Phytomolecules as a Source for Drug Discovery

Phytomolecules as a Source for Drug Discovery:

Antimicrobials from Medicinal Plants

Edited by

Harish C. Upadhyay

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Phytomolecules as a Source for Drug Discovery: Antimicrobials from Medicinal Plants

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PREFACE

Medicinal plants have always provided new and important leads against various pharmacological targets. Nature, the master craftsman of molecules, has created almost an inexhaustible array of molecular entities. The compounds isolated from medicinal plants already have biological functions with better biologically relevant chemistry, hence fewer side effects. The identification of novel biologically active molecules is the very first, but crucial step in the larger process of drug discovery where these newly identified bioactive phytomolecules are to be used as raw material for the next stage of research. This strategy has given more than half of the modern pharmaceuticals to humans for the treatment of almost all types of diseases and debilities. Nature has created almost an inexhaustible array of chemotypes in terms of plant secondary metabolites, standing as an infinite resource for drug development for amplification into efficacious drugs for a multitude of disease indications and other valuable bioactive agents.

After the discovery and development of antibiotics, penicillin, and streptomycin from natural sources, the industry of chemotherapy for infectious diseases finds momentum. These antibiotics were miracles that saved millions of lives from many diseases, including deadly pneumonia and tuberculosis. However, due to emerging infections and the global spread of drug-resistant bacterial pathogens, antimicrobial therapy is facing serious challenges. There is a huge emergence of new strains of organisms that are multidrug-resistant (MDR) with novel penicillin-binding proteins (PBPs), enzymatic mechanisms of drug modification, mutated drug targets, enhanced efflux pump expression, and altered membrane permeability. The discovery and development of new antimicrobial agents have become an urgent priority in the global health landscape due to the rising threat of antibiotic resistance and emerging infectious diseases. In this context, medicinal plants have garnered significant attention as a prolific and sustainable source of bioactive compounds, or phytomolecules, with the potential to inspire novel therapeutic solutions.

This book, *Phytomolecules as the Incessant Inspiring Source for Drug Discovery: Antimicrobials from Medicinal Plants*, aims to provide a comprehensive and in-depth exploration of the vast potential of phytomolecules in antimicrobial drug discovery. These phytomolecules offer a rich repository of structural diversity and biological activity, making them invaluable leads for the development of new antimicrobial agents. Our focus is on the unique chemical structures and diverse biological activities of these phytomolecules, which have evolved over millions of years as plants adapted to survive and thrive in various environments.

We begin with an overview of the 'current status and challenges in antimicrobial drug discovery and development' covering from traditional use of medicinal plants and their role in modern medicine, highlighting the importance of ethnopharmacology in identifying potential drug candidates, to the present scenario and future perspectives of natural product-based drug discovery and development. We then delve into the phytochemical analysis, the latest advancements in the bioactivity of plant extracts, isolation and characterization of antimicrobial phytomolecules, and success stories of natural product-based antimicrobials including those in clinical practice. Subsequent chapters are dedicated to specific plants or classes of phytomolecules and their potential against various pathogens, including bacteria, viruses, fungi, and parasites including malaria. We also examine the challenges and opportunities in translating these findings into clinical applications.

Our goal with this book is to inspire and inform researchers, pharmacologists, and healthcare professionals about the immense possibilities offered by medicinal plants in the quest for new antimicrobial agents. By providing a detailed and multidisciplinary perspective, we hope to foster collaboration and innovation in the field of phytochemistry and drug discovery.

I am grateful to the many contributors who have shared their expertise and insights, enriching this work with their diverse perspectives and experiences. I also acknowledge the growing community of scientists and researchers dedicated to exploring the therapeutic potential of natural products.

I invite you to embark on this journey through the world of phytomolecules, as we explore their role as an incessant and inspiring source for drug discovery, with the hope of addressing some of the most pressing health challenges of our time.

Editor
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Assistant Professor
Rajkiya Engineering College Sonbhadra
August, 2024

About the Editor

Dr. Harish C. Upadhyay

Dr. Harish C. Upadhyay is a distinguished academician and researcher in the field of phytochemistry and pharmacology, specializing in the study of bioactive compounds from medicinal plants. With a career spanning more than a decade, Dr. Upadhyay has made significant contributions to the understanding and application of phytomolecules in drug discovery and development. Dr. Upadhyay earned his Ph.D. in medicinal chemistry from CSIR-Central Institute of Medicinal and Aromatic Plants, where he conducted pioneering research on the antimicrobial properties of plant-derived compounds. His work has been instrumental in identifying novel phytochemicals with potential therapeutic applications, particularly in the fight against antibiotic-resistant pathogens. As a prolific author, Dr. Upadhyay has published numerous peer-reviewed articles and book chapters, and he has presented his findings at international conferences and symposia. His research interests include natural product chemistry, chemical transformation of bioactive leads and structure-activity relationship study, design and synthesis of plant-based hybrid leads, and computer-aided drug design for the development of sustainable pharmaceuticals.

Dr. Upadhyay is currently a faculty member at the Department of Applied Sciences at Rajkiya Engineering College, Churk, Sonbhadra, a government college affiliated with Dr. A.P.J. Abdul Kalam Technical University, Lucknow, India, where he is actively involved in mentoring the next generation of scientists and is committed to promoting interdisciplinary collaboration in the field of natural product research for exploring the chemical diversity and biological activity of medicinal plants.

His work continues to inspire and guide efforts in the search for innovative solutions to global health challenges, emphasizing the importance of harnessing the power of nature in modern medicine.

CHAPTER 1

CURRENT STATUS AND CHALLENGES IN ANTIMICROBIAL DRUG DISCOVERY AND DEVELOPMENT

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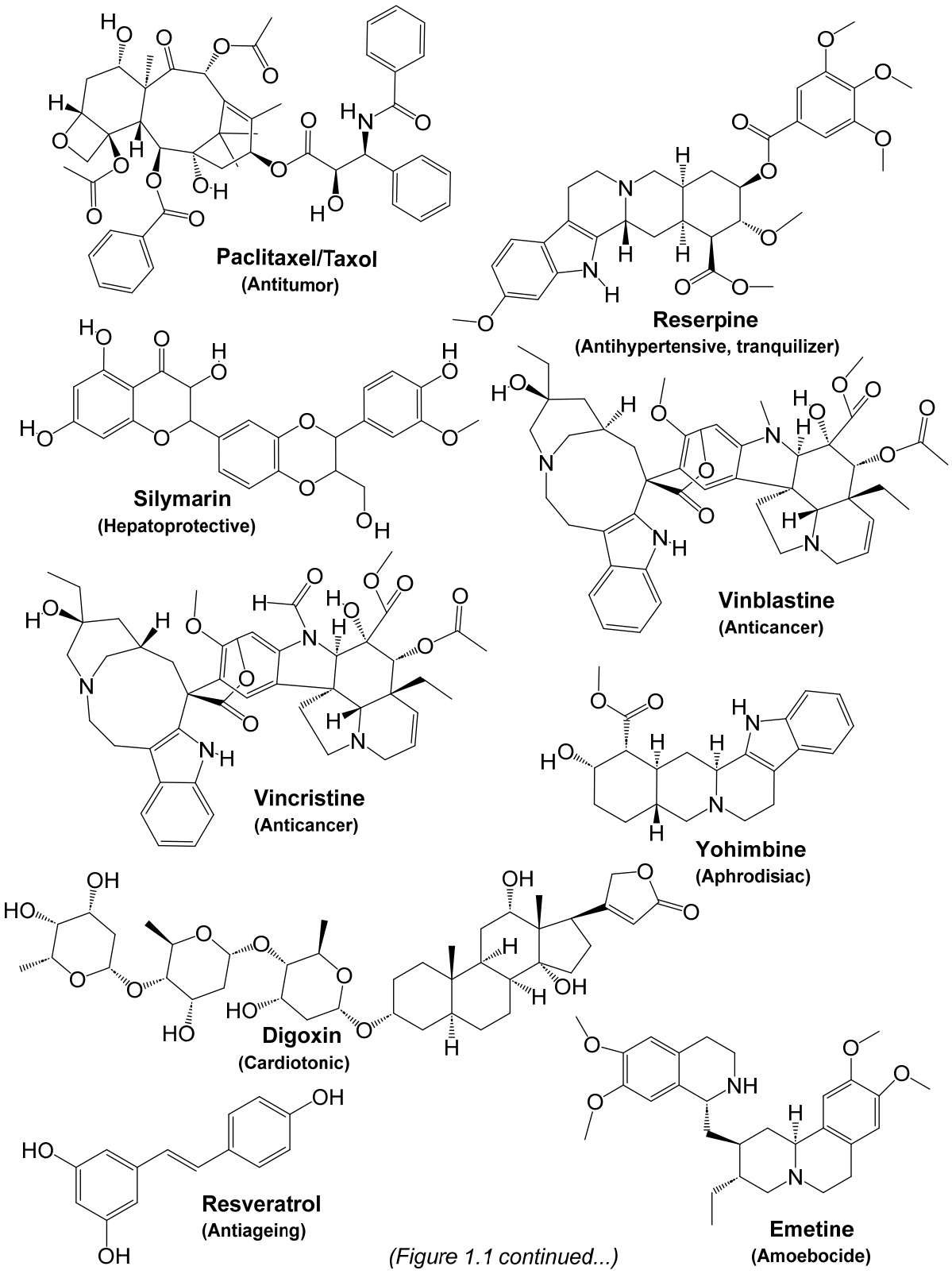
1.1 Introduction

Medicinal plants are the incessant source of modern drugs. The moieties of natural origin have provided several life-saving drugs for the treatment of various types of infections, cancer, and debilities like diabetes (Cragg *et al.* 1997; Harvey 2008)**.** The chemical diversity of structures in natural molecules provides a unique opportunity for drug discovery, as it allows researchers to identify new compounds with specific biological activities (Dias *et al.* 2012; Lahlou 2013). Since the early nineteenth century, organic chemistry has gradually advanced, making it possible to develop the processes for isolating, characterizing, and purifying the active components from medicinal plants. After the Second World War, the field of natural product chemistry underwent a more rapid revolution as a result of the development of a variety of advanced tools and methods for the isolation and characterization of plant secondary metabolites. This led to the quick identification of various plant-natural products, which in turn led to the discovery of new drug candidates. Since then, several active principles have been extracted from plants, enriching contemporary medicine and serving as crucial building blocks for developing new drugs.

Various phytomolecules have served as clinically approved drugs of the last century; notable are artemisinin as antimalarial, quinine as antimalarial and antipyretic, digoxin as cardiotonic, and morphine as analgesic (Figure 1.1). While, a majority of drugs of today are phytomolecule derivatives. Agrimophol is a naturally obtained drug from the *Agrimonia pilosa.* Agrimophol is an effective anthelmintic used to treat trichomonas enteritis, vaginitis, and other diseases(Jin *et al.* 2018). Artemisinin is a naturally obtained drug from the *Artemisia annua* (sweet wormwood) plant. It has robust antimalarial characteristics. This drug has been used to treat malaria, particularly in situations when the illness has proven resistant to other drugs (Ma *et al.* 2020; Wang *et al.* 2019). Atropine is a naturally obtained drug from *Atropa belladonna* belongs to a class of drugs called anticholinergics or antimuscarinics used to treat certain types of nerve agents and pesticide poisonings(Aldossary 2022; Upadhyay & Beuerman 2020). Camptothecin is a naturally obtained drug from the Chinese tree *Camptotheca acuminata*. It has powerful anticancer activities (El-Sayed *et al.* 2022a; Mei *et al.* 2020). Codeine is a naturally obtained drug from *Papaver somniferum* (poppy). This drug belongs to a class of drugs called Analgesic (pain-relieving) and antitussive(El-Sayed *et al.* 2022a; Mei *et al.* 2020). Ephedrine is a natural product of the ephedra plant, *Ephedra sinica*. This drug belongs to a class of drugs called Sympathomimetic, antihistamines (Eskandr *et al.* 2021; Martin *et al.* 1971; Park *et al.* 2020; Stohs *et al.* 2020). Hesperidin is a naturally obtained drug from *Citrus species* (e.g., oranges) citrus fruits that exhibits antioxidant, neuroprotective, and anti-inflammatory activities(Choi *et al.* 2022; Mas-Capdevila *et al.* 2020; Pyrzynska 2022). Morphine is a naturally obtained drug from *Papaver somniferum* (poppy). Morphine is a very efficacious drug for the relief of moderate to severe pain and is the standard by which all other agents are measured (Listos *et al.* 2019; Ofoegbu & B. Ettienne 2021). Quinine is a naturally obtained drug from *Cinchona ledgeriana* (quinine tree). It has powerful Antimalarial and antipyretic properties (Achan *et al.* 2011; Saeheng & Na-Bangchang 2022). Reserpine is a naturally obtained drug from *Rauvolfia serpentina.* It has powerful Antihypertensive, tranquilizer drugs (Li *et al.* 2022; Strawbridge *et al.* 2023). Resveratrol is a naturally obtained drug from *Vitis vinifera.* It is Chemopreventive and chemotherapeutic in different types of cancer. Also used as an antidiabetic, in cardiovascular complications, metabolic syndromes, and antioxidants (Barber *et al.* 2022; Breuss *et al.* 2019; Meng *et al.* 2021; Ren *et al.* 2021). Silymarin is a naturally obtained drug from *Silybum marianum* (milk thistle) It is a powerful Antihepatotoxic drug (Fallah *et al.* 2021; Gillessen & Schmidt 2020; Koltai & Fliegel 2022). Taxol, also known by its generic name paclitaxel, is a naturally obtained drug from *Taxus brevifolia* (Pacific yew). It is a powerful Antitumor agent (El-Sayed *et al.* 2020; Kumar *et al.* 2019; Yang & Horwitz 2017). Vinblastine is a naturally obtained drug from *Catharanthus roseus* (Madagascar periwinkle). It is an Antitumor, an Antileukemic agent (Caputi *et al.* 2018; Murray *et al.* 2022; Zhang *et al.* 2022). Vincristine is a naturally obtained drug from *Catharanthus roseus* (Madagascar periwinkle). It is an Antitumor, Antileukemic agent (Herradón *et al.* 2021; Madsen *et al.* 2019; Triarico *et al.* 2021). Yohimbine is a naturally obtained drug from *Pausinystalia yohimb*e (yohimbe).

It is an Aphrodisiac drug (Jabir *et al.* 2022; Mueller-Schoell *et al.* 2021; Sperl *et al.* 2022). L-DOPA, or levodopa, is a naturally obtained drug from *Vicia faba*. It is commonly used to treat Parkinson's disease and dopamine-responsive dystonia (Breitel *et al.* 2021; Soares *et al.* 2014). Digoxin is a naturally obtained drug from *Digitalis lanata.* Digoxin is a medication used primarily to treat certain heart conditions, particularly atrial fibrillation and congestive heart failure (CHF). It belongs to a class of drugs known as cardiac glycosides and has been used for several decades to manage heart-related problems (Chan *et al.* 2022; Lopes *et al.* 2018; Ode 2019).



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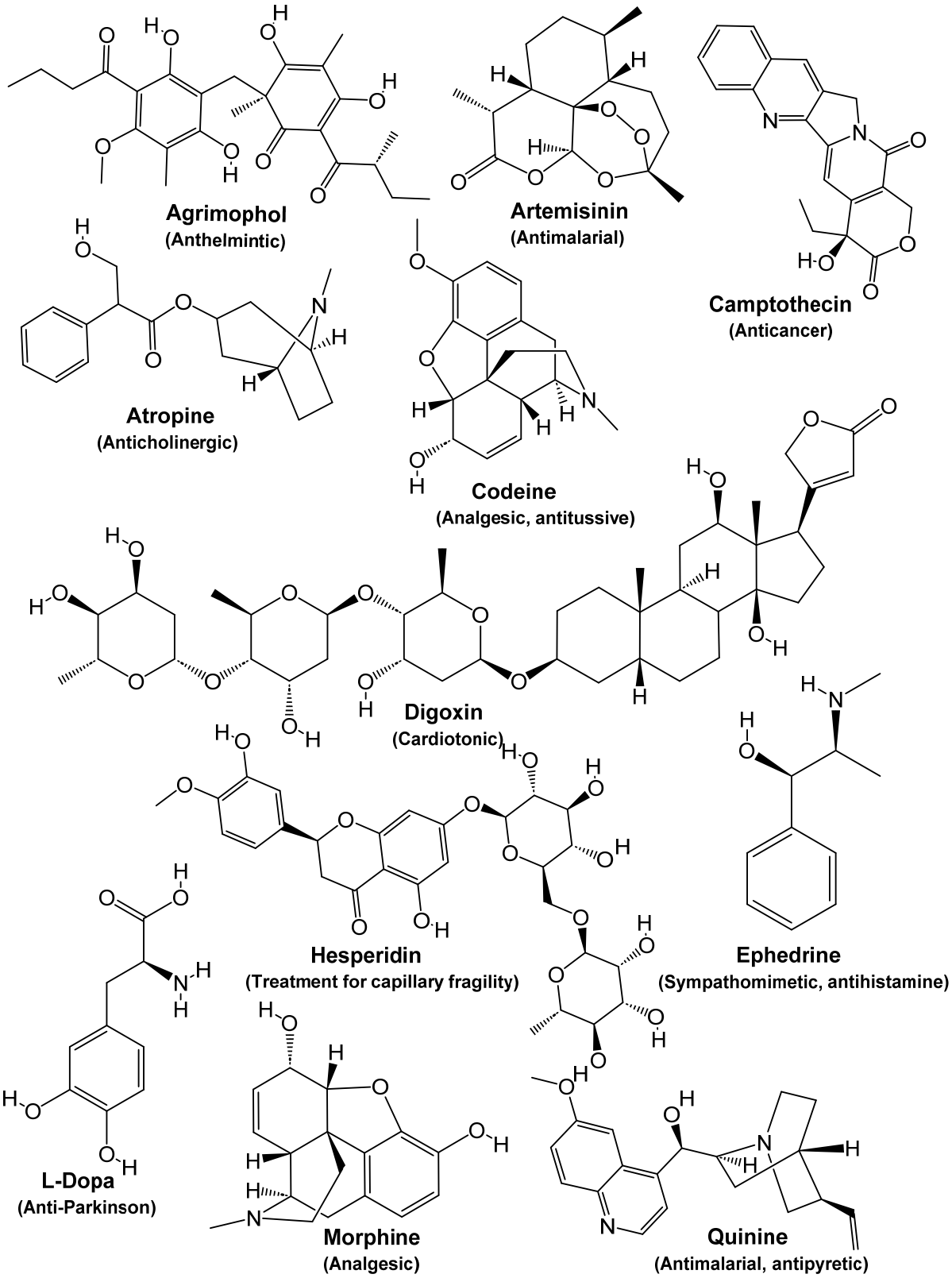

Figure 1.1: Structure of clinically approved drugs originating from natural sources

The isolation and characterization of pharmacologically active compounds from plants continue today. Being safe due to their sync with nature, they are the consistent inspiration for medicinal chemists (Cragg & Newman 2013; G.M. & D.J. 2013; Lahlou 2013).

1.2 Natural Products as a Source of Antimicrobial Drugs

With the discovery of penicillin from *Penicillium spp.* by Alexander Fleming in the early 20th century, antibiotics have revolutionized modern medicine by providing effective treatments for bacterial infections (M. Wainwright 1990). Then

after, numerous antibiotics have been discovered, and they have become a critical tool in treating infectious diseases (Calixto 2019). Due to targeting specific structures or processes within bacteria, either by inhibiting their growth or by killing them outright, antibiotics have had a profound impact on modern medicine, and their continued development remains an important area of research (M. Wainwright 1990). At present, directly or indirectly, more than half of the drugs in the market have their basic skeleton from natural products, the status is the same in the case of antibacterial drugs also (Figure 1.2) (Cragg *et al.* 1997; Newman & Cragg 2016, 2020).

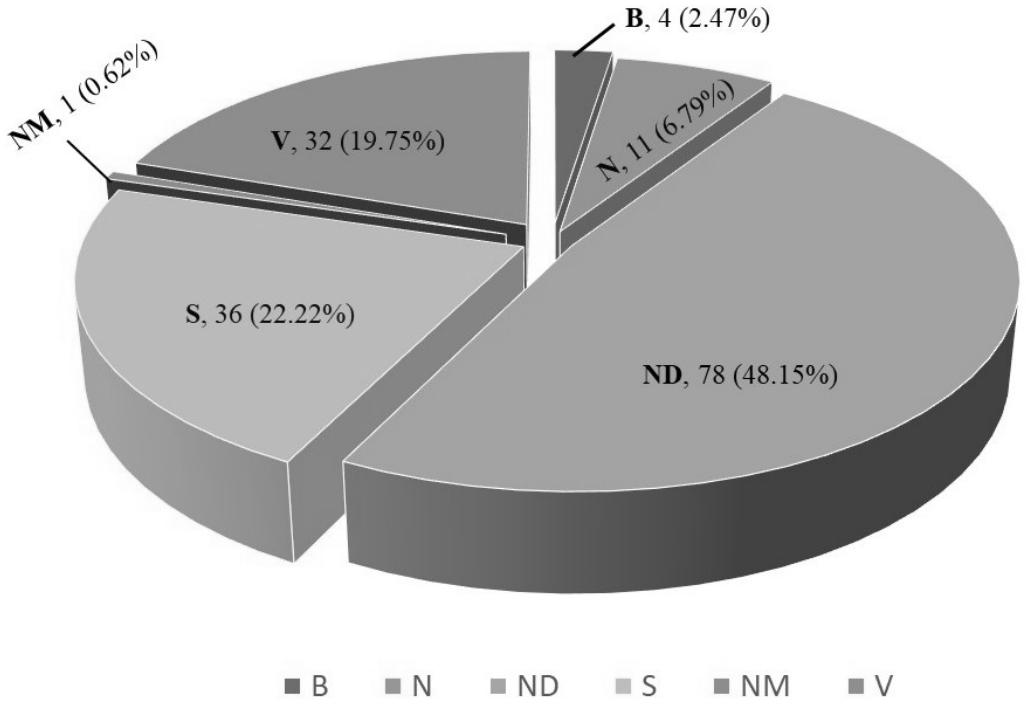

Figure 1.2: Antibacterial drugs by source (approved during January 1981-September 2019)

The greatest medical discovery of the 20th century may have been the development of antibiotics for clinical use that revolutionized the field of medicine, providing effective treatments for infectious diseases and saving countless lives. From the discovery of penicillin by Alexander Fleming to the antibiotics of the Golden Age, these drugs have been instrumental in reducing mortality rates and improving public health (Kardos & Demain 2011). This era saw the isolation and development of several key antibiotics, including streptomycin, chloramphenicol, tetracycline, and erythromycin (Kardos & Demain 2011). The representative antibiotics from natural sources are summarized in Table 1.1 (Chiu 2013; Ebimieowei & Ibemologi 2016; Kohanski *et al.* 2010).

Table 1.1: Notable antibiotics from natural source

S.No	Class	Representative	Organism	Molecular target
A.	**Antibiotics from actinomycetes**			
1.	Aminoglycosides	Kanamycin A	*Streptomyces kanamyceticus*	Protein synthesis: 30S ribosomal
2.	Tetracyclines	Tetracycline	*S. aureofaciens*	Protein synthesis: 30S ribosomal subunit
3.	Amphenicols	Chloramphenicol	*S. venezuelae*	Protein synthesis: 50S ribosomal subunit
4.	Macrolides	Erythromycin	*Saccharopolyspora erythraea*	Protein synthesis: 50S ribosomal subunit
5.	Tuberactinomycins	Viomycin	*Streptomyces puniceus*	Protein synthesis: 30S and 50S ribosomal subunits
6.	Glycopeptides	Vancomycin	*Amycolatopsis orientalis*	Cell wall synthesis: D-Ala-D-Ala termini of lipid II

7.	Lincosamides	Clindamycin	*Streptomyces lincolnensis*	Protein synthesis:50S ribosomal subunit
8.	Ansamycins	Rifamycin SV	*Amycolatopsis rifamycinica*	Nucleic acid synthesis: RNA polymerase
9.	Cycloserines	Seromycin	*Streptomyces orchidaceus*	Cell wall synthesis: inhibition of alanine racemase and D-alanine-D-alanine ligase
10.	Streptogramins	Pristinamycin	*Streptomyces pristinaespiralis*	Protein synthesis: 50S ribosomal subunit
11.	Phosphonates	Fosfomycin	*Streptomyces fradiae*	Cell wall synthesis: MurA (UDP-GlcNAc-3-enolpyruvyltransferase) inhibition
12.	Carbapenems	Meropenem	*Streptomyces cattleya*	Cell wall synthesis: penicillin-binding proteins
13.	Lipopeptides	Daptomycin	*Streptomyces roseosporus*	Cell wall: cell membrane disruption.
14.	Lipiarmycins	Fidaxomicin	*Dactylosporangium aurantiacum subsp. hamdenesis*	Nucleic acid synthesis: RNA polymerase
B.	**Antibiotics from other bacteria**			
15.	Polypeptides	Gramicidin A	*Bacillus brevis*	Cell wall: forms ion channels that increase the permeability of the bacterial cell membrane
16.	Bacitracin	Bacitracin A	*Bacillus subtilis*	Cell wall synthesis: inhibition of dephosphorylation of C 55-isoprenyl pyrophosphate
17.	Polymyxins	Colistin	*Paenibacillus polymyxa*	Cell wall: cell membrane disruption
18.	Mupirocin	Mupirocin	*Pseudomonas fluorescens*	Protein synthesis: isoleucyl t-RNA synthetase
19.	Monobactams	Aztreonam	*Chromobacterium violaceum*	Cell wall synthesis: penicillin-binding proteins
C.	**Antibiotics from Fungi**			
20.	Penicillin	Amoxicillin	*Penicillium chrysogenum*	Cell wall synthesis: penicillin-binding proteins
21.	Fusidic acid	Fusidic acid	*Fusidium coccineum*	Protein synthesis: elongation factor G
22.	Enniatins	Fusafungine	*Fusarium lateritium*	Cell wall: cell membrane disruption
23.	Cephalosporins	Cefacetrile	*Acremonium chrysogenum*	Cell wall synthesis: penicillin-binding proteins
24.	Pleuromutilins	Retapamulin	*Pleurotus mutilus*	Protein synthesis: 50S ribosomal subunit

1.3 Challenges with Existing Antimicrobial Drugs

Till now, natural products, mainly plant extracts have been the major source of new antimicrobials (Butler 2005, 2008). Drug discovery from medicinal plants is much tedious, time-consuming, costly, and risky process (Galm & Shen 2007). The diminishing approach towards natural sources due to complexities, there is a lack of novel scaffolds to be used as lead molecules. The synthetic molecules usually do not have compatibility with the biological system and often lead to

adverse effects, also have rather limited functionality against antimicrobial targets (Upadhyay 2023)Most of the leads are rejected due to failures in various drug development stages. Hence, a depleted antibiotic pipeline and high failure rates in clinical studies are the major challenges in antimicrobial drug discovery.

Another burning challenge with the existing antimicrobial drugs is antimicrobial resistance (AMR). As a consequence of selective pressure of use and misuse, pathogenic bacteria, fungi, and other parasites have developed the ability to resist the effects of antimicrobial drugs, rendering them ineffective in treating infections (Maddocks 2013; Yang & Buttery 2018). Even before penicillin was used in medical applications, penicillinase, a -lactamase capable of degrading penicillin, was discovered. *S. pyogenes* and *S. aureus*, which were resistant to penicillin and sulfonamides, respectively, emerged in the 1930s and 1940s as a result of heavy usage in hospitals. Since then, a large number of bacterial strains have developed resistance, some of which are resistant to multiple antibiotics (Jasovský *et al.* 2016; Laxminarayan *et al.* 2013).

Antimicrobial resistance (AMR) is a global health challenge that has emerged as a consequence of the widespread use and misuse of antibiotics (Yang & Buttery 2018; Zaman *et al.* 2017). Infections caused by resistant microorganisms are more difficult to treat, often requiring more expensive and toxic drugs, and leading to longer hospital stays and increased healthcare costs (Morrison & Zembower 2020; WHO 2020). Acinetobacter baumannii, *Pseudomonas aeruginosa*, and Enterobacteriaceae are among the dirty dozen of human pathogenic bacteria that urgently need innovative antibiotics due to drug resistance and a lack of available treatments (Upadhyay 2021). The economic impact of AMR is also significant, with estimates suggesting that it could cost the global economy up to $100 trillion by 2050 if left unchecked (Dadgostar 2019; Spellberg *et al.* 2013). AMR is a critical issue that requires urgent attention and action for which the discovery and development of novel antimicrobial leads with broad spectrum functionality is the need of hour (Laxminarayan *et al.* 2013)Only 38% of the antibiotics currently under research are predicted to be effective against the ESKAPE infections, which have been given high priority for more than ten years and include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and *Enterobacter*.

1.4 Modern Approaches in Nature-inspired Antimicrobial Drug Discovery

1.4.1 Application of CADD approach

Computer-aided drug design (CADD) is a rapidly growing field that involves the use of computational methods and tools to design and discover new drug molecules with improved therapeutic properties (E. N. 2017; Macalino *et al.* 2015). CADD involves various steps, including ligand-based and structure-based drug design, virtual screening, molecular dynamics simulations, and cheminformatics analysis (Lee *et al.* 2022; Lionta *et al.* 2014). CADD techniques such as molecular docking, molecular dynamics simulations, and quantitative structure-activity relationship (QSAR) modeling have a significant impact on the antibiotic drug design industry by enabling the rational design and optimization of new antibiotics with improved potency and selectivity (David *et al.* 2021; Kitchen *et al.* 2004; Xavier Suresh 2010). One of the major applications of CADD is in the design of small-molecule inhibitors of enzymes, which play crucial roles in the life cycle of pathogenic microbes, various diseases such as cancer, Alzheimer's disease, and HIV (Lee *et al.* 2022). Another important application of CADD is in the optimization of drug molecules to improve their pharmacokinetic and pharmacodynamic properties, such as bioavailability, solubility, and potency (Xiang *et al.* 2012). This can be achieved through structure-based drug design, where the three-dimensional structure of the target protein is used to design ligands with optimal binding affinity and selectivity (Seidel *et al.* 2020). In recent years, CADD has revolutionized the process of drug discovery and development by reducing both the time and cost of the drug development process as well as a number of drug failures (Baig *et al.* 2017; Dwivedi *et al.* 2015; Surabhi & Singh 2018; Upadhyay *et al.* 2021).

With the increasing threat of antibiotic resistance, CADD is likely to play an even more important role in the development of new antibiotics in the future (Macalino *et al.* 2015). Finding new antibiotic targets that might stand in for innovative survival strategies for bacteria is a significant alternative to addressing the problem of antibiotic resistance. This has been rapidly achieved by using CADD/bioinformatics approach (Schneider & Fechner 2005). For example, in the case of the gram-positive bacterium *Staphylococcus aureus*, bioinformatics approaches have been used to computationally screen different databases and identify seven enzymes involved in bacterial metabolic pathways as well as 15 non-homologous proteins located on membranes, thereby indicating them as potential targets (O'Daniel *et al.* 2014). These findings could prove helpful in overcoming this bacterium's resistance to well-known antibiotics like methicillin, fluoroquinolones, and oxazolidinones (Hasan *et al.* 2016). Researchers led by Chang et al. discovered a new class of non-lactam antibiotics called oxadiazoles via in silico database screening. These antibiotics can block the penicillin-binding protein 2a (PBP2a) of methicillin-resistant *S. aureus* (MRSA), which is the main source of infections in hospitals (Velvadapu *et al.* 2011; Wagh *et al.* 2013). Yu *et al.*, in collaboration with Andrade and colleagues, looked at third-generation ketolide antibiotic telithromycin analogs using ligand-based drug design (LBDD) as a potential solution to the issue of bacterial resistance to that class of antibiotics (Velvadapu *et al.* 2011; Wagh *et al.* 2013).

Overall, CADD can be used in combination with wet-lab methods to develop emerging antibiotics by elucidating the mechanism of drug resistance, finding new antibiotic targets, and creating novel antibiotics for both well-known and

undiscovered targets. Notably, CADD techniques might produce an atomic level structure-activity relationship (SAR) that is used to speed up and reduce the cost of the discovery and development process of antimicrobials.

1.4.2 Design and synthesis of multifaceted hybrid molecules

It has been noted that medications that only target one target frequently cause resistance and are linked to a wide range of adverse effects (Sausville 2012). However, medications that target several targets may be more effective, less likely to cause resistance, and have fewer side effects. Multidrug therapy, which entails taking two or more medications at the same time or co-formulating two or more medications into a single tablet, is a common strategy used to combat drug resistance these days (Upadhyay 2019, 2021). Despite its many advantages, the combinatorial technique has a number of significant drawbacks. Because the medications in a combination have varied pharmacokinetic properties, the efficacy of a given combination therapy in vitro may not necessarily translate into a definite response in vivo (Mishra & Upadhyay 2022). Moreover, this approach is unable to deal with the issue brought about by MDR strains that show resistance to both medication classes used together. Consequently, the pattern of drug molecules has changed from "one molecule-one target" to "one molecule-multi-target." Various multitargeting compounds' polypharmacology can be used to treat various multifactorial ailments (Mishra *et al.* 2023; Mishra & Upadhyay 2022). This can be accomplished efficiently by creating hybrid compounds, which are created by combining many physiologically significant moieties (Bérubé 2016). This allows for the generation of leads with a wide variety and improvement in pharmacological qualities due to the leads' ability to influence multiple targets. The development of drugs with broad spectrum functionality can be facilitated by the hybridization of biologically active molecules, which presents an opportunity to create drugs with enhanced pharmacological properties for the treatment of various illnesses and diseases such as MDR bacterial infections, malaria, tuberculosis, and cancer (Pokrovskaya & Baasov 2010). Over the past twenty years, there has been a significant increase in the synthesis of hybrid compounds in the drug development sector to create scaffolds with remarkable and enhanced capabilities. In medicinal chemistry, the idea of a hybrid molecule offers several benefits, including the ability to cure co-infection, delay the onset of resistance, lower toxicity, and preclinical evaluation costs (Decker 2011; El-Sayed *et al.* 2022b). Furthermore, hybrid compounds reduce drug-drug interactions, improve dosage compliance, and lower the cost of preclinical study. Natural products played a significant role in the development of drugs in the early history of pharmacology. The combination of numerous naturally occurring scaffolds with different biological profiles to create natural product-based hybrid molecules has proven to be a successful strategy in the development of drugs to treat diseases with complex etiologies.

Conclusion and outlook

The traditional process of drug discovery entails the separation of bioactive molecules from plants or other natural resources, or the synthesis of lead molecules; the evaluation of bioactivities and structural modifications for lead optimization; clinical evaluations; and approval for marketing by the drug administration. More than half of the antimicrobials have been discovered and developed in this way. The discovery and development of antibiotics from natural resources have saved billions of lives by effectively controlling bacterial infections, but the development of resistance by pathogenic bacteria, viruses, fungi, and other parasites is making the present antimicrobial drugs ineffective. Several highly efficient antibiotics of the time have now become inactive due to the ever-increasing rise in drug-resistant strains of pathogenic microbes. The existing challenges in antimicrobial drug discovery can be set by the development of nature-inspired multifaceted hybrid molecules able to show broad-spectrum functionality. Further, in recent years, the application of computer-aided drug design (CADD) techniques such as molecular docking, molecular dynamics simulations, and quantitative structure-activity relationship (QSAR) modeling has had a significant impact on antibiotic drug design by enabling the rational design and optimization of new antibiotics with improved potency and selectivity. Overall, designing hybrid molecules with natural scaffolds with the application of CADD is the need of the hour to meet the current requirement for antimicrobials.

References

Achan, J., Talisuna, A. O., Erhart, A., … D'Alessandro, U. (2011). Quinine, an old anti-malarial drug in a modern world: Role in the treatment of malaria. *Malaria Journal*. doi:10.1186/1475-2875-10-144

Aldossary, S. A. (2022). Review on Pharmacology of Atropine, Clinical Use and Toxicity. *Biomedical and Pharmacology Journal*. doi:10.13005/bpj/2408

Baig, M. H., Ahmad, K., Rabbani, G., Danishuddin, M., & Choi, I. (2017). Computer Aided Drug Design and its Application to the Development of Potential Drugs for Neurodegenerative Disorders. *Current Neuropharmacology*, **16**(6). doi:10.2174/1570159x15666171016163510

Barber, T. M., Kabisch, S., Randeva, H. S., Pfeiffer, A. F. H., & Weickert, M. O. (2022). Implications of Resveratrol in Obesity and Insulin Resistance: A State-of-the-Art Review. *Nutrients*. doi:10.3390/nu14142870

Bérubé, G. (2016). An overview of molecular hybrids in drug discovery. *Expert Opinion on Drug Discovery*. doi:10.1517/17460441.2016.1135125

Breitel, D., Brett, P., Alseekh, S., Fernie, A. R., Butelli, E., & Martin, C. (2021). Metabolic engineering of tomato fruit enriched in L-DOPA. *Metabolic Engineering*, **65**. doi:10.1016/j.ymben.2020.11.011

Breuss, J. M., Atanasov, A. G., & Uhrin, P. (2019). Resveratrol and its effects on the vascular system. *International Journal of Molecular Sciences*, **20**(7). doi:10.3390/ijms20071523

Butler, M. S. (2005). Natural products to drugs: Natural product derived compounds in clinical trials. *Natural Product Reports*. doi:10.1039/b402985m

Butler, M. S. (2008). Natural products to drugs: Natural product-derived compounds in clinical trials. *Natural Product Reports*. doi:10.1039/b514294f

Calixto, J. B. (2019). The role of natural products in modern drug discovery. *Anais Da Academia Brasileira de Ciencias*, **91**. doi:10.1590/0001-3765201920190105

Caputi, L., Franke, J., Farrow, S. C., … O'Connor, S. E. (2018). Missing enzymes in the biosynthesis of the anticancer drug vinblastine in Madagascar periwinkle. *Science*, **360**(6394). doi:10.1126/science.aat4100

Chan, B. S., Isbister, G. K., Chiew, A., Isoardi, K., & Buckley, N. A. (2022). Clinical experience with titrating doses of digoxin antibodies in acute digoxin poisoning. (ATOM-6). *Clinical Toxicology*, **60**(4). doi:10.1080/15563650.2021.1968422

Chiu, C. (2013). Definitions, classifications, and antibiotics. In *Urinary Tract Infection: Clinical Perspectives on Urinary Tract Infection*. doi:10.1007/978-1-4471-4709-1_1

Choi, S. S., Lee, S. H., & Lee, K. A. (2022). A Comparative Study of Hesperetin, Hesperidin and Hesperidin Glucoside: Antioxidant, Anti-Inflammatory, and Antibacterial Activities In Vitro. *Antioxidants*, **11**(8). doi:10.3390/antiox11081618

Cragg, G. M., & Newman, D. J. (2013). Natural products: A continuing source of novel drug leads. *Biochimica et Biophysica Acta - General Subjects*. doi:10.1016/j.bbagen.2013.02.008

Cragg, G. M., Newman, D. J., & Snader, K. M. (1997). Natural products in drug discovery and development. *Journal of Natural Products*. doi:10.1021/np9604893

Dadgostar, P. (2019). Antimicrobial resistance: implications and costs. *Infection and Drug Resistance*. doi:10.2147/IDR.S234610

David, L., Brata, A. M., Mogosan, C., … Popa, S. L. (2021). Artificial intelligence and antibiotic discovery. *Antibiotics*. doi:10.3390/antibiotics10111376

Decker, M. (2011). Hybrid Molecules Incorporating Natural Products: Applications in Cancer Therapy, Neurodegenerative Disorders and Beyond. *Current Medicinal Chemistry*, **18**(10). doi:10.2174/092986711795328355

Dias, D. A., Urban, S., & Roessner, U. (2012). A Historical overview of natural products in drug discovery. *Metabolites*. doi:10.3390/metabo2020303

Dwivedi, G. R., Maurya, A., Yadav, D. K., Khan, F., Darokar, M. P., & Srivastava, S. K. (2015). Drug resistance reversal potential of ursolic acid derivatives against nalidixic acid- and multidrug-resistant Escherichia coli. *Chemical Biology and Drug Design*, **86**(3). doi:10.1111/cbdd.12491

E. N., Dr. S. (2017). CADD: PHARMACOLOGICAL APPROACHES IN DRUG DESIGN AND DRUG DISCOVERY. *World Journal of Pharmacy and Pharmaceutical Sciences*. doi:10.20959/wjpps20174-8999

Ebimieowei, E., & Ibemologi, A. (2016). Antibiotics: Classification and mechanisms of action with emphasis on molecular perspectives. *International Journal of Applied Microbiology and Biotechnology Rsearch*, **4**(September 2017).

El-Sayed, A. S. A., Fathalla, M., Yassin, M. A., … Sitohy, B. (2020). Conjugation of Aspergillus flavipes taxol with porphyrin increases the anticancer activity of taxol and ameliorates its cytotoxic effects. *Molecules*, **25**(2). doi:10.3390/molecules25020263

El-Sayed, A. S. A., Hassan, W. H. B., Sweilam, S. H., … Abdelaziz, S. (2022a). Production, Bioprocessing and Anti-Proliferative Activity of Camptothecin from Penicillium chrysogenum, “An Endozoic of Marine Sponge, Cliona sp.”, as a Metabolically Stable Camptothecin Producing Isolate. *Molecules*, **27**(9). doi:10.3390/molecules27093033

El-Sayed, W. A., Alminderej, F. M., Mounier, M. M., Nossier, E. S., Saleh, S. M., & Kassem, A. F. (2022b). Novel 1,2,3-Triazole-Coumarin Hybrid Glycosides and Their Tetrazolyl Analogues: Design, Anticancer Evaluation and Molecular Docking Targeting EGFR, VEGFR-2 and CDK-2. *Molecules*, **27**(7). doi:10.3390/molecules27072047

Eskandr, A. M., Ahmed, A. M., & Bahgat, N. M. E. (2021). Comparative Study Among Ephedrine, Norepinephrine and Phenylephrine Infusions to Prevent Spinal Hypotension During Cesarean Section. A Randomized Controlled Double-Blind Study. *Egyptian Journal of Anaesthesia*, **37**(1). doi:10.1080/11101849.2021.1936841

Fallah, M., Davoodvandi, A., Nikmanzar, S., … Mirzaei, H. (2021). Silymarin (milk thistle extract) as a therapeutic agent in gastrointestinal cancer. *Biomedicine and Pharmacotherapy*. doi:10.1016/j.biopha.2021.112024

Galm, U., & Shen, B. (2007). Natural Product Drug Discovery: The Times Have Never Been Better. In *Chemistry and Biology*, Vol. 14. doi:10.1016/j.chembiol.2007.10.004

Gillessen, A., & Schmidt, H. H. J. (2020). Silymarin as Supportive Treatment in Liver Diseases: A Narrative Review. *Advances in Therapy*. doi:10.1007/s12325-020-01251-y

G.M., C., & D.J., N. (2013). Natural products: A continuing source of novel drug leads. *Biochimica et Biophysica Acta - General Subjects*.

Harvey, A. L. (2008). Natural products in drug discovery. *Drug Discovery Today*. doi:10.1016/j.drudis.2008.07.004

Hasan, M. A., Khan, M. A., Sharmin, T., Hasan Mazumder, M. H., & Chowdhury, A. S. (2016). Identification of putative drug targets in Vancomycin-resistant Staphylococcus aureus (VRSA) using computer aided protein data analysis. *Gene*, **575**(1). doi:10.1016/j.gene.2015.08.044

Herradón, E., González, C., González, A., Uranga, J. A., & López-Miranda, V. (2021). Cardiovascular Toxicity Induced by Chronic Vincristine Treatment. *Frontiers in Pharmacology*, **12**. doi:10.3389/fphar.2021.692970

Jabir, N. R., Firoz, C. K., Zughaibi, T. A., … Tabrez, S. (2022). A literature perspective on the pharmacological applications of yohimbine. *Annals of Medicine*. doi:10.1080/07853890.2022.2131330

Jasovský, D., Littmann, J., Zorzet, A., & Cars, O. (2016). Antimicrobial resistance—a threat to the world’s sustainable development. *Upsala Journal of Medical Sciences*. doi:10.1080/03009734.2016.1195900

Jin, G. M., Du, L. Da, & Lu, Y. (2018). Agrimophol. In *Natural Small Molecule Drugs from Plants*, Springer Singapore, , pp. 563–568.

Kardos, N., & Demain, A. L. (2011). Penicillin: The medicine with the greatest impact on therapeutic outcomes. *Applied Microbiology and Biotechnology*. doi:10.1007/s00253-011-3587-6

Kitchen, D. B., Decornez, H., Furr, J. R., & Bajorath, J. (2004). Docking and scoring in virtual screening for drug discovery: Methods and applications. *Nature Reviews Drug Discovery*. doi:10.1038/nrd1549

Kohanski, M. A., Dwyer, D. J., & Collins, J. J. (2010). How antibiotics kill bacteria: From targets to networks. *Nature Reviews Microbiology*. doi:10.1038/nrmicro2333

Koltai, T., & Fliegel, L. (2022). Role of Silymarin in Cancer Treatment: Facts, Hypotheses, and Questions. *Journal of Evidence-Based Integrative Medicine*. doi:10.1177/2515690X211068826

Kumar, P., Singh, B., Thakur, V., … Chand, D. (2019). Hyper-production of taxol from Aspergillus fumigatus, an endophytic fungus isolated from Taxus sp. of the Northern Himalayan region. *Biotechnology Reports*, **24**. doi:10.1016/j.btre.2019.e00395

Lahlou, M. (2013). The Success of Natural Products in Drug Discovery. *Pharmacology & Pharmacy*, **04**(03). doi:10.4236/pp.2013.43a003

Laxminarayan, R., Duse, A., Wattal, C., … Cars, O. (2013). Antibiotic resistance-the need for global solutions. *The Lancet Infectious Diseases*. doi:10.1016/S1473-3099(13)70318-9

Lee, J. W., Maria-Solano, M. A., Vu, T. N. L., Yoon, S., & Choi, S. (2022). Big data and artificial intelligence (AI) methodologies for computer-aided drug design (CADD). *Biochemical Society Transactions*. doi:10.1042/BST20211240

Li, Y., Yin, Q., Wang, B., Shen, T., Luo, W., & Liu, T. (2022). Preclinical reserpine models recapitulating motor and non-motor features of Parkinson's disease: Roles of epigenetic upregulation of alpha-synuclein and autophagy impairment. *Frontiers in Pharmacology*, **13**. doi:10.3389/fphar.2022.944376

Lionta, E., Spyrou, G., Vassilatis, D., & Cournia, Z. (2014). Structure-Based Virtual Screening for Drug Discovery: Principles, Applications and Recent Advances. *Current Topics in Medicinal Chemistry*, **14**(16). doi:10.2174/1568026614666140929124445

Listos, J., Łupina, M., Talarek, S., Mazur, A., Orzelska-Górka, J., & Kotlińska, J. (2019). The mechanisms involved in morphine addiction: An overview. *International Journal of Molecular Sciences*. doi:10.3390/ijms20174302

Lopes, R. D., Rordorf, R., De Ferrari, G. M., … Wallentin, L. (2018). Digoxin and Mortality in Patients With Atrial Fibrillation. *Journal of the American College of Cardiology*, **71**(10). doi:10.1016/j.jacc.2017.12.060

M. Wainwright, M. C. (1990). The Story of Penicillin and the Golden Age of Antibiotics. *Blackwell*.

Ma, N., Zhang, Z., Liao, F., Jiang, T., & Tu, Y. (2020). The birth of artemisinin. *Pharmacology and Therapeutics*. doi:10.1016/j.pharmthera.2020.107658

Macalino, S. J. Y., Gosu, V., Hong, S., & Choi, S. (2015). Role of computer-aided drug design in modern drug discovery. *Archives of Pharmacal Research*. doi:10.1007/s12272-015-0640-5

Maddocks, S. (2013). Antimicrobial resistance: Global problems need global solutions. *Medical Journal of Australia*. doi:10.5694/mja13.c0318

Madsen, M. L., Due, H., Ejskjær, N., Jensen, P., Madsen, J., & Dybkær, K. (2019). Aspects of vincristine-induced neuropathy in hematologic malignancies: a systematic review. *Cancer Chemotherapy and Pharmacology*. doi:10.1007/s00280-019-03884-5

Martin, W. R., Sloan, J. W., Sapira, J. D., & Jasinski, D. R. (1971). Physiologic, subjective, and behavioral effects of amphetamine, methamphetamine, ephedrine, phenmetrazine, and methylphenidate in man. *Clinical Pharmacology and Therapeutics*, **12**(2). doi:10.1002/cpt1971122part1245

Mas-Capdevila, A., Teichenne, J., Domenech-Coca, C., … Crescenti, A. (2020). Effect of hesperidin on cardiovascular disease risk factors: The role of intestinal microbiota on hesperidin bioavailability. *Nutrients*. doi:10.3390/nu12051488

Mei, C., Lei, L., Tan, L. M., … Liu, Z. Q. (2020). The role of single strand break repair pathways in cellular responses to camptothecin induced DNA damage. *Biomedicine and Pharmacotherapy*. doi:10.1016/j.biopha.2020.109875

Meng, T., Xiao, D., Muhammed, A., Deng, J., Chen, L., & He, J. (2021). Anti-Inflammatory Action and Mechanisms of Resveratrol. *Molecules*. doi:10.3390/MOLECULES26010229

Mishra, K. N., Singh, S., Upadhyay, H. C., … Dwivedi, G. R. (2023). Synergy Potential of Ursolic Acid-Based Hybrid Molecules. *Letters in Drug Design & Discovery*, **20**(4), 469–478.

Mishra, K. N., & Upadhyay, H. C. (2022). Coumarin-1,2,3-triazole hybrids as leading-edge anticancer agents. *Frontiers in Drug Discovery*, **2**. doi:10.3389/fddsv.2022.1072448

Morrison, L., & Zembower, T. R. (2020). Antimicrobial Resistance. *Gastrointestinal Endoscopy Clinics of North America*. doi:10.1016/j.giec.2020.06.004

Mueller-Schoell, A., Michelet, R., Weinelt, F., Kloft, C., & Mikus, G. (2021). CYP2D6 phenotype explains reported yohimbine concentrations in four severe acute intoxications. *Archives of Toxicology*, **95**(8). doi:10.1007/s00204-021-03082-4

Murray, M. J., Moleron, R., Adamski, J., … Nicholson, J. C. (2022). Vinblastine monotherapy induction prior to radiotherapy for patients with intracranial germinoma during the COVID-19 pandemic. *Pediatric Blood and Cancer*, **69**(1). doi:10.1002/pbc.29359

Newman, D. J., & Cragg, G. M. (2016). Natural Products as Sources of New Drugs from 1981 to 2014. *Journal of Natural Products*. doi:10.1021/acs.jnatprod.5b01055

Newman, D. J., & Cragg, G. M. (2020). Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *Journal of Natural Products*. doi:10.1021/acs.jnatprod.9b01285

O'Daniel, P. I., Peng, Z., Pi, H., … Chang, M. (2014). Discovery of a new class of non-β-lactam inhibitors of penicillin-binding proteins with gram-positive antibacterial activity. *Journal of the American Chemical Society*, **136**(9). doi:10.1021/ja500053x

Ode, H. (2019). Digoxin. *Neuro-Ophthalmology Japan*, **36**(3). doi:10.11476/shinkeiganka.36.291

Ofoegbu, A., & B. Ettienne, E. (2021). Pharmacogenomics and Morphine. *Journal of Clinical Pharmacology*. doi:10.1002/jcph.1873

Park, J. H., Shim, J. K., Hong, H., & Lim, H. K. (2020). The efficacy of intramuscular ephedrine in preventing hemodynamic perturbations in patients with spinal anesthesia and dexmedetomidine sedation. *International Journal of Medical Sciences*, **17**(15). doi:10.7150/ijms.48772

Pokrovskaya, V., & Baasov, T. (2010). Dual-acting hybrid antibiotics: A promising strategy to combat bacterial resistance. *Expert Opinion on Drug Discovery*. doi:10.1517/17460441.2010.508069

Pyrzynska, K. (2022). Hesperidin: A Review on Extraction Methods, Stability and Biological Activities. *Nutrients*, **14**(12). doi:10.3390/nu14122387

Ren, B., Kwah, M. X. Y., Liu, C., … Goh, B. C. (2021). Resveratrol for cancer therapy: Challenges and future perspectives. *Cancer Letters*, **515**. doi:10.1016/j.canlet.2021.05.001

Saeheng, T., & Na-Bangchang, K. (2022). Clinical pharmacokinetics of quinine and its relationship with treatment outcomes in children, pregnant women, and elderly patients, with uncomplicated and complicated malaria: a systematic review. *Malaria Journal*, **21**(1). doi:10.1186/s12936-022-04065-1

Sausville, E. A. (2012). Drug Discovery. *Principles of Clinical Pharmacology, Third Edition*, 507–515.

Schneider, G., & Fechner, U. (2005). Computer-based de novo design of drug-like molecules. *Nature Reviews Drug Discovery*. doi:10.1038/nrd1799

Seidel, T., Wieder, O., Garon, A., & Langer, T. (2020). Applications of the Pharmacophore Concept in Natural Product inspired Drug Design. *Molecular Informatics*. doi:10.1002/minf.202000059

Soares, A. R., Marchiosi, R., de Cássia Siqueira-Soares, R., de Lima, R. B., dos Santos, W. D., & Ferrarese-Filho, O. (2014). The role of L-DOPA in plants. *Plant Signaling and Behavior*. doi:10.4161/psb.28275

Spellberg, B., Bartlett, J. G., & Gilbert, D. N. (2013). The Future of Antibiotics and Resistance. *New England Journal of Medicine*, **368**(4). doi:10.1056/nejmp1215093

Sperl, M. F. J., Panitz, C., Skoluda, N., … Mueller, E. M. (2022). Alpha-2 Adrenoreceptor Antagonist Yohimbine Potentiates Consolidation of Conditioned Fear. *International Journal of Neuropsychopharmacology*, **25**(9). doi:10.1093/ijnp/pyac038

Stohs, S. J., Shara, M., & Ray, S. D. (2020). p-Synephrine, ephedrine, p-octopamine and m-synephrine: Comparative mechanistic, physiological and pharmacological properties. *Phytotherapy Research*. doi:10.1002/ptr.6649

Strawbridge, R., Javed, R. R., Cave, J., Jauhar, S., & Young, A. H. (2023). The effects of reserpine on depression: A systematic review. *Journal of Psychopharmacology*. doi:10.1177/02698811221115762

Surabhi, S., & Singh, B. (2018). COMPUTER AIDED DRUG DESIGN: AN OVERVIEW. *Journal of Drug Delivery and Therapeutics*, **8**(5). doi:10.22270/jddt.v8i5.1894

Triarico, S., Romano, A., Attinà, G., … Ruggiero, A. (2021). Vincristine-induced peripheral neuropathy (Vipn) in pediatric tumors: Mechanisms, risk factors, strategies of prevention and treatment. *International Journal of Molecular Sciences*. doi:10.3390/ijms22084112

Upadhyay, A., & Beuerman, R. W. (2020). Biological Mechanisms of Atropine Control of Myopia. *Eye and Contact Lens*. doi:10.1097/ICL.0000000000000677

Upadhyay, H. C. (2019). Medicinal chemistry of alternative therapeutics: Novelty and hopes with genus ammannia. *Current Topics in Medicinal Chemistry*, **19**(10). doi:10.2174/1568026619666190412101047

Upadhyay, H. C. (2021). Coumarin-1,2,3-triazole Hybrid Molecules: An Emerging Scaffold for Combating Drug Resistance. *Current Topics in Medicinal Chemistry*, **21**(8). doi:10.2174/1568026621666210303145759

Upadhyay, H. C. (2023). Exploring Nature's Treasure for Drug Discovery. *Letters in Drug Design & Discovery*, **20**(4), 373–374.

Upadhyay, H. C., Mishra, A., Pandey, J., … Srivastava, S. K. (2021). In vitro, in vivo and in silico Antihyperglycemic Activity of Some Semi-synthetic Phytol Derivatives. *Medicinal Chemistry*, **17**. doi:10.2174/1573406417666201216124018

Velvadapu, V., Paul, T., Wagh, B., … Andrade, R. B. (2011). Desmethyl macrolides: Synthesis and evaluation of 4,8,10-tridesmethyl telithromycin. *ACS Medicinal Chemistry Letters*, **2**(1). doi:10.1021/ml1002184

Wagh, B., Paul, T., Debrosse, C., … Andrade, R. B. (2013). Desmethyl macrolides: Synthesis and evaluation of 4,8,10-tridesmethyl cethromycin. *ACS Medicinal Chemistry Letters*, **4**(11). doi:10.1021/ml400337t

Wang, J., Xu, C., Wong, Y. K., … Tu, Y. (2019). Artemisinin, the Magic Drug Discovered from Traditional Chinese Medicine. *Engineering*, **5**(1). doi:10.1016/j.eng.2018.11.011

WHO. (2020, October 13). WHO: Antimicrobial resistance.

Xavier Suresh, M. (2010). Computer aided drug design strategies for the development of novel beta lactam analogs targeting penicillin binding proteins. In *Proceedings of the 2nd International Conference on Trendz in Information Sciences and Computing, TISC-2010*. doi:10.1109/TISC.2010.5714614

Xiang, M., Cao, Y., Fan, W., Chen, L., & Mo, Y. (2012). Computer-Aided Drug Design: Lead Discovery and Optimization. *Combinatorial Chemistry & High Throughput Screening*, **15**(4). doi:10.2174/138620712799361825

Yang, C. P. H., & Horwitz, S. B. (2017). Taxol®: The first microtubule stabilizing agent. *International Journal of Molecular Sciences*. doi:10.3390/ijms18081733

Yang, Y. H., & Buttery, J. (2018). Antimicrobial resistance: a global one-health problem for all ages. *World Journal of Pediatrics*. doi:10.1007/s12519-018-0194-y

Zaman, S. Bin, Hussain, M. A., Nye, R., Mehta, V., Mamun, K. T., & Hossain, N. (2017). A Review on Antibiotic Resistance: Alarm Bells are Ringing. *Cureus*. doi:10.7759/cureus.1403

Zhang, J., Hansen, L. G., Gudich, O., … Keasling, J. D. (2022). A microbial supply chain for production of the anti-cancer drug vinblastine. *Nature*, **609**(7926). doi:10.1038/s41586-022-05157-3

CHAPTER 2

THE HERBS AND PHYTOMOLECULES AS KEY ASSISTERS TO ANTIBIOTICS FOR COMBATING MULTIDRUG-RESISTANT BACTERIAL INFECTIONS

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2.1 Introduction

Countless lives have been saved from numerous bacterial infections by antibiotics. However, the improper and widespread use of antibiotics has provided sufficient evidence for certain pathogenic bacteria to develop as resistant microbial populations (Hutchings *et al.* 2019). Antimicrobial resistance (AMR) is a major global health threat that occurs when bacteria, viruses, fungi, and parasites evolve to resist the effects of medications that were previously effective against them (Morrison & Zembower 2020). This resistance renders standard treatments ineffective, leading to persistent infections and an increased risk of spread to others. AMR, also known as drug resistance, has been observed at three escalating levels: multidrug resistance (MDR), extensive drug resistance (XDR), and pan-drug resistance (PDR). Multidrug resistance (MDR) is the failure to respond to at least one drug in three or more antimicrobial classes. In comparison, extensive drug resistance (XDR) indicates resistance to at least one drug in all but two or fewer antimicrobial classes. Lastly, pan-drug resistance (PDR) entails non-susceptibility to all antimicrobial agents across all available classes (Abdallah *et al.* 2023; Upadhyay 2023).

According to the WHO, antibacterial resistance is an endangering problem worldwide. Antibiotic resistance may be identified as natural resistance, acquired resistance, cross-resistance, and multidrug resistance, and generally, it follows five common mechanisms, including destructive enzymes, modifying antibacterial targets, efflux-reducing permeability, and creating alternative metabolic pathways (Laxminarayan *et al.* 2013; Upadhyay 2021). The total human consumption of antibiotics in Asian countries, including India and China, increased by 30% compared to European countries, such as the United States and the United Kingdom, from 2010 – 2015. The most recent worldwide global antibiotics resistance was published by World Health Organization in 2015 has listed *Escherichia coli*, *Klebsiella pneumonae*, *Cirtrobacter freundii*, and *Staphylococcus aureus* as the four greatest concern, associated with both hospital and community-acquired (Upadhyay 2021; Upadhyay *et al.* 2014a). In the United States alone, each year, at least two million people acquire serious infection, mainly *Mycobacterium tuberculosis* and MDR. *A. baumannii*, *Neisseria gonorrhea*, *P. aeruginosa*, and *Enterobacteriaceae*, leading to the death of 23,000 people, may be due to carrying extended spectrum β-lactamases (ESBLs) in bacteria which confers resistance to penicillin and cephalosporin (Koch *et al.* 2015; Pletz *et al.* 2015).

Medications with low bioavailability often fail to reach effective drug concentrations in the bloodstream, leading to inadequate therapeutic effects unless very high doses are administered, which can result in significant side effects (Atal & Bedi 2010; Kumar-Sarangi *et al.* 2018). Several factors can lead to poor oral bioavailability, such as inadequate dissolution and solubility in water, limited permeability through intestinal barriers, degradation in gastrointestinal and intestinal fluids, and metabolism in the intestines and liver, collectively contributing to drug resistance (Kumar-Sarangi *et al.* 2018). Biopotentiation or bioenhancement is the process of increasing the presence of a chemical substance in the biological fluid and systemic circulation, as well as the secondary chemicals responsible for raising the plasma drug concentration of the main component. Bioenhancers are herbal formulations, herbs, or phytomolecules that do not have significant biological activity but enhance the bioavailability of a drug when used in combination (Randhawa *et al.* 2011; Upadhyay *et al.* 2012a). Ideally, bioenhancers must be nontoxic, simple to formulate, should show their effect when combined with the drug at a very low concentration, and should increase the absorption and activity of the drug (Randhawa *et al.* 2011).

Plants remain a primary source of naturally occurring substances that are biologically active and either function as economically relevant entities in and of themselves or serve as lead structures for the production of modified derivatives with phytomolecules (Upadhyay *et al.* 2012a). Since plants are the main source of antimalarial medications, two novel herbal drugs for treating malaria are quinine and artemisinin. Herbal bioenhancers are a gift for mankind through medicinal plants. Based on the ancient knowledge of Ayurveda, herbal bio-enhancers are widely used nowadays due to their multifaceted nature (Singh *et al.* 2016). They are easy to make, effective at low doses, safe for both humans and animals, and they improve the drug's or nutrient's absorption and bioactivity (Kumar-Sarangi *et al.* 2018). Ayurveda

medicine contains several active medicinal elements that are being investigated by contemporary researchers even now; it is expected that these elements will change the way medicine is dispensed today by improving the bioavailability of existing drugs and thereby lowering the cost of drug development (Upadhyay *et al.* 2014a). This chapter encompasses the role of herbal formulations, herbs, and phytomolecules as key assisters to antibiotics for combating multidrug-resistant bacterial infections.

2.2 History of Bioenhancers

The scientists at the Regional Research Laboratory, Jammu (now known as the Indian Institute of Integrative Medicine) have conducted extensive research on molecules that improve the bioavailability of specific drugs. They have named these molecules "bioavailability enhancers". C.K. Atal, the Chairman of the Regional Research Laboratory at Jammu, coined the term "bioavailability enhancer" in 1979 when Piperine was identified and proven to be the world's first bioavailability enhancer (Atal & Bedi 2010). According to him, bioenhancers are compounds that, when ingested orally, do not have any independent pharmacological effects but instead augment biological activity or the absorption of the active ingredient, thus increasing bioavailability when used in combination therapy (Atal *et al.* 1981; Atal & Bedi 2010; Zutshi *et al.* 1985).

The Ayurvedic medical system's old wisdom is where the idea of bio-enhancers—herbal origins—came from as part of a multidrug prescription; black pepper, long pepper, and ginger are commonly prescribed for various ailments. This Ayurvedic mixture is called "Trikatu'' a Sanskrit word that means "three acrids" (Kumar-Sarangi *et al.* 2018).

2.3 Mechanism of Action of Bioenhancers

Various mechanisms have been proposed to explain the mode of action of bioenhancers. They may enhance a drug's efficiency by inhibiting drug efflux pumps, modifying drug-metabolizing enzymes, enhancing drug permeation by modifying cell membrane permeability, or inhibiting enzymes that degrade drugs. Most of them enhance the drug's bioavailability in tissues by one or more of the proposed modes of action. The mechanism of action suggested for the most explored bioenhancer, piperine, includes binding to DNA receptors, influencing cell signal transduction, and inhibiting drug efflux pumps.

2.3.1 Efflux Pump Inhibitors

Bacterial efflux pumps play a significant role in antimicrobial resistance (AMR). These pumps are membrane proteins that actively expel toxic substances, including antibiotics, out of bacterial cells. This reduces the intracellular concentration of these drugs and enables bacteria to survive despite antibiotics. For instance, in recent times, the complete genome sequence of *S. aureus* explored 30 efflux pump genes that belong to the major facilitator superfamily (MFS) (Stephen *et al.* 2023). The multidrug efflux pumps identified in *S. aureus* can be categorized into six groups: the resistance nodulation division (RND) superfamily, the ATP-binding cassette (ABC) superfamily, the drug metabolite 63 transporter (DMT) superfamily, the multidrug and toxin extrusion (MATE) family, and the major facilitator superfamily (MFS).(Hassanzadeh *et al.* 2020). In *S. aureus*, the ABC transporter family is the primary active system, whereas the MFS, SMR, and MATE transporter families are part of the secondary active system (Hassanzadeh *et al.* 2020).

Gram-negative *E. coli* utilizes several major efflux pumps that contribute to its ability to resist various antibiotics. The most common ones include AcrAB and TolC. Several tetralones and their semisynthetic analogs have been found as potent inhibitors of AcrA, AcrB, and TolC efflux pumps (Upadhyay *et al.* 2012b). In another study, phytol derivatives have been reported as potential bioenhancers that reduced tetracycline's minimum inhibitory concentration (MIC) up to 16 folds. The detailed study showed that the phytol derivatives inhibited bacterial efflux pumps (Upadhyay *et al.* 2014b).

2.3.2 Enzyme inhibitor

Bioenhancers that inhibit Cytochrome P450 (CYP-450) enzymes can significantly impact the metabolism and bioavailability of co-administered drugs. The CYP-450 family consists of several isoenzymes responsible for the oxidative metabolism of various endogenous and exogenous substances, including drugs. Inhibiting these enzymes can increase plasma concentrations of drugs metabolized by the same pathways, potentially enhancing their therapeutic effects. Several bioenhancers, including Naringin, Gallic acid and its ester derivatives, and Quercetin, increase the bioavailability of the drug by inhibiting the CYP-450 enzyme and isoenzyme.

2.3.3 Absorbance Enhancers

Reportedly, certain bioenhancers can boost drug absorption in the gastrointestinal tract or hinder enzymes that metabolize drugs, particularly in the liver, once the drug has been absorbed from the gastrointestinal tract. In rats,

administering piperine orally significantly suppressed hepatic aryl hydrocarbon hydroxylase (AHH) and UDP-glucuronyltransferase activities (Atal *et al.* 1985).

2.4 Classification of Bioenhancers

Bioenhancers derived from natural sources have been extensively studied because of their safety and compatibility with the animal metabolic system. Bioenhancers can be categorized into natural, semi-synthetic, or synthetic based on their origin. There are limited examples of synthetic Bioenhancers. Surfactants that include polysorbates (e.g., Tween 80) and sodium lauryl sulfate are compounds that can enhance drug solubility and permeability. Few of the cyclic oligosaccharides (e.g. cyclodextrins) can form inclusion complexes with drugs, improving their solubility and stability, and hence may be considered bioenhancers. However, using synthetic compounds as bioavailability enhancers to assist the drugs and antibiotics is rare.

2.4.1 Natural Bioenhancers

Most of the bioenhancers are nature's gift. Natural bioenhancers may be either plant-derived or derived from other natural resources. The most explored plant-derived bioenhancers are herbal formulations, herbs, or phytomolecules.

2.4.1.1 Herbs and Herbal Formulations as bioenhancers

Trikatu

The three ingredients found in trikatu are black pepper (*Piper nigrum*), long pepper (*Piper longum*), and ginger (*Zingiber officinale*). Trikatu is a potent decoction that can balance kapha, vatta, and pitta. The Ayurvedic approach focuses on the three humors of the body and how imbalances can lead to various ailments (Sadanandan & Prasad 2021). Piper species have been used internally to treat fevers, gastric and abdominal disorders, and urinary difficulties, as well as externally to treat rheumatism, neuralgia, and boils. *P. Iongum* and *P. nigrum* are also known as folklore remedies for asthma, bronchitis, dysentery, pyrexia, and insomnia (Kaushik *et al.* 2018). In Chinese folkloric medicine, *P. nigrum* is used to cure epilepsy (Kumar-Sarangi *et al.* 2018).

Zingiber officinale

Ginger (Zingiber officinale), a member of the Zingiberaceae family, strongly affects the gastrointestinal mucous membrane. Ginger improves absorption by regulating intestinal function, making it an excellent bioenhancer; the effective dose ranges from 10 to 30 mg/kg body weight. Ginger has also been observed to increase the bioavailability of the drug moieties of several drug classes, including antibiotics (cephalexin, 85%; cloxacillin, 90%; amoxicillin, 90%; rifampicin, ethionamide, 56%), antifungals (ketoconazole, 125%), antiretrovirals (zidovudine, 105%), and anticancer drugs (5-fluorouracil, 110%) (Kumar-Sarangi *et al.* 2018).

Piper nigrum

Piperine (1-piperoyl piperidine), an alkaloidal component of both Piper nigrum Linn. and Piper longum Linn. (or mixtures containing the same), enhances the bioavailability and efficacy of several drugs and other substances, including vasaka leaves vaccine, sparteine, phenytoin, rifampicin, sulfadiazine, and propranolol. Piperine's enhancing effect was first noted in the treatment of human tuberculosis (Waykar *et al.* 2019). We know now that when given in conjunction with piperine, the bioavailability of the antituberculosis drug rifampicin increases by about 60%, resulting in significant reductions in drug dosages (Zutshi *et al.* 1985). Piperine, in addition, has been demonstrated to bolster the effects of the non-nucleoside HIV-1 reverse transcriptase inhibitor nevirapine, as well as those of other antiretroviral agents (Dudhatra et al., 2012).

Curcuma longa

Curcumin, the active principle of turmeric (Curcuma longa) (a natural bioenhancer similar to piperine), has long been curative in several traditional medicinal systems. The yellow compound is isolated from the plant Curcuma longa and sees regular use in traditional Indian medicine to combat various respiratory conditions (asthma, bronchial hyperactivity, allergies and sinusitis, coryza, cough) but is also commonly employed to treat anorexia and hepatic disease. Evidence from numerous sources shows that this agent promotes anti-inflammatory, antioxidant, antimicrobial, and wound-healing activities. Documented also is it radio-sensitizing, chemotherapeutic, and chemosensitizing properties (Dudhatra et al., 2012). Notably, its bioavailability is increased in the presence of piperine: A 20 mg dose of piperine co-administered with curcumin has been found to increase the bioavailability of the latter as much as 20-fold in humans.

Glycyrrhiza glabra

The active component of licorice, glycyrrhizin, is a known bioenhancer that increases Taxol's cell-division inhibitory activity by fivefold, thereby inhibiting the growth and multiplication of the MCF-7 cancer cell line (4); the inhibition of

cancer cell growth using Taxol was found to be greater when combined with glycyrrhizin than when Taxol was used alone. Glycyrrhizin has also been shown to improve the transport across the intestinal membrane of many antibiotics (rifampicin, tetracycline, nalidixic acid, and ampicillin), vitamin B1, and vitamin B_{12}(Kumar-Sarangi *et al.* 2018).

Moringa oleifera

Niaziridin, a nitrile glycoside, was isolated from *Moringa oleifera* pods (Pareek *et al.* 2023). It has been found to enhance the bioactivity of commonly used antibiotics against both Gram-positive and Gram-negative bacteria, including *E. coli* (Murthy *et al.* 2011). When combined with rifampicin, ampicillin, and nalidixic acid, the anti-Grampositive properties of these drugs are enhanced by 1.2 to 19 times; the potency of the azole antifungal drug clotrimazole against Candida albicans is increased by 5- to 6-fold; and finally, niaziridin increases the absorption of vitamin B12 (40). An in vitro study that used fractioned hydroalcoholic extracts of *M. oleifera* to investigate this glycoside's protective effect against Mycobacterium tuberculosis (H37Ra) found initially that it has no such effect (Kumar-Sarangi *et al.* 2018).

Cuminum cyminum

Within a dosage range of 0.5 to 25 mg/kg body weight, the active ingredients found in hydroalcoholic extracts made from black cumin (Cuminum cyminum) have been demonstrated to improve the bioavailability of erythromycin, cephalexin, amoxicillin, fluconazole, ketoconazole, zidovudine, and 5-fluorouracil. Black cumin is not just a potent bioenhancer but also a carminative, anthelmintic, and stomach stimulant. Additional medicinal applications include its usage as a galactagogue, diuretic, and anti-diarrhea; in addition, hoarseness has been reported to respond well to its application (Kumar-Sarangi et al. 2018).

Aloe vera

Lawrence et.al. (2009) combined 2 different Aloe vera preparations (an inner-fillet gel and a whole-leaf extract) with either vitamin C or vitamin E. Their results showed that the 2 preparations improved the absorption of both vitamins, leading to their longer retention in blood plasma; that being the case, it seems probable that Aloe vera has a role as a nutritional herbal bioenhancer (Lawrence *et al.* 2009). The gel and whole leaf extracts of *Aloe vera* have been demonstrated to enhance the oral absorption of vitamin C and vitamin E (Chelu *et al.* 2023).The bioenhancing mechanism of action of these products is thought to be attributed to their polysaccharide's constituents. Polysaccharides of natural origin, such as chitosan, act as penetration enhancers through a process of a transient opening of the tight junctions between adjacent epithelial cells(Tariq *et al.* 2019). Thus, they can enhance the intestinal absorption of concurrently administered drugs. It has been shown that the *Aloe vera* gel and whole leaf extract significantly increased the transport of the macromolecular peptide drug, insulin, across the Caco-2cell monolayers (Kumar-Sarangi *et al.* 2018). Limited information is currently available on the drug absorption enhancement activities of *A. vera* extracts.

Carum carvi

Carum carvi (family Apiaceae), also called Caraway/cumin, has carvone as its major constituent, obtained from the dried and crushed seeds. At a dose in the range of 5 – 100 mg/kg body weight, this extract significantly enhanced the bioavailability of a wide range of drugs of various therapeutic classes, including antimicrobial, antileprosy, anti-inflammatory, anti-arthritic, cardiovascular, antihistaminics, CNS drugs, antiulcer, nutraceuticals, and herbal formulations. Its mode of action is by inhibition of P-gp efflux pump (Kumar-Sarangi *et al.* 2018) .

2.4.1.2 Phytomolecules and their derivatives as bioenhancers

Piperine

Plants belonging to the *Piperaceae* family, such as Piper longum (long pepper) and Piper nigrum (black pepper), contain the amide alkaloid piperine (1-piperoyl piperidine) (Zutshi *et al.* 1985). Piperine's bioenhancing ability was initially applied to treating tuberculosis in humans. It was discovered that piperine reduced the dosage of rifampicin from 450 mg to 200 mg by increasing its bioavailability by roughly 60% (Zutshi *et al.* 1985). In human medicine, it is permissible to use piperine with antitubercular medications (Dudhatra *et al.* 2012). When taken with Nevirapine, a strong non-nucleoside inhibitor of HIV-1 reverse transcriptase that is used in conjunction with other antiretroviral medications to treat HIV-1 infection, piperine also shown increased bioavailability. The active ingredient in turmeric, curcumin, is also more bioavailable when piperine is present (Figure 2.1).

Curcumin

The active ingredient found in the rhizome (also known as turmeric) of the herb Curcuma longa is known as curcumin, also known as diferuloylmethane. Due to its broad range of pharmacological actions, this hydrophobic molecule has traditionally been used to treat several illnesses. Curcumin is a generic term for structurally related chemicals called curcuminoids. Three main curcuminoids are typically found in commercially accessible curcumin: bisdemethoxycurcumin (3%), demethoxy curcumin (20%) and curcumin (77%), respectively. Early investigations into

the pharmacokinetics of curcumin in animal models have demonstrated that the substance is poorly absorbed from the gut following oral administration of various dosages (Figure 2.1) (Dudhatra *et al.* 2012).

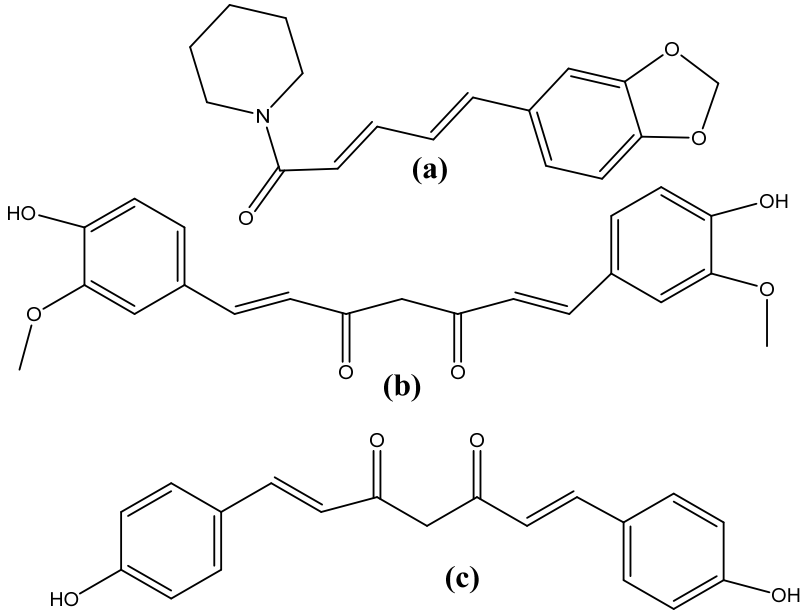


Figure 2.1: Phytomolecules as bioenhancers: (a) Piperine, (b) Curcumin, (c) Bisdemethoxycurcumin

Quercetin

Ubiquitous throughout nature, the plant-derived flavonoid quercetin is the aglycone form of several other flavonoid glycosides (e.g., rutin, quercitrin, kaempferol) and is found in Thuja occidentalis, Morus alba, and Quercus tinctoria (Islam *et al.* 2012; Wadhwa *et al.* 2022). Possessing antioxidant, radical scavenging, anti-inflammatory, and antiatherosclerotic capabilities, quercetin is also an inhibitor of CYP3A4 as well as a modulator of P-glycoprotein(Islam *et al.* 2012). Quercetin has been observed to enhance the bioavailability of and—therefore—increase the therapeutic efficacy of several drugs: epigallocatechin gallate (an anticancer component of green tea), diltiazem (used to treat angina pectoris, hypertension, and some types of arrhythmia), and digoxin (widely used for atrial fibrillation, atrial flutter, and heart failure) to name a few (Figure 2.2) (Kumar-Sarangi et al., 2018).

Lysergol

Lysergol is a bioactive alkaloid isolated from medicinal plant *Rivea corymbosa, Ipomoea violacea*, and *Ipomoea muricata* (Figure 2.2). Lysergol enhances the antimicrobial effects of different antibiotics, thus showing promising herbal bioenhancer (Kumar-Sarangi et al., 2018).

Genistein

Dietary plants that contain genistein, a phytoestrogen member of the isoflavone class of flavonoids, include kudzu (Pueraria lobata) and soybean (Glycine max). Breast cancer resistance protein (BCRP), multidrug resistance protein 2 (MRP2), and efflux function are among the effects of genistein that have been documented (Figure 2.2). Co-administering genistein with paclitaxel resulted in a considerable increase in intestinal absorption of the drug, which is a substrate for efflux transporters such P-gp and MRP2 (Kumar-Sarangi et al., 2018).

Naringin

Naringin is a CYP3A4 inhibitor. It is the predominant flavonoid glycoside in onions, apples, and grapefruit. Because of its potential to inhibit intestinal CYP3A4, this molecule has been shown to enhance the bioavailability of several CYP3A4 and P-gp substrates, including paclitaxel, diltiazem, verapamil, saquinavir, and cyclosporine (Dwivedi *et al.* 2019). It is a naturally occurring flavonoid found in citrus fruits (bergamot, orange, lemon, mandarin), and grapes and is responsible for the sour flavor of these fruits. It has potent anti-inflammatory and antioxidant properties and is used to treat obesity, excessive blood sugar, and high blood pressure (Dwivedi *et al.* 2019). This is also used in the culinary sector as a flavoring agent and to improve the texture of foods. It is also used to prevent bitterness in canned citrus foods (Atal & Bedi 2010).

Niaziridin

Niaziridin is a glycoside extracted from the leaves, pods, and bark of *Moringa oleifera*. Medicinally it is well known to be used in the treatment of arthritic pain, and hyperlipidemia, along with its anti-teratogenic potential to provide

protection to the embryo and fetus (Dwivedi *et al.* 2019). As per the Indian traditional system of medicine, it is also used for its potential activities such as hepatoprotection, anti-fertility, antimicrobial, anti-fungal, anti-cancer, anti-inflammatory, spasmolytic, anti-ulcer, and antioxidant profiles (Figure 2.2). It is found to have a bioenhancing effect on anti-fungal drugs, etc (Dwivedi *et al.* 2019).

Gallic acid

Gallic acid is a CYP3A4 inhibitor and its esters, propyl gallate, octyl gallate, and lauryl gallate, have been shown to en hance the bioavailability of orally taken medicinal drugs that are CYP3A4 substrates (Singh *et al.* 2016).

Sinomenine

Sinomenine, a monoterpene glucose alkaloid extracted from *Sinomenium acutum* is known to be a P-gp inhibitor (Dudhatra *et al.* 2012). Co-administration of sinomenine markedly increased the oral absorption of paeoniflorin through decreased efflux transport of the drug by P-gp in the small intestine (Figure 2.2). Paeoniflorin, used in treating inflammation and arthritic conditions, has a very low oral bioavailability of 3 – 4%.

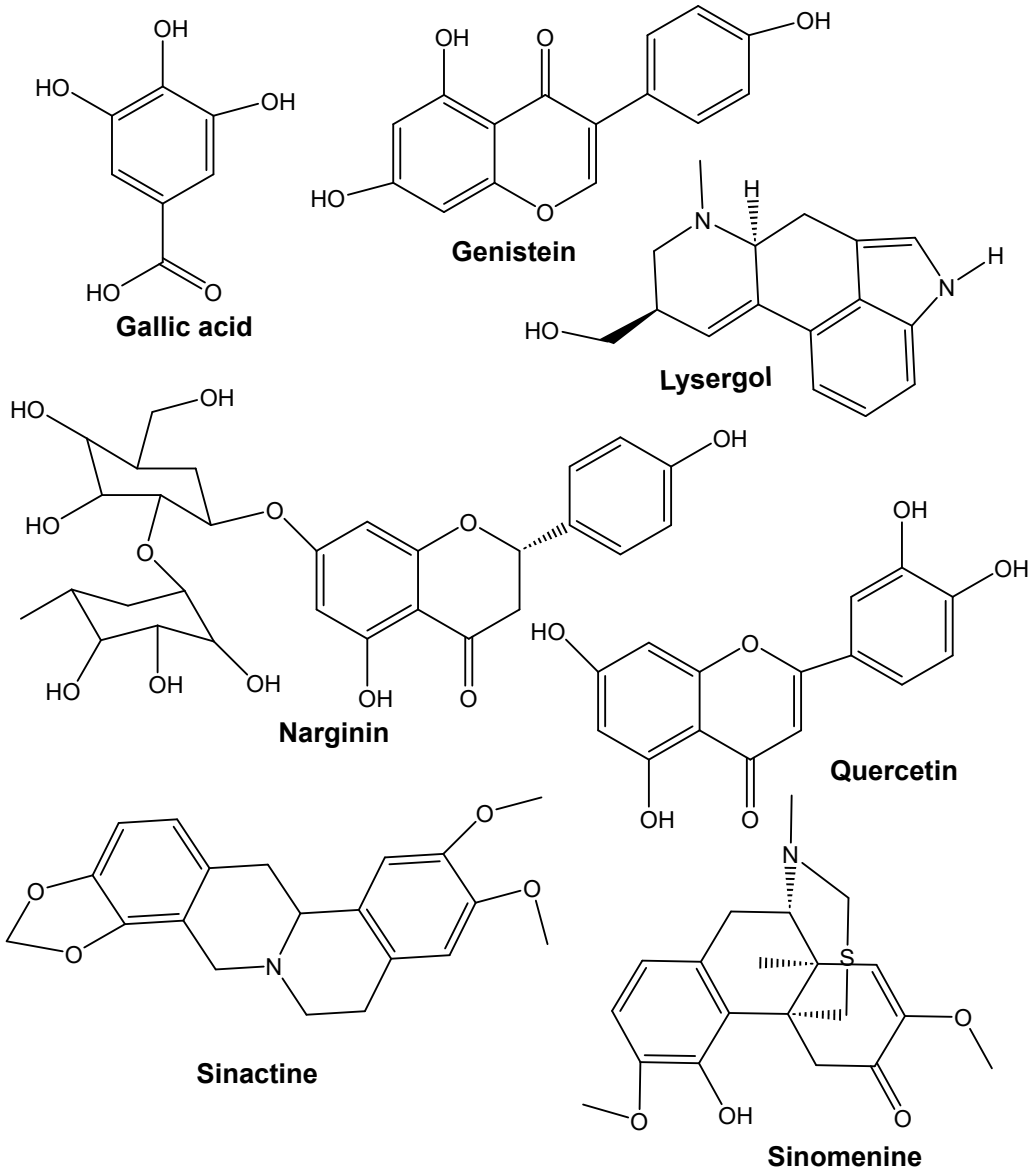


Figure 2.2: Some more phytomolecules as bioenhancers

Ginger phytoconstituents

Ginger contains many active components, including phenolic and terpene compounds (Figure 2.3). The primary phenolic compounds in ginger are gingerols, schools, and parasols. The major polyphenols in fresh ginger are gingerols, such as 6-gingerol, 8-gingerol, and 10-gingerol. Gingerols, Zingeberols, and Zingeberene are reported to have bioenhancing activities (Braga 2019).

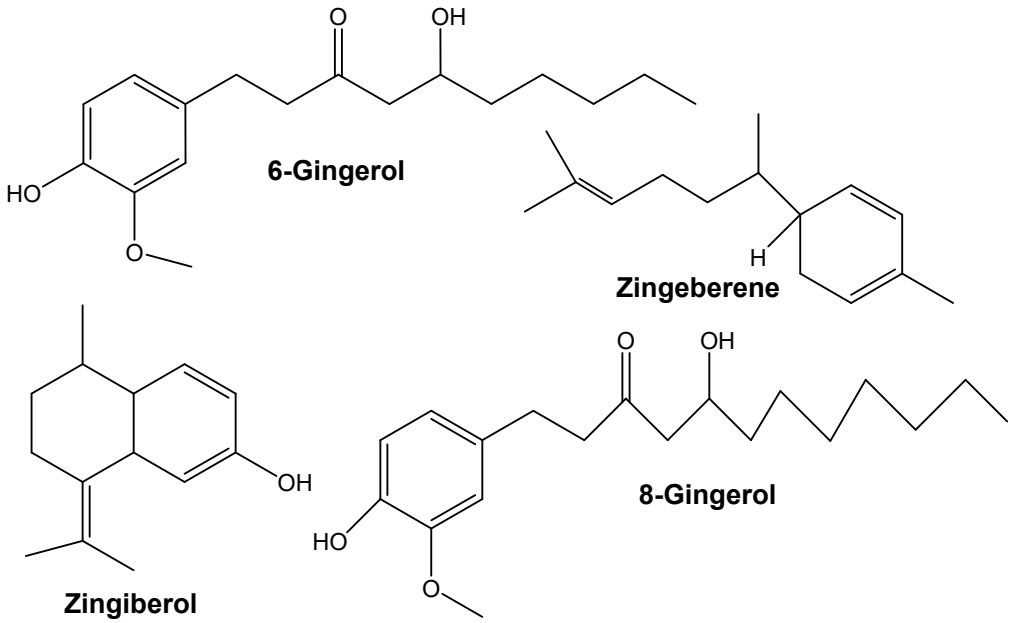


Figure 2.3: Ginger phytoconstituents as bioenhancers

2.5 Bioenhancing potential of *Ammannia* species

The genus *Ammannia* (family Lythraceae) is primarily found in damp or marshy areas known by most of the people as "Red stems," in both the temperate and worldwide tropical zones (Upadhyay 2019). Dymock *et al.* in 'Pharmacographia Indica' stated that *A. baccifera,* which is known as 'Dadmari' in local vernacular, has been utilized by the natives as a blistering agent (Upadhyay *et al.* 2012c). Nadkarni in 'Indian Materia Medica' has reported that leaves of *A. baccifera* are used to raise blisters in rheumatism when applied on the skin for half an hour or a little longer. The leaves or ashes of the plant, mixed with oil, are applied to cure herpetic eruptions (Upadhyay 2019). Perry, in 'Medicinal Plants of East and South Asia' has cited that *A. baccifera* has been used as an epispastic and has also been administrated to treat biliousness (Upadhyay 2019). In India, the dried leaves of *A. baccifera* are utilized for treating venereal diseases and also as an ingredient of the betel leaf chewing mixture by adult human (Upadhyay 2019). The hot water extract of *A. baccifera* when used orally by human adults, was found to be effective for renal or urinary calculi (Upadhyay *et al.* 2013b). The tribals of southern Rajasthan use *A. baccifera* as an herbal drug to treat guinea worm diseases (Upadhyay 2019; Upadhyay *et al.* 2013b). Traditional Chinese and Indian medicine makes extensive use of several species of the *Ammannia* genus. *Ammania baccifera*, one of this genus's species, is frequently used in traditional Chinese herbal remedies to treat spine disorders, gastroenteropathies, human female infertility, hamorrhoids, urethritis, common cold, abscess, sore, itching, and other skin diseases (Upadhyay et al., 2013; Upadhyay, Verma, et al., 2012).

Upadhyay et al. worked extensively on four species of this genus: *A. multiflora*, *A. baccifera*, *A. verticillata*, and *A. coccinea*. The phytochemical investigation of *A. multiflora* revealed the presence of many bioactive classes of compunds including flavonoids, reducing sugar, saponins, steroids, and phenolic glycosides (Upadhyay *et al.* 2011). These plant species and their isolates have shown potential antimalarial activity (Upadhyay *et al.* 2014c). The isolates of *A. multiflora*, *A. baccifera* and their semi-synthetic analogs have showed potential antihyperglycemic activity and antituberculosis activity (Upadhyay *et al.* 2012c, 2013b, 2013c).

2.5.1 Bioenhancing potential of Extracts of Ammannia multiflora

The methanolic extract of *A. multiflora* was evaluated for its antimicrobial activity against CA8000 and DH5 strains of *E. coli*. However, the extract was inactive against both strains (MIC > 500 µg/mL). Under efforts to study the bioenhancing potential, when 10 µg/mL of the methanol extract was used in combination with nalidixic acid (NAL), the MIC of NAL decreased to half i.e. 3.125 and 50 µg/mL than the NAL alone, which was 6.25 and 100 µg/mL for CA8000 and DH5α strains of *E. coli* respectively.

2.5.2 Bioenhancing Potential of Compounds isolated from Ammannia species and their Semi-synthetic Analogs

Upadhyay et al. isolated several compounds from *A. multiflora* and *A. baccifera*, and semi-synthetic analogs were synthesized to study their bioactivities. They isolated 4-Hydroxy-α-tetralone, 3,3'-(2R,5R)-tetrahydrofuran-2,5-diyldiphenol, 4-Hydroxy-α-tetralone glucoside, Quercetin-3-O-glucoside and Kempferol-3-o-rutinoside from *A. multiflora* (Figure 2.4).

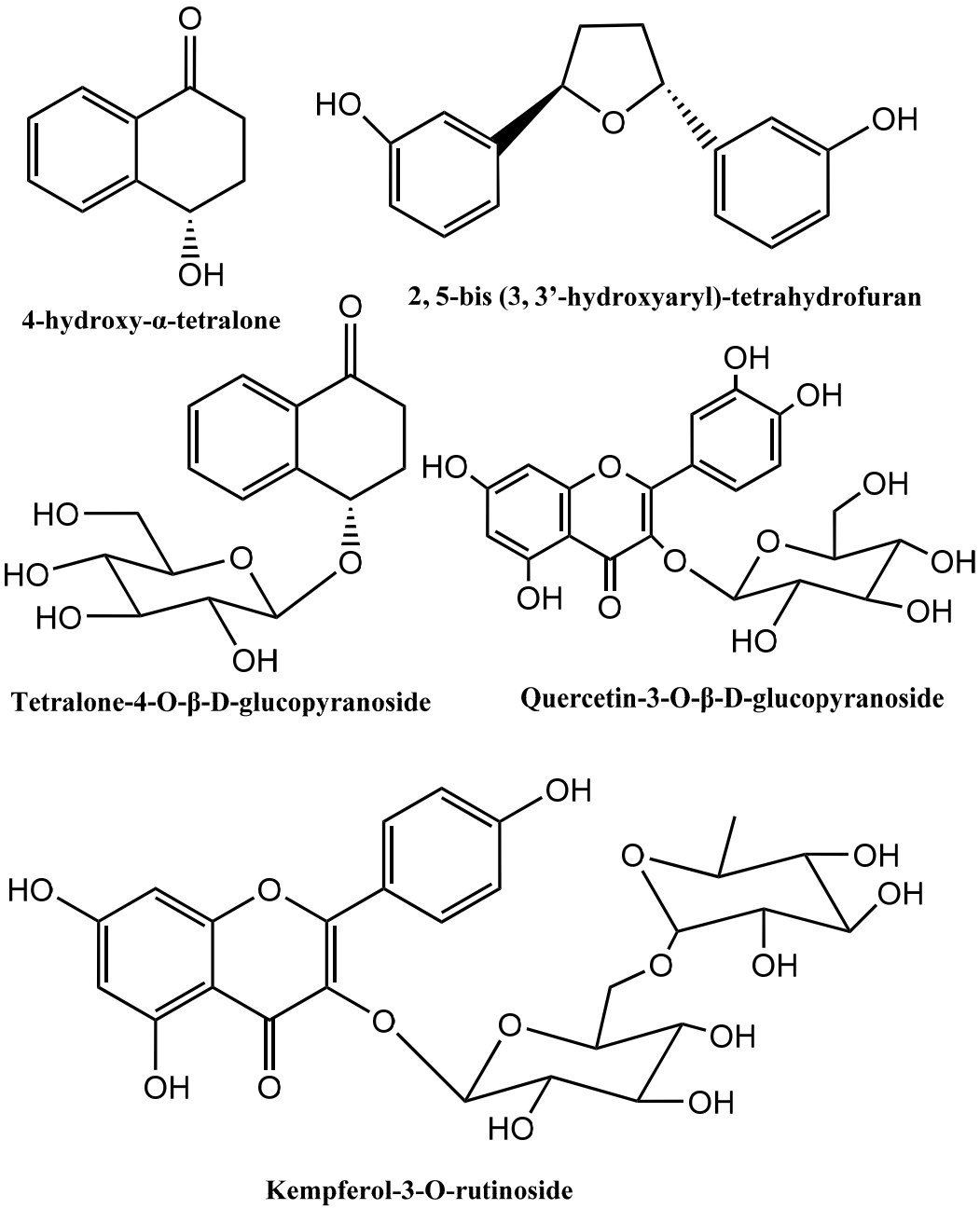
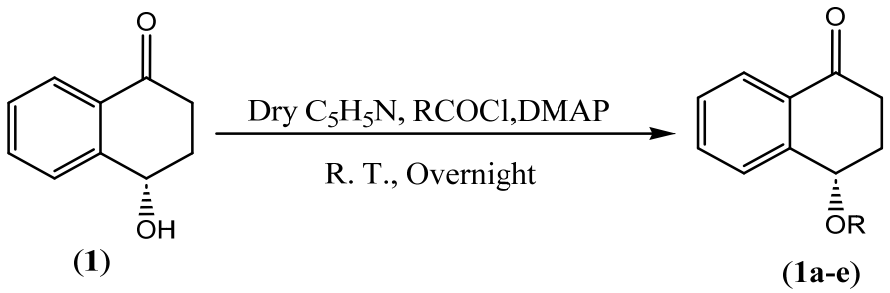


Figure 2.4: Bioenhancer compounds isolated from *A. multiflora*

Further, they applied a semi-synthetic approach to the major compound 4-Hydroxy-α-tetralone to obtain lipophilic ester derivatives. Finally, five derivatives 4-O-m-Anisyl-α-tetralone, 4-O-Cinnamoyl-α-tetralone, 4-O-Palmitoyl-α-tetralone, 4-O-(3,4,5-Trimethoxybenzoyl)-α-tetralone and 4-O-Myricitoyl-α-tetralone of 4-Hydroxy-α-tetralone were synthesized (Scheme 2.1).



(1) **(1a-e)**

1a: R= m-anisoyl **1b:** R= cinnamoyl

1c: R= palmitoyl **1d:** R= 3,4,5-trimethoxybenzoyl

1e: R= myristoyl

Scheme 2.1: Procedure for synthesis of 4-Hydroxy-α-tetralone ester analogs

All the isolated and semi-synthetic derivatives were individually evaluated for their *in vitro* antibacterial activity against CA8000 and DH5 strains of *E. coli* and bioenhancing activities in combination with NAL (Table 2.1).

Table 2.1: MIC of compounds alone and in combination with standard drug, Nalidixic acid (NAL)

S. No.	Compound	MIC of compound alone against *E. coli* strains (µg/mL)		MIC of NAL (µg/mL) in combination with 10 µg/mL of compound and fold reduction (FR) in MIC of NAL			
		CA8000	DH5α	CA8000		DH5α	
				MIC	FR	MIC	FR
1.	NAL	6.25	100	-	-	-	-
3.	2,5-Bis-(3,3′-hydroxyaryl) tetrahydrofuran	> 500	> 500	3.125	2	50	2
4.	4-Hydroxy-α-tetralone	500	500	1.56	4	25	4
5.	4-Hydroxy-α-tetralone glucoside	> 500	> 500	3.125	2	50	2
6.	Quercetin-3-O-glucoside	> 500	> 500	1.56	4	25	4
7.	Kempferol-3-o-rutinoside	> 500	> 500	3.125	2	50	2
8.	4-O-m-Anisyl-α-tetralone	> 500	> 500	3.125	2	50	2
9.	4-O-Cinnamoyl-α-tetralone	> 500	> 500	3.125	2	50	2
10.	4-O-Palmitoyl-α-tetralone	500	500	3.125	2	50	2
11.	4-O-(3,4,5-Trimethoxybenzoyl)-α-tetralone	500	500	3.125	2	50	2
12.	4-O-Myricitoyl-α-tetralone	> 500	> 500	3.125	2	50	2

It has been observed that new strains of multi-drug resistant organisms are frequently developing due to various mechanisms, among which efflux pumps are considered the major culprit. In a real-time polymerase chain reaction (RT-PCR) study, these tetralone derivatives inhibited ATP-dependent efflux pumps by down-expression of the efflux pump gene (yojI) encoding multidrug ATP-binding cassette (ABC) transporter protein (Dwivedi *et al.* 2014). The in silico docking studies further supported the study, which revealed a significant binding affinity of compounds with YojI (Dwivedi *et al.* 2014; Upadhyay *et al.* 2012a). In our studies, bioenhancer compounds reduced the antibiotic dose significantly, which can be related to the down-regulation of the gene yojI, which is responsible for the efflux pump mechanism in developing multidrug resistance in bacteria. The compounds substantially delay the resistance development process and can enhance the lifespan of novel and existing antibiotics.

Conclusion and Outlook

The current chapter acknowledges the various features of herbal bioavailability enhancers, including historical aspects, distinct philosophies, and methodologies employed in Ayurveda, as well as their chemical and pharmacological basis for herbal therapy. In developing countries like India, the cost of modern medications is a serious worry. The scientific community is focused on reducing dosage and, indirectly, the overall expense of treatment. Bio-enhancers minimize drug resistance and toxicity, leading to shorter treatment periods. Traditional herbal medicines could be quite helpful in

improving the bioavailability of allopathic medications. The herbs and phytoconstituents substantially delay the resistance development process and can enhance the lifespan of novel and existing antibiotics. It may be concluded that developing antibacterial combinations using bio enhancers for managing MDR infections may set a milestone in reducing the dose and cost burden on the common people.

References

Abdallah, E. M., Alhatlani, B. Y., de Paula Menezes, R., & Martins, C. H. G. (2023, July). Back to Nature: Medicinal Plants as Promising Sources for Antibacterial Drugs in the Post-Antibiotic Era. *Plants*, Multidisciplinary Digital Publishing Institute (MDPI). doi:10.3390/plants12173077

Atal, C. K., Dubey, R. K., & Singh, J. (1985). Biochemical basis of enhanced drug bioavailability by piperine: Evidence that piperine is a potent inhibitor of drug metabolism. *Journal of Pharmacology and Experimental Therapeutics*, **232**(1).

Atal, C. K., Zutshi, U., & Rao, P. G. (1981). Scientific evidence on the role of Ayurvedic herbals on bioavailability of drugs. *Journal of Ethnopharmacology*, **4**(2). doi:10.1016/0378-8741(81)90037-4

Atal, N., & Bedi, K. L. (2010). Bioenhancers: Revolutionary concept to market. *Journal of Ayurveda and Integrative Medicine*, **1**(2). doi:10.4103/0975-9476.65073

Braga, S. S. (2019). Ginger: Panacea or consumer's hype? *Applied Sciences (Switzerland)*. doi:10.3390/app9081570

Chelu, M., Musuc, A. M., Aricov, L., … Moreno, J. C. (2023). Antibacterial Aloe vera based biocompatible hydrogel for use in dermatological applications. *International Journal of Molecular Sciences*, **24**(4), 3893.

Dudhatra, G. B., Mody, S. K., Awale, M. M., … Chauhan, B. N. (2012). A comprehensive review on pharmacotherapeutics of herbal bioenhancers. *The Scientific World Journal*. doi:10.1100/2012/637953

Dwivedi, G. R., Maurya, A., Yadav, D. K., … Srivastava, S. K. (2019). Comparative Drug Resistance Reversal Potential of Natural Glycosides: Potential of Synergy Niaziridin & Niazirin. *Current Topics in Medicinal Chemistry*, **19**(10). doi:10.2174/1568026619666190412120008

Dwivedi, G. R., Upadhyay, H. C., Yadav, D. K., … Darokar, M. P. (2014). 4-Hydroxy-α-tetralone and its derivative as drug resistance reversal agents in multi drug resistant Escherichia coli. *Chemical Biology and Drug Design*, **83**(4). doi:10.1111/cbdd.12263

Hassanzadeh, S., ganjloo, S., Pourmand, M. R., Mashhadi, R., & Ghazvini, K. (2020). Epidemiology of efflux pumps genes mediating resistance among Staphylococcus aureus; A systematic review. *Microbial Pathogenesis*. doi:10.1016/j.micpath.2019.103850

Hutchings, M., Truman, A., & Wilkinson, B. (2019). Antibiotics: past, present and future. *Current Opinion in Microbiology*, Elsevier Ltd, , pp. 72–80.

Islam, M., Al-Amin, M., Siddiqi, M. M. A., … Chowdhury, A. S. (2012). Isolation of Quercetin-3-O-beta-d-glucopyranoside from the Leaves of *Azadirachta Indica* and Antimicrobial and Cytotoxic screening of the Crude Extracts. *Dhaka University Journal of Science*, **60**(1). doi:10.3329/dujs.v60i1.10328

Kaushik, R., Jain, J., Khan, A. D., & Rai, P. (2018). Trikatu - A combination of three bioavailability enhancers. *International Journal of Green Pharmacy*, **12**(3).

Koch, A. M., Nilsen, R. M., Eriksen, H. M., Cox, R. J., & Harthug, S. (2015). Mortality related to hospital-associated infections in a tertiary hospital; repeated cross-sectional studies between 2004-2011. *Antimicrobial Resistance and Infection Control*, **4**(1). doi:10.1186/s13756-015-0097-9

Kumar-Sarangi, M., Chandra-Joshi, B., & Ritchie, B. (2018). Natural bioenhancers in drug delivery: An overview. *Puerto Rico Health Sciences Journal*.

Lawrence, R., Tripathi, P., & Jeyakumar, E. (2009). Isolation, purification and evaluation of antibacterial agents from Aloe vera. *Brazilian Journal of Microbiology*, **40**, 906–915.

Laxminarayan, R., Duse, A., Wattal, C., … Cars, O. (2013). Antibiotic resistance-the need for global solutions. *The Lancet Infectious Diseases*. doi:10.1016/S1473-3099(13)70318-9

Morrison, L., & Zembower, T. R. (2020). Antimicrobial Resistance. *Gastrointestinal Endoscopy Clinics of North America*. doi:10.1016/j.giec.2020.06.004