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Original Article

BIOLOGICAL SCREENING OF *ARAUCARIA COLUMNARIS*

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ABSTRACT

Objective: The present research is biological screening of aerial part of *Araucaria columnaris* (Araucariaceae). There were investigation for their antibacterial, antifungal, phytotoxic and cytotoxic activities of Dichloromethane and methanol extract.

Methods: Anti-bacterial, Anti-fungal, cytotoxicity and phytotoxicity activity was performed by Disc diffusion method, Agar tube dilution assay, Brine Shrimp Lethality bioassay and Lemna bioassay respectively.

Results: Dichloromethane and methanolic extract exhibited significant phytotoxicity against Lemna minor having Paraquat as standard drug and incubation condition (28±1 °C). None of extracts presented any significant antibacterial and cytotoxic activity having Imipenum and Etoposide as standard drug respectively. Both extract had non-significant antifungal activity but it has been noted that MeOH and DCM extract of *Araucaria columnaris* showed 10% and 20% inhibition with linear growth at 90 mm and 80 mm respectively, when compared with control; against *Fusarium solani* and *Aspergillus flavus* respectively.

Conclusion: *Araucaria columnaris* exhibited significant phytotoxicity bioassay. The phytotoxicty assay is a useful primary screen for weedicide research. Synthetic weedicides are expensive, toxic and non-specific. This study will help to discover the phytotoxic constituents of the plant by isolation, purification and structure elucidation to find out as effective herbicidal.

Keywords: *Araucaria columnaris,* Biological screening, Phytotoxicity, Weedicide research.

INTRODUCTION

Araucaria columnaris also known as *Cupressus columnaris J. R. Forst* is indigenous to New Caledonia, Polynesia [1]. It is belonging to genus Araucaria. Almost 19 species belong to the genus. It is indigenous to North America. Biflavanoid, diterpene, phenyl propanoid and lignans are abundant in the genus. It is recognised as well known ornamental plant all over the world. Its genus possesses other pharmacological activity such as anti-inflammatory, anti-ulcer, anti-viral, neuro protective and anti depressent [2]. Present study is conducted to document the *in vitro* biological activities of *Araucaria columnaris*. Antibacterial, antifungal, phytotoxic and cytotoxic bioassay has been studied.

Experiment

Plant material

Araucaria columnaris were collected from an ornamental shop and identified by Dr. Altaf Hussain Dasti, Professor, Institute of pure and applied Biology, Bahauddin Zakariya University, Multan giving the Catalog number 12-fci. Total wet weight of plant collected was 6 kg. It was then reduced to 3 kg of dried plant. The plant was then ground till it becomes powder. The total weight of powder drug was 600 grams.

Extraction

Maceration is the technique for extraction for finely ground plant material. Measured quantity of plant material was taken in a glass bottle. After that quantified volume of dichloromethane was added to it with constant sonication in the ultrasonic bath. It takes 24 hours to be settle down and then filtration was performed. Repeat the process three times with dichloromethane and then methanol. The Dichloromethane used during first, second and third soaking was 1000 ml, 700 ml and 600 ml respectively and 600 ml, 400 ml and 400 ml for methanol respectively. Rotary evaporator was used for the concentration of both extracts under reduced pressure labeled with codes as ACAPD and ACAPM respectively.

Anti-bacetrial bioassay

Escherichia coli (NCTC 10418), *Bacillus subtilis* (NCTC 8236), *Staphylococcus aureus* (NCTC 6571), *Pseudomonas aureginosa* (ATCC 10145) *Shigella flexinari* and *Salmonella typhi* are tested microorganism. The Petri plates are prepared with an inoculated media. Three wells of 8 mm diameter on one plate are cut with a borer and sealed with a drop of inoculated sterile media. All the solutions i.e., the extract, solvent and reference standard (Imipenum 10µg/disc.) was poured into their respective well by sterilized pipette. The petri dishes were incubated at 37°C for 24-48 hrs. Zones of inhibition were measured with vernier caliper [3].

Anti-fungal bioassay

Test fungi such as *Candida albicans, Aspergillus flavus, Microsporum canis Fusarium solani, Candida glabrata* were employed for preliminary screening. Extracts were dissolved in sterile DMSO to serve as stock solution. Sabouraud dextrose agar was prepared by mixing Sabouraud 4% glucose agar and agar in distilled water. Known amount of media was dispensed into screw capped test tubes. Test tubes containing media were autoclaved 121°C for 15 minutes. Tubes were allowed to cool to 50°C and the desired concentration of extract was added into non-solidified media. The tubes were allowed to solidify at room temperature. Each tube was inoculated with a 4 mm diameter piece of inoculum removed from a seven-day-old culture of fungi. All culture-containing tubes were inoculated at optimum temperature of 28-30°C for growth for 7-10 days. Culture was examined at least twice a weekly during the incubation. With no visible growth of microorganisms is taken to represent the MIC of the test sample which is expressed in µg [3].

Phytotoxicity bioassay

Prepared inorganic medium of 5.5-6.0 pH attained with KOH pellets. 10 vials per dose 500, 50, 5 and control were prepared. 15 mg of the extract was dissolved in 15 ml of the solvent. 1000, 100 and 10 µl of solution to vials for testing allow the solvent to

evaporate overnight. 2 ml of medium was added in each vial containing a single plant a rosette of three fronds. Placed the vials in a glass dish filled with 2 cm of water, sealed the container with stopcock grease and glass plate. Placed the dish along with vials in the growth chamber for seven days at 25 °C under fluorescent and incandescent light. Count the number of fronds per vials on day 3 and 7. Analyzed the data as percent of control with ED 50 computer program [3].

Brine shrinp lethality bioassay

Brine shrimp cytotoxicity assay was accomplished according to the standard procedure described by Mc Laughlin, (1991). Three concentrations (1000, 100, and 10 ppm) of the plant extracts were used in this assay. Brine shrimp larvae were hatched in a small partitioned tank in artificial seawater. Illumination was provided on one side to attract newly hatched larvae. Brine shrimp larvae with second instar stage were used in this assay.

Plant extracts of the respective concentrations were added to dram vials. To each dram vial ten brine shrimp larvae were added. Negative control was prepared by evaporating 0.5 ml of methanol in dram vials and then by adding sea salt solution to it. Following 24 h of incubation, survivors were counted by using the magnifying glass. The experiment was repeated three times. Mortality data were transformed by probit analysis in finny computer program to estimate ED50 value. Percentage of mortality was also calculated at all concentrations [4].

RESULTS AND DISCUSSION

Dichloromethane and methanol extracts of the aerial plant of Araucaria columnaris were studied for their antibacterial, antifungal, phytotoxic and Brine Shrimp lethality bioassay. Antibacterial activity of the extracts was performed against *Eschericha coli, Bacillus subtilis, Shigella flexenari, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi*. Both the extracts exhibited no activity at the concentration of 0.3mg/ml (table 1).

Table 1: Results of *In vitro* antibacterial bioassay of *Araucaria columnaris*

Extract	Name of bacteria	Zone of inhibition of sample (mm)	Zone of inhibition of standard drug (mm)
MeOH	Eschericha coli	0	25
	Bacillus subtilis	0	50
	Shigella flexinari	0	28
	Staphylococcus aureus	0	48
	Pseudomonas aeruginosa	0	23
	Salmonella typhi	0	28
	Eschericha coli	0	25
DCM	Bacillus subtilis	0	50
	Shigella flexinari	0	28
	Staphylococcus aureus	0	48
	Pseudomonas aeruginosa	0	23
	Salmonella typhi	0	28

Conc of sample: 0.3mg/ml of DMSO, Size of well: 6 mm. (diameter) Std, Std. drug: Imipenum (10µg/disc).

Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani and Candida glabrata were employed for fugitoxic effect of the extracts. It has been noted that MeOH extract of and DCM extract of Araucaria columnaris showed 10% and 20% inhibition with linear growth at 90 mm and 80 mm respectively, when compared with control; against *Fusarium solani* and *Aspergillus flavus* respectively.

Dichloromethane and methanolic extracts of the aerial plant of *Araucaria columnaris* showed significant phytotoxicity at concentrations of 1000 µg/ml, 100 µg/ml and 10 µg/ml against Lemna minor.

Dichloromethane and methanolic extract of aerial part of *Araucaria columnaris* does not showed cytotoxicity even at the highest level having Etoposide as standard drug containing 28°±1°C as incubation condition.

Table 2: Results of *In vitro* antifungal bioassay of *Araucaria columnaris*

Extract	Name of fungus	Linear growth (mm)		% Inhibition	Standard drug	Mic (µg/ml)
		Sample	control			
MeOH	Candida albicans	100	100	0	Miconazole	110.8
	Aspergillus flavus	100	100	0	Amphotericin B	20.20
	Microsporum canis	100	100	0	Miconazole	98.4
	Fusarium solani	90	100	10	Miconazole	73.25
	Candida glabrata	100	100	0	Miconazole	110.8
DCM	Candida albicans	100	100	0	Miconazole	110.8
	Aspergillus flavus	80	100	20	Amphotericin B	20.20
	Microsporum canis	100	100	0	Miconazole	98.4
	Fusarium solani	100	100	0	Miconazole	73.25
	Candida glabrata	100	100	0	Miconazole	110.8

Conc. of Sample: 400 µg/ml of DMSO, Incubation Time: 27 (28±1 °C), Incubation period: 7 (7-10 days)

Table 3: Results of *in vitro* phytotoxic bioassay of *Araucaria columnaris*

Extract	Plant name	Conc. of compound (µg/ml)	No. of fronds		% Growth regulation	Conc. of standard drug (µg/ml)
			Sample	Control		
MeOH	*Lemna minor*	1000	0	20	100	0.015
		100	6		70	
		10	10		50	
DCM		1000	10	20	50	
		100	11		45	
		10	12		40	

Std Drug: Paraquat, Incubation condition: (28±1 °C)

Table 4: Results of In vitro cytotoxic bioassay of *Araucaria columnaris*.

Extract	Dose(µg/ml)	No. of Shrimp	No. of Survivors	LD 50 (µg/ml)	STD Drug	LD 50 (µg/ml)
	1000	30	22			
MeOH	100	30	28	10246.36	Etoposide	7.4625
	10	30	29			
	1000	30	24			
DCM	100	30	26	377166.8		
	10	30	28			

CONCLUSION

The Lemna assay is a quick measure of phytotoxicity investigation. The phytotoxicty assay is a useful primary screen for weedicide research. As weeds is one of the major factors of poor agricultural productivity in the developing countries. Synthetic weedicides are expensive, toxic and non-specific. Weedicides from natural sources having improved characteristics could have a promising future. Furthermore, studies may be carried out to explore the phytotoxic components of the plant by isolation, purification and structure determination leading to the development of an effective herbicide.

CONFLICT OF INTERESTS

Declared none

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