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Antimicrobial Activity of Condiments

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1. Introduction

The phenomenon of antibiosis, life prevents life, observed by Goubert and Pasteur in 1877, gave rise to the use of antibiotics in therapy. In fact, on that date has been found that certain microorganisms were sensitive to the action of products produced by other microorganisms. Unfortunately, many of these products were toxic to the cells of higher animals, and only in 1943, the first antibiotic isolated and studied by Sir Alexander Fleming - penicillin G – was introduced in clinic. Penicillin was discovered in 1929 when Fleming sought potential antibacterial compounds. He noted that a colony of the fungus *Penicillium notatum* had grown up on a plate containing the bacterium *Staphylococcus aureus* and around the fungus had a zone where the bacteria did not grow. The active substance, Fleming called penicillin, but could not isolate it. Several years later, in 1939, Ernst Chain and Howard Florey developed a way to isolate penicillin and used it to treat bacterial infections during the Second World War. The new drug came into use in the clinic in 1946 and had a huge impact on public health. Its discovery and development revolutionized modern medicine and paved the way for the development of many more antibiotics of natural origin.

Antimicrobial activity is understood as the ability of some agents to eliminate microorganisms (aiming at different metabolic or structural targets, as nucleic acid synthesis disruption or peptidoglycan synthesis inhibition) or by inhibiting their growth.

Before the introduction of antibiotics in the 1940s, infections were rare, but rapidly increased in frequency as increased the use of antibiotics. In fact, most antibiotics that were first used in the 1940s and 1950s are no longer used clinically because nowadays the resistance of infectious beings to these antibiotics is very common. Over time they have been developing new antibiotics and with the introduction of each, new drug-resistant bacteria appeared rapidly. Today, we moved the mode of use and prescription of antibiotics in order to try to slow the relentless pace of bacterial evolution, but not yet found a solution to this problem. Microbiologists continue to study how bacteria evolve so that we can predict how they will respond to medical treatment and so we can better manage the evolution of infectious diseases.

This microbiocidal or microbiostatic activity is, on one's mind, usually related with therapeutic objectives or sanitizing activities within the food or pharmaceutical industries. Nevertheless, in our daily routine, and also linked with food microbiology, we are faced with a number of substances, which we use only as culinary additives, that may work as antimicrobial agents or may turn to be a good source of new antimicrobial molecules for industrial application and, therefore, may turn to have an increased economic value. The search for new antibacterial molecules in spices and herbs is particularly important. Multidrug-resistant strains are becoming increasingly common, both in hospitals and community, raising the need for expanding research. Moreover, the effective life duration of classic antibiotics is decreasing, probably due to overconsumption and misuse. The global attention on infectious diseases going epidemic, like human immunodefficiency virus (HIV), raises even more the interest on drugs of non-microbial origin.

In many regions of the world a large variety of plants and herbs are used for their medicinal value, treating various diseases, some of infectious nature. Historically, the use of plants, in particular edible plants, is widely reported since ancient times and is associated with some sort of medicinal effect, and these plants were used (some still are) against bacteria, fungi, viruses and even helmiths (Cowan, 1999). The therapeutic use of such products in some areas is related not only with cultural aspects, traditional medicine, availability of these products, with some plants and herbs endemic to very specific areas, as parts of Latin America, Africa and Asia, but also related with economic issues. Some regions are empoverished and modern medicine and antibiotic-based therapies are not largely available to most people. Most of the very specific plants used in some regions are largely unknown to western medicine and are object of study in the field of ethnopharmacology, especially for research of their antimicrobial properties. Nevertheless, even in some western countries, plant and herb-based treatments are used in “unconventional” therapies, though seen with mistrust by “conventional” medicine.

The use of plants for medicinal purposes involves the use of extracts more or less complex and sometimes it is hard to blame the healing power of a single substance. Although in some parts of the world population is still dependent on ancestral knowledge to alleviate their sufferings, the so-called western medicine world prefers the use of pure substances. The attempt to rationalize the "healing process" by Western medicine led to the study and attempt to isolate the active ingredients of medicinal plants that is to identify the chemical responsible for its healing power.

Many of the plants used in traditional medicine have been well evaluated and several compounds isolated that serve today as "models" to the pharmaceutical industry for drug development. So many of the drugs used today have a story that involves their isolation from plant material, identification in chemical terms, chemical synthesis and evaluation of the pharmacological properties of the pure compound. Often this assessment leads to the need of modifying the chemical structure first identified in the compound extracted from the plant, which is done to improve its pharmacological properties often reducing the adverse effects associated with the initial chemical structure.

This whole process was initiated in the nineteenth century with the investigation of plants used in treating some diseases, and the identification of compounds isolated in terms of its chemical structure was made much later, back in the twentieth century. The first compounds that were isolated were pure morphine (isolated from opium which is obtained from the white poppy capsules) and quinine of *Chinchona* spp. (family of plants used in South America to treat malaria). Both compounds are still used for therapeutic purposes. This process started in the nineteenth century never stopped and is now easier due to improved techniques for the isolation and structural determination of chemical compounds.

Despite the success of conventional medicine, based on the use of pure compounds, it is undeniable the current increased use of "natural medicines" (natural remedies) in Western societies, even though these products are sometimes viewed with suspicion by the professionals linked to the administration health care. One of the problems associated with the use of drugs of plant origin comes from the fact that in most cases, these products are sold without any control. The concentration of the pharmacologically active compounds depends on the season that was harvested, the state of maturation and the conditions under which the plant grew. The lack of regulation could result in the same plant product that was purchased at different times will have different biological activity.

The drug resistance of human and animal pathogens is one of the best documented in biological evolution and a serious problem in both developed and developing countries. The consumption of more than one ton daily antibiotics in some European countries has resulted in resistance to bacterial populations, thus causing a serious public health problem. In view of this scenario, the search for new antimicrobial substances from natural sources, including plants, has gained importance in pharmaceutical companies.

In the past, herbal medicine was taken over by poor people in rural or urban area, due to easy availability and lower costs. Currently, the use of plants as a source of drugs is prevalent in developing countries as an alternative solution to health problems and is well established in some cultures and traditions, especially in Asia, Latin America and Africa. It was through the recognition by man of the healing power of certain plants that was born and developed the pharmacy as we know it today.

All cultures in different parts of the world have developed knowledge of local plants that enabled them to its use for therapeutic purposes. Ancient written sources from Babylon, Egypt, India and China reached us, where the procedures are described for the collection, handling and use of different plant materials for recovery of its healing power.

Herbs, spices, condiments and their essential oils are usually seen merely as a way to season and add flavour or colour to foods, so its chemical complexity is often forgot and, therefore, some of its molecules ignored.

The prediction of specific antimicrobial effects of spices and condiments may be difficult to ascertain, as well as the minimal inhibitory concentration (MIC), not only because the specific mechanism is possibly different in different products, but also because of the amount of spice or condiment, the food to which is added, the cooking process or because the bacterial target, type and concentration, may influence the result, and also differences between *in vitro* (using culture media) and food experiments.

The present chapter intents to show the main results in terms of antimicrobial activity of plants, herbs and condiments, the main techniques envolved and future prospects in this field.

2. History

There are more than 300,000 species of plants, ranging from green algae to seed plants. Only a relatively small amount of these plants has been used since human populations were still collectors. With the beginning of agriculture and the expansion of mankind, the number of plants employed as nourishment or for medicinal purpose increased. The use of plants as a way to ease human ailments dates back to prehistory. In some parts of the World the medicinal practices envolving vegetable products, conventionally called ethnomedicine, may not have changed very dramatically since those ancient times, particularly in tribal areas of South American rain forest, Africa or South East Asia. These areas have a common characteristic – an overwhelming diversity and amount of plant material to be used.

There are many historical records on the use of plants for treating ailments ranging from 4.000 B.C. The first medical record filed in the Pennsylvania Museum is dated 2.100 B.C. and includes a collection of thirty different formulas of drugs of plant origin, animal or mineral. The Egyptian manuscript *Ebers Papirus* (1.500 B.C.), contains 811 prescriptions and 700 drug and the first Chinese text on herbal (500 B.C.) reports names, doses and indications for the use of plants to treat disease. Some of these plants are still used, such as ginseng (*Panax* spp.), *Ephedra* spp., *Cassia* spp. and *Rheum palmatum L.*, including as sources for pharmaceuticals industries.

Early reports, from various civilizations, mentioned several medicinal plants and their effects. The Greek physician Hippochrates (V century B.C.) described 300 to 400 medicinal plants and their effects. Another Greek physician Pedanius Dioscorides (I century A.D.) wrote a catalog of medicinal plants with the Latin title "De Materia Medica", in what is nowadays considered to be one of the world's first pharmacopeias. Even religious documents, as the Christian Bible, indicated some products of vegetable origin with antiseptic properties, namely frankincense and myrrh that were used in Ancient Middle East (including Ancient Egypt) as mouthwashes. In imperial China, we assisted the discovery of tea and other infusions described as having soothing properties and other medicinal characteristics.

As time went by and Man began accumulating more knowledge, critical analysis and observation of results began to follow the true scientific method. The real therapeutic success of many of these plants was mixed. In some cases, cure was achieved or symptoms were truely relieved. Other plants have shown to be only ideal for nutricional purposes. The more dramatic cases were of plants being simply considered source of poisoning.

Since the advent of antibiotics, in the 1920's, following Alexander Fleming's findings, researchers focused in microorganisms to obtain new ways to kill other microorganisms. The development of microbial resistance as well as economic incentives triggered the research and development of new antibiotics in order to maintain a pool of effective drugs in all situations. Although the development of resistant strains is inevitable, the way we manage and use antibiotics has exacerbated the process. The growing emergence of resistance phenomenon led to new classes of antibiotics of synthetic origin. Unfortunately, the rate of new and more efficient antibiotics is not as fast as microorganisms finding new ways to survive. Thus, in the beginning of the 21st century we assist to a race to plants, trying to discover innovative natural weapons to fight infectious diseases and microbial contaminations in food.

Since medicinal plants produce a variety of substances with antimicrobial properties, it is expected that screening programs discover candidate compounds for the development of new antibiotics. However, scientific research to determine the therapeutic potential of plants is limited, there is a lack of scientific studies that confirm the possible experimental antibiotic properties of a large number of these plants. It is expected that compounds that reach targets different from those used by known antibiotics may be active against resistant pathogens.

3. Plants, herbs and condiments

3.1 Conventional products

Worldwide, among the most used and studied spices and condiments known for the proven or supposed antibacterial activity we find aniseed (*Pimpinella anisum*), bay leaf (*Laurus nobilis*), black pepper (*Piper nigrum*), cinnamon (*Cinnamonum verum*), clove (*Syzygium aromaticum*), coriander (*Coriandrum sativum*), garlic (*Allium sativum*), ginger (*Zingiber officinale*), lime (*Citrus aurantifolia*), onion (*Allium cepa*), oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*). All of these are, generally, widely available in nearly every corner of the world.

Apart from the world areas associated with great vegetable diversity, like tropical regions, densely forested areas of South America, sub-Saharian Africa or Southeast Asia, we also can find a great number of plants/herbs with medicinal use. In an academic study made in 2002, in a Portuguese National Park, with a relatively small area, 346 hectare, 140 different plants were found, with 124 different medicinal uses, 15 aromatic, 16 categorized also as condiments and 20 others, including 2 specimens classified as toxic/poisonous (Rodrigues, 2002).

Table 1 lists a number of well-known plants and herbs, many used daily worldwide, and their most important antimicrobial component.

Common name	Scientific denomination	Antimicrobial component
Alfalfa	*Medicago sativa*	-
Allspice	*Pimenta dioica*	Eugenol
Aloe	*Aloe barbadensis / Aloe vera*	Latex
Apple	*Malus sylvestris*	Phlosetin (flavonoid)
Basil	*Ocimum basilicum*	Terpenoids
Bay	*Laurus nobilis*	Terpenoids
Black pepper	*Piper nigrum*	Piperine
Caraway	*Carum carvi*	Coumarin
Cashew	*Anacardium purshiana*	Polyphenols
Chamomile	*Matricaria chamomilla*	Phenolic acid
Chili peppers	*Capsicum annuum*	Capsaicin
Clove	*Syzygium aromaticum*	Eugenol
Coriander	*Coriadrum sativum*	-
Dill	*Anethum graveolens*	Terpenoids
Eucalyptus	*Eucalyptus globulus*	Tannin
Fava bean	*Vicia faba*	Fabatin
Garlic	*Allium sativum*	Allicin
Olive	*Olea europaea*	Hexanal
Onion	*Allium cepa*	Allicin
Peppermint	*Mentha piperita*	Menthol (terpenoid)
Rosemary	*Rosmarinus officinalis*	Terpenoids
Thyme	*Thymus vulgaris*	Caffeic acid, thymol, tannins
Turmeric	*Curcuma longa*	Curcumin (terpenoid)

Table 1. Plants and herbs and their main antimicrobial components (adapted from Cowan, 1999).

There are some generally accepted conclusions about the antimicrobial abilities of these natural products: spices are less effective in foods than in culture media, probably because of the higher concentration *in vitro* and non-existing co-factors, and Gram-positive bacteria are more sensible than Gram negatives, although some Gram negative strains may be extremely affected as well (Jay, 1982; Zaika *et al*., 1983), as *Escherichia coli* to varying concentrations of cumin, for example (Allahghadri *et al*., 2010). Within the Gram positive group, lactic bacteria are considered the less sensitive (Zaika *et al*., 1983).

Confirming the greater susceptibility to herbs and condiments of Gram positive bacteria when compared with Gram negative, an experiment using forty-six methanolic extracts of dietary spices and medicinal herbs reached results in which *Staphylococcus aureus* was the most sensitive bacteria and *Escherichia coli* the less sensitive bacteria to the extracts tested. The other test species included *Bacillus cereus*, *Listeria monocytogenes* and *Salmonella anatum*. The experiment also has shown a strong positive correlation between antibacterial activity and the total phenolic content of the herbs (Shan *et al*., 2007).

Garlic and clove are widely used for culinary purposes due to their strong flavour and aroma. They are also some of the most widely refered condiments in scientific literature when comes to antibacterial activity. Some bacteria that show resistance to conventional antibiotics are susceptible to decoctions or extracts of garlic and clove. Garlic, in particular, was able to inhibit *Staphylococcus epidermidis* and *Salmonella typhi* after one and three hours, respectively. In terms of yeast inhibition, it took one hour to achieve it, and in terms of inhibition diameter, reached larger areas of inhibition for *Candida* than those produced by nystatin. Clove took five hours to completely inhibit yeasts, including *Candida* species (Arora & Kaur, 1999).

There are indications that some spices, or their components may be active not only against resistant strains, but also against clinical isolates (usually studies are carried on academic collection strains). Rahman *et al*., proved the effectiveness of spice extracts on multi-resistant *Escherichia coli*. In spite the fact each extract, alone, caused no inhibition, the combination of three different aqueous extracts (1:1:1) of ginger, lime and garlic had a synergistic effect, inhibiting the bacteria. The incorporation of 0.3% in culture media was sufficient to inhibit 21 of the 24 Gram positive bacteria tested (Shelef *et al*., 1980). In a study conducted by Masood et al., using oral isolates as test targets, black pepper decoction shown bacterial toxicity against 75% of the samples, bay leaf was effective against 53% and aniseed against 18%. Coriander did not show any inhibiting activity.

Many herbs and spices also have antifungal activity, which is important to relate, as fungi and its toxins also have a role as origin of food spoilage and food contaminants. Out of 29 spices and herbs studied, clove (genus *Caryophyllus*), star anise (*Illicium anisatum*) and allspice (genus *Pimenta*) completely inhibited the growth of 3 different *Aspergillus* species (*Aspergillus ochraceus*, *Aspergillus versicolor* and *Aspergillus flavus*) and also inhibited the toxin production. Eugenol extracted from clove and anethol extracted from star anise were incorporated in PDA (Potato Dextrose Agar) medium to test its growth inhibition ability. The concentrations required for total inhibition were 0.4 and 2.0 mg/mL, respectively (Hitokoto *et al*., 1980).

Dietary and medicinal plants and spices have been constantly studied for their antimicrobial properties, in particular for their antibacterial activity. In a study aimed at finding solutions against diarrhoegenic bacteria, onion, garlic, ginger, black pepper, clove, mint, cumin and turmeric were tested. The microorganisms used in the experiment were *Salmonella typhi*, *Salmonella typhimurium*, *Shigella flexneri*, *Shigella dysenteriae*, *Escherichia coli* O102 and *Escherichia coli* O157, *Yersinia enterocolitica* and *Campylobacter jejuni*. The most efficient plant was clove, with maximum inhibition diameters for all bacteria, followed by black pepper that showed a high inhibition of *Shigella dysenteriae*, *Campylobacter jejuni* and both strains of *Escherichia coli*. The less efficient plants were ginger and mint (Vaishnavi *et al*., 2007).

3.2 Ethnic products

Beyond the more conventional vegetable products known worldwide, there is a great number of plants and herbs that have a great importance in cooking and medicinal practices, especially in Eastern Asian countries. For some plants, the biological properties described include not only antimicrobial activity but also the ability to influence the immune system.

Among these properties, there is the ability to stimulate the production of cytokines, increase the activation of macrophages, lymphocytes and NK cells (Natural killer cells). Chinese traditional medicine often uses plants like *Aloe vera*, *Angelica* spp., *Astragalus membranaceus*, *Ganoderma lucidum*, *Panax ginseng*, *Scutellari*a spp. and *Zingiber officinale*, some of which, beyond the immunomodulatory activity refered, also show some antimicrobial activity, like toxicity to *Aspergillus candidus* (*Angelica* spp.) and toxicity to MRSA (methicillin-resistant *Staphylococcus aureus*), shown by *Scutellaria* spp. (Tan & Vanitha, 2004).

The purpose of using plants and herbs may not be only a medicinal or therapeutic purpose. The presence of some herbs with antimicrobial activity in some dishes may act as food preservatives. Curry leaf (*Murraya koenigii*) and Vietnamese coriander (*Persicaria odorata*) have shown to be effective against some bacteria isolated from fish. Methanolic extracts of curry leaf and Vietnamese coriander were effective against *Streptococcus agalactiae* (MIC= 0.39 mg/mL) and *Staphylococcus aureus*, respectively (MIC= 3.13 mg/mL) (Najiah et al., 2011).

Asafoetida (*Ferula assafoetida*), a spice used in Indian cuisine, was tested against endemic pathogens in India and shown antibacterial activity against *Salmonella typhi* and *Escherichia coli* O157, leading the authors of the study to conclude that the presence of some spices and condiments acts not only as flavouring agents but also as some sort of protection against gastroentestinal diseases (Vaishnavi *et al*., 2007).

In Thai cuisine and traditional medicine, galangal (genus *Alpinia*) has a special relevance. Studies have shown that the ryzome of this plant has antimicrobial properties, in particular against *Vibrio paraheamolyticus*. Bacterial inhibition was performed using disks with 10 μL freshly squeezed galangal, although the chloroform extract also shown activity. One of the molecules envolved in the inhibition mechanism was identified as being 1′-acetoxychavicol acetate (Vuddhakul *et al*., 2006).

Ayurvedic medicinal tradition uses many plants and herbs to treat several diseases, including those of infectious nature. *Ocimum sanctum*, *Eugenia caryophillata*, *Achyranthes bidentata* and *Azadirachta indica* are plants used in Nepal and India and were tested for their antibacterial properties against *Escherichia coli*, *Salmonella typhi*, *Salmonella paratyphi*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* through cup diffusion method. The experimental results have shown that both *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were resistant to any of the four plant extracts, and *Achyranthes* *bidentata* extract was ineffective against all bacterial strains. The largest inhibition area was obtained against *Salmonella typhi* by *Eugenia caryophillata* extract (Joshi *et al*., 2011).

As refered before, the unavailability of conventional antibiotics drives people to search in their own local products solutions for bacterial contamination of food and for infectious diseases. In a study conducted in Algeria, the antibacterial properties of four types of local berries (*Crataegus azarolus*, *Crataegus monogyna*, *Ziziphus lotus* and *Eleagnus angustifolia*) and three date (*Phoenix dactylifera*) varieties were analyzed. The targets were seven strains of *Salmonella* isolated from poultry industry. The bacterial strains were shown to be resistant to ticarcillin, amoxicillin, chloramphenicol and co-trimoxazole. Despite local berries extracts were ineffective against the bacteria, the date fruits results have shown moderate inhibition of *Salmonella*. An *Escherichia coli* strain was used as control and its inhibition diameters were smaller than those of *Salmonella* (Ayachi *et al*., 2009).

Cassia tora, *Momordica charantia* and *Calendula officinalis* are herbs used by ayurvedic medicine to treat psoriasis and other dermatological episodes. Aqueous and organic extracts of these plants were tested against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*. Results have shown that aqueous extracts were more effective than extracts obtained using organic solvents. *Staphylococcus aureus* was the most susceptible bacteria to any of the four herbs tested. However, none of inhibition diameters produced was larger than the diameter produced by the antibiotic used for comparison, streptomycin (Roopashree *et al*., 2008).

In Brazilian folk medicine, a very large number of plants is used for treatmente of various diseases. This is due not only to ancestral tribal tradition, but mainly to the extreme abundance of vegetable raw materials in tropical environment. One of the plants employed with therapeutic objectives is *Plectranthus ornatus*, one of the many species of *Plectranthus* genus. In a study conducted in Portugal aimed at isolating the antimicrobial components of this plant, MIC were determined by microdilution. Extracts obtained by using different solvents were tested against bacterial strains, Gram positive and Gram negative and *Candida albicans*. The main experimental results show an MIC of 31.25 μg/mL against *Streptococcus faecalis*, using an acetone extract and an MIC of 125 μg/mL against *Staphylococcus aureus*, using a methanol/water extract. Extracts obtained with other organic solvents were devoid of antimicrobial activity (Rijo *et al*., 2010).

3.3 Others

Beyond the traditional plants and herbs, other "vegetable" edible products are becoming object of attention. Seaweeds are part of oriental food habits for long, like the use of seaweeds in preparation of sushi-like dishes in Japanese cuisine, but the interest in its antimicrobial and other biological activity is recent. Methanolic extracts of six species of edible Irish seaweeds proved to be effective against food pathogenic bacteria like *Listeria monocytogenes*, *Salmonella abony*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*, with brown algae more effective than green and red algae (Cox *et al*., 2010).

4. Molecules involved in antimicrobial activity

Plants, herbs, spices and other products of vegetable origin must be seen as multi ingredient mixtures. Thus, there is a high probability that a product may have different compounds that may act as antimicrobial agents, although present in low concentrations. However, we often relate a plant with a specific molecule. For instance, piperine in pepper, eugenol in clove, allicin in garlic, cinnamic aldehyde in cinnamon stick or thymol in thyme leafs. In other cases, even if the specific molecule is unknown, there seems to be a significant correlation between antibacterial activity and high content of phenolic components (Shan *et al*., 2007), flavonoids and terpenoids (Cowan, 1999; Rios *et al*., 1987) which, among other effects, may be associated with membrane disruption.

The membrane disruption, specifically a rupture of the phospholipid bilayer, is the main cause of cell death when natural antimicrobials are concerned. However, cell death can also be precipitated by other factors, including the disruption of enzyme systems. The inhibition of flagellin synthesis in *Escherichia coli* O157:H7, promoted by carvacrol, seems to be of relevance (Tajkarimi *et al*., 2010).

One of the issues that raises interest, not only scientific but also economic, is the possibility to obtain a source of new anti-retrovirals in the nature. Terpenes, phenols and polysaccharides obtained from several medicinal plants could act as inhibitors of HIV replication, the majority of them targeting HIV reverse transcriptase (Jung *et al*., 2000).

Some spices and herbs may have the same type of active molecule, and consequently the same bactericidal mechanism. Zaika *et al*. shown that oregano, rosemary, sage and thyme had the same effects. The resistance development observed in their test strains (lactic bacteria) when exposed to one of the herbs, allowed resistance to the other three. The same herbs, nonetheless, were considered to be among the most antimicrobial.

As said previously, plants have a great number of molecules that are responsible for the colour, odour and flavour of vegetable products. Most of these phytochemicals are secondary metabolites, and among those that have antimicrobial activity we find (Cowan, 1999): phenols and polyphenols, terpenoids, essential oils, alkaloids and, finally, lectins and polypeptides.

This list is an atempt to systematise the phytochemicals envolved in antimicrobial activity. From a strictly chemical point of vue, there are phytochemicals that could be put in different groups. As an example of the “confusing” chemical denomination, we have terpenoids, phenols and phenolic terpenoids. However, we chose to follow this list to better organize the available information.

The chemistry envolved in these antimicrobial mechanisms is complex and the diversity of molecules is great. There are other types of natural antimicrobials whose inclusion in any of above mentioned groups is not easy. A group of molecules, obtained from garlic, the thiosulfinates are active against Gram negative strains. Vegetables like broccoli, Brussels sprouts, cabbages but also mustard and horseradish are rich in glucosinolates that have a wide range of antibacterial and antifungal activity.

4.1 Phenols and polyphenols

This group comprises a large number of different molecules like simple phenols, phenolic acids, quinones, flavonoids, flavones, flavonols, tannins and coumarins. They have in common the fact of participating in the aromatic characteristics of plants and are very common.

Phenols like catechol and epicatechin or cinnamic acid (phenolic acid) participate in membrane disruption (Figure 1). Because phenols are very common, the antibacterial activity of plants and herbs is very often related with this phenomenon.

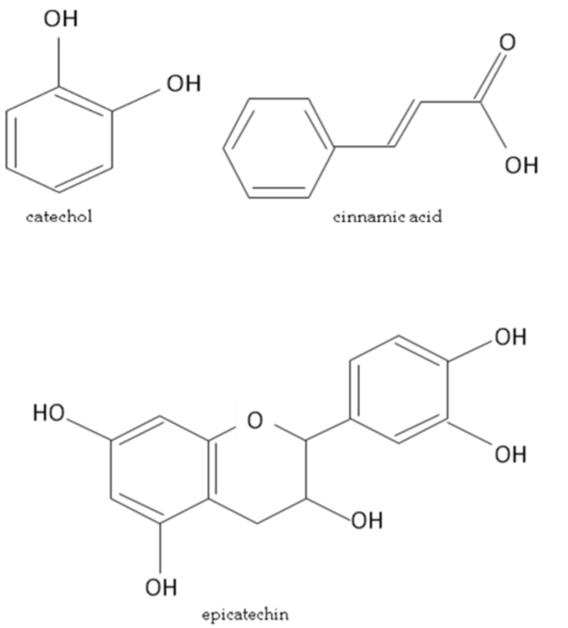

Fig. 1. Structural formulae of some phenols with antimicrobial properties.

The antibacterial activity of quinones, flavonoids, flavones and flavonols seems to be similar. Some examples are hyperacin, chrysin, and abyssinone (Figure 2). Their mechanism of inhibition includes ability to bind to adhesins and specially, enzymatic inhibition.

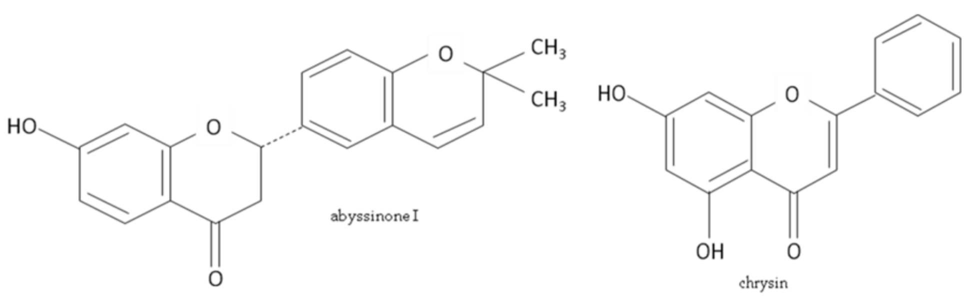

Fig. 2. Chemical structures of abyssinone and chrysin.

Additionally, abyssinone has the ability to inhibit HIV reverse transcriptase, pointing out the therapeutic value that some phytochemicals could have for some infectious diseases (Cowan, 1999). Tannins are one of the most commom chemical groups present in plants and vegetable material. It is easily found in seeds, bark, and other parts of the plant. In terms of organoleptic characteristics, tannins are responsible for astringency. An example of tannins with antimicrobial activity is ellagitannin, and the mechanism of inhibition is related with their ability to bind to proteins, blocking some metabolic pathways. Finally, we have coumarins, like warfarin, whose medicinal/therapeutic use is related to the ability to interact with eucaryotic DNA, which implies some antiviral activity (Figure 3) (Cowan, 1999).

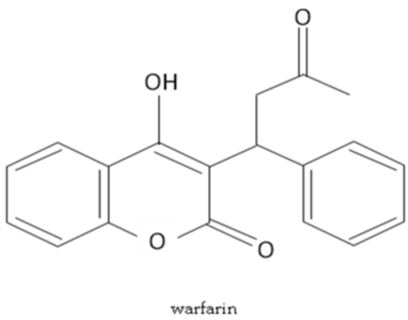

Fig. 3. Chemical structure of a coumarin, warfarin.

4.2 Terpenoids

Like phenols, terpenoids are one of the most common constituents in plants, herbs and spices. As plant metabolites, terpenoids play a role in growth and development but also in the process of resistance against environmental stresses (Figure 4). Their inhibition ability is based on membrane disruption, a mechanism widely refered in scientific literature, but yet not fully understood. Diterpene metabolites like totarol and abietic acid are active against Gram positive bacteria. In fact, diterpenes is one of the largest groups of plant-derived natural products with anti-staphylococcal activity. This is justified by their ability to cross the bacterial cytoplasmatic membrane (due to their amphipathic character).

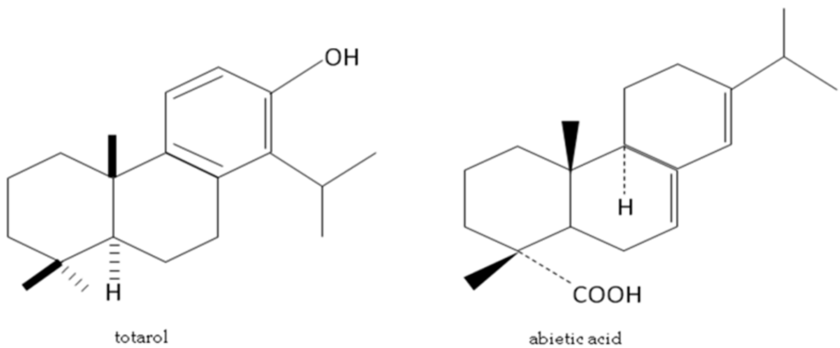

Fig. 4. Chemical structures of totarol and abietic acid.

Totarol also behaves as an efflux pump inhibitor, stopping one of the pathways bacteria can follow to resist antimicrobial molecules (Rijo *et al.*, 2010). Other example of an antimicrobial terpenoid is capsaicin, a phytochemical found in chilli pepper seeds (Cowan, 1999).

4.3 Essential oils

Essential oils do not constitute a separate molecular group. In fact, many of the molecules present in essential oils are terpenoids. However, they are treated separately because scientific literature studies them intensively and they are commercially traded as medicinal products, perfume ingredients or food additives. Essential oils are aromatic oily liquids extracted from plants. As said, essential oils are mixtures, but among the most common molecules present in some essential oils we find carvacrol, thymol, eugenol, perillaldehyde, cinnamaldehyde, cinnamic acid, camphor or linalool (Figure 5). Different plants can be used for the extraction of essential oil, like coriander, cinnamon, oregano, rosemary, sage, clove or thyme, among others. The methods of oil extraction include the traditional steam distillation but also hydrodistillation and more recent supercritical fluid extraction. Some essential oils constituents like carvacrol, thymol, eugenol, perillaldehyde, cinnamaldehyde and cinnamic acid have been shown to have antibacterial activity against such food pathogens like *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli* O157:H7, *Shigella dysenteriae*, *Bacillus cereus* and *Staphylococcus aureus* with MIC ranging 0.05 µL/mL to 5 µL/mL *in vitro*. In terms of application on foods (as preservative agents) or as constituents in washing solutions for fruits and vegetables, however, MIC of essential oils increases, with values ranging 0.5 to 20 µL/g in first case and 0.1 to 10 µL/mL, in the second (Burt, 2004).

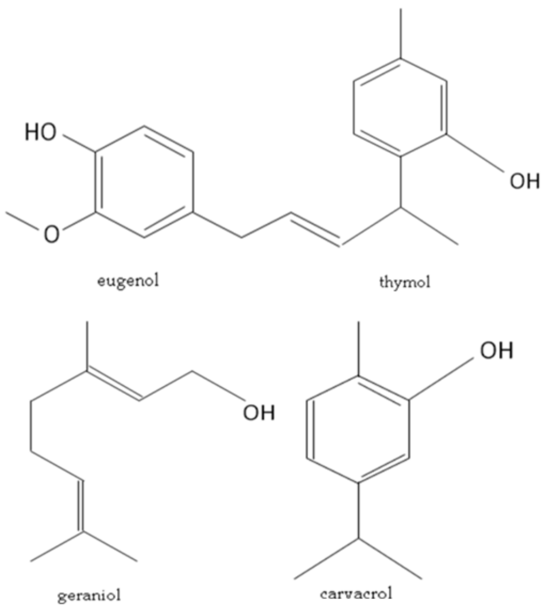

Fig. 5. Chemical structures of some essential oils components.

4.4 Alkaloids

Alkaloids constitute a chemical group that includes many molecules of vegetable origin that are very well known, such as caffein or cocaine. In terms of antimicrobial properties, molecules like berberine and piperine seem have the ability to intercalate into cell wall or DNA (deoxyrribonucleic acid) (Figure 6). Some activity against protozoa is also refered, mainly anti-*Plasmodium* and anti-trypanossome, although *Giardia* and *Entamoeba* infections, common in HIV patients, can also be eliminated through the consumption of some alkaloids. Solamargine, a glycoalkaloid extracted from *Solanum khaniasum* is helpeful in HIV infections (Cowan, 1999).

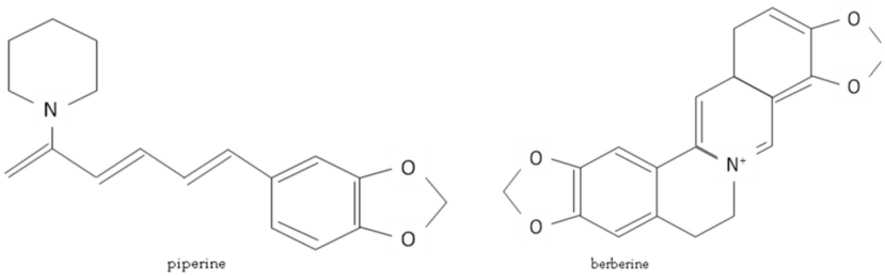

Fig. 6. The piperine and berberine molecules.

4.5 Lectins and polypeptides

Antimicrobial activity of phytochemicals is not only against bacteria, fungi or protozoa. Lectins and polypeptides, whose main characteristic is the ability to form disulfide bridges, are targeted to virus. These molecules are able to block viral fusion or adsorption, probably through competition for cellular binding spots. An example of such phytochemicals is fabatin, found in fava beans (Cowan, 1999). There are vegetables (including potatos) and seeds producing protease inhibitors that are involved in the innate defense response of the host against phytopathogens. This antimicrobial activity could, in the future, be applied in clinical research. Antimicrobial proteins and peptides are produced by various plants and its presence is correlated with plant resistance to some microbial infections. These peptides include lectins, protease inhibitors and antifungal peptides (Kim *et al.*, 2009).

5. Methodologies

Medicinal use of plants is conducted through different ways, according to geographical availability, historic tradition and known efficacy and may include preparation of aqueous infusions and beverages using dried plants, tinctures (plants in alcoholic solutions), inhalation of steam from boiling preparations, ingestion of fresh or cooked parts as ingredients, preparation of topic ointments, essential oils, among others. However, the real scientific and therapeutic value of the plant properties, like antimicrobial potential, needs to be evaluated through proper techniques. The screening of antimicrobial activity may be pursued on two different pathways:

- Analysis and identification of individual components, after extraction;
- Evaluation of inhibition properties on sample microorganisms.

5.1 Analysis and identification of individual components

For the chemical analysis of plants and herbs, one must choose the part of the plant containing the active compounds, and depending on the plant it may be the root (ginseng, for instance), the flowers (lavender, for example), the leaves (like bay leaf and thyme), the bark (cinnamon), the seeds, the fruit or the stem. It is also possible to observe variation of content of antimicrobial molecules in each plant due to different factors as: influence of seasonal harvest, geographic location and altitude and extraction procedures.

Fresh products can be used in order to obtain aqueous mixtures, but before extraction using organic solvents, samples are usually dried and grinded. The samples may be prepared as crude extracts or aqueous and organic solvent extracts. Further operations may include filtration and centrifugation (for clarification). Besides water, extraction is made by use of organic solvents, because most active components are not totally water-soluble. In fact, only a small portion is water-soluble, like polypeptides and polysaccharides (starch). The main organic solvents are ethanol, methanol, chloroform, dichloromethane, diethyl ether, petroleum ether and acetone. The techniques for identification of molecules and description of molecular structure include chromatography (from simple thin-layer chromatography to high-performance liquid chromatography), radioimmunoassay, mass spectrometry, nuclear magnetic resonance and X-ray crystallography.

5.2 Evaluation of inhibition properties

The differences with respect to the techniques employed to investigate the action of plant compounds and a wide variation in the chemical composition of some plant preparations can result in data difficult to compare between surveys. There is also no consensus on acceptable levels of inhibition for plants compounds, when compared with standard antibiotics. The methodologies used for the evaluation of antimicrobial properties of plants, herbs, spices and condiments do not differ significantly from those used in classic clinical microbiology. The inhibitory effects on bacteria and yeasts can be tested by broth dilution assay (evaluation can be performed using optical density or colony viable count), which allows determination and comparison of MIC and susceptibility tests by disk or agar-well diffusion. For classic antibiotics, CLSI (Clinical Laboratories Standards Institute) establishes the exact concentrations to be studied, whereas with spices or herbs there are no such specifications.

A problem that may be encountered when reviewing some scientific literature is the difference between concepts. Although MIC is a well-defined and internationally accepted concept, there are several uses of the term. In her review, Burt (Burt, 2004) gathers a number of possible definitions for MIC, MBC (minimum bactericidal concentration), bacteriostatic concentration and bactericidal concentration. These possible differences in the concepts must be taken into consideration when performing laboratorial assays. Moreover, it is also important when comparing results, in order to know with what values each researcher is comparing his results to. MIC is defined as the lowest concentration resulting in maintenance or reduction of inoculum viability; the lowest concentration required for complete inhibition of test organism up to 48 hours incubation; or as the lowest concentration resulting in a significant decrease in inoculum viability (>90%). MBC is established as being the concentration where 99.9% or more of the initial inoculum is killed or the lowest concentration at which no growth is observed after subculturing into fresh broth. Bacteriostatic concentration is defined as the lowest concentration at which bacteria fail to grow in broth but are cultured when broth is plated onto agar and the bactericidal concentration is considered to be the lowest concentration at which bacteria fail to grow in broth and are not cultured when broth is plated onto agar (Burt, 2004).

For susceptibility tests controls must be performed simultaneously, usually using the antibiotics most suited for the conventional treatment. Tetracycline was used for comparison with thirteen Thai condiments against *Vibrio parahaemolyticus* (Vuddhakul et al., 2007) and fifteen antiobiotics were used when testing the antimicrobial activity of condiments against multidrug resistant *Escherichia coli* isolated from water in Bangladesh (Rahman *et al*., 2011). Comparison with conventional antibiotics is necessary to categorize the sensibility of a specific microorganism to a plant, herb or condiment as sensible or resistant.

A rigorous evaluation of the individual MIC is sometimes difficult because of the ambiguity of results. Eugenol inhibited 9 out of 14 Gram negative and 12 out of 20 Gram positive bacteria, at a concentration of 493 ppm, by incorporating in Plate Count Agar. But the same paper reported a MIC of 32 ppm for *Candida glabrata* and *Aspergillus niger*, and a MIC of 63 ppm for *Staphylococcus aureus* and *Escherichia coli* (Cowan, 1999).

The variation in concentration of antimicrobial substances is usual and expected. This, coupled with testing different preparations contributes for the difficulty of comparing results from study to study. For instance, allicin in garlic ranges from 0.3 to 0.5%, whereas eugenol in clove ranges from 16 to 18% (Shelef, 1984).

The problem is worsened by the fact each researcher may use different methods for preparing the samples: crude, aqueous extracts, ethanolic or methanolic extracts, chloroform or other solvents, as refered above. The results from testing the inhibitory effect of essential oils is not easy to ascertain, as the hydrophobic nature of such preparations may alter the inhibition areas because of the irregular diffusion, when compared with the more hydrophilic antibiotics. Some researchers add emulsifiers, as Tween 20 or Tween 80, to the oils, but the quantity and nature of the latter must not interfere with the results, like producing false-positive ones. Thus, standardisation of methods becomes difficult to achieve (Nascimento *et al*., 2006).

There are other methodologies available to use in order to ascertain the effects of antibacterial activity of certain phytochemicals. The rate of inhibition and cell death can be observed through time-kill analysis and survival curves. The physical aspects of antibacterial activity concerning the structural modifications achieved can be observed by the use of scanning electron microscopy (Burt, 2004).

Yeasts susceptibility can be evaluated by techniques similar to those used for bacteria, however the evaluation of antifungal ability of phytochemicals must be performed by other methods. One of these methods is the spore germination assay. Spores are put in contact with the testing compound for a period of time; afterwards they are observed microscopically in a slide (usually fixed with lactophenol cotton blue) and spore germination (or its absence) is observed.

In terms of antiviral ability of plant products, they can be determined by observation of cytopathic effects or plaque formation in cells infected and put in contact with the phytochemicals. Other option is, in the same conditions, to use molecular techniques for detection of products resulting from viral replication, as nucleic acids.

Researchers must have in mind, as mentioned earlier, that different plants may have the same antimicrobial phytochemicals, although they may be in different concentrations, resulting in different MIC for the same molecule. Moreover, the same plant may have more than an antimicrobial molecule, resulting in effects that can not be easily evaluated. An inhibition or decrease in bacterial population may be due to different mechanisms.

6. Herbs, condiments and spices as food preservatives

Safety and high-quality characteristics of food products are some of the attributes growingly demanded by consumers worldwide. Despite technological advances, either by chemical preservatives or mechanical equipment for inactivation or inhibition of microorganisms, there are still problems concerning food spoilage of biological origin. Attention is concentrated on psychrophiles, halophiles and toxigenic foodborne pathogens. Consequently, natural antimicrobial molecules are interesting tools to control microbial food contamination, in addition to their already well-known flavouring properties. The main commercial objective of adding these compounds to foodstuff is extending their shelf-life and increasing, if possible, their nutricional and organoleptic value.

As mentioned earlier, these natural antimicrobials derive from plant products and, historically, are been in use for a long time in areas as China or India. Nowadays the concentrated use of a large number of condiments/spices is mainly performed in hot climate regions. Virtually every group of food raw materials can be added with one or more plants/herbs/spices that work as food preservatives, as shown in Table 2.

Among the species used for food preservation or that have shown to produce inhibition of food contaminants we find: thyme (*Thymus eigii*), ginger (*Zingiber officinalle*), galangal (*Alpinia galanga*), turmeric (*Curcuma longa*) and fingerroot (*Boesenbergia pandurata*). Cinnamon, cloves and cumin also show antimicrobial effects against pathogens, some of which of foodborne origin, like *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Mycobacterium smegmatis*, *Micrococcus luteus* and even *Candida albicans*. We also find condiments as allspice, bay leaf, caraway, coriander, cassia bark and liquorice as a way to eliminate or inhibit foodborne pathogens. In particular, olive tree leaves (*Olea europaea*) shows effects against important species as *Campylobacter jejuni*, *Helicobacter pylori* and *Staphylococcus aureus*.

The molecules interviening in the antimicrobial process have been object of a specific section within this chapter, but among thje most commom we usually find α-pinene, cineole, limonene, linalool and geranyl acetate.

In terms of use for food preservation, these natural antimicrobial agents may pose problems that must be addressed, namely the existence of antagonism between different agents, degradation of organoleptic profile and the existence of toxic effects for the consumer. These issues have to be considered seriously and the solutions may be in continuing research of the health effects and mainly in finding ways to lower the sensorial perception of some spices/herbs (optimization of food formulation) or by guaranteeing food preservation by combining different methods (conventional techniques plus addition of natural antimicrobials).

Food Group	Plant/spice/herb or active compound	Microbial target
Meat Products	Clove oil, eugenol+coriander, oregano, thyme oils, rosemary oil, clove, tea tree	*Listeria monocytogenes*, *Aeromonas hydrophila*, *Escherichia coli* O157:H7
	Oregano+thyme, oregano+marjoram, thyme+sage	*Bacillus cereus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*
	Chinese cinnamon	Pathogenic microorganisms
	Oregano, pimento, oregano+pimento	*Escherichia coli* O157:H7, *Pseudomonas* spp.
	Marjoram oil	Several species
	Eugenol	*Listeria monocytogenes*, *Aeromonas hydrophila*
	Sage oil	*Bacillus cereus, Staphylococcus aureus, Salmonella typhimurium*
	Mint oil	*Listeria monocytogenes*, *Salmonella enteritidis*
	Cilantro oil	*Listeria monocytogenes*
	Oregano oil	*Clostridium botulinum* spores
	Rosemary	*Listeria monocytogenes*
Fish	Thyme oil, cinnamaldehyde	*Pseudomonas putida*
	Carvacrol, citral, geraniol	*Salmonella typhimurium*
	Oregano oil	*Photobacterium phophoreum*
	Mint oil	*Salmonella enteritidis*
	Eugenol, linalool, oregano	Increased shelf-life
Dairy	Clove oil, carvacrol	*Listeria monocytogenes*
	Clove, cinnamon, cardamom, peppermint oil	*Streptococcus thermophilus*
	Mint oil	*Salmonella enteritidis*
Vegetables	Thyme oil	*Escherichia coli* O157:H7
	Basil methyl chaviol (BMC)	Natural flora
	Oregano oil	*Escherichia coli* O157:H7
	Cinnamaldehyde	*Escherichia coli* O157:H7
Rice	Carvacrol	Natural flora
	Sage oil	*Bacillus cereus, Staphylococcus aureus, Salmonella typhimurium*
	Basil	Rice storage pests
Fruits	Carvacrol, cinnamic acid	Natural flora

Table 2. List of herbs/spices/condiments or natural antimicrobial molecules used in different groups of foodstuff and their potential targets (adapted from Tajkarimi *et al.*, 2010).

7. Commercial availability and legislation

Some essential oils have been registered by the European Commission as flavouring agents to be used in foodstuff. In terms of legislation related to foodstuff, both the European Union (through European Food Safety Agency and its national branches) and the United States (through Food and Drug Administration) have been continually issuing and updating a number of regulations and list of food additives and supplements that have been authorised and considered safe for human consumption. In particular, some antimicrobial essential oils like cinnamon oil, clove oil, lemon grass oil and their respective active substances (cinnamaldehyde, eugenol and citral) have been generally recognized as safe (GRAS denomination) by the American FDA as food additives.

However, the classification of plant-based products as pharmacologically active substances or medicaments is less clear. They can only be included in foodstuff legislation if not proven as being medicaments. The main obstacle on the legal establishment of these molecules or mixtures originates from potential risks to the consumers health. The risks arise from any toxic effect, unknown to date. Several studies have to be performed on the metabolic effects, physical and chemical characterization of the molecules, microbiological studies, safety assays, and the cost of performing these experiments is high. This financial cost can be an excessive burden to small companies.

Medicaments are molecules with properties of treatment or prevention of human diseases, or with pharmacological, immunological or metabolic action. Plant-based medicaments are regulated, within European Union, by Directive 2004/24/CE. Each member country has to integrate onto their own legislation the contents of this Directive. It comprises not only antimicrobial herbs and plants, but any pharmacologically active vegetable product, in particular those utilized in Chinese traditional medicine and ayurvedic medicine. The European Commission issued it in order to establish concepts and regulate the trade. They define traditional plant-based medicaments as products, of vegetable origin, targeted to treat some illnesses, in use for at least 30 years (including 15 years of use within European Union borders) and that are to be employed without medical supervision and whose administration does not include injection or parenteral use. This usage must be proven by documentation. Some examples of species employed in the production of plant-based medicaments have already been listed along this chapter, as *Calendula officinalis*, *Echinea purpurea* or *Pimpinella anisum*.

Although being natural products (and sometimes with a long tradition of medicinal use), some of these substances may be harmful to patients and this is why European Union requires specific authorization for these products (which are included in general pharmaceutical legislation) to be placed in the market. The objective is to guarantee quality, safety and efficiency. Neverthless, taking in account the financial burden that some laboratory tests and clinical assays represent, the European Union has introduced a more simplified registration procedure without forgeting the forementioned requirements of quality, safety and efficiency. The companies that produce or trade the plant-based medicaments must present unquestionable documentation proving the innocuity to human health and the established therapeutic use (30 years of use and 15 years within European Union).

8. Conclusion

Spices, condiments and herbs, used fresh or as extracts have a very much reported ability to inhibit some microorganisms. However, analysis of scientific literature shows that researchers must take care when comparing results because experimental standardization has not been achieved yet.

The use of natural medicines has undeniably increased in western societies. The suspicion raised by conventional health care professionals is due to lack of legislation and control. The therapeutic results and the active molecules of these natural products have a variability caused by seasonal conditions, leading consequently to variable biological activity.

In several regions of the world traditional culinary habits and medicinal pratices use plants and herbs in daily routine. Modern conventional medicine is challenged today by the ever growing bacterial resistance to classic antibiotics. More research is necessary to ascertain the real therapeutic value of these products, but these natural resources must not be despised because their clinical and economic value may be greater than has been supposed up to this day.

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