Biotechnological strategies for natural bioactive compounds: From discovery to therapeutic applications (Review)

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Abstract. Bioactive compounds, derived from plants, microorganisms and marine organism biomolecules have been long known to have varied therapeutic properties and to be the future development of drugs. The discovery, production and optimisation of these compounds have undergone major progress through advancements in biotechnology, which have allowed these compounds to be used sustainably and efficiently to utilise natural resources. The present review identifies the key biotechnological approaches used in the discovery and development of natural bioactive compounds (NBCs), such as plant tissue culture, microbial fermentation, metabolic engineering, synthetic biology and omics-based approaches. Such technologies enable the discovery of new biosynthetic pathways, the activation of biosynthetic gene clusters and metabolic flux optimisation to improve the production and stability of high-value secondary metabolites. Moreover, newer methods of extraction and characterisation have enhanced the isolation and structural examination of bioactive molecules of various biological sources. NBCs exhibit antimicrobial, antioxidant, anti-inflammatory and anticancer properties through mechanisms, such as the inhibition of microbial cell wall synthesis, the scavenging of reactive oxygen species, the modulation of inflammatory signalling pathways and the induction of the apoptosis of cancer cells. Lastly, the issues related to large scale production, regulatory licensure and sustainable use of natural resources are presented and discussed, as well as future opportunities in incorporating state of art biotechnological devices to hasten the discovery and development of NBCs to be used in pharmaceutical and biomedical applications.

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Abbreviations: NBCs, natural bioactive compounds; NPs, natural products; PKS, polyketide synthase; CRISPR, clustered regularly interspaced short palindromic repeats; BGCs, biosynthetic gene clusters; ROS, reactive oxygen species; PI3K, phosphoinositide-3-kinase; HPLC, high performance liquid chromatography; CC, column chromatography; TLC, thin layer chromatography; DNA, deoxyribonucleic acid

Key words: natural products, bioactive compounds, metabolic engineering, phytochemicals, drug discovery, therapeutic applications

Contents

1. Introduction

Natural products (NPs) obtained from plants, animals, microorganisms and marine organisms contain a wide range of bioactive compounds that exhibit critical pharmacological properties (1). The earliest evidence of the use of medicinal plants for the preparation of drugs was found on a Sumerian clay slab from Nagpur, ~5,000 years old. It comprised 12 formulas for drug preparation referring to >250 various plants, some of which were alkaloids, such as poppy, henbane and mandrake (2). Due to their distinct chemical diversity and millions of years of evolution, NPs exhibit a wide range of biological activity and drug-like qualities. These materials are currently among the most crucial resources used for creating

new scaffolds and lead compounds. NPs will continue to be used to address the pressing need to create effective medications, and they will take the lead in the development of medications to treat human illnesses, particularly critical diseases (3). Ayurveda, the traditional Indian medicine, and traditional Chinese medicine are still the oldest and most active medical traditions. The experiential, philosophical and experimental foundations of these two 'great traditions' are solid. The interest of the public in complementary and alternative medicine has resurfaced due to several factors, including increased side-effects of chemical drugs, the lack of a cure for a number of chronic illnesses, the high cost of new medications, microbial resistance and emerging disorders (4).

The creation of structure-activity libraries can be aided by combining the advantages of knowledge derived from conventional systems, such as Ayurveda and other medical systems, with the potency of combinatorial chemistry and high-throughput screening. The three primary obstacles in drug development time, cost and toxicity, can be decreased by using fresh functional leads from traditional knowledge and experience-based databases. Drug research still benefits from the marked chemical variety that is observed in NPs. Numerous strategies have been created to capture the intrinsic value of NPs, despite the fact that the drug discovery engine used at present functions at a more rapid pace than in the past when they were the primary suppliers of drug leads. The difficulties in screening mixtures of structurally complex compounds have been reduced by significant advances in the separation and structure determination technology (5).

Bioactive NPs will continue to be key active ingredients and model molecules for the discovery and validation of therapeutic targets in the future. The most effective method with which to enhance drug discovery and development productivity in recent decades has been a multidisciplinary approach that combines combinatorial organic synthetic chemistry techniques with the creation of novel molecular diversity from natural product sources.

Biotechnology has enabled the development of contemporary medical devices for both preventive and diagnostic purposes, including vaccines, diagnostic test kits and radio-labelled biological treatments used for imaging and analysis. Due to infectious diseases, human health is currently a major global concern. Through the development of promising technologies, biotechnology has the flexibility to reduce global health inequities and has significantly improved human health issues. Globally, the advantages of biotechnology have increased life expectancy, health and quality of life (6). A number of different compounds, including phenolic compounds, nitrogen compounds, vitamins, terpenoids and certain other secondary metabolites, have been found in medicinal plants. These compounds are rich in valuable bioactivities, such as antioxidant, anti-inflammatory, antitumor, antimutagenic, anti-carcinogenic, antibacterial or antiviral properties. Researchers, including chemists, biochemist, and pharmacologists, currently focus widely on medicinal plants (7).

Ancient Indian texts reference the use of aquatic species in Indian therapeutic systems, which amply demonstrates their indispensability. Since the calcium and carbon in corals, shells and pearls do not form calcium carbonate, they are regarded as distinct calcium salts that are utilised in the treatment of a variety of illnesses. The chemistry of marine NPs is a relatively novel and promising area for drug discovery. The capacity of microorganisms to create bioactive small compounds for medication development is yet unparalleled. However, there is a lack of new therapeutic leads as the fundamental technologies used to find microbial natural compounds have not markedly changed over the previous few decades (8).

The present review paper focuses on natural bioactive compounds (NBCs) and also highlights the role of modern biotechnology in their systematic exploration, production and therapeutic potential. Bioactive compounds that can be derived from plants, microorganisms, as well as marine organisms are also discussed. The present review is based on the recent developments in the fields of biotechnology, metabolic engineering and omics technologies that have been integrated to provide a broad view of the discovery and sustainable production of NBCs to be used in therapeutic applications. In addition, the key challenges related to safety, scalability and regulatory aspects are briefly addressed, followed by an outlook on future prospects.

A comprehensive search of academic databases for peer-reviewed literature published until the current year was performed. Search terms, including plant tissue culture, microbial fermentation, metabolic engineering, synthetic biology, omics and bioactive secondary metabolites were used. Articles describing biotechnological mechanisms, biosynthetic pathways and industrial production were included, whereas non-peer-reviewed sources, duplicates and irrelevant studies were excluded.

2. Sources of natural bioactive compounds

NBCs can be derived from a wide range of biological and natural sources, which may include plants, microorganisms, marine organisms, animals and minerals. These sources produce primary and secondary metabolites which are structurally diverse. Among all natural sources, plants, marine organisms and microorganisms have emerged as the most reliable reservoirs of bioactive compounds, and hence can contribute substantially to medicine and pharmaceutical development (9). The natural sources of bioactive compounds are depicted in Fig. 1.

Plant-based bioactive constituents. Plant-based functional foods are derived from natural or processed plant sources and contain both known and unknown bioactive compounds. Functional foods can be systematically classified into six principal categories according to their dominant bioactive components, as follows: Steroidal saponins, polyphenols, flavonoids, alkaloids, polysaccharides and miscellaneous phytochemicals (10). The elevated concentrations of bioactive constituents and demonstrated health-promoting properties of these compounds have led to substantial growth in their consumption over recent years (11).

Microorganism-based constituents. Microorganisms play a crucial role in the development of NPs and medical therapy. Currently, a number of microbial-originated antibiotics are available in the market, and >120 of the key medicines

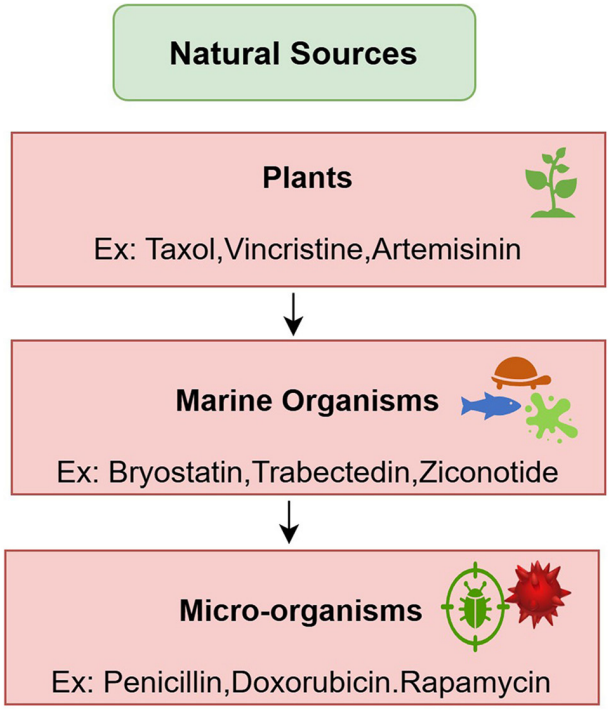

Figure 1. Natural sources of bioactive compounds.

in use are obtained from terrestrial microorganisms (12). A large number of bioactive metabolites are used in medicine, agriculture and industry, whereas ~100 of them are used for therapeutic purposes, herbicidal activity, growth-promoting agents, or tools for biochemistry (13).

In recent years, there has been immense interest in NPs from unexplored microbial sources, particularly actinomycetes (14), marine ecosystems (15) and microorganisms associated with plants (16), mammals (17) and invertebrates (18) from marine and terrestrial habitats. Even though the chief antibiotics currently in use are derived from cultivable microorganisms, only a minute fraction of microorganisms can be cultivated in routine laboratory cultures (19). The majority of microorganisms found in biological samples cannot be cultivated under normal laboratory conditions; these microorganisms are termed uncultivated microorganisms. These types of microorganisms can be cultivated using systems developed specifically for the organisms, such as synthetic medium mimicking the biosystem conditions and numerous other *in situ* cultivation strategies (20).

Marine organism-based constituents. It was discovered that compared to terrestrial microorganisms, marine microorganisms are a more valuable source of physiologically active secondary metabolites. This is explained by the fact that marine creatures have survived every stage of development and have developed the capacity to create a wide range of compounds with distinctive structural and functional characteristics (21). However, only 179 novel bioactive substances in marine bacteria have been identified. Thus, it is evident that researching the types and characteristics of secondary metabolites in marine microbes is critical. Previous number of studies have summarised research on the antibacterial, antimalarial, anticarcinogenic and anti-inflammatory properties of marine microorganisms and provide information on the types of NPs made from marine microorganisms (22,23). Fungi and bacteria are among the marine microorganisms whose properties and mechanisms of antibacterial action have been investigated. Therefore, from the perspective of human and animal health, there is a great demand for scientific and applied studies on the beneficial secondary metabolites of marine microorganisms (24). Furthermore, using marine microorganisms for drug discovery is a sustainable and eco-friendly option. Thus far, the results have been very positive. Therefore, substances derived from marine microorganisms may hold the key to combating antibiotic-resistant bacteria and numerous terrible illnesses, including cancer, acquired immune deficiency syndrome (AIDS), and even the current major worldwide pandemic, COVID-19, which is caused by the virus SARS-CoV-2 (25).

3. Biotechnological strategies for the production of natural bioactive compounds

Biotechnological approaches are vital in increasing the process of discovery and production of NBCs by allowing the manipulation of biosynthetic pathways, metabolic flux optimisation and the massive growth of biological systems. Such methods provide effective platforms for the sustainable production of high-value secondary metabolites that have pharmaceutical and biomedical uses.

Plant tissue culture. Plant tissue culture is a potent method for the development of novel, industrially significant plant characteristics and varieties, as well as large-scale, high-yield production to meet demand, frequently to create plant clones (26).

This methodology includes a wide variety of methods intended to accomplish certain commercial or scientific objectives. Through various morphogenic pathways, these methods enable regeneration by taking advantage of the developmental plasticity of plant cells and tissues, with the final choice of method depending on the explant type, plant species and intended use (27).

Callus culture. In callus culture, explants grown on media supplemented with suitable growth regulators are used to induce an unorganised, proliferative mass of cells. Cellular dedifferentiation is usually the cause of callus formation, which provides a versatile foundation for physiological research, genetic modification and regeneration. Hormone composition and nutrition availability have an impact on callus properties, such as texture, colour and growth rate. Callus cultures are particularly helpful for researching cellular metabolism and for commencing organogenesis or somatic embryogenesis. However, extended callus maintenance may increase the possibility of somaclonal variation; thus, the length of the culture needs to be carefully managed (28,29).

Organogenesis. The process by which organs, such as shoots or roots, develop from explants or callus tissue is known as organogenesis. It can occur directly from the explant surface or indirectly through a callus phase in between. Since direct organogenesis reduces genetic variation, it is typically selected for clonal propagation. Organogenesis is frequently used in genetic engineering and micropropagation as regenerated shoots are easily rooted and acclimated. Growth regulator

balance, explant competency and environmental factors all play a major role in the process (30,31).

Somatic embryogenesis. The process of developing embryo-like structures from somatic cells without fertilisation is known as somatic embryogenesis. The developmental stages and bipolar structure of these somatic embryos are comparable to those of zygotic embryos. The benefits of somatic embryogenesis include automation, large-scale propagation and the creation of synthetic seeds. For woody plants and species that are challenging to propagate using traditional methods, this method is extremely beneficial. However, exact hormonal and environmental regulation is frequently necessary for somatic embryo induction and maturation (32,33).

Cell suspension. Cell suspension cultures are formed by putting friable callus into liquid media and agitating continuously to disperse cells. These cultures provide uniform cell populations and are ideal for researching stress responses, metabolic control and cell physiology. Cell suspension systems are also commonly employed for the generation of secondary metabolites, since culture conditions may be modified to improve biosynthesis. A critical step toward industrial applications is the scaling up of suspension cultures in bioreactors (34).

Micropropagation. The most economically critical tissue culture method is micropropagation, which entails a number of distinct phases, including culture establishment, shoot multiplication, rooting and acclimatisation. This method allows for the production of genetically uniform, disease-free plants rapidly and on a large scale. Micropropagation has been used successfully on a variety of crops, including ornamentals, fruit trees, plantation crops and medicinal plants. Despite its benefits however, the physiological differences between *in vitro* and *ex vitro* environments render plantlet acclimatisation a crucial challenge (35).

Moreover, precursor feeding methods, as well as the optimization of nutrient composition, can be used to promote metabolic flux into the desired metabolites to yield higher yields and consistency (36). Hairy root cultures, suspension cultures and callus cultures have thus been extensively utilized in the manufacture of useful compounds, including paclitaxel, artemisinin, as well as vincristine. These biotechnological systems can ensure the stable production of large quantities of bioactive molecules of plant origin and may reduce reliance on natural plant resources (37).

Microbial fermentation. The term fermentation, which was formerly used to describe the anaerobic conversion of sugar by yeast to carbon dioxide and alcohol, currently refers to the technique used in the industry to produce a wide range of metabolites and biomaterials utilising microorganisms or mammalian cells in a controlled culture environment. Depending on the desired product, fermentation can be carried out in combinatory, fed-batch mode, batch mode, or continuous mode. Antibiotics, solvents such as ethanol, intermediate molecules such as citric acid, probiotics such as yoghurt, and other medically significant goods have long been produced using fermentation technology. Monoclonal antibodies, therapeutic recombinant proteins and deoxyribonucleic acid (DNA), as well as antiviral medications are examples of new generation fermentation products. In addition to pharmaceuticals, fermentation is employed in the commercial production of materials required for the creation of medical devices, drug delivery systems and diagnostic kits. The current rapidly expanding biopharmaceutical business, which is predicted to develop even more so in the near future in tandem with advancements in unique, focused medication discovery, is still centred on fermentation technology (38).

Microbial fermentation provides a highly regulated platform of production of bioactive secondary metabolites, since these metabolic networks are highly regulated in microorganisms. Bioactive compounds (e.g. antibiotics, polyketides, non-ribosomal peptides) are produced in specialized biosynthetic gene clusters (BGCs) which encode enzymes, including polyketide synthases (PKS) and non-ribosomal peptide synthetases (39). Nutrient availability, oxygen availability and pH are some of the factors in the environment, which affect the metabolism of cells and regulate the level of these gene clusters during fermentation. It is the optimization of fermentation conditions that can thus cause metabolic flux to be rerouted, to produce the desirable compounds and produce the undesirable by-products to a minimum. Recent developments in the systems biology and the study of metabolic pathways have further made it possible to manipulate the microbial metabolism in order to enhance productivity (40). Due to this, microbial fermentation is currently a stable means of producing pharmaceutically useful molecules in large quantities, including antibiotics, anticancer agents and immunosuppressive agents. The biotechnological strategies, mechanistic modifications of natural sources and applications of natural sources are illustrated in Fig. 2.

Metabolic engineering. The use of genetic manipulation to reroute metabolic pathways is known as metabolic engineering. Currently, metabolic engineering is crucial to the production of fuels from renewable resources, the transformation of agricultural raw materials (such corn syrup) into bulk and speciality chemicals, and the identification, creation, and expansion of products that have medicinal value (41).

Metabolic engineering is an effective technique for the discovery and development of drugs from natural products, including lead optimisation and industrial-scale production. Advances in the understanding of natural product biosynthesis pathways and metabolic networks at the genome level have led to the rational discovery of novel chemicals and high-producing strains, replacing the conventional empirical methods. Plant aromatic polyketides, including flavonoids and stilbenes, were effectively synthesised in *Escherichia coli* by Horinouchi (42) using an artificial pathway construction strategy. This involved combining genes from different plant pathways to create a functioning pathway (Horinouchi) (38) A metabolite produced by a fungal synthesis in a heterologous host is 6-methylsalicylic acid, which is produced by the multifunctional PKS 6-methylsalicylic acid synthase. In previous studies, heterologous expression was successfully achieved in *Streptomyces coelicolor* CH999 (43), *Escherichia coli*, *Saccharomyces cerevisiae* (44) and *Aspergillus nidulans* (45).

The integration of developing systems with synthetic biology is predicted to make metabolic engineering applications more powerful. Certain natural compounds that are only available in small amounts, such as those from certain plants and marine animals, can currently be synthesised in

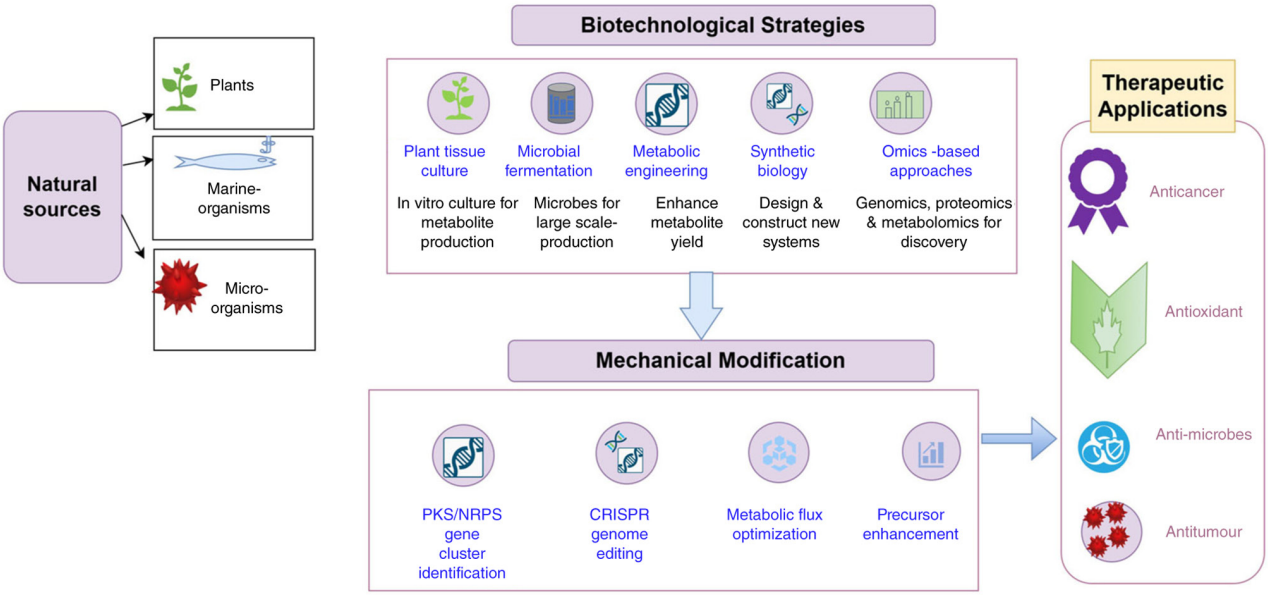

Figure 2. Biotechnological strategies, mechanistic modifications of natural sources.

modified heterologous hosts in amounts adequate for industrial use thanks to metabolic engineering. Through processes such as gene dosage (46), transcriptional regulation (47), or protein engineering (48), metabolic engineering enables one to precisely control the expression of certain enzymes. For instance, it was discovered that aminoadipyl-cysteinyl-valine synthetase (ACVS) was the bottleneck enzyme during the biosynthesis of penicillin in *Aspergillus nidulans*. The overexpression of the gene encoding ACVS led to a 100-fold increase in ACVS expression and a corresponding 30-fold increase in penicillin (49). Penicillin only slightly increased when genes encoding the downstream tailoring enzymes, isopenicillin N synthetase and acyltransferase, were overexpressed (50).

CRISPR technology allows for precise metabolic engineering by reprogramming biosynthetic pathways in plants, fungi and bacteria. It enhances the biosynthesis of bioactive compounds by optimising gene expression, altering regulatory elements and activating silent biosynthetic gene clusters (51,52). It is a versatile tool for metabolic engineering with precision, flexibility and multiplexing ability. The use of guide RNA (gRNA) to guide the Cas endonuclease to specific genomic loci, allows researchers to perform targeted modifications to reprogram metabolic flux and improve the production of bioactive compounds (53,54). The key methodologies include the following: Genome editing, pathway optimization, multiplex editing and dynamic regulation.

The integration of clustered regularly interspaced short palindromic repeat (CRISPR) into metabolic engineering provides a sustainable and efficient approach to meeting industrial demand for 'superfoods' and therapeutic agents. The characterisation of genes in elucidated pathways and the identification of limiting steps can enable the development of plants and microbes with tailored metabolic profiles (55,56). Along with the clearly increased yields, CRISPR technology has enabled the identification of novel compounds, such as the previously unknown alkaloids found in edited opium poppy plants. Challenges such as off-target effects and delivery limitations continue to exist; however, the integration of CRISPR with machine learning-aided gRNA design and synthetic biology continues to expedite the design-build-test-learn cycle in biomanufacturing (53,54).

There have not been sufficient metabolic engineering experiments being performed on marine natural drugs. A native organism was used in one effective case of metabolic engineering for the development of marine natural drugs (57). The salinosporamide A-specific pathway regulatory gene, salR2, from the marine bacterium *Salinispora tropica* was previously characterised and expressed, allowing the modified strain to manufacture salinosporamide A at a 2-fold higher level than the wild-type strain (58). Metabolically engineered heterologous microbes have also been used to yield microbial marine natural medicines. The lyngbyatoxin biosynthesis pathway from *Moorea producens* was heterologously expressed in *Escherichia coli* and *Anabaena* sp (59,60). Similarly, *Streptomyces lividans* and *Streptomyces avermitilis* expressed the lyngbyatoxin biosynthesis pathway from Streptomyces blastmyceticus in a heterologous manner (61). Metabolic engineering can be a complementary strategy which can be used to reduce the time required to optimise a strain, streamline downstream chemical processing, or create analogues that would be challenging or costly to obtain by chemical methods alone. By combining all these strategies, the rich biochemical diversity that nature has to offer can be taken advantage of; the traditional methods of chemical diversification and classical strain improvement remain potent techniques and currently outperform metabolic engineering approaches in terms of the size of combinatorial libraries produced or the titer achieved for industrial microorganisms (62).

Synthetic biology. Synthetic biology represents an emerging and cross-disciplinary branch of biotechnology that combines systems such as biology, chemistry, computer science and genetic engineering technology. The main aim of synthetic biology is the design, modelling and fabrication of new

biological systems or the modification of old ones to carry out new functions (63-65).

Synthetic biology technologies may generate bioactive molecules from scratch by creating heterologous metabolic pathways inside microbial cell factories. This approach is far safer and 'greener' than conventional chemical synthesis and plant extraction, providing a sustainable mode of production. It also allows for large-scale sustainable cultivation, which is consistent with modern notions of green, low-carbon and high-quality development, and is unaffected by the external environment. Berberine (16.9 mg/l), (+)-borneol (753 mg/l) and β-elemene (4.7 g/l) are examples of the substantial progress made in the manufacture of pharmaceutical bioactive chemicals using synthetic biology technology (66-68).

Synthetic biology applications in drug delivery remain in their early stages. However, it has already had a significant influence in reorienting pharmaceutical studies. The plethora of experimental chemical proteomics data has revealed the presence of numerous biological targets for a single medication. This introduces a systemic perspective on polypharmacology that is perfectly consistent with the cell-based phenotypic and holistic approaches of synthetic biology. Rational-based genetic design is analogous to rational-based drug design in medicinal chemistry. The significant success of synthetic biology in bioproduction, combined with the success of artemisinin, is likely to affect the early stages of drug delivery. Future research will most likely focus on the rational design of new biochemicals via genetic shuffling of biosynthetic modules in order to meet the demands of large-scale production within microorganisms.

Synthetic cells have evolved not only into a bio factory for creating added-value compounds or developing novel NP-like derivatives, but also into a wet laboratory where therapeutic targets or cell signalling pathways may be investigated. Additionally, this provides a rational approach for creating cell-based assays to search for substances that will cause the designed disease phenotype. Synthetic cellular models can also be utilised to discover disease causes and investigate medication modes of action. The use of luminous or fluorescent reporter genes is particularly beneficial for tracking phenotypic biomarkers in connection with physiological conditions. Optogenetics has considerable applications in the research of psychiatric disorders (69) as well as immuno-oncology, which aims to reactivate immune responses in tumours (70,71). The 'holy grail' of synthetic biology in the field of drug delivery is to develop a universal cell in which NP production may be selectively activated by disease characteristics. Lead optimisation could be accomplished via selective pressure caused by specific substrates. The modification of genome expression through environmental conditions is another notable property of living organisms, which can be modulated by synthetic biology and transformed into a therapeutic strategy to trigger molecular switches within pathological tissues (72).

Omics-based approaches (bioactive compounds). Traditional bioactive compound discovery was based on bioassay-guided fractionation, which frequently resulted in the re-isolation of known metabolites, producing a 'discovery bottleneck (73). The introduction of omics technologies, namely genomics, transcriptomics, proteomics and metabolomics has transformed this discipline by focusing on a systems-level knowledge of the metabolic potential of an organism (74). These techniques identify silent or cryptic biosynthetic gene clusters (BGCs) that remain unexpressed under typical laboratory settings, providing a blueprint for targeted discovery.

Genome mining uses bioinformatics platforms to predict chemical structures directly from DNA sequences. By screening sequenced genomes for essential biosynthetic enzymes, researchers can identify novel routes for experimental validation (75,76). The primary accomplishment of this approach was the discovery of salinosporamide K from the marine actinomycete, *Salinispora pacifica*. The anti-SMASH pipeline was used to conduct a systematic screening of sequenced genomes as part of the discovery process. Researchers discovered a unique BGC that did not correspond to any known metabolites in the *Salinispora* genus. The subsequent targeted fermentation and chemical analysis validated the synthesis of this new proteasome inhibitor (77). That study demonstrates how genome sequencing can reveal a massive database of BGCs that are undetectable under typical laboratory conditions but can be detected using their genetic fingerprint (77).

Since >99% of environmental microorganisms are unculturable, metagenomics provides a culture-independent approach by directly sequencing DNA from environmental samples (78,79).The discovery of ET-743 (trabectedin), a therapeutic anticancer drug, was a landmark moment in metagenomics. Initially isolated from the tunicate *Ecteinascidia turbinata*, the limited yield from the host animal rendered pharmaceutical development difficult. Metagenomic sequencing of the microbial population of the tunicate revealed that the real producer is the symbiotic bacteria, *Candidatus Endoecteinascidia frumentensis*. Similarly, the bioactive metabolite bryostatin 1 was linked to its symbiont using metagenomic binning and assembly (73,77). These examples demonstrate the ability of metagenomics in unlocking the chemical potential of symbiotic organisms that cannot be produced alone. The types of biotechnological strategies for the synthesis of bioactive compounds, along with their applications, advantages and limitations are presented in Table I.

Metabolomics provides the high-throughput profiling of low-molecular-weight metabolites, allowing for the more rapid investigation of chemical variety than previous approaches. Genomics indicates potential, proteomics and transcriptomics validate actual metabolite synthesis. The proteomic investigation of secondary metabolism (PrISM) technique, as opposed to bioassays, identifies biosynthetic proteins. Using GE-PrISM, scientists found griseobactin and rakicidin D in *Streptomyces lilacinus* (80). Furthermore, transcriptomics has proven critical in understanding the production of artemisinin in *Artemisia annua*. Omics-based approaches have transformed natural product discovery into a molecularly guided pipeline. Landmark molecules have been successfully identified using genome mining, metagenomics, and molecular networking, providing a strong foundation for next-generation therapeutic leads.

4. Extraction and characterisation of bioactive compounds

In order to separate and characterise the desired chemical components from the plant materials, extraction is an

Table I. Summary of various biotechnological strategies for bioactive compound production, along with their key applications, advantages and limitations.

Strategies	Types	Key products	Potential	Advantages	Limitations	(Refs.)
Plant tissue culture	Callus, organogenesis, somatic embryogenesis, cell suspension, micropropagation	Paclitaxel, artemisinin, vincristine	High-yield cloning and industrial mass production	Continuous yield without wild plant depletion	Somaclonal variation and acclimatisation issues	(26,27)
Microbial fermentation	Batch, fed-batch, continuous, BGC regulation (PKS/NRPS)	Antibiotics, citric acid	Biopharmaceuticals and targeted drug discovery	Controlled and scalable large-volume output	Needs strict pH and O_2 optimization	(38-40)
Metabolic engineering	Heterologous expression, gene dosage, pathway rerouting, protein engineering	Flavonoids, 6-MSAS, salinospora mide A	Strain optimization and custom analogue creation	Overcomes pathway bottlenecks (e.g., ACVS)	Lower titers vs. classical strain improvement	(42-46)
Synthetic biology	*De novo* pathway assembly, genetic shuffling, optogenetics, biosensors	Berberine, (+)-borneol, β-elemene	Green cell-factories and smart drug delivery	Sustainable, green and weather-independent	Early stage for drug delivery	(66-71)
Omics approaches	Genomics, transcriptomics, proteomics, metabolomics + bioinformatics	BGC mapping, biomarker discovery	Precision medicine and BGC discovery	Systemic and holistic cellular insights	High data complexity	(73,77-80)

BGC, biosynthetic gene cluster; PKS, polyketide synthase; NRPS, non-ribosomal peptide synthetase; 6-MSAS, 6-methylsalicylic acid synthase; ACVS, aminoadipyl-cysteinyl-valine synthetase.

essential initial step in the investigation of medicinal plants. Pre-washing, freeze-drying plant materials, grinding to create a homogeneous sample, and frequently enhancing analytical extraction kinetics and sample surface contact with the solvent system are all part of the fundamental operation. During the manufacture of the extract from plant samples, appropriate measures need to be taken to ensure that possible active elements are not lost, altered, or destroyed. In order to replicate the traditional herbal medication as accurately as possible, the extract needs to be prepared according to the instructions of the traditional healer in the event that the plant is selected based on its traditional usage. Furthermore, fresh green plants or dried powdered plant material are macerated or percolated in water and/or organic solvent systems to create a plant extract (81). The extraction methods of bioactive compounds are depicted in Fig. 3.

Extraction of marine sources. Compounds from marine sources can be extracted and isolated using methods that are relatively similar to those for terrestrial plants or microbial NPs. Thin-layer chromatography (TLC), column chromatography (CC), solid-phase extraction, ion-exchange chromatography, size-exclusion chromatography, counter-current chromatography, medium or low-pressure chromatography, and high-performance liquid chromatography (HPLC) are some of these methods. Additionally, the solvent partitioning approach is employed. When collecting marine invertebrates, handling them is crucial. It is necessary to take precautions against the breakdown of compounds. To aid with recall and the subsequent taxonomic identification, details about the organism and the location of collection should be meticulously documented (82).

Extraction of micro-organism sources. The first steps in a bioprospecting procedure are typically the isolation of a native microbe, the extraction of chemicals from a culture sample, or the isolation of environmental DNA for sequencing or heterologous expression. While DNA from isolated microorganisms or the environment can be screened using genome mining or sequence-based screening techniques, extracts from living microorganisms can be examined for bioactivity using function-based screening tests. The bioactive chemical should be purified, its structure clarified, and ideally, its mechanism of action explained if a desired activity has been identified (83).

Identification and characterization. The process of identifying and characterising bioactive compounds continues to face significant challenges as plant, marine, microorganisms extract typically consist of a mixture of different types of bioactive compounds or phytochemicals with varied polarity.

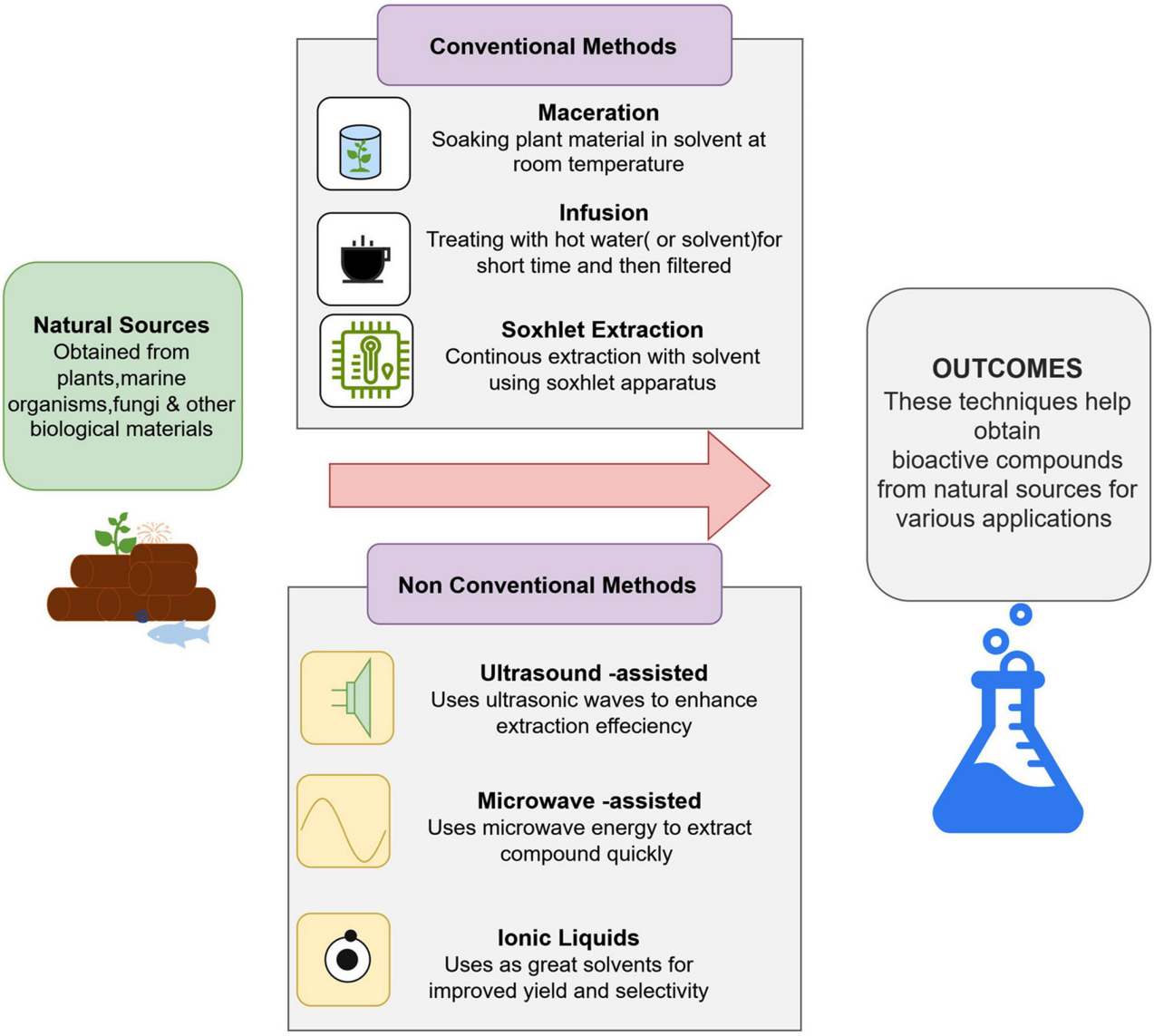

Figure 3. Extraction of bioactive compounds.

Several separation techniques, including TLC, CC, flash chromatography, Sephadex chromatography and HPLC, are frequently utilised in the isolation of these bioactive chemicals in order to get pure molecules. The structure and biological activity of the pure chemicals are subsequently ascertained. In addition, non-chromatographic methods, including phytochemical screening assays, immunoassays using monoclonal antibodies and Fourier-transform infrared spectroscopy can be utilised to identify the bioactive chemicals (84).

NBCs of plants, microorganisms and marine creatures have various pharmacological effects that vary in terms of their molecular mechanisms. These compounds have the potential to react with cellular targets, including enzymes, receptors and signalling pathways, to mediate biological processes that involve the occurrence of diseases. Indicatively, a variety of natural metabolites exhibit antimicrobial effects by destabilising microbial cell membranes or damaging key biosynthetic pathways and reactive oxygen species (ROS), and prevent oxidative cell damage. Moreover, various natural compounds can control the inflammatory signalling pathways and cause apoptosis in cancer cells, which promotes their role in the prevention and treatment of a wide range of diseases (85).

5. Therapeutic applications

Natural materials derived from plants, animals and marine life provide a variety of therapeutic treatments for infectious diseases and cancer. These substances function via intricate signalling pathways, which frequently call for advanced biotechnological production techniques to overcome issues with bioavailability and scalability.

In contemporary pharmacology, plant-derived chemicals are essential, particularly for the treatment of infections and cancer (86). Famous examples of these bioactive substances are curcumin, paclitaxel and artemisinin. Artemisinin, originally an antimalarial, displays anticancer effects by causing cell cycle arrest and apoptosis via ROS, as well as preventing tumour angiogenesis (87). Paclitaxel is basically a

Table II. Therapeutic applications of the bioactive compounds derived from plants, marine life and microorganisms.

A, Therapeutic applications of bioactive compounds derived from plants

Origin	Bioactive compound	Therapeutic application	Specific disease indication	(Refs.)
Artemisia annua	Artemisinin	Antimalarial	Malaria (particularly *Plasmodium falciparum*)	(50)
Taxus brevifolia	Paclitaxel	Anticancer	Ovarian, breast, and non-small cell lung cancer	(104)
Curcuma longa	Curcumin	Anti-inflammatory, antioxidant, anticancer	Osteoarthritis, metabolic syndrome, adjuvant cancer therapy	(105)

B, Therapeutic applications of bioactive compounds derived from marine life

Origin	Bioactive compound	Therapeutic application	Specific disease indication	(Refs.)
Brown seaweeds	Fucoidan	Anti-inflammatory, anti-viral	Metabolic syndrome, viral infections (e.g., influenza)	(106)
Dolabella auricularia	Dolastatin (DOLA)-10	Antitumour	Hodgkin's lymphoma, soft tissue sarcoma	(107)
Marine sponges	Cytosine arabinoside	Anticancer	Acute myeloid leukaemia (AML), acute lymphocytic leukaemia (ALL)	(108)

C, Therapeutic applications of bioactive compounds derived from microorganisms

Origin	Bioactive compound	Therapeutic application	Specific disease indication	(Refs.)
Fungal compounds	Polyketides, peptides, steroids	Anti-microbial. anti-viral, cytotoxic, anti-microbial, anti-viral, cytotoxic	Anti-microbial, anti-viral, cytotoxic	(109)
Marine microbial alkaloids	Alkaloids, peptides	Anti-bacterial, anti-fungal	Anti-bacterial, anti-fungal	(110)
Endophytic fungi metabolites	Secondary metabolites	Anti-microbial, anticancer, antioxidant	Anti-microbial, anticancer, antioxidant	(111)

terpenoid which functions as a potent cancer agent. It treats bladder cancer by modulating the phosphoinositide-3-kinase (PI3K)/Akt/mammalian target of rapapmycin (mTOR) signalling pathway. Curcumin, on the other hand, is a polyphenol that inhibits several carcinogenic pathways. It has anti-proliferative properties and regulates the PI3K/Akt/mTOR pathway, inhibiting cancer cell survival (88,89). To assure scalability, the production of these substances has moved from classical extraction to enhanced microbial cultivation, synthetic biology, and genome mining (90).

Marine species provide an extensive variety of secondary metabolites with various structural and biological functions. Cytarabine and eribulin are two major marine-derived agents. They are both successful marine-derived medications, especially in oncology. Marine-derived peptides and macrolides exhibit antiviral, anti-HIV and antimalarial effects (91). Marine anticancer peptides often induce apoptosis, disrupt the tubulin-microtubule equilibrium and prevent angiogenesis. These medicines are known for their selective targeting of cancer cells and ability to overcome multi-drug resistance (92). Technological developments in biotechnology, metabolomics and proteomics support marine therapies research (93). The therapeutic applications of bioactive compounds are presented in Table II.

Microorganisms and animal defence mechanisms serve as key sources for drug development, providing potent bioactive molecules refined by evolutionary constraints. Microorganisms are essential in the manufacturing of antibiotics. Advanced microbial cultivation techniques and synthetic biology have increased the possibility of creating new compounds at scale, addressing the worldwide antibiotic resistance challenge. Animal-derived substances include toxins such as bee venom (*Apis mellifera*), chlorotoxin (Israeli deathstalker scorpion), and Huachansu (*Bufo* toads). These chemicals are largely investigated for their potent anticancer properties. Various animal-derived medicines are being tested for their capacity to target cancer pathways. These, as with other natural compounds, are rigorously investigated to reconcile their high biological activity with safety and efficacy in therapeutic settings (94).

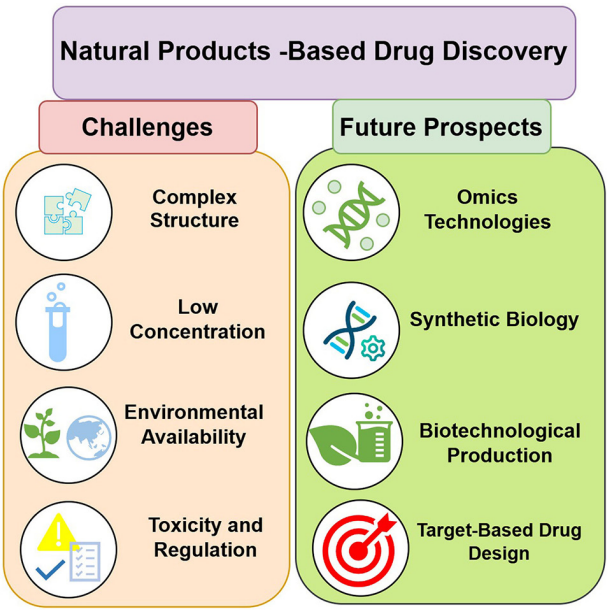

Figure 4. Challenges and future prospects in natural products-based drug discovery.

6. Challenges and future prospects

A number of bioactive compounds and natural products have poor pharmacokinetic profiles, which greatly restrict their clinical utility. As a result, these substances do not reach effective therapeutic concentrations *in vivo*, even though they are highly potent *in vitro* (95,96). The low bioavailability is primarily due to complex composition, physicochemical limitations, metabolic instability and the gastrointestinal tract. Furthermore, these chemicals are prone to chemical instability and rapid metabolism, limiting their duration in the body and preventing effective accumulation in target areas, such as the tumour microenvironment (97). A summary of the challenges and future prospects of natural products is depicted in Fig. 4.

Regulatory and systemic constraints further impede the translation of these drugs from the laboratory to clinical practice. The key issues include the following: The lack of standardisation in formulation poses a risk of contamination and adulteration in herbal goods (98), and the lack of globally accepted protocols for evaluating nano-phytopharmaceuticals and challenges in fulfilling FDA and EMA standards hinder clinical translation (99). While long-term toxicity assessments remain critical, they are not sufficient, and more rigorous safety profiling is required prior to their widespread clinical adoption (100). A key technical barrier remains the cost-effective scaling up of production of bench-scale formulations to large-scale manufacturing. Ethical commercialisation and sustainable harvesting practices are essential to prevent depletion of natural resources (101).

In order to overcome these limitations, the future of bioactive medicine is increasingly associated with nanotechnology, in which nanocarriers, such as liposomes and polymeric nanoparticles offer protected and targeted delivery systems that enhance therapeutic efficacy while minimising systemic side effects (102). Emerging computational frameworks, especially artificial intelligence and machine learning, are revolutionising the field by accelerating the discovery of novel compounds (101) and allowing the discovery of novel compounds and the prediction of their biological interactions with unprecedented accuracy. Furthermore, the convergence of synthetic biology and personalised medicine is opening the door for tailored nutraceutical and pharmaceutical therapies, permitting the customised use of bioactive compounds from plants and animals, according to individual genetic and metabolic profiles (103).

7. Conclusion

Since ancient times, NBCs have been a primary source of therapeutic agents, and they continue to be essential for the development of new drugs at present. The enormous chemical diversity found in microbes, plants and other natural sources holds tremendous promise for the creation of innovative and strong medications. Improved yield, purity and reproducibility have been made possible by the systematic discovery, manufacture and optimisation of bioactive chemicals enabled by the combination of biotechnology with traditional ways. The search for novel therapeutic leads has been accelerated by developments in methods, including tissue culture, microbial fermentation, metabolic engineering and omics-based approaches. Natural product-derived therapies are becoming safer, more effective, and supported by evidence because of ongoing technical advancements and interdisciplinary cooperation and have a promising future. Although major achievements have been made in the discovery and production of NBCs, there certain challenges remain in the way of large-scale, cost-effective and sustainable production systems. Recent technologies, such as genome editing, synthetic biology and multi-omics integration, are likely to be instrumental in enhancing the optimization of biosynthetic pathways and metabolic control further. Specifically, through the combination of systems biology, computational systems and more sophisticated systems based on metabolic engineering, it may be possible to precisely manipulate complex biosynthetic networks to provide superior yield and bioactivity. Future studies are warranted to focus on the integration of such advanced biotechnology methods in order to hasten the process of NBC engineering into effective therapeutic and functional biomolecules.

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Authors' contributions

AG was involved in literature review and in the writing the original draft of the manuscript. KY was involved in the review of the literature, and in the writing and editing of the manuscript. RS designed the structure of the review, and supervised the overall preparation of the review. ST supervised the review and edited the manuscript. AKM supervised the study and designed the structure of the review. PG and KS were involved in editing and revising the manuscript. KJ edited and supervised the manuscript. BB designed and supervised the manuscript. All authors have read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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