An introduction to natural products and phytochemicals with special reference to its antimicrobial activity

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Abstract: This review describes the plants as sources of food and medicine in various parts of the worlds including the developed countries. It also includes their various uses in the traditional medicines followed by their applications in the ailments resulting from microbial infestations. This section also briefly glimpses the secondary metabolites that have been marked for their anti-infective potentials and their presumed mechanisms that are nearly the same as those of prevalent antibiotics.

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Introduction

Introduction to natural products

Humans and animals have always used plants. The initial use of plants as medicines by humans is thought to have been the result of instinctive dowsing. Animals in the wild provide evidence that this phenomenon still occurs: they eat plants that heal them, and avoid plants that do them harm. Presumably humans also possessed this instinct at one time. Murray (1995b) reported that plants cannot run away from their enemies nor get rid of troublesome pests as humans or other animals do, so what have they evolved to protect themselves? Whatever this protection is it must be successful, for the diversity and richness of green plants is extraordinary, and their dominance in most ecosystems of the world is unquestioned. Plant successes are closely intertwined with the evolution and production of highly diverse compounds known as secondary metabolites, compounds that are not essential for growth and reproduction, but rather, through interaction with their environment, enhance plant prospects of survival. There are twenty thousand known secondary plant metabolites, all exhibiting a remarkable array of organic compounds that clearly provide selective advantage to the producer, which outweighs their cost of production These metabolites are therefore plant agents for chemical warfare, allowing plants to ward off microorganisms, insects, and other animals acting as predators and pathogens.

Humans benefit from their production by using many of them for medicinal purposes to fight infections and diseases. An estimated two-fifths of all modern pharmaceutical products in the United States contain one or more naturally derived ingredients, the majority of which are secondary metabolites, such as alkaloids, glycosides, terpenes, steroids, and other classes grouped according to their physiological activity in humans or chemical structure. The term herb refers to a plant used for medicinal purposes. To the uninformed, herbs are generally thought of as ineffective medicines used prior to the advent of more effective synthetic drugs. To others, herbs are simply sources of compounds to isolate and then market as drugs. But to some, herbs and crude plant extracts are effective medicines to be respected and appreciated. For many people herbal medicines are the only therapeutic agents available. In 1985, the WHO estimated that perhaps 80%of the world population relies on herbs for primary health care needs (Farnsworth et al., 1985).

This widespread use of herbal medicines is not restricted to developing countries, as it has been estimated that 30-40% of all medical doctors in France and Germany rely on herbal preparations as their primary medicines (Wagner, 1988).

Herbal medicine is almost as old as human civilization itself. Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain as active ingredients parts of plants, or other plant materials, or combinations. Herbal medicines have been used in traditional medicine for thousands of years. Traditional use of herbal medicines refers to the long historical use of these medicines. Their use is well established and widely acknowledged to be safe and effective, and may be accepted by national authorities and in many countries are still used in mainstream medicine. Because of their diverse range of uses, plants have a wide impact on the different functions of the body. Most of us eat plants in some form, every day so we are used to the effects they have - tea and coffee as stimulants; peppermint as a digestive; senna as a laxative; camomile as a relaxant. In the same way, by using the appropriate plant extracts, herbal medicines can be used for treating a wide range of common

health problems. They can also be taken as a preventative measure against catching infections such as coughs and colds. Taken regularly, some herbal medicines can help maintain the body's equilibrium in functions, which are subject to recurring imbalances, such as digestion. Because our metabolism is well suited to digesting plants and herbal medicines have a gentle, cumulative effect, they have no side effects for most people. However, like all medicines, dosage instructions should be followed.

Herbal remedies are not new. All cultures possess folk medicine traditions using plants and plant products. Out of these traditions individuals have methodically developed well-defined herbal pharmacopoeias. From these collections of drugs, efforts were made to treat every ailment of the body one can think of, such as infections, respiratory tract problems, digestive tract difficulties, cardiovascular system troubles, nervous system disorders, metabolic and endocrine problems, arthritis, problems of the skin, cancer and cataracts. After hundreds of years, the traditions of medicine changed, especially in industrialized nations, from herbs being the main source of medicinal to chemical formulations. These chemical formulations are developed, standardized and scientifically tested for safety and efficacy.

Regulations influenced the decline in the use of herbal products in the U. S. earlier this century. The Food, Drug, and Cosmetic Act of 1938 and the Kefauver-Harris Drug Amendment of 1962 require that pharmaceutical companies prove their products to be safe and effective in order to be marketed. When these laws were enacted, some companies complied and provided the required proof while others discontinued manufacturing some herbal medications. Yet some other manufacturers began marketing herbs as nutritional supplements, since no claims of efficacy were necessary. This legislative loophole places herbal remedies outside of the purview of the Food and Drug Administration (FDA). The Dietary Supplement Health and Education Act (DSHEA) of 1994 classifies herbal products as dietary supplements, thus claims pertaining to disease prevention, treatment or diagnosis cannot be made on the labeling of herbal products. As civilizations developed, medicine men and women were responsible for transmitting the information on herbs to their successors. Before the advent of written language, this information was handed down by verbal and experimental means. It was commonly believed that the creator with some sort of clue that would indicate its therapeutic use had signed plants. This concept was commonly referred to as the" Doctrine of Signatures."

Common examples of this doctrine include ginseng (*Panax ginseng*), whose roots bear strong resemblance to a human figure and whose general use is as a tonic; blue cohosh (*Caulophyllum thalictroides*), whose branches are arranged like limbs in spasm, indicating its usefulness in the treatment of muscular spasm; bloodroot (*Sanguinaria canadensis*), whose roots and sap are a beautiful blood color corresponding to its traditional use as a blood purifier (Murray, 1995c). Fossil records date human use of plants as medicines at least to the Middle Paleolithic age some 60,000 years ago (Farnsworth, 1990). The number of higher plant species (angiosperms and gymnosperms) on this planet is estimated at 250,000 (Farnsworth, 1994), with a lower level at 215,000 (Burton and Ch'en, 1983; Caspi et al., 2001) and an upper level as high as 500,000. Of these, only about 6% have been screened for biologic activity, and a reported 15% have been evaluated phytochemically (Colegate and Molyneux, 1993; Swain, 1972).

While it is impossible to know exactly when we first started using herbs and plants for medicinal purposes, archaeological remains from early civilizations have revealed that plants were used in burials and other rituals. The knowledge of drugs goes back to prehistoric times. Records of ancient civilizations shows that a considerable number of drugs, utilized in modern practice, were already used in one form or another in ancient times. The Babylonians, Egyptians, Chinese, Greeks, Romans and the people of subcontinent of India and Pakistan had developed their respective material medica, characterized by the local customs and methods. Chen Nung in China and sekhet Enanch in Egypt were the first to persue studies in the medicinal uses of herbs around 3000 BC for the treatment of various human ailments (Marse, 1934; Withington, 1984). The other earlier known references of medicine are in "Rigveds" (4500-1600BC) and the Ayurvedas (2500-600BC).), the science of life, prevention and longevity is the oldest and most holistic or comprehensive medical system available. It was placed in written form over 2,000 years ago in India, it is said to be a world medicine.. Medical knowledge from all areas of the world gathered in India, and the famous sage Vyasa, put into writing the complete knowledge of Ayurveda, along with the more directly spiritual insights of ethics, virtue and Self-realization. Ayurveda is an intricate system of healing that originated in India thousands of years ago we can find historical evidence of Ayurveda in the ancient books of wisdom known as the Vedas The Rig Veda was written over 6,000 years ago, but really Ayurveda has been around even longer than that.

There is a pharmacopoeia-like compilation in Chinese tradition called "Peutsao" or "the great herbal" (about 1500 BC), containing thousands of prescriptions. The famous Egyptians material media

"Pabyrus Ebers", dating back to about sixteenth century BC, is among the early manuscripts or literature pertaining to pharmacy and medicine which contains a chapter on remedial agents and methods for compounding. The actual history of medicine and pharmacy begins from Hippocrates (Finlayson, 1934), "The father of medicine" (460 BC) and Theophrastus (287-370 BC) (Hort, 1916). Hippocrates reported nearly 400 samples as medicine substances. Hippocrates is often called the "Father of Medicine." He lived between 468-377 BC, and strongly believed that natural laws governed health and well-being. He believed that health was directly influenced by the environment. One of his most famous quotes were that we should "let food be our medicine and medicine be our food." It was the Greek philosopher-physician Hippocrates, who freed Medicine from the realm of superstition and magic, and gave it the status of Science. The theoretical framework of Unani Medicine is based on the teachings of Hippocrates.

However, the most significant pharmacological compilation of the Greeks was the authoritative text of Dioscordies (Withington, 1984). Dioscorides wrote an important herbal during the first century AD. His herbal, *Peri hulas iatrikes (About Medicinal Trees)*, remained a standard text on the subject for hundreds of years. After him, Pliny the Elder (23-79 AD) wrote "Natural History" in 37 volumes. After a number of other Greek scholars had enriched the system considerably, Galen (131-210 AD) stands out as one who stabilized its foundation. Galen wrote some 30books on pharmacology beside Galenicals" his medical formulae (Walsh, 1940). After Galen, the work of early Greek physicians were transferred to Romans and then to Muslims. Europe descended into the Dark Ages when the Roman Empire fell. During that time, literate monks maintained the Greek and Roman traditions of healing, as they were the only people capable of translating and transcribing Latin texts. There is virtually no documentation of new works during that period, but rather the recopying of older and already established works. Many people who needed healing did not have access to a monastery, and generally relied on the medicinal techniques of a local healer. The Dark Ages was published by the English Master-surgeon John Gerard in 1597. In that herbal, he addressed over 3500 medicinal plants that the English were only just beginning to recognize in 1100sAD Arab world had major influence on medicine and healing practices. In the Muslim period of civilization we find a treasure of valuable medical knowledge. The great physician and philosopher Bu-Ali-Sina (Avicenna, 908 1037 AD) has described 700 herbal drugs in his famous book "Qanun fi al Tibb", the principles of medicines (Chatard, 1908). He wrote the Canon of Medicine.

In 1200sAD Black Death spread across Europe; 'qualified' apothecaries tried bleeding, purging, mercury and arsenic to stem the epidemic with no more success than traditional herbalists. In 1500sAD Henry VII promoted herbal medicine in the face of the growing number of untrained apothecaries and other 'medical practitioners' flourishing in London Various Acts of Parliament were passed to introduce some regulation of medical practices including protection for 'simple herbalists' to practice without fear of prosecution. In 1600sAD the first two-tier health system emerged - herbs for the poor and exotics (plant, animal or mineral extracts) or 'drugs' for the rich. Nicholas Culpepper wrote his famous herbal: The English Physician, explaining in simple terms the practice of herbal medicine. Almost a hundred years after Culpeper became a prominent herbalist, Dr. William Withering isolated the first active constituent from foxglove, which was found to have beneficial effects on dropsy. In 1700sAD Preacher Charles Wesley advocates a sensible diet, good hygiene and herbal medicine as the keys to a healthy life. Samuel Thomson was an individual critically influential in spreading and accumulating the herbal knowledge of the Native Americans. He was known as one of the most influential herbalists during his lifetime, 1769-1843. As a sick child, he had been effectively treated by the herbal medicines of the Natives.. He set up the first multi-level marketing program.

The sources of Indian medicine are derived from "Rigvedas" and "Ayurvedas". They are mainly based on the use of drugs of plant origin. The Ayurvedic system of medicine is mainly attributed to Charaka (Kaviratana, 1912) and Sushruta (Kunja Lal, 1907), who cited about 700 medicinal plants. The Muslim rulers introduced their traditional system of medicine in India and incorporated it in the native Ayurvedic medicine. This mixture is known as Unani medicine or Eastern medicine. Higher plants have been the source of medicinal agents since earliest times, and today they continue to play a dominant role in the primary health care of about 80% of the world population (Farnsworth et al., 1985). Natural products, and medicinal agents derived there from, are also an essential feature in the health care system of the remaining 20% of the population residing mainly in developed countries; with more than 50% of all drugs in clinical use have a natural product origin (Balandrin et al., 1993). Of the world's 25 best-selling pharmaceutical agents, 12 are natural product derived (Neill and Lewis, 1993). Natural products continue to play an important role in drug discovery programs of the pharmaceutical industry and other research organizations (Kinghorn and Balandrin, 1993; Michael, 1993; Berdy, 1989). Research into the chemical and biological properties of natural products

over the past two centuries has not yielded drugs for the treatment of human ailments, but has provided the stimulus for the development of modern synthetic organic chemistry, and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents.

Numerous phytomedicines are registered and extensively used in Europe, and more than 600 botanical items have been recognized in various editions of the *United State Pharmacopoeia* (Tyler, 1993), inspite of legislative ban on some of the marketable items as drugs. Of the 119 plant-derived drugs commonly in use in one or more countries, 74 were discovered as a result of chemical studies directed at the isolation of the active constituents of plants used in traditional medicine (Farnsworth et al., 1985). Well known examples include the cardiac glycosides from *Digitals purpurea* L., the antihypertensive agent and tranquilizer, reserpine, from the east Indian snakeroot, *Rauvolfia serpentina* L. Bentham ex Kurz. The antimalarial agent, quinine, from Cinchona sp. And the analgesics, codeine and morphine, from *Papaver somniferum* L. (Balandrin et al., 1993). Secondary metabolites isolated from medicinal plants have also served as precursors or models for the preparation of effective agents through semi-syntheses or lead-based total syntheses. Examples include the anticancer agent, etoposide, a semi-synthetic derivative of epipodophyllotoxin isolated from Podophyllum sp. (Cragg et al., 1993), and anticholinergic drugs modeled on the belladonna alkaloids (e.g. atropine) isolated from *Atropa belladonna* L. and other medicinal plant species (Gentry, 1993). Very little is known about the secondary metabolites of the estimated 250,000 currently known higher plant species. This is particularly true for tropical flora, which constitute over 60% of this estimated number (Balandrin et al., 1993; Norse, 1994). Even less is known about the far more abundant (though taxonomically relatively unexplored) insect and microbial worlds (Norse, 1994) as well as the biologically rich and enormously diverse marine environment (Chadwick and Marsh, 1994). Considering that the 119 drugs were isolated from only about 90 plant species (Farnsworth et al., 1985), the potential for drug discovery from plants and other natural sources are enormous. Although the long-established traditional medicinal systems, such as existing in China and India, have recorded much of their knowledge, including the use of many medicinal plants, in written text, ethnobotanists and anthropologists have expressed alarm at the rapid loss of the knowledge of the traditional healers, particularly among the indigenous groups in the Neotropics (Norse, 1994). Before the late 1980s, the developed world displayed little interest in such indigenous knowledge, and minimal effort was expended to assist indigenous communities in preserving their unique knowledge and traditions. With the resurgence of interest in the screening of plants and other natural resources for potential medicinal properties, western research organizations are beginning to place greater value on such knowledge (Gentry, 1993).

Throughout the world, but especially in Europe and Asia, a tremendous renaissance in the use and appreciation of herbal medicine has taken place. In Germany, estimates show that over billion dollars are spent on herbal products each year. In Japan, the figure is thought to be even higher. Herbal products are a major business in the United States as well, with an estimated annual sales figure of $1.3 billion dollars for 1992 and climbing. However, it is interesting to note that while annual sales of ginseng products in the United States in 1992 were roughly $10 million dollars, over 3 million pounds (roughly $100 million dollars) of American ginseng were exported. The rebirth of herbal medicine, especially in developed countries, is largely based on the renewed interest of scientific researches. During the last 10 to 20 years their efforts have yielded an explosion of scientific information concerning plants, crude plant extracts and various substances from plants as medicinal agents (Murray, 1995b). In many parts of the world including both developed and under-developed countries, a revival of herbal medicine, and other complementary therapies, such as traditional Chinese medicines, osteopathy, and homeopathy, is occurring In general, people are becoming more aware of the harmful effects of artificial commodities and are realizing the benefits of a more natural way of life. The already acknowledged side-effect and symptomatic (instead of causative) treatment by drugs, along with the concern of the harmful effects of chemical farming and genetically modified food, trends are changing to incorporate natural products that are kinder to, and more compatible with our bodies. Medicinal plants, as a source of medicine nowadays are a recognized system of medicine throughout the world named by complementary or alternative medicine. For centuries, plants with medicinal properties have been utilized successfully in the treatment of ailments of varying degrees of severity. The Greek physician, Hippocrate was quoted as saying in 377BC, “Let medicine be your food and food your medicine” and many of the medicinal herbs that he used in his practice are still popular with medical Herbalists today (Bartram, 1995; Niemi and Duodecim, 1997; Fabricant and Farnsworth, 2001).

It is also important to recognize that plant extracts and products contribute in four major areas to human health and welfare, as foodstuffs, as flavoring

agents and spices, as perfumes and cosmetics and as pharmaceutical and biological agents. In the latter area we found that there are at least 120 compounds from over 90 different plant materials, which are available globally as single entity prescription products. In addition, because of widely divergent views of what constituents a medicinal agent in a particular culture, there are many thousands of plant extracts and plant materials, which are employed commercially in various parts of the world. For approximately 88% of the world population (5.3 billion of 6 billion people) it is to these plants materials, which are primary source of health care (Fabricant and Farnsworth, 2001). The extraction and characterization of active compounds from medicinal plants have resulted in the discovery of new drugs with high therapeutic value (Tippo and Stern, 1997; Schultes, 1972). Research in this direction has been greatly facilitated by the use of modern physico-chemical techniques of isolation and structure elucidation. In this connection particular attention has been paid to studies involving correlation of structure and biological activity on selected pharmacologically active constituents. Such phytochemical screening of medicinal plants has served the dual purpose of discovering new therapeutic agents and providing precedence for chemotherapeutic studies directed towards the synthesis of drugs modeled on the structure of natural products. Moreover, these studies promote work on the correlation of the chemical structure and pharmacological activity through functional variations in the active components of the plant material. Sometimes the isolation of pure compounds are also helpful to plant taxonomists. Now day many taxonomists are interested in the distribution of secondary metabolites in plants. Certain types of compounds are restricted to some particular classes of genera and are regarded as taxonomic markers. As a rule, herbal preparations are less toxic than their synthetic counterparts and offer less risk of side effects (obviously, there are exceptions to this rule). In addition, the mechanism of action of an herb is often to correct the underlying cause of ill health. In contrast, a synthetic drug is often designed to alleviate the symptom or effect without addressing the underlying cause. It has also been demonstrated with many plants that the whole plant or crude extract is much more effective than isolated constituents. Herbal medicine will certainly play a major role in future medicine. As modern medicine gains more knowledge and understanding about health and disease it is adopting therapies that are more natural and less toxic. Lifestyle modification, stress reduction, exercise, meditation, dietary changes and many other traditional naturopathic therapies are becoming much more popular in standard medical circles. This illustrates the paradigm shift that is occurring in medicine. What were once scoffed at is now becoming generally accepted as effective alternatives. In fact, in most instances these natural alternatives offer significant advantages over standard medical practices (Murray, 1995c). Appreciation is growing for the harmonious healing properties that herbal medicines possess, particularly in Europe and Asia. The United States is becoming more aware of the medicinal value of herbs as well. Without doubt, future medicine will make good use of herbs: the medicine of the past will be the medicine of the future. The difference will be due to the growing sophistication of herbal medicine. With the continuing advancement in science and technology there has been a great improvement in the quality of herbal medicines available. Improvements in cultivation techniques, coupled with improvements in quality control and standardization of potency, will continue to increase the effectiveness of herbal medicines.

Antimicrobial agents from medicinal plants

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 (Casley-Smith and Casley-Smith, 1997). 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 (Alper, 1998). 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 (Chaurasia and Vyas, 1977). It was reported that in 1996, sales of botanical medicines increased 37% over 1995 (Klink, 1997). 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 reacted to the overuse of bleeding, purging, and calomel (Yankauer, 1997).

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 phenylpropane-derived compounds which are in the highest oxidation state. The common herbs tarragon and thyme both contain caffeic acid, which is effective against viruses (Geissman, 1963), bacteria (Schultes, 1978; Wild, 1994), and fungi (Brantner, 1996). Catechol and pyrogallol both are hydroxylated phenols, shown to be toxic to microorganisms. Catechol has two OH 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 (Thomson, 1978). In addition, some authors have found that more highly oxidized phenols are inhibitory (Duke, 1985; Geissman, 1963). The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Scalbert, 1991). Phenolic compounds possessing a C3 side chain at a lower level of oxidation and containing no oxygen are classified as essential oils and often cited as antimicrobial as well. Eugenol is a well-characterized representative found in clove oil. Eugenol is considered bacteriostatic against both fungi (Urs and Dunleavy, 1975) and bacteria (Wild, 1994).

Quinones.

Quinones are aromatic rings with two ketone substitutions. 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 (Mason, and Wasserman, 1987). Their presence in henna gives that material its dyeing properties (Duke, 1985). The switch between diphenol (or hydroquinone) and diketone (or quinone) occurs easily through oxidation and reduction reactions. The individual redox potential of the particular quinone-hydroquinone pair is very important in many biological systems; witness the role of ubiquinone (coenzyme Q) in mammalian electron transport systems. Vitamin K is a complex naphthoquinone. Its antihemorrhagic activity may be related to its ease of oxidation in body tissues (Schmidt, 1988). Hydroxylated amino acids may be made into quinones in the presence of suitable enzymes, such as a polyphenoloxidase (Fessenden and Fessenden, 1982).

In addition to providing a source of stable free radicals, quinones are known to complex irreversibly with nucleophilic amino acids in proteins (Harris, 1963), 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. Sakanaka et al., (1989) 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 (Urs and Dunleavy, 1975).

Flavones, flavonoids, and flavonols.

Flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinones) (Fig. 1). The addition of a 3-hydroxyl group yields a flavonol (Duke, 1985). Flavonoids are also hydroxylated phenolic substances but occur as a C6-C3 unit linked to an aromatic ring. Since they are known to be synthesized by plants in response to microbial infection (Fessenden and Fessenden, 1982), 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 (Stern et al., 1996). 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 (Kazmi et al., 1994) and that they contain a mixture of catechin compounds. These compounds inhibited in vitro *Vibrio cholerae* (Tsuchiya, 1996), *Streptococcus mutans* (Fessenden and Fessenden, 1982; Toda et al, 1989; Borris, 1996.), *Shigella (*Batista et al., 1994) and other bacteria and microorganisms (Borris, 1996; Sakanaka et al., 1992). The catechins inactivated cholera toxin in *Vibrio* (Tsuchiya, 1996) and inhibited isolated bacterial glucosyltransferases in *S. mutans* (Vijaya et al., 1995), possibly due to complexing activities described for quinones above. This latter

activity was borne out in in vivo tests of conventional rats.

When the rats were fed a diet containing 0.1% tea catechins, fissure caries (caused by *S. mutans*) was reduced by 40% (Thomson, 1978). Flavonoid compounds exhibit inhibitory effects against multiple viruses. Numerous studies have documented the effectiveness of flavonoids such as swertifrancheside (Nakahara et al., 1993), glycyrrhizin (from licorice) (Ooshima et al., 1993), and chrysin (Pengsuparp et al., 1995) against HIV. More than one study has found that flavone derivatives are inhibitory to respiratory syncytial virus (RSV) (Watanbe et al., 1996; Critchfield et al., 1996). Kaul et al. (1985) has provided a summary of the activities and modes of action of quercetin, naringin, hesperetin, and catechin in in vitro cell culture monolayers. While naringin was not inhibitory to herpes simplex virus type 1 (HSV-1), poliovirus type 1, parainfluenza virus type 3, or RSV, the other three flavonoids were effective in various ways. Hesperetin reduced intracellular replication of all four viruses; catechin inhibited infectivity but not intracellular replication of RSV and HSV-1; and quercetin was universally effective in reducing infectivity. The authors propose that small structural differences in the compounds are critical to their activity and pointed out another advantage of many plant derivatives: their low toxic potential. The average Western daily diet contains approximately 1 g of mixed flavonoids (Barnard et al., 1993) pharmacologically active concentrations are not likely to be harmful to human hosts. An isoflavone found in a West African legume, alpinum isoflavone, prevents schistosomal infection when applied topically (Kaul et al., 1985). Phloretin, found in certain serovars of apples, may have activity against a variety of microorganisms (Kuhnau, 1976). Galangin (3,5,7-trihydroxyflavone), derived from the perennial herb *Helichrysum aureonitens*, seems to be a particularly useful compound, since it has shown activity against a wide range of gram-positive bacteria as well as fungi (Perrett et al., 1995) and viruses, in particular HSV-1 and coxsackie B virus type 1 (Hunter and Hull, 1993). Delineation of the possible mechanism of action of flavones and flavonoids is hampered by conflicting findings. Flavonoids lacking hydroxyl groups on their -rings are more active against microorganisms than are those with the OH groups (Afolayan and Meyer, 1997), this finding supports the idea that their microbial target is the membrane. Lipophilic compounds would be more disruptive of this structure. However, several authors have also found the opposite effect; i.e., the more hydroxylation, the greater the antimicrobial activity (Meyer et al., 1997). This latter finding reflects the similar result for simple phenolics. It is safe to say that there is no clear predictability for the degree of hydroxylation and toxicity to microorganisms.

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 (Chabot et al., 1992), and they are found in almost every plant part: bark, wood, leaves, fruits, and roots (Duke, 1985). They are divided into two groups, hydrolyzable and condensed tannins. 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 (Sato et al., 1996). This group of compounds has received a great deal of attention in recent years, since it was suggested that the consumption of tannin containing beverages, especially green teas and red wines, can cure or prevent a variety of ills (Haslam, 1996).

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 (Geissman, 1963). One of their molecular actions is to complex with proteins through so-called nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation (Geissman, 1963; Harris, 1963). Thus, their mode of antimicrobial action, as described in the section on quinones (see above), may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, etc. They also complex with polysaccharide (Serafini et al., 1994). The antimicrobial significance of this particular activity has not been explored. There is also evidence for direct inactivation of microorganisms: low tannin concentrations modify the morphology of germ tubes of *Crinipellis perniciosa* (Haslam, 1996). Tannins in plants inhibit insect growth (Ya et al., 1988) and disrupt digestive events in ruminal animals (Brownlee et al., 1990). Scalbert (1991) 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 (Schultz, 1988). Although this is still speculative, tannins are considered at least partially responsible for the antibiotic activity of methanolic extracts of the bark of *Terminalia alata* found in Nepal (Butler, 1988). This

activity was enhanced by UV light activation (320 to 400 nm at 5 W/m2 for 2 h). At least two studies have shown tannins to be inhibitory to viral reverse transcriptases (Critchfield, 1996; Jones et al., 1994).

Coumarins.

Coumarins are phenolic substances made of fused benzene and pyrone rings (Taylor et al., 1996). They are responsible for the characteristic odor of hay. As of 1996, at least 1,300 had been identified (Nonaka et al., 1990) their fame has come mainly from their antithrombotic (O'Kennedy and Thornes, 1997), anti-inflammatory (Hoult and Paya, 1996), and vasodilatory (Thastrup et al., 1985) activities. Warfarin is a particularly well-known coumarin which is used both as an oral anticoagulant and, interestingly, as a rodenticide (Piller, 1975). It may also have antiviral effects (Namba et al., 1988). Coumarins are known to be highly toxic in rodents (Keating and O'Kennedy, 1997) and therefore are treated with caution by the medical community. However, recent studies have shown a "pronounced species dependent metabolism", so that many in vivo animal studies cannot be extrapolated to humans. It appears that toxic coumarin derivatives may be safely excreted in the urine in humans (Berkada, 1978). 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*. (During subsequent in vivo tests on rabbits, the coumarin-spiked water supply was inadvertently given to all the animals in the research facility and was discovered to be a potent contraceptive agent when breeding programs started to fail (U.S. Department of Health and Human Services, 1992). Its estrogenic effects were later described (Weinmann, 1997).

As a group, coumarins have been found to stimulate macrophages, which could have an indirect negative effect on infections. More specifically, coumarin has been used to prevent recurrences of cold sores caused by HSV-1 in humans (Thornes, 1997) but was found ineffective against leprosy (U.S. Department of Health and Human Services, 1992). Hydroxycinnamic acids, related to coumarins, seem to be inhibitory to gram-positive bacteria (Soine, 1964). 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 (Berkada, 1978). General antimicrobial activity was documented in woodruff (*Galium odoratum*) extracts [44]. 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 (Bose, 1958; Hoult and Paya, 1996; Bose, 1958). 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. 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). Artemisin and its derivative -arteether, also known by the name qinghaosu, find current use as antimalarials. In 1985, the steering committee of the scientific working group of the World Health Organization decided to develop the latter drug as a treatment for cerebral malaria. Terpenenes or terpenoids are active against bacteria (Butler, 1988), fungi (Hamburger and Hostettmann, 1991), viruses (Scheel, 1972), and protozoa (Suresh et al., 1997). In 1977, it was reported that 60% of essential oil derivatives examined to date were inhibitory to fungi while 30% inhibited bacteria (Xu et al., 1996). The triterpenoid betulinic acid is just one of several terpenoids which have been shown to inhibit HIV. The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds. Accordingly, (Mendoza et al., 1997) found that increasing the hydrophilicity of kaurene diterpenoids by addition of a methyl group drastically reduced their antimicrobial activity. Food scientists have found the terpenoids present in essential oils of plants to be useful in the control of *Listeria monocytogenes* (Chaurasia, and Vyas, 1977). Oil of basil, a commercially available herbal, was found to be as effective as 125 ppm chlorine in disinfecting lettuce leaves (Mendoza et al., 1997).

Chile peppers are a food item found nearly ubiquitously in many Mesoamerican cultures (Anonymous, 1997). Their use may reflect more than a desire to flavor foods. Many essential nutrients, such as vitamin C, provitamins A and E, and several B vitamins, are found in chiles (Wan et al., 1998). A terpenoid constituent, capsaicin, has a wide range of biological activities in humans, affecting the nervous, cardiovascular, and digestive systems (Coe, 1994) as well as finding use as an analgesic (Bosland, 1994).

The evidence for its antimicrobial activity is mixed. Recently, Cichewicz and Thorpe (Virus and Gebhart, 1979) found that capsaicin might enhance the growth of *Candida albicans* but that it clearly inhibited various bacteria to differing extents. Although possibly detrimental to the human gastric mucosa, capsaicin is also bactericidal to *Helicobacter pylori* (Cordell and Araujo, 1993). Another hot-tasting diterpene, aframodial, from a Cameroonian spice, is a broad-spectrum antifungal (Cichewicz and Thorpe, 1996). 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* (Jones et al., 1997). Two diterpenes isolated by Batista et al. (1994) and Ayafor et al. (1994) were found to be more democratic; they worked well against *Staphylococcus aureus*, *V. cholerae*, *P. aeruginosa*, and *Candida* spp. When it was observed that residents of Mali used the bark of a tree called *Ptelopsis suberosa* for the treatment of gastric ulcers, investigators tested terpenoid-containing fractions in 10 rats before and after the rats had ulcers chemically induced. They found that the terpenoids prevented the formation of ulcers and diminished the severity of existent ulcers. Whether this activity was due to antimicrobial action or to protection of the gastric mucosa is not clear (Hufford et al., 1993). Kadota et al. (1997) found that trichorabdal A, a diterpene from a Japanese herb, could directly inhibit *H. pylori*.

Alkaloids

Heterocyclic nitrogen compounds are called alkaloids. The first medically useful example of an alkaloid was morphine, isolated in 1805 from the opium poppy *Papaver somniferum* (De Pasquale et al., 1995), the name morphine comes from the Greek Morpheus, god of dreams. Codeine and heroin are both derivatives of morphine. Diterpenoid alkaloids, commonly isolated from the plants of the Ranunculaceae, or buttercup (Kadota et al., 1997) family (Fessenden and Fessenden, 1982), are commonly found to have antimicrobial properties (Jones and Luchsinger, 1986). Solamargine, a glycoalkaloid from the berries of *Solanum khasianum*, and other alkaloids may be useful against HIV infection (Atta-ur-Rahman and Choudhary, 1995; Lee-Huang et al., 1995) as well as intestinal infections associated with AIDS (McMahon et al., 1995). While alkaloids have been found to have microbiocidal effects (including against *Giardia* and *Entamoeba* species (Sethi, 1979), the major antidiarrheal effect is probably due to their effects on transit time in the small intestine. Berberine is an important representative of the alkaloid group. It is potentially effective against trypanosomes (McDevitt et al., 1996) and plasmodia (Ghoshal et al., 1996). The mechanism of action of highly aromatic planar quaternary alkaloids such as berberine and harmane (Freiburghaus et al., 1996) is attributed to their ability to intercalate with DNA (Omulokoli et al., 1997).

Lectins and Polypeptides

Peptides which are inhibitory to microorganisms were first reported in 1942 (Hopp et al., 1976). They are often positively charged and contain disulfide bonds. Their mechanism of action may be the formation of ion channels in the microbial membrane (Phillipson and O'Neill, 1987; Balls et al., 1942) or competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors (Zhang and Lewis, 1997). 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 (Terras et al., 1993). Thionins are peptides commonly found in barley and wheat and consist of 47 amino acid residues (Sharon and Ofek, 1986; De Bolle et al., 1996). They are toxic to yeasts and gram-negative and gram positive bacteria (Colilla et al., 1990). Thionins AX1 and AX2 from sugar beet are active against fungi but not bacteria (Mendez et al., 1990). Fabatin, a newly identified 47-residue peptide from fava beans, appears to be structurally related to -thionins from grains and inhibits *E. coli*, *P. aeruginosa*, and *Enterococcus hirae* but not *Candida* or *Saccharomyces* (Phillipson and O'Neill, 1987).

The larger lectin molecules, which include mannose-specific lectins from several plants (Fernandes de Caleya et al., 1972), MAP30 from bitter melon (Kragh et al., 1995), GAP31 from *Gelonium multiflorum* (Balzarini et al., 1991), and jacalin (Lee-Huang et al., 1995), are inhibitory to viral proliferation (HIV, cytomegalovirus), probably by inhibiting viral interaction with critical host cell components. It is worth emphasizing that molecules and compounds such as these whose mode of action may be to inhibit adhesion will not be detected by using most general plant antimicrobial screening protocols, even with the bioassay-guided fractionation procedures (Bourinbaiar and Lee Huang, 1996; Favero et al., 1993) used by natural-products chemists. It is an area of ethnopharmacology which deserves attention, so that initial screens of potentially pharmacologically active plants (Lewis and Elvin-Lewis, 1995) may be made more useful.

1.3.10 Mixtures

The chewing stick is widely used in African countries as an oral hygiene aid (in place of a toothbrush) (Rinehart et al., 1990). Chewing sticks come from different species of plants, and within one stick the chemically active component may be

heterogeneous (Vlietinck and Vanden Berghe, 1991). Crude extracts of one species used for this purpose, *Serindeia werneckei*, inhibited the periodontal pathogens *Porphyromonas gingivalis* and *Bacteroides melaninogenicus* in vitro (Norton and Addy, 1989). The active component of the Nigerian chewing stick (*Fagara zanthoxyloides*) was found to consist of various alkaloids (Akpata and Akinrimisi, 1977). Whether these compounds, long utilized in developing countries, might find use in the Western world is not yet known. Papaya (*Carica papaya*) yields a milky sap, often called a latex, which is a complex mixture of chemicals. Chief among them is papain, a well-known proteolytic enzyme (Rotimi et al., 1988). An alkaloid, carpaine, is also present (Odebiyi and Sofowora, 1979). Terpenoids are also present and may contribute to its antimicrobial properties (Oliver-Bever, 1986). Osato et al. (1993) and Burdick. (1971) found the latex to be bacteriostatic to *B. subtilis*, *Enterobacter cloacae*, *E. coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Proteus vulgaris*.

Ayurveda is a type of healing craft practiced in India but not unknown in the United States. Ayurvedic practitioners rely on plant extracts, both "pure" single-plant preparations and mixed formulations. The preparations have lyrical names, such as Ashwagandha (*Withania somnifera* root) (Thomson, 1978), Cauvery 100 (a mixture) (Osato et al., 1993), and Livovet (Dhuley, 1998). These preparations are used to treat animals as well as humans (Dhuley, 1998). In addition to their antimicrobial activities, they have been found to have antidiarrheal (Manonmani et al., 1995), immunomodulatory (Kumar and Singh, 1992; Manonmani et al., 1991), anticancer (Dhuley, 1998), and psychotropic [167] properties. In vivo studies of Abana, an Ayurvedic formulation, found a slight reduction in experimentally induced cardiac arrhythmias in dogs (Dwivedi and Abu-Ghazaleh, 1997]. Two microorganisms against which Ayurvedic preparations have activity are *Aspergillus* spp. (Shah et al., 1997) and *Propionibacterium acnes* (Gautam et al., 1993). (The aspergillosis study was performed with mice in vivo, and it is therefore impossible to determine whether the effects are due to the stimulation of macrophage activity in the whole animal rather than to direct antimicrobial effects.) The toxicity of Ayurvedic preparations has been the subject of some speculation, especially since some of them include metals. Prpic-Majic et al. identified high levels of lead in the blood of adult volunteers who had self-medicated with Ayurvedic medicines (Dhuley, 1998). Propolis is a crude extract of the balsam of various trees; it is often called bee glue, since honeybees gather it from the trees. Its chemical composition is very complex: like the latexes described above, terpenoids are present, as well as flavonoids, benzoic acids and esters, and substituted phenolic acids and esters (Paranjpe and Kulkarni, 1995). Synthetic cinnamic acids, identical to those from propolis, were found to inhibit hemagglutination activity of influenza virus (Prpic-Majic et al., 1996). Amoros et al. found that propolis was active against an acyclovir resistant mutant of HSV-1, adenovirus type 2, vesicular stomatitis virus, and poliovirus (Paranjpe and Kulkarni, 1995). Mixtures of chemicals found in latex and propolis, may act synergistically. While the flavone and flavonol components were active in isolation against HSV-1, multiple flavonoids incubated simultaneously with the virus were more effective than single chemicals, a possible explanation of why propolis is more effective than its individual compounds (Amoros et al., 1992). Of course, mixtures are more likely to contain toxic constituents, and they must be thoroughly investigated and standardized before approved for use on a large-scale basis in the West.

1.3.11 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) (Hudson et al., 1993), isothiocyanates (Amoros et al., 1992), thiosulfinates (Flayeh and Sulayman, 1987), and glucosides (Iwu et al., 1991). Polyacetylenes deserve special mention. Estevez-Braun et al. isolated a C17 polyacetylene compound from *Bupleurum salicifolium*, a plant native to the Canary Islands. The compound, 8Sheptadeca-2(*Z*),9(*Z*)-diene-4,6-diyne-1,8-diol, was inhibitory to *S. aureus* and *B. subtilis* but not to gram-negative bacteria or yeasts (Tada et al., 1988). 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 (Rucker et al., 1992).

Much has been written about the antimicrobial effects of cranberry juice. Historically, women have been told to drink the 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 (Estevez-Braun et al., 1994). Clinical studies have borne out the protective effects of cranberry juice (Brandao et al., 1997). 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 (Estevez-Braun et al., 1994).

Experimental Approaches

Extraction Methods

Advice abounds for the amateur herbalist on how to prepare healing compounds from plants and herbs. Water is almost universally the solvent used to extract activity. At home, dried plants can be ingested as teas (plants steeped in hot water) or, rarely, tinctures (plants in alcoholic solutions) or inhaled via steam from boiling suspensions of the parts. Dried plant parts can be added to oils or petroleum jelly and applied externally. Poultices can also be made from concentrated teas or tinctures (Zafriri et al., 1989; Avorn, 1996). Scientific analysis of plant components follows a logical pathway. Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found (Brantner and Grein, 1994).

Initial screenings of plants for possible antimicrobial activities typically begin by using crude aqueous or alcohol extractions and can be followed by various organic extraction methods. Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction. In fact, many studies avoid the use of aqueous fractionation altogether. The exceptional water-soluble compounds, such as polysaccharides (e.g., starch) and polypeptides, including fabatin (Phillipson and O'Neill, 1987) and various lectins, are commonly more effective as inhibitors of pathogen (usually virus) adsorption and would not be identified in the screening techniques commonly used. Occasionally tannins and terpenoids will be found in the aqueous phase, but they are more often obtained by treatment with less polar solvents. Compounds which, according to the literature, partition exclusively in particular solvents are indicated in boldface type in the table.

Solvents used for active component extraction

Any part of the plant may contain active components. For instance, the roots of ginseng plants contain the active saponins and essential oils, while eucalyptus leaves are harvested for their essential oils and tannins. Some trees, such as the balsam poplar, yield useful substances in their bark, leaves, and shoots (Oliver-Bever, 1986). For alcoholic extractions, plant parts are dried, ground to a fine texture, and then soaked in methanol or ethanol for extended periods. The slurry is then filtered and washed, after which it may be dried under reduced pressure and redissolved in the alcohol to a determined concentration. When water is used for extractions, plants are generally soaked in distilled water, blotted dry, made into slurry through blending, and then strained or filtered. The filtrate can be centrifuged (approximately 20,000 × *g*, for 30 min) multiple times for clarification (Thomson, 1978; Butler, 1988). Crude products can then be used in disc diffusion and broth dilution assays to test for antifungal and antibacterial properties and in a variety of assays to screen for antiviral activity, as described below. Natural-products chemists further purify active chemicals from crude extracts by a variety of methods. Petalostemumol, a flavanol from purple prairie clover, was obtained from the ethanol extract by partitioning between ethyl acetate and water, followed by partitioning between *n*-hexane and 10% methanol. The methanol fraction was chromatographed and eluted with toluene (Martin, 1995). Terpenoid lactones have been obtained by successive extractions of dried bark with hexane, CHCl3, and methanol, with activity concentrating in the CHCl3 fraction (Cichewicz, and Thorpe, 1996). The chemical structures of the purified material can then be analyzed. Techniques for further chemical analysis include chromatography, bioautography, radioimmunoassay, various methods of structure identification, and newer tools such as fast atom bombardment mass spectrometry, tandem mass spectroscopy (Hufford et al., 1993) high-performance liquid chromatography, capillary zone electrophoresis, nuclear magnetic resonance spectroscopy, and X-ray crystallography (Rao et al., 1993). Recently, (Eloff, 1998) examined a variety of extractants for their ability to solubilize antimicrobials from plants, as well as other factors such as their relative ranking as biohazards and the ease of removal of solvent from the fraction. The focus of the study was to provide a more standardized extraction method for the wide variety of researchers working in diverse settings. Although it is not one of the more frequently used extractants in studies published to date, acetone received the highest overall rating. In fact, in a review of 48 articles describing the screening of plant extracts for antimicrobial properties in the most recent years of the *Journal of Natural Products*, the *Journal of Ethnopharmacology*, and the *International Journal of Pharmacognosy*, only one study used acetone as an extractant. Of the solvents listed in Table 3, which are reported in the recent literature with the highest frequency, Eloff ranked them in the order methylene dichloride, methanol, ethanol, and water.

The row indicating the number of inhibitors extracted with each solvent points to two implications: first, that most active components are not water soluble and second, that the most commonly used solvents (ethanol and methanol, both used as initial extractants in approximately 35% of the studies appearing in the recent literature) may not demonstrate the greatest sensitivity in yielding

antimicrobial chemicals on an initial screening. This disparity should be examined as the search for new antimicrobials intensifies.

In vitro analysis.

Initial screening of potential antibacterial and antifungal compounds from plants may be performed with pure substances (Borris, 1996) or crude extracts (Eloff, 1998). The methods used for the two types of organisms are similar. The two most commonly used screens to determine antimicrobial susceptibility are the broth dilution assay (Klopoukh et al., 1997) and the disc or agar well diffusion assay (Silva et al., 1996), clinical microbiologists are very familiar with these assays. Adaptations such as the agar overlay method (Taniguchi and Kubo, 1993) may also be used. In some cases, the inoculated plates or tubes are exposed to UV light (Navarro et al., 1996) to screen for the presence of light-sensitizing photochemicals. Other variations of these methods are also used. For instance, to test the effects of extracts on invasive *Shigella* species, noncytotoxic concentrations of the extracts can be added to Vero cell cultures exposed to a *Shigella* inoculum (Mayr-Harting et al., 1972). The decrease in cytopathic effect in the presence of the plant extract is then measured.

In addition to these assays, antifungal phytochemicals can be analyzed by a spore germination assay. Samples of plant extracts or pure compounds can be added to fungal spores collected from solid cultures, placed on glass slides, and incubated at an appropriate temperature (usually 25°C) for 24 h. Slides are then fixed in lactophenol cotton blue and observed microscopically for spore germination (Taylor et al., 1996). Of course, after initial screening of phytochemicals, more detailed studies of their antibiotic effects should be conducted. At this stage, more specific media can be used and MICs can be effectively compared to those of a wider range of currently used antibiotics. The investigation of plant extracts effective against methicillin-resistant *S. aureus* (Vijaya et al., 1995) provides an example of prospecting for new compounds which may be particularly effective against infections that are currently difficult to treat. Sato et al. (1996) examined the activity of three extracts from the fruiting bodies of the tree *Terminalia chebula* against methicillin-sensitive and methicillin-resistant *S. aureus* as well as 12 other gram-negative and gram-positive bacteria. They found that gallic acid derivatives were more effective against both types of *S. aureus* than they were against other species.

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