PLANT PRODUCTS AS ANTIMICROBIAL AGENTS

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Abstract: Plants produce a diverse array of secondary metabolites, many of which have antimicrobial activity. Some of this compounds are constitutive, existing in healthy plants in their biologically active forms. Others such as cyanogenic glycosides and glucosinolates, occur as inactive precursors and are activated in response to tissue damage or pathogen attack.

INTRODUCTION

Finding healing powers in plants is an ancient idea. People on all continents have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory. There is evidence that Neanderthals living 60,000 years ago in present-day Iraq used plants such as hollyhock (12); these plants are still widely used in ethno medicine around the world. Historically, therapeutic results have been mixed; quite often cures or symptom relief resulted. Poisonings occurred at a high rate, also. Currently, of the one-quarter to one-half of all pharmaceuticals dispensed in the United States having higher-plant origins, very few are intended for use as antimicrobials, since we have relied on bacterial and fungal sources for these activities. Since the advent of antibiotics in the 1950s, the use of plant derivatives as antimicrobials has been virtually nonexistent.

Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First, it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans. It is reported that, on average, two or three antibiotics derived from microorganisms are launched each year [2]. After a downturn in that pace in recent decades, the pace is again quickening as scientists realize that the effective life span of any antibiotic is limited. Worldwide spending on finding new anti-infective agents (including vaccines) is expected to increase 60% from the spending levels in 1993 . New sources, especially plant sources, are also being investigated. Second, the public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics. In addition, many people are interested in having more autonomy over their medical care. A multitude of plant compounds (often of unreliable purity) is readily available over-the-counter from herbal suppliers and natural-food stores, and self-medication with these substances is commonplace. The use of plant extracts, as well as other alternative forms of medical treatments, is enjoying great popularity in the late 1990s. Earlier in this decade, approximately one-third of people surveyed in the United States used at least one “unconventional” therapy during the previous year . It was reported that in 1996, sales of botanical medicines increased 37% over 1995 . It is speculated that the American public may be reacting to over prescription of sometimes toxic drugs, just as their predecessors of the 19th century (see below) reacted to the overuse of bleeding, purging, and calomel [15].

Historic Use of Plants as Antimicrobials

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Their role is two fold in the development of new drugs: first: they may become the base for the development of a medicine, a natural blueprint for the development of new drugs, or; second: a phytomedicine to be used for the treatment of disease. There are numerous illustrations of plant derived drugs. Some selected examples, including those classified as anti-infective, are presented below. The isoquinoline alkaloid emetine obtained from the underground part of *Cephaelis ipecacuanha*, and related species, has been used for many years as and amoebicidal drug as well as for the treatment of abscesses due to the spread of *Escherichia histolytica* infections. Another important drug of plant origin with a long history of use, is quinine. This alkaloid occurs naturally in the bark of *Cinchona* tree. Apart from its continued usefulness in the treatment of malaria, it can be also used to relieve nocturnal leg cramps. Currently, the widely prescribed drugs are analogs of quinine such as chloroquine. Some strains of malarial parasites have become resistant to the quinines, therefore antimalarial drugs with novel mode of action are required. Similarly, higher plants have made important contributions in the areas beyond antiinfectives, such as cancer therapies. Early examples include the antileukaemic alkaloids, vinblatine and vincristine, which were both obtained from the Madagascan periwinkle (*Catharanthus roseus* syn. *Vinca roseus*) [9].

Present use of plants as antimicrobial

It is estimated that today, plant materials are present in, or have provided the models for 50% Western drugs [10]. Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activity. The primary benefits of using plant

derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment.

Therapeutic Benefit

Much of the exploration and utilization of natural products as antimicrobials arise from microbial sources. It was the discovery of penicillin that led to later discoveries of antibiotics such as streptomycin, aureomycin and chloromycetin. [14]. Though most of the clinically used antibiotics are produced by soil microorganisms or fungi, higher plants have also been a source of antibiotics [14]. Examples of these are the bacteriostatic and antifugicidal properties of *Lichens,* the antibiotic action of allinine in *Allium sativum* (garlic), or the antimicrobial action berberines in goldenseal (*Hydrastis canadensis*) [14].

Economic Benefit

World wide, there has been a renewed interest in natural products. This interest is a result of factors such as: consumer's belief that natural products are superior; consumer's dissatisfaction with conventional medicines; changes in laws allowing structure-function claims which results in more liberal advertising; aging baby boomers; national concerns for health care cost. The potential for developing antimicrobials into medicines appears rewarding, from both the perspective of drug development and the perspective of phytomedicines. The immediate source of financial benefit from plants based antimicrobials is from the herbal products market.

MAJOR GROUPS OF ANTIMICROBIAL COMPOUNDS FROM PLANTS

Over the centuries man made use of medicinal plants even though he was unable to find a rational explanation for their effects. It was not until the 19th century and the rapid development of organic chemistry and pharmacology, that man determined which active principles of group of principles are responsible for a given therapeutic effect. [11]

All plants containing active compounds are important. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, which are synthesized and deposited in specific parts or in all parts of the plant. These compounds are more complex and specific and are found in certain taxa such as family, genus and species, but heterogeneity of secondary compounds is found in wild species . The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct.

The plants secondary products may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential target sites. Therefore, random screening of plants for active chemicals is as important as the screening of ethnobotanically targeted species .

Phenolics and Polyphenols Simple phenols and phenolic acids.

Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Cinnamic and caffeic acids are common representatives of a wide group of phenyl propane-derived compounds which are in the highest oxidation state (Fig. 1).

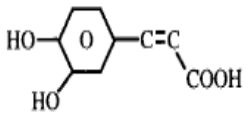

Fig. 1 Structures of caffeic acid

The common herbs tarragon and thyme both contain caffeic acid, which is effective against viruses, bacteria , and fungi . Catechol and pyrogallol both are hydroxylated phenols, shown to be toxic to microorganisms. Catechol has two 2OH groups, and pyrogallol has three. The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity . In addition, some authors have found that more highly oxidized phenols are more inhibitory. The mechanisms thought to be responsible for phenolic toxicity to microorganism.

Quinones.

Quinones are aromatic rings with two ketone substitutions (Fig.2). They are ubiquitous in nature and are characteristically highly reactive. These compounds, being colored, are responsible for the browning reaction in cut or injured fruits and vegetables and are an intermediate in the melanin synthesis pathway in human skin.

Fig. 2 Structure of quinone

Vitamin K is a complex naphthoquinone. Its antihemorrhagic activity may be related to its ease of oxidation in body tissues . In addition to providing a source of stable free radicals, quinones are known to complex irreversibly with nucleophilic amino acids in proteins , often leading to inactivation of the protein and loss of function. For that reason, the potential range of quinone antimicrobial effects is great. Probable targets in the microbial cell are surface-exposed adhesins, cell wall polypeptides, and membrane-bound enzymes. Quinones may also render substrates unavailable to the microorganism. As with all plant-derived antimicrobials, the possible toxic effects of quinones must be thoroughly examined. Kazmi et al. [7] described an anthraquinone from *Cassia italica*, a Pakistani tree, which was bacteriostatic for *Bacillus anthracis*, *Corynebacterium pseudodiphthericum* , and *Pseudomonas aeruginosa* and bactericidal for *Pseudomonas pseudomalliae*. Hypericin, an anthraquinone from St. John’s wort (*Hypericum perforatum*), has received much attention in the popular press lately as an antidepressant, and Duke reported in 1985 that it had general antimicrobial properties .

Flavones, flavonoids, and flavonols.

Flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinones). Flavonoids are also hydroxylated phenolic substances but occur as a C6-C3 unit linked to an aromatic ring (Fig.3). Since they are known to be synthesized by plants in response to microbial infection , it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls, as described above for quinones. More lipophilic flavonoids may also disrupt microbial membranes .

Catechins, the most reduced form of the C3 unit in flavonoid compounds, deserve special mention. These flavonoids have been extensively researched due to their occurrence in oolong green teas. It was noticed some time ago that teas exerted antimicrobial activity and that they contain a mixture of catechin compounds. These compounds inhibited in vitro *Vibrio cholerae*, *Streptococcus mutans,*, *Shigella*, and other bacteria and microorganisms.

Flavonoid compounds exhibit inhibitory effects against multiple viruses. Numerous studies have documented the effectiveness of flavonoids such as swertifrancheside, glycyrrhizin (from licorice), and chrysin [3] against HIV.

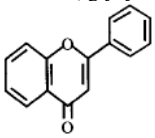

Fig.3 General structures of flavones

Tannins.

“Tannin” is a general descriptive name for a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency. Their molecular weights range from 500 to 3.000 , and they are found in almost every plant part: bark, wood, leaves, fruits, and roots . They are divided into two groups, hydrolyzable and condensed tannins(fig. 4). Hydrolyzable tannins are based on gallic acid, usually as multiple esters with D-glucose, while the more numerous condensed tannins (often called proanthocyanidins) are derived from flavonoid monomers . Tannins may be formed by condensations of flavan derivatives which have been transported to woody tissues of plants. Alternatively, tannins may be formed by polymerization of quinone units [5].

Fig.4 Structure of tannin

condensed tannin hydrolyzable tannin

This group of compounds has received a great deal of attention in recent years, since it was suggested that the consumption of tannin-containing bever.

Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions, have been assigned to tannins . Thus, their mode of antimicrobial action, as described in the section on quinones may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, etc. The antimicrobial significance of this particular activity has not been explored. Scalbert reviewed the antimicrobial properties of tannins in 1991. He listed 33 studies which had documented the inhibitory activities of tannins up to that point. According to these studies, tannins can be toxic to filamentous fungi, yeasts, and bacteria. Condensed tannins have been determined to bind cell walls of ruminal bacteria, preventing growth and protease activity [6].

Coumarins.

Coumarins are phenolic substances made of fused benzene and a-pyrone rings (Fig.5). They are responsible for the characteristic odor of hay.

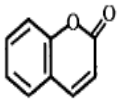

Fig.5 Structure of coumarins

As of 1996, at least 1,300 had been identified . Their fame has come mainly from their antithrombotic, anti-inflammatory , and vasodilatory [8] activities. Several other coumarins have antimicrobial properties. R. D. Thornes, working at the Boston Lying-In Hospital in 1954, sought an agent to treat vaginal candidiasis in his pregnant patients. Coumarin was found in vitro to inhibit *Candida albicans*. Its estrogenic effects were later described .

Also, phytoalexins, which are hydroxylated derivatives of coumarins, are produced in carrots in response to fungal infection and can be presumed to have antifungal activity. General antimicrobial activity was documented in woodruff (*Galium odoratum*) extracts [13]. All in all, data about specific antibiotic properties of coumarins are scarce, although many reports give reason to believe that some utility may reside in these phytochemicals . Further research is warranted.

Terpenoids and Essential Oils

The fragrance of plants is carried in the so called quinta essentia, or essential oil fraction. These oils are secondary metabolites that are highly enriched in compounds based on an isoprene structure. They are called terpenes, their general chemical structure is C10H16, and they occur as diterpenes, triterpenes, and tetraterpenes (C20, C30, and C40), as well as hemiterpenes (C5) and sesquiterpenes (C15). When the compounds contain additional elements, usually oxygen, they are termed terpenoids (Fig.6).

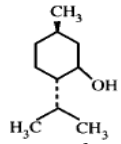

Fig. 6 Structure of menthol

Terpenoids are synthesized from acetate units, and as such they share their origins with fatty acids. They differ from fatty acids in that they contain extensive branching and are cyclized. Examples of common terpenoids are methanol and camphor (monoterpenes) and farnesol and artemisin (sesquiterpenoids). Terpenenes or terpenoids are active against bacteria, fungi, viruses , and protozoa . In 1977, it was reported that 60% of essential oil derivatives examined to date were inhibitory to fungi while 30% inhibited bacteria . The ethanol-soluble fraction of purple prairie clover yields a terpenoid called petalostemumol, which showed excellent activity against *Bacillus subtilis* and *Staphylococcus aureus* and lesser activity against gram-negative bacteria as well as *Candida albicans* . Two diterpenes isolated by Batista et al. [1] were found to be more democratic; they worked well against *Staphylococcus aureus*, *V. cholerae*, *P. aeruginosa*, and *Candida* spp.

Lectins and Polypeptides

Peptides which are inhibitory to microorganisms were first reported in 1942. Recent interest has been focused mostly on studying anti-HIV peptides and lectins, but the inhibition of bacteria and fungi by these macromolecules, such as that from the herbaceous *Amaranthus*, has long been known. Thionins are peptides commonly found in barley and wheat and consist of 47 amino acid residues . They are toxic to yeasts and gram-negative and gram-positive bacteria [4]. Fabatin, a newly identified 47- residue peptide from fava beans, appears to be structurally related to g-thionins from grains and inhibits *E. coli*, *P. aeruginosa*, and *Enterococcus hirae* but not *Candida* or *Saccharomyces.*

Alkaloids

Alkaloids rank among the most efficient and therapeutically significant plant substances. They are chemically very diverse group of organic nitrogen compounds(fig.7).

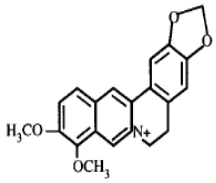

Fig. 7 Structure of an alkaloid (berberine)

Generally they are extremely toxic though they do have a marked therapeutic effect in minute quantities. For this reason plants containing alkaloids were not often used in folk medicine and then for external application only. Pure, isolated plant alkaloids and their synthetic derivates are used as basic medicinal agents all over the world for their analgesic, antispasmodic, and bactericidal effects. [11]

Other Compounds

Many phytochemicals not mentioned above have been found to exert antimicrobial properties. This review has attempted to focus on reports of chemicals which are found in multiple instances to be active. It should be mentioned, however, that there are reports of antimicrobial properties associated with polyamines (in particular spermidine) , isothiocyanates, thiosulfinates , and glucosides . Polyacetylenes deserve special mention. Acetylene compounds and flavonoids from plants traditionally used in Brazil for treatment of malaria fever and liver disorders have also been associated with antimalarial activity.

Much has been written about the antimicrobial effects of cranberry juice. Historically, women have been told to drink he juice in order to prevent and even cure urinary tract infections. In the early 1990s, researchers found that the monosaccharide fructose present in cranberry and blueberry juices competitively inhibited the adsorption of pathogenic *E. coli* to urinary tract epithelial cells, acting as an analogue for mannose. Clinical studies have borne out the protective effects of cranberry juice . Many fruits contain fructose, however, and researchers are now seeking a second active compound from cranberry juice which contributes to the antimicrobial properties of this juice [16].

CONCLUSIONS

Scientists from divergent fields are investigating plants anew with an eye to their antimicrobial usefulness. A sense of urgency accompanies the search as the pace of species extinction continues. Laboratories of the world have found literally thousands of phytochemicals which have inhibitory effects on all types of microorganisms in vitro. More of these compounds should be subjected to animal and human studies to determine their effectiveness in whole-organism systems, including in particular toxicity studies as well as an examination of their effects on beneficial normal microbiota. It would be advantageous to standardize methods of extraction and in vitro testing so that the search could be more systematic and interpretation of results would be facilitated. Also, alternative mechanisms of infection prevention and treatment should be included in initial activity screenings. Disruption of adhesion is one example of an anti-infection activity not commonly screened for currently. Attention to these issues could usher in a badly needed new era of chemotherapeutic treatment of infection by using plant-derived principles.

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