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Antibacterial, Antifungal, Insecticidal and Phytotoxic activities of *Abies pindrow* leaves

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Abstract

The current study was carried out to investigate the bioactivities of various extract fractions of *Abies pindrow* leaves. All fractions exhibited no anti-bacterial activity against all the tested bacterial strains. In case of antifungal study, *n*-hexane, ethyl acetate, chloroform, and residue fractions showed 20, 30 and 10% minimum inhibition at concentration of 20µg/mL, against *Microsporum canis* and *Fusarium solani*. The data obtained from insecticidal assay revealed that all the tested fractions shows no activity against the insects *Tribolium castaneum*, *Rhyzopertha dominica* and *Callos bruchuanalis*, except *n*-hexane extract fraction which display 20% bioactivity against *Callos bruchuanalis*. Chloroform fraction shows 20% activity against *Tribolium castaneum, Rhyzopertha dominica* and 40% against *Callos bruchuanalis* respectively. The water fraction which revealed 40% activity against *Rhyzopertha dominica* and 20% against *Callos bruchuanalis* respectively. The residue fraction shows 20% activity against *Rhyzopertha dominica*. Results of phytotoxicity revealed that the *n*-hexane, ethyl acetate, chloroform, water and residue fractions showed good phytotoxic activity at 1000µg/ml concentration. All the fractions showed moderate activity at 100 µg/ml concentrations except the *n*-hexane and residue fractions which showed 60 and 35% activity at 100 µg/ml concentrations respectively. At concentration of 10 µg/ml all the fractions showed low activity except chloroform fraction which shows no activity at 10µg/ml at all.

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Introduction

The genus *Abies* (family Pinaceae) consists of about 50 species, distributed in Asia, Europe, North and Middle America as well as in North Africa (Zheng and Fu., 1978). In Pakistan this genus is represented by two species, i.e. *Abies pindrow* and *Abies spectabilis* (Radcliffe *et al.,* 1986). *Abies pindrow* locally known as 'Partal, Palundar', is widely spread in various regions of Pakistan such as Islamabad, Rawalpindi, Khyber agency, Hazara, Muree, Dunga Gali, Shogran etc. With their high medicinal value. The other species of the same genus are widely distributed in Afghanistan, Kaghan, Himalaya from Chitral eastward to C. Nepal (Radcliffe *et al.,* 1986).

A large number of compounds have been isolated from genus *Abies*. Most of them are flavonoids, fatty acid, alcohol, triterpenes and lignin's (Raldugin, *et al.,* 1990). Some species have been used as folk medicines against various diseases such as indigestion, colds, stomachache, pulmonary and venereal diseases (Yesilada *et al.,* 1995; Fujita, *et al.,* 1995). This genus has also shown many kinds of activities such as insect juvenile hormone (Bowers *et al.,* 1966; Manville *et al.*1977) antiulcer genic (Singh *et al.,* 1998; Singh *et al.,* 2000), anti-inflammatory (Singh *et al.,* 1998; Singh *et al.,* 1997; Singh *et al.*, 1997), antihypertensive (Singh, *et al.,* 1998; Bhakuni, *et al.,* 1971), antitussive (Singh *et al.,* 2000; Nayak *et al.,* 2003) and CNS (central nervous system) activities (Singh *et al.,* 1998; Kumar *et al.*, 2000; Nayak *et al.,* 2004).

The data given above revealed that this genus is very important from medicinal point of view. In the ongoing studies the specie *Abies pindrow* was selected to investigate the antibacterial, antifungal, insecticidal and phytotoxicity activities of *Abies pindrow* leaves to further explore the medicinal potential of this genus.

Material and methods

Plant material

The leaves of medicinal plant *A. pindrow* were collected in May 2010 from Mukhspori regions northern areas of Pakistan. Taxonomic identification of the plant was done at the Department of Botany, Islamia College University, Peshawar, Pakistan. A voucher specimen (No.Sj-38) was deposited at the herbarium of the University.

Extraction and fractionation

Air dried, powdered leaves (20kg) were subjected to extraction (hot extraction) with methanol as a solvent. The methanol extract was filtered, separated and concentrated by vacuum rotary evaporator. The greenish combined extract obtained weighed (5.4kg), was further subjected to fractionation by using *n*-hexane. The *n*-hexane fraction was allowed to concentrate by vacuum rotary evaporator; afforded *n*-hexane fraction weighed (1.8kg). The water fraction obtained weighed (3.5kg) which was further subjected to fractionation with ethyl acetate resulted in to ethyl acetate fraction weighed (1.2 kg). The water fraction obtained was further subjected to fractionation with chloroform resulted into three fractions. The chloroform fraction obtained weighed (1kg). The residue obtained weighed (0.8kg) and the water fraction obtained weighed (0.4kg) respectively. All these fractions were tested for antimicrobial, Insecticidal and phytotoxic activities.

Antibacterial assay

In this biological evaluation, a total of six bacterial strain were chosen to be used. The bacterial strain used were classified as *Escherichia coli* ATCC 25922, *Shigella flexenari* (clinical isolate), *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Salmonella typhi* ATCC and 19430 *Pseudomonas aeruginosa* ATCC 27853. All these strains were sustained on agar slant at 4°C and the slant was allowed to activate at a temperature of 37°C for one day on nutrient agar (NA), before any screening is carried out. The microorganisms; *B. subtilis* ATCC 6633, *S. flexenari (clinical isolate), E. coli* ATCC 25922, *S. aureus* ATCC 25923 *P. aeruginosa* ATCC 27853 and *S. typhi* ATCC 19430 were castoff for appraisal of antibacterial activity. The organisms were kept in Muller hantin agar in the refrigerator at 4°C prior to subculture. Antibacterial testing was conceded out on the already developed agar well diffusion method to study the effectiveness of the extract fractions of *A. Pindrow* leaves.

Broth media were primed and the test organisms were moved to the broth media from agar plate and were grown-up at 37°C for one day. After 24 hours 25ml of MHA were discharged into each petri plate and cooled in sterile condition. The fresh culture was primed from day old culture, after solidification of MHA in plate, 0.6ml of fresh culture of test organism were emptied on to MHA. Wells of 6mm diameter were digged in to the medium by using sterile borer and 22mg of dissimilar fractions of the extract of *A. Pindrow* leaves were used against each organism. DMSO and standard antibiotic (imipenum) were mixed into other wells. The plates were kept in pasteurized inoculation chambers for 60 minutes to facilitate diffusion of the antimicrobial agent into the medium. The plates were then incubated at 37°C for one day and the diameters of the zone of inhibition of microbial progression were measured in millimeters (Carron *et al.,* 1987).

Antifungal assay

The microorganisms; *Trichophyton longifusis, Aspergilus flavus* ATCC 32611, *Candida albicans* ATCC 2091, *Fusarium solani* 11712, *Microsporum canis* ATCC 11622 and *Candida glaberata* ATCC 2091 were used for antifungal potency. All these strains were maintained on agar slant at 4°C, the slant was endorsed to activate at a temperature of 37°C for duration of 4 days on nutrient agar (NA), for fungi, before any screening is done. The crude extract fractions were liquefied in DMSO (24mg/ml) and sterile medium (5ml) was positioned in a test tube and inoculated with the sample solution (400µg/ml) which was then retained in a slanting position at room temperature for whole night. The tubes were inoculated by a piece of fungus (4mm diameter) from one week old culture. The samples were then incubated for one week at 28°C and the fungal strain starts growth on the slant. The growth reticence was detected and percentage growth inhibition was firm by calculating with reference to the positive control by applying the formula

$$\% \text{ Inhibition} = \frac{\text{100-linear growth and test (mm)}}{\text{Linear growth in control (mm)}} \times 100$$

Amphotericin B and miconazole were used as standard antibiotics (Choudhary *et al.,* 1995).

Insecticidal assay

Different fractions of *A. Pindrow* leaves were investigated against various Insects viz, *Tribolium castaneum, Rhyzopertha dominica* and *Callos bruchuanalis*. The test sample was prepared by dissolving crude fractions in 3ml acetone and kept in a Petri dish with the filter papers covered. After a total time of 24 hours, 10 test insects were mixed in each plate and incubated at temperature of 27°C for a total duration of 24 hours with 50% relative humidity in growth chamber. Insecticidal activity was done by direct contact application of the test compounds by using filter paper (Ahn *et al.,* 1995). In this experiment 3ml of all extract/fractions (1mg/ml) were applied to the filter papers having (90 mm diameter). After drying, each filter paper was allowed to place in individual Petri dish along 10 adults of each of *Tribolium castaneum, Callos bruchuanalis* and *Rhyzopartha dominica*. Permethrin (235.71µ/ cm^3) was used as a reference insecticide in this experiment. These entire insects were kept to stand without food for 24hours after which the mortality number was calculated.

Phytotoxicity assay

In this bioactivity study the various crude fractions were tested against *Lemna minor* (Ahn *et al.,* 1995). In this experiment three flasks were inoculated with a sufficient stock solution of (20mg/ml) to obtain a final concentration of 1000, 100, and 10µg/ml respectively. Each flask was then mixed to a 20ml medium sized 10 plants, each one containing rosette of three fronds. Parquet was used as a standard growth inhibitor in this experiment. The whole 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

All these crude fractions were tested for their bioassay evaluation which included antibacterial, antifungal, Insecticidal and phytotoxic activities. The results obtained are depicted in tabular form as follow.

Antibacterial activity

All the fractions exhibited no antibacterial activity against all bacterial strains used for antibacterial activity.

Antifungal activity

The *n*-hexane, ethyl acetate, chloroform, water and residue fractions were investigated (Table 1) for their antifungal bioassay against the *Trichophyton longifusis, Candida albicans, Aspergilus flavus, Microsporum canis, Fusarium solani,* and *Candida glaberata*. All these fractions showed no bioactivity against selected fungal strains except *n*-hexane, ethyl acetate and chloroform fractions which showed 20, 30 and 10% bioactivity against *Microsporum canis*.

Residue fraction which displayed 10% bioactivity against *Microsporum canis*. All the fractions exhibited no activities against the remaining fungal strains.

Table 1. Antifungal activity of crude fractions of *Abies pindrow* leaves.

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

Key: A = *n*-Hexane, B = EtOAc, C = Chloroform, D = Water, E = Residue.

Insecticidal activity

Results were determined as percentage mortality, which was calculated with reference to the positive and negative controls. In this bioactivity, Permethrin was used as a standard drug, while Permethrin, acetone and test insects were used as positive and negative controls. All the tested fractions showed no activity against the insects *Tribolium castaneum, Rhyzopertha dominica* and *Callos bruchuanalis,* except *n*-hexane which showed 20% bioactivity against *Callos bruchuanalis*. Chloroform fraction which displayed 20% activity against *Tribolium castaneum, Rhyzopertha dominica* and 40% against *Callos bruchuanalis* respectively. The water fraction which showed 40% activity against *Rhyzopertha dominica* and 20 % against *Callos bruchuanalis* respectively. The residue fraction showed 20% activity against *Rhyzopertha dominica* (Table 2).

Table 2. Insecticidal activity of crude fractions of *Abies pindrow* leaves.

Insect	% Mortality						
	+ve control (Permethrin)	-ve control	A	B	C	D	E
Tribolium castaneum	100	0	0	0	20	0	0
Rhyzopertha dominica	100	0	0	0	20	40	20
Callos bruchuanalis	100	0	20	0	40	20	0

Key: A = *n*-Hexane, B = EtOAc, C = Chloroform, D = Water, E = Residue.

Phytotoxic activity

Results of Phytotoxic activity of various fractions of the leaves of *A. Pindrow* are shown in the (Table 3). The *n*-hexane, ethyl acetate, chloroform, water and residue fractions showed good phytotoxic activity at 1000µg/ml concentration. All the fractions showed moderate activity at 100µg/ml concentration except the *n*-hexane and residue fractions which showed 60 and 35% activity at 100µg/ml concentration. At concentration of 10µg/ml all the fractions showed low activity except chloroform fraction which shows no activity at 10µg/ml at all.

Table 3. Phytotoxic activity of crude fractions of *Abies pindrow* leaves.

Conc.of sample (µg/ml)	% Growth regulation						
	Paraquat (0.015 µg/ml)	-ve control	A	B	C	D	E
1000	100	0	100	85	60	25	75
100	100	0	60	15	10	10	35
10	100	0	05	05	-	05	05

Key: A = *n*-Hexane, B = EtOAc, C = Chloroform, D = Water, E = Residue.

Conflicts of interest

Authors have none to declare.

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