Review Article

Mechanism of Antimicrobial Activities of Medicinal Plants Extracts, From Traditional Knowledge to Scientific Insights

Abstract

Background: The therapeutic properties of plants have been acknowledged for numerous years as a vital source of active ingredients in therapeutic agents. In particular, the use of active ingredients derived from plants for use in microbial natural products have long been used before the dawn of modern medicine.

Discussion: From ancient times, the efficacy of medicinal plant products has been associated with the chemistry, biochemistry and synthetic activities of the plant products. Thus, with scientific advancement in modern molecular and cellular biology, analytical chemistry and pharmacology, the unique properties of these plant products are being harnessed in order to exploit the chemical and structural diversity and biodiversity of these types of products in relation to their therapeutic effect.

Conclusion: Often, new molecules of interest in drug design units focus on the rearrangement of chemical entities or structural isomers of naturally occurring products in order to generate new molecules; these may be formulated into clinically useful therapies.

Keywords: Medicinal plants; Extracts;efficacy; pharmacology

INTRODUCTION

Nigeria is a developing country with many rural areas where orthodox medicines are not available or not accessible; traditional medicine is, therefore, the first line of defense for many ailments such as coughs, fevers, headaches, psychosis, etc. Traditional medicine has been reported to be of unquantifiable importance to locals around the world [1], but as important as these traditional medicines are, there have been several scientific reports of their side effects [2], which led to the prescription of standardization of herbal medicines by the World Health Organization [3]. The standardization process consists of several steps, the first of which is the systematic documentation of the medicinal plants and their uses in rural communities around the world.

The World Health Organization (WHO) has stated that 80% of the developing world still benefits from the use of traditional medicines derived from medicinal plants [4]. The total estimated number of plants is approximately 374,000 [5] in comparison to 28,187 medicinal species used by humans [6]. WHO has also recorded the names of over 20,000 species of medicinal plants [7] and described medicinal plants as one of the potential sources of new drugs. More than 100 countries have developed regulations for medicinal plants. There are over 1340 plants with defined antimicrobial activity and over 30,000 antimicrobial compounds have been isolated from plants]8[. Moreover, it has been estimated that 14–28% of higher plant species are medicinal and that 74% of bioactive plant-derived compounds were discovered based on ethnomedicinal uses [9].

Different pathogens, such as virus, bacteria, or protozoans, have been and continue to be the cause of major pandemics and epidemics around the world, some considered relatively mild, such as influenza, and some others qualified as severe, such as the aforementioned COVID-19. Some examples are Vibrio cholerae, cholera-causing bacteria; Aedes aegypti, the dengue

transmitting mosquito; or Morbillivirus, a genus of paramyxoviruses which causes measles. One of the biggest challenges of medicine is the growth of multidrug-resistant microorganisms endangering the effectiveness of common drugs used in the health system [10]. Although multidrug resistance is a natural phenomenon, the inappropriate use of antimicrobial drugs, inadequate sanitary conditions, improper food handling, and poor infection prevention and control practices contribute to the emergence and promote the spread of resistance to several drugs [11]. Without an effective action to reverse current trends, we could face a return to the pre-antibiotic era, with simple wounds and infections causing significant harm and even death, and routine medical procedures becoming a very high risk. It has been estimated that antimicrobial resistance (AMR) might cause more deaths than cancer by 2050 [12]. This emerging trend is concerning and considered by the WHO to be perhaps the most urgent issue facing medical science. Given the increase in these diseases and the limited effectiveness of antibiotics, traditional knowledge can constitute a useful tool to address these new health challenges. One of the most important recent drug designs from ethnobotanically used plants, having deserved a Nobel Prize in Physiology or Medicine, is the discovery of artemisinin [13], which began as a response to the resistance developed by plasmodia to quinine-derived drugs.

Antimicrobial Activities of Medicinal Plant Extracts

Extracts isolated from medicinal plants have been reported exhibit various biological activities such as antimicrobial, anti-inflammatory, and antioxidant activities. The antimicrobial compounds from medicinal plants may inhibit the growth of bacteria, fungi, viruses, and protozoa by different mechanisms than those of presently used antimicrobials and may have a significant clinical value in the treatment of resistant microbial strains [14]. There are several mechanisms that underlie antimicrobial action of plant-derived compounds. Phytochemicals can act by disrupting microbial membranes (carvacrol, thymol, eugenol, *etc.*) or impairing cellular metabolism (cinnamaldehyde). They can also control biofilm formation (trans-cinnamaldehyde, carvacrol, thymol, geraniol, *etc.*). Plant antimicrobials can inhibit bacterial capsule production (salicylic acid and its derivatives). Some plant compounds can attenuate bacterial virulence by controlling quorum-sensing. Another mechanism of plant metabolites' antimicrobial action is the reduction of microbial toxin production (dihydroisosteviol, RG-tannin, *etc.*) [15]. Some of those active compounds show both intrinsic antibacterial activity and antibiotic resistance-modifying activities and some of them, while not effective as antibiotics by themselves, when combined with antibiotics, can help overcome antibiotic resistance in bacteria. Chemically complex compounds have great therapeutic potential as they have fewer side effects compared to synthetic drugs and also low chances of developing resistance [16]. Bacteria may develop resistance to medicinal plants treatment if only one active ingredient with a specific target is involved, a condition similar to an antibiotic. However, since the literature on bacteria developing resistance plants is limited then further research on resistance mechanisms is required [17]. Furthermore, the effectiveness of medicinal plant extracts to inhibit bacteria growth is also related to the synergistic effect between the active compounds of the extracts. The synergism action come from different effects, namely the emergence of multi-target mechanisms, the existence of compounds capable of suppressing bacterial resistance mechanisms, pharmacokinetic or physicochemical effects resulting in enhanced bioavailability, solubility and resorption rate, neutralization of adverse effects and reduction of toxicity [18].

Phytochemical studies identified the presence of different compounds such as spermidine, rutin, quercetin, tocopherol, and carotenoids, derived from caper (Capparis sp.) responsible for antimicrobial, antioxidative, anti-inflammatory, and antiviral activities. Seed extracts of

Capparis decidua showed antibacterial, antifungal, and antileishmanial activity probably due to quaternary ammonium and glucosinolate [19]. The use of bearberry (Arctostaphylos ura-ursi) and cranberry juice (Vaccinium macrocarpon) to treat urinary tract infections have been published, while species such as lemon balm (Melissa officinalis), garlic (Allium sativum), and tea tree (Melaleuca alternifolia) are described as broad-spectrum antimicrobial agents. Phenolic, alkaloids, flavonoids, triterpenes, and steroids from Cameroonian plants were the most bioactive compounds revealing significant antimicrobial activity [20]. The active ingredient of Fulyzaq (crofelemer, a proanthocyanidin oligomer0, was isolated from the plant Croton lechleri (Euphorbiaceae) found in the Western Amazonian regions of South America. The leaf extracts of Myrtus communis and Verbena officinalis exhibited good antibacterial activity against Staphylococcus aureus, Escherichia coli, and Salmonella typhi. Myrtus communis also displayed remarkable activity against Pseudomonas aeruginosa. Carrot (Daucus carota) seed oil, and tea tree (Melaleuca alternifolia) oil show antimicrobial activity against Helicobacter pylori and Mycoplasma pneumoniae, respectively[21]. Methanol extracts of Oxalis corniculata, Artemisia vulgaris, Cinnamomum tamala, and Ageratinaadenophora exhibited antimicrobial activities against Escherichia coli, Salmonela Typhi, Klebsiella pneumoniae, Staphylococcus aureus, and Citrobacter koseri [22] Also, hydromethanolic extracts of Berberis vulgaris, Cistus monspeliensis, and Punica granatum demonstrated high activity against Staphylococcus aureus, Enterococcus faecalis, and Enterobacter cloacae [23].

An endophytic fungus isolated from the medicinal plant Hypericum acmosepalum contained some compounds including hyperenone A, hypercalin B, and hyperphorin and emodin, responsible for antibacterial activity on resistant Staphylococcus aureus, on Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enterica, Escherichia coli, Mycobacterium tuberculosis, upon the fungal strains Aspergillus niger and Candida albicans [24]. The Hypericum olympicum contains numerous essential oil compounds, with the main components being E-anethole, β-farnesene and spathulenol, while other components included E-caryophyllene, germacrene D, terpenes and new type of acylphloroglucinol. The crude methanol extract of Hypericum olympicum showed a broad spectrum of very strong antimicrobial activity, with the highest activity observed against Klebsiella pneumoniae and Salmonella enteritidis [25]. Natural resins derived mostly from medicinal plants and their compounds revealed antibacterial and antiprotozoal activity [26]. In particular, the extract of propolis richer in flavonoids (pinocembrin and galangin) was more active against Streptococcus pyogenes strains. The antimicrobial effect of Korean propolis was studied against Streptococcus mutans. The compound diaporthalasin yielded from the fungus Diaporthaceae spp. from a marine sponge displayed potent antibacterial activity against both Staphylococcus aureus and methicillin-resistant Staphylococcus aureus (MRSA) [27]. Essential oils derived from aromatic medicinal plants, like fennel, peppermint, thyme, lavender, and containing mixtures of volatile substances, such as monoterpenes, sesquiterpenes, and phenylpropanoids, have been reported to be active on Gram-positive and Gram-negative bacteria and on fungi and viruses [28].

HISTORICAL PERSPECTIVE OF TRADITIONAL MEDICINAL PLANTS

African traditional medicine

African traditional medicine is ancient and perhaps the most diverse of all medicinal systems. Africa is considered to be the cradle of humankind, with a rich biological and cultural diversity and marked regional differences in healing practices. Unfortunately, even today the systems of medicines are poorly recorded. The documentation of medicinal uses of African plants is becoming increasingly urgent because of the rapid loss of the natural habitats of

these plants due to human activities. The African continent is reported to have one of the highest rates of deforestation in the world. This loss is all the greater because the continent has a high rate of endemism, with Madagascar topping the list at 82% [29]. African traditional medicine in its varied forms is holistic, involving both the body and the mind. The healer typically diagnoses and treats the psychological basis of an illness before prescribing medicines to treat the symptoms. Well known African medicinal plants include Acacia senegal(gum arabic), Agathosmabetulina(buchu), Aloe ferox (Cape aloes), Aloe vera (north African origin), Artemisia afra(African wormwood), Aspalanthus linearis (rooibos tea), Boswellia sacra (frankincense), Catha edulis (khat), Commiphora myrrha(myrrh), Harpagophytum procumbens (devil's claw), Hibiscus sabdariffa (hibiscus, roselle), Hypoxishemerocallidea(African potato), Prunus africana(African cherry). Madagascar has contributed Catharanthus roseus (rosy periwinkle) and has the potential of contributing more in view of the diversity of the flora and fauna [30].

North American traditional medicine

In the USA, just like in many other cultures, the indigenous healer or Shamantreated illnesses by addressing both the physical and spiritual dimension of diseases. These Shamanistic ceremonies involve chanting, dancing and other rituals aimed at expelling evil forces so that the patient or the community as a whole can be healed [31]. Early settlers learnt from native practices and they eventually adopted many of the herbal remedies, which later formed the basis of the early United States Pharmacopoeia. Among the well-known medicinal plants of the United States are Echinacea (*Echinacea purpurea*) and Goldenseal (*Hydrastis canadensis*). During most of the 20th century, herbs or botanicals have been regarded with skepticism and the practice of herbal medicine went into decline. Plants were viewed mainly as a potential source of pure chemical compounds for the development of medicine. In recent years, herbs and botanicals have become very popular in the USA and Canada but they are still considered as nutritional supplements rather than medicines in their own rights [32].

Australian and Southeast Asian medicine

This region has witnessed a resurgence of interest in traditional medicine and many countries now promote research into medicinal plants as a potential source of new remedies. The Aborigines had a complex healing system but much of the traditional knowledge in Australia was lost before it could be systematically recorded. In contrast, many healing practices such as those of Malaysia, Thailand, Vietnam, New Zealand, Borneo, and the Polynesian Islands remain intact and are being recorded and developed. A strong Chinese influence is being observed in most countries. Among the well-known medicinal products originating from this region are *Croton tiglium* (purging croton), *Duboisiahopwoodii*(pituri), *Eucalyptus globulus* (bluegum), *Melaleuca alternifolia* (tea tree), *Myristica fragrans* (nutmeg and Mace), *Piper methysticum*(kava kava), *Strychnos nux-vomica* (strychnine), *Styrax benzoin* (benzoin) and *Syzygium aromaticum* (cloves) [32]

Indian traditional medicine

Ayurveda is perhaps, the most ancient of all medicinal traditions. It is probably older than traditional Chinese medicine and is considered to be the origin of systemized medicine. It is actually a practical and holistic set of guidelines to maintain balance and harmony in the system. Dioscorides (who influenced Hippocrates) is thought to have taken many of his ideas from India. Ancient Hindu writings on medicine contain no references to foreign medicines whereas Greek and Middle Eastern texts refer to concepts and drugs of Indian origin [33]. Ayurveda is derived from the Indian words '*Ayar*' (life) and '*veda*' (knowledge or science) and hence means the science of life. Following the system would help ensure a long life,

which is considered to be the instrument for achieving righteousness (*dharma*), wealth (*artha*) and happiness (*sukha*). In India, knowledge and wisdom have been passed on from one generation to the next through songs and poems, which scholars and physicians had to learn and recite by heart [33].

The Veda is an ancient text in four parts (Rig Veda, Sama Veda, Yajur Veda and Atharva Veda), the earliest of which date back to 2000 years BC. The principles of Ayurvedic medicine and the medicinal uses of plants are contained in thousands of poetic hymns in the Rig Veda. The first school to teach Ayurvedic medicine was at the University of Banaras in 500 BC where the great Samhita (or encyclopedia of medicine) was written. Another great enclyclopedia was written 700 years later, and these two together form the basis of the Ayurveda [33]. Ayurveda is similar to Galenical medicine in that it is based on body humours (*dosas*) and the inner life force (*prana*) that is believed to maintain digestion and mental activity. The living and the non-living environment, including humans, are considered to be elements: earth (prithvi), water (jada), fire (tejac), air (vaju) and space (akasa). For an understanding of these traditions, the concept of impurity and cleansing is also essential. Illness is the consequence of imbalance between the various elements and it is the goal of the treatment to restore this balance [34]. Famous India medicinal plants include *Azadirachta indica* (neem), *Centella asiatica* (gotu kola), *Cinnamomum camphora*(camphor), *Elettariacardamomum* (*ela*or cardamomum), *Rauwolfia serpentina* (Indian snake root), *Santalum album* (sandalwood), *Terminalia* species (myrobolan) and *Withaniasomnifera*(aswargandha) [32].

Chinese traditional medicine

The civilizations of China and India were flourishing when only modestly sophisticated cultures were developing in Europe. Expectedly writings on medicinal plants and the aesthetics of vegetation were numerous. This ancient system of medicine, believed to be more than 5 000 years old, is based on two separate theories about the natural laws that govern good health and longevity, namely *yin* and *yang*, and the five elements (*wuxing*). The legendary emperor Shen Nung discussed medicinal herbs in his works— which were probably written 2 500 years B.P. (Before Present) and not the traditional date of 3 500 B.P. The Traditional Chinese medicine was systematized and written between 100 and 200 BC (Before Christ). The most complete reference to Chinese herbal prescription is the Modern-Day Encyclopedia of Chinese *materia medica* published in 1977. It lists nearly 6000 medicines out of which 4 800 are of plant origin [34]. Treatment is based on symptoms and on a pattern of imbalances, often detected by taking the pulse or observing the patient's tongue. Warming or hot herbs, such as ginger and cinnamon, are used to treat ailments associated with cold symptoms such as cold hands, abdominal pains and indigestion [35].

In common with Western and African traditional medicines, Chinese herbs are usually given in fixed mixtures or formulas of up to 20 herbs, carefully prepared according to traditional recipes. There are hundreds such recipes being used alongside with Western medicines. As in other healing cultures, traditional recipes are used preferentially against chronic illnesses while acute or serious illnesses are cured by Western medicines. The spread of traditional Chinese medicine to most continents has undoubtedly contributed to the current popularity of herbal medicines throughout the world. Examples of famous Chinese medicinal plants are *Angelica polymorpha* var. *sinensis* (dang gui), *Artemisia annua* (qing hao), *Ephedra sinica*(ma huang), *Paeonia lactiflora*(bai shao yao), *Panax ginseng* (ren shen) and *Rheum palmatum*(da huang) [32].

Table 1: Botanical drugs used in traditional medicine which led to useful modern drugs [32].

Botanical names	**English Names**	**Indigenous use**	**Origin**	**Uses in biomedicine**	**Biologically active compounds**
Adhatodavasica	--------	Antispasmodic, antiseptic, insecticide, fish poison	India, Sri Lanka	Antispasmodic, oxytocic,Cough suppressant	Vasicin (lead molecule for Bromhexin and Ambroxol)
Aloe barbadensis miller	Aloe vera	Healing of burn injuries, treatment of skin irritations	Nigeria	Hypoglycaemia, anti-diabetic, wound healing	Anthranol, emodin, chrysophanic acid
Catharanthus roseus	Periwinkle	Diabetes, fever	Madagascar	Cancer chemotherapy	Vincristine, Vinblastine
Condrodendrontomentosum	--------	Arrow poison	Brazil, Peru	Muscular relaxation	D-Tubocurarine
Gingko biloba	Gingko	Asthma, anthelmintic (fruit)	Eastern China	Dementia, cerebral deficiencies	Ginkgolides
Harpagophytum procumbens	Devil's claw	Fever, inflammatory	Southern Africa	Pain, rheumatism	Harpagoside, Caffeic acid

		Conditions			
Piper methysticum	Kava	Ritual stimulant, tonic	Polynesia	Anxiolytic, mild stimulant	Kava pyrones
Podophyllum peltatum	May apple	Laxative, skin infections	North America	Cancer chemotherapy, warts	Podophyllotoxin and lignans
Prunus Africana	African plum	Laxative, 'Old man's disease'	Tropical Africa	Prostate hyperplasia	Sitosterol

Scientific insight of traditional medicinal plants

Drug discovery from medicinal plants has evolved to include numerous fields of inquiry and various methods of analysis. The process typically begins with a botanist, ethnobotanist, ethnopharmacologist, or plant ecologist who collects and identifies the plant(s) of interest. Collection may involve species with known biological activity for which active compound(s) have not been isolated (i.e. traditionally used herbal remedies) or may involve taxa collected randomly for a large screening program. It is necessary to respect the intellectual property rights of a given country where plant(s) of interest are collected [36]. Phytochemists (natural product chemists) prepare extracts from the plant material, subject these extracts to biological screening in pharmacologically relevant assays, and commence the process of isolation and characterization of the active compound(s) through bioassay-guided fractionation.

Molecular biology has become essential to medicinal plant drug discovery through the determination and implementation of appropriate screening assays directed towards physiologically relevant molecular targets. Pharmacognosy encapsulates all of these fields into a distinct interdisciplinary science. Numerous methods used to acquire compounds for drug discovery include: isolation from plants and other natural sources; synthetic chemistry; combinatorial chemistry, and molecular modeling. Despite the recent interest in molecular modelling, combinatorial chemistry, and other synthetic chemistry techniques by pharmaceutical companies and funding organizations, the natural products, and particularly that of medicinal plants, remain an important source of new drugs, drug leads, and chemical entities [37]. In both 2001 and 2002, approximately one quarter of the best-selling drugs worldwide were natural products or were derived from natural products (Butler, 2004). An example is Arteether, a potent antimalaria drug. It is derived from artemisinin, a sesquiterpene lactone isolated from *Artemisia annua* (Asteraceae), a plant used in traditional Chinese medicine (TCM) [38].

Despite evident successes of drug discovery from medicinal plants, future endeavors face many challenges. Pharmacognosists, phytochemists, and other natural product scientists will need to continuously improve the quality and quantity of compounds that enter the drug development phase to keep pace with other drug discovery efforts [37]. The process of drug discovery has been estimated to take an average of 10 years upwards and cost more than 800 million US dollars [39]. Much of this time and money is spent on the numerous leads that are discarded during the drug discovery process. It has been estimated that only one in 5000 lead compounds will successfully advance through clinical trials and be approved for use. Lead identification is only the first step in a lengthy drug development process (Fig. 1). There is also lead optimization (involving medicinal and combinatorial chemistry), development (including toxicology, pharmacology, pharmacokinetics, ADME [absorption, distribution, metabolism, and excretion], and drug delivery), and clinical trials which all take a considerable length of time [37].

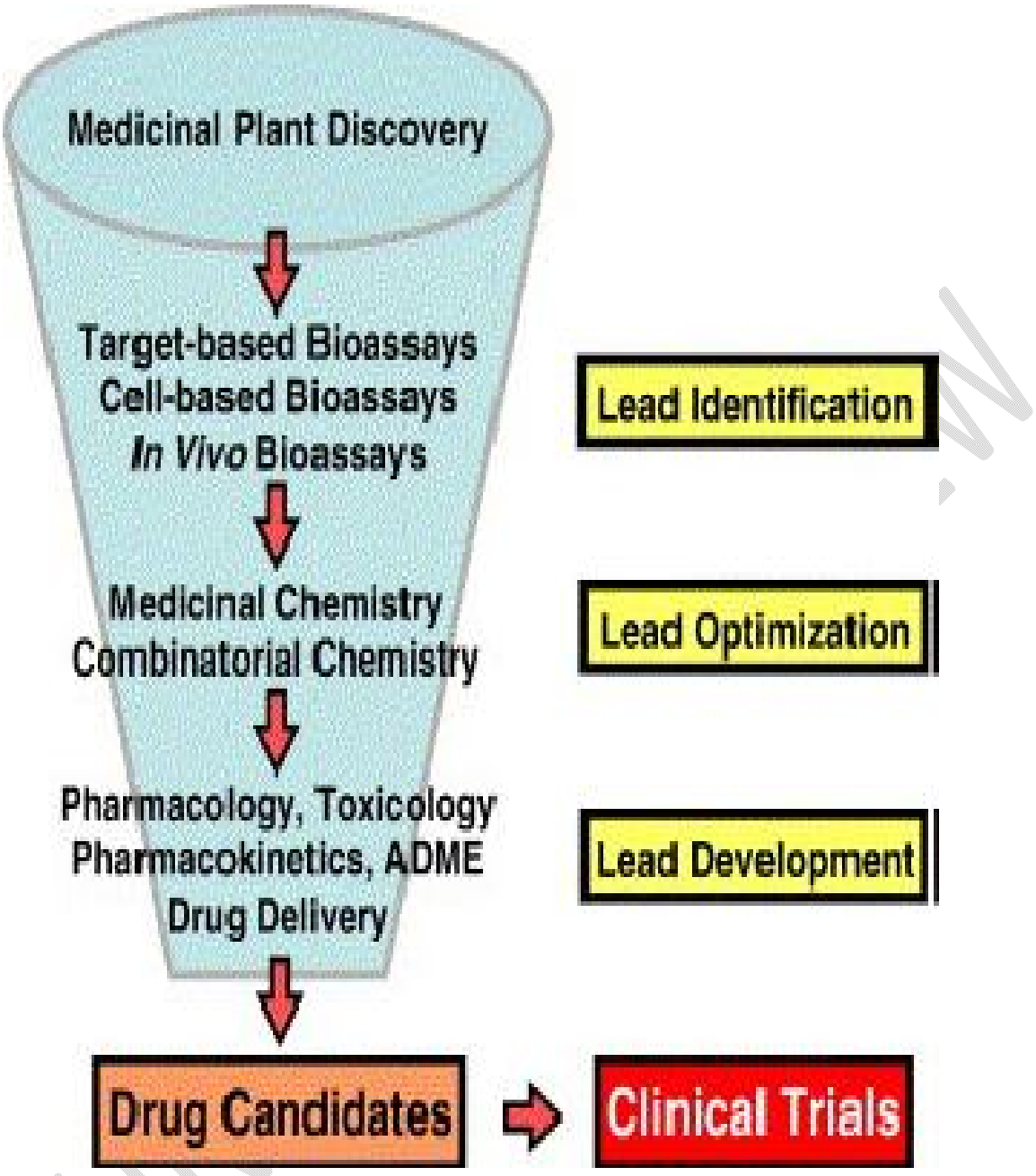

Figure 1: Schematic representation of a typical medicinal plant drug discovery process and development [40].

Drug discovery from medicinal plants has traditionally been lengthier and more complicated than other drug discovery methods. Therefore, many pharmaceutical companies have eliminated or scaled down their natural product research [37].

Interaction between Medicinal Plant Extracts and Conventional Antibiotics

There is already evidence for the enhancement of the activity of conventional antibiotics when acting synergistically with plant-derived compounds. The combination of β-lactams with α-mangostin isolated from mangosteen fruit, substantially increase the efficacy of the therapy in β-lactam resistant bacterial strains. It is likely that the mangosteen- derived compounds of those combinations may inhibit the bacterial β-lactamase enzyme, thus reactivating the antibiotic [41]. Several in vitro studies have reported the use of plant extracts in combination with antibiotics with a significant reduction in the minimum inhibitory concentration (MIC) of the antibiotics against some resistant strains. The curative effect of plant extracts in this combination studies has been variably referred to as resistance-modifying activity (RMA) [42] pharmacokinetics and pharmacodynamics, since combination confirmed in vitro may not have the same effect on humans. Pharmacokinetic interactions occur mainly by increasing the permeability of antibiotics to the bacterial cell membrane or by inhibiting or inducing antibiotic-metabolizing enzymes and transporters, which adversely affect absorption, distribution, metabolism, and excretion of concurrently administered antibiotics. Pharmacodynamic plant extract-antibiotics interactions such as synergism, additive, and antagonist effects are also known to occur [42].

Camellia sinensis dried leaves extract, together with nalidixic acid, reflected the inhibition of Salmonella Typhi. With this combination (Cextract = 0.62 mg/mL), nalidixic acid presented a MIC value that was 8-fold lower (32 µg/mL) than when used alone (256 µg/mL). Moreover, pyridine isolated from Jatropha elliptica by bioassay- guided fractionation, at a concentration of 75 µg/mL, was shown to increase by 4-fold the activity of ciprofloxacin and norfloxacin against nor A Expressing Staphylococcus aureus when tested at sub-inhibitory concentrations [43]. Isoflavones isolated from the plant Lupinus argentens, potentiate the activity of the natural plant antibiotic berberine and the synthetic fluoroquinolone antibiotic norfloxacin. The isoflavone allows a greater concentration of berberine to accumulate in Staphylococcus aureus cells by inhibiting the EP mechanism. A study reported that carsonic acid isolated from Rosmarinus officinalis L. potentiated the activity of erythromycin [44]. That study determined that the increased erythromycin activity was due to an inhibition of the MDR EP's by carbonic acid. Similarly, reserpine, a plant alkaloid, isolated from the Rauwolfia vonitona. Afzelalso demonstrated effective EP inhibition activity against the bacterial MDR EP, which mediates tetracycline efflux in Bacillus subtilis [45]. Fungi have also been evaluated for synergism between plant extracts and antifungals. Synergism has been reported between ketoconazole and Agastache rugosa essential oil against Blastischizomycescapitatus and between Pelargonium graveolens essential oil and amphotericin B plus ketoconazole on strains of *Aspergillus spp*. Furthermore, metronidazole showed potentiation of its antifungal effect when combined with Eugenia Jambolana L. [46]. Plant extract-antibiotic combinations not only enhance the antimicrobial effect but also can act as resistance modifying/modulating agents. A study reported that Salvia spp. and Martiaria recutita had synergistic effects with oxacillin, greatly enhancing its efficacy. The authors postulated that it was due to damage to the cytoplasmic membrane of the resistant bacteria and loss of intracellular components [47]. Many medicinal plants acting as MDR EP inhibitors become significant tools when used in combination with some previously ineffective resistance-prone antibiotics. For instance, synergistic activities have been reported for several plant tannins-conventional antibiotic combinations against both resistant and sensitive strains of Acinetobacter baylyi [47].

Most studies on the interaction between plant extracts and antibiotics have been focused on the identification and isolation of potential resistance modifiers from medicinal plants. However, it is

likely that such combinations could produce antagonistic interactions that many studies have considered irrelevant and thus ignored. However, elucidating synergism and antagonism between plant extracts and antimicrobial drugs is very important. Typical examples are as follows: Synergism assays between terpenes and penicillin against MRSA and Escherichia coli revealed a synergistic effect produced by the interaction between carvone and penicillin whereas an antagonistic effect between thymol and penicillin was detected against MRSA strains [48]. Ampicillin, cephalothin, and tetracycline presented synergistic interactions with some essential oils whereas gentamycin mostly had antagonistic interactions. Four essential oils in combination with ciprofloxacin against *Staphylococcus aureus* and *Klebsiella pneumoniae* and with amphotericin B against *Candida albicans* strains revealed synergism or antagonism depending on the type of essential oil and the concentration assayed [49]

Antimicrobial Activity Mechanisms of Medicinal Plant-Derived Chemical Compounds

Although synthetic antimicrobial agents are already approved in many countries, the usage of medicinal plant-derived natural compounds continues to attract the attention of many researchers. Medicinal plants have enormous potential for the discovery of new bioactive compounds which can fight against resistant microorganisms [50]. Medicinal plant-derived chemicals are a wide group of chemical compounds that have been found naturally in plants. They can restore the clinical application of older antibiotics by increasing their potency and therefore, avoid the fact of resistance [51]. Plant-derived bioactive compounds (phytochemicals) of therapeutic value are mostly secondary metabolites used for medicinal purposes. Secondary metabolites are the results of secondary plant metabolism and can occur as intermediate or end products. They have a wide antimicrobial activity range according to the structure, number, and position of substituent groups, presence of glycosidic linkages alkylation of OH groups, and the topography and climate of the country of origin. Indeed, variations in the quality and quantity of bioactive secondary metabolites modify their antimicrobial activity against different microbial strains [51].

In most cases, bioactive plant extracts contain complex mixtures of ingredients, and their synergistic action can yield an enhanced effect. The microbial cell can be affected by these compounds in several ways. In general, bioactive compounds primary target site is the cytoplasmic membrane, affecting its structure and integrity, permeability, or functionality in different ways [52]. It has been suggested that plant extracts may contain inhibitors of EP in their composition. In addition, inhibition of normal cell communication [quorum sensing (QS)] has been also described as one of the most promising mechanisms of action of bioactive compounds against MDR pathogens. QS inhibitors should have the ability to decrease the expression of QS-controlled genes and being chemically stable to resist the metabolic and disposal processes of the host organism [52]. Certain compounds can modify or inhibit the protein-protein interactions, thus presenting themselves as effective modulators of immune response, mitosis, and apoptosis. Moreover, they have the ability to interfere with intermediary metabolism, to induce the coagulation of cytoplasmic constituents and disrupt or inhibit the formation of biofilms, which confer a protective advantage to pathogens during infection. The presence of multiple antiviral components in medicinal plant extracts interfaces with different viral proteins at various stages of viral replication [53]

Phytochemicals as antimicrobial agents

In plants, as a result of metabolic processes, many different kinds and types of organic compounds or metabolites are produced. These metabolites are grouped into primary and secondary metabolites. The primary metabolites like chlorophyll, amino acids, nucleotides, simple carbohydrates or membrane lipids, play recognized roles in photosynthesis, respiration, solute transport, translocation, nutrient assimilation and differentiation. The secondary metabolites also differ from primary metabolites in having a restricted distribution in the plant kingdom. That is, particular secondary metabolites are often found in only one plant species or a taxonomically related group of species, whereas the basic primary metabolites are found throughout the plant kingdom. During the past few decades, experimental and circumstantial evidence has made it clear that many secondary metabolites do indeed have functions that are vital for the fitness of a plant producing them. The main roles are:

• Defence against herbivores (insects, vertebrates)

• Defence against fungi and bacteria

• Defence against viruses

• Defence against other plants competing for light, water and nutrients

• Signal compounds to attract pollinating and seed dispersing animals

• Signals for communication between plants and symbiotic microorganisms

(e.g. N-fixing Rhizobia or mycorrhizal fungi)

• Protection against UV-light or other physical stress [54]

Phenolic Compounds

Phenolic compounds are well known bioactive phytochemical molecules, scientific reports evaluated up-to-date about 8000 phenolic compounds, halve of them are flavonoids; Phenolic compounds have versatile benefits for human health, for example, but not limited, antioxidants, anti-inflammatory, anti-cancer, antibacterial, immune system promoting, cardio-protective agents, and skin protection from UV radiation,. With regard to antibacterial activity, hundreds of plants showed antibacterial phenolic compounds extracted from different plant parts, such as Pomegranate peels the methanol, ethanol and aqueous extracts showed high content of phenolic compounds and exhibited remarkable antibacterial activities against *Staphylococcus aureus*, *Enterobacter aerogenes*, *Klebsiella pneumoniae* and *Salmonella typhi*. The leaves extracts using deep eutectic solvents of Rue (*Ruta graveolens*) revealed the highest antibacterial activity against gram-negative *Pseudomonas aeruginosa*, and it was also active but with lesser degrees against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* [55].

Alkaloids

Alkaloids are one of the largest and diverse phytochemical group, there are about 12000 alkaloids extracted from various plants and extensively studied; Alkaloids have many benefits for human health, for example, but not limited, muscle relaxant, narcotic analgesics, anti-cancer and antimicrobial agents, also some drugs were derived from alkaloids such as morphine, apomorphine and codeine. The following are few examples of plants rich in alkaloids and showed effective antibacterial properties; The alkaloids extracted from leaves of Callistemon citrinus exhibited highest antibacterial activity against *Staphylococcus aureus* (ATCC 9144) and

good activity against *Pseudomonas aeruginosa* (ATCC 27853) the mode of action of the extracted alkaloids showed that these molecules inhibiting ATP-dependent transport of compounds across the bacterial cell membrane. Alkaloids from leaf extract of Eclipta alba was tested against some human pathogenic bacteria, *Staphylococcus aureus* and *Escherichia coli* were among the most susceptible bacteria and it was found that the inhibitory action of the alkaloid increased with the increase in concentration against all tested pathogens [56].

Terpenoids

Terpenoids are an important diverse class of phytochemical compounds. Based on scientific literature, it has been confined about 40 000 compounds belonging to terpenoids which makes it one of the largest classes of phytochemicals. Terpenoids are widely used as flavors, fragrances, insecticides, pharmaceutical and industrial compounds. A lot of studies claimed that many terpenoid compounds have potent antibacterial activity. Twelve pure terpenoid compounds extracted from the wood and bark of an aromatic cypress tree (Pilgerodendronuviferum) exhibited varied degrees of remarkable antibacterial activities against twelve different microorganisms including *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes, Escherichia coli, Pseudomonas aureginosa, Fusarium graminearum, Ophiostomapiliferum, Rhizoctonia solani, Phragmidiumviolaceum, Schizophyllum commune, Pythium irregulare and Botrytis cinerea*. Terpenoids extracted from leaves of *Eremophila lucida* possess good antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213 and *Staphylococcus aureus* ATCC 25923) but did not show activity against the Gram-negative bacteria (*Escherichia coli* ATCC 25922) [57].

Carotenoids

Carotenoids are lipid phytochemical metabolites; they are an important phytochemical class of bioactive diverse properties and till now there are up to 600 carotenoids have been identified and isolated from plants. One of its main roles in the plant is the production of pigments for coloring fruits and vegetables; Scientific studies showed that they possess many protective roles against some diseases and disorders such as cancer, aging-associated diseases, cardiovascular diseases, antioxidant properties and many more. Unfortunately, investigations on the antibacterial potential of carotenoids from plants are little and many studies focusing on carotenoids of some fungal species and Streptomyces. Although, some interesting investigations stated that carotenoids exhibited a remarkable antibacterial activity; Galls of Guiera plant (Guiera senegalensis) from Burkina Faso showed good contents of carotenoids which recorded significant antibacterial activities against *Staphylococus aureus ATCC 6538, Bacillus cereus 13061, Escherichia coli ATCC 25922, Salmonella typhimurium ATCC 13311, and no effect with Proteus mirabilis ATCC 35659*. Carotenoids extracted from fruits of Annato (Bixa orellana L., growing in Philippine, revealed high antibacterial activity against *Staphylococus aureus* [58].

Laboratory Techniques use in the study of antimicrobial plant extracts

Antimicrobial susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome. After the revolution in the “golden era”, when almost all groups of important antibiotics (tetracyclines, cephalosporins, aminoglycosides and macrolides) were discovered and the main problems of chemotherapy were solved in the 1960s, the history repeats itself nowadays and these exciting compounds are in danger of losing their efficacy because of the increase in microbial resistance (Mayers *et al.*, 2009). Currently, its impact is considerable

with treatment failures associated with multidrug-resistant bacteria and it has become a global concern to public health [59].

1. Diffusion methods

(a) Agar disk-diffusion method

Agar disk-diffusion testing developed in 1940, is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria and yeasts testing [60]. In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at a desired concentration, are placed on the agar surface. The Petri dishes are incubated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured which shows the growth media, temperature, period of incubation and inoculum size required by CLSI standards.

Moreover, the agar disk-diffusion method is not appropriate to determine the minimum inhibitory concentration (MIC), as it is impossible to quantify the amount of the antimicrobial agent diffused into the agar medium. Nevertheless, an approximate MIC can be calculated for some microorganisms and antibiotics by comparing the inhibition zones with stored algorithms. Nevertheless, disk-diffusion assay offers many advantages over other methods: simplicity, low cost, the ability to test enormous numbers of microorganisms and antimicrobial agents, and the ease to interpret results provided. Moreover, several studies have demonstrated the great interest in patients who suffer from bacterial infection of an antibiotherapy based on the antibiogram of the causative agent. This fact is due to the good correlation between the in vitro data and the in vivo evolution [61]

(b) Agar well diffusion method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts [62]. Similarly, to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 μL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested

(c) Agar plug diffusion method

Agar plug diffusion method is often used to highlight the antagonism between microorganisms [62] and the procedure is similar to that used in the disk-diffusion method. It involves making an agar culture of the strain of interest on its appropriate culture medium by tight streaks on the plate surface. During their growth, microbial cells secrete molecules which diffuse in the agar medium. After incubation, an agar-plot or cylinder is cut aseptically with a sterile cork borer and deposited on the agar surface of another plate previously inoculated by the test microorganism. The substances diffuse from the plug to the agar medium. Then, the antimicrobial activity of the

microbial secreted molecules is detected by the appearance of the inhibition zone around the agar plug

2. Cross streak method

Cross streak method is used to rapidly screen microorganisms for antagonism . The microbial strain of interest is seeded by a single streak in the center of the agar plate. After an incubation period depending upon the microbial strain, the plate is seeded with the microorganisms tested by single streak perpendicular to the central streak. After further incubation, the antimicrobial interactions are analyzed by observing the inhibition zone size

3. Poisoned food method

Poisoned food method is mostly used to evaluate the antifungal effect against molds [63]. The antifungal agent or the extract is incorporated into the molten agar at a desired final concentration and mixed well. Then, the medium is poured into Petri dishes. After overnight pre-incubation, the inoculation can be done by a mycelia disc ranging from 2 to 5 mm, which is deposited in the center of the plate. After further incubation under suitable conditions for the fungal strain tested, the diameters of fungal growth in control and sample plates are measured, and the antifungal effect is estimated by the following formula:

Antifungal activity (%)

$$= ((Dc - Ds)/Dc) \times 100$$

Where Dc is the diameter of growth in control plate and Ds is the diameter of growth in the plate containing tested antifungal agent. Sporulation can be also compared to the control. Generally, when standardization of the method used fails, the researcher must carry a positive control with known antimicrobial molecule to compare the results found and assert the experimental approach right.

4. Thin-layer chromatography (TLC)–bio autography

In 1946, Goodall and Levi combined paper chromatography method (PC) with contact bio autography to detect different penicillin for their determination. Thereafter, Fischer and Lautner introduced TLC in the same field. This technique combines TLC with both biological and chemical detection methods. Several works have been done on the screening of organic extracts, mainly plant extracts, for antibacterial and antifungal activity by TLC–bio autography [64]. As shown below, three bioautographic techniques, i.e., agar diffusion, direct bio autography and agar-overlay assay, have been described for the investigation of antimicrobial compounds by this approach.

Conclusion

Medicinal plant antimicrobial activity is a new hope to combat the dangerous threats posed by increasing evidence of antimicrobial resistance. Therefore, there is an urgent need to identify and isolate new bioactive compounds from medicinal plants, which have yet to be adequately explored. The large diversity of these compounds has proved to have therapeutic potentials as antimicrobials and as antimicrobial resistance modifiers.The potential use of new bioactive compounds is still challenging. It is essential to emphasize that extensive in vitro and in vivo

tests must be conducted to assure the selection of active and nontoxic antimicrobial plant-derived compounds. It is also a major challenge to exploit the potential synergistic or antagonistic effects of compounds within and between medicinal plant extracts. As biotechnology advances, it is obvious that we will be able to search further into the chemical composition of medicinal plants and develop more sophisticated techniques for the extraction, fractionation, and identification of bioactive compounds which are characterized by diverse chemical structures and mechanisms of action. It would be advantageous to standardize methods of extraction and in vitro testing in order that the search might be more systematic, and interpretation of results would be facilitated. Additionally, reference models have yet to be employed in studying plant extract mixtures and future studies will reveal their applicability for this approach.Studies on the mechanisms of action, interactions with antibiotics or other medicinal plants or compounds, and the pharmacokinetic and pharmacodynamics profile of the extracts should be given high priority. It is expected that this review and the main challenges that were identified in this field would be helpful in the use of more efficient, successful, and straightforward methods to get to the use of new therapeutic medicinal plants more quickly against microbes.

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