Research Article

ANTIMICROBIAL ACTIVITY OF *EUCALYPTUS TERETICORNIS* AND COMPARISON WITH DAILY LIFE ANTIBIOTICS

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ABSTRACT

Eucalyptus is a fast growing tree which has shown to possess high degree of resistance against stressed environmental conditions. *Eucalyptus tereticornis* is widely cultivated in various parts of the world even in Pakistan. The medicinal properties of this tree reside in its oil. The main aim of our study is to check the antimicrobial activity of this valuable tree and to compare it with commercially available antibiotics. *Eucalyptus tereticornis* oil was extracted from the fresh leaves and branch tips during flowering season from surrounding areas of Hazara University, Pakistan. Different concentrations of oil were checked against Gram positive bacteria *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 49452), Gram negative bacteria including *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028) and *Pseudomonas aeruginosa* (ATCC 27853), and also against yeast *Candiada albican* (ATCC 2091). The oil was significantly active against all the microbes studied. The activity of *E. tereticornis* oil was compared with standard antibiotics Ciprofloxacin (CIP-5 µg), Chloramphenicol (C- 30 µg), Tetracycline (TE-30 µg) and Ampicillin (AMP 25-µg). The comparison gives the significant results and proves the antimicrobial efficiency of this valuable plant.

Keywords: *Eucalyptus tereticornis*, leave extracted oil, Antimicrobial activity, Antibiotics.

INTRODUCTION

Plants based medicines are important therapeutic weapon to cure human diseases. Plants are of relevance to pharmacology. Pharmacological properties of medicinal plants may be used as leads in developing novel therapeutic agents. For thousands of years, traditional plant derived medicines have been used in most parts of the world and their use in fighting microbial disease is becoming the focus of intense study.[1-2] Much of the research into traditional medicinal plant use has focused on Asian[3] and South American[4] plants.

A medicinal plant might contain one or more different compounds that might have medicinal activity. These pure compounds could be used or mixed together to make very effective medicines. The trend of growing interest in using medicinal plants is due to the awareness of the effectiveness of traditional medicines over and above orthodox medicines used for the managements of chronic ailments like Rheumatism, Diabetes, Hypertension, Sickle-cell anemia, Cancers, etc. in addition, our flora is a rich reservoir for new molecules which can be tapped in the discovery of new drugs molecules. This has economic advantage of combating the high cost of research on the discovery of new drugs. Major pharmaceutical houses have therefore, on-going research programs to discover potential molecules from natural resources[5].

Today herbal products and extracts are widely used to control various human diseases. Medicinal plants are providing an efficient local aid to the health care and disease free life and they contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various alignments[6].

Eucalyptus is a diverse genus of trees in the family Myrtaceae. Of the more than 700 species that comprise this genus, most are endemic to Australia. A smaller number are also native to New Guinea, Indonesia and the Phillipines. Eucalyptus can be found in almost every region of the Australian continent. They have also been widely introduced into drier subtropical and tropical regions in areas as diverse as Africa, the Middle East, India, USA and South America. In many of these areas these trees are considered invasive, whilst in other areas they are prized for their commercial applications. Eucalyptus are valued for their wood and some are also valuable sources of proteins, tannins, gum, and dyes although their most valuable product is the eucalyptus oil that is readily distilled from their leaves[7-8].

Essential oils from some Eucalyptus species (e.g *Eucalyptus pulverulenta*) comprise up to 90% cineol[9]. Essential oils from other plants containing cineol have been previously demonstrated antimicrobial properties[10]. Eucalyptus oil is used extensively in cleaning and de-odourising products as well as in cough drops and decongestants[7]. Eucalyptus oil has insect pest repellent properties and is a component in many commercial pesticides[11].

Eucalyptus tereticornis is a fast growing tree that can reach 30 to 45 m in height and 1 to 2 m in diameter. The species grows in open forests or as scattered trees in alluvial plains and along streams, including brackish

waters. It grows better in deep, well drained, light textured, neutral, or slightly acid soils. Outside its natural range, the tree has been planted in a great variety of places, including alluvial, muddy, and sandy clay soils. It tolerates seasonal floods for short periods and can endure up to 15 freezes per year in the southern part of its natural range. In the South of China and Pakistan the species survives temperatures of -7°C. The tree is planted amply in areas with summer rainfall and moderate to harsh dry seasons, although it does not tolerate long periods of drought. It thrives where annual precipitation is 800 mm to 1500 mm, but trees have been planted in areas with less rainfall (400 mm in India, 550 mm in Israel, and 580 mm in Zimbabwe) and in areas with considerably more rainfall (2180 mm in Colombia and 3500 mm in Papua New Guinea). It is found at elevations between 0 and 1000 m.[12]

The use of essential oils for the testing of antimicrobial activity is not without problems. The relative insolubility of many of the oil components retards their diffusion through agar gels in agar dilution or disc diffusion studies. Many studies have utilized solubilising agents to aid oil component diffusion, resulting in variable results.[13, 14] Solubilising agents appear to increase the susceptibility of some bacteria to antimicrobial agents, decrease the susceptibility of others, whilst having no effect on yet other bacteria. A recent study[15] has demonstrated the antibacterial activity of methanolic extracts of *Eucalyptus baileyana* leaves and Eucalyptus major leaves and flowers against a limited panel of bacteria.

Since multi drug resistance of microorganisms is major medical problem, screening of natural products and in search for new antimicrobial agents that would be active against these organisms[16] is the need of the hour. Development of microbial resistance to antibiotics is a global concern. Isolation of microbial agents less susceptible to regular antibiotics and recovery of increasing resistant isolates during antibacterial therapy is rising throughout the world which highlights the need for new principles[17]. Actually, essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers and their exploitation for potential multipurpose functional use[18-19]. Keeping in view of all these aspects, the main objective of present research is to determine the antimicrobial activity of locally available *Eucalyptus tereticornis* and to compare the antimicrobial activity with commercially available antibiotics.

MATERIALS AND METHODS

Collection of plant Material

Eucalyptus tereticornis (dark grey bark) leaves were collected during the flowering season from Harzara University, Pakistan. Fresh leaves were dried in an incubator at 40°C for 24 hours and the dried material was ground to a coarse powder. 200g of the powdered sample was subjected in 500ml round bottom flask for oil extraction through steam distillation. 1 ml of volatile oil was obtained by steam distillation and rectification from the fresh leaves (Indian Pharmacopoeia, 1996). This active material was used for the antibacterial assay.

Test Microorganisms

The in-vitro activity of the extracts was assayed against the bacterial strains which were obtained from Microbiology Laboratory of Hazara Univeristy. All the ATCC (American type culture collection) strains were maintained on Nutrient agar slants (Oxoid) at 4°C which were purchased from MicroBioLogics. All strains were identified according to the techniques described in the Manual of Clinical Microbiology. The bacterial strains on which the antibiotic efficacy of the plant extracts were evaluated are as *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 49452, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028 and *Pseudomonas aeruginosa* ATCC 27853.

Purity Testing of Each Organism

Each organism is inoculated form working culture of Nutrient Broth (Merk) on their respective selective media for control as well as for purity testing i.e *Pseudomonas aeruginosa* on (PCA) Pseudomonas Cetrimide Agar (Oxoid, CM0579), *Salmonella typhimurium* on (XLD) Xylose Lysine Deoxycholate Agar (Oxoid, CM0469), *Staphylococcus aureus* on (MSA) Mannitol Salt Agar (Oxoid, CM0085), *Enterococcus faecalis* on (S&B) Slanetz & Bartley (Oxoid, CM0377), *Escherichia coli* on (EMB) Eosin Methylene Agar (Oxoid, CM0069) and incubated at 37°C for 24hr.

Evaluation of Antimicrobial Activity on Different Concentrations

After the incubation time, one colony of each bacterium from their respective selective agar medium was inoculated into 5ml nutrient broth and incubated for 4-6hrs at 37°C. The inoculums were standardized by matching its turbidity with McFarland No 1 standard. The test culture was spread evenly on the surface of pre-sterilized plastic petri dish containing solidified (MHA) Mueller Hinton Agar (Oxoid CM 0337) with a sterile cotton swab. Wells were made in the MHA agar plate using a sterile cork borer of 6 mm. With the help of a sterile micropipette tips five different doses of oil i.e. 50µl, 60µl, 70µl, 80µl, 90µl and 100µl doses of *E.tereticornis* oil were poured in the wells. The plates were incubated at 37°C for 24hr. After 24hrs, the diameter of the resulting zone of inhibition was measured and the average values were recorded. Each antimicrobial assay was performed in at least triplicate. Mean values are reported in this manuscript.

Comparison with standard antibiotics

Standard discs (7mm diameter) of ampicillin 'AMP' (25µg), chloramphenicol 'C' '(30µg), ciprofloxicin 'CIP' (5 µg) and tetracycline 'TE' (30µg) obtained from Oxoid Ltd, were used as positive controls for antimicrobial activity against five different Gram positive and Gram negative

bacteria. The results of *E.tereticornis* were compared among the lowest and the highest concentration of the oil as well as with the standard antibiotics.

RESULTS

The oil from E. *tereticornis* was extracted by steam distillation according to the method described in literature.[20-22] The activity of oil was evaluated by well diffusion method previously mentioned in literature.[23-24]

The results obtained by antimicrobial analysis of *E.tereticornis* leaves oil are presented in table (1, 2 and 3).

Antimicrobial activity was assayed at quantities of 50µl, 60µl, 70µl, 80µl, 90µl, and 100µl against three Gram negative bacteria (*P.aeruginosa*, *E.coli*, *S.typhimurium*) in Table 1, two Gram positive spp. (*S.aureus*, *E.faecalis*) in Table 2 as well as against yeast (*C.albican*) in Table 3. The antimicrobial activity was compared with four standard antibiotics (CIP 5 µg, TE30 µg, AMP 25µg, C30µg). Different quantities of oil were used against each microbe lowest quantity was 50µl, and maximum quantity 100µl was used with the interval of 10µl increase. Oil showed significant results against all microbes studied.

Table 1: Zones of Inhibitions (mm) produced by *Eucalyptus tereticornis* against Gram Negative bacterial strains in comparison with standard antibiotic discs.

Bacterial strains	Different concentrations of Eucalyptus oil						Antibiotics			
	50µl	60 µl	70 µl	80 µl	90 µl	100 µl	CIP 5µg	TE 30µg	AMP 25µg	C 30µg
Pseudomonas aeruginosa ATCC®27853	14.55	17.33	16.97	17.01	17.07	18.06	23.89	10.91	00	00
Escherichia coli ATCC®25922	18.90	21.68	19.35	20.13	20.80	21.49	31.31	20.69	19.46	22.68
Salmonella tuphimurium ATCC®14028	14.59	17.08	14.79	15.22	15.55	16.69	21.82	16.40	25.06	23.66

Table 2: Zones of Inhibitions (mm) produced by *Eucalyptus tereticornis* against Gram Positive bacterial strains in comparison with standard antibiotic discs

Gram Positive Bacterial strains	Different concentrations of Eucalyptus oil						Antibiotics			
	50µl	60 µl	70 µl	80 µl	90 µl	100 µl	CIP 5µg	TE 30µg	AMP 25µg	C 30µg
Enterococcus faecalis ATCC®49452	20.97	23.09	23.69	26.12	25.24	26.65	21.49	24.41	30.50	23.10
Staphylococcus aureus ATCC®6538	22.28	25.86	26.53	26.72	27.82	30.27	20.87	25.64	30.98	22.73

Table 3: Zones of Inhibitions (mm) produced by *Eucalyptus tereticornis* against *Candida albican*

Fungus (Yeast)	Different concentrations of Eucalyptus oil						Antifungal Drug
	50µl	60 µl	70 µl	80 µl	90 µl	100 µl	---------
Candida albican ATCC®2091	13.47	15.30	17.49	17.88	18.17	20.31	--------

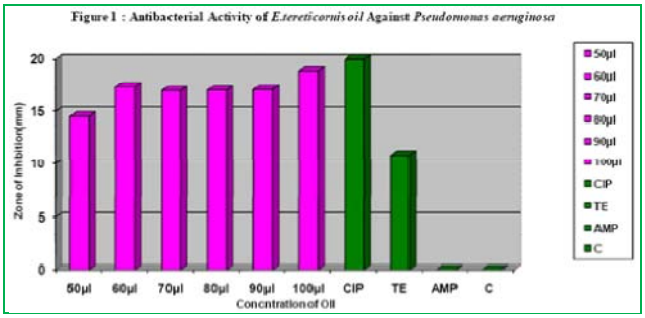
*Diameter of the disc is 7 mm (oxoid), and the results shown are the mean of three replicates.

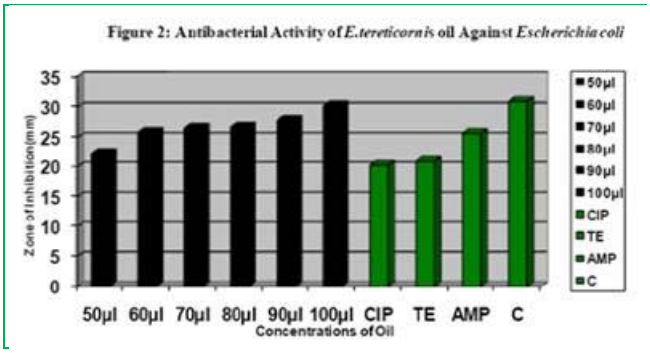
Antimicrobial activity against Gram Negative Bacteria

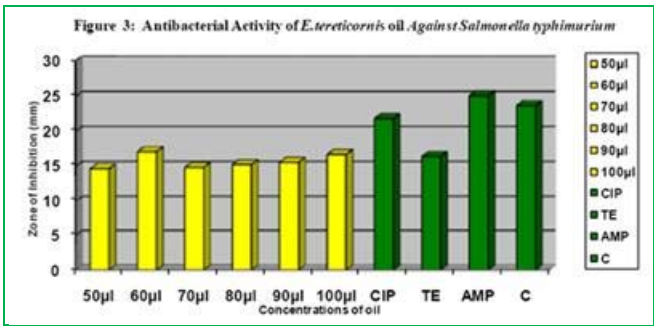
In case of Gram negative bacteria *P.aeruginosa* showed resistance against two broad spectrum antibiotics i.e. AMP and Chloramphenicol while it was sensitive to the oil with zone of inhibition 14.55mm to 18.49mm (Fig. 1 & 7). The most sensitive Gram negative spp. was *E.coli* with zone of inhibition from 18.90mm to 21.53mm at quantity of 50µl to 100µl (Fig. 2 & 8). *S.typhimurium* was least sensitive to the oil among all bacterial species studied with zone of inhibition from 14.59mm to 16.15mm (Fig.3 & 9). Among positive control drugs CIP and Chloramphenicol was sensitive to *E.coli* and *S.typhimurium* with zone of inhibition of 31.3mm and 23.66mm respectively.

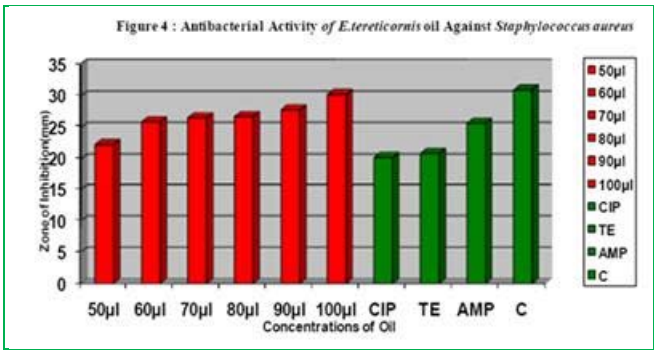
Antimicrobial activity against Gram Positive Bacteria

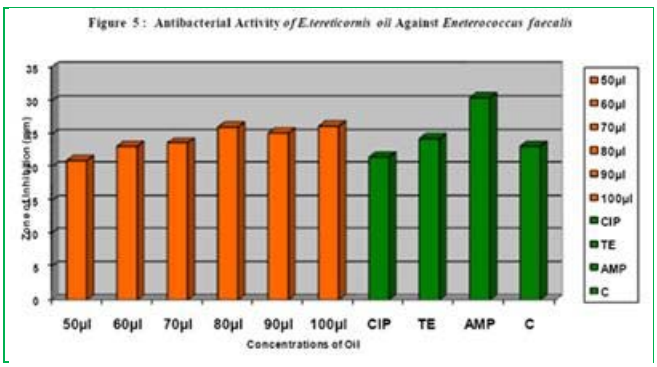
While Gram positive bacteria were more susceptible to the oil, the zone of inhibition increased from 22.28mm to 30.27mm and from 20.97mm to 26.65mm with increase in quantity from 50µl to 100µl against *S.aureus* and *E.faecalis* (Fig 4 & 5).While TE and AMP were sensitive to *S.aureus* with zone of inhibition 25.64mm and 30.98mm (Fig 10 & 11). From the current study it has been revealed that the difference in activity of oil at 50, 60 and 70µl was significant while the variation of activity among the quantities 70, 80 and 90µl was comparatively stable.





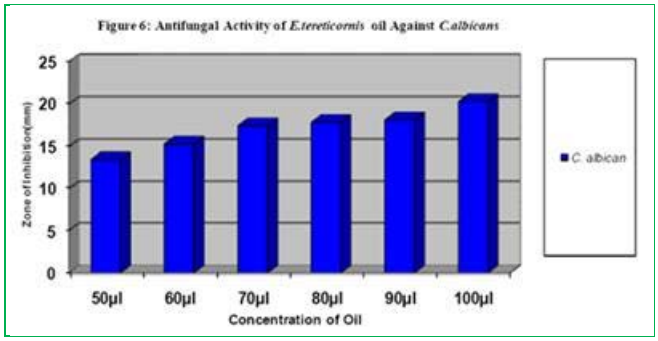






Antimicrobial activity against *Candida albican*

This was the only fungus specie, whose activity was tested against *E. Tereticornis* and positive results were obtained. *E.tereticornis* oil showed significant results against *Candida albican.* The zone of inhibition at 50µl was 13.47 and this zone increases with the concentration of oil (Fig. 6 & 12).



DISCUSSION

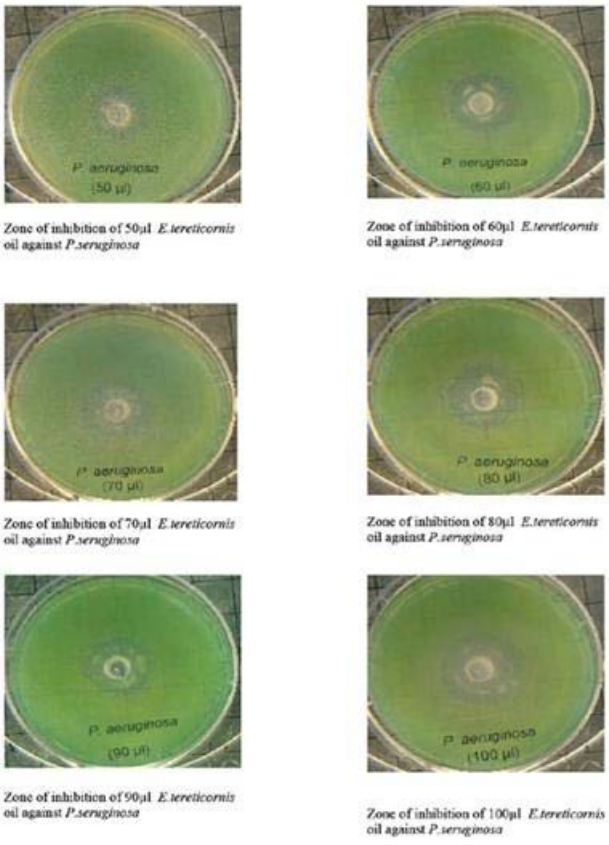
The current study reports on the broad spectrum antimicrobial activity of *Eucalyptus tereticornis* leaf oil. The ability of *Eucalyptus* oil to inhibit the growth of both gram positive, gram negative bacteria and fungus is in agreement with previous reports of the antibacterial activity of other *Eucalyptus* species[7, 15-27]. This study also reported the susceptibility of both gram positive and gram negative bacteria towards *E. tereticornis* oil. The greater susceptibility of gram positive bacteria is in conformity with reported results for a wide variety of South American[4], African[28-29] and Australian[15, 30] plant oil. The gram negative bacterial cell wall outer membrane is thought to act as a barrier to many substances including antibiotics[31]. The uptake of the *Eucalyptus* oil antibiotic agents by gram negative bacteria is presumably affected by the cell wall outer membrane of some bacteria.

Since multi drug resistance of these microorganisms is major medical problem, screening of natural products in search for new antimicrobial agents that would be active against these organisms[16] is the need of the hour.

Development of microbial resistance to antibiotics is a global concern. Isolation of microbial agents less susceptible to regular antibiotics and recovery of increasing resistant isolates during antibacterial therapy is rising throughout the world which highlights the need for new principles[17, 32]. Actually, essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers and their exploitation for potential multipurpose functional use[18-19]. During this study, the oil was significantly active against bacterial and fungal strains. Most resistant bacterial strains was *P. aeruginosa* showed different zone of inhibition (14.55mm, 16.33mm, 18.21mm, 18.35mm, 18.44mm, and 18.49mm) on different concentrations of *E. tereticornis* oil 50µl, 60µl, 70µl, 80µl, 90µl and 100µl respectively. *P. aeruginosa* showed resistant against two standard antibiotics Ampicillin and Chloramphenicol while CIP showed 23.89mm and TE showed 10.91mm zone of inhibition *P. aeruginosa* which is the most resistant strain, evidences are provided by previous studies that *P. aeruginosa* has intrinsic resistance to several antibiotics and capability to acquire resistance during antibiotic therapy[33-35].

Figure 7

Zones of inhibition at different concentrations of *E. tereticornis* oil against *Pseudomonas aeruginosa* on Mueller Hinton Agar



Zone of inhibition of 50µl *E.tereticornis* oil against *P.seruginosa*

Zone of inhibition of 60µl *E.tereticornis* oil against *P.seruginosa*

Zone of inhibition of 70µl *E.tereticornis* oil against *P.seruginosa*

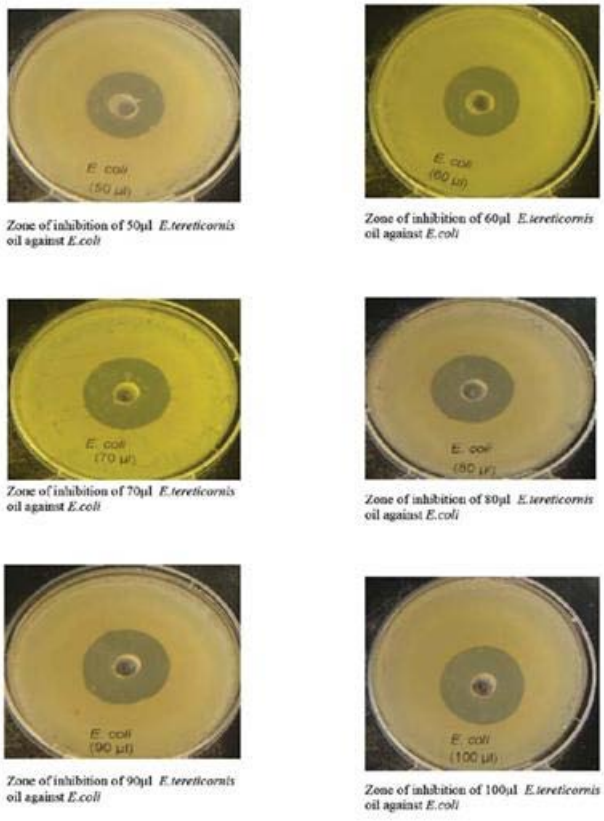
Zone of inhibition of 80µl *E.tereticornis* oil against *P.seruginosa*

Zone of inhibition of 90µl *E.tereticornis* oil against *P.seruginosa*

Zone of inhibition of 100µl *E.tereticornis* oil against *P.seruginosa*

Figure 8

Zones of inhibition at different concentrations of *E. tereticornis* against *Escherichia coli* on Mueller Hinton Agar



Zone of inhibition of 50µl *E.tereticornis* oil against *E.coli*

Zone of inhibition of 60µl *E.tereticornis* oil against *E.coli*

Zone of inhibition of 70µl *E.tereticornis* oil against *E.coli*

Zone of inhibition of 80µl *E.tereticornis* oil against *E.coli*

Zone of inhibition of 90µl *E.tereticornis* oil against *E.coli*

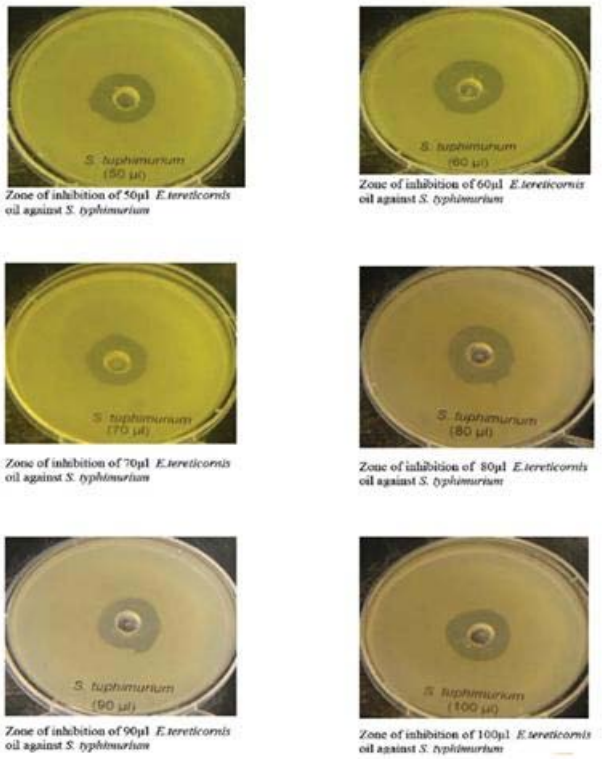
Zone of inhibition of 100µl *E.tereticornis* oil against *E.coli*

Amongst the gram negative strains studied, the oil was highly active against *E. coli* on different concentrations of oil 50µl, 60µl, 70µl, 80µl, 90µl and 100µl showed different zones of inhibition 18.9mm, 19.68mm, 20.48mm, 20.98mm, 21.43mm and 21.53mm respectively. The most sensitive drug against *E. coli* was CIP 5µg with zone of inhibition 31.31mm. Increasing concentration of oil gave a distinct zone of inhibition. These results are similar to those found by previous reported literature. [36-38]

The least sensitive gram negative organism was *S. typhimurium* showed different zones of inhibition 14.45mm, 15.08mm, 15.58mm, 15.89mm, 16.09mm and 16.15mm with different quantity of oil 50µl, 60µl, 70µl, 80µl, 90µl and 100µl respectively. AMP was most sensitive drug against *S. typhimurium* with zone of inhibition of 25.06mm. With higher concentration of oil showed greater zone of inhibition. These results are similar to those found by previous reported literature. [20, 26] Gram positive *S. aureus* showed antibacterial activity against different quantities (50µl, 60µl, 70µl, 80µl, 90µl and 100µl) of *E. tereticornis* oil was evaluated with zones of inhibition 22.28mm, 24.30mm, 26.53mm, 28.53mm, 29.61mm and 30.27mm respectively. Four standard antibiotics CIP, TE, AMP, and C were also checked against *S. aureus* produced zone of inhibition 20.87mm, 25.64mm, 30.98mm and 22.73mm respectively. AMP 25µg produced zone of inhibition 30.98mm which was equivalent to zone of inhibition against 100µl of oil. Increasing amount of essential oil however, gave a diverse zone of inhibition. These results are similar to those found by using other species of *Eucalyptus* oil. [8, 39-40]

Figure 9

Zones of inhibition at different concentrations of *E. tereticornis* oil against *Salmonella typhimurium* on Mueller Hinton Agar



Zone of inhibition of 50µl *E.tereticornis* oil against *S. typhimurium*

Zone of inhibition of 60µl *E.tereticornis* oil against *S. typhimurium*

Zone of inhibition of 70µl *E.tereticornis* oil against *S. typhimurium*

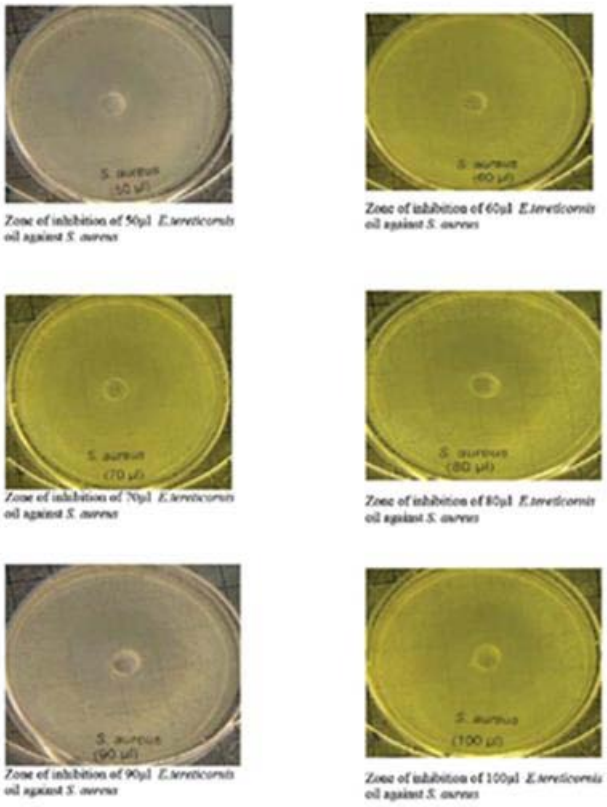
Zone of inhibition of 80µl *E.tereticornis* oil against *S. typhimurium*

Zone of inhibition of 90µl *E.tereticornis* oil against *S. typhimurium*

Zone of inhibition of 100µl *E.tereticornis* oil against *S. typhimurium*

Figure 10

Zones of inhibition at different concentrations of *E. tereticornis* oil against *Staphylococcus aureus* on Mueller Hinton Agar



Zone of inhibition of 50µl *E.tereticornis* oil against *S. aureus*

Zone of inhibition of 60µl *E.tereticornis* oil against *S. aureus*

Zone of inhibition of 70µl *E.tereticornis* oil against *S. aureus*

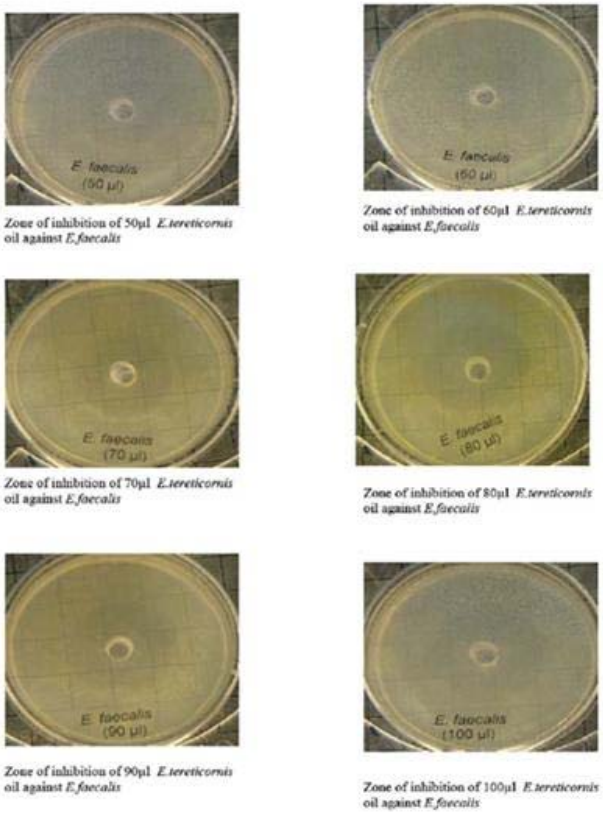
Zone of inhibition of 80µl *E.tereticornis* oil against *S. aureus*

Zone of inhibition of 90µl *E.tereticornis* oil against *S. aureus*

Zone of inhibition of 100µl *E.tereticornis* oil against *S. aureus*

Figure 11

Zones of inhibition at different concentrations of *E. tereticornis* oil against *Enterococcus faecalis* on Mueller Hinton Agar



Zone of inhibition of 50µl *E.tereticornis* oil against *E.faecalis*

Zone of inhibition of 60µl *E.tereticornis* oil against *E.faecalis*

Zone of inhibition of 70µl *E.tereticornis* oil against *E.faecalis*

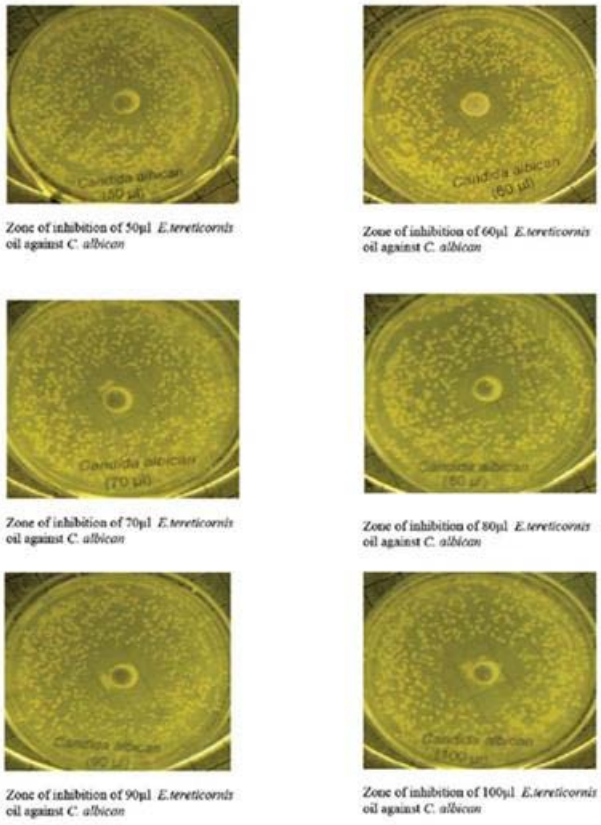
Zone of inhibition of 80µl *E.tereticornis* oil against *E.faecalis*

Zone of inhibition of 90µl *E.tereticornis* oil against *E.faecalis*

Zone of inhibition of 100µl *E.tereticornis* oil against *E.faecalis*

Figure 12

Zones of inhibition at different concentrations of *E. tereticornis* oil against *Candida albican* on Potao Dextorse Agar



Zone of inhibition of 50µl *E.tereticornis* oil against *C. albican*

Zone of inhibition of 60µl *E.tereticornis* oil against *C. albican*

Zone of inhibition of 70µl *E.tereticornis* oil against *C. albican*

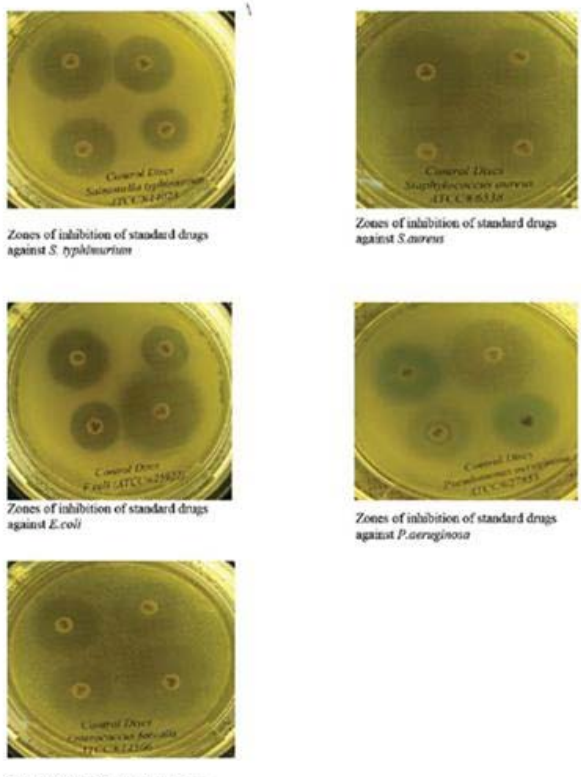
Zone of inhibition of 80µl *E.tereticornis* oil against *C. albican*

Zone of inhibition of 90µl *E.tereticornis* oil against *C. albican*

Zone of inhibition of 100µl *E.tereticornis* oil against *C. albican*

Figure 13

Antimicrobial Activity of Standard Antibiotic Discs against few Clinically Important Bacteria on Mueller Hinton Agar



Zones of inhibition of standard drugs against *S. typhimurium*

Zones of inhibition of standard drugs against *S.aureus*

Zones of inhibition of standard drugs against *E.coli*

Zones of inhibition of standard drugs against *P.aeruginosa*

Zones of inhibition of standard drugs against *E. faecalis*

Similarly gram positive *E. faecalis* produced different zones of inhibition (20.97mm, 23.09mm, 25.11mm, 26.2mm, 26.5mm and 26.65mm) against different quantities of oil 50µl, 60µl, 70µl, 80µl, 90µl and 100µl respectively. The maximum zone of inhibition was produced against 100µl of oil. The most sensitive standard drug against *E. faecalis* was AMP 25µg with zone of inhibition of 30.5mm. This diverse zone of inhibition was similar on other species of *Eucalyptus* oil. [41-42]

In association with bacterial susceptibility against different concentrations of *E. tereticornis* oil, unicellular fungal strain (*C. albican*) also showed sensitivity. The oil also showed its activity against *C. albican* the different zone of inhibitions of 13.47mm, 15.3mm, 17.49mm, 19.88mm, 20.08mm and 20.31mm were produced against different concentrations 50µl, 60µl, 70µl, 80µl, 90µl and 100µl of oil respectively. The minimum activity was seen on 50µl and maximum on 100µl. The antifungal activity increases with increase of concentration. [43-44]

The broad range of microbial susceptibilities indicates the potential of these extracts as a surface disinfectant as well as for medicinal purposes and possibly as food additives to inhibit spoilage. However, further studies are needed before these extracts can be applied to these purposes. Particular toxicity studies are needed to determine the suitability of these extracts for the use as antiseptic agents and as a food additive.

The study suggests that isolation of the active compound from the oil would give more satisfactory and promising results. Furthermore, isolation and identification of active compounds present in the oil could be useful in understanding the relations between traditional cures and current medicines.

Chemotherapeutic agents, used topically or systemically for the treatment of microbial infections of humans and animals, possess varying degrees of selective toxicity. Although the principle of selective toxicity is used in agriculture, pharmacology and diagnostic microbiology, its most dramatic application is the systemic chemotherapy of infectious diseases. Plant products which have been tested appear to be effective against a wide spectrum of microorganisms, both pathogenic and non-pathogenic. Administered orally, these compounds may be able to control a wide range of microbes, but there is also the possibility that they may cause an imbalance in the gut micro flora, allowing opportunist pathogenic bacteria, such as coliforms, to become established in the gastrointestinal tract with resultant deleterious effects. Further studies on therapeutic applications of volatile oils, including those from Eucalyptus, are needed to investigate these issues, and to complement the substantial number of analytical and in vitro bioactivity studies that are being carried out on these natural products. The potential of eucalyptus oils for use as practical antimicrobial agents remains to be proven. Some results have been encouraging but others have been less so. In vitro studies have shown that oils from some Eucalyptus species are effective against a range of pathogens, non-pathogens and spoilage organisms. More comprehensive (and standardized) tests of oils from a greater number of Eucalyptus species are needed to determine whether such oils, or formulations containing them, have a major role to play as antimicrobial agents. If they have, then in vivo studies are needed to assess their efficacy under clinical conditions. With an increasing public awareness of "green issues", plant volatile oils, including those from Eucalyptus, offer a more eco friendly alternative to conventional formulations in a number of sectors where antimicrobial action is desirable.

CONCULSION

The study suggests that isolation of the active compound from *E. tereticornis* oil would give more satisfactory and promising results in near future that would save millions of life around world with this cheap plant. The study is the first report of comparison with commercially available antibiotics with plant based oil. Chemotherapeutic agents, used topically or systemically for the treatment of microbial infections of humans and animals, possess varying degrees of selective toxicity. Plant products are now getting popular and have tested to be effective against a wide spectrum of microorganisms, both pathogenic and non-pathogenic. *E. tereticornis* oil gives promising results and proves the effectiveness of plants based products.

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