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Medicinal Plants As Antibacterial Agents

Dr. Hoger Sabah Sedeeq
Erbil Medical Technical Institute, Erbil Polytechnic University,
Erbil, Kurdistan Region in Iraq

ABSTRACT

The richest bio-resource of drugs for traditional systems of medicine are Medicinal plants . The most common source of antimicrobial agents are plants . Their usage as traditional health remedies is the most popular in Asia, Latin America and Africa. Important subclasses of compounds include phenols, phenolic acids, quinines, flavones, flavonoids, flavones, tannins and coumarone are extracted from medicinal plants .

Keywords: Medicinal plants, Extraction, Antibacterial.

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1.INTRODUCTION

The important therapeutic aid for various ailments are medicinal plants (1) and are the richest bio-resource of drugs for traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

Spices and herbs have played a significant part in civilization and nation-building. Spices are vital in the making of delectable dishes because of their delectable flavor and pungency. Furthermore, they are said to have a variety of medical and pharmacological qualities, and as a result, they are used in the creation of a variety of medicines. Over the last decade, pharmacologists and herbalists have been more interested in alternative medical therapy. Plants have long served as a source of inspiration for new therapeutic molecules, as plant-derived medications have improved human health and well-being (2 and 3).

Drugs made from plants are currently attracting a lot of attention. The assumption that green medicine is safe and dependable, as opposed to expensive synthetic medications with side effects, is driving this enthusiasm. Plants continue to be the most prevalent source of antimicrobial compounds; scientific studies on the antibacterial capabilities of plants have been conducted. (1 and 4)

Their use as customary wellbeing cures is the most famous for 80% of worldpopulation in Asia, Latin America and Africa and is accounted for to have insignificant incidental effect (5). The antibacterial compound from plants might hinder bacterial development by various components (5 and 6).

Clinical microbiologists have two motivations to be keen on the subject of antibacterial plant removes. To start with, almost certainly, these phytochemicals will discover their direction into the munititions stockpile of antibacterial medications endorsed by doctors; a few are as of now being tried in people. All things considered, a few anti-microbials got from microorganisms are dispatched every year. After a downturnin that speed in

recentdecades, the speed is again enlivening as researchers understand that the successful life expectancy of any anti-toxin is restricted. Overall spending on discovering new enemy of infective specialists (counting immunizations) is relied upon to increment 60% from the spending levels in 1993. New sources, particularly plant sources, are additionally being explored. Second, the general population is turning out to be progressively mindful of issues with the over solution and abuse of conventional anti-microbials (7).

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Major Compounds in Spices and Herbs

Spices impart aroma, color and taste to food preparations and sometimes mask undesirable odors. Volatile oils give the aroma, and oleoresins impart the taste. Aroma compounds play important role in the production of flavorants, which are used in the food industry to flavor, improve and increase the appeal of their products. They are classified by functional groups, e.g. alcohols, aldehydes, amines, esters, ethers, ketones, terpenes, thiols and other miscellaneous compounds. In spices, the volatile oils constitute these components (3, 8, 9and 10).

Plants have limitless ability to synthesize aromatic secondary metabolites, most of which are phenols or their oxygen-substituted derivatives. Important subclasses in this group of compounds include phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins (11 and 12).

Flavones, flavonoids and flavonols are phenoplast structure with one carbonyl group. they're synthesized by plants in response to microbic infection and are usually found effective in vitro as antimicrobial substance against a large array of microorganisms. Tannins are chemical compound phenolic substances possessing the astringent property. These compounds are soluble in water, alcohol and dissolvent and provides precipitates with proteins. Coumarins are phenolic substances manufactured from amalgamated aromatic hydrocarbon and α-pyrone rings. they need a characteristic odor and several other of them have antimicrobial properties (7 and 12). Then again, plant fundamental oils are a conceivably helpful wellspring of antimicrobial mixtures. Every one of these plant parts have been screened by specialists and demonstrated their antibacterial exercises against a wide range of kinds of microbes, including foodborne microorganisms (13).

In cardamom, the oil has next to no mono-or sesquiterpenic hydrocarbons and is overwhelmed by oxygenated compounds, which are all potential fragrance compounds. While a significant number of the recognized mixtures (alcohols, esters and aldehydes) are normally found in many flavor oils (or even volatiles of various food sources), the strength of the ether, 1, 8-cineole, and the esters, α-terpinyl and linalyl acetic acid derivations in the structure make the cardamom volatiles an interesting blend (14 and 15).

Cinnamon has a fragile, fiery fragrance, which is credited to its unpredictable oil. Unpredictable parts are available in all pieces of cinnamon and cassia. The significant synthetic constituents of Cinnamomumzeylanicumoil are cinnamaldehyde and eugenol (16 and 17). The oil from the stem bark contains 75% cinnamaldehdyde and 5% cinnamyl acetic acid derivation, which add to the character (18).

Among the seed flavors, cumin natural products have an unmistakable harsh character and solid, warm fragrance because of their plentiful fundamental oil content. Of this, 40–65% is cuminaldehyde (4-isopropylbenzaldehyde), the significant constituent and significant fragrance compound, as likewise the sharpness compound announced in cumin. The smell is best portrayed as infiltrating, aggravating, greasy and overwhelming, curry-like, weighty, fiery, warm and constant, even in the wake of drying out (19). The trademark kind of cumin is likely due to dihydrocuminaldehyde and Mono-terpenes (3).

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In the developed product of fennel, up to 95% of the fundamental oil is situated in the organic product, more noteworthy sums being found in the completely ready organic product. Hydro-refining yields 1.5–3.5%.

By and large, anethole and fenchone are found more in the waxy and ready organic products than in the stems and leaves (20) Anethole has flavoring properties and is distinctly sweet, being 13 times sweeter than sugar.
As for coriander, in the unripe fruits and the vegetative parts of the plant, aliphatic aldehydes predominate in the steam- volatile oil and are responsible for the peculiar aroma. On ripening, the fruits acquire a more pleasant and sweet odor and the major constituent of the volatile oil are the monoterpene alcohol, linalool. Sotolon (also known as sotolone, caramel furanone, sugar lactone and fenugreek lactone) is a lactone and an extremely powerful aroma compound and is the major aroma and flavor component of fenugreek seeds (21).

Extraction and formulation of Medicinal Plants
Extraction (as the term is chemically utilized) is the partition of restoratively dynamic segments of plant (and creature) tissues utilizing specific solvents through standard techniques. Such extraction procedures separate the solvent plant metabolites and leave behind the insoluble cell marc. The items so acquired from plants are generally intricate combinations of metabolites, in fluid or semisolid state or (in the wake of eliminating the dissolvable) in dry powder structure, and are expected for oral or outer use. These incorporate classes of arrangements known as decoctions, implantations, liquid concentrates, colors, pilular (semisolid) removes or powdered concentrates (10) The modern preparing of therapeutic and sweet-smelling plants begins with the extraction of the dynamic parts utilizing different innovations. The overall procedures of therapeutic plant extraction incorporate maceration, implantation, permeation, assimilation, decoction, hot constant extraction (Soxhlet), watery alcoholic extraction by aging, counter-ebb and flow extraction, microwave-helped extraction, ultrasound extraction (sonication), supercritical liquid extraction, and phytonic extraction (with hydrofluorocarbon solvents). For aromaticplants, hydrodistillation procedures (water refining, steam distillation,water and steam refining), hydrolytic maceration followed by distillation,expression and enfleurage (cold fat extraction) might be utilized. Some ofthe most recent extraction strategies for fragrant plants incorporate headspace trapping,solid stage miniature extraction, protoplast extraction, miniature distillation,thermo-miniature refining and sub-atomic refining (22).

Therapeutic plants are utilized as unrefined substances for extraction of dynamic constituents in unadulterated structure (eg. alkaloids like quinine and quinidine from cinchona bark, emetine from ipecacuanha root, glycosides from digitalis leaves, sennosides from senna leaves), as antecedents for engineered nutrients or steroids, and as arrangements for home grown and native meds. Items like ginseng, valerian and liquorice roots are important for the natural and wellbeing food market, just as the food flavors, scent and corrective enterprises. Certain plant items are mechanically taken advantage of

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like liquorice in candy parlor and tobacco, papaine as meat tenderiser, quinine as soda pop tonic and cinchona as wine flavor. An enormous amount of restorative plant material is utilized in the readiness of natural and therapeutic teas, eg. chamomile. These home grown and food utilizes are critical, additionally to the exporters from emerging nations. Many therapeutic plants are things of business, but generally little nations are utilized in planned home grown cures. A few definitions like home grown teas, extricates, decoctions, implantations, colors, and so forth are ready from therapeutic plants (22).

Homogeneity for the definitions is vital, especially where the dynamic fixing is available in lower focus. (22):

1. Pre-processing: Washing, particle size optimization, moisture reduction, refinement or concentration..
2. Primary extraction: Primary contaminations removal.
3. Purification: Secondary contaminations removal, decolorization, concentration, recemization.
4. Derivatization (optional): Chemical modification.
5. Drying (optional): Lyophilization or spray drying.

Solublization: Insoluble removal, product stabilization.

6. Primary extraction: Primary contaminations removal.
7. Purification: Secondary contaminations removal, decolorization, concentration, recemization.
8. Derivatization (optional): Chemical modification.
9. Drying (optional): Lyophilization or spray drying.

Types of Extraction

Aqueous extracts: Depending on the type of plant parts, the following degrees of commination are used for the extraction. Blooms and herbs are destroyed (4000mm); forests, barks, and roots are destroyed (2800mm); natural goods and seeds are destroyed (4000mm) (2000mm). Powdered alkaloid-containing medications (700mm) (22).

Decoctions The crude medication is boiled in a specific amount of water for a specific amount of time, then cooled and strained or filtered. Water-soluble, heat-stable components can be extracted using this method. The beginning ratio of crude drug to water is set, for example, at 1:4 or 1:16, and the volume is reduced to one-fourth of its original volume throughout the extraction phase by boiling. The concentrated extract is then filtered and utilized as is or further processed (10).

Infusions: are often used for fragile herbs, foliage, and vulnerable plants in their fresh state. Making an infusion is similar to brewing a cup of tea. Water is brought to a low boil, then poured over a herb (or mix of herbs), covered, and set aside to steep for 10-15 minutes. It can be made in a drinking cup (by simply pouring warm water over the herb in the cup) or in a pot (by dropping the herb into the pot where the water was heated). Some herb merchants even sell empty gauze tea bags that may be filled with herbs and then sealed with an iron. It's recommended to use a ceramic pot with a lid when making an infusion in a heating pan/pot (avoid metal pots). Adding a couple times of stirring).

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Depending on the cure, the plant, and whether sliced or powdered herb is used, the herb to water ratio can change. In a 6-8 ounce cup of water, 1 teaspoon of powdered herb or 2 teaspoons of more bulky sliced herb is usually sufficient. If using a powdered herb, mix halfway during the seeping time and allow the powder to settle to the bottom of the cup before drinking the infusion (leaving the sediment in the bottom of the cup). If using a cut herb, filter the infusion after seeping with a tea strainer. Infusions should be made as needed and consumed the same day. They can be consumed hot, warm, or cold. Infusion dosages are usually one teacup (6-8 ounces).

Macerates: The entire or coarsely powdered crude medicine is placed in a stoppered container with the solvent and left to stand for at least 3 days at room temperature with frequent agitation until the soluble stuff has dissolved. After standing, the combination is strained, the marc (damp solid material) is pressed, and the combined liquids are purified by filtration or decantation (23).

Tinctures are extracts from medicinal plants made with variable concentrations of ethanol, ether, or combinations of these, maybe with additions.that one part of the medication is extracted using more than two parts of extraction liquid, but no more than ten parts (23).

Dry extracts: Because they are usually quite hygroscopic, they should be ground and blended in settings that keep moisture out as much as possible. Both the intermediate and final product must be stored in a dry environment (24).

Mechanisms of Action against Bacteria

Phenolic and polyphenols: Catechol and pyrogallol are examples of hydroxylated phenols that have been demonstrated to be harmful to microorganisms. The amount of hydroxyl groups on the phenol group and their location(s) on the phenol group are assumed to be connected to their relative toxicity to microorganisms, with evidence that increasing hydroxylation causes increased toxicity. Furthermore, more highly oxidized phenols are reported to be more inhibitory. Enzyme inhibition by oxidized chemicals, potentially by reactivity with sulfhydryl groups, or more nonspecific interactions with proteins are hypothesized to be responsible for phenolic toxicity to microbes. Quinones have been shown to form irreversible complexes with nucleophilic amino acids in proteins.

Flavones, flavonoids, and flavonols: As with quinones, their activity is most likely related to their propensity to combine with extracellular and soluble proteins as well as bacterial cell walls. More lipophilic flavonoids may disrupt microbial membranes or inactivate toxins or enzymes in S. mutans, such as glucosyltransferases. Flavonoids with no hydroxyl groups on their b-rings are more active against bacteria than those with 2OH groups, implying that the membrane is their microbiological target. Lipophilic chemicals are more likely to disturb this structure.

Tanins: The ability of tannins to inactivate microbial adhesins, enzymes, cell envelope transport proteins, and other proteins, as well as their ability to combine with polysaccharide, may be associated to their antibacterial effect (7 and 25). This activity's

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antibacterial significance has yet to be investigated. Ruminal bacteria's cell walls have been found to bind condensed tannins, inhibiting growth and protease activity..

Terpenes: The exact method of action of terpenes is unknown, however it is thought that the lipophilic molecules disrupt membranes. Mendoza et al. (26) discovered that enhancing the hydrophilicity of kaurenediterpenoids by adding a methyl group significantly lowered their antibacterial action (7 and 27).

Lectins and Polypeptides: The development of ion channels in the bacterial membrane or competitive suppression of microbial protein adhesion to host polysaccharide receptors could be the mechanism of action (7).

Essential oils and terpenoids: Inhibition of key enzymes, chelation of essential trace elements like iron, interference with cell membrane production, and cell membrane disruption are all possible mechanisms (13 and 28). Terpenoids and phenylpropanoids are thought to be able to enter the cell membrane.

Because of their lipophilicity, as well as their structural and aromatic features, bacteria can enter the cell's interior (29).

The content and solubility of the oil, the microorganism, and the method of cultivating and counting the surviving bacteria are all elements that can influence the outcome of a test of plant oil's antibacterial effectiveness. The diameter of the zone of inhibition of bacterial growth on solid medium is a typical unit for measuring antibacterial activity. The zone of inhibition for plant essential oil samples will be determined by the oil's ability to diffuse uniformly through an agar medium and the effect of any oil vapors generated on bacteria. The presence of two or more active components that may interact in plant antibacterial compound assays is another variable. Changes in the antibacterial activity of oils in complex test samples (e.g., food) compared to the activity of the oils alone; and substances present in complex sample reaction mixtures that may stimulate or inhibit the growth of the test microorganisms independent of the test sample (due to the partitioning of active components between the lipid and aqueous phases). To allow comparisons of results obtained in different research, it is necessary to standardize test procedures and examine factors that influence antibacterial agent potencies (13).

Low toxicity, ease of evaporation at low heat, promotion of quick physiologic absorption of the extract, preservation activity, and inability to cause the extract to complex or dissociate are all characteristics of a good solvent in plant extractions. The solvent should be non-toxic and should not interfere with the bioassay because the end product of extraction will contain traces of residual solvent (12). The decision will also be influenced by the substances to be extracted. Plants are commonly screened for antimicrobial activity using crude or alcohol extractions, which can then be followed by other organic solvent extraction methods. Water is a universal solvent that can be used to extract antibacterial plant compounds. Despite the fact that traditional healers mostly utilize water, plant extracts from organic solvents have been demonstrated to have more consistent antibacterial activity than water extract (30). Furthermore, water soluble flavonoids (mainly anthocyanins) have no antibacterial properties, whereas water soluble phenolics

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are solely useful as antioxidant compounds.. According to one study, aqueous acetone extracts tannins and other phenolics better than aqueous methanol. In another study, chloroform was determined to be the best solvent for extracting non-polar biological active chemicals out of twenty various solvents tested (12).
Extraction procedures, on the other hand, vary depending on the length of the extraction time, the solvent employed, the pH of the solvent, the temperature, the particle size of the plant tissues, and the solvent-to-sample ratio. Eloff's research found that (31), Extractions of very small particles with a diameter of 10 m took only 5 minutes, whereas results obtained after 24 hours in a shaking machine with less finely ground material yielded higher quantities. In previous investigations, a solvent to sample ratio of 10:1 (v/w) solvent to dry weight ratio was recommended.

Standard antibacterial susceptibility tests are divided into diffusion and dilution procedures for ease of use. Agar well diffusion and agar disk diffusion are examples of diffusion tests, while agar dilution, broth micro-dilution, and broth macro-dilution are examples of dilution methods (12).

2. AGAR DISK DIFFUSION ASSAY

In 1940, the Agar disk diffusion method of antimicrobial testing was created (32). The procedure that was recognized by NCCLS and is now extensively used is a modification of Bauer, Kirby, Sherris, and Truck's (commonly known as Kirby-Bauer test) Plant extracts have long been tested for antibacterial activity using the Agar disk diffusion method (12). Filter sterilized plant extract of desired concentration is saturated on 6 mm sterilized filter papers disks (Whatman No. 1) in this procedure. After that, the impregnated discs are placed on top of a solid agar medium such as Mueller Hinton (Mueller and Hinton, 1941), Trypton soy agar, or Nutrient agar. The media has played a role in this. For inoculating diffusion plates, the standard inoculum size is 1 x 108 CFU/ml of bacteria, which is the same as the McFarland 0.5 turbidity standard. Some scientists prefer to impregnate the paper disk with plant extract before placing it on the inoculation plates, while others prefer to do it afterward. Under a laminar flow cabinet, the drying period of impregnated paper disks varies between researchers, ranging from 2 hours to overnight. After that, the plates are incubated for 24 hours at 37°C (bacteria) and 48 hours at 25°C (fungi) (fungi). After incubation, the diameter of the zone is measured to the nearest full millimeter at the point where growth is reduced by 80%. (33).

3. AGAR WELL DIFFUSION

On the surface of the gelled agar plate, a standardized concentration of inoculum with a defined volume is dispersed equally. A hole between 6 and 8 mm in diameter is punched aseptically in the centre using a sterile cork borer. Then, depending on the test microorganism, a set volume of plant extract is injected into the bored agar well and incubated at the appropriate temperature and duration (34).).

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4. BROTH MICRODILUTION ASSAY

The micro-titer plate or broth micro-dilution method has shown promise in measuring the Minimum Inhibitory Concentration (MIC) of a large number of test materials. In microbiology, the minimum inhibitory concentration (MIC) is the lowest concentration of antimicrobials that inhibits observable microorganism growth during an overnight incubation period. In diagnostic laboratories, MIC is used to confirm microorganism resistance to antimicrobial agents and to monitor the activity of new antimicrobial agents. In the microtiter plate method, the extract is first made into a stock solution in solvent or DMSO. In the wells of the micro-titer plate, Mueller Hinton broth or water is frequently employed as a diluent.

5. BROTH MICRODILUTION ASSAY

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To obtain a concentration range, two fold serial dilutions are made from the first well. The MIC-5-8 concentrations can represent the antimicrobials' attainable concentrations (26). For this method, the inoculum size is commonly 1 x 106 CFU/ml. Some researchers have utilized a 12 h broth culture adjusted to a 0.5 McFarland turbidity standard or a microbial culture with an optical density of 0.4 at 620 nm. The wells are filled with an equivalent volume of microbial culture and incubated at 37°C for 24 hours. Plates are checked for changes in turbidity as a growth indication after incubation. The MIC of the extract is determined by the first well that becomes clear. Some studies utilize tet as an indicator.

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5. BROTH MACRODILUTION ASSAY

This assay works on the same principles as the broth micro-dilution assay. However, the test is carried out in a test tube. A series of test tubes with varied quantities of plant extract and the same volume are prepared for the macro-dilution experiment. As previously

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indicated, tubes are infected with tested microorganisms at defined concentrations. Tubes will be inspected for changes in turbidity as a growth indication after incubation. The above-mentioned procedures can be used to determine the MIC of a plant extract or a test phytochemical (37).

6.AGAR DILUTION METHOD

This approach, which has been approved by the NCCLS, is used to determine the MIC and involves creating various quantities of plant extract. After autoclaving, the plant concentration is integrated into the agar, which is subsequently solidified. The plate is inoculated with 1 l of the inoculum (prepared to 0.5 McFarland) once it has solidified (37).

7.CONCLUSION

Plants include a wide range of ingredients and are important sources of novel, physiologically active compounds with antibacterial properties.

Plant products with antibacterial characteristics are now being researched by scientists. Standardizing extraction procedures and in vitro antimicrobial activity testing would be beneficial in order to make the search for new biologically active plant compounds more systematic and the interpretation of results easier. Plant pathologists and microbiologists are critical to seeing a promising lead molecule evolve into a finished product. These plant extracts were also tested for antibacterial effectiveness against microorganisms.

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