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Evaluation of antioxidant and antimicrobial activities on various extracts of Himalayan medicinal plants

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Abstract: The DPPH radical scavenging potentials of the fractions were determined in comparison to positive controls such as quercetin with EC_{50} = 4.12±1.27, ascorbic acid with EC_{50} = 6.20±1.67, gallic acid with EC_{50} = 4.75±1.24 and α-tocopherol with EC_{50} = 32.50±1.57 μg/mL. The experiment showed that aqueous fractions of the bark extracts of *Abies pindrow* (fraction: C_2) and *Cedrus deodara* (fraction: E_2) showed significantly lower EC_{50} values of 2.5±0.5 and 2.5±0.6 μg/mL, respectively. In reducing power assay, lower EC_{50} values of 5.5 and 4.5μg/mL were recorded for the aqueous fraction (fraction: C_2) and final residue (fraction: C_5), of *Abies pindrow*, respectively. The ethyl acetate, acetone and final fractions of knot wood of *Picea smithiana* were found significantly active against all bacterial strains. Of the most sensitive fractions towards all the fungal strains was ethyl acetate fraction obtained from the bark of *Cedrus deodara* with a zone of inhibition ranging from 75 to 88 % that was more than the standard fluconazole.

Keywords: *Picea smithiana, Abies pindrow, Cedrus deodara,* antioxidant and antimicrobial activities.

INTRODUCTION

Plants belonging to family Pinaceae are especially important due to their different biological activities. For example, turpentine exudes of *Pinus nigra* were shown to possess antioxidant and analgesic properties (Gülçin *et al.,* 2003). The study of chemical constituents of *P. abies* (Slimestad *et al.,* 1994), *P. glauca* (Kraus *et al.,* 1997) and *P. glehni* (Nabeta *et al.,* 1994) revealed that they contain lignans, flavonoids and diterpenoids (Kuo *et al.,* 2004). These constituents are considered to be responsible for their antioxidant activities and antimicrobial activities (Cosentino *et al.,* 2007). The volatile oils of *Cedrus deodara* were evaluated for anti-inflammatory and analgesic potentials. Similarly, the alcoholic extract of the stem of the same plant was shown to have anticancer activity. The constituents separated from the heartwood of *C. deodara* also exhibited radical scavenging activities (Tiwari *et al.,* 2001).

MATERIALS AND METHODS

Collection of plant material

The subject parts of *Picea simithiana*, *Abies pindrow* and *Cedrus deodara* were collected in Swat, Khyber Pakhtunkhwa, Pakistan. Botanical identification was done by Dr. Habib Ahmad, Dean of Sciences, Hazara University, Manshra, Pakistan. Voucher samples listed in table 1 were submitted to the herbarium of the Department of Botany, Hazara, University, Mansehra, Pakistan.

Preparation of extracts and fractions

The bark and knot wood of *Picea smithiana, Abies pindrow* and *Cedrus deodara* were extracted with ethanol by Soxhlet apparatus. The extracts obtained were first filtered and then concentrated under vacuum at 40^{o}C. Solvent extraction was done to obtain different fraction. Details of the fractions are listed in table 1.

Chemicals

All the experiments were conducted using chemicals of analytical grade. Quercetin and gallic acid were obtained from Acros (USA), sodium carbonate, potassium ferricyanide, α-tocopherol, sulfuric acid,"ascorbic acid, Folin-Ciocalteu reagent and ferric chloride from Merck (Germany), trichloroacetic acid from Riedel-de Haen (Germany), sodium phosphate of Panreac (Spain), ammonium molybdate of ABSCO (UK) and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) of Fluka (Germany). SP-3000 PLUS spectrophotometer (Optima, Japan) was used to record UV spectra. Solvents of commercial grade were distilled before extraction.

DPPH radical-scavenging activity

Discolouration of the purple colour of DPPH methanolic solution was used as a measure of radical scavenging potential of the extracts/fractions and standards. Blois with a slight modification was applied to experiments. A DPPH solution of 1 mM concentration and sample

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solutions (3 mL) in ethanol having 20-100 μg samples were mixed. A control reading was taken for the solution containing no sample at all.

These solutions were kept in the dark for 30 minutes in order to react. Absorbance was measured for each sample after 30 minutes at a wavelength of 517 nm. - Percentage of radical scavenging activity (% RSA) was calculated by the following formula:

$$\% \text{ RSA} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

All the tests were repeated for three times and the results were shown as average values ± standard deviations. EC_{50} (effective concentration 50 %) was determined from the graph of percent radical scavenging activity versus concentrations of extract, ascorbic acid, gallic acid, quercetin and α-tocopherol applied as standards.

Reducing power determination

Oyaizu "method (Oyaizu 1986) was followed to determine reducing power. 1mM sample solutions containing 5-25μg/mL extracts were combined with 2.5 mL of phosphate buffer (pH 6.6) and 2.5mL potassium ferricyanide solution (10 g/L) and the mixture incubated for 30 minutes at 50^{o}C. Aliquots of trichloroacetic acid of volume 2.5mL and strength 100g/mL were mixed to the above solution and centrifuged at 650 rpm for 10 minutes. The supernatant solution with volume 2.5mL was added to 2.5mL of distilled water and 0.5mL of ferric chloride (1 g/L) and the absorbance was measured at 700 nm. Absorbance was proportional to reducing power. Assays were repeated in triplicate and results were expressed as mean values ± SD. The EC_{50} value was fixed for extract concentration that showed an absorbance of 0.4 at 700 nm."

Evaluation of total antioxidant capacity

The "extracts and fractions were tested for total antioxidant potential using a slightly modified protocol (Prieto *et al.,* 1999). Methanol was used to prepare the sample solution. 0.3 mL aliquot of the sample solution was transferred to Eppendorf tube and mixed with 2.7 mL of the reagent solution (28 mM sodium phosphate, 4 mM ammonium molybdate and 0.6 M sulphuric acid). An effective concentration of the sample solution was 50 μg/mL. The reagent of volume 2.7 mL was combined with ethanol to prepare the blank. The tubes were capped and kept for incubation in a water bath at 95^{o}C for 90 min. The samples were cooled to room temperature and absorbance for each measured at 695 nm against a blank. The antioxidant capacity of the samples was expressed in units of μmol/mg. The readings were repeated three times and expressed as mean values ± standard deviations."

Determination of total phenolic compounds

Compounds containing phenolic groups show antioxidant properties. For this purpose, the quantities of phenolic compounds in the ethanolic extract were compared to get more information about those fractions having antioxidant potential. A previously described method was adopted for this purpose. 1 mL extract solution with the strength of 500 μg was added to 50 mL volumetric flask and the total volume was made to 46 mL by addition of distilled water. Then, 1 mL of Folin-Ciocalteu Reagent (FCR) was mixed with this mixture followed by the addition of 3 mL of aqueous Na_2CO_3 (2%) after 3 minutes. The prepared mixture was shaken at room temperature for two hours and the absorbance noted at 760 nm. The phenolics were estimated in triplicate and results given as average values ± SD in μg/mg units. The following equation was used for estimation which was derived from the standard curve."

A = 1156C + 0189

In the above equation, A stands for absorbance and C stands for gallic acid equivalents (mg/g). The test solutions were prepared by adding 500μg of dried extracts and the final volumes used were 50mL.

Antimicrobial activities

Test organisms for bioassays

Antimicrobial "activities of different plant extracts were evaluated by using bacterial Gram (+ive) strain: *Staphylococcus aureus* and Gram (-ive) strains: *Proteus vulgaris*, *Escherichia coli*, *Enterobacter aerogenes* and *Salmonella typhi*; strains of fungi used: *Aspergillus flavus*, *Alternaria solani*, *Helminthosporium maydis* and *Aspergillus niger*. These microbial strains were obtained from the Centre of Biotechnology and Microbiology, University of Peshawar, Pakistan. Nutrient agar and Sabouraud dextrose agar from Oxide, UK was used to maintain bacterial and fungal strains, respectively. Bacterial cultures (2 to 3 colonies) were prepared in a tube containing 20 mL of nutrient broth (Oxide, UK) and allowed to grow for a night at 37^{o}C. Cultures of fungi were made in SDA. The cultures suspensions in fresh form were inoculated at McFarland 0.5 density (10^{8} CFU/mL)."

Antibacterial screening

Agar-well "diffusion method (Atta-ur-Rahman. *et al.,* 1999; Mathabe *et al.,* 2006; Taous *et al.,* 2005) was implied for antibacterial screening. Nutrient agar was used in making agar plates. For spreading bacterial cultures over the surface of agar plates the use of sterile swab sticks was adapted. A sterile borer was used to dig five wells of 6 mm diameter in each agar plate. 100 μL of ethanol, aqueous, ethyl acetate, acetone and final extracts (strength equal to 10 mg/mL each) of the bark and knot wood of the selected plants were placed in each of the wells. Absolute alcohol (100 μL) was used as negative control in the wells. For positive control, Streptomycin (100 μL with 2 mg/mL strength) was used. The extract and controls were allowed for diffusion at room temperature for one hour in a laminar flow cabinet then

the plates were covered with lids and incubated for 24 hours at 37°C. The inhibition of bacterial growth was noticed by the appearance of a clear zone surrounding the wells. The diameters of inhibitory zones were measured as average diameters in units of millimeters. The absence of activity was noted as the absence of a zone of inhibition.

Antifungal activity assay

Tube "dilution method (Paxton 1991; Taous *et al.,* 2005) was adapted for antifungal activity. For this purpose, 5 mL of SDA medium was taken in a test tube and screw-capped and autoclaved for 15 minutes at 121°C. The sterile medium was then added to ethanol, aqueous, ethyl acetate, acetone and final extracts (each with 400μg/mL strength) and fuconazole (200μg/mL) in absolute ethanol. The sterility of the tubes was checked by maintaining them in salutation position for a night long time. The tubes were inoculated with fungal strains on the next day in the salutation position at 27-30°C for 10 days. The negative control consisted of 1mL absolute alcohol, 5mL SDA and 1mL fungal culture while positive control was made of 1mL absolute alcohol, 5mL SDA and 200μg/mL of fluconazole in 1mL absolute alcohol and fungal cultures." The following formula was used to calculate % growth inhibition:

$$\% \text{ growth inhibition} = \frac{\text{Linear growth of negative control} - \text{Linear growth of sample}}{\text{Linear growth of negative control}} \times 100$$

The degree of activity was recorded in four grades according to the % inhibition of growth: inactive (0), low (0-30 %), moderate (30-50 %), Good (50-70 %) and significant (70% and above).

STATISTICAL ANALYSIS

Statistical calculations were done using Minitab 11.0 software. Data were evaluated through analysis of variance. Values at $P<0.05$ were significant, values at $P<0.01$ very significant and at $P>0.05$ non-significant. The calculated p value for all antioxidant analyses was found less than 0.05 whereas $P<0.05$ was found for antibacterial activity.

RESULTS

In vitro antioxidant activity

For screening antioxidant potential of the different extracts and their fractions, four different assays were performed.

DPPH radical scavenging activity

The "radical scavenging potentials of ethanol, water, ethyl acetate, acetone and final residue of the bark and knot wood of the selected plants and different standards are listed in table 2. All the fractions demonstrated excellent anti-DPPH activities which were almost close to the standards and in some cases even higher activities than α-tocopherol (standard) were observed. The highest activity (94.22% at 100μg/mL and with 2.5μg/mL EC_{50}) was shown by the aqueous extract (C2) of the bark of *Abies pindrow*. The significant DPPH radical scavenging activity of fractions was due to their corresponding phenolic contents, the fraction with higher phenolic contents showed greater scavenging activity (table 2 and 3).

Reducing power

Relative reducing powers of the extracts and fractions prepared from the bark and knot wood of *P. smietiana, A. pindrow* and *C. deodara* were evaluated in comparison to selected standards (table 2). The highest activity (1.260 ± 0.0100 at 25μg/mL; with 5.5 + 0.9μg/mL EC_{50}) was shown by the aqueous extracts of the bark of *A. pindrow* while the lowest activity (0.225 + 0.00451; with 45.5 + 1.2 μg/mL EC_{50}) was observed in the final fraction of the knot wood of *C. deodara*. Phenolic contents might be responsible for the reducing capabilities of the tested materials because a correlation can be found in the phenolic contents, reducing power and DPPH radical scavenging activity as shown in table 2 and 3."

Total antioxidant activity

The"antioxidant capacity was determined through quantitative phosphomolybdate method. The antioxidant activities of the extracts and fractions of the bark and knot wood of *P. smithiana*, *A. pindrow* and *C. deodara* were compared with standards (table 2). Significant activities were recorded for most of the fractions. Even activities closer to or higher than standard compounds were observed in some cases. A similar trend was observed as in case of radical scavenging activity (RSA) and reducing power. Thus the aqueous fraction of the bark of *A. pindrow* was noticed with maximum antioxidant activity (1907.1 + 160.0 μmole/mg) while the final fraction of the bark of *P. smithiana* showed minimum activity (92.3 + 27.4 μmole/mg)."

Determination of total phenols

The "estimation of total phenols was carried out using Folin Ciocalteu reagent. The amounts of total phenols in different fractions were found to be dependent on the type of solvent used and were expressed in milligrams of gallic acid equivalents (GAE). A summary of the total phenolic compounds found in different fractions is given in table 2. The phenolic contents ranged from 23.05±2.49mg/g for final fraction of knot wood of *P. smithiana* to 168.38 ±2.18mg/g for an aqueous fraction of bark *A. pindrow* expressed as gallic acid equivalents (GAE)."

Antibacterial activity

The "crude extract from the bark of *Abies pindrow* showed good activity against all the bacterial strains

Table 1: Relevant data on *Picea smithiana, Abies pindrow, Cedrus deodara* and the yields and percent yields (% y) of the crude extracts and fractions (fr.).

Plant	V. No.	Date of collection	Locality	Part	Fractions (fr)	Code	Wt. of fr.	% y of fr.
Picea smithiana	HUH-001	Sep, 2005	Swat, Khyber Pakhtunkhwa, Pakistan	Bark (A)	Ethanol	A1	7.00g	14.0%
					Aqueous	A2	4.20g	8.4%
					Ethyl acetate	A3	0.25g	0.50%
					Acetone	A4	1.30g	2.6%
					Final residue	A5	0.50g	1.0%
				Knotwood (B)	Ethanol	B1	2.30g	4.6%
					Aqueous	B2	0.085g	0.17%
					Ethyl acetate	B3	0.455g	0.91%
					Acetone	B4	1.20g	2.4%
					Final residue	B5	0.450g	0.90%
Abies pindrow	HUH-009	Sep, 2005	Swat, Khyber Pakhtunkhwa, Pakistan	Bark (C)	Ethanol	C1	9.25g	18.50%
					Aqueous	C2	4.275g	8.55%
					Ethyl acetate	C3	1.65g	3.3%
					Acetone	C4	1.535g	3.07%
					Final residue	C5	0.565g	1.130%
				Knotwood (D)	Ethanol	D1	12.25g	24.50%
					Aqueous	D2	0.275g	0.55%
					Ethyl acetate	D3	4.165g	8.33%
					Acetone	D4	5.74g	11.48%
					Final residue	D5	0.865g	1.73%
Cedrus deodara	HUH-011	Sep, 2005	Swat, Khyber Pakhtunkhwa, Pakistan	Bark (C)	Ethanol	E1	7.50g	15.0%
					Aqueous	E2	3.42g	6.82%
					Ethyl acetate	E3	0.410g	0.842%
					Acetone	E4	1.122g	2.244%
					Final residue	E5	0.750g	1.50%
				Knotwood (D)	Ethanol	F1	6.00g	12.0%
					Aqueous	F2	0. 07g	0.14%
					Ethyl acetate	F3	4.105g	8.21%
					Acetone	F4	0.684g	1.368%
					Final residue	F5	0.075g	0.15%

ranging from 12 mm zone of inhibition for *Salmonella typhi* to 18 mm for *Staphylococcus aureus* and *Pseudomonas aeruginosa* (table 4). Similarly, Crude, aqueous and ethyl acetate fractions of the knot wood of the same plant remained active against *Escherichia coli, Enterobacter aerogenes and Pseudomonas aeruginosa.* All fractions of the bark of *Cedrus deodara* showed significant activity (19-23 mm zone of inhibition) against *Staphylococcus aureus* and good activity against *Enterobacter aerogenes (12-18 mm) and Pseudomonas aeruginosa* (15-20 mm).The zones of inhibition in case of ethyl acetate fraction of the knot wood of *p.s* were equal to or even more sensitive towards *Escherichia coli* (30 mm), *Staphylococcus aureus* (33mm) and *Enterobacter aerogenes* (28mm) as compared to the standard streptomycin. All the bacteria in the study were sensitive to streptomycin with *Staphylococcus aureus* and *Pseudomonas aeruginosa* being the most sensitive (inhibition zone values of 31 and 32mm, respectively).

Antifungal activity

In "antifungal assay, *C. deodara* remained the most active, *A. pindrow* moderately active and *P. smithiana* the least active as shown in table 5. In case of *C. deodara*, the ethyl acetate fraction of the bark showed a zone of inhibition (75-88%) which was more than reference standard (fluconazole). Aqueous and final fractions of the bark of *C. deodara* and crude, aqueous, ethyl acetate and acetone fractions of the knot wood of the same plant were found significantly active against *Aspergillus niger* (table 5).

DISCUSSION

This work is based on studies searching for indigenous plants with the best antioxidant, antibacterial and antifungal potentials. For this purpose, *P. smithiana*, *A. pindrow* and *C. deodara* were collected from Khyber Pakhtunkhwa province of Pakistan.

Table 2: Antioxidant activities and total phenolic contents of various fractions of the knot wood and bark of *Picea smithiana, Abies pindrow* and *Cedrus deodara*

Plant	Part studied	Fractions/ Standards	[a]DPPH assay % RSA (at 100 μg/ml)	[b]Reducing Power (at 25 μg/ml)	[c]Total Antioxidant Phosphomolybdate assay as gallic acid equivalents (μmole/mg of extract)	[d]Total phenolic contents as gallic acid equivalents (mg/g of extract)
Picea smithiana	Bark (A)	A1	87.97+1.97	0.973+0.01429	1828.96±85.2	152.52+4.36
		A2	91.12+0.150	0.651+0.00751	1782.1±107.9	73.51+0.865
		A3	89.83+0.618	0.766+0.01079	917.32±62.1	103.79+0.87
		A4	92.06+0.069	0.599+0.00721	1255.28±236	66.88+0.502
		A5	90.87+0.435	0.547+0.0060	1469.7±267	51.60+1.80s
	Knotwood (B)	B1	92.22+0.573	0.858+0.0083	2317.44+163.2	97.16+2.64
		B2	82.83+0.619	0.679+0.0225	1932.62+190	164.05+1.32
		B3	78.42+0.375	0.721+0.01531	1273.74+147	145.89+0.50
		B4	79.70+1.673	1.034+0.0170	1574.78+108	158.29+0.87
		B5	43.76+2.22	0.263+0.00200	92.3+27.4	23.05+2.49
Abies pindrow	Bark (C)	C1	92.62+0.177	0.925+0.00451	867.62+98.3	94.85+1.80
		C2	94.22+0.10	1.260+0.0100	1907.1+160.0	168.38+2.18
		C3	92.02+0.380	0.939+0.0278s	113.00+85	97.73+1.730
		C4	91.710.207	0.853+0.00520	1718.2+113.5	88.50+2.64
		C5	90.43+0343	1.230+0.0100	1256.7+101.8	123.11+2.64
	Knotwood (D)	D1	83.86+0.122	0.939+0.00361	1491+230	166.65+2.18
		D2	87.49+0.261	1.045+0.0225	1421.4+224	145.31+3.12
		D3	83.70+0.600	0.883+0.01058	1056.5+148.4	135.79+1.73
		D4	81.89+0.427	0.794+0.01518	1449.8+147.1	148.48+2.65
		D5	82.60+0.525	0.729+0.0176	1150.2+110.8	133.49+2.64
Cedrus deodara	Bark (E)	E1	93.25+0.115	1.211+0.0101	1499.52+111.0	134.35+2.78
		E2	93.99+0.023	1.252+0.0247	556.64+113.9	157.71+2.64
		E3	85.43+0.767	0.780+0.00902	948.56+98.5	69.76+2.17
		E4	92.35+0.160	0.792+0.01021	1218.36+73.9	91.10+3.90
		E5	93.41+0.108	0.655+0.01114	981.22+72.5	78.12+2.17
	Knot wood (F)	F1	29.86+1.334	0.279+0.00603	1326.28+42.7	49.29+1.730
		F2	36.44+0.391	0.471+0.01150	1456.92+53.4	123.97+2.49
		F3	27.51+0.599	0.287+0.00666	1331.96+45.9	38.91+1.730
		F4	27.51+0.599	0.248+0.01026	1373.14+102.6	48.71+2.18
		F5	32.75+0.574	0.225+0.00451	1131.74+90.4	38.91+1.730
Standards		Quercetin	98.28±0.257	1.638+0.024	2058.70±180.1	370.18±14.11
		Ascorbic acid	97.60±0.689	1.692+0.020	2470.30±146.8	----------
		Gallic acid	98.03±0.503	1.653+0.019	2173.50±194.6	322.66±22.2
		α-tocopherol	92.48±0.68	0.468+0.088	557.70 ±54.56	67.40±5.51

[a,b,c,d] The assays were carried out in triplicate and the results are expressed as mean values ± standard deviations.

The selected plants were evaluated for their antioxidant activities using four different types of assays namely free radical scavenging assay, reducing power, total antioxidant capacity and total phenolic contents. DPPPH was used as a radical scavenger. Reaction progress was followed by seeing a decrease in absorbance at 517 nm. For each sample, reducing power was determined by reacting it with ferric compounds and then measuring the absorbance. Higher absorbance of a sample indicates greater reducing power and vice-versa. For total antioxidant and total phenolic contents, Folin-Ciocalteu's and Phosphomolybdenum reagents were used, respectively.

All the fractions demonstrated significant radical scavenging activity against DPPH radical. Some of the fractions were even noticed with activities greater than α-tocopherol (one of the reference standards). The highest

Table 3: EC_{50} values [a,b] (µg/mL) of various fractions of the knot wood and bark of *Picea smithiana, Abies pindrow* and *Cedrus deodara* in reducing power and DPPH radical scavenging assays

Plant species	Part	Fractions/ Standards	DPPH Radical scavenging assay (EC_{50}[a])	Reducing Power (EC_{50}[b])
Picea smithiana	Bark (A)	A1	20.5+1.3	8.5+1.5
		A2	24.0+2.5	14.0+1.8
		A3	23.5+3.1	13.5+2.3
		A4	19.3+1.4	15.0+2.0
		A5	35.45+3.2	18.0+3.1
	Knot wood (B)	B1	21.5+1.5	9.5+1.5
		B2	26.5+3.0	13.5+2.1
		B3	39.0+2.5	19.9+1.5
		B4	35.5+3.4	9.0+1.3
		B5	110+3.5	35.5+3.5
Abies pindrow	Bark (C)	C1	18.5+2.4	9.5+1.8
		C2	2.5+0.5	5.5+0.9
		C3	14.5+1.2	8.5+1.9
		C4	16.5+2.1	11.5+1.4
		C5	3.5+1.8	4.5+1.5
	Knot wood (D)	D1	28.5+1.4	9.0+1.5
		D2	22.5+1.9	8.5+2.1
		D3	29.6+1.6	9.5+1.4
		D4	30.5+2.3	11.3+2.5
		D5	23.0+3.0	12.5+1.6
Cedrus deodara	Bark (E)	E1	3.5+0.9	7.6+1.5
		E2	2.5+0.6	6.5+1.2
		E3	49.0+2.4	13.0+1.6
		E4	21.5+3.0	11.0+1.8
		E5	22.5+0.537	17.5+0.4
	Knot wood (F)	F1	140.5+1.5	40.5+3.5
		F2	130.0+2.8	19.5+2.1
		F3	145.5+3.5	32.5+1.5
		F4	120.0+1.9	39.5+2.7
		F5	132.5+2.8	45.5+1.2
Standards		Quercetin	4.12+ 1.27	1.88+ 0.032
		Ascorbic acid	6.20+ 1.67	3.31+ 0.041
		Gallic acid	4.75+ 1.24	1.20+ 0.025
		α-tocopherol	32.50+ 1.57	21.50+ 0.085

[a] EC_{50} (mg/mL): effective concentration at which 50 % of DPPH radicals are scavenged.
[b] EC_{50} (mg/mL): effective concentration at which the absorbance is 0.4.

RSA was shown by *A. pindrow* while the lowest RSA was noted for *C. deodara*.

Reducing the power of a sample is a significant test to demonstrate its antioxidant capability and was shown to be a function of concentration. Significant statistical differences ($P<0.05$) were present amongst the same fractions of different plant parts. The bark of a plant was found to be more active than its knot wood. The reducing power might be correlated to the phenolic contents or some other reducing agents in the plant (table 2 and 3). The fraction having more reducing power showed the more significant concentration of phenolics and vice versa.

The total antioxidant capacities of the samples were calculated by their reaction to form a green phosphate/Mo (V) complex at acidic pH. A good deal of total antioxidant capacity was observed for all the fractions in the range of 92.3 ± 27.4-2317.44 ± 163.2 μmole/mg green phosphate/Mo (V) (table 2). This capability of the extracts and fractions may attribute to the presence of phenolics and other reducing agents in them (Jayaprakasha *et al.,* 2008).

Table 4: Antibacterial activities (diameter in mm of growth inhibition zone) of ethanol, water, ethyl acetate, acetone and final extracts (10 mg/mL) of the knot wood and bark of *Picea smithiana, Abies pindrow* and *Cedrus deodara*

Plant	Part	Bacteria[a] tested zone of inhibition (mm)						
		Fractions/Standards	Ec	Sa	Ea	St	Pv	Pa
Picea smithiana	Bark (A)	A1	11	14	11	13	12	14
		A2	11	12	11	14	13	16
		A3	11	14	11	19	11	18
		A4	10	12	10	11	13	20
		A5	11	13	11	12	11	14
	Knot wood (B)	B1	25	14	16	11	10	17
		B2	10	25	12	12	11	23
		B3	30	33	28	25	11	12
		B4	11	27	15	16	22	17
		B5	32	19	20	21	18	17
Abies pindrow	Bark (C)	C1	16	18	13	12	16	18
		C2	14	17	14	16	17	18
		C3	12	14	13	12	13	17
		C4	13	12	12	20	15	18
		C5	11	13	10	15	15	16
	Knot wood (D)	D1	16	14	16	12	11	22
		D2	17	12	13	13	12	18
		D3	15	18	13	16	14	25
		D4	12	13	18	13	13	20
		D5	13	13	14	12	13	16
Cedrus deodara	Bark (E)	E1	11	21	18	12	12	17
		E2	11	22	15	12	15	18
		E3	11	21	12	11	13	16
		E4	12	23	14	13	13	20
		E5	10	19	14	15	14	15
	Knot wood (F)	F1	14	17	12	11	12	23
		F2	13	15	11	9	11	13
		F3	14	18	13	11	12	17
		F4	16	17	13	11	11	16
		F5	15	16	13	11	11	17
		streptomycin	30	31	25	30	28	32

[a]Bacteria: Sa, Staphylococcus aureus; Pa, Pseudomonas aeruginosa; St, Salmonella *typhi; Ec*, Escherichia coli; Pv, Proteus vulgaris; Ea, *Enterobacter aerogenes.*

Table 4 enlists the antibacterial activities of the tested samples. All the extracts were found active against different strains of gram-positive and gram-negative bacteria. The diameters of inhibition zones were measured to be in the range of 10 to 33 mm. No significant statistical differences (P>0.05) were noticed in the activities of the test samples.

For antifungal activity, the % zone of inhibition was found in the range of 1.3 to 88%. No statistical differences were found amongst the different fractions. *C. deodara* was found to be the most active, *A. pindrow* moderately active and *P. smithiana* least active against the tested strains of fungi.

CONCLUSION

This preliminary screening shows that the plants of family Panacea, possess significant antioxidant and antimicrobial activities. Their use as a readily accessible source of natural antioxidants and as possible supplements in pharmaceutical industries might be justified by activities

Table 5: Antifungal screening (% growth inhibition) of ethanol, water, ethyl acetate, acetone and final extracts (400 μg/mL) of the knot wood and bark of *Picea smithiana, Abies pindrow* and *Cedrus deodara*.

Plant	Part	Fungi[a] tested zone of inhibition (mm)				
		Fractions/Standards	*An*	*Hm*	*Af*	*As*
Picea smithiana	Bark (A)	A1	12.5	18.1	6.3	12.2
		A2	37.5	13.3	15.0	17.1
		A3	15.0	3.6	15.0	8.5
		A4	12.5	12.0	18.8	8.5
		A5	6.3	21.7	10.0	32.9
	Knotwood (B)	B1	0.0	3.6	6.3	8.5
		B2	6.3	3.6	-1.3	14.6
		B3	15.0	15.7	0.0	2.4
		B4	8.8	30.1	13.8	26.8
		B5	2.5	9.6	6.3	2.4
Abies pindrow	Bark (C)	C1	37.5	45.8	25.0	45.1
		C2	37.5	27.7	37.5	32.9
		C3	50.0	51.8	25.0	26.8
		C4	25.0	27.7	37.5	39.0
		C5	50.0	27.7	37.5	39.0
	Knotwood (D)	D1	15.0	24.1	8.8	17.1
		D2	12.5	21.7	6.3	20.7
		D3	25.0	21.7	15.0	14.6
		D4	18.8	22.9	25.0	13.4
		D5	15.0	18.1	27.5	20.7
Cedrus deodara	Bark (E)	E1	43.8	39.8	25.0	51.2
		E2	62.5	33.7	12.5	63.4
		E3	87.5	75.9	62.5	87.8
		E4	0.0	33.7	50.0	63.4
		E5	58.8	45.8	68.8	39.0
	Knotwood (F)	F1	62.5	45.8	87.5	39.0
		F2	60.0	39.8	43.8	26.8
		F3	72.5	39.8	50.0	69.5
		F4	67.5	27.7	87.5	39.0
		F5	12.5	63.9	25.0	63.4
		Fuconazole	76.47	70.59	72.94	74.12

[a]Fungi: An, Aspergillus niger; Af, Aspergillus flavus; Hm, Helminthosporium maydis; As, Alternaria solani.

mentioned above. Also, the selection of plants by ethnobotanical approach can be underlined in the discovery of new bioactive substances and places these species amongst the most promising of indigenous drugs. The above studies of bioactivities also demonstrate that antioxidant and antimicrobial activities are not correlated. A plant species having antioxidant activity may or may not possess antimicrobial potential.

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