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Study the Anti-microbial property of Clove Oil [CO] and Eucalyptus Oil [EO]

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Abstract
Whenever we're feeling stuffy because of a cold and cough, we typically opt for home remedies first, and eucalyptus oil stands out as one of the most effective ones. Similarly, Clove oil used as a natural dental anesthetic to improving gut health, it is also part of traditional and folk medicines in Ayurveda treatments. The existent study covenants with the anti-microbial activity of Clove Oil [CO] and Eucalyptus Oil [EO]. It was found to be present terpenoids, lipids, steroids, polyphenols and glycerides in CO. Steroids, polyphenols, and glycerides present in EO. Both the oils CO and EO inhibit the microbial cultures such as Pseudomonas, S. aureus, E.Coli, Shigella and Salmonella. The zone of inhibition varies from 1 to 2.5mg of CO and EO. Apart from CO and EO anti-microbial property, fortunately both CO and EO found that non-toxic property as it is unable to cleave packed RBC in in-vitro study.

Key words: *CO (Clove Oil), Eucalyptus Oil (EO), Antibacterial property and Non- toxic property.*

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I. Introduction

Medicinal Herbal and aromatic plants (MAPs) make up a significant portion of the natural plant kingdom and are regarded as a valuable asset in various sectors, including pharmaceuticals, flavor and fragrance, perfumery, and cosmetics [1]. The essential oils derived from MAPs are known for their aromatic qualities, thanks to a blend of diverse chemical compounds from different chemical groups, such as terpenes, aldehydes, alcohols, esters, phenols, ethers, and ketones [2]. These essential oils, or aromatic plant extracts, are volatile and have a characteristic oily texture, typically produced by plants. They can exist in a liquid state at room temperature, although some are solid or resinous, displaying a variety of colors from light yellow to deep emerald green and from blue to dark brownish red [3]. Essential oils have been found to contain bioactive compounds, including antioxidants and antimicrobial agents. Numerous parts of plants are capable of yielding oils for extraction: leaves, seeds, bark, resin, berries, flowers, roots, or fruits [4]. For instance, clove oil is extracted from Eugenia caryophyllata, a species of the Myrtaceae family. Clove oil is a type of aromatic oil obtained from the buds and leaves of clove trees, which is extensively used in antibacterial and food preservation due to its broad-spectrum bactericidal, biodegradable, safe, and nontoxic characteristics [5]. Clove oil is considered an excellent essential oil for enhancing wound healing and reducing infection, thanks to its antioxidant properties and ability to neutralize free radicals, its antistress effects, and its antimicrobial activity. Eucalyptus oil, on the other hand, is derived from Eucalyptus globulus of the Myrtaceae family [6]. This eucalyptus oil exhibits a wide range of biological activities, including antimicrobial and antifungal properties. The antimicrobial effects of eucalyptus oil have been demonstrated in various studies [7]. The oil has shown to have a significant impact on microorganisms, with several Gram-positive and Gram-negative bacteria being sensitive to it. This is evident from clear zones observed on agar plates where the essential oil inhibits the growth of specific microorganisms [8]. Additionally, some studies have determined the minimal inhibitory concentration and minimal bactericidal concentration of essential oils in liquid solutions. The antibacterial properties of essential oils have been observed in numerous research studies, with many focusing on the direct impact of essential oils on various microorganisms [9]. For example, essential oils have been found to affect a wide range of bacteria, including both Gram-positive and Gram-negative species, by creating clear zones on agar plates that indicate the essential oil's ability to inhibit the growth of a particular microorganism.

II. Materials and Methods

All the chemicals used were of analytical grade. Microbial cultures were purchased from MTCC.

Preliminary phytochemical screening of CO and EO

CO and EO were initially screened for terpenoids, phytosterol, tannin, phenolic, glycoside, saponins, flavonoids, carbohydrates, proteins, steroids and lipids [10].

Direct hemolytic activity of CO and EO

Direct hemolytic activity was determined by using washed human erythrocytes. Briefly, packed human erythrocytes and Phosphate Buffer Saline (PBS) (1:9v/v) were mixed; 1mL of this suspension was incubated independently with the various concentrations of CO and EO (100µL & 200µL) for 1hr at 37°C. The reaction was terminated by adding 9mL of ice cold PBS and centrifuged at 1000g for 10min at 37°C [11]. The amount of hemoglobin released in the supernatant was measured at 540nm. Activity was expressed as percent of hemolysis against 100% lysis of cells due to the addition of water (positive control), whereas PBS served as negative control.

Antimicrobial assay of CO and EO

The bacterial cultures (E. coli, Salmonella, Pseudomonas, Shigella and S. aureus) were grown in Muller Hinton nutrient agar medium that contain peptone (1%), beef extract (1%) and NaCl (1%) at pH 6.8. Sterile nutrient agar petri plates were prepared and 0.1mL of the overnight grown bacterial culture was spread on the solidified agar plates evenly with the help of a glass spreader. Wells were made on the solidified agar using a cork borer. The test solution was made by dissolving 50mg of CO and EO in 1.0mL of water to get 50mg/mL concentration followed by sonication for 2min. The 100µL of this test solution containing 5mg of CO and EO were added into the respective wells by varying the concentration (1-10mg). The standard antibiotic drug Amoxycillin was kept as positive control and tested against all the pathogens. These plates were incubated at 37°C for 24hr. The diameter of 'zone of inhibition' at each well was measured and recorded [12]. The minimum inhibitory concentration (MIC) assay was carried out in triplicate and the average values were reported.

III. Results and Discussion

Characterization of CO and EO

It was found to be present terpenoids, lipids, steroids, polyphenols and glycerides in CO. Steroids, polyphenols, and glycerides present in EO (Table 01). In addition, both the oils show the presence of several minerals such as iron, nickel, zinc and etc., (Table 02).

SL NO	Phytochemical Analysis	CO	EO
01	Terpenoid	Present	Absent
02	Lipids	Present	Absent
03	Tannin	Absent	Absent
04	Poly phenols	Present	Present
05	Glycerides	Present	Present
06	Saponin	Absent	Absent
07	Flavonoid	Absent	Absent
08	Carbohydrates	Absent	Absent
09	Proteins	Absent	Absent
10	Alkaloid	Absent	Absent
11	Steroids	Present	Present

Table :01

SL.NO.	Name of The Metal	CO (ppm)	EO (ppm)
01	Aluminium	0.00	0.06
02	Boron	0.00	0.03
03	Barium	0.00	0.00
04	Cadmium	0.00	0.00
05	Copper	0.00	0.00
06	Iron	0.05	0.08
07	Manganese	0.00	0.00
08	Molybdenum	0.00	0.00
09	Nickel	0.01	0.01
10	Lead	0.00	0.00
11	Zinc	0.04	0.29

Table: 02

Moreover, CO and EO did not hydrolyze RBC suggested its nontoxic property (Fig.01).

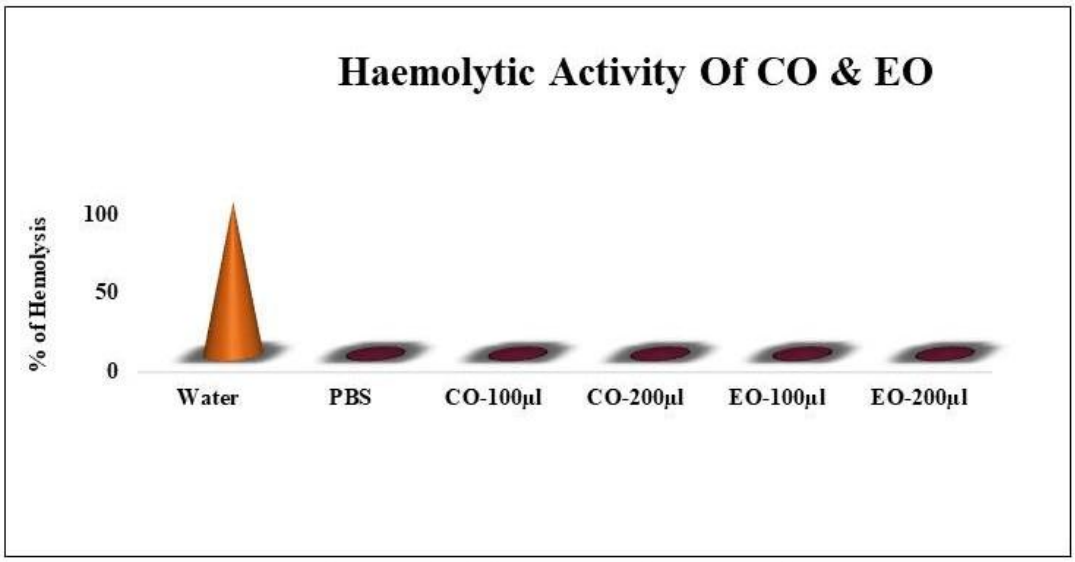

Fig.01: Haemolytic Activity Of CO & EO

Antimicrobial activity of CO and EO

CO and EO antimicrobial property were performed with several pathogenic strains namely E.coli, S. aureus, Salmonella, Pseudomonas and Shigella. Surprisingly, both the oils CO and EO exhibited anti-microbial property and the Minimum Inhibitory of Concentration value was demonstrated individually (Fig.02). Numerous studies have shown that, in comparison to synthetic compounds, herbal extracts and extracts coupled with nanoparticles have a wider range of antibacterial activity [13].

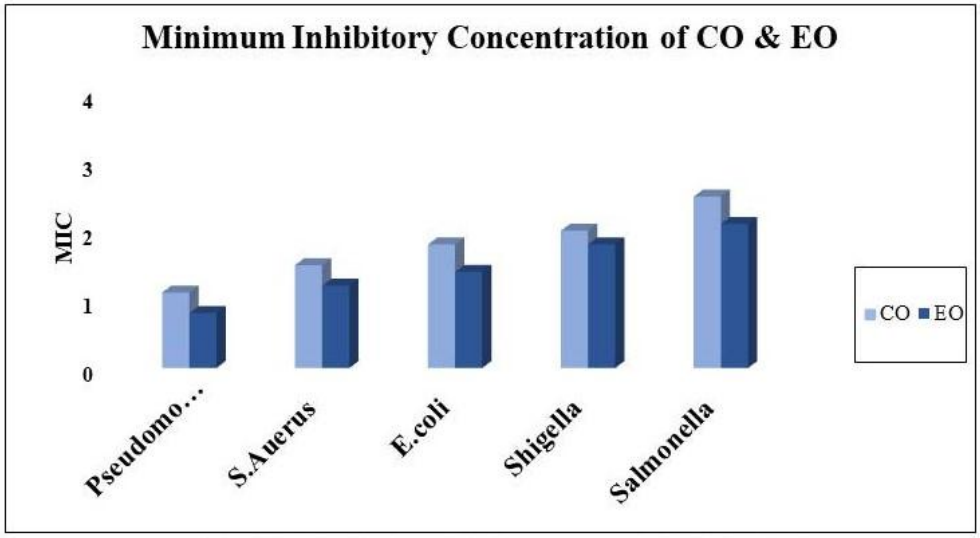

Fig.02: Antimicrobial Property Of CO & EO

IV. Conclusion

In conclusion, this study demonstrates the preliminary characterization of GASM and its antimicrobial property which is very specific to gram negative bacteria.

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Declaration of Conflict of Interest

The authors declared no potential conflict of interest with respect to the authorship and publication.

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