PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTIBACTERIAL EFFECTS OF SEA BUCKTHORN BERRIES

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ABSTRACT

Sea buckthorn berries are therapeutically used as folk medicine for a variety of diseases, however, the scientific evidence is hardly available to support their role. This study explored their chemical constituents and their role as antioxidant and antibacterial agents.

Three common solvents such as petroleum ether (40º - 60ºC), chloroform and methanol were successively used for the extraction of active principles from sea buckthorn berries. Five major fractions (F1-F5) were isolated from the active methanol extract by column and thin layer chromatography. An attempt was made to identify the chemical nature of pooled fractions by available spectral means. Antioxidant potential of methanol extract and its fractions was measured by DPPH, formation of phosphomolybdenum complex and TBA methods. The hole-plate diffussion method was used to find out the antibacterial activity. A very brief structure-activity relationship of the potent antioxidant and antimicrobial compounds is discussed. Methanolic extract and its fractions contain numerous phenolic compounds such as flavonoids, which may be responsible for antioxidant and antibacterial effects.

Keywords: Antibacterial, antioxidant, phytochemical, sea buckthorn.

INTRODUCTION

Sea buckthorn (*Hippophae rhamnoides)* berries are abundant in nutrients and therapeutic compounds, such as flavonoids, carotenes, volatile oils, carbohydrates, vitamins, amino- and mineral-acids (Sabir *et al*., 2005). The dietary intake of these berries may have a profound impact on human well being (Seeram, 2008).

The flavonols of sea buckthorn berries such as, rutin, quercetin, myricetin, kaempferol, isorhamnetin (Hibasami *et al*., 2005), tocopherols and tocotrienols (Kallio *et al*., 2004; Luhua *et al*., 2004) and carotenoids (Weller and Breithaupt, 2003; Pintea *et al*., 2005) have shown antioxidant properties. Moreover, various extracts of sea buckthorn have also exhibited marked antioxidant activity (Suleyman *et al*., 2002; Negi *et al*., 2005; Chauhan *et al*., 2007).

The phenolic compounds from the berries of sea buckthorn have been reported to minimally inhibit gram-negative bacteria, though gram-positive bacteria remained unaffected (Puupponen-Pimiä *et al*., 2001). Nagi *et al*. (2005), however, showed profound sensitivity of gram-positive than gram-negative bacteria to different organic extracts of sea buckthorn. The present study was designed to investigate chemical components of sea buckthorn, which may contribute to the antioxidant and antibacterial effects.

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MATERIALS AND METHODS

Plant and Chemicals

Sea buckthorn berries were obtained from the northern parts of Pakistan. The berries were dried in the shade and subsequently pulverized to a fine powder before stored appropriately. Throughout the study, all the chemicals and solvents employed were of analytical grade.

Test microorganisms

Pure cultures of *Staphylococcus aureus* ATCC 1234, *Bacillus subtilis* ATCC 1235, *Streptococcus pneumoniae* ATCC 1236 (Gram +); *Pseudomonas aeruginosa* ATCC 1237, *Escherichia coli* ATCC 1238, *Klebsiella pneumoniae* ATCC 1239 (Gram -) were obtained from the diagnostic laboratories of Fatima Memorial Hospital, Lahore. All the bacterial strains were maintained at 37ºC.

Solvent extraction

The pulverized dried sea buckthorn berries (250 g) were extracted successively in petroleum ether (40°- 60°C), chloroform and methanol by using 500 ml of each solvent for soaking. Maceration was carried out in each solvent for four days at room temperature (25° ± 2.5°C). The solvent of each extracted material was removed under reduced pressure and the residues were weighed.

Aqueous extract

Aqueous extract was also prepared by macerating the sea buckthorn berry powder (250 g) in distilled water (500 ml).

Thin Layer Chromatography

Silica gel G-60 (Merck, Germany) thin layer plates (0.25 mm thick) were prepared with the help of moving spreader (Dosga applicator). For a comparative TLC analysis, the solvent system (chloroform/methanol) in different ratios (95:5, 90:10 and 30:70 v/v) were used for crude residue obtained from petroleum ether, chloroform and methanol extraction respectively. Visualization of the chromatograms was achieved by UV light and iodine vapours.

Column Chromatography

The fractionation of methanol extract (20 g) was carried out on silica gel G-60 (70-230 mesh) column (300 g). Chloroform was used for packing the column. The column was eluted first with chloroform. Then polarity of the system was raised by increasing the quantity of methanol in chloroform. 20 ml fractions were collected and the fractions having similar compounds were pooled together after monitoring with thin layer chromatography. The isolated compounds were further subjected to phytochemical screening, spectral analysis, antioxidant and antimicrobial studies.

Phytochemical screening

Pooled column fractions were tested for the presence of flavonoids, polyphenols, terpenes and sterols (Egon, 1969).

Spectral analysis

UV spectra of isolated compounds were measured on spectrophotometer (Shimadzo-1700) using ethanol as a solvent and IR spectra on spectrophotometer (Pye-Unicam SP-8-400) using thin film on NaCl disc.

Antioxidant activity

Antioxidant activity of petroleum ether, chloroform, methanol and aqueous extracts of the berries and isolated fractions from methanol extract was determined using radical scavenging ability by DPPH (2,2-diphenyl-1-picrylhydrazyl) method (Erasto *et al*., 2004), by the formation of phosphomolybdenum complex (Prieto *et al*., 1999) and thiobarbituric acid (TBA) method (Osawa *et al*., 1981). Butyl hydroxyl toluene (BHT) was employed as a standard. In DPPH method, the absorbance values of extracts and isolated fractions were measured at 517 nm. The decline in absorbance at 517 nm was measured and the results were evaluated as percentage scavenging of radicals and finally IC_{50} values were calculated. In phosphomolybdenum assay, reduction of Mo (VI) phosphate to Mo (V) phosphate produced green colour and its absorbance was determined spectrophoto-meterically at 695 nm. In TBA method, the absorbance of supernatant was measured at 532 nm.

Antibacterial activity

The crude extracts and purified fractions were studied for antibacterial activity. Suspensions of bacteria were prepared by suspending a loopfull of the 24 hour old culture in sterile water (10 ml). Bacterial suspensions (1 ml) were individually mixed with 14 ml of nutrient agar medium in labelled petri-dishes under aseptic conditions. The media in all petri-dishes were allowed to solidify at room temperature. The hole-plate diffusion method was employed to test the antibacterial potentials of the above mentioned extracts. The results were compared with broad spectrum antibiotics, such as ampicillin and streptomycin.

The mean diameter of inhibition zones against the microorganisms produced by six replicates of different extracts and isolated compounds were calculated along with their effective ranges.

RESULTS

The polar components extracted in methanol were in the highest yield (13.10%), followed by 5.20% of non-polar component, extracted in petroleum ether and 3.7% of the components with intermediate polarity, extracted with chloroform.

All the three solvent extracts were subjected to a comparative TLC analysis using different solvent systems. The best solvent system, which resolved the mixture of methanolic extract into five major components, seems to be chloroform/methanol (30:70 v/v). Whereas, petroleum ether and chloroform extracts were segregated maximally into three components with the above solvent system in the ratio 95:5 and 90:10 v/v respectively. Methanolic extract was further subjected to column chromatographic analysis to isolate the active compounds using an increasing quantity of methanol in chloroform. The elution process was monitored by thin layers. Five pooled fractions designated as F1, F2, F3, F4 and F5 were further subjected to spectral analysis.

Isolated fraction F1

Fraction F1 was isolated and purified from second pooled column fraction, as from first pooled column fraction, no compound was detected. It was yellowish orange, sticky and chromatographically pure. Under UV light it appeared as a light blue spot, while it gave dark brown colour with iodine vapours. It also gave dark brown spot on thin layers when treated with ceric sulphate in HNO_3, but showed no reaction with ninhydrin. UV spectrum of isolated fraction F1 showed strong absorption at λ_{max}=204nm and 207nm, and weak absorption at 291 nm. IR-spectrum of the fraction showed strong absorption at 3645 cm^{-1}, 2927 cm^{-1} and 2859 cm^{-1} (Table 1).

Isolated fraction F2

Fraction F2 was isolated and purified from fourth pooled column fraction. It was yellow, sticky and chromatographically pure. Under UV light it appeared as

blue spot, while it gave yellow colour with iodine vapours. It also gave light brown spot on thin layers when treated with ceric sulphate in HNO_3, but showed no reaction with ninhydrin. UV spectrum showed strong absorption at λ_{max}=260nm, while weak absorption at 364 nm. IR spectrum of this fraction showed absorption at 3688 cm $^{-1}$, 3022 cm^{-1}, 2810 cm^{-1}, 1732 cm^{-1}, 1279 cm^{-1} and 1076 cm^{-1} (Table 1).

Isolated fraction F3

Fraction F3 was isolated and purified from seventh pooled column fraction. It was light yellow, and gummy in appearance, and chromatographically pure. Under UV light it appeared as pink spot. It gave yellow colour with iodine vapors when observed under ordinary light conditions. It also gave light brown spot on thin layers when treated with ceric sulphate in HNO_3 reagent but showed no reaction with ninhydrin reagent. UV spectrum showed strong absorption at λ_{max}=216nm and 213nm, while weak absorption at 383nm. The IR spectrum showed absorption bands at 3584 cm -1, 3021 cm-1, 2923 cm-1, 2256 cm^{-1}, 1722 cm^{-1}, 1459 cm^{-1}, 1294 cm^{-1} and 1142 cm^{-1} (table 1).

Isolated fraction F4

Fraction F4 was isolated and purified from eighth pooled column fraction. It was light yellow crystalline solid and chromatographically pure. Under UV light it appeared as blue spot. It gave brown colour with iodine vapours. It gave no reaction on thin layers when treated with ceric sulphate in HNO_3 or with ninhydrin. UV spectrum showed strong absorption at λ_{max}=204 and weak absorption at 383nm.

Isolated fraction F5

Fraction F5 was isolated and purified from ninth pooled column fraction. It was off- white amorphous solid and chromatographically pure. No spot appeared under UV light. It gave dark brown colour with iodine vapours. It gave purple spot on thin layers when treated with ninhydrin reagent, but showed no reaction with ceric sulphate in HNO_3. UV spectrum showed strong absorption at λ_{max}=234nm and weak absorption at 383nm. IR spectrum exhibited absorption bands at 3222 cm^{-1}, 3035 cm^{-1}, 2915 cm^{-1}, 2734 cm^{-1}, 2370 cm^{-1}, 1753 cm^{-1}, 1271 cm^{-1}, 1071 cm^{-1}, 986 cm-^{1} and 756 cm^{-1} (Table 1).

Antioxidant activity

In DPPH radical scavenging assay, methanol extract showed maximum antioxidant potential (IC_{50} 0.11±0.04), followed by extracts of water, petroleum ether and chloroform respectively (Table 2). Whereas, isolated fraction F1 showed better radical scavenging activity than other fractions and methanol extract (Table 3). In phosphomolybdenum method, methanol extract again showed the better activity than other extracts, followed by petroleum ether, water, and chloroform respectively (table 2). Isolated fraction F1 exhibited approximately equal antioxidant potential to methanol extract, followed by remaining fractions (table 3). In TBA method, inhibition of peroxides of methanol extract was found to be greater than other extracts and fractions (F1-F5) (tables 2-3).

Antibacterial activity

Preliminary antibacterial tests were performed with all three types of solvent extracts. The results (Table 4) indicated that the methanol extract (100mg/ml) produced a comparatively marked antibacterial response, while other extracts showed a weak zone of inhibition against all types of bacteria. Low doses (5 and 50mg/ml) of either extracts failed to show any inhibitory effect on test organisms. Isolated fractions (F1-F5) showed inhibitory activity against all organisms but zones of inhibition were comparatively greater in gram negative bacteria (Table 5).

DISCUSSION

Above results indicate that sea buckthorn berries contain a larger proportion of methanol soluble polar compounds, which may be polyphenolic in nature (Egon, 1969).

Isolated fraction F1

The UV spectrum of the isolated fraction F1 may indicate that strong absorption was probably due to n→π* and π→π* transitions which suggested a carbonyl group either of a ketone or an aldehyde or a carboxylic acid along with some conjugated diene chromophoric system present in its molecule. IR-spectrum of this fraction exhibited a strong absorption at 3645 cm^{-1}, indicating -OH group, due to O-H stretching vibration. Alkane group might be responsible for absorption at 2927 cm^{-1} and appeared due to C-H stretching vibration. Aldehyde functionality was indicated at λ_{max} 2859 cm^{-1} involving C-H bending vibrations. CO_2 moiety might be responsible for absorption at 2365 cm^{-1} indicating -COOH functionality. Absorption band at λ_{max} 1734 cm^{-1} suggested presence of carbonyl group; this might belong to ketone, aldehyde, ester or anhydride. The -CH_2 group might be responsible for absorption at 1459 cm^{-1} involving C-H bending vibration. Weak absorption at 1256 cm^{-1} might indicate aromatic amine because of C-N stretching vibration. Medium absorption at 1085 cm^{-1} indicated presence of mononuclear aromatic system.

The spectral analysis showed that F1 was probably a conjugated diene containing, alkane, alkene, hydroxyl, ketonic, carboxylic acid , acid anhydride, aromatic amine group and mononuclear aromatic system (Silverstein and Webster, 1997).

Isolated fraction F2

From the chemical properties of this fraction, it also appears to be polyphenolic in nature (Egon, 1969). The strong absorption at UV level was probably due to n→π*

and π→π* transitions which suggested the presence of a carbonyl group either of a ketone or an aldehyde or a carboxylic acid along with some conjugated diene chromophoric system present in its molecule. IR-spectrum showed strong absorption at 3688 cm^{-1} indicating -OH group. Variable absorption at 3022 cm^{-1} might be due to aromatic system involving C-H stretching vibration. A weak band at 2810 cm^{-1} suggested aldehyde group, which might be due to C-H bending vibration. Carbonyl group was possibly responsible for absorption at 1732 cm^{-1} and might belong to ester, ketone, and aldehyde or anhydride group. A weak absorption at 1279 cm^{-1} suggested presence of aromatic amine involving C-N stretching vibration. Mononuclear aromatic system might be responsible for absorption at 1076 cm^{-1}.

The spectral analysis showed that F2 isolated fraction was probably a conjugated diene containing hydroxyl, aldehyde, ketonic, carboxylic acid, acid anhydride, aromatic amine group and mononuclear aromatic system (Silverstein and Webster, 1997).

Isolated fraction F3

The chemical characteristics of this fraction most likely indicate that it may contain phenol related compounds (Egon, 1969). At UV level, the strong absorption was probably due to n→π* and π→π* transitions which suggested the presence of a carbonyl group either of a ketone or an aldehyde or an carboxylic acid along with some conjugated diene chromophoric system present in its molecule. The IR spectrum exhibited a weak band at 3584 cm^{-1} demonstrating -OH group, because of O-H stretching vibration. A strong absorption band at λ_{max} 3021 cm^{-1} suggested mono, or di-substituted alkene group and arose due to C-H stretching vibration. Absorption band at λ_{max} 2923 cm^{-1} might indicate alkane group. Unsaturated nitrogenous compound might be responsible for absorption at λ_{max} 2256 cm^{-1}. Carbonyl functionality might be present at λ_{max} 1722 cm^{-1} belonging to aldehyde, ester or ketone group. Absorption at 1459 cm^{-1} indicated aromatic group, possibly involving C-C stretching vibration or alkane group due to C-H bending vibration. Absorption band at λ_{max} 1294 cm^{-1} indicated di-substituted alkene and may be because of C-H bending vibration. A strong peak at 1142 cm^{-1} suggested mono nuclear aromatic system involving C-H stretching vibration.

The spectral analysis showed that F3 was probably a conjugated diene containing mono or di-substituted alkene, alkane, unsaturated nitrogen along with ester, ketone or aldehyde, -OH group and mono nuclear aromatic system (Silverstein and Webster, 1997).

Isolated fraction F4

The UV spectrum exhibiting strong absorption at 204nm was probably due to n→π* and π→π* transitions, which suggested a carbonyl group either of a ketone or an aldehyde or a carboxylic acid along with some conjugated diene chromophoric system present in its molecule. IR spectrum showed a strong peak at λ_{max} 3021 cm^{-1} indicated presence of mono-, or di-substituted alkene groups, which may involve C-H stretching vibration. λ_{max} 2925 cm^{-1} suggested alkane group. Peak 2256 cm^{-1} suggested that compound might be an un-saturated N-compound. Medium band at 1592 cm^{-1} indicated aromatic system and arose due to C-C stretching vibration within skeleton. Strong absorption band at λ_{max} 1141 cm^{-1} was possibly due to mono nuclear aromatic system involving C-H stretching vibration.

The spectral analysis showed that F4 was probably a conjugated diene containing mono or disubstituted alkene, alkane, unsaturated nitrogen along with mono nuclear aromatic system (Silverstein and Webster, 1997).

Isolated fraction F5

The physic-chemical properties of this fraction probably indicated presence of amino acid(s) (Egon, 1969). At UV level, the strong absorption was probably due to n→π* and π→π* transitions which suggested the presence of a carbonyl group either of a ketone or an aldehyde or an carboxylic acid along with some conjugated diene chromophoric system present in its molecule. In IR spectrum, the medium and broad bands at λ_{max} 3212 cm^{-1} indicated presence of either -OH or amide group which may be due to O-H bending vibration and N-H stretching vibration respectively. A weak absorption peak at 3035 cm^{-1} might indicate alkene group because of C-H stretching vibration. Absorption peak at 2915 cm^{-1} suggested presence of alkane group and might appear due to C-H stretching vibration. Aldehyde function group was indicated at 2734 cm^{-1} because of C-H bending vibration. CO_2 group functionality was indicated at 2370 cm^{-1} absorption band suggested presence of -COOH group. Medium peak absorption at 1753 cm^{-1} indicated presence of carbonyl group. This might be belonged to aldehyde, ester or anhydride group. Weak band at 1435 cm^{-1} appeared, might be due to presence of mononuclear aromatic system involving C-C stretching vibration within the ring or due to presence of $–CH_2-CH_3$ group because of C-H bending vibration. A hydroxyl group, involving O-H bending vibration, or amine group because of C-H vibration might be responsible for absorption at λ_{max} 1271cm^{-1}. λ_{max} 1071cm^{-1} suggested presence of mononuclear aromatic system. Absorption band at 986cm^{-1} pointed towards the presence of mono substituted alkane group due to C-H bending vibration. Aromatic group, involving C-H bending vibration might be responsible for absorption at 756 cm^{-1}.

The spectral analysis showed that F5 was probably a conjugated diene containing -OH or amine group, alkane, alkane, aldehyde, -COOH, ester, or anhydride, aromatic

Table 1: Physico-chemical characteristics of purified isolated fractions (F1-F5) from the methanol extract of sea buckthorn berries

Characteristics	F1	F2	F3	F4	F5
Colour	Yellowish orange	Yellow	Light yellow	Light yellow	Off-white
Physical appearance	Viscous	Viscous	Gummy	Crystalline solid	Amorphous solid
Fluorescence	Light blue	Blue	Pink	Blue	No colour
Reaction with iodine	Dark brown	Yellow	Yellow	Brown	Dark brown
Reaction with cerric sulphate+HNO_3	Dark brown	Light brown	Light brown	No reaction	No reaction
Reaction with ninhydrine	No reaction	No reaction	No reaction	No reaction	Purple
UV absorption (λ_{max})nm	204, 207	260	213, 216	204	234
IR absorption (cm^{-1})	3645(s), 2927(s), 2859 (s), 2365 (s), 1734 (s), 1459 (s), 1256 (w), 1085(m)	3688(s), 3022(m), 2810(w), 1732(s), 1279(w), 1076(s)	3584(w), 3021(s), 2923(s), 2256(s), 1722(s) 1429(s), 1294(s), 1142(s)	3021(s), 2925(s), 2256(s), 1592(w), 1141(s)	3222(m), 3035(w), 2915(s), 2734(s), 2370(s), 1753(m), 1271(s), 1071(s), 986(s), 756(s)

Table 2: Antioxidant activity of various extracts of sea buckthorn berries by different methods

Extracts	Scavenging activity against DPPH in IC_{50} mg/ml	Relative activity in phosphomolybdenum complex	% inhibition of peroxidation by TBA method
Petroleum ether	3.59 ±0.01	0.41±0.03	49.71±0.21
Chloroform	3.80±0.01	0.22±0.01	26.53±0.20
Methanol	0.11±0.04	0.74±0.01	60.12±1.03
Water	2.27±0.02	0.37±0.02	47.17±0.14
BHT	0.036±0.01	0.56±0.01	51.21±0.21

The values are Mean±SE (n=6)

Table 3: Antioxidant activity of isolated fractions of methanol extract by different methods

Isolated Fractions	Scavenging activity against DPPH in *IC_{50} mg/ml	Relative activity in phosphomolybdenum complex (Absorbance at 695 nm)	% inhibition of peroxidation by TBA method
Fraction 1	0.09±0.01	0.70±0.01	57.41±0.01
Fraction 2	0.095±0.00	0.65±0.01	50.52±0.14
Fraction 3	0.10±0.03	0.63±0.02	48.92±0.12
Fraction 4	0.11±0.01	0.5 7±0.01	47.51±0.14
Fraction 5	0.15±0.02	0.25±0.00	30.43±0.21
**BHT	0.036±0.00	0.56±0.02	51.21±0.11

The values are Mean±SE (n=6)

amine group and mononuclear aromatic system (Silverstein and Webster, 1997).

The nature and complete identity of above isolated fractions could not be interpreted without other spectral analytical aids such as mass spectrometry, proton- and C-NMR and possibly by the X-ray crystallographic analysis of their stable derivatives.

Antioxidant activity

Methanol extract had shown maximum activity in all above mentioned antioxidant methods. The data are in compliance with the findings of Negi *et al*., (2005). The capacity of a compound to act as an antioxidant depends upon its molecular structure and the position of hydroxyl and prenyl groups (Buhler and Mirinda, 2000). Methanol being polar solvent, extracted total phenolic and ascorbic

Table 4: Comparison of antibacterial activity of ampicillin and streptomycin with various solvent extracts of sea buckthorn berries

Microrganisms	Zones of Inhibition (mm)					
	Antibiotics (1mg/ml)		Solvent extracts 100mg/ml			
	Ampicillin	Streptomycin	Petroleum ether	Chloroform	Methanol	Water
Staphylococcus aureus	18.70±0.05	24.30±0.01	-	-	4.01±0.10	3.01±0.10
Bacillus subtilis	18.51±0.04	25.03±0.05	-	2.51±0.04	6.21±0.04	4.11±0.14
Streptococcus pneumoniae	17.12 ±0.03	24.06±0.31	-	2.61±0.06	5.61±0.16	3.11±0.14
Pseudomonas aeruginosa	14.30±0.12	29.06±0.31	2.00±0.03	4.21±0.03	9.51±0.03	6.71±0.23
Escherichia coli	14.02±0.01	28.30±0.21	2.45±0.13	4.45±0.13	10.45±0.13	7.45±0.23
Klebsiella pneumoniae	13.01±0.01	28.01±0.31	-	3.5±0.02	9.20±0.12	6.5±0.12

All values are Mean±SE (n=6)

Table 5: Comparison of antibacterial activity of ampicillin and streptomycin with various isolated fractions (F1-F5) of methanol extract of sea buckthorn berries

Microrganisms	Zones of inhibition (mm)						
	Ampicillin	Streptomycin	F1	F2	F3	F4	F5
Staphylococcus aureus	18.23±0.04	24.50±0.020	4.10±0.10	4.00±0.02	3.00±0.02	2.50±0.14	3.4±0.02
Bacillus subtilis	18.20±0.04	25.06±0.06	6.10±0.14	6.50±0.20	4.40±0.40	3.40±0.40	3.40±0.40
Streptococcus pneumoniae	17.12±0.03	24.02±0.21	5.10±0.06	4.30±0.02	4.50±0.21	2.50±0.21	3.40±0.02
Pseudomonas aeruginosa	14.02±0.12	29.0±0.10	10.45±0.23	9.70±0.04	6.7 0±0.02	6.50±0.02	5.20±0.05
Escherichia coli	14.26±0.03	28.30±0.02	12.50±0.30	10.00±0.03	6.20±0.05	6.20±0.05	6.70±0.04
Klebsiella pneumoniae	13.0±0.02	28.01±0.03	8.00±0.12	7.2±0.20	5.50±0.02	5.50±0.02	4.20±0.2

All values are Mean±SE (n=6)

acid contents of sea buckthorn, which were responsible for its maximal antioxidant activity (Gao *et al*., 2000). Similarly, F1-F5 also showed radical scavanging activity with DPPH; their IC_{50} values were, however, lower than methanol extract. Total antioxidant and TBA methods also assured the antioxidant potential of isolated fractions but the activity was lesser than methanol extract. Further investigations are required to assess the synergistic effects of methanol and its fractions. Antioxidant potential of isolated fractions may indicate involvement of conjugated dienes containing methyl, aryl, ketonic, carboxylic acid, acid anhydride, amine or secondary amide group along with -OH groups due to some alcohol or phenols. Petroleum ether extract had shown antioxidant activity next to methanol extract with total antioxidant and TBA methods, which might be due to non polar componets like vitamins, carotenoids, lipids and fatty acids (Gao *et al*., 2000). Water extract showed better antioxidant activity than methanol and petroleum ether extract in DPPH method but in other two methods activity was comparatively less. DPPH radical scavenging method is less time consuming and dependable, which is used for the preliminary screening of antioxidant potential.

Antibacterial activity

The isolated fractions may contain dienes, having methyl, aryl, ketonic, carboxylic acid, acid anhydride, amine or secondary amide group along with -OH groups due to some alcohol or phenol in their molecules probably penetrated through the bacterial cell wall and inhibited their growth or killed them. This antibacterial effect might be due to flavonoids, as these have been reported to possess antifungal, antiviral and antibacterial activity (Cushnie and Lamb, 2005). Current data suggest a relationship between flavonoids and antioxidant/ antibacterial properties. The antibacterial action of methanol extract might indicate involvement of (+)-catechin (Kajiya *et al.,* 2004). The results are also in compliance with the findings of Nair *et al*. (2005).

In conclusion, the study suggests that methanol extract and its isolated fractions may probably have a role as antioxidant and antibacterial agents. However, further investigations are required for identification of active principle(s), responsible for these effects.

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