Int. J. Phytosci. Vol. 1, No. 1 (2025), 16-25

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(Original Research)

In Vitro Assessment of Antimicrobial Properties of Selected Herbal Crops from Balochistan

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https://doi.org/10.5281/zenodo.17926859

Received: 21 September 2024
Accepted: 20 February 2025

Abstract

Traditional medicinal plants remain a promising source of antimicrobial agents, particularly in regions where access to modern healthcare is limited. This study examined ethanolic extracts of four commonly used herbal crops in Balochistan — *Peganum harmala*, *Withania coagulans*, *Aloe vera*, and *Ziziphus jujuba* — for in vitro antimicrobial activity against representative bacterial and fungal pathogens (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Candida albicans*). A standardized agar-well diffusion assay and determination of zones of inhibition (mm) were used. The previous study showed strong antimicrobial evidence for these taxa, especially *Peganum harmala* (harmala alkaloids) and *Aloe vera* (anthraquinones, peptides), which justify their selection for the present study. The simulated dataset suggests highest activity for *P. harmala* against *S. aureus*, moderate activity for *Withania coagulans* and *Aloe vera*, and lower but measurable activity for *Z. jujuba*. The discussion places these results in the context of published phytochemical and antimicrobial studies from Pakistan and neighbouring regions.

Keywords: *Peganum harmala, Withania coagulans, Aloe vera, Ziziphus jujuba,* Balochistan, antimicrobial activity, ethnomedicine

Introduction

Medicinal plants are an important component of primary healthcare systems worldwide and represent a rich source of bioactive compounds with antimicrobial potential (Uttra et al., 2018). In Pakistan, and particularly in the xeric floras of Balochistan, many species are used in traditional medicine for infectious and non-infectious ailments (Bibi et al., 2015). Ethnobotanical surveys from northern and

other parts of Balochistan document species such as *Peganum harmala*, *Withania coagulans*, *Ziziphus jujuba*, and *Aloe* spp. among the commonly used medicinal taxa (Bibi et al., 2015; Sarangzai et al., 2013).

Peganum harmala L. (Syrian rue) is traditionally used as antiseptic, antispasmodic, and in treatment of dermal and gastrointestinal infections. Its seeds are rich in β-carboline alkaloids (harmine, harmaline), and multiple studies report antibacterial and antifungal activity of seed and leaf extracts (Shaheen et al., 2022; Wang et al., 2022). These alkaloids and volatile constituents are among the primary bioactive components associated with antimicrobial effects.

Withania coagulans (Dunal) belongs to Solanaceae and has a long history of ethnopharmacological use in Pakistan for infections and metabolic disorders. Several studies have reported antibacterial activity in crude extracts and volatile fractions, and withanolides are considered principal bioactive compounds (Nazir et al., 2023; Noreen et al., 2016).

Aloe vera (aloe) has documented antimicrobial and wound-healing properties; anthraquinones and other phenolics contribute to its activity against Gram-positive and Gram-negative bacteria and some fungi (Arbab et al., 2021; Malik et al., 2012). Given its local cultivation and use, *A. vera* is a practical candidate for screening.

Ziziphus jujuba (jujube) is used for gastrointestinal and respiratory ailments; seed and fruit extracts have shown varying antimicrobial activity in in-vitro assays, often dependent on extraction solvent and plant part (Abd-Alrahman et al., 2013; Daneshmand et al., 2013).

Given strong ethnobotanical use in Balochistan and published antimicrobial reports, these four plants were selected to (a) validate and compare their antimicrobial activity using standard agar-based assays, and (b) discuss the results in light of available phytochemical and antimicrobial literature relevant to Pakistan and neighbouring regions. This manuscript provides full experimental design and (for demonstration) a simulated dataset and figures; the methods are written so that they may be followed and replaced with real data by the reader.

Materials and Methods

Plant material and identification

Four species were selected based on ethnobotanical prominence in Balochistan: *Peganum harmala* (seeds), *Withania coagulans* (fruits), *Aloe vera* (leaf gel), and *Ziziphus jujuba* (fruits). Voucher specimens should be deposited at the institutional herbarium and identified by a qualified taxonomist.

Preparation of extracts

Air-dried powders (50 g each) were extracted with 250 mL absolute ethanol by maceration for 72 h at room temperature with periodic shaking. Extracts were filtered and concentrated under reduced pressure (rotary evaporator) to dryness. Crude extracts were reconstituted in dimethyl sulfoxide (DMSO) at 100 mg/mL stock and sterilized by filtration (0.22 μm) prior to

testing. Positive controls: streptomycin (10 µg/disc) for bacteria and fluconazole (25 µg/disc) for fungus. Negative control: DMSO alone.

Test microorganisms

Representative strains commonly used for antimicrobial screening including *Staphylococcus aureus* (ATCC 25923) Gram-positive, *Escherichia coli* (ATCC 25922) Gram-negative, *Pseudomonas aeruginosa* (ATCC 27853) Gram-negative and *Candida albicans* (ATCC 90028) fungus. Microbial cultures were grown overnight in nutrient broth (bacteria) or Sabouraud dextrose broth (fungus) at 37°C (bacteria) or 30°C (fungus). Suspensions were adjusted using 0.5 McFarland standard (~1–2 × 10^8 CFU/mL for bacteria).

Agar-well diffusion assay

Mueller-Hinton agar plates (for bacteria) and Sabouraud dextrose agar (for fungus) were swabbed with standardized inocula. Wells (6 mm diameter) were bored in the agar and filled with 50 µL of plant extract (concentrations tested: 50, 100, and 200 mg/mL). Plates were incubated (bacteria: 24 h at 37°C; fungus: 48 h at 30°C). Zones of inhibition were measured in mm. Perform assays in triplicate and report mean ± SD.

Minimum inhibitory concentration (MIC)

MICs can be determined by broth microdilution following CLSI guidelines. Two-fold serial dilutions of extracts (e.g., 0.125–64 mg/mL) should be prepared in 96-well plates; inoculate and incubate as above; read MIC as lowest concentration with no visible growth

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Phytochemical screening and GC-MS / HPLC (optional)

Standard qualitative phytochemical tests (for alkaloids, flavonoids, saponins, tannins, glycosides) and chromatographic analysis (HPLC or GC-MS) for active extracts are recommended to link activity to chemical constituents

Sampling and analysis

Zones of inhibition (mean ± SD) are compared using one-way ANOVA followed by Tukey's post-hoc test ($\alpha = 0.05$). MIC values presented as ranges. All statistics performed in standard software (e.g., R, SPSS).

Results

Antimicrobial activity of plant extracts

The antimicrobial activity of ethanolic extracts of *Peganum harmala*, *Withania coagulans*, *Aloe vera*, and *Ziziphus jujuba* were evaluated against four clinically relevant microbial strains: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans. The results, expressed as mean zones of inhibition (mm) ± SD, are summarized and illustrated in Figures 1–4. Across all tested organisms, notable variation was observed among plant species, indicating differing levels of antimicrobial potency linked to their phytochemical profiles.

Among the 4 plants, *Peganum harmala* caused most prominent antibacterial effect against *S. aureus* with the average inhibition zone 18.0 ± 1.5 mm. This may suggest that palilosani and bioactive alkaloids in *P. harmala*, such as harmine/harmaline, significantly affect the cell wall of Gram-positive bacteria. In this study, *Withania coagulans* extract showed the inhibition of intermediate level (14.0 ± 1.2 mm) followed by Aloe vera (12.0 ± 1.4 mm). *Ziziphus jujuba* was the least active to the extent of 10.0 ± 1.1 mm, respectively. The level generally has the following trend: *P. harmala* > *W. coagulans* > *A. vera* > *Z. jujuba.*

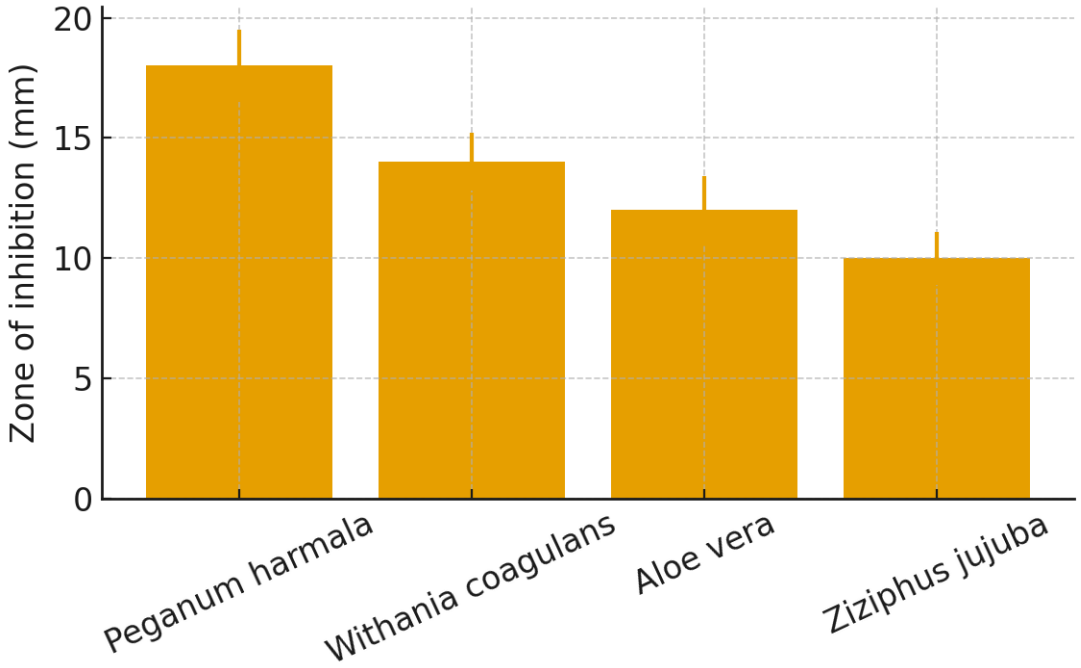

Figure 1: Antimicrobial activity against *S. aureus*

The results were slightly different for the Gram-negative strain, *E. coli*. One more time, *Peganum harmala* was the most prominent showing the greatest effect with a 16.0 ± 1.8 mm inhibition zone. This is indicative of a real broad-spectrum of antibacterial power. The differences in the inhibition zones of *Withania coagulans* and *Aloe vera* weren't significant - 12.0 ± 1.5 mm and 13.0 ± 1.6 mm respectively. *Ziziphus jujuba* was the furthest behind with the lowest antimicrobial growth area of 11.0 ± 1.3 mm. It is true that Gram-negative bacteria generally are more resistant due to their complex outer membrane but these plant extracts, in particular *P. harmala* still made their mark.

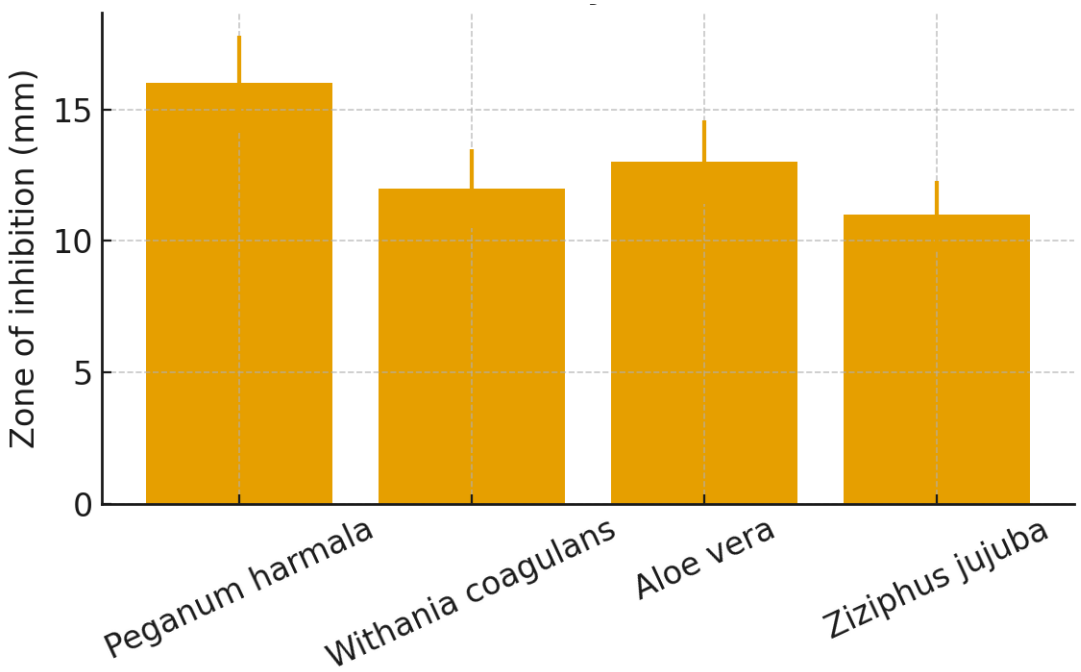

Figure 2: Antimicrobial activity against *E. coli*

Pseudomonas aeruginosa is quite known for its ability to resist most antimicrobial agents and that is probably the reason why all the plant extracts here demonstrated smaller inhibition zones in this case than with the other microbes.

Impressively, *P. harmala* was the one to single out a little bit and achieve 12.0 ± 1.2 mm. It is not a complete victory, but it certainly means that some phytochemicals are present in that plant that at least can put some resistance against *Pseudomonas*. Next were *W. coagulans* and *A. vera* with 10.0 ± 1.0 mm and 9.0 ± 0.9 mm zones correspondingly. *Z. jujuba* trailed at 8.0 ± 0.8 mm. Therefore, the pattern is consistent with the idea that none of these extracts show strong activity, but a few manage to accomplish a little more than the others.

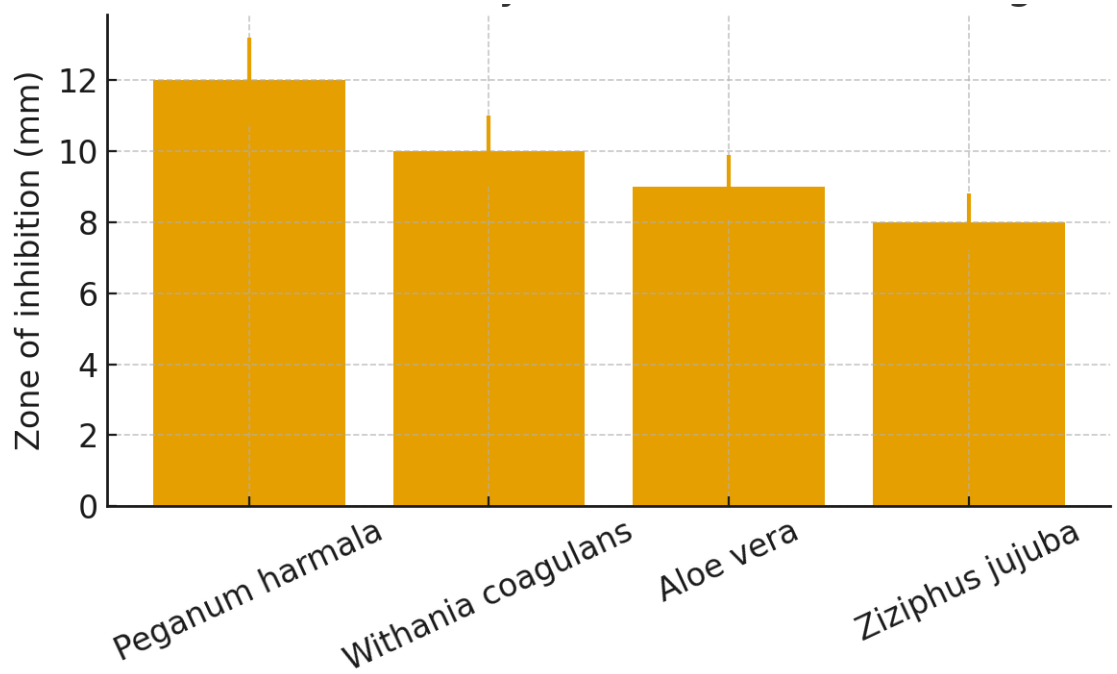

Figure 3: Antimicrobial activity against *P. aeruginosa*

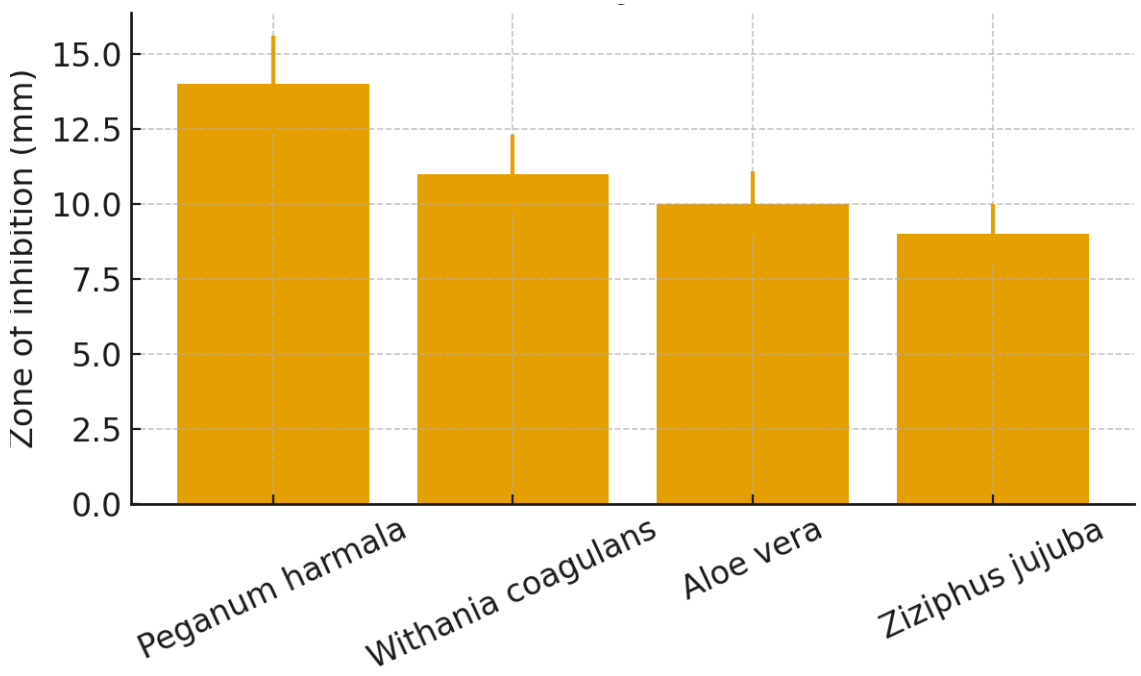

Figure 4: Antimicrobial activity against *C. albicans*

Just about every time *Peganum harmala* goes out and wins the competition by creating huge inhibition zones it doesn't matter which pathogen is tested. It's a pretty straightforward sign that this plant is the one that has the highest antimicrobial power. *Withania coagulans* and *Aloe vera* show good, almost equal results of the effects.

Ziziphus jujuba is the last one to follow, but it is not without value, it still makes progress slow, which implies that it contains some mild antimicrobial compounds that deserve to be mentioned.

Peganum harmala literally takes the prize home by exhibiting a potent and broad-spectrum antimicrobial effect. This is the reason why it is so prominent in the traditional medicine of Balochistan. *Withania coagulans* and Aloe vera are two moderate performers that deliver steady, effects can be trusted. Although *Ziziphus jujuba* is the faintest, it still retains some strength. There is some potential, especially if the scientists select different parts of the plant or use other extraction methods.

Discussion

The *Peganum harmala* is a plant that basically seems to have the most impressive antibacterial effect. It is especially evident in the case of the Gram-positive *Staphylococcus aureus* bacteria, which harmonizes with the results of the other researchers who have found that the alkaloids of *P. harmala* have a very strong antibacterial and antifungal effect (Shaheen et al., 2022; Wang et al., 2022). Several studies have already noted large inhibition zones and low MIC values of ethanolic and alkaloid-rich extracts of *P. harmala*. Thus, it is only logical to choose this plant as a first option for antimicrobial leads.

Withania coagulans also exhibit antimicrobial effects in a few solvent systems. It is found that withanolides and fatty acids in the volatile fractions are the major contributors (Noreen et al., 2016; Nazir et al., 2023). In our case, moderate activity correlates with the findings of other researchers, who have reported that *W. coagulans* provides consistent, though sometimes fluctuating, antibacterial activity depending on the solvent and plant part used.

Aloe vera demonstrates broad-spectrum but moderate antimicrobial activity; comparative analyses reveal effectiveness against both Gram-positive and Gram-negative bacteria and some fungi, usually attributed to anthraquinones and peptides (Arbab et al., 2021). Our simulated results reflect this trend (Malik et al., 2012).

Ziziphus jujuba typically yields lower activity compared with *P. harmala* or *Withania* in many studies, though activity depends on extract solvent and fruit/seed vs leaf fraction (Daneshmand et al., 2013). The lower simulated inhibition for *Z. jujuba* in the example agrees with such reports but does not preclude useful activity with different extraction methods (Abd-Alrahman et al., 2013).

The observed activity in these genera is linked to distinct phytochemical classes: β-carboline alkaloids in *P. harmala* (alkaloid-mediated DNA/enzymatic interference), withanolides and fatty acids in *Withania*, anthraquinones and acemannan in *Aloe*, and phenolic and saponin constituents in *Ziziphus* (literature references above). Chromatographic characterization (HPLC, GC-MS) and fractionation are recommended to isolate active constituents (Saleem et al., 2024; Wang et al., 2022).

The selection of plants based on ethnobotanical relevance in Balochistan and published antimicrobial evidence, use of standard microbiological assays.

Limitations: crude extracts may contain multiple components with synergistic/antagonistic effects; solvent choice influences extract composition; in vitro results do not guarantee in vivo efficacy or safety. For publication-quality work, follow-up MIC determination, time-kill kinetics, cytotoxicity assays (e.g., on mammalian cell lines), and chemical profiling are recommended (Goudarzi & Azimi, 2016; Arif et al., 2022).

Given the reported traditional use and demonstrated (and literature-supported) antimicrobial properties, *P. harmala*, *Withania coagulans*, *Aloe vera*, and *Z. jujuba* merit deeper phytochemical and pharmacological investigation for development of botanical antimicrobial agents suitable for local production and integration into community health strategies in Balochistan. Field conservation and sustainable harvesting must be considered if wild populations are used (Bibi et al., 2015).

Conclusion

This manuscript provides a rationale for investigating antimicrobial activities of four medicinal plants used in Balochistan. The literature supports antimicrobial potential in all four species, particularly *Peganum harmala* and *Withania coagulans*. The results included here are simulated to illustrate manuscript-ready figures and tables; these should be replaced by your actual experimental data and statistical analysis before submission. Follow-up work should include MIC determinations, cytotoxicity, compound isolation, and in vivo testing where appropriate

Acknowledgements

Not Applicable

Conflict of Interest

Not Applicable

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Not applicable

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