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"The effect of Eucalyptus oil on the growth of selected micro-organisms: in vitro study"

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Abstract: *It is an essential oil, obtained from the leaf of Eucalyptus. It is commonly known as Nilgiri oil. Eucalyptus oil is used to control several diseases derived from microbial infections. It has anti-inflammatory and antibacterial activities and can be used as a vehicle for intracanal medicaments. Studies have reported that the antibacterial activity of eucalyptus oil only in pure concentration on Escherichia coli as well as on klebsiella, proteus and staphylococcus aureus.*

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I. Introduction

Eucalyptus oil is the generic name for distilled oil from the leaf of Eucalyptus, a genus of the plant family Myrtaceae native to Australia and cultivated worldwide. It is commonly known as **Nilgiri oil**. Eucalyptus oil has a history of wide application, as a pharmaceutical, antiseptic, repellent, flavouring, fragrance, and industrial uses. Eucalyptus oil is used to control several diseases derived from microbial infections. Eucalyptus oil and its major component 1,8-cincole have antimicrobial effects.

II. Materials And Methods

This study conducted in the Department of Conservative Dentistry & Endodontics, Institute of Dental Science Bareilly and collaborated with Department of Microbiology Rohilkhand Medical College, Bareilly. The main purpose of this study is to evaluate the Anti-bacterial activity of eucalyptus oil against micro-organisms which are commonly found in infected root canals.

Armamentarium: The armamentarium was used during this study. The following materials are;

1. Micro-organisms
- E. coli
- Staphylococcus aureus
- Klebsiella
- Proteus

2. Materials
- Eucalyptus oil
- Peptone water
- Distilled water

3. Culture Media
- Blood Agar (HiMedia Laboratories Pvt.Ltd.)
- Muller Hiltons Agar (HiMedia Laboratories Pvt. Ltd.)

4. Instruments
- Sterile container
- Culter Plates
- Test Tube (borosil**)**
- Test tube stand
- Flask
- Spatula
- Inoculating loup
- Culture swab

- Divider
- Gloves and mouth mask
- Autoclave
- Incubator (Yorco bacteriological incubator)
- Durham tube

Procedures:

- Take 2 ml of peptone water in two test tubes.
- Bacteria taken from blood agar plate with the help of nichrome wire loop and pour into two test tubes. Then incubate for two hours in incubator.
- Take two plates of Muller Hilton Agar and punch at the center of each plates about 7 mm diameter with the help of Drams Tube.
- Take suspension from test tubes and swap in whole plates with the help of swap.
- Drop the eucalyptus oil at punching area.
- Incubate it at 37 degree centigrade and zone of inhibition measured after 24 hours.
- The antibacterial sensitivity pattern represented as zone of inhibition at its maximum diameter was measured around each well using a caliper and results obtained were tabulated.

Statistical analysis: The data were entered on a Microsoft Excel spreadsheet and imported into Statistical Package for Social Sciences (SPSS) version 22 for statistical analysis. Data was present in mean and standard deviation. One Way Anova test was used to find significant difference in different microorganism in zone of inhibition and Tukey post hoc test was performed to find significant difference in different microorganism in zone of inhibition in multiple groups. Paired t-test was performed to find significant difference in different microorganism in zone of inhibition from day-1 to day-2. A P-value less than 0.05 was considered statistically significant.

III. Result

Table-1: Antibacterial activity of eucalyptus oil day -1 of incubation.

Micro-Organism	No of Samples	Mean	S.D.	Minimum	Maximum
E-Coli	10	8.00	1.05	6	9
Staphylococcus Aureus	10	1.00	0.47	0	2
Klebsiella	10	2.00	0.82	1	3
Proteus	10	4.00	0.71	3	5

Graph-1: Antibacterial activity of eucalyptus oil day -1 of incubation.

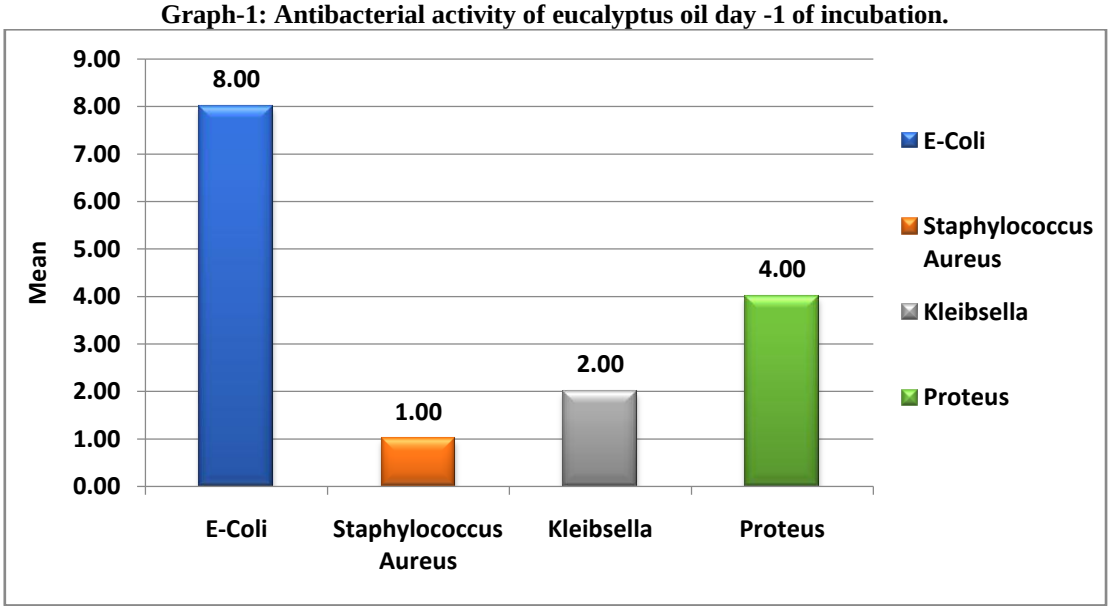

Table-2: Antibacterial activity of eucalyptus oil day -2 of incubation.

Micro-Organism	No of Samples	Mean	S.D.	Minimum	Maximum
E-Coli	10	8.5	0.53	8	9
Staphylococcus Aureus	10	2.0	0.47	1	3
Klebsiella	10	2.5	0.47	2	3
Proteus	10	6.0	0.85	5	7

Graph-2: Antibacterial activity of eucalyptus oil day -2 of incubation.

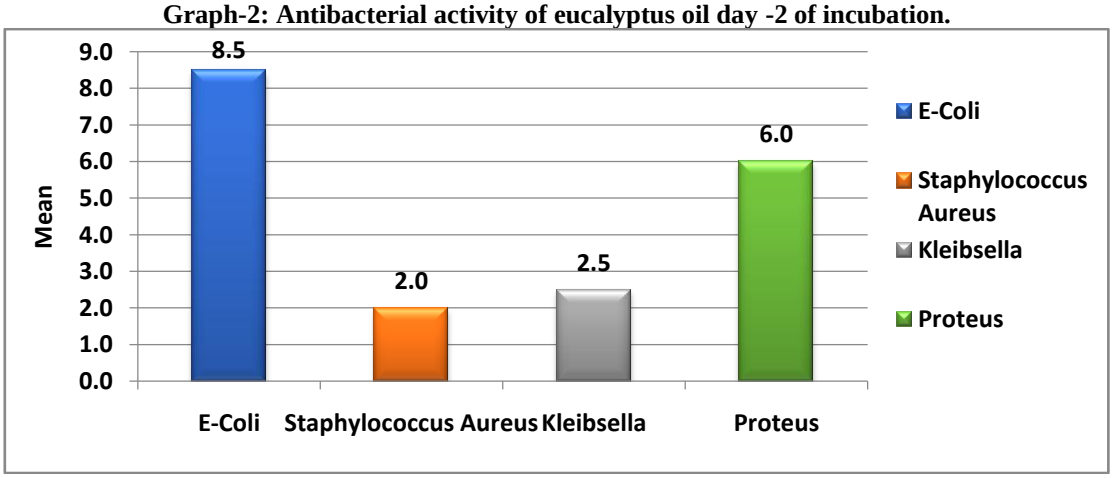

Table-3: Comparison of Zone of Inhibition in antibacterial activity of eucalyptus oil day -1 of incubation in different Microorganism.

Micro-Organism	No of Samples	Mean ± S.D.	F-Value	P-Value
E-Coli	10	8.0 ± 1.05	153.3	< 0.001*
Staphylococcus Aureus	10	1.0 ± 0.47		
Klebsiella	10	2.0 ± 0.82		
Proteus	10	4.0 ± 0.71		

***. The mean difference is significant at the 0.05 level.**

Table-4 Comparison of Zone of Inhibition in antibacterial activity of eucalyptus oil day -2 of incubation in different Microorganism.

Micro-Organism	No of Samples	Mean ± S.D.	F-Value	P-Value
E-Coli	10	8.5 ± 0.53	260.8	< 0.001*
Staphylococcus Aureus	10	2.0 ± 0.47		
Klebsiella	10	2.5 ± 0.47		
Proteus	10	6.0 ± 0.85		

***. The mean difference is significant at the 0.05 level.**

IV. Discussion

Escherichia coli (E. coli) bacteria normally live in the intestines of healthy people and animals. Most varieties of E. coli are harmless or cause relatively brief diarrhoea. But a few particularly nasty strains, such as E. coli O157:H7, can cause severe abdominal cramps, bloody diarrhoea and vomiting.

In group I study shows that maximum zone of inhibition was seen in case of E.coli which was 8 mm in diameter in first day of incubation and 8.5 mm in diameter in day of second.

Staphylococcus aureus is Gram-positive bacteria (stain purple by Gram stain) that are cocci-shaped and tend to be arranged in clusters that are described as "grape-like." On media, these organisms can grow in up to 10% salt, and colonies are often golden or yellow (aureus means golden or yellow). These organisms can grow aerobically or anaerobically (facultative) and at temperatures between 18 C and 40C.

In group II study shows that zone of inhibition was seen by staphylococcus aureus which was mean diameter of 1 mm in day of first and mean diameter of 2 mm in day of second.

Klebsiella is non motile, gram negative, non-sporulating, facultative anaerobic bacilli which is 0.3-0.5µ by width and 2-5µ in length. In teeth where endodontic therapy has been compromised or grossly inadequate for instance: repeated but inadequate antibiotic treatment, multiple openings of root canal, inadequate periapical surgical treatment, in such instances Klebsiella species and yeast are recovered. In group III study shows that zone of inhibition was seen by Klebsiella which was mean diameter of 2 mm in first day of incubation and 2.5 mm of mean diameter in second day of incubation.

Proteus species are part of the Enterobacteriaceae family of gram-negative bacilli. Proteus species are most commonly found in the human intestinal tract as part of normal human intestinal flora, along with Escherichia coli and Klebsiella species.

In group IV study shows that zone of inhibition was seen by Proteus which was mean diameter of 4 mm in first day of incubation and 6 mm of mean diameter in second day of incubation.

V. Conclusion

Among all the microorganisms maximum zone of inhibition seen in case if E.Coli.In eucalyptus oil it has antibacterial and antiinflamatory property.Eucalyptus oil can be considered as alternative intracanal medicament for conventional drugs and are affordable effective and nontoxic.They providing comprehensive and effective herbal intracanal medicaments.Thereby potential side effects and safety aspect using herbs as a substitute may seen to be more viable option.Eucalyptus oil along its efficacy in vivo require further investigation.

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