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In vitro Evaluation of Antioxidant Activity of *Picea smithiana* Growing in Bhaderwah Region of Jammu and Kashmir

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Research Article

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Abstract

Picea smithiana is one of the plants belongs to family pinaceae that has not been explored scientifically from pharmaceutical point of view. The present study targeted to explore the antioxidant potential of *Picea smithiana* growing in Bhadewah region (Shoj dhar) at the altitude of 11000 feats of Jammu and Kashmir, India. Three types of extracts were prepared from leaf and Bark part of the plant viz. chloroform, methanol and aqueous and analyzed for their total phenols and flavonoid content. Antioxidant potential of the extracts were analyzed by four different method viz., DPPH radical scavenging method, Fe^{2+} ion chelating method, FRAP assay and Potassium ferric cyanide reduction method.

The results of the study determined that methanolic extract of leaf part contained good content of phenolic compound (70.4 ± 2.1mg GAE/g dw) which contributed as good antiradical (IC_{50} value 228 ± 3.2µg/ml), chelation activity (55 ± 1.5% at 500µg), FRAP (494 ± 5.2µmol Fe (II)/g) and Potassium ferric cyanide reduction activity (EC_{50} value of 978µg/ml). A correlation between the antioxidant activity (FRAP) and the phenolic content of *Picea smithiana* extracts has also been drawn and found significant (R^2=0.965). In comparison, bark extracts possess less poly phenols that confer poor antioxidant potential.

Keywords: *Picea smithiana*; Essential Oil; Antimicrobial Compounds; Bio autography

Introduction

The role of free radicals and reactive oxygen species has been recognized in pathogenesis of many human diseases like cardiovascular disease, cancer, diabetes, cataracts, aging and neurodegenerative diseases including alzheimer's disease, parkinson's disease, huntington's disease [1,2]. Free radicals can also cause deterioration of food by oxidation that constitutes a serious problem in food industries. However, antioxidants would be an option which prevent or delay auto oxidation either by inhibiting the formation of free radicals or by interrupting their proliferation. Some synthetic antioxidants such as BHT and BHA are commercially available but these compounds are suspected to cause side effects [3].

This concern has resulted in an increased interest in the investigation of the effectiveness of naturally occurring compounds with antioxidant properties. Thus the natural antioxidants present in foods and other biological materials have attracted considerable interest because of their safety and potential nutritional and therapeutic effects.

Picea smithiana (Pinaceae) is an evergreen tree commonly called Morinda Spruce, usually found in the Himalayan range at an altitude of 2400-3600m. The plant also has some commercial application like timber, paper, and food additives. Its edible parts like, Young male and female cones used as a flavoring agent. Dried bark of this plant is used as a thickener in soups or added to cereals when making bread. Moreover, it's essential oil also being used in room spray, deodorants [4].

Essential oil Picea species are used in the treatment of catarrhal diseases of children by inhalation with hot water and for rheumatic and neuralgic [5]. Plants belonging to Pinaceae family, like Pinus roxburgii, Pinus wallichaina and Cedrus deodara have abundant commercial uses and have also been scientifically explored for various biological activities. However, limited scientific reports on *Picea smithiana* are available to support its biological activity and its active components. The present aims to explore the phytochemicals and antioxidant properties of different extracts of *Picea smithiana*.

Material and Methods

Plant Material

Plant material was collected in the month of August (2012), from meadows of Seoj dhar region (11000 fts) of Bhaderwah, Jammu and Kashmir. Identification of the plant was done by taxonomist of Department of Botany, University of Jammu, Jammu and a voucher specimen has been deposited in the herbarium of the department of botany, University of Jammu (Accession no: 14613).

Preparation of Extracts

Plant material (leaf and bark, separately) was shade dried and powdered in an electronic grinder. Three types of extracts were prepared in three different solvents viz., chloroform, methanol and water. 100g of dried plant material was extracted in 500 ml of solvent and the process was repeated thrice. Resulting extracts were pooled, filtered and the volume was reduced to 50 ml using rotary vacuum evaporator and finally lyophilized to dried powder [6]. Percentage yield of the extracts of *Picea smithiana* was given in Table 1.

Extract of *Picea smithiana*	%age yield	TPC (mg GAE/g dry weight)	TFC (mg QE/g dry weight)
Methanolic (Leaf)	11.3	70.4 ± 2.1	16.2 ± 0.7
Aqueous (Leaf)	8.4	57.5 ± 1.8	21.6 ± 0.9
Chloroform (Leaf)	3.1	12 ± 0.4	-
Methanolic (Bark)	12.4	40.6 ± 1.9	15.1 ± 0.7
Aqueous (Bark)	9.3	14.5 ± 0.6	26.3 ± 1.2
Chloroform (Bark)	2.6	-	-

Table 1: Percentage yield, total phenol and flavonoid content in *Picea smithana* extracts
Experiment was conducted in triplicate and the values were given as mean ± SD

Total Phenols and Flavonoids in Extracts

Total poly-phenol content in the extracts was determined by Folin-Ciocalteu method [7]. Briefly, 0.5ml of extract was mixed with 0.5ml of 1N Folin-Ciocalteu reagent and 1ml of 20% Na_2CO_3. After 10 min of incubation, the absorbance was measured at 750nm. The amount of total phenolic compounds was determined using a standard curve prepared from gallic acid (Make: Himedia) and expressed as milligram gallic acid equivalents per gram fresh weight (mg GAE/g) of sample.

Total flavonoid content of the extracts was determined by $AlCl_3$ colorimetric method [8]. Plant extracts were diluted with distilled water to a volume of 3.5ml and added 150 µl of a 5% $NaNO_2$ solution. After 5 min, 300 µl of 10% $AlCl_3$ solution was added. After 6 min, 300 µl of 1M NaOH and 550 µl of distilled water were added. The mixture was well shaken and absorbance was measured at 510nm in UV-VIS spectrophotometer. The amount of total flavonoid content was determined using a standard curve prepared from Quercetin (Make: Sigma aldrich) and expressed as milligram Quercetin equivalents per gram fresh weight (mg QE/g) of sample.

DPPH Radical Scavenging Activity

Free radical scavenging activity of the extracts was determined by DPPH method given by Abe N et al. [9] with little modifications. A total of 1 ml from a 0.5m methanol solution of the DPPH radical was mixed to 2 ml sample and to this 2 ml of 0.1 M sodium acetate buffer

(pH 5.5) was added. The mixtures were well shaken and kept at room temperature in the dark for 30 min. The absorbance was measured at 517 nm using a UV-VIS spectrophotometer. BHT was used as reference antioxidant compound.

The radical scavenging activity (RSA) was calculated as a percentage of DPPH radical discoloration, using the equation:

$$\%RSA = [(A_0-A_S)\ A_0] \times 100$$

Where, A_0 is the absorbance of the control and A_S is the absorbance of the test compound.

FRAP Assay

The FRAP assay was carried out according to the method followed by Li HB et al. [10]. FRAP reagent was prepared in acetate buffer (300mM) by adding 10 mM 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ) solution in 40mM HCl and 20 mM $FeCl_3$ solution in proportion of 10:1:1 (v/v), respectively. FRAP reagent was prepared fresh at the time of use. 50µl of the sample was added to 1.5 ml of the FRAP reagent and after 3-4 min, absorbance was measured at 593nm. The standard curve was prepared by using $FeSO_4$ (100-2000 µM) and the result was expressed as µmol Fe (II)/gm dry weight of extract. Gallic acid was used as reference antioxidant compound.

Chelation Activity

The chelating potential of the *Picea smithiana* extracts on ferrous ions was estimated by the method given by Dinis TCP et al. [11]. Extract (0.5mg) in methanol was added to 20 µl of 2 mM $FeCl_2$. The reaction was initiated by the addition of 40 µl of 5 mM ferrozine into the mixture. The reaction mixture was incubated at room temperature for 10 min and finally the absorbance was measured at 562 nm. EDTA was used as reference chelating agent. The ratio of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the equation:

$$\%\ Inhibition = [(I_0 - I_S)\ I_0] \times 100$$

Where, I_0 is the absorbance of the control and I_S is the absorbance of the test compound.

Potassium Ferric Cyanide Reduction Method

The reducing power of the samples was also assessed by potassium ferric cyanide reduction method with slight modifications [12]. Different dilutions of extracts/fractions were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6). 2.5 mL of 1% potassium ferricyanide ($K_3Fe[CN]_6$) (Make: Himedia) was added and the mixture was incubated at 50 ºC for 20 min. After incubation, trichloroacetic acid was added to the mixture.

The mixture was then centrifuged at 1036 × g for 10 min. The upper layer of the solution (2.5 mL) was taken and mixed with 2.5 mL of distilled water. To this, 2.5 mL of 0.1% ferric chloride (Make: Himedia) solution was added and the absorbance was noted at 700 nm. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against extract/fraction concentration.

Results and Discussion

The present study determined the antioxidant properties of different extracts of *Picea smithiana* leaf and bark. Three types of extracts were prepared viz., chloroform, methanol and aqueous, and their extractive yields has been depicted in Table 1. Maximum yield was observed in methanolic extracts of both the parts of *Picea smithiana* (leaf: 11.3% and bark: 12.4%). Poor yield was observed in chloroform, in both parts of the plants. Antioxidant potential of *Picea smithiana* was analyzed by four methods viz., DPPH radical scavenging activity, and metal ion chelating activity, FRAP assay and reducing power.

The DPPH radical scavenging activity was determined on the basis of concentration providing 50% effectiveness or radical scavenging activity (EC_{50}). Results of the study determined that methanol and aqueous extracts of leaf displayed highest antiradical efficiency with EC_{50} values 228 ± 3.2µg/ml and 331 ± 4.1µg/ml, respectively Table 2. In comparison bark methanol extract showed poor radical scavenging activity with EC_{50} values 457.3 ± 5.2 µg/ml. Aqueous and chloroform extracts of bark have shown negligible radical scavenging activity. BHT as standard showed effective radical scavenging activity with EC_{50} value 23.5 ± 0.5 µg/ml.

Extract of *Picea smithiana*	DPPH Assay (EC_{50} in µg/ml)	FRAP Assay (µmol FeII /g)	Reducing power (EC_{50} in µg/ml)	%age Chelation
Methanolic (Leaf)	228 ± 3.2	494 ± 5.2	978 ± 8.1	55 ± 1.5
Aqueous (Leaf)	331 ± 4.1	423 ± 8.3	1235 ± 11.1	14 ± 0.6
Chloroform (Leaf)	-	157 ± 5.1	4832 ± 14.4	16 ± 0.4
Methanolic (Bark)	457 ± 8.6	250 ± 6.3	1653 ± 15	49 ± 1.8
Aqueous (Bark)	-	185 ± 4.3	1652 ± 14.2	37 ± 1.4
Chloroform (Bark)	-	-	4947 ± 17.2	30 ± 0.9
BHT	23.5 ± 0.5	-	-	-
Gallic acid	-	19320 ± 51.2	150 ± 4	-
EDTA	-	-	-	98 ± 1.8

Table 2: Free radical scavenging, reducing and metal ion chelating activity of *Picea smithiana* extracts. Experiment was conducted in triplicate and the values were given as mean ± SD.

Chelation of metal ions is one of important mechanism of antioxidant activity. Metal ion chelation capacity was determined at 0.5mg/ml concentration. Again, methanol leaf extract of *Picea smithiana* showed a better chelating effect on ferrous ions (55 ± 1.5%) than all the leaf and bark extracts. Methanol bark extract also showed good chelating capacity with 49 ± 1.8% at 0.5mg/ml. Aqueous and chloroform extracts of leaf part showed poor chelating activity. EDTA, used as positive reference showed 98 ± 1.8% chelating activity at the given concentration Table 2.

FRAP is the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ, forming an intense blue (Fe^{2+}-TPTZ complex) [10]. The results of reducing power were expressed in µmol Fe (II)/g dry weight as given in Table 2. Strongest antioxidant activity to reduce the Ferric ions (Fe^{3+}) was observed in leaf methanol extract of *Picea smithiana* (494 ± 5.2 µmol Fe (II)/g) followed by its aqueous extract (423 ± 8.3 µmol Fe (II)/g) similar to the radical scavenging activity. Bark of *Picea smithiana* showed moderate reducing activity in methanol and aqueous extracts (250 ± 6.3µmol and 185 ± 4.3 µmol Fe (II)/g respectively). Gallic acid was used as a standard reducing agent that determined extremely high reducing activity (19320 ± 51.2 µmol Fe (II)/g).

Reducing potential of the extracts was also analyzed by potassium ferric cyanide reduction method. Presence of reducing components in the test sample results into reduction of Fe^{3+}/Ferric cyanide complex. Different concentrations of the test samples were tested and the results were expressed as EC_{50} Table 2. Analysis showed that methanol extracts of leaf part of *Picea smithiana* displayed better reducing power with EC_{50} value of 978 µg/ml. Methanol and aqueous extracts of bark showed low reducing activity with EC_{50} value of 1653 µg/ml and 1652 µg/ml respectively.

Phenolic compounds have already been known to possess various biological activities like, antioxidant, antimicrobial etc. Table 1 represented total phenol and total flavonoid content in leaf and bark extracts of *Picea smithiana*. Highest amount of poly phenols was observed in methanol extract of leaf (70.4 ± 2.1mg GAE/g dry weight) followed by aqueous extract of leaf (57.5 ± 1.8 mg GAE/g dry weight). Bark extracts possess comparatively less phenolic content whereas, least amount was observed in chloroform extracts of both leaf and bark. Aqueous extracts of both leaf and bark contained relatively higher amount of flavonoids (21.6 ± 0.9 mg QE/g and 26.3 ± 1.2 mg QE/g dry weight, respectively) than methanol extracts of leaf and bark (16.2 ± 0.7 mg QE/g and 15.1 ± 0.7 mg QE/g dry weight, respectively). Like total phenolics, chloroform extracts of *Picea smithiana* contained trace amount of flavonoids. Many studies have shown that the antioxidant activities in the plants are associated with their phenolic contents. This may be due to their redox properties of the phenolic compounds that make them good reducing, scavenging and chelating agent [7-14] investigated the phytochemicals and antioxidant activity of Pinus cembra L. bark and needles.

Their results determined that the bark extract had higher concentrations of total phenolics, flavonoids and

proanthocyanidins than needle extract. Bark extract also showed better radical scavenging activity with EC_{50} value of 71.1µg/mL than needles [15] have reported the antioxidant activity of the essential oil of *Picea smithiana* growing in Kashmir (India). They observed moderate DPPH radical scavenging effect (35%) of *Picea smithiana* essential oil at the given concentration of 100µg/ml, in comparison to standard α-tocopherol (100µg/ml) that showed 78% antiradical effect.

Antioxidant and phytochemical investigation of *Pieca smithiana* extracts revealed that there is significant correlation between the antioxidant activity and phenolic and flavonoid. Total phenolic content determined using Folin-Ciocateu method and reducing activity showed significant correlation (R^2 = 0.965) between FRAP and the total phenolic content of *Picea smithiana* extracts. Similar correlation between total phenolic content and DPPH EC_{50} values (R^2 = 0.982) of the active extracts of Pieca smithiana has been observed. Therefore, it is possible that phenolic compounds are the major contributor for the antioxidant activity of the *Picea smithiana*.

Conclusion

The present study demonstrated the chemical composition and antioxidant potential of different extracts of *Picea smithiana* growing in Bhaderwah region of Jammu and Kashmir. The results conclude that methanol extract of *Picea smithiana* leaf contained good content of poly phenolic compound and that contributed as good radical scavenger and reductant as compare to other extracts.

Conflict of Interest

The authors have no conflict of interest regarding this paper.

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