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IN VITRO ANTI-PSEUDOMONAL POTENTIAL OF *JUGLA NS REGIA* AND *OTOSTEGIA LIMBATA* LEAVES EXTRACT AGAINST PLANKTONIC AND BIOFILM FORM OF *PSEUDOMONAS AERUGINOSA*

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Abstract

In the present study the anti-pseudomonal potential of crude methanolic extracts, hexane, ethyl acetate and water fractions of the leaves of *Juglans regia* L. and *Otostegia limbata* (Benth.) Boiss.against planktonic and biofilm form of clinical strains (P1, P2 and P3 strains) of *Pseudomonas aeruginosa* (*P. aeruginosa*) were evaluated. Agar well diffusion and minimum inhibitory concentration (MIC) assays were used against planktonic, whereas pellicle inhibition and static biofilm inhibition assays were performed against biofilm form of *P. aeruginosa*. In well diffusion assay, the crude methanolic extract of *J. regia* showed good bacterial inhibition than *O. limbata*. The *J. regia*crude methanol extract had significant (+; complete breakage of pellicle layer), good (++; partial breakage of pellicle layer) and moderate (+++; uniformthin layer of pellicle formation) pellicle inhibition activity, while *O. limbata* had moderate (+++; uniformthin layer of pellicle formation) to weak (++++; loose thick layer pellicle formation) pellicle inhibition effect. In MIC assays, hexane and water fractions of *J. regia* had high (86 *vs.* 77%) antibacterial activity, while crude methanolic extract of *O. limbata* showed 51% inhibition against the most resistant P3 strain at 1000 µg/ml concentration. In static antibiofilm assay, hexane fraction of *J. regia* had high (63%) inhibition compared to crude methanolic extract of *O. limbata*(31%) against P3 strain. The present study highlights that *J. regia* extracts possesses high anti-pseudomonal properties as compared to *O. limbata*.

Key words: Biofilm; Planktonic; *Juglans regia*; *Otostegia limbata*; *Pseudomonas aeruginosa*.

Introduction

Rapid increase in antibiotic resistant bacteria has been observed over the past several decades. The increasing frequency of resistant bacteria all over the world is mostly due to the over and misuse of antibiotics. Interestingly, traditional medicine (including herbal medicine) has been used in the healthcare for many years in the developing countries (World health Organization, 2002) and a number of reports have verified their effective role for the control of various infectious diseases. Plant extracts prepared from leaves, stems and roots represent an important pool for the search of potent and novel drugs against bacterial and biofilm form (Essawi & Srour, 2000).

Bacteria exists either in a free-floating planktonic state or growing in a biofilm having similar or different bacterial species enclosed in a complex exopolymeric substance consisting of carbohydrates, proteins and nucleic acids (Costerton *et al*., 1999; Mah & O'Toole, 2001). Recent study shows that biofilm forming bacteria are responsible for approximately 60% human infections (Begun *et al*., 2007). The most important attribute of biofilm cell is its resistance to many antibiotics which significantly reduces the therapeutic effects of antibiotics (Flemming *et al*., 2007). Therefore, it is important to develop an alternative antimicrobial source such as screening of local medicinal plants for potential antimicrobial activity (Essawi & Srour, 2000). *Pseudomonas aeruginosa* (*P. aeruginosa*) is a gram-negative bacterium used as a model organism for biofilm studies. It is an important opportunistic human pathogen involved in both nosocomial and community acquired infections (Gales & Jones, 2002; Wong & O'Tool, 2011). Therefore, it is highly significant to identify an effective antibiofilm agent against *P. aeruginosa* (Abidi *et al*., 2014), in order to design suitable biofilm control strategies.

Juglans regia L. is about 25 m tall deciduous tree (Fig. 1A). It is a member of family *Juglandaceae,* and commonly known as Akhrot and also "Ghuz" in Lower Dir, Pakistan. The fruit is edible and from the bark and roots, a product called "dandasa" is produced that are used for brushing of teeth. The bark is generally used as an astringent and digestive diuretic (Ahmad *et al*., 2011).

Otostegia limbata (Benth.) Boiss. is profusely branched, 40-60 cm tall, spiny shrub (Fig. 1B). Locally (in Lower Dir) it is called as "spin azghay" and grows best in dry habitats (Hedge, 1990). It is commonly grown all over Pakistan and in Kashmir (Kale *et al*., 2011). *O. limbata* is effective against ophthalmia, gum and skin diseases, and it is also used for wounds healing (Abbasi *et al*., 2010). This study provides a comprehensive data set on the *In vitro* anti-pseudomonal potential of crude methanolic extracts, water and hexane fractions, of aerial parts (leaves) from *J. regia* and *O. limbata*against both planktonic and biofilm form of *P. aeruginosa*.

Materials and Methods

Extraction and Fractionation of Plant Material: Leaves of *J. regia* and *O. limbata* were collected from Chakdara, Lower Dir (34°38'59.99" N 72°01'60.00" E), KhyberPakhtunkhwa, Pakistan during March-April 2013. Voucher specimens of both plants were deposited in the Herbarium of Hazara University. For extraction, the shade-dried leaves of each plant (1.5 kg) were

ground into fine particles (0.5 mm). Methanolic extract of each plant sample was prepared by soaking 600 g in 1000 ml methanol for 3 days with vigorous agitation of five times a day (Bibi *et al*., 2011). Each extract was filtered, and solvent was evaporated by rotary evaporator at 40 °C (Rotavapor R-200 Buchi, Switzerland). The residues were dissolved in n-hexane and ethyl acetate and water was added. The mixture was subjected to partition into different layers (Fig. 2). The hexane and ethyl acetate layers were collected; solvents were evaporated (Haq *et al*., 2013). Each extract was kept at 4°C for further analysis.

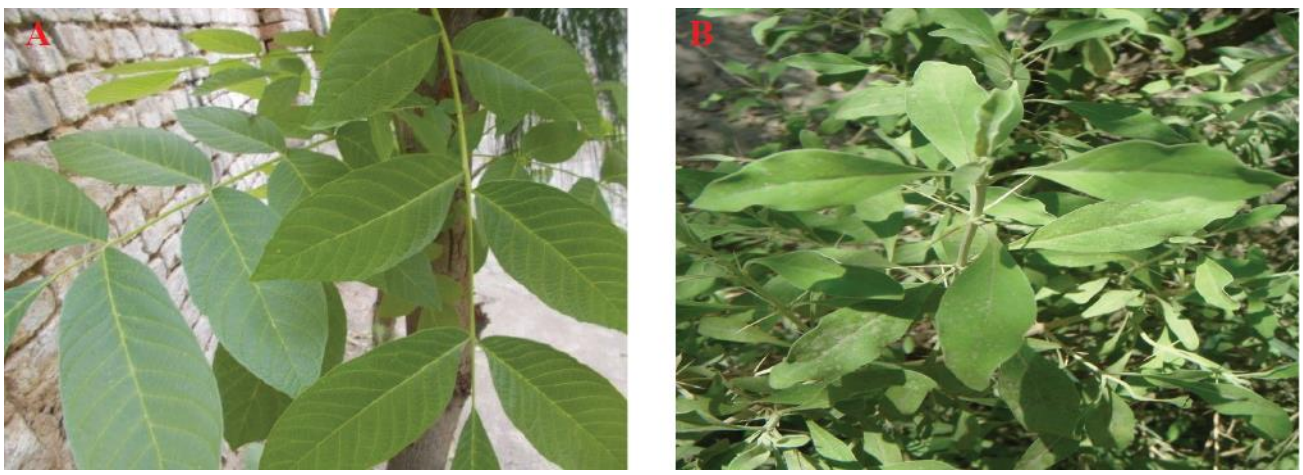

Fig. 1.Images showing the structure of *Juglans regia* (A) *Otostegia limbata* (B) used in the study.

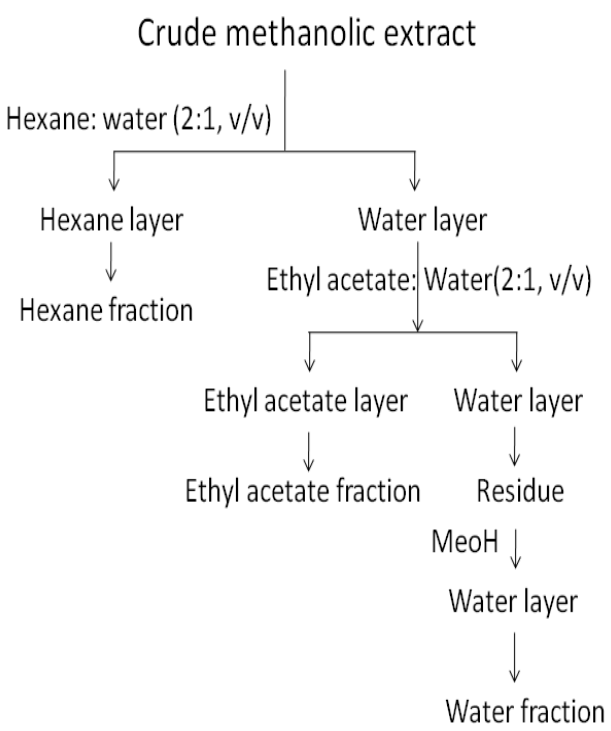

Fig. 2. Schematic fractionation for crude extracts of *J. regia* and *O. limbata*.

Tested microorganisms: Three pre-identified clinical strains of *P. aeruginosa* were collected from infected wounds and classified as P1, P2 and P3 on the basis of its biofilm formation ability by the Pathology Laboratory of Pakistan Institute of Medical Sciences, Islamabad, Pakistan, with P2being more resistant than P1, and P3 being more resistant than P2. Bacterial cultures were inoculated individually, incubated at 37°C and cell number was adjusted to approximately 10^8 CFU/ml.

Antibiotic sensitivity test: Antibiotic sensitivity testing was carried out by Kirby-Bauer disc diffusion technique. Pure colonies (3-4) were transferred with sterile cotton swab to sterile Mueller–Hinton broth (MH) to make direct colony suspension of the three tested strains of *P. aeruginosa*. The turbidity of test suspension was standardized to match that of 0.5 McFarland standard solution. Petri plates were then incubated at 37ºC for 24 h. The zone interpretation of each antibiotic was used in accordance with clinical and laboratory standards institute guidelines (CLSI, 2013) and was measured in millimeter.

Antibacterial activity: The antibacterial potential of methanolic crude extracts of *J. regia* and *O. limbata* was carried out using agar well diffusion method, as previously reported by Naz & Bano (2012). Seventy five milliliter of sterilize nutrient agar was added to agar plate, and incubated at 37ºC for 24 h. After incubation, six wells (6 mm) were made in seeded agar plate using cork borer, and 100 µl of extract was transferred into each well. The plates were then incubated at 37°C for 24 h. The antibacterial potential was determined by recording the diameter of zone of inhibition (ZOI) in millimeter (mm). Dimethyl sulfoxide was used as negative control. The assay was done in triplicate for each sample.

Anti-pellicle assay: The effect of crude methanol extracts on biofilm growth was determined by anti-pellicle activity according to a modified protocol as described by Joshua *et al*., (2006). Five different concentrations *i.e.* 7.5, 10, 12.5, and 15 mg/ml of all extracts were used against the tested strains of *P. aeruginosa*. Test tubes were prepared by adding 6 ml of nutrient broth (NB) medium into it, and labeled as P1, P2 and P3, respectively. Into to each tube, 60 µl of inoculum and 100 µl of extract were added. Extract free NB plus bacteria and NB were used as positive and negative control, respectively. The tubes

were kept at room temperature (7 days) without agitation and the anti-pellicle potential of each extract was observed by naked eye and represented as (+,-) signs, and denoted as: "-, no biofilm; + significant biofilm inhibition (complete breakage of pellicle layer); ++, good biofilm inhibition (partial breakage of pellicle layer); +++, moderate biofilm inhibition (uniform thin layer); ++++, weak biofilm inhibition (loose thick layer); and +++++ no biofilm inhibition (compact mature pellicle layer)".

Minimal inhibitory concentration (MIC) assay: The MIC assay was performed for the crude methanolic extracts, hexane and water fractions of each plant using a modified 96 well micro-dilution method as reported by Chan *et al*., (2013). Five different concentrations (62.5, 125, 250, 500, 1000 µg/ml) of the extract were used in the assay. Bacterial cells (10^8 CFU/ml) were inoculated into Mueller–Hinton broth (MH) adding appropriate concentrations of extract and then 200 µl (100 µl extract + 100 µl inoculum) per well was put in 96-well micro titer plates and were incubated for 24 h. After incubation, optical density (OD) was recorded at 620 nm by using micro plate absorbance reader (Multimode Detector DTX 880, Beckman Coulter, USA). Imipenem (4 µg/ml), MH broth plus cells and MH were used as positive, negative and sterility controls. The assay was performed in triplicate for each sample. For each extract, the MIC ≥ 70 (the amount required to kill bacteria 70% or greater) was calculated (Al-Mariri *et al*., 2014). Percentage inhibition was determined by using the following formula:

$$Percentage\ Inhibition = 1 - \frac{\text{ODE 24} - \text{ODE 0}}{\text{ODNc 24} - \text{ODNc 0}} \times 100$$

where, ODE 24 is optical density (620 nm) experimental at 24 h; ODE 0 is optical density (620 nm) experimental at 0 h; ODNc 24 is optical density (620 nm) negative control at 24 h; ODNc0 is optical density (620 nm) negative control at 0 h.

Static biofilm formation assay: A modified static antibiofilm assay as described previously by Kim & Park (2013) was performed for methanol crude extract and fractions of both plants in sterile flat-bottom 96-well polystyrene plates. Overnight culture of P3 strain (10^8 CFU) in Brain-heart infusion (BHI) medium was diluted with BHI medium containing extract and the dilution (200 µl) was put into each well and incubated at 37°C for 24 h without agitation. Initially, OD at 595 nm of the suspended culture was measured. The plates were washed with phosphate-buffered saline to remove any remaining suspended cells in the wells. The biofilm was then stained with 1.0% crystal violet for 15 min, and washed with phosphate-buffered saline to remove unbound dye. The crystal violet bound to the biofilm was re-dissolved using absolute ethanol and quantified by measuring OD at 540 nm using microplate absorbance reader (Multimode Detector DTX 880, Beckman Coulter, USA) and divided with reference OD at 595 nm. The assay was performed in triplicate for each extract sample. Imipenem (4 µg/ml), BHI plus cells and BHI were used as positive, negative and sterility control. The effect on biofilm was determined by using the following formula:

$$Percentage\ Inhibition = \frac{\text{OD negative control} - \text{OD experimental}}{\text{OD negative control}} \times 100$$

Statistical analysis: All experiments reported in the study were carried out in triplicates. The quantitative results are presented as means ± standard error (SE) using graphPad prism v.4 software (Graph-Pad Inc., CA, USA).

Results and Discussion

In the last few decades the antimicrobial potential of plants has been extensively reported from different parts of the world. According to the World Health Organization, 80% population of the world commonly used plant extracts or their active ingredients as medicine, mostly for the control of bacterial infections in developing nations (Essawi & Srour, 2000). A rapid increase in the development of resistant microbes due to over and often misuse of antibiotics has triggered a growing research into the exploitation of medicinal plants for the control of resistant microbes (Bibi *et al*., 2011). As such, the screening of natural extracts from these medicinal plants as new antimicrobial agents is important in both local and international context (O'Toole & Kolter, 1998). Furthermore, compared to the antibiotics, the natural agents have been reported to have no side effects and available at lower price. The present study reports the first data set on the anti-pseudomonal potential of crude methanol extracts and fractions of *J. regia* and *O. limbata* leaves against planktonic and biofilm form of three (P1, P2 and P3) clinical strains of *P. aeruginosa*. Initially crude methanol extract of each plant was used to screen out its antibacterial (Agar well diffusion) and antibiofilm (Pellicle inhibition) potential. In addition, to crude methanol, hexane, ethyl acetate and water fraction of each plant was used in MIC and static biofilm inhibition assay.

Other advantages of the use of these antimicrobial extracts is the development of the phytotherapics and its production in large-scale can be performed by biotechnological approaches, mainly using plant tissue culture, such as calli culture, cell suspension culture (Costa *et al.*, 2015) and hairy root culture (Habibi *et al.*, 2016). It is also possible to obtain the maximum yield of the biocompounds using controlled conditions *In vitro* (Gollo *et al.*, 2016). Moreover, the uses of medicinal plants in traditional medical system are more popular due to its immense pharmacological properties like anti-inflammatory and immune-modulant. A variety of different phytochemicals such as alkaloids, glycosides, flavonoids, phenols and essential oils are found in medicinal plants that have wide range of therapeutic potential with no or less side effects (Charis, 2000; Saroya, 2011). Due to these advantages, medicinal plants can be considered as a potential alternative of antimicrobial sources.

Antibacterial activity of crude methanolic extracts of *J. regia* and *O. limbata*: Data representing the antibacterial activity of extracts against the tested strain of *P. aeruginosa* are shown in Fig. 3 and 4. For *J.*

regia, maximum (18.5 mm) antibacterial activity was recorded against P2 strain than P1 (15.8 mm) and P3 (14.9 mm) strains. Similarly, the *O. limbata* showed maximum (15.8 mm) activity against P2 strain as compared to P1 (14.2 mm) and P3 (13.7 mm) strain at 15 mg/ml. Furthermore, P3 strains had more resistance to the herbal treatment. Data showing (ZOI) against the tested strains by standard antibiotics served as positive control (Table 1). Our results are supported by the earlier findings of antibacterial potential of different parts of *J. regia*. Zakavi *et al*., (2013) reported that ethanolic extract of *J. Regia* bark was active against the tested oral bacteria than its aqueous extract. Similarly, high antibacterial potential of green husks of different cultivars of *J. regia* was reported against gram positive bacteria than gram negative (Oliveira *et al*., 2008; Chaieb *et al*., 2013). The antibacterial potential of *J. regia* might be due to the fact that it contains high content of phenolic compounds (Pereira *et al*., 2007). According to our analysis, *O. limbata* had moderate activity at 8 mg/ml which contradicted the findings of Anwar *et al*., (2009) who reported that methanol extracts of *O. limbata* had no activity against *P. aeruginosa*. Discrepancy in the findings of the two studies might be due to the differences in the bacterial strain used.

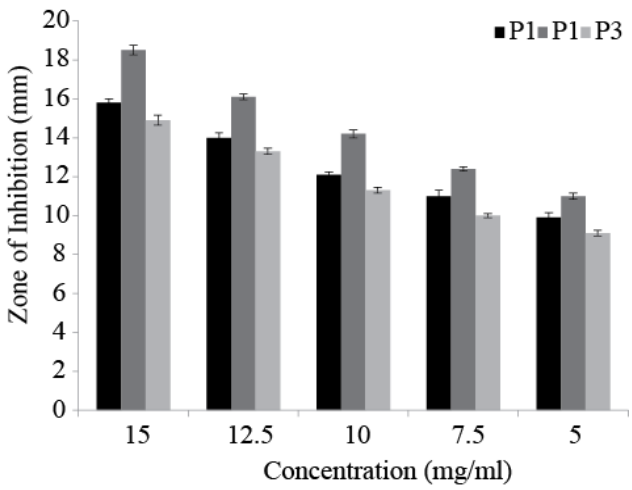

Fig. 3. Zone of Inhibition (mean ± SE) against different *P. aeruginosa* strains by the crude methanolic extracts of *Juglans regia* after 24 h incubation
P1, P2 and P3: *Pseudomonas aeruginosa* strain 1, 2, and 3; mm: millimeter

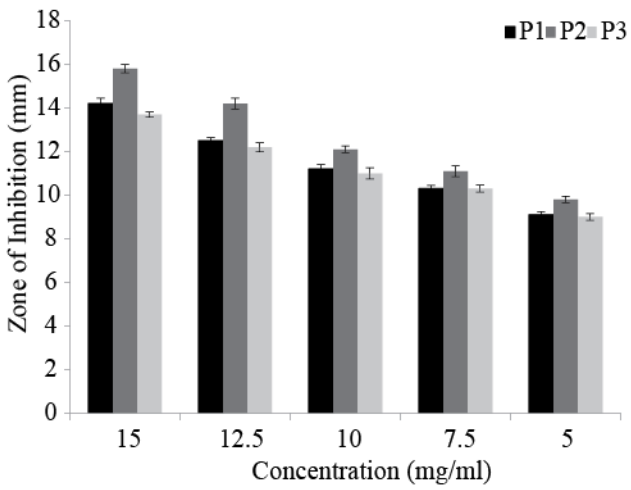

Fig. 4. Zone of inhibition (mean ± standard error) against different *P. aeruginosa* strains by the crude methanolic extract of *Otostegia limbata* after 24 h incubation
P1, P2 and P3: *Pseudomonas aeruginosa* strain 1, 2, and 3; mm: millimeter

Table 1. Effect of antibiotics on different strains of *P. aeruginosa*. Data represented as mean ± standard error (n = 3).

Tested Antibiotics	Concentration (µg/ml)	Zone of Inhibition (mm)		
		P1 strain	P2 strain	P3 strain
Gentamicin	10	19.2 ± 0.12	21.1 ± 0.20	18.4 ± 0.11
Piperacillin/Tazobactam	100/10	29.1 ± 0.20	30.7 ± 0.15	28.2 ± 0.25
Ceftazidime	30	29.1 ± 0.17	30.2 ± 0.2	28.1 ± 0.17
Ciprofloxacin	5	39.0 ± 0.11	39.6 ± 0.11	38.1 ± 0.23
Cefepime	30	25.2 ± 0.2	26.9 ± 0.17	24.1 ± 0.15
Imipenem	10	21.1 ± 0.17	22.1 ± 0.17	20.0 ± 0.11
Ticarcillin / Clavulanate	75/10	19.2 ± 0.2	19.8 ± 0.11	18.2 ± 0.25
Levofloxacin	5	29.2 ± 0.20	30.1 ± 0.15	28.1 ± 0.20

Table 2. Pellicle inhibition of *J. regia* against different strains of *P. aeruginosa* after 7 days of incubation.

Concentration (mg/ml)	Bacterial strains		
	P1 strain	P2 strain	P3 strain
7.5	+++	+++	+++
10	+++	+++	+++
12.5	+++	++	+++
15	+	+	++
†Positive control	+++++	+++++	+++++

+, complete breakage of pellicle layer; ++, partial breakage in pellicle layer; +++, uniform thin layer; +++++, compact mature pellicle layer, †Extract free.

Table 3. Pellicle inhibition of *O. limbata* against different strains of *P. aeruginosa* after 7 days of incubation.

Concentration (mg/ml)	Bacterial strains		
	P1 strain	P2 strain	P3 strain
7.5	++++	++++	++++
10	+++	++++	++++
12.5	+++	+++	+++
15	+++	+++	+++
†Positive control	+++++	+++++	+++++

+++, uniform thin layer; ++++, loose thick layer; +++++, compact mature pellicle layer, †Extract free

Pellicle inhibition of methanolic crude extracts: The antibiofilm strength of the crude methanol extracts of *J. regia* (Table 2) and *O. limbata* (Table 3) was assessed through its pellicle formation potential at 7.5 to 15 mg/ml concentrations. A significant (+) antibiofilm activity was recorded for *J. regia* against pellicle formation of P1 and P2 strains by the complete breakage of pellicle layer, while against P3 strain good antibiofilm (++) effect was observed at 15 mg/ml concentration by partially inhibited the pellicle layer. A moderate activity (+++) was recorded against P1 and P3 strains at 7.5 to 12.5 mg/ml concentration. From the results against P1 and P2 strains it is clear that with increase in concentration from 7.5 to 12.5 mg/ml, there is no increase in activity on biofilm inhibition, indicating 7.5 mg/ml to be the minimum dose for moderate antibiofilm effect. It has been reported that if a low concentration of antibacterial drug is effective to stop initial attachment of bacteria to surfaces, the later steps of biofilm formation will also be inhibited (Guarrera, 2005).At a concentration of 12.5 mg/ml good antibiofilm activity (++) was recorded against P2 strain, while at 7.5 mg/ml and 10 mg/ml, moderate activity (+++) was observed.

For *O. limbata*, moderate antibiofilm (+++) activity was observed against P1 strain at 10 to 15 mg/ml and weak antibiofilm effect (++++) was recorded at 7 mg/ml respectively (Table 3). Against P2 and P3 strains, moderate antibiofilm (+++) activity was recorded at 12.5 mg/ml and 15 mg/ml, while a weak antibiofilm effect was observed at 10 mg/ml and 7.5 mg/ml respectively. Pattiyathanee *et al*., (2009) and Joshua *et al*., (2006) earlier reported the antibiofilm potential of different plants extracts on bacterial pellicle inhibition. According to the author knowledge, very limited information is present on antipellicle effect of *J. regia* and *O. limbata* extracts against *P. aeruginosa*. After the preliminary antibacterial and antibiofilm assays, P3 strain of *P. aeruginosa* was used in the MIC and static antibiofilm assays based on its high resistance compared to P1 and P2 strains.

Minimum inhibitory concentration of crude extracts and fractions: The real extent of antibacterial potency of crude extract and fractions of *J. regia* and *O. limbata* was evaluated by calculating the MIC ≥ 70 values. The 1 mg/ml maximum concentration used in the present study was based on the earlier findings, showing that MIC values equal or less than 1 mg/ml are significant (Rios & Recio, 2005). Hexane fraction of *J. regia* was the most active fraction showing MIC ≥ 70 value of 1000 µg/ml with 86% bacterial inhibition. The water fractions and crude methanol extract of *J. regia* showed MIC ≥ 70 values of 1000 µg/ml with 77% and 72% inhibition respectively, as shown in Table 4. Meanwhile, the MIC ≥ 70 values of ethyl acetate fraction of both plants and crude extract of *O. limbata* exceeded 1000 µg/ml. Imipenem used as positive control completely inhibit the tested bacterial strains. Quave *et al*., (2008) reported a similar kind of activity of fruits and leaves extracts of *J. regia* against methicillin resistant *Staphylococcus aureus*. Pereira *et al*., (2007) also documented a similar activity of *J. regia* aqueous leaves extracts against *Staphylococcal* species. In this study the respective fractions of *J. regia* exhibited high activity compared to the crude methanol extract. This might be due to the fluctuation of active compounds in that fraction based on its nature of solubility (Bibi *et al.,* 2011).

Effect of crude methanolic extracts on biofilm formation: The extracts that showed 50% or more bacterial inhibition in MIC assay were used in antibiofilm assay. All the extracts tested exhibited good antibiofilm activity at 1000 µg/ml with hexane fraction being the most active with 63% biofilm inhibition and 56% inhibition by water fraction. For crude methanolic extracts of *J. regia* and *O. limbata* 50% and 31% biofilm inhibition was recorded, respectively. At 500 µg/ml *J. regia* hexane, water and crude methanolic extract showed 25%, 20% and 18% biofilm inhibition, respectively, and for *O. limbata*, 8% biofilm inhibition was recorded as shown in Table 5. The hexane, water and crude methanolic fraction at 250 µg/ml of *J. regia* had 19%, 15% and 11% biofilm inhibition, respectively; while for *O. limbata* negative inhibition was recorded. Imipenem showed 95% biofilm inhibition compared to 100% inhibition in MIC assay against planktonic form. Thus *P. aeruginosa* in biofilm mode showed increase resistance. In this study the effect of plant extracts to inhibit cell attachment was investigated that confirm results of the previous investigation that inhibition of cell attachment to a substrate is easier and effective to achieve than growth of preformed biofilm (Cerca *et al*., 2005). A similar trend in biofilm inhibition for *J. regia* leaves and fruits were reported by Quave *et al*., (2008) against methicillin resistant *S. aureus*. The antibiofilm potential of different plant extracts against clinical strains of *P. aeruginosa* was

reported by Abidi *et al*., (2014). It is often essential to inhibit initial cell attachment for preventing bacteria to colonize surfaces and form biofilm (Bavington & Page, 2005), and also in agreement to our study. This is the first report ofantibiofilm activity for *J. regia* and *O. limbata* leaves extracts against clinical strains of *P. aeruginosa*. From the results of MIC and static antibiofilm it was concluded that P3 strain of *P. aeruginosa* had higher resistance in biofilm mode as compared to planktonic mode and an average increase of 20-23% in resistance for biofilm form was recorded at 1000 µg/ml as compared to its planktonic counterparts which confirmed that bacteria in biofilm form had higher resistance than planktonic form (Donlan & Costerton, 2002).

Table 4. Minimum inhibitory inhibition (MIC) values of *Juglansregia* and *Otostegia limbata* extracts on P3 strain† after 24h incubation.

Plant extracts tested	MIC ≥ 70 value (µg/ml)	Inhibition (%)
Juglans regia crude	1000	72 ±2.08
Juglans regia hexane	1000	86 ± 2.00
Juglans regia ethyl acetate	>1000	33 ± 2.00
Juglans regia water	1000	77 ± 1.52
Otostegia limbata crude	>1000	51 ± 2.08
Otostegia limbata ethyl acetate	>1000	46 ± 2.51
††Imipenem	4	100

Data represented as mean ± standard error, † 3rd (most resistant) strain of *Pseudomonas aeruginosa*used in the assay, †† used as positive control @ 4µg/ml

Table 5. Biofilm inhibition of *Juglansregia* and *Otostegialimbata*extracts on P3 strain† after 24h incubation.

Plant extracts tested	Biofilm inhibition (%)		
	1000 (µg/ml)	500 (µg/ml)	250 (µg/ml)
Juglans regia crude	51 ± 1.39	18 ± 2.25	11 ± 2.34
Juglans regia hexane	63 ± 1.52	25 ± 1.73	19 ± 2.02
Juglans regia water	56 ± 1.73	20 ± 2.20	15 ± 1.78
Otostegia limbata crude	31 ± 1.80	08 ± 1.16	00†††
††Imipenem	95.0	95.0	95.0

Values are shown mean ± standard error,† 3rd (most resistant) strain of *Pseudomonas aeruginosa* used in the assay, †† used as a positive control @ 4µg/ml; †††negative inhibition

Conclusions

Findings of the present study demonstrate that crude methanol extract of *J. regia* showed good antibacterial activity than *O. limbata* against both planktonic and biofilm form of *P. aeruginosa*, thus highlighting an opportunity for the control strategies against *P. aeruginosa*. The hexane, water fraction and crude methanol extract of *J. regia* possessed high activity at 1000 µg/ml against both planktonic and biofilm form than *O. limbata*.

Conflict of interest

The authors have no conflict of interest to disclose.

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