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Antibacterial Effect of Fixed and Volatile Oils against Gram-positive and Gram-negative Bacteria

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Abstract

Antibiotic resistance phenomena among pathogenic bacteria considered as a major health problem and associated with increased mortality or long-term hospitalization, which lead to open a new era by using plant and herbal extracts as an alternative source of various chemotherapeutic drugs, also to increase antibiotic efficiency by combining with plant extract for obtaining a powerful and broad spectrum action. The current investigation aims to investigate antibacterial actions of fixed oils of (Olea europaea L., Ricinus communis L. and Linum usitatissimum) and volatile oil of (Nigella sativa, Curcuma longa L and Zingiber officinale) against both Staphylococcus aureus strain (6734151) and Escherichia coli strain (5344572). This study conducted on antibacterial effect of six different extracted oils from medical herbs. The findings revealed that the oil extracts have different antibacterial activities with efficacy. Bacterial inhibition zone was detected by using disk diffusion method. Furthermore, volatile oil of N. sativa showed a great inhibitory action against resistant S. aureus, which was (27.7± 1.2 mm). The antimicrobial effects of other fixed and volatile oils against S. aureu, the inhibition zone was (10 ± 1.0 mm) for (Zingiber officinale), (9 ± 1.0 mm) for Ricinus communis L., (7.7 ± 0.6 mm) for Olea europea L., (7.3 ± 0.6 mm) for Linum usitatissimum and for Curcuma longa L. was (6.7 ± 0.6mm). Moreover, antimicrobial effect of N. sativa against E. coli was more active in comparison with other oils, while other oils showed a slight antibacterial effect. In conclusion, volatile oil of N. sativa reveals great antibacterial activities in comparison with other extracted oils.

Keywords*:* Black seed, antibacterial activity, fixed oil and volatile oil.

1. INTRODUCTION

Medical plants have a popular and long history utilization backdrop; the World Health Organization (WHO) reported that most inhabitance in the world depends mainly on traditional therapies, which include the use of their natural products and plant extracts [1]. Furthermore, many herbal plant products have been applied as an alternatives therapy due to their antibacterial characteristics [2,3]. A number of phytochemical and pharmacological studies have been conducted on *Nigella sativa* seeds because of its important biological activities. Also, the antimicrobial actions of fixed and volatile oils of various plant extracts have been completely investigated its prospect antibacterial action toward a wide range of bacterial species which collected from diarrheal stool samples [4]. Therefore, the producing of new antibiotics that is expensive and a consuming process, and pathogens are able to develop a rapid antibiotic resistance. Hence, extracts of herbal plants appeared to be a good candidate as an alternative antimicrobial drug [5].

Olive trees (*Olea europaea L.*) is considered as the crucial trees in the Mediterranean countries for producing olive oil, which has the important usage in aspect of nutrition and medicine during the history, the antimicrobial compounds in olive fruits have been observed after the beginning of the olive fermentation. Also, it contains many potentially bioactive compounds that may have antibacterial, anti-inflammatory and antioxidant activity [6,7]. Moreover, other investigations on the antimicrobial effect of phenolic compounds in olive productions showed to have antimicrobial effects against pathogenic bacteria and viruses [8]. This is might be due to that olive oil has a high content of monounsaturated fat and polyphenols [9]. Extracted Castor oil from seeds of *Ricinus communis L.* has been used commercially for several purposes such as in intestinal obstructions [10]. Furthermore, Castor oil has utilized in minimal doses in a clinical setup for a wide range of medical purposes; for example, gallbladder disturbances, and liver abscesses. Also, it possess many potentially bioactive compounds that may have an effective antibacterial action against skin diseases and diarrhea [11]. Moreover, Castor seeds also contain toxic natural compounds, which is ricin toxin and it has a rich source of plant phytochemicals, with important biological actions; for instance, antioxidant, antimicrobial, antifungal, antiviral, anticoagulant and anti-carcinogenic characterestics [12]. Flax seed (*Linum usitatissimum L.*) is considered to be an effective food due to owing abundant of bioactive compounds, such as lignin, polysaccharide, and fatty acid especially (omega-3), which is very important for cardiovascular disease [13]. Also, it is one of great sources for protein and phenols [14]. Antimicrobial activity of flax seed against microbial food poisoning has been reported [15]. moreover, it has antimicrobial activity with very low minimum inhibitory concentrations (MICs) for some bacteria [16]. Application of plant extracts as antiviral, antifungal and antibacterial activities has been used world widely [17]. Genetically, modified flax might be the active source of antibacterial compounds and substitution to antibiotic therapy[18]. *Turmeric Curcuma longa L.* is a genus of 1200 species of rhizomatous herbs belongs to the family *Zingiberaceae*, which is an aromatic plant and has multiple uses and particularly in medicine, especially in India as a volatile oil [19]. The tuber roots of a few species of the genus *Curcuma* are economic sources of phytochemicals, pharmaceutical and perfumery compounds, which have antimicrobial and antioxidant compounds and exclude harmful organisms [20]. Many plant extracts have been extensively examined and abundant of reports have been documented outlining the uses of plant extracts to control the human disease such as urinary tract infection, liver infection and serious skin infections due to its anti-inflammatory, antioxidant, and antimicrobial activities [21].

Antibacterial activity of turmeric against *S. aureus* is due to phenolic compounds and against *E. coli* due to its alkaloid, turmerol and veleric acids [22]. Ginger (*Zingiber officinale)* is belong to *Zingiberaceae* family, it is a perennial herb; the fleshy aromatic roots are a rhizome as a volatile oil, which is rich in phenolic compounds [23]. Chemical analysis of ginger clarified that

Ginger ingredients consist of various chemical compounds, the most of of them are lipids, phenolic, terpenes, and carbohydrates, which have anti-angiogenesis and anti-cancer properties. It has a lineal anti-bacterial action and thus can be applied for treating bacterial infection and inhibits multiplication of intestinal bacteria [24,25]. *Nigella sativa* which has a common name (black seeds or black cumin), is a promising herb, which grows in many countries around the Mediterranean sea [26]. *Nigella sativa* seed has been applied for treating a number of health problems such as asthma, inflammation, jaundice, hypertension, diabetes, cough, headache, eczema, influenza and gastrointestinal problems [27]. It has also antitumor activity and a stimulatory effect on the immune system [28]. In the recent years, both its seeds and extracted oil have been widely investigated and reported to exhibit a wide range of pharmacologic actions such as analgesic, anti-inflammation, and antibacterial activity because it contains saponins, alkaloids, and essential oils. *Nigella sativa* has a significant antibacterial effect against a resistant strain of *Staphylococcus aureus* [29,30].

S. aureus is the most common cause for food poisoning and also can causes skin, gastrointestinal tract, and urinary tract infection. It is normal human skin bacterial flora and also presents on mucous membranes of digestive system. Asymptomatically, it has the ability to be present on mucous membranes from weeks to months. Therefore, it is a cause of community-acquired infections [31]. Since the discovery of penicillin, the world's first antibiotic, and was considered that this antibiotic will be leading to removing of infectious diseases. However, the overuse and misapplication of these antibiotics have led to develop multidrug resistant bacteria. Latterly, antibiotic resistance has reached an alarm point that some infectious agents have developed resistance to even vancomycin, which is considered as the 'antibiotic of last resort [32]. Thence, this has commenced an interlined the development of alternative antibacterial agents and the reevaluation of the curative uses of the herbal extracts and essential oils that are rich in phytochemicals, such as tannins, flavonoids, and phenolics [33]. They are sources of natural antioxidants and provide preservation against ferity by microbial pathogens[34]. Furthermore, another investigation has showed a significant relationship between the phytochemical content and the antimicrobial activity of the herbs [35].

In spite of that, the effective antibacterial activity of antibiotics, but it has a number of side effects on human body. However, plant products have a milder antimicrobial action but importantly, it shows to have no harmful effect on human body in comparison with commercially available antibiotics [36]. Some studies revealed that herbal drugs pose various mechanisms by which bacteria are usually unable to be resistant to herbs drug. This may be due to that it has synergistic interaction with other drugs in killing bacteria by inactivating bacterial enzymes that responsible for inactivation of antibiotics, and also has the ability to inhibit the efflux pumps action that leads to accumulate antibiotics within bacterial cell [37]. Therefore, the aims of the current investigation are firstly to determine antibacterial activities of extracted fix oil from (*Olea europaea L., Ricinus communis L. and Linum usitatissimum*) and volatile oil from (*Curcuma longa L., Zingiber officinale* and *Nigella sativa* against *S. aureus* strain (6734151) and *E. coli* strain (5344572) respectively by using a disk diffusion technique. Finally, to determine the minimum inhibition concentration (MIC) for extracted plant oils that shows a maximum inhibition zone.

2. METHODS AND MATERIALS

2.1. Isolation of bacteria

Samples were collected from 6 clinical patients from (Shar and Emergency hospitals) for isolation and identification of bacteria (*S. aureus and E. coli*). The collected samples were inoculated onto nutrient agar, and then the plates were incubated for 24-36 hours at 35-37ºC, for identifying bacterial characteristics, the standard microbiological techniques are applied. For

sub-culturing bacteria, colonies obtained from cultures diluted using McFarland standard [38] by putting one colony in normal saline, the turbidity was 0.5 using differential media such as mannitol salt agar and MacConkey agar.

2.2 Identification of Staphylococcus aureus

Samples were collected from 6 clinical patients from (Shar and Emergency hospitals) for isolation and identification of bacteria (*S. aureus and E. coli*). The collected samples were inoculated onto nutrient agar, and then the plates were incubated for 24-36 hours at 35-37ºC, for identifying bacterial characteristics, the standard microbiological techniques are applied. For sub-culturing bacteria, colonies obtained from cultures diluted using McFarland standard [38] For identification of *S. aureus*; firstly, a Gram stain technique has been used and gram-positive cocci bacteria observed, then catalase test also performed to differentiate *Staphylococcus* from *streptococcus* [39], selective and differential media such as mannitol salt agar (MSA) were used for identification of *S. aureus* [38]; also, coagulase test was done for differentiating *S. aureus* from other *Staphylococcus species* [39]. Lastly, api 20 Staph was used for emphasizing the result, the microorganisms were suspended in a sterile distilled water and incubated for 24 hours at 37°C and the suspension was pipetted in 20 microtubes of the strip for detecting enzymatic activity and fermentation result of biochemical tests [40].

2.3 Identification of E. coli

Firstly, *E. coli* identified by Gram's stain [41] , then MacConkey agar was used as a selective and differential medium to differentiate it from lactose fermenter enteric-bacteria, which produced deep red colonies; after that, indole test was performed, the bacteria produce red layer at the top of the tube after the addition of Kovács reagent indicates the presence of indole, For detecting ability of bacteria to utilize citrate the Simmons citrate agar used to differentiate *E. coli* from *Citrobacter*. Lastly, api 20E was used for emphasizing the result, the bacterial suspensions were incubated for 24 h at 37°C and the suspension was pipetted in 20 microtubes of the strip for detecting enzymatic activity and fermentation result of biochemical test. (Appendix, Picture 1).

2.4. Disk diffusion method

For detecting antimicrobial activity for each oil, the disk diffusion method was used; firstly, inoculating bacterial colony from mannitol salt agar and MacConkey agar into nutrient broth for 12 hours, pre-inoculated plates of Mueller Hinton agar by using pour plate method. 0.9 ml of nutrient broth allocated in the center of the plate then spread by L-shape, then 6 mm sterilized filter paper disks (Whatmann No.1) cut as a form of disk then the 4 impregnated disks were placed onto the surface of Mueller Hinton agar[42], the disks are saturated with oils by putting 0.7 ml of the oils on the disk, after absorption oils by filter paper, the plates were incubated for 24 hours at 37 °C. After incubation, susceptibility of bacteria was determined by measuring the zone of inhibition (ZOI) of bacterial growth in millimeters. Inhibition zone diameter is measured to the nearest whole millimeter at the point wherein there is a prominent reduction of 80% growth, These values were then compared to the National Committee for Clinical Laboratory Standards (NCCLS) antimicrobial sensitivity values for each organism [43].

2.5. Minimum Inhibitory Concentration (MIC)

MIC was performed for *Nigella sativa* oil by using disk diffusion technique; for this purpose, 10 different volumes of *N. sativa* oil from (0.1-10 ml) were used against *S. aureus*; ethanol was used as a solvent. MIC was recorded as the lowest concentration of the extract oil at which a bacterial growth was completely inhibited according to CLSI [44].

2.6. Extraction of volatile oil

Hot oil extraction technique was used to extract oils**.** Dried seeds or new rhizomatous of (*Curcuma longa L., Zingiber officinale and Nigella sativa*) volatile oil plants was collected from local markets in Sulaimani city. It was subjected to hydro-distillation for 3 hours using a

Clevenger-type apparatus (100 gm) was milled and extracted by adding (500 ml) distilled water (Clevenger, 1928). The volatile oil content was calculated as a relative percentage (v/w). Later, essential oil was taken from 100 gm of the milled sample in hydro-distillation method with the help of Clevenger set in 500 mL of distilled water, and kept at 4 °C until use [45].

2.7. Extraction of fixed oil

Hot oil extraction technique was used to extract oils according to AOAC (1970), of (Olea *europaea L., Ricinus communis L. and Linum usitatissimum*) fixed oil plants were (100 gm) was milled and extracted by adding (500 ml) of ethanol (96%) and Boiled between 70–78 °C with a soxhlet extractor for 3 hrs. Whatman No.1 filter paper was placed in the thimble of the Soxhlet extractor. The oil was extracted with ethanol (1:5 w/v) and then the mixture was filtered and the liquid part was evaporated by using a rotary evaporator to remove the excess solvent used in the oil, cooled and kept at 4 °C until use [46].

2.8 Data analysis

Statistical analysis for the results in the current investigation was achieved by using one-way analysis of variance (ANOVA). Mean differences between medicinal plants extracts were separated by Fisher's [Fisher, 1932] test significant difference (LSD) at 5% significant probability level [47].

3. RESULTS

To determine antibacterial action of extracted oil of (*Curcuma longa L., Zingiber officinale* and *Nigella sativa*) as a volatile oil and (*Olea europea L., Ricinus communis L.* and *Linum usitatissimum*) as a fixed oil, disc diffusion test was performed. The result shows that essential oil extracts of (*Curcuma longa L., Zingiber officinale* and *Nigella sativa*) as a volatile oil have antimicrobial effect against *S. aureus*. Interstingly, *Nigella sativa* extract revealed a great antibacterial activity (27.7 ±1.2 mm), while others such as *Curcuma longa L* was (6.7 ± 0.6 mm) and *Zingiber officinale* was (10.0 ± 1.0 mm) both of them they had no antimicrobial effect as show in (Figure 1).

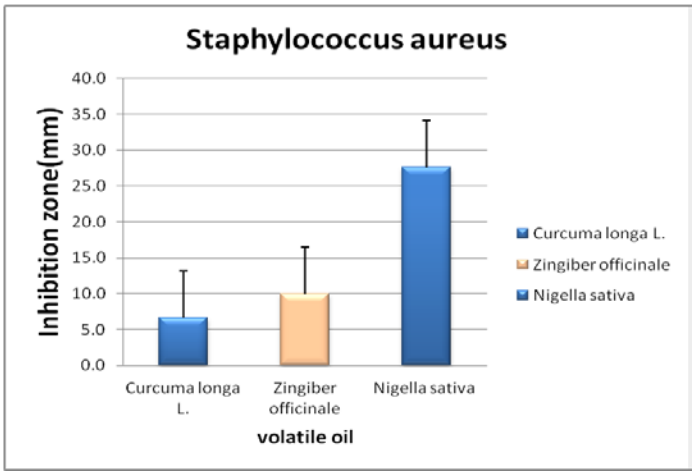

Figure 1: Antimicrobial effect of essential oil for three different plants (*Curcuma longa L., Zingiber officinale* and *Nigella sativa*) as volatile oil plants by measuring zone of inhibition (ZOI) of *S. aureus.*

To determine antibacterial effects of fixed oil of (*Olea europea L., Ricinus communis L. and Linum usitatissimum*) on *S. aureus.* The result showed that antimicrobial effects of *Ricinus communis L., Olea europea L.* and *Linum usitatissimum* were (9 mm ±1.0), (7.7 mm ± 0.6) and (7.3 ± 0.6mm) respectively as it is shown in (Figure 2).

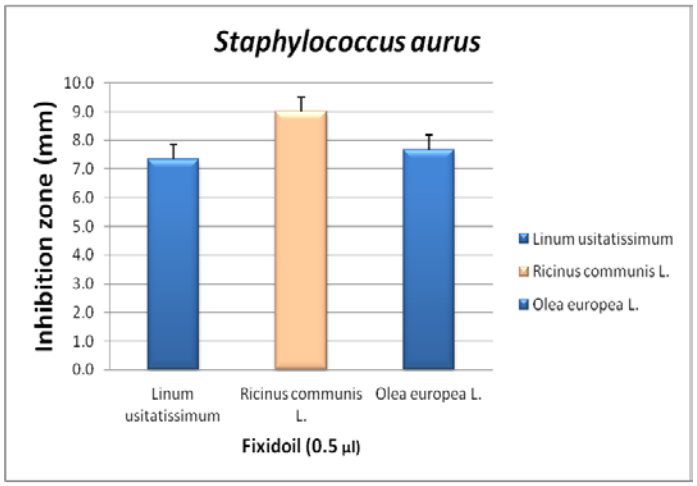

Figure 2: Antimicrobial effect of fixed oil (*Olea europea L., Ricinus communis L.* and *Linum usitatissimum)* against *S. aureus*.

Bacterial inhibition comparison of *S. aureus* by both volatile oil and fixed oil. The result showed that antimicrobial effect of *Nigella sativa* oil showed a higher inhibition zone (27.7 ±1.2) mm; whereas, oils from (*Zingiber officinale, Ricinus communis L., Olea europaea L., Linum usitatissimum, Curcuma longa L.* showed a lower inhibition zone and were (10 ±1.0) mm, (9 ±1.0) mm, (7.7 ±0.6) mm, (7.3 ±0.6) mm and (6.7 ± 0.6) mm respectively as it is shown in (Figure 3).

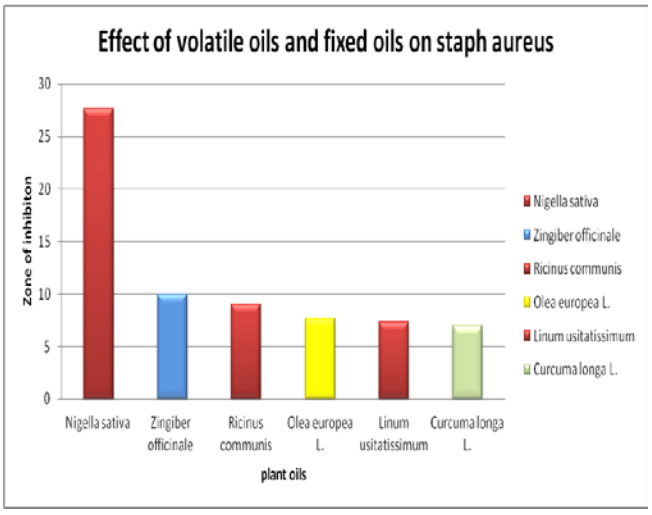

Figure 3: Comparison of bacterial inhibition of fixed and volatile oils extracted from different plants on *S. aureus*.

Obtained results for Essential oil of new rhizomes and seed of (*Curcuma longa L., Zingiber officinale* and *Nigella sativa*) as a volatile oil plants, showed that antimicrobial effect of *Nigella sativa* extract was more significant effect which was (11.7 ±0.6) mm to E. coli, while *Zingiber officinale* oil (10 ± 1.0) mm and *Curcuma longa L.* oil was (9.3 ±0.6) mm both of them had no effect after interpretation of data as determined in (Figure 4).

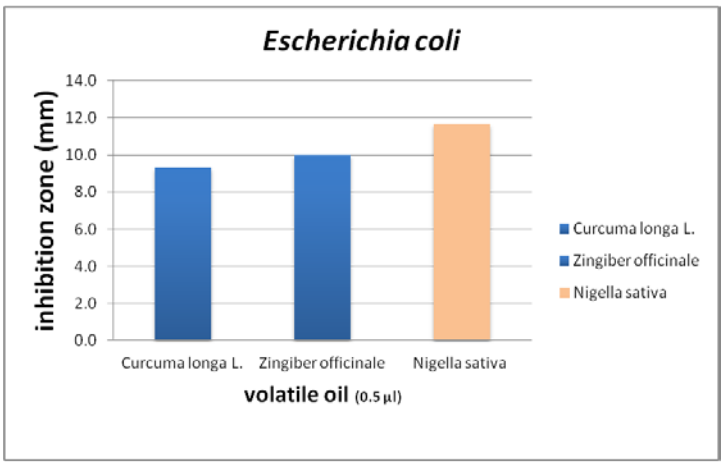

Figure 4: Inhibition zone of *E. coli* by volatile oil from plants (*Curcuma longa L., Zingiber officinale and Nigella sativa*).

The data in Figure 5 showed the results of inhibition zone of *E. coli* by fixed oils from seeds of (*Olea europaea L., Ricinus communis L. and Linum usitatissimum*), the antimicrobial effect of *Olea europaea L.* extract was more significant which was (10 ±1.0) mm to *E. coli*, when compared to other oils *Ricinus communis L.* (8.3 ±0.6) mm and *Linum usitatissimum* (8 ±1.0) mm. All extract had no antimicrobial effect to *E. coli.*

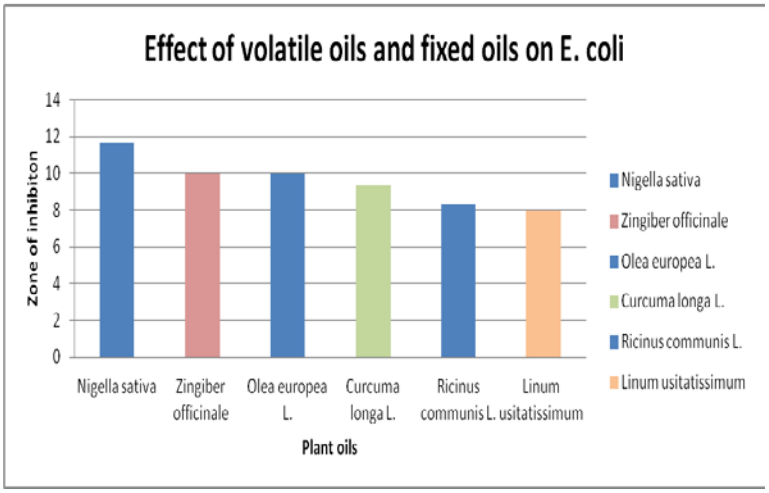

Figure 5: *E. coli* inhibition by fixed oils extracted from seeds of (*Olea europea L., Ricinus communis L. and Linum usitatissimum).*

After comparison of both used oils volatile oils of (*Curcuma longa L., Zingiber officinale and Nigella sativa*), and fixed oils of (*Olea europaea L., Ricinus communis L. and Linum usitatissimum*) our results showed that antimicrobial effect of *Nigella sativa* was more significant than other which was (11.7 ±0.6) mm to *E. coli*, while *Zingiber officinale* was (10 ±1.0) mm *Olea europea L.* was (10 ±1.0) mm they have same effect inhibition zone. On another hand, inhibition zone of *Curcuma longa L.* was (9.3 ±0.6) mm, *Ricinus communis L.* (8.3 ± 0.6) mm and *Linum usitatissimum* (8 ±1.0) mm. All extract had no antimicrobial effect to *E. coli*, when interpretation of data as indicated in (Figure 6).

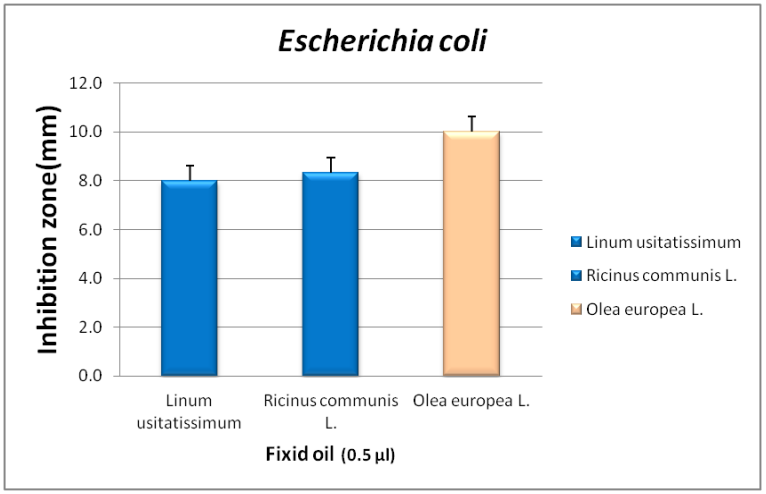

Figure 6: Comparison of antimicrobial effective against *E. coli* by (*Curcuma longa L., Zingiber officinale and Nigella sativa*) as a volatile oils and fixed oils of *(Olea europea L., Ricinus communis L. and Linum usitatissimum)*.

The greater inhibition zone obtained after using volatile oil extracted from *Nigella sativa* seeds to gram positive bacteria *S. aureus*, for determining minimum inhibition zone by this oil, microdilution technique used from dilution (0.1, 0.2, to 1 µl). Our results indicated that just dilution of 0.1 has no antimicrobial effect which was (8.7 ± 0.6) while all other dilution showed significant effect to bacteria *S. aureus.* The *p* value was significant which less than 0.05 as shown in (Table 3).

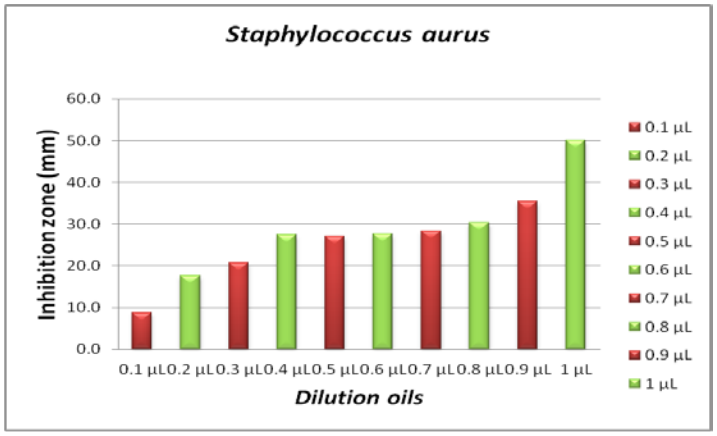

Figure 7: Determining minimum inhibition zone of *Nigella sativa* as a volatile oil to bacteria *S. aureus*, showing that 0.1µL dilution has less antimicrobial effect, Fisher analysis of the differences between the categories with a confidence interval of 95%.

Because of *Nigella sativa* has significant antimicrobial effect on gram positive bacteria *S. aureus*, as positive control 8 antibiotic disks were used to comparing with the results of *Nigella sativa* oil. Our results showed that inhibition zone of *Nigella sativa* was (27.7 ±1.2) mm which has more effect than standard antibiotics to *S. aureus*, inhibition of antibiotic disk for Ciprofloxacin (CIP) was (23 ±0.9) mm, Vancomycin (VA) (21 ±0.6) mm, Rifampin (RA) (20 ±0.3) mm, Amikacin (AK) (13 ± 0.7) mm, Meropenem (MEM) (11 ±0.1) mm, Piperacillin (PRL) (6 ±0.4) mm, Impenem (IPM) (0 ± 0) mm, and Gentamicin (CN) (0 ±0) mm which illustrated in (Figure 8).

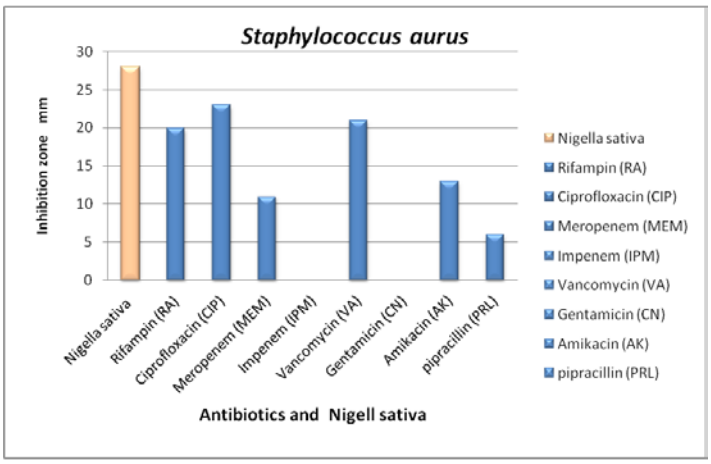

Figure 8: Comparing inhibition zone between *Nigella sativa* and standard antibiotic as positive control to bacteria *S. aureus*, Fisher analysis of the differences between the categories with a confidence interval of 95%.

Although *Nigella sativa* has no antimicrobial effect on *E. coli*, to comparing our results the 8 antibiotic disks were used as positive control. Our results revealed that inhibition zone of *Nigella sativa* was (11.7 ±0.9) mm which has less effect than standard antibiotics to *E. coli*, inhibition of antibiotic disk for Meropenem (MEM) (36 ±1.1) mm, Piperacillin (PRL) (24 ±0.5) mm, Gentamicin (CN) (22 ±0.3) mm Rifampin (RA) (18 ±0.3) mm, Amikacin (AK) (13 ±0.7) mm, Ciprofloxacin (CIP) was (12 ±0.9) mm, Vancomycin (VA) (11 ± 0.8) mm, Impenem (IPM) (0 ± 0) mm which illustrated in Figure 9.

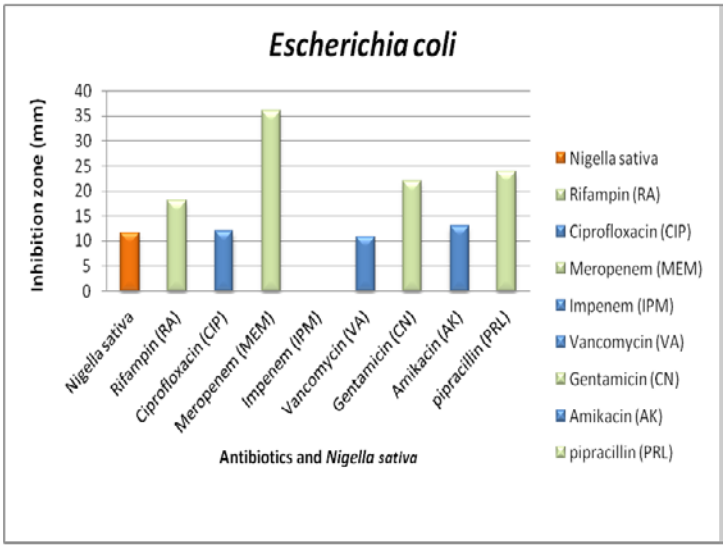

Figure 9: Comparing inhibition zone between *Nigella sativa* to bacteria *E. coli* and standard antibiotic as positive control, Fisher analysis of the differences between the categories with a confidence interval of 95%.

4. DISCUSSION

Increasing and development of antibiotic resistance phenomena among pathogenic bacteria has reached an alarming status; therefore, the needs for discovering new antimicrobial agents are greatly required. In the current investigation, antimicrobial activities of six different extracted oils from a number of medical plants were performed against both *S. aureus* and *E. coli*. The results showed that the extracted oils reveal a great antimicrobial activity against *S. aureus* and *E. coli*. More interestingly, extracted oil of *Nigella sativa* (black seed) shows a higher bacterial inhibition toward *S. aureus* in comparison with other extracted oils. However, such a great effect was not observed with *E. coli.* For instance, in the current results, a crude oil of *Nigella sativa* showed antimicrobial effect against *S. aureus*; whereas, both *Curcuma longa L* and *Zingiber officinale* extracted oils showed a very lower inhibitory effect in comparison with *Nigella sativa* extracted oil (Figure 1). These findings are in agreement with other researchers who have showed that volatile oil of *Nigella sativa* shows the inhibitory action against antibiotic-resistant *S. aureus* [4,48]. It may be regarding to that *S. aureus* ATCC 25923 are more sensitive to antibacterial effect of extracted oils in comparison to Gram-negative *E. coli*, this controversy result may be due to the difference in their cell wall structure [49]. Furthermore, antibacterial effect of fixed oils from (*Olea europaea L., Ricinus communis L. and Linum usitatissimum*) on *S. aureus* revealed that the inhibition zone by *Ricinus communis L.* was (9 ±0.1 mm); and for *Olea europaea L. and Linum usitatissimum* were (7.7 ±0.04 mm and 3.7 ±0.09 mm) respectively[8] . A study by Hossein Hosseinzadeh, in 2007 showed a supportive outcome to our results; volatile oil of *Nigella sativa* and fixed oil of *Ricinus communis L.* were effective against Gram-positive bacteria, while a lower inhibitory activity was observed with the fixed oil of *Linum usitatissimum* against both *S. aureus* and *E. coli* [50]. Similar findings were observed with other researchers, in which that extracted oils show antimicrobial property and this property may be due to its chemical compounds, structure, composition and functional group of essential oils that may has a crucial properties for posing its antibacterial action [51].

Moreover, an investigation on the action of *Nigella sativa* extracted oil with different extraction methods on the result revealed that *Nigella sativa* oils can be further safe for clinical application and pharmacokinetic parameters such as metabolism or allocation does not interfere with the antibacterial activity of *Nigella sativa*, due to the oil ability to enter through the bacterial membranes and damage bacterial cell and finally leading to cell death [52]. For the comparison between antibacterial effect of volatile and fixed oils, the results showed that volatile oil of *Nigella sativa* exhibits a potent antibacterial activity against *S. aureus*, in which the inhibition zone by black seed oil was (27.7 ±1.2 mm), while other extracted oils showed such as ginger, castor, olive, linseed and turmeric oils revealed a lower inhibition zone of bacteria between (6.7 ±0.6 mm – 10 ±1.0 mm) in comparison with antibacterial activity by black seed oil (Figure 3). Furthermore, other investigators also reported that the essential oils of *Nigella sativa* offer a complete growth inhibition against *S. aureus* by the agar disk diffusion method [53]; moreover, Khan in 2011 identified several compounds such as sterols and phenolic constituents were found in *Nigella sativa* seed, its oils have been recommended as food preservative agents in food production; moreover, it is also showed at (2.0%) concentration has the ability to inhibit bacterial growth for 24 pathogenic, spoilage and lactic acid bacteria[26].and application of herbal extracts as an alternative to chemical preservatives in fish aquaculture [54].

A fresh essential oil extraction of new rhizomes and seed of (*Curcuma longa L., Zingiber officinale and Nigella sativa*) as a volatile oil was performed, and it showed a high antimicrobial effect of *Nigella sativa* (11.7 ±0.6) mm against *E. coli*, while both *Zingiber officinale* and *Curcuma longa L.* oils revealed a lower effect (10 ±0.1) mm and (9.3 ±0.6) mm respectively (Figure 4). This result is in agreement with Alam et al. in 2010, who described that *Nigella sativa* extract showed the diverse activity caused by the ingredients contained in the extracts [55]. Moreover, phytochemical analysis showed that Zanjabel zerumbet contains alkaloids, flavonoids, steroids and carbohydrates, which suggest having inhibitory effect on bacterial

growth. Furthermore, bacteria growth can be also inhibited by sterols, eugenol and phenolic [56,57]. In addition to that, a diversity of plant species is able to synthesizing many substances with antibacterial activity such as alkaloids, tannins, flavonoids, phenols or steroids [58]. For instance, Hayes & Berkovitz in 1979 investigated the role of fatty acids on bacterial cell and it showed that fatty acids have a harmful effect on bacterial cell wall, and more specifically at low temperatures, fatty acids are thought to harm the structure and function of cell membrane and cell wall of bacterial cell [59].

Another study by Seher et al, in 2006 who reported that the extract from Linum usitatissimum has an effective inhibitory action against bacteria [60]. However, results of the current investigation reveals that the plant oil extracts show a greater inhibitory effect toward bacteria. Another supportive study by Shoaib A in 2014 [61]. who revealed that olive extracts were effective in inhibition against *S. aureus* and *Bacillus cereus* but a low inhibitory activity was revealed against the Gram-negative bacteria, these results aren't agree with our results. Inass L., et.al. in 2015 showed that the oils are enriched with pure biophenolsphenols; for example, eugenol has a high antioxidant activity against bacterial [62].

Furthermore, Hatice Z., et. al. 2014, who reported that essential oil has an antioxidant property and antimicrobial activity has been refer to the presence of phenolic compounds, due to their action on the cell membrane [63]. In the current results, after comparison of both used volatile oils of (*Curcuma longa L*., *Zingiber officinale* and *Nigella sativa*), and fixed oils of (*Olea europaea L*., *Ricinus communis L*. and *Linum usitatissimum*) showed that antimicrobial activity of *Nigella sativa* was more significantly higher which is (11.7 ± .6) mm toward *E. coli* than other extracts, while all other oil extracts showed a lower antimicrobial effect against *E. coli*. Moreover, Salah G. et al, reported that a number of substances which presents in plant extracts; for instance, thyme, clove, cinnamon, and rosmarinus hydrous, methanol and ethanol have antibacterial effects on Gram-positive and Gram- negative bacteria, these results support our results, in which the plant extracts revealed antibacterial action on *S. aureus*; however, such effect was weakly observed against *E. coli* [64].

Investigation shows that Gram-negative bacteria are more likely to be resistant to antibiotics than the Gram-positive bacteria; this is due to the difference property in their cell wall or to the membrane cumulating mechanism [65]. Lambert et al., in 2001 described that some fixed and essential oils show antimicrobial activity against bacteria at different concentrations, this is due to different mode of actions by which fixed oils can inhibit the bacterial growth [49]. Moreover, Cerdeiras et al. 2000, described that such action is due to the presence of fatty acids that showed an interesting antibacterial activity, such as the antibacterial activity of linoleic and oleic acids being active against *E. coli*, *Bacillus subtilis* and *S. aureus* [66]. But our results showed a lower antimicrobial activity for fixed oils. Furthermore, N. H. Georgopapadakou in 1993, revealed β-lactam antibiotics are currently in use are ineffective for treating of bacterial skin infections; vancomycin is the pre-eminent glycopeptidic antibiotic and is the "last resort" of antibiotic against multidrug ressistant bacteria [67].

From the investigation, the inhibition zone by Ciprofloxacin (CIP) was (23 ±0.9 mm), a greater zone of inhibition by Vancomycin (VA) was recorded after *Nigella sativa*. Ciprofloxacin (CIP), Gentamicin (CN), Amikacin (AK) are used for both Gram-positive and Gram-negative bacteria. Finally, Meropenem (MEM) and Imipenem (IPM) are used for Gram-negative bacteria. The current investigation showed that the inhibition zone by *Nigella sativa* extract was greater (27.7 ±1.2 mm) than the used antibiotics against *S. aureus*, whereas a lower inhibitory activity (11.7 ±0.9 mm) was not observed in comparison with antibiotics toward Gram-negative bacteria.

4. CONCLUSION

In the current study, it has been concluded that the extracted oil from *Nigella sativa* has a powerful antibacterial effect against antibiotic-resistant *S. aureus* in comparison with the commonly used antibiotics; however, a lower antibacterial activity with *Nigella sativa* oil extract observes against Gram-negative *E. coli*. Therefore, we can recommend that *Nigella sativa* oil can be used as a superficial cream for treatment of skin infections that is caused by *S. aureus* such as carbuncles, wound infections, furuncles, boils, and impetigo. Furthermore, it may show to has no any side effects on human body.

Further work

Further investigation will be required to establish the antibacterial properties of extracted oil from *Nigella sativa*; for instance, estimating the quality and quantity of chemical components in *Nigella sativa* oil by using Gas Chromatography/ Mass Spectrometry analysis. Furthermore, it is important to determine the mode of action of extracted oil from *Nigella sativa* on bacterial cell.

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