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Biological activities of *Prunus persica* L. batch

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Biological screening of extracts from bark of *Prunus persica* was carried out. The crude methanolic extract and its various fractions namely petroleum ether, dichloromethane, chloroform and ethyl acetate were investigated for their antibacterial, antifungal, phytotoxic and insecticidal properties. The methanolic extract showed significant antibacterial activity against *Klebsiella pneumonia* and *Enterococcus faecalis*. The petroleum ether fraction also demonstrated significant antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The dichloromethane fraction inhibited the growth of *E. coli* and *K. pneumonia*. The chloroform fraction showed no significant activity against *E. coli, K. pneumonia* and *Bacillus subtilis*. The ethyl acetate fraction indicated significant activity against *E. faecalis*. Among the tested fungal species, *Aspergillus flavus*, *Microsporum canis*, and *Fusarium solani* were more susceptible to dichloromethane, chloroform and methanol fractions. Only methanol fraction indicated moderate phytotoxic activity at high dose, while other fractions showed low activity. Crude extract and its fractions exhibited low insecticidal activity.

Key words: *Prunus persica*, biological activities, antimicrobial, phytotoxic, insecticidal.

INTRODUCTION

Majority of people in developing countries still uses traditional folk medicines obtained from plant resources (Farnsworth, 1994; Srivastava et al., 1996). Medicinal plants contain active constituents to cure human diseases (Stary and Hans, 1999) and possess great potential for the production of new drugs. Therefore, the exact potentials for screening of medicinal plants are required to be explored through proper scientific investigations. Keeping in view the medicinal properties of *Prunus persica* and its wide applications in folk medicine system, it has been investigated for its various biological activites.

P. persica L. Batch (Peach) named as *Amygdalus persica* is a deciduous tree of the subfamily Prunoideae of the family Rosaceae with a height of 5 to 10 m and is commonly cultivated in West Asia, Europe, Himalayas and India up to an altitude of 1000 ft. There are about 100 genera and 3,000 species in Rosaceae family (Judd et al., 1999). *Prunus* has nearly 200 species cultivated for their edible fruits and seeds (Rheder, 1940). The leaves are anthelmintic, insecticidal, sedative, diuretic, demulcent, expectorant, vermicidal and are used in leucoderma and in piles. Leaf paste is used to kill worms in wounds and fungal infections. The treatment of gastritis, whooping cough and chronic bronchitis is carried out internally with leaves (Kritikar and Basu, 1984). The flowers are considered as laxative and diuretic and are used to treat constipation and oedema. The fruit is used as a demulcent, an anti-scorbutic and a stomachic. Fruit being aphrodisiac, anti-pyretic, act as a tonic to the brain, enhance the blood, removes bad smell from the mouth. The seeds are used as an anthelmintic and emmenagogue. The oil extracted from seeds is known as “kapha”, used as an abortifacient, good in deafness, piles, stomach troubles of children and earache. Peach kernels are used for blood diseases, menstrual disorders, coughs and rheumatism in China and Malaya (Kritikar and Basu, 1984). The kernel oil is applied to impetigo. The bark is used in leprosy and jaundice.

The seed contains ‘laetrile’, a substance also called

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vitamin B_{17}. Leaves of *P. persica* have been investigated for their antioxidant (Deb et al., 2010) and anti-inflammatory (Shin et al., 2010) activities in the past. However, its detail biological activities are hitherto unreported. Therefore, the present study was undertaken to evaluate the biological activities spectrum of *P. persica*.

EXPERIMENTAL PROCEDURE

Collection and extraction method

The plant was collected from Jhandgran paein, Azad Kashmir, Pakistan. The plant was identified by a plant taxonomist at the Department of Botany, University of Azad Jammu and Kashmir. The voucher specimens of the plant were placed in the herbarium of the department. The dried plants materials (bark) were grinded to the powdered form. About 200 g of the powdered material was separately soaked in the methanol for 7 days. The extracts were then filtered and evaporated on rotary evaporator under reduced pressure to dryness. The dried plant materials were extracted in various organic solvents (petroleum ether, dichloromethane, chloroform, methanol and ethyl acetate) to avoid the solvents soluble material. Antibacterial activity of crude extract and its fractions were determined by using disc diffusion method, also known as the zone of inhibition method (Bauer et al., 1966).

Antibacterial activity

Nutrient broth medium was inoculated with single colony of bacterial culture and incubated at 37°C for 24 h. After 24 h, nutrient agar medium was taken and autoclaved at 121°C for 15 min. This medium was then melted and cooled up to 45°C. About 10 µl of fresh bacterial culture was added, shaked and then poured on to the nutrient agar plate. The plate was rotated to make even distribution of the culture and allowed to solidify. The test samples were added in agar plates by using 5 mm sterile filter paper discs. The plates were incubated at 37°C for 24 h. The next day the results were noted in terms of zone of inhibition (expressed in mm). Reference antibacterial drug (ampicilline) and dimethylsulfoxide (DMSO) were used as positive and negative controls.

Antifungal activity

Agar tube dilution protocol was used for analysis. Sabouraud dextrose agar (SDA) with acidic pH 5.5 to 5.6 was prepared by mixing 32.5 g of glucose (2%) in 500 ml distilled water. It was then steamed to dissolve and dispense 4 ml amount in to screw capped tubes. The tubes containing media were autoclaved at 121°C for 15 min. The extracts dissolved in sterile DMSO (400 mg/ml) served as a stock solution. The tubes were allowed to cool to 50°C and solidified Sabouraud agar media was poisoned with 66.6 µl of test sample pipette out from the stock solution which provided a final concentration of 200 µg/ml of SDA for test samples. Tubes were then allowed to solidify in slanted position at room temperature. Each tube was inoculated with piece of inoculums removed from a seven days old culture of fungus, for non mycelia growth, an agar surface streak was employed. Other media supplemented with DMSO and reference antifungal drugs used as negative and positive control, respectively. The standard drugs used in the assays were miconazole and amphotericin B. The tubes were incubated at 27 to 29°C for 7 to 10 days. The growth in the compound containing media was determined by measuring the linear growth in mm and growth inhibition with reference to the negative control. The percentage inhibition was determined as prescribed by Atta-ur-Rahman et al. (1991).

Phytotoxic bioassay

Phytotoxic bioassays were analyzed by modified protocol of *Lemna minor* (McLaughlin et al., 1991). The medium was prepared by mixing various constituents in 1000 ml distilled water and the pH was adjusted between 6.0 and 7.0 by adding KOH solution. The medium was then autoclaved at 121°C for 15 min. The extracts (30 mg) dissolved in methanol served as stock solution. Three flasks were inoculated with 10,100 and 1000 µl of stock solution. The solvent was allowed to evaporate overnight under sterile conditions. Now 20 ml of the medium and the plant of *L. minor* each containing a rosette of two to three fronds, were added to each flask (total 20 fronds). All flasks were plugged with cotton and kept in growth cabinet for seven days. The number of fronds per flasks were counted and recorded on day 7. Finally, the results were calculated as growth regulation in percentage. The results were calculated with reference to the positive and negative control. Paraquat was used as a standard drug, while paraquat and volatile solvent were used as positive and negative control.

Insecticidal activity

Contact toxicity method was used for analysis of plant extracts and fractions. The test sample was prepared by dissolving 200 mg of crude fractions in 3 ml volatile solvent and loaded in petri plate. After 24 h of complete evaporation of the solvent, 10 test insects were placed in each plate (test and control) and plates were incubated at 27°C for 24 h with 50% relative humidity in growth chamber. The results were analyzed as percentage inhibition or percentage mortality, calculated with reference to the positive and negative controls. The percentage mortality was determined. Permethrin was used as a standard drug, while permethrin, acetone and test insects were used as positive and negative controls (Ali et al., 2009).

RESULTS AND DISCUSSION

Antibacterial activity

The crude methanolic extracts as well as its fractions were investigated against eight bacterial strains namely, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *S. aureus*, *Bacillus subtilis*, *Salmonella typhi* and *Shigella flexenari*. The petroleum ether fraction indicated significant activity against *E. coli*, while it exhibited moderate activity against *S. aureus* and low activity against *K. pneumonia* and *E. faecalis.* The fraction had no effect on the growth of *P. aeruginosa, B. subtilis, S. typhi and S. flexenari.* The dichloromethane fraction displayed good activity against *E. coli, K. pneumonia* and *S. aureus*. The chloroform *glabrata.* The petroleum ether fraction indicated no activity against *C. albicans*, *A. flavus, F. solani, M.* canis and *C. glabrata*. The dichloromethane fraction indicated significant activity against *M. canis*. The fraction exhibited showed low activity against *E. coli*, *B. subtilis* and *K. pneumonia*. The ethyl acetate fraction showed significant

Table 1. Antibacterial activities of different fractions of *P. persica*.

Bacterial strain	Extract	Activity	ZI of Std. drug
E. coli	P. E.	33	40
	DCM	17	40
	$CHCl_3$	8	40
	E. A.	10	40
	MeOH	-	40
K. pneumonia	P. E.	8	42
	DCM	21	42
	$CHCl_3$	6	42
	E. A.	37.6	42
	MeOH	28	42
P. aeruginosa	P. E.	-	22
	DCM	-	22
	$CHCl_3$	-	22
	E. A.	-	22
	MeOH	-	22
E. faecalis	P. E.	9	41
	DCM	8	41
	$CHCl_3$	-	41
	E. A.	55	41
	MeOH	33	41
S. aureus	P. E.	17	38
	DCM	22	38
	$CHCl_3$	-	38
	E. A.	-	38
	MeOH	-	38
B. subtilis	P. E.	-	36
	DCM	-	36
	$CHCl_3$	6	36
	MeOH	-	36
S. typhi	P. E.	-	40
	DCM	-	40
	$CHCl_3$	-	40
	MeOH	-	40
S. flexenari	P. E.	-	36
	DCM	9	36
	$CHCl_3$	-	36
	MeOH	-	36

ZI, Zone of inhibition, Std. drug, ampicilline; petroleum ether, P. E.; dichloromethane, DCM; chloroform, $CHCl_3$, ethyl acetate, E.A; methanol, MeOH; '-', indicated no activity.

activity against *K. pneumonia* and *E. faecalis.* The crude methanolic fraction of *P. persica* significantly inhibited the growth of *K. pneumonia* and *E. faecalis*, while no activity was indicated by *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, *S. typhi and S. flexenari*. The results of antibacterial bioassays are presented in the Table 1.

Table 2. Antifungal activities of different fractions of *P. persica*.

Name of fungus	Extract	Linear growth (mm)		Inhibition (%)	Std. drug	Std. drug conc. (µg/ml)
		Sample	Control			
C. albicans	DCM	100	100	0	Miconazole	110.8
	$CHCl_3$	100	100	0	Miconazole	110.8
	MeOH	100	100	0	Miconazole	110.8
A. flavus	DCM	85	100	15	Amphotericin B	20.20
	$CHCl_3$	100	100	0	Amphotericin B	20.20
	MeOH	100	100	0	Amphotericin B	20.20
M. canis	DCM	60	100	40	Miconazole	98.4
	$CHCl_3$	70	100	30	Miconazole	98.4
	MeOH	100	100	0	Miconazole	98.4
F. solani	DCM	75	100	25	Miconazole	73.25
	$CHCl_3$	100	100	0	Miconazole	73.25
	MeOH	80	100	20	Miconazole	73.25
C. glabrata	DCM	100	100	0	Miconazole	110.8
	$CHCl_3$	100	100	0	Miconazole	110.8
	MeOH	100	100	0	Miconazole	110.8

Concentration of sample, 400 µg/ml of DMSO; incubation time, 27 (28±1°C); incubation period, 7 days.

Table 3. Phytotoxic activities of different fractions of *P. persica*.

Plant	Fraction	Conc. (µg/ml)	No. of fronds		Growth regulation (%)	Std. drug (µg/ml)
			Sample	Control		
L. minor	Petroleum ether	1000	15	18	17	0.015
		100	18		5.5	
		10	19		0	
	Dichloromethane	1000	15	19	21	0.015
		100	18		5	
		10	19		0	
	Chloroform	1000	13	18	27.7	0.015
		100	16		11.11	
		10	19		5.5	
	Methanol	1000	12	18	33.3	0.015
		100	18		11.11	
		10	20		0	

Antifungal activity

The various extracts of plants were investigated against five fungal strains like *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida* moderate activity against *F. solani* and low activity against *A. flavus*.

The chloroform fraction indicated moderate activity against *M. canis*. The fraction showed no activity against *C. albicans*, *A. flavus*, *F. solani* and *C. glabrata*. The methanolic fraction of *P. persica* exhibited low activity against *F. solani*. The activity profile is presented in the Table 2.

Phytotoxic bioassay

These plant extracts were also investigated for their phytotoxic activity against *L. minor*. The petroleum ether fraction showed low activity. The dichloromethane fraction exhibited low activity against *L. minor*. The chloroform fraction also indicated low activity. The crude methanolic extract showed low activity against *L. minor*. The activity profile is presented in the Table 3.

Insecticidal bioassay

The plant extracts of *P. persica* were also examined for

Table 4. Insecticidal activities of different fractions of *P. persica*.

Insect	Mortality (%)		Sample mortality (%)			
	Std.	NC	Petroleum ether (%)	Dichloromethane (%)	Chloroform (%)	Methanol (%)
T. castaneum	100	0	0	0	0	0
R. dominica	100	0	20	20	20	0
C. analis	100	0	0	0	20	20

Concentration of sample = 1019.1 µg/cm^2; concentration of Std. drug = 239.5 µg/cm.

their insecticidal activity against three insects, namely:

Tribolium castaneum, *Rhyzopertha dominica* and *Callosbruchus analis*. The methanolic and petroleum ether fraction exhibited moderate activity against *C. analis* and *R. dominica*. The dichloromethane fraction showed moderate activity against *R. dominica*, while it indicated no activity against *T. castaneum* and *C. analis*. The chloroform fraction exhibited moderate activity against *R. dominica* and *C. analis*, while it showed no activity against *T. castaneum*. The activity profile is presented in the Table 4.

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