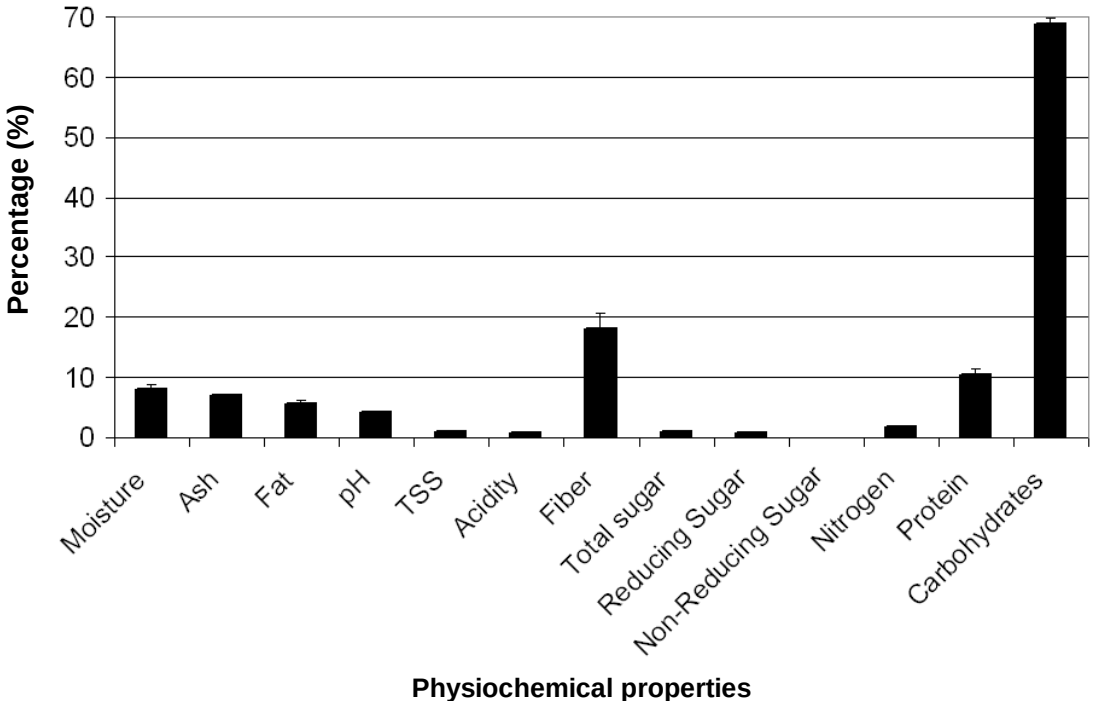

Figure 1. Physiochemical analysis of *Hippophae rhamnoides L.* leaves extracts.

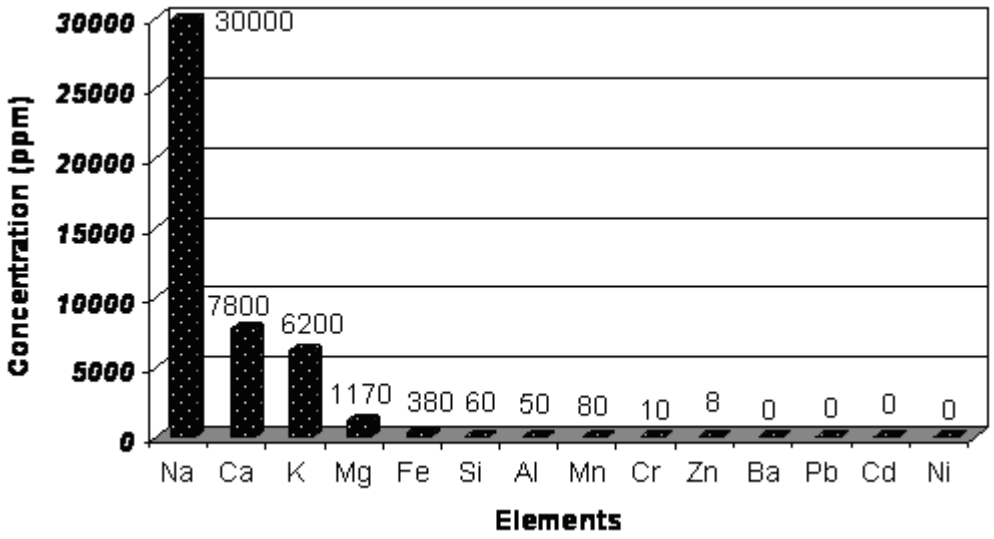

Figure 2. Minerals analysis of *Hippophae rhamnoides L.* leaves extracts.

tration (30,000 ± 1 ppm) and zinc was found in lowest concentration (8 ± 1 ppm), while the reaming elements were calculated (ppm) in the order Ca > K > Mg > Fe > Si > Al > Mn > Cr. Barium, cadmium, lead and nickel were not detected in seabuckthorn leaves. Extractive values of sea-buckthorn leaves are presented in Figure 3. Acetone showed the highest yield (22.95%) followed by methanol > ethanol > aqueous > chloroform > ethyl acetate > n-hexane.

Phytochemicals

As can be seen from Table 1, the content of phenols and flavonoids were found high while flavones, tannins and amino acids detected in the aqueous extract were in the average range. The terpenoids, glycosides, alkaloids and saponins were not present, and the quantity of steroids noted was very low in the aqueous extract of seabuckthorn leaves. In the methanolic extract of sea-

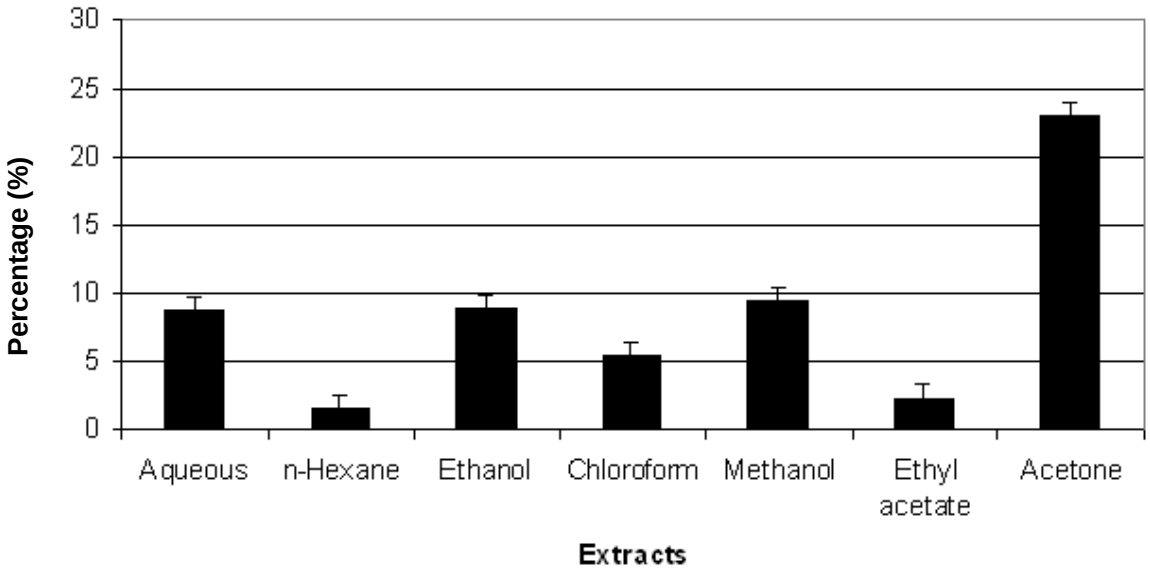

Figure 3. Extractive values of *Hippophae rhamnoides L.* leaves extracts.

buckthorn leaves, phenols, flavonoids, flavones and tannins were present in high concentration followed by terpenoids and amino acids, while saponins were not found in the methanolic extract. The Table 1 data shows that phenols, flavonoids and tannins concentrations were recorded maximum, while the least contents of terpenoids, steroids glycosides and alkaloids were noted in the ethanolic extract. Glycosides, flavones and saponins were absent in the ethyl acetate extract while average amount of flavonoids and tannins were noted in acetone extract. The minimum concentration of steroids, alkaloid, flavonoids and amino acids were present in the chloroform extract. Average contents of terpenoids were recorded in hexane extract of SBL, while the concentration of steroids, alkaloids and tannin were found in low range.

Antimicrobial activities

The mean zones of inhibition of different extract against 10 bacterial species are summarized in Table 2. The aqueous extract of sea-buckthorn leaves were found more active against *S. aureus* with 21 ± 1 mm zone of inhibition followed by 20 ± 2 mm against *E. coli,* while the lowest value 11 ± 0 mm was recorded for *S. typhi*. The methanolic extract showed the highest value of 21 ± 1 mm against *V. cholerae* while 19 ± 1 mm zone of inhibition was noted against *E. coli*. The zone of inhibition 16 ± 1 mm was measured against both *B. cereus* and *C. freundii* while the methanolic extract was found less active and showed 09 ± 0 mm zone of inhibition against *S. typhi*. The highest zone (20 ± 1 mm) of inhibition was recorded against *P. aeruginosa*, while *S. typhi* were found more resistant against the ethanolic extract, with 08 ± 0 mm zone of inhibition. The ethyl acetate extract was found more resistant against both *P. aeruginosa* and *B. cereus*, with 15 ± 1 mm zone of inhibition, and the least value 10 ± 0 mm zone of inhibition was recorded against *E. coli* by acetone extract. The chloroform extract zone of inhibition values were found in the range of 08 to 16 mm, while all the tested bacteria showed strong resistance against the hexane extract. Ciproxin was used as standard, ranging the value of zone of inhibition from 20 to 28 mm.

Table 3 data shows the MIC and MBC of the different extracts of SBL. The aqueous extract of SBL was found more active against S. *aureus*, with MIC and MBC values of 40 and 60 mg/ml, respectively. *P. aeruginosa* showed high resistance, with 100 and 110 mg/ml of MIC and MBC, respectively against the methanolic extract while 40 mg/ml MIC and 60 mg/ml MBC showed the highest activity of the ethanolic extract against *E. coli*. The MIC of the ethyl acetate extract were in the range of 65 to 120 mg/ml. MBC of the acetone extract ranged from 85 to 135 mg/ml, and the lowest MIC of the chloroform extract, 70 mg/ml, was recorded against *K. pneumoniae*.

The antifungal activity of the aqueous extract was observed, with maximum 16 ± 1 mm zone of inhibition against *F. oxysporum* and the minimum value 9 ± 0 mm was observed in methanolic extract against *A. oryzae*. Ethanolic extract showed the highest 14 ± 1 mm zone of inhibition against both *A. niger* and *C. albicans*, while ethyl acetate extract was found more active, with 15 ± 0 mm zone of inhibition against *R. arrhizus*. Extract of acetone and chloroform showed the same value (10 ± 1 mm) of zone of inhibition against *C. albicans* and the n-hexane extract was found inactive against all the tested

Table 1. Phytochemical analysis of *Hippophae rhamnoides L.* leaves extracts.

Chemical constituents	Aqueous	Methanol	Ethanol	Ethyl acetate	Acetone	Chloroform	n-Hexane
Terpenoids	ND	+ +	+	++	+	+ +	+ +
Steroids	+	+	+	+	+	+	+
Glycosides	ND	+	+	ND	+	ND	ND
Alkaloids	ND	+	+	+	+	+	+
Phenols	+ + +	+ + +	+ + +	+ +	+	+ +	ND
Flavonoids	+ + +	+ + +	+ + +	+ +	+ +	+	ND
Flavones	+ +	+ + +	+ +	ND	ND	ND	ND
Tannins	+ +	+ + +	+ + +	+ + +	+ +	+ +	+
Saponins	ND	ND	ND	ND	ND	ND	ND
Amino Acids	+ +	+ +	+ +	+	+	+	ND

+ sign shows detection level of the phytochemicals present in extracts. + = small quantity, + + = average quantity, + + + = large quantity, ND = not detected.

Table 2. Antibacterial activities of *Hippophae rhamnoides L*. leaves extracts.

Tested bacteria	Zone of Inhibition (mm)								
	Aqueous	Methanol	Ethanol	Ethyl acetate	Acetone	Chloroform	n-Hexane	C^+	C^-
S. aureus	21±1	14±1.7	13±0	12±1	11±1	11±0	0.0	28±1	0.0
E. coli	20±2	19±1	18±1	13±1	10±0	16±0	0.0	26±1	0.0
E. faecalis	15±1.7	14±1.7	16±0	08±0	16±1	13±0	0.0	24±0	0.0
K. pneumoniae	14±1	17±2	17±1	12±1	13±0	11±0	0.0	25±1	0.0
P. aeruginosa	14±1	11±1.7	20±1	15±1	18±0	08±0	0.0	23±0	0.0
V. cholerae	13±0	21±1	14±0	07±0	16±0	09±1	0.0	24±0	0.0
S. typhi	11±0	09±0	08±0	10±0	17±0	10±1	0.0	22±0	0.0
B. cereus	14±1	16±1	13±1	15±1	11±0	13±1	0.0	25±1	0.0
B. subtilis	12±1	14±0	15±0	09±0	12±1	12±0	0.0	23±0	0.0
C. freundii	13±1	16±1	15±1	10±1	16±0	11±0	0.0	20±1	0.0

C^+ = Positive Control (Ciproxin 0.5 mg/ml), C^- = Negative Control (DMSO), each value represents mean ± standard deviation (SD) (n = 3). 0 = no zone of inhibition.

fungi. Fluconazole was used as a standard antifungal agent, showing the values of zone of inhibition from 16 to 24 mm (Table 4).

The aqueous extract of SBL was found highly active against *F. oxysporum*, with 50 mg/ml MIC value and 65 mg/ml MFC value (Table 5). The maximum (90 mg/ml) MIC value of the methanolic extract was observed against *A. oryzae*, while the least value (90 mg/ml) of MFC of ethanolic extract was noted against *P. digitatum*. Ethyl acetate and acetone extracts of SBL showed poor activity as reflected by the high MIC and MFC values as compared to methanol, ethanol and aqueous extracts. The MFC of the chloroform extract of SBL were in the range of 100 to 150 mg/ml.

FTIR analysis

The FTIR spectra analysis was utilized to identify the functional group of the active ingredients on the basis of peak value in the vicinity of infrared radiation. The results of FTIR peak values and functional groups of SBL aqueous extract are represented in Figure 4. IR-spectrum shows strong absorption peaks at 3242.34 (broad), 2929.87 (small short) and 1593.20 cm^{-1} (sharp), which correspond to the presence of hydroxyl (OH), alkane (C-H) and aromatic rings (C=O) functional groups, respectively. SBL methanol extract (Figure 5) showed peak values 2922.16, 2852.72, 1693.50 and 1600.92 cm^{-1} which could be attributed to the existence of functional groups alkanes (sharp peak)**,** alkanes, carboxylic and amides, respectively. Ethanol extracts (Figure 6) showed broad peak values (3315.63 cm^{-1}) of OH groups, sharp and strong peak (2924.09 cm^{-1}) of alkane, small peak (2854.65 cm^{-1}) for alkane, and small and weak peaks of alkene (1735.93 and 1604.77 cm^{-1}). Ethyl acetate peak (cm^{-1}) data are shown in (Figure 7) and confirm the presence of hydroxyl, alkane and carboxyl group.

Table 3. Antibacterial MIC and MBC of *Hippophae rhamnoides L.* leaves extracts.

Tested bacteria	MIC/MBC	Concentration (mg/ml)						
		Aqueous	Methanol	Ethanol	Ethyl acetate	Acetone	Chloroform	Ciproxin
S. aureus	MIC	40.0	60.0	55.0	70.0	60.0	80.0	0.10
	MBC	60.0	80.0	70.0	80.0	95.0	100.0	0.10
E. coli	MIC	65.0	55.0	40.0	120.0	60.0	85.0	0.10
	MBC	75.0	70.0	60.0	125.0	90.0	90.0	0.20
E. faecalis	MIC	70.0	60.0	45.0	100.0	115.0	75.0	0.20
	MBC	80.0	80.0	66.0	120.0	135.0	90.0	0.20
K. pneumoniae	MIC	80.0	55.0	70.0	65.0	80.0	70.0	0.20
	MBC	90.0	70.0	80.0	75.0	110.0	90.0	0.20
P. aeruginosa	MIC	80.0	100.0	65.0	70.0	65.0	85.0	0.20
	MBC	85.0	110.0	75.0	125.0	85.0	100.0	0.20
V. cholerae	MIC	90.0	50.0	75.0	110.0	70.0	85.0	0.20
	MBC	95.0	60.0	80.0	130.0	90.0	110.0	0.20
S. typhi	MIC	90.0	50.0	55.0	65.0	100	85.0	0.22
	MBC	100.0	55.0	75.0	75.0	130	125.0	0.24
B. cereus	MIC	75.0	55.0	70.0	80.0	75.0	85.0	0.20
	MBC	80.0	70.0	70.0	120.0	85.0	100.0	0.20
B. subtilis	MIC	80.0	60.0	65.0	70.0	80.0	80.0	0.20
	MBC	90.0	90.0	80.0	85.0	120.0	95.0	0.25
C. freundii	MIC	80.0	50.0	50.0	90.0	90.0	85.0	0.22
	MBC	110.0	70.0	70.0	125.0	110.0	100.0	0.25

Each value represents mean (n = 3).

Acetone extract FTIR spectra (Figure 8) peak values were 3350.35 (OH group), 2924.09 cm^{-1} (CH3-CH2 alkane stretch), 2852.72 cm^{-1} (alkane) and C=O group (1703.14, 1693.50 cm^{-1}). Four considerable peaks were observed in chloroform (Figure 9) and found as an OH, alkane and (C=O) functional groups. Two sharp and strong peaks (2922.16 and 2850.79 cm^{-1}) attributed the existence of alkane group in n-hexane (Figure 10) extract and another two peaks values seen in the region of 3398.57 and 1716.65 cm^{-1} were OH and C=O groups.

DISCUSSION

Plants are always known to provide shelter, food, medicine etc. to human beings and other living organisms. Medicinal plants hold some pharmacologically active values which are used from the ancient times and were exploited in phyto drugs for the cure of different types of diseases.

The physiochemical analysis indicated that sea-buckthorn leaves are very rich source of protein. Due to high protein content of fiber and protein, it can be used as animal feed, and its purified form is also used for human consumption. In sea-buckthorn leaves, protein is one of the important components (chemically) that has an important nutritional value in animal feed and can be used as a source of unconventional protein for human food (Pirie, 1986). The results (Figure 1) showed 10.45/100 g proteins in the sea-buckthorn leaves while *H. rhamnoides* subsp. sinensis leaves were reported to contain an average of 17.1/100 and 16.2/100 g protein in dried leaf (Li and Wardle, 2003). Sea-buckthorn seeds moisture content was 5.5% and ash content was 2.05% (Alam and Ijaz, 2009), which revealed that in our findings, the leaves have high moisture and ash content (Figure 1). Similarly, total sugar content in sea-buckthorn pulp was 2.86% (Tsering et al., 2010), which was very high as

Table 4. Antifungal activities of *Hippophae rhamnoides L.* leaves extracts.

Tested fungus	Zone of inhibition (mm)								
	Aqueous	Methanol	Ethanol	Ethyl acetate	Acetone	Chloroform	n-Hexane	C^-	C^+
A. niger	13±1	15±1	14±1	10±1	08±0	12±0	0.0	0.0	18±0
A. parasiticus	14±0	17±1	12±1	09±0	09±0	11±1	0.0	0.0	22±0
A. flavus	13±1	16±1	10±1	08±1	10±0	09±0	0.0	0.0	20±0
A. fumigatus	09±0	13±0	11±0	11±1	09±0	08±1	0.0	0.0	23±0
A. oryzae	12±0	09±1	11±1	12±0	08±0	09±1	0.0	0.0	24±0
F. oxysporum	16±1	13±0	13±0	13±1	12±1	10±0	0.0	0.0	22±0
R. arrhizus	15±0	14±0	09±0	15±0	11±0	8±0	0.0	0.0	16±0
A. alternata	11±1	18±0	10±0	11±1	09±0	07±1	0.0	0.0	19±0
C. albicans	12±1	14±1	14±1	09±0	10±1	10±1	0.0	0.0	17±0
P. digitatum	07±0	13±1	10±0	08±0	09±1	12±0	0.0	0.0	21±0

C^+ = positive control (Fluconazole 0.5 mg/ml), C^- = negative control (DMSO), each value represents mean ± SD (n = 3). 0 = no zone of inhibition.

Table 5. Antifungal MIC and MFC of *Hippophae rhamnoides L. leaves* extracts.

Tested fungus	MIC/MFC	Concentration mg/ml						
		Aqueous	Methanol	Ethanol	Ethyl acetate	Acetone	Chloroform	C^+
A. niger	MIC	60	50	80	120	120	135	0.35
	MFC	75	80	100	130	145	150	0.40
A. parasiticus	MIC	100	55	90	110	115	95	0.30
	MFC	130	70	100	115	135	105	0.35
A. flavus	MIC	120	85	120	100	100	80	0.35
	MFC	145	90	160	120	140	100	0.40
A. fumigatus	MIC	90	70	100	80	110	100	0.30
	MFC	100	75	115	90	115	115	0.35
A. oryzae	MIC	60	90	130	120	120	110	0.25
	MFC	75	135	140	140	135	115	0.30
F. oxysporum	MIC	50	60	90	100	85	130	0.30
	MFC	65	70	120	120	105	145	0.35
R. arrhizus	MIC	65	55	110	100	105	120	0.50
	MFC	75	85	130	135	115	135	0.50
A. alternata	MIC	80	40	100	120	120	130	0.40
	MFC	100	50	120	130	130	140	0.40
C. albicans	MIC	100	80	80	120	95	95	0.30
	MFC	120	140	95	180	100	105	0.35
P. digitatum	MIC	110	80	80	120	105	100	0.25
	MFC	130	120	90	135	130	150	0.35

Each value represents mean (n = 3).

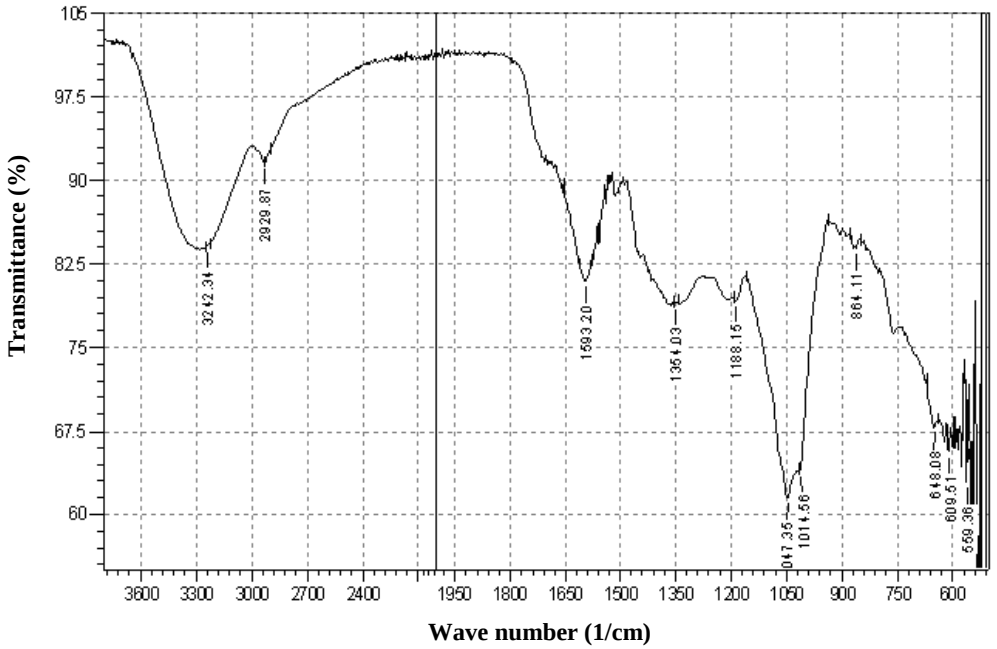

Figure 4. FTIR spectra analysis of aqueous extract of *Hippophae rhamnoides L.* leaves.

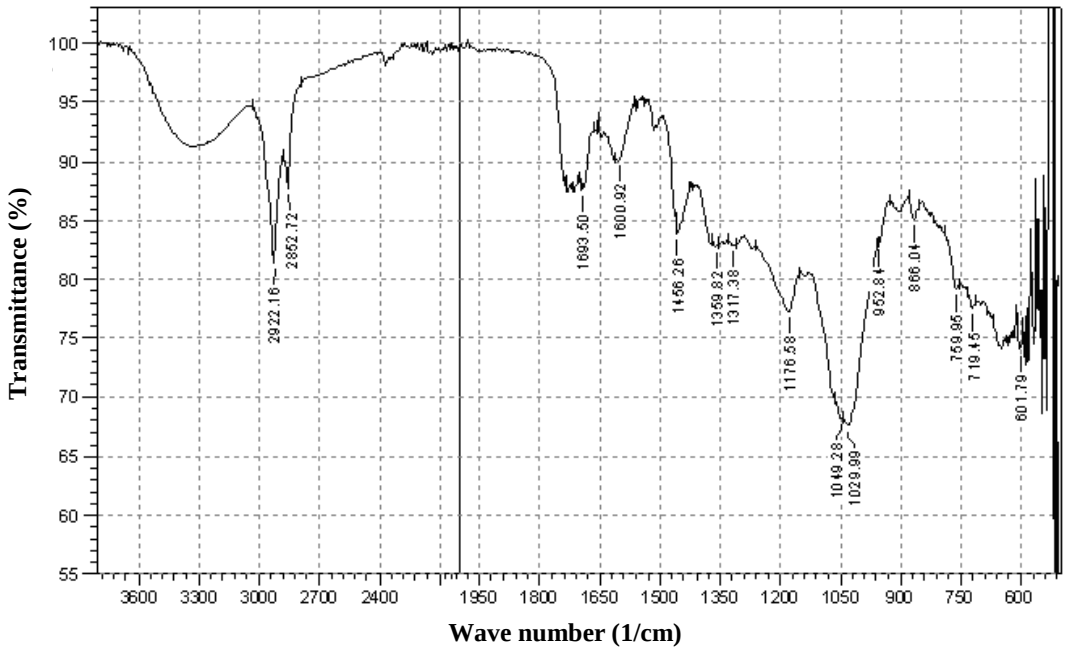

Figure 5. FTIR spectra analysis of methanol extract of *Hippophae rhamnoides L.* leaves.

compared to the sea-buckthorn leaves (Figure 1). Minerals play vital and important role in our body; sodium and potassium normalize acid base balance and osmotic pressure of fluid (body). Similarly, Ca is an important component for the teeth, enzyme (cofactor) and skelton (Okolo et al., 2012). Magnesium and Zn help in the enhancement of muscle regeneration, growth and prevent cardiomyopathy (Chaturvedi et al., 2004).

Previous studies (Alam and Ijaz, 2009) on elemental analysis of sea-buckthorn ssp turkestanica seeds revealed that Na, K, Ca and Fe were found very less as compared to the present study on the seabuckthorn leaves which showed high contents of these elements (Figure 2). In sea-buckthorn pulp, the mineral content

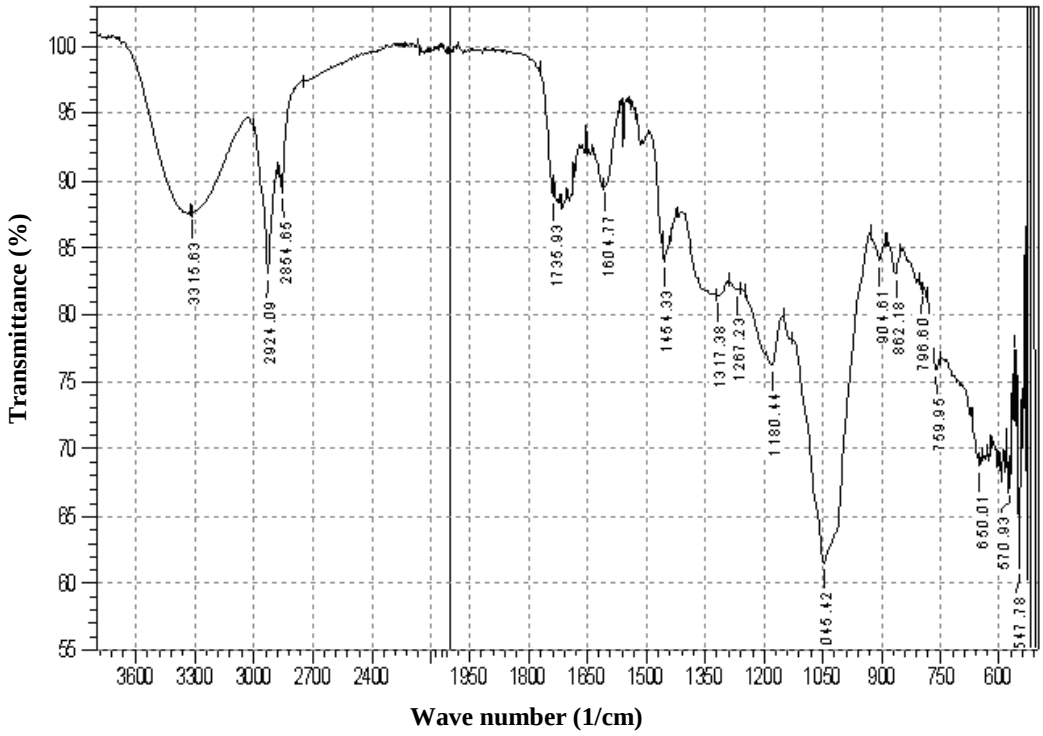

Figure 6. FTIR spectra analysis of ethanol extract of *Hippophae rhamnoides L.* leaves.

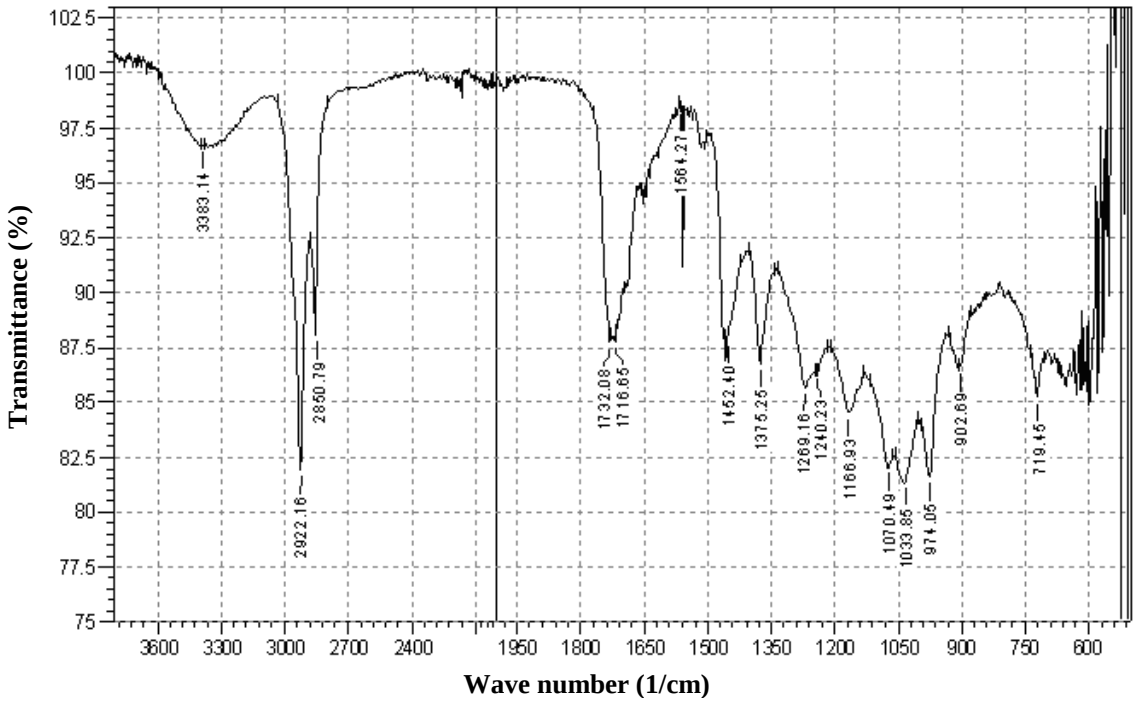

Figure 7. FTIR spectra analysis of ethyl acetate extract of *Hippophae rhamnoides L. leaves*.

(mg/l) of K, Ca, Fe, Mg, Na, Zn and Mn were 647.2, 176.6, 30.9, 22.5, 414.2, 1.4 and 1.06, respectively (Tsering et al., 2010), in sea-buckthorn leaves these minerals values were found high (Figure 2). The present study revealed that the sea-buckthorn leaves are a good source of sodium, potassium and calcium, so it can be

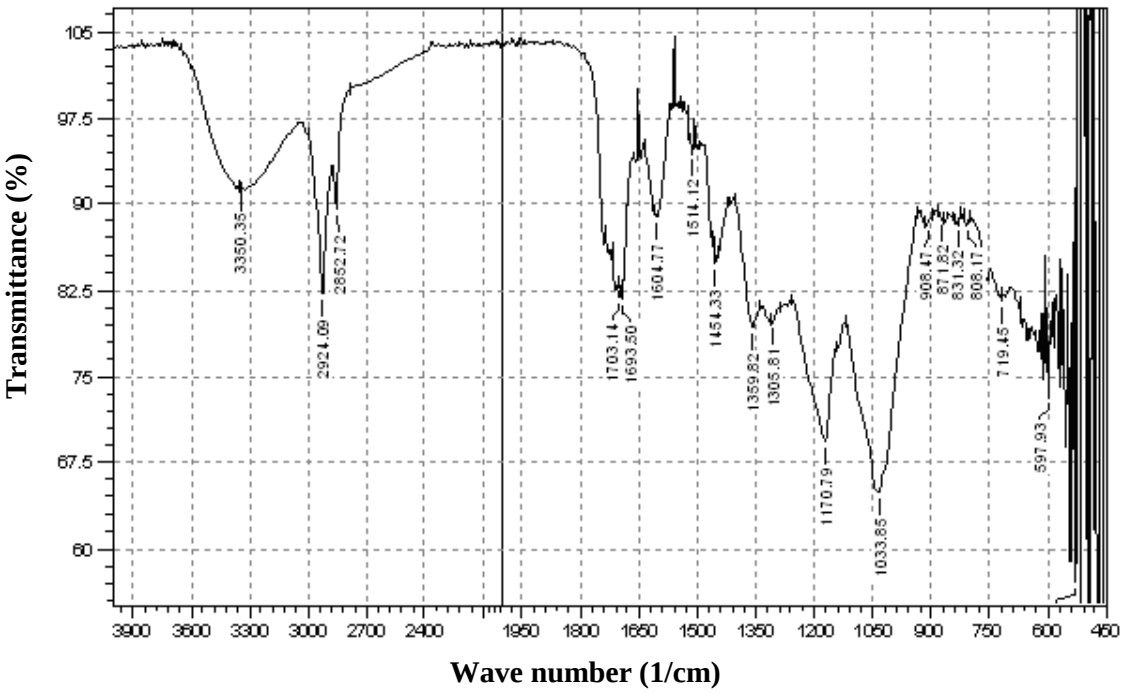

Figure 8. FTIR spectra analysis of acetone extract of *Hippophae rhamnoides L.* leaves.

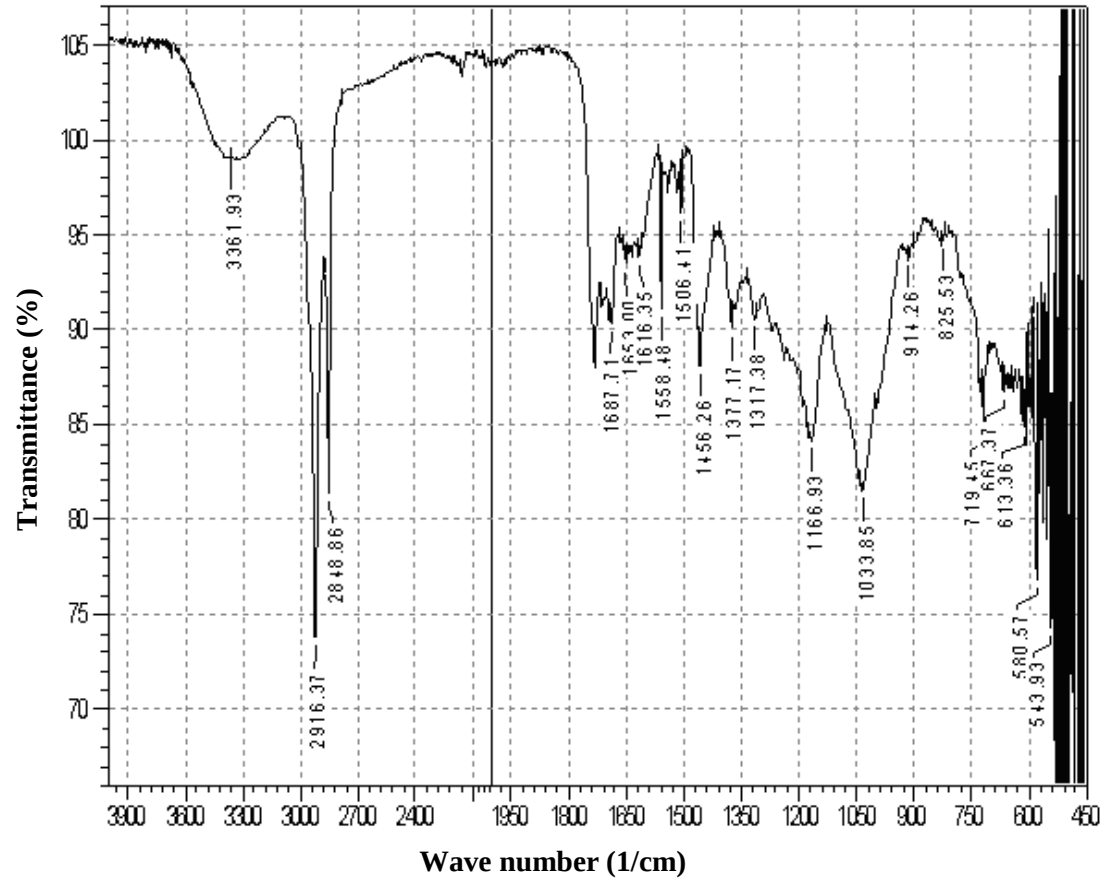

Figure 9. FTIR spectra analysis of chloroform extract of *Hippophae rhamnoides L.* leaves.

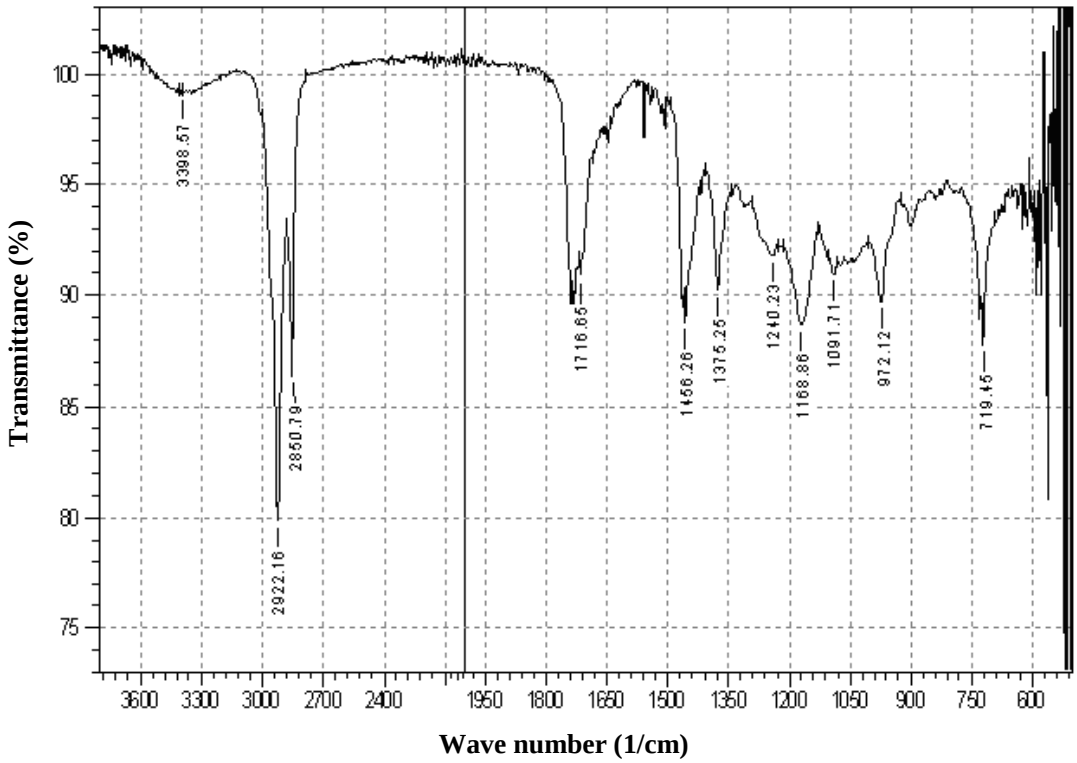

Figure 10. FTIR spectra analysis of n-hexane extract of *Hippophae rhamnoides L*. leaves.

used as animal feed additives as well as for malnutrition cases in human beings.

Phytochemicals

Different types of disease are on the raise globally particularly in the developing countries, and their treatment by synthetic drugs are always associated with many side effects. The use of phytochemical could be a safe way to solve these problems. Phytochemicals commonly produce their antimicrobial activities through special principles which may be different from synthetic drugs (Scalbert, 1991). The tannins containing plants as their main component are astringent in nature and are used for the treatment of stomach disorders such as diarrhea and dysentery (Dharmananda, 2003). Flavonoids are considered as the main phytochemicals present in plant, and are used for the treatment of various types of microbial infections (Xiao, 1980). Alkaloids were absent in methanolic and chloroform + methanolic extracts of sea-buckthorn twigs (Keshab et al., 2010), while present in the leave extracts (Table 1).

Antimicrobials activities

Nowadays, bacterial resistance has increased against a number of valuable antibiotics and it has become essential to search for new sources of antibiotics. Presently, most of the antibiotics available in the market are derived from the natural sources, particularly from different types of plant. The current study has been designed to assess the MIC, MBC and MFC of different extracts of sea-buckthorn leaves against various strains of bacteria and fungi. The susceptibility of the bacteria and fungi to the extracts on the basis of inhibition zones growth varies according to extracting solvent and microorganism. It has been observed that the inhibition zone diameter changed from one another due to variation from plant to plant and organism to organism at diverse concentrations (Mann et al., 2008; El-Mahmood et al., 2008). The hexane extracts however did not exhibit any zone of inhibition against all the test microorganisms; it can be attributed to poor extractive value and absence or low quantity of phytochemicals (Figure 3 and Table 1). Highest activity was verified by the standard antibiotic ciproxin and fluconazole (control). It is not surprising that reference antibiotics have high zone of inhibition, least MIC, MBC and MFC values as compared to the SBL extracts. Because the antibiotic is synthesized by high standard techniques, common plant origin medicines are produced from crude sources which are mostly time exposed to degradation and contamination (El-Mahmood and Amey, 2007).

The MIC values of sea-buckthorn root and stem

extracts against *B. subtilis, S. aureus, E. coli and C. albicans* were reported (Jong et al., 2010) very low as compared to the sea-buckthorn leave extracts (Tables 3 and 5). The sea-buckthorn (*H. salicifolia* D. Don) seed oil, concentrated crude leaf and fresh crude leaf extracts showed no antibacterial and antifungal activity (Sanjay et al., 2011), whereas *H. rhamnoides L*. leave extracts were found active against both bacteria and fungi (Tables 2 to 5). The sea-buckthorn aqueous and methanolic extracts of seeds and pomace showed low zone of inhibition against *B. cereus, S. aureus, E. coli, E. faecalis, K. pneumoniae and P. aeruginosa* reported by Richa et al. (2012), while sea-buckthorn leaves aqueous and methanolic extracts were observed for high zone of inhibition against the studied bacteria. The manifestation of activity against both gram-positive and gram-negative bacteria and fungi is a sign that the plant can be a source of bioactive substance that could be of broad spectrum of activity.

FTIR analysis

FTIR analysis of the SBL extracts shows a strong presence of hydroxyl group which is common in all phenolic compounds. All SBL extracts absorption bands were attributed to (OH) stretching vibrations from phenols, a group of compounds (chemical) containing hydroxyl functional groups (-OH) attached to an aromatic hydrocarbon. Phenolic compounds from natural resources displayed antifungal activity (Soundararajan et al., 2012). The location site(s) and the amount of hydroxyl groups found in the phenols are related to their relative toxicity towards microorganisms, with evidence that increased hydroxylation is directly proportion to toxicity (Geissman, 1963). Also, carboxylic acids were found to be linked with many antimicrobial and antifungal activities which are found to exist in various plant metabolite molecular structures such as ursolic acid, which had been reported as a strong antibacterial agent (Sultana et al., 2010). Many active compounds were produced by plants which contained these active groups (secondary metabolites). Certainly, other chemical components of the extracts could also contribute, although lack of chemical profiling has never been reported on this. It is possible that these compounds are mainly responsible for the antifungal activities observed in this study.

Conclusion

The findings of our study revealed that SBL is a rich source of protein as well as mineral, which could be beneficial to human being, while the extracts strongly inhibited many pathogenic bacterial strains and fungi which can be used for the development of new broad spectrum antibiotics. The SBL leaves are still in use as a traditional herbal medicine, containing a number of useful phytochemicals and providing a scientific data base for further primary health care system.

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