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Proximate composition, mineral contents, phytochemical constituents, antimicrobial activities and Fourier transforms infrared spectroscopy analysis of bark, stem and seed of *Hippophae rhamnoides* Linn

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ABSTRACT

Objective: To compare the proximate composition, mineral contents, antimicrobial, phytochemical and Fourier transforms infrared (FTIR) spectroscopy analysis of bark, stem and seed of *Hippophae rhamnoides*.

Methods: Proximate composition was determined according to the described methods. Mineral analysis was carried out by atomic absorption spectroscopy and flame photometer. Antimicrobial activities were evaluated according to the agar well diffusion method. Phytochemical qualitative analysis was carried out according to the described methods and functional groups were determined by FTIR Prestige-21 Shimadzu Japan.

Results: The proximate analysis showed high content of protein and fiber in stem and bark. High content of Na (900 mg/L) and K (670 mg/L) was found in bark powder, while in seed, high contents of Ca (800 mg/L), Mg (725 mg/L), Fe (250 mg/L) Zn (90 mg/L) and Mn (65 mg/L) were found compared to stem and bark. Phenols, flavonoids and tannins showed high contents in stem and bark of all extracts. The bark aqueous extract showed high zone of inhibition against *Staphylococcus aureus* (21 mm) and *Escherichia coli* (20 mm), while methanol extract of stem showed high zone of inhibition (14 mm and 13 mm) against *Enterococcus faecalis* and *Escherichia coli* respectively. The aqueous extract of bark documented high zone of inhibition against *Aspergillus niger* (21 mm) and *Aspergillus parasiticus* (20 mm). FTIR spectra revealed the presence of OH, C-O and C=O functional groups.

Conclusions: The study concludes that bark, stem and seed extracts will be useful guideline for the new syntheses of feed, food supplements and herb drugs with various combination, which can be used for the treatment of many diseases at global level especially in tropical regions as well as the male nutrition problems in these areas.

1. Introduction

Since prehistoric times, human beings have been relying on plants for their healing and health. Plants and their products are not just food products now, but are being utilized and explored for each probable opportunity. This has evoked researchers to discover each and every plant with fresh new ideas towards its possible use. Key metabolites *i.e.* hormones, carbohydrates, vitamins, proteins, and lipids, are essential for plants to live and reproduce. These primary metabolites provide food, feed and basis of nutrition for the entire living world[1]. Quantification of nutritive value gives important data as it denotes the ability of any plants or plant parts for being utilized as a drug and food[2]. The nutritional value describes chiefly the percentage of main nutritional constituents like fiber, carbohydrates, lipids, proteins and minerals and their food value[1].

Hippophae rhamnoides (*H. rhamnoides*), commonly known as seabuckthorn, is a deciduous shrub with yellow or orange fruits. Such shrub is being domesticated in several countries like China, Russia, Germany, Finland, Romania, France, Pakistan, Nepal and India[3]. Seabuckthorn leaves, seed and pulp were reported with high amounts of vitamins, fatty acids, lipids, carbohydrates, phenolic

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compounds with antibacterial, antifungal and free radical scavenging activities[4,5]. Seabuckthorn has been used conventionally for cure of stomachache, cancers, liver disorder, thrombosis, ulcer, tendon and ligament injuries[6]. In the present study, *H. rhamnoides* bark, stem and seed were evaluated for proximate composition, minerals, phytochemical, antimicrobial activities and Fourier transforms infrared spectroscopy (FTIR) spectra determination to evaluate its nutritional, medicinal values as well as its activity against bacterial and fungal strains causing different diseases.

2. Materials and methods

2.1. Sample collection

The bark, stem and seed of fully grown healthy *H. rhamnoides* were collected from Pakistan Council of Scientific and Industrial Research Skardu, Pakistan. The bark, stem and seed were dried in shade, powdered and transferred into air tight plastic bags until used.

2.2. Extraction

Fifty grams powder of *H. rhamnoides* bark, stem and seed were extracted in 250 mL of distiled water, ethanol and methanol for 48 h. These extracts were then filtered under vacuum through filter paper and the process was repeated for each extract three times. The extracts were concentrated in rotary evaporator to obtain dried residue. Finally crude extracts were stored in air tight bottles and kept at 4 ˚C until used.

2.3. Physiochemical analysis

Physiochemical analysis was performed to determine the moisture, ash, fat, crude fibers and proteins of *H. rhamnoides* bark, stem and seed powder in accordance with procedures described by Maisarah *et al*[7].

2.4. Mineral analysis

Elemental compositions were determined by wet digestion procedure. Minerals like Na and K were determined with the help of Flame Photometer (Jenway PFP7). The concentrations of Ca, Mg, Fe, Mn, Zn, Cu and Ni were determined by atomic absorption spectrophotometer, Hitachi Zeeman Japan Z-8000[8].

2.5. Phytochemical qualitative screening

Phytochemicals were analyzed qualitatively using the described methods[8].

2.6. Tested microorganisms

Bacterial cultures of *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Enterococcus faecalis* (*E. faecalis*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and fungus cultures of *Aspergillus niger* (*A.niger*), *Aspergillus parasiticus* (*A. parasiticus*), *Aspergillus flavus* (*A. flavus*), *Aspergillus fumigatus* (*A. fumigatus*) and *Aspergillus oryzae* (*A. oryzae*) were obtained from Pakistan Council of Scientific and Industrial Research Laboratories Complex Peshawar Khyber Pakhtunkhwa, Pakistan. 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.

2.7. Antibacterial activity

One milliliter of tested bacterial strains (1.0×10^8 CFU/mL) was inoculated into each plate. The nutrient agar at 45 ˚C was added into each plate and homogeneous mixing of the culture and media was made. The dried seabuckthorn extracts (1 000 mg) were dissolved in 5 mL of dimethyl sulfoxide (DMSO). Fifty micro liters of the 200 mg/mL of each extracts were pipetted into each well (6 mm). Fifty micro liters of DMSO and Ciproxin (0.5 mg/mL) solutions were used as negative control and positive control respectively. The plates were incubated at 37 ˚C for 18 h. The antibacterial activity was calculated by determining the zones of inhibition diameters[8].

2.8. Antifungal activity

One milliliter of each spore suspension (10^5 spores/mL) was poured on the surface of Sabouraud dextrose agar plates. Seabuckthorn extracts (1 000 mg) were dissolved in 5 mL of DMSO (200 mg/mL) and 50 μL of each extract was tested into each particular well (6 mm). Fluconazole (0.5 mg/mL) and DMSO (50 μL) each were used as positive and negative control respectively. The plates were kept in incubator for 48 h at 30 ˚C and then anti-fungal activities were calculated in term of inhibition zone[9].

2.9. FTIR analysis

A small quantity (5 mg) of each extract was placed one by one in a sample cup and IR band was gated using FTIR Prestige-21 Shimadzu Japan. The sample was scanned from 3 900 to 500 cm^{-1} and operated at a resolution of 4 cm^{-1} with 10 number of scan.

2.10. Statistical analysis

SPSS version 14 was used to analyze the data. The data were presented in the form of mean ± SD. Values with $P < 0.005$ were considered statistically significant.

3. Results

Moisture is the quality parameter for herbs used as medicine. The bark, stem and seed moisture values were 4.2% ± 0.4%, 3.5% ± 0.1% and 7.0% ± 0.5% respectively. Ash, another quality controlling parameter for herbal medicine, shows the inorganic and organic material present in the herbs. The bark ash value was 5.0% ± 0.5%, stem 4.0% ± 0.3% and seed 3.0% ± 0.2% respectively. Fat or oil has great importance in food. Usually seed contains the greatest amount of fats. The fat content recorded was 1.5% ± 0.1%, 1.0% ± 0.1% and 8.0% ± 0.5% for bark, stem and seed respectively. Crude fiber is very important for healthy life. It plays an important role for the quick removal of waste products from body. The maximum crude fiber (30.0% ± 0.1%) was found in stem and minimum (20.0% ±

0.1%) in seed, while 25.0% ± 0.1% was noted in bark. The crude protein values of 3.0% ± 0.2%, 3.5% ± 0.2% and 1.2% ± 0.1% were calculated in bark, stem and seed respectively.

Metallic elements constitute the inorganic part of herbs. The content of Na was noted for bark, stem and seed with the values of (900 ± 20), (455 ± 10) and (72 ± 4) mg/L. Values for K were (670 ± 20) mg/L for bark, (543 ± 10) mg/L for stem and (52 ± 4) mg/L for seed. Ca was found in bark, stem and seed with the values of (745 ± 20), (567 ± 10) and (800 ± 30) mg/L respectively. The values obtained regarding Mg content were (445 ± 2) mg/L for bark, (248 ± 8) mg/L for stem and (725 ± 20) mg/L for seed. Fe content for stem, bark and seed was (35 ± 2), (70 ± 3), and (250 ± 10) mg/L respectively. Zn was found in bark, stem and seed with the values of (56 ± 2), (75 ± 3) and (90 ± 3) mg/L respectively. Mn content was (48 ± 2) mg/L for bark, (60 ± 3) mg/L for stem and (65 ± 3) mg/L for seed.

Table 1 shows the antibacterial activity of bark, stem and seed extracts. The bark aqueous extract showed highest antibacterial activity against *S. aureus* followed by *E. coli*, *E. faecalis*, *K. pneumoniae* and *P. aeruginosa*. The bark methanolic extract showed maximum activity against *E. coli* (19 mm), and minimum activity against *P. aeruginosa* (11 mm). Ethanolic extract of bark showed antibacterial activity against *S. aureus*, *E. coli*, *E. faecalis*, *K. pneumonia* and *P. aeruginosa* with the zone of inhibition of 13, 18, 16, 17, and 20 mm respectively. The stem extract also showed the trend of increasing activity from methanolic to aqueous extract. The aqueous extract of stem showed maximum activity against *K. pneumoniae* (11 mm) and no activity against *E. faecalis* or *P. aeruginosa*. The methanolic and ethanolic extract of stem showed highest antibacterial activity against *E. faecalis* and *K. pneumoniae* respectively. No tested bacterial strains showed susceptibility to the seed extract.

Table 2 shows the antifungal activities of *H. rhamnoides* extracts. Among the tested extracts, the aqueous extract of bark showed the maximum antifungal activity against *A. niger* (21 mm), and the minimum antifungal activity was showed by methanolic and ethanolic extract of the stem against *A. oryzae*.

Phytochemicals analysis of seed, stem and bark of *H. rhamnoides* is shown in Table 3. Maximum terpenoids content was found in methanolic extracts of seed, while minimum was in aqueous extract of seed. No terpenoids were found in the aqueous extract of stem and all extracts of bark. Moderate content of steroids was detected in methanolic and ethanolic extracts of seed and stem while aqueous extract of seed and all bark extracts contained no steroids. All extracts of seed, stem and bark contained glycosides. Alkaloids were absent in all the tested eztracts. Phenols were detected in all extracts of the seed, stem and bark of *H. rhamnoides*, and maximum phenolic content was found in methanolic and ethanolic extracts of bark. Similarly, Moderate content of flavonoids were detected in stem and bark extracts, while minimum in all seed extracts. Tannins were also found in all three parts of seabuckthorn, but saponins were not detected in all three parts.

The IR spectra of dried powdered materials of bark, stem and seed are shown in Figures 1, 2 and 3 respectively. Different dominant peaks were noted in different ranges, which showed the presence of different constituents in bark, stem and seed of seabuckthorn. The spectra of dried powdered bark were observed with values of 3375.43, 2885.51, 1625.99, 1519.91, 1417.68 and 1060.85 cm^{-1} (Figure 1). The stem showed peak values of 3344.57, 1645.28, 1558.48, 1373.32, 1319.31, 1060.85 and 1018.41 cm^{-1} (Figure 2). Intense band vibrations occurred at 3300.20, 2922.16, 2850.79, 1747.51, 1456.26, 1166.93 and 1041.56 cm^{-1} for seed (Figure 3). All these above prominent peak showed the presence of different functional groups like OH, O-H, C=O, C-H and C-O. These groups or bands are usually present in phenols, flavonoids, tannins, steroids, terpenoids and glycosides.

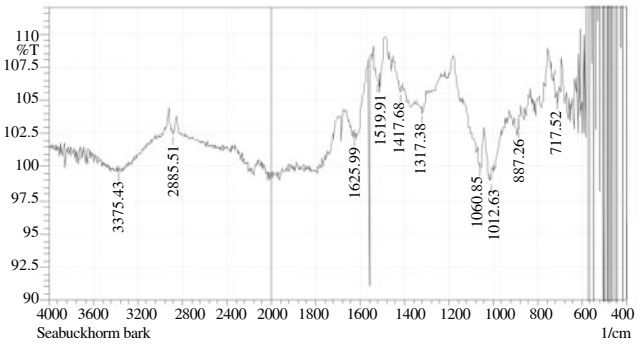

Figure 1. FTIR spectra of *H. rhamnoides* bark.

Table 1

Antibacterial activities of *H. rhamnoides* (mm).

Bacteria	Bark			Stem			Seed			Control	
	Aqueous	Methanol	Ethanol	Aqueous	Methanol	Ethanol	Aqueous	Methanol	Ethanol	C+	C-
S. aureus	21.0 ± 1.0	14.0 ± 1.7	13 ± 0	10 ± 1	12 ± 0	0	-	-	-	28 ± 1	-
E. coli	20.0 ± 2.0	19.0 ± 1.0	18 ± 1	9 ± 0	13 ± 0	10 ± 0	-	-	-	26 ± 1	-
E. faecalis	15.0 ± 1.7	14.0 ± 1.7	16 ± 0	0	14 ± 0	9 ± 0	-	-	-	24 ± 0	-
K. pneumoniae	14.0 ± 1.0	17.0 ± 2.0	17 ± 1	11 ± 0	10 ± 0	13 ± 0	-	-	-	25 ± 1	-
P. aeruginosa	14.0 ± 1.0	11.0 ± 1.7	20 ± 1	0	9 ± 0	0	-	-	-	23 ± 0	-

C+: Positive control; C-: Negative control; -: No zone of inhibition. Each value represents mean ± SD (n = 3), $P < 0.005$.

Table 2

Antifungal activities of *H. rhamnoides* (mm).

Fungi	Bark			Stem			Seed			Control	
	Aqueous	Methanol	Ethanol	Aqueous	Methanol	Ethanol	Aqueous	Methanol	Ethanol	C+	C-
A. niger	21 ± 1	14.0 ± 1.0	13 ± 0	15 ± 0	14 ± 0	12 ± 0	-	-	-	28 ± 1	-
A. parasiticus	20 ± 2	19.0 ± 1.0	18 ± 1	13 ± 0	11 ± 0	13 ± 0	-	-	-	26 ± 1	-
A. flavus	15 ± 1	14.0 ± 1.0	16 ± 0	11 ± 0	13 ± 0	10 ± 0	-	-	-	24 ± 0	-
A. fumigatus	14 ± 1	17.0 ± 2.0	17 ± 1	10 ± 0	10 ± 0	9 ± 0	-	-	-	25 ± 1	-
A. oryzae	14 ± 1	11.0 ± 1.7	20 ± 1	9 ± 0	8 ± 0	8 ± 0	-	-	-	23 ± 0	-

C+: Positive control; C-: Negative control; -: No zone of inhibition. Each value represents mean ± SD (n = 3), $P < 0.005$.

Table 3

Phytochemicals analysis of seeds, stem and bark of *H. rhamnoides*.

Phytochemicals	Seed			Stem			Bark		
	Methanol	Ethanol	Aqueous	Methanol	Ethanol	Aqueous	Methanol	Ethanol	Aqueous
Terpenoids	+++	++	+	+ +	+ +	-	-	-	-
Steroids	++	++	-	+ +	+	+	-	-	-
Glycosides	++	+	+	+ +	+ +	+	+	+	+
Alkaloids	-	-	-	-	-	-	-	-	-
Phenols	+	+	+	+ +	+ +	+ +	+++	+++	++
Flavonoids	+	+	+	+ +	+ +	+ +	++	++	++
Tannins	+	+	+	+ + +	+ +	+ +	++	++	++
Saponins	-	-	-	-	-	-	-	-	-

+: Small quantity; ++: Average quantity; +++: Large quantity; -: Not detected.

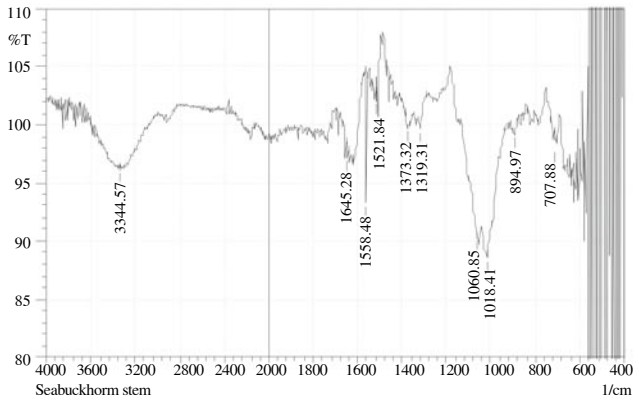

Figure 2. FTIR spectra of *H. rhamnoides* stem.

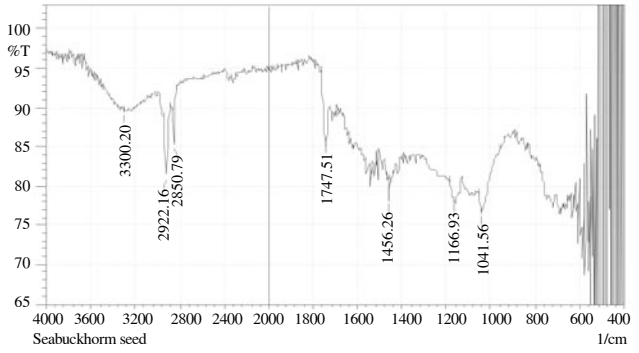

Figure 3. FTIR spectra of *H. rhamnoides* seed.

4. Discussion

The physiochemical analysis indicated that the stem, bark and seed of seabuckthorn were a chief source of proteins and fibers, due to which it can be used as animal feed and its purified form can also be used for human consumption. The presence of higher protein levels in the plants increase food value or that protein base bioactive compounds could also be isolated in future[10].

Minerals play a vital and important role in our body; Na and K normalize acid base balance and osmotic pressure of body fluid. Ca is a vital constituent for skeleton, teeth and enzyme[11]. Zn is needed for normal insulin secretion, wound healing, normal growth and development, brain health and behavioral development. Fe is an important trace element. It is part of hemoglobin and plays a role in the metabolism of lipids, carbohydrates and proteins. Its deficiency can lead to anemia[12]. Mn is needed for proper functioning of enzymes[12]. The high amount of these minerals found in *H. rhamnoides* indicated that the plant could be a good source of dietary minerals to overcome nutritional deficiency of specific element, if supplemented in the diet.

Phytochemical constituents such as alkaloids, glycosides, reducing sugar, flavonoids, tannins, saponins and several other organic compounds are secondary metabolites of medicinal plants that serve as defense mechanism against many microorganisms and insects[13]. These secondary metabolites exert antimicrobial activity through different mechanisms. Tannins have been found to form irreversible complexes with prolin-rich proteins resulting in the inhibition of cell protein synthesis[9]. These days, microbial infections have blown up in spite of numerous expensive antibiotics, and it has become necessary to find out novel antibiotics. Nowadays, most antibiotics launched in the marketplace are originated from natural origin, especially from diverse kinds of plants. The susceptibility of the fungi and bacteria to the extracts on the basis of inhibition zones differs due to microbes and extracts. It was concluded that inhibition zone diameter differed from one another due to difference from organism to organism and plant to plant at varied quantity[14,15]. *K. pneumoniae* is non-motile, rod shaped, Gram negative and causes a bacterial pneumonia. *E. coli* is the rod Gram negative bacteria which causes diarrhea. *E. fecalis* is blamed for the illnesses of urinary tract and surgical wounds. *S. aureus* has the liability to produce numerous poisons, which adds the bacterium's pathogenicity through increasing its capacity to attack the body and injure tissues[16].

Fungi of the genus *Aspergillus* are filamentous microorganisms that are saprophyte in nature and generate spores in the air. Rarely do they turn pathogenic in man and be responsible for diverse of lesions, especially pulmonary ailment[16]. It is not amazing that standard antibiotics have high zone of inhibition as compared to stem, bark and seed extracts of seabuckthorn. For the reason that the antibiotic is manufactured by standard of the art technology, ordinary phyto medicines are synthesized from raw sources which generally exposed to contamination and degradation[17]. The alkaloids, flavonoids, tannins and phenolic compounds are toxic to microbial cells[9]. The differences observed in the antibacterial activities of the extracts as observed in the present work could be due to the differences in their phytochemical

composition[18].

The FTIR spectra of all leaf extracts of seabuckthorn showed the existence of diverse functional groups ranging from hydroxyl (OH) stretching for hydroxyl group, alkenes, aromatic rings, alkanes, carboxylic and amides[8]. Phenolic constituents derived from plant origin exhibited fungicidal activity[19]. The hydroxyl groups amount and location site(s) found in the phenolic compound are linked to lethality towards bacteria and fungi[20]. It was found that carboxylic acids were associated with a lot of bactericidal and fungicidal properties which exist in many plant metabolites molecular framework[21]. Numerous dynamic components were originated from plants which exhibited these bioactive compounds, which chiefly are accountable for the antimicrobial potency examined in this research.

The current study shows that seabuckthorn stem, bark and seeds are a rich source of fibers, proteins as well as minerals, which could be beneficial to human beings, while stem and bark extracts strongly inhibit many pathogenic bacterial strains and fungi, and can be used for the development of new broad spectrum antibiotics. Our research provides a scientific data base for further primary health care system.

Conflict of interest statement

We declare that we have no conflict of interest.

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