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ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANT AGAINST PLANT AND HUMAN PATHOGENS

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ABSTRACT
Plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases including epidemics. The knowledge of their healing properties has been transmitted over the centuries within and among human communities. Active compounds produced during secondary vegetal metabolism are usually responsible for the biological properties of some plant species used throughout the globe for various purposes, including treatment of infectious diseases. Currently, data on the antimicrobial activity of numerous plants, so far considered empirical, have been scientifically confirmed, concomitantly with the increasing number of reports on pathogenic microorganisms resistant to antimicrobials. Products derived from plants may potentially control microbial growth in diverse situations and in the specific case of disease treatment, numerous studies have aimed to describe the chemical composition of these plant antimicrobials and the mechanisms involved in microbial growth inhibition, either separately or associated with conventional antimicrobials. Thus, in the present work, medicinal plants with emphasis on their antimicrobial properties are reviewed.

KEYWORDS: Medicinal plants, Antibacterial activity, Minimal Inhibitory Concentration.

INTRODUCTION
For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Now a day, the use of phytochemicals for pharmaceutical purpose has gradually increased in many countries. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants.[1] The use of crude extracts of plants parts and phytochemicals, of known antimicrobial properties, can be of great significance in the therapeutic treatments. In recent years, a number of studies have been conducted in various countries to prove such efficiency.

Many plants have been used because of their antimicrobial traits, which are due to the secondary metabolites synthesized by the plants. These products are known by their active substances like, phenolic compounds which are part of the essential oils, as well as in tanning. The screening of plant products for antimicrobial activity has shown that the higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003). There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants.[2]

Plant produces a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However, very little information is available on such activity of medicinal plants and out of the 4,00,000 plant species on earth, only a small number has been systematically investigated for their antimicrobial activities.[3] Plants and plant based medicaments are the basis of many of the modern pharmaceuticals we use today for our various oilments. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of much intractable disease.[4]

Bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration varies according to the plant parts, season climate and particular growth phase. Leaf is one of the highest accumulated plant part of such compounds and people are generally preferred it for therapeutic, purposes some of the active compounds inhibit the growth of disease causing microbes either singly or in combination.[5] Medicinal plants have been tested for biological, antimicrobial and hypoglycemic activity. They have also tested for antiulcerogenic, antihelminthic, hepatoprotective, analgesic, antipyretic, antileishmania and insecticidal activities.[6] Some plants such as, Ocinum gratissimum and Eugenia uniflora have been reported to be rich in volatile oils. It which contain up to 75% thymol which has antimicrobial effect and mainly used in the treatment of diarrhea and ear infection in human beings, besides it also has antimicrobial properties against Staphylococcus sp., Escherichia coli and Shigella sp.[7]

ANTIMICROBIAL PROPERTIES OF MEDICINAL PLANTS

The antimicrobial actions of “carqueja” (Baccharis trimera Less.) decoction on gram-positive (Staphylococcus aureus and Streptococcus uberis) and gram-negative (Salmonella gallinarum and Escherichia coli) bacterial strains were evaluated and it was found that the former microorganisms are more sensitive to this herb than the latter, which corroborates previous studies.[8] Similarly, antimicrobial assays with plant extracts used in Asia (Ruta graveolens and Zingiber officinale) revealed an inhibitory capacity against Bacillus cereus strains.[9]

In another study, the inhibitory activity of concentrates from 14 Brazilian plants against methicillin-resistant Staphylococcus aureus (MRSA) strains was analyzed.[10] The substances that demonstrated inhibitory activity were ethanol extract and its fractions (n-hexane, water, chloroform, dichloromethane, ethyl acetate and n-butanol) from Punica granatum fruit (pomegranate) and parts of T. avellanedae wood (purple trumpet tree). The greatest activities were found in the ethyl acetate fraction from P. granatum and hexane and chloroform fractions from T. avellanedae.

As to the common yarrow (Achilleamillefolium), its essential oil (obtained from stem and leaves) presents higher antimicrobial activity than its respective extracts (methanol extract separated by chloroform into parts that were not all soluble). The oils prevented the growth of Streptococcus pneumoniae, Clostridium perfringes and Candida albicans and slightly inhibited Mycobacterium smegmatis, Acinetobacter lwoffii and Candida krussei.[11]

A study evaluated the effects of some plant extracts (aqueous and 40% hydroalcoholic) against bacteria found in the oral cavity of dogs.[12] It found that standard S. aureus strain and isolated Streptococcus oralis and Streptococcus mitis strains were sensitive to extracts from garlic (Allium sativum), “espinheira santa” (Maytenus ilicifolia) and guava tree leaves (Psidium guajava).

Similarly, antibacterial properties against Staphylococcus aureus were found in chamomile. The phenolic compounds present in its ethanol extract are responsible for this activity.[13] It was also reported that the aqueous extract from the artichoke (Cynara scolymus) and the ethanol extracts (80%) from both artichoke and “macela” (Achyrocline satureioides) inhibited the growth of Bacillus cereus, B. subtilis, Pseudomonas aeruginosa and S. aureus.[14] In Argentina, terpene compounds (eugenol, geraniol, thymol and carvacrol) derived from essential oils of native plants showed inhibitory effects on MRSA.[15]

In another work, essential oils from 28 plants were tested against ETEC (enterotoxigenic E. coli) and EPEC (enteropathogenic E. coli) serotypes, and the results indicated that palmarosa (Cymbopogon martinii), a highly common plant in Brazil, presents a wide spectrum of action against three ETEC and two EPEC serotypes, whereas Java citronella grass (Cymbopogon winterianus) inhibited one EPEC and two ETEC serotypes.[16] The concentration responsible for the microbial inhibition varied between 100 and 500 µg/mL, while other plants caused inhibition only at higher concentrations.

Studies on the antimicrobial action of 70% methanol extracts from leaves of Mikania glomerata (“guaco”), P. guajava (guava), Baccharis trimera (“carqueja”), Mentha piperita (peppermint) and Cymbopogon citratus (lemongrass), and A. sativum (garlic), Syzygium aromaticum (clove) and Zingiber officinale (ginger) plants in natura were performed all showed some activity against S. aureus, and the most effective extracts were those from clove at the concentration of 0.36 mg/mL and guava at 0.56 mg/mL.[17]

In a study carried out in an indigenous community, it was reported that the hydroalcoholic extracts from Vernonia polyanthes (“assa-peixe”), Aristolochia triangularis (“cipó mil-homens”), Tabebuia avellanedae (purple trumpet tree) and Stryphnodendron adstringens (“barbatimão”) presented a significant antimycobacterial effect and that a local beverage, similar to rum (with an ethanol content of 30%), was employed to prepare the extracts.[18]

Furthermore, the results of a recent study described a potent inhibitory activity of Vernonia polyanthes extract against Leishmania strains.[19] However, its concentrate had no antifungal action under the same conditions. Similarly, Baccharis dracunculifolia oil (“alecrim-do-campo”) at a 10-µL dose prevented microbial growth of E. coli, S. aureus and P. aeruginosa in antimicrobial assays.[20]

The essential oils from Pelargonium graveolens (geranium) present low values of minimum inhibitory concentration against B. cereus (0.36 mg/mL), B. subitilis (0.72 mg/mL) and S. aureus (0.72 mg/mL), whereas Origanum vulgare (oregano) oils also show antimicrobial activity against the same bacteria, in addition to E. coli; however, in the latter, a concentration of 0.35 mg/mL is required to inhibit B. subitilis whereas 0.70 mg/mL is necessary to inhibit the other bacteria.[21]

In a recent study carried out at the Department of Microbiology and Immunology, Botucatu Biosciences Institute, UNESP, tests were performed utilizing extracts from A. sativum (bulbs), Z. officinale (rhizomes), Caryophyllus. aromaticus (flower buds), C. citratus (leaves), P. guajava (leaves) and M. glomerata (leaves) against Enterococcus sp., E. coli, S. aureus and Salmonella. The extracts from garlic (A. sativum) and ginger (Z. officinale) presented the most intense activity against gram-negative bacteria; for garlic, concentrations ranged from 1.38 to 1.61 mg/mL while for ginger it was 6.97. Gram-positive strains were more susceptible to guava extracts at concentrations between 0.77 and 1.74 mg/mL, and to clove extracts at concentrations from 0.46 to 1.24 mg/mL.[22]

Costa et al. tested the inhibitory capacity of essential oils from Croton zehntneri (wildcinnamon) leaves against Shigella flexneri, Salmonella Typhimurium, E. coli, S. aureus and Streptococcus β-hemolyticus strains and found antimicrobial activity against all bacteria, except Salmonella. Moreover, the inhibitory action against S. flexneri was highly significant, with a minimal inhibitory concentration of 25 μg/mL.[23]

Remarkable results using the disc methodology were established in tests on the leaf, phloem and latex of Croton urucurana ("urucuana") against the bacteria Enterococcus faecalis, S. aureus, Staphylococcus epidermidis, Streptococcus pyogenes, E. coli, Klebsiella pneumoniae, P. aeruginosa, Salmonella Typhimurium and S. flexneri.[24] It was found that latex inhibited all tested bacteria except E. coli and showed strong activity against K. pneumoniae and P. aeruginosa (0.125 to 1 mg/disc), whereas leaf hexane extract presented the widest spectrum against gram-negative bacteria and strong action against K. pneumoniae and P. aeruginosa (0.25 mg/disc). The dichloromethane extract was active only against S. pyogenes (0.5 mg/disc), whereas hydroalcoholic extract acted against gram-positive bacteria and revealed a potent action against Salmonella Typhimurium (0.5 to 1 mg/disc). On the other hand, the ethyl acetate extract was inactive. As to phloem, the hexane and dichloromethane extracts were active against S. aureus, S. epidermidis and P. aeruginosa at concentrations from 0.5 to 1 mg/disc; chloroform extract was the most potent against E. faecalis, S. aureus, S. pyognes and K. pneumoniae, at concentrations ranging from 0.25 to 1 mg/disc, but was ineffective against S. flexneri and Salmonella Typhimurium. Furthermore, ethyl acetate extract presented an antimicrobial effect only against K. pneumoniae and S. epidermidis, whereas 75% ethanol extract had a wide activity against E. faecalis, S. pyogenes and P. aeruginosa strains; for both extracts the concentration on the disc was between 0.25 and 1 mg.

More et al., studying extracts from eight South African plants frequently used against human oral cavity pathogens (Actinobacillus actinomycetemcomitans, Actinomyces naeslundii, Actinomyces israelii, Candida albicans, Porphyromonus gingivalis, Prevotella intermedia and Streptococcus mutans), found that six of the eight plants (Annona senegalensis, Englerophytum magalismontanum, Dicerocarym senecioides, Euclea divinorum, Euclea natalensis, Solanum panduriforme and Parinari curatellifolia) had antimicrobial effect against those microorganisms, of which gram-negative ones were more resistant.[25]

In another work, the antimicrobial activities of hexane, chloroform, acetone, ethanol, methanol and aqueous extracts from roots and leaves of bushy lippia (Lippia alba) at the concentration of 2 mg/disc were evaluated against S. aureus, B. subtilis, E. faecalis, Micrococcus luteus, E. coli, P. aeruginosa, Serratia marcescens, Mycobacterium smegmatis, Monilia sitophila and C. albicans.[26] The results indicated that chloroform, acetone and ethanol extracts from roots prevented the growth of S. aureus, M. luteus, B. subtilis, M. smegmatis, C. albicans and M. sitophila, while hexane, ethanol and methanol extracts from leaves inhibited S. aureus, M. luteus, B. subtilis, M. smegmatis and M. sitophila.

The ethanol extract from Hyptis martiusii suppressed the growth of E. coli and MRSA strains (at concentrations between 128 and 512 μg/mL) and was more effective when compared with gentamicin and methicillin.[27] Likewise, the ethanol extract and essential oil from Myrtus communis (myrtle) presented antibacterial effect against B. subtilis and S. aureus, but not against E. coli.[28] Extracts from the peel of Punica granatum fruit (pomegranate) were inhibitory against 38 S. aureus strains.[29]

Rosmarinus officinalis Linn. (rosemary) hydroalcoholic extract was assayed against Streptococcus mitis, Streptococcus sanguinis, Streptococcus mutans, Streptococcus sobrinus and Lactobacillus casei standard strains, and its antimicrobial activity was proven in all tests, except against S. mitis.[30]

Although there are numerous previous studies on medicinal uses of natural products, their applicaton as food additive has only been reported in recent years. Thus, assays with Listeria monocytogenes, S. aureus, E. coli and Salmonella Enteritidis strains isolated from food and essential oils from leaves of oregano, thyme, basil, marjoram, lemongrass, ginger rhizomes and clove flower buds were performed.[31] These bacterial strains were susceptible to the oils, among which lemongrass was the most effective against gram-positive microorganisms, followed by clove and ginger; the concentrations (%v/v) were respectively 0.05, 0.09 and 0.09. In addition, thyme and clove were the most effective against gram-negative bacteria at the concentration of 0.10%v/v. The potential use of these essential oils from aromatic plants to conserve meat and meat derivatives deserves further research.

The hexane, dichloromethane, ethyl acetate and ethanol extracts from phloem of Bowdichia virgilioides ("sucupira"), Calophyllum brasiliense (guanandi), Cariniana rubra ("jequitibá"), Lafoensia pacari ("dedaleira") and Stryphnodendron obovatum; Simaba ferruginea rhizomes; and C. urucurana latex were tested against several bacteria and fungi.[32] Ethyl acetate and hexane extracts from C. brasiliense phloem showed marked antibacterial activity against gram-positive bacteria such as S. aureus, S. epidermidis and S. agalactiae. Ethyl acetate and ethanol extracts from L. pacari phloem and B. virgilioides extracts produced some effects on fungi. Silva Jr. et al. claimed that it was the first report on antifungal activities of extracts from C. rubra and S. ferruginea.

The antibacterial activity of essential oils from oregano (Origanum vulgare) against multiresistant bacteria – including E. coli, E. faecalis, Acinetobacter baumannii, K. pneumoniae, P. aeruginosa and MRSA – was analyzed by Costa et al.[33] Concentrations of at least 0.125% showed higher antimicrobial efficiency on the studied bacterial strains, although P. aeruginosa was found to be more resistant to the essential oil because it was only inhibited at the concentration of 0.5%.[33]

Zampini et al.[34] examined multiresistant bacteria under the influence of ethanol extracts from 11 Argentinean plant species (Baccharis boliviensis, Chiliotrichiopsis keidelii, Chuquiraga atacamensis, Fabiana bryoides, Fabiana densa, Fabiana punensis, Frankenia triandra, Parastrephia lucida, Parastrephia lepidophylla, Parastrephia phyliciformis, and Tetraglochin cristatum). They observed growth inhibition in at least one of the following tested strains: S. aureus, E. faecalis, E. coli, K. pneumoniae, Proteus mirabilis, Enterobacter cloacae, Morganella morganii and P. aeruginosa.[34]

In another study, essential oils from rosemary (R. officinalis), clove (Caryophyllus aromaticus L.), ginger (Z. officinalis), lemongrass (C. citratus), peppermint (M. piperita) and cinnamon (Cinnamomum zeilanicum Blume) were tested against S. aureus and E. coli strains. The oils showed some antimicrobial action; ginger essential oil was the most efficient against S. aureus while cinnamon and clove were the most effective against E. coli at 0.09%v/v.[35] Additionally, the cyclohexane extract of Monodora myristica fruit and the ethyl acetate extract from the stem bark of Albizia gummifera were effective in inhibiting C. albicans and Candida krusei.[36]

As in previous studies, the antimicrobial activity of essential oils from oregano (Origanum vulgare), thyme (Thymus vulgaris), marjoram (Origanum majorana) and basil (Ocimun basilicum) was evaluated in meat products against strains of L. monocytogenes and Salmonella Enteritidis.[37] It was reported that all oils showed antibacterial activity and that, particularly, gram-positive bacteria were more susceptive to them.

Based on the aforementioned data, it is possible to conclude that the literature on testing antimicrobial activity of plant products is broad, including an increasing number of publications per year. Therefore, it is difficult to integrate all those numerous studies on the antimicrobial action of plant products into the present review; a multidisciplinary approach to this theme is increasingly required.

ANTIMICROBIAL ACTIVITY MECHANISMS OF NATURAL PRODUCTS

Most plants contain several compounds with antimicrobial properties for protection against aggressor agents, especially microorganisms. The chemical structures of some antimicrobial compounds, according to Cowan (2), obtained from plants are shown in Fig 1.

Active compounds found in some plants have antiseptic action; for example, thyme has thymol and carvacrol, clove has eugenol and isoeugenol, and oregano has carvacrol and terpinenol-4. In some cases, terpenes from essences that are soluble in water have higher antibacterial power than others.[38] The sites or structures of the bacterial cell that are considered targets for action by the components of natural products are illustrated.

Important characteristics responsible for the antimicrobial action of essential oils include hydrophobic components that allow the participation of lipids from the bacterial cell membrane, which disturbs cell structures and make them more permeable.[40]

Chemical compounds from essential oils also act on cytoplasmic membrane proteins.[41] Cyclic hydrocarbons act on ATPases, enzymes known to be located at the cytoplasmic membrane and surrounded by lipid molecules. In addition, lipid hydrocarbons may distort the lipid-protein interaction, and the direct interaction of lipophilic compounds with hydrophobic parts of the protein is also possible.[42] Some essential oils stimulate the growth of pseudo-mycelia, evidencing that they may act on enzymes involved in the synthesis of bacterium structural components.[43]

Carvacrol and thymol

The structure of thymol is similar to that of carvacrol; however, they differ as to the location of the hydroxyl group in the phenolic ring. Both substances seem to make the membrane permeable.[44] Their structure disintegrates the external membrane of gram-negative bacteria, releasing lipopolysaccharides (LPS) and increasing the permeability of the cytoplasmic membrane to ATP. The presence of magnesium chloride does not influence this action, suggesting a chelating mechanism of different cations on the external membrane.[45]

Eugenol

Different concentrations of eugenol may inhibit the production of amylase and protease by B. cereus. Furthermore, cell wall degradation and cell lysis were also reported.[46]

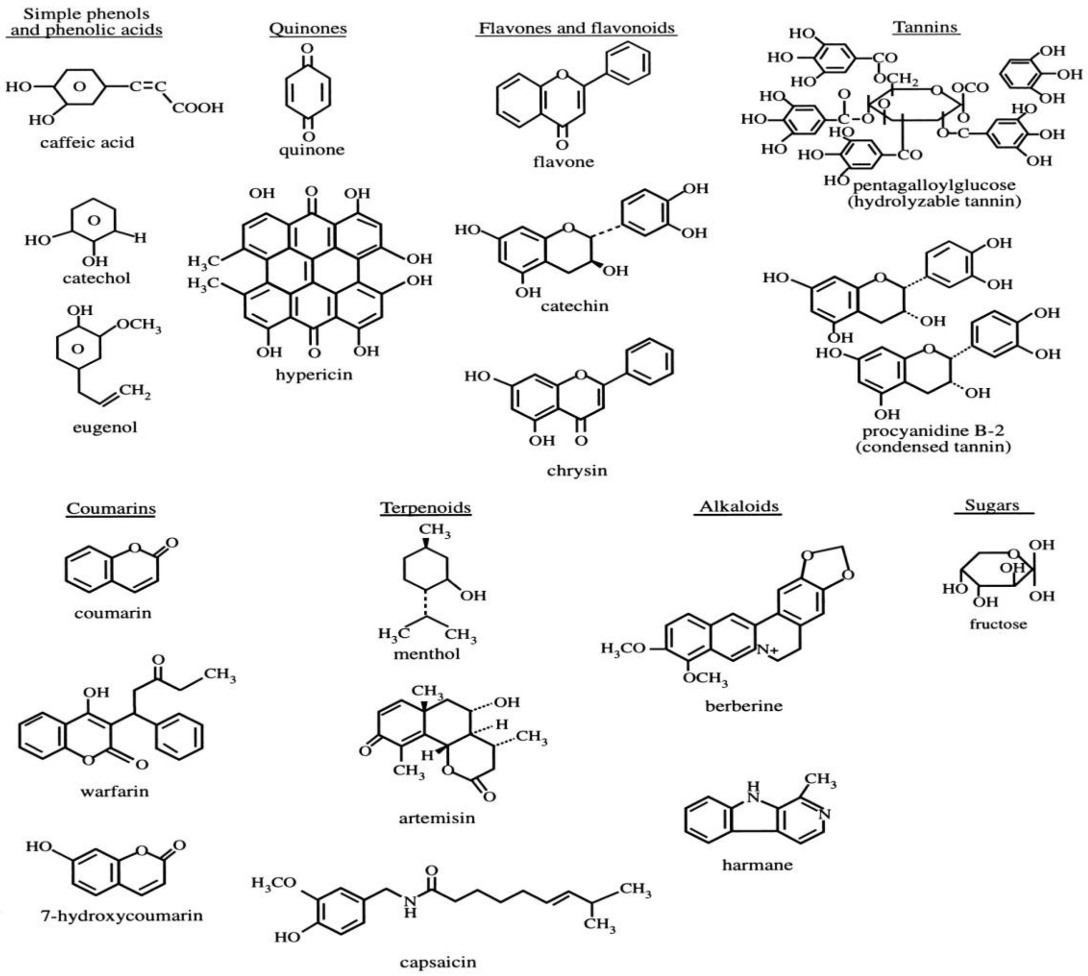

Figure 1. Chemical structures of antimicrobial compounds (2).

In Figure 2. The action mechanisms of natural compounds are related to disintegration of cytoplasmic membrane, destabilization of the proton motive force (PMF), electron flow, active transport and coagulation of the cell content. Not all action mechanisms work on specific targets, and some sites may be affected due to other mechanisms.[39]

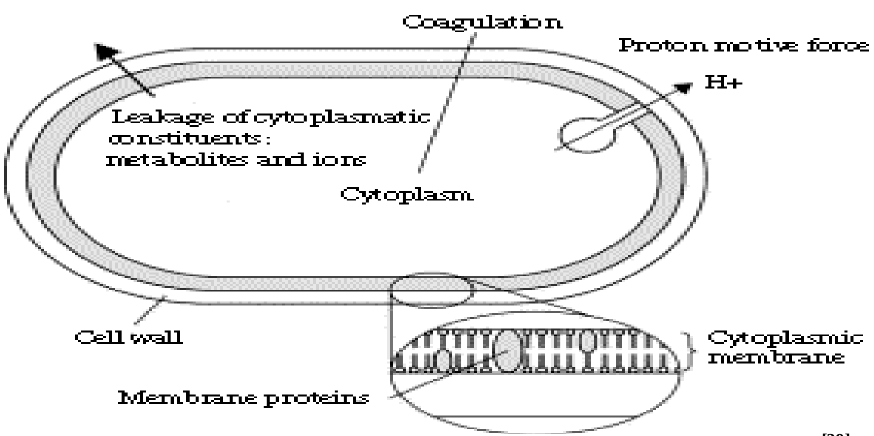

Figure 2. Sites in a bacterium where natural compounds are active.[39]

p-Cymene: A precursor of carvacrol, this hydrophobic compound provokes greater swelling of the cytoplasmic membrane compared to carvacrol.[47]

Carvone: When tested at concentrations higher than its minimum inhibitory concentration, carvone dissipates gradient pH and cell membrane potential. The growth of E. coli, Streptococcus thermophilus and Lactococcus lactis may decrease according to the concentrations of carvone, suggesting that it acts by disturbing the general metabolic status of the cell.[48]

Cinnamaldehyde: Cinnamaldehyde is known to inhibit E. coli and Salmonella Typhimurium growth at concentrations similar to those of carvacrol and thymol. However, it neither disintegrates the outer membrane nor weakens the intracellular ATP. Its carbonyl group has affinity for proteins, preventing the action of decarboxylase amino acids on E. aerogenes.[48]

CONCLUTION

Since ancient times, plants have been used by several communities to treat a large number of diseases, including infections. Numerous studies on the pharmacology of medicinal plants have been accomplished, since they constitute a potential source for the production of new medicines and may enhance the effects of conventional antimicrobials, which will probably decrease costs and improve the treatment quality. However, several plants may present antagonistic effects during antibiotic therapy.

An important aspect comprises the search for new compounds that have antimicrobial action and synergism with currently available antimicrobial drugs, since bacteria resistant to conventional medicines are increasingly frequent; consequently, medicinal plants constitute an alternative for infection treatment.

The antimicrobial activity of plants was proven by various examples, in the form of both essential oils and extracts. Thus, this property can be a promising ally in the development of medicines necessary to combat the increasing number of bacterial strains that become resistant to conventional antibiotics.

Therefore, given that the literature on tests for the antimicrobial action of plant products is broad, including an increasing number of publications per year, it is highly difficult to relate the countless reports on the antimicrobial action of these products in this review article about a subject of such a great complexity, which requires a multidisciplinary approach.

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