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Research Article

Antibacterial Effect of Essential Oils (Clove Oil, Castor Oil and Ginger Oil) Against Human Pathogenic Bacteria

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Abstract

Essential oils are volatile, natural, complex compounds which are produced as secondary metabolites by plants for their protection against various microorganisms as well as pests. A wide range of plants have been explored for their essential oils in the past few decades. The study was conducted to determine the antibacterial activity of essential oils against human pathogenic bacteria which were gram positive (*Staphylococcus aureus* and *Streptococcus pyogenes*) as well as gram negative (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Shigella sonnei*). Five ml of three different oils, i.e. clove oil, castor oil, and ginger oil, were taken in a test tube so that each oil had four different concentrations. Four concentrations of (0, 25, 50 and 75) µL of oils were mixed with 1000, 975, 950 and 925 µL of DMSO respectively to make it a volume of 1ml. It was observed that clove oil was effective against the entire gram positive as well as gram negative bacteria that were used. The inhibition zone was greatest in the case of clove oil at 75 µL against *P. aeruginosa* (23 mm) and the smallest zone of inhibition was shown by castor oil against *K. pneumoniae* (12 mm). Other oils were sensitive as well as resistant to the bacteria. Hence, it is found that different oils have shown inhibitory activity towards different pathogens to a variable extent. However, clove oil was inhibitory to all the bacteria in all concentrations.

Keywords: Antibacterial activity; essential oil; pathogenic bacteria

Introduction

Aromatherapy, which believes that essential oils and other aromatic molecules have therapeutic benefits, has sparked a surge in interest in essential oils in recent decades. They are frequently used in fragrances, cosmetics, soaps, cleaning goods, and other items, as well as in food and beverage flavoring (Alizadeh, 2013; Al-Qudah *et al.*, 2014). As contemporary antibiotic treatments, higher plant products with evidence-based activities against fungus and harmful bacteria are playing an increasingly important role (Gundidza, 1993). Furthermore, many researchers are still interested in finding novel antibacterial natural compounds. Despite the fact that their antibacterial and antifungal effects have been demonstrated to be significantly less potent than commercially available synthetic medicines (Bidlack *et al.*, 2000; Giamperi *et al.*, 2002), plant oils are a source of very promising natural ingredients for the production of new antimicrobial drugs. Essential oils (EOs) are volatile liquids derived from herbs, spices, and various plants, and can include up to 50 distinct components in various ratios (Burt, 2004). They are volatile chemicals of terpenoid and non-terpenoid origins that are produced via separate biosynthetic pathways and have different main metabolic precursors (Bakkali *et al*., 2008).

Infectious illnesses caused by bacteria are still one of the major sources of morbidity and death in people and animals today. Bacteria are known to have the genetic capacity to acquire and spread resistance to therapeutic drugs (Nascimento *et al*., 2000). Multiple drugs resistance to currently available medicines is reducing their efficacy, resulting in severe failures in the treatment of infectious diseases (Hancock, 2005). Methicillin resistance in Staphylococci, penicillin resistance in Pneumococci, vancomycin resistance in Enterococci, and various gram-negative bacteria resistances are examples (Norrby *et al*, 2005). In light of microorganism resistance and the possible absence of new antimicrobial medicines, new chemicals capable of inhibiting bacteria's resistance mechanism must be developed, therefore aiding disease management, treatment, and eradication (Oluwatuyi *et al*., 2004). As a result, there is a strong desire to develop natural and efficient cures for such diseases through the use of essential oils. The ability of different essential oils to suppress certain infections varies depending on the kind of essential oil, the concentration utilized, and the pathogen against which the oil is employed (Friedman *et al*., 2004). This study aims to unravel the antibacterial effect of Clove oil [*Syzygium aromaticum* (L.) Merr. & Perry], Castor oil (*Ricinus communis* L.) and Ginger oil (*Zingiber officinale* Roscoe) available in the market against gram positive (*Staphylococcus aureus* and *Streptococcus pyogenes*) and gram negative (*Escherichia coli*, *Pseudomonas aeruginosa. Klebsiella pneumoniae* and *Shigella sonnei*. Therefore, the present study has tried to figure out the antimicrobial efficacy of essential oils available in the market of Dharan.

Materials and Method

Preparation of Oils of Different Concentration

Three essential oils (Clove oil, Castor oil, and Ginger oil) were obtained at a market in Dharan, Nepal. The branding of those essential oils, as well as the name of the business, are, however, kept out of the current study. The microbiology lab at the Central Campus of Technology in Dharan, Nepal, was used to investigate its antibacterial properties.

Five milliliters of essential oils were placed in a test tube to create sample oils in four different concentrations. To create a volume of 1ml, four concentrations of 0, 25, 50, and 75 µL of oils which were freshly prepared and were combined with 1000, 975, 950, and 925 µL of Dimethyl sulfoxide (DMSO), respectively (Table 1). The experiment was carried out at room temperature. In screw-capped test tubes, different concentrations of oils were produced. The oil samples were then ready for antibacterial activity testing.

Preparation of Standard Inoculum of Test Organisms

Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Shigella sonnei, Staphylococcus aureus, and *Streptococcus pyogenes* were used to assess essential oils' antibacterial effectiveness. The organisms were inoculated on Nutrient Agar plates from their stock culture, and the organisms produced on the NA plates were stored on Nutrient Agar slants at 4°C. To match the turbidity of 0.5 McFarland standards, Nutrient Broth was injected with newly sub-cultured bacteria and incubated at 37°C for 5 hours. To construct a bacterial lawn, sterile cotton swabs were used to distribute the inoculums onto Mueller Hinton Agar.

Screening of Antibacterial Activity

The antibacterial activity was determined using the agar well diffusion technique. Mueller Hinton Agar (MHA) plates were utilized to culture each bacterial species in this test. In order to evaluate the antibacterial activity of six distinct bacterial species for three different oils, a total of 18 MHA plates were constructed. Extract was loaded into each of the 6 mm diameter wells created in the inoculated agar media with a sterile corkborer, incubated at 37°C for 24 hours, and the plates were checked for the appearance of a zone of inhibition around the well to determine the effect of the extract on the desired bacteria.

Data Collection

Data collection was based in experimental outcomes. The zone of inhibition was measured in millimeter (mm) using scale.

Data Analysis

In MS Excel 2007, the data was processed, inferred, and displayed in tables and figures. The MIC values were determined by analyzing the data. The zone of inhibition on different bacteria and the amount of essential oils used were compared to compare the effects of essential oils on bacteria.

Table 1: Concentration of oil with DMSO

Oils	**0 µL**	**25 µL**	**50 µL**	**75 µL**
Clove oil	0 µL oil + 1000 µL DMSO	25 µL oil + 975 µL DMSO	50 µL oil + 950 µL DMSO	75 µL oil + 925 µL DMSO
Castor oil	0 µL oil + 1000 µL DMSO	25 µL oil + 975 µL DMSO	50 µL oil + 950 µL DMSO	75 µL oil + 925 µL DMSO
Ginger oil	0 µL oil + 1000 µL DMSO	25 µL oil + 975 µL DMSO	50 µL oil + 950 µL DMSO	75 µL oil + 925 µL DMSO

Results

Oils have a hydrophobic nature, and hence the solvent DMSO is used to allow diffusion of oils from the wells so that the organisms present around the wells can be acted upon by the oils and inhibit their growth. The zone of inhibition (ZOI) was measured in millimeters (mm) in four different concentrations (0, 25, 50, and 75 µL). Hence, the antibacterial activity of the essential oils was determined.

Effects of Oils on Test Bacteria

Tests of different oils against the test bacteria were performed by the agar well diffusion method. It was observed that clove oil was effective against the entire gram positive as well as gram negative bacteria that were used (Fig. 1). Clove oil showed a highest inhibition zone against *P. aeruginosa* (23 mm), which was followed by *E. coli* (22 mm), *S. pyogenes* (18 mm) (Fig. 4) and *S. aureus* (17 mm). Clove oil was less effective against *S. sonnie* (16 mm) and *K. pneumonia* (16 mm) (Fig. 1). Overall, clove oil was found effective against all bacteria. However, castor oil was found effective against three bacteria and ineffective against the remaining three bacteria. *P. aeuroginosa* was highly affected by castor oil with a ZOI of 15 mm (Fig. 4), followed by *S. pyogenes* (13 mm) and *K. pneumonia* (12 mm) in a 75 µl concentration (Fig. 2). Moreover, ginger oil was found to be effective against all the sample bacteria except *P. aeruginosa*. *K. pneumonia* was highly inhibited by ginger oil with an inhibition zone of 16 mm, which was followed by *S. aureus* (14 mm), *S. sonnie* (12.5 mm), *E. coli* (12 mm) and *S. pyogenes* (11 mm) (Fig. 4) in 75 µl concentration (Fig. 3).

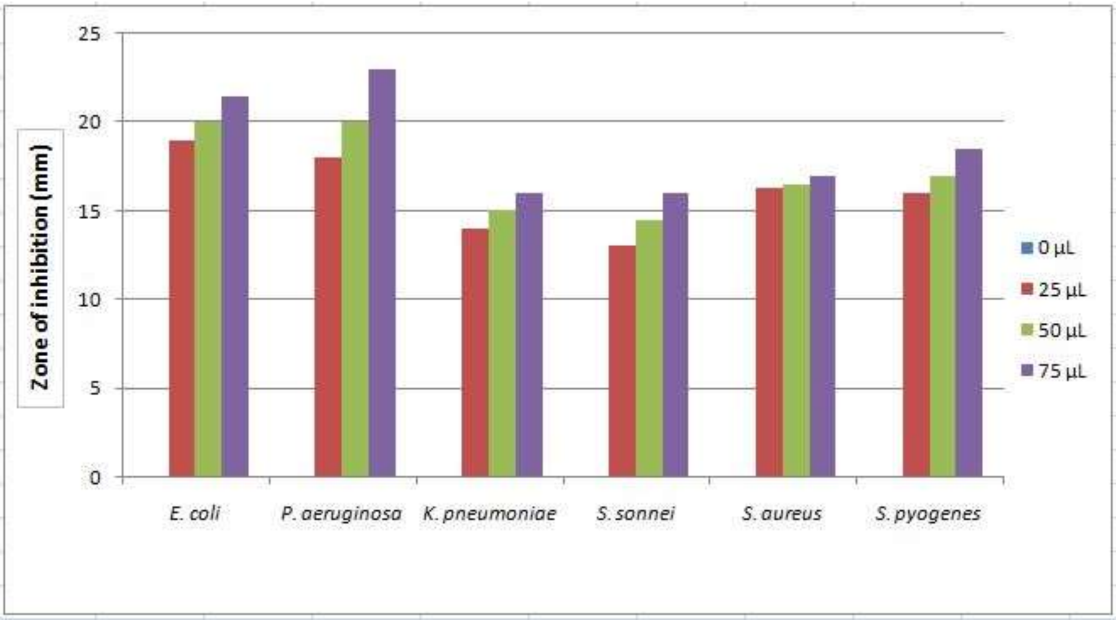

Fig 1: Effect of clove oil against sample bacteria with inhibition zone (mm).

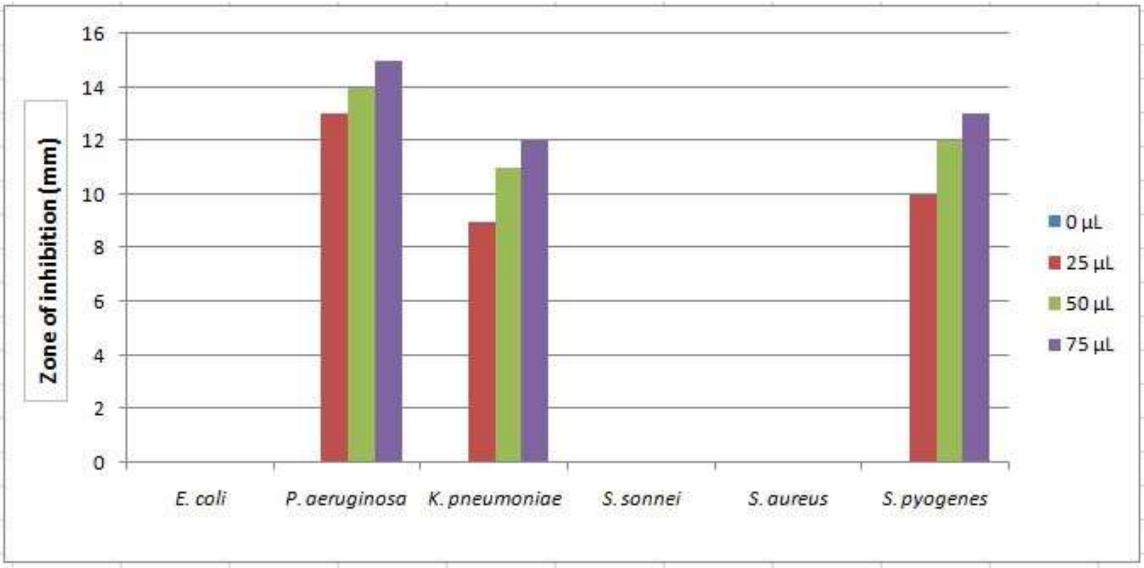

Fig 2: Effect of castor oil against sample bacteria with inhibition zone (mm).

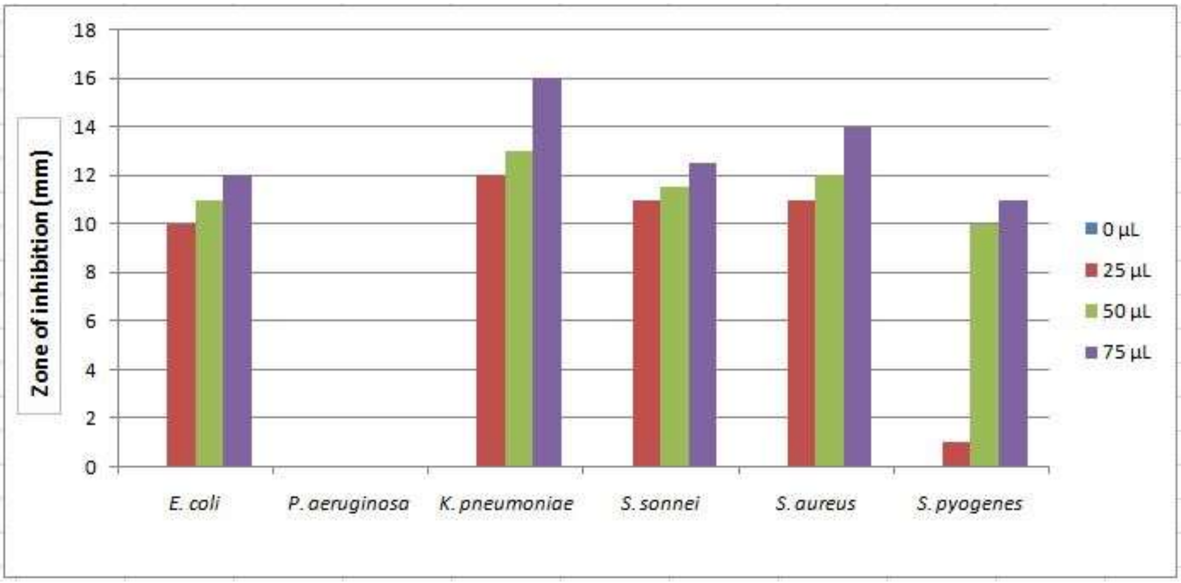

Fig 3: Effect of ginger oil against sample bacteria with inhibition zone (mm).

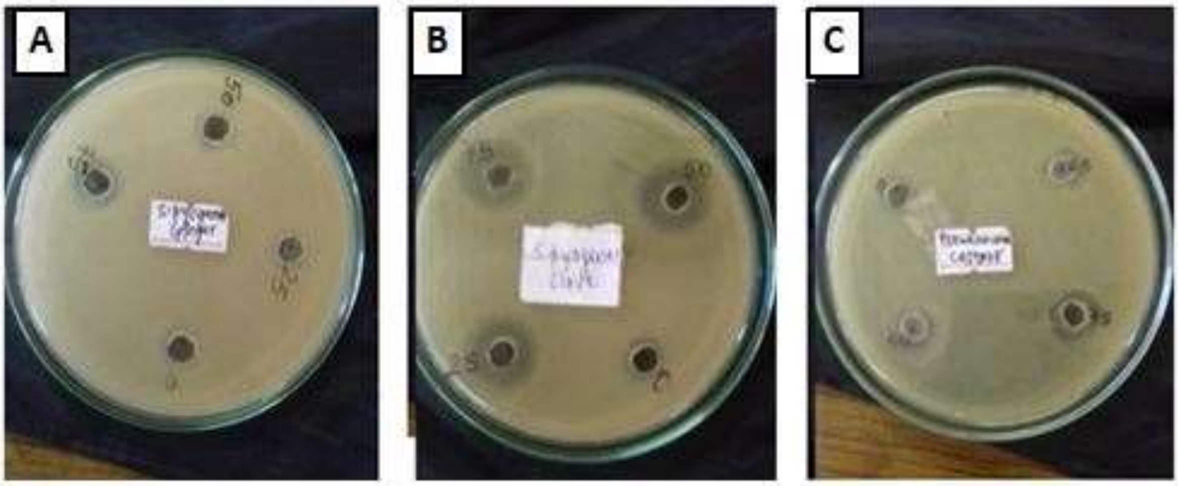

Fig 4: Inhibitory effects of ginger (A), clove (B), and castor oil (C) against *P. aeruginosa*, *S. pyogenes* and *S. pyogenes* respectively.

Discussion

Many essential oils have antibacterial action, as evidenced by numerous studies conducted in recent years. Because essential oils are highly permeable through the cell wall and cell membrane due to their lipophilic properties, they are predicted to be widely used as antibacterial and antifungal agents (Burt, 2004). The high concentration of terpenes, benzene derivatives, oxygenated compounds, and phenolic components such as thymol and carvacol, which are known to have significant antibacterial characteristics, might be linked to the oil's antimicrobial capabilities (Burt, 2004). The interaction of essential oil components with polysaccharides, fatty acids, and phospholipids causes membrane breakdown, cellular content leakage, proton pump interference, and cell death (Edris, 2007; Oussalah *et al*., 2006). The results of multiple researches are difficult to compare, owing to the various test techniques, bacterial strains, and antibiotic sample sources employed. The essential oil of herbs and spices can have a wide range of compositions depending on the geographical location, variety, age of the plant, drying technique, and oil extraction method (Jerkovic *et al*., 2001). After the samples were put in corresponding wells created on MHA that had been swabbed with human pathogenic test bacteria, a zone of inhibition was detected in the research.

Due to the presence of a peptidoglycan layer beyond the outer membrane, essential oils are often more active against gram-positive bacteria (Sokovic *et al*., 2005). Gram negative bacteria, on the other hand, showed better sensitivity in this research than gram positive bacteria. This might be owing to the fact that the study used a smaller amount of gram-positive bacteria than gram negative bacteria for testing. The results indicated that clove oil was efficient at all concentrations in suppressing all of the test bacteria. It's possible that eugenol and phenolic compounds are the major components responsible for clove oil's antibacterial properties (Dorman and Deans, 2000). Hydrocarbon monoterpenes have the lowest antibacterial action, but oxygenated compounds, particularly phenol-type compounds like thymol and carvacol, have a greater potential (Dorman and Deans, 2000). According to Knobloch *et al.* (1986), oxygenated monoterpenes have significant antibacterial action, especially on entire cells, whereas hydrocarbon derivatives have reduced antimicrobial activity due to their poor water solubility, which restricts their diffusion through the medium. As a result, the interaction of eugenol and phenolic compounds with bacteria's cell membrane phospholipids, which affects their permeability, might be the major stumbling block to their high antibacterial activity. They also maintain the ability to denaturate proteins (Chaieb *et al*., 2007).

Castor oil was not found effective against three pathogens (*Shigella sonnei*, *Staphylococcus aureus* and *Escherichia coli*) and showed inhibition zone against rest of three pathogens. Zarai *et al.* (2012) have reported the effect of castor oil against *S aureus, P. aeruginosa, K. pneumonia* and *E. coli* to be 24 mm, 8.2 mm, 6.2 mm and 4.2 mm respectively, which was self-extracted in laboratory. The antibacterial effects of this essential oil may be attributed to the comparatively high content of -pinene (16.88%), which is thought to actively impede microbe development (Dorman and Deans, 2000). However, *P. aeruginosa* was tolerant to ginger oil in our study, compared to Zarai *et al*. (2012). This might be due to the different strain of *P. aeruginosa*, as we did not differentiate the *P. aeruginosa* strains. Zarai *et al.* (2012) applied this oil against *P. aeruginosa* 27853 strain.

Except for *P. aeruginosa*, ginger oil showed the inhibitory zone against all microbes tested. Ginger oil was shown to be extremely susceptible to *K. pneumoniae* (ZOI of 16 mm). -Zingiberene is ginger oil's main sesquiterpene hydrocarbon (Parthasarathy *et al*., 2008) and might be the reason for higher antibacterial effect. Citral, Zingiberene, -sesquiphellandrene, and -curcumene, according to Salzer (1975), may be used to evaluate the quality of ginger oil and are also the main source of the strong antibacterial action.

Conclusion

Different oils have been discovered to have varying degrees of inhibitory effect against various infections. Clove and ginger oils were shown to be highly efficient against the infections tested in humans. As a result, the oils that have demonstrated inhibitory effects are extremely important in the treatment of many human diseases. As a result, essential oils might be a promising source of alternative antimicrobial agents in the near future, and could play a key role in the identification of novel medicines for the treatment of a wide spectrum of pathogenic bacteria.

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Authors' Contributions

Sraddha Dulal, Shiv Nandan Sah, and Sujan Chaudhary designed the manuscript. Laboratory work and data collection was carried out by Sraddha Dulal. Shiv Nandan Sah supervised the whole research. Manuscript preparation was done by Sujan Chaudhary and Chiranjibi Dangi. Final draft was critically revised and approved by all the authors.

Conflict of Interest

Authors declare to have no any conflict of interest with the present study.

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