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## Research Article

### ANTIBACTERIAL INVESTIGATIONS OF CRUDE EXTRACTS OF ANACARDIUM OCCIDENTALE ON PSEUDOMONAS AERUGINOSA

Gulhane Pranita A\*, Marar Mamta P and Chandekar C.J

Department of Microbiology, S.S.E.S.A's Science College, Nagpur (MS) India-440012

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#### ABSTRACT

In the traditional civilization, one of the plants which have been used for ethno medical purposes is *Anacardium occidentale*. It has been reported to inhibit the growth of medically important microorganisms. *Pseudomonas aeruginosa* is an opportunistic pathogen. It takes advantage of an individual's weakened immune system to create an infection. Therefore aim of the present work was to investigate the antibacterial activity of methanol extracts of different parts of *Anacardium occidentale* on *Pseudomonas aeruginosa* strains. Methanol extract of leaves, bark, shell and nut was tested against 5 strains of *Pseudomonas aeruginosa*. It was found that all *Pseudomonas aeruginosa* strains (100%) were sensitive to *Anacardium occidentale* shell extract and nut extract followed by leaves extract (20%). However bark extract was not effective against it. Antibiotic resistance profile showed that all *Pseudomonas aeruginosa* (100%) were sensitive to Gatifloxacin, which was the highest among all other antibiotics tested followed by Meropenem (80%) and Piperacillin (20%). The synergistic activity of extract and antibiotic Gatifloxacin, showed that, the organisms were much sensitive to this combination. *Pseudomonas aeruginosa* (80%) was found to be sensitive each to leaves extract and bark extract. The present study highlighted remarkable antibacterial activities of *A. occidentale* extracts on *Pseudomonas aeruginosa*.

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#### 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 the scientific knowledge. Due to the difficulty of combating antibiotic resistant microorganisms, plants become a low-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 which have been used for ethno medical purposes is *Anacardium 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 to possess 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 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. *Pseudomonas 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. *Pseudomonas aeruginosa* is frequently resistant to many commonly used antibiotics (Wu *et al.*, 2011). Due to the fact that the plant *Anacardium occidentale* is very useful, as found by above mentioned reports, there is a need to find out more about the potentiality of this plant as an antimicrobial agent. The present study is, therefore, designed to assess the potency of methanolic extracts of *Anacardium occidentale* on *Pseudomonas aeruginosa*.

#### MATERIALS AND METHODS

**Preparation of Methanol extract:** For the preparation of methanol extract *Anacardium 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 hours at room temperature. After 72 hours, it was filtered by using Whatman's No. 1 filter paper and then crude extracts were obtained by filtration were used for further process (Cheeseborough, 2000; Tambekar *et al.*, 2009).

**Test organisms:** Skin infection causing *Pseudomonas aeruginosa* were collected from pathology laboratory in Nagpur

\*Corresponding author: Gulhane Pranita A

Department of Microbiology, S.S.E.S.A's Science College, Nagpur (MS) India-440012

and were identified on the basis of morphological, cultural and biochemical characteristics (Collee and Marr, 1996).

**Antibiotic sensitivity test:** Antibiotic sensitivity test was performed by Kirby Bauer Disc Diffusion method (Bauer *et al.*, 1966). Five different types of antibiotics were used in the study (Table 1). *Pseudomonas aeruginosa* strains were grown on nutrient agar at 37°C for 24 hours and the colonies were suspended in sterile saline water equivalent to a 0.5 McFarland standard (1.5X10<sup>8</sup>CFU/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 hours. 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 *Pseudomonas aeruginosa*:** Antibacterial activity of extracts of different parts of *A. occidentale* was performed by well diffusion technique. *Pseudomonas 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 (1.5×10<sup>8</sup> 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. Then 20µl each of the extracts were added separately into the separate wells. The plates were incubated at 37°C for 18 hours. The diameter of the zone of inhibition around each well was measured and recorded (Bauer *et al.*, 1966).

**Antibacterial activity of combination of antibiotic and extract against *Pseudomonas aeruginosa*:** Extracts were used in combination with antibiotic, against *Pseudomonas aeruginosa* by agar well diffusion method. Here, in well, with 20 µl extract, an antibiotic disc was also kept to see the anti-bacterial activity against *Pseudomonas aeruginosa* strains.

## RESULTS AND DISCUSSION

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

*Pseudomonas aeruginosa* strains were further tested against 5 different antibiotics (Table 1).

**Table 1** Antibiotics used in study

Sr. No.	Antibiotics	Abbreviation	Concentration
1	Gatifloxacin	GF	10 mcg
2	Cefepime	CPM	30 mcg
3	Piperacillin	PC	10 mcg
4	Carbenicillin	CB	100 mcg
5	Meropenem	MR	10 mcg

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

Sr. No.	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	-

It was found that all *Pseudomonas aeruginosa* (100%) were sensitive to Gatifloxacin, which was the highest among all other antibiotics tested followed by Meropenem (80%) and Piperacillin (20%) (Table 3).

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

Sr. No.	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%

When studying the synergistic activity of extract and the antibiotic Gatifloxacin, it was found that, the organisms were much sensitive to this combination. *Pseudomonas 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) *Anacardium occidentale* has good antimicrobial activity against *Pseudomonas aeruginosa*.

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

Sr. No.	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%

## CONCLUSION

*A. occidentale* extracts exhibited antimicrobial activity against *Pseudomonas 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 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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