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Original Research Article

Efficacy of plant-derived extracts against multidrug resistant pathogens: A potential alternative to conventional antibiotic therapy

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Abstract

Background: Antimicrobial resistance (AMR) poses a global threat and reported as a leading cause for morbidity and mortality. It is mediated by several factors like irrational use of antibiotics for treatment in various diseases, the misuse of antibiotics, especially in livestocks, improper hygiene and infection control practices and environmental contaminants. As a result, the efficacy of antibiotics has been inhibited and there is an urgent need for newer antibiotics. Therefore our study is aimed to explore the anti-bacterial activity of herbal extracts which would act as an alternate herbal therapy to fight against the multidrug-resistant (MDR) pathogens.

Materials and Methods: The multidrug resistant (MDR) bacterial isolates were obtained from various clinical specimens from wound swab, urine, pus aspirates, blood, endotracheal aspirates, and sputum samples according to standard microbiological protocols. In our study 15 herbs were selected for methanolic extraction by standard Soxhlet extraction method. The extracts were then tested against the multi-drug resistant pathogens by well diffusion method and interpreted.

Results: The resistant bacterial strains such as *Methicillin-Resistant Staphylococcus aureus (MRSA), Escherichia coli, Proteus species, Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter species* were used as the test strains in the study. Remarkably 7 Out of the 15 herbal extracts tested for anti-bacterial activity showed susceptibility to the resistant strains. Among the 7 effective herbal extracts *Juglans regia* had effectiveness even in ethanol.

About 60% of the *MRSA* strains were susceptible to several herbal extracts which includes *Phyllanthus edulis, Phyllanthus debilis, Juglans regia, Justicia adathoda, Ormocarpum cochinchinense, Mentha* and *Andrographis alata*. *Proteus species* showed susceptibility to *Phyllanthus edulis, Phyllanthus debilis, Justicia adathoda*. *Acinetobacter species* showed susceptibility to *Phyllanthus edulis, Mentha* and *Justicia adathoda*. *Pseudomonas aeruginosa* strains were susceptible to *Phyllanthus edulis* and *Justicia adathoda*.

Conclusion: The preliminary findings of this study is that certain herbal extracts exhibit antibacterial activity against multidrug-resistant pathogens. And so that such extracts may have potential as natural therapeutic agents. Further studies are required to isolate the active phytochemicals and their clinical applicability.

Keywords: Anti-bacterial agent, multi-drug resistance, herbal extracts, Phytochemicals.

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1. Introduction

As a global concern, antimicrobial resistance effects significantly the health care community and the economy with a high cost for treating infections caused by the multidrug resistant pathogens.[1,2] There are insufficient therapeutic options and so it has led to increased mortality meanwhile, behind the need for the development of urgent therapeutic agents AMR has been reported as the third leading to cause for global death.[3]

Considering the current AMR and the prevalence of resistant isolates globally, there is a need for an immediate

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need for novel antimicrobial agents. The researchers are now focussing in developing newer antimicrobials in natural sources like the plant extracts for reliable potency against multi-drug resistant (MDR) bacterial strains.[4] Earlier several plants have shown good antimicrobial property for various infections.[5] Approximately 10% of all the herbs are flowering and are used traditionally worldwide as a treatment of several infections. And only about 1% of these herbs have been scientifically investigated.[6] The traditional method of using the herbal plants as an antimicrobial agent has now brought the search for herbs that contain antimicrobial phytochemicals which remains as intense research in drug discovery.

The phytochemical compounds such as flavonoids, alkaloids, and tannins, has significant antimicrobial properties in scientific research and traditional use. Since it is a plant-based origin it is believed to have a lower toxicity and milder side effects compared to the other synthetic antibiotics. The herbal extracts contain multiple phytocompounds, which can both act as synergism and antagonism reactions.

The efficacy of the phytochemical can be enhanced by nanotechnology and in a natural way (green synthesis) by introducing nano materials that are been extracted from herbal plants itself, which also possess promising synergistic effect.

The plant-based nanoparticles can be customized precisely based on the biological activity in targeted delivery, as anti-cancer agents, leading to develop sustainable antimicrobial agents. Hence, this study is carried out to understand the antimicrobial potency of several herbal extracts that combat infections caused by the multidrug resistant pathogens.

2. Materials and Methods

2.1. Study design

This invitro experimental study was carried out in the Department of Microbiology in our college. The clinical bacterial isolates were collected over a period of 6 months. Institutional human Ethics Committee clearance was obtained before initiating the study.

2.2. Inclusion criteria

Clinical isolates obtained from clinical specimens such as wound swabs, pus, urine, endotracheal aspirates, and sputum were included in the study. Clinical bacterial isolates exhibiting multidrug resistance confirmed using standard microbiological procedures and interpreted according to CLSI guidelines were included in the study. Carbapenem-resistant Gram-negative isolates and *Methicillin resistant Staphylococcus aureus* (MRSA) strains representing Gram positive bacteria were included in this study.

2.3. Plants extraction preparation

A total of 15 plant extracts were included in the current study (**Table 1**). These plants were sourced from our college campus and Dr. Y.S. Parmar University Campus Nauni, Solan *(Juglans regia)*. After collection, the parts of the selected plants were washed with distilled water and air-dried. The dried samples were ground into fine powder and passed through a 100 mm mesh sieve. Ten grams of powdered material were soaked in 100ml of methanol for 7 days. The crude material was filtered by using Whatman filter paper No.1 to obtain the filtrate. The filtrate was concentrated under reduced pressure using rotatory vacuum evaporator.[7] The final extract yields were weighed and the crude extracts were suspended in dimethyl sulfoxide (DMSO) at a concentration of 1g/ml.

2.4. Antibacterial activity of the selected plant extracts

Multiple strains of each Carbapenem resistant organisms included in the study were *Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii, Methicillin Resistant Staphylococcus aureus (MRSA), Escherichia coli* and *Proteus mirabilis.*

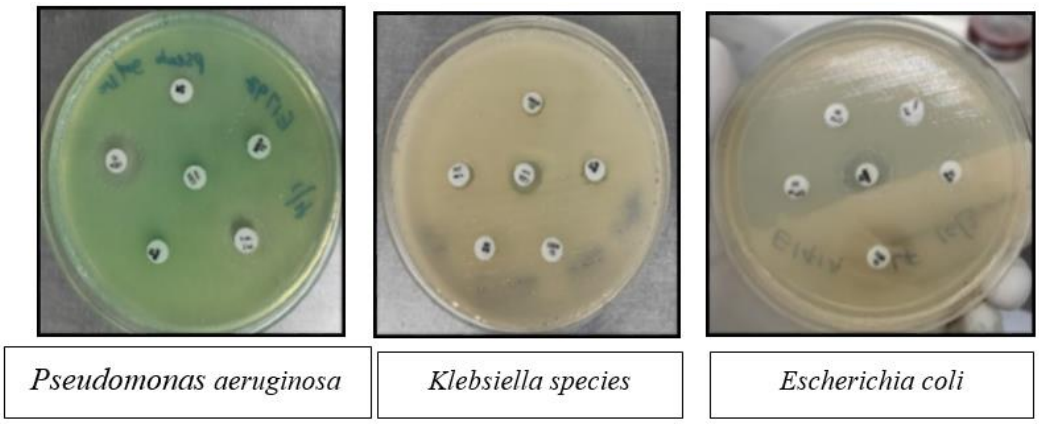

Figure 1: Multi drug resistant bacterial isolates included in the study

Table 1: Ethnobotanical profiles of medicinal plants assessed for antibacterial properties

S. No.	Local name	English name	Botanical name	Parts
1.	Indian sorrel	Creeping wood sorrel	*Oxalis corniculata*	leaf
2.	Kanaa pillai	Benghal dayflower	*Commelina benghalensis*	leaf
3.	Kalmegh	King of bitters	*Andrographis paniculata*	leaf
4.	Nela nelli	Bhuamla	*Phyllanthus edulis*	leaf
5.	Nela Nelli	Nela nelli	*Phyllanthus debilis*	leaf
6.	Gokharu	Puncturevine	*Goksura*	leaf
7.	Moringa	moringa	*Murraya*	leaf
8.	Pudhina	Pudina	*Mentha leaf*	leaf
9.	Tilo	Fresh-cut justicia	*Justicia pectoralis*	leaf
10.	Adathoda	Malabar nut	*Justicia adathoda*	leaf
11.	Akhrot	Walnut	*Juglans regia*	Root
12.	Aniyarathi	Aniyarathi	*Ormocarpum cochinchinense*	leaf
13.	Apamarga	Prickly chaff flower	*Achyranthes aspera*	leaf
14.	Vembu	Neem	*Azadirachta indica*	leaf
15.	Kalpanath	Eagle Wood	*Andrographis alata*	leaf

2.5. Bacterial inoculum preparation

The confirmed multidrug resistant (MDR) bacterial isolates were preserved in glycerol broth and stored at -20°C. For antimicrobial testing of the plant extracts, the MDR isolates were revived on nutrient agar and checked for their sterility and purity. They were then inoculated into brain heart infusion broth and incubated until they reached a turbidity to 0.5 McFarland standard, corresponding to approximately 1.5x 10^8 CFU per ml.

2.6. Anti-bacterial activity (Agar well diffusion assay)

The test strains were evenly inoculated onto Muller Hinton agar (MHA) plates using a sterile swab to ensure uniform distribution of the bacterial suspension. Upto 6 Wells (6mm diameter) were aseptically created on MHA agar plate using sterile cork borer and each well was filled with 50 µl of crude plant extract using a sterile micropipette. Standard antibiotic discs such as Meropenem (10mcg) and Linezolid (30mcg) was procured from Himedia was used as positive control and dimethyl sulfoxide (DMSO) as negative control. The plates were incubated at 37°C for 48-hours. The zone of inhibition around the wells was interpreted as sensitivity of the bacterial strain to the plant extract, whereas the absence of a zone indicates resistance.

3. Results

The antibacterial activity and potency of the extracts were evaluated based on the presence or absence of inhibition zones, as detailed in **Table 2**.

Our study findings indicated that out of the 15 plants only seven plants exhibited significant antibacterial activity which was susceptible against Methicillin-*Resistant Staphylococcus aureus* (MRSA) as clearly evidenced by their respective zones of inhibition: *Phyllanthus edulis* (25 mm), *Juglans regia* (24 mm), *Mentha* spp. leaf (21 mm), *Justicia adathoda* (21 mm), *Andrographis alata* (21 mm), *Phyllanthus debilis* (16 mm), and *Ormocarpum cochinchinense* (14 mm). Similarly, only three plant extracts demonstrated notable antibacterial activity against *Proteus species* with *Phyllanthus edulis*, Phyllanthus *debilis*, *Justicia adathoda* showed inhibitory zones of 22 mm, 24mm, and 19 mm respectively. Against *Acinetobacter species*, *Phyllanthus edulis*, *Mentha leaf* and *Justicia adathoda* showed an inhibitory zone of 18 mm, 12 mm, and 17 mm respectively.

Against *Pseudomonas aeruginosa*, *Phyllanthus edulis* and *Justicia adathoda* showed inhibitory zones of 14mm and 11 mm respectively. The plant extracts evaluated for the antibacterial activity represent as promising alternate sources of antimicrobial compounds and they exhibit varying degrees of activity against multidrug resistant (MDR) bacterial strains. Among the tested plant extracts, *Phyllanthus* spp., *Justicia adathoda*, *Mentha*, and *Juglans regia* demonstrated significant broad -spectrum of antibacterial activity.

Although various studies have reported the antimicrobial potential of medicinal plants based on the traditional knowledge and usage, investigations specifically targeting their efficacy against MDR clinical isolates remain limited.

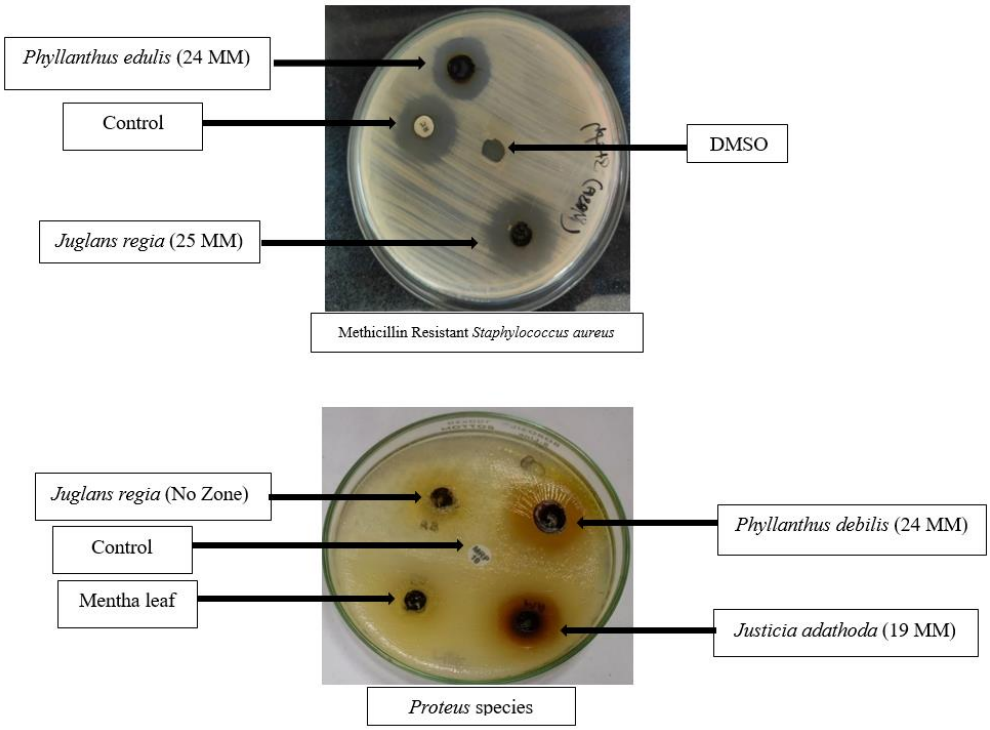

Figure 2: Zone of inhibition against MDR *Proteus species* and *MRSA* strains

Table 2: Efficacy of herbal extracts against MDR isolates

Herbal extracts	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Acinetobacter baumannii***	***MRSA***	***Proteus species***
Phyllanthus edulis		14mm	18 mm	25 mm	22 mm
Phyllanthus debilis	-	-	-	16 mm	24mm
Mentha leaf	-	-	12 mm	21 mm	-
Justicia adathoda	-	11 mm	17 mm	21 mm	19 mm
Juglans regia	13/12 mm	-	-	24mm	-
Ormocarpum cochinchinense	-	-	-	14mm	-
Andrographis alata	-	-	-	21 mm	-

4. Discussion

The plant extracts evaluated in this study demonstrated significant anti-bacterial activity against both gram positive and gram-negative bacteria. The test bacterial strains were isolated from clinical samples, including wound swabs, pus aspirates, sputum, endotracheal aspirates and urine. Among the plant extracts tested, *Justicia adathoda* exhibited notable efficacy against MRSA with an inhibitory zone of 21mm. These findings are consistent in studies which reported that essential oils derived from *Justicia adathoda* possess significant activity against MRSA strains.[9]

Several studies have investigated the antibacterial efficacy of various herbal extracts in different combinations and have reported an enhanced activity, unlike other studies, which often emphasize the enhanced efficacy of combined or synergistic plant extracts, our findings revealed that the individual plant extract itself displayed significant antibacterial activity. This highlights the distinct significance of our current study findings particularly against multidrug resistant (MDR) pathogens.

Furthermore, in the current study, *Juglans regia* exhibited significant antibacterial activity, with a greater inhibitory zone of 24 mm against *Methicillin-Resistant Staphylococcus aureus (MRSA).* Also, the root extract of *Juglans regia* showed the highest zone of inhibition among the tested plant extracts.[8] To our knowledge, this is the only study reporting the use of *Juglans regia* root extract against the multidrug resistant (MDR) strains.[10]

In this study *Phyllanthus edulis* showed a wider zone of inhibition of about 25mm for MRSA and had a significant antimicrobial effect against other MDR strains, like *Proteus species* (22 mm), *Acinetobacter species* (18mm) and *Pseudomonas aeruginosa* (14mm). As mentioned by Bernaitis *et al.*, *Phyllanthus debilis* shows broad spectrum of activity against Gram positive MDR strains. The overall

study clearly indicates significant antibacterial potency of various herbal extracts against the clinical MDR isolates.

Similarly, *Pseudomonas aeruginosa*, a bacterial pathogen well known in the hospital environment causing nosocomial infection and intrinsic resistance to certain antibiotics was inhibited by two plant extracts which includes *Phyllanthus edulis* (14 mm) and *Justicia adathoda* (11 mm).

Our study shows that the selected herbal extracts possess significant antibacterial activity which can be an alternative antimicrobial agent in reducing the antibiotic resistance[11]. Although the traditional method of using the herbs as therapeutic agent, there is a lack of scientific evidence in confirming their antibacterial activity especially against the MDR isolates remains limited.[12-14]

Therefore, this study will help to bridge the gap by providing basic evidence for selecting appropriate herbal extracts for further investigation and validation. Research should focus on determination of minimum inhibitory concentration (MIC), performance of time-kill kinetics and identifying the active phytochemicals are required. Also, in exploring the synergistic effects of these plant extracts in combination with the conventional antibiotics may contribute valuable new ways to enhance treatment efficiently against MDR strains.[15]

5. Conclusion

The current study has assessed the antibacterial activity of selected plant extracts derived from the roots and leaves of various indigenous plant species. Preliminary phytochemical screening of these crude extracts lay a strong foundation for future research. Also, synergistic combinations of validated herbal extracts with conventional antibiotics may help reduce side effects and curb the emergence of antibiotic resistance. Further research including, the phytochemical isolation, elucidation of mechanisms of action and in vivo evaluations would advance the use of bioactive compounds as an alternative or complementary antimicrobial agents.

6. Limitations

The current study was conducted with limited sample size and primarily focused on evaluating the antibacterial activity. Synergistic interactions of bioactive compounds and antibiotic based combinations remain unexplored and deeper understanding in this area would support the development of such extract -based combinations for disease management and could reduce the antibiotic use and related side effects. Additional studies on green-synthesized nanoparticles can help to combat antibiotic resistance.

Therefore, further research on the plant based bioactive compounds hold significant promising tools in addressing the global challenge of multidrug-resistant (MDR) infections, contributing to the development of more effective and sustainable antimicrobial therapies.

7. Source of Funding

None.

8. Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

9. Ethical Approval

This study was approved by Institutional ethical approval committee with IHEC reference No: IHEC-II/0386/23.

10. Author Contributions

This research work was carried out through the collective effort of all authors. All authors contributed equally to the study and manuscript preparation and have approved the final version for publication.

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