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Antimicrobial Properties of *Prosopis cineraria* stem bark

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ABSTRACT

Infectious diseases are the most common causes of morbidity and mortality in developing countries. Nowadays, medicinal plants play a major role in treatment of infectious diseases and they are easily available and more affordable as compared to synthetic compounds. The emerging trends of multidrug resistance among several groups of microorganisms against different classes of antibiotics led different researchers to develop efficient drugs from plant sources to counter multidrug resistant strains. This study investigated two different concentration of methanol extracts of stem bark of *Prosopis cineraria* to determine their efficacy against multidrug resistant microbes.

Powdered barks of the tree were treated with methanol using hot extraction method. Crude methanol extracts of the bark of P. cineraria was investigated for their antibacterial activity against a wide range of bacteria (both gram-positive and gram-negative) by disc diffusion method. Ciprofloxacin was used as standard.

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) were used in the study. The methanolic bark 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 stem bark 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*, antibacterial, ciprofloxacin, bark, microorganisms

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1. Introduction

Infectious diseases are the most common causes of morbidity and mortality in developing countries (Food, medicine, 2009). Deaths from infectious diseases occur disproportionately in the developing world, where they are the biggest killer of children and young adults (Andrew et al 2011). 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. Globally, use of antibiotics has contributed to the dramatic fall in morbidity and mortality from communicable and infectious diseases over the last 50 years. However, the control of infectious diseases is seriously threatened by the steady increase in the number of microorganisms that are resistant to antimicrobial agents (Food, medicine, 2009). 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. Medicinal plants are now getting more attention than ever because they have potential of numerous benefits as a source of drugs to all mankind. 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.

Drug resistance to pathogenic microorganisms has been commonly reported worldwide. Antibiotic resistance refers to the ability of a microorganism to withstand the effects of an antibiotic. The increasing frequency of microorganisms that are resistant to common and generally accepted antibiotics is on the increase. Furthermore, the rate of resistance to these drugs is higher in developing countries as compared to developed countries because of extensive and indiscriminate use of antibiotics over the last few decades (Akram et al 2007) and people's ability to self-medicate without a prescription from a physician.

Medicinal plants are natural resources for valuable products that can be used in the treatment of various ailments. Plant materials remain an important resource for combating illnesses, including infectious diseases, and many plants have been investigated for novel drugs for the development of new therapeutic agents. These natural compounds are the foundations of modern drugs as we know today (Krishnaiah et al 2009).

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). Therefore, to continue our further research to detect the potency of ghaf 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 bark of ghaf. This research was carried out as an awareness of medicinal value of ghaf tree in pharmaceutical.

2. Material and methods

2.1 Plant material collection

The stem bark of ghaf (Three samples) were collected from Dahan garden, Ras Al Khaimah, UAE in the month of March 2020. The barks were sun dried for 5-7 days or more and then oven dried for better grinding. The dried barks 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 bark sample. Weighed accurately 0.1 gm of residue in two different test tubes and added 0.5 mL of methanol in one test tube [20 % (w/v) solution (E1)] and 1.0 mL of methanol [10 % (w/v) solution (E2)] in other test tube. These were two different final concentration of extracts for experiment. All the extracts were then stored in refrigerator till use.

2.3 Chemicals

The chemicals used in the present investigation were of analytical grade and of high purity from Merck. Standard 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* stem bark 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. Two wells were bored in agar plates for two different concentrations. The concentrations of the extract were 20% (w/v) and 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).

3. Results and Discussion

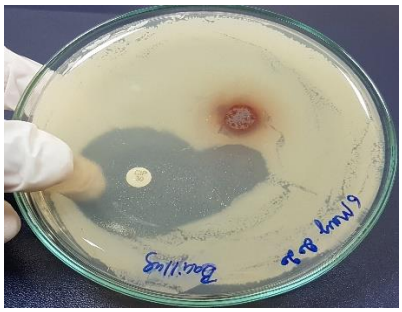
According to the present research findings, the methanolic and aqueous extracts of the stem bark of *Prosopis cineraria* exhibited antibacterial activity with all the tested strains of microorganisms on comparison with the standard 30 mcg ciprofloxacin. Antibacterial activity of stem bark extracts using agar well diffusion. The extract of both concentrations showed antibacterial activity as indicated by the zone of growth inhibition ranged from 2 ± .000 – 36 ± .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 in both the concentrations and showed zone of inhibition 11mm and 2mm at 20% and 10% (w/v) 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 (31.00 ± 0.05 mm) at concentration of 20% (w/v) and (8.00 ± 0.05 mm) at concentration of 10% (w/v) respectively followed by *P. aeruginosa* showed significant difference with the positive control ciprofloxacin and showed 36mm of zone of inhibition at 20% (w/v) and 9mm at 10% (w/v) and *E.coli* showed 35mm and 20mm at a concentration of 20% (w/v) and 10% (w/v) respectively while *S. aureus* had the zone of inhibition (16 ± .000 mm) at a concentration of 20% (w/v) and 11mm at 10% (w/v). (Table 1). Similar results were reported by Begashaw et al 2017 and Kapoor et al 2013.

Table 1: Ratio of diameters of the inhibition zone to extracts under observation (I) and diameter of inhibition zone due to standard reference antibiotic Ciprofloxacin (C)

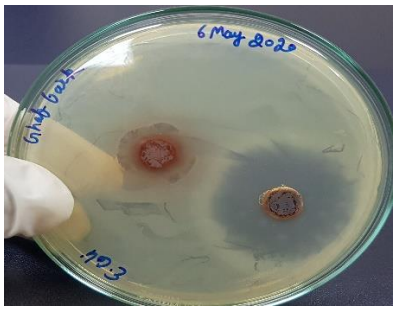
S. No	Microorganisms	I/C[a]	
		E1	E2
1.	*Bacillus subtilis* (ATCC 6633)	0.26	0.05
2.	*E.coli* (ATCC 8739)	1.09	0.63
3.	*Salmonella enterica* (ATCC 14028)	0.97	0.25
4.	*Staphylococcus aureus* (ATCC 6538)	0.52	0.35
5.	*Pseudomonas aeruginosa* (ATCC 27853)	0.95	0.24

a = Ratio of diameters of the inhibition zone to extracts under observation (I) and diameter of inhibition zone due to standard reference antibiotic Ciprofloxacin (C) Extraction of Bark 20 %(w/v) solution (E1); Extraction of Bark 10 %(w/v) solution (E2)



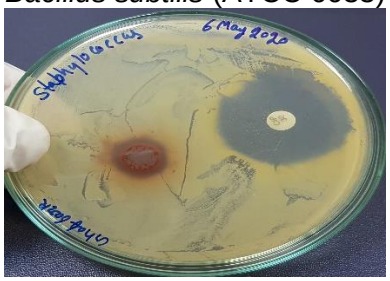
Bacillus subtilis (ATCC 6633)

Bacillus subtilis (ATCC 6633)



E.coli (ATCC 8739)

Salmonella enterica (ATCC 14028)



Staphylococcus aureus (ATCC 6538)

Pseudomonas aeruginosa (ATCC 27853)

Figure 1: Extracts (E1 and E2) of stem bark 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 stem bark of *Prosopis cineraria* against *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella enterica* whereas moderate antibacterial activity was observed in *Bacillus subtilis* and *Staphylococcus aureus.*

4. Conclusion

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. The present study on stem bark of *P. cineraria* indicates that medicinal tree species growing in arid region have definitely some antimicrobial principles as secondary products, which are responsible for antibacterial activity. Thus, the activity of all these extracts against bacterial pathogens, indicate that these arid plants are more resistant to bacterial attacks due to the presence of some biologically active substances, so these can be used in pharmaceutical and drug industries.

Multiple drug resistance has developed due to indiscriminate use of commercial antimicrobial drugs that are commonly used in the treatment of infectious diseases, making it a global growing problem. There is an urgent need to develop new antimicrobial drugs for the treatment of infectious diseases from medicinal plants, which may be less toxic to humans and possibly with a novel mechanism of action.

5. Abbreviations

P. cineraria, *Prosopis cineraria*; **SD**, standard deviation; **MDR**, Multidrug resistant; **ATCC**, American Type Culture Collection; **E1**, Extract 1; **E2**, Extract 2; **h**, hours; **C**, ciprofloxacin

6. Ethics approval and consent to participate

Not applicable.

7. Consent for publication

Not applicable.

8. Availability of data and materials

The relevant data and materials are available in the present study.

9. Competing interests

The authors declare that they have no competing interests. All procedures followed were in accordance with the ethical standards (institutional and national).

10. Funding

Not applicable.

11. Acknowledgements

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12. Authors' contributions

SAG supervised the entire project. VB performed all the experiments. The supervision of the laboratory work was performed by VB. PK assisted in experiments. VB analysed the data and wrote the manuscript.

13. References

1. Akram M, Shahid M, Khan AU (2007) Etiology and Antibiotics Resistance Pattern of Community Acquired Urinary Infections in J N M C Hospital Aligarh India. Ann Clin Microbiol Antimicrob 6: 4.
2. Al Ghais S, Bhardwaj V and Kumbhar P (2020a). Prosopis cineraria (Ghaf): An Unconventional Desert protein rich supplement. American Journal of Agricultural Research, 5:94. https://escipub.com/ajar-2020-04-1805/
3. Al Ghais S, Bhardwaj V and Kumbhar P (2020b). Prosopis cineraria (Ghaf): A potential desert nutraceutical. International Journal of Development Research, Vol. 10, Issue, 03, pp. 34162-34165.
4. Andrew LH, et al. Rapid analysis of Pharmacology for infectious diseases. Curr Top Med Chem. 2011;11:1292–300.
5. Begashaw B, Mishra B, Tsegaw A and Shewamene Z (2017). Methanol leaves extract Hibiscus micranthus Linn exhibited antibacterial and wound healing activities. BMC Complementary and Alternative Medicine 17:337.
6. Food, Medicine and Health Care Administration and Control Authority (FMHACA). Antimicrobial use, resistance and containment baseline survey, syntheses of findings, Addis Ababa, Ethiopia, 2009.
7. Gibbons S (2005) Plants as a source of bacterial resistance modulators and anti-infective agents. Phytochem Rev 4: 63-78.
8. Gottlieb OR, Borin MR, Brito NR (2002) Integration of ethnobotany and phytochemistry: dream or reality? Phytochemistry 60: 145-52.
9. Kapoor B. B. S., Bansal R (2013). Antimicrobial Screening of Some Medicinal Tree Species of Nagaur District of Rajasthan. International Journal of Herbal Medicine; 1 (4): 10-11.

10. Krishnaiah D, Devi T, Bono A, Sarbatly R. (2009). Studies on phytochemical constituents of six Malaysian medicinal plants. Journal of Medicinal Plants Research.;3(2):67–72.
11. Khan R, Islam B, Akram M, Shakil S, Ahmed A, Ali SM, Siddidui M, Khan AU (2009) Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin. Molecules 14: 586-97.
12. Okwori AE (2007). Antibacterial activities of Ageratum conyzoides extracts on selected bacterial pathogens. Internet J Microbiol.;4(1):34–56.
13. Singh B and Bhat TK (2003) Potential therapeutic applications of some antinutritional plant secondary metabolites. J Agric Food Chem 51: 5579–97.
14. Sohel A, (2010). Antibacterial activity of the ethanol extracts of Hibiscus rosasinensis leaves and flowers against clinical isolates of bacteria. Bangladesh J Life Sci.;22(2):65–73.
15. Tomoko N, Takashi A, Hiromu T, Yuka I, Hiroko M, Munekazu I, Tsutomu N, Kazuhito W (2002) Antibacterial activity of extracts prepared from tropical and subtropical plants on methicillin-resistant Staphylococcus aureus. J Health Sci 48: 273–76.
16. Uddin, B., Nahar, T., Khalil, M.I. and Hossain, S. In vitro antibacterial activity of the ethanol extracts of Paederia foetida L. (Rubiaceae) leaves. Bangladesh J. life Sci. 2007;19(2): 141–143.
17. Velmurugan V, Arunachalam G and Ravichandran V (2010). Antibacterial activity of stem bark of Prosopis cineraria (Linn.) druce. Archives of Applied Science Research, 2 (4): 147-150