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Research Article

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Pharmacological investigation of leaves extracts of *Picea smithiana*

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ABSTRACT

Picea smithiana is a medicinal plant, which is extensively used in traditional medicines. In the current study, pharmacological investigation of n-hexane, ethyl acetate, chloroform, water and residue extract fractions of Picea smithiana leaves was carried out. All fractions showed no antibacterial activity against the tested bacterial strains. The results of antifungal test revealed that the ethyl acetate (B), chloroform (C) and water (D) fractions have promising antifungal activities. In case of insecticidal activity, all fractions were found inactive against Tribolium castaneum, Rhyzopertha dominica and Callosbruchu analis, except the residue (E) fraction, which exhibited 18 % mortality against Rhyzopertha dominica. In case of phytotoxicity, all fractions showed significant phytotoxic activity at 1000 µg/ml concentration. All fractions except aqueous extract, exhibited moderate activity at the concentration level of 100 µg/ml and lowest activity at 10 µg/ml. This data supports the conclusion that extensive research should be conducted to explore the bioactive phytochemicals of the plant.

Keywords: *Picea smithiana*, antifungal, antibacterial, insecticidal, phytotoxicity, leaves

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INTRODUCTION

Picea smithiana locally known as 'Kachal' is widely spread in various regions of Pakistan such as Hazara, Mokhspuri and Kashmir. Some species of the genus *Picea* are widely distributed in Afghanistan, Himalaya from Chitral eastward to Nepal [1]. In Pakistan, the genus *Picea* is represented by one species, i.e. *Picea smithiana* [1]. A large number of compounds have been isolated from genus. Most of these are lignans, flavonoids, glucosides, and abietane type diterpenoids and norabietane derivatives [2]. Lignans are found almost in all parts of the plants, such as leaves, roots, stem, seeds and fruits. Higher amounts of lignans (6-24% w/w) were found in the knots of *Picea abies*, with hydroxymatairesinol ranging from 65–85% of the total lignan contents [3]. Traditionally this plant is used in various diseases such as body pains, inflammations and diabetes.

In the current study, we have made an effort to investigate its antibacterial, antifungal, insecticidal and phytotoxic activities profile of the plant.

EXPERIMENTAL SECTION

Plant Collection

Plant materials were collected from the Thandiani region, Abbottabad, Khyber Pakhtunkhwa province of Pakistan, in May, 2009. Taxonomic identification of the plant was done at the Department of Botany, Hazara University, Mansehra, Khyber Pakhtunkhwa province of Pakistan. A voucher specimen was deposited in the herbarium of the Department. The leaves of the plant were air-dried under shade for two consecutive months at room temperature. The dried plant materials were later finely grinded and stored in a polyethylene bags for bioassays.

Extract preparation

The dried and powdered leaves, 20 kg were soaked in ethanol with occasional stirring at room temperature for a period of one week and filtered. After filtration, the filtrates were evaporated under reduced pressure at temperature 45^{o}C. The process was repeated thrice. The combined ethanol extracts of leaves (1.9 kg) was suspended in water and fractionated with different solvents *n*-hexane, ethyl acetate, chloroform, water and residue etc. to obtain different fractions A to E. These fractions were further studied for antifungal, antibacterial, insecticidal and phytotoxic activities.

Fungal and bacterial strains

Six fungal and six bacterial strains were used for antimicrobial assays [4-6]. The bacterial strain used were *Escherrchia coli* ATCC 25922, *Bacilus subtilis* ATCC 6633, *Shigella flexeneri* (clinical isolate), *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhi* ATCC19430. The fungal strains included *Trichophyton Longifusus* (clinical isolate), *Candida albicans* ATCC2091, *Aspergillus flavus* ATCC 32611, *Microspoum canis* ATCC11622, *Fusarium solani* 11712 and *candida glaberata* ATCC 90030. All these strains were maintained on agar slants at 4 ^{o}C. The bacterial and fungal strains activated nutrient agar (NA) and Sabouraud glucose agar (SGA), prior to any screening.

Hole diffusion method

Anti microbial test was made by the hole diffusion method [7], by making use of a cell suspension of 1.5 x 10^6 CFU/mL, keeping in view the Farland turbidity standard No.0.5. The suspension concentration was standardized by adjusting the optical density to 0.1 at 600 nm (Shimadzu UV Visible spectrophotometer. Holes with 6 mm diameter were bore on Mueller Hinton agar (MHA) plate (8 mm thick) and were filled with 15 µL of extract fractions or standard drug (s) in DMSO. The plates were then allowed to incubate at 37 ^{o}C for 24 hours. The anti microbial activity was assessed by measuring the diameter of zones of inhibition around the holes. The bioassay was repeated thrice and then the mean diameter was calculated. Imipenem was used as standard antibiotics to compare the activity of extract fractions.

Determination of antifungal activity

In this method, extracts (10 mg/mL) were dissolved in DMSO and serially diluted with sterile water in microplates, in a laminar flow cabinet. An equal volume of actively growing cultures of the test fungi were mixed to the different wells and cultures were allowed to grow overnight in 100% humid environment at 37^{o}C. At morning, tetrazolium violet was mixed to all wells and the growth was observed by the appearance of violet color of the culture. The lowest concentration of the test solutions, which cause growth inhibition of the culture, was taken as the minimum inhibitory concentration (MIC). In this bioassay, amphotericin B and miconazole were used as positive controls.

Insecticidal activity

Insecticidal bioassay was carried out by direct contact application of the test compounds [8-9]. In the experiment, 3 mL of all extract fractions (1mg/mL) were applied to the filter papers having (90 mm diameter). After drying, each filter paper was placed in individual petri dish along with 10 adults of each of *Tribolium castaneum, Callosobruchus analis* and *Rhyzopartha dominica*. Permethrin (235.71 µ/cm^3) was used as a reference insecticide in this experiment. These insect were kept without food for 24 hours after which the % mortality was calculated.

Phytotoxic assay

In this phytochemical investigation, the crude extracts (A-E) were tested against *Lemna minor* [10-13]. In the assay, stock solutions (20 mg/mL) of extract fractions (A-E) were diluted and taken in three flasks to get final concentrations of 1000, 100, and 10μg/mL. Each flask was then mixed to a 20 mL medium and 10 plants, each one containing rosette of three fronds. Parquet was used as a standard growth inhibitor for the assay. All flasks were

allowed to keep in growth cabinet for incubation up to seven days. After this growth regulation in percentage was calculated with reference to the negative control.

RESULTS AND DISCUSSION

Antibacterial activity

All fractions (A-E) exhibited no antibacterial activity against the bacterial strains, used for antibacterial activity.

Antifungal activity

The *n*-hexane (A), ethyl acetate (B), chloroform (C), water (D) and residue (E) fractions *of Picea smithiana* were checked for antifungal activity against selected fungi including *Trichophyton longifusis, Candida albicans, Microsporum canis, Fusarium solani, Candida glabrata* and *Aspergillus flavus*. The results are presented in **Table 1**. The data showed that the ethyl acetate fraction (B) exhibited minimum inhibition at the concentration of 20 µg/mL against *Microsporumcanis*, while chloroform fraction(C) displayed minimum inhibition at the concentration of 10 µg/mL against *Microsporum canis*. The water fraction (D) was found active against *Fusarium solani* and minimum inhibition was recorded for 30 µg/mL concentration. All other fractions were found inactive against the selected fungi.

Insecticidal assay

Different fractions of extracts of leaves of *Picea smithiana* were investigated against various insects viz, *Tribolium castaneum, Rhyzopertha dominica* and *Callos bruchucanalis.* Permethrin was used as a standard drug in this assay. All the tested fractions show no activity against the insects *Tribolium castaneum, Rhyzopertha dominica* and *Callosbruchu analis* except the residue fraction (E), which showed 18% mortality with reference to *Rhyzopertha dominica* **(Table 2).**

Phytotoxic assay

Results of phytotoxicity assay (**Table 3**) of various fractions of the *Picea smithiana* leaves extract revealed that the *n*-hexane fraction (A), ethyl acetate fraction (B), chloroform fraction (C), water fraction (D) and residue fraction (E) showed promising phytotoxicity at the concentration levels of 1000 μg/ml. The *n*-hexane fraction showed 73, o8 and 04% growth regulation at the concentrations of 1000, 100 and 10 μg/ml. The ethyl acetate fraction revealed maximum value for growth regulation (74%) at the concentration of 1000μg/ml, while the aqueous fraction showed the low activity (29%) for the same concentration. The chloroform fraction exhibited the highest (22%) growth regulation at 100 μg/ml concentration. The aqueous fraction exhibited no activity against *Lemna minor* at 100 and 10 μg/ml.

Table-1: Antifungal activity of the leaves extract fractions of *Picea smithiana*

Fungal species	**Minimum Inhibitory Concentration (µg/mL)**					
	Miconazole	A	B	C	D	E
Trichophyton longifusis	70.08	-	-	-	-	-
Candida albicans	110.8	-	-	-	-	-
Microsporum canis	98.4	-	20	10	-	-
Fusarium solani	73.10	-	-	-	30	-
Candida glabrata	110.8	-	-	-	-	-
	Amphotericin B					
Aspergillus flavus	20	-	-	-	-	-

Key words: *A= n-hexane, B = ethyl acetate, C = chloroform, D = aqueous, E = residue*

Table 2: Insecticidal activities of the extract fractions of *Picea smithiana* leaves

Insect	% Mortality						
	+ve control (Permethrin)	**-ve control**	**A**	**B**	**C**	**D**	**E**
Tribolium castaneum	100	0	0	0	0	0	0
Rhyzopertha dominica	100	0	0	0	0	0	18
Callosbruchu analis	100	0	0	0	0	0	0

Key words*: A = n-hexane, B = ethyl acetate, C = chloroform, D = aqueous, E = residue*

Table 3: Phytotoxic activities of the extract fractions of *Picea smithiana* leaves

Conc. of sample (µg/ml)	% Growth regulation						
	Paraquat (0.015 µg/ml)	-ve control	A	B	C	D	E
1000	100	0	73	74	29	42	73
100	100	0	08	18	22	-	08
10	100	0	04	05	17	-	04

***Key words**: A = n-hexane, B = ethyl acetate, C = chloroform, D = aqueous, E = residue*

CONCLUSION

Picea smithiana is an important medicinal plant, which is abundantly found in Pakistan. The current study revealed that the various extracts of leaves of plants posses antifungal, phytotoxic and cytotoxic activities. However, no antibacterial activity was recorded for the tested microorganisms. This data supports the ethno pharmacological importance of this plant.

REFERENCES

[1] A. Radcliffe-smith Royal botanical garden, Kew, Fl. pak., **1986**, (182), 6-15.
[2] KUO Yueh-Hsiung; *Chem. Pharm. Bull.*, **2004**, (52), 861-863.
[3] S Willfor; J Hemming; C Eckerman; *Holzforschung.*, **2003**, (57), 27-34.
[4] AK Jan; MR Shah; I Anis; IK Marwat; *J. Enzyme Inhib Med Chem*., **2009,** 24 (7), 192-196.
[5] M Nisar; SA Tariq; IK Marwat; MR Shah; IA Khan; *J Enzyme Inhib Med Chem.*, **2009**, 24(4), 224-229.
[6] M Shakirullah; H Ahmad, MR; AI Shah; M Ishaq; N Khan; A Badshah; I Khan; *J Enzyme Inhib Med Chem.*, **2011**, 26(4), 468-471.
[7] VA Berghe; AJ Vlietinck;. *Method Plant. Biochem*., **1999**, (6) 47-68.
[8] R Rashid; M Farah; MN Mirza; *Pak J Bot.*, **2009**, 41(3), 1453-1462.
[9] AU Rehman; MI Choudhary; JT William; Bioassay techniques for drug development. *Harward academic Publisher, Singapore.* **2001**, 8(4), 67-68.
[10] AU Rehman; Studies in Natural Product Chemistry. *Elsevier Science Publishers, Netherlands*; **1991**, (9), 383-384.
[11] DJ, Finny Probit Analysis. 3rd edition, *Cambridge Uni Press, Cambridge.* **1971**, 333-341.
[12] IY Hideji; A Oshida; IH Inatomi; T Adachi; *Phytochemistry.*, **1982**, 21(8), 1935-1937.
[13] MA Lewis; *Environ Pollut.*, **1995**, 87(3), 319-336.