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

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Background: Over the years, the medicinal properties of plants have been recognized as important sources of active ingredients in herbal medicine. In particular, plant-derived active ingredients have been used in microbial natural products long before the advent of modern medicine.

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Discussion: Since ancient times, the potency of medicinal plant products has been linked to the chemical, biochemical and synthetic activities of the plant product. Therefore, with the advancement of modern science in molecular and cellular biology, analytical chemistry and pharmaceuticals, the unique properties of these plant products are used to exploit the chemical and structural diversity and diversity. of the biology of this type of fruit in relation to their medicinal properties.
Conclusion: New molecules are of interest to drug designers who are interested in modifying the chemical entities or structural parameters of natural products to produce new molecules that can be made into effective medicine.

Keywords: Medicinal plants; Extracts; efficacy; pharmacology; traditional knowledge, orthodox medicine.

1. INTRODUCTION

Nigeria is a developing country where orthodox medicine is not available or not available in many rural areas; therefore, traditional medicine is the first line of treatment for coughs, fevers, headaches, mental illness, and many other ailments. These drugs are traditional medicines, and there are many scientific reports about their side effects [1], which led the World Health Organization to decide on herbal prescriptions [2]. The standardization process has several components, the first of which is the systematic documentation of medicinal plants and their uses in rural communities around the world.

According to the World Health Organization (WHO) 80% of developing countries still benefit from the use of traditional medicines derived from medicinal plants [3]. The number of plant species is around 374,000 species [4], 28,187 medicinal species are used by humans [5]. The World Health Organization has also listed the names of more than 20,000 medicinal plants [6] and states that medicinal plants are one of the new sources of medicine. More than 100 countries have laws against medicinal plants. More than 1,340 plant species have established pesticidal activity, and more than 30,000 pesticidal compounds have been isolated from plants [7]. In addition, it is estimated that 14-28% of higher plant species have medicinal properties and 74% of biological compounds obtained from plants are found in ethnomedicinal use [8].

Pathogens, such as viruses, bacteria or protozoa, have been and continue to be the cause of major epidemics and epidemics throughout the world. Some pathogens are considered benign, such as the flu, while others are considered dangerous, such as the previously mentioned COVID-19. Examples include *Vibrio cholerae*, the bacterium that causes cholera; *Aedes aegypti*, the mosquito that spreads dengue; or morbillivirus, a genus of paramyxovirus that causes measles. One of the major challenges facing the world of medicine is the growth of microorganisms that are resistant to many drugs, thereby compromising the effectiveness of drugs used in the healthcare system [9]. Although multidrug resistance is a natural phenomenon, inappropriate use of antimicrobial drugs, poor hygiene, improper food management, and inadequate infection prevention and control methods that prevent the effect of multidrug resistance and promote its spread [10]. Without effective action to change the current situation, we may return to the pre-antibiotic era, where simple wounds and infections can cause serious illness, including death, and high-risk medical procedures. It is estimated that tuberculosis (AMR) will become more common than cancer by 2050 [11]. This emerging trend has caught the attention of the World Health Organization, which believes it may be the biggest problem facing the medical world. Given the rise of these diseases and the limited effectiveness of antibiotics, traditional knowledge can be a useful tool to address these new health challenges. One of the most important examples of recent ethnobotanical drugs, worthy of the Nobel Prize in Physiology or Medicine [12], is the discovery of artemisinin, which was initially developed in response to the development of resistance in malaria parasites to quinine drugs.

1.1 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" [13]. "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.*)" [14]. "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" [15]. "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" [16]. 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" [17].

"Phytochemical studies identified the presence of different compounds such as spermidine, rutin, quercetin, tocopherol, and carotenoids, derived from caper (*Capparis spp*.) 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" [18]. "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" [19]. "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" [20]. "Methanol extracts of *Oxalis corniculata, Artemisia vulgaris, Cinnamomum tamala,* and *Ageratina adenophora* exhibited antimicrobial activities against *Escherichia coli, Salmonela Typhi, Klebsiella pneumoniae, Staphylococcus aureus,* and *Citrobacter koseri"* [21] Also, "hydromethanolic extracts of *Berberis vulgaris, Cistus monspeliensis,* and *Punica granatum* demonstrated high activity against *Staphylococcus aureus, Enterococcus faecalis,* and *Enterobacter cloacae"* [22].

"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"* [23]. "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*" [24]. "Natural resins derived mostly from medicinal

plants and their compounds revealed antibacterial and antiprotozoal activity" [25]. 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) [26]. 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 [27].

2. HISTORICAL PERSPECTIVE OF TRADITIONAL MEDICINAL PLANTS

2.1 African Traditional Medicine

African traditional medicine is an ancient and perhaps the most unique system of medicine. Considered the cradle of humanity, Africa is rich in diversity and culture, and there are significant regional differences in recovery efforts. Unfortunately, until now, the medical system has not been well documented. The literature on the medicinal use of African plants is increasing rapidly due to the rapid loss of their natural habitats due to human activities. The African continent is said to have one of the highest rates of deforestation in the world. These diseases are stronger than the highest levels in the country, Madagascar is at the top of the list at 82% [28]. African traditional medicine is holistic and involves both the body and the mind. Psychiatrists usually investigate the psychological basis of the illness before prescribing medication to treat the symptoms. The most famous African medicinal plants are Acacia Senegal (*gum arabic*), Agathosma betulina (*buchu),* Aloe vera (*Aloe vera*), Aloe vera (native from North Africa), *Artemisia abrasicae* (African bitter weed), Camellia sinensis (Louis sinensis) Boss tea), frankincense, khat, myrrh, devil's claw, hibiscus, roselle, African potato, African cherry. Madagascar's Catharanthus roseus (Periwinkle roseus) contributes significantly to plant and animal diversity [29].

2.2 North American Traditional Medicine

In the United States, as in many other cultures, indigenous shamans or healers treat illness by addressing the physical and spiritual aspects of illness. These shamanic techniques include music, dance, and other techniques designed to dispel evil forces and heal patients and entire communities [30]. Early settlers learned from local practices and eventually adopted many of the herbs that later became the basis of the early United States Pharmacopoeia. The most popular medicinal plants in America are echinacea purpurea and goldenrod (*Hydrastis canadensis*). Over the course of the 20th century, society became skeptical of herbs or herbal medicine, and the practice of herbal medicine declined. Most plants are considered as sources of pure compounds for medicinal development. Herbal and botanical medicines have become popular in the United States and Canada in recent years, but are still considered non-prescription dietary supplements [31].

2.3 Australian and Southeast Asian Medicine

Interest in traditional medicine is also rising in the region, and many countries are promoting research into medicinal plants as a potential source of new treatments. Indigenous healing systems are complex, but much of Australia's traditional knowledge was lost before it was systematically recorded. However, many treatments are still available, documented and developed in countries such as Malaysia, Thailand, Vietnam, New Zealand, Kalimantan and the Polynesian Islands. China's influence can be seen in most countries. The most popular medicinals from this region are Croton tiglum (*puring croton*), Duboisia hopwoodii (*pituri*), bluegulus, Melaleuca alternifolia (tea tree), pala (*nutmeg* and *nutmeg*), Piper methysticum (*kava*), Strychnos nux-vomica (*strychnine*), Styrax benzoin (*frankincense*), and cloves [31].

2.4 Indian traditional Medicine

"Ayurveda is probably the oldest system of medicine. It is probably older than traditional Chinese medicine and is considered the origin of systemic medicine. It is meant to be a useful and comprehensive set of guidelines for maintaining a balanced and efficient system. It is believed that Dioscorides (who was influenced by Hippocrates) got many ideas from India. There is no mention of foreign medicines in ancient Hindu

medical texts, but Greek and Middle Eastern texts mention Indian theories and remedies" [32]. "*Ayurveda* is derived from the Indian words "Ayar" (life) and "veda" (knowledge or science), so it means the science of life. Following this system helps in longevity and is considered as a way to attain truth (dharma), wealth (*artha*) and happiness (*sukha*). In India, knowledge and wisdom were passed down from generation to generation through songs and poems for the study of scholars and doctors" [32].

"The Vedas are ancient texts divided into four parts (Rig Veda, Sama Veda, Yajur Veda and Atharva Veda), the first of which dates back to 2000 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 Banaras University in 500 BC, where the great Samhita (Medical Encyclopedia) was written. Another major treatise appeared 700 years later, and these two treatises formed the foundation of *Ayurveda" [32]. "Ayurveda* is similar to Galenic medicine based on the humors (dosa) and internal life force (prana) believed to support digestion and mental function. Living and non-living things, including humans, are called elements: earth (*prithvi*), water (*jada*), fire (*tejac)*, air (*vaju*), and space (*akasa)*. To understand these principles, the concepts of pollution and purification are also important. Disease is the result of an imbalance between various elements, the goal of treatment is to restore this balance [33]. The most popular medicinal plants in India are neem (*Neem*), Centella asiatica (*Centella asiatica*), camphor (*camphor*), cardamom (ela or cardamom), *Rauwolfia vulgaris* (Indian snake root), sandalwood (*cendalwood*), *terminalia* (terminal) and withania somnifera. (*aswargandha*)" [31].

2.5 Chinese Traditional Medicine

While Europe developed a very complex culture, the civilizations of China and India grew rapidly. We hope to write a lot about medicinal plants and their properties. This ancient medical system, believed to be over 5,000 years old, is based on two distinct concepts of the natural laws of health and longevity, Yin Yang and the Five Elements. The famous Emperor Shen Nong mentioned herbal medicine in his writings - probably written before 2500 BC. (before) rather than the usual date of 3,500 B.P. Traditional Chinese medicine was formulated and written down between 100 and 200 BC (BC). The definitive reference for traditional Chinese medicine recipes is the "Encyclopedia of Modern Chinese Medicine" published in 1977. There are approximately 6000 medicinals, of which 4800 are plant extracts [33]. The treatment is based on the symptoms and the pattern of the imbalance, which can be seen by measuring the swelling or looking at the patient's tongue. Warming or hot herbs, such as ginger and cinnamon, are used to treat conditions associated with cold symptoms, such as cold hands, stomach pain, and incontinence.

Like traditional Western and African medicine, Chinese medicine is usually administered in the form of a fixed mixture or formula of up to 20 herbal remedies, slowly according to traditional recipes. There are hundreds of recipes that can be used in Western medicine. As in other healing cultures, traditional recipes are central to the treatment of chronic illnesses, while acute or chronic illnesses are treated by Western medicine. The spread of Chinese medicine across countries has greatly contributed to the popularity of herbal medicine throughout the world. An example of a popular Chinese medicinal plant is *Angelica sinensis*. *Angelica sinensis, Artemisia annua, Ephedra,* White Peony*, Ginseng and Rhubarb* [31].

2.6 Scientific Insight of Traditional Medicinal Plants

"The discovery of drugs from medicinal plants has extended to many research areas and various analytical methods. The process usually begins with a botanist, ethnobotanist, ethnopharmacologist, or plant ecologist, who collects and identifies plants of interest. Collections may include species with known biological activity but whose active compounds have not yet been isolated (i.e., traditional herbal medicines), or may include taxa that are randomly collected for important screening purposes. Respect the intellectual property rights of the country from which the interest is collected" [34]. "Phytochemists (natural product interpreters) prepare extracts from plant materials, biologically screen these extracts in medically relevant tests, and begin the process of isolation and characterization of compounds. active compounds through the bio-lead fraction. Molecular biology is the key to drug discovery in medicinal plants through the identification and use of accurate screening tests that target the molecular levels of interest. Pharmacognosy summarizes all these fields into a separate science. Various methods used to obtain

compounds for drug discovery include: isolation from plants and other synthetic chemical sources; Despite the interest of pharmaceutical companies and financial institutions in molecular modeling, synthetic chemistry, and other synthetic chemical methods, natural products, especially those derived from medicinal plants, remain an important source. for new drugs, drugs, and chemical entities" [35]. In 2001 and 2002, nearly a quarter of the world's best-selling medicines were natural products or derived from natural products (Butler, 2004). An example is Artemether, an antimalarial drug. It is derived from artemisinin, a sesquiterpene lactone isolated from Artemisia annua (*Asteraceae*), a plant used in traditional Chinese medicine (TCM) [36].

Although the discovery of drugs from medicinal plants has been successful, there are still many challenges in the future. Pharmaceutical, phytochemical, and other natural product scientists should continue to improve the quality and quantity of compounds involved in drug development to pursue further drug discovery [35]. Drug discovery is estimated to take more than 10 years and cost more than $800 million [37]. Much time and money is spent on many raw compounds that are discarded during drug discovery. It is estimated that only one in 5,000 lead compounds successfully pass clinical trials and are approved for use. Identifying lead compounds is the first step in the long drug development process (Fig. 1). It also leads optimization (involving drugs and combination chemistry), development (including toxicology, pharmacology, pharmacotherapy, ADME [adsorption, distribution, manufacturing and extraction] and drug administration) and clinical trials, which take a long time [35].

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 [35].

Table 1. Botanical drugs used in traditional medicine which led to useful modern drugs [31]

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
Condrodendron tomentosum	---------	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 Conditions	Southern Africa	Pain, rheumatism	Harpagoside, Caffeic acid
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

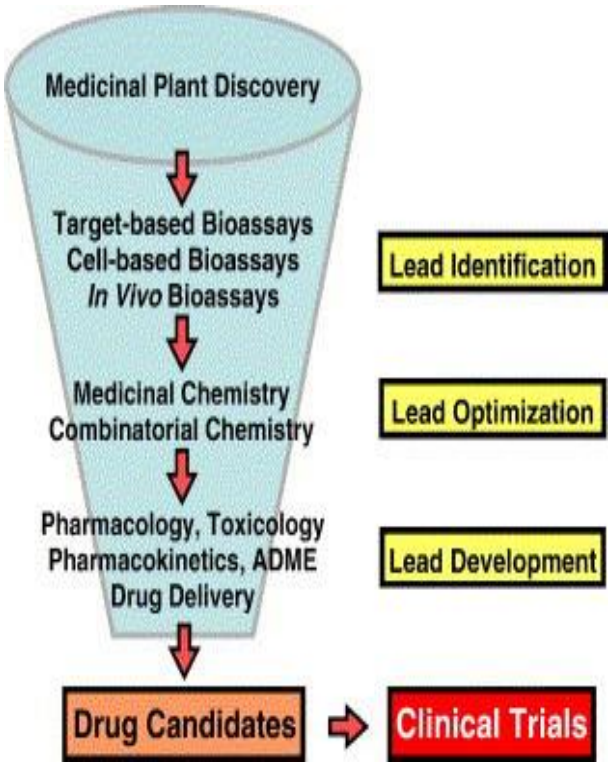

Fig. 1. Schematic representation of a typical medicinal plant drug discovery process and development [38]

2.7 Interactions between Medicinal Plant Extracts and Conventional Antibiotics

There is evidence that the efficacy of traditional antibiotics increases when combined with plant-derived compounds. The combination of β-lactam and α-mangostin isolated from mangosteen fruits significantly increases the therapeutic effect of β-lactam-resistant strains. Mangosteen-derived compounds in this combination can inhibit bacterial β-lactamase, thereby activating the antibiotic [39]. Several in vitro studies suggest that combining plant extracts with antibiotics can significantly reduce the minimum inhibitory concentration (MIC) of antibiotics against some resistant bacterial strains. The strength of plant extracts in these combination studies has been called resistance-modifying activity (RMA) [40] pharmacokinetics and pharmacodynamics, because combinations tested in vitro may not produce side effects of man. Pharmacokinetic interactions occur primarily by increasing the penetration of antibiotics into bacterial cell membranes, or by inhibiting or activating metabolic enzymes and antibiotic transporters, which adversely affect the absorption, distribution, metabolism and elimination of administered antibodies. Pharmacodynamic interactions between plant extracts and antibiotics, such as synergism, additivity, and antagonism are also known to occur [40].

Dry leaf extract of tea tree and nalidixic acid showed inhibitory activity against *Salmonella typhi.* With this combination (Cextract = 0.62 mg/mL), the MIC value of nalidixic acid (32 µg/mL) is 8-fold lower than when used alone (256 µg/mL). Furthermore, when pyridine was isolated from Jatropha curcas by bioassay-guided fractionation at a concentration of 75 µg/mL, ciprofloxacin and norfloxacin inhibited non-A-expressing *Staphylococcus aureus* in when testing low-resistance packages. Work increased 4 times. Isoflavones isolated from silver lupine plants enhance the activity of the natural plant antibiotic berberine and the fluoroquinolone antibiotic norfloxacin. Isoflavones increase the accumulation of berberine in *Staphylococcus aureus* cells by inhibiting the EP mechanism. One study reported that carnosic acid isolated from rosemary increased the activity of erythromycin [41]. This study concluded that the increased activity of

erythromycin was due to inhibition of MDR EP by carbonic acid. Similarly, reserpine is an alkaline plant isolated from Rauwolfia rauwolfia. Afzel also demonstrated strong EP inhibitory activity against MDR EP bacteria mediated tetracycline efflux in *Bacillus subtilis* [42]. The combination between mushroom phytoextracts and antifungal drugs was also evaluated. Ketoconazole and patchouli oil were reported to be effective against B. capitatum, and geranium essential oil and amphotericin B and ketoconazole were effective against the Kojima strain. In addition, the use of metronidazole and Eugenia Jambolana L can increase its antifungal effect. [43]. The phytoextract-antibiotic combination not only enhances the antibacterial effect but also acts as a resistance modifier/modulator. A study reported that *Salvia spp.* Matia and oxacillin act synergistically and are more potent. The authors suggest that this is due to the damage of the plasma membrane of drug-resistant bacteria and the loss of intracellular components [44]. Many medicinal plants act as MDR EP inhibitors and can be an important tool when used with many antibiotics that were previously ineffective and prone to the development of resistance. For example, several combinations of plant tannin-derived antibiotics have been reported to have synergistic activity against susceptible and susceptible *Acinetobacter burnetii strains* [44].

Most studies on interactions between plant extracts and antibiotics have focused on the identification and isolation of resistance modifiers from medicinal plants. However, this combination may result in an antagonistic interaction, which many studies consider to be minimal. However, it is important to clarify the synergistic and antagonistic relationships between plant extracts and antimicrobials. A typical example is as follows: Synergistic tests of terpenes and penicillin against MRSA and *E. coli* showed that the interaction between carvone and penicillin produced a synergistic effect, but it was found that antagonism between thymol and penicillin against MRSA strains [45]. Ampicillin, cephalothin, and tetracycline act synergistically with some essential oils, but gentamicin is mostly antagonistic. Four essential oils showed synergistic or antagonistic effects with ciprofloxacin against *Staphylococcus aureus* and *Klebsiella pneumoniae* and amphotericin B against *Candida albicans* strains, depending on the type of essential oil and concentration at tested [46]

2.8 Antimicrobial Activity Mechanisms of Medicinal Plant-Derived Chemical Compounds

Although synthetic antimicrobials have been approved in many countries, the use of natural compounds derived from medicinal plants continues to attract the attention of many researchers. Medicinal plants have great potential to find new bioactive compounds against drug-resistant microbes [47]. Medicinal substances are a type of compound found in many plants. They can save the clinical use of old antibiotics by increasing their potency, thus avoiding the fact that resistance occurs [48]. Plant biological compounds (phytochemicals) with medicinal value are mostly secondary metabolites used for medicinal purposes. Secondary metabolites are the products of secondary metabolism in plants that occur as intermediate or end products. There is a wide range of antimicrobial activity depending on the structure, the number and location of the substituents, the presence of glycosidic alkylation of the hydroxyl groups, and the topography and climate of the country of origin. In fact, changes in the quality and quantity of biological secondary metabolites alter their antibacterial activity against different microbial strains [48].

In most cases, organic herbs contain a complex mixture of nutrients that work together to produce better results. These compounds can affect microbial cells in different ways. In general, the main site of biological compounds is the cytoplasmic membrane, which affects its structure and integrity, permeability or action in different ways [49]. Plant extracts are thought to contain EP inhibitors in their composition. In addition, inhibition of normal cell interactions [quorum sensing (QS)] has also been described as one of the most promising mechanisms of action of biological compounds against MDR pathogens. QS inhibitors should be able to reduce the expression of genes controlled by QS, and have a chemical effect on the metabolic and excretory processes of the host organism [49]. Some compounds can alter or inhibit protein-protein interactions, potentially altering the immune response, mitosis, and apoptosis. In addition, they can disrupt mediator exchange, activate cytoplasmic components and disrupt or prevent biofilm formation, thereby benefiting pathogens during infection. Several antiviral components in medicinal plant extracts interact with various viral proteins at different stages of viral replication [50]

2.9 Phytochemicals as Antimicrobial Agents

In plants, different types and types of organic compounds or metabolites are produced through metabolic processes. These metabolites are divided into primary metabolites and secondary metabolites. Important metabolites such as chlorophyll, amino acids, nucleotides, simple carbohydrates, membrane lipids play an important role in photosynthesis, respiration, solute transport, transport, synthesis and differentiation of nutrients. Secondary metabolites also differ from primary metabolites because of their limited distribution in the plant world. Secondary metabolites are usually found only in a single plant species or group of taxonomically related species, whereas primary metabolites are found throughout the plant kingdom. In the past decades, experimental and experimental evidence has clearly shown that many secondary metabolites have functions that are very important to the health of the plants that produce them. The main characters 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 [51]

2.10 Phenolic Compounds

Phenolic compounds are biologically active phytochemical molecules. Scientific Reports reviewed nearly 8,000 phenolic compounds, half of which are phenolic compounds that have many benefits for human health, such as but not limited to antioxidants, anti-inflammatories, and antioxidants. anti-cancer, antibacterial, immune booster, cardioprotective and protects the skin from UV rays. In terms of antibacterial activity, phenolic compounds extracted from various plant leaves have antibacterial properties. For example, the extract of pomegranate peel, methanol, ethanol and water contain higher levels of phenolic compounds, which are effective against *Staphylococcus aureus* and *Enterobacter aerogenes*, *Klebsiella pneumoniae*, etc. showed great antibacterial activity. Leaf extracts using eutectic solvents in Rutagravolens have high antibacterial activity against Gram-negative *Pseudomonas aeruginosa* and are also active against *Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli*, but at low levels [52].

2.11 Alkaloids

Alkaloids are one of the most abundant types of chemicals. About 12,000 alkaloids are extracted from various plants and extensively studied. Alkaloids have many benefits for human health, such as but not limited to muscle relaxants, antiseptic and protective, anticancer and antibiotic properties, and many drugs in derived from alkaloids such as morphine, apomorphine and codeine. Here are some examples of plants that are rich in alkaloids and show strong antimicrobial properties; Alkaloids extracted from leaves of Callistemon citrinus showed strong antimicrobial activity against *Staphylococcus aureus* (ATCC 9144) and *Pseudomonas aeruginosa* (ATCC 27853). These molecules block the ATP transport of compounds. The alkaloids in Eclipta leaf extract were tested against many human pathogenic bacteria, with Staphylococcus aureus and *Escherichia coli* being the most susceptible bacteria, and the inhibitory effect of the alkaloids on all pathogens has been test was found to increase with increasing levels [53].

2.12 Terpenoids

Terpenoids are an important part of phytochemical compounds. According to the scientific literature, terpenoids are limited to only 40,000 compounds, making them one of the most abundant types of chemicals. Terpenes are widely used as perfumes, fragrances, pesticides, medicines, and industrial compounds. Many studies suggest that many terpenoids have strong antibacterial activity. Twelve pure terpenoids extracted from the wood and bark of the fragrant spruce (*Pilgerodendron uviferum*), are active against *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes, Escherichia coli, Pseudomonas aureus, Fusarium graminearum Ophiostoma piliferum, Rhizoctonia solani, Phragmidium violaceum, Schizophyllum commune, Pythium irregulare and Botrytis cinerea*. Terpenoids extracted from Eremophila lucida leaves have good antibacterial activity against Gram-positive bacteria

(*Staphylococcus aureus* ATCC 29213 and *Staphylococcus aureus* ATCC 25923) but no activity against Gram-negative bacteria (*Escherichia coli* ATCC 25922).

2.13 Carotenoids

Carotenoids are phytochemical metabolites of lipids; an extremely important chemical group with diverse biological activities, up to 600 carotenoids have been identified and isolated from plants. One of its main functions in plants is to produce pigments to color fruits and vegetables; According to scientific studies it has many protective effects against some diseases and conditions, such as cancer, diseases related to aging, cardiovascular diseases, antioxidant properties, etc. Unfortunately, there is little research on the antibacterial potential of carotenoids in plants, with most studies focusing on carotenoids from certain fungi and the genus *Streptomyces*. However, some interesting studies show that carotenoids show significant antibacterial activity; Guiera plant gall (*Guiera senegalensis*) from Burkina Faso is rich in carotenoids and has antimicrobial activity 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].

3. LABORATORY TECHNIQUES USE IN THE STUDY OF ANTIMICROBIAL PLANT EXTRACTS

Antimicrobial susceptibility testing can be used in drug discovery, epidemiology, and predicting treatment outcomes. After the "Golden Age" revolution, almost all-important antibiotics (tetracyclines, cephalosporins, aminoglycosides, and macrolides) were discovered and the major problems of chemotherapy were solved in the 1960s, history is repeating itself, and these interesting compounds are in trouble. it loses its potency due to increased microbial resistance (Mayers et al., 2009). Currently, there is a large impact on the lack of treatment associated with bacteria-resistant to many drugs, and it has become a public health problem [54].

3.1 Diffusion Method

(a) Agar disk diffusion method: The disc debris test was developed in the 1940s and is the standard method used in many clinical microbiology laboratories for antimicrobial susceptibility testing. Currently, the Clinical and Laboratory Standards Institute (CLSI) publishes a number of test standards for bacteria and yeast [55]. In this popular method, agar plates are inoculated with a standard inoculum of test microorganisms. Then, a disc of filter paper (about 6 mm in diameter) containing the test compound at the desired concentration is placed on the agar surface. Incubate the Petri dish under appropriate conditions. Basically, an antimicrobial agent is spread on the agar and inhibits the growth and growth of the test microorganism, then the diameter of the inhibited growth zone is measured, thus indicating the growth medium, temperature, incubation time, and inoculum required by CLSI. standard.

In addition, the agar disk diffusion method is not suitable for determining the minimum inhibitory concentration (MIC) because it is not possible to measure the amount of antimicrobial agent dispersed in the agar medium. However, by comparing inhibition zones and therapeutic algorithms, MICs can be calculated for specific microorganisms and antibiotics. However, the immunoassay test has several advantages over other methods: simplicity, low cost, the ability to test a large number of microorganisms and antimicrobial agents, and the ease of interpretation of results. products are delivered. In addition, many studies have shown that patients with bacterial infections are more sensitive to antibiotic therapy depending on the antimicrobial type of the pathogen. This fact is due to the good correlation between in vitro data and in vivo development [56].

(b) Agar well diffusion method: The diffusion method is widely used to evaluate the antimicrobial activity of plants and microbial extracts [57]. Similar to the technique used in the disc diffusion method, the surface of the agar plate is inoculated by spreading a large amount of microbial inoculum across the agar surface. Next, a 6- to 8-mm-diameter hole is made with a syringe or a blunt tip, and a double volume (20-100 µL) of the antimicrobial agent or extract is injected. required concentration. Agar plates are cultured under conditions appropriate to the test microorganism. Antimicrobial agents are dispersed in the agar medium and inhibit the growth of the test microbial strain.

(c) Agar plug diffusion method: The plug agar diffusion method is used to increase the

antagonism between microorganisms [58], and the process is similar to the disc diffusion method. This provides an opportunity to grow agar of the desired strain on a positive medium using lines attached to the surface of the dish. During growth, microbial cells release molecules that are dispersed in the agar medium. After incubation, cut the block or cylinder using a sterile blue screw and place it on the surface of the agar in another plate inoculated with the test organism. These substances diffuse from the plug into the agar medium. The antimicrobial activity of molecules secreted by microorganisms can be seen by the appearance of the inhibitory zone around the agar plug.

3.2 Cross Streak Method

The cross-sectional method is used to rapidly screen microorganisms for their harmful effects. The desired microorganism strain is seeded with a single pellet in the center of an agar plate. After the incubation period according to the microbial strain, the tested microorganisms are injected in a line perpendicular to the center line in the plate. After incubation, antimicrobial interactions were analyzed by examining the size of the inhibition zone.

3.3 Poisoning Food Method

The toxic food method is widely used to evaluate antifungal effects on fungi [58]. The antifungal agent or extract is added to the liquid agar at the final desired concentration and mixed well. The culture medium was then poured into a Petri dish. After the night before the culture, it can be fixed with a mycelium disc 2-5 mm and placed in the middle of the plate. After incubation in the appropriate conditions for the test fungal strain, the diameter of the fungal growth was measured on the control plates and the samples and the antifungal effect was determined by the following formula:

$$\text{Antifungal activity (\%)} = ((Dc - Ds)/Dc) \times 100$$

where Dc is the growth diameter on the control plate and Ds is the growth diameter on the plate containing the test antifungal agent. Sporulation can also be compared to controls. Of course, when the methods used fail to compare, researchers should perform positive controls using known antimicrobial molecules to compare findings and confirm the accuracy of the test method.

3.4 Thin Layer Chromatography (TLC) – Bio Autography

In 1946, Goodall and Levi combined paper chromatography (PC) with biochemical methods to detect and determine penicillins. After that, Fischer and Lautner introduced TLC in the same field. This technology combines thin layer chromatography with biological and chemical detection techniques. Several studies have been carried out to investigate organic extracts (mostly plant extracts) for their antibacterial and antifungal activity by thin-layer chromatography-bio autoradiography [59]. As shown below, three bio autoradiographic methods, namely diffusion agar, direct bio autoradiography, and overlay agar, have been described to study antimicrobial compounds by this method [60-64].

4. CONCLUSION

The antimicrobial activity of medicinal plants is a new hope to combat the threat posed by the increasing evidence of antimicrobial resistance. Therefore, there is an urgent need to identify and isolate new bioactive compounds from medicinal plants that have not been fully explored. This diversity of compounds has shown therapeutic potential as antibiotic agents and to modulate antimicrobial resistance. Ample in vitro and in vivo testing should be emphasized in order to select effective and non-toxic antimicrobial phytogenic compounds. Exploiting the synergistic or antagonistic effects of compounds within and between medicinal plant extracts is also a major challenge. As biotechnology advances, it is clear that we can relearn the chemical composition of medicinal plants and develop more sophisticated methods to extract, fractionate, and identify biological compounds. and various chemical structures and methods of operation. Extraction methods and in vitro testing are useful for better detection and interpretation of results. In addition, reference samples have not been used to study mixtures of plant extracts and future research may prove useful for this approach. Attention should be paid to the study of the mechanism of action, interactions with antibiotics, other plants or medicinal products, and the pharmacokinetic and pharmacodynamic characteristics of the extract. It is hoped that this review, together with the main challenges identified in this field, will contribute to better, more successful and easier methods of using

new medicinal plants to be faster against microorganisms.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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