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Research Article

Phytochemical and Bioactivity of Commercially Available *Eucalyptus* Oil against Human Pathogens

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ABSTRACT

Many species of Eucalyptus are used as a folk medicine. Traditionally, they are used in the treatment of many respiratory tract infections like bronchitis, sinusitis and pharyngitis as it is rich with the antimicrobial inhibitory agent. The present study aimed to determine the phytochemical and bioactivity of commercially available Eucalyptus oil against human pathogens which cause many infections in human population. The "AXE Brand Eucalyptus oil" which are used for the relief of colic and stomachache show significant bioactivity against *Staphylococcus aureus, Escherichia coli, Salmonella paratyphi A, Salmonella paratyphi B, Shigella dysenteriae, Bacillus licheniformis and Pseudomonas aeruginos*, which is seen as a significant pathogen. However, Highest bioactivity observed against *Bacillus licheniformis* whereas no activity or zeroes zone of inhibition shows against *Bacillus subtilis* and *Salmonella typhi*. Phytochemical tests to know about the secondary metabolites show the presence of phytosterols, phenols, tannins, flavanoids, protein and amino acid. On the bases of this result, the oil of eucalyptus can be effectively utilized in the treatment of infectious disease as well as in Sanitizers and disinfectant as an innate and environmental friendly product.

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INTRODUCTION

In today's world large numbers of medicinal plants and their compound have shown beneficial therapeutic activity (Aqil et al., 2006). These medicinal plants are used for the mitigation of various diseases and characterize as a rich source of antimicrobial agent. In different countries plants are used medicinally for the extraction of potential active and powerful drugs (Srivestava et al., 1996).

The Eucalyptus belongs to the Myrtaceae family and includes about 900–700 species over the entire world (Broker and klerining 2006, 2004). It is the native genus of Australia, but few numbers of these plants are also the native of New Guinea, Indonesia and the Phillipines. The importance of eucalyptus is due to their wood, proteins, tannins, gum, and dyes, but the most important product of eucalyptus is their oil. Approximately 300 species of this genus have volatile oils in their leaves. Less than 20, within these species, have a high content of 1,8– (more than 70% and up to 90%), which is commercially applied for the synthesis of essential oils in cosmetic and pharmaceutical industries (Pino et al., 2002). There are many studies that support the antimicrobial activity of eucalyptus oil. One of the best study showed that the *Eucalyptus* oil is effective against 22 kinds of bacteria and 11 kinds of fungus (Pattnaik et al., *1995*). According to one of the researcher three different species of Eucalyptus – *Eucalyptus globulus*, *Eucalyptus tereticornis* and *Eucalyptus robusta* showed excellent activity against *Escherichia coli, Salmonella typhi, Streptococcus Lactobacillus, Pseudomonas aeruginosa and Staphylococcus aureus* (Bachheti et al., 2011). *For* instance, E. *globules* contain 1,8–channel which showed toxicity against Pediculus humanus capitis (human head lice) (Yang et al., 2004), it has also strong antibacterial activity against *Es, Streptococcus pyogenes, Candida albicans Acinetobacter baumannii, and Klebsiella pneumoniae* (Biljana et al., 2011).

Eucalyptus contains many chemical complexes that play significant roles in the plant. These chemicals consist of the many chemical compounds that are providing safety against UV radiation, vertebrate, insects and cold stress. The main phytochemical present in Eucalyptus oil are steroids, alkaloids, tannins, saponins, phenolics, flavonoids, terpenoids glycosides (Saxena et al., 2010, Pathmanathan et al., 2010, Jain et al., 2010). Among of these chemicals the better–known chemicals constituted are the terpenoids, which giving eucalyptus foliage, its natural smell and synthesis most of the essential oil (Lassak and MacCarthy 1992). In many countries Eucalyptus species are traditionally used as folk medicine. The Aborigines (native Australians) have used eucalyptus leaves to cure wounds and fungal infections (Takahashi 2004). While in Tunisian essential oil of eucalyptus species has been used for the treatment of respiratory tract disorders such as sinusitis, pharyngitis, and bronchitis (Boukef 1986).

Today these essential oils have great value in the market (Ajaj 1995) since they are employ for the treatment of chest pain, or cough, influenza, and skin rashes while

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their vapour is inhaled to cure the inflammation (Musyimi and Ogur 2008).There application are also in the stringent, anesthetic, anodyne, deodorant, diaphoretic, expectorant, febrifuge , disinfectant, fumigant, hemostat, rubefacient,cancer, diabetes, diarrhea, diphtheria encephalitis, enteritis, laryngitis,erysipelas,leprosy, malaria, mastitis, miasma, rhinitis, laryngalgia, sores, sore throat, and trachalgia.((Elliot and Jones 1980).To the best of our information there are no such studies on phytochemical and bioactivity of market available eucalyptus oil, therefore the main objective of this study was conducted it and evaluate its activity.

MATERIALS AND METHOD

Product and Culture

The synthesis oil of eucalyptus of "AXE brand universal oil "was purchased from the local market of Saudia Arabia. The microbial culture used for the antibacterial test was *Bacillus subtilis,Staphylococcus aureus,Escherichia coli,Salmonella typhi,Salmonella paratyphi A,Salmonella paratyphi B,Shigella dysenteriae, Bacillus licheniformis and Pseudomonas aeruginosa* which was obtained from the Institute of Environmental studies and Department of Microbiology, University of Karachi.

Antibacterial Assay

The antibacterial sensitivity of eucalyptus oil was performed by using two methods the disc diffusion method (Anonymous 1993) and by agar well diffusion method (Irobiet et al., 1994). For control Cefixime antibiotic disc was applied. The plate was incubated at 37 °C for 24 hours. The antibacterial activity was measured through the diameter of the zone of inhibition, which is expressed in millimeter (mm). For more accuracy the test was conducted in triplicate.

Phytochemical Screening

To investigate the different compound, the oil of eucalyptus was tested by follow the following procedures.

Detection of Alkaloids

Wagner`s Test: Few drops of oil were dissolved in 1 ml of hydrochloric acid than filter it. In Filtrated with a few drops of Wagner`s reagent added (Iodine in potassium Iodide). Brown/reddish color precipitate indicates the presence of alkaloids (Tiwari et al., 2011)

Detection of Saponins

Foam Test: 2ml of oil was treated with 6ml of water into a test tube. The mixture was trembling vigorously and observed in the formation of persistent foam that verifies the presence of saponins (Ugochukwu et al., 2011).

Detection of Phytosterols

Salkowski's Test: 1 ml of oil was treated with chloroform and later filtered. The filtrates were treated with a few drops of Concentrated Sulfuric acid, shaken for some times and allowed to stand. The presences of golden yellow color indicate the oil contain triterpenes (Tiwari et al., 2011).

Detection of Tannins

Gelatin Test: 1% gelatin solution containing sodium chloride was added in a few drops of oil. White precipitate in tube confirms the presence of tannins (Tiwari et al., 2011).

Detection of Flavonoids

Ferric chloride Test: Ferric chloride solution treated with drops of oil in the test tube that result in the formation of blackish red color point out the presence of flavonoids (Bhandary et al., 2012).

Detection of Proteins and Amino Acids

Xanthoproteic Test: 5–6 drops were treated with a few drops of conc. Nitric acid. Appearance of yellowish color verifies the presence of proteins and amino acid (Tiwari et al., 2011).

Detection of Quinones

Few drops of oil were treated with concentrated Hydrochloric acid and observed for the appearance of yellow precipitation (or coloration) (Ugochukwu et al., 2011).

Detection of Terpenoids

1 ml of chloroform was treated with 2ml of oil, then added a few drops of Concentrated Sulfuric acid. A reddish brown precipitation generates immediately indicated the presence of terpenoids (Ugochukwu et al., 2011).

Detection of Phenol

Ferric Chloride Test: 0.5 ml of ferric chloride solution was added in a test tube containing oil. The appearance of bluish black color indicates the presence of phenols (Tiwari et al., 2011).

Data Analysis

All the date and graphs which is given, analysis on Microsoft Office Excel–2007 and all the zones of inhibitions were expressed as mean ± standard deviation (SD).

Table 1: Zone of inhibition of *eucalyptus oil* from Disc Diffusion method

Bacteria Spp.	Gram reaction	Zone of inhibition in mm (0.025 mg)
Bacillus subtilis	Gram positive	NE
SalmonellaTyphi	Gram negative	NE
Staphylococcus aureus	Gram positive	7.2±0.4
Salmonella Paratyphi A	Gram positive	5.8±0.4
Salmonella Paratyphi B	Gram positive	3.9±0.1
Shigella dysenteriaes	Gram negative	4±0.2
Bacillus lichniformis	Gram negative	10.5±0.2
Pseudomonas aeruginosa	Gram negative	1.1±0.2
Escherichia coli	Gram negative	7.9±0.2
Proteus spp	Gram negative	3.9±0.2

*For control Cefixime antibiotic used; *NE = No zone of inhibition

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RESULT AND DISCUSION:

Plants are important sources for the investigation of many drugs against different diseases (Jean et al., 2005). Essential oils have many volatile constitute natures, which have strong antibacterial activities (Cowan 1999). Eucalyptus plant also has a volatile compound in their oil, therefor its shows significant antimicrobial activity. The results in Table 1 and 2 revealed that the commercially available eucalyptus oil showed antibacterial activity with different magnitudes, depending on the size of inoculums and the concentration of oil. Diameter of inhibition zone oil varied from 1 to 7mm.In disc diffusion method *Bacillus lichniformis* shows highest susceptibility with eucalyptus oil and in agar well diffusion method highest susceptibility shows by *Salmonella paratyphi B.* whereas there is no effect of Eucalyptus oil is measure against *Bacillus.subtilis* and *Salmonella typhi* by both the techniques i.e. agar well diffusion and disc diffusion method.Eucluptus oil also shows significant restrain activity against *Escherichia coli and Staphylococcus aureus* that are consider as opportunistic pathogens that cause life–threatening and severe infections in immunocompromised patients (Lestari, 2004).

Table 2: Zone of inhibition of *Eucluptus oil* from agar well diffusion method

Bacteria Spp.	Gram reaction	Zone of inhibition in mm (0.1 ml)
Bacillus. subtilis	Gram positive	NE
SalmonellaTyphi	Gram negative	NE
Staphylococcus aureus	Gram positive	4.1±0.6
Salmonella Paratyphi A	Gram positive	2.9±0.2
Salmonella Paratyphi B	Gram positive	6.9±0.2
Shigella dysenteriaes	Gram negative	4.6±0.2
Bacillus lichniformis	Gram negative	3.5±0.5
Pseudomonas aeruginosa	Gram negative	1.9±0.2
Escherichia coli	Gram negative	3±0.5
Proteus spp	Gram negative	3.3±0.3

*For control Cefixime antibiotic used ; *NE = No zone of inhibition

This result is more conformed with the previous research on eucalyptus oil which result showed the significant inhibition effect on *Escherichia coli and Staphylococcus aureus* (Bachir and Benali 2012) *however, Farah* et al., *10, Babayi et al.,. 11, Gamal and Sabrin12 and Nair et al.,4 who also reported the inhibitory activity of eucalyptus essential oil against S. aureus and E. coli.* In both the test inhibition rate of gram negative bacteria and gram positive bacteria are almost similar however, according to the reports of some authors gram–negative bacteria are slightly more sensitive to essential oils of eucalyptus species when compared to gram–positive (Bachir and Benali 2012). Although the amount of inoculum and the concentration of oil may also affect the growth of the organisms. This antibacterial activity of Eucalyptus extracts has been due to the presence of many chemical components like citronella, 1,8–cineole, Citronellol, p–cymene, alloocimene, eucamalol, limonene, citronellyl acetate, linalool, β – pinene, γ –terpinene, α – terpinol, and aroma dendrene (Nezhad et al., 2009).

Table 3: Phytochemical investigation of *Eucalyptus oil*

Phytochemical	Eucalyptus oil
Alkaloids	–
Phytosterols	+
Phenols	+
Tannins	+
Flavanoids	+
Proteins and amino acid	+
Saponins	–
Quinones	–
Terpenoids	+

Key: Present = +, Absent =–

Phytochemical Analysis

Preliminary phytochemical testing for the presence of various compounds in eucalyptus oil reveals the positive presence of Phytosterols, Phenols, Tannins, Flavanoids, Terpenoids, Proteins and amino acid and negative presence of Saponins, Quinones and Alkaloids. Phytochemicals are aromatic compounds synthesized by plants which have been used as protective agents to microbial infection, insect and herbivore killer. The macromolecules produced by plants have been divided into primary and secondary metabolites (Rauha et al., 2005). The secondary metabolites act as, repellent, antibacterial agent or even toxic agents to pests and herbivores (Dewick, 1997). Our finding is somehow verifying with the result of Pathmanathan et al., the presence of tannins in eucalyptus specie was also reported in one of the previous study (Babayi et al., 2004).The eucalyptus oil, which is under tested used against many diseases. The plants which have terpenoids and tannins are used in anti inflammatory activities (Okwu and C. Jasiah 2006). Tannins are also responsible for antimicrobial characteristics in different plants (Chung 1998). Phenol is also present in eucalyptus oil. The presence of phenolic compounds in plant is responsible for the protection of carcinogen, inflammation, atherosclerosis and cardiovascular. Flavanoides also positive in eucalyptus oil, this is synthesized by plants in response to microbial infection (Nyarko and add 1990). Hence, the inhibitory mechanism of these oils on the microorganisms may therefore be due to the presence of the above phytochemical components.

The commercially available eucalyptus oil showed varying degrees of antibacterial activity against a reference strain of bacteria. The presence of important phytochemical in this oil also reveals its medical and herbal importance. This information provides a scientific ground for the application of this oil in the drug formulation for the prevention and treatment of bacterial infections caused by various pathogenic bacteria.

CONCLUSION

Eucalyptus oil has been shown a wide spectrum antimicrobial activity and has important phytochemical compound. After few modifications it is effectively used

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against human pathogens and this oil will use in manufacturing of different disinfectant and Sanitizers.

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CONFLICT OF INTEREST

There is no conflict of interest in authors.

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