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Research Paper

Effect of Antibacterial activity of crude extracts of *Anacardium occidentale* on *Pseudomonas aeruginosa*

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ABSTRACT

Anacardium occidentale is one of the plants that has been used for ethno medical purposes in traditional civilization. Some extracts from this plant parts especially the apple, bark, leaves, gum and nut have been reported to inhibit the growth of medically important microorganisms. *Pseudomonas aeruginosa* is an opportunistic pathogen. The bacteria takes advantage of an individual's weakened immune system to create an infection and this organism also produces tissue-damaging toxins. Therefore, the aim of the present study was to investigate the antibacterial activity of methanol extracts of different parts of *A.occidentale*on *Pseudomonas aeruginosa* strains. Methanol extract of *A.occidentale* parts, such as leaves, bark, shell and nut, was tested against 5 strains of *P.aeruginosa*. It was found that all *P. aeruginosa* strains (100%) were sensitive to *A. occidentale* shell extract and nut extract followed by leaves extract (20%).However, thebark extract was not effective against it. *P.aeruginosa* strains were further tested against 5 different antibiotics. It was found that all *P.aeruginosa* strains (100%) were sensitive to Gatifloxacin, which was the highest among all other antibiotics tested followed by Meropenem (80%) and Piperacillin (20%). When studying the synergistic activity of extract and the antibiotic Gatifloxacin, it was found that, the organisms were much sensitive to this combination. *P.aeruginosa*(80%) was found to be sensitive each to leaves extract and bark extract. The present study highlighted the remarkable antibacterial activities of the *A. occidentale* extracts on *P.aeruginosa* strains.

Key words: *Anacardium occidentale, Pseudomonas aeruginosa,* antibacterial activity.

INTRODUCTION

The increase in the use of medicinal plants by the population to treat diseases makes it necessary to carry out pharmacological studies in order to contribute to scientific knowledge. Due to the difficulty of combating antibiotic resistant microorganisms, plants have become alow-cost and effective alternative (Baptista et al., 2018).Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Cashew, *Anacardium occidentale*, belongs to the family Anacardiaceae. It originates from South and central America. In the traditional civilization, one of the plants that has been used for ethno medical purposes is *A.occidentale*. It produces a pseudocarp on which the nut is attached. Some extracts from cashew plant parts, especially the apple, bark, leaves, gum and nut, have been reported to inhibit the growth of medically important microorganisms (Goncalves et al., 2005; Kannan et al., 2009; Goncalves and Gobbo, 2012; Olife et al., 2013). It has also been reported topossess anti-diabetic, anti-bacterial, anti-inflammatory and anti-ulcerogenic activities (Akinpelu, 2001).

Skin infections are caused by a wide variety of germs, and symptoms can vary from mild to serious. Mild infections

Table 1: Antibiotics used in study.

S/N	Antibiotics	Abbreviation	Concentration (mcg)
1	Gatifloxacin	GF	10
2	Cefepime	CPM	30
3	Piperacillin	PC	10
4	Carbenicillin	CB	100
5	Meropenem	MR	10

may be treatable with over-the-counter medications and home remedies, whereas other infections may require medical attention. *Pseudomonas aeruginosa* is an opportunistic pathogen. The bacteria takes advantage of an individual's weakened immune system to create an infection and this organism also produces tissue-damaging toxins. *P.aeruginosa* causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed. It is frequently resistant to many commonly used antibiotics (Wu et al., 2011). Due to the fact that the plant *A. occidentale* is very useful, as found by above mentioned reports, there is a need to determine further the potentiality of this plant as an antimicrobial agent. The present study was, therefore, designed to assess the potency of methanolic extracts of *A.occidentale* on *P.aeruginosa*.

MATERIALS AND METHODS

Preparation of methanol extract

For the preparation of methanol extract *A.occidentale* parts such as leaves, bark, shell and nut were rinsed with water and dried. Tree parts were ground into fine particles and 5 g each of powder was added in 50 ml methanol in respective conical flasks. The conical flasks were kept in rotary shaker for 72 H at room temperature. After 72 h, it was filtered using Whatman's No. 1 filter paper and then crude extracts obtained by filtration were used for further process (Cheeseborough, 2000; Tambekar et al., 2009).

Test organisms

Skin infection causing *P.aeruginosa* were collected from pathology laboratory in Nagpur and were identified on the basis of morphological, cultural and biochemical characteristics (Collee and Marr, 1996).

Antibiotic sensitivity test

Antibiotic sensitivity test was performed using Kirby Bauer Disc Diffusion method (Bauer et al., 1966). Five different types of antibiotics were used in the study (Table 1). *P.aeruginosa* strains were grown on nutrient agar at 37°C for 24 h and the colonies were suspended in sterile saline water equivalent to a 0.5McFarland standard (1.5X108CFU/ml). Hi-sensitivity agar plate was uniformly seeded by adding 100µl inoculated broth and was spread by means of spreader. The discs were placed on each inoculated Hi-sensitivity agar plate. The plates were incubated at 37°C for 18 h. The diameter of the zone of inhibition was observed in mm and the isolates were classified as "resistant" or "sensitive" based on the standard interpretative chart according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2007).

Antibacterial activity of *A. occidentale* against *P.aeruginosa*

The antibacterial activity of extracts of different parts of *A. occidentale* was performed using well diffusion technique. *P.aeruginosa* strains were grown overnight on nutrient agar at 37°C, and the colonies were suspended in sterile saline water equivalent to a 0.5 McFarland standard (1.5×108 CFU/ml). The suspension (100 µL) was spread over the Hi-Sensitivity agar. The wells of 6 mm diameter were cut into the agar medium with a sterilized cork borer. Thereafter, 20µl each of the extracts were added separately into the separate wells. The plates were incubated at 37°C for 18 h. The diameter of the zone of inhibition around each well was measured and recorded (Bauer et al., 1966).

Antibacterial activity of the combination of antibiotic and extract against *P.aeruginosa*

Extracts were used in combination with antibiotic against *P.aeruginosa*by agar well diffusion method. Here, in well, with 20 µl extract, an antibiotic disc was also kept to determine the anti-bacterial activity against *P. aeruginosa*

Table 2: Effect of *A. occidentale* extracts on *Pseudomonas aeruginosa*(n=5).

S/N	Samples	Resistant		Sensitive	
		No.	%	No.	%
1.	Leaves	4	80	1	20
2.	Bark	5	100	0	-
3.	Shell	0	-	5	100
4.	Nut	0	-	5	100
5.	Ampicillin (+ve control)	0	-	5	100
6.	Solvent (-ve control)	5	100	0	-

Table 3: Antibiotic susceptibility test of *Pseudomonas aeruginosa*(n=5).

S/N	Antibiotics	Resistant		Sensitive	
		No.	%	No.	%
1.	Cefepime	5	100	0	-
2.	Piperacillin	4	80	1	20
3.	Carbenicillin	5	100	0	-
4.	Meropenem	1	20	4	80
5.	Gatifloxacin	0	-	5	100

Table 4: Synergistic effect of *A. occidentale* extracts and Gatifloxacin against *Pseudomonas aeruginosa*(n=5).

S/N	Extract + antibiotic (Gatifloxacin)	Resistant		Sensitive	
		No.	%	No.	%
1.	Leaves	1	20	4	80
2.	Bark	1	20	4	80
3.	Shell	0	-	5	100
4.	Nut	0	-	5	100

strains.

RESULTS AND DISCUSSION

The present study was conducted to evaluate the effect of *A.occidentale* extract on strains of *P.aeruginosa*. Different extracts of *A.occidentale* tree such as leaves, bark, shell and nut extracts were used in the study. All *P.s aeruginosa* strains (100%) were sensitive to *A.occidentale* shell extract and nut extracts each, followed by leaves extract (20%). However, the bark extract was not effective against it (Table 2). The antimicrobial activity of different parts of *A.occidentale* extracts has also been reported by previous researchers (Belonwu et al., 2014). The ability of methanol to extract a wider range of antibacterial principles has been reported (Brittos, 2001). In one of the previous study, a wide range of human pathogenic microorganisms were examined, includingnot only Gram-positive and Gram-negative bacteria, butalso fungi. The study indicated thatthe *A.occidentale* extracts have broad inhibitory activities against pathogenic microorganisms and promising in acting as potential antibacterial and antifungal agents from natural plant sources (Dahake et al., 2009).

P. aeruginosa strains were further tested against 5 different antibiotics (Table 1). It was found that all the strains (100%) were sensitive to Gatifloxacin, which was the highest among all other antibiotics tested, followed by Meropenem (80%) and Piperacillin (20%) (Table 3). When studying the synergistic activity of the extract and the antibiotic Gatifloxacin, it was found that, the organisms were much sensitive to this combination. *P.aeruginosa* was found to be 80% sensitive each to leaves extract and bark extract (Table 4). This may be due to the fact that the active ingredient in Gatifloxacin is a refined and purified form, whereas the active ingredient in the plant extract is in a crude, impure, unrefined form. According to Kudi et al.(1999), *A.occidentale*has good antimicrobial activity

against*P.aeruginosa*.

CONCLUSION

A. occidentale extracts exhibited antimicrobial activity against *P. aeruginosa* strains. Moreover, the *A. occidentale* shell extract and nut extract were more effective on tested organisms. When all the extracts were used in combination with antibiotic Gatifloxacin, it was found that the leaves and bark extracts were more effective on the organisms used in the study. The study supports the use of *A. occidentale* plant in traditional medicine to treat skin infections which is expected to be a renowned source of antimicrobial agents for the future endeavours.

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