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Review Article

The Journey of Natural Products: From Isolation Stage to Drug's Approval in Clinical Trials

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Abstract
Nature serves as an excellent inspiration for researchers in the fields of chemistry and medicine. Terrestrial or marine organisms produce billions of natural products with unique chemical and physical properties. Some of them have been used in traditional therapy for specific diseases since ancient times. Although their exact chemical structures have yet to be elucidated clearly in that time, investigations in medicinal chemistry have been well documented. Furthermore, utilizing natural products in drug design and development offers advantages such as high biocompatibility, low toxicity, fewer side effects, wide bioactivities, and large structure diversity. Nowadays, rational drug research using computer-aided drug design is well established to cut the long way of drug discovery and overcome the resistance cases and the increment in the number of active patients. This review will highlight some natural products to comprehensively understand their journey from unknown natural products, isolation, purification, characterization, *in silico* evaluation, bioactivity screening assay, drug modifications, *in vitro* investigation, *in vivo* examination, and clinical trial.

Keywords: natural products, drug, *in vitro*, *in vivo*, clinical, toxicity, biological assay

1. INTRODUCTION

Natural products have infiltrated many aspects of industrial and our daily activities, including biomaterials [1]-[7], bioenergies [8][9], nanomaterials [10][11], bioelectronics [12][13], and pharmaceuticals [14]-[22]. They could be isolated from terrestrial and marine sources. Since the ancient era, natural products have been utilized in the traditional medicine and ethnopharmacology fields [23]. World Health Organization (WHO) defines traditional medicine as "a number of knowledges, skills, and practices based on indigenous beliefs and experiences, whether explained or not, used in health care, and in prevention, diagnosis, improvement or treatment of physical and mental illness". As of today, traditional medicines are still being applied in Indonesia, China, Egypt, Japan, Korea, India, and other countries [24].

Indonesia is a tropical country with the second largest global biodiversity after the Amazon rainforest. The National Agency for Drug and Food Control in Indonesia reported that at least 283 plants have been registered in the national database for medical applications [25]. In Indonesia, the use of herbs has been recorded since the 13th century. At that time, the drug design and development had not been established well. One of the main challenges was the few available technologies to predict the chemical structure of natural products; thus, the linkage between structure and activity relationship was not well connected. Nevertheless, people could find specific applications of herbs to treat a certain disease [26].

Both terrestrial and marine species produce billions of natural products with outstanding biochemical activities. Traditional herbs from various terrestrial plant extracts have been discovered since hundred years ago, and some people in urban areas still use these traditional herbs for some diseases. For example, the aqueous extract of *Kaempferia galanga* L. has been used to suppress the aches, while the aqueous extract of *Curcuma domestica* is helpful in decreasing blood pressure and menstrual pain. On the other hand, artemisinin from *Artemisia annua*, bakuchiol from *Psoralea corylifolia*, resveratrol from *Veratrum grandiflorum*, curcumin from *Curcuma longa*, and lycopene from *Solanum lycopersicum* have been used in global as antimalarial, anticancer, anti-

Table 1. Summary of the medicinal applications of natural products.

Source	Chemical compound	Application	Ref.
Terrestrial environment			
Curcuma domestica	curcumin	anticancer and antiinflammation	[29][30]
Curcuma xanthorrhiza	xanthorrhizol	anti-HIV	[31]
Zingiber officinale	gingerol	anticancer	[32]
Kaemferia pandurata	panduratin A	antibacterial	[33]
Alpinia galanga	ethyl 4-methoxycinnamate	antigastritis	[34]
Rheum palmatum	rhapontin	antidiabetic	[35]
Andrographis paniculata	xanthone	antimalarial	[36]
Phyllanthus sp.	elargic acid	anti-HIV and antimalarial	[37][38]
Glycyrrhiza glabra	liquiritin	antitussive	[39]
Abelmoschus moschatus	myricetin	antidiabetic	[40]
Taxus brevifolia	paclitaxel	anticancer	[41]
Cinnamomum camphora	camphor	analgesic	[42]
Pinus longifolia	longifolene	antibacterial	[43]
Cinchona ledgeriana	quinine	antimalarial	[44]
Digitalis purpurea	digoxin	antidiuretic	[45]
Artemisia annua	artemisinin	antimalarial	[46]
Rauwolfia serpentine	reserpine	antihypertensive	[47]
Quercus quercetum	quercetin	antioxidant	[48]
Syzygium aromaticum	eugenol	antifungal	[49]
Garcinia hanburyi	gambogic acid	anticancer	[50]
Marine environment			
Bulgula neritina	bryologs	antineoplastic	[51]
Halichondria okadai	halichondrin B	anticancer	[52]
Cryptotheca crypta	spongouridine	antiviral	[53]
Dictyota dichotoma	nordictyolide	antitumor	[54][55]
Ecteinascidia turbinata	ectrinascidin 743	anticancer	[56]
Microorganism			
Penicillium rubens	penicillin	antibacterial	[57]
Actinoplanes sp.	acarbose	antidiabetic	[58]

Table 1. *Cont.*

Source	Chemical compound	Application	Ref.
Streptomyces spectabilis	streptomycin	antitubercular	[59]
Streptomyces kanamyceticus	kanamycin	antibacterial	[60]
Aspergillus terreus	lovastatin	anticardiovascular	[61]

Alzheimer, anti-inflammatory, and antioxidant agents, respectively [27]. The marine sources, sponges, and algae are the primary sources of marine natural products. Ecteinascidin 743 was the first marine anticancer drug approved in 2006 [28]. A brief summary of the usage of natural products for medicinal application is listed in Table 1.

Sometimes, the combination of herbs can be used for traditional medical treatment due to the synergistic effect. Diabetin, a commercial name for a mixture of *Tinosporae caulis*, *Andrographidis herba*, *Curcuma rhizoma*, *Polyanthi folium*, *Alstoniae cortex*, and *Orthosiphonis folium*, is commercially used to treat diabetes mellitus in Indonesia. This example undoubtedly shows that nature has been an eminent inspiration for many researchers over the past several years [25]. Furthermore, natural products offer various advantages such as high biocompatibility, low toxicity, less side effects, wide bioactivities, and large structure diversity in medical treatment [62].

Recently, WHO reported that at least 65% of the world's population is concerned about traditional medicines. Now, we have modern sciences and technologies that can help the renaissance development of approved drugs based on natural products. Therefore, some natural products are undergoing clinical trials to understand the mechanism of action as the drug compound. Until 2019, at least 1602 natural products have been approved as drugs to treat diseases such as cancers (247 drugs), viral infections (186 drugs), bacterial infections (162 drugs), hypertension (82 drugs), diabetes (63 drugs), inflammation (53 drugs), fungal infections (34 drugs), Parkinson's disease (14 drugs), and Alzheimer's disease (6 drugs) [63].

Even though herbs from natural sources exhibit wide and excellent bioactivities, people shall be aware of the "blind" application of natural extracts for health treatment just by believing in "natural" and "herbal" brandings. Careless consumption of herbs or traditional medicines may lead to serious side effects and mortality as they consist of a mixture of bioactive, inactive, and (maybe) toxic compounds [64]. WHO encourages people to consider the modern era of health science based on reliable clinical trials and safe pharmaceutical formulations. To accelerate the drug discovery progress, this review briefly describes a rational drug journey from unknown natural products, isolation, purification, characterization, *in silico* evaluation, bioactivity screening assay, drug modifications, *in vitro* investigation, *in vivo* examination, clinical trial and approval by authorized organizations such as United States Food and Drug Administration. The drug discovery process for natural products is shown in Figure 1.

2. NATURAL PRODUCT ISOLATION, PURIFICATION, AND CHARACTERIZATION

The first step on this journey is the isolation of natural products. Natural products could be isolated from the sources using an extraction process [65]. Extraction is a mass transfer process of a chemical from the natural sample's matrix to the suitable solvent. This process consists of four steps, i.e., penetration of solvent to the sample's matrix, dissolution of the natural products to the used solvent, diffusion of natural products from the sample's matrix to the liquid phase, and collection of the extract [66]. This process depends on the nature of the solvent, such as polarity, dielectric constant, boiling point, viscosity, and surface tension. Commonly used solvents that are being used for the extraction process are acetone, *n*-butanol, chloroform, dichloromethane, ethyl acetate, ethanol, *n*-hexane, isopropanol, methanol, petroleum ether, tetrahydrofuran, and water, depending on the polarity and solubility of the target compounds [67].

The extraction process could be conducted in many ways, such as maceration, percolation, Soxhlet extraction, steam distillation, supercritical fluid extraction, ultrasound-assisted extraction, and microwave-assisted extraction. Maceration is the most straightforward extraction technique by soaking the samples in a solvent. However, it suffers from time-consuming and poorly extracted chemicals due to the strong or tight matrix in the samples. Percolation is a continuous maceration process in which the saturated solvent is replaced by fresh solvent for several times. Compared to the maceration process, percolation gives a higher extraction percentage but is more expensive due to using a larger volume of solvents. Even though the solvents could be recycled, solvent loss is inevitable during the evaporation and distillation processes [65].

Soxhlet extraction is another form of percolation technique in which the recycling happens within a system. The sample is placed in a chamber. Then, the solvent is heated and condensed to reach the chamber. Because of that, the sample is flooded by warm solvent, and the extraction occurs. When the chamber is full of the solvents, the solvents are flown through the siphon and reach the distillation flask. This process continuously happens until the targeted natural products are completely extracted. The main drawback of Soxhlet extraction is the usage of heating, which consumes more energy. It could damage the natural products as not all natural products are stable in high temperatures. Steam distillation is an isolation technique using water steam, especially for essential oils. The steam extracts the volatile compounds, and then both phases are collected in a separatory funnel [67].

Supercritical fluid extraction is a kind of extraction using a supercritical fluid such as carbon dioxide and water. Supercritical fluid offers great solubility to the desired chemical and large diffusivity into the sample matrix. Supercritical carbon dioxide is non-toxic, inert, low critical temperature (304 K), and non-polar media that meets the polarity of almost all natural products. Ultrasound-assisted extraction is an extraction process utilizing ultrasonic wave that improves the mass transfer of chemicals to the solvent. Meanwhile, microwave-assisted extraction exploits the mass transfer process by dipole rotation and ionic conduction mechanisms [68].

After the extraction process, a lot of natural products may be extracted in the solvent. Therefore, separation and purification of the targeted natural product are necessary [69]. The separation and purification stages can be performed by using a bio-guided fractionation strategy. In this method, each extract was tested for a certain biological application. Extracts that give no activity or poor activity are discarded and not further investigated. In contrast, the extract that gives a promising biological activity is collected, and then further separation and purification stages are conducted to find the lead compound that is responsible for the biological activity [70]. The scheme for bio-guided fractionation is shown in Figure 2.

Chromatography is the most used separation technique due to its high efficiency. The separation process is based on the difference in chemical affinities between the stationary and mobile phases [71]. Another technique is solvent partitioning by using an immiscible solvent. The separation principle happens through the different partition

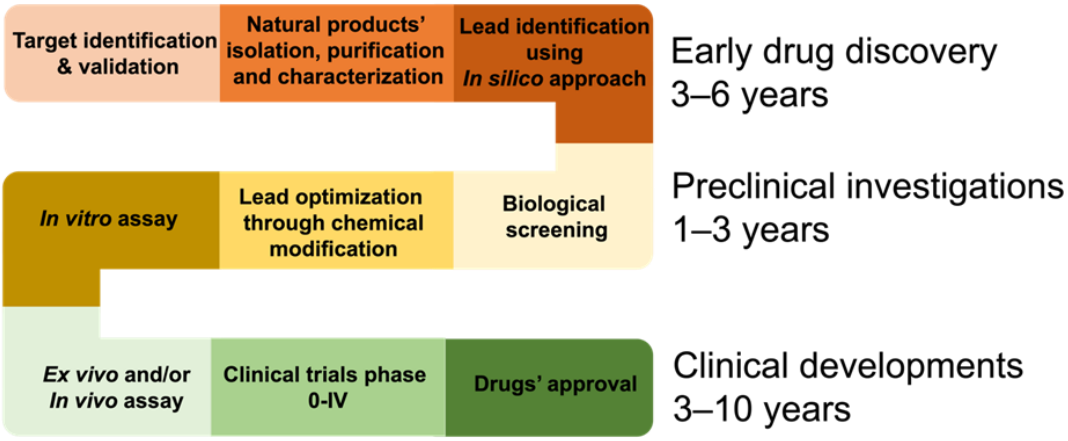

Figure 1. Drug discovery process for natural products.

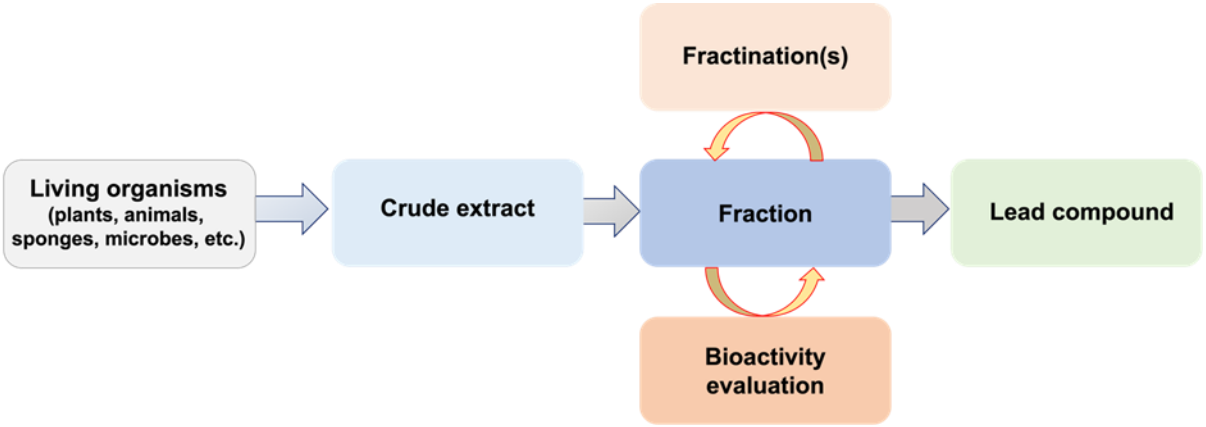

Figure 2. The principle of bio-guided fractionation.

coefficients in both solvents. Using a repetitive partition makes it possible to isolate pure natural products simultaneously [72]. On the other hand, membrane filtration can be performed if there is adequate information on the chemical composition of the samples. Smaller chemicals will pass through the membrane, while larger chemicals will be retained on the membrane. A similar principle is applied in the gel filtration chromatography. In gel filtration chromatography, smaller chemicals will be retained in the stationary phase; thus, they will have a longer retention time. Preparative thin-layer chromatography and recrystallization are other techniques that are sometimes powerful for the purification of natural products [68].

After the pure natural product is isolated, characterization of the natural product is required to elucidate its chemical structure. The characterization of natural products can be conducted using infrared, ultraviolet-visible, mass, and nuclear magnetic resonance spectroscopies. Infrared spectra are useful to identify the existing functional groups in the natural product, while ultraviolet-visible spectra are essential to understand the electronic levels due to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions. Mass spectra offer an investigation of the molecular ion of the natural product and its fragmentation on the chemical structure. On the other hand, nuclear magnetic resonance serves as an excellent tool to elucidate all protons and carbons in the natural product. Elemental analysis, single X-ray spectroscopy, and circular dichroism are also helpful for the final assessment of the determination of the chemical structure [73][74].

3. IN SILICO EVALUATION

After the clear chemical structure of natural products is obtained, *in silico* evaluation employing computer-aided drug design can be conducted to have fewer trials and errors in the laboratory [75]. In this stage, the three-dimensional structure of the natural product could be obtained from the available online database, as listed in Table 2 [76]. In the case of a novel natural product, its three-dimensional structure could be built in a computer and optimized using computational chemistry tools to observe the physicochemical properties such as total energy, atomic charge, dihedral angle, electron density surface, electrostatic potential surface, electronegativity, energy gap between the highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), electron affinity, dipole moment, and ionization potential.

After that, the observed parameters (descriptors) can be used to predict the experimental biological activity through a quantitative structure-activity relationship (QSAR) study. QSAR is a mathematical model that connects the molecular descriptors with the biological activity and could be found in the literatures based on the structure similarity. For example, the anticancer activity of halogenated xanthone against MCF-7 breast cancer cells can be estimated using the following equation:

$$\log(IC_{50}) = 100(LUMO) + 0.36(DM) - 3.02(qC6) - 1.71(qC4) - 7.98(qC1) + 7.85 \quad \textbf{(1)}$$

whereas IC_{50}, LUMO, DM, and qCi represent the half-maximal inhibitory concentration, LUMO

Table 2. Available online database for in silico screening of natural products [76].

Database	Hyperlink	Application
InterBioScreen Natural Products Database	https://www.ibscreen.com/natural-compounds	This database provides the chemical structure of more than 400,000 natural products
Super Natural II	https://bioinformatics.charite.de/supernatural	
PubChem	https://pubchem.ncbi.mlm.nih.gov/	
TCM@Taiwan	http://tcm.cmu.edu.tw/	Traditional Chinese medicine database
Indian Medicinal Plants, Phytochemistry	http://cb.imsc.res.in/imppat/home	Indian medicinal plants
SystematX	https://sistematx.ufpb.br/	Natural products database including species and taxonomy data with reported biological activity and pharmacokinetics data
NuBBE Database	https://nubbe.iq.unesp.br/portal/nubbe-search.html	
Dr. Duke's Phytochemical & Ethnobotanical	https://phytochem.nal.usda.gov/phytochem/search/list	
KNApSAcK	http://www.knapsackfamily.com/KNApSAcK	
DrugBank	https://www.drugbank.ca/	Database of proteins and nucleotides related to a specific disease with biochemical pathways for drug target
Therapeutic Targets Database	https://db.idrblab.org/ttd/	
Search Tool for Interacting Chemicals	http://stitch.embl.de/	
Medicinal Plant Genomics Resource	https://medicinalplantgenomics.msu.edu	Database for metabolome and genome of natural
METLIN Metabolomics	https://metlin.scripps.edu	

energy, dipole moment, and charge of a carbon atom, respectively [77].

Molecular docking is the next step in computer-aided drug design. Molecular docking can be performed utilizing the available three-dimensional structure of the protein receptor in the protein databank. Afterward, the natural product is placed in the same position as the native ligand or in the same position of its binding pocket. The possible orientations and conformations are estimated using computational algorithms to find the best complex structure. The best complex structure is indicated by the lowest root-mean-square deviation, the highest binding energy, the lowest inhibition constant, and more accessibility to the key amino acid residues of the targeted protein [78].

Then, molecular dynamic simulation is carried out to observe the stability of non-covalent interactions between the natural product and protein receptors during hundred nanoseconds at a certain temperature and pressure. Strong binding energy and stable complex from molecular dynamic simulation indicate that the natural product is promising for biological applications [79]. However, almost 80% of natural products fail in the experimental trials due to toxicity and poor pharmacology properties. Lipinski's rule of five is simple but valuable for evaluating orally active natural products. This rule does not allow (a) more than five hydrogen bond donors, (b) more than ten hydrogen bond acceptors, (c) higher molecular mass from 500 Daltons, and (d) higher octanol-water partition coefficient (log P) from 5. All criteria are multiples of five, which is the origin of the rule's name [80]. On the other hand, ADMET assessment examines the pharmacokinetic

properties of the natural product that stands for absorption, distribution, metabolism, excretion, and toxicity profiles [81].

4. BIOACTIVITY SCREENING ASSAY

After finding the best candidate of natural products through *in silico* assay, bioactivity screening is necessary to confirm and validate the expected biological activity of the proposed compounds. The type of bioactivity screening depends on the targeted applications. For antimicrobial assay, Kirby Bauer disc diffusion method is a standard protocol to screen the antibacterial activity for either Gram-positive bacteria or Gram-negative bacteria or fungi. A petri dish containing the cultured microbes in agar media was inoculated with the tested compound for a certain time. When the tested compound shows a strong antimicrobial activity, a wide clear zone (called as inhibition zone) will be formed. In contrast, a restricted clear zone will be observed when the tested compound shows weak antimicrobial activity. In that clear zone, the microbes are not able to grow due to the antimicrobial activity of the tested compounds [82].

In another case, antimalarial activity could be evaluated through heme polymerization inhibitory assay. This assay uses hematin due to its structural similarity with hemozoin in the Plasmodium parasites. When the tested natural products show promising antimalarial activity, the polymerization of hematin will be suppressed; thus, the concentration of hematin inside the Plasmodium vacuole will be high enough to cause the mortality of the parasites. Therefore, this initial screening is pivotal before *in vitro* screening using real Plasmodium parasites [83].

5. DRUGS MODIFICATION

Sometimes, it is necessary to modify the chemical structure of the natural products to obtain a higher biological activity, better pharmacological properties, lower toxicity to normal cells, higher solubility and partition coefficient, better metabolism, higher chemical and physical stabilities, or less undesired side effects [84][85]. As an example, halichondrin B is a natural macrolide produced by *Halichondria okadai* sponge. The first isolation of this compound was reported in 1986 by Hirata and Uemura [86]. In 1991, this natural product was highly prioritized by the United States National Cancer Institute due to its superior anticancer activity against murine cancer cells. In 1992, Aicher et al. reported a total

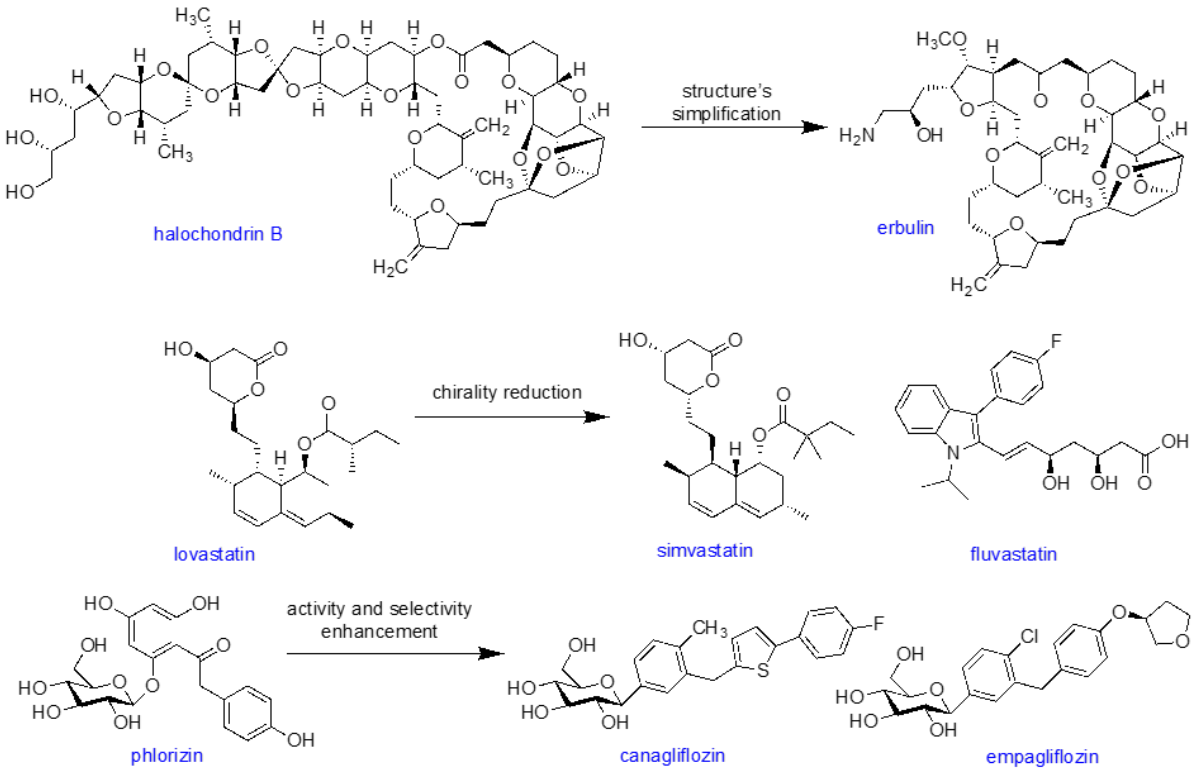

Figure 3. Modification of natural products-based drugs through chemical reactions.

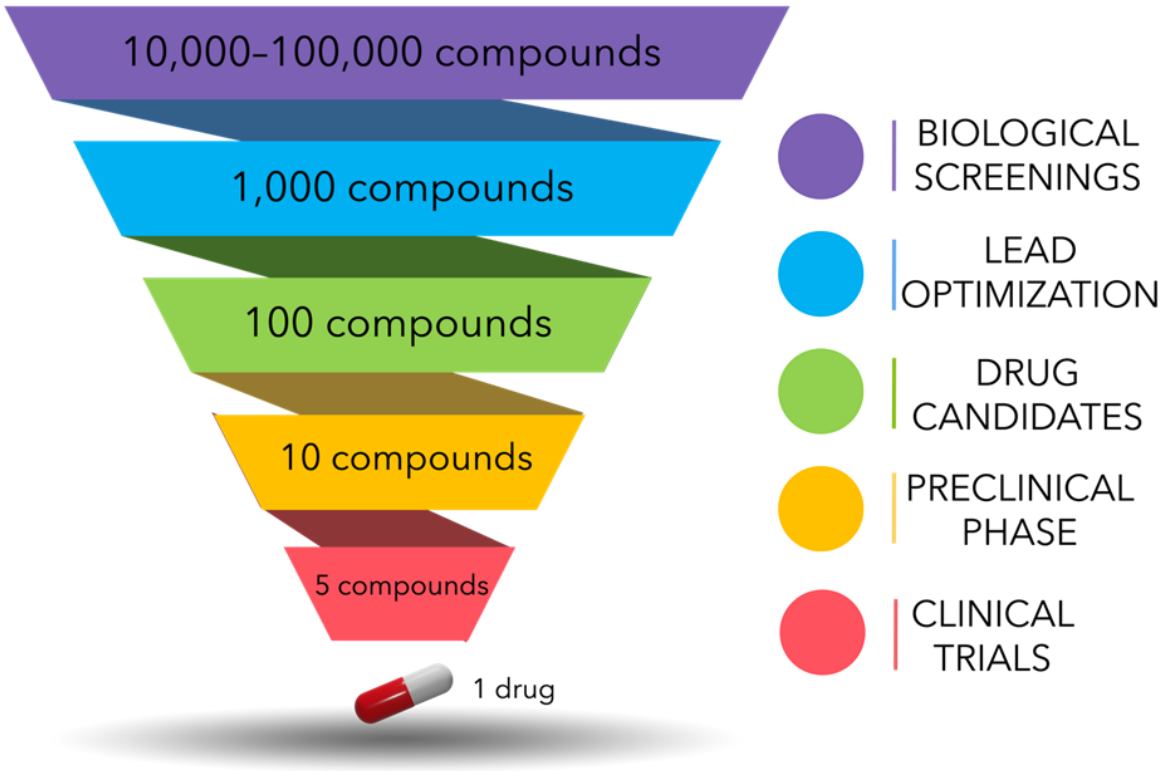

Figure 4. The journey of natural products in the drug discovery process.

synthesis of eribulin, a simplified structure of halichondrin B with similar bioactivity. This structure simplification is beneficial to significantly reduce the synthesis cost in the commercialization process. In 2010, the United States Food and Drug Administration approved eribulin for treating metastatic breast cancer patients [87]. The chemical modification strategy is shown in Figure 3.

Another approach for structure simplification has been applied to lovastatin in suppressing the blood cholesterol level and cardiovascular disease. Lovastatin had its approval in 1987. However, lovastatin contains 8 chiral centers; thus having 256 possible stereoisomers, which complicates the chiral resolution stage. The additional methyl group at carbon-alpha of lovastatin gives simvastatin with one chiral center reduction. Further modification of lovastatin yielded fluvastatin, having only two chiral centers with the same biological activity to suppress hypercholesterolemia in cardiovascular disease. Both simvastatin and fluvastatin have been approved in 1992 and 1994, respectively [88].

Phlorizin is an antidiabetic drug in *Mallus pumila* (apple) that works through the inhibition of SLT1 and SGLT2. However, its activity and selectivity as an antidiabetic agent are not satisfying. Chemical modification of phlorizin to canagliflozin and empagliflozin dramatically increased the activity and selectivity as the antidiabetic agent. Both canagliflozin and empagliflozin were approved in 2013 and 2014, respectively, in the United States, the European Union, and Australia [89].

6. IN VITRO AND IN VIVO INVESTIGATIONS

Investigation of chemically modified natural products is necessary to confirm no significant change in biological activity, but there is a critical improvement in the targeted properties [90]. For antimalarial agents, the *in vitro* antimalarial activity could be examined by testing on *Plasmodium falciparum* or *Plasmodium vivax* parasites on the blood samples. The half-maximal inhibitory concentration (abbreviated as IC_{50}) is a commonly used parameter to evaluate the bioactivity of the drug candidates. A higher IC_{50} represents a weaker antimalarial agent and vice versa. Another parameter, i.e., resistance index is an indicator to evaluate the ability of drug candidates for the resistant strains. The resistance index can be determined by dividing the IC_{50} of the resistant strain by the IC_{50} of the parent strain. A lower

resistance index shows a higher selectivity to the resistant strain [91].

A similar approach can be used to evaluate drug candidates as anticancer agents. The cancer cell lines are cultured in the selected media and incubated with the drug candidates for a specific time. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent is mostly used *in vitro* assay due to its simple observation by using an ELISA reader at a fixed wavelength of 570 nm. MTT assay observes the reaction between MTT and oxidoreductase enzyme in living cells; thus, the color absorbance of the solution directly reflects the cell viability. The IC_{50} value is estimated using a probit analysis. Further investigation of the anticancer agent can be performed by dividing the IC_{50} value for normal cells with the IC_{50} value of the cancer cells, called the selectivity index. A safe anticancer agent shall be toxic for cancer cells only and not give significant toxicity to the normal cells. The selectivity index shall be higher than 3 to be said as a selective anticancer agent [92].

Ex vivo assay is also possible to be examined when the disease is located in a tissue or an organ with a similar biological condition in the living organisms. This assay can be performed when there is no possibility of ethical approval in the living organisms, such as gene therapy, surgical model, or bioimaging purposes [93]. On the other hand, *in vivo* assay is the next stage for drug discovery after the *in vitro* assay. In *in vivo* assay, the experiment is conducted in a whole living organism such as mices or rabbits. *In vivo* gives a more comprehensive map of the bioactivity of natural products because it concerns all effects in the living organism. A drug candidate can be very effective and selective in the *in vitro* assay; however, it could be non-active in the *in vivo* assay due to fast catabolism in the liver or other reasons [94].

7. CLINICAL TRIALS AND DRUG APPROVAL

The last stage in the drug discovery is clinical trials. In this stage, the experiment involves a small group of living humans as a representative of the world's population. The main purpose of this stage is to confirm that the drug candidates are safe and give minimal side effects to the human body [95]. Four stages in the clinical trials should be passed before the drug approval. The first level is clinical trial phase 0. In this phase, around 10 to 20 people with specific diseases are given a drug candidate in a certain dose to confirm that the treatment is not harmful to the human body. This stage is also useful in preliminary investigating whether the drugs could reach the targeted cells. The second stage is clinical trial phase I, involving 20 to 50 people. This stage aims to find the best drug dose and medical treatment, as well as observe the side effects during the therapy. Next, clinical trial phase II uses a random sampling for more than 100 people to better understand the optimum dose and medical treatment that gives the best bioactivity with the most negligible side effects as a function of sex, age, genes, and health conditions. In clinical trial phase III, hundreds of people are treated with the new drug candidate to know its efficiency compared to the commercial drugs and/or standardized treatments. In the final stage, clinical trial phase IV is designated the last stage for hundreds to thousands of people to observe the long-term effects after consuming the drug candidates [96]. Unfortunately, almost 80 to 90% of natural products fail in clinical trials due to unsatisfying results [97]-[101]. Figure 4 shows that generally, a drug is approved from the thousands of natural products in the biological screening stage. Ultimately, the health organization will review the results of the clinical trials, and then the board will decide whether the drug approval can be given for commercialization, health, and safety purposes [102].

Once drug approval by authorized organizations and/or the government has been given, the commercial production of the approved drug compound is started. At this level, research and development groups are working to have a simpler isolation and modification method, a higher production yield, a higher purity, and a robust large-scale production. These research optimizations aim to find low-cost raw materials, suppress the production cost, inexpensive but safe drug formulation, limit the side effects, establish a continuous flow production rather than batch process, fasten the production process, and meet a wide consumer diversity [103][104]. This industrial research needs serious and continuous monitoring

because small mistakes in the drug production process could lead to fatal health problems in the communities [105]. From the industrial researchers, hundreds of patents are available as the results of industrial research; thus, the same drug compound may have some commercial names due to the unique and special recipe of each industry. For example, paracetamol is sold in the Tylenol brand by McNeil Consumer Healthcare from Johnson & Johnson in the United States, Switzerland, Portugal, Canada, and Brazil. In Turkey, Hong Kong, and Great Britain, paracetamol has been commercialized in the Calpol brand by Johnson & Johnson since 2006. On the other hand, Panadol containing paracetamol is available in the United Arab Emirates, Thailand, Taiwan, Singapore, Netherlands, Korea, Italy, Indonesia, and Australia. Other brand names of paracetamol, such as Acamol, Acetaminophen, Adol, Dexamol, Hedanol, Pacol, Paracitol, Paramol, Pyrenol, and Sanmol, are found in other countries as well [106].

8. FUTURE PROSPECTS IN DRUG DESIGN BASED ON NATURAL PRODUCTS

As of today, we are grateful that some powerful tools have been available for drug design based on natural products. They are the detailed reports of lead compounds on the published articles and online databases, available software's for *in silico* screenings, advanced isolation and purification techniques with robust instruments, well-established biological assay in the medicinal field, and a systematic evaluation for clinical trials. However, researchers are still widely open for drug design and development because some standard drugs have been resistant due to irregular usage of drugs and mutations in the key biomolecules in a specific disease. Furthermore, there is no available technology on cell, tissue, and organ simulations for a specific drug with similar parameters to biological conditions as of today. Drug design using machine learning and artificial intelligence may overcome that issue, but this field is still limitedly investigated recently. Drug design and development based on natural products calls on all talented researchers to give their best efforts to find more active and selective drug candidates and faster drug discovery to suppress the number of active and death cases in the future.

9. CONCLUSIONS

This review offers a comprehensive perspective on the drug discovery process based on natural products. The isolation, separation, purification, and characterization stage is the first step in finding the expected bioactive compounds. The natural products are then screened through *in silico* approach employing quantitative structure-activity relationship, molecular docking, molecular dynamic simulation, and ADMET assessments. This computer-aided drug design is constructive to suppress the laboratory's experimental cost, trials, and errors. The bio-guided assay is then subjected to focus the research on the targeted application. Structural modification can be conducted in order to have stronger biological activity, better pharmacological properties, lower toxicity to normal cells, higher solubility and partition coefficient, better metabolism, higher chemical and physical stabilities, and less undesired side effects. Preclinical investigations, including *in vitro*, *ex vivo*, and *in vivo* assays, are needed to eliminate unsuitable drug candidates. In the final stage, natural products shall pass through the clinical trials phases 0, I, II, III, and IV to obtain drug approval for an actual medical application.

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Conflicts of Interest

The authors declare no conflict of interest.

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