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ANTIBACTERIAL, ANTIFUNGAL, PHYTOTOXIC AND BRINE SHRIMP LETHALITY BIOASSAY OF ACACIA JACQUEMNOTII AND RHAMNUS PERSICA

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Abstract:

The aim of this study was to investigate antibacterial, antifungal, phytotoxic and Brine shrimp lethality bioassay of Acacia jacquemnotii and Rhamnus persica. Dichloromethane and methanol extracts of the plants were evaluated for described activities. Agar tube diffusion method was employed for study of antibacterial and antifungal potential. Methanol extract of Acacia jacquemontii exhibited moderate antibacterial activity against some of the tested microorganisms. Non significant antifungal activity was shown by both plant extracts. Phytotoxic potential of both plant extracts was analyzed by using Lemna minor phytotoxic bioassay. The methanol extract of root bark of Acacia jacquemontii showed 35% growth inhibition of Lemna minor where as both dichloromethane and methanol extract of aerial parts of Rhamnus persica showed 40 % growth inhibition of Lemna minor at higher concentration. In Brine shrimp Lethality Bioassay, The dichloromethane extract of aerial parts of Rhamnus persica exhibited 56.66 % mortality. Similarly methanol extract of (root bark) of Acacia jacquemontii and aerial parts of Rhamnus persica showed 53.33 and 66.66 mortality respectively at dose of 1000µg/ml. These results confirm that both plant extracts possess significant phytotoxic as well as cytotoxic potential, where as moderate antibacterial effect was also observed, thereby providing worthy justification for isolation of novel bioactive compounds. Further study is needed to screen out cytotoxic potential of both plants against various cell lines.

Keywords: *Acacia jacquemontii, Rhamnus persica, Antibacterial, Antifungal, Phytotoxic, Cytotoxic*

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INTRODUCTION:

The genus *Acacia* (Fabaceae) is comprised of almost 1380 species. A majority of these are native to Australia and rest belongs to tropical and subtropical parts of the world [1]. *Acacia jacquemontii* Benth. (Fabaceae), known as Banolii (Hindi) and Chhoti kikrii (Urdu) is native to "Thar desert" of Indo-Pak subcontinent. It is an erect shrub with multiple branches coming from below ground [2]. Traditionally, the decoction of the plant bark is used to combat fever, muscular pain and cough [3].Various species of genus *Acacia* have been reported with variety of pharmacological potential. Ethanol and aqueous extracts of aerial parts of *Acacia hockii*, were tested for antimicrobial potential by using micro dilution and agar diffusion methods. Both extracts showed strong antibacterial activity by inhibiting growth of Gram-positive (*Staphylococcus aureus Enterococcus faecalis, Staphylococcus aureus* and *Staphylococcus epidermidis* and methicillin resistant) and Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*). The results exhibited that the MICs ranged from 0.62 mg/ml to 5 mg/ml. Similarly in another study, Methanol extract of *Acacia karroo* produced the best antibacterial activity against *Staphylococcus aureus* with MIC of 7.5mg/ml [4]. Ethanolic and methanolic extracts of *Acacia baileyana*, *Acacia dealbata* exhibited significant antifungal potential against *Candida albicans* and *Candida parapsilosis* [5]. Aqueous and chloroform fractions from *Acacia modesta* when studied by using Brine shrimp lethality assay exhibited lethal effects of 40% were observed at 1000 µg/ml [6].

Rhamnaceae, the Buck thorn family contains 50 genera and about 900 species of flowering plants distributed globally but mostly in tropical and subtropical areas. Represented in Pakistan by 6 genera and 21 species. *Rhamnus* fairly a large genus of 160 species, cosmopolitan in distribution. Represented in Pakistan by 6 species only. *Rhamnus persica*, belongs to the genus *Rhamnus* and family Rhamnaceae, is widely distributed in Pakistan and Iran. In Pakistan it is distributed in the area of Baluchistan and surroundings [7]. Several species of *Rhamnus* are used in different parts of the world by the peoples for treatment of various ailments. The bark and fruit of *Rhamnus* species have been used for centuries in folk and official medicine as purgatives and for blood detoxication [8] an infusion prepared from the fruits of *Rhamnus cathartica* is used in Bulgarian folk medicine as an antiseptic for wounds. In folk medicine in Bosnia and Herzegovina the bark of *Rhamnus fallax* is used to treat skin diseases [9]. Biological assays have validated the traditional uses of various *Rhamnus* species.

The crude methanol extract of leaves of *Rhamnus prinoides* when investigated for antimicrobial assay against various bacterial strains, showed MBC of 2.03 mg/ml against *S. aureus*, 4.06 mg/ml against *S. pyogen*, 8.13 mg/ml against *S. pneumoniae* and 4.06 mg/ml against *S.* typhi [10]. Significant antibacterial activity was observed by methanol extract of leaves of *Rhamnus prinoides* against *Escheri coli*, *Staphylococcus aureus*, and *Streptococcus pneumonia* with MIC of 97.5mg/ml, 97.5 mg/ml and 390 mg/ml and MBC of 195 mg/ml, 196 mg/ml and 390 mg/ml respectively against all tested bacterial strains [11]. Cytotoxic potential of both methanol and aqueous extracts of *Rhamnus alaternus* bark was studied against human monocytic leukemia cells (U937) lines and in normal PBMCs. The result showed that the aqueous extract showed significant decrease in the viability of PBMCs, with IC_{50} of 38.81 µg/ml where as the methanol extract illustrated a weak cytotoxic effect on normal PBMCs, with IC_{50} of 220.35 µg/ml [12].

MATERIAL AND METHOD:

Plant Collection and extraction

The plant *Acacia jacquemontii* was collected from the surroundings of Muzaffargarh power plant, District Muzaffargarh while Aerial parts of *Rhamnus persica* from Fort Manro.The plants were identified as *Acacia jacquemontii* and *Rhamnus persica* by taxonomist at The Institute of pure and applied Biology of Bahauddin Zakariya University Multan and voucher Specimen was deposited in the herbarium of the department for future reference. The root bark of *Acacia jacquemontii* was separated from the underground parts of the collected plant, chopped into small pieces and dried at room temperature for 15 days. Similarly the aerial parts of *Rhamnus persica* were shade dried. The dried material was powdered and 400 g each of the powder was macerated in dichloromthane and methanol respectively and filtered; the filtrate were concentrated under vacuum using a rotary evaporator to obtain both extracts.The extracts were assigned the codes for plant *Acacia jacquemontii* AJRBD, AJRBM and for *Rhamnus persica* RPAPD and RPAPM respectively.

Antibacterial and antifungal activity:

The agar diffusion assay was employed to study the antibacterial and antifungal potential of plant extracts. Test organisms were inoculated with ten ml of aliquots of the nutrient broth. After that these were incubated for 24 hours in oven at 37ºC.With the help of a sterile

pipette 0.6ml of broth culture of the test organism was added to sixty ml of molten agar. The mixture was poured into a sterile petridish (for the 9 cm Petridish, 0.2ml of culture is added to 20 ml of agar). Duplicate plates were prepared for each organism. With the help of a sterile cork bore required number of holes was cut, when the agar had become harden. It was ensured that there was proper distribution of holes in periphery. After that Agar plugs were removed. Test sample was dissolved in suitable solvent. With the help of 0.1ml pipette, 100 microliter of test sample was poured into appropriate labeled cups. Standard antimicrobial agents i.e. *Roxithromycin* and Clotrimazole were used at concentration of 50µg/ml. The solvent was taken as control. In order to ensure proper diffusion of sample, the plates were kept at room temperature for two hours. After that these were incubated (face upwards at) 37ºC for 24 hours. Zones of inhibition were measured in mm [13].

Phytotoxicity bioassay:

For growth of *Lemna minor*, an inorganic medium (E-medium) comprising of blend of twelve inorganic salts was prepared. By addition of pellets of KOH, the pH was adjusted to 5.6-6.0. Four sets each containing ten vials were designed as 500, 50, 5 ppm and control, respectively. The plant extract was dissolved in suitable solvent. 1000, 100, and10 microliter of extract solution was added to 500, 50, 5 ppm vials, correspondingly. The solvent was allowed to evaporate. *Lemna minor* kept in each vial. 2 ml of E-medium was added to each vial. These vials were kept in a glass dish, which had been filled up to 2 cm level with water. Glass dish was sealed, kept in growth chamber under fluorescent and incandescent light for seven days at room temperature. The no. of fronds in each vial was counted on 3rd and 7th day. With the help of computer programme, data was analyzed to obtain ED_{50}. Standard drug used was Paraquat (0.015 µg/ml) [13].

Brine shrimp lethality bioassay:

In one liter distilled water, sea salt (3.8 g) was dissolved, filtered. Solution was kept in inequitably divided tank. In larger compartment of the tank, Brine shrimp eggs were placed and and darkened by suitable covering. The shrimp larvae (*nauplii*) were attracted to smaller illuminated compartment through perforations in dividing partition. Three vials each of 1000, 100, and 10µg/ml were prepared. Plant extract (20 mg) was made soluble in two ml of organic solvent. Aliquots were transferred (500, 50 and 5 µL) to respective vials (1000, 100, or 10 µg/ml). Solvent was allowed to evaporate. Sea water (5ml) was added to each vial. After that 10 shrimps per vial were introduced. Vials were placed at room temperature under illumination. Total number of surviving shrimps was counted. LC_{50} and 95% confidence intervals was calculated by using Probit analysis [13].

RESULTS:

Antibacterial ctivity:

Dichloromethane and methanol extracts of both plants were tested by using Agar tube diffusion method against *Klebsiella pneumonia, Bacillus subtilis, Salmonella agona, Pseudomonas aeruginosa*. Methanol extract of Acacia jacquemontii exhibited moderate antibacterial activity against all strains of bacteria except *Salmonella agona* . Results are described in table 1.

Table 1: Antibacterial activity of dichloromethane and methanol extracts of *Acacia jacquemontii* and *Rhamnus persica:*

Extract/standard	*Antibacterial activity* (Zone of inhibition (mm) at concentration of 50 µg)				
	Salmonella agona	***Bacillus* subtilis**	***Escheria coli***	***Klebsiella pneumonia***	***Pseudomonas aeruginosa***
AJRBD	–	6± 0.43	7± 0.34	7±0.25	8±0.14
AJRBM	–	9± 0.13	9± 0.19	9± 0.13	17± 0.29
RPAPD	–	6±0.34	8± 0.29	8± 0.14	6± 0.15
RPAPM	–	–	–	–	–
Roxithromycin	23± 0.9	24± 0.4	25± 0.41	22± 0.34	26± 0.15

(–) = inactive

Antifungal activity:

Both plant extracts were tested for antifungal activity against *Aspergillus niger, Aspergillus fumigates, Mucor racemosus, Fusarium solani, and Aspergillus flavus.* Agar tube diffusion method was employed for this study. Non significant antifungal activity was shown by both plant extracts. Results are described in table 2 given below.

Table* 2: Antifungal activity of dichloromethane and methanol extracts of *Acacia jacquemontii* and *Rhamnus persica:

Extract/standard	*Antifungal activity* (Zone of inhibition (mm) at concentration of 50µg)				
	Aspergillus niger	***Aspergillus fumigatus***	***Mucor racemosus***	***Fusarium solani***	***Aspergillus flavus***
AJRBD	–	–	12± 0.16	–	–
AJRBM	–	–	10± 0.29	7±0.21	–
RPAPD	–	–	–	–	6±0.35
RPAPM	–	–	–	–	–
Clotrimazole	32± 0.20	30± 0.23	42± 0.34	24± 0.41	33± 0.15

(–) = inactive

In vitro phytotoxic bioassay:

Phytotoxic potential of both plant extracts was analyzed by using *Lemna minor* phytotoxic bioassay. The methanol extract of root bark of *Acacia jacquemontii* showed 35% growth inhibition of *Lemna minor* where as both dichloromethane and methanol extract of aerial parts of *Rhamnus persica* showed 40 % growth inhibition of *Lemna minor* at higher concentration i:e 1000 µg/ml. The result of phototoxic bioassay is given below in table 3

Table 3: Results of In vitro *Lemna minor* phytotoxic bioassay of dichloromethane and methanol extracts of *Acacia jacquemontii* and *Rhamnus perisca:*

Extract/standard	Concentration (µg/ml)	No. of Fronds		% Growth Regulation
		Taken	Survived	
AJRBD	1000	20	15	25
	100	20	16	20
	10	20	17	15
AJRBM	1000	20	13	35
	100	20	17	15
	10	20	19	5
RPAPD	1000	20	12	40
	100	20	14	30
	10	20	15	25
RPAPM	1000	20	12	40
	100	20	16	20
	10	20	18	10
Control	–	20	20	0
Paraquat	0.015	20	0	100

Brine shrimp Lethality Bioassay:

The dichloromethane extract of root bark of *Acacia jacquemontii* and aerial parts of *Rhamnus persica* exhibited 46.66 and 56.66 % mortality respectively at dose of 1000 µg/ml. Similarly methanol extract of (root bark) *Acacia jacquemontii* and aerial parts of *Rhamnus persica* showed 53.33 and 66.66 mortality respectively at dose of 1000µg/ml. Result of Brine shrimp lethality bioassay is summarized in table 4.

Table 4: Results of Brine shrimp lethality bioassay of dichloromethane and methanol extracts of *Acacia jacquemontii* and *Rhamnus persica*:

Extract/standard	Concentration (µg/ml)	No of shrimps taken	No of shrimps survived	Mortality Percentage
	10	30	25	16.66
AJRBD	100	30	24	20.00
	1000	30	16	46.66
AJRBM	10	30	23	23.33
	100	30	21	30.00
	1000	30	14	53.33
RPAPD	10	30	22	26.66
	100	30	20	33.33
	1000	30	13	56.66
RPAPM	10	30	23	23.33
	100	30	22	26.66
	1000	30	10	66.66
Etoposide	7.46	30	16	46.66

DISCUSSION:

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay [14]. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants [15]. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. However, not many reports are available on the plants for developing commercial formulations for applications in crop protection. In the present study the Methanol extract of Acacia jacquemontii exhibited moderate antibacterial activity against all strains of bacteria except *Salmonella agona.*

Klebsiella pneumoniae is a bacterium that normally lives inside human intestines, where it doesn't cause disease. However, if *K. pneumoniae* gets into other areas of the body, it can cause a range of different illnesses which includes, Pneumonia , bloodstream infections, wound infections, surgical site infections, meningitis and urinary tract infections (UTI) [16]. *Pseudomonas aeruginosa* has become an important cause of gram-negative infection, especially in patients with compromised host defense mechanisms. It is the most common pathogen isolated from patients who have been hospitalized longer than 1 week, and it is a frequent cause of nosocomial infections. Pseudomonal infections are complicated and can be life-threatening [17]. In the present study the methanol extract of *Acacia jacquemontii* exhibited moderate antibacterial activity against *Klebsiella pneumonia* and *Pseudomonas aeruginosa.*

The brine shrimp lethality test is used to predict compounds or extracts that may have anticancer activity .For the bioactive compound of either natural or synthetic origin, this is a rapid and comprehensive test. It is also an inexpensive and simple test as no aseptic techniques are required. It easily utilizes a large number of organisms for statistical validation and requires no special equipment and relatively small amount of sample (2-20 mg or less) is necessary [18]. The outcome of the study showed that the both plant extracts showed significant mortality at higher doses. Further studies should be going on fractionation and identification of bioactive constituent to human cell line culture of cytotoxic effect.

Herbicides, originating from plant's origin are often environment friendly. Therefore search for plant's origin herbicides, is sensible. The results of the phytotoxic activity of the both plant extracts against *Lamna minor* are promising as the methanol extract of root bark of *Acacia jacquemontii* showed 35% growth

inhibition of *Lemna minor* whereas both dichloromethane and methanol extract of aerial parts of *Rhamnus persica* showed 40 % growth inhibition of *Lemna minor* at higher concentration i:e 1000 µg/ml. This indicates that the plant extracts do have phytotoxic constituents which should be isolated.

CONCLUSION:

In light of above findings, we conclude that both the plants showed significant phyotoxic and cytotoxic activity. The outcomes of antibacterial study showed that the methanol extract of *Acacia jacquemontii* exhibited moderate antibacterial activity against all strains of bacteria except *Salmonella agona.* Both plants were found to be inactive regarding antifungal potential. The isolation of biologically active compounds responsible for the observed effects is under way in our laboratory.

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