Horticulture and Forestry Society of Transylvania

Asha S and Radhakrishnan Radhamoni D (2023)
Notulae Scientia Biologicae
Volume 15, Issue 3, Article number 11575
DOI:10.15835/nsb15311575
Research Article

Notulae Scientia Biologicae

Analysis of the antibacterial potentials of the leaf extracts of selected medicinal plants against pathogenic bacterial strains

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Abstract

Medicinal plants render herbal remedies for human ailments and hence prevail over traditional healthcare practices. This study has analysed the antibacterial potential of the leaf extracts of four selected medicinal plants namely, *Azadirachta indica*, *Ocimum tenuiflorum*, *Biophytum sensitivum*, and *Mimosa pudica,* using Kirby-Bauer's disc diffusion method. Six bacterial species, namely *Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, Bacillus megaterium, Bacillus subtilis,* and *Brevibacillus choshinensis* were used to assess the growth inhibitory potentials of the aqueous and ethanolic extracts of the selected plant leaves. The study revealed that the leaf extract of *B. sensitivum* had the most pronounced antibacterial potential, followed by *A. indica* and *O. tenuiflorum*. The least inhibitory potential was showed by the extracts of *M. pudica*. The findings of the investigation state that the aqueous extract had more consistent antibacterial activity than the ethanolic extracts, confirming that the antibacterial agents present in these leaf extracts are hydrophilic in nature. In terms of growth inhibition, the susceptibility of the pathogenic, virulent strains of bacteria toward these extracts was found comparatively lower than that of the non-virulent, environmental bacteria. With the aqueous extracts, *A. baumannii* was found to be the most susceptible bacterial species while *E. coli* was the most resistant. With the ethanolic extracts, *E. coli* was found to be the most susceptible and *B. subtilis* the least. The current study supports the usage of the screened medicinal plants in alternative medical practices, as a measure to avoid opportunistic infections by environmental bacteria.

Keywords: antibacterial; antibiotics; opportunistic; resistance; susceptibility

Introduction

Infections induced by harmful microorganisms have become a major reason for morbidity and mortality. The spread of antibiotic resistance among the pathogens, along with its clinical manifestations including vomiting, hypersensitivity, immune suppression, and allergic reactions, experienced in humans, demands an urge for developing alternative antimicrobial drugs that are more active and safer (Bottalico *et al*., 2022). Plants are preferred sources, mainly due to their lesser side effects, low cost, ease in availability, and faith of people (Pretorius and Watt, 2001), and a major part of the total population in developing countries still seek herbal

Received: 26 May 2023. Received in revised form: 29 Aug 2023. Accepted: 18 Sep 2023. Published online: 26 Sep 2023.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

health remedies (Sen and Chakraborty, 2017). Medicinal plants can serve as an excellent source to control and reduce microbial infections, as they contain a diverse array of secondary metabolites like tannins, alkaloids, phenolic compounds, and flavonoids (Manandhar *et al*., 2019; Khan *et al*., 2019), which improve innate immune responses and imparts disease resistance in humans (Wink, 2015). Furthermore, reports on antibiotic agents such as rosmarinic acid from *Melissa officinalis*, allicin from *Allium sativum,* and terpinene-4-ol from *Melaleuca alternifolia* (Heinrich *et al.*, 2004), are highly encouraging in finding novel, efficient, yet-to-be characterized bioactive molecules from medicinal plants, for the development of new therapeutic agents.

However, a majority of the ethnopharmacological studies tend to view the antimicrobial activity as a complementary aspect of the study, without showing interest in the original antibiotic potential of the plant and the nature of the bioactive compound. The growth response of non-pathogenic, but opportunistic environmental bacteria towards such compounds also remains greatly elusive (Bonnet *et al*., 2017). But such studies will be decisive in drug development, helping to optimize dosage fixation with minimal non-target effects. Hence this study has been carried out to test the antimicrobial potential of the leaf extracts of four medicinal plants, namely *Azadirachta indica, Ocimum tenuiflorum, Biophytum sensitivum,* and *Mimosa pudica* on selected strains namely, *Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, Bacillus subtilis, Brevibacillus choshinensis* and *Bacillus megaterium.* Here the antimicrobial potential of two types of leaf extracts (aqueous and ethanolic) was tested and compared to determine the most suitable solvent for extraction.

Materials and Methods

The antibacterial potential of the leaf extracts of four medicinal plants was tested on six bacterial species. The solvents used for the extraction were distilled water and ethanol.

Preparation of plant extracts

<u>Collection</u>

The selected medicinal plants, *A. indica, O. tenuiflorum, B. sensitivum,* and *M. pudica* were collected from the garden of NSS College, Pandalam, Pathanamthitta, Kerala, India (Table 1; Figure 1). The plant leaves were thoroughly washed and air dried for two weeks. The dried leaves were finely powdered and placed in moisture-free containers for extraction.

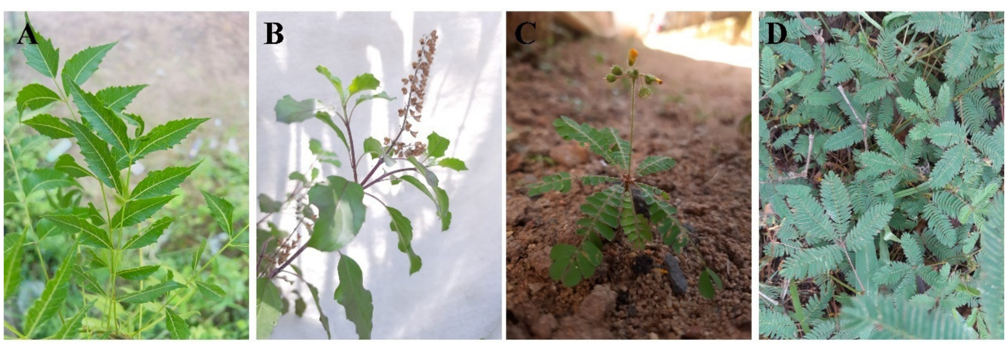

Figure 1. The medicinal plants used for the study

<u>Extraction</u>

10 g of leaf powder was soaked separately in 100 ml of distilled water, and ethanol, in distinct beakers for a period of 24 hours. The mixture was stirred regularly using a sterile glass rod. Muslin cloth and Whatman filter paper No. 1 were used to filter the solvent. The resulting filtrates were concentrated at 40 °C under

reduced pressure in a rotary evaporator (Rotavapor® R-100, Buchi, Switzerland), leaving a dark brown residue (Figure 2). It was stored at 4 °C for further analysis (Nath *et al.,* 2018).

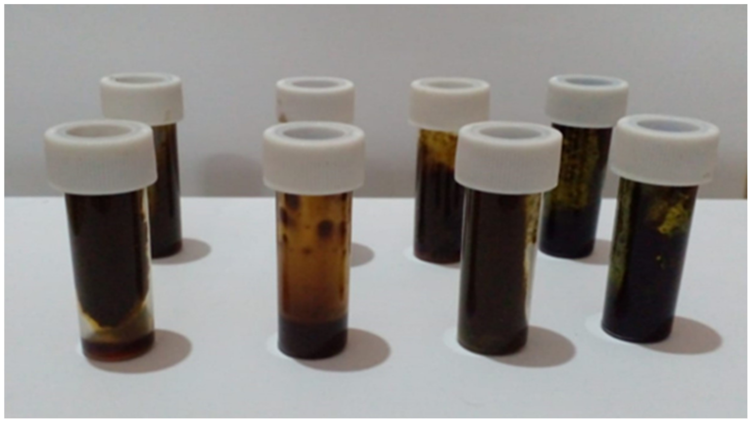

Figure 2. Leaf extracts of selected plants

Table 1. The systematic position of the selected medicinal plants used for antimicrobial efficacy screening

Sample code	Botanical name	Class	Order	Family	Genus	Species	Plant parts used
A	*Azadirachta indica*	Magnoliposida	Sapindales	Meliaceae	*Azadirachta*	*indica*	Leaf
B	*Ocimum tenuiflorum*	Magnolioposida	Lamiales	Lamiaceae	*Ocimum*	*tenuiflorum*	Leaf
C	*Biophytum sensitivum*	Magnoliposida	Oxalidales	Oxalidaceae	*Biophytum*	*sensitivum*	Leaf
D	*Mimosa pudica*	Magnoliposida	Fabales	Fabaceae	*Mimosa*	*pudica*	Leaf

Culture of selected bacteria

Culture media

The culture media used were Nutrient agar for maintaining the bacterial culture and Mueller-Hinton agar for the antibacterial susceptibility test.

Test microorganisms

The bacterial species selected for the study were *E. coli, B. megaterium, B. subtilis, P. aeruginosa, B.choshinensis, and A. baumannii* (Table 2). All the test microorganisms were received from Microlab Scan & Diagnostics, Pandalam, Pathanamthitta, Kerala, India. The pure line colonies were sub-cultured on agar slants by streaking, for further analysis.

Table 2. The systematic position of the selected bacterial species

Bacteria	Phylum	Class	Order	Family	Genus	Species
E. coli	Proteobacteria	Gamma proteobacteria	Enterobacterales	Enterobacteriaceae	*Escherichia*	*coli*
P. aeruginosa	Proteobacteria	Gamma proteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*aeruginosa*
B.megaterium	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	*megaterium*
B. subtilis	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	*subtilis*
A. choshinesis	Firmicutes	Bacilli	Caryophanales	Paenibacillaceae	*Brevibacillus*	*choshinensis*
A. baumannii	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*	*baumanni*

Antibacterial susceptibility test

Kirby-Bauer's disc diffusion method was employed to assess the growth inhibitory efficacy of the selected plant extracts. The bacterial colonies were uniformly inoculated on the entire surface of individual Nutrient Agar plates, following the spread plating technique, with sterile cotton swabs. Sterile, circular discs of Whatmann filter paper 1 (6 mm diameter), were impregnated with 10 µl volume of the chosen extracts. Discs impregnated with distilled water and ethanol were used as a negative control. Subsequently, the discs were positioned onto the bacterial inoculum present on the agar plates using sterile forceps. The plates were then incubated in an inverted position at 37 °C. The diameter of the zone of inhibition was measured after 24 hours of incubation.

Statistical analysis

The significance of variation in the inhibitory effects of the aqueous and the ethanolic leaf extracts of the selected plants and the significance of variation of the zone of inhibition across the selected bacterial species were assessed using ANOVA test (two-way).

Results

As per the observation on the Mueller Hinton agar plates, the antibacterial efficacy of the aqueous and the ethanolic extracts of *A. indica, O. tenuiflorum, B. sensitivum,* and *M. pudica* was evaluated against the pathogenic (*E. coli, P. aeruginosa, A. baumannii*) and the non-pathogenic (*B. megaterium, B. subtilis, B. choshinensis*) bacterial strains. The inhibition zone produced on the agar plates ranged from 2 to 31 mm and depicted the antibacterial potential of the respective plant extracts (Figures 3 and 4). The larger the observed zone of inhibition, the greater the antibacterial potential (measured in millimetre).

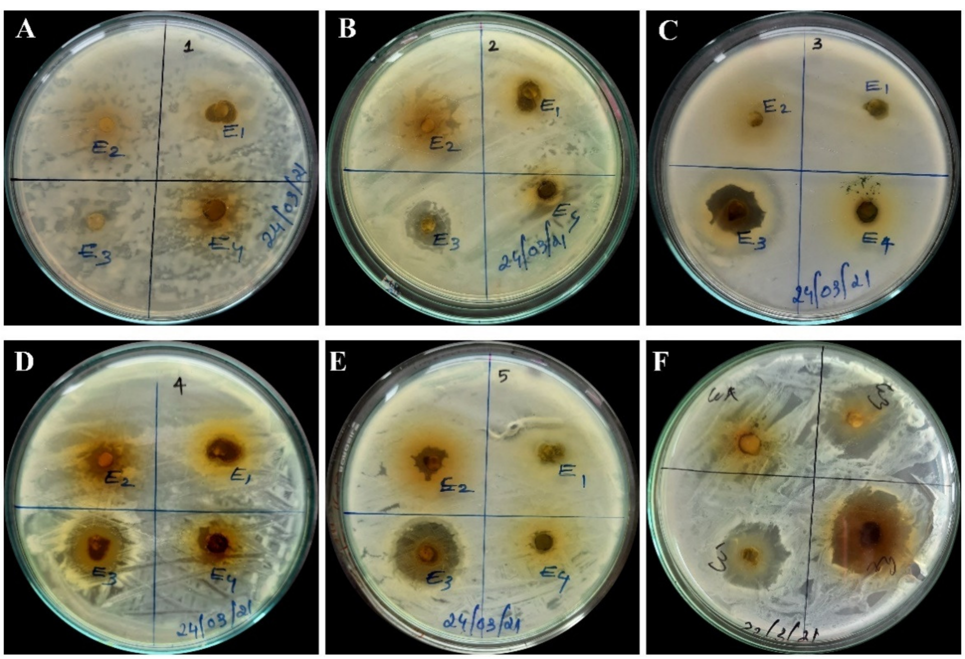

Figure 3. Inhibition zone produced by the ethanolic extracts of selected medicinal plants against bacterial strains like (A) *P. aeruginosa* (B) *B. megaterium* (C) *B. subtilis* (D) *B. choshinensis* (E) *A. baumannii* and (F) *E. coli*

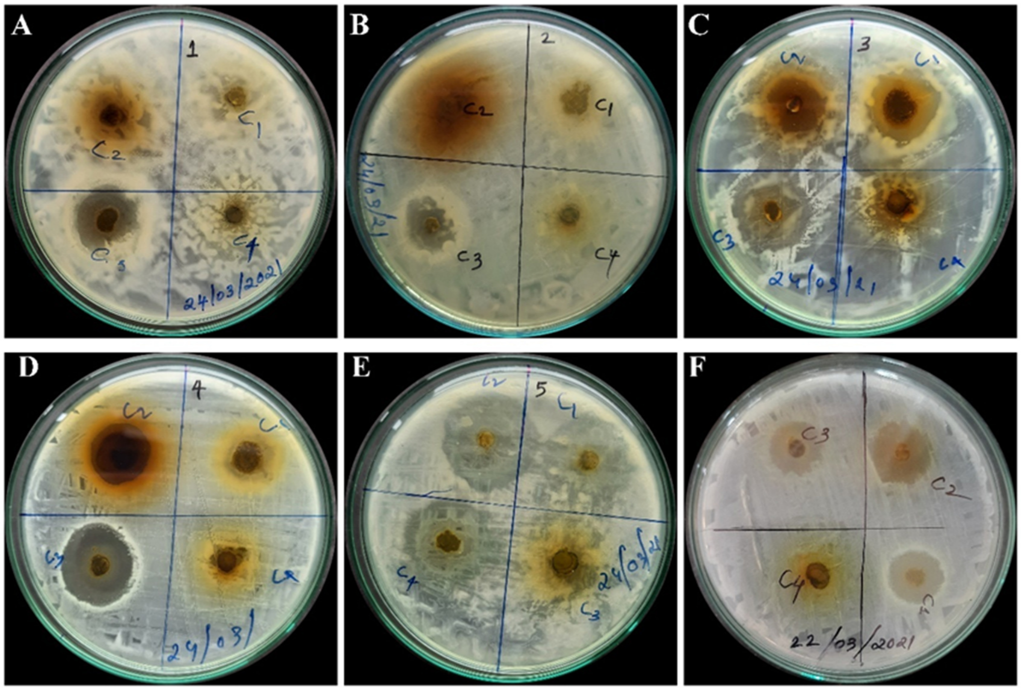

Figure 4. Inhibition zone produced by the aqueous extracts of selected medicinal plants against bacterial strains like (A) *P. aeruginosa* (B) *B. megaterium* (C) *B. subtilis* (D) *B. choshinensis* (E) *A. baumannii* and (F) *E. coli*

Statistical analysis showed that the antibacterial potential of the selected leaf extracts varied significantly among each other in terms of effectiveness, with the F-value (4.08) being greater than the F-crit value (2.9), at five degrees of freedom and at P value 0.015. This observation is in alignment with the studies of Chadha *et al.* (2021), confirming the ethnomedical applications of the selected plants. The inhibitory effect of the aqueous extracts of all the tested plants was found significantly greater than their ethanolic counterparts ($p \leq 0.05$), and this finding aligns with the traditional practice of employing water-based extraction methods for these plant components in the treatment of human ailments (Perumalsamy and Gopalakrishnakone, 2010). On the other hand, the variation observed in the inhibitory effects of the extracts across the tested bacterial species was statistically insignificant ($p \geq 0.05$).

Among the tested leaf extracts, *B. sensitivum* was found to be the most effective bacterial growth inhibitor while *M. pudica* was the least effective. The observed antibacterial potentials indicate the presence of bioactive constituents such as bioflavonoid, amentoflavone, and cupressoflavone in the extracts, as reported by Bharati and Sanu (2012) and Lithshabin *et al*. (2020). The inefficiency of *M. pudica* extract to inhibit bacterial growth agrees with the findings of Sukanya *et al.* (2009), where the extracts of *M. pudica* were reported ineffective against *E. coli* and *S. aureus*.

A. baumannii, which is a common environmental bacterium, causing opportunistic infections in immune-compromised individuals (Kyriakidis *et al*., 2021), was found to be the most susceptible towards all the tested aqueous extracts, while *E. coli* remained the most resistant. In contrast, concerning ethanolic extracts, *E. coli* exhibited the highest susceptibility, whereas *B. subtilis* emerged as the most resistant (Table 3). This reported susceptibility of *A. baumannii* validates the traditional practice of using medicinal plants to prevent the accidental invasion and disease manifestation of the strain. The observed resistance of *E. coli* towards the aqueous extracts confirms the reports about the ability of *E. coli* to resist and counteract the intake of inhibitory molecules from the environment, through porin diameter restriction (Ongsakul *et al*., 2009), and efflux pump activation (Rana *et al*., 2023). The study highlights the diversity of bacterial responses toward different plant extracts and attests the herbal medicinal practices.

Table 3. Measurements of the zone of inhibition (in mm) produced by the selected plant extracts

Plant extracts		Inhibition zones (mm) (M+_SD)					
		E. coli	*P. aeruginosa*	*A. baumannii*	*B. subtilis*	*B. megaterium*	*B. choshinensis*
Azadirachta indica	Aqueous	15.8±0.62	15.5 ±0.24	22.5±1.04	20.±1.04	14± 0.94	21.5±0.24
	Ethanol	21 ± 1.44	15.6 ±0.43	16.8±0.19	0±0	8.5±0.22	15 ± 0.94
Ocimum tenuiflorum	Aqueous	17.6±0.52	11±0.12	30 ± 1.8	15.±0.94	9.8±0.35	17.5±0.74
	Ethanol	25.8±0.92	9±0.11	12.8±0.72	0± 0	8.5±0.42	12.5±0.69
Biophytum sensitivum	Aqueous	15.6±0.11	20.5±1.6	21.5±1.01	21.± 1.02	21± 1.26	24.5± 1.3
	Ethanol	27.5± 1.3	9.6±0.13	21± 0.98	19.± 0.86	18± 0.84	19± 0.98
Mimosa pudica	Aqueous	0 ± 0	10±0.36	27 ±1.1	17.8±0.77	11.5±0.74	9.8± 0.12
	Ethanol	0 ±0	12±0.71	11± 0.61	11±0.55	10.5±0.54	19±0.90

Discussion

The rise of infections, along with increasing multidrug resistance among pathogens, poses a significant challenge to healthcare, jeopardizing current treatment methods. Consequently, there is an urgent need for alternative approaches, and plants show promising potential. Our study confirms the varying degrees of antibacterial activities elicited by selected plant leaf extracts against tested bacteria, highlighting their therapeutic efficacy. Aqueous extracts outperformed their ethanolic counterparts in bacterial growth inhibition, aligning with the traditional water-based extraction methods. Research suggests that antimicrobial phytochemicals are more soluble in polar solvents (Vittaya *et al*., 2023). Aqueous extracts owe their antimicrobial activity to anionic components like thiocyanates, nitrates, chlorides, and sulphates in plants (Omorodion *et al*., 2022). Notably, the antimicrobial efficacy of the plant extract *B. sensitivum* matched standard antibiotics, indicating the presence of bioactive constituents like bioflavonoids, amentoflavone, and cupressoflavone in their leaves (Negi & Mirza, 2020), consistent with the findings of Mary and Raj (2017) and Natarajan *et al*. (2010).

The resistance of *E. coli* towards the aqueous leaf extracts aligns with the previous findings on bacterial adaptation to environmental changes, including temperature, pH, chemicals, and osmolarity, which can affect porin function and the intake of aqueous molecules (Guilhemelli *et al*., 2013; Ongsakul *et al*., 2009). In contrast, *B. subtilis* exhibited resistance to ethanolic extracts, attributable to its well-documented ability to withstand harsh environment (Kovács, 2019). This resistance is not an alarming concern since *B. subtilis* is non-virulent and not associated with human infections or pathologies in domestic flora and fauna (LaJeon *et al*., 2012). On the other hand, *A. baumannii,* a common environmental bacterium known to cause opportunistic infections in immunocompromised individuals (Howard *et al*., 2012), was highly susceptible to aqueous extracts. This susceptibility supports the traditional use of medicinal plants in preventing its invasion and disease manifestation. This study underscores the value of herbal medicinal practices and suggests isolating and characterizing bioactive components from these extracts for therapeutic applications.

Conclusions

This work was devoted to assess the antibacterial efficacy of *A. indica, O. tenuiflorum, B. sensitivum* and *M. pudica* against *E. coli, P. aeruginosa, A. baumannii, B. subtilis, B. choshinensis* and *B. megaterium.* The study revealed that the leaf extract of *B. sensitivum* had the most pronounced antibacterial potential, followed by *A. indica* and *O. tenuiflorum*. The least inhibitory potential was showed by the extracts of *M. pudica*. In case of growth response towards the aqueous extracts, *A. baumannii* was found to be the most susceptible bacterial species while *E. coli* was the most resistant. With the ethanolic extracts, *E. coli* was found to be the most susceptible and *B. subtilis* the least. From this study it is clear that the aqueous extracts of the selected plants exhibited more consistent antibacterial activities than their ethanolic counterparts, indicating the hydrophilic nature of the antibacterial agents that may be present in the extracts. The study also confirms that the virulent, obligate and opportunistic pathogenic bacteria are more resistant towards such bioactive compounds than the environmental, non-virulent strains. These findings validate these plants as candidates for the development of new medicines with activities against resistant pathogens.

Authors' Contributions

Both authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

The authors are thankful to NSS College Pandalam, Pathanamthitta (Dist.), Kerala, India, for the infrastructural support.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Bharati AC, Sahu AN (2012). Ethnobotany, phytochemistry and pharmacology of *Biophytum sensitivum* DC. Pharmacognosy Reviews 6(11):68. *https://doi.org/10.4103/0973-7847.95893*

Bonnet SI, Binetruy F, Hernández-Jarguín AM, Duron O (2017). The tick microbiome: why non-pathogenic microorganisms matter in tick biology and pathogen transmission. Frontiers in Cellular and Infection Microbiology 7:236. *https://doi.org/10.3389/fcimb.2017.00236*

Bottalico L, Charitos IA, Potenza MA, Montagnani M, Santacroce L (2022). The war against bacteria, from the past to present and beyond. Expert Review of Anti-infective Therapy 20(5):681-706. *https://doi.org/10.1080/14787210.2022.2013809*

Chadha J, Gupta M, Nagpal N, Sharma M, Adarsh T, Joshi V, ... Diwan P (2021). Antibacterial potential of indigenous plant extracts against multidrug-resistant bacterial strains isolated from New Delhi region. GSC Biological and Pharmaceutical Sciences 14(2):185-196. *https://doi.org/10.30574/gscbps.2021.14.2.0053*

Guilhelmelli F, Vilela N, Albuquerque P, Derengowski LDS, Silva-Pereira I, Kyaw CM (2013). Antibiotic development challenges: the various mechanisms of action of antimicrobial peptides and of bacterial resistance. Frontiers in Microbiology 4:353. *https://doi.org/10.3389/fmicb.2013.00353*

Heinrich M, Barnes J, Gibbons S, Williamson EM (2004). Fundamentals of Pharmacognosy and Phytotherapy. Churchill Livingstone, Edinbrugh, pp 245-252.

Howard A, O'Donoghue M, Feeney A, Sleator RD (2012). *Acinetobacter baumannii*: an emerging opportunistic pathogen. Virulence 3(3):243-250. *https://doi.org/10.4161/viru.19700*

Khan SU, Anjum SI, Ansari MJ, Khan MHU, Kamal S, Rahman K, … Khan D (2019). Antimicrobial potentials of medicinal plant's extract and their derived silver nanoparticles: A focus on honey bee pathogen. Saudi Journal of Biological Sciences 26(7):1815-1834. *https://doi.org/10.1016/j.sjbs.2018.02.010*

Kyriakidis I, Vasileiou E, Pana ZD, Tragiannidis A (2021). *Acinetobacter baumannii* antibiotic resistance mechanisms. Pathogens 10(3):373. *https://doi.org/10.3390/pathogens10030373*

Kovács ÁT (2019). *Bacillus subtilis*. Trends in Microbiology 27(8):724-725. *https://doi.org/10.1016/j.tim.2019.03.008*

La Jeon Y, Yang JJ, Kim MJ, Lim G, Cho SY, Park TS, … Lee HJ (2012). Combined *Bacillus licheniformis* and *Bacillus subtilis* infection in a patient with esophageal perforation. Journal of Medical Microbiology 61(12):1766-1769. *https://doi.org/10.1099/jmm.0.042275-0*

Manandhar S, Luitel S, Dahal RK (2019). *In vitro* antimicrobial activity of some medicinal plants against human pathogenic bacteria. Journal of Tropical Medicine 2019:1895340. *https://doi.org/10.1155/2019/1895340*

Mary L, Raj T (2017). Antibacterial activity of root extract of *Biophytum sensitivum* (L.) DC. International Journal of Biology Research 2(3):16-19.

Natarajan D, Shivakumar MS, Srinivasan R (2010). Antibacterial activity of leaf extracts of *Biophytum sensitivum* (L.) DC. Journal of Pharmaceutical Sciences and Research 2(11):717-720.

Nath K, Talukdar AD, Bhattacharya MK, Bhowmik D, Chetri S, Choudhury D, Bhattacharjee A (2018). Antibacterial activity of certain ferns against multi drug resistant organisms. Journal of Natural Remedies 17(4):144-153.

Negi K, Mirza A (2020). Nephroprotective and therapeutic potential of traditional medicinal plants in renal diseases. Journal of Drug Research in Ayurvedic Sciences 5(3):177-185. *https://doi.org/10.5005/jdras-10059-0079*

Omorodion NJ, Ujoh EC (2022). Antimicrobial activities of aqueous and ethanolic extracts of some natural spices (garlic, turmeric, thyme and onions) on some clinical isolates. GSC Biological and Pharmaceutical Sciences 19(1):335-345. *https://doi.org/10.30574/gscbps.2022.19.1.0094*

Ongsakul M, Jindarat A, Rojanaworarit C (2009). Antibacterial effect of crude alcoholic and aqueous extracts of six medicinal plants against *Staphylococcus aureus* and *Escherichia coli*. Journal of Health Research 23(3):153-156.

Perumal Samy R, Gopalakrishnakone P (2010). Therapeutic potential of plants as anti-microbials for drug discovery. Evidence-Based Complementary and Alternative Medicine 7(3):283-294. *https://doi.org/10.1093%2Fecam%2Fnen036*

Pretorius CJ, Watt E (2001). Purification and identification of active components of *Carpobrotusedulis* L. Journal of Ethnopharmacology 76:87-91. *https://doi.org/10.1016/s0378-8741(01)00197-0*

Rana T, Farooq U, Kaur N, Khan A, Khan A (2023). Plants derived efflux pump inhibitors: an approach against multidrug-resistant gram-negative bacteria *Klebsiella pneumoniae*. Pharmacophore 14(1). *https://doi.org/10.51847/7FQXFNYnT5*

Sen S, Chakraborty R (2017). Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future. Journal of Traditional and Complementary Medicine 7(2):234-244. *https://doi.org/10.1016/j.jtcme.2016.05.006*

Sharma A, Gumber K, Gohain A, Bhatia T, Sohal HS, Mutreja V, Bhardwaj, G. (2023). Importance of essential oils and current trends in use of essential oils (aroma therapy, agrofood, and medicinal usage). In: Essential Oils 2023:53-83. *https://doi.org/10.1016/B978-0-323-91740-7.00002-5*

Sukanya SL, Sudisha J, Hariprasad P, Niranjana SR, Prakash HS, Fathima SK (2009). Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. African Journal of Biotechnology 8(23). *http://dx.doi.org/10.4314/ajb.v8i23.66376*

Vittaya L, Chalad C, Ratsameepakai W, Leesakul N (2023). Phytochemical characterization of bioactive compounds extracted with different solvents from *Calophyllum inophyllum* flower and activity against pathogenic bacteria. South African Journal of Botany 154:346-355. *https://doi.org/10.1016/j.sajb.2023.01.052*

Wink M (2015). Modes of action of herbal medicines and plant secondary metabolites. Medicines 2(3):251-286. *https://doi.org/10.3390/medicines2030251*