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Biological properties of *Eucalyptus* L sps. extracts against microbial pathogens: An updated review

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Abstract

Plants allelopathic ingredients being non-hazardous and environmental friendly are uses as a natural substitute of pesticides. Keep in view this aspect of plants; present study aims to review published work of *Eucalyptus* for its antimicrobial properties against different disease causing agents from last eight years. Diseases due to biotic agents are among, the most significant factors that cause severe losses to agriculture foodstuffs every year. Plants produce active compounds during secondary metabolism that are usually responsible for the biological properties. *Eucalyptus* has an extensive record of curative uses with a variety of important beneficial properties such as boost respiratory health, eliminate inflammation, strengthen the immune system, lower blood sugar, protect skin health, relieve tension and anxiety, and fight against pathogens infection. Moreover, the most important compounds of the plant are under investigation for possible benefits in the treatment of cancer. However, apart from its uses as a medicinal plant the pesticides properties of several *Eucalyptus* species were proven against certain pests in the form of both essential oils and extracts by research data from the international literature. Thus, this property can be confidently used in the development of natural pesticides to combat those bacterial and fungal strains that become resistant to conventional pesticides. This method of pest management of agricultural importance can significantly contribute to minimize the risk of toxic chemical pesticides, especially sprayed on economic crops and vegetables.

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Introduction

Eucalyptus L. is a native genus of Australia, belongs to family *Myrtaceae* with about 900 species throughout the world (Brooker and Keing 2004). It is distributed on an area of more than eighteen million hectares (Grattapaglia 2008). A French botanist Heritier proposed the name *Eucalyptus* and published first description about this plant in 1789. This name (*Eucalyptus*) has given due to a special character of eucalyptus that is an operculum (calyptras) covered its flower bud. Most of the species of *Eucalyptus* are evergreen range from the size of a small shrub to a medium-tall flowering tree.

Crop damages caused by plant pathogens have become major concern in agriculture. The frequent and misuse chemical applications for the control of these pathogens results several problems like residual effect of chemicals in food, resistance in target pathogens for chemicals and environmental pollution. Today, there are strict regulations on chemical pesticide use, and there is political pressure to remove the most hazardous chemicals from the market. A lot of work has verified in laboratory trials that different plant tissues, such as roots, leaves, seeds and flowers containing natural compounds of known therapeutic properties against bacteria, fungi and insects (Davicino *et al.* 2007). Medicinal plants possess a variety of chemical constituent for this reason they are used as excellent antimicrobial agents that's why great attention has going towards extracts and biologically active compounds isolated from popular plant species.

Biological screening of *Eucalyptus* L. extract is carried out throughout the world for the determination of their antimicrobial activity. The efficiency of this plant extract and Essential oil as pesticides has been reported by different researcher in different times. Different compositions of oil are produce by the oil glands present in the leaves (Grieve 1992). Essential oil distillation takes place from the leaves and young twigs either fresh or dry.

The main components of essential oil recorded are 1, 8-cineol and limonene. *Eucalyptus* oil is used as natural pesticides have an important role in view of the environmental and toxicological inference of the haphazard use of artificial pesticides and reducing the trouble of increasing pest resistance (Batish *et al.* 2000).

Aim and motivations

Presently, different extracts have been studied, in attempts to identify defensive effects against agents that cause disease. In this paper, we conduct a review of different Eucalyptus species which, by means of their pesticidal activity, protect the plants and animals from the damage caused by agents such as fungi, bacteria and viruses *etc.* In addition to these studies, observed evidence for the use of natural compounds for the managements of pest diseases has a long history, and this field has become an innovative field of study, with the principal aim of analyzing the consumption of medicinal plants by a great number of people and the different phytochemicals that are extracted from these plants. In general, plants contain a variety of chemical compounds, such as phenols, coumarins, lignans, essential oils, monoterpenes, glycosides, alkaloids, carotenoids, flavonoids, organic acids, and xanthenes Bhawna and Kumar (2009).

This present review had as its objective to organize and evaluate previously published data based on works that have demonstrated antimicrobial capacity, as well as analysis of some phytochemicals extracted from Eucalyptus essential oil that have been evaluated in different models of toxicity. With these goals in mind, the authors of this paper have attempted to provide information and bibliographic support to pest management researchers who are exploring compounds with this potential and encourage focusing their efforts on developing alternative inputs to acquire new technologies for the extraction of plant extract that can be used for controlling pests and diseases.

The pharmaceutical companies have been motivated to develop new antimicrobial drugs in recent years, especially due to the constant emergence of microorganisms resistant to conventional antimicrobials and its awareness about the safety of its utilization without any harmful effects.

Materials and methods

Study area

The study was done in all relative articles from 2008-2015, that were collected by performing Google search. Searches were made by means of different key words i.e. *Eucalyptus* species, extracts, essential oil, antimicrobial, antifungal, antibacterial and antivirus. Without duplication total of 169 papers were explored, used at least one of the key observation proposed. Only 50 of them made useful investigation and were integrated in the analysis.

This review summarizes all on hand data about the effects of *Eucalyptus* different Sps against disease causing microorganisms.

Results

Screening as fungicidal properties

Plant pathogens are serious panic, as they cause huge injury to economic crops and results yield losses. Research from the world wide shown that *Eucalyptus* L. oil has marked antiseptic action against a wide variety of infectious bacteria, viruses and fungi (Inouye *et al.* 2001). Bashir and Tahira (2012) uses the aqueous, methanol and n-hexane extracts of *Eucalyptus* L. leaves stem and bark as natural fungicides because it contains antifungal constituents. The *Eucalyptus* crude aqueous extracts offer a potential antifungal property against *Candida albicans* (Uzama *et al.* 2011).

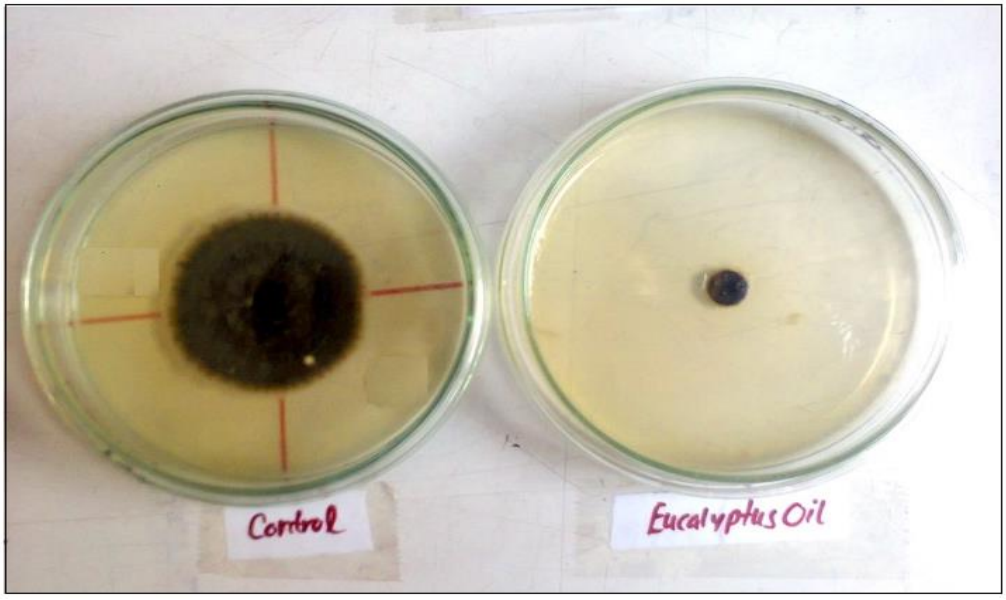

Fig. 1. Effect of Eucalyptus E.oil treatment.

Crude extracts of *E. citriodora* very effective in suppressing the growth of fungus *Didymella bryoniae* (Fiori *et al.* 2000).

The alcoholic and chloroform extracts of *E. citriodora* leaves having strong antifungal activity against *A. Rabia* (Jabeen and Javaid, 2008). The organic solvent extracts of *E. citriodora* especially chloroform extracts contain antifungal constituents and can effectively be used for the management of *M. phaseolina* (Javaid and Rahman 2011).

The *E. citriodora* methanol extract has maximum fungal growth inhibition against *Aspergillus flavus, Aspergillus fumigates, Aspergillus nidulans* and *Aspergillus terreus* followed by essential oil, chloroform extract and aqueous extract (Javed *et al.* 2012).

The foliar spray of *Eucalyptus cameldulensis* L. Leaf extracts on tomato plants results, significant reduction in early blight disease under greenhouse and field conditions (Nashwa and Abo-Elyousr 2012).

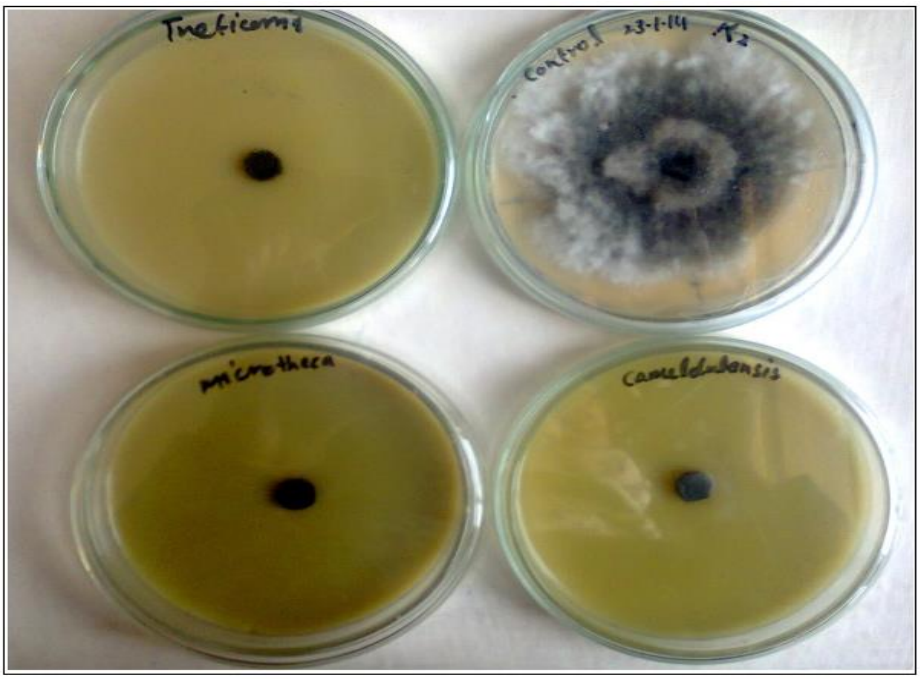

Fig. 2. Effect of Eucalyptus different species crude extracts.

E. camaldulensis Dehnh and *E. citriodora* Hook, extracts showed the fungicidal activity against three pathogenic fungi, *A. alternate*, *Drechslera hawaiiensis* and *D. tetramera*. E. *camaldulensis* exhibited highly pronounced antifungal potential. Higher concentrations of both the *Eucalyptus* species were generally less effective as compared to lower concentrations of the employed extracts (Bajwa and Iftekhar, 2005) For instance, the mycelia growth of *Colletotrichum sublineolum*, *Phytophthora* sp. and *Sclerotium rolfsii* are 100% inhibited by 20% fresh leaves aqueous extract of *E.citriodora*. (Stangarlin *et al.* 2011).

Fig. 3. Hydrodistilation of E.oil.

Most of the studies reports that the essential oils of *E. camaldulensis* having some active compounds that show antibacterial properties (Cimanga *et al*. 2002). The E. oil of *E. camaldulensis* is strong antifungal agent especially against the seedling blight pathogens, domestic molds and wood decay fungi (Siramon *et al*. 2013). More interestingly, the mycelial growth of wood decay fungi is drastically inhibited by the *E. camaldulensis* essential oils (Su *et al*. 2006). According to some results,

Eucalyptus citriodorahas shown moderate fungicidal response to the *Rhizoctonia solani* (Sehajpal 2009). *E. citriodora* oil efficacy is more towards fungi, Gram-positive and drug resistant mutants of *C. albicans* as well as *E. coli* as compared to Gram-negative and wild type microbes (Luqman *et al*. 2008). The *E. citriodora* E. oil found to be non-phytotoxic at morphological level. It has likewise been found effective in the control of fruit rot of M. *pumilo* (Sushi and Shahi 2011).

Fif. 4. Effect of E.oil in mircroenvironment.

Methanol bark extract of *Eucalyptus tereticornis* is more effective in inhibiting *Candida albicans* as compared to methanol leaf extract (Jain *et al*. 2010). *E.unigera* and *E. darlympleana* effectively inhibited mycelia growth of the *B.dothidea* and *B. cinere*. However, *E. gunii* and *E. globulous* have no inhibitory effect on the mycelial growth of *B. dothidea*. Among the pathogenic fungi, *D. actinidiae* is highly sensitive to crude extracts of *E. darlympleana*, *E. globules*, *E. gunii* and *E.unigera* (Soon *et al*. 2008).

The *E. globulus* essential oil has rather strong antimicrobial activity, especially against *Candida albicans* (Biljana *et al*. 2011). *Eucalyptus* crude and boiled extract show good inhibition of *Alternaria brassicae*. Essential oil extract is less effective as compared to crude and boil extracts (Sasode *et al*. 2012). The *Eucalyptus* L. methanol extracts remarkably inhibiting the mycelial growth as well as germination of conidia of *Alternaria alternata*.

According to some results, the methanol extracts are more effective than mixture of methanol and water extracts Zaker and Mosallanejad (2010). Essential oil of *E. sargentii* more actively eliminates Gram-positive growth than the Gram-negative bacteria and exhibited significant antifungal activities against *Aspergillus flavus* and *Candida albicans*. (Bardaweel *et al*. 2014).

Chemical identification evidenced that *Eucalyptus* oil has oxygenated monoterpenes molecules that are responsible for the antimicrobial potential against food spoilage yeasts (S. cerevisiae SPA) in fresh fruit juices. (Amit *et al*. 2014). Similarly *A. Niger and R. solani* are effectively inhibited by *E. citriodora* oil while *E. microtheca* E.oil is found significant against *A. Niger*. These inhibitory properties might be associated to the good chemical contents of oils such as phenolic, alcoholic or aldehyde, which could be linked to the ecological allocation and environmental changes on making of chemicals in plants (Ghaffar *et al*. 2015).

Fig. 5. Crude extract preparation.

Antibacterial potential of eucalyptus

The antibacterial effects of different plant extracts have been studied in different times from all over the world (Reddy *et al*. 2001). *Eucalyptus* species are counted in those plants that are most competent in antibacterial treatments of infections i.e. upper respiratory tract infections (Ben-Arye *et al*. 2011). The Ethanol extracts of *Eucalyptus* exhibit potent antibacterial activity against cariogenic bacteria such as *streptococcus mutant, streptococcus sobrinus* (Amrutesh 2011).

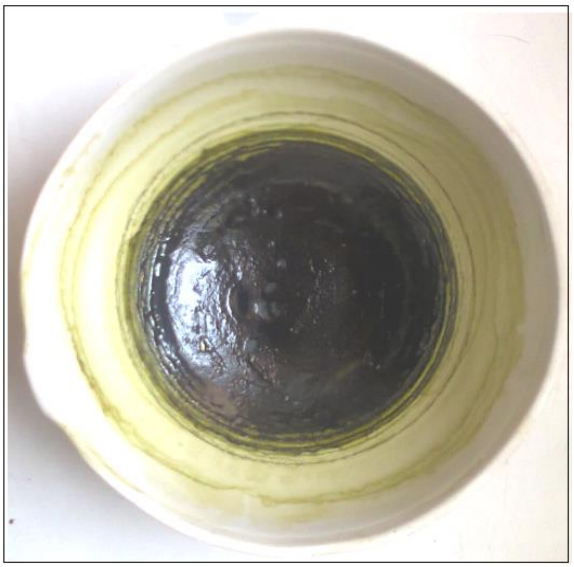

Fig. 6. Dried Crude extract.

Crude extracts of *E. globulus* show the most active antibacterial potency (Oswaldo *et al*. 2012). The methanol extract of E. globules drastically inhibit the bacterial growth (Egwaikhide *et al*. 2007). Both the Gram negative bacteria (E. coli) and gram positive bacteria (*S. aureus*) are affected by the water-distilled extracts of *E. globulus* leaves Ghulam and Mohamed (2012). *Eucalyptus globulus* fruit oil exerts the most prominent activity against methicillin-resistant *Staphylococcus aureus* (Chhetri *et al*. 2008). Similarly, the *Eucalyptus globulus* essential oil having antibacterial activity against *Staphylococcus aureus, Klebsiella* species and *Escherichia coli* (Mulyaningsih *et al*. 2011). *E. globulus* and *E. Camaldulensis* leaf essential oils show an excellent inhibitory effect on *S. aureus* than that of *E. coli* (Ghulam and Mohamed 2008).

The effectiveness of *Eucalyptus* essential oils are significantly varied within species and strains. Some Gram-positive bacteria such as *S. aureus* and *Enterococcus faecalis* are the most sensitive, while some Gram-negative bacteria such as

P. aeruginosa and *Escherichia coli* are the most resistant. A similar antibacterial effect due to essential oils of *Eucalyptus odorata* having the best action against *S. aureus* followed by *S. agalactiae, H. influenzae, S. pyogenes* and *S. pneumoniae*. *E. maidenii* oil shows a comparatively good activity against *S. aureus* (Ameur *et al.* 2012). The essential oil of three *Eucalyptus* Sps *Eucalyptus tereticornis, Eucalyptus globules Eucalyptus robusta* can be used as natural antibiotic against infectious diseases caused by *Staphyllococcus aureus, Streptococcus, Lactobacillus* (Bechheti *et al.* 2011).

The ethanol extract of *Eucalyptus tereticornis* is a more potent inhibitor against *Pseudomonas aeruginosa, Bacillus thurangenisis* and *Bacillus cereus,* compared to methanol extracts (Badrunnisa *et al.* 2011). Similarly the methanol extract of *Eucalyptus camaldulensis* inhibited the growth of *Bacillus subtilis* and *Staphylococcus aureus* but had no inhibitory effects on *Escherichia coli* (Jouki and Khazaei 2010). The essential oil of *E. globules* leaves efficiently good to use for the cure of urinary tract infection and typhoid fever caused by *Salmonella typhi, K. pneumoniae, P. mirabilis, and S. aureus* and urinary tract pathogens (Ogbuanya *et al.* 2013). The major component of *Eucalyptus* oil, 1.8- cineole (90.4 %) and P-cymene (39.4%) seem to have a synergistic effect against the inhibition of *Candida albicans, Staphylococcus aureus* and *Escherichia coli.* (Nah *et al.* 2014). *Eucalyptus* E oil successfully treats the *P. aeruginosa* that is most difficult bacteria in hospital infections. This bacterial strain has a strong resistance to the E oil of peppermint and orange (Dias *et al.* 2014). It is more interesting that usually Gram-negative bacteria are more resistant to essential oils than Gram-positive bacteria. But *E. camaldulensis* essential oil has more inhibitory property on Gram-negative bacteria than Gram-positive bacteria (Ghulam and Benali 2014).

Fig. 7. Extract drying under redusced pressure.

The essential oils *from E. maideni, E. astrengens, E. cinerea*, *E.bicostat mostly uses in* pharmaceutical and food products due to very significant inhibition against *Listeria ivanovii and Bacillus cereus***.** These properties are credited to α-pinene (1.27 to 26.35%) and 1, 8-cineole (49.07 to 83.59%) the major components present in the oil of *Eucalyptus*. (Sebei *et al.* 2015).

E. globulus having very effective antibacterial properties against both Gram-positive and Gram-negative bacterial strains especially against *Listeria monocytogenes* (Dezsi *et al.* 2015). Similar results observed for *Eucalyptus globules* essential oil against strains of different microorganisms and possibly will be feasible to alternatively use as germicidal agent (Valeria *et al.* 2015).

Using *Eucalyptus* extract containing chewing gum possibly will recover gingival index, reduce blood loss during probing, and reduce periodontal diseases. In this way formation of biofilm of plaques is inhibited and prevents tooth decay (Nagata *et al*. 2008).

Antiviral potential

Cotton leaf curl virus disease incidence is significantly cut down by *Eucalyptus globules* extract compared to untreated crops (Ali *et al*. 2010).

Discussion

The literature about antimicrobial achievements of plant based products is very extensive, with an increasing figure of publications per year. It is very easier said than done, to describe the countless articles about the pesticides properties of these natural products in this review, regarding a matter of such a large complexity and requires multidiscipline study. However, the experimental data from the international literature, as reviewed here, indicated that *Eucalyptus* has been found effective in controlling certain agricultural and animal pests due to the presence of a variety of bioactive constituents that interfere with pathogens behavior and growth**.**

Plants extracts and essential oils are composed of wide variety of components and their biological properties are therefore linked to their composition, pattern, quantity and their potential interaction. These special effects can be *Additive*: the joint effect of the components is equal to the sum of the individual effects, *Antagonist*: the activity of components in mixture is inferior in contrast when they are useful individually and *Synergetic*: the action of the combined substances is higher than the sum of the individual activities. (Lis-Balchin *et al*. 1998).

The action of E.oil against bacteria may be bacteriostatic (only slow down the growth of bacteria) or bactericide (turn down in the number of cells of bacteria). In bacteriostatic state, the microbial cells get well their reproductive capability after neutralization of the agent.

While the other one (bactericidal) has a stable result and microbial cells are not able of growth and replication, even the agent are neutralized (Bloomfield 1991).

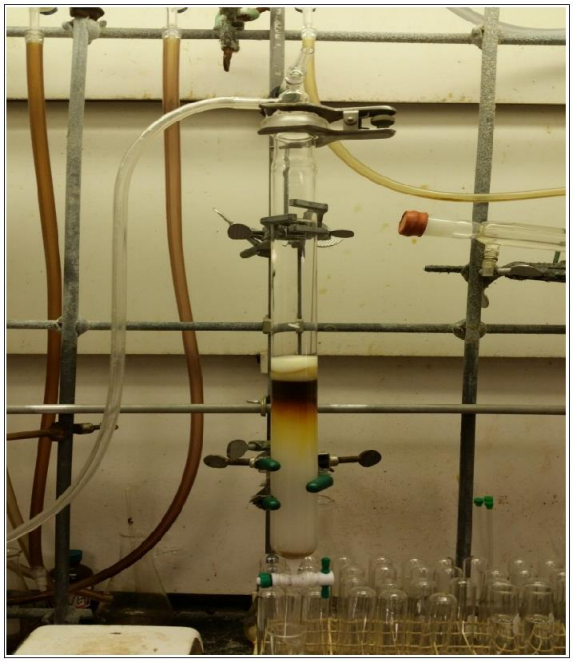

Fig. 8. Separation of Extract.

A lot of work has been done on the antimicrobial activity of *Eucalyptus* E. Oil and extracts (Bardaweel *et al*. 2014; Dezsi *et al*. 2015; Dias *et al*. 2014; Ghaffar *et al*. 2015; Ghulam and Mohamed 2012). The Eucalyptus E. oil and extracts inhibited pathogens growth but their effectiveness varied. Some species are more active i.e. *E. bosistoana, E. botryoidal, E. camaldulensis, E. cinerea and E. citriodora,* and causes total inhibition of some pathogen like *L. monocytogenes* and *S. aureus* while *E. citriodora* and *E. radiate* caused a total inhibition of every strain of bacteria tested (Lis- Balchin and Deans 1997). Usually Gram-positive bacteria are more susceptible to the natural compounds in general than Gram-negative bacteria. But some eucalyptus spa having oil that the most significantly inhibit the gram-negative bacteria. There are two possibilities for these inhibitions, either it may be due to differences in cell wall structure of Gram positive and Gram-negative bacteria (Gootz 2010) or it may be the heterogeneous chemical composition of E. oil because one major compound of E.oil 1, 8-cineole is founded in very rich quantity in *E. camaldulensis* and *E. citriodora* has only 1.2% of this molecule.

On the other hand, citronellal that is another compound of eucalyptus oil is found in *E. citriodora* in (72.7%) but does not exist in the E.oil of *E. camaldulensis* (Cimanga *et al*. 2002). So it is possible that this pesticides result is due to some minor compound being there in the *Eucalyptus* E. oil such as globulus a minor component of E.oil, extracted from leaf of *E. globulus* has a strong antifungal activity against *Fusarium graminearum, Alternaria solani, Rhizoctoniasolani, Venturia pirina* and some minor compounds that are used as a pharmacological active component or as antiseptic sanitizer. The oxygenated monoterpenes evidenced for the antimicrobial activity during the characterization of different chemical molecules of eucalyptus oil (Amit *et al*. 2014).

The antimicrobial properties of essential oils depends on some important characters, include hydrophobic mechanism that permit the contribution of lipids from cell membrane of bacteria to disturb cell structures and make them more permeable (Sikkema *et al*. 1994). Cell wall and cell membrane disorders are linked with the components of E. oils. They discharge lipopolysaccharides from Gram negative bacteria, following increase of cell membrane permeability and loss of ATP. (Gaunt *et al*. 2005; Caillet *et al*. 2005; Nguefack *et al*. 2004; Oussalah *et al*. 2006). Hydrophobicity is a special characteristic of essential oil that enhanced cell permeability and resulting cell constituents' leakage (Mulyaningsih *et al*. 2010; Turgis *et al*. 2009). This loss of cell constituents severely damage cell membrane. Cell membrane disruption by any antimicrobial agent (E.oil) cooperate several very important function, i.e. energy exchange, nutrient processing, structural macromolecules synthesis, growth enzymes secretion. The cytoplasmic membrane proteins are affected by components that are present in essential oils. The growth of pseudo-mycelia in bacteria is stimulated by some essential oils that gave confirmation regarding to act on enzymes concerned in the synthesis of bacterium structural mechanism (Conner and Bechet 1984).

When phenolic compounds and carboxylic acids are in non-charged form can penetrate the microbial cell membrane. Essential oil indirectly acts on the cell membrane in case of *Staph. Aureus and B. cereuses* by secreting toxins.

In prokaryotes ATP produce both in the cell wall and in the cytosol. When E.oil acts on cell membrane probably the intracellular and extracellular ATP balance would be affected. The intracellular ATP losses are thought to be taking place due to disturbed membrane by e. oil (Turgis *et al*. 2009). The compounds of E.oil affect synthesis of protein. When bacterial cells were treated with two important components caracole and cymene of E.oil heat shock proteins (HSPs) is synthesized (Burt *et al*. 2007). The pH in bacterial cells may be weakening by the act of E.oil on the membrane that loses its ability to block protons (Lambert *et al*. 2001; Turgis *et al*. 2009; Oussalah *et al*. 2006). E.oil may act on bacterial DNA attached to the cell membrane. It quantifies the genotoxicity and antimutagenic effects of antimicrobial agents (De Martino *et al*. 2009).

Conclusion

From the above outcome it is summarized that, the crude mixture either they are extracted from leaves, bark or flowers of different species of *Eucalyptus* L. can be used as biological control agent against different pathogens. Thus, this property can be a confidently used in the development of natural pesticides to combat those bacterial and fungal strains that become resistant to conventional pesticides. This method of pest management of agricultural importance can significantly contribute to minimize the risk of toxic chemical pesticides, especially sprayed on economic crops and vegetables. Further research is needed for the isolation, purification and characterization of these active ingredients that are responsible for biological activities. Because once the structure of those active ingredients are identified that can be used as a lead to form the new synthetic pesticides that would be less harmful, easily degradable and environmentally sound.

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