Plant Extracts: Search for New Alternatives to Treat Microbial Diseases

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Abstract: Medicinal plants constitute the base of health care systems in many societies. The recovery of the knowledge and practices associated with these plant resources are part of an important strategy linked to the conservation of biodiversity, discovery of new medicines, and the bettering of the quality of life of poor rural communities. Research in phytosciences, an emerging multidisciplinary science, is almost unlimited, with several aspects to be discussed. Therefore, the focus of the present review is mainly on the antimicrobial and antioxidant properties of bioactive phytocompounds resultant of our research with crude plant extracts and essential oils of medicinal plants belonging to different families, used in various infectious disorders. The results obtained in the last years warrant the present review, discussing not only the use of several medicinal plants against bacteria, yeast, filamentous fungi and protozoa, but also their mechanisms of action, interactions with macromolecules and potential for toxicity in mammalian cells. Problems related to the efficacy of the isolation techniques and stability of bioactive compounds are also commented on. In addition, this review aims to emphasize the greatest importance to investigate plant species that have not been the subject of pharmacological studies, although their popular uses have been reported.

Keywords: Medicinal plants, antimicrobial activity, antioxidant activity

INTRODUCTION

Since ancient times, mankind has used plants to treat common diseases and some of these traditional medicines are still included as part of the habitual treatments of various maladies [1]. Folk medicine, mainly based on plants, enjoy a respectable position today, specially in the developing countries, where the availability of modern health services is limited. However, in the absence of a scientific base, such practices may generate serious adverse effects. The analyses of the pharmacological activity of plant extracts may therefore make possible the design of less expensive therapies to be used in economically unprivileged regions [2].

Mainstream medicine is increasingly receptive to the use of antimicrobials and other drugs derived from plants, as traditional antibiotics become ineffective and as new, particularly viral, diseases remain intractable to this type of drug. Another driving factor for the renewed interest in plant antimicrobials in the past 20 years has been the rapid rate of plant species extraction [3]. However, the full acceptance of phytopharmaceuticals and the integration of phytotherapy into the concept of classical medicine can be achieved only if they meet the same criteria of quality as synthetic pharmaceuticals. Moreover, the ideal procedures for standardization of the phytopharmaceuticals are based on knowledge of the major pharmacological and toxicological assays [4].

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In the last years, the use of plant extracts, as well as other alternative forms of medical treatments, is enjoying great popularity all over the world. Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First, few antibiotics derived from microorganisms are launched each year. Second, self-medication with these substances is common place [3]. In addition, the overuse of antibiotics and consequent antibiotic selection pressure is thought to be the most important factor contributing to the appearance of different kinds of resistant microbes [5]. Antimicrobial resistance is one of the biggest challenges facing global public health. The emergence of multidrug-resistant isolates in several infections, often referred to as the diseases of poverty, has had its greatest toll in developing countries. Antibiotic resistance can occur via three general mechanisms: prevention of interaction of the drug with target; efflux of the antibiotic from the cell; and direct destruction or modification of the compound. The increasing prevalence of multidrug resistance in pathogenic microorganisms, as well as, undesirable side effects of certain antibiotics have triggered immense interest in the search for new antimicrobial drugs of plant origin. Common side effects are allergic or toxic reactions or mutagenic substances. The allergic reactions can vary from transient dermatitis to anaphylactic shock. Therefore, medicinal plant extracts offer considerable potential for the development of new agents effective against infections currently difficult to treat [4]. Among several examples, Schelz and co-workers [6] proved the antiplasmid activity of peppermint oil and its main constituent, menthol, which means that menthol-containing substances are potential agents that could eliminate the resistance plasmids of bacteria. The main point of this menthol-induced plasmid elimination is a special mechanism of action and confirmed

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the relevance of peppermint oil and menthol as adjuvant antimicrobial agents [6].

Research laboratories worldwide have found literally thousands of phytochemicals which have *in vitro* inhibitory effects on all types of microorganisms. These *in vitro* screening programs, using the ethnobotanical approach, are important in validating the traditional use of herbal remedies and for providing leads in the search for new active substances. Whereas activity identified by an *in vitro* test does not necessarily confirm that a plant extract is an effective medicine, nor a suitable candidate for drug development, it does provide basic understanding of a plant efficacy and in some cases toxicity. However, more of these compounds should be subjected to animal and human studies to determine their effectiveness in whole organism systems, including particular toxicity studies as well as an evaluation of their effects on normal microbiota. The non prescription use of medicinal plants is cited today as an important health problem, in particular their toxicity to the kidneys [7].

Aromatic plants have been used for generations not simply as food ingredients but also to treat a plethora of ailments and, in recent times, scientific data are accumulating that demonstrate for many herbs and related essential oils medicinal properties useful in the prevention of diseases or in the relieve of their symptoms [8]. Essential oils, mixtures of natural volatile compounds deriving from plant secondary metabolism, mainly monoterpenes, sesquiterpenes, and their oxygenated derivatives (alcohols, aldehydes, esters, ethers, ketones, phenols and oxides), are isolated by steam distillation and have been known since antiquity to possess antibacterial and antifungal properties [9]. Generally, the oil composition is a balance of various compounds, although in many species one constituent may prevail over all others. Changes in the essential oil compositions might arise from several environmental, chemical, seasonal, geographical and genetic differences [10]. Essential oils antimicrobial, antioxidant, anti-inflammatory, antispasmodic and relaxing properties have been described both in animals and humans [8]. Therefore, crude plant extracts in the form of decoction, infusion or tincture are traditionally more used by the population for the treatment of several diseases, as well as an anti-inflammatory and healing agent [11].

When infection or physical damages happen, many processes of plant defense are activated. Some compounds are produced immediately, whereas phytoalexins are present only after two to three days. Phenolic substances generally have significant antimicrobial activity as well as flavonoids [12-14]. The complex chemical composition of plant extracts is generally a very limiting obstacle to the isolation of bioactive compounds. Nevertheless, the use of bioautography agar overlay bioassay allows the detection of antimicrobial components in a crude plant extract [15]. Any consideration, however, of the future commercial exploitation of the plants found to show significant activities must take into account not only biological properties, including acceptable levels of toxicity, but also the growing habits, ease of cultivation and availability of the plants to the local population [16].

Many essential oils are known to exert antimicrobial activity but the mechanism of action is often not entirely understood. Membrane disruption by the lipophilic constituents may be involved in this mechanism [6]. An important characteristic of essential oils and their constituents is their hydrophobicity, which enables them to partition in the lipids of bacterial cell membrane and mitochondria thus disturbing the structures and rendering them more permeable [17]. Although, essential oil of oregano and its component carvacrol slightly increased the incidence of apoptotic cell death, they showed extensive antimicrobial activity even at lower concentrations [18]. Relatively high cytotoxicity was demonstrated by thyme oil, which increased both apoptotic and necrotic cell death incidence. In contrast, its component thymol showed no cytotoxic effect as well as greatly-reduced ability to inhibit visible growth of the chosen pathogen, in the doses used. On the other hand, the addition of other essential oils and their components at lower doses, with the exception of thyme oil, to bacterial suspension significantly reduced the cytotoxic effect of *Escherichia coli* on Caco-2 cells after 1h culture. In conclusion, it is possible to find appropriate doses of essential oils showing both antimicrobial activity and very low detrimental effect on mammalian cells [18]. In fact, the synergistic effects of the diversity of major and minor constituents present in the essential oils should be taken into account for their biological activity.

Free radicals/reactive oxygen species (ROS) are related to inflammation, aging, and cancer. However, living systems have essential antioxidant mechanisms by which these harmful radicals can be scavenging, i.e., free radical scavenging activity (FRSA). Oxidative stress is the condition in which a lack of balance exists between the levels of oxidant stimuli and the various antioxidants in biological systems. Currently, a large number of antioxidants are being investigated. However, it remains controversial whether ingestion of foods and supplements with antioxidant actions directly contributes to the antioxidant status in biologic systems. The human body is always exposed to stress producing ROS. The smell of lavender or rosemary kinds of aroma decreased cortisol which is a kind of stress hormone, but increased FRSA in human saliva [19]. The antimicrobial and antioxidant properties of essential oils have been known for a long time, and a number of investigations have been conducted into their activities [18]. Essential oils in broad spectrum of plant species have been shown to have cytotoxic and/or antitumoral activity. There has been accumulating evidence supporting a role of antioxidant mechanisms on the anti-cancer potential of dietary essential oils [2].

A number of aromatic medicinal plants used for treating infectious diseases have been mentioned in different phytotherapy manuals due to their availability, fewer side effects, and reduced toxicity. However, only limited research had been done in Brazil on the use of natural medicine, although in the last 15 years more work has been initiated on the use of medicinal plants by communities living in diverse ecosystems [20]. Lately, our research group has been contributing to this area evaluating the bioactive potential of several extracts and essential oils isolated from different organs of Brazilian plants. In this review, we analyze the *in vitro* antimicrobial activity and antioxidant potential of medicinal plants extracts based specially on Brazilian folk medicine [21]. Pharmaceutical companies have demonstrated renewed interest in investigating higher plants as sources for new lead structures and also for the development of stan-

dardized phytotherapeutic agents of proven efficacy, safety and quality [4].

ANTIBACTERIAL ACTIVITY

The effectiveness of alternative strategies for controlling bacterial growth is extremely important nowadays. [22]. Bacterial plaque, or biofilm accumulated on teeth surfaces and composed of native oral microbiota, is the primary aetiological agent for periodontal disease and dental caries which may result in teeth loss if left untreated [23, 24]. Corrective treatment for such infectious diseases requires the reduction and/or elimination of bacterial accumulations in the retentive sites on the top of the teeth (occlusal surfaces) and between teeth by daily toothbrushing and frequent dental cleanings or prophylaxys [25]. Several antiseptic agents including chlorhexidine, cetyl pyridinium chloride, fluorides and phenol derivatives have been used widely in dentistry to inhibit bacterial growth. However, these substances have several adverse effects such as vomiting, diarrhea and teeth staining. In addition, the development of antimicrobial resistant strains is a growing cause of concern. These drawbacks justify further research and development of natural antimicrobial agents targeting specific oral pathogens while being safe for the host [26].

Antibacterial activities of *Aristolochia cymbifera* (rhizomes), *Cocos nucifera* (husk fiber), *Caesalpinia pyramidalis* (leaves) and *Ziziphus joazeiro* (inner bark) extracts were evaluated, based on the northeast of Brazil popular knowledge to treat oral diseases [27]. Besides the main etiological agents of caries disease, *Streptococcus mutans* and *Lactobacillus casei*, other important oral bacteria such as the periodontal pathogens *Fusobacterium nucleatum*, *Prevotella intermedia* and *Porphyromonas gingivalis* were used. *A. cymbifera* alcoholic extract was the most effective, inducing high bactericidal effects against all tested strains. *C. pyramidalis* and *C. nucifera* aqueous extracts showed to be also effective against the tested species, whereas *Z. joazeiro* aqueous extract was the less effective. However, if considered the crude nature and low toxicities of the extracts, as well justified by Al-hebshi and co-workers [28], which demonstrated the antimicrobial activities of aqueous crude extracts against oral microorganism, our results allow us to conclude that the crude extract from *Z. joazeiro* exhibited significant antimicrobial activity and properties that support folkloric use in the treatment of some oral diseases, corroborating the importance of ethnopharmacological surveys in the selection of plants for bioactivity screening.

Biofilms are much more resistant to antimicrobial agents than planktonic cells. In this context, artificial biofilms from non-stimulated saliva were prepared and then exposed to the four crude extracts selected. The *in vitro* biofilms were very sensitive to *A. cymbifera*, *C. pyramidalis* and *C. nucifera* extracts after 30 min of incubation. However, no effect against the biofilm system was detected when the *Z. joazeiro* extract was assayed. Confirming the results obtained with planktonic cells, the extract from *A. cymbifera* inhibited all the microorganisms present in the human saliva biofilm. The results of this preliminary study, focused in these four plant species, tend to reinforce the use of these extracts as antimicrobial agents in folk medicine. Accordingly, the results corroborate that the part of the plant used, as well as the extraction procedure, are crucial to reveal the plant bioactive potential, increasing the value of popular knowledge. Since these plant extracts have low toxicity for mammalian cells, their use in rinse or gel preparations produced by pharmaceutical industries certainly has also a clinical application in the treatment of oral diseases. In addition, besides their antimicrobial potential, *C. nucifera*, *C. pyramidalis* and *A. cymbifera* plant extracts may be used as adjuvant antioxidants in mouthwashes and may provide new treatment strategies for periodontal diseases in the future [27].

Antimicrobial activity of khat (*Catha edulis*) crude aqueous extract against a several oral microorganisms and the test of their ability to modify bacterial resistance to tetracycline and penicillin were evaluated *in vitro*. Khat extract has water soluble constituents possessing selective antimicrobial activity against oral bacteria. The presence of khat extracts at a sub-minimal inhibitory concentration (sub-MIC) resulted in a 2-4 fold potentiation of the tested antibiotics against the resistant strains. There is preliminary evidence for the presence of an antibiotic resistance-modifying component, but the identification of khat extract active substances is needed to assess its clinical relevance [28].

Different scientific works with extracts from several plants used in Brazilian traditional medicine presented antibacterial activity against susceptible and or resistant strains of *Staphylococcus aureus*, a particularly virulent organism that cause a broad of health conditions including, osteomyelitis, endocarditis, and bacteremia. Of major concern is the problem of *S. aureus* infection in hospitals, especially when so-called multi-drug resistant (MDR) strains are involved [29]. The hexane extracts fractions of *A. cymbifera* and *Myroxylon balsamum* showed significant results against methicillin-sensitive and resistant *S. aureus* and *Pseudomonas aeruginosa* strains. Bioassay-guided fractionation of hexane extracts of *A. cymbifera* and *M. balsamun* led to the identification of the diterpene 2-oxo-populifolic acid and the chalcone isoliquiritigenin, respectively, as antimicrobial compounds [4].

The decoction of *C. nucifera* L. husk fiber has been used in northeast of Brazil traditional medicine for treatment of diarrhea and arthritis [30]. *S. aureus* was selected as the first bacterial target for *C. nucifera* fractions due to its importance as a human pathogen. Catechin and epicatechin, together with condensed tannins (β-type procyanidins), were demonstrated to be the components of the water extract responsible for the high antimicrobial activity against *S. aureus*. The crude extract and one of the fractions rich in catechin also showed inhibitory activity against acyclovir-resistant herpes simplex virus type (HSV -1- ACVR) [31].

It is known that *Punica granatum* is rich in hydrolysable tannins [32] and this class of compounds has remarkable antimicrobial activity. *Tabebuia avellanedae* is rich in naphtoquinones [33], which also has antibacterial, antifungal, antiviral and antineoplasic activities [34-37]. Ellagitannins are the principal components responsible for the antimicrobial action of *P. granatum* and naphtoquinones for that of *T. avellanedae*. These polyphenols are known to form with proteins soluble complexes of high molecular weight. Thus, after being absorbed, the polyphenols will react with the pro-

tein moiety of cellular enzymes (oxidoreductases) in the cytoplasm and in the cell wall. They may also bind to bacterial adhesins and so, interfering with availability of receptors on the cell surface [38, 39].

Recently, the synergism between antimicrobial drugs and eight plant extracts - "guaco" (*Mikania glomerata*), guava (*Psidium guajava*), clove (*Syzygium aromaticum*), garlic (*Allium sativum*), lemongrass (*Cymbopogon citratus*), ginger (*Zingiber officinale*), "carqueja" (*Baccharis trimera*) and mint (*Mentha piperita*) against *S. aureus* was evaluated. The antimicrobial activities of plant extracts on *S. aureus* strains were confirmed and synergism was detected with all the antimicrobial drugs tested. Tetracycline presented good synergism with all the extracts. The possible activities of substances found in plant extracts on ribosome structure and bacterial enzymes inhibition appear to be related with synergism profile between plant extracts and inhibitors of protein synthesis. However, the understanding of the synergism mechanism is fundamental to the development of pharmacological agents to treat diseases caused by *S. aureus* using medicinal plants [40].

The antimicrobial activity of essential oils and their derivatives against a wide spectrum of bacteria has been recognized for a long time. In general, this activity is more pronounced against Gram-positive than against Gram-negative bacteria, which could be ascribed to the presence of their outer phospholipidic membrane, almost impermeable to lipophilic compounds. The absence of this barrier in Gram-positive bacteria allows the direct contact of the essential oils hydrophobic constituents with the phospholipid bilayer of the cell membrane, where they bring about their effect, causing either an increase of ion permeability and leak of vital intracellular constituents, or impairment of the bacterial enzyme systems [10].

Kalemba and Kunicka [41] showed that the susceptibility of a given microorganism to essential oils depends, first of all, on the properties of the essential oil and the microorganism itself. Factors that may contribute to divergent results include differences between the types and numbers of bacterial isolates tested in each study, and the methods used, including the criteria for determining MICs. Recent isolates may exhibit an increased resistance against the antimicrobial compounds, which possibly derives from their recent interactions with host cells.

Dental caries is a multifactorial disease usually associated with increased numbers of *S. mutans* at the site of infection. Essential oils have been formulated into several over-the-counter oral hygiene products, concerning their efficacy against oral pathogens. In folk medicine, *Hyptis pectinata* (L.) Poit (Lamiaceae) has been extensively used as mouth rinses by the population in the state of Sergipe, Brazil. This herbaceous plant with aromatic leaves, popularly known as "sambacaitá", or "canudinho", is also recommended for several conditions, among them rinopharyngitis, nasal congestion, certain skin diseases [42], gastric disorders, fever, and also bacterial infections [43, 44]. In a recent study, *H. pectinata* essential oil exhibited considerable inhibitory effect against either all the clinical isolates obtained from patient's saliva or the ATCC strains tested of *S. mutans*, with minimum inhibitory and bactericidal concentrations (MBC) of 200μg/ml. In addition, the efficiency of the emulsifying agents tween 20, tween 80, dimethylsulphoxide and propylene glycol in *H. pectinata* essential oil was evaluated. The data obtained confirmed the better inhibitory effect of the oil when using all tested diluents, although tween 80 seemed to be more suitable for the emulsification. According to these preliminary results, *H. pectinata* essential oil can be considered a promising alternative to chlorhexidine for the control of oral bacteria related diseases. Since this essential oil is not toxic for mammalian cells, its use in rinse or gel preparations produced by pharmaceutical industries certainly has a clinical application in the treatment of oral diseases [22].

In 1937, Penfold and Morrison [45] reported that oral conditions such as thrush, aphthous stomatitis, mouth ulcers, gingivitis and pyorrhea all responded favorably to treatment with tea tree oil. *In vitro* susceptibility data showed that the bacteria found in the mouth are susceptible to, and rapidly killed by, tea tree oil. As such, tea tree oil, incorporated into appropriate oral hygiene products such as mouthrinses, dentifrices and dental gels or irrigators may be suitable for use in oral cavity [46].

In 2005, Alviano and co-workers [47] demonstrated that a linalool-rich essential oil from *Croton cajucara* inhibits the growth of cell suspensions of strains of *L. casei, S. aureus, Streptococcus sobrinus, P. gingivalis* and *S. mutans*, all of then associated with oral cavity disease. Microbes of saliva specimens from human individuals with fixed orthodontic appliance, as well as reference strains, were used to construct an artificial biofilm which was exposed to linalool or to the essential oil. As in microbial suspensions, the essential oil was toxic for all the bacteria. The compounds of the essential oil were separated by thin layer chromatography and exposed to the above-cited microorganisms. In this analysis, the proliferation of the bacterial cells was inhibited by still uncharacterized molecules component of the essential oil. The crude essential oil is not toxic to mammalian cells [48] which suggest that the essential oil or its purified components may be useful to control the microbial population in patients with fixed orthodontic appliances [47].

The mechanisms of action of essential oils or their components are unclear, as the possible existence of resistance mechanisms impairing their antimicrobial activity [49]. This condition makes mandatory the development of experimental models evaluating the pharmacological applicability of essential oil components as antimicrobial agents [47].

Lippia alba, locally known as "erva-cidreira", and *Lippia alba* f. intermedia, known as "carmelitana", are two species characterized by a variability in morphology and in the chemical composition of the essential oil. The essential oils from these two chemotypes were active against all microorganisms assayed showing large inhibition zones, superior or similar to those obtained for the positive controls (vancomycin and methicillin) (Table **1**).The good antimicrobial activities of both essential oils may partly explain and support the use of the decoction of these *Lippia* species in traditional medicine. Their chemical analyses allowed the identification of *L. alba* as a myrcene-citral chemotype (15%, 37.1% and 1%, respectively) and *L. alba* f. intermedia as a citral chemotype (22.1%). The essential oils of both species were active against all microorganisms assayed, probably due to the high

Table 1. Growth Inhibition Zones of Bacteria Species by Essential Oils in Millimeters[•]

Microorganisms	Inhibition zone diameter (mm)				
	Lippia alba f. intermedia[¨]	*Lippia alba*[¨]	*Lippia origanoides*[¨]	Vancomycin[¨¨]	Methicillin[¨¨]
Staphylococcus aureus ATCC 25923	20	25	25	21	20
Staphylococcus aureus MRSA (BMB9393)	30	18	25	18	08
Lactobacillus casei ATTC 4646	40	20	20	10	15
Streptococcus mutans ATCC 25175	11	20	26	10	10

[•]Antimicrobial assay was carried out using the drop agar diffusion method [49].
[¨]The essential oils were diluted 1:1 with Tween 80 (0.5% in water).
[¨¨]Reference antibiotics as positive controls – 1 mg/ml in Tween 80 (0.5% in water)
Negative control – Tween 80 (0.5% in water) [50, 52].

content of oxygenated monoterpenes (51% and 40.1%, respectively), specially represented by aldehydes and alcohols. The chemical and pharmacological data are in agreement with the ethnobotanical survey [50].

Duarte and co-workers [51] demonstrated the antimicrobial activity of essential oils from two chemotypes of *L. alba*. Carvone-chemotype has been shown to be active against all tested microorganisms, especially against Gram-positive ones and linalool-chemotype was active against *Candida albicans*.

Lippia origanoides H.B.K. (Verbenaceae) is a plant known in Oriximiná (Brazil) as "sálvia-de-Marajó". The essential oil of *L. origanoides* inhibits all bacteria assayed (Table **1**). The major constituents in the essential oil were carvacrol and thymol. Due to its essential oil yield and composition, *L. origanoides* can be considered as promising for future utilization as a spice and condiment. In addition, the extensive use of this plant, by the local people of Oriximiná, as carminative, as a general antiseptic (mouth, skin and vagina) and to treat respiratory diseases can be related to its antimicrobial activity [52].

Several studies have demonstrated the above activity for many species of the genus *Lippia* rich in thymol and carvacrol. This is the case of *L. sidoides* [53], *L. graveolens* [54], *L. chevalieri* [55], *L. multiflora* [55], and *L. origanoides* [56]. Moreover, the antimicrobial activities of carvacrol and thymol have also been demonstrated [57, 54]. Both substances appear to make the cell membrane permeable, and are able to disintegrate the outer membrane of Gram-negative bacteria, releasing lipopolysaccharides and increasing permeability of the cytoplasmic membrane to ATP [58]. The inhibitory effect of several terpenoids on microbial oxygen uptake and oxidative phosphorylation has also been demonstrated. In particular, phenolic and non-phenolic alcohols exhibited the strongest inhibitory effects, followed by aldehydes and ketones. The monoterpene hydrocarbons were less active and it has been suggested that this behavior depends on the free hydroxyl group from the alcohols [59].

The essential oils of *Salvia officinalis* and *Salvia triloba* cultivated in south Brazil were analyzed by GC-MS, and the major constituents of oil of *S. officinalis* were α–thujone, 1,8-cineole, camphor, borneol and β-pinene, whereas those of *S. triloba* were α-thujone, 1,8-cineole, camphor and β-caryophyllene. The essential oils of both species exhibit remarkable bacteriostatic and bactericidal activities against *Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Aeromonas hydrophila, Aeromonas sobria,* and *Klebsiella oxytoca*. Moreover, the essential oil of *S. triloba* efficiently inhibited the growth of *S. aureus* [10].

The chemical composition and the antibacterial properties of the essential oils obtained from the aerial parts of the four Lamiaceae species, wild oregano (*Origanum minutiflorum*), oregano *(Origanum onites*), black thyme (*Thymbra spicata*), and wild savory (*Satureja cuneifolia*), with commercial importance in Turkey, were evaluated against *A. hydrophila, Bacillus amyloliquefaciens, Bacillus. brevis, B. cereus, B. subtilis, Corynebacterium xerosis, Enterococcus faecalis, E. coli, Klebsiella pneumoniae, Lysteria monocytogenes, Micrococcus luteus, Mycobacterium smegmatis, Proteus vulgaris, S. aureus, and Yersinia enterocolitica*. The major constituent of the oils determined by GC was carvacrol. All essential oils inhibited all bacteria. The essential oil of *T. spicata* was the most active. *B. amyloliquefaciens* was the most sensitive microorganism. These results confirmed the possibility of using these four essential oils in food systems to prevent the growth of foodborne bacteria and extend the shelf life of processed foods [60].

A study was made to evaluate harmful effects of clove, cinnamon, oregano and thyme commonly used essential oils and their major components on intestinal cells. Antimicrobial activity of selected plant extracts against enteroinvasive *E. coli* was dose dependent. However, doses of essential oils with the ability to completely inhibit bacterial growth showed relatively high cytotoxicity to intestinal-like cells cultured *in vitro*. Lower doses of essential oil had only partial antimicrobial activity and their damaging effect on Caco-2 cells was only modest. Cell death assessment based on

morphological and viability staining followed by fluorescence microscopy showed that essential oils of cinnamon and clove and their major component eugenol had almost no cytotoxic effect at lower doses.

Although essential oil of oregano and its component carvacrol slightly increased the incidence of apoptotic cell death, they showed extensive antimicrobial activity even at lower concentrations. Relatively high cytotoxicity was demonstrated by thyme oil, which increased both apoptotic and necrotic cell death incidence. In contrast, its component thymol showed no cytotoxic effect as well as greatly-reduced ability to inhibit visible growth of the tested pathogen in the doses used. On the other hand, the addition of all essential oils and their components at lower doses, with the exception of thyme oil, to bacterial suspension, significantly reduced the cytotoxic effect of *E. coli* on Caco-2 cells after 1h culture. In conclusion, it is possible to find appropriate doses of essential oils showing both antimicrobial activity and very low detrimental effect on intestinal cells [18].

ANTIFUNGAL ACTIVITY

In humans, fungal infections range from superficial to deeply invasive or disseminated and have increased dramatically in recent years. Failure of drug treatment in fungal infections combined with improvements in performance and standardization of antifungal susceptibility testing have drawn attention to the problem of antifungal resistance and its underlying mechanisms. HIV-positive patients have developed resistance to treatment with fluconazole, the most currently used antifungal. In some cases, resistance to fluconazole triggers cross-resistance to other azoles or pathogen shift from *Candida albicans* to less sensitive species such as *Candida glabrata* and *Candida. krusei* [61]. Despite the existence of potent antifungal agents, the appearance of resistant or multi-resistant strains imposes the need for a permanent search and development of new drugs [15]. In the quest for new antifungal drugs, medicinal plants must not be overlooked. Many plant extracts and essential oils possess antifungal or antibacterial activity and have been proposed for use in complementary medicine. Although they are not widely used in conventional healthcare, these extracts and essential oils have been recommended for use as home remedies for the treatment of fungal infections by numerous books and articles in the popular press [62]. In spite of the rich flora, from Brazilian biomes, only few data from some species of medicinal plants are available, including both native and exotic species.

A survey of medicinal plants to treat common mycoses was done in Curituba district, Sergipe State, Brazil. One hundred inhabitants were interviewed by health agents and traditional healers. Four different plants of *Ziziphus joazeiro, Caesalpinia pyramidalis, Bumelia sartorum,* and *Hymenea courbaril* were the most cited. Considering that candidiasis and dermatophytosis were the main mycoses cited in the interviews, *C. albicans, Candida guilliermondii, Trichophyton rubrum* were selected, and other important pathogenic fungi were also evaluated, such as *Fonsecaea pedrosoi* and *Cryptococcus neoformans*. The aqueous extracts obtained following traditional methods, and using different parts of the plants, were submitted to drop agar diffusion tests for primary antimicrobial screening. Only the water infusion extract of *Z. joazeiro* and *C. pyramidalis* presented a significant antifungal activity against *T. rubrum, C. guilliermondii, C. albicans, C. neoformans,* and *F. pedrosoi,* when compared to the antifungal agent amphotericin B. Moreover, the best antimicrobial activity was detected against *T. rubrum* and *C. guilliermondii* with a MIC of 6.5 μg/ml for both extracts. The clinical isolate from *C. guilliermondii* was less sensitive than the ATCC sample, ratifying the literature data about the higher resistance of strains recently isolated [63]. In addition, *Z. joazeiro* and *C. pyramidalis* extracts presented low acute toxicity in murine models. Accordingly, these results corroborate that the extraction procedure and the part of the plant utilized are crucial to reveal the plant bioactive potential [64]. The cited study validates the folk use of these plant extracts and indicates that they can be effective potential candidates for the development of the strategies to treat fungal infections [65].

In the search for bioactive compounds, bioautography and ethanol precipitation of macromolecules (proteins, polysaccharides, etc.) of plant aqueous extracts of *Xanthosoma sagittifolium, Anacardium occidentale, Solanum* sp., *Momordica charantia, Schinus molle,* and *Schinus terenbinthifolius* were associated in an antifungal screening. Thus, the supernatants, precipitates (obtained by ethanol precipitation) and aqueous extracts of medicinal and fruit bearing plants used against skin diseases by the Brazilian population were investigated. Among the microorganisms tested, the plant extracts showed activity against three fungi: *C. albicans, T. rubrum* and *C. neoformans.* The most susceptible fungus was *T. rubrum* and the best antifungal activity was shown by *X. sagittifolium* supernatant. The separation of macromolecules from metabolites, as in the case of *X. sagittifolium, A. occidentale* and *Solanum* sp., enhanced antifungal activity. In other cases, the antifungal activity was abolished, as observed for *M. charantia, S. molle* and *S. terenbinthifolius.* All the crude aqueous extracts were tested on peritoneal mouse macrophages and on a transformed cell line (VERO). No cytotoxic effects were observed on mammalian cells at the concentrations used [15].

Different studies showed the antimicrobial activity of several essential oils against bacteria, yeasts, and filamentous fungi [59, 66, 67]. In addition, their therapeutic potential, mainly in diseases involving mucosal, cutaneous and respiratory tract infections, was also demonstrated. The major constituents of these essential oils are phenolic compounds (terpenoids and phenylpropanoids) like thymol, carvacrol or eugenol, of which antimicrobial and antioxidant activities are well documented [68]. Nevertheless, aromatic plants producing non-phenolic essential oils, like some *Artemisia* species, are also used as spices and in folk remedies as antiseptics. *Artemisia* oils had inhibitory effects on the growth of bacteria (*E. coli, S. aureus*, and *S. epidermidis*), yeasts (*C. albicans* and *C. neoformans*), dermatophytes (*T. rubrum, Microsporum canis, and Microsporum gypseum*), *F. pedrosoi*, and *Aspergillus niger*. *A. biennis* oil was the most active against dermatophytes, *C. neoformans, F. pedrosoi,* and *A. niger* and *A. absinthium* oil, the most active against *Staphylococcus* strains. In addition, antioxidant (beta-carotene/ linoleate model) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities were determined, and weak

activities were found for these oils. The oils obtained from *Artemisia* species investigated are quite interesting from a pharmaceutical standpoint because of their antifungal properties. For instance, *M. gypseum* is known to cause skin, hair and scalp infections in humans and domestic animals [69] and thus *A. biennis* oil can be a good candidate for medicated skin and hair care formulations. Further investigations are in progress to compare the levels of activity in *Artemisia* oils and some of their constituents with the objective of identifying plant substances for future antifungal formulations [9].

Lippia alba is an aromatic herb that occurs in practically all regions of Brazil and has a large importance in Brazilian folk medicine. The chemical composition of *L. alba* and *Lippia alba* f. *intermedia* is very similar. Although quantitative differences are verified, *L. alba* has a much higher content of monoterpene hydrocarbons (21.7%) than *L. alba* f. *intermedia* (8.7%) due to a considerable presence of myrcene in its essential oil, leading to their separation into different chemotypes. Concerning antifungal activity, both essential oils were active against the fungi assayed showing large inhibition zones, larger or similar to those obtained for the positive control (amphotericin B) in all cases (Table **2**). Thus, it can be suggested that the antimicrobial activity of essential oils of *L. alba* and *L. alba* f. *intermedia* is probably related to the high content of oxygen-containing monoterpenes [50]. In a different study, the antifungal activity of *L. origanoides* essential oil, with high carvacrol content (38.6%), was demonstrated (Table **2**) [52].

The essential oil of *Dracocephalum foetidum*, a popular essential oil used in Mongolian traditional medicine, was examined for its antimicrobial activity against *C. albicans* and *Saccharomyces cerevisiae* and exhibited strong activity. The chemical composition of this oil analyzed by GC-MS identified several major components, including *n*-mentha-1,8-dien-10-al, limonene, geranial, and neral [17].

The eugenol-rich essential oil of *Ocimum gratissimum*, plant known as "alfavaca" (Labiatae family), has been reported with *in vitro* activity against several fungi as *T. rubrum, T. mentagrophytes, C. neoformans* [70], *C. albicans, C. krusei, C. parapsilosis*, and *C. tropicalis* [71]. These results are in agreement with the studies from Boonchird and Flegel [72] that showed the *in vitro* activity of eugenol against *C. albicans* and *C. neoformans*. Eugenol is a component of sealers and pastes used in endodontics, and it has been associated with antibacterial, antifungal and antiviral activity. Although many studies have focused on showing the antifungal activity of plant extracts or essential oils, few have demonstrated their effects on the morphology and ultrastructure of the fungi. In the study of *O. gratissimum* essential oil against *Candida* species, analyses of the ultrastructure of yeast cells revealed changes in the cell wall and in the morphology of some subcellular organelles. Bud formation in the yeasts was impaired in treated cells. For comparison, the yeasts were also tested with nystatin, which was more effective than this essential oil at lower concentrations. But, differently from nystatin, the essential oil showed to be an effective fungicide and a potential candidate as a phytotherapeutic agent in some fungal diseases and for the control of fungi in the environment [73].

The anti-Candida activity was also described to essential oils of medicinal plants commonly used in Brazil including *Aloysia triphylla, Anthemis nobilis, Cymbopogon martini, Cymbopogon winterianus, Cyperus articulatus, Cyperus rotundus, L. alba, Mentha aryensis, Mikania glomerata, Mentha piperita, Mentha* sp., *Stachys byzantina,* and *Solidago chilensis.* Whereas the ethanolic extracts from the leaves and /or roots of these plants were not effective at any of the concentrations tested, chemical analyses of these essential oils showed the presence of compounds with known antimicrobial activity, including 1,8-cineole, geranial, germacrene-D, limonene, linalool, and menthol [51].

The use of essential oils as antimicrobial agents has been described qualitatively for many years, but little is known about its spectrum of action against oral microorganisms.

Table 2. Growth Inhibition Zones of Fungi Species by Essential Oils in Millimeters[*]

Microorganisms	Inhibition zone diameter (mm)			
	Lippia alba f. intermedia[¨]	***Lippia alba***[¨]	***Lippia origanoides***[¨]	**Amphotericin B**[¨¨]
Candida albicans Serotype B ATCC 36802	35.6	23	25	20
Candida albicans	23.6	29	27	16
Candida guilliermondii	40	35	40	24
Candida parapsilosis	12	25	35	18
Cryptococcus neoformans T1-444 Serotype A	20.6	21	24	20
Trichophytum rubrum T-544	50	30	30	20
Fonsecaea pedrosoi 5VPL	15	18	40	18

[*]Antimicrobial assay was carried out using the drop agar diffusion method [49].

[¨]The essential oils were diluted 1:1 with Tween 80 (0.5% in water).

[¨¨]Reference antibiotics as positive controls – 1 mg/ml in Tween 80 (0.5% in water)
Negative control – Tween 80 (0.5% in water) [50, 52].

The dental plaque is a complex biofilm accumulated onto the surface of hard tissues (teeth), to which the growing microbial population adheres [74]. After the initial bacterial colonization, plaque evolves to contain a variety of microorganisms, including *Candida* species, many of which are potential periodontal pathogens. *C albicans* is one of many *Candida* species isolated from humans and is responsible for the majority of oral yeast infections [75]. It is a pleomorphic, opportunistic pathogen that exhibits a number of different morphological forms under different environmental conditions [76]. Antimicrobial effects of a linalool-rich essential oil from *Croton cajucara* demonstrated that linalool is the major component involved in the antifungal activity of this essential oil. Comparing the MIC's of *C. cajucara* essential oil and purified linalool for the growth of *C albicans*, with the standard drug chlorhexidine, it was concluded that the linalool has a potent antifungal effect. Artificial biofilms were prepared and then exposed to linalool or to the essential oil. Confirming the results above, the profiles of inhibition from the essential oil and the purified linalool fraction were similar to that observed for the standard drug chlorhexidine against the reference strain and the human isolate.

Accordingly, by bioautography, the purified linalool fraction was confirmed as the antifungal component of the essential oil. The effects of linalool on the cell biology of *C. albicans* were evaluated by electron microscopy, which showed that linalool induced the reduction in cell size and abnormal germination (Fig. (**1**)). Neither the crude essential oil nor the purified linalool fraction is toxic to mammalian cells, suggesting its use as mouthwash to control the oral microbial population. This condition makes mandatory the development of experimental models evaluating the pharmacological applicability of essential oil components as antimicrobial agents. In addition, repeated use of antimicrobial agents on biofilms can select drug-resistant microbes. In this context, new agents that can inhibit the growth of biofilm-associated microorganisms are greatly needed and would enhance the number of effective therapeutic alternatives. It has been demonstrated that mouthwashes with essential oils can be a beneficial, safe component of daily oral health routines, but the antimicrobial activity and selective toxicity of these preparations, as well as their chemical composition, are poorly known [47].

The envelope of *C albicans*, with its outermost array of macromolecules protruding towards the environment, is pivotal to the expression of major virulence factors such as adhesiveness and the morphological transition to hyphal form. Eugenol, main component of clove oil, and thymol, main component of thyme oil, alone or in combination interfered on the morphogenesis of the envelope, but the effects of thymol were more pronounced than those of eugenol. The potentiated antifungal action has also been observed when thymol is combined with amphotericin B. Interference with the morphology of the envelope of *C. albicans* is a useful feature because it means interfering with the important virulence factor of adhesiveness and the morphological transition to hyphal form thus reducing the ability of the fungus to colonize host tissues and decreasing its infectiousness [77]. Therefore, a search for new antifungal drugs is extremely necessary and urgent.

Recently, a comparative study evaluated the chemical composition, antioxidant and antimicrobial activities of *Anacardium occidentale* L. (fruits), *Xanthosoma sagittifolium* (L.) Schott (cormel), *Malpighia glabra* L. (fruits), and *Rosmarinus officinalis* (leaves) aqueous extracts obtained after ethanol precipitation [14]. Samples were evaluated as radical scavengers by the DPPH radical scavenging method. High pressure liquid chromatography – dioxide array detector (HPLC-DAD) chromatograms were performed to identify the flavonoids and the broth dilution method, to determine the antimicrobial activity of plant extracts against three different pathogenic fungi, *C. neoformans, C. albicans*, and *T. rubrum. A. occidentale* and *M. glabra* showed DPPH radical scavenging activity above 90% at 100 μg/ml. The content of flavonoids can influence the antioxidant activity as it was the case for *A. occidentale* and *X. sagittifolium*. In relation to *M. glabra*, the antioxidant activity may be due to its content of vitamin C and anthocyanins not detected under our HPLC-DAD conditions. The flavonoid content of *A. occidentale* and *X. sagittifolium* influenced antimicrobial activity, but flavonoids in *M. glabra* and *R. officinalis* had no antimicrobial activity. In relation to *M. glabra* and *R. officinalis*, the antioxidant activity may be due to other classes of compounds such as vitamin and anthocyanins, not detected under the HPLC-DAD used conditions. The antimicrobial activity does not seem to be connected to the flavonoid content. However, the species studied have great potential as nutraceutical due to their antimicrobial and antioxidant activities [14].

ANTIPROTOZOAL ACTIVITY

The number and efficacy of drugs available for the treatment of human and animal trypanosomiasis, leishmaniasis, amoebiasis and malaria are limited. Considering the side effects and resistance that pathogenic protozoan parasites develop against these drugs, more attention should be given to the extracts and biologically active compounds isolated from plant species commonly used in herbal medicine [47].

Leishmaniasis is a group of diseases with a large spectrum of clinical manifestations caused by protozoans of the genus *Leishmania*, which affect about 12 million people worldwide and are responsible for high rates of mortality in tropical and subtropical countries [78]. The control of leishmaniasis remains a problem because no vaccines exist and the available chemotherapy still relies on the potentially toxic pentavalent antimonials which cause side effects, require long-term treatment and systemic use [79]. The rise in the rates of *in vitro* antimonial resistance due to intermittent drug exposure, the isolation of antimonial –resistant *Leishmania* strains from patients with unresponsive cutaneous leishmaniasis [80], and recently, the numerous cases of visceral leishmaniasis among patients infected with the human immunodeficiency virus make the search for new agents for the treatment of leishmaniasis urgent [81]. Extensive studies of new drugs with antileishmanial activities, including both natural products and synthetic compounds, have been undertaken worldwide, although problems with the side effects of the chemotherapies used at present have not yet been solved. In recent years, there has been growing interest in alternative therapies and in the use of natural products, especially those derived from plants [47].

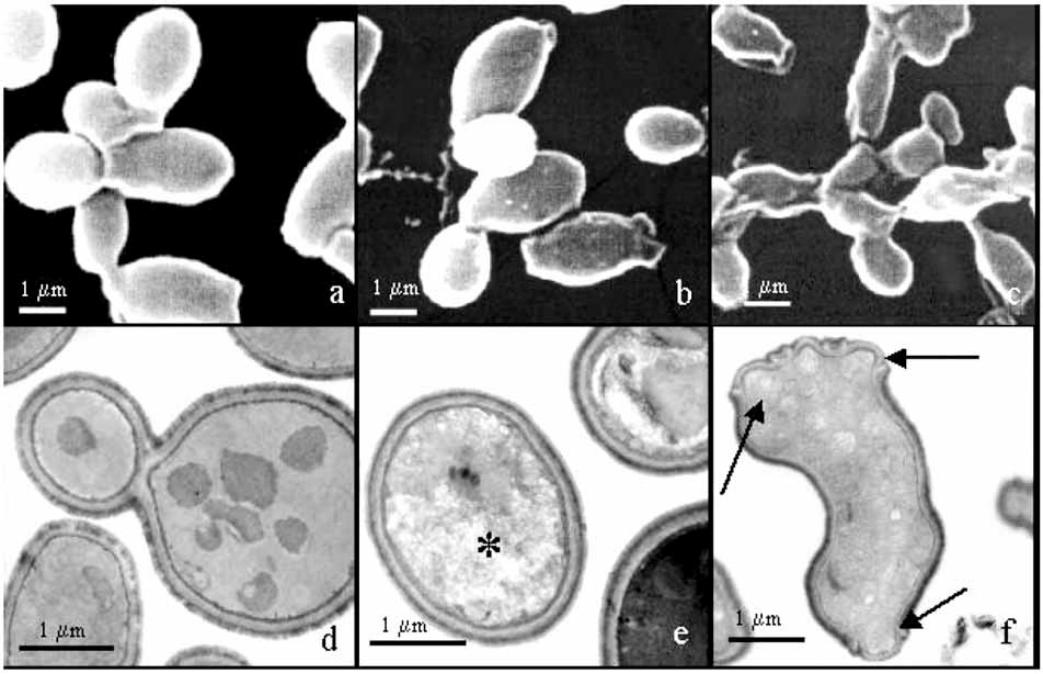

Fig. (1). Scanning electron microscopy (SEM) **(a-c)** and transmission electron microscopy (TEM) **(d-f)** analyses of non-stimulated **(a** and **d)**, essential oil-treated **(b** and **e)**, and linalool-stimulated **(c** and **f)** *C. albicans*. The asterisk denotes rarefaction of the cytoplasmic matrix in yeast cells exposed to the essential oil. Treatment of *C. albicans* with purified linalool showed an irregular aspect and a disordered profile of germination (arrows) [47].

Clinical reports indicate that a large proportion of cases are becoming unresponsive to chemotherapy. Variable efficacy, toxicity, requirement for long courses of parenteral administration, resistance or a combination of these factors have been reported [82, 83]. The recognition and validation of traditional medical practices and the search for plant-derived drugs could lead to new strategies for leishmaniasis control [84].

In recent years, an alarming increase in the incidence of resistance to pentavalent antimonials has led to the introduction of newer antileishmanial agents that include Miltefosine, Amphotericin B, Ketoconazole and Paramomycin. However, as none of these drugs are free of adverse effects, the search for alternative therapeutic agents is essential.

Although the amastigote-macrophage model is considered as the gold standard for establishing the drug sensitivity profile of an antileishmanial compound [85], it is a relatively cumbersome procedure. Recently, a modified MTT [3-(4,5-dimethylthiazol -2-yl) -2,5 –diphenyl tetrazolium bromide] assay was proposed and could be applied for preliminary screening of field isolates and screening of new antileishmanial agents thus representing a useful tool in *Leishmania* research [86].

The eugenol rich essential oil of *Ocimum gratissimum* progressively inhibited *L. amazonensis* growth, one of the major agents of diffuse cutaneous leishmaniasis, which is usually unresponsive to all known treatments. The IC50 (sub inhibitory concentration) of the essential oil for promastigotes and amastigotes were respectively 135 and 100 μg/ml and the IC50 of eugenol was 80 μg/ml for promastigote forms. All *L. amazonensis* parasites were destroyed by both essential oil and eugenol (at 100 μg/ ml), after 60 min treatment, regardless of the developmental form of the parasites (promastigote or amastigote). Mitochondrial/morphological alterations occurred at the ultrastructural level in the *L. amazonensis* parasites after treatment with *O. gratissimum* essential oil, shown by transmission electron microscopy (Fig. (**2A**)). *Leishmania* promastigotes are susceptible to killing by exposure to superoxide and radicals generated from hydrogen peroxide. A second anti-leishmanial oxidant produced by macrophages is nitric oxide (NO), which is generated after macrophages activation by interferon-γ (IFN- γ) and tumor necrosis factor- α (TNF-α) and is most important to killing of established intracellular amastigote [87]. Despite the sound knowledge of NO-dependent killing of *Leishmania* by macrophages, little is known of the effect of plant extracts or their purified compounds on this type of macrophage response. The minimum inhibitory concentration of *O. gratissimum* essential oil for both promastigotes and amastigotes was 150 μg/ml. Pretreatment of mouse peritoneal macrophages with 100 and 150 μg/ml essential oil reduced the indices of association between promastigotes and the macrophages, followed by an increase in nitric oxide production by infected macrophages. The essential oil showed high toxicity to *L. amazonensis*, with no cytotoxic effects against mammalian cells. These results suggest that *O. gratissimun* essential oil and its compounds could be used as a source for new antileishmanial drugs [78].

The *in vitro* leishmanicidal effects of a linalool-rich essential oil from of *Croton cajucara* against *L. amazonensis* promastigotes treated with 15ng/ml of essential oil were observed by transmission electron microscopy. Leishmanial nuclear and kinetoplast chromatin destruction, followed by

cell lysis, was observed within 1h (Fig. (**2B**)). Pretreatment of mouse peritoneal macrophages with 15 ng/ml of essential oil reduced by 50% the interaction between these macrophages and *L. amazonensis*, with a concomitant increase by 220% in the level of NO production by the infected macrophages. Treatment of pre-infected macrophages with 15 ng/ml of essential oil reduced by 50% the interaction between these cells and the parasites, which led to a 60% increase in the amount of NO produced by the pre-infected macrophages. This linalool-rich essential oil is a strikingly potent leishmanicidal plant extract, which inhibited the growth of *L. amazonensis* promastigotes at very low concentrations (MIC 85.0 pg/ml) and which presented no cytotoxic effects against mammalian cells [47].

The minimal inhibitory concentration of the polyphenolic extract from the husk fiber of *C. nucifera* Linn. (Palmae) to completely abrogate parasite growth was 10 μg/ml. Corroborating this result, microscopic observation showed complete lysis of promastigote cells after treatment of parasites with 10 or 20 μg/ml of *C. nucifera* polyphenolic-rich extract for 60 min**.** The same profile and morphological alterations were observed in the amastigote forms. Pretreatment of peritoneal mouse macrophages with 10 μg/ml of *C. nucifera* polyphenolic-rich extract reduced approximately in 44% the association index between these macrophages and *L. amazonensis* promastigotes, with a concomitant increase of 182% in NO production by the infected macrophages in comparison to no treated macrophages. This extract of *C nucifera* at 10 μg/ml

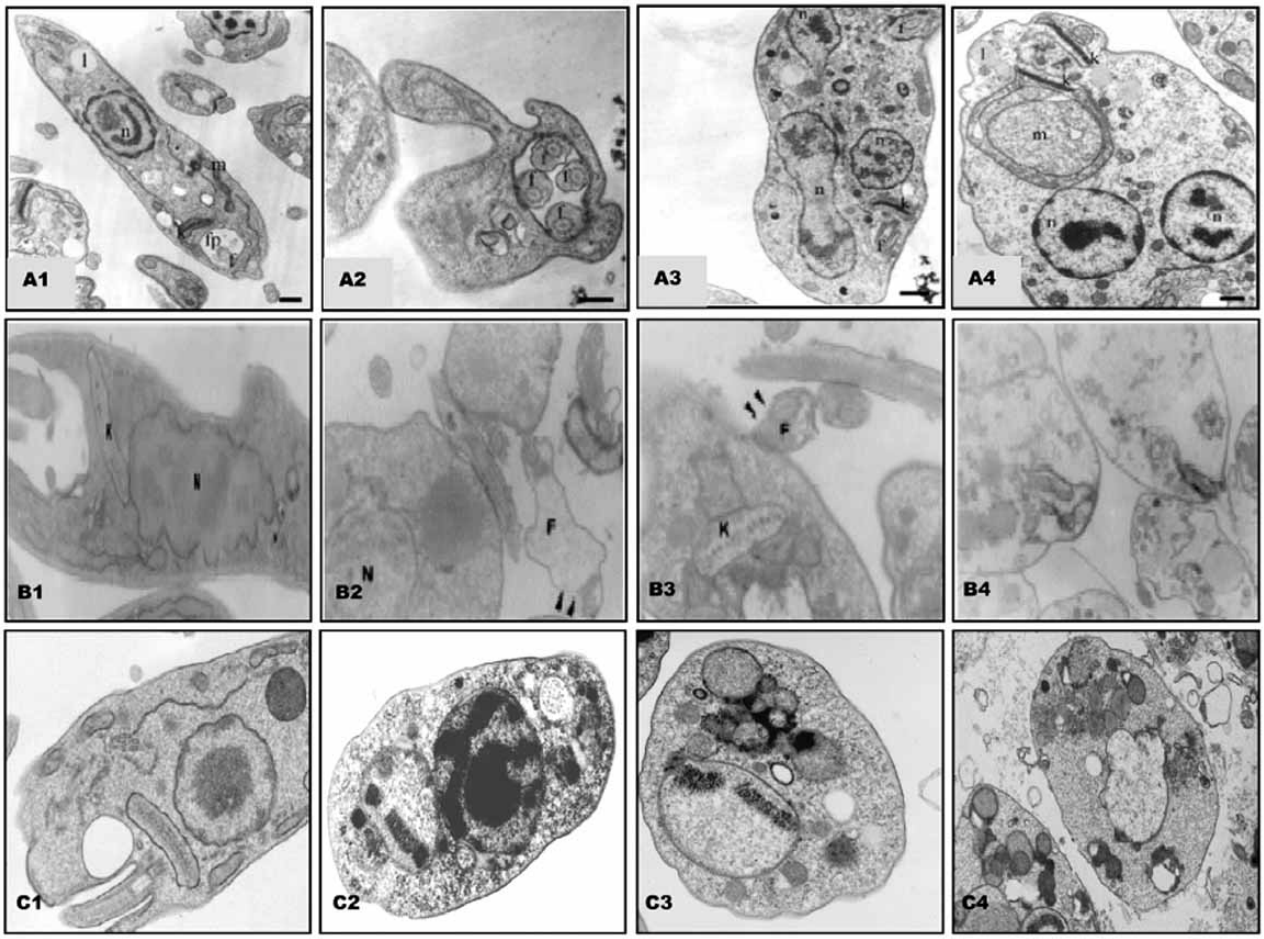

Fig. (2). Effects of different crude extracts from Brazilian plants on Trypanosomatidae species observed by transmission electron microscopy. **(A)** *L. amazonensis* promastigotes cultivated in Warren medium **(A1**, **A2**, and **A3)** and amastigotes cultivated in Schneider's Drosophila medium **(A4)**, treated with *Ocimum gratissimum* essential oil at 100 μg/ml (IC50 for amastigotes). **(A1)** Untreated promastigotes; **(A2** and **A3)** promastigotes treated with essential oil at 135 μg/ml (IC50 for promastigotes) showing four flagella or four nucleuses; **(A4)** Note amastigote form with mitochondrial swelling and two nucleuses; m, mitochondrion; fp, flagellar pocket; f, flagellum; l, lipid inclusion; n, nucleus; k, kinetoplast [78]. **(B)** Antileishmanial activity of linalool-rich essential oil (15.0 ng/ml) extracted from *C. cajucara* on promastigote forms of *L. amazonensis.* **(B1)** Control parasites; **(B2** to **B5)** parasites treated for 5 **(B2)**, 10 **(B3)**, and 30 **(B4)** min, showing promastigotes with different degrees of damage. Note the disruption of flagellar membranes **(**arrowheads in panels **B2** and **B3)**, the mitochondrial swelling **(B3)**, and the gross alterations in the organization of the nuclear and kinetoplast chromatins **(B3)**. After 30 min in the presence of essential oil, the parasites were completely destroyed **(B4)**. N, nucleus; K, kinetoplast, F, flagellum [47]. **(C)** Effects of linalool-rich essential oil (30 ng/ml) extracted from *Croton cajucara* on epimastigote forms of *Trypanosoma cruzi*. **(C1)** Control parasites; **(C2** to **C4)** parasites treated for 2 **(C2)**, 5 **(C3)**, 20 **(C4)** min, showing epimastigotes damage. Disruption of flagellar membranes, mitochondrial swelling and the gross alterations in the cellular organization could also been note [I. A. Rodrigues, unpublished].

is a strikingly potent leishmanicidal substance, presenting no *in vivo* allergenic reactions or *in vitro* cytotoxic effects in mammalian systems [84].

Despite the great advances to control the American trypanosomiasis transmission, like vector chemical attack or systemic screening of blood donors, more effective chemotherapy is needed for people who are already infected [88]. The primary drugs nifurtimox and benznidazol have been in use for 40 years and are effective only during the early acute stage of infection and are poorly tolerated [89].

Chagas' disease, or American Trypanosomiasis, is caused by infection with the protozoan parasite *Trypanosoma cruzi*. The prevalence of the infection is estimated in 16-18 million cases and approximately 100 million people are at risk of infection in the Americas [90]. There is no efficient treatment and the drugs that are approved for clinical use, benznidazole and nifurtimox, reduce symptoms and mortality only in acute illness. Once the disease has progressed to later stages, no medication has been proven to be effective [91]. Moreover, Chagas' disease chemotherapy is still a challenge, since both available drugs are highly toxic [92]. Mitochondrial swelling and important alterations in the organization of the nuclear and kinetoplast chromatin could be seen in electron microscopy, when *T. cruzi* parasites were treated for 2-20 min with 30 ng/ml essential oil from *C. cajucara* (Fig. (**2C**)) [I.A. Rodrigues, unpublished].

Mamani-Matsuda and co-workers. [93] described the trypanocidal activity of quercetin, a food-derived flavonoid that directly promoted the death by apoptosis of *Trypanosoma brucei gambiense*, the causative agent of sleeping sickness [93]. The essential oil from *C. cajucara* was able to promote macrophage activation with an improvement of NO production. When infected macrophages were previously treated with the essential oil, 15 and 30 ng/ml, NO production was respectively 47 and 181% higher than that of the non-treated control. Besides, when both macrophages and parasites were post-treated with 30 ng/ml of the essential oil, the NO production were 168% higher than control. These results are in agreement with those obtained with association index assay, where we could observe that low index corresponded to high levels of NO suggesting that NO could be an important tool for the clearance of the infection [I.A. Rodrigues, unpublished]. Trypanosome-derived products were also shown to activate the generation by macrophages of various pro-inflammatory mediators including TNF-α, NO, and interleukin-1 (IL-1) [94]. TNF-α and NO fulfill important functions in host-parasite interactions as they control infections by various pathogens, including *T. cruzi* [95]. Then, the finding that macrophages pretreated with *C. cajucara* essential oil produced approximately twice the amount of NO as compared to the non-treated macrophages is hardly surprising. Linalool purified fraction was extremely potent when used directly on *T. cruzi* parasites but it had little effect when used on the assays for measuring the association between macrophages and parasites, as well as for measuring the NO production by the infected macrophages [I.A. Rodrigues, unpublished]. These data could be explained by synergistic effects of different compounds of the plant extracts used in this work, which is a phenomenon largely known in several other systems [96]. On the other hand, the decrease in the association between macrophages and parasites, concomitant with the increase in NO production by the infected macrophages when both cell types were pretreated with essential oil, was less than when macrophages alone were pretreated. The extreme toxicity of *C. cajucara* leaf extracts to *T. cruzi*, with no effect upon mammalian cells, enables linalool-rich essential oil as a source of a new lead compound for novel antitrypanosomal drugs [I.A. Rodrigues, unpublished].

*Giardia lamb*lia, the causative agent of giardiasis, is one of the most common causes of intestinal infections in humans worldwide. The current treatments of giardiasis include nitroimidazoles (usually metronidazole), nitrofurans, quinacrene or paromomycin. However, all of these drugs are reported to have unpleasant side effects, and potential carcinogenic, teratogenic and embryogenic questions are being addressed. In developed nations, besides being the most common intestinal protozoan parasite of humans, a rising incidence of giardiasis has been noted for children in day care centers, which has led to the designation of giardiasis as a "reemerging" infectious disease [97]. In the developing world however, there is a very high prevalence and incidence of this infection, and data suggest that long-term growth retardation can result from chronic giardiasis [98]. In the young, elderly, and immunocompromised, giardiasis can lead to morbidity and even death [99]. Current antigiardial treatments are reported to have unpleasant side effects, and resistant strains are increasingly developing. In this regard, the search for new effective alternative treatments with low- or non-toxicity is extremely important. The effect of *Ocimum basilicum* essential oil on *G. lamblia* and on the modulation of these parasites ingestion by peritoneal mouse macrophages and NO production shows that this oil (2 mg/ml) was able to kill almost 80% *G. lamblia* trophozoites in a time course of 2 h. Linalool (69%) and eugenol (10%) are the principal components of essential oil of *O. basilicum*. Linalool (300 μg/ml), however, was able to kill 100% parasites after 1h of incubation, which demonstrated its high antigiardial potential. Pretreatment of peritoneal mouse macrophages with 2 mg/ml essential oil dilution reduced in 79% the association index between these macrophages and *G. lamblia*, with a concomitant increase by 153% on NO production by *G. lamblia*-ingested macrophages**.** The proteolitic activity, mainly of cysteine proteases, was clearly inhibited by the essential oil (2 mg/ml) and the purified linalool (300 μg/ml) .These results suggest that, with *G lamblia*, the essential oil from *O basilicum* and its purified compounds, specially linalool, have a potent antimicrobial activity [100].

ANTIOXIDANT ACTIVITY

Peroxidative processes and free radicals have been the subject of intense research in recent years, since they have been implicated in numerous diseases, including cardiovascular disease, some forms of cancer, inflammatory ailments and neurodegenerative disorders [101, 102]. Free radicals released during oxidative stress are among the widespread intracellular DNA modifiers [103].

The deteriorative effects of the free radicals can be diminished by natural antioxidants available in foods. There has been a growing interest in plants as natural sources for

the prevention of oxidation, controlling pathogens and/or toxin-producing microorganisms in foods, and in the treatment of several diseases as therapeutics. Search for novel antioxidants acting in chemoprevention of relevant biomolecules oxidation is a promising field in phytotherapy [103].

Medicinal plants are often more or less unknown scientifically and therefore of potential interest as new sources of medicinally active antioxidants [104, 105]. Several naturally occurring compounds, such as flavonoids and carotenoids, are antioxidants. Proanthocyanidins and other flavonoids are polyphenols, a class of substances that seems particularly effective in this respect, and conceivably may play an important role as dietary antioxidants. It has been suggested that, in many cases, proanthocyanidins are active principles in medicinal plants [106]. For these reasons, polyphenols have attracted widespread attention in the fields of nutrition and health, and it has been suggested that they may modulate key biological pathways *in vivo* in mammals [107].

Proanthocyanidins [108] as well as other antioxidants, e.g. vitamin E [109], are used extensively in therapy of skin diseases. Ailments such as dermatitis and itching are treated topically with *Lannea velutina* bark preparations. The alcoholic extracts of root bark of *L. velutina* are effective 15-lipoxygenase inhibitors and excellent radical scavengers in non-cellular and cellular systems. This effect is mainly due to their high content of proanthocyanidins. These results can explain the use of *L. velutina* in ailments such as gastric ulcer, dysentery and dermatitis [110].

C. nucifera extracts are mainly composed of catechin and epicatechin together with condensed tannins (ß- type procyanidins) [31]. These classes of molecules have been associated with antinociceptive and free radical scavenging activities [16]. Procyanidins from grape seeds were also shown to protect endothelial cells which may be correlated with the free radical scavenging properties. Additional *in vivo* assays indicate that *C. nucifera* aqueous extracts possess low toxicity and do not induce dermic or ocular reactions [16].

The free radical scavenging properties of the *C. nucifera* (husk fiber), *Z. joazeiro* (inner bark), *C. pyramidalis* (leaves) aqueous extracts, and *A. cymbifera* (rhizomes) alcoholic extract were analyzed using the DPPH photometric assay, a rapid and inexpensive antioxidant assay (Table **3**). The effective concentration (EC50) values were determined, and the results obtained for *C .nucifera* and *C. pyramidalis* were comparable to that obtained using extracts from *Hyptis tetracephala, Lantana camara, Lantana trifolia, Verbena litoralis, Vitex polygama* and *Ginkgo biloba*. All these species are Brazilian plants with proved antioxidant activity, as detected by the use of the same technique [27]. Concerning the free radical scavenging activity, the superiority of the *C. nucifera* extract could be attributed to the presence of polyphenols, confirming the results obtained in a previous work [16].

Flavonoids and other plant phenolics are reported to have multiple biological activities and one of these is their antimicrobial property in addition to their free radical scavenging activity [111]. Studies on edible plants such as *Anacardium occidentale, Xanthosoma sagittifolium, Malpighia glabra,* and *Rosmarinus officinalis*, previously evaluated as antimicrobials, showed that they may also be potential sources of flavonoids with antioxidant activity. Concerning *A. occidentale* and *X. sagittifolium* extracts, the content of flavonoids has influence on the antioxidant and antimicrobial activities, but in *M. glabra* and *R. officinalis* the antimicrobial activity does not seem to be connected to the flavonoid content [14].

Table 3. Free Radical Scavenging Properties of the Plant Extracts Analyzed Using the DPPH Photometric Assay

Plant extracts	Antioxidant EC50 values (µg/ml)*
Aristolochia cymbifera	124.5 ± 6.6
Caesalpinia pyramidalis	15.2 ± 1.0
Cocos nucifera	10.0 ± 0.7
Ziziphus joazeiro	821.4 ± 35.3

*EC50 values represent the mean and standard deviation obtained from three independent assays [16].

Many medicinal properties, including antiseptic, bacteriostatic, antimycotic, antiprotozoan, antiviral, spasmolytic, astringent, anti-inflammatory and immuno-stimulatory activities, have been ascribed to propolis [112, 113]. Propolis contains a variety of chemical compounds such as polyphenols, flavonoids, sesquiterpenes, quinones, coumarins, steroids, amino acids and inorganic compounds. It has been suggested that the biological activities mainly depend on the presence of a large number of flavonoids. In addition, some propolis produce allergic contact dermatitis due to the presence of cinnamic acid and caffeic acid esters [114]. The correlation between flavonoid content and scavenging of free radical activity is significant, but obviously, other factors are involved. These might be different flavonoid composition or the presence of non-flavonoid scavengers. Moreno and coworkers [115] also determined that propolis possesses free radical-scavenging activity, which justify its use as a source of natural antioxidants.

Melissa officinalis L. (lemon balm), popularly known in Brazil as erva-cidreira or melissa, is a traditional herbal medicine used widely as a mild sedative, spasmolytic and antibacterial agent. However, in the absence of a scientific basis, such practices may generate serious adverse effects. Sousa and co-workers [2] focused on the analysis of the chemical composition and the biological activities of *M. officinalis* essential oil. The results pointed that this oil possessed antitumoral and antioxidant activities, indicating, thus, that the antioxidant properties of *M. officinalis* essential oil might contribute to its antitumoral activity. The antitumor properties of several antioxidants have been described.

Origanum acutidens is an endemic, herbaceous and perennial plant and its essential oil possesses compounds with antimicrobial and antioxidant properties, although no antiviral effect can be observed. The free radical scavenging activity of the *O. acutidens* essential oil was high, and this was obviously related to the high content of carvacrol (72%,v/v), main oxidant constituents of the oils isolated from several *Origanum* species. This observation confirmed the findings

Table 4. Some Biologically Active Compounds from Plants Described by Our Group

Plants	Part used	Extract	Bioactive compounds	Biological activities	References
Aristolochia cymbifera	Rhizomes	Alcoholic extract	ND*	Antibacterial, antioxidant	[27]
		Hexane extract	Diterpene 2-oxo-populifolic acid	Antibacterial	[4]
Cocos nucifera	Husk fiber	Aqueous extract	ND*	Antibacterial	[27]
				Antiprotozoal	[84]
			Polyphenols	Antioxidant,	[27]
			Catechin-rich fraction	Antiviral (HSV -1- ACVR)	[31]
Caesalpinia pyramidalis	Leaves	Aqueous extract	ND*	Antibacterial, antioxidant	[27]
				Antifungal	[65]
Ziziphus joazeiro	Inner bark	Aqueous extract	ND*	Antibacterial, antioxidant	[27]
				Antifungal,	[65]
Hyptis pectinata	Leaves	Essential oil	ND*	Antibacterial	[22]
Croton cajucara	Leaves	Essential oil	Linalool	Antibacterial, antifungal	[47]
				Antiprotozoal	[48]
Lippia alba mircene citral-chemotype	Leaves	Essential oil	ND*	Antibacterial, antifungal	[51]
Lippia alba linalool-chemotype					
Lippia origanoides	Leaves	Essential oil	Carvacrol-rich essential oil	Antifungal	[52]
Xanthosoma sagittifolium	Leaves, stalks and roots	Aqueous extract	Flavonoids	Antifungal	[15]
				Antioxidant	[14]
Anacardium occidentale	Leaves and fruits	Aqueous extract	Flavonoids	Antifungal	[15]
				Antioxidant	[14]
Solanum sp.	Leaves, stalks and roots	Aqueous extract	ND*	Antifungal	[15]
Momordica charantia	Leaves, stalks and roots	Aqueous extract	ND*	Antifungal	
Schinus molle	Leaves	Aqueous extract	ND*	Antifungal	[15]
Schinus terenbinthifolius	Leaves, stalks and roots	Aqueous extract	ND*	Antifungal	
Malpighia glabra	Fruits	Aqueous extract	ND*	Antifungal	[14]
			Vitamin C and anthocyanins	Antioxidant	
Rosmarinus officinalis	Leaves	Aqueous extract	ND*	Antifungal	
			Vitamin and anthocyanins	Antioxidant	
Ocimum gratissimum	Leaves	Essential oil	Eugenol	Antiprotozoal	[78]
Ocimum basilicum	Leaves	Essential oil	Linalool and eugenol	Antiprotozoal	[100]
Melissa officinales	Leaves	Essential oil	ND*	Antioxidant	[2]

*Bioactive compound not identified (ND) yet.

in the previous studies reporting that the essential oils contain remarkable antimicrobial substance (carvacrol in particular) compared to plant extracts [116, 117]. The remarkable antioxidant and antimicrobial properties of *O. acutidens* essential oil against some bacteria and fungi are quite interesting for the prevention of oxidation, controlling pathogens

and/or toxin-producing microorganism in foods, as well as therapeutics for the treatment of several diseases [118].

Aromatic plants producing non-phenolic essential oils, like some *Artemisia* species, are also used as spices and in folk remedies as antiseptics. Antioxidants retard oxidation and are sometimes added to meat and poultry products to prevent or slow oxidative degradation of fats. The potential antioxidant activity of the oils was determined on the basis of the scavenging activity of the stable free radical DPPH and inhibition of the coupled oxidation of linoleic acid and β-carotene. This was the first study to provide data that these essential oils possess antibacterial and antifungal activities, with weak antioxidant abilities [9].

Liskmann and co-workers [119] evaluated the levels of various antioxidants in saliva to identify differences between the saliva of patients with healthy peri-implant tissues and patients with peri-implant disease. They also examined whether the whole saliva of those with peri-implant disease conditions had lower levels of antioxidants than that of healthy individuals. As results, the total antioxidant status of saliva and concentration of uric acid and ascorbate, which are the main salivary antioxidants, were significantly decreased in patients with peri-implant disease. Besides the importance for these data for the better understanding of the processes involved in the pathogenesis of peri-implant disease, the authors also suggest that the treatment of peri-implant disease may involve adjuvant antioxidants supplementation together with the recommended treatment. Numerous antioxidants have been tried and tested both by systemic administration and as mouthwashes. These include synthetic products, like vitamins, and natural products like wine and green tea. As described previously, naturally occurring substances in higher plants have antioxidant activity, which are of great application in the control of chronic disorders caused by oxygen containing free radicals [119].

CONCLUSION

This review records several results about the use of essential oils, crude extract and fractions of medicinal plants (with potential bioactive properties that exhibited significant antimicrobial and antioxidant properties) that support their folkloric use in the treatment of some diseases as broad-spectrum antimicrobial agents (see Table **4**). Attention to this issue could usher in a badly needed new area of chemotherapeutic treatment of infection by using plant-derived principles. However, different kinds of studies on the mechanisms of action, interactions with antibiotics or other medicinal plants or compounds, and the pharmacokinetic profile of the extracts should be given high priority. Finally, additional tests, including development of experimental models evaluating the pharmacological applicability, are required before considering these plants extracts real promising compounds.

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ABBREVIATIONS

CG-MS	=	Chromatography gas - mass spectrometry
DPPH	=	2,2-diphenyl-1-picrylhydrazil
EC50	=	Effective concentration (required to induce a 50% effect)
FRSA	=	Free radical scavenging activity
HPLC-DAD	=	High pressure liquid chromatography – dioxide array detector
HSV-1-ACVR	=	Acyclovir-resistant herpes simplex virus
IC50	=	The 50% inhibitory concentration
IL-1	=	Interleukin-1
INF- γ	=	Interferon-γ
MBC	=	Minimal bactericidal concentration
MIC	=	Minimal inhibitory concentration
MTT	=	[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenil tetrazolium bromide]
NO	=	Nitric oxide
ROS	=	Reactive oxygen species
SEM	=	Scanning electron microscopy
TEM	=	Transmission electron microscopy
TNF-α	=	Tumor necrosis factor- α

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