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Review Article

Natural Antioxidant and Antibacterial Agents from Medicinal Plants: A Comprehensive Review

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Abstract

The research of antibiotics and antiviral medicines has received more attention in recent years as public health and hygiene issues have grown. However, because to their possible toxicity and side effects, synthetic antimicrobial medicines have not been widely used. Because they provide safer options with fewer side effects, the usage of herbal therapies has grown in popularity. By blocking virulence factors and specifically targeting pathogenic cells, medicinal plants and their bioactive components demonstrate antimicrobial action. Numerous bioactive substances originating from plants have also demonstrated the capacity to overcome antibiotic resistance and improve the synergistic effects of currently available medications, giving them attractive options for the creation of novel pharmacological treatments to combat resistant illnesses. Moreover, oxidative stress brought on by an excess of free oxygen radicals damages cells by causing DNA hydroxylation, protein denaturation, and lipid peroxidation. Antioxidants are essential for preserving cellular integrity and shielding cells from oxidative damage. Antioxidants have historically been obtained from medicinal fruits, herbs, and spices; but, because microbes can develop quickly in controlled environments, they may also be a source. Many medicinal plants contain phytochemicals like terpenes and phenolic compounds, which have antibacterial and antioxidant properties and may be used in food preservation. It is possible to efficiently extract these bioactive compounds and use them as natural additives to extend the shelf life and safety of food. The origins, workings, and uses of herbal bioactive chemicals as sustainable substitutes for synthetic antioxidants and antibacterials are reviewed in this chapter.

Keywords: Medicinal Plants, Bioactive Compounds, Antimicrobial Activities, Oxidative Stress, Antimicrobial Resistance.

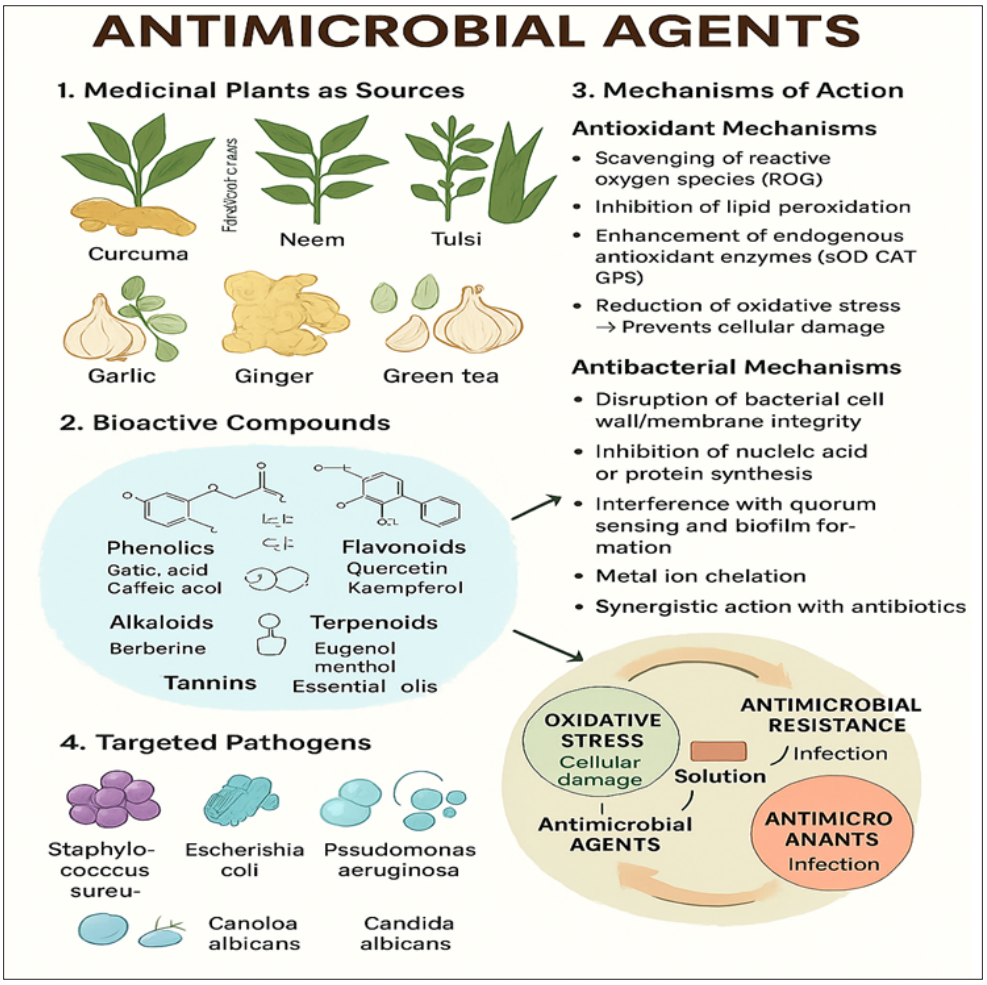

Figure 1: Graphical Abstract

1. Introduction

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. Examples of antioxidants include beta-carotene, lycopene, vitamins C, E, A and other substances[1,2].

Antioxidants help prevent oxidation, which can cause damage to cells and may contribute to aging. They may improve immune function and perhaps lower the risk for infection, cardiovascular disease, and cancer. Antioxidants exist as vitamins, minerals and other compounds in foods. A diet containing plenty of fruits and vegetables, whole grains and nuts can supply all the antioxidants your body needs. Diets rich in antioxidants can be very beneficial. A few of the better known antioxidants include carotenoids (a form of vitamin A) — the substance that gives fruits and vegetables their deep rich colors. Apricots, broccoli, pumpkin, cantaloupes, spinach and sweet potatoes are good choices. Foods containing vitamins like vit. C and vit. E are also good sources of antioxidants, as well as selenium and zinc. The increased risk of adverse effect due to synthetic drugs increased the scope and utilization of the compounds derived from natural bioactive. Bioactives compounds from the plant origin are considered more secure as they produce less harmful metabolites[3].

The use of herbal plant products is growing in many segments of the population. According to an estimate, 80–90% of the world's population lead to herbal plants for their medicine[4]. Herbal products play a very important role in the treatment of life-threatening conditions[5]. This review helps us about the application of scientific technological approach in traditional medicine has been used to

achieve the objective[6]. Plant-derived bioactives possess a variety of biological activities including antioxidants, antimicrobial, antitumor activity, vasorelaxant activity, immunosuppressive activity, antimalarial activity and many more[7].

2. Characteristics of Antioxidants

The major antioxidants currently used in foods are monohydroxy or polyhydroxy phenol compounds with various ring substitutions. These compounds have low activation energy to donate hydrogen. Hence, the resulting antioxidants radical does not initiate another free radical due to the stabilization of the delocalized radical electron. Propagation and initiation of free radicals chain reaction can be delayed or minimized by the donation of hydrogen from the antioxidants and metal chelating agent. The resulting antioxidant free-radical is not subject to rapid oxidation due to its stability. Antioxidants free-radicals can also react with lipid free radicals to form a stable complex compound thereby preventing some of their damages[2].

A. Source of Antioxidants: Vit. C, Vit. E, α-carotene, Licopein, Selenium, Polyphenol, Glutathione, Proxidase, Cystine are main sources of antioxidants. Fruit juices, beverages and hot drinks contain high amounts of antioxidants, like polyphenols, vitamin C, vitamin E, Maillard reaction products, â-carotene, and lycopen. The consumption of fruit juices, beverages and hot drinks was found to reduce the morbidity and mortality caused by degenerative diseases. The recommendations based on epidemiological studies are such, that fruits, vegetables and less processed staple foods ensure the best protection against the development of diseases caused by oxidative stress, such as cancer, coronary heart disease, obesity, type 2 diabetes, hypertension and cataract. The explanation consists in the beneficial health effect, due to antioxidants present in fruit and vegetables[8].

B. Function of Antioxidants: The Food and Drug Administration (FDA) define antioxidants only as dietary supplements to be taken in addition to normal food consumption in an effort to prevent these diseases.

Function of Vitamin C: Vitamin C intake is inversely related to cancer, with protective effects shown for cancer of the lung, breast, pancreas, stomach, cervix, rectum and oral cavity. In stressful situations adrenal glands react by releasing hormones that trigger the "fight or flight" reaction. It has been indicated that 200mg of vitamin C a day may reduce the levels of stress hormones. Stress suppresses the immune system. Mega doses of vitamin C increase the levels of antibody that fights against germs and viruses in both stressed and unstressed rats, with greater antibody increase in the unstressed rats[9]. Vitamin E: One of the most significant lipid-soluble primary defensive antioxidants is vitamin E. It is a general name for a number of tocopherols and tocotrienols found in nature. In its function as a chain-breaking antioxidant, vitamin E rapidly transfers its phenolic H-atom to a lipid peroxyl radical, converting it into a lipid hydroperoxide and a vitamin E radical. Tocopherols (vitamin E) and tocotrienols (provitamin E) are powerful antioxidants that confer oxidative stability to red palm olein (RPO) as well as help to keep the carotenoids and other quality parameters of the oil stable. The various function are maintains normal conditions of cells, and healthy skin and tissues, Protects red blood cells, antioxidation, enhance immunity. The important sources of vitamin E include wheat germ, nuts, seeds, whole grains, green leafy vegetables, vegetable oil and fish-liver oil.

β-Carotene: Beta-carotene has antioxidant properties that can help neutralize free radicals – reactive oxygen molecules potentially damaging lipids in cell membranes and genetic material, which may lead to the development of cardiovascular disease and cancer[10]. At present, it is unclear whether some beneficial effects of beta-carotene and other carotenoids in humans are a result of their antioxidant activity or other non-antioxidant mechanisms. The relevance of deactivating reactive oxygen species to human health, potentially preventing diseases such as cancer and coronary heart disease, is not clear. In vitro studies indicate that carotenoids can also inhibit the oxidation of fats under certain

conditions. They may have anti-atherosclerotic potential, but their effects in humans appear to be more complex[11]. Selenium: Selenium is mostly known for its potential antioxidant properties. Indeed, it is a required oligoelement for the synthesis and function of about 20-40 enzymes, among which most of them help prevent cellular damage from natural by-products of oxygen metabolism, called reactive oxygen species/free radicals. Selenium is also essential for the proper function of the immune system and is known to have antiviral properties. Effects on inflammatory responses are among the other key activities identified for selenoproteins[12].

C. Polyphenol Antioxidant

Current research strongly suggests that polyphenols may prevent heart disease, cancer, and osteoporosis. There is also evidence that they may help prevent neurological diseases and diabetes. Significant progress has been made in the ares of cardiovascular diseases, and today it is well established that some polyphenols, administered as supplements or with food, do improve health status, as indicated by several biomarkers closely associated with cardiovascular risk. Vita J.A 2005 and Arts et al. 2005 reported that epidemiologic studies tend to confirm the shielding effects of polyphenol consumption against cardiovascular diseases[13,14].

Glutathione: Dolas and Gotmare 2025 reported that Glutathione protects cells from toxins such as free radicals. The human body produces glutathione from the synthesis of three key amino acidscysteine, glycine and glutamic acid. Food sources with the highest amounts of naturally occurring glutathione include; asparagus, avocado, grapefruit, squash, potato, cantaloupe, peach, zucchini, spinach, broccoli, watermelon, and strawberries. Fish, meat, and foods which yield sulfur containing amino acids (e.g. eggs) are the preferred sources for maintaining and increasing bodily glutathione levels.

D. Peroxidase: Dolas and Gotmare 2015 said that an enzyme found mostly in plants, milk, and white blood cells is made up of a protein complex containing hematin groups that speeds up the oxidation of different substances. Horseradish root, soybeans, mango fruit, and turnip are all foods that contain peroxidase[15].

E. Flavonoids: Flavonoids promote antioxidant activity, cellular health and normal tisse growth and renewal throughout the body. They also work with vitamin C to lower oxidative stress in the cell's water-based part, which may help slow down some of the impacts of aging. There are more than 4,000 unique flavonoids and they are most effective when several types are consumed together. Food sources include: cranberries, kale, beets, berries, red and black grapes, oranges, lemons, grapefruits and green tea[16].

2.1 Oxidative Stress and Illnesses in Humans

An important relationship has been observed between oxidative stress and a wide variety of human diseases.[17] Such diseases include:

A. Cardiovascular Disease: Oxidative stress caused by reactive oxygen species is a major cause of many cardiovascular disorders, including atherosclerosis, ischemic heart disease, high blood pressure, cardiomyopathy, cardiac hypertrophy, and congestive heart failure. The induction of Ca2+ ion excess by ROS (reactive oxygen species) results in alterations in membrane permeability, breakdown of the lipid bilayer, functional modifications of many cellular proteins, and anomalies in myocyte function and endothelial dysfunction[18].

B. Diabetes: Oxidative stress in normal cell contribute to both type- 1(insulin dependent) and type-2 (non-insulindependent) diabetes mellitus. Stimulation of reactive species could come from cytochromeP450 monooxygenases, glucose auto-oxidation, NADPH-oxidase, lipooxygenase, nitric oxide synthase and oxidative phosphorylation[19].

C. Neurodegenerative Diseases: The diseases are categorized by loss of nerve cells from brain and spinal cord, leading to either functional loss (ataxia) or sensory dysfunction (dementia), mitochondrial dysfunctions and apoptosis. Examples of such disorders are Parkinson's disease, Alzheimer's

disease, multiple sclerosis, amyolotrophic lateral sclerosis, Huntington's disease, cognitive dysfunction in the elderly, schizophrenia and tardive dyskinesia. Esposito[20,21].

D. Cancer: Deoxyribonucleic acid (DNA) damage and mutation play critical roles in carcinogenesis. Redox potentials of reactive species (RNS, ROS) may play important roles in sequence-specific DNA damage arising from activation of activator protein (AP1) and nuclear factor kappa B (NF-κB) signal transduction pathways leading to the transcription of genes involved in cell growth regulatory pathways and pathogenesis of cancer[22].

E. Rheumatoid arthritis: Is a chronic inflammatory autoimmune disease characterized by erosive, progressive and chronic polyarthritis. The pathogenesis of rheumatoid arthritis arises from the development of the free radicals at the site of inflammation leading to lipid peroxidation modification of low density lipoprotein, inactivation of alpha-1-protease inhibitor, DNA damage, activation of neutorphil, NADPH oxidase and endothelial cell xanthine dehydrogenase. Decrease in the concentrations of whole blood glutathione and total thiols also contribute to rheumatoid arthritis[23].

F. Gastrointestinal Disorder: Oxidative stress has been found as a causative factor for almost all gastrointestinal diseases. This arises probably from the enzymatic processes in gastrointestinal tract (GIT) necessary to form large amounts of oxygen radicals[24].

G. Renal System Disorder: ROS (Reactive oxygen species) can hurt the renal system. These reactive species are generated by glomerular (endothelial), vascular (endothelial and smooth muscle cells) and tubular (proximal, distal and collector) cells of renal structure by stimulating potential factors[25].

H. Pulmonary disorder: Oxidative stress-induced airway inflammation is a key factor in the chronic pulmonary illnesses, including asthma and COPD(chronic obstructive pulmonary disease). The risk of pulmonary disorders is further amplified by free radicals generated by ozone, cigarette smoking (air pollutants), infections, and other allergens. These ROS (reactive oxygen species) act by causing direct oxidative damage to epithelial cells of the airways or may evoke bronchial hyper-reactivity and stimulation of histamine release from mast cells of the airways epithelial cells[26].

I. Eye Disorder: Human eye is most susceptible organs to oxidative damage caused by atmospheric oxygen, abrasion, light and toxins. Ultraviolet light also increases generation of ROS (reactive oxygen species) such hydrogen peroxide, superoxide and hydroxyl radicals. Oxidative stress caused by these ROS is responsible for eye disorders such as cataracts, glaucoma and macular degeneration. Conversion of light energy into a nerve impulse by the photoreceptors could be the mechanism of action[27].

J. Infertility and Pregnancy: Oxidative stress has been associated in male and female infertility.[28] ROS (reactive oxygen species) can exhibit beneficial or detrimental effects on reproductive system depending on the location, concentration, also the length of exposure to these reactive species. Reactive oxygen species are being generated from spermatozoa, leucocytes and an increased concentration levels in semen correlate negatively with sperm concentration, motility, fusion events associated with fertilization and subsequently leads to infertility in male. Oxidative stress also influences multiple physiological processes such as oocyte maturation, fertilization, implantation, embryo development and associated in endometriosis, polycystic ovarian disease, unexplained infertility and recurrent pregnancy loss[29].

K. Aging: Aging is defined as a progressive decline in the physiological functions of an organism after the reproductive phase of life. Oxidative stress is responsible for damage of different cell components and is the key components of age related disorders. Among the genetic theory and damageaccumulation theories used to explain the aging process, free

radical theory is probably the most complex approach. It is based on the fact that the random deleterious effects of free radicals produced during aerobic metabolism cause damage to DNA, lipids and proteins[17].

2.2 Classification of Antioxidants:

Antioxidants can be categorized into two types. Non-enzymatic antioxidants Non-enzymatic antioxidants interrupt free radical chain reactions. For example, vitamin E interrupts a chain of free radical activity after only five reactions. Other examples include vitamin C, plant polyphenols, carotenoids, Se, and GSH.

GSH (cysteine containing natural antioxidant) is called as the "master antioxidant" and is found in every single cell of your body, maximizing the action of all the other antioxidants.

GSH is a tripeptide with a gamma peptide linkage between the amine group of cysteine (which is attached by a normal peptide linkage to a glycine) and the carboxyl group of the glutamate side-chain.

GSH exists in both reduced (GSH) and oxidized (GSSG) states. In the reduced state, the thiol group of cysteine is able to donate a reducing equivalent ($H^{++} e^{-}$) to other unstable molecules such as ROS. In donating an electron, GSH itself becomes reactive but readily reacts with another reactive GSH to form GSH disulfide (GSSG). Such a reaction is probable due to the relatively high concentration of GSH in cells (up to 5 mM in the liver).

GSH is regenerated from GSSG by the enzyme GSH reductase (GSR). In healthy cells and tissue, more than 90% of the total GSH pool is in the reduced form (GSH) and <10% exists in the disulfide form (GSSG). An increased GSSG-to-GSH ratio is considered indicative of oxidative stress.

2.3 Antioxidant – Mechanism of Action

Antioxidants are substances that, even at low concentrations, can retard the oxidation of easily oxidizable biomolecules such as lipids and proteins in food products, thereby improving their shelf life by protecting them from oxidative deterioration. The mechanism of antioxidant action involves several pathways. Firstly, antioxidants act as radical scavengers, neutralizing free radicals; common examples include flavonoids, phenolic compounds, ligands, and phenolic acids. Secondly, some antioxidants react with transition metals to form stable complexes, preventing these metals from catalyzing oxidation reactions. Thirdly, certain antioxidants decompose peroxides into stable, non-radical products—an example being selenium-containing glutathione peroxidase, an antioxidative enzyme that inactivates free radicals and oxidants like hydrogen peroxide. Fourthly, antioxidants can inactivate the singlet form of oxygen that is produced in the occurrence of photosensitizers like chlorophyll and pheophytins under light exposure. Lastly, antioxidants inhibit the enzymatic activity required for auto-oxidation; for instance, flavonoids, phenolic acids, and gallates deactivate lipoxygenase, thereby preventing lipid oxidation[30].

3. Antimicrobial Agent

Antimicrobial agents are compounds that kill microorganisms or stop them from growing. They are grouped based on the sort of pathogens they fight, like bacteria, viruses, fungus, or parasites. There are two main groups of these agents based on their chemical makeup. Synthetic antimicrobial agents are the first group. These are chemicals made in a lab, like antibiotics and metal or metal-oxide nanoparticles like silver and silver oxide. All of these compounds are very good at destroying germs. The second type is herbal antimicrobial agents, which come from plants and have natural bioactive components that can stop or kill dangerous microorganisms[31].

Synthetic antimicrobial agents, like as antibiotics and other chemicals, are very important for treating illnesses caused by microbes. However, they often have serious adverse effects. One of the most serious side effects is the production of reactive oxygen species (ROS), which are very poisonous and have been connected to the growth of cancer.

On the other hand, herbal antimicrobial agents like clove, portulaca, tribulus, eryngium, cinnamon, turmeric, ginger, thyme, pennyroyal, mint, fennel, chamomile, burdock, eucalyptus, primrose, lemon balm, mallows, and garlic have natural bioactive compounds that are very good at getting rid of free radicals. These herbal compounds assist lower oxidative stress and stop ROS-related cell damage by neutralizing ROS.[32]

3.1 Antimicrobial Mechanism of Bioactive Substances against Clinically Important Pathogens

Plant antimicrobial secondary metabolites are generally classified into three broad classes namely such as phenolic compounds, terpenes, and alkaloids. Research indicates that plant extracts and their bioactive constituents demonstrate antimicrobial properties by compromising cell walls, producing reactive oxygen species, inhibiting biofilm formation, obstructing cell wall synthesis, impeding DNA replication and energy production, and mitigating bacterial toxin release[33,34,35,36]. In addition, these compounds may prevent antibacterial resistance as well as synergetics to antibiotics, which can ultimately kill pathogenic organisms.

Table:1 Antimicrobial mechanism of bioactive substances against clinically important pathogens

Name of bioactive molecules	Antimicrobial mechanism of bioactive substances against clinically important pathogens	References
Phenolic compounds	Although increased hydroxylation reactions lead to microbial action because they encourage cell wall breakdown and lysis, the hydroxyl functional group (-OH), which is claimed to be absolutely poisonous to germs. Examples include 2, 4, 6-trihydroxy-30-methyl chalcon, quercetin, rutin, naringenin, sophoraflavanone etc.	37
Flavonoids	Flavonoids are strong inhibitors of biofilm development because they cause multicellular composites of bacteria to aggregate and then suppress bacterial growth following aggregate formation. Examples include epicatechin, isovitexin, and galangin etc.	38, 39, 40 , 41,42, 37
Catechins	Additionally, these catechins support the inhibition or inactivation of the synthesis of intracellular and extracellular enzymes. Antibacterial drugs can effectively stop bacterial growth by inhibiting the enzymes involved in fatty acid production; in particular, the inhibitor of the main enzyme fatty acid synthase II (FAS-II) is important as an antimicrobial medication. Examples include quercetin, apigenin, kaempferol, myricetin, baicalein, luteolin, and fisetinetc.	43, 44, 45 46, 47, 48, 49, 50, 51
Alkaloids	The alkaline nature of alkaloids, which are nitrogenous substances, helps to impede cell respiration, intercalates with DNA, and inhibits a number of enzymes involved in replication, transcription, and translation. Examples of plant-based bioactive substances include chrysin, nobiletin, myricetin, tangeritin, genistein, and apigenin etc	52,53,54,55, 56,57,58,59
Flavonoids such as isobavachalcone	Reduced Energy Production Since these chemicals are the primary component of living systems, energy production, or ATP synthesis, is the most essential necessity for the existence and development of bacteria. Examples include the flavonoids silymarin, baicalein, morin, silibinin, quercetin, isoquercetin, and other flavonoids etc.	33, 36, 60,36
Catechins and other flavonoids	Biological Toxin Inhibition Catechins like pinocembrin, kaempferol, and others are effective at neutralising the harmful effects of bacteria like S. aureus, E. coli, and V. cholerae. Hyaluronic acid lyase inhibitory flavonoids in Streptococcus equisimilis and Streptococcus agalactiae include myricetin and quercetin.	61, 62, 63, 64, 65, 66, 67,

Quinone and phenolic compounds	Bacteria produce different enzymes to deactivate antibiotics through the process of phosphorylation, adenylation, or acetylation; (b) damage or alteration of the drug compound; (c) prevent the interaction of the drug with the target; and (d) efflux of the antibiotic from the cell. These are the mechanisms by which bacteria develop resistance to antibacterial agents. Examples include baicalein, phenolic compounds, and emodin (1, 2, 8-trihydroxy-6-methylanthraquinone) etc.	68, 69,70, 71,72,73
Catechins and lipophilic bioactive compounds	ROS (reactive oxygen species) production in conjunction with antimicrobial action Reactive oxygen species (ROS) can be created when molecular oxygen is partially reduced with the goal of inhibiting the action of certain pathogens that cause disease by exerting antibacterial activity. Examples are Catechins and lipophilic bioactive compounds	74, 75

3.2 Methods for in Vitro Evaluating Antimicrobial Activity

Antimicrobial susceptibility testing is important for finding new drugs, studying the spread of diseases, and guessing how well therapy will work. This study looked at in vitro antimicrobial testing methods to see if plant extracts and pure chemicals may be employed as antimicrobial agents. Natural sources yield a vast array of structurally varied molecules. Recent studies have progressively investigated plant and microbial extracts, essential oils, and synthetic chemicals for their antibacterial properties. While numerous plant extracts exhibit intriguing antibacterial properties, the necessity for trustworthy and comparable data is imperative to guarantee significant outcomes, rather than relegating antimicrobial testing to a mere adjunct of phytochemical investigations.

A. Aagar disk-diffusion method: The agar disk-diffusion test, developed in 1940, is a widely used standard method in clinical microbiology for assessing antibiotic resistance. It is commonly applied to fastidious bacteria such as streptococci, Haemophilus influenzae, H. parainfluenzae, Neisseria gonorrhoeae, and N. meningitidis. In this method, agar plates are inoculated with a uniform amount of the test microorganism, and filter paper disks containing the test substance are placed on the surface. During incubation, the antimicrobial agent diffuses into the agar and inhibits microbial growth, forming measurable zones of inhibition[76].

B. Antimicrobial gradient technique (Etest): To find the MIC, the antimicrobial gradient approach uses both dilution and diffusion procedures to produce a concentration gradient of the test drug in agar. The commercial Etest®, made by bioMérieux, has a plastic strip with an increasing amount of the antibiotic on it. This strip is put on an agar plate that has the test microorganism on it. After incubation, the MIC is read at the point where the inhibition ellipse crosses the strip. This straightforward and dependable technique is extensively employed for ascertaining the MICs of antibiotics, antifungals, and antimycobacterial drugs[77].

C. Other diffusion methods: When studying microbial conflict or testing the antibacterial effects of extracts, fractions, or pure compounds, researchers in the field of microbiology often turn to alternative diffusion methods. Here are some of the most common approaches to this problem.

D. Agar well diffusion method: The agar well diffusion method is a common way to test the antibacterial properties of plant or microbial extracts. In the same way as the disk-diffusion method, the test microorganism is first added to the agar plate, and then a sterile tool is used to make wells that are 6 to 8 mm wide. A specific amount of the extract or antimicrobial solution is poured to each well, and the plates are then put in an incubator. The chemical

spreads through the agar and stops microbes from growing, creating quantifiable inhibition zones[78,79].

E. Plug diffusion method: The agar plug diffusion method, which is like the disk-diffusion approach, shows how microbes can fight each other. To start, the producer strain is cultivated on the right kind of agar, which lets it release antimicrobial chemicals into the medium. After incubation, a sterile cork borer is used to cut off an agar plug, which is then put on another agar plate that has already been inoculated with the test microorganism. As the compounds move away from the plug, an inhibitory zone emerges around it. This shows that the compounds are antimicrobial[80,81].

F. Method of cross streak: The cross streak method is a simple way to see if bacteria are fighting each other. To start the microbial strain of interest, a single streak is made in the middle of the agar plate. After a particular amount of time, which varies depending on the type of bacteria being tested, a single streak is made across the middle of the plate to add the microbes. The size of the inhibition zone is utilized to look at the antimicrobial interactions after more time in the incubator[82].

G. Poisoned food method: This is a standard way to test how well antifungal drugs work. Mix the antifungal agent or extract into hot agar at the right amount, pour it into Petri plates, and let it cool. A 2–5 mm mycelial disc is put in the middle after being pre-incubated overnight. After the plates have been incubated in the right circumstances, the diameters of the fungal growth on both the treated and control plates are measured. The antifungal impact is then evaluated using the following formula**:**

- Antifungal activity (%) = ((Dc−Ds)/Dc) × 100
- Dc: Diameter of growth in control plate
- Ds: Diameter of growth in the plate containing tested antifungal agent.
- Sporulation can be also compared to the control[83,84,85].

H. Agar diffusion: The agar diffusion or agar contact method is the least popular way to test for antimicrobial activity. You put antimicrobial chemicals from a chromatography plate (PC or TLC) onto an agar plate that already has the test microorganisms on it. After a short time of contact to let the molecules spread out, the chromatogram is taken off and the plate is put in an incubator. Then, zones of inhibition show up where the chemicals spread into the agar[86].

I. Direct bioautography: It is the most common of these three procedures. You dip or spray a microbiological suspension onto the produced TLC plate. After that, the bioautogram is kept at 25 °C for 48 hours in a humid environment. Tetrazolium salts are often used to see how microbes grow. The dehydrogenases in live cells change these salts into formazan, which has a very bright hue. The best reagent for detection is p-Iodonitrotetrazolium violet. The bioautogram is sprayed with these salts and then put back in the incubator at 25 °C for 24 hours or at 37 °C for 3–4 hours. Adding agar to Mueller Hinton Broth has been suggested as a way to make a medium that has enough fluid for the optimal observance to the TLC plate and keeps the right amount of humidity for bacteria to grow[87].

There can use either fungus or bacteria for direct bioautography, which is one of the easiest and most reliable ways to find antifungal chemicals. It is very effective against fungi that make spores, like Aspergillus, Penicillium, and Cladosporium. Bacillus subtilis, Staphylococcus aureus, and Escherichia coli are popular test organisms for antibacterial screening[88].

J. Agar overlay bioassay: This combined method overlays a molten, inoculated agar medium onto a TLC plate, allowing antimicrobial compounds to diffuse into the agar. After cooling and incubation, tetrazolium dye may be used to visualize clear inhibition zones. Suitable for bacteria, molds, and Candida albicans, TLC–bioautography is a simple, low-cost technique that separates complex mixtures and pinpoints active antimicrobial components on the TLC plate[89].

K. Dilution methods: This method are the most accurate way to find MIC values since they detect the exact amount of an antimicrobial drug in agar (agar dilution) or broth (macro- or microdilution). These methods give quantitative in vitro data for bacteria and fungi, with the MIC being the lowest concentration that stops growth, commonly given in µg/mL or mg/L. The CLSI and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) establish standardized recommendations for testing fastidious and non-fastidious bacteria, yeasts, and filamentous fungus. This makes sure that all microbiology labs follow the same steps[90].

L. The broth dilution method: The broth dilution technique is one of the easiest ways to test how sensitive microbes are to antibiotics. To do this, you need to make two-fold serial dilutions of the antimicrobial agent (for example, 1, 2, 4, 8, 16, 32 µg/mL) in a liquid growth medium. You can do this in test tubes that hold at least 2 mL (macrodilution) or in smaller amounts using a 96-well microtitration plate (microdilution). Then, a standardized microbial solution that has been changed to the 0.5 McFarland scale is added to each tube or well, mixed, and incubated at the right conditions for the test organism, usually without shaking[91].

M. Agar dilution method: To use the agar dilution method, you mix varying amounts of an antimicrobial agent into melted agar, usually using two-fold serial dilutions. Then, you put a standardized microbiological sample on the surface. The MIC is the lowest concentration that stops observable growth altogether. This technique is appropriate for assessing antibacterial and antifungal properties and demonstrates a robust connection with the Etest for both Gram-positive and Gram-negative bacteria. Comparative studies show that agar dilution, disk diffusion, and broth microdilution techniques work very well together[92].

N. Time-kill test (time-kill curve): The time-kill test is an important way to find out if an antimicrobial agent kills bacteria or fungus. It shows how the agent works in real time, whether it works better at higher concentrations or over time. According to CLSI recommendations, the test is done in broth with three tubes, each containing 5×10^5 CFU/mL. Two of the tubes include the antimicrobial agent at 0.25×MIC and 1×MIC, and the third tube is a growth control. Samples are incubated for predetermined durations (0–24 hours), and viable cell counts (CFU/mL) are assessed by agar plate counts to compute kill rates. Bactericidal action is shown by a 90% death rate in 6 hours or 99.9% in 24 hours. It is also excellent for testing drug interactions and has been used a lot in investigations of antifungal drugs[93,94].

O. ATP bioluminescence assay: The ATP bioluminescence assay quantifies the production of adenosine triphosphate (ATP) by bacteria or fungi, as ATP is a universal component of live cells and indicates their vitality. This process uses ATP to turn D-luciferin into oxyluciferin, which makes light. A luminometer sees this light as relative light units (RLU), which can be changed to RLU per mole of ATP. So, the amount of light is directly related to the quantity of living microbial cells[95,96,97].

P. Flow cytometric technique: Recently, flow cytometry has been looked at as a good way to test how susceptible microbes are because it can quickly find injured cells with certain fluorescent dyes. Propidium iodide (PI) is often used to show that a membrane has been damaged, and when used with cFDA, it has been useful in testing the antibacterial properties of essential oils against Listeria monocytogenes. This method checks for cell viability, membrane integrity, and overall damage in 2 to 6 hours, which is substantially faster than older microdilution methods. But flow cytometry equipment is still not widely used since it is expensive and hard to get [76, 98, 99]

Table 2: Common potential antioxidants and antimicrobial containing herbal plants and their bioactive compounds

S. No.	Plant Name (Scientific & Common Name)	Bioactive Molecules	Mechanism of Antioxidant and Antimicrobial Action	
1.	*Cymbopogon citratus* (D.C.) Stapf. *(Lemongrass)*	Citral, Geraniol	Its antioxidant and antibacterial properties mostly stem from inhibiting the generation of ROS(reactive oxygen species). The herb has stomachic, antihypertensive, carminative, antispasmodic, relaxing, and antidepressant effects.	100, 101, 102
2.	*Euphorbia stenoclada* Baill. *(Silver thicket)*	Quercetin, Quercitrin, Phenolic compounds	Quercetin, one of the key constituents, controls lipid peroxidation in phospholipids and inhibits DNA gyrase, showing strong antimicrobial activity.	103, 104, 105. 106
3.	*Geranium mexicanum* Kunt *(Geranium)*	Flavonoids, Tannins, Lignans, Essential oils, Epicatechin, Flavan-3-ols	Polyphenolic bioactive substances act as hydroxyl radical scavengers due to their aromatic structure. The plant exhibits antioxidant, antimicrobial, antidiabetic, and gastrointestinal protective effects.	107, 108, 109, 110, 111
4.	*Helianthemum glomeratum* Lag *(Clustered frostweed)*	Tiliroside, Quercitrin, Kaempferol, Epigallocatechin, Isoquercitrin, Phenolic compounds	Phenolic compounds show antioxidant potential and are useful as food additives. The plant's bioactives also possess analgesic, antidiarrheic, antiparasitic, and antidysenteric properties.	112, 113
5.	*Gnaphalium oxyphyllum* DC. *(Gordolobo)*	Diterpenoids, Flavonoids, Acetylenic compounds, Carotenoids, Luteolin, 3-Methoxyquercetin, β-Sitosterol, Stigmasterol	Luteolin and 3-methoxyquercetin exhibit potent antibacterial effects, inhibiting *S. aureus* and *B. cereus*. Traditionally used to treat wounds, inflammation, and infections.	114, 115, 116, 117, 118, 119
6.	*Chenopodium ambrosioides* L. *(Wormseed)*	Flavonoids, Terpenes, Steroids	Exhibits ABTS radical scavenging activity and fungicidal effects. Bioactive compounds are effective against parasites, menstrual disorders, fibroids, and uterine haemorrhage.	120, 121, 122, 123
7.	*Larrea tridentata* (DC.) Coville *(Creosote bush)*	Flavonoids, Triterpenes, Lignans (NDGA), 4-Epi-larreatricin, Dihydroguaiaretic acid	Bioactive compounds such as NDGA reduce ROS(reactive oxygen species) and show antifungal and antiviral activity against polio, AIDS, and herpes viruses. Traditionally used for diabetes, menstrual cramps, cancer, and tuberculosis.	124, 125, 126, 127
8.	*Marrubium vulgare* L. *(Horehound)*	Vitexin, Vicenin II, Lutein 7-Glucoside, Apigenin, Chrysoeriol, Apigenin 7-(6″-p-coumaroyl) Glucoside	Flavonoids provide antioxidant and antibacterial effects, particularly against Gram-positive bacteria. The plant exhibits tonic, diuretic, expectorant, and antidiabetic activities.	128, 129

4. Potential Bioactive Components used as Antimicrobial and Antioxidant Agents

Terpenes and phenolics, which are abundant in different plant tissues and most prevalent bioactive components of medicinal plants, have demonstrated their efficacy in preventing microbial growth and/or oxidation reactions in a diversity of food matrices. The claim that extracts from medicinal plants with comparable composition can work well as food additives can be supported by this premise. Carvacrol is a bioactive substance with antibacterial and antioxidant properties that is present in *C. ambrosioides*, used to treat parasite illnesses, uterine haemorrhage, and menstrual abnormalities[130,131]. The addition of carvacrol or thymol as a food preservative was undesirable to the panellists, who also ranked the sensory acceptability of carrot treated with carvacrol, thymol, and cinnamaldehyde negatively due to their powerful flavours[132]. Phenolic chemicals, which are widely dispersed in medicinal plants, are gaining interest as potential food additives. Commercial meat products such pig sausage, raw and roasted ham, bacon, and hamburgers have been found to contain catechin oligomers from apple tissues that are effective at inhibiting the oxidation of cholesterol.[133] Similar to this, other flavonoids, including quercetin, which are present in many plant tissues, including those of medicinal plants, are successfully used to prevent fish oil oxidation[134] Gallic acid has the benefit over other sweeteners in that the induced sweetness is noncaloric, persistent, and leaves no aftertaste that isn't sweet (for example, aspartame has a metallic aftertaste). Several technologies must be taken into consideration in order to treat food products more successfully (sensorially accepted) utilising extracts with components from medicinal plants: nanoemulsions, nanocapsules, vapours, and edible films to maximise the reactions (antimicrobial, antioxidant, and sensorial). The generation of this data will have a significant impact.

5. Conclusions

Numerous herbal plants have been traditionally utilised as food additions by common people and may have antioxidants and antibacterial characteristics. When compared to other plants that have tissues like those found in fruits, vegetables, spices, and herbs, it is negligible. These properties could be attributed to terpenes and phenolic content. It is primarily responsible for the antioxidant and antibacterial properties of plant materials. This study support to the idea that some medicinal plants may be a reliable source of antioxidants and antimicrobials for the food industry. Researchers should look into the different medical plants that have historically been used as antibacterial and/or antioxidant remedies. The chemical composition of the medicinal plants might be examined using various extraction methods, is needed advanced research to uncover novel chemicals. To incorporate medicinal plant extracts into food matrices, a variety of technologies can be used, including nanoemulsions, edible films or coatings, controlled release from encapsulation systems, and others. Last but not least, adding extracts from medicinal plants may be regarded as creating functional foods; nonetheless, a number of experimental methods support this assertion. The usage of natural extracts will be encouraged by this suggestion, which will also meet customer desire for healthier foods.

6. Authors' Contribution Statement

All authors contributed significantly to the preparation and completion of this review article titled "Natural Antioxidant and Antibacterial Agents from Medicinal Plants: A Comprehensive Review." Their individual contributions are as follows:

1. Ajay Kumar Shukla: Conceptualization, literature collection on antioxidant mechanisms, and drafting of introduction and background sections.
2. Vijay Singh Kachawa :Data compilation.
3. Vijay Kumar Bansal: Editing, refinement of manuscript structure, and referencing.
4. Chetna Baregama:Collection and analysis of literature related to bioactive phytoconstituents and their pharmacological significance.

5. Ayush Garg: Data organization, tabulation of plant species and active compounds, and figure preparation.
6. Mohamad Junaid Alam Mansuri: assisting in manuscript formatting.
7. Suresh Kumar Dev: Corresponding author; overall supervision, conceptual framework, manuscript design, critical revision, and final approval of the article for submission.
8. Yogesh Kumar Apurva : Review of antimicrobial mechanisms, literature verification, and technical editing.
9. Akhil Mangal: Assistance in reviewing recent literature and compiling references on herbal antimicrobial agents.
10. Vaibhav Rathore: Contribution to proofreading and quality assurance of data presentation.
11. Mohini Vishwas: Support in graphical representation and manuscript formatting.

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