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Research Article
Antimicrobial Activity of Tamarind Seeds Extract on *Pseudomonas aeruginosa* Biofilm Forming Isolates

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Abstract

Background and Objective: *Tamarindus indica* (*T. indica*) fruit is used as traditional medicine for the treatment of bacterial and parasitic infections in many tropical countries. *Pseudomonas aeruginosa* (*P. aeruginosa*) is a problematic bacterium that causes nosocomial/opportunistic infections. Planktonic, as well as biofilm forms of this bacterium, are resistant to a wide range of antibiotics. Hence, this study aimed to determine the antimicrobial potential of *T. indica* against *P. aeruginosa* forming bacteria from different clinical specimens. **Materials and Methods:** This was a descriptive case study conducted to determine the antimicrobial activity of different concentrations of methanolic extract of *T. indica* seeds on biofilm-forming *P. aeruginosa* clinical isolates. Sixty *P. aeruginosa* from urine, wounds, blood, eye swabs, ear swabs and sputum were tested against the methanolic extract of *T. indica* seeds (50, 100, 150 and 200 μg mL^{-1}) through the microtiter plate method. **Results:** The highest anti-pseudomonal activity of *T. indica* methanolic extract was among *P. aeruginosa* biofilm-forming isolates from blood as the 4 concentrations were significantly effective, followed by urine and wounds were 3 (100, 150 and 200 μg mL^{-1}) concentrations were highly effective ($p = 0.00$) and the lowest effects were among eye swabs, ear swabs and sputum with insignificant statistical correlation ($p>0.05$). **Conclusion:** Methanolic extract of *T. indica* seeds exhibited antimicrobial activity and inhibited the biofilm formation of *P. aeruginosa* isolates at various concentrations. Tamarind could be a potential source for antimicrobial substances against biofilm-forming *P. aeruginosa* isolates.

Key words: *Tamarindus indica*, biofilm, antimicrobial, *P. aeruginosa*, microtiter plate method, cell cytotoxicity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tamarindus indica (Tamarind) belongs to the dicotyledonous family Leguminosae and subfamily Caesalpiniaceae[1]. It is used as traditional medicine in Africa, India, Pakistan, Bangladesh, Nigeria and other tropical countries for the treatment of abdominal pain, diarrhoea, dysentery, helminths infections, constipation, inflammation, cell cytotoxicity, gonorrhoea, eye disease[2], various ailments, jaundice and stomach disorders[3].

T. indica chemical contents revealed the existence of several active compounds such as fatty acids and many vital elements such as arsenic, calcium, cadmium, copper, iron, sodium, manganese, magnesium, potassium, phosphorus, lead and zinc, according to a phytochemical analysis[4]. The major fatty acids of the seeds include palmitic acid, oleic acid, linoleic acid and eicosanoic acid. The unsaponifiable matter from the seeds oil of *T. indica* showed the presence of b-amyrin, campesterol, β-sitosterol and seven hydrocarbons. *T. indica* seeds and pericarp contain phenolic antioxidants, procyanidin, represented mainly by oligomeric procyanidins tetramer, procyanidin hexamer and procyanidin pentamer with a lower amount of procyanidin B2 epicatechin[5].

This bacterium is found on and in medical equipment, especially catheters, because it thrives on damp surfaces, causing cross-infection in hospitals and clinics. It's linked to hot tub rash[6]. *P. aeruginosa* can colonize large areas and form long-lasting biofilms[7]. These biofilms protect these bacteria from adverse environmental factors and often cannot be treated effectively with traditional antibiotic therapy[8]. Recently the inhibitory effect of different plant extracts on anti-adherence activity or other virulence factor production in bacteria has been reported[9-11].

There is an urgent need to find alternative treatments that could eliminate biofilms formed by *P. aeruginosa* and reports claim that *T. indica* was effective against planktonic forms of this bacterium). Therefore, to investigate the antimicrobial activity of *Tamarindus indica* seeds extract on *P. aeruginosa* biofilm-forming bacteria.

MATERIALS AND METHODS

Study area: This was a descriptive case study carried out in Khartoum City, Sudan, from June, 2018-November, 2019 on clinical isolates of *P. aeruginosa* obtained from different hospitals in Khartoum, Sudan. The study was approved by the Ethical Committee of the College of Medical Laboratory Science, Sudan University of Science and Technology. A total of 60 clinical isolates of *P. aeruginosa* isolates from different clinical specimens were collected from Khartoum city hospitals.

Preparation of *Tamarindus indica* methanolic seeds extract: *T. indica* fruits were collected from Khartoum local market. The seeds were separated from the fruit, washed well to remove adhering matter, dried in oven 60°C and powdered using a blender. The seeds powder was subjected to soxhlet extraction and extracted with methanol then filtered through 4-fold muslin cloth followed by Whatman No. 1 filter paper. Finally, the extract was concentrated and dried in an oven at 60°C[12].

Confirmation of bacterial identity and inoculum standardization: *P. aeruginosa* isolates were sub-cultured on MacConkey's agar and blood agar aerobically at 37°C overnight. Organisms showed typical colonial morphology of *P. aeruginosa* was re-identified by conventional methods[13]. Pure colonies of bacterial isolates were suspended in normal saline and the turbidity was compared with 0.5 McFarland standards corresponding to 1.5×10^8 CFU mL^{-1} approximately. The standardization of culture was done according to clinical and Laboratory Standard Institute[14].

Inhibition of biofilms formation: Approximately 100 μL of different concentrations of *T. indica* seeds extract (50, 100,150 and 200 μg mL^{-1}) was dispensed with 100 μL nutrient broth into 96 well microtiter plates and inoculated with 5 μL of a 1 in a 5-diluted overnight culture of the test organism (population density of 1.5×10^8 (CFU mL^{-1}). Each microtiter plate included wells without inoculum (negative control), wells without added *T. indica* seeds extract (positive control). Microtiter plates were incubated statically at 37°C for 24 hrs. After incubation, the contents were removed carefully, wells were washed with distilled water, dried at room temperature and finally stained with 0.1% crystal violet solution for 30 min. The crystal violet solution was removed and the plate was washed twice thoroughly with distilled water and allowed to air-dry completely. A 0.1 mL 30% acetic acid was added to each well for 10 min after that the content of wells was removed and the biofilm biomass was measured using a microtiter plate reader at a wavelength of 592 nm[15].

Statistical analysis: Data analysis was done through the Statistical Package of Social Sciences (SPSS) software version 14.0. Chi-square test and one-way ANOVA were tested and a $p<0.05$ was considered statistically significant.

Table 1: Distribution of *P. aeruginosa* isolates

Isolates	Frequency	Percentages
Urinary tract isolates	18	30.0
Wound swab isolates	25	41.6
Eye swab isolates	4	6.6
Blood isolates	10	9.8
Respiratory Tract isolates	4	6.6
Otitis media isolates	3	5.0
Total	60	100.0

Table 2: Means+SD of the density of biofilms biomass of *P. aeruginosa* clinical isolates treated with various concentrations of *T. indica* extract

Concentration of *T. indica* methanolic extract (μg mL^{-1})	Density of biofilms biomass of *P. aeruginosa* clinical isolates Means+SD
50	0.047±0.036
100	0.035±0.029
150	0.027±0.027
200	0.020±0.023

Table 3: Association between the inhibitory effects of different concentrations and *T. indica* seeds extract with various *P. aeruginosa* clinical isolates

	Number of isolates					
Biofilm biomass at Zero (μg mL^{-1}) (Mean±SD)	Urinary isolates 18	Wound isolates 25	Eye isolates 4	Otitis media isolates 3	Blood isolates 6	Respiratory tract isolates 4
Biofilm biomass (Mean±SD)	0.043±0.024	0.058±0.037	0.056±0.026	0.060±0.052	0.063±0.043	0.064±0.044
50	0.039±0.025	0.058±0.037	0.076±0.048	0.083±0.028	0.034±0.019	0.059±0.046
p-value	0.43	0.96	0.18	0.34	0.04	0.44
100	0.014±0.007	0.022±0.016	0.035±0.006	0.019±0.001	0.012±0.006	0.015±0.006
p-value	0.00	0.00	0.20	0.31	0.03	0.09
150	0.011±0.007	0.019±0.017	0.032±0.013	0.016±0.003	0.007±0.005	0.013±0.002
p-value	0.00	0.00	0.13	0.30	0.02	0.10
200	0.008±0.006	0.014±0.013	0.031±0.10	0.012±0.002	0.005±0.007	0.010±0.002
p-value	0.00	0.00	0.12	0.26	0.02	0.08

Biofilm biomass absorption means of various *P. aeruginosa* clinical isolates against different concentrations of *T. indica* seeds extract in comparison to Imipenem

RESULTS

The distribution of the isolated *P. aeruginosa*, according to clinical specimens, shows that the most frequent isolates were wound swabs while the least isolates were respiratory tract isolates Table 1.

T. indica seeds extract showed antibacterial inhibitory effect against different *P. aeruginosa* biofilm formation. The biofilm biomass absorption means of different *P. aeruginosa* against different concentrations of *T. indica* seeds extract were shown in Table 2.

The highest anti-pseudomonal activity of *T. indica* methanolic extract was among *P. aeruginosa* biofilm-forming isolates from blood as the four concentrations were significantly effective with p-value (0.04, 0.03, 0.02 and 0.02), respectively. Three *T. indica* concentrations (100, 150 and 200 μg mL^{-1}) were highly effective against isolates from urine and wounds with (P = 0.00) for the three concentrations (Table 3). While the lowest effects of *T. indica* methanolic extract was among isolates from eye swabs, ear swabs and sputum with insignificant statistical correlation (p>0.05).

DISCUSSION

The results of this study showed that methanolic *T. indica* seeds extract exhibited effective antimicrobial activity against biofilm-forming *P. aeruginosa was* indicated by lowering the biofilm biomass density after treatment with tamarind extract. The biofilm biomass density has had decreased when the optical density of tamarind biofilm treated isolates (100 (0.035), 150 (0.027) and 200 μg mL^{-1} (0.020)) was compared with the optical density of none treated (zero μg mL^{-1} (0.049)) isolates. Similar results were reported in Iraq, where the biofilm biomass density decreased after treatment with *T. indica* seeds extract concentrations 100 (0.70) and 200 μg mL^{-1} (0.61) compared with the absorption before treatment (1.25)[16].

Methanolic *T. indica* seeds extract has antimicrobial inhibitory activity against *P. aeruginosa* biofilm-forming isolates from different clinical specimens, this result is similar to results reported by Rasheed in Iraq where *T. indica* seeds extract inhibit bacterial adhesion to the microtiter plate surface and consequently decrease the absorbance value of biofilm[1,16].

In this study higher concentrations led to a lower density of *P. aeruginosa* biofilms. This reflects that the mode of action of *T. indica* seeds extracts is concentration dependant as 100, 150, 200 μg mL^{-1} yielded (p = 0.025, 0.004, 0.001), respectively. Concentration dependant antibiofilm activity was reported among anti-*Streptococcus pyogens* biofilm [concentration dependant ref][17].

The inhibitory effects of 100, 150 and 200 μg mL^{-1} *T. indica* extract on *P. aeruginosa* biofilm-forming isolates yielded significant results against *P. aeruginosa* biofilm-forming isolates from the urinary tract and wound infections p-value (0.00). While blood isolates needed lower concentrations of *T. indica* seeds extract to give significant results as all concentrations (50, 100, 150 and 200 μg mL^{-1}) were effective yielding (p-value (0.04, 0.03, 0.02 and 0.02), respectively [Relate efficacy to the site of infection]. This result could be due to the complex, problematic nature and diversity of *P. aeruginosa* [18].

This study sheds the light on the possible use of *T. indica* seeds extract as an adjuvant in antimicrobial combinations. Many studies highlighted the benefits of using antibiotics in combination with chelating agents[19] or herbal remedies[20] as an alternative to a combination of two antibiotics. This approach has two main advantages; the degradation of the biofilm[21] and minimizing the chances of the emergence of resistant strains[22]. A study conducted by Tré-Hardy *et al*.[23] elucidated the need to treat biofilms with combinations of antimicrobial agents, since combination therapy against various biofilms was superior to monotherapy. This finding was common among planktonic as well as biofilm forms of *P. aeruginosa*[23].

Moreover, the antibiofilm activity of *T. indica* seeds extract could be used in the interference with Quorum-Sensing (QS) of *P. aeruginosa* biofilm. QS could be defined as "a cell-to-cell communication mechanism known as Quorum Sensing (QS) that has been found to play a role in *P. aeruginosa* bio lm formation"[24]. Methanolic *T. indica* seeds extract could be useful in hindering and interfering with QS of Pseudomonas aeruginosa biofilms to ease the degradation and eradication of the biofilm matrix[25].

Following this study future studies involving Minimum Biofilm Eradication Concentrations (MBECs), Minimum Biofilm Inhibitory Concentrations (MBICs), time-kill kinetics and synergistic studies could be conducted to determine the effective combinations and dosing regimens. Thus this study elucidated the promising future of the use of medicinal plants in hindering the growth and development of biofilms: hence, leading to the possible eradication of biofilm-forming pathogens.

CONCLUSION

In conclusion, methanolic *T. indica* seeds extract has antimicrobial inhibitory activity against *P. aeruginosa* biofilm-forming isolates from different clinical specimens. The higher concentration of *T. indica* seeds extract has a significant inhibitory effect against *P. aeruginosa* biofilm-forming bacteria, particularly those isolated from urine, wounds and blood. These results elucidate that Tamarind extracts could be a useful tool in eliminating the threats of biofilm-forming *Pseudomonasa eruginosa*. Further studies are required to confirm these results through other methods and determine the MIC and MBC of Tamarind extract.

SIGNIFICANCE STATEMENT

The ability of certain pathogens to form a matrix known as biofilm adds to the existing burden of antimicrobial resistance by making it difficult for the antimicrobial agent to pass through the matrix, thus making it difficult to eliminate the existing threat. This study illustrates the possible antimicrobial effects of tamarind extracts in reducing the density of *P. aeruginosa* biofilms, thus making it easy to be eradicated and removed or inhibited either by the extracts alone or more easily in association with effective antibiotics and/or chelating agents such as ethylenediaminetetraacetic acid (EDTA). This study will pave the road for the introduction of tamarind extracts and other herbal remedies to limit the current threats of biofilm-forming pathogens.

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