Antimicrobial activity of *Eucalyptus globulus* oils

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Plant essential oils are complex mixtures of volatile organic compounds which may possess antimicrobial activities of interest in the food and cosmetic industries as well in the human health field. Consequently, studies on the antimicrobial activities of essential oils have becomes increasingly important in the search for natural and safe alternative in the last decades. This review discusses the antimicrobial activities of essential oils of *Eucalyptus globulus* that have been reported in scientific references. At the same time a survey of the important methods generally used for the evaluation of antimicrobial activity and some of the mechanisms involved in the antimicrobial activities of essential oils are also reported.

Keywords: essential oils; *Eucalyptus globulus*; antimicrobial activity

1. Introduction

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases and growing problem of antimicrobial resistance has become a significant public health concern worldwide and especially in developing countries as a result of overuse and misuse of antibiotics [1]. Essential oils obtained from aromatic plants have recently gained popularity and scientific interest. Many plants are used for different industrial purposes such as food, drugs, and perfumery manufacturing [2]. Their use have taken place since ancient times, and despite many of them were substituted by synthetic ones, the demand for natural products is increasing [3]. They have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties [4,5]. They are the most promising natural antimicrobials, because they do not cause microbial resistance due to the diversity of mechanisms of action. They have a GRAS status given by the U.S. Food and Drug Administration, meaning that they are generally recognized as safe for human consumption without limitations on intake and commonly accepted by consumers [6].

Eucalyptus is one of the diverse genus of flowering plants in the world belongs to the family *Myrtaceae* (subfamily *Myrtoideae*) and comprises about 800 species. It has been used in folk medicine throughout the world as anti-inflammatory, analgesic and antipyretic remedies for the symptoms of respiratory infections, such as cold, flu, and sinus congestion [7,8].

Essential oils from *Eucalyptus* species have been approved as food additives, and the extracts also widely used in modern pharmaceutical, and cosmetic industries[9]. In addition, the oil possesses a wide spectrum of biological activity including antimicrobial, fungicidal, insecticidal/ insect repellent, herbicidal, acaricidal and nematicidal [10]. The main of this review article is to focus on the characteristics of essential oils of *Eucalyptus globulus*, their antimicrobial activities and the mechanisms involved in the inhibition of these pathogenic microorganisms.

2. Taxonomy, botanic and ecology characteristics

The family *Myrtaceae* is composed of at least 3,000 species in 130-150 genera [11]. They have a wide distribution in tropical and sub-tropical areas, and are cultivated in many other climates [12]. *Eucalyptus globulus* is a tree of the genus *Eucalyptus* from *Myrtaceae* family[13].

The Tasmanian blue gum, southern blue gum or blue gum Eucalyptus (*Eucalyptus globulus*), is an evergreen, typically grow from 30 to 55 m tall. The tallest currently known specimen in Tasmania is 90.7 m tall [14], up to 200cm in diameter [15].

Root system deep and spreading. Bark smoothish, mottled gray, brown, and greenish or bluish, peeling in long strips, at base becoming gray, rough and shaggy, thick and finely furrowed; inner bark light yellow within thin green layer.

Leaves alternate, drooping on flattened yellowish petioles 1.5-4 cm long, narrowly lanceolate, 10-30 cm long, 2.5 - 5 cm wide, mostly curved, acuminate at tip, acute at base, entire, glabrous, thick, leathery, with fine straight veins and vein inside marlin, shiny dark green on both surfaces [16].

Flowers bisexual, regular, whitish; pedicel up to 8 mm long; flower buds top-shaped, divided into an oboconical, ribbed or smooth hypanthium (lower part) 5-12 mm x 5-17 mm, and flattened, hemispherical operculum (up-per part) 3-15 mm x 3-17 mm, having a short knob, stamens numerous, ovary inferior, 3-5 -celled [15].

The fruits are woody and range from 1.5 to 2.5 cm in diameter. Numerous small seeds are shed through valves (numbering between 3 and 6 per fruit) which open on the top of the fruit. It produces roots throughout the soil profile, rooting several feet deep in some soils. They do not form taproots [14].

The Eucalyptus tree consists with fragrant foliage rich in oil glands and is an excellent source of commercially important eucalyptus oil that finds extensive use in pharmaceutical, perfumery and industry [17].

The most important type of eucalyptus oil is the medicinal type derived primarily from *Eucalyptus globulus* Labill (Tasmanian blue gum) and *E. exserta* F. Muell. (Queensland peppermint)[18]. The essential oil extracted from leaves of *Eucalyptus globulus* Labill is known to be a rich source of traditional medicines with a variety of biological activities. It is widely used to treat pulmonary tuberculosis, diabetes, asthma and also used as disinfectant, antioxidant agent, and antiseptic agent especially in the treatment of respiratory tract infections and certain skin diseases [19].

3. Physico-chemical properties of oil

E.globulus oil is colourless to light yellow with camphoraceous odour and the following properties.
Specific gravity at 20 °C 0.9065-0.9155
Optical rotation -9 39 ' to + 5 27 '
Refractive index at 20 °C 1.463-1.466
Acid value 0.18- 1.04
Saponification value 8.90- 12.0
Saponification value after acetylation 17.00-21.68 [20, 21].

The eucalyptus oil is a complex mixture of a variety of monoterpenes and sesquiterpenes, and aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketones; however, the extract composition and proportion of which varies with species [17]. Wide-ranging studies on *Eucalyptus globulus* have been achieved which report the isolation of various phytoconstituents from the plant. The leaves have been reported to possess various volatile constituents aromadendrene, γ-cadienene, 1,8-cineole, α-gurjunene, globulol, linalool oxide, eremorphilene, β-pinene, pipertone, α-,β- and γ-terpinen-4-ol, and alloaromadendrene. Moreover, borneol, bornylacetate, camphene, caproic acid, citral, eudesmol, fenchone, isoamylalocohal, ρ-menthane, myrecene, myrtenol, trans-pinocarveol, sabinene, α-terpineol, α and β-thujone, thymol, transverbinol, verbinone, asparagine, cysteine, glycine, glutamic acid, norvaline, ornithine, threonine have been found in fruits of the plant [13].

The chemical composition of the essential oil varies with season, location, climate, soil type, age of the leaves, fertility regime, the method used for drying the plant material, and the method of oil extract [22].

4. Anti-microbial agents from *Eucalyptus globulus* essential oils

People in different parts of the world traditionally use essential oils and their components for various microbial infections related to skin, fever, gut and respiratory tract [23].

In spite of production of a number of new antibiotics by pharmaceutical industries in the last three decades, resistance development by microorganisms limited the use of these drugs for the treatments of diseases. The growing problem of antibiotic resistance has made it compulsory to look for suitable alternatives [24].

Many plants have been used because of their antimicrobial traits and antimicrobial properties of plants have been investigated by a number of researchers worldwide. Ethno-pharmacologists, botanists, microbiologists and natural product chemists are searching the world for phytochemicals which could be developed for treatment of infectious diseases [25].

Essential oils from higher and aromatic plants have shown growth inhibitory potential against microbes due to the presence of certain secondary metabolites [26], most of which are phenols or their oxygen-substituted derivatives [27].

Eucalyptus essential oils and their major constituents possess toxicity against wide range of microbes including bacteria and fungi, both soil-borne and post-harvest pathogens. They have been found to reduce mycelial growth and inhibit spore production and germination [28].

4.1. Antibacterial actions of *E. globulus* essential oils

Previous antibacterial studies showed that *E. globulus* essential oil had antibacterial effect on the growth of Gram-negative and Gram positive bacteria.

Several aromatic plants, mainly Eucalyptus spp. (E. camaldulensis, E.tereticornis, E.alba, E.citiodora, E.deglupta, E.globulus, E.saligna and E.robusta), had potentially useful medicinal effects against Pseudomonas aeruginosa, although the effectiveness of different plants could not be correlated with the content of any major constituent such as 1,8-cineole, α- pinene, and ρ-cymene of the oils [29].

Mounchid et al. (2005) [30] examined the antibacterial effect of *E. globulus* essential oils on *Escherichia coli* CIP54127 and *E. coli* isolated from urine and resistant to several antibiotics by micro-atmospheric technique and reported that oils were effective against the two strains bacteria with Minimal inhibitory quantity of 60 µl. Salari et al.

(2006) [12] used *Eucalyptus globulus* leaf extract to evaluate their activity on 56 isolates of *Staphylococcus aureus*, 25 isolates of *Streptococcus pyogenes*, 12 isolates *Streptococcus pneumonia* and seven isolates of *Haemophilus influenzae* obtained from 200 clinical specimens of patients with respiratory tract disorders. MIC_{50s} for these species were 64, 32 and 16 mg/ml, respectively; MIC_{90s} were 128, 64, 32 and 16 mg/ml, respectively; and MBCs were 512, 128, 64 mg/l, respectively. In the study conducted by Cermelli et al. (2008) [31] to evaluate the antibacterial property of *Eucalyptus globulus* essential oil on 120 isolates of *Streptococcus pyogenes*, 20 isolates of *S pneumoniae*, 40 isolate of *Staphylococcus aureus*, 40 isolates of *Haemophilus influenzae*, 30 isolates of *H. parainfluenzae*, 10 isolates of *Klebsiella pneumoniae*, 10 isolates of *Stenotrophomonas maltophilia*, they found that *H. influenzae,* and *S.maltophilia* were most susceptible, followed by to this oils. Our previous *in-vitro* experiments revealed the activity of *Eucalyptus globulus* essential oil against *E.coli* and *S.aureus* [32, 33]. The essential oils extracted from *Eucalyptus globulus* were tested by Bachheti et al. (2011) [34] against *E.coli*, *P.aeruginosa*, *Streptococcus*, *Lactobacillus* and *S.aureus* using agar diffusion method. The diameter of inhibition zone ranged between 3 (*P. aeruginosa*) and 14 mm (*E. coli*). Damjanovic-Vratnica et al. (2011) [35] reported that MICs of eucalyptus oil from Montenegro against 17 microorganisms, including food poisoning and spoilage bacteria and human pathogens, varied between 0.3 and 3.13 mg/ml. Moreover, Ait-Ouazzou et al. (2011) [36] demonstrated that Moroccan *E. globulus* essential oils displayed a bacteriostatic and bactericidal effect against seven pathogenic and spoilage bacteria of significant importance. Sharma et al. (2014) [37] tested *Eucalyptus globulus* essential oils against *Sphingobium indicum*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* using disk diffusion method and found that Eucalyptus oil exhibited inhibited growth on *S. indicum* and *E. coli* but *Staphylococcus* and *Bacillus* strains were completely insensitive to this oil. The essential oil from the fresh leaves of Indian *Eucalyptus globulus* Labill showed significant inhibitory activity against gram positive *Bacillus subtilis*, *Staphylococcus aureus* and gram negative bacteria *Pseudomonas aeruginosa*, and *Escherichia coli* [38]. A study carried out by Nadjib et al., (2014) [39] on the antibacterial activity of essential oil of *Eucalyptus* against 20 clinical bacterial isolates (7 gram-positive bacteria and 13 gram-negative strains) revealed potent antimicrobial activity against Gram-positive more than Gram -negative bacteria. The diameter of inhibition zone varied from 69 mm to 75 mm for *Staphylococcus aureus* and *Bacillus subtilis* (Gram +) and from 13 to 42 mm for Enterobacter sp and Escherichia coli (Gram-), respectively. A study by Pombal et al., (2014) [40] including a Portuguese *Eucalyptus globulus* EO, reported this oil to be active against *Escherichia coli* and *Staphylococcus aureus*. Bachheti (2015) [41] tested essential oil of *E. globulus* on two Gram-positive strains (two Gram-positive strains (*Staphylococcus aureus* MTCC 3160 and *Staphylococcus epidermidis* MTCC 435) and two Gram-negative strains (*Pseudomonas aeruginosa* MTCC 7453 and *Klebsiella pneumonia* MTCC 4030). The results of this study showed that this oil has strong effect on these bacteria with diameter zone inhibition ranged between 23 and 28 mm and Minimum inhibitory concentration (MIC) for the oil ranged from 0.72 to 2.75 µl/ml. Dezsi et al. (2015) [42] found that the essential oils from the leaves of *E. globulus* exhibited weak activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogenes* and *Escherichia coli* with diameter zone of inhibition ranged between 2.3 and 10.1 mm and MIC ranged between 30 and >100 µg\mL. Madouri et al. (2015) [43] observed that *Eucalyptus globulus* oils exhibited a marked antibacterial activity against Gram negative bacteria, mainly for *Fusobacterium nucleatum* ATCC 25586 (MIC = 1.14 mg/mL) and *Porphyromonas gingivalis* ATCC 33277 (MIC = 0.28 mg/mL).

Leaf oils from Brazilian-grown Eucalyptus globulus, xylitol and papain substances were tested by Mota et al. (2015) [44] against Pseudomonas aureginosa, Salmonella sp, Proteus vulgaris, Escherichia coli and Staphylococcus aureus. The Eucalyptus globulus oil showed higher inhibition than control (chlorohexidine) when applied to Staphylococcus aureus and equal inhibition when applied to the following microorganisms: Escherichia coli, Proteus vulgaris. Most recently, a Tunisians researchers team investigated the antimicrobial activity of 19 essential oils on 11 bacterial species (6 Gram positive, 5 Gram negative) and 7 fungal species (2 dermatophytes, 1 mould, 4 yeasts) by microdilution assays., and they found that MIC of the effect of Eucalyptus globulus essential oils ranged between 0.90 and 4.50 mg/mL for bacterial strains [45]. Another Portuguese researchers' team examined the antibacterial effect of Eucalyptus globulus essential oil on P.aeruginosa, E.coli, K. pneumoniae, Salmonella Typhimurium, Acinetobacter baumannii and reported that essential oil had high antibacterial activity against all test bacteria with MIC and MBC value ranged between 4 and 32 µl/ml[46].

The in vitro antimicrobial activities of *E. globulus* essential oil incorporated in chitosan films was evaluated b essential oil incorporated in chitosan films was evaluated by Hafsa et al. (2016)[47] against bacterial strains that commonly contaminate food products. Results showed that the rate of inhibition was greater, on gram negative bacteria (*E.coli*, *P.aeruginosa*) than that observed on gram positive bacterium (*S.aureus*). Mekonnen et al. (2016)[48] screened some essential oils and their constituents for their antibacterial activity against *S. typhi*, *S. paratyphi*, *S. thphimurium, Shigella species, P. aeruginosa, S.aureus* and *E.coli*. The results showed that *E. globulus* oil displayed strong antibacterial effects with diameter inhibition zone ranging between 10 and 32 mm.

Further, studies haves also documented that eucalyptus essential oils are effective even against resistant strains of microbes. For example, Sherry et al. (2001)[49] demonstrated that a topical application of eucalyptus oil can effectively remove the methicillin resistant *Staphylococcus aureus* infection. Trivedi and Hotchandani (2004)[50] examined the antibacterial activity of *Eucalyptus* oils against multidrug resistant *E.coli*, *Proteus*, *Klebsiella*, *Pseudomonas* and

S.aureus. The results of the study revealed that oil of eucalyptus has antibacterial activity against gram positive as well as gram negative bacteria. Mulyaningsih *et al*. (2011) [51] reported that the eucalyptus (*globulus*, *radiata* and *citriodora*) oils and the components (Aromadendrene, citronellol, citronellal and 1,8-cineole) were hardly active against multidrug-resistant Gram-negative bacteria.

4.2. Antifungal actions of *E. globulus* essential oils

The increasing incidence of drug-resistant pathogens and the toxicity of existing antifungal compounds have drawn attention towards the antimicrobial activity of natural products. The small number of drugs available for fungal treatment, most of which are fungistatic, and the emerging resistance to antifungal agents encourage the search for alternative treatments [52]. Plants therefore constitute an excellent source for substances that can be used in the formulation of new antifungal agents [53]. Among this plants, many studies are focused on the search of antifungal agents from *Eucalyptus globulus,* which its oil has demonstrated varying amount of antimicrobial effectiveness.

Benjilali *et al.* (1984) [54] tested antifungal properties of six essential including *E. globulus* against 39 mold strains (13 from the genus *Penicillium,* nine from *Aspergillus* and 17 others). Overall, the eucalyptus oils showed a weak activity on the test organisms. Similar results were obtained with the same test oils but by using an alternative method of testing. *Eucalyptus globulus* oil demonstrated a moderate activity against *Byssochlamys nivea*, *Geotrichum candidum*, *Paecilomyces variotii*, *Penicillium purpurogenum* and *Stachybotrys* sp., but was the least effective on all spoilage organisms, among the oils [55]. Singh and Dwivedi (1987) [56] reported that among five different oils tested, *Eucalyptus globulus* and *Ocimum americanum* (syn. *O. canum*) were the most effective in the control of Sclerotium rolfsii, the causative organism of foot-rot of barley, with MICs of <4000 ppm. However, in other studies conducted by same researchers' team [57, 58] neem oil (from *Azadirachta indica)* exhibited more activity against *S. rolfsii* than both *Eucalyptus globulus* and *0. americanum* oils. Nevertheless, *Eucalyptus globulus* oil showed considerable activity towards ten soil fungi, including the mycotoxigenic *Penicillium citrinum;* it was most active against *Trichoderma viride* [57]. The effect of oregano (*Origanum compactum* Benth.), mugwort (*Artemisia herba-alba* Asso) and eucalyptus (*Eucalyptus globulus* Labill.) oils on spore germination, mycelial elongation and sporulation were studied by Tantaoui-Elaraki et al.(1993) [59] in three fungi (*Zygorrhynchus sp.,Aspergillus niger and Penicillium italicum*). Oregano oil was observed to be the most active on the three phenomena studied, followed by mugwort at spore germination and sporulation stages and eucalyptus oil when mycelial elongation was considered. Lis-Balchin et al. (1998) [60], and Montes- Belmont and Carvajal(1998) [61] observed a lack of antifungal activity when *E.globulus* essential oil and 1,8-cineole solution on *A. flavus* growth were compared. Ramezani et al. (2002a,b) [62, 63] reported that volatile oils from lemon scented eucalyptus and its major constituents monoterpene citronellal possessed a wide spectrum of fungicidal activity and inhibited the growth of fungal pathogens.

Tan et al. (2008) [64] analyzed antimicrobial activity globulol separated from the extract of *Eucalyptus globulus* Labill (*Myrtaceae*) fruits against *Alternaria solani*, *Fusarium oxysporum* f.sp. *niverum*, *F. graminearum*, *Rhizoctonia solani* and *Venturia pirina*. The median effective inhibitory concentration (IC_{50})values were 47.1 µg mL^{-1}, 114.3µg mL^{-1},53.4 µg mL^{-1}, 56.9 µg mL^{-1}, 32.1 µg mL^{-1} and 21.8 µg mL^{-1}, respectively. Bansod and Rai (2008) [65] screened some essential oils for their antifungal activity against *Aspergillus fumigatus* and *Aspergillus niger*. The results showed that *Eucalyptus globulus* oils displayed strong antifungal effects with diameter inhibition zone ranging between 18 and 22 mm. Vilela et al. (2009) [66] tested *E. globulus* EO and its major compound 1,8-cineole against *A. flavus* and *A. parasiticus* and found a complete fungal growth inhibition of both species with the essential oil by contact and volatile assays. Martins et al. (2010) [67] evaluated the in vitro antifungal activity of *E.globulus* essential oils against *Mucor hiemalis*, *Alternaria alternaria*, *Penicillium sp*., *Penicillium glabrum and Fusarium roseum.* The results found that Eucalyptus essential oils were lethal at concentration between 2.5-20 Eucalyptus essential oils were lethal at concentrations between 2.5–20 µL/mL and inhibited growth of all fungal species between 1.25-5 µL/mL. The in *vitro* antifungal activity of a combination of some essential oils extracted from the herbs (*Thymus vulgaris*, *Salvia officinalis*, *Eucalyptus globulus* and *Mentha piperita*) against some filamentous fungal strains (*Metrhizium* sp., *Ophiostoma* sp., *Trichoderma* sp. and *Penicillium expansum*) was determined by Mousavi and Raftos (2012) [68]. The fungal strains were sensitive to this combination and MIC and MFC values were, respectively, 0.022 and 0.064 mg/ml for *Metrhizium* sp., 0.02 and 0.064 mg/ml for *Ophiostoma* sp., 0.018 and 0.048 mg/ml for *Trichoderma* sp. and 0.03 and 0.085 mg/ml for *Penicillium expansum*.

Several studies have been conducted to evaluate the antifungal activity of the *E.globulus* essential oils against *Candida* spp wherein the oils used in these studies have demonstrated varying degrees of antifungal effectiveness [69, 70, 71, 72, 73, 74, 75, 76, 77, 44]. Essential oils of *Eucalyptus globulus* L. were evaluated for their efficacy to control *Aspergillus parasiticus* and *Fusarium moniliforme* growth and their ability to produce mycotoxins by López-Meneses et al. (2015) [78] who suggest that Essential oils affect *F. moniliforme* and *A. parasiticus* development and mycotoxin production. Mekonnen et al. (2016) [48] showed a good antifungal activity of *E.globulus* essential oil against *Trichophyton* spp1 (27.3 mm) and *Aspergillus* spp1(11 mm).

4.3. Antiviral actions of *E. globulus* essential oils

Eucalyptus oils not only show toxicity against a wide range of fungi and bacteria but also possess antiviral activity. Australian *Eucalyptus globulus* essential oil demonstrated weak antiviral activity against HSV-1,2. It affected the virus before or during adsorption but not after penetration into the host cell [79]. The antiviral effect of 12 essential oils including *Eucalyptus globulus* oils on herpes simplex virus type-1 (HSV-1) replication was examined *in vitro*. The replication ability of HSV-1 was suppressed by incubation of HSV-1 with 1 % *Eucalyptus globulus* essential oils at 4°C for 24hr [80]. The activity of *Eucalyptus globulus* essential oil was determined by Cermelli et al. (2008) [31] for a strain of adenovirus and a strain of mumps virus. The antiviral activity assessed by means of virus yield experiments tittered by the end-point dilution method for adenovirus, and by plaque reduction assay for pumps virus, disclosed only a mild activity on mumps virus. Essential oils from eucalyptus, tea tree and thyme and their major monoterpene compounds alpha-terpinene, gamma-terpinene, alpha-pinene, ρ-cymene, terpinen-4-ol, alpha-terpineol, thymol, citral and 1,8-cineole were examined by Astani et al. (2010) [81] for their antiviral activity against herpes simplex virus type 1 (HSV-1) in vitro. These essential oils including Eucalyptus were able to reduce viral infectivity by > 96 %, the monoterpene inhibited HSV by about > 80%. Essential oils from *Cymbopogon citratus*, *Mentha piperita*, *Melaleuca alternifolia*, *Eucalyptus globulus*, *Ocimum basilicum*, *Pelargonium graveolens*, and *Thymus vulgaris*) and three ethanolic extract of *Glyzirrhiz glabra*, *Plantago major* and *Zizyphus spina christi* were screened by Shaheen Aly (2012) [82] for their inhibitory effect against Herpes simplex virus type one (HSV-1) and Hepatitis A virus (HAV) *in vitro* on vero cells using plaque reduction assay. The results showed that Herpes simplex virus was more sensitive towards plant extracts than Hepatitis A virus.

Davood et al. (2012) [83] reported that methanolic extracts of *Eucalyptus globulus* had a significant inhibitory effect against HSV-1 and concentration (200, 150, 50 μg/mL) has the best effect and (> 200 μg/mL) has the lowest effect on HSV-1. In other study, Ethanol extract of 21 samples derived from 19 species of plants that were explored from East Java region were tested by Wahyuni et al.(2013) [84] against Anti Hepatitis C virus (HCV) activities by cell culture method using Huh 7.5 cells and HCV J6/JFH1. The results showed that *Eucalyptus globulus* stem with IC_{50}: 15.1 μg/ml is one from the 6 of 21 samples which have potential activity against HCV.

Recently, Vimalanathan and Hudson[85] evaluated several essential oils and some of their major constituents for their possible anti-influenza virus properties in both liquid and vapor phases. Among them, *Eucalyptus globulus* showed excellent activity at higher concentrations, but were much less effective at the lower concentrations by liquid phase, and significant activity against influenza virus following exposures of only 10 minutes in vapor phases. In other study, Versiati et al. (2014) [86] evaluated the anti-hepatitis virus C activity of extract and fractions of *Eucalyptus globulus* stems against 2a strain of JFH1a hepatitis virus. The results showed that the ethanol extract of *Eucalyptus globulus* stems, dichloromethane, ethyl acetate, and butanol fraction have activity in inhibiting the viral infection of cells with IC_{50} value of 10.19 μg/mL; 1.64 μg/mL; 10.49 μg/mL; and 18.78 μg/mL respectively.

4.4. Mechanism of Action of essential oil

The diversity of essential oil constituents is enormous and presents a wide range of compounds. Some have low or no efficiency against microorganisms while others are potent antimicrobials. The majority of antimicrobial compounds found in essential oils are terpenoids and phenylpropenes with most active being phenols, although some aldehydes and non-phenolic substances also present promising antimicrobial activity [87]. The antimicrobial action of essential oil components is determined by lipophilicity of their hydrocarbon skeleton and the hydrophilicity of their major functional groups. The antimicrobial action of essential oil components has been ranked as follows: phenols> aldehydes> ketones> alcohols> ethers> hydrocarbones [88]. Therefore, essential oils with phenols as main compounds express the highest activity against microorganisms, and their activity spectrum is the broadest, while oils with ethers and alcoholic compounds are slightly less active [89].

Antimicrobial compounds may target various cell structures or chemical pathways, such as cell wall degradation, membrane damage, dissipation of the proton motive force, decrease in extracellular protease activity, o-lipopolysaccharide rhamnose content, ergosterol content or unsaturated fatty acids [90].

The mechanisms by which essential oils can inhibit microorganisms involve different mode of action, and in part may be due to their hydrophobicity[91]. As typical lipophiles, they pass through the cell wall and cytoplasmic membrane, disrupt the structure of their different layers of polysaccharides, fatty acids and phospholipids and permeabilize them.

Cytotoxicity appears to include such membrane damage[92]. In bacteria, the permeabilization of the membranes is associated with loss of ions, reduction of membrane potential, collapse of proton pump and depletion of the adenosine triphosphate (ATP) pool [93]. Essential oils can coagulate the cytoplasm and cause damages to lipids and proteins [94]. In the eukaryotic cells, essential oils can provoke depolarization of the mitochondrial membranes by decreasing the membrane potential, affecting ionic Ca^{++} cycling and other ionic channels, and reduce the pH gradient, affecting (as in bacteria) the proton pump and the ATP pool [95].

Antimicrobial properties of essential oils reveal that Gram-positive bacteria are more vulnerable than Gram-negative bacteria. This greater resistance could be attributed to the outer membrane surrounding the cell wall, which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering [96]. Zaika(1988) [97] proposed that Gram-positive bacteria are more resistant then Gram-negative bacteria to the antibacterial properties of plant volatile oils which is in contrast to the hypothesis proposed by Deans that the susceptibility of bacteria to plant volatile oils and the Gram reaction appears to have little influence on growth inhibition [98, 99].

4.5. Antimicrobial susceptibility testing

The currently available screening methods for the detection of antimicrobial activity of natural products fall into three groups, including bioautographic, diffusion, and dilution methods. The bioautographic and diffusion methods are known as qualitative techniques since these methods will only give an idea of the presence or absence of substances with antimicrobial activity. On the other hand, dilution methods are considered quantitative assays once they determine the minimal inhibitory concentration [100].

a) Dilution methods

Dilution assays are standard methods used to compare the inhibition efficiency of antimicrobial agents [101]. The test extracts or compounds are mixed an appropriate medium that has been previously inoculated with the test microorganisms [102]. The main advantage of dilution methods is possibility to estimate the concentration of the test compound in the agar medium or the broth suspension [103]. In the agar-dilution method, the Minimal Inhibitory Concentration (MIC) is usually the lowest concentration able to inhibit any visible microbial growth [104] In liquid or broth -dilution methods, turbidity and redox-indicators are most frequently used. Turbidity can be estimated visually or obtained more accurately by measuring the optical density at 405 nm. However, test samples that are not entirely soluble may interfere with turbidity readings, emphasizing the need for a negative control or sterility control, i.e. extract dissolved in blank medium without microorganisms [104, 105, 102]. The major benefit of this assay is that it allows determining whether a compound or extract has a microbicidal or microbistatic action at tested concentration [105]. The minimal bactericidal or fungicidal concentration (MBC or MFC) is determined by plating-out samples of completely inhibited dilution cultures and assessing growth (static) or no-growth (cidal) after incubation. In most investigations, the redox indicators, 3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) and resazurin are frequently used to quantify bacterial [106, 107] and fungal growth [108, 109]. In general, dilution methods are appropriate for assaying polar and non-polar extracts or compounds for determination of MIC and Minimal Bactericidal Concentration (MBC)/ Minimal Fungicidal Concentration (MFC) values. Using redox indicators or turbidimetric endpoints, dose-response effects allow calculation of IC_{50}- and IC_{90}-values, which are the concentrations required to produce 50 and 90 % growth inhibition [104].

b) Agar-diffusion methods

The agar diffusion method is the most widely used technique for assaying plant extract for their antimicrobial activity [105]. In the diffusion technique, a reservoir containing the test compound or extract, at a known concentration, is brought into contact with an inoculated medium and the diameter of the clear zone around the reservoir () is measured at the end of incubation period [104]. In order to enhance the detection limit, the inoculated system is kept at lower temperature for several hours before incubation to favour compound diffusion over microbial growth, thereby increasing the inhibition diameter. Different types of reservoirs can be used, such as filter paper discs, stainless steel cylinders placed on the surface and holes punched in the medium.

The hole-punch method is the only suitable diffusion technique for aqueous extracts, because interference by particulate matter is much less than with other types of reservoirs. To ensure that the sample does not leak under the agr layer, fixed agar is left on the bottom of the hole [110]. The small sample requirements and the possibility to test up to six extracts per plate against a single microorganism are specific advantages [111].

The diffusion method is not appropriate for testing non-polar samples that do not easily diffuse into agar. In general, the relative antimicrobial potency of different samples may not always be compared, mainly because of differences in physical properties, such as solubility, volatily and diffusion characteristics in agar. Furthermore, agar-diffusion methods are difficult to run on high capacity screening platforms [104].

c) Bioautographic methods

Antimicrobial activity can be used by bioautography that localizes on a chromatogram using three approaches:

- direct bioautography, where the microorganism grows directly on the thin-layer-chromatographic (TLC) plate;
- contact bioautography, where the antimicrobial compounds are transferred from TLC plate to an inoculated agar plate through direct contact;
- agar-overlay bioautography, where a seeded agar medium is applied directly onto the TLC plate [112, 113].

These procedures are based on the agar diffusion technique, whereby the antimicrobial agent is transferred from the thin layer or paper chromatogram to an inoculated agar plate through a diffusion process [112]. The inhibition of bacterial growth by compounds separated on the TLC plate is visible as white spots against a deep red background [114]. The red background is as a result of p-iodonitrotetrazolium chloride reduction by bacteria into formazan.

5. Conclusion

A literature-based survey of *Eucalyptus globulus* and their essential oils with antimicrobial activity was carried out. A number of studies have reported that *Eucalyptus globulus* essential oils and many of their components possess and exhibited different degrees of antimicrobial activity against a wide spectrum of bacteria, fungi and virus. These differences may be explained by susceptibility testing conditions, method of extraction, physicochemical characteristics of the oil, and even strain to strain differences. The antimicrobial studies reported in the present review confirm the therapeutic value *Eucalyptus globulus* and support the use of this plant in folk medicine. It could be used as a potential antimicrobial in animal breeding, as anti-infective and therapeutic agents, and in agriculture to the fight against phytopathogenic microorganisms and insects. In addition, *Eucalyptus globulus* oils, as plant secondary metabolites, offer many possibilities as natural preservatives in perfumery and food industries.

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