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Antifungal activity of essential oils of *Chenopodium ambrosioides* and *Lippia alba*

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ABSTRACT: Since ancient times, folk medicine and agro-food science have benefitted from the use of plant derivatives, such as essential oils, to combat different diseases, as well as to preserve food. In Nature, essential oils play a fundamental role in protecting the plant from biotic and abiotic attacks to which it may be subjected. Many researchers have analyzed in detail the modes of action of essential oils and most of their components. The purpose of this brief review is to describe the properties of essential oils, principally as antifungal agents, and their role in blocking cell communication mechanisms, fungal biofilm formation, and mycotoxin production.

KEYWORDS: Antifungal activity , essential oils , *Chenopodium ambrosioides* , *Lippia alba,* folk medicine, mycotoxin

I. INTRODUCTION

There is an increasing demand to reduce the use of chemicals as antimicrobial agents in the field of nutrition and to combat various infections due to increasingly aggressive and increasingly endogenous microorganisms that are resistant to the use of synthetic antimicrobials. In this direction, substances derived from plants, such as hydro-alcoholic extracts or essential oils, can certainly play a fundamental role. The versatility of such substances is enormous; the same plant can provide a pool of substances with a very broad spectrum of action due to their different chemical structure. Furthermore, the hypersensitivity and toxicity to the drugs, because of their improper and excessive application, represent some of the major problems of the conventional medicine consequences of the presently excessive use of synthetic antimicrobials. Public awareness has therefore generated interest in the application of natural substances already used throughout the ages for the treatment of certain diseases transmitted by organisms. The use of essential oils is common, even since the earliest civilizations, firstly in the East and the Middle East, then in North Africa and Europe [1]. The term “Essential Oil” (EO) was coined in the 16th century by the Swiss reformer of medicine, Paracelsus von Hohenheim. Plant EOs are usually complex mixtures of natural compounds, both polar and non-polar [1,2]. Well-known for their antiseptic and medicinal properties (analgesic, sedative, anti-inflammatory, spasmolytic, local anesthetic, anti-carcinogenic), they are also used in embalmment, and, due to their antimicrobial and antioxidant activity, as natural additives in foods and food products [3,4,5,6]. The International Organization for Standardization (ISO) (ISO/D1S9235.2) defines an essential oil as a product made by distillation with either water or steam or by mechanical processing or by dry distillation of natural materials.[3,4] They appear as liquid, volatile, limpid and colored mixtures of several aromatic compounds. EOs are obtained from all plant parts, mainly from herbs and spices, although at present new sources of EOs are examined, for example from food and vegetal wastes [7,8]. About 3000 EOs are known, 300 of which are commercially important, mainly used in the flavors and fragrances market [6]. In nature, EOs play an important role in the protection of plants against undesirable enemies. As for other plant metabolites, the role of EOs is the protection of the plant organism against some pathogenic microorganisms, the exertion of a repel action towards insects that act as plague vectors, and the reduction of the appetite of some herbivores (by inducing unpleasant taste to the plant). On the other hand, they also may attract some insects to promote the dispersion of pollens and seeds. Thus, EOs can play a role in mediating the interactions of plants with the environment [4]. The main categories of compounds are terpenes and terpenoids; rarely nitrogen- and sulphur-containing compounds, coumarins and homologues of phenylpropanoids can also be found [9,10]. Terpenes are a large class of naturally occurring hydrocarbons, deriving from the isoprene unit (C_5H_8), with various chemical features and biological properties. They are synthesized in the cytoplasm of plant cells through the pathway of mevalonic acid starting from acetyl CoA. Monoterpenes ($C_{10}H_{16}$) and sesquiterpenes ($C_{15}H_{24}$) are generally the principal terpenes, but longer chains such as diterpenes ($C_{20}H_{32}$), triterpenes ($C_{30}H_{40}$), etc., also exist. Examples of terpenes include *p*-cymene, limonene, terpinene, sabinene and α- and β-pinene. Monoterpenes are constructed from the coupling of two isoprene units. They

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constitute 90% of the essential oils and allow a great variety of structures. Some of the major compounds include monoterpene hydrocarbons (such as limonene, *p*-cymene, α-pinene, and α-terpinene), [5,6]and oxygenated monoterpenes (such as carvacrol, thymol and camphor). Sesquiterpenes are formed from the assembly of three isoprene units. The extension of the chain increases the number of cyclizations permitting a great variety of structures. The structure and function of sesquiterpenes are comparable to those of the monoterpenes. Terpenoids are compounds related to terpenes, with some oxygen functionality or some rearrangement.[7,8] Thymol, carvacrol, linalyl acetate, linalool, piperitone, citronellal, geraniol and menthol are considered as the most known terpenoids. Within each group, molecules can be simply unsatured hydrocarbons, or contain functional groups; in this last case, they are acids, alcohols, aldehydes, ketones, esters [10,11]. Chemical composition of plant essential oils differ among species; it is affected by factors including the geographical location, environment, the stage of maturity and method of extraction. This chemical difference is directly correlated to differences in biological activities [12,13]. EOs and their components have a variety of targets, particularly the membrane and cytoplasm, and in certain situations, they completely alter the morphology of the cells [13]. Both humans and plants are susceptible to fungal infections by pathogenic fungi and some synthetic fungicides are known to be effective in their control. However, the use of synthetic fungicides is limited by the emergence of resistant fungus strains and some fungicides possess considerable toxicity. Moreover, there is a growing public concern over the increased health and environmental hazard associated with synthetic molecules. For this reason, alternative, safe and natural methods to develop new antifungal agents are actively studied [11]. Recently, there has been a great interest in using essential oils as possible natural substitutes for conventional synthetic fungicides [14].

Chenopodium ambrosioides L. (Chenopodiaceae), commonly known as Mexican tea, is a polymorphic annual, and perennial herb growing to a height of over 1 m and covered with aromatic glandular hair. It is widely distributed in West Africa especially in Nigeria, Senegal, Ghana, and Cameroon [9]. This herb is used in folk medicine in the form of teas, poultices, and infusions for inflammatory problems, contusions, and lung infections, and as purgative, analgesic, as a vermifuge to expel round-worms and hook-worms, and as an antifungal [11] [10]. Chronic toxicity studies in albino rats with high doses of the plant extract ranging from 12.31 to 31.89 g/kg for 42 days revealed pathological features such as congestion of the lungs, metaplastic changes in the mucosal surface of the stomach, and necrosis of the kidney tubules were noticed [12]. This suggests that caution should be taken when using this plant in any chronic treatment

Young (A) and flowering (B) *Chenopodium ambrosioides*.

Lippia is a straggling shrub about 4-6 ft tall, with a close resemblance to Lantana. Young branches are velvet-hairy, hairless when mature, branches 4-angular, furrowed, round at nodes. Leaves are decussate-opposite, lanceshaped-oblong 3-10 x 1.5-3.5 cm across, base narrowed to pointed, margin sawtoothed, tip pointed, chartaceous, sparsely velvet-hairy above, silvery velvet-hairy beneath, lateral veins 5-9 on either side of the midvein, more prominent beneath. Leaf-stalk are velvet-hairy, slender, about 1 cm long, exstipulate. [13,14]

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Lippia alba

Flowers are borne in leaf-axils, in a solitary spike or cylindric head, flower-cluster-stalk hairy, round, about 0.5-2.5 cm long. Flowers are stalkless, aromatic, calyx cup-shaped, 2 lobed bracts lanceshaped, base fused, forming a circular cup, tip 2 toothed, hairy, velvet-hairy. Flower hypocrateriform, bluish purple, 4 lobed, upper 2 lobes notchedd, lower 2 lobes deflexed, Flower tube narrow, cylindric, tip ampliate 5 x 1 mm, Stamens are 4, didynamous, filaments hairless, slender, anthers ovoid or spherical, stigma oblong, Fruit is a drupe, pear-shaped, about 2 x 1.5 mm hard, bony, separating into 2-seeded pyrenes. *Lippia* is native of South America, naturalized in parts of India.

A tea made from the leaves is a favourite domestic remedy in Central America for both intestinal and respiratory disturbances, including influenza. A well-sugared infusion is drunk to bring relief of heart problems and to soothe tachycardia. Used externally, the aromatic leaves are used in herbal baths, to cure fevers and severe stomach pain, and to cleanse the bladder[15,16]

II. DISCUSSION

The essential oil of the aerial part (leaves, flowers and stem) of *Chenopodium ambrosioides* was obtained by hydrodistillation and its chemical composition analyzed by GC and GC/MS, which permitted the identification of 14 components, representing 98.8% of the total oil. Major components were α-terpinene (51.3%), *p*-cymene (23.4%) and *p*-mentha-1,8-diène (15.3%). The antifungal properties of this essential oil were investigated *in vitro* by the well diffusion and broth microdilution methods. The *in vitro* antifungal activity was concentration dependent and minimum inhibitory concentration values varied from 0.25 to 2 mg/mL. The *in vivo* antifungal activity was evaluated on an induced vaginal candidiasis rat model. The *in vivo* activity of the oil on mice vaginal candidiasis was not dose-dependent. Indeed, all the three tested doses; 0.1%, 1% and 10% led to the recovery of mice from the induced infection after 12 days of treatment. The effect of the essential oil on *C. albicans* ATCC 1663 fatty acid profile was studied. This oil has a relatively important dose-dependent effect on the fatty acids profile.[17]

Several volatile natural compounds produced by plant secondary metabolism have been proven to present antimicrobial action, enabling their use in phytopathogen control. They also present low environmental impact when compared to conventional pesticides. Essential oils contain these compounds and can be found in several plant species, such as *Lippia alba* (Mill.) N.E. Brown (Verbenaceae). Essential oils of four chemotypes of L. alba, characterized by their major compounds, namely camphor, citral, linalool and camphor/1,8-cineole, were tested against the phytopathogen *Alternaria solani* Sorauer (Pleosporaceae), which causes early blight on tomatoes and is responsible for great economic losses regarding production. Essential oils antifungal action was tested in vitro using potato dextrose agar medium with essential oil concentrations at 0.1, 0.5, 1.0, 1.5 and 2.0 µL mL-1. The chemotype that had the best performance was citral, showing significant inhibition compared to the others, starting at the 0.5 µL mL-1 concentration. The essential oil belonging to the linalool chemotype was efficient starting at the 1.5 µL mL-1 concentration. Conversely, the camphor chemotype did not show any action against the phytopathogen. Moreover, the essential oils had no remarkable effect on tomato germination and growth. In conclusion, these essential oils presented fungicidal action against *A. solani*.[18]

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III. RESULTS

Lippia alba is a plant that has antifungal activity against *Aspergillus, Penicillium* and *Trichoderma* genera as well as against human pathogenic microorganisms; however, there are no records on its effect on basidiomycetes which are responsible for white rot of wood. The objective of this study was to evaluate the antifungal activity of *L. alba* for the control of the white-rot fungus *Pleurotus ostreatus.* From *L. alba* leaves, essential oil (EO) was extracted by hydrodistillation, alcoholic extract (AE) was obtained through alcoholic maceration, and aqueous extract (QE) from aqueous infusion. Each extract was added to several culture media to evaluate the fungicidal effect on *P. ostreatus*. AE and QE do not have fungicidal activity. *P. ostreatus* does not survive EO when concentrations are higher than 1,0 mL L-1 in malt extract liquid culture medium, or higher than 9,0 mL L-1 in particulate sawdust solid culture medium. The physical state of the cultivation medium affects the fungicidal action of EO. In surfaces subject to greater volatility, the minimum fungicidal concentration of the EO is 25,0 mL L-1. Altogether, *L. alba* EO is a potential alternative of biological control of basidiomycetes of white rottenness in wood.[19]

Sclerotium rolfsii is a soil-borne fungal plant pathogen that causes diseases in more than 500 plant species. Chemical fungicides used to control this disease cause environmental pollution, therefore, plant derived compounds can be used as alternative to synthetic fungicides to reduce environmental pollution. *Chenopodium album* is a weed of family Chenopodiaceae that is used as food and also has medicinal importance. In the present study, antifungal activity of methanolic root extract of *C. album* was evaluated against *S. rolfsii* using six concentrations viz. 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 g 100 mL-1 amended in malt extract as growth medium. All the root extract concentrations significantly reduced fungal biomass by 15-58% over control. Gas chromatography-mass spectrometry (GC-MS) analysis of the methanolic root extract of *C. album* was performed. Six compounds were identified in methanolic root extract through GC-MS analysis. The most abundant compound was 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (58.56%) followed by 9-octadecenoic acid (Z)-, methyl ester (12.75%) and 9-octadecenoic acid (Z)-, methyl ester (10.27%), which might be responsible for antifungal activity of methanolic root extract of *C. album.*[20]

IV. CONCLUSIONS

Pulse crops such as chickpeas, lentils, and dry peas are grown widely for human and animal consumption. Major yield- and quality-limiting constraints include diseases caused by fungi and oomycetes. The environmental and health concerns of synthetic fungicides used for disease management, emergence of fungicide-resistant pathogens, and demand for organic pulse crop products necessitate the search for effective alternatives. Safe and environmentally friendly plant-derived essential oils (EOs) have been reported effective against some pathogenic fungi. Growth on EO-amended growth medium and an inverted Petri plate assay were used to determine the effects of 38 oils and their volatiles on mycelial growth and spore germination of important pathogenic fungi and oomycetes: *Aphanomyces euteiches, Botrytis cinerea, Colletotrichum lentis, Didymella pisi, D. rabiei, D. lentis, Fusarium avenaceum, Stemphylium beticola, Sclerotinia sclerotiorum, and Pythium sylvaticum.* These essential oils incorporated in media inhibited mycelial growth of all the pathogens by 100% at 1:1,000 to 1:4,000 dilution. In addition, (1:500 dilution) showed complete inhibition of conidial germination (0% germination) of *F. avenaceum* and *D. pisi.* All seven EO volatiles inhibited mycelial growth of all pathogens by 50 to 100% except for *B. cinerea* and *S. sclerotiorum*. EO effects on mycelial growth were fungistatic, fungicidal, or both and varied by EO. EOs show potential for management of major crop diseases in organic and conventional production systems.[20]

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