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RESEARCH ARTICLE

INHIBITORY EFFECT OF LEAF AND BARK OF *ANACARDIUM OCCIDENTALE* AGAINST CLINICAL ISOLATES OF *STAPHYLOCOCCUS AUREUS* AND *STREPTOCOCCUS MUTANS*

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ABSTRACT

The present study was undertaken to determine inhibitory activity of methanolic extract of leaf and bark of *Anacardium occidentale* L. (Anacardiaceae) against clinical isolates of *Staphylococcus aureus* and *Streptococcus mutans* recovered previously from burn and dental caries patients respectively. Agar well diffusion method was employed to investigate antibacterial activity. The extracts were found to be effective in inhibiting all clinical isolates. Leaf extract was found to inhibit bacteria to higher extent than bark extract. The inhibition of clinical isolates by the extracts could be ascribed to the presence of bioactive components present in the extracts.

Key words: *Anacardium occidentale*, Agar well diffusion, Burn, Dental Caries, *Staphylococcus aureus*, *Streptococcus mutans*

INTRODUCTION

Cashew (*Anacardium occidentale* L., Anacardiaceae) is a native to Brazil and is one of the most important plantation crops in countries such as India, Brazil, Nigeria and Vietnam. It is largely grown for the nuts (true fruit) having an exclusive fine taste and a commercial importance. The edible cashew apple (the thick receptacle or 'false fruit' to which the cashew nut or true fruit is attached) has high nutritive values and has found several applications in food industries and in breweries[1,2,3]. Various parts such as leaf, pseudo-fruit, stem, nut shell, gum of the plant *A. occidentale* have been shown to possess several biological activities such as antimutagenic[4], anti-inflammatory[5,6], analgesic[5,6], hypoglycemic[7,8], antimicrobial[9-12], antioxidant[13], antidiabetic[14], renal protective[8], aphrodisiac[15], insecticidal[9], antiulcerogenic[16], hypocholesterolemic[17], β-Lactamase inhibitory[18] and acetylcholinesterase inhibitory activity[19]. In the present study, we report antibacterial activity of methanolic extract of leaf and bark of *A. occidentale* against clinical isolates of *Staphylococcus aureus* and *Streptococcus mutans* isolated previously from burn and dental caries patients respectively.

MATERIALS AND METHODS

Collection and identification of plant material

Leaves and barks of *A. occidentale* were collected at Maragalale, Thirthahalli (taluk), Shivamogga (district), Karnataka during June 2013. The plant materials were washed thoroughly, shade dried and powdered in a blender. The powdered leaf bark materials were stored in air-tight containers.

Extraction

About 10g of dried and powdered leaf and bark of *A. occidentale* were added to 100ml of methanol (HiMedia, Mumbai), sonicated for 30 minutes and left at room temperature overnight. The extracts were filtered through Whatman No. 1 filter paper, concentrated in vacuum under reduced pressure and dried in the desiccator[20].

Antibacterial activity of leaf and bark extracts

Agar well diffusion assay was carried out to determine antibacterial activity of leaf extract (LE) and bark extract (BE) against five isolates of *S. aureus* (recovered previously from burn patients) and five isolates *S. mutans* (isolated from dental caries subjects previously). The isolates of *S. aureus* and *S. mutans* were grown in sterile Nutrient broth (HiMedia, Mumbai) and sterile Brain heart infusion broth (HiMedia, Mumbai) respectively for 24 hours at 37^{o}C. The broth cultures of *S. aureus* and *S. mutans* were aseptically swabbed on sterile Nutrient agar (HiMedia, Mumbai) and sterile Brain heart infusion agar (HiMedia, Mumbai) plates respectively using sterile cotton swabs. Wells of 6mm diameter were made in the inoculated plates using sterile cork borer. 100µl of LE and BE (25mg/ml of 25% dimethyl sulfoxide [DMSO]), standard antibiotic (Chloramphenicol, 1mg/ml of sterile distilled water) and DMSO (25%, in sterile water) were filled into labeled wells. The plates were incubated at 37^{o}C for 24 hours and the zone of inhibition was measured using a ruler[21].

Statistical analysis

The results are represented as Mean±Standard deviation (n=3).

RESULTS AND DISCUSSION

Table 1 shows the inhibitory activity of LE and BE of *A. occidentale* against clinical isolates of *S. aureus*. Among extracts, LE was more effective (zone of inhibition ranging 1.5 to 1.8cm) in inhibiting bacterial isolates when compared to BE (zone of inhibition ranging 1.2 to 1.6cm).

Chloramphenicol showed higher inhibition of bacteria than LE and BE. DMSO did not cause inhibition of bacteria (not shown in table).

Table 1: Inhibitory activity of LE and BE of *A. occidentale* against *S. aureus* isolates

Test Bacteria	Zone of inhibition in cm		
	Solvent extract		Standard
	LE	BE	
Sa-01	1.8±0.2	1.6±0.2	3.1±0.5
Sa-02	1.5±0.1	1.2±0.1	2.7±0.3
Sa-03	1.7±0.2	1.2±0.2	2.4±0.1
Sa-04	1.5±0.2	1.2±0.1	3.3±0.2
Sa-05	1.5±0.2	1.2±0.1	3.2±0.2

Table 2 shows the antibacterial effect of LE and BE of *A. occidentale* against clinical isolates of *S. mutans*. Here also, among extracts, LE was more effective (zone of inhibition ranging 1.5 to 2.2cm) in inhibiting bacterial isolates when compared to BE (zone of inhibition ranging 1.3 to 1.5cm). Chloramphenicol caused higher inhibition of bacteria than LE and BE. DMSO did not cause inhibition of bacteria (not shown in table).

Table 2: Inhibitory activity of LE and BE of *A. occidentale* against *S. mutans* isolates

Test Bacteria	Zone of inhibition in cm		
	Solvent extract		Standard
	LE	BE	
Sm-01	1.6±0.2	1.3±0.1	3.5±0.2
Sm-02	1.9±0.2	1.4±0.2	3.3±0.3
Sm-03	1.7±0.1	1.3±0.1	2.9±0.2
Sm-04	1.5±0.1	1.5±0.1	3.6±0.3
Sm-05	2.2±0.2	1.4±0.1	3.1±0.1

DISCUSSION

Burn wounds are more vulnerable and suitable sites for multiplication of pathogenic bacteria. These wounds are ideal sources of infection than surgical wounds because of the larger area involved and longer duration of patient stay in the hospital. The major cause of morbidity and mortality in hospitalized burn patients is infection caused by a number of pathogenic microbes. It is estimated that more than 75% of deaths following burn is related to infections. The bacteriology of burn wounds is usually poly-microbial in nature. The most common bacterial pathogens isolated from burn wounds are *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes* and various coilform bacilli. Among these, *Staphylococcus aureus* is frequently isolated in both community and hospital practices. *S. aureus* is one of the greatest causes of nosocomial infection in burn patients. The pattern of antimicrobial susceptibility of *S. aureus* is changing and therefore antimicrobial agents are becoming ineffective. Majority of strains of *S. aureus* in community as well as hospitals have developed resistance to commonly used antibiotics such as penicillin, methicillin and vancomycin. Infection of burn wounds caused by multidrug resistant pathogens is a serious threat for successful treatment[22-27]. It has been found that plants and their components are effective against *S. aureus* isolates including drug resistant strains[21,25,28-33]. In the present study, the extracts of *A. occidentale* were shown to be potent in causing inhibition of isolates of *S. aureus* recovered previously from burn patients.

Dental infections namely dental caries and periodontal diseases are among the most common human diseases. The ubiquitousness and non-life threatening nature have minimized their significance in overall health of humans. Mutans streptococci are found in highest numbers on teeth. Mutans streptococci are the streptococci found in plaque, ferment mannitol and sorbitol, produce extracellular glucans from sucrose and are cariogenic. *Streptococcus mutans* is often considered as the most important and primary aetiological agent among mutans streptococci responsible for causing dental caries in humans. *S. mutans* possesses the property to adhere to pellicle-coated tooth surfaces and to form acids[34-40]. Prevention and treatment of dental caries involves the use of antimicrobial mouth rinses such as triclosan and chlorhexidine and antibiotics such as penicillin, erythromycin, and ampicillin. However, the antimicrobial mouth rinses are reported to cause some undesirable side effects such as tooth staining, taste alteration and development of hypersensitivity reactions whereas antibiotics suffer from the major drawback that oral microflora are getting resistance against them[33,37,41,42]. This has triggered interest in searching alternatives for treatment of dental caries. Plants have shown to be effective in the treatment of dental caries and a number of studies have shown the potential of plants and their metabolites to inhibit cariogenic flora[21,33,39,42-49]. In our study, leaf and bark extracts of *A. occidentale* displayed inhibition of *S. mutans* isolates recovered from dental caries subjects.

CONCLUSION

The extracts from leaf and bark *A. occidentale* displayed inhibition of clinical isolates of *S. aureus* and *S. mutans*. The inhibitory effect could be ascribed to the presence of bioactive components present in extracts. The plant can be employed as a potential candidate for the development of inhibitory agents active against clinical isolates.

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