1

Introduction

Nature have bestowed upon the humankind with resources far more than one can utilize. These resources have been utilized by man since antiquity and several of these evidences have been found through preserved and written documents, monumental evidences and original plants medicines. Various civilizations throughout history have been instrumental in the uses and development of medicinal plants [1,2]. The uses of these natural resources have been vital for the survival of the human species on earth. The use of medicinal plants has been reported as a pivot for primary healthcare in over 80% of the world's population and approximately 80% of all the synthetic drugs were derived from such plants [3]. Plant based bioactive molecules could be seen being used in the betterment of human society since morphine, the first alkaloid isolated by H.E. Merck in Germany in 1826. As such medicinal plants have been at the forefront of human aid in terms of combating various disorders [4].

1.1 Medicinal plants

Plants, which are vital for the survival of the human race and have benefits in one or more of its parts and from which various substances can be isolated for the treatment of human ailments are referred to as medicinal plants [5]. Medicinal plants are known to possess potent therapeutic properties and impart various pharmacological benefits. The traditional therapeutic use of medicinal plants can be traced back to over thousandsof years ago which were used for the treatment of various ailments worldwide. In folk medicines, varied indigenous system of medicine like Siddha, Ayurveda and Unani were in existence since ages and were used in treatment of several ailments [6]. Medicinal plants form the backbone of traditional medicine where around 3.3 billion population relies on its use for curing health ailments on persistent basis in less developed nations [7]. (WHO) World Health Organization reported the use of

medicinal plants by about 80% of the world's population living in the developing nations. It is estimated that there are approximately 3,74,000 species of plants [8] of which 28,187 have medicinal properties [9]. In the Indian context, the Botanical Survey of India (BSI) reported the presence of approximately 44,500 species of plant. It accounts for about 7% plant species of the world and approximately 28% plant species are endemic to the region [10]. The bioactive composition of plants are the major components that aids in the treatment of various ailments. These compounds once isolated and identified can be used further as a new lead molecule and aid in the drug discovery process [11].

The WHO has promoted the addition of herbal drugs in National Health Care programs as they are environment friendly, cost effective and safer to use with lesser side effects compared to the modern synthetic drugs [12]. Therefore, scientific research on pharmacologically active compounds derived from the screening of natural sources like plant extracts can lead to the discovery of pharmaceutically valuable drugs which may play a key role in the treatment of various human diseases. Some examples of plants being used are as follows: serpentine isolated from the Indian plant *Rauwolfia serpentine* root is used in the treatment of hypertension and lowering of blood pressure. Another potent medicine called vinblastine which is used for the treatment of leukaemia in children, Hodgkin's choriocarcinoma, non- Hodgkin's lymphomas, testicular and neck cancer was isolated from the *Catharanthus roseus*. Indian indigenous tree of *Nothapodytes nimmoniana* is now being frequently used in Japan for the treatment of cervical cancer [13].

1.2 History of medicinal plants

Since primeval times, society looked upon for drugs in nature for the treatment of diseases. With time, the reasons for the use of traditional medicinal plants were being discovered which gradually abandoned the empiric framework, and from the 16th century, plants have been used as the source of treatment and prophylaxis of various diseases [14,1].

The oldest written evidence of medicinal plants' usage for preparation of drugs was found on a "Sumerian clay slab" which was obtained from Nagpur and was approximately 5000 years old. The slab was found to comprise 12 different varieties of drug formulations using over 250 various plants [14]. The Ayurveda, Indian holy book

on plants discusses about the treatment of various ailments by using plants [15]. “Pen T’Sao,” written by the Chinese Emperor Shen Nung circa 2500 BC, is a Chinese book on roots and grasses and it details about the uses of 365 drugs which were obtained from the dried parts of medicinal plants. Some of these plants are *Rhei rhisoma, Theae folium, Podophyllum,* camphor, cinnamon bark, ginseng, jimson weed and ephedra [16].

Various writers throughout history documented different medicinal plants. One of the most prolific writers was Dioscorides, also known as “the father of pharmacognosy,” studied several medicinal plants in places wherever he travelled with the Roman Army [1]. In circa 77 AD, he documented the book “*De Materia Medica”* where he elaborated about 657 drugs from plant origin [17]. Preceding that, Theophrastus (Father of Botany), a Greek scientist, founded botanical sciences through his books “*De Causis Plantarium”* and “*De Historia Plantarium”.* In both the books he classified more than 500 plant species [18,19].

The Republic of Macedonia enacted a new law on Drugs and Medical Devices in September, 2007 where it was stated that dry or fresh parts of medicinal plants or any herbal substances may be used in the preparation of various herbal drugs and other processed products. In the same law, it was enacted that herbal substances may also be implemented in the preparation of homeopathic drugs so as to enhance the efficacy [1].

1.3 Natural products derived from medicinal plants

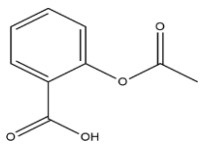
Medicinal plants are known to possess various chemical molecules necessary for survival against various exogenous and endogenous stresses. These chemicals, collectively known as secondary metabolites or natural products are usually synthesized by plants to tolerate any environmental changes without disrupting their normal cellular and physiological processes. Approximately, more than 100,000 natural products are found in Plant Kingdom, which are involved in plant defence mechanism against innumerable biotic and abiotic stresses [20].

Plant based natural products (NPs) play a vital role in drug discovery. These natural products may be bioactive compounds which deliver a host of pharmacological activities that are useful in the treatment of various diseases. They are further characterized by enormous biological diversity and structural complexity, serving as a major source of oral drugs. However, natural products must follow certain important rules to be considered as drugs. One of the major regulations they must follow is the

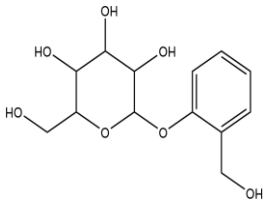
Lipinski's rule of five. It has been observed that natural products, that follow the Lipinski's rule, demonstrate better efficacy [21].

Once morphine was isolated, it paved the way for further isolation of purified bioactive molecules from plants. Salicylic acid, a plant metabolite with diverse medical uses was first isolated from the bark of the Willow plant. Further, acetylation of salicylic acid paved the way for the discovery of aspirin which was a revolutionary drugand the Nobel Prize in Physiology or Medicine was awarded for discovery of the mechanism of action of aspirin to Sir John Vane in 1982 (Fig. 1.1) [22].

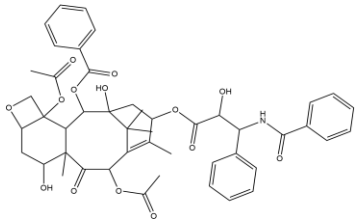
Studies on medicinal plants led to the isolation of various other drugs such as cocaine, digitoxin, codeine, quinine and pilocarpine which are in use till date. Apart from the above, paclitaxel from *Taxus brevifolia,* artemisinin from traditional Chinese plant *Artemisia annua* and silymarin from the seeds of *Silybum marianum* are used in the treatment of lung, ovarian and breast cancer, combating against multidrug-resistant malaria and treatment of liver diseases. These are among the few examples of natural product isolated from medicinal plants for treatment of several ailments [4].



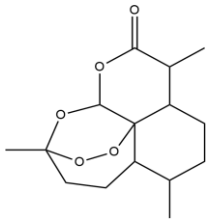
1. **Aspirin**



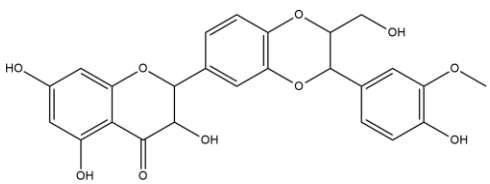
2. Salicin



3. Paclitaxel



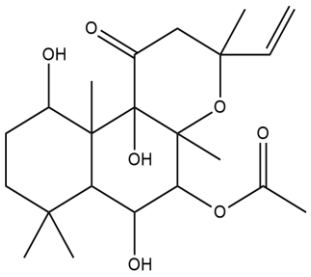
4. Artemisinin



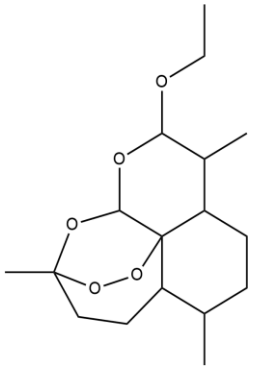
5. Silymarin

Fig. 1.1 Structures of first natural product derived from medicinal plants

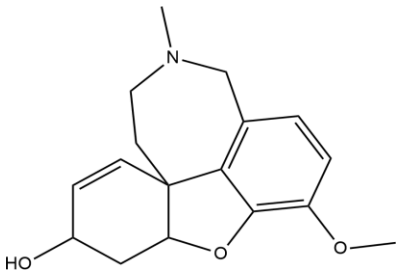
With the advent of modern pharmacology, molecular techniques and clinical observations; various old molecules previously deemed as redundant were found to have new applications. Forskolin, an alkaloid obtained from the plant *Coleus forskohlii* and various bioactive compounds derived from *Stephania glabra*, were rediscovered as potential adenylate cyclase and nitric oxide activators. Such molecules are useful in the prevention of conditions such as obesity and atherosclerosis. In the last couple of decades, a few plant-derived drugs were launched in the market. For the treatment of malaria, bioactive molecules such as arteether, endoperoxide sesquiterpene lactone and semisynthetic natural product, derived from artemisinin are used; nitisinone, derived from the natural product leptospermone (*Callistemon citrinus*) is used in the treatment of anti-tyrosinemia; galantamine, a natural alkaloid derived from the plant *Galanthus nivalis* has been found to be promising for the treatment of Alzheimer's; apomorphine, a semisynthetic compound obtained from morphine, derived from the plant *Papaver somniferum,* is used in the treatment against Parkinson's disease (Fig. 1.2). Besides the above, various other compounds such as tiotropium which is a derivative of atropine originally obtained from *Atropa belladonna*, is used in the treatment of chronic obstructive pulmonary disease; dronabinol and cannabidiol isolated from *Cannabis sativa* and capsaicin isolated from *Capsicum annuum* are used as effective painrelievers [4].



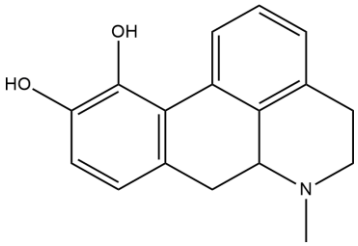
1. Forskolin



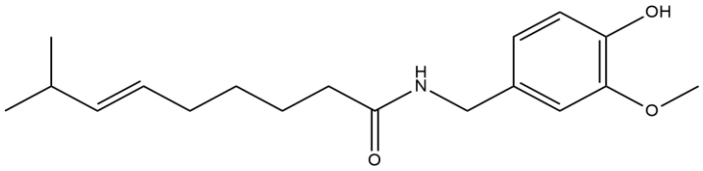
2. Arteether



3. Galantamine



4. Apomorphine



5. Capsaicin

Fig. 1.2 Some examples of natural product isolated from medicinal plants as therapeutic agents

Plant secondary metabolites are usually found to be accumulated at cellular, organ and tissue levels through various biosynthetic pathways. The plant natural products are classified into three major classes: phenolics, terpenoids and alkaloids. The metabolites belonging to these classes possess wide applications in nutraceuticals, pharmaceuticals and cosmetics.

Various pathways are involved in the synthesis of these compounds of which, the shikimic acid pathway is responsible for the production of phenolics [20, 23]. Mevalonic acid and methylerythritol phosphate pathways are responsible for the production of terpenoids while the amino acid and isoamylene biosynthetic pathway is responsible for the production of alkaloids. Various important pharmacological properties are associated with these secondary metabolites, *viz,* the phenolics have defense properties and are antioxidant compounds while, the terpenoids have been found to contain anticarcinogenic, antimalarial, diuretic properties [23]. Alkaloids have been found to possess anti-hypertensive, anti-malarial, anti-arrhythmic and anti-cancer properties [24]. A comprehensive example of other medicinal plants with antibacterial activities against MRSA are listed in Table 1.1

Table 1.1 List of medicinal plants with antibacterial properties

Medicinal plants	Phyto-active compounds	Antibacterial assay method	MIC (µg/mL)	Reference
Psidium guineense Swartz	Tannins Flavonoids Saponins Alkaloids Condensed proanthocyanidins Leucoanthocyanidins Phenylpropanoids, Iridoids	MIC MBC	250-500 µg/ml	[274]
Cocos nucifera	Procyanidins	MIC, MBC Agar diffusion method	1024 µg/ml	[275]
Chelidonium majus Linn	Alkaloids	MIC, MBC	0.49-15.63 µg/ml	[276]
Camellia sinensis	Polyphenols	MIC	50-180 µg/ml	[277]
Blechnum orientale Linn	Flavonoids Terpenoids Tannins	MIC, MBC Disc diffusion test	15.6-250 µg/ml	[278]
Mentha longifolia	Corilagin Tellimagrandin	MIC, MBC MIC, MBC MIC MBC	3.125-12 mg/ml 12.50-25.00 mg/ml 0.395-0.780 µg/ml 1.563-3.125 mg/ml	[277]
Heicanthus elastica	Phenolic compounds	MIC Agar diffusion method	250-500 µg/ml	[279]

1.4 Essential oil derived from medicinal plants

Essential oils (EOs) are a complex mixture of molecules containing more than 20 different components. These compositions are of low molecular weight and are usually obtained from aromatic medicinal plants. They are a volatile mixture of chemical compounds with an odour. Alternatively, they are also known as volatile oil [25]. The major components of essential oils are monoterpenes and sesquiterpenes along-with diterpenes and phenylpropanoids, thus making up to 70% of the total oil imparting potent biological properties [26,27]. The essential oils are generally extracted from the medicinal and aromatic medicinal plants by using either hydro-distillation or Soxhlet extraction methods [28]. The essential oils are regarded as one of the major plant products. They serve varieties of roles in agriculture, and exhibits anti-bacterial, anti-

fungal, anti-oxidant, anti-diabetic, anti-cancer, anti-viral, insect repellent, and anti-inflammatory properties [26]. Essential oil obtained from *Thymbra spicata* is known to possess good antioxidant and antimicrobial properties against a host of microorganisms such as *Streptomyces murinus, Staphylococcus aureus*, *Micrococcus luteus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Proteus vulgaris*, *Candida albicans, Yersinia enterocolitica and Aspergillus niger*. Ginger oil is also found to possess prominent antioxidant and anti-inflammatory properties and it has been proved that the essential oil isolated of *Curcuma* herbs imparts anti-carcinogenic activity against LNCaP and HepG2 cells [29].

Essential oils play a vital role in possessing cytotoxic property. The various mechanisms by which the cytotoxic effect of essential oils is exhibited are as follows:

1. By induction of cell death through activation of apoptosis or necrosis.
2. Arrest of cell cycle, and
3. Through loss of function of essential organelles.

The above mechanisms exhibited by essential oils are due to the lipophilic nature and low molecular weight of the various constituents present. The presence of these properties enables the components to cross the cell membrane and alter the membrane composition and increasing membrane fluidity thus causing leakage of ions and cytoplasmic molecules. The alteration caused in the cellular membranes can trigger reduced ATP production, alteration of pH and loss of mitochondrial potential resulting in cell death. Besides, there are certain essential oils which acts as pro-oxidant elements which can alter the cellular redox state of the cells and therefore compromise cellular survival [27].

*1.5*Medicinal Plants as antimicrobial agents against *Staphylococcus aureus*

Plant-derived bioactive compounds, primarily secondary metabolites, are used for medicinal purposes due to their wide antimicrobial activity range. Bioactive plant extracts contain complex mixtures of ingredients that can enhance their effects on microbial cells [30]. These compounds target the cytoplasmic membrane, affecting its structure, integrity, permeability, and functionality [31]. Some plant extracts may contain inhibitors of exopolysaccharide (EP) and inhibition of normal cell communication, known as quorum sensing (QS), which can be effective against MDR pathogens in particular *Staphylococcus aureus*. Medicinal plants can modify or inhibit

protein-protein interactions, acting as effective modulators of immune response, mitosis, and apoptosis. They can interfere with intermediary metabolism, induce coagulation of cytoplasmic constituents, and disrupt biofilm formation, providing a protective advantage to pathogens during infection [32].

Green tea, *Camellia sinensis*, a plant known for its antioxidant and antimicrobial properties, has been found to be effective against *S. aureus* [33]. Catechin, a polyphenol found in green tea, is responsible for its activity. The plant contains four forms of catechin, including epicatechin, epigallocatechin, EC-3-gallate, and EGC-3-gallate. Catechin has metal-chelating properties, making it more effective as an antioxidant. Catechin gallates, with two distinct binding sites in the NorA substrate, effectively counteract *S. aureus* resistance by inhibiting the efflux pump at high concentrations [34,35].

Berberine, an isoquinoline alkaloid found in various herbs like *Hydrastis canadensis* L., *Berberis aristata* DC., *Coptis chinensis* Franch., *Coptis japonica* (Thunb.) Makino., and *Phellodendron amurense* Rupr., has been known for its antimicrobial activity in China for thousand years, possibly due to interaction with bacterial DNA. This antibacterial action may be mediated by interaction with bacterial DNA, inhibition of bacterial protein FtsZ responsible for cell division, and enzyme targeting in *S. aureus* [36,9].

Essential oils have a killing effect on bacteria due to their hydrophobicity. They cause cell permeability changes and rupture when entering cells. *Chenopodium ambrosioides* L. essential oil and α-terpinene, can inhibit *S. aureus* by inhibiting the NorA efflux pump [37].

1.6 Production of biofilm by *Staphylococcus aureus* and its mechanism of resistance

Staphylococcus aureus produces a multilayered biofilm within a glycocalyx or slime layer, with heterogeneous protein expression. The solid component is primarily composed of teichoic acid, staphylococcal and host proteins. Later, a specific polysaccharide antigen called polysaccharide intercellular antigen (PIA) was isolated, composed of β-1,6-linked N-acetylglucosamine residues and an anionic fraction with phosphate and ester-linked succinate (15-20%) [38,39]. *S. aureus* create three-dimensional biofilms through a complex process consisting of four stages: adhesion, aggregation, maturation, and dispersion (Fig. 1.3) [40].

1. **Adhesion stage**: *S. aureus* planktonic cells use various factors and regulatory mechanisms during the adhesion stage to promote host-bacterial combination. These include cell wall-anchored protein, adhesin, and eDNA expression, and microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), which mediate bacterial adhesion to natural tissues and biomaterial surfaces [41,42].
2. **Aggregation stage**: Bacterial cells adhere to surfaces, divide, and accumulate, regulating biofilm formation through environmental signals. They proliferate and thicken, providing resistance against the human immune system and antibiotics. Bacterial cells in the matrix depend on cell-cell and cell-EPS adhesion [43,44].
3. **Maturation stage**: Mature biofilms have a highly structured, compact mushroom or tower structure, with numerous pipes for nutrient transport, and unique metabolic structures that resist environmental factors and stress [45]. EPS, attached by bacterial cells, forms microcolonies, which thicken and disperse biofilm under genetic or external factors. This process involves exoenzymes and surfactants, and physiological changes. Dispersed bacteria form planktonic bacteria, which can colonize and form biofilm under specific conditions [46-48].
4. **Dispersion stage**: The dispersion step in the biofilm life cycle involves the use of surfactant phenol-soluble modulins (PSMs) as key effector molecules. PSMs, characterized by their amphiphilicity α-helical secondary structure, destroy non-covalent forces in the biofilm matrix, transport nutrients, and spread biofilm masses [49, 50]. They aggregate into amyloid fibers, eliminating biofilm-degrading activity and stabilizing the biofilm structure [51]. Biofilms, formed by *S. aureus* and other bacteria, pose a significant challenge to medical professionals due to their inherent immune response and antibiotic resistance [44].

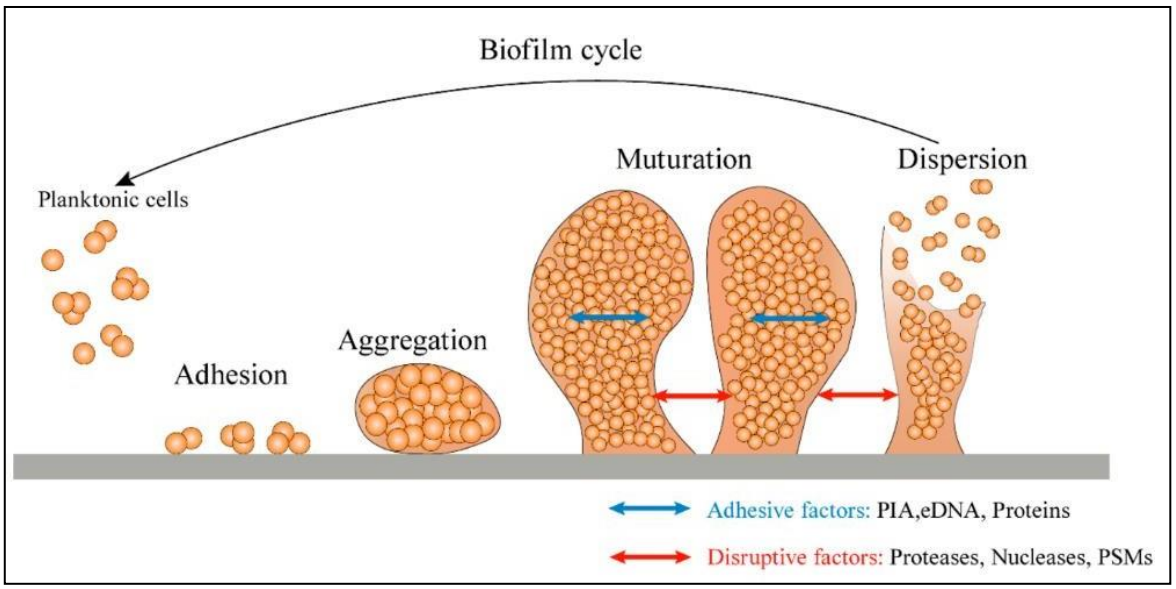


Fig. 1.3 The growth cycle of biofilm involves planktonic cells attaching to the surface, aggregating, producing ECM (extracellular matrix), forming microcolonies, cell division, and finally, enzymes promote dispersion, allowing bacterial cells to detach and colonize new ecological niches [52,44].

1.7 Methicillin resistant *Staphylococcus aureus* (MRSA) and its causes

Staphylococcus aureus, is one of the most famous microorganisms responsible for a host of infections in humans. It is a gram-positive, coccoid bacterium and appears as grape-like clusters. It belongs to the family, *Staphylococcaceae* which is inherent as normal flora in humans and animals. It resides in the skin and nasal membrane of humans and causes various infections which can be mild as well as life-threatening. Some of the diseases observed are, skin and wound infections, nosocomial infections especially pneumonia, food poisoning, osteomyelitis, sepsis, infectious endocarditis and BSI (bloodstream infection) infections. Thus, *S. aureus* through its various infectious mechanisms has managed to establish itself as a potent cause of community acquired infections [53].

The resistance against methicillin was first observed in 1961 when the antibacterial agent was introduced clinically. The Scanning electron microscope (SEM) image of MRSA is shown in Fig. 1.4. Over the years, the resistant strain, Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a global epidemic in both

healthcare and community acquisition raising serious concerns amongst healthcare professionals [54].

MRSA strains have acquired resistance to methicillin and various other β-lactam antibiotics such as penicillin and cephalosporin. The mecA gene is found on a large mobile genetic element called the *Staphylococcal* chromosomal cassette mec (SCCmec), which encodes for a penicillin binding protein, PBP2a. This protein renders the cell wall of the bacteria resistant to the β-lactam antibiotics by interfering with the activity of the antibiotics. MRSA causes serious nosocomial infections which includes community-associated MRSA and healthcare-associated MRSA strains. Further the MRSA strains associated with the community-based infection are referred to as community-acquired MRSA (CA-MRSA) and the strains associated with hospital-based infections are referred to as hospital-acquired MRSA (HA-MRSA) [55,56]. MRSA has been observed to be the leading cause of various skin and soft tissue infection causing septic shock (56%), pneumonia (32%), endocarditis (19%), bacteraemia (10%), and cellulitis (6%) in invasive care units [55].

MRSA has also been found to colonise animals leading to the emergence of various zoonotic strains. It has been observed that pet animals such as cats and dogs can be infected with MRSA through transmission from the owners and subsequently, they act as reservoirs for infection or re-infection of humans [57].

The treatment of MRSA includes a list of various drug regimen consisting of vancomycin, linezolid, daptomycin SMZ-TMP (Sulfamethoxazole and trimethoprim), clindamycin, quinupristin-dalfopristin, and tetracycline. However, with the surge in the formation of resistance against the drugs, there is a growing alarming situation. It calls for an exhaustive study to understand the mechanism of resistance against these antibiotics and also requires new drugs to be developed. A major resource for the development of new novel drugs are medicinal plants which includes a huge diversity of bioactive compounds [58,59].

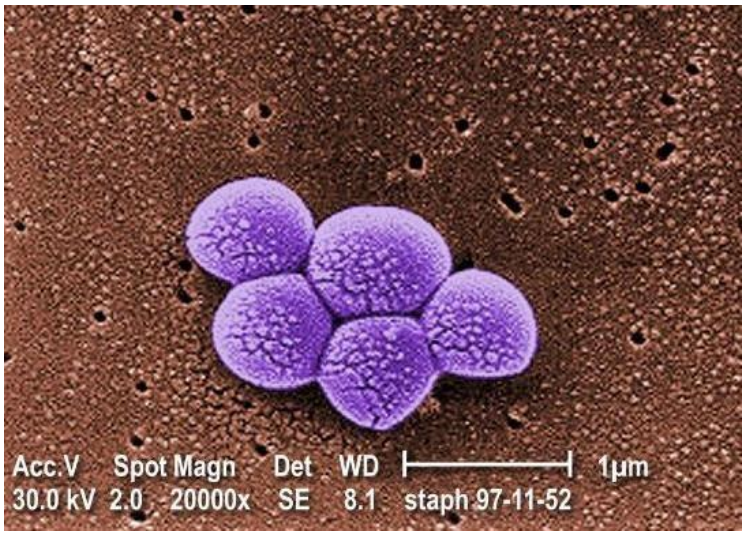


Fig. 1.4 SEM micrograph showing MRSA [60]

1.8 Activity of medicinal plants against *S. aureus* and MRSA proteins

The World Health Organization (WHO) has acknowledged the necessity for development of novel approaches in controlling the havoc caused by MRSA. This has further led to the keen interest in medicinal plants for their rich storehouse of phytochemicals as an alternative source of drug discovery [54].

Various medicinal plants from around the world have been investigated for their role in the inhibition of growth of *S. aureus* as well as MRSA strains, such as *Vernonia amygdalina* and *Azadirachta indica*, *Perganum* harmala, Grapefruit, *Lithospermum erythrorhizon* [61]. *Lippia sidoides, Ocimum gratissimum* Linn., *Scoporia dulcis* Linn, *Spilanthes filicaulis*, *Bauhinia kockiana*, and *Kalanchoe crenata* showed antibacterial efficacy against *S. aureus* [62]. *Cissus quadrangularis*, *Euphorbia heterophylla*, *Euphorbia prostrate* showed promising antibacterial activity against *S. aureus* (Fig. 1.5) [63]. Some of the natural product compounds having bactericidal properties against MRSA are summarised in Table 1.2.

Table 1.2 Antibacterial natural products against methicillin-resistant *Staphylococcus aureus* (MRSA)

Natural product compound	Test organism	MIC (µg/ml)	References
Curcumin	MRSA	217 µg/ml	[280]
Thymoquinone	MRSA	8-16 µg/ml	[281]
Micromonohalimanes B	MRSA	40 µg/ml	[282]
Roemerine	MRSA	32-64 µg/ml	[283]
Baicuru	MRSA	39 µg/ml	[284]
Endophenzine G	MRSA	2-128 µg/ml	[285]
Solanioic acid	MRSA	1 µg/ml	[286]
Emodin	MRSA	32-64 µg/ml	[287]

Lippia sidoides

***Ocimum gratissimum* Linn.**

Bauhinia kockiana

***Scoporia dulcis* Linn.**

Euphorbia heterophylla

Euphorbia prostrate

Fig. 1.5 Some medicinal plants exhibiting antibacterial activity against *S. aureus* [288-293]

A major source of resistance in MRSA is the presence of efflux pump proteins which eliminates any metabolites or drugs that are toxic to the microorganism. Therefore, synergistic application of natural products along with antibiotics can prove as a major effort in demonstrating anti-efflux pump activities. In one such study, it was found that genistein in combination with norfloxacin demonstrated a much better activity in inhibiting the NorA efflux protein [64,65].

1.9 *In-silico* docking

A major study in the establishment of any natural product as a drug requires the search for the potential target in the target microorganism. Few medicinal plants and their respective bioactive compounds targeting *S. aureus* are tabulated in Table 1.3. Studies in the area of genome sequencing has led to an increase in the number of new therapeutic targets [66]. The use of robust instrumentation and techniques have led to the advances in structural biology. Of these, nuclear magnetic resonance (NMR) and X-ray crystallography, have contributed in the analysis and provided valuable 3D information about enzymes and receptors and protein-ligand interaction at a large scale [66,67]. Understanding of the three-dimensional structure of the target is very important as the most common and frequently used method for designing lead molecules is structure-based drug design (SBDD). Of the different SBDD methods, the most common and widely followed is molecular docking. The term docking was coined in the late 1970s and was referred to as the modification of a model with complex structure. The structure is optimized with fixed relative orientations. *In silico* molecular docking is of two types: (i) rigid docking, where the relative orientation varies while the internal geometry remains fixed and (ii) flexible docking, where the internal geometry changes prior to formation of complex by the interaction of the molecule [66].

Molecular docking is therefore a primary tool for reviewing protein–ligand interaction. They efficiently combine the molecular features of both the targeted proteins and the ligands, thus, increasing their binding affinity [68,69]. Combining of algorithms is carried out to generate different poses and scoring functions which helps in determining the protein– ligand interactions [70]. The docking methods have been potentially used to predict the energetically favourable conformations and also the orientations of the ligands within the binding site of the protein [67]. The molecular docking approaches are also being used for prediction of the quaternary structure of a biological protein complex [66, 71]. In the last two decades, more than 60 different docking tools have been developed which can be used both academically and commercially, few of them are: DOCK, AutoDock, FlexX, Glide, Cdocker, Surflex, GOLD, ICM, LigandFit, MOE-Dock, LeDock, AutoDock Vina, MCDock, FRED, rDock, UCSF Dock and many others. Of all these tools, AutoDock Vina, GOLD, and MOE-Dock are known to predict top ranking poses with best scores [72].

Therefore, it can be inferred that *in-silico* docking tools are an essential requirement for the analysis of large databases of chemical compounds which can be used for the identification of possible drug candidates. Information on the interactions accountable for binding can be extracted from the results that has been generated by these docking programs and can be further used to design successful lead compounds [67].

Table 1.3 Representative medicinal plants and their bioactive compounds targeting *S. aureus* [294]

Medicinal plant	Bioactive compound	Target protein of S. aureus
Chelidonium majus-Beberis vulgaris	Berberine	SarA
Terminalia chebula	Corilagin	DNA gyrase
Rheum ribes	Galangin	CrtM
Hypericum perforatum-Hibiscus sabdariffa	Hypericin	PBP2a

1.10 Target identification and its importance

The identification of a potential target and validation of the activity of the bioactive compounds and their synthetic analogues is a very promising research field in medicinal chemistry and pharmacology [73,74]. Therefore, both these steps provide a crucial role in drug discovery and has posed a challenge acting as a cost-barrier towards chemical biology and phenotypic screening. *In-silico* studies are thus carried out to identify potential biological targets for phytochemical and chemical analogues offering an alternative method of ligand–target interactions, understanding their biochemical mechanisms, as well as for investigating a drug [75.76]. It is observed that the interaction between the identified lead molecule and the potential target have improved the likelihood of the therapeutic success and consequently plays a key role in drug discovery. Studies involving investigations conducted on Astra-Zeneca's drug research

and development programs revealed that 82% of terminations in pre-clinical studies were majorly due to safety issues, of which 25% were target-related and 48% of safety failures were observed in clinical trials. As such, proper guidance on the selection of potential candidate targets is required which can help improve the success rate of the interaction in pre-clinical and clinical trials which can be time and cost effective in nature [77,78].

Information about the new drugs and their disease targets are being methodically deposited in public databases such as Drug Bank database, launched in 2006. It is known to be a systematic collection of drug-protein interactions and has information on more than 760 Food and Drug Administration (FDA)-approved drugs and approximately 2000 drug target proteins. Moreover, this database contains drug-target interactions along with gene annotations retrieved from Swiss-Prot [75].

The drug discovery and development process are known to be a very intricate and inflated venture which include various peripherals including disease selection, target identification and validation, lead discovery and its optimization, both prior to pre-clinical and clinical trials. The advent of *in-silico* methods in the recent decades, has led to a drastic increase in the introduction of multiple new chemical entities that has been approved by the U.S. Food and Drug Administration (FDA) [79, 80]. It has been established through various studies that many drug candidates cease to become proper drug molecules due to lack of efficacy and safety, which are the two major causes leading to the drug failure. It can also be emphasized that absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties play a vital role in every stage of drug discovery and development [81, 82]. Therefore, it is essential to analysis and find effectual molecules with better ADMET properties. In the evaluation of drug likeliness of a compound, Lipinski and his co-workers proposed the “Rule of Five” in 1997, the most well-known rule-based filter of drug likeness which distinguishes whether a molecule is orally absorbed well or not. The rule depicts the following:

- Molecular mass less than 500 Dalton,
- High lipophilicity (expressed as LogP less than 5),
- Less than 5 hydrogen bond donors,
- Less than 10 hydrogen bond acceptors,

- Molar refractivity should be between 40-130.

According to the Rule of Five, a molecule will not be orally active if it violates any of the two or more of the above five rules. At the same time, the rules are strictly not confined to complicated natural products as they are usually derived from relatively simple small molecules [82,83].

1.11 Drug formulation using natural products

Topical dermatologic products either solid to liquid, are available in the market for the treatment of various ailments. The majority of the ointments consist of a base that primarily acts as a carrier during the formulation of the drug. The nature of the base is the main factor in controlling the performance and therefore selection of the ointment base is a very important aspect of drug formulation [84,85].

The traditional ointment bases are greasy in nature, due to the presence of hydrocarbons like petrolatum, beeswax, and vegetable oils which do not allow any addition of water molecules. The ointments exhibit topical aided value in terms of being protective, emollient, antipruritic, antiseptic, keratolytic and astringent in nature. Thus, the foremost importance for the finished product is the base of an ointment, exhibiting the above-mentioned properties. The ointment base composition governs the extent of penetration in the human body and controls its transfer from the base to the other body tissues [86].

Due to the presence of various limitations, many formulated drugs that were applied have been found to be non-suitable for topical applications because of poor solubility, bad penetration, stability problems and poor spreadibility [87]. However, at the same time, the use of medicinal plants and natural products can be an alternative strategy for minimising such problems. Medicinal plants contain biologically active volatile organic components such as phenylpropenes, aromatic hydrocarbons, aliphatic and cyclic terpenoid chemicals, thereby enabling its use in the formulation of a topical formulation of ointment [88]. As per reports of 2008, 68% of all pharmaceutical products that are derived from medicinal plants are due to the presence of secondary metabolites imparting potent antioxidant, anti-nociceptive, anticonvulsant, neuroprotective, and anti-inflammatory properties, as observed in pre-clinical study trials [89].

1.12 Treatment of infection in animal model by formulated drug

Animal models have been in the forefront of any drug discovery research. They are widely used in determining whether a drug has safety and desired activity. Animal model study provides a highly meticulous and lucrative technique that ensures efficacy of a drug in humans and helps in enhancement of the treatment and distribution of the same. Research in animal models also helps in bridging the gap between the *in-vitro* drug discovery process and clinical studies. Studies on animal models have helped investigators in analysing the safety of various antimicrobial agents against explicit human pathogens [90,91].

The animal models are mostly used for the evaluation of new antimicrobial agents and is a fundamental part of pre-clinical studies during the drug development phase. Various preliminary information like safety, pharmacokinetic, and pharmacodynamic can be obtained for a drug through animal model studies. These data are of utmost importance before proceeding for conduct of subsequent clinical studies [91]**.** It has been observed that drugs formulated from plant-based natural products are safe, cost-effective and have potent biologic alternatives and can be used to treat various ailments without posing hazardous impact [92].

Various plant derived formulations and active molecules have been investigated for their efficacy and safety using animal models. Therefore, it can be observed that plant-derived metabolites can be promising alternative agents for various human ailments including wound healing and other inflammatory issues. Additionally, plant-based formulations are in high demand due to their easier availability, non-toxic in nature, easy administration, bearing lesser side effects and are also effective as crude preparations [94].

Aim and objectives of the present study

In the premises of the above introduction, the following research objectives werechosen for the present work.

Research objectives

The research objectives of the proposed study are-

i. Isolation and characterization of essential oil from *Kaempferia galanga* Linn andits anti-staphylococcal activity.

ii. Investigation into *in-silico* docking and molecular dynamic simulation ofcompounds identified from the essential oil.

iii. Study on wound healing efficacy of essential oil.