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Antioxidant and antibacterial activity of leaf, bark, pulp and seed extracts of seabuckthorn (*Hippophae salicifolia* D. Don) of Sikkim Himalayas

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The present study was conducted to evaluate the antioxidant and antibacterial activities of methanol, acetone, chloroform and petroleum ether extracts of leaf, bark, pulp and seed of Seabuckthorn (*Hippophae salicifolia* D.Don) species found in Sikkim Himalayas. Antioxidant activity was measured using total phenolic content, reducing power and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays. The extraction yield and total phenolic contents were significantly higher in seed extracts with methanol being the most efficient solvent. Methanolic extract of seeds showed the highest reducing power and DPPH-radical scavenging activity among all the extracts. Antibacterial activity was tested against six pathogenic strains viz. *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Enterobacter aerogenes* and *Pseudomonas aeruginosa* by agar diffusion and broth macrodilution methods. The extracts were found to exert low to strong antibacterial activity compared to a standard antibacterial agent. The results showed that seed and pulp extracts were better radical scavengers than leaf and bark extracts. Both antioxidant and antibacterial activities were found to be higher in methanolic extract. Strong correlation ($P < 0.05$) was observed between total phenolic content versus antioxidant and antibacterial activity from the extracts under study.

Keywords: *Hippophae salicifolia*, antioxidant activity, antibacterial activity, phenolic content, methanolic extract.

INTRODUCTION

Oxidation is a natural process in organisms, for the production of energy to fuel biological cycles. Conversely, the uninhibited production of oxygen-derived free radicals is involved in the onset of many diseases such as arthritis, atherosclerosis, rheumatoid and cancer, as well as in many degenerative diseases related with ageing (Halliwell and Gutteridge, 1984). Plants contain a large variety of compounds that provide unlimited opportunities for new drug discoveries because of the unmatched chemical diversity they can provide (Cos et al., 2006). These compounds exhibit a wide range of biological activity, antimicrobial activity and antioxidant properties (Ren et al., 2003; Tapiero et al., 2002). Therefore in recent years, considerable attention has been directed towards identification of new bioactive compounds from natural sources that may be used for human consumption. *Hippophae salicifolia* D. Don. (Vernacular - Tarubo), a deciduous tree, which belongs to the family Elaeagnaceae, is restricted to the Himalayan region between 1,500 to 3,500 m amsl (Hooker, 1894; Gaur 1999). It has been reported as one of the best species of genus Hippophae in terms of high quality fruit, high yield and less thorns (Lu et al., 2001). There are a total of five species based on morphological variations: *H. rhamnoides* L.,

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H. salicifolia D. Don, *H. neurocarpa* Liu and He, *H. tibetana* Schlecht and *H. goniocarpa* Y. S. Lian et al. Three species of Hippophae namely *H. rhamnoides*, *H. salicifolia* and *H. tibetana* are found in India. Out of these, only *H. salicifolia* D. Don has been reported in Lachen and Lachung Valley of Sikkim (Baisistha et al., 2010).

Various parts of Seabuckthorn (SBT) plant, especially the berries, were used in traditional medicine, mainly in the Tibetan, Mongolian, Chinese and Central Asian systems (Cheng et al., 2003). Seabuckthorn oils, juice, leaves and bark are well known for their medicinal properties, and they have been used to treat high blood lipid symptoms, gingivitis, eye and skin ailments, and cardiovascular diseases (Liu et al., 1980; Yang et al., 2000). The berries have been used as a raw material for foods and medicines for decades in China and Russia (Yang et al., 2000; Cheng et al., 2003). High amounts of vitamin C, flavonoids, oils and oil-soluble compounds, as well as minerals, are present in the berry (Kallio et al., 2000). In addition to medicinal use, the berries are processed into various products such as juice and marmalade, and used for flavouring of dairy products because of their unique taste (Gao et al., 2000).

Berry products of SBT are among popular foods in the United States, Canada, Finland, Germany, and some other European countries (Li and Schroeder, 1996). Leaves are also reported to possess anti-inflammatory properties (Padwad et al., 2006). In recent years, extracts from this plant have been used increasingly in the U.S. as a dietary supplement. Seabuckthorn contains a series of compounds including carotenoids, tocopherols, sterols, flavonoids, lipids, ascorbic acid, and tannins. These compounds are of interest not only from the chemical point of view, but also because many of them possess biological and therapeutic activity including, antioxidant, antitumor, hepato-protective, and immunomodulatory properties (Cheng et al., 2003). The leaves of *H. rhamnoides* were considered for their antioxidant potential correlated to flavonoides and phenolic acids derivatives (Kim et al., 2011; Sharma et al., 2008; Upadhyay et al., 2011). Antimicrobial activities have also been reported for Seabuckthorn leaves (Upadhyay et al., 2011). The proximate composition of SBT has been well documented (Gao et al., 2000). There are reports on chemical composition, characterization and health benefits of SBT fruit and its oil (Chen et al., 1990; Yang et al., 2000). Also there have been reports on antioxidant and antibacterial activities of various SBT (*H. rhamnoides* L.) seed extract (Negi et al., 2005; Michel et al., 2012). However, there is no information available on antioxidant and antibacterial activity of *H. salicifolia* found in North East India, except a preliminary report on antioxidant activity of leaf and bark by Goyal et al. (2011). Therefore, the objective of the present study was to evaluate the comparative account of antioxidant properties of *H. salicifolia* leaf, bark, pulp and seed, and determine their antibacterial activity against different food-borne and clinical microorganisms for their therapeutic potential.

MATERIALS AND METHODS

Chemicals and reagents

DPPH (2,2-diphenyl-1-picryl-hydrazyl) and catechin were purchased from Sigma–Aldrich (USA). Gallic acid was obtained from HiMedia Laboratories (India). Sodium carbonate (Na_2CO_3), phosphate buffer, potassium ferricyanide, trichloroacetic acid, ferric chloride, ascorbic acid, Mueller Hinton agar, dimethylsulfoxide (DMSO) and methanol were purchased from Merck (India). Folin-Ciocalteu reagent was from SRL (India). All chemicals and solvents were of analytical grade.

Plant extracts

Leaf, bark, pulp and seed of *H. salicifolia* were collected from Lachen valley of North Sikkim district lying between 27° 46′ 31″ and 27° 42′ 30″ North latitude and 88° 30′ 00″ and 88° 35′ 00″ East longitude. Leaf and bark samples were collected during the month of July, and ripe fruits were collected in November. The species was authenticated with the voucher specimen at the Botanical survey of India, Shillong. The plant extracts were prepared based on the method described previously by Haq et al. (2011), with minor modifications. The samples collected were washed using tap water and dried in an incubator at 40°C. Dried samples were ground to produce fine homogenous powder using an electric blender and the powder (10 g) was soaked in 100 ml of selected solvents (methanol, acetone, chloroform and petroleum ether) at room temperature in the dark, for three days. Each sample was filtered through Whatman no. 1 filter paper (Whatman International, England) and the filtered solutions were then evaporated to dryness using water bath at 40°C overnight. The plant extracts were then stored at 4°C.

Determination of the total phenolic contents

The amounts of phenolic compounds in the extracts were determined using Folin-Ciocalteu method, with certain modifications (Waterman and Mole, 1994), and Gallic acid was used as the standard phenolic compound. The extract solution in appropriate solvent (0.1 ml) was transferred to a volumetric flask containing 3 ml of distilled water. After that, 0.5 ml of Folin-Ciocalteu reagent was added. Three minutes later, 2 ml of 20% sodium carbonate solution was added. Subsequently, the shaken mixture was placed in boiling water for exactly one minute, cooled and then measured at 650 nm. The experiment was carried out in triplicate and the content of total phenolic compounds was calculated using a standard curve prepared with gallic acid.

Determination of antioxidative activity

Reducing power

The reducing power was based on the method described previously by Yildirim et al. (2001). Different concentrations of extracts and ascorbic acid in 1 ml of methanol were mixed with 2.5 ml of phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 ml of 10 g/L potassium ferricyanide. The mixture was incubated at 50°C for 30 min. An aliquot (2.5 ml) of 100 g/L trichloroacetic acid was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. Finally, 2.5 ml of the upper layer solution was mixed with 2.5 ml of

distilled water and 0.5 ml of 1 g/L $FeCl_3$, and the absorbance of the resulting solution was measured at 700 nm.

DPPH- radical scavenging activity

The stable free radical scavenging activity was determined by the 1,1-diphenyl-2-picryl-hydracyl (DPPH) method of Shyur et al. (2005), with minor modifications. The assay was performed in 3 ml reaction mixture containing 2 ml of 0.1 mM DPPH methanol solution, 0.9 ml of 50 mM tris-HCl buffer (pH 7.4) and 0.1 ml of test extract at different concentrations or catechin and ascorbic acid (standard reference).

The mixture was incubated at room temperature for 30 min and then the absorbance was measured at 517 nm. Radical scavenging activity is represented as inhibition (%) of DPPH radical which is calculated by the following formula:

$$\text{Inhibition (\%)} = [(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}) / \text{Absorbance}_{\text{control}}] \times 100$$

IC_{50} is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color) (Molyneux, 2004). IC_{50} of reference antioxidant compound, catechin, was used for comparison with IC_{50} of the extracts.

Determination of antibacterial activity

Test microorganisms

All the microbial cultures used for antimicrobial screening were produced from the "Microbial Type Culture Collection and Gene Bank" (MTCC), Chandigarh, India. A total of six bacterial species were tested including, *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 739), *Klebsiella pneumonia* (MTCC 432), *Enterobacter aerogenes* (MTCC III) and *Pseudomonas aeruginosa* (MTCC 424). The bacterial culture was maintained on nutrient agar slants which were stored at -4°C.

Antibacterial assays

The antibacterial activity was based on agar well diffusion method using bacterial cell suspension whose concentration was equilibrated to a 0.5 McFarland standard. A 100 µl of each bacterial suspension was spread on a Mueller Hinton agar plate. Wells (6 mm diameter) were impregnated with 10, 30 and 50 µl of each extract dissolved in DMSO at a concentration of 100 mg/ml. The wells were allowed to dry and then placed in the incubator at 37°C for 24 h.

Wells with the solvent used for dissolution were used as negative control and 1 mg/ml amoxicillin were used as positive controls. After incubation time, zone of inhibition was measured. The experiment was performed in triplicate.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibition concentrations of the extracts were evaluated for the bacterial strains which were determined as being sensitive to the extracts in the well diffusion assay. A broth macrodilution method was used, as previously described by Nakamura et al. (1999), with a slight modification. Serial two-fold dilutions of each extract were prepared in DMSO at a concentration of 2 mg/ml, and 2 ml of each dilution was added to 2 ml of nutrient broth. These were inoculated with 50 µl of culture of the test bacterial strains. After incubation of the cultures at 37°C, the MIC value was determined as the lowest concentration of the extract that demonstrated no visible growth.

Statistical analysis

All experimental results were expressed as means ± standard deviation (SD). Analysis of variance was performed by ANOVA procedures. Correlation coefficient (R) was used to determine two variables. The results with $P < 0.05$ were regarded to be statistically significant.

RESULTS AND DISCUSSION

Extraction yield

The extraction yields (g/100 g) from various solvents, that is methanol, acetone, chloroform and petroleum ether are presented in Table 1. The extraction yield depends on solvents, time and temperature of extraction, as well as the chemical nature of the sample.

Under the same time and temperature conditions, the solvent used and the chemical property of the sample are the two most important factors (Shimada et al., 1992). In the present study, the obtained extraction yields of *H. salicifolia* for the different tested solvents were ranked in the following order: Methanol > Acetone > Chloroform > Petroleum ether.

Methanol is a polar solvent and is known to extract a wide range of molecules including sugar, glycoside and weakly polar compounds. Negi et al. (2005) already reported for better extraction of SBT seeds using methanol. The extraction yield was found maximum for pulp, however significant extraction was found for leaf and seed compared to bark (Table 1). This result was similar to the result reported by Michel et al. (2012) where extraction was more efficient for leaves and seeds.

Total phenolics content

The total phenolic contents of the extracts were determined by Folin-Ciocalteu method. It is well known that plants contain many phenolic compounds which contain a hydroxyl group on an aromatic ring. These phenolic compounds interrupt chain oxidation reactions by donation of a hydrogen atom or chelating metals. So they act as reducing agents and antioxidants (Bursal and Koksal, 2011).

The high amounts of phenolic compounds of the leaf, bark, pulp and seed extracts were found to be in the following order: methanol extract > acetone extract >

chloroform extract > petroleum ether extract (Table 1). The assays were performed in the whole extracts, as that could be more beneficial than isolated constituents because of the additive and synergistic effects, and considering that a bioactive individual component can change its properties in the presence of other compounds present in the extracts (Liu, 2003). A significant variation of phenolic content was observed in different extracts. The phenolic content of SBT extracts ranged from 11.2 to 156.7 mg/g.

The total phenolic content of seed and pulp extracts were significantly higher than leaf and bark extracts. Variations in the yields and phenolic contents of various extracts are attributed to polarities of different compounds present in the leaf, bark, pulp and seed, and such differences have been reported in the literature for other fruit seeds (Jayaprakasha et al., 2001).

Reducing power

The extracts obtained by various solvent extractions were determined for their antioxidant activities. The extracts were investigated for the reductive capabilities by using the potassium ferricyanide reduction method. The reducing ability may serve as a significant indicator of potential antioxidant activity (Meir et al., 1995). It appears that antioxidative activity may have a mutual correlation with the reducing effect. The leaf, bark, pulp and seed extracts increased in reducing powers with increasing concentration (Figure 1), and the seed and pulp extracts exhibited higher reducing ability than the leaf and bark extracts. The methanolic extract showed the highest activity, followed by the acetone, chloroform and petroleum ether extracts. When compared to the ascorbic acid, the methanolic fraction showed higher activity ($P < 0.05$) at all concentrations.

In the present study, the correlation coefficient (R^2) between the total phenolic content versus reducing power of *H. salicifolia* leaf, bark, pulp and seed extracts was found to be 0.93, 0.95, 0.96 and 0.98, respectively. This correlation coefficient suggests that the reducing power of different *H. salicifolia* extracts were positively correlated with their corresponding antioxidant activities. Yen and Chen (1995) reported that the extract which showed a reducing power could function as an donor and also could reduce the oxidized intermediates generated from the lipid peroxidation reaction.

DPPH radical scavenging activity

The free radical scavenging capacity of the extracts against common free radicals (DPPH) *in vitro* were further determined. The results indicated that the extracts exhibited a potential free radical scavenging activity. The inhibition percentage of the radical scavenging activity of the leaf, bark, pulp and seed extracts were calculated and are illustrated in Figure 2. The results revealed that the extract with the highest effective radical scavenging activity was the methanol extract, followed by the acetone and chloroform extract, while lower activities were found in the petroleum ether extracts. The seed and pulp extracts had significantly higher antioxidant properties han leaf and bark extracts. This result was correlated with reducing power assay where seed and pulp extracts were also found more significant than leaf and bark extracts. The antioxidant potential was determined to be in the following order: seed > pulp > leaf > bark extracts. This result is in correlation with data found in Sharma et al. (2008) and Michel et al. (2012), where seeds have more antioxidant capacity than leaves.

The total phenolic contents could be regarded as an important indication of antioxidant properties of plant extracts (Wang et al., 2010). In the present study, R^2 between the total phenolic content versus free radical scavenging activity of *H. salicifolia* leaf, bark, pulp and seed extracts was found to be 0.90, 0.92, 0.94 and 0.90, respectively. This correlation coefficient suggests that the phenolic compounds of *H. salicifolia* extracts contributed 90 to 98% to their antioxidant activities. The significant correlation between the phenolic content and the antioxidant activity of various vegetable extracts has been previously observed (Velioglu et al., 1998). There are many types of compounds possessing antioxidant activity in higher plants (Larson, 1988) and the phenolic compounds were highlighted to be the potential antioxidants (Yu et al., 2005). The fact that phenolic compounds possess a high potential to scavenge radicals can be explained by their ability to donate a hydrogen atom from their phenolic hydroxyl groups (Sawa et al., 1999). The inhibitory effects on lipid peroxidation and autoxidation of linoleic acid have been attributed to the radical scavenging activity (Hatano et al., 2002). Thus, it can be noted that the strong antioxidant properties may be attributed to the phenolic components in the extracts.

Antibacterial activity

The results of the antibacterial activity of methanol, acetone, chloroform and petroleum ether extracts of *H. salicifolia* leaf, bark, pulp and seed are given in Table 2. The obtained antibacterial activities were categorized as follows: (a) strong: for inhibition above 70%; (b) moderate: for inhibition within 50 to 70% or; (c) weak: for inhibition below 50% (Chan et al., 2007). The antimicrobial activity of the tested extracts showed different selectivity for each microorganism. The results revealed that *S. aureus* showed strong inhibition for seed (81%) and leaf (75%) extracts, *E. aerogenes* showed moderate inhibition for leaf (66%), pulp (53%) and seed (66%) extracts and *B. subtilis* showed moderate inhibition for leaf (52%) extracts, respectively whereas weak inhibition was found for *P. aeruginosa, K. pneumonia* and *E. coli* for all organs extracts. Antimicrobial activity of SBT extracts

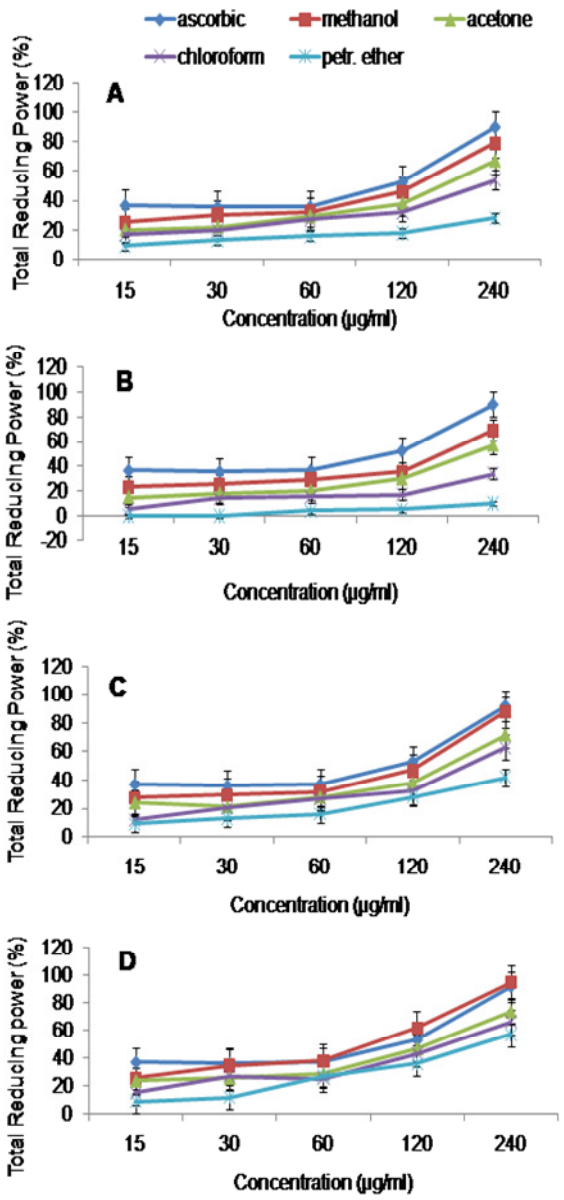

Figure 1. Total reducing power of leaf (A), bark (B), pulp (C) and seed (D) extracts of *Hippophae salicifolia* D. Don. Data are presented as mean ± SD of triplicate determinations.

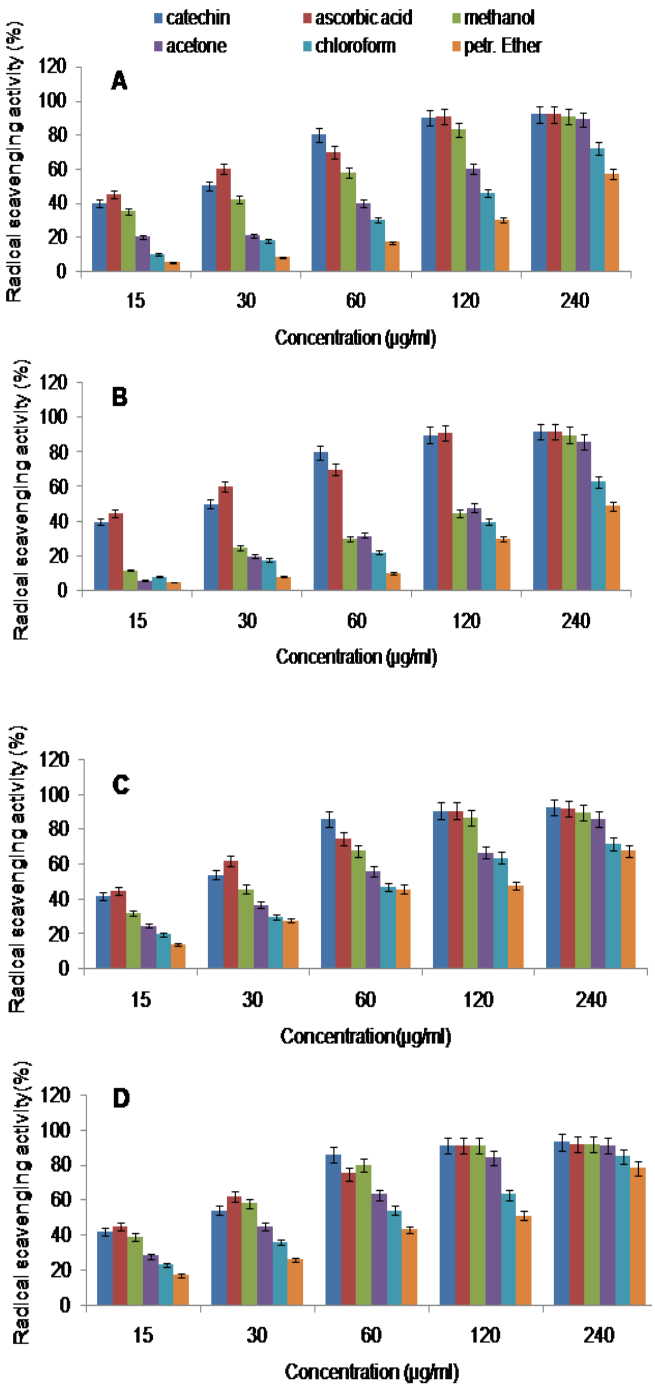

Figure 2. Percentage inhibition concentration for DPPH radical scavenging activity of leaf (A), bark (B), pulp (C) and seed (D) extracts of *Hippophae salicifolia* D. Don. Data are presented as mean ± SD of triplicate determinations.

was found against both Gram negative and Gram positive strains which was already found by Negi et al. (2005) when they worked on *H. rhamnoides* seed. The activity of methanolic extract was the most efficient for *S. aureus* and *E. aerogenes.* The petroleum ether extract of leaf and bark showed no activity against all microorganisms tested. However *E. coli* was not affected by all extracts tested.

There were substantial differences between the MIC of various extracts. The methanolic extracts had the most effective antibacterial activity, followed by acetone, chloroform and petroleum ether extracts (Table 3). These results verify the earlier studies that methanol is the better solvent for more consistent extraction of antimicrobial substances compared to the other solvents (Ahmad et al., 1998; Natarajan et al., 2005). Gram negative strains were most resistant to all the extracts, and higher MIC values were obtained for it. It is worth noting that all

Table 1. Extraction yield and total phenolics content of *Hippophae salicifolia* extracts of leaf, bark, pulp and seed extracts.

Plant extract	Extraction yield (g/100 g)	Total phenolics (mg/g) GAE
Leaves		
Methanol	20.1	98.7±1.5[a]
Acetone	15.2	64.6±1.2[c]
Chloroform	8.64	42.4±1.7[d]
Pet. Ether	1.02	25.9±0.2[a]
Bark		
Methanol	18.7	85.3±1.8[e]
Acetone	12.5	45.8±0.2[a]
Chloroform	7.95	21.6±0.5[b]
Pet. Ether	0.84	11.2±0.5[a]
Pulp		
Methanol	26.5	123.5±1.2[c]
Acetone	21.8	105.6±0.2[a]
Chloroform	11.4	76.3±0.2[a]
Pet. Ether	4.8	32.4±1.6[d]
Seed		
Methanol	17.2	156.7±1.8[e]
Acetone	14.9	117.6±0.2[a]
Chloroform	7.7	53.9±0.4[b]
Pet. Ether	2.1	42.3±0.2[a]

Mean values ± standard deviations (n = 3) with the same letter are not significantly different ($P < 0.05$). GAE: garlic acid equivalent.

Table 2. Antimicrobial activity of the extracts of *Hippophae salicifolia* leaf, bark, pulp and seed (50µL/well) against the tested microorganisms based on agar well diffusion method.

Plant extract	Zone of inhibition; mm (inhibition %)					
	S. aureus	***B. subtilis***	***E. aerogenes***	***P. aeruginosa***	***K. pneumonia***	***E. coli***
Leaf						
Methanol	24 (75)$^{++}$	15 (44)$^{+}$	14 (46)$^{+}$	14 (42)$^{+}$	12 (37)$^{+}$	10 (27)$^{+}$
Acetone	22 (68)$^{++}$	18 (52)$^{++}$	13 (43)$^{+}$	13 (39)$^{+}$	12 (37)$^{+}$	-
Chloroform	14 (43)$^{+}$	10 (29)$^{+}$	20 (66)$^{++}$	14 (42)$^{+}$	11 (34)$^{+}$	-
Pet. Ether	-	-	-	-	-	-
Bark						
Methanol	21(65)$^{++}$	16 (47)$^{+}$	14 (46)$^{+}$	14 (42)$^{+}$	11 (34)$^{+}$	9 (24)$^{+}$
Acetone	20 (62)$^{++}$	15 (44)$^{+}$	13 (43)$^{+}$	11 (33)$^{+}$	12 (37)$^{+}$	-
Chloroform	13 (40)$^{+}$	11 (32)$^{+}$	14 (46)$^{+}$	12 (36)$^{+}$	9 (28)$^{+}$	-
Petr. Ether	-	-	-	-	-	-
Pulp						
Methanol	18 (56)$^{++}$	10 (29)$^{+}$	16 (53)$^{++}$	12 (36)$^{+}$	12 (37)$^{+}$	10 (27)$^{+}$
Acetone	16 (50)$^{++}$	15 (44)$^{+}$	15 (50)$^{++}$	10 (30)$^{+}$	12 (37)$^{+}$	9 (24)$^{+}$
Chloroform	12 (37)$^{+}$	9 (26)$^{+}$	12 (40)$^{+}$	9 (27)$^{+}$	11 (34)$^{+}$	-
Pet. Ether	10 (31)$^{+}$	9 (26)$^{+}$	10 (33)$^{+}$	9 (27)$^{+}$	10 (31)$^{+}$	-

Table 2. Contd.

Seed						
Methanol	26 (81)$^{++}$	16 (47)$^{+}$	20 (66)$^{++}$	12 (36)$^{+}$	12 (37)$^{+}$	12 (32)$^{+}$
Acetone	19 (59)$^{++}$	15 (44)$^{+}$	17 (56)$^{++}$	10 (30)$^{+}$	11 (34)$^{+}$	9 (24)$^{+}$
Chloroform	16 (50)$^{++}$	9 (26)$^{+}$	14 (46)$^{+}$	10 (30)$^{+}$	10 (31)$^{+}$	-
Petr. Ether	16 (50)$^{++}$	10 (29)$^{+}$	15 (50)$^{++}$	9 (27)$^{+}$	9 (28)$^{+}$	-
Standard						
Amoxicillin	32	34	30	33	32	37

Values in parentheses are the inhibition percentages compared to standard antibacterial agent. +Weak inhibition; ++moderate inhibition; - no inhibition zone.

Table 3. The minimum inhibition concentration (MIC) values of the extracts of *Hippophae salicifolia* leaf, bark, pulp and seed extract.

Plant extract	Concentration of extract (µg/ml)					
	S. aureus	***B. subtilis***	***E. aerogenes***	***P. aeruginosa***	***K. pneumonia***	***E. coli***
Leaf						
Methanol	250	250	250	250	500	500
Acetone	250	250	250	500	500	ND
Chloroform	250	250	500	500	500	ND
Pet. Ether	ND	ND	ND	ND	ND	ND
Bark						
Methanol	250	250	250	250	500	500
Acetone	250	250	250	500	500	ND
Chloroform	250	250	500	500	500	ND
Pet. Ether	ND	ND	ND	ND	ND	ND
Pulp						
Methanol	250	250	250	250	250	500
Acetone	250	250	250	500	500	500
Chloroform	250	250	500	500	500	ND
Pet. Ether	500	500	ND	ND	ND	ND
Seed						
Methanol	250	250	250	250	250	250
Acetone	250	250	250	250	250	500
Chloroform	250	250	500	500	500	ND
Pet. Ether	500	500	ND	ND	ND	ND

ND- Not determined.

the extracts showed greater potent antibacterial activity against Gram-positive bacteria than Gram-negative. This result is supported by the fact that Gram-negative bacteria have an outer membrane consisting of lipoprotein and lipopolysaccharide, which is selectively permeable and thus regulates access to the underlying structures (Chopra and Greenwood, 2001). This renders the Gram-negative bacteria generally less susceptible to plant extracts than the Gram-positive bacteria (Chan et al., 2007).

The antibacterial activity of the plant extracts might be attributed to the presence of bioactive plant compounds such as tannins, phenolic compounds, polyphenols and flavonoids (Ouattara et al., 2011). Among these bioactive compounds, Fernandez et al. (1996), Shoko et al. (1999), Baydar et al. (2004) and El-Chaghaby et al. (2011) confirmed that phenolics were the most important active compounds against bacteria. Thus the results of antibacterial activities obtained in the present study of *H.*

salicifolia leaf, bark, pulp and seed extracts were correlated to their total phenolic contents.

Positive correlations were obtained between the concentrations of phenolic compounds in the different *H. salicifolia* extracts and inhibition of all of the tested bacteria. The R^2 values of leaf extracts were found to be: 0.88 and 0.90 for the Gram positive bacteria *S. aureus* and *B. subtilis*, respectively whereas for the Gram negative bacteria *E. aerogenes, P. aeruginosa, K. pneumonia* and *E. coli*, the corresponding R^2 values were 0.88, 0.71, 0.71 and 0.86. The R^2 values of bark extracts for *S. aureus* and *B. subtilis* were found to be 0.87 and 0.85, respectively, whereas for *E. aerogenes, P. aeruginosa, K. pneumonia* and *E. coli*, the corresponding R^2 values were 0.74, 0.83, 0.83 and 0.86, respectively. The R^2 values of pulp extracts were found to be 0.96 and 0.74 for the Gram positive bacteria *S. aureus* and *B. subtilis*, respectively and for the Gram negative bacteria *E. aerogenes, P. aeruginosa, K. pneumonia* and *E. coli*, the corresponding R^2 values were 0.98, 0.95, 0.98 and 0.88. The R^2 values of seed extracts for *S. aureus* and *B. subtilis* were found to be 0.93 and 0.98, respectively, whereas for *E. aerogenes, P. aeruginosa, K. pneumonia* and *E. coli*, the corresponding R^2 values were 0.97, 0.92, 0.97 and 0.99, respectively. Strong antibacterial properties may be attributed to the phenolic components in the extracts. Thus, it may be concluded that the phenolic compounds in the *H. salicifolia* extracts could be the main components which possess the antioxidant and antibacterial properties.

Conclusion

This study has demonstrated the comparative account of antioxidant and antibacterial activities of various solvent extracts of leaf, bark, pulp and seed of *H. salicifolia*. Methanol was found as a better solvent for extraction of antioxidant and antibacterial substances that showed high extraction yields as well as strong antioxidant and antibacterial activities compared to the other solvents. SBT leaf, bark, pulp and seed extracts were found to possess good antioxidant and antibacterial activity. The study revealed that the seed and pulp extracts contain a considerable quantity of phenolic compounds than the leaf and bark extracts that were found to be the major contributor for their antioxidant and antibacterial activities. Thus, the *H. salicifolia* leaf, bark, pulp and seed can be considered as a potent source of natural antioxidants and antibacterial agents and can be exploited for developing nutraceutical and pharmaceutical products.

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