

ANTIMICROBIAL ACTIVITY OF AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF ANACARDIUM OCCIDENTALE

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ABSTRACT

Objective: The objective of this study was to evaluate the antimicrobial activity of leaves of *Anacardium occidentale* (*A. occidentale*) against microorganisms including multidrug-resistant (MDR) bacteria.

Methods: Agar well diffusion method was employed to demonstrate the antimicrobial activity of leaves *A. occidentale*. Ethanol and aqueous extracts of the leaves were used against microorganisms, which included American type culture collection strains of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* and the clinical isolates of *Streptococcus pneumoniae*, *Candida albicans*, MDR *Escherichia coli*, and MDR *Klebsiella pneumoniae*.

Results: The ethanolic extract of leaves of *A. occidentale* showed significant antimicrobial activity. Aqueous extract had mild antifungal activity.

Conclusion: Ethanolic extract of leaves of *A. occidentale* could be a good source for the antibacterials to combat MDR bacterial infections. Further studies are necessary for these potent plant extracts to evaluate the *in vivo* efficacy and toxicity.

Keywords: Leaves of *Anacardium occidentale*, Antimicrobial susceptibility testing, Multidrug-resistant bacteria.

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INTRODUCTION

Multidrug resistance (MDR) is increasing both in community and health-care associated bacterial infections worldwide. This has impaired the current antimicrobial therapy, thereby creating a demand for other alternatives. There is a demand for medicinal plants in health-care system and the use of these has been associated with fewer side effects [1]. Nowadays, bacteria such as *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), and *Enterobacter species* have acquired resistance to the commonly used antibiotics [2-5]. The treatment options for these microorganisms are less and treating physicians are compelled to use expensive drugs like colistin that is associated with significant side effect to the patient's health [6]. Therefore, there is a need for the development of alternate drugs to prevent the infections caused by these organisms. Medicinal plants are rich sources of antimicrobial agents. Herbal plants have attained a significant role as therapeutic agents [7]. *Anacardium occidentale* (*A. occidentale*) a native of Brazil, a tropical evergreen tree, almost all the plant parts, namely bark, leaves, fruit, flower, and nut oil, have been reported to be useful to cure many diseases such as venereal diseases, diarrhea, skin diseases, stomatitis, bronchitis, psoriasis, toothaches, and gum problems [8-10]. Leaves of which contain pharmacologically rich components such as alkaloids, essential oils, tannins, saponins, and cardenolides [11, 12] and are known to have cosmetic [13], antimicrobial [14], and antioxidant [15] properties. In this study, antimicrobial activity against selected bacterial and fungal strains was performed using ethanol and aqueous extract of *A. occidentale* leaves.

MATERIALS AND METHODS

Plant collection

The fresh leaves of *A. occidentale* were collected from the plantation area of Udupi, Karnataka, India. Its botanical identity was authenticated by a botanist.

Aqueous and ethanol extraction

Leaves of *A. occidentale* were washed with distilled water and air dried at room temperature for 10 days. Fine powder was made by pulverizing with clean mortar and pestle, then stored in a sterilized glass container at room temperature (25–30°C).

By Soxhlation method, the ethanol extract of leaves was prepared [16]. The aqueous extract of the leaves was prepared by crushing the leaves in mortar and pestle using sterile distilled water in the ratio of 1:1.

Antimicrobial activity

Agar well diffusion method was employed to study the antibacterial and antifungal susceptibility [17]

Antimicrobial susceptibility was determined using American type culture collection (ATCC) strains, MDR strains, and fungus. The ATCC strains were *S. aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *K. pneumoniae* ATCC 700603, *E. coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 and clinical strains of *Streptococcus pneumoniae* (*S. pneumoniae*), MDR *E. coli*, MDR *K. pneumoniae*, and *Candida albicans* (*C. albicans*). Ampicillin disc (10 µg), oxacillin disc (1 µg), gentamicin disc (10 µg), and ketoconazole disc (10 µg), obtained from Hi-Media, Mumbai, were used as controls.

Culture media used were Sabouraud's dextrose agar (SDA), Muller-Hinton agar (MHA), and blood agar (for *S. pneumoniae*) which were procured from Hi-Media, Mumbai. The above-mentioned bacterial and fungal strains were identified by standard procedure. Isolated bacterial colonies were transferred to sterile Mueller-Hinton broth, and *C. albicans* was inoculated to Sabouraud's dextrose broth and incubated at 37°C overnight. The concentration of growth of microorganisms to 10⁵ CFU/ml was adjusted using 0.5 McFarland's turbidity standard.

Determination of antibacterial activity

Blood agar and MHA measuring 20 ml each was poured into Petri dishes. The bacterial culture was spread over the surface of the MHA plate and blood agar. Wells of 6 mm diameter were punched into the agar and filled with 100 µl of the solution. The inoculated plates were incubated at 37°C for 18 h. Tests were performed in triplicates and the average of the three was considered for the study.

Determination of antifungal activity

20 ml of SDA was poured into each Petri dish. Culture of the *C. albicans* was spread over the surface of the SDA plate. Wells were punched into the agar plate measuring 6 mm in diameter and filled with 100 µl solution. The plates were further incubated for 18 h at 37°C. Tests were done in triplicates and the average of the three was considered for the study.

RESULTS

Diameter of zone of inhibition was measured for antimicrobial activity. Ethanol extract of *A. occidentale* leaves exhibited antibacterial activity against all the bacterial strains. The zone of inhibition for *E. coli* ATCC 25922 was 20 mm and *S. aureus* ATCC 25923 was 18 mm. Zone of inhibition for MDR strains of *E. coli* was 18 mm and *K. pneumoniae* 15 mm. All the bacterial strains were resistant to aqueous extract of *A. occidentale* leaves. Both the aqueous and ethanolic extracts of *A. occidentale* leaves were found to have antifungal activity $p < 0.05$ [Table 1].

DISCUSSION

Antimicrobial resistance is the most common prevailing problem in the present scenario. The microorganisms employ new mechanisms to survive [18]. *S. aureus*, *K. pneumoniae*, *E. coli*, and *Streptococcus pneumoniae* are some of the most important organisms exhibiting MDR. Development of alternative drugs to treat infectious diseases caused by such organisms is essential. The herbal drugs obtained from the medicinal plants have fewer adverse effects than the conventional ones. In this study, the ethanolic extract of *A. occidentale* leaves showed significant antibacterial activity against clinically isolated MDR microorganisms. Although the mechanism of the action of these plant constituents is not yet fully known, we observed that ethanolic extract provided antimicrobial activity.

Venkatadri et al. and Girish et al. [19,20] in their study reported that the effectiveness of extracts largely depends on the type of solvent used and ethanolic extracts exhibited greater antibacterial activity than the corresponding aqueous extracts. Our study was in concordance with their study. These findings could be due to the ability of the solvent to extract more active ingredients from the plant materials [21]. Most of the antibiotic compounds such as plant alkaloids and tannins were reported as antibacterial substances [22] and were better extracted using ethanol than water. Belonwu et al. and Krishnananda

et al. [23,24] on systemic evaluation of antibacterial activity of *A. occidentale* reported that alkaloids, saponins, flavonoids, tannins, hydrogen cyanide, phenols, and anthocyanin present in the leaf were responsible for the antibacterial activity. They showed that *S. aureus*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae* were greatly inhibited by ethanolic leaf extract. Dahake et al. [14] also reported similar results.

In our study, both the aqueous and ethanolic extract of *A. occidentale* leaves showed antifungal activity against *C. albicans*. This bioactivity might be due to antifungal components such as alkaloids, lectins, terpenes, and saponins which is easily extracted by water [25,26].

CONCLUSION

This study shows that ethanolic extract of *A. occidentale* leaves had antimicrobial activity against pathogenic microorganisms including MDR strains. Further studies are necessary to evaluate the component responsible for antimicrobial activity of this plant for pharmaceutical applications.

AUTHORS' CONTRIBUTION

All the authors have substantially contributed to the research and publication of this study.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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Table 1: Zone of inhibition of ethanol and aqueous leaf extract of *A. occidentale* against various microorganisms

Name of the organism	Zone of inhibition (mm)	
	Ethanol extract	Aqueous extract
<i>Staphylococcus aureus</i> ATCC 25923	18	-
<i>Enterococcus faecalis</i> ATCC 29212	12	-
<i>Klebsiella pneumoniae</i> ATCC 700603	18	-
<i>Escherichia coli</i> ATCC 25922	20	-
<i>Pseudomonas aeruginosa</i> ATCC 27853	16	-
<i>Streptococcus pneumoniae</i>	17	-
MDR <i>Escherichia coli</i>	18	-
MDR <i>Klebsiella pneumoniae</i>	15	-
<i>Candida albicans</i>	17	9

(-): Indicates no zone of inhibition, ATCC: American type culture collection, MDR: Multidrug resistant

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