2

Review of Literature

2.1 Medicinal plants

Nature is blessed with immense resources nurturing a huge diversity of life forms which include plants, animals and microorganisms. Human beings since time immemorial have been using medicinal plants for various uses. A large spectrum of these plants is being used widely as a system of medicine for treatment of various human ailments [94]. Medicinal plants are used in various systems of medicine and mostly used in rural areas of the developing countries where they continue to be used as the primary source of medicine. According to the World Health Organization (WHO), it has been estimated that about 80% of the people in developing countries use medicinal plant-based traditional/ folk medicines for their general health care [95]. With the discovery of the multitude of benefits, medicinal plants presently occupy a key position in plant research and drug discovery [96].

2.1.1 History of medicinal plants

For thousands of years, medicinal plants have been used as a safe therapeutic modality in various cultures worldwide. This natural medical paradigm has been inherited from ancestors, healer-to-healer transfer, or developed through personal experiences. No modern system of medicine can claim to it as traditional systems like allopathic, homeopathic, ayurvedic, and Chinese systems have evolved over time. Most civilized nations have developed their own Materia Medica, compiling details about various plants used for therapeutic purposes. The merging of this human pharmacopoeia with modern medical sciences provides the foundation for a revolution in the existing healthcare system [97]. Archaeological studies have provided evidence that the healing properties of plants were known to people since prehistoric times. Some of the oldest

references to medicinal plants include Artharvaveda, Ayurvedic medicine, Clay tablets, Eber Papyrus, De Materia Medica, and Pen Ts'ao Ching Classic of Materia Medica [98, 2].

Greek civilization was a time of scientific and philosophical advancement, with significant contributions to pharmaceutical sciences, particularly in phytopharmaceuticals. Aristotle described 500 crude drugs used for various pathological conditions, while Hippocrates, known as the "Father of Allopathic Medicine", formulated the first scientific medical paradigm of treatment. He proposed that many pathological conditions were caused by disturbances in human system physiology. Theophrastus, a student of Aristotle, also mentioned 500 crude drugs in his book. Claudius Galen Pergamum prepared vegetable drugs using extraction techniques called Galenicals and introduced the concept of pharmaceutical formulation [99].

Traditional Chinese Medicine is a unique and influential system of treatment that has gained global recognition due to its evidence-based approach, pioneered by Fu His (2953 BC) and later developed by Emperors Shen Nung and Hong Ti. The Chinese pharmacopoeia Pen Tsao contains numerous remedies for various medical problems. Shen Nong Ben Cao Jin (22-250 AD) is considered the "Crown of Written Chinese Medicine". CaoYuan Fang's Zhu Bing Yuan Ji Lun is a standard reference book for Chinese medical students. Wang Tao's Waitai Miyao described 600 prescriptions, focusing on tongue color and status during different pathological conditions. Li Shizen's Ben Ca Gang Mu, published in 1596, has 1894 prescriptions and is still used as a reference for research and schooling in China and other communities [100,101].

Ayurveda, the oldest healthcare system on Earth, is a natural healing system combining physiologic and holistic medicine. Originating around 5000 BC, it describes humans as a matrix of seven basic tissues, with disease resulting from imbalances in these components. Ayurveda, a Sanskrit word meaning life knowledge, is a holistic approach to health [2].

Over the years, significant advancements in chemistry have led to the use of medicinal plants as a starting point for synthesizing new compounds with different structural parameters. This has simplified the development of plant-derived drugs, leading to the discovery of clinically useful molecules [102]. From 2000 to 2003, 15 compounds of natural origin were launched, with the same number in phase III clinical trials. However, only 1 molecule out of 5000 successfully completed all stages of development and obtained registration for clinical applications [103, 2].

Medicinal plants are considered a rich source of ingredients which can be profusely used in drug development and synthesis. They play a vital role in the development of human cultures worldwide. Some of the plants are regarded as important sources of nutrition having high therapeutic values *viz* ginger (*Zingiber officinale*), garlic (*Allium sativum*), turmeric (*Curcuma longa*), giloi (*Tinospora cordifolia*), pepper (*Piper nigrum*), neem (*Azadirachta indica*), tulsi (*Ocimum sanctum*), etc., [104]. Medicinal plants form the basic raw materials for extraction of various active ingredients that can be used in the synthesis of different drugs such as taxol (*Taxus brevifolia*), vincristine (*Catharanthus roseus*), artemisinin (*Artemisia annua*), quinone (*Parthenium integrifolium*) and morphine (*Papaver somniferum*) [103].

Medicinal plants are believed to possess various characteristics which explains its use in the treatment of ailments, some of which are described as follows:

a) Synergistic medicine- The ingredients of the plants interact simultaneously and their use can neutralize any possible negative effects which could have occurred if used alone.
b) Support of the standard official medicine- Medicinal plants and the molecules isolated from them have been found to be helpful in the treatment of various complex diseases. At the same time these products were found to be very effective and safe.
c) Preventive medicine- It has been proved that the effective components of the medicinal plants have the ability to prevent the advent of certain diseases. This also helps in reduction of the use of the chemical drugs; thus, it helps in the reduction of the side effect of synthetic treatment [105].

Medicinal plants are known to contain a huge diversity of bioactive compounds. Different families of medicinal plants like Solanaceae, Asteraceae, Caesalpiniaceae, Liliaceae, Apocynaceae, Piperaceae, Rutaceae and Sapotaceae serves as a major source harbouring plant products like flavonoids, alkaloids, coumarins, fatty acids, cucurbitacin, diarylheptanoids, iridoids, lignans, limonoids, naphthoquinones, and phenanthrene derivatives [106].

Approximately, 100 plant-derived new drugs were introduced into the market in between 1950-1970 namely vincristine, reseinnamine, vinblastine, deserpidine and reserpine [107]. During the period of 1971-1990, medicines like artemisinin, ginkgolides, lectinam, teniposide, ectoposide, plaunotol and nabilone was introduced globally. The rest 2% were introduced during 1991-1995 which includes irinotecan,

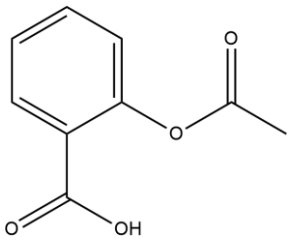
toptecan, paclitaxel and gomishin [13]. The manufacture of morphine industrially on a large scale by Merck, Germany in 1826 marked the beginning of commercialization of plant-derived drugs which led to the onset of the use of medicinal plants and natural products or their derivatives in pharmaceutical industries [94].

The distribution pattern of the medicinal plants shows that they are widely distributed across diverse habitats and landscape elements. About 70% of the medicinal plants in India are found in tropical forests in Eastern and Western Ghats, Chota Nagpur plateau, Aravalis, Vindhyas and the Himalayas. Among the Himalayas, the Kashmir Himalayan region is huddled within the North-western folds of the recently designated global biodiversity hotspot of the Himalayas. Some of the plant species found in this part are Caraway (*Carum carvi*), Saffron (*Crocus sativus*), Siya zira (*Bunium persicum*), Garlic (*Allium sativa*), Coriander (*Coriandrum sativum*), Mint (*Mentha* spp.), Fennel (*Foeniculum vulgare*) and Hare's foot (*Trigonella foenum-graecum*) [13].

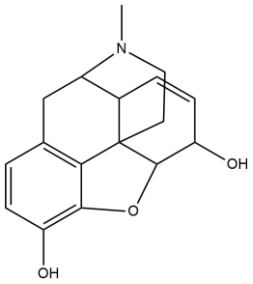
2.2 Natural product

Natural products are chemically active components formed by a naturally occurring living organism bearing pharmacological properties and they are vital for the process of drug discovery and design. Natural products play an immense role in the pharmaceutical and biotechnology industries as the vast range of modern medicines are mostly based on either naturally occurring molecules, or their derivatives [108,109]. Moreover, the therapeutic agents that are used in the treatment process and usually inhaled, ingested, and injected are a mixture of various complex therapeutic compounds which are derived from medicinal plants. It has been reported that in the industrialized nations, more than 60% of all medicines are produced from natural products or secondary metabolites derived from them [110].

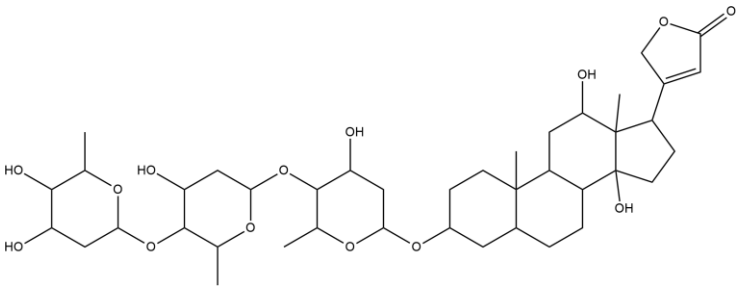
The structures of some of the most widely used natural products are presented in the Fig. 2.1:



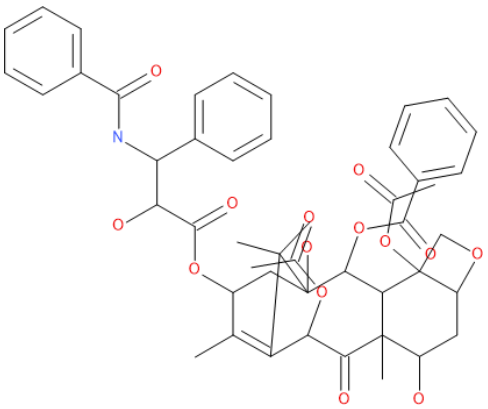
1. Aspirin



2. Morphine



3. Digoxin



4. Paclitaxel

Fig. 2.1 Structures of medicines derived from natural products

Natural products are produced by plants as a form of secondary metabolites. These secondary metabolites, are small molecules which are organic in nature.

Although, they are not directly involved in the growth and development, and reproduction, yet, they facilitate certain functions that increases the survival and fertility of the plant [94]. The basic process of photosynthesis, glycolysis and the Krebs cycle serves as biosynthetic intermediates resulting in the formation of secondary metabolites. The major significant building blocks or pathways acquired in the biosynthesis of secondary metabolites are derived from the intermediates such as acetyl coenzyme A (acetyl-CoA), shikimic acid, mevalonic acid and 1-deoxyxylulose-5-phosphate. They are effortlessly involved in numerous biosynthetic mechanisms and reactions (e.g., alkylation, decarboxylation, aldol, Claisen and Schiff base formation) providing an array of vital biological activities [111]. Plant based natural products (PNPs) are commonly divided into three main classes which are, phenolics, terpenoids and alkaloids which are

used in pharmaceutical and nutraceutical industry [20].

Natural products serve as a better and safe alternative for pharmaceutical research. It has been observed that extensive research in natural products provides a novel framework for drug discovery of lead compounds in the pharmaceutical industry. Drugs produced from synthetic sources demonstrate lesser therapeutic impact along with adverse side effects [109]. Contrary to that, therapeutic agents derived formed from natural sources show lesser side effects, as they exert physiological and pharmacological impact within living cells by interacting with proteins, enzymes, and other biological molecules [110,112].

2.2.1 Natural product in drug discovery

Natural products are a valuable source of drugs and play a key role in the drug discovery process [108]. With the advancement in the field of drug discovery and medicinal chemistry, the pharmaceutical and biotech industries are utilizing natural products to formulate new useful derivatives in the therapeutic industry as there lies a surging need for treating severe clinical conditions such as cancer, obesity and infection stimulated by multi-resistant pathogens. Medicinal plants are a rich source of natural products in the drug discovery process and approximately, 5-15% of terrestrial plants out of 250,000 species have been pharmacologically studied in resourceful mode for its use as therapeutic agents [108].

Natural products have been a crucial part of drug discovery and development, with many drugs currently available in the market being discovered from natural sources. Aspirin, the world's most widely used medicinal agent, is an example of a

natural product originating from plant genera *Salix* spp. and *Populus* spp., related to salicin. Penicillin, an antibiotic, was discovered in the laboratory from the fungus *Penicillium notatum*. Other examples include Paclitaxel, isolated from the bark of the Pacific yew tree *Taxus brevifolia*, and hypericin and pseudohypericin, isolated from *Hypericum perforatum* [113]. These compounds inhibit the release of reverse transcriptase by stabilizing the HIV capsid structure, preventing the uncoating process. Overall, natural products from plants and microorganisms hold significant importance in modern medicine [114].

2.2.2 Approval of natural products as new drug entities

The main objective of any drug discovery projects is to discover and formulate the most promising lead compounds, that can be used as a potent therapeutic agent to combat against various ailments [108]. In the approval of any naturally-derived lead compounds, various steps are involved: (i) Initially, in the drug design process, isolation, and purification of the lead compounds from their natural source is carried out by utilizing various methods depending on the structural diversity, stability and quantity of the compound required. (ii) High throughput screening of the lead compounds against specific targets. (iii) Once the promising lead molecules are identified, modifications of the molecule are usually carried out to enhance the efficacy and various biochemical and pharmacological tests are carried out. (iii) If the suggested modifications increase the selectivity, then the promising compounds are subjected for *in vitro* and *in vivo* testing [109]. Once the compounds are found to have the desired results, various safety tests on the selected compounds are conducted using pharmacokinetics to know the mechanism by which the drug is absorbed, distributed, metabolized, and excreted. This procedure indicates the mechanisms that occur when the therapeutic agent enters the body. If all the results and optimizations are positive, then lead compounds becomes potential candidate drugs [110].

Approximately, one-third of the successful drugs worldwide are either natural products or their derivatives. Food and Drug Administration (FDA) approved 520 new drugs between 1983 and 1994, among which, 39% were derivatives of natural products and about 60 - 80% of antibiotics and anti-cancer drugs were also are obtained from natural products [115]. Lately, Newman and Cragg (2016) evaluated the importance of natural products in the drugs that were approved by the FDA between 1981 and 2014. It was observed that during the period, the FDA approved 1,562 drugs, 64 (4%) were unaltered natural products, 141 (9.1%) were botanical drugs (mixture), 320 (21%) were

natural product derivatives and 61 (4%) were synthetic drugs but with natural products pharmacophore contributing to the enforcement of medicinal plants [116].

Some examples of naturally derived drugs from medicinal plants are listed below:

1. **Artemisinin** and its derivatives, such as dihydroartemisinin, artemether, and artesunate, are being used worldwide as active antimalarial drugs targeting against multidrug-resistant *Plasmodium falciparum*. It is known as *Qinghaosu* in Chinese, and is described as a highly oxygenated sesquiterpene. A unique 1,2,4-trioxane ring structure is present in artemisinin, which is responsible for its antimalarial activity. Antimalarial drugs developed from *Artemisia annua* is also widely used for the treatment of malaria [117].
2. **Bicyclol**, an active natural product obtained from Chinese magnolia vine fruit or orange magnolia vine fruit was officially approved in 2001 as a therapeutic agent for hepatitis in China. It also obtained patent protection in 15 countries and regions. According to Traditional Chinese Medicine theory, Chinese magnolia contains five varieties of flavours, namely, pungent, sweet, sour, bitter, and salty, which signifies widely its therapeutic potential [118].
3. **Berberine**, a potent antibiotic and anti-inflammatory compound, was isolated from goldthread (*Coptis chinensis* Franch), a plant used in traditional Indian and Chinese medicine for over 3,000 years to treat inflammation, infections, diabetes, cancer, depression, hypertension, and hypercholesterolemia, among other conditions [119].
4. **Morphine**, an alkaloid derived from the poppy plant *Papaver somniferum*, is an effective pain reliever and standard for other drugs. It is used preoperatively to reduce anxiety, sedate, and reduce anesthetic doses. Morphine's vasodilatory and bradycardic effects make it useful in treating myocardial infarction and pulmonary edema. However, it can cause respiratory depression and gastrointestinal effects at therapeutic doses. Repeated use can lead to physical dependence, making it subject to abuse and controlled by national and international regulatory agencies [120].
5. **Vinca alkaloids,** vincristine and vinblastine, are the first plant-derived anticancer drugs to enter clinical use. Originating from Madagascar's periwinkle (*Catharanthus roseus*), they exhibit antiproliferative activity by disrupting microtubules, causing cell arrest, and apoptotic death. They were used as

templates for developing effective semisynthetic derivatives like vindesine, vinorelbine, and vinflunine [119,121].

Natural products are found to have various advantages over synthetic products or molecules due to which they are found to be a major source of drug discovery. Therefore, it has been possible for the extraction of various lead components ranging from simple chemical structures to highly complex structures from natural products for treatment of various diseases afflicting human kind. The present drug discovery processes ascending from natural resources is mainly focused on the isolation, purification, screening, and discovery of novel drug candidates. Therefore, to work with any potential lead compounds, large scale extraction or production is required in order to make the lead compounds clinically feasible [122].

Natural products can resemble distinctive metabolites of biological functionality such as hormones and naturally occurring ligands as well as possessing great structural diversity, which is necessary for the drug discovery process [110].

2.2.3 Current status of natural product in clinical trials

The annual global market of medicines is worth about 1.1 trillion US dollars worldwide. 35 percent of these medicines are created directly or indirectly from natural products which includes plants (25%), microorganisms (13%) and animals (about 3%). Thus, it can be observed that nature-derived products contribute a major source for global pharmaceutical companies working on the development of new medicines [116, 123].

Natural products are thereby used in the following ways,

i. As a direct source of therapeutic agents either in the form of pure drugs or phytomedicines;
ii. As a source of raw material for the development of complex, semi-synthetic drugs;
iii. As prototypes for design of lead molecules and;
iv. As taxonomic markers for discovery of new drug entities.

The advancement in genomics, proteomics, transcriptomics, and metabolomics has enhanced the contribution of natural products in drug discovery. Twenty-five natural products and its derived drugs were approved for marketing from January 2008 to December 2013. Few of new human drug classes were marketed successfully, namely: romidepsin isolated from *Chromobacterium violaceum*, eribulin (halichondrin B) isolated from Japanese sponge *Halichondria okadai*, dapagliflozin (phlorizin) isolated from root bark of Apple tree [103].

Since the late 90s, with the advancement in science novel technologies like combinatorial chemistry, high-throughput screening, genomics, proteomics, and bioinformatics have arisen, which are being widely used in the field of drug discovery studies from natural products. Further various new techniques have been evolved since the beginning of the 21st century which led the rise in use of natural products. Some of these widely used techniques are molecular diversity, compound library design, protein 3D-structures, NMR- based screening, 3D QSAR in modern drug design, physicochemical concepts, and computer-aided prediction of drug toxicity and metabolism. Therefore, with the upliftment in the technology, there is an increase in the drug discovery and development processes leading to increase in use of natural products in pharmaceutical industry [108].

2.3 MRSA and resistance mechanism

Staphylococcus aureus (*S. aureus*), a nosocomial pathogen is responsible for causing various infectious diseases mostly of the skin and soft tissue, endocarditis, osteomyelitis, bacteraemia, mastitis, and fatal pneumonia [124]. *S. aureus* is particularly known for its infections in people with burns and surgical wounds, where it secretes various toxins that can induce toxic shock syndrome leading to fever, sickness and in severe cases, can lead to death. *S. aureus* is also responsible for enterotoxin production that leads to food poisoning [125]. Alexander Ogston first isolated *S. aureus* in 1880 in Aberdeen, Scotland, from patients with ulcerated sores. *S. aureus* belongs to the genus *Staphylococcus*, Firmicutes; is gram-positive in nature, ~0.8μm in diameter, arranged in a "string of grapes" when observed under a microscope, is either aerobic or anaerobic in nature; and found to grow optimally at 37°C, at pH 7.4 [126]. *S. aureus* is also found to infect domestic cats and dogs, horses, goats, sheep, cattle, rabbits, pigs, and poultry apart from human microflora.

Although various infections have been reported in these species, the most economically significant are mastitis in dairy cattle and other ruminants, lethal systemic infections in farmed rabbits, and bumblefoot (ulcerative pododermatitis) in poultry [127].

With the discovery of antibiotics, the treatment of *S. aureus* infections before the 1950s involved the use of benzylpenicillin (penicillin G), a β-lactam antibiotic, however by the late 1950s, strains resistant to benzylpenicillin was observed which were capable of inactivating the β-lactam ring by producing an enzyme, called a β-

lactamase [125]. The enzyme is encoded by *blaZ*, which is found on a large transposon on a plasmid. Therefore, with increasing rate of penicillin resistance in humans by *S. aureus* resulted in withdrawal of the drug for treatment of the bacteria and other associated infections [127].

A semisynthetic penicillinase-resistant β-lactam drug methicillin was developed to prevent the emergence and spread of penicillin resistance in *S. aureus*. This drug was marketed as Celbenin and was clinically introduced in 1959. Nonetheless, methicillin resistance by *S. aureus* was detected in 1961 soon after the antibacterial agent was introduced clinically [127].

The British scientist named, Jevons, reported that, there has been a global epidemic of Methicillin-resistant *Staphylococcus aureus* (MRSA) whose resistance against antibiotics was produced by a gene encoding the penicillin-binding protein2a (PBP2a) called *mec*A [128]. It was also found that, MRSA was turning out to be the most recurrently resistant pathogen identified in many parts of the world, including Europe, the United States, North Africa, the Middle East, and East Asia in comparison to other resistant pathogens in both healthcare and community acquisition [54, 129 130]. Initially, MRSA was classified into hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA) [52, 131]. The infectivity of *S. aureus* was such that, in China, it was reported that hospital-acquired MRSA infection has reached 50.4% leading to its severeness and concern [132].

The Centres for Disease Control and Prevention (CDC), USA reported that, MRSA infection has surpassed the mortality rate in comparison to that of Acquired Immune Deficiency Syndrome (AIDS), Parkinson's disease and other health threatening infections [133]. Therefore, it is essential to study the molecular characteristics of *S. aureus*, which can aid in understanding the prevalence and monitoring the evolution of *S. aureus*, leading to the discovery of new molecular features of *S. aureus*, and finally, providing information for developing novel drugs against *S. aureus* thereby solving a major global health concern [134].

The origin of MRSA was mainly due to Staphylococcal Cassette Chromosome *mec* (SCC*mec*) genes which harbours the *mec*A gene encoding the penicillin-binding protein (PBP2a) that evokes resistance to all β-lactam antibiotics [135]. The active efflux system of the bacteria was discovered in 1980 by Ball and McMurry which play a vital role in providing resistance to multiple drugs. There are three types of multidrug pumping proteins that contributes to the resistance of the *Staphylococcus aureus* cell

membrane: QacA, NorA, and Smr. Reports have conveyed that QacA is considered an important factor in MRSA [126]. Multidrug pumping proteins are all considered as proton kinesins and does not rely on ATP hydrolysis to release energy, and, material exchange is performed by an electrochemical gradient formed on both sides of the cell membrane [137] thereby providing an active efflux system in MRSA resistance [134].

2.3.1 Prevalence of MRSA infection worldwide

The prevalence of MRSA has increased in both health-care and community environments. In 2014, the percentage of intrusive MRSA isolates in Europe ranged from 0.9% in the Netherlands to 56% in Romania, with a mean population of 17.4%. Although the proportion of MRSA isolates in Europe has decreased over time, 7 of the 29 European Union countries still report 25% or more of intrusive *S. aureus* isolates as MRSA for instance, 0.4% in Sweden, 25% in western part to 50% in southern India, 33%–43% in Nigeria, 37–56% in Greece, Portugal, and Romania in 2014 [138]. In 2005, the invasion of intrusive MRSA infections in the Unites States occurred at a rate of 31.8 per 100,000 people in accordance of their respective age, race, and, gender, and around 75% of such MRSA infections involved *S. aureus* bacteraemia [139]. It was also found that in between 2011–2012, approximately, 12.3% of all healthcare-associated infections in Europe were caused by *S. aureus*. In Cyprus, Italy, Portugal, and Romania, more than 60% of healthcare-associated *S. aureus* infections were solely identified as MRSA [140].

High prevalence of MRSA with rates greater than 50% has also been observed in hospitals worldwide including in Asia, Malta, North and South America. Such variation in the prevalence rates of MRSA was mainly due to different epidemiological factors such as geographical and health system capability in running infection control program. Other countries like Australia, Korea, Hong Kong, Scotland, Israel, Thailand, South Africa, and many other have also reported MRSA resistance with prevalence ranging from 0–74% [54].

2.3.2 Percentage of population affected by MRSA in India

Methicillin resistant *S. aureus* is associated with poor clinical consequences in ICUs and is therefore a major concern for causing nosocomial infections in India. Patients in an ICU, specifically a surgical ICU, have wounds, and, invasive monitoring devices that cause skin breach exposed which makes the patients more prone in developing an infection. Furthermore, patients having impaired neutrophil properties underlying due

to health conditions like chronic liver disease, diabetes, or pertaining to any steroid therapy may make such patients more vulnerable to MRSA [141].

Prevalence of MRSA in human from various regions of India at 37% was reported during 2015–2019 [141]. Twenty-two states of India had outlined the prevalence of MRSA where, Jammu and Kashmir showed the highest occurrence of MRSA at 55% and Maharashtra showed the lowest occurrence of MRSA at 21%. Various studies conducted in different zones of the country exhibited that east zone (West Bengal and Odisha) showed highest pooled pervasiveness of 43%. The lowest occurrence of MRSA was recorded by west zone (Rajasthan, Maharashtra, and Gujarat) at 33%. In the north zone (Uttar Pradesh, Haryana, Jammu and Kashmir, Himachal Pradesh, Punjab, New Delhi, and Uttarakhand) pooled prevalence of MRSA was reported to be 41%, while in the south zone (Tamil Nadu, Telangana, Karnataka Andhra Pradesh, Kerala and, Puducherry) reported a pooled pervasiveness of MRSA as 34%, the central zone (Madhya Pradesh) showed a pooled pervasiveness of 36% and north east zone (Assam, Tripura, and Sikkim) showed a pooled occurrence of MRSA as 40%. The yearly analysis showed the pooled prevalence of MRSA was more during 2016 (39%), succeeding 38% prevalence during 2015. Fig. 2.2 shows the prevalence of MRSA in different states of India. It appears that there was a consistency in reporting of prevalence rate of MRSA in all zones of India due to the homogenous behaviour of population [142].

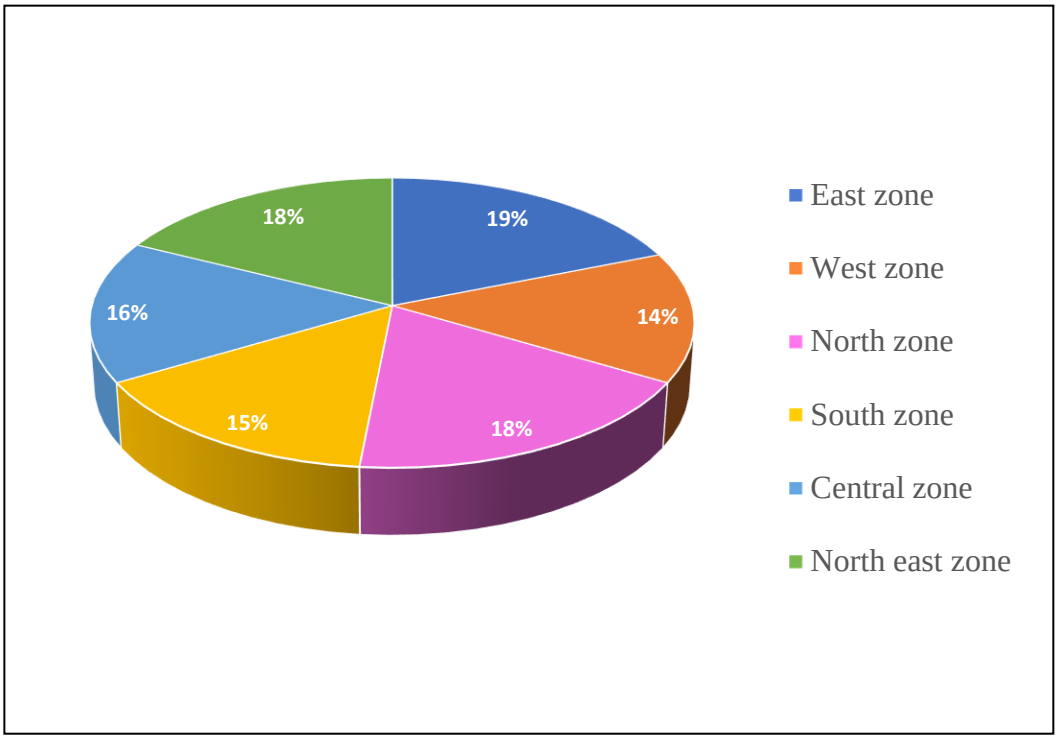


Fig. 2.2 Prevalence of MRSA in different regions of India

2.3.3 Comorbidity

It has been found that people who are infected with MRSA infections have various comorbidities like decubitus, diabetes, ulcers, chronic renal disease, prior stroke, or dementia [143]. Other risk comorbidity factors also include chronic hepatities, cardiovascular diseases, pulmonary diseases and even cancer [144].

Staphylococci mainly *S. aureus*, is one of the most virulent human pathogens which results in the colonization and infection of immune-compromised patients. Individuals having pre-existing diseases have a high risk for occurrence of MRSA infections as their immune system is compromised [144].

All comorbidities in association with MRSA that are involved in the original Chronic Disease Score (CDS) are respiratory illness, heart disease, rheumatism, rhinitis, rheumatoid arthritis, cancer, diabetes, Parkinson's disease, hypertension, epilepsy, acne, peptic ulcers, glaucoma, migraines, gout/hyperuricemia, high cholesterol and tuberculosis. Risk-factor studies of MRSA have revealed that four additional comorbidities that are associated with MRSA are human immunodeficiency virus (HIV)/acquired immune deficiency syndrome, transplant (solid organ or bone marrow), kidney disease, and injection-drug addiction [146, 147].

Recently, during the surge of SARS-CoV-2 virus, patients suffering fromCovid-19 were found to suffer from nosocomial infections (NIs), acquired during hospitalisation within 48-72 hours. MRSA infection is known to be endemic to healthcare acquisition. During the pandemic, it was observed that many immune-compromised patients and other Covid-19 patients who were hospitalised got detected with secondary illnesses, thereby, exposing them to MRSA infection posing greater risk of patient's death during hospitalisation. Studies suggest that around 63 samples out of 494 (12.6%) of SARS-Co-V-2 positive patients were found to be infected with MRSA [148].

Research conducted in the US found 34% increase in MRSA infections during COVID-19 which was comparatively greater than the cases reported in 2019 and this might be due to the poor management methods and central line insertion and duration of their hospitalization [148]. It was also found that on examining the blood samples of Covid-19 patients, traces of bloodstream infection were observed which was mainly caused due to co-infection by MRSA in the first 48 hrs duration of hospitalization [150]. In Brazil, the incidence density of MRSA increased up to 94% among healthcare-associated infections, during COVID-19. In general, all studies demonstrated the surge rise in secondary infection caused by MRSA thereby, alerting a major concern in pandemic era of COVID-19 [151].

2.4 *In- silico* approach on drug discovery

The term '*in silico'* stems from the computer component silicium: *in- silico* method. Therefore, it refers to the methods or prediction using computational approaches [152]. *In-silico* pharmacology, also known as computational pharmacology is looked upon as a rapidly growing area running on application of software to apprehend, analyse and integrate biological and medical information from varying sources. Precisely, it deals with the conception of computational simulations used to make predictions, propose hypotheses, aiding in discoveries in medical and therapeutical field [153].

Amongst all the *in-silico* approaches, the most important is target discovery in which, bioinformatics plays a major role. *In-silico* methods have advantages over other computational approaches which can be summarized as follows:

(i) Rapid predictions for a large array of compounds via high-throughput mode.

(ii) Prediction based on only the availability of the structure of a compound prior to its synthesisation.

Therefore, *in-silico* approach is suggested to be used at a very early stage in the drug development process, such that compounds are readily available to be synthesized and no impurities are present later in drug development process [152]. Drug design, discovery and development is a complex and gruelling process. The Drug discovery process involves an array of steps necessary for identification of new compound which can efficiently bind to a specific target for effective treatment of a disease [154]. Briefly, the steps are summarized in Fig. 2.3

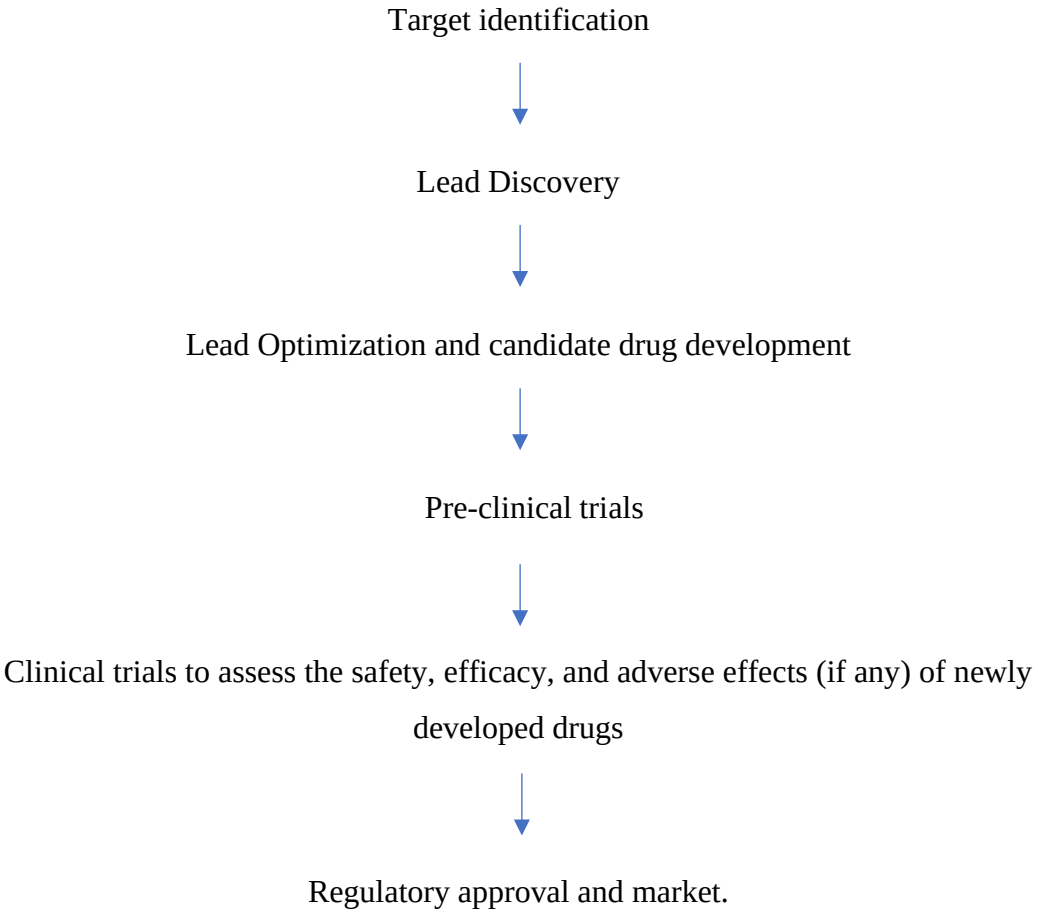


Fig. 2.3 Flowchart of *in-silico* drug design process

A major limitation of the drug discovery process is the long duration of time taken of around 12–15 years to introduce a new drug into the market having a cost value of up to $2 billion. Again, if any default arises in the later stages of drug design process, it will lead to great loss both in terms of money and the time taken during research. Hence, to overcome such barrier, inexpensive technologies that shorten the length of the drug discovery process in a reliable way are required [155].

Computer aided drug design (CADD) is one such technique which is helpful in the search for new drug and drug targets, thus playing a key role in the drug discovery process, right from the initial stage to the last stage of clinical development of a compound. During such studies, in-depth analysis of drug receptor interactions is accelerated, which is an important requirement for efficient designing of novel drugs and further development of the existing drugs [155,156]. The important factors that enable CADD in designing an effective drug includes: identification of target, analysis of a sequence, comparative structure modelling, virtual high throughput screening, drug-receptor interactions, physicochemical modelling, drug optimization and ADMET [154].

The properties of pharmacokinetics, toxicity, and effectiveness is critical for the production and release of effective lead molecules and drugs in the market. The efficacy of a pharmacokinetic profile of any compound is determined by its absorption, distribution, metabolism and excretion (ADME) properties along with toxicity (T). Application of ADMET analysis resulted in identification of drugs and consideration of the same in early-stage drug development, leading to a significant reduction in the number of compounds that failed in clinical trials due to poor ADMET properties [157].

ADMET-related *in-silico* models are generally used for fast and preliminary screening of compounds before carrying out their studies in *in-vitro* conditions. At present, there are several free and commercial computational tools for predicting ADMET properties namely, admetSAR, pkCSM, eMolTox, SwissADME, other databases and web servers, all of which aids in the prediction of absorption, distribution, metabolism, excretion, and toxicity properties of a drug molecule before it can be given the status of a lead drug [158]. ADMET properties are reliant on each other and therefore, their optimization in pre-clinical trials is done individually for drug development.

Various properties like logP, logD, and logS, along with the human intestinal absorption rate, oral bioavailability, blood-brain barrier permeation rate, Caco-2 permeability, human intestinal absorption, drug interactions, P-glycoprotein, plasma protein binding rate, CYP metabolic enzymes, and kidney clearance, toxicity are first screened using different softwares [159]. Human intestinal absorption (HIA) is amongst the important parameters of ADMET property. It determines the transport of the compound (drug) to the desired target site. Another important parameter is blood-brain barrier (BBB) that predicts several features of central nervous system (CNS)

microcirculation. Endothelial cell of CNS is pore-less and regulates the transport of ions, molecules, and cells which make CNS vessels impermeable in nature acting as an interference for the delivery of compounds into the central nervous system [160]. These properties are amongst the several other properties of ADMET that are widely studied as they play a major role in predicting the drug-likeliness of a compound [161].

Another major area of computational lead molecule standardization is molecular docking which helps in understanding the binding orientation of protein targets with respective ligands to predict the affinity and activity of the drug. The main aim of docking is to computationally stimulate orientations of ligands inside the protein binding site, visualize intermolecular chemical interactions, and estimate binding energies or affinities. Over the last decades many docking tools have been introduced to the scientific community that aid in drug discovery and development process. Some of these *in silico* molecular docking tools are AutoDock, GOLD, FlexX, DOCK, AutoDock Vina and Glide [162,163]. The process of molecular docking in brief is explained in Fig. 2.4.

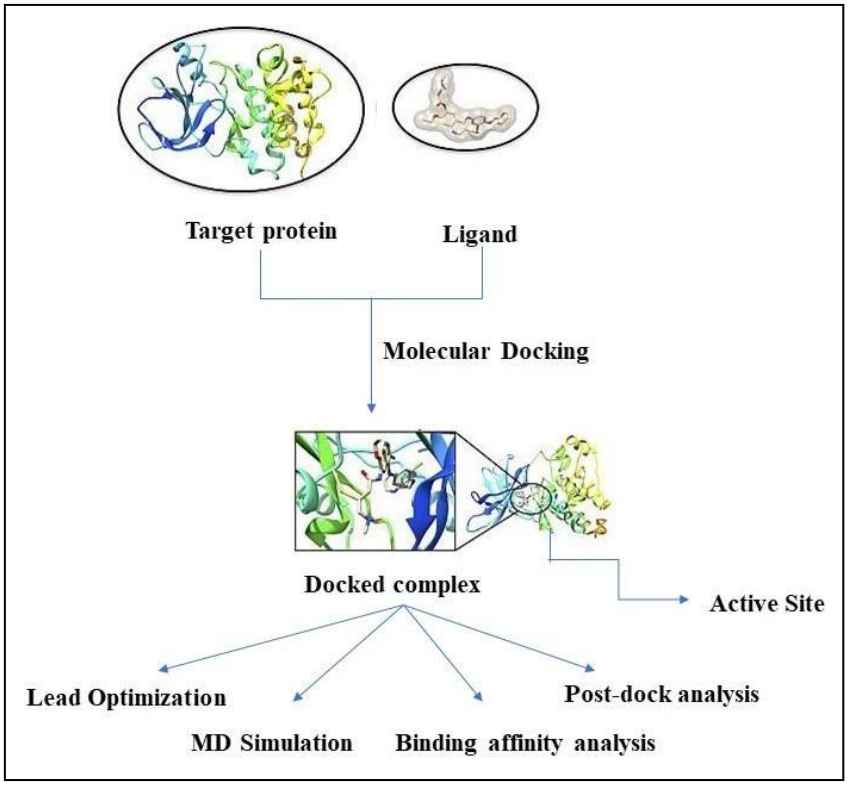


Fig. 2.4 Flowchart of Molecular Docking

Molecular dynamics (MD) simulations predict the movement of atoms in proteins or other molecular systems over time, using a general model of interatomic interactions. They can capture biomolecular processes like conformational change, ligand binding, and protein folding, revealing atom positions at femtosecond resolution. MD simulations can also predict how biomolecules respond to perturbations like mutation, phosphorylation, protonation, or ligand addition or removal. They are often used in conjunction with experimental structural biology techniques like X-ray crystallography, cryo-EM, NMR, EPR, and FRET [164, 165].

Molecular dynamics (MD) simulations, developed in the late 1970s, use Newtonian physics approximations to simulate atomic motions, reducingcomputational complexity. They begin by creating a computer model from NMR, crystallographic, or homology-modeling data. Forces on atoms are estimated using equations involving interactions between bonded and non-bonded atoms. Modelsinclude chemical bonds, atomic angles, dihedral angles, non-bonded forces due to vander Waals interactions, and charged interactions using Coulomb's law [166].

The energy terms in molecular dynamics simulations are parameterized to fit quantum-mechanical calculations and experimental data. This includes identifying ideal stiffness and lengths of springs, determining the best partial atomic charges for electrostatic-interaction energies, and identifying proper van der Waals atomic radii [165]. These parameters are called a 'force field', describing the contributions of various atomic forces that govern molecular dynamics. Common force fields used in molecular dynamics simulations include AMBER, CHARMM, and GROMOS [167, 168].

Molecular dynamics simulations provide valuable insights into protein motion, which is crucial in drug discovery. Protein conformations, like a single protein conformation, do not provide a complete understanding of protein dynamics. While static models like NMR, X-ray crystallography, and homology modeling offer insights into macromolecular structure, molecular recognition and drug binding are dynamic processes. When a small molecule like a drug approaches its target, it encounters a macromolecule in constant motion [169]. In some cases, protein motions are limited, and the ligand may fit into a static binding pocket. Typically, the ligand binds and stabilizes a subset of the many conformations sampled by its dynamic receptor, causing the population of all possible conformations to shift towards those that can best accommodate binding. Receptor motions play an essential role in the binding of most small-molecule drugs. Several techniques have been developed to exploit the

information provided by molecular dynamics simulations. Molecular dynamics simulations are crucial in filling the details in experimental methods, and with advancements in computer power and algorithm design, they are expected to play a more significant role in developing novel pharmacological therapeutics [165].

2.5 Ointment formulation and its activity against wound healing

Once the lead molecules are identified, various products can be developed for specific uses. A major use of plant based natural products including essential oils is in the development of topical formulations for treatment of wounds including bacterial infections. Topical ointments are semisolid substances usually behaving as viscoelastic materials when shear stress is applied. They contain medicaments and are generally intended for application in the external region of the body or the mucous membrane. Various medicaments already present are meant for topical application to intact or broken skin or to the mucous membranes, have been presented in the form of semisolid consistency and designated as ointment, creams, salves, pastes etc., and used mainly as protective or emollient for the skin [170].

Ointments constitutes a mixture of homogenous, viscous semisolid substance, having a greasy, oily texture bearing oil-80% and water-20% components. It is highly viscous in nature and is externally applied to skin or mucous membranes. Ointments are also used topically for several purposes in the form of antiseptics, emollients, antipruritic, keratolytic and astringents. The active ingredients present in the ointment when applied to the skin imparts protective and therapeutic effects [171].

Ointments consist of a base which acts as a conveyer for further formulation. Ointment bases are generally non-medicated substances required for the preparation of medicated ointments. Therefore, proper selection of the ointment base is a very crucial characteristic of its formulation which can result in proper drug absorption [172]. Some of the ideal properties of ointment bases that is required for consideration while formulating any drug are:

1) Wound healing ability should not be retarded
2) They must be able to bear low sensitization index
3) Should be pharmaceutically well-designed
4) Must be able to release the active ingredient resourcefully at the site of application
5) Bear low index of irritation

6) They must be non-dehydrating, non-greasy and neutral in reaction
7) Companionable with the skin
8) Easily washable with water
9) Should adhere to minimum number of ingredients
10) They must be inert, odourless, smooth, and,
11) They must be physically and chemically stable [173].

Ointments are also of various types depending upon the base material. The ointment bases are of following types as per Unique Selling Proposition (USP), namely:

1) Hydrocarbon ointment base (Water in Oil)- The base contains minute amount of aqueous component imparting emollient effect keeping the medicament effect for a long period of contact with the skin thereby acting as occlusive dressing. Examples- white petrolatum, yellow ointment, white ointment.
2) Absorption ointment bases- They are further categorized into two groups. Firstly, the base allows the incorporation of aqueous solution with the formation of water in oil type of bases, example- hydrophilic petrolatum lanolin. Secondly, other type being already w/o type of bases allowing the additional amount of aqueous solution, example- anhydrous lanolin.
3) Water removable bases- They are also termed as emulsifying bases. These are water washable bases, example- hydrophilic ointment, vanishing cream.
4) Water soluble base- It is a greaseless base containing water soluble constituents due to the absence of any oleaginous materials, example- polyethylene glycol ointment [171].

In the recent years, herbal drugs and products obtained from medicinal plants are also used in the formulation of ointment. The active constituents are incorporated along with the ointment base at an effective ratio through pulverization. Once the formulated drug is prepared, quality assurance of the ointment is evaluated in terms of irritancy, spreadibility, diffusion and stability [170].

Cranberry Fruit from the plant *Vaccinium macrocarpon*, a native plant grown in North America, is formulated as capsules. Due to the presence of proanthocyanidins, it is effectively used to prevent urinary tract infections, mainly caused by *Escherichia coli*. *Pedilanthus tithymaloides* (PTL) leaves was used as ointment and assessed for wound healing rat models. The results demonstrated that the methanol extract of PTL

showed a significant wound healing activity of the topical ointment formulations. The histological analysis revealed epithelialization with increased formation of collagen [174]. The leaves of *Entada africana* is commonly used in Africa for the treatment of wounds and skin infections where the extract was primarily used in the form of microcapsules. A prominent cytoprotective effect was found for the extractincorporated in microcapsules against indomethacin and ethanol induced gastro ulceration, signifying its potential use as a pharmaceutical formulation for the treatmentof peptic ulcer [175].

In traditional Indian medicine, polyherbal formulation is commonly used as hepatoprotective medicines possessing substantial effect on liver enzymes, lipid profiles, urea, and creatinine level. Polytoxinol, a steam-distilled hydrocarbon is extracted from five herbs namely, *Melaleuca alternifolia*, *Eucalyptus globulus*, *Syzygium aromaticum*, *Thymus sp*., and *citrus* species. It can significantly inhibit the biofilm formation at sub-inhibitory concentrations against clinical isolates of coagulase-negative *Staphylococci*. Polytoxinol when added in an essential oil-based topical formulation treats post-operative methicillin-resistant *Staphylococcus aureus* (MRSA) infection, serving as an alternative to long-term systemic antibiotic therapies. Bioavailability of the natural products promote the formulation of drugs in more effective way into modern therapeutics due to their exclusive advantages such as good therapeutic efficacy, low side effects and cost effectiveness over synthetic products thereby, encouraging the use of pharmaceutical products in the market soon [176].

2.5.1 Animal model for in-vivo studies

An effective ointment for the treatment of various ailments in humans can be validated only if proper scientific animal models' investigations are carried out. However, various studies indicate the significant features by which animals differ from man in features that affect percutaneous absorption specifically the thickness and nature of stratum corneum, the density of hair follicles and sweat glands, the papillary blood supply, and various biochemical aspects [177]. The animal models are valuable for studying the anatomy, physiology, and biochemistry of skin and for screening of topical agents. It is also used in the detection of possible hazards, and for carrying out various biopharmaceutical investigations thereby serving as a good candidate for pre-clinical trials. Thus, the animal models also serve as a precursor to study the wound healing process of various formulated topical ointments [172].

The estimation of transdermal absorption of molecules is a significant phase for evaluation of any topical formulation and therefore, studies using animal models are essential for evaluation of various experimental data [178]. Laboratory rats and mice offer ideal animal models for carrying out biomedical research and comparative medicine studies, since they share many resemblances with humans in terms of anatomy and physiology. Studies reveal that rats, mice, and humans, possess approximately 30,000 genes of which almost 95% are shared by all three species. Rodents are used for research purposes as they have economic advantages over other animals such that they require little space or resources for their maintenance, have short gestation times producing a large number of offspring, pertaining to rapid development into adulthood, and having short life spans [179,180].

Wounds, particularly in the skin, offer a great chance for many microorganisms to infect, which if not treated can lead to increased economic costs. Improper wound care can therefore lead to infections, bleeding, inflammation, longer healing process and complications related to regeneration of tissues [181, 27].

During the study of wound healing in animal models, the most common site where topical ointment formulations are applied is the skin. Skin is amongst the largest organ of the human body and forms the main basis for outer covering of the living body. It is made up of multiple layers of the ectodermal tissues which protects fundamental set of muscles, bones, ligaments, and internal organs. It also protects against various environmental stress factors like microbes, heat, light and injury. In conducting wound healing studies, a wound is incised on the normal structure of the skin. Wound healing is an intricate process which is a response to the disruption in the anatomical structure and normal function of a skin tissue. Wound healing process is characterized by a sequence of events like granulation, inflammation, wound contraction, epithelialization, collagen formation and cicatrisation. Hence, with the smooth and coordinated development of all the wound healing events eventually leads to a successful completion of the process followed by restoration of the disrupted anatomical and functional state of the skin [182].

Various studies have indicated that wound healing process can be enhanced by the incorporation of natural products infused with medicinal properties. The various important wound healing attributes of such plant-based bioactive compounds can be attributed due to alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, and phenolic compounds. These bioactive compounds impart wound healing properties

through various mechanisms. For example, saponins play a role in enhancement of synthesis of pro-collagen, while tannins and flavonoids possess antiseptic property, and are readily absorbed by the apparent layers of the skin. Due to their properties, natural products, and their phytochemical constituents play an important role in wound healing [182].

Some of the medicinal plants imparting wound healing property are listed as follows:

Turmeric (*Curcuma longa*) is traditionally used for the treatment of asthma, respiratory diseases, liver disorders, diabetes, and skin injury. A major bioactive compound present in turmeric is curcumin which plays a vital role in wound healing. Various *in-vivo* trials and *in-vitro* experiments demonstrates that by altering the pericellular and extracellular matrix in the infected wound area, curcumin stimulates fibroblast proliferation, granulation deposition of collagen formation in cutaneous wound healing [183].

Ginseng (*Panax ginseng*) is amongst the popular medicinal plant consumed in China, Japan, Korea, and Eastern Siberia. The root extracts of *Panax ginseng* demonstrated notable improvement in the wound healing process by strengthening keratinocyte migration, proliferation induction and *in vitro* surge in collagen production in human dermal fibroblasts [184,185].

Comfrey is a perennial herb belonging to the genus Shikonin from the family Boraginaceae**,** has been found to have wound healing properties. Different studies revealed that in clinical trials, the rate of wound healing contraction time was remarkably shorter after the topical application of the ointment formulated using comfrey [186].

German chamomile (*Chamomilla recutita*) belonging to Asteraceae family is comprised of bioactive compounds like phenolics, flavonoids, apigenin, quercetin, luteolin, and their glucosides. Apigenin, is the main constituent of chamomile flora and has a notable effect on the wound healing process demonstrating the accumulation of re-epithelization and collagen in the dermis tissue, hence accelerating wound healing process [185].

Centella asiatica belonging to the Umbellifere family is found to be abundantly growing throughout India and parts of Pakistan and Madagascar [187]. Asiaticoside, the main constituent of *C. asiatica* has been found to be useful in the improvement of the healing of small and hypertrophic wounds by exerting anti-inflammatory efficacy.

It accelerates wound healing ability by fibroblast proliferation, leading to migration of tissue cells around the wounded area thereby activating certain growth factors uplifting wound healing [188].

Thus, in the process of wound healing, various events take place such as multiple cell formation, formation of extracellular matrix and action of soluble mediators like growth factors and cytokines in the wounded area. In the process of healing of the wounds various topical formulations are applied which aids in the healing process. With the advent of new technology and incorporation of bioactive components from medicinal plant, various composition of the topical formulations is prepared which are widely being explored by researchers for developing cost effective, efficient, stable, and sustainable delivery system for the treatment of wounds. Studies have demonstrated that bioactive compounds in medicinal plants have resulted in effective wound healing activity by promoting antioxidant effect, faster collagen deposition, and other connective tissue constituent formation [182,188]. Therefore, in the current perspective, the importance of medicinal plants is receiving wide importance for the formulation of topical application ointments. The wound healing ability of the essential oil from plants are particularly more investigated for their efficient activity in restoration of the skin.

A major solution for wound healing by bioactive compounds from plants is through the process of reducing the formation of scar. They also help in the stimulation of blood coagulation, fighting against infection and thus, help in the process of speeding up the rate of healing of wounds [189,190].

Although, the role and use of natural products derived from plants is well supported for the formulation of several topical applications, yet they also provide a lot of challenges. Some of these challenges are low yield of extraction [191] and use of organic solvents in the extraction process which may lead to irritation and cytotoxicity [192,193]. Another area of concern is the thermolabile property of bioactive compounds because of which many of these molecules are degraded during the preparation process of the ointments when heat is applied [194,195]. Even though these challenges posed a threat, however with the emergence of supercritical fluid technology a lot of bioactive compounds could be isolated. Also, being non-toxic, supercritical fluids posed major success for the isolation of such compounds [196]. Besides, several biomaterials have been developed over the decades which could prolong the stability of the compounds [181,197].

Therefore, the bioactive compounds isolated from various plants, due to their anti-oxidant, antimicrobial and anti-inflammatory properties have been a major source of the ingredients in formulation of topical ointments used for wound healing. They can be used in all the four stages of wound healing: haemostatis, inflammation, proliferation and remodelling [198,199]. It has been observed that, plant bioactive compounds help in the normal development of the wound healing process and supports the proper functioning of the growth factors and components of the extracellular matrix. Besides it also prevents the deterioration of the granulation tissues [181].

As such bioactive compounds present in plants can serve as a major boost in the formulation of novel topical ointment formulations which can pave the way for a fast wound healing process, however further research in the area needs to be carried out. Table 2.1 summarizes the wound healing properties of various natural products, revealing that potent components found in extracts or isolated from these products exhibiting *in vivo* wound healing effects.

Table 2.1 *In vivo* studies of the wound healing properties of the natural products.

Sl. No	Plant Name	Component Used	Wound healing model	References
1	*Thymus vulgaris*	Essential oils	Wistar rats (180–200 g, 3–5 months) were induced to acute dermal toxicity. Rabbits were used for wound healing test	[200]
2	*Artemisia judaica*	Piperitone, terpinene-4-ol, α-thujone, β-thujone, 1,8-cineole, camphor, linalool	Skin burn induction model was used on Sprague Dawley female rats (150 g, 3 months old)	[201]
3	*Rosmarinus officinalis*	Essential oils	Mice	[202]
4	*Zizyphus mauritiana*	Fruit extract	Full-thickness excisional wounds in adult male New Zealand Dutch strain albino rabbits	[203]
5	*Ephedra ciliata*	Methanol extract and quercetin	Albino male and female rat model with excision and burn wounds was used	[203]
6	*Moringa oleifera*	Hydroethanolic extract of seeds	Excision and incision wound Male Swiss albino mice models	[205]
7	*Pistacia vera*	Oleoresins	Circular wound excision New Zealand albino rabbits' model	[206]
8	*Curcuma longa*	Curcumin	Sprague-Dawley male rats	[182,207]
9	*Vitis labrusca*	Rutin, quercetin, nicotiflori, and astragalin	Excision wounds in male albino rats which was infected by *B. cereus*	[208]
10	*Nigella sativa*	Essential oil	The full-thickness excisional wound in female Sprague Dawley rats	[209,210]

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