INTRODUCTION

In the past two decades, the frequency of antimicrobial-resistant infections has increased in both the hospital and community. Pharmacological industries are producing many new antibiotics, and resistance to these drugs by microorganisms has increased [1,2]. Hence, there is a need for the development of drugs to prevent the infections caused by these organisms. Medicinal plants are used in the world and more so in India and this contributes significantly to primary healthcare system [3].

Synthetic drugs are not only expensive but also inadequate for the treatment of infections. It can have adventitious and side effects. In the developing countries, a major challenge seen nowadays in the global health care is the need for novel, effective, and affordable medicines to treat microbial infections [4,5]. For a long time, India among other countries forms the richest hub for the widest application of the medicinal plants. A number of such plants have found their applications in treating a wide range of human ailments. Drugs which are derived from natural sources have a significant role in the prevention and treatment of infections in humans. Hence, there is a need to search for new strategies to fight and control microbial infections [6].

Medicinal plants and herbs are used for the treatment of infections. Hence, their therapeutic potential, biological properties, and safety have to be considered before we take the decisions to use them. Acacia species are a rich source of polyphenolic compounds due to antioxidant property, and it is used in the prevention and therapy of various diseases including cardiovascular, neurodegenerative, and cancer [7,8]. Acacia auriculiformis (A. auriculiformis) also called black wattle is a common Indian medicinal plant. The present investigation focuses on in vitro antimicrobial properties of aqueous and ethanolic leaf extracts of A. auriculiformis tested on Gram-positive cocci, Gram-negative bacilli, multidrug-resistant (MDR) Gram-negative bacilli, and fungus.

METHODS

Aqueous and ethanolic extracts of the leaves of A. auriculiformis were prepared. Agar well diffusion was the method for antimicrobial susceptibility. Freshly grown standard strains of Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae (K. pneumoniae), Escherichia coli (E.coli) and Pseudomonas aeruginosa, clinical strains of Streptococcus pneumoniae, Candida albicans (C. albicans), and MDR E. coli, and MDR Klebsiella pneumoniae were used. Ampicillin disc (10 µg) was used as control.

RESULTS: The zone of inhibition was measured to determine the antimicrobial activity. Ethanolic extract of A. auriculiformis exhibited antibacterial activity against all the strains including MDR strains of K. pneumoniae and E. coli. Antifungal activity was exhibited by both aqueous and ethanole leaf extracts of A. auriculiformis.

Conclusion: Ethanolic extract could be used against MDR K. pneumoniae and MDR E. coli. Similarly, aqueous and ethanolic extract can be the drug of choice for C. albicans infection. Further study is necessary to evaluate the accurate compound responsible for antibacterial and antifungal activity for pharmaceutical applications.

Keywords: Acacia auriculiformis, Antimicrobial susceptibility testing, Multidrug-resistant bacteria, Klebsiella pneumoniae.
determined against following strains. The American Type Culture Collection (ATCC) strains of bacteria used were *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 700603, *Escherichia 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*.

Sabouraud Dextrose Agar (SDA), Mueller-Hinton agar (MHA), and blood agar (for *S. pneumoniae*) procured from HiMedia, Mumbai, were used. The above-mentioned bacterial and fungal strains were revived by plating on nutrient agar, blood agar, and SDA, respectively. After overnight incubation at 37°C, isolated colonies were selected. Identification of the organisms was done by standard procedure. Isolated bacterial colonies were then transferred to sterile Mueller-Hinton broth, and *C. albicans* was transferred to Sabouraud Dextrose Broth and incubated overnight at 0.5 McFarland’s turbidity standard was used to adjust the concentration of the growth of microorganisms to 10⁵ CFU/mL. Ampicillin disc (10 μg), oxacillin disc (1 μg), gentamicin disc (10 μg) and ketoconazole disc (10 μg) obtained from HiMedia, Mumbai, were used as controls [13].

**Determination of antibacterial activity**

Blood agar and MHA measuring 20 ml each were 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 solution of test compound. The inoculated plates were incubated in an incubator for 18 h at 37°C. Tests were done in triplicates, and the average of the three was considered for the study.

**Determination of antifungal activity**

Nearly 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 of test compound. The plates were then kept in the incubator 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 the zone of inhibition was measured for the antimicrobial activity. Ethanol extract exhibited antibacterial activity against all the strains, and MDR *K. pneumoniae* and MDR *E. coli* strain had zone of inhibition measuring 13 mm and 14 mm, respectively, whereas they were resistant to ampicillin. *Staphylococcus aureus* ATCC 25923 showed a zone of inhibition of 18 mm and no zone of inhibition with aqueous extract. Antifungal activity was exhibited by aqueous and ethanol extracts with zone of inhibition measuring 9 mm and 17 mm diameter. Both the aqueous and ethanol extracts of the leaves were found to have antifungal activity (p<0.05) [Table 1].

**DISCUSSION**

In the present scenario, antimicrobial resistance is very common. Bacteria and fungi continue to develop drug resistance by employing various mechanisms to survive in the lethal environment created by antimicrobials [14]. It is now the requirement of developing the alternative drug line to treat infectious diseases caused by MDR organisms such as *Staphylococcus aureus, K. pneumoniae*, and *E. coli*.

Attention has been given more toward the extracts and biologically active compounds isolated from plants. Plant-derived drugs have been reported to be safe and without side effects; hence, much attention has been given to these natural products as new therapeutic agents [15]. Medicinal plants play a key role in the basic health needs in developing countries. These plants offer new source of antibacterial, antifungal, and antiviral agents with significant activity against infective microorganisms [16-18].

Saha Riaz et al. showed that the pods of *Acacia* are commonly used in the traditional therapy of various diseases [19]. Our study supports the valuable use of *A. auriculiformis* against organisms.

**Table 1: Zone of inhibition of ethanol and aqueous leaf extract of *Acacia auriculiformis* against various microorganisms**

| Name of the organism | Zone of inhibition (mm) |
|----------------------|------------------------|
|                      | Ethanol extract | Aqueous extract |
| *Staphylococcus aureus* ATCC 25923 | 18 | - |
| *Enterococcus faecalis* ATCC 29212 | 13 | - |
| *Klebsiella pneumoniae* ATCC 700603 | 7 | - |
| *Escherichia coli* ATCC 25922 | 10 | - |
| *Pseudomonas aeruginosa* ATCC 27853 | 15 | - |
| *Streptococcus pneumoniae* | 10 | - |
| MDR *Escherichia coli* | 14 | - |
| MDR *Klebsiella pneumoniae* | 13 | - |
| *Candida albicans* | 17 | 9 |

MDR: Multidrug resistant, ATCC: American Type Culture Collection, (-): Indicates no zone of inhibition

The present study observation was that the alcoholic extracts showed significant antibacterial activity against clinically isolated multidrug-resistant microorganisms when compared to aqueous extracts and this was supported by other investigators [20,21]. The effectiveness of the extracts mainly depends on the type of solvent used and the ability of the solvent to extract more active ingredients from the plant materials [22].

Antifungal activity against *C. albicans* was exhibited by both aqueous and ethanol leaf extracts of *A. auriculiformis* in this study. This might be due to the fact that the antifungal bioactive components such as alkaloids, lectins, terpenes, and saponins can be easily extracted by water. Ethanol extract of the leaves showed more inhibitory activity than that of the control drug used, and this was supported by other investigators [23,24].

**CONCLUSION**

The result of the present study suggests for further investigation, as ethanol leaf extract of *A. auriculiformis* showed antibacterial effects, especially on MDR *K. pneumoniae* and MDR *E. coli* which can be used against these strains as they are a threat to the community. Aqueous or ethanol extract can be the choice for infections caused by *C. albicans*. Further, purification of the bioactive principle will result in more significant activity. Our results also support the idea that herbal medicines with medicinal value have a promising future in regard to the discovery of new substances.

**AUTHORS' CONTRIBUTIONS**

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

**CONFLICTS OF INTEREST**

Authors have no conflicts of interest to declare.

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