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Traditional knowledge, medicinal plants, bioactive constituents, and prospecting technology: potential control of fungi

Conhecimento tradicional, plantas medicinais, constituintes bioativos e tecnologia de prospecção: potencial de controle de fungos

Conocimientos tradicionales, plantas medicinales, componentes bioactivos y tecnología de prospección: control potencial de hongos

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Antonio Fávero de Carvalho
ORCID: https://orcid.org/0000-0002-7339-5758
Universidade Federal de Sergipe, Brazil
E-mail: tonyfavero@hotmail.com
Allívia Rouse Carregosa Rabbani
ORCID: https://orcid.org/0000-0003-0564-7113
Instituto Federal de Educação, Ciência e Tecnologia da Bahia, Brazil
E-mail: alliviarouse@ifba.edu.br
Sheila Valéria Álvares-Carvalho
ORCID: https://orcid.org/0000-0003-0545-2703
Universidade Federal de Sergipe, Brazil
E-mail carvalhosva@gmail.com
Renata Silva-Mann
ORCID: https://orcid.org/0000-0001-5993-3161
Universidade Federal de Sergipe, Brazil
E-mail: renatamann@gmail.com

Abstract

The use of plants with medicinal properties for fungi control has led to a continuous exploration of new compounds that could contribute towards promising studies in the development of new drugs and the knowledge of how this control is performed on microorganisms. The objective of this review has been to report on the potential use of medicinal plants to control the pathogenic fungi of a host of plants and animals, which can contribute to the achievement of new formulations for botanical fungicides. Many authors have demonstrated antifungal and general antimicrobial activities for Brazilian flora species through well-established methods, such as by microdilution, agar diffusion, and disk diffusion, while determining a minimum inhibitory concentration (MIC), minimum fungicidal concentrations (MFC), and the inhibition potential of essential oils, extracts and fractions. In this review, 68 species were cited for occurring in Brazil, with 25 being in the north-northeastern part of the country. Thus, most studies about the antimicrobial activities of medicinal plants bring an 'initial understanding' of their potential, particularly of some species, genera, and even families. Nevertheless, more data that is exceedingly specific is mandatory by focusing on new and more accurate approaches, such as the action mechanisms, toxicity, the active components, and the verification of the existence of synergic effects. These criteria would be the minimum required to develop new natural products as alternative treatments for the various infectious pathologies that affect plants, animals, and human beings.

Keywords: Botanic fungicide; Mycoses; Patents; Essential oils.

Resumo

A utilização de plantas com propriedades medicinais para o controle de fungos tem levado a uma exploração contínua de novos compostos que podem contribuir para estudos promissores no desenvolvimento de novos fármacos e para o conhecimento de como esse controle é realizado em microrganismos. O objetivo desta revisão foi informar sobre o potencial uso de plantas medicinais no controle de fungos patógenos de um grande número de plantas e animais, o que pode contribuir para o alcance de novas formulações de fungicidas botânicos. Muitos autores demonstraram atividades antifúngicas e antimicrobianas gerais para as espécies da flora brasileira por meio de métodos bem estabelecidos, como microdiluição, difusão em ágar e difusão em disco, determinando a concentração inibitória mínima (CIM), as concentrações fungicidas mínimas (CFM) e o potencial de inibição de óleos essenciais, extratos e frações. Nesta revisão, 68 espécies foram citadas como estando presentes no Brasil, 25 das quais são encontradas nas regiões norte-nordeste do país. Assim, a maioria dos estudos sobre as atividades antimicrobianas das plantas medicinais traz uma "compreensão inicial" de seu potencial, particularmente de algumas espécies, gêneros e até famílias. No entanto, mais dados que sejam excessivamente específicos são obrigatórios, focando em abordagens novas e mais precisas, como os mecanismos de ação, a toxicidade, os componentes ativos e a verificação da existência de efeitos sinérgicos. Esses critérios seriam o mínimo necessário para desenvolver novos produtos naturais como tratamentos alternativos para as diversas patologias infecciosas que afetam plantas, animais e seres humanos.

Palavras-chave: Fungicida botânico; Micose; Patentes; Óleos essenciais.

Resumen

El uso de plantas con propiedades medicinales para el control de hongos ha llevado a una exploración continua de nuevos compuestos que pueden contribuir a estudios prometedores en el desarrollo de nuevos fármacos y al conocimiento de cómo se lleva a cabo este control en microorganismos. El objetivo de esta revisión fue informar sobre el uso potencial de plantas medicinales en el control de patógenos fúngicos de un gran número de plantas y animales, que pueden contribuir al alcance de nuevas formulaciones de fungicidas botánicos. Numerosos autores han demostrado actividades antifúngicas y antimicrobianas generales para especies de la flora brasileña a través de métodos bien establecidos como la microdilución, difusión en agar y difusión en disco, determinando la concentración mínima inhibitoria (CMI), las concentraciones mínimas de fungicida (MFC) y el potencial de inhibición de Aceites esenciales, extractos y fracciones. En esta revisión, se citaron 68 especies presentes en Brasil, 25 de las cuales se encuentran en la parte noreste del país. Por tanto, la mayoría de los estudios sobre las actividades antimicrobianas de las plantas medicinales aportan una "comprensión inicial" de su potencial, en particular de algunas especies, géneros e incluso familias. Sin embargo, es obligatorio contar con más datos excesivamente específicos, centrándose en enfoques nuevos y más precisos, como los mecanismos de acción, la toxicidad, los componentes activos y la verificación de la existencia de efectos sinérgicos. Estos criterios serían los mínimos requeridos para desarrollar nuevos productos naturales como tratamientos alternativos para las diversas patologías infecciosas que afectan a plantas, animales y seres humanos.

Palabras clave: Fungicida botánico; Tiña; Patentes; Aceites esenciales.

1. Introduction

Humans acquired information about their environment in such a way as to build and establish tools that facilitate their survival. One field has been extensively exploited since ancient peoples, tribes, and communities know about plant species, their relationship to food, and medicinal purposes, with this know-how still being maintained and disseminated in the modern era. Despite a gradual loss of traditional knowledge, most of the world's population still enjoys these nutritional benefits, especially when dealing with medicinal plants (Quinlan & Quinlan, 2007). About 80% of developing countries still employ traditional folk knowledge in treating symptoms and illnesses. In addition, modern pharmacopeia has 25% of its drugs derived from plants. Given the current global scenarios, the quest for sustainability has increased a great deal of interest in "green" products for both industries and developed countries (Setshogo & Mbereki, 2011).

The so-called traditional medicines are a habit in local communities around the world. About 3.5 billion people use these resources in some way. About 70%-80% of traditional healers use traditional medicine in routine health care (Bekalo et al., 2009). In Asia, the number of plant species used for medicinal purposes is over 6,500 (Batharrai et al., 2010), with over 3,000 of them occurring in India alone (Mazid et al., 2012).

In Brazil, flora and fauna are an undeniable wealth, and a great interest in deepening and developing the knowledge of plants with medicinal properties, as shown by the increasing number of scientific papers on Brazilian medicinal plants, demonstrating that traditional knowledge has been seriously valued by the scientific community (Brito & Senna-Valle, 2011). The SUS (Sistema Único de Saúde - Unified Health System), better known as Brazil's publicly funded health care system, in 2005 proposed the inclusion of herbal plants as therapeutic alternatives for health problems. However, herbal medicines' production is still considered incipient (Argenta et al., 2011).

For instance, 62.4% of patients in a community in Passo Fundo, Rio Grande do Sul, RS, Brazil, say that they use herbal teas and natural compounds, such as Marcela (*Achyrocline satureioides* Lam.), Lemon (*Melissa officinalis* L.), Pata de Vaca (*Bauhinia* spp.) and Tanchagem (*Plantago* spp.), as complementary therapies (Silva & Hahn, 2011). Mint (*Mentha piperita* L.), Bilberry (*Boldus peumus* Molina), Chamomile (*Matricaria chamomilla* L.), Fennel (*Pimpinella anisum* L.), and Lemongrass (*Cymbopogon citratus* Stapf.) were the most frequently cited among 36 species as a use for any type of alternative treatment in São Paulo-SP, Brazil (Santos & Sebastiani, 2011). In a neighborhood of Porto Alegre-RS, Brazil, 150 species from 59 different families, with 23 species in the Asteraceae family and 21 species in the Lamiaceae family, were cited as having herbal properties (Vendruscolo & Mentz, 2006).

There are numerous therapeutical applications of medicinal plants in Brazil, emphasizing antidiuretic, expectorant, healing, anti-inflammatory, and anti-rheumatic properties (Argenta et al., 2011). Other possible effects that are also widely exploited in alternative therapies are soothing remedies, analgesic, antipyretic, anti-cough, bronchitis, influenza, and pneumonia curatives, as well as for contusion injuries in general (Brito & Senna-Valle, 2011). In a small Brazilian community, the very same species was cited for more than 40 applications, ranging from asthma and heartburn to prostate cancer, as well as for the strengthening of hair (Vendruscolo & Mentz, 2006). Infectious diseases kill approximately 50,000 people daily. Pathogens, especially in humans, have developed various resistance mechanisms to commercial drugs, generating a series of clinical complications and health policies. In addition, there are innumerable side effects and adverse reactions caused by these aforesaid commercial drugs (Namita & Mukesh, 2012).

Lamentably, only a matter of hundreds of almost 500,000 species of plants has been tested for their antimicrobial activities, with many inadequately assessed (Vashist & Jindal, 2012). Nevertheless, antimicrobial activities against several species of microorganisms have already been positively demonstrated worldwide. *Cassia alata* L., *Semecarpus anacardium* Linn., *Annona glabra* L., *Eugenia caryophyllus* Spreng., *Thymus vulgaris* L., *Cinnamomum zeylanicum* Blume., and *Cuminum cyminum* L., have been effective against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus* spp., *Salmonella* spp. and *Corynebacterium* spp. In contrast, *Azadirachta indica* A. Juss, *Catharanthus roseus* L., *Ocimum sanctum* Linn., *Ricinus communis* L., and *Lawsonia inermis* L., have shown antifungal activities. In addition, *Phyllanthus amarus* Schum., *Glycyrrhiza glabra* L., *Rhizophora* spp., and *Excoecaria agallocha* L. have inhibited certain viruses, while *Parthenium hysterophorus* L., *Artemisia* spp., and *Swertia chirata* Buch.-Ham. ex Wall. have shown significant effects against some genera of protozoa (Sher, 2009).

Several diverse plants in Brazil have been reported to present antimicrobial activities, with over 20% of plant species described. For instance, gram-positive and gram-negative pathogens were susceptible to the decoction of *Baccharis trimera* Less. and *Achillea millefolium* L. has been effective against bacteria and fungi, as has been previously demonstrated in extracts of *Allium sativum* L., *Maytenus ilicifolia* Schrad., and *Psidium guajava* L. Extracts of *Cynara scolymus* L. and *Achyrocline satureioides* Lam. have inhibited the growth of *Bacillus* spp. and *Pseudomonas*, *Staphylococcus aureus*, whereas *Vernonia polyanthes* Less. has been effective against *Leishmania* spp., even though it has not demonstrated antibacterial or antifungal effects (Silva & Fernandes-Junior, 2010). For antimicrobial activities of plants, quite a few methods have been commonly applied. Tests and protocols selected for an evaluation in the literature vary greatly, providing different approaches to study. However, they are mostly discussing a wide variety of plant groups with compounds that can be exploited. Polyphenols and other phenolic compounds, such as catechol and pyrogallol have been analyzed, demonstrating an enzyme inhibition. Quinones are aromatic rings that bind irreversibly, reacting with the nucleophilic amino acids, inactivating the proteins.

However, antimicrobial activities have not been fully described for many chemical compounds, especially for essential oils. The secondary metabolites are composed of a complex mixture of compounds, such as monoterpenes, sesquiterpenes, phenylpropanoids, fatty acids, and their derivatives. They can be found in many different secretory structures of plants, and they play an essential role as signaling molecules while also attracting pollinators (Zuzarte et al., 2011). Their complex composition of the primary compounds as concentrations of each component in the essential oils gives uniqueness to the oils. Due to these unique characteristics, these compounds have received a certain number of scientific approaches in recent years. Their market value has been raised upwards by various manufacturers like the pharmaceutical, agricultural, food, health, and cosmetics industries (Zuzarte et al., 2011).

The antimicrobial activities of essential oils have been widely covered in studies from around the entire world. For instance, on the website for the CAPES (Brazilian Federal Agency for the Improvement of Higher Education) journals, the "antimicrobial" terms of "essential" and "oils" show an outcome of more than 8,000 results. Researchers have shown a keen interest in evaluating plants' essential oils while considering their antimicrobial properties in traditional medicines and alternative therapies (Luangnarumitchai et al., 2007). In Brazil, this trend is very significant. There are countless studies, from all regions of the country, analyzing and finding the antimicrobial potentials of various genera and species, many of them already known by the country's population and the scientific community (Borges, 2012; Costa, 2008; Zacaria, 2006; Vilela, 2008).

2. Methodology

This study is descriptive and exploratory with a quantitative approach (Sakamoto & Silveira, 2019). Use bibliometric analysis of articles contained in the Scopus (http://www.scopus.com) and Web of Science (http://www.webofknowledge.com) databases that were prospected in title or abstract of scientific articles. The present research is a meta-analysis, a quantitative method that combines the information obtained from previous independent studies and critically analyzes these results (Hernandez et al., 2020).

Thus, this current prospection has aimed to present data confirming the antimicrobial potentials for various species of medicinal plants in Brazil, mainly focusing on their fungal control, either phytopathogenic or upon human pathogens, such as viruses and bacteria prions, or fungi.

The data for this work was obtained by using scientific papers that have been previously published in indexed journals and which were internationally recognized in the CAPES periodical database. The technological prospection was realized based upon patent applications in the European Patent Office (Espacenet), the World Intellectual Property Organization (WIPO), the United States Patent and Trademark Office (USPTO), and the National Institute of Industrial Property (INPI).

3. Results

The terms “antifungal,” “essential,” and “oil” when using “quick search” tools resulted in 62, 43, and one application in the Espacenet, WIPO, and USPTO databases, respectively. In the INPI database, for the terms “oils,” “essential,” and “fungi” two patents were found. The antimicrobial activities of plants for the pathogens in humans are presented in Table 1.

Table 1. Medicinal Plants applied in Control of Human Pathogenic Microorganisms.

Species	Microorganism control efficiency
Achyrocline satureioides (Lam.) DC.	*Bacillus* spp., *Pseudomonas*, *Staphylococcus aureus*
Alternanthera brasiliana Kuntze	*Candida* sp., *Cryptococcus neoformans*, *Sporothrix schenckii*
Annona glabra L.	*Bacillus* spp., *Corynebacterium* spp., *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp.
Azadirachta indica A. Juss.	*Bacillus* spp., *Corynebacterium* spp., *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp.
Baccharis dracunculifolia D. C.	*Candida* sp., *Cryptococcus neoformans*, *Sporothrix schenckii*
Calendula officinalis L.	*Candida dubliniensis*, *Candida guilliermondii*, *Rhodotorula* sp.
Campomanesia eugenioides (Cambess.), D. Legrand *ex* Landrum	*Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Leishmania amazonensis*
Eleutherine plicata Herb.	*Candida albicans*
Herissantia crispa L. Briz	*Candida albicans, Cryptococcus neoformans*, *Sporothrix schenckii*
Hyptis ovalifolia Benth.	*Microsporum canis*, *Microsporum gypseum*, *Trichophyton mentagrophytes*
Luehea paniculata Mart.	*Bacillus subtilis*, *Candida albicans*, *Pseudomonas aeruginosa*
Piper malacophyllum (C. Presl) C. DC	*Acinetobacter baumannii*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Bacillus cereus*, *Candida albicans*, *Epidermophyton floccosum*, *Escherichia coli*, *Microsporum canis*, *Microsporum gypseum*, *Cryptococcus neoformans*, *Leishmania braziliensis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trypanosoma cruzi*
Piper regnellii (Miq.) C. DC.	*Sporothrix schenckii*
Rubus urticaefolius Poir	*Sporothrix schenckii*, *Candida* sp., *Cryptococcus neoformans*
Rumex acetosa L.	*Sporothrix schenckii*, *Candida* sp., *Cryptococcus neoformans*
Schinus terebinthifolia Raddi.	*Alternaria* spp., *Bacillus subtilis, Cryptococcus neoformans, Candida krusei, Candida glagrata, Candida albicans, Fusarium* spp., *Pseudomonas aeruginosa*, *Sporothrix schenckii*
Syzygium aromaticum (L.) Merr. & L. M. Perry	*Candida albicans*
Vernonia Polyanthes Less.	*Leishmania* spp.
Zanthoxylum rhoifolium Lam.	*Leishmania amazonensis*

Source Authors.

Lippia spp. was a genus with over 200 species, with most of these widely used in popular medicine. Countless works have already been carried out regarding the antimicrobial properties of these essential oil species, backed up with assertive solid data, reinforcing the high potentials of genera against a significant number of microorganisms of sanitary, ecological, veterinary, agronomic, or human relevance. The *Lippia sidoides* Cham. the essential oil was tested with the Candida spp. and

Microsporum canis pathogens. The tests were with agar disk diffusion and broth microdilution. At first, the strains were cultivated in potato dextrose agar. The receiving wells were made in the center of an agar medium plate. 100 µL of essential oils were added at concentrations ranging from 25 to 100 mg mL^{-1} and then diluted in mineral oil. The results were observed after five days.

A two-fold serial dilution was performed for the microdilution tests, with concentrations of between 5 mg mL^{-1} and 0.002 mg mL^{-1}. In addition to the minimum inhibitory concentrations (MIC), the minimum fungicidal concentrations (MFC) were determined by 100µL of inoculum in the wells and whose concentrations caused a total inhibition, while at the same time, lacking any turbidity.

The inhibition zone of *M. canis* was significant for the lowest concentration, totally restraining growth from 50 mg mL^{-1}. For *Candida*, although effective, the highest oil concentration induced a lower inhibition than it did for all of those used for *M. canis*. The MIC levels and the MFC levels were visibly lower for *M. canis* as well. Thus, the *L. sidoides* essential oils presented fungicidal effects for *M. canis*, and their high potential was apparent due to the large percentage of timol (Fontenelle et al., 2007).

For the *Piper malacophyllum* (C. Presl) C. DC essential oil, in vitro tests were performed to evaluate its activities. The antibacterial effects over *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* were estimated by the MIC levels a Muller-Hinton medium with different concentrations of essential oils. In these tests, the colorimetric reagent triphenyl tetrazolium chloride (TTC) was applied, aimed at identifying its metabolic activities and its minimum bactericidal concentrations (MBC) (Rebelo et al., 2012)

The antifungal potential of *P. malacophylum* essential oil over the *C. albicans*, *Cryptococcus neoformans*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Rhizopus* sp., *Epidermophyton floccosum*, *M. canis*, *M. gypseum*, *Trichophyton mentagrophytes*, and *T. rubrum* strains were also analyzed. The simple cultivations of these strains in a medium with dilutions of the essential oil in DMSO defined the MIC levels, which came to be the lowest concentration in which there was fungal growth. Moreover, the oil's potential against *Leishmania braziliensis* and *Trypanosoma cruzi* was evaluated by applying essential oil solutions from 500 to 0.8 µg mL^{-1}, and after 48 hours, the IC50 was determined the MTT assay.

In the antibacterial assays, the *P. malacophyllum* essential oil had the potential to be considered weakly effective. Its antifungal activity was classified as being moderately effective only against *T. mentagrophytes* and *C. neoformans*. Its anti-parasite effects were taken as being generally low once the IC50 was over 300 µg mL^{-1} (Rebelo et al., 2012).

The potential of the *Calendula officinalis* L. essential oils against 23 fungi strains, some of them clinically isolated, were tested against 22 Candida sp. strains and one *Rhodotorula* sp. strain. The essential oils were trialed by disc diffusion tests, with the paper discs soaked in essential oil and laid over a Muller-Hinton medium (Gazim et al., 2008). High inhibitory levels were observed in the *C. dubliniensis* and *C. guilliermondii* strains, whereas the variances of the oil effects were more elevated than Nystatin. It must be underlined that a high sensibility range of this microorganism can be a valuable feature for using the *C. officinalis* essential oil.

The amount of information concerning the activities of tropical medicinal plants remains limited in the face of the increasing number of pathogenic fungi being resistant to the usual drugs and treatments. Hence, the eight Brazilian plants commonly used in traditional medicine were contemplated in scientific work to evaluate their effects against *Candida* sp., *Cryptococcus neoformans*, and *Sporothrix schenckii*. The extracts of *Inga dulcis* (Cell.) Mart., *Schinus terebinthifolia* Raddi, *Alternanthera brasiliana* Kuntze, *Piper regnellii* CDC, *Herissantia crispa* L. Briz, *Rubus urticaefolius* Poir, *Rumex acetosa* L. and *Baccharis dracunculifolia* DC. were obtained. It was used in broth microdilution tests, with eight different concentrations (1,000 to 7.8 µgmL^{-1}) acquired by a two-fold dilution. After that, the MIC levels were defined by concentration, in whose wells were seemingly straightforward. All appointed plants significantly affected at least one tested strain (Johann et al., 2007).

The oils of *S. terebinthifolia* showed higher activities against *C. krusei*, *C. glagrata*, and *S. schenckii*. The same MIC levels were found for *C. neoformans* from the *B. dracunculifolia* and *P. regnellii* extracts; the latter was also effective against *C. tropicalis*, while *R. urticaefolius* inhibited the growth of *S. schenckii* and *H. crispa* restrained the growth of *C. neoformans*. The studied species held strong antifungal properties (Johann et al., 2007).

Work was developed with malva-do-cerrado (*Hyptis ovalifolia* Benth.) to evaluate the antifungal activities of its essential oil against the *M. canis*, *M. gypseum*, and Trichophyton mentagrophytes strains, as well as the clinically isolated *T. rubrum* strain. The chosen approach was by using an agar diffusion. When placed into a solid medium, these dispersions were added to a still liquid Sabouraud-Dextrose medium and a later fungi suspension of inoculum. The MIC levels were checked with 31.2 µg mL^{-1}, and all of the tested fungi were inhibited, testifying the antifungal activities to be equal or better than control (Itraconazole) (Oliveira et al., 2004).

Aqueous and hydroalcoholic extracts of *Zanthoxylum rhoifolium* Lam., *Campomanesia eugenioides* Cambess., *S. terebinthifolia* Raddi., *Cordia americana* (L.) Gottshling and J. E. Mill., *Luehea paniculata* Mart., and *Ocimum gratissimum* L. were tested against *E. coli*, *Pseudomonas aeruginosa*, *S. aureus*, *Bacillus subtilis*, *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *L. amazonensis*. The broth micro-dilution tests were performed to verify their effects on bacteria and fungi. The extracts were diluted in the Muller-Hinton and RPMI-1640 mediums. To evaluate their effects over *L. amazonensis* promastigotes, the broth micro dilutions were tested in 24-well plates by a viable cell counting with a Neubauer chamber and then compared to the negative control. None of the extracts showed activity against E. coli. The *Z. rhoifolium* extract did not present an inhibitory action for any bacteria. *S. terebinthifolia* was effective for *P. aeruginosa* and *B. subtilis*, with growth suppressed for the L. *paniculata* extract. *C. eugenioides, S. terebinthifolia,* and *L. paniculata* inhibited the *C. albicans* strains. Referring to *O. gratissimum*, and *C. americana*, no relevant activities against the presented fungi were found. All the trialed *Candida* sp. showed a sensitivity to the *C. eugenioides* extract. *Z. rhoifolium* presented a superior potential against *L. amazonensis*. Given these results, the current study has noted that the antimicrobial activities of the deliberated species were promising, yet further studies ensuing a notably greater specificity, together with cytotoxicity tests, are required (Moura-Costa et al., 2012).

Pereira et al. (2011) verified the antifungal activities of the *Cymbopogon winterianus* Jowitt ex Bor essential oil, which is usually known as *Citronella*, against the *Trichophyton mentagrophytes* strains. The agar disk-diffusion method was applied by using the pure essential oil soaked into filter paper disks. These were seated in a Sabouraud-Dextrose medium containing 1 mL of standardized conidia inoculum. The MIC levels were determined by a broth micro dilution when using 96-well plates to apply a two-fold dilution of the oils to the fungi-inoculated wells. The MFC levels were obtained by employing 20 µL aliquots from the wells, resulting in no apparent growth to the clean culture plates. These pure essential oil dilutions could inhibit the strains. The lowest MIC level obtained was 156 µgmL^{-1}. The MFC levels that were found were, generally, eight times higher than the MIC levels. In the viability cell tests, the fungi solution was exposed to various oil concentrations and after 3.6, 9 and 12 days, it was observed that there was a gradual decrement of viability over time, reaching 100% after nine days. It was confirmed that the antifungal activities of *C. winterianus* were related to the damage of the cell membrane's integrity and the functionality of the *T. mentagrophytes*, possibly due to their lipophilic properties.

Another multidisciplinary study was adopted that focused on *C. albicans* of the Amazon. The exotic species that stood out for their intense widespread use were *Copaifera multijuga*, *Carapa guianensis* Aubl., *Piper aduncum, Piper hispidinervum*, *Annona glabra* L., *Azadirachta indica* A. Juss, *Bryophyllum calycinum* Salisb., *Eleutherine plicata* Herb., *Mammea americana* L., *Psidium guajava* var. and *Syzygium aromaticum* L. A 32% solution of the essential oils was prepared, and it was serially diluted until it had a concentration of 2%.

Likewise, the extracts were tested by agar disk-diffusion, except for the filter paper disks, which were centrally placed on the yeast-cultivated plates. For MIC microdilution tests were performed. Of the seven tested extracts, *E. plicata*, *P. guajava* and *S. aromaticum* showed the highest effects. None of the essential oils presented any significant activities. However, most of the plants that have been related to this work have previous reports concerning antimicrobial activities of their essential oils or their isolated compounds (Menezes et al., 2009).

A lack of methodology standardization is a limitation in reported studies of essential oils. It is common to see expressive approach variations among the works, such as when the dilutions have been made by percentages (%) or by the mass over final volume (µg mL^{-1}). In These cases, density oils vary within and between the species, mainly because of the differences in composition and the compound's concentrations. Therefore, the percentages of oils in each concentration ensures a parity in the volume of oils used, but this is not the same as the oil's mass, which may complicate the evaluation of each oil's potential, as suggested by Menezes et al. (2009). Variations in compounds composition and concentrations, the usage results for any given dilution in distinct surveys may diverge due to the contrast in compositions of the samples in each study.

Another fragility in the standardization of antimicrobial tests with essential oils is in their physical-chemical features. For a solid or a liquid culture media, both have a hydrophilic character. Thus, when solubilizing oils, which are essentially hydrophobic, it is necessary to perform such actions in a medium. Different alternatives must be considered, either in choosing the solvent or by merging the oils in a medium. In the main, these alternative methodologies are likely to be efficient, for they allow for verification and quantification of the oil's potential, as reported by Sarmento-Brum et al. (2013). However, the diversity of the applied approaches prejudices a result's comparison, as the used parameters and resources are not always co-relatable.

The antimicrobial activities of plants for pathogens are presented in Table 2. The chemical compounds for a phytopathogen control in agriculture is an easily confirmed fact. However, in the same way as these compounds are increasingly used, the risk of their application in crops, has in many cases, been underestimated. On the other hand, there is a growing commercial demand for food produced with organic certification, especially when using natural products for disease control. Thus, this research using plant medicinal compounds may be interesting for the agricultural industries (Table 2).

Table 2. Medicinal Plants applied in Control of Plant Pathogen Microorganisms.

Species	Microorganism control efficiency
Achyrocline satureioides (Lam.) DC.	*Colletotrichum gloeosporioides*, *Glomerella cingulata*, *Pseudomonas*
Ageratum conyzoides L.	*Sclerotinia sclerotiorum*
Calendula officinalis L.	*Colletotrichum gloeosporioides*, *Glomerella cingulata*
Cymbopogon citratus [DC] Stapf.	*Colletotrichum gloeosporioides*, *Glomerella cingulata*
Cymbopogon martinii Motia Bruno	*Alternaria* spp., *Rhizoctonia solani*
Cymbopogon nardus L.	*Fusarium subglutinans*, *Rhizoctonia solani*
Cymbopogon winterianus Jowitt ex Bor	*Trichophyton mentagrophytes*
Eremanthus erythropappus (DC.) Macleish	*Alternaria* spp., *Rhizoctonia solani*
Lippia alba [Mill] N. E. Brown	*Colletotrichum gloeosporioides*
Melia azedarach L.	*Sclerotinia sclerotiorum*
Piper aduncum L.	*Sclerotinia sclerotiorum*
Piper callosum Ruiz and Pav.	*Crinipellis perniciosa*, *Phytophthora capsici*, *Phytophthora palmivora*
Piper dilatatum Rich.	*Crinipellis perniciosa*
Piper enckea (Miq.) C. DC.	*Phytophthora palmivora*
Piper malacophyllum (C. Presl) C. DC	*Acinetobacter baumannii*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Bacillus cereus*, *Rhizopus* sp.
Piper marginatum var. *anisatum* Jacq.	*Crinipellis perniciosa*
Rosmarinus officinalis L.	*Alternaria* spp., *Rhizoctonia solani*
Ruta graveolens L.	*Sclerotinia sclerotiorum*
Schinus molle L.	*Alternaria* spp., *Fusarium* spp., *Colletotrichum* spp., *Botrytis* spp.
Schinus *terebinthifolia* Raddi.	*Colletotrichum gloeosporioides*, *Fusarium* spp., *Botrytis* spp., *Alternaria* spp.
Syzygium aromaticum (L.) Merr. & L. M. Perry	*Colletotrichum gloeosporioides*, *Glomerella cingulata*
Tagetes minuta L.	*Colletotrichum gloeosporioides*, *Glomerella cingulata*

Source Authors.

The essential oils from *Eremanthus erythropappus*, *Cymbopogon martinii*, and *Rosmarinus officinalis* were tested against the mycelial growth of *Alternaria* spp, *Rhizoctonia solani* (Hillen et al., 2012). Different aliquots were tested on the BDA medium. Mycelial discs were laid on the center of the plates, and the halo diameters were measured daily. The Mycelial Growth Index was estimated based upon the growing and the negative control. All the phytopathogens were inhibited by the *E. elytropappus* oils, with more significant effects over *R. solani* and *Alternaria* spp. In just the higher concentrations, the *R. officinalis* oils inhibited the fungi as well. However, the best inhibitory effect was observed by *C. martinii*, which suppressed the growth of all the concentration strains tested. Therefore, it is conclusive that the essential oils of the three species mentioned above are distinctly efficient against *Alternaria* spp. and *R. solani*.

In pursuit of a solution to control *Sclerotinia sclerotiorum* in a soy stem without any agro-toxic effects, Garcia et al. (2012) evaluated the impact *Azadirachta indica* and *Pongamia glabra* associated oils and their vegetal extracts against the cultures. The oils' dilutions were transferred in distinct proportions into the BDA medium, which had mycelial discs placed over it to analyze the mycelial growth inhibitions. For the extracts, the species of *Schinus molle* L., *Ageratum conyzoides* L., *Ocimum gratissimum* L., *Artemisia absinthium* L., *Syzygium cumini* L., *Ruta graveolens* L., *Manihot esculenta* Crantz., *Melia azedarach* L. and *Piper aduncum* L were used. The vegetal extracts blended in a culture medium and 6 mm mycelial discs were used. The first assay resulted in an inhibition of the second species oil concentrations. The synergies between the oils were demonstrated. Concerning the extracts of *M. azedarach* L., *A. conyzoides* L., *R. graveolens* L., and *P. aduncum* L., they reduced mycelial growth by approximately 25%. At the same time, the other tested species did not show any significant effects.

Crinipellis perniciosa Stahel, *Phytophthora palmivora* E.J. Butler, and *Phytophthora capsici* Leonian are pathogens that attack the cocoa tree in the Amazon region. *In vitro* fungal toxic activities of the Piper spp. essential oils, Silva and Bastos (2007) developed a work in the Institute of Rural Development of Amapá (Instituto de Desenvolvimento Rural do Amapá). The samples of *Piper dilatatum* Rich., *P. cyrtopodon* Miq., *P. hostmannianum* Miq., *P. callosum* Ruiz and Pav., *P. tuberculatum* Jacq., *P. divaricatum* G. Mey., *P. nigrispicum* C. DC, *P. hispidum* Sw., *P. marginatum* var. *anisatum* Jacq. and *P. enckea* C. DC. were collected, and their oils were extracted by hydro distillation. The agar diffusions and the dilutions of the oils that were incorporated into the founding medium were tested. Their effects against the germination of *C. perniciosa* were also analyzed. *C. perniciosa* was inhibited by low concentrations of the P. callosum oils and 1 μL mL^{-1} of the *P. marginatum* oils. When relating to *P. palmivora*, only *P. callosum* and *P. enckea* showed any effectiveness, and *P. callosum* induced a 100% inhibition of *P. capsici*. In the *C. perniciosa* basidiospores assays, the *P. dilatatum, P. callosum*, and *P. marginatum* oils were effective, inducing a complete inhibition of the germination at low concentrations.

Another study evaluated the essential oils of *Cymbopogon nardus* (L.) Rendle against the *Rhizoctonia solani* fungus. The micelle discs were laid in every plate, and the fungal growth was observed during ten days of incubation. By the end of the tests, all treatments presented lower growth rates when compared to control until the eighth day (Sarmento-Brum et al., 2013).
Oliveira Junior et al. (2013) investigated the in vitro and in vivo antifungal activities of the *S. terebinthifolia* Raddi. essential oil over the *Colletotrichum gloeosporioides* strain that was cultivated in BDA. The essential oils were obtained by hydro distillation. The in vitro dilution of the oils was realized directly in the medium. Paper filter discs were placed in the center of the plates, and the spore suspensions were inoculated onto the discs and plates. Measuring the colony diameters were performed 2, 4, 6, and 8 days after the inoculation. For the in vivo tests, the papaya solo-type fruits were harvested and disinfected with calcium hypochlorite. The injuries were produced on the fruits using entomological needles, and 3μL spore solutions were inoculated. These fruits were wrapped with starch biofilm with 0.5% of the essential oil. It was found that the highest concentration could inhibit 79% of the mycelial growth of *C. gloeosporioides*. In the in vivo tests, the fresh mass loss was 70% when using the oil, against 40% for control.

With the Faculty of Chemistry of Uruguay (Faculdade de Química do Uruguai), Brazilian researchers tested the effects of the essential oils of several *Schinus molle* L. and *S. terebinthifolia* Raddi. Samples against the phytopathogens of *Fusarium* spp., *Colletotrichum* spp., *Botrytis* spp., and *Alternaria* spp. They were tested using the essential oils in distinct concentrations added onto the culture plates and spread over the medium's surface. The dilutions of the *S. terebinthifolia* oils were lower than 50%. They did not inhibit *Fusarium* spp. For the other fungi, a decreasing growth halo was observed for the 25% concentrations. It was more effective against *Botrytis* spp., with a complete inhibition in all concentrations at every period. *S. molle* was effective for all the fungi in all concentrations, showing their lethality to *Botrytis* spp. and *Fusarium* spp. for dilutions lower than 10% and all the fungi at 25% (Santos et al., 2010).

A study was performed to analyze the antifungal activities of the *Cymbopogon nardus* L. Rendle essential oils over the mycelia of *Fusarium subglutinans* that were isolated from pineapples. Aliquots of the oils were added onto the surfaces of the BDA. The evaluations occurred every 48 hours by measuring the culture halos, totaling five checks per plate. The three highest aliquots performed 100% growth inhibition until the fourth evaluation day. A 25 μL aliquot pointed to a mycelial growing rate of 1.55 mm day^{-1}, while the control showed an 8.56 mm day^{-1} growing rate. The citronellal majority chemical compound effect was evaluated. It presented a growth rate of 3.36 mm day^{-1}, suggesting that the synergism possibly gave the highest efficiency of the essential oils among most oil compounds (Seixas et al., 2011).

Rozwalka et al. (2008) tested the extracts and the oils of plants against *C. gloeosporioides* and *Glomerella cingulata*. *R. officinalis* L., *O. basilicum* L., *Arctium lappa* L., *Calendula officinalis* L., *Chamomilla recutita* (L.) Rauschert, *Cymbopogon citratus* (DC) Stapf., *Equisetum* sp., *Maytenus ilicifolia* (Schrad.) Planc, *Foeniculum vulgare* Mill., *Zingiber officinale* Roscoe, *M. piperita* L., *L. alba* (Mill) N. E. Brown, *Achyrocline Bacteroides* (Lam.) DC, *Phyllanthus* sp., *Sambucus nigra* L., *Plantago australis* L., *Tagetes minuta* (L.), *Syzygium aromaticum* (L.) Merr. And L.M. Perry, *P. guajava* L. and Orange *Citrus sinensis* (L.) Osbeck bark and aqueous extracts in 10% concentrations were obtained and the essential oils by hydro distillation.

The extracts were blended in the BDA medium, and essential oils were inoculated at three equidistant points on the medium's surface. The *S. aromaticum* aqueous extracts inhibited 100% of the pathogens. The *P. australis* and elderberry extracts did not show any inhibitory effects on any of the fungi. *Lippia*, lemongrass, and calendula extracts were not effective against *G. cingulate*, and the extracts of "cavalinha," basil, and mint were not effective against *C. gloeosporioides*. The clove and lemongrass essential oils inhibited the growth of *C. gloeosporioides* until the fifth day. At the same time, the lemongrass oil also suppressed the growth of *G. cingulata*, reducing its inhibition potential from 100% to 62.8% on the eighth day. This reduction might have been due to the volatilization of some of the oil compounds.

The properties of the essential oils suffer from several variations, with differences in their pH, density, coloring, volatility, fusion point, and viscosity. These are only a few examples of the features directly associated with the chemical compositions of the essential oils. On the other hand, the compound varies to many factors, such as geographic localization diversity, age, extraction methods, planting season, and more.

Various antimicrobial activities of the essential oils were determined primarily by their chemical composition, either from the isolated compounds or from the synergism between the majority and minority compounds, resulting from their intervention in the pathogen metabolic pathways. Therefore, the conditions that influence the composition variations of the essential oils are significant for upcoming studies about their antimicrobial activities. Hence, it is essential to consider such conditions before developing new assignments or comparing literature data that discusses certain species and their potential. The essential oils that have been reviewed may have presented countless positive results, but some samples did not render any equivalent efficiencies.

The substances mentioned therein have been tested, and the traditional knowledge associated with them has been proved. However, little is known about how fungal control is established, given a molecular and genetic outlook. Thus, most studies about the antimicrobial activities of medicinal plants bring an 'initial understanding' of their potential, particularly of some species, genera, and even families. Nevertheless, more data that is exceedingly specific is mandatory by focusing on new and more accurate approaches, such as the action mechanisms, toxicity, the active components, and the verification of the existence of synergic effects. These criteria would be the minimum required to develop new natural products as alternative treatments for the various infectious pathologies that affect plants, animals, and human beings. Sixty-eight species were mentioned in the literature, all occurring in Brazil, with 25 occurring in the Brazilian north-northeastern region.

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