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UNPRECEDENTED ANTIMICROBIAL PROPERTIES OF *PROSOPIS CINERARIA* LEAVES

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Abstract— The antibacterial effect of *Prosopis cineraria* leaves was evaluated on multidrug resistant (MDR) strains of *Bacillus subtilis* (ATCC 6633), *E. coli* (ATCC 8739), *Salmonella enterica* (ATCC 14028), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 27853). Powdered leaves of the tree were treated with methanol for the extraction. Crude methanol extracts of the leaves of *P. cineraria* was investigated for their antibacterial activity against a wide range of bacteria (both gram-positive and gram-negative) by agar well diffusion method. Ciprofloxacin was used as standard.

The methanolic leaves extracts of *P. cineraria* showed a remarkable inhibition of the microorganisms. The potency shown by these extracts recommends their use against multidrug resistant microorganisms. The present study suggests that the methanol extract of the leaves of *P. cineraria* exhibited a potential antibacterial activity against the tested microorganisms and could be a potential source of new antimicrobial agents.

Keywords— *Prosopis cineraria*, antimicrobial, antibiotic, multidrug resistant, microorganisms

I. INTRODUCTION

Infectious diseases are responsible for millions of global deaths annually (Walsh, 2003) and amongst them, bacterial infections are a major threat (Westh et al, 2004). Traditionally, indigenous people at this part of the world uses what the nature produces to heal them and then treat the diseases, they exposed to. Folk medicines did not address treatment as we mentioned in our research (AlGhais et al, 2020c). The only solution to this problem is use of antibiotics or chemicals. However, the increasing failure of chemotherapy and antibiotic resistance exhibited by bacterial pathogens has prompted researchers for screening of plants for their antimicrobial activity (Scazzocchio et al, 2001). Thus, there is an urgent need to discover new antimicrobials for new and re-emerging bacterial diseases. In general, bacterial infections are one of the main problems in the world and should be treated by antimicrobial agents. The increased prevalence of known resistant organisms and the emergence of newly resistant organisms has resulted in delayed effective therapy, increase length of hospitalization and have led to increased cost for patients (Andrew et al 2011). Many efforts have been

made to discover new antimicrobial compounds from various kinds of sources such as plants, animals and microorganisms (Khan et al 2009; Gibbons 2005; Gottlieb 2002). On the other hand, the world is rich with natural products including medicinal plants. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Medicinal plants have recently gained much attention from research groups worldwide. The need for new, safer, and effective therapeutic agents represent the main targets for clinical investigators (Kujawska et al 2015). Plants produce certain chemicals which are naturally toxic to bacteria (Singh et al 2003) and many plants have been investigated for the development of novel drugs with therapeutic properties (Tomoko et al 2002). As opposed to synthetic drugs, antimicrobials of plant origin are not associated with many adverse effects and have an enormous therapeutic potential to heal many infectious diseases.

In our previous study we investigated that ghaf is a potential desert nutraceutical and compared the nutrients and protein of ghaf with spinach, lettuce and different species of fish (AlGhais et al 2020 a, b). Also, we investigated that ghaf bark has potential of antimicrobial properties and can be use in pharmaceuticals (AlGhais et al 2020c). Therefore, to continue our further research to detect the potency of ghaf leaves as source of new antimicrobial agent and also to meet the increasing demand of antimicrobial agent, alternative strategies, this study have been considered recently. Therefore, Hence, the objectives of the study were to study the antimicrobial activity of methanolic extract of leaves of ghaf. This research was carried out as an awareness of medicinal value of ghaf tree in pharmaceutical.

II. MATERIAL AND METHODS

2.1 Plant material collection

The leaves of ghaf (Three samples) were collected from Khuzam road, Ras Al Khaimah, UAE in the month of March 2021. The leaves were sun dried for 5-7 days or more and then oven dried for better grinding. The dried leaves were then ground to a coarse powder using high capacity of grinding machine and then stored in airtight bottles.



2.2 Preparation of the extracts

About 5 g of the coarse powder was extracted with 25.0 ml of methanol followed by continuous hot extraction method. Stirred well and kept for incubation in closed container. Centrifuged the tubes at 4000 rpm for 30 min. Transferred the supernatant extract for drying for 10 min and finally got residue of leaves sample. Weighed accurately 0.1 gm of residue in test tube and added 1.0 mL of methanol [10 % (w/v) solution]. The final concentration of extracts used for further experiment. All the extracts were then stored in refrigerator till use (AlGhais et al 2020b).

2.3 Chemicals

The chemicals used in the present investigation were of analytical grade and of high purity from Merck. Standard kits and reagents used for analysis were purchased from Germany and USA.

2.4 Test organisms

In the present study, the bacterial strains used were *Bacillus subtilis* (ATCC 6633), *E. coli* (ATCC 8739), *Salmonella enterica* (ATCC 14028), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 27853) obtained from the American Type Culture Collection (ATCC) to determine the antibacterial activity of *P.cineraria*. The bacterial strains were procured from LTA srl Italia. Pure culture of bacteria was maintained at 4 °C on nutrient agar slants.

2.5 Methodology for detection of antibacterial activity

2.5.1 Inoculums preparation

The bacterial isolates were first grown in 5 ml of nutrient broth in to sterile test tubes for 18 h before use.

2.5.2 Agar well diffusion assay

The antibacterial activity of methanolic extracts of *P. cineraria* leaves was tested against isolates by agar-well diffusion method. An aliquot of 100 µl inoculum for each bacterial isolate was evenly spread by a sterile glass spreader onto Muller Hinton Agar using sterilized cotton swab and was allowed at room temperature. A Cork borer of 6 mm diameter was used to punch well in agar plates to cut uniform wells. Wells were bored in agar plates. The concentration of the extract was 10% (w/v), prepared using methanol as solvent. Subsequently, 30 µl extracts of bark were poured into the wells. Ciprofloxacin 30 µg was used as positive control. DMSO was used as a negative control. Then the plates were kept at 2-8 °C in a refrigerator to allow diffusion of the extracts in to the agar and further incubated at 37 °C for 24 h. The diameter of zone of inhibition was measured to the nearest millimeter (Sohel 2010; Uddin et al 2007). The

formation of clear inhibition zone of ≥ 7 mm diameters around the wells was regarded as significant susceptibility of the organisms to the extract (Okwori 2007). The effect was compared to those of antibiotic discs. The tests were performed in triplicates and the mean was taken. The whole experiments were performed under strict aseptic conditions.

2.6 Statistical analysis

The tests were performed in triplicates. Data are expressed as mean. Pair wise comparisons were performed. Experimental error was determined for triplicate and expressed as standard deviation (SD).

III. RESULTS AND DISCUSSION

According to the present research findings, the methanolic and aqueous extracts of the leaves of *Prosopis cineraria* exhibited antibacterial activity with all the tested strains of microorganisms on comparison with the standard 30 mcg ciprofloxacin. Antibacterial activity of leaves extracts using agar well diffusion. The extract showed antibacterial activity as indicated by the zone of growth inhibition ranged from $10 \pm .000 - 25 \pm .000$ mm (Figure 1). Similar work was reported by Velmurugan et al 2010. According to present research finding *B. subtilis* showed significant difference with the positive control ciprofloxacin and showed zone of inhibition 10mm and *S. enterica* strain in a concentration dependent fashion which showed significant difference with the positive control ciprofloxacin and had the large zone of inhibition (14.00 ± 0.05 mm) respectively. *P. aeruginosa* showed significant difference with the positive control ciprofloxacin and showed 12mm of zone of inhibition and *E. coli* showed 15mm while *S. aureus* had the largest zone of inhibition ($25 \pm .000$ mm) (Table 1). Similar results were reported by Begashaw et al 2017 and Kapoor et al 2013.

S. No	Microorganisms	Zone of Inhibition (mm)
1	<i>Bacillus subtilis</i> (ATCC 6633)	10
2	<i>E.coli</i> (ATCC 8739)	15
3	<i>Salmonella enterica</i> (ATCC 14028)	14
4	<i>Staphylococcus aureus</i> (ATCC 6538)	25
5	<i>Pseudomonas aeruginosa</i> (ATCC 27853)	12

Table 1: Diameters of the inhibition zone to extracts

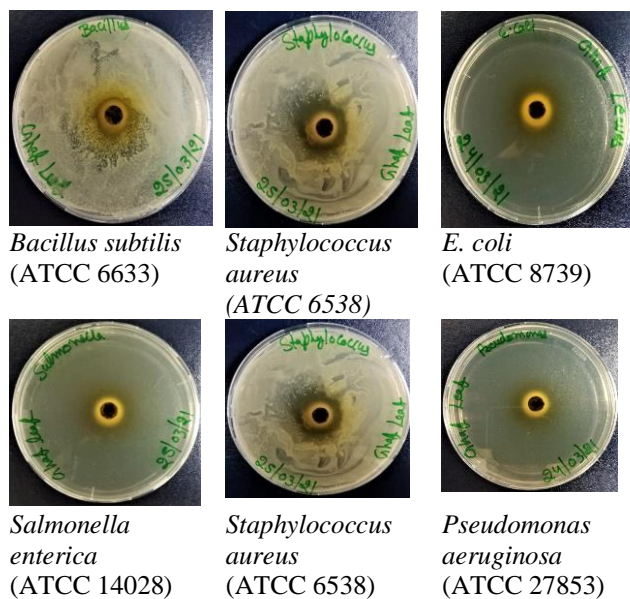


Figure 1: Extracts of leaves of *P. cineraria* showed antibacterial activity as indicated by the zone of inhibition against different microorganism's strain

Maximum antibacterial activity was exhibited by the extracts of leaves of *Prosopis cineraria* against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enterica* whereas moderate antibacterial activity was observed in *Bacillus subtilis* and *Pseudomonas aeruginosa*.

IV. CONCLUSION

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu et al., 1999). Since ancient times, plants have been a veritable source of drugs.

However, modern societies tend to ignore the importance of herbal medicine. Recently, much attention has been directed towards extracts and biologically active compounds of plants. In conclusion, *P. cineraria* extracts possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds.

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