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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF CLOVE AND BLACK CUMIN OILS AGAINST GRAM-POSITIVE AND NEGATIVE BACTERIA

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ABSTRACT

The aims of this study wereto investigatethe antibacterial activity of clove and black cumin oils against Gram-positive and negative Bacteria.Clove and black cumin oils were purchased from the local market in Riyadh Saudi Arabia. The antibacterial activity of clove and black cumin oils was determined by the agar diffusion method.The tube dilution method was done to determine the minimum inhibitory concentration of the oils.The results of the antibacterial activity of clove oil and black cumin oilagainst *Staphylococcus aureus*. Both oils inhibited the growth of *Staphylococcus aureus*. black cumin oilshowed the highest [15mm] whereas clove oil was [13mm]antibacterial activity. Where *E. coli* black cumin oilshowed the highest [32mm] whereas clove oil was [16mm].The minimum inhibitory concentration [MIC] of clove oil against microorganisms. The MIC of clove oil ranged from [1.5-2 mg/ml]. When The MIC of black cumin oil ranged from [0.9-1.3 mg/ml]. The lower MIC is an indication of the high effectiveness of the oil.In conclusion, the results of this study showed that black cumin oil was more potent than clove oil against gram-positive and negative bacteria. So it could be used as antimicrobial agent against *Staphylococcus aureus* and *E.coli*bacteria.

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INTRODUCTION

Essential oils are abundant in plants (EOs). Because of their antioxidant, antimicrobial, phytotoxic, neuroprotective, and anti-inflammatory properties, they are used in a variety of industries, particularly the food industry. EOs are also low in cytotoxicity, which lowers the risk of intoxication. They are natural volatile fractions extracted from aromatic plants that are formed during secondary plant metabolism. Several classes of volatile substances, such as fatty acid esters, monoterpenes, sesquiterpenes, phenylpropanoids, alcohols, aldehydes, and, in some cases, aliphatic hydrocarbons, can be found in their chemical composition. The composition varies due to physiology, environmental conditions, geographic variations, seasonality, collection period, genetic factors, and plant evolution (Siyuan, 2002). The efficacy of clove oil against vaginal candidiasis. conclusions:From this study, we evaluated that the clove oil was highly effective in reducing the fungal load in vaginal tissues. Thus, clove oil being nontoxic and quite efficacious may find a place as a constituent in the new generation of antifungal drugs (Anis Ahmad, 2013). Clove oil is a popular toothache remedy due to its antiseptic and analgesic properties. It also has powerful antioxidant and antiviral properties. Clove oil is primarily composed of eugenol. Several studies reported the fatty acid and tools composition of cold-pressed clove oil; however, major constituents such as eugenol, eugenol acetate, and other compounds were not quantified in detail.

Cold-pressed clove oil has been shown to have higher levels of radical scavenging and antimicrobial activity than virgin oil. Similarly, cold-pressed clove oil has been shown to have hepatoprotective activity in experimental animals, as well as other biological activities (Hari Prasad Devkota, 2020). The combination of cinnamon bark/clove and cinnamon bark/cinnamon leaves EOs had an additive effect on L. monocytogenes but had no effect on *E. coli* O157:H7. Cinnamon bark EO (85 ppm)/vanillin (910 ppm) and clove EO (121 ppm)/vanillin (691 ppm) combinations inhibited L. monocytogenes the best. The inhibitory effects of clove EO (104 ppm)/vanillin (1006 ppm) and cinnamon leaves EO (118 ppm)/vanillin (979 ppm) combinations on *E. coli* were significant. Some of the tested combinations increased the antimicrobial effect and allowed for lower effective doses, potentially opening up new applications for food and active food packaging (Cava-Roda, 2021). The complex structure of EOs, as well as the diverse chemical nature of their constituents, are responsible for a wide range of biological interactions, many of which are of growing interest in the field of food preservation (Mancianti, 2020; Shaaban, 2020). However, the use of essential oils in food can be hampered by the following factors: (a) the need for high concentrations to achieve bacteriostatic (the inhibition of bacterial growth without killing cells) or bactericidal (the destruction of bacterial cells) effects; (b) adverse effects after EO treatment (e.g., changes in the physicochemical and sensory characteristics of the subject of application); and (c) cost increases due to higher essential oil concentrations (Cho, 2020).

Thymoquinone (TQ) is one of the most active constituents and has different beneficial properties in *Nigella sativa* seeds. It has been reported to have significant effects against many ailments such as skin diseases. In terms of antimicrobial effects, different N. sativa extracts and TQ have a broad antimicrobial spectrum that includes Gram-negative and Gram-positive bacteria, viruses, parasites, schistosoma, and fungi. The efficacy of *N. sativa* seeds and TQ varies depending on the species of target microorganisms. The current review paper attempts to describe all antimicrobial activities conducted by various researchers (Forouzanfar, 2014). The antimicrobial activity of BSO was studied against various types of bacteria. Strong bacterial inhibitory effects were observed, especially against *Bacillus subtilis*, with an average inhibition zone of 15.74 mm (Sewara, 2019). Several studies have been conducted on the anti-ischemic activity of black cumin and its active constituents on various organs such as the brain, kidneys, heart, and liver. Through inhib, black cumin exerts its beneficial effects as an antioxidant, anti-inflammatory, anti-apoptosis, and anti-necrosis agent (Oskouei, 2018).

The diethyl ether extract of Nigella sativa seeds (25-400 micrograms extract/disc) inhibited Gram-positive bacteria represented by Staphylococcus aureus in a concentration-dependent manner. The extract synergized with streptomycin and gentamicin and had an additive antibacterial activity with spectinomycin, erythromycin, tobramycin, doxycycline, chloramphenicol, nalidixic acid, ampicillin, lincomycin, and sulphamethoxazole-trimethoprim combination. When injected at the site of infection, the extract successfully eradicated a non-fatal subcutaneous staphylococcal infection in mice[11].The mean diameters of growth inhibition zones caused by Nigella sativa nanoparticle nanoemulsion were close to each other in different bacteria (p=0.665). Furthermore, there was no significant difference between these values due to different nanoemulsion dilutions in different microbial species (p=0.778). The MIC and lethal concentrations of Nigella sativa nanoemulsion were similar to those of Enterococcus faecalis and Streptococcus mutans, but higher than those of other bacteria. In comparison, all bacteria in chlorhexidine had lower MIC and MBC values than the nanoemulsion (Nazemi Salman, 2021). The aims of this study were to investigatethe antibacterial activity of clove and black cumin oils against Gram-positive and negative Bacteria.

MATERIALS AND METHODS

Materials

Clove and black cumin oils were purchased from the local market in Riyadh Saudi Arabia.

Antibacterial activity of clove and black cumin oils assay: Theantibacterial activity of clove and black cumin oilswas determined by the agar diffusion method. For this, fresh [overnight] isolated colonies of *Staphylococcus aureus*and*E. coli* were suspended in sterile saline to get turbidity of 0.5 McFarland standards. o.1 ml. of this suspension was spread aseptically on a sterile Muller Hinton agar medium [Hi media]. Then the wells [6 mm diameter] were bored by a sterile cork borer. 0.2 ml. of each oil was added to the wells. It was allowed to diffuse,and 10 % DMSO in one of the wells was used as a negative control. After the diffusion of oils, the plates were incubated at 370c for 24 hours. Zonesof inhibition were then measured in mm. For each oil, three replicates were maintained.

Determination of minimum inhibitory concentration [MIC]: The tube dilution method was done to determine the minimum inhibitory concentration of the oils. A series of two-fold dilutions of each oil ranging from 10 m *Staphylococcus aureus*and*E. coli* g /ml to 0.3 mg/ml was made in Muller Hinton broth. 0.1 ml of a suspension of *Staphylococcus aureusand E. coli* matched to 0.5 McFarland standard was seeded into each dilution. Two controls were maintained for each test batch. This included tubes containing oil and growth medium without inoculum and organism control i.e.tubes containing the growth medium and inoculum. The tubes were incubated at 37^0c for 24 hours and checked for turbidity. Minimum inhibitory concentration was determined as the highest dilution of the oils that showed no visible growth.

Statistical analysis: Data fromthe experiment for assay of the anti-bacterial activity of oils were statistically analyzed using analysis Excel Program 2019, and presented as average, standard error.Means were obtained using t-tests and making gr

RESULT

Antibacterial Activity: Figure1&2shows the results of the antibacterial activity of clove oil and black cumin oilagainst *Staphylococcus aureus*. Both oils inhibited the growth of *Staphylococcus aureus*. black cumin oilshowed the highest [15mm] whereas clove oil was [13mm]antibacterial activity.

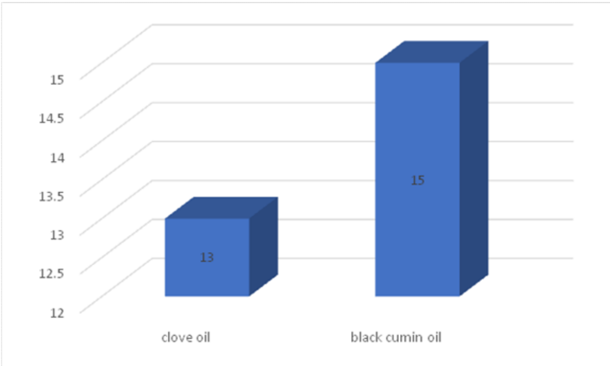


Figure 1. Theantibacterial activity of clove oil and black cumin oilagainst *Staphylococcus aureus*

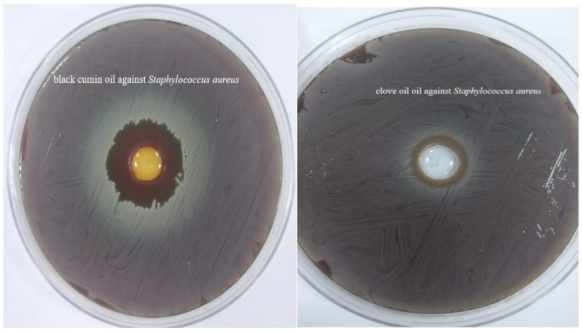


Figure 2. The antibacterial activity of clove oil and black cumin oilagainst Staphylococcus aureus

Figure 3 & 4 shows the results of the antibacterial activity of clove oil and black cumin oilagainst *E.coli*. Both oils inhibited the growth of *E.coli*. black cumin oilshowed the highest [32mm] whereas clove oil was [16mm]antibacterial activity.

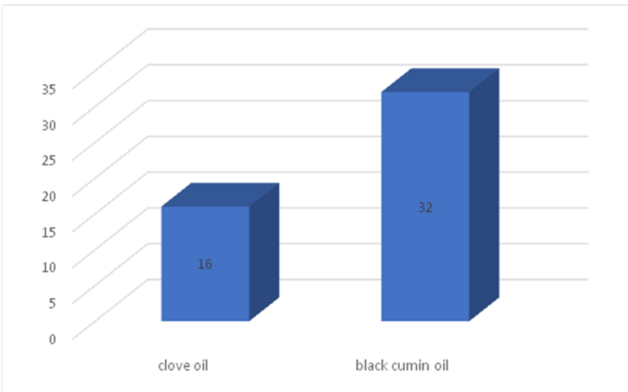


Figure 3. The antibacterial activity of clove oil and black cumin oilagainst *E.coli*

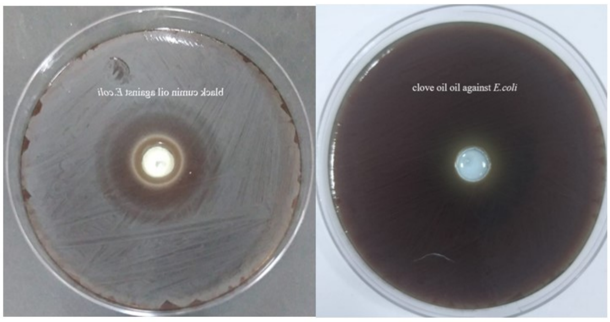
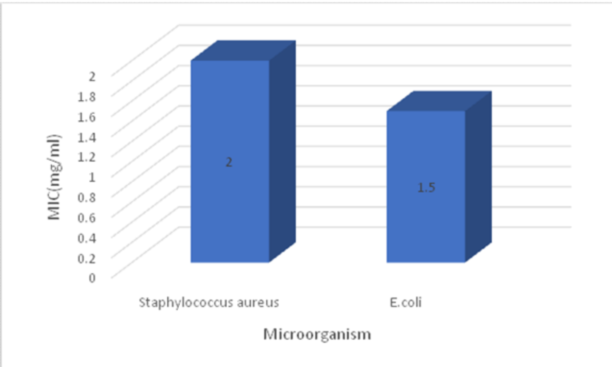


Figure 4. Theantibacterial activity of clove oil and black cumin oilagainst *E.coli*

Figure.5 shows the minimum inhibitory concentration [MIC] of clove oil against microorganisms. The MIC of clove oil ranged from [1.5-2 mg/ml]. The lower MIC is an indication of the high effectiveness of the oil.



The minimum inhibitory concentration of clove oil against testedmicroorganisms Figure.5. Figure.6 shows the minimum inhibitory concentration [MIC] of black cumin oil against microorganisms. The MIC of black cumin oilranged from [0.9-1.3 mg/ml]. The lower MIC is an indication of the high effectiveness of the oil.

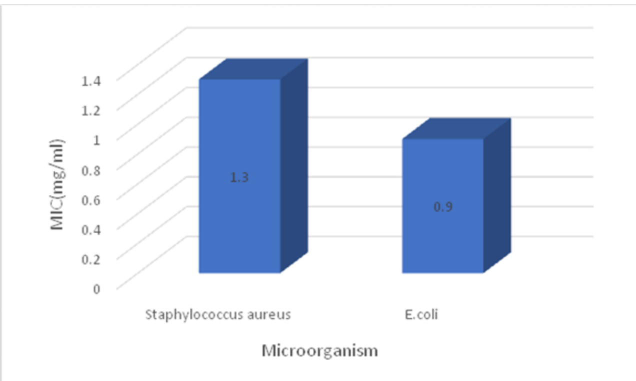


Figure 6. The minimum inhibitory concentration of black cumin oil against testedmicroorganisms

DISCUSSION

Organic material reduces antibacterial activity while conserving bactericide efficacy. Clove essential oil has the potential to be an antimicrobial agent for external use**.**Clove essential oil can be considered a potential antimicrobial agent for external use because it is not markedly inactivated by dilution or affected by organic matter. The design of clinical trials will determine its effectiveness in medical and dental practice. bacterial sensitivity to clove essential oil at 21°C was 1.514 (*Escherichia coli*) > 1.334 (*Pseudomonas aeruginosa*) > *Staphylococcus aureus* (0.995) (Nuñez, 2012).

This study confirms clove oil's efficacy as a natural antimicrobial against MDR S. suis and suggests that it could be used as a promising alternative product for the control of infectious diseases caused by *S. suis* in both animal and human patients[14].The results were obtained by a current studythat showed the antibacterial activity of clove oil and black cumin oil*Staphylococcus aureus*. Both oils inhibited the growth of *Staphylococcus aureus*. black cumin oil showed the highest [15mm] whereas clove oil was [13mm]antibacterial activity. Where E. *coli* inhibited the growth of black cumin oilwasthe highest [32mm] whereas clove oil was [16mm]antibacterial activity this result was consistently with the result obtained by the previous study. Furthermore, the antimicrobial activity of black cumin oil against various types of bacteria was investigated. With an average inhibition zone of 15.74 mm, strong bacterial inhibitory effects were observed, particularly against *Bacillus subtilis*[9]. Researchers measured the growth of the microbes Malassezia furfur, *Candida albicans*, and *Staphylococcus aureus*, which can be found in healthy skin and gut microbiota but are also infection-inducing microorganisms that can disrupt the microbiome's homeostasis when overgrown. The results showed that the extract with 3% TQ and 2% FFA inhibited M. furfur and *C. albicans* the most, while all oils inhibited S. aureus significantly[15]. Black seed oil demonstrated significant antibacterial activity against all strains of *L. monocytogenes*, with a significantly (P<0.01) larger inhibition zone than gentamicin.

Due to the unique chemical properties of unconventional oilseeds constituents, they are important and may be introduced as new edible oil sources. Furthermore, these oilseeds have several features beneficial for human health and to produce new patents (Saeid Hazrati, 2022). The result obtained by a current study of the minimum inhibitory concentration of black cumin and clove oils against tested microorganismsreflected that black cumin oil was more potent than clove oil against all two tested organisms. The comparable activity of Saudi and Syrian black cumin oil seeds to certain antibiotics, only S. *aureus* was found to be sensitive in black cumin oil samples. Saudi black cumin seeds were more active than Syrian black cumin seeds. Syria's seed oil performed almost identically to one of the marketed oils (M1). Among all types of oils, another marketed black cumin oil (M2) demonstrated the highest antibacterial activity (Rashid, 2022). As a result, the presence of alkaloids, steroids, and fatty acids may be responsible for the antibacterial activity of black cumin oil samples. Differences in antibacterial activity can be attributed to a variety of factors, including extraction method, drying, storage, geographical, agricultural, and harvesting conditions, and so on, all of which result in chemical variation[19]. Clove oil and its applications, such as food and health products, have been tested and shown inhibitory activity against a variety of pathogens, including *Listeria monocytogenes*, *Campylobacter jejuni, Salmonella enteritidis, Bacillus cereus, Escherichia coli*, and *Staphylococcus aureus* (Gill, 2006; Xu, 2016; Devi, 2010; Vieira, 2019).

CONCLUSION

In conclusion, the results of this study showed thatblack cumin oil was more potent than clove oil against gram-positive and negative bacteria. So it could be used asantimicrobial agent against *Staphylococcus aureus*and *E.coli*bacteria. Also, butter to usefor the treatment of some infectious bacterial diseases. The study recommended further investigation of the effect of organic oils as antiviral agents.

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