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International Journal of Current Research in Life Sciences
Vol. 07, No. 08, pp.2622-2624, August, 2018

ISSN: 2319-9490

RESEARCH ARTICLE

ANACARDIUM OCCIDENTALE EXTRACTS: A PROPOSED MULTIFACETED APPROACH TO COMBAT *STAPHYLOCOCCUS AUREUS* INFECTION

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

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

Received 24th June, 2018; Accepted 17th July, 2018; Published 30th August, 2018

ABSTRACT

Anacardium occidentale (Cashew) is a member of genus Anacardium belonging to family Anacardiaceae, which has its roots in different parts of the world. Va rious parts of the plant possess curative qualities like antidiabetic, antiinflammatory, antibacterial and antifungal properties. Therefore aim of the present work was to investigate the antibacterial activity of methanol extracts of different cashew tree parts on Staphylococcus aureus strains. Methanol extract of Anacardium occidentale parts such as leaves, bark, shell and nut was tested against 5 strains of S.aureus. It was found that all S.aureus strains (100%) were sensitive to Anacardium occidentale shell extract while leaves extract, bark extract and nut extract were not effective against it. S. aureus strains were further tested against 5 different antibiotics. It was found that all S. aureus (100%) were sensitive to Ampicillin and Gatifloxacin each, which was the highest among all other antibiotics tested followed by Azithromycin (80%), Clindamycin (60%) and Erythromycin (40%). When studying the synergistic activity of extract and the antibiotic Gatifloxacin, it was found that, the organisms were much sensitive to this combination. S. aureus was found to be 100% sensitive each to leaves extract, bark extract and shell extract while 60% to nut extract. The present study highlighted the remarkable antibacterial activities of the A. occidentale extracts on S. aureus strains.

Key words: Anacardium occidentale, S.aureus, Antibacterial activity.

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Citation: Mamta Marar, Pranita A. Gulhane and Chandekar, C.J. "Anacardium occidentale extracts: A proposed multifaceted approach to combat Staphylococcus aureus Infection" *International Journal of Current Research in Life Sciences*, 7, (08), 2622-2624.

INTRODUCTION

The cashew tree (*Anacardium occidentale*) is a tropical evergreen tree that produces cashew apple and cashew nut. The cashew tree is a native of Brazil and the lower Amazons. The cashew has been introduced and is a valuable cash crop in the Americas, the West Indies, Madagascar, India and Malaysia (Frankel, 1991). It can tolerate a wide range of conditions including drought and poor soil, but cannot withstand cold frost. The major producing countries of cashew are Tanzania, India, Mozambique, Sri Lanka, Thailand Indonesia, Senegal, Kenya, Madagascar, Malaysia, Nigeria, Angola and Malawi (Rosengarten, 1984). The cashew nut, also called a cashew, is consumed widely. It is used in recipes, eaten in own, processed into cashew butter or cashew cheese. The cashew nut shells can be used in making lubricants, paints, etc. The cashew apple which is light reddish or yellow fruit, its pulp is processed into a sweet astringent fruit drink, etc. The fruit of the cashew tree is an accessory fruit also called pseudo carp or false fruit. It appears in oval or pear-shaped structure, a hypocarpium that develops from the pedicel and the receptacle of the cashew apple (Varghese and Pundir, 1964), called cashew apple.

****Corresponding author:*** **Pranita A. Gulhane,**
Department of Microbiology, S.S.E.S.A's Science College, Nagpur (MS), India.

It is edible and has strong 'sweet' smell and a sweet taste. Its pulp is juicy, but its skin is fragile so it is unsuitable for transport. A fruit drink is made from this cashew apple, it has very refreshing taste. Skin flora is usually non-pathogenic, and either commensal (are not harmful to their host) or mutualistic (offer a benefit). The benefits bacteria can offer include preventing transient pathogenic organisms from colonizing the skin surface, either by competing for nutrients, secreting chemicals against them, or stimulating the skin's immune system (Cogen *et al.,* 2008). However, resident microbes can cause skin diseases and enter the blood system, creating life-threatening diseases, particularly in immunosuppressed people. *Staphylococcus aureus* can cause various skin and soft tissue infections, particularly when skin or mucosal barriers have been breached. *Staphylococcus aureus* is a gram positive, round shaped bacterium that is mostly found in in the respiratory tract and also on the skin. *S. aureus* is not always pathogenic; it is a common cause of skin infections, such as skin abscess, respiratory infections. The strains which are pathogenic, they often promote infections by producing virulence factors like potent protein toxin. The emergence of antibiotic resistant strains of *S. aureus* such as Methicillin resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine. Skin infections are the most common forms of *S. aureus* infection. They include folliculitis, small benign boils, cellulitis, impetigo and more severe, invasive soft tissue invasions. *S. aureus* is extremely prevalent in persons with atopic dermatitis. It is mostly found in fertile, active places, including the hair, scalp and armpit. Large pimples that appear in those areas may exacerbate the infection. This may thus lead to staphylococcal scalded skin syndrome, a severe form of which can be seen in neonates (Curran and Al-Salihi, 1980). Plant extracts have the anti-microbial activity against the bacterial pathogens. Plant derived medicines have made significant contributions towards human health. However, plants are a great source of novel drug compounds. Traditional healing systems around the world that utilize herbal remedies are an important source of discovery of new antibiotics (Okpekon *et al.,* 2004); some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria (Kone *et al.,* 2004). It indicates the need for further research into traditional health systems (Romero *et al.,* 2005). It also facilitates pharmacological studies leading to synthesis of a more potent drug with reduced toxicity. The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents (Nostro *et al.,* 2000). Phyto chemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds (Krishnaiah *et al.,* 2007). Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities (Mahato and Sen, 1997). Terpenoids are very important in attracting useful mites and consume the herbivorous insects (Kappers *et al.,* 2005). Alkaloids are used as anaesthetic agents and are found in medicinal plants (Herouart *et al.,* 1988). Therefore aim of the present work was to investigate the anti-bacterial activity of methanol extracts of different cashew tree parts on *Staphylococcus aureus* strains.

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 *Staphylococcus aureus* 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 by Kirby Bauer Disc Diffusion method (Bauer *et al.,* 1966). Five different types of antibiotics were used in the study (Table 1). *S.aureus* strains were grown on nutrient agar at 37^0C for 24 hours 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 37^0C 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 *S.aureus*: Antibacterial activity of extracts of different parts of *A. occidentale* was performed by well diffusion technique [19]. *S.aureus* strains were grown overnight on nutrient agar at 37^0C, 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. Then 20µl each of the extracts were added separately into the separate wells. The plates were incubated at 37^0C 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 *S. aureus*: Extracts were used in combination with antibiotics, against *S.aureus* 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 *S.aureus* strains.

RESULTS AND DISCUSSION

The present study was conducted to evaluate the effect of *Anacardium occidentale* extract on strains of *Staphylococcus aureus*. Different extracts of *Anacardium occidentale* tree such as leaves, bark, shell and nut extracts were usstudy. All *S.aureus* strains (100%) were sensitive to *Anacardium occidentale* shell extract while leaves extract, bark extract and nut extract were 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). *S. aureus* strains were further tested against 5 different antibiotics (Table 1). It was found that all *S. aureus* (100%) were sensitive to Ampicillin and Gatifloxacin each, which was the highest among all other antibiotics tested followed by Azithromycin (80%), Clindamycin (60%) and Erythromycin (40%) (Table 3). When studying the synergistic activity of extract and the antibiotic Gatifloxacin, it was found that, the organisms were much sensitive to this combination. *S. aureus* was found to be 100% sensitive to each leaves extract, bark extract and shell extract while 60% to nut extract (Table 4). This may be due to the fact that the active ingredient in Ampicillin and Gatifloxacin is a refined and purified form whereas the active ingredient in the plant extract is in a crude, impure, unrefined form and in a mixture with unknown compounds. Therefore, further studies on extracts of *A. occidentale* should involve refining or purifying and concentrating the active ingredient to determine its real effect on pathogenic microorganisms.

Table 1. Antibiotics used in study

Sr. No.	Antibiotics	Abbreviation	Concentration
1	Erythromycin	E	15 mcg
2	Ampicillin	A	10 mcg
3	Clindamycin	CD	2 mcg
4	Azithromycin	AT	15 mcg
5	Gatifloxacin	GF	10 mcg

Table 2. Effect of *A. occidentale* extracts on *Staphylococcus aureus* (n=5)

Sr. No.	A. occidentale Extract	Resistant		Sensitive	
		No.	%	No.	%
1.	Leaves	5	100%	0	-
2.	Bark	5	100%	0	-
3.	Shell	0	-	5	100%
4.	Nut	5	100%	0	-
5.	Ampicillin (+ve control)	0	-	5	100%
6.	Solvent (-ve control)	5	100%	0	-

Table 3. Antibiotic susceptibility test of *Staphylococcus aureus* (n=5)

Sr. No.	Antibiotics	Resistant		Sensitive	
		No.	%	No.	%
1.	Erythromycin	3	60%	2	40%
2.	Ampicillin	0	-	5	100%
3.	Clindamycin	2	40%	3	60%
4.	Azithromycin	1	20%	4	80%
5.	Gatifloxacin	0	-	5	100%

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

Sr. No.	A. occidentale Extract + Gatifloxacin	Resistant		Sensitive	
		No.	%	No.	%
1.	Leaves	0	-	5	100%
2.	Bark	0	-	5	100%
3.	Shell	0	-	5	100%
4.	Nut-natural	2	40%	3	60%

Conclusion: The present study highlighted the remarkable antibacterial activities of the *A. occidentale* extracts on *S. aureus* strains. Moreover, the *A. occidentale* shell extracts 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. These results partly justify the use of this plant by traditional healers in the treatment of certain diseases such as skin diseases, burn injuries, blood stream infection, and food poisoning. More research is required to fully establish the usefulness of this plant. The extracts of *A. occidentale* could be a possible source to obtain new and effective herbal medicines. There is need for the development of new antimicrobials to combat the problem of the spread of resistance more importantly from natural sources as this delays the emergence of resistance.

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