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# *In vitro* and *In vivo* Anti-Microbial Effects of *Nigella sativa* Linn. Seed Extracts Against Clinical Isolates from Skin Wound Infections

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**Abstract: Problem statement:** The developing microbial resistance to the existing anti-microbial agents has become a real challenge and a serious problem facing patients suffering from skin infections. Seeds of *Nigella sativa* have been used for a long time in folk medicine for the treatment of such infections. Production of new potent agents is urgently needed, especially for hospitals and health centers. Therefore, the anti-microbial effect of aqueous, diethyl ether, chloroform and petroleum ether extracts of the seeds against four standard microbial strains and seven clinical isolates from patients with skin wound infections were investigated. **Approach:** The *in vitro* anti-microbial effect of the extracts at a concentration of 20% on standard strains and clinical isolates was assessed and compared with standard drugs, chloramphenicol and amphotericin B using agar well diffusion assay. The *in vivo* anti-bacterial effect of petroleum ether extract was studied in male BALB/c mice infected subcutaneously with *S. aureus* (ATCC 25923) or a clinical isolate (0.1 mL from $10^9$ colony forming units $mL^{-1}$ suspension) and immediately treated at the infected site by subcutaneous injection of 0.1 mL of pure extract (fixed oil) or chloramphenicol or normal saline. Counts of viable bacteria present in the skin area corresponding to the infected site were determined, after 24 and 48 h of infection and treatment. **Results:** The aqueous extract did not show any inhibitory effect against all the tested microorganisms. The diethyl ether and chloroform extracts indicated significant inhibitory effect only against Gram-positive bacteria. However, petroleum ether extract was proved to be the most powerful one against these bacteria and also against other clinical isolates like one Gram-negative bacterium (*Klebsiella pneumonia*) and the yeast (*Candida albicans*). Moreover, the extract revealed a superior effect over the standard drug, chloramphenicol, on the clearance of subcutaneous staphylococcal infection in mice when injected at the site of infection. Counts of viable bacteria were decreased at highly significant level in mice infected with *S. aureus* (ATCC 25923) or a clinical isolate. **Conclusion/Recommendations:** The results of this study revealed clear potentiality of *N. sativa* fixed oil as a source for anti-microbial drugs and support its use in folk medicine for the treatment of microbial skin infections.

**Key words:** *Nigella sativa*, fixed oil, clinical isolate, skin wound, staphylococcal infection

## INTRODUCTION

*Nigella sativa* (black cumin) Linn. (Ranunculaceae) is an herbaceous indigenous plant in the Mediterranean region. Seeds of this plant have been used for centuries as a spice and food preservative, as well as a traditional medicine for the treatment of various diseases, including skin infections[1,2].

The main component of a seed is oil (about 31-36%), however other reserves are also found like carbohydrates (33-34%), proteins (16-20%) and fiber (4.5-6.5%). The major component of oil is the fixed oil, while the volatile oil ranged between 0.4-0.7 percent[3].

Crude extracts and seed constituents of *N. sativa*, in particular thymoquinone, have been reported to possess a number of pharmacological properties[4], like anti-oxidant[5], anti-tumor[6,7], anti-parasitic[8], anti-inflammatory[9], anti-diabetic[10], protection against nephrotoxicity[11] and hepatotoxicity[12] and acting as promoter of skin wound healing[13].

The anti-microbial effects of *N. sativa* seeds against different pathogenic microbes were investigated. The diethyl ether extract was found to cause concentration-dependent inhibition of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and a pathogenic yeast *Candida albicans*[14]. The methanol and chloroform extracts have high inhibitory effects against *S. aureus*, *P. aeruginosa* and *C. albicans*[15]. The essential oil of the seeds have also dose-dependent anti-bacterial effects on Gram-positive (*S. aureus*) and

Gram-negative (*E. coli*) bacteria[16]. The volatile oil of *N. sativa* seeds, prepared by steam distillation, was proved to be more effective against many strains of bacteria, including those known to be highly resistant to drugs[17]. The anti-microbial agent has been isolated from the volatile oil, identified as thymohydroquinone and was found to be active against Gram-positive bacteria and yeasts[18].

The *in vivo* anti-fungal effect of the aqueous extract of *N. sativa* seeds was also investigated. Treatment of mice with the extract 24 h after intravenous injection of *C. albicans* caused a considerable inhibitory effect on the growth of the fungus in the liver, spleen and kidneys[19]. Moreover, the anti-fungal effects of ether extract and thymoquinone of *N. sativa* seeds against many species of dermatophytes, revealed the potentiality of such seeds as a source for anti-fungal drugs and support their use in folk medicine for the treatment of fungal skin infections[20-22].

The skin represents the first line of defense against microbial colonization and infection simply indicates the presence of microbes, which may be pathogens, opportunist or commensals. However, the skin supports the growth of commensally bacteria, which protect the host from pathogenic bacteria. In fact, environmental factors, host immunity, organism adherence and virulence are strongly related to cutaneous infection[23]. *S. aureus* and *Streptococcus pyogenes* are among the most common causative agents of primary and/or secondary skin infections in human[23,24]. These infections may lead to serious local and systemic complications like skin lesions[25].

The developing microbial resistance to the existing anti-microbial agents has become a serious problem. Therefore, production of new potent agents is urgently needed, especially for hospitals and health centers, keeping in mind that studies on strains of pathogenic microbes are scarce. Furthermore, most studies on the anti-microbial potential of plant extracts, active ingredients and/or chemical drugs have been tested on experimental animals. So, the present study was carried out to investigate the anti-microbial effect of *N. sativa* crude (aqueous and organic) extracts against clinical isolates from patients with skin wound infections and to elucidate the effectiveness of petroleum ether extract on the clearance of subcutaneous staphylococcal infection in mice.

## MATERIALS AND METHODS

**Selection of *Nigella sativa*:** Seeds of *N. sativa* were purchased in February, 2008 from an herbal shop in Irbid, Jordan. The seeds were authenticated at the Herbarium of the Department of Biological Sciences, Al al-Bayt University, Mafraq, Jordan.

**Preparation of seed extracts:** Seeds of *N. sativa* were ground into slightly coarse powder using electric blender. Organic extracts were prepared by soaking three portions of 150 g each of the dried powder separately in 600 mL of analytical organic solvents (chloroform 95%, diethyl ether 99% and petroleum ether 40-60°C), using a conical flask plugged with cotton wool. The mixture was kept at room temperature for 48 h under continuous shaking (memmert shaker SV 1422, Germany). Aqueous extract was prepared by soaking 50 g of the dried powder in 300 mL distilled water and heating for 30 min at 50°C. Then, the mixture was kept at room temperature under continuous shaking for 48 h. The viscous mixture was centrifuged for 5 min at 2000 rpm to collect the supernatant. The aqueous supernatant and organic extracts were filtered separately through whatman filter paper no. 2 under vacuum. The filtrates were evaporated to dryness by Rota vapor (Rüchl R-114, Switzerland). The rotary water bath (Rüchl B-480) was adjusted to 55°C. The organic extracts were kept overnight under vacuum fume hood to obtain a constant dry weight. The extracts were weighed and stored in closed vessels at 4°C in a refrigerator for further use.

**Determination of *in vitro* anti-microbial effect:**

**Microbial strain:** The aqueous and organic extracts of *N. sativa* seeds were tested against four standard microorganisms (*Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6051), *E. coli* (ATCC 11229) and *Candida albicans* (ATCC 18804)) and seven clinical isolates (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumonia*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia* and *Candida albicans*) from patients with skin wound infections in El-basher hospital and Islamic hospital, Amman- Jordan, from February to June 2008.

**Inoculum's preparation:** Microbial strains and clinical isolates were re-identified and characterized by standard microbiological techniques prior to use. The test microorganisms were maintained at 4°C on nutrient agar slants (bacteria) and potato dextrose agar slants (yeast). Active cultures for each bacterial species were prepared by transferring a loopful of cells from the stock cultures to test tubes of nutrient broth and potato dextrose broth for yeast. The inoculated tubes were incubated without agitation for 24 h at 37°C. The cultures were diluted with fresh nutrient or potato

dextrose broth to achieve optical densities corresponding to $1\times10^8$ colony forming units per milliliter (cfu $mL^{-1}$) for bacteria and $1\times10^6$ spore $mL^{-1}$ for yeasts[26].

**Agar well diffusion assay:** The agar well diffusion method was used to test the anti-microbial effect of *N. sativa* crude extracts[27]. All media plates (9 cm in diameter) were prepared according to the manufacturer recommendations (Difco Laboratories, USA). After agar solidification, the well (7 mm in diameter) was cut from the agar to produce a total of three wells per each agar plate. For preliminary test, six concentrations of each extract (10, 20, 30, 40, 50 and 60% v/v) were prepared in a total volume of 100 μL per well using 99.5% analytical Dimethyl Sulphoxide (DMSO) as an organic solvent. Chloramphenicol (10 μg/well) and amphotericin B (10 μg/well) were used as positive standard antibiotics. One hundred μL of each diluted microbial suspension were inoculated on nutrient and/or dextrose agar plates using sterile cotton swab. The inoculums were allowed to dry for 5 min. Then, 100 μL of each extract solution, blank (DMSO or water) and positive control was added separately to each well of agar plate and allowed to diffuse at room temperature for 15-20 min. After incubation at 37°C for 24 h, all plates were examined for any zones of growth inhibition and the diameter of these zones was measured. Preliminary test revealed that the extract concentration of 20% was the most effective. Therefore, the assay was repeated six times at such concentration for each extract. The anti-microbial effect was recorded as the mean diameter of the resulting inhibition zones of growth in millimeter.

**Determination of *in vivo* anti-microbial effect:**

**Animals:** Male BALB/c mice six-week of age were purchased from the animal house of the Department of Biological Sciences, Yarmouk University, Irbid, Jordan. All animals were housed, fed and treated in accordance with the in-house guidelines for animal care[28]. Animals were kept for two weeks to be acclimatized prior to the investigation. During this time they were given standard pellet diet and water *ad libitum*. At the time experiment was initiated, mice have a mean ± SD body weight of 23.4±1.2 g.

**Bacterial strain:** Based on results obtained from agar well diffusion assay, the bacterial strain selected for this experiment was *S. aureus* (ATCC 25923) and a clinical isolate. Bacterial cultures were routinely grown in nutrient broth at 37°C for 24 h. Active cultures were harvested by centrifugation, washed twice and re-suspended in sterile saline. Bacterial density was determined by measuring the absorbance at 600 nm. The bacterial suspension was then diluted with sterile saline to $1\times10^9$ cfu $mL^{-1}$ using a standard growth curve to relate measured $A_{600}$ to the bacterial concentration[29].

**Infection of animals:** Skin infection of BALB/c mice with *S. aureus* (ATCC 25923) or a clinical isolate was conducted as described by Godin *et al*.[30]. The assay was run on a total of forty-eight mice randomly divided into two groups. In the first experimental group, twenty-four mice were injected subcutaneously with 100 μL of *S. aureus* (ATCC 25923) at a concentration of $1\times10^8$ cfu/mouse into the previously shaved mid-dorsal skin of the mice. The infected mice were randomized into four sub-groups, with six mice in each sub-group. Three sub-groups were treated with petroleum ether extract, chloramphenicol and the vehicle (normal saline). Sub-group four was left untreated as control. Post-infection immediate treatment occurred through subcutaneous injection of the infected site with 0.1 mL of pure extract (fixed oil), antibiotic solution (10 μg/mouse) and the vehicle using insulin syringes. In the second experimental group, twenty-four mice were injected subcutaneously with 100 μL of *S. aureus* (clinical isolate) at a concentration of $1\times10^8$ cfu/mouse. The experimental protocol was followed as described in the first group.

The mice were monitored for the development of skin abscesses and lesions characterized by swelling, redness and other inflammatory reaction on the infected site through 48 h after infection.

**Bacterial counts:** Three mice from each treated and/or untreated sub-group were sacrificed after 24 and 48 h of infection. Then, after disinfection with 70% ethanol, the skin area corresponding to the infection site and underlying tissue were removed and homogenized in 2 mL sterile saline. To quantify the bacterial numbers, samples were diluted to 1:10, plated on nutrient agar plates and incubated for 24 h at 37°C. Bacterial counts were expressed as numbers of *S. aureus* colony forming units per gram (cfu $g^{-1}$) of tissue[29,30].

**Statistical analysis:** Data were presented as means ± SEM (Standard Error of the Mean). Comparisons between groups were performed by using paired students t-test on a Statistical Software Package (SPSS). Differences were considered significant, if p-value is less than 0.05 and 0.01.

## RESULTS

**Preliminary assessment and *in vitro* anti-microbial effect:** The preliminary assessment of the *in vitro* anti-microbial effect of *N. sativa* crude extracts revealed three basic outcomes (Table 1 and 2).

Table 1: The anti-microbial effect of *N. sativa* crude extracts against standard microbial strains

| | Mean diameter of inhibition zone in mm (mean ± SEM)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Extracts (20% v/v) | | | | Standard antibiotic (10 μg/well) | | Vehicles (100 μL/well) | |
| Microorganisms | Aqueous | Diethyl ether | Chloroform | Petroleum ether | Chloramphenicol | Amphotericin B | DMSO | Water |
| **Gram positive:** | | | | | | | | |
| *S. aureus* (ATCC 25923) | NZ | 17.00±0.17 | 15.66±0.33* | 38.66±0.34** | 22.33±0.32 | NZ | NZ | NZ |
| *B. subtilis* (ATCC 6051) | NZ | 24.66±0.21 | 33.50±0.31 | 35.17±0.31* | 22.33±0.33 | NZ | NZ | NZ |
| **Gram negative:** | | | | | | | | |
| *E. coli* (ATCC 11229) | NZ | NZ | NZ | NZ | 20.21±0.22 | NZ | NZ | NZ |
| **Yeast:** | | | | | | | | |
| *C. albicans* (ATCC 18804) | NZ | NZ | NZ | NZ | NZ | 18.50±0.26 | NZ | NZ |

[a]: (n = 6), *: p<0.05; **: p<0.01 were considered significant in comparison to the standard antibiotic control group; NZ: No Zone of inhibition; DMSO: Dimethyl Sulfoxide

Table 2: The anti-microbial effect of *N. sativa* crude extracts against clinical isolates from patients with skin wound infections

| | Mean diameter of inhibition zone in mm (mean ± SEM)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Extracts (20% v/v) | | | | Standard antibiotic (10 μg/well) | | Vehicles (100 μL/well) | |
| Microorganisms | Aqueous | Diethyl ether | Chloroform | Petroleum ether | Chloramphenicol | Amphotericin B | DMSO | Water |
| **Gram positive:** | | | | | | | | |
| *S. aureus* | NZ | 09.83±0.31* | 09.50±0.34* | 41.33±0.22** | 21.00±0.26 | NZ | NZ | NZ |
| *S. epidermidis* | NZ | 25.66±33 | 30.16±0.31* | 36.83±0.17** | 22.33±0.21 | NZ | NZ | NZ |
| *S. pneumonia* | NZ | 07.50±0.22 | 09.66±0.21 | 18.50±0.22 | 21.50±0.22 | NZ | NZ | NZ |
| *B. subtilis* | NZ | 24.33±0.21 | 38.66±0.31 | 38.83±0.31** | 22.83±0.17 | NZ | NZ | NZ |
| **Gram negative:** | | | | | | | | |
| *E. coli* | NZ | NZ | NZ | NZ | 22.33±0.21 | NZ | NZ | NZ |
| *K. pneumonia* | NZ | NZ | NZ | 10.33±0.21* | 22.17±0.40 | NZ | NZ | NZ |
| **Yeast:** | | | | | | | | |
| *C. albicans* | NZ | NZ | NZ | 11.33±.0.21 | NZ | 18.00±0.21 | NZ | NZ |

[a]: (n = 6), *: p<0.05 and **: p<0.01 were considered significant in comparison to the standard antibiotic control group, NZ: No Zone of inhibition, DMSO: Dimethyl Sulfoxide

First, the aqueous extract at a concentration of 20% did not show any inhibitory effect against all standard strains and the clinical isolates tested.

Second, the organic extracts (fixed oil) at a concentration of 20% were proved to be effective under the conditions of the present investigation. The diethyl ether and chloroform extracts were found active against Gram-positive bacteria, both standard and clinical isolates. However, petroleum ether extract was proved to be the most powerful one against these bacteria and also against some other clinical isolates like one Gram-negative bacterium (*K. pneumonia*) and the yeast (*C. albicans*). The extract also revealed superior inhibitory effect over the standard drug, chloramphenicol, when testing against *S. aureus* (standard and clinical isolate), *S. epidermidis* (clinical isolate) and *B. subtilis* (standard and clinical isolate). Third, two standard antibiotics or drugs were used; chloramphenicol and amphotericin B and the former was proved to be effective against Gram-positive and Gram-negative bacteria of standard strains and clinical isolates from patients with skin wound infections. On the other hand, amphotericin B was effective against both standard and clinical isolate of *C. albicans*.

**I*n vivo* anti-microbial effect:** The petroleum ether extract was proved to have potent anti-bacterial effect against Gram-positive bacteria, based on results obtained from Table 1 and 2. The most significant effect was observed against *S. aureus*, as compared with other organic extracts. In the *in vivo* experiments, the anti-bacterial effect of petroleum ether extract in mice infected with *S. aureus* (ATCC 25923) or a clinical isolate was conducted.

The effect of the extract on the growth of *S. aureus* (ATCC 25923) in the skin of infected mice was shown in Fig. 1. Counts of viable bacteria present in the skin after 24 and 48 h of infection, showed that there was no significant difference between the infected untreated (control group) and the saline-treated mice.

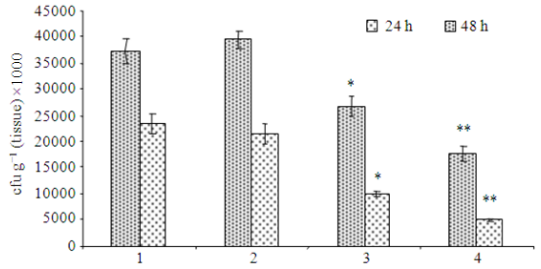


Fig. 1: Counts of viable *S. aureus* (ATCC 25923) in the skin of infected untreated mice (bars labeled "1"), saline-treated mice (bars labeled "2"), chloramphenicol-treated mice (bars labeled "3") and petroleum ether extract-treated mice (bars labeled "4") after 24 and 48 h of subcutaneous infection and treatment. Data were expressed as means ± SEM for three mice in each group, *: $p<0.05$ and **: $p<0.01$ were considered significant in comparison to the infected untreated control group.

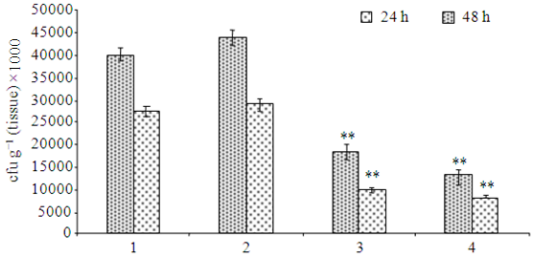


Fig. 2: Counts of viable *S. aureus* (clinical isolate) in the skin of infected untreated mice (bars labeled "1"), saline-treated mice (bars labeled "2"), chloramphenicol-treated mice (bars labeled "3") and petroleum ether extract-treated mice (bars labeled "4") after 24 and 48 h of subcutaneous infection and treatment. Data were expressed as means ± SEM for three mice in each group, **: $p<0.01$ was considered significant in comparison to the infected untreated control group

However, chloramphenicol-and extract-treated mice had bacterial counts much lower than the control group. In the chloramphenicol-treated mice, viable bacteria decreased to a significance level ($p<0.05$), after 24 and 48 h of infection, to around $2.7\times10^7$ and $1\times10^7$ cfu, respectively. In the extract-treated mice, bacterial numbers decreased considerably to a highly significant level ($p<0.01$), after 24 and 48 h of infection, to around $1.8\times10^7$ and $5\times10^6$ cfu, respectively.

Similar effect on the growth of clinically isolated strain of *S. aureus* in the skin of infected mice was observed in Fig. 2. After 24 and 48 h of infection, the saline-treated mice had bacterial numbers slightly higher, but was not statistically significant, than the infected untreated mice. On the other hand, bacterial counts in chloramphenicol-and extract-treated mice declined to a highly significant level ($p<0.01$), after 24 h of infection, to around $1.8\times10^7$ and $1.4\times10^7$ cfu, respectively. The bacterial numbers were further declined to a highly significant level ($p<0.01$), after 48 h of infection and reached around $9.5\times10^6$ and $8.6\times10^6$ cfu, respectively.

In all groups of animals subcutaneously injected with $1\times10^8$ cfu of *S. aureus* (ATCC 25923) or a clinical isolate, development of small abscesses in the skin was observed within 24-48 h of infection in the infected untreated and saline-treated mice.

These findings have clearly demonstrated that the clearance of *S. aureus* from the site of infection in mice by petroleum ether extract was significantly high ($p<0.01$), as compared with the infected untreated mice. Furthermore, it was more effective than the standard drug, chloramphenicol, in treating a non-fatal subcutaneous staphylococcal infection in mice when injected at the site of infection.

## DISCUSSION

The data of the preliminary assessment of the *in vitro* anti-microbial effect of *N. sativa* seeds revealed that aqueous extracts did not contain anti-bacterial and/or anti-fungal constituents, at least under the conditions of the current study, which are in fully agreement with other studies on the same plant species. The extract was found to be ineffective on standard and hospital strains of *C. albicans*, *S. aureus* and *P. aeruginosa*[15]. However, other studies have shown that such extracts of these seeds could have an inhibitory effect on the growth of the yeast *C. albicans* in the liver, spleen and kidneys of infected mice[19]. This controversial results can be explained by: (a) The different techniques used for extraction[14,15,17], (b) The differences between *in vivo* and *in vitro* studies[15,19], (c) The sensitivity and the accuracy of the anti-microbial test, (d) The concentration and the effectiveness of the constituents in the extracts, (e) The conditions of seed collections and the season[15], (f) The storage and the preservation method of the extracts and (g) Most previous studies stressed on the volatile oil and its constituent, thymoquinone[3,4,18,20-22].

Three organic solvents were used to extract fixed oil constituents from seeds of *N. sativa*, these included: diethyl ether, chloroform and petroleum ether. The first

two solvents have been used widely[14-16], while the third one was never used before at least with seeds of *N. sativa*. The fixed oil of organic solvents had more effective anti-bacterial effect against Gram-positive than Gram-negative bacteria. Gram-negative bacteria have an outer membrane, which make their cell wall impenetrable to anti-microbial agents. It is possible that the apparent ineffectiveness of *N. sativa* organic extracts was largely due to this permeability barrier. On the other hand, Gram-positive bacteria are only composed of peptidoglycan cell wall and so are more susceptible to anti-microbial agents[17,31]. Although petroleum ether extract was a crude one, it proved superior over the standard antibiotic, chloramphenicol, when testing against *S. aureu*s, *S. epidermidis* and *B. subtilis*. This could be explained by the presence of potent anti-bacterial constituents in high concentrations in the extract. Moreover, the specific mode of action of active chemical constituent against microorganisms is attributed to their chemical composition and morphology[31]. The extract was also active on *K. pneumonia* rather than *E. coli*, which indicates a higher concentration of the extract is required to inhibit the growth of *E. coli*. Since, both are Gram-negative bacteria with effective permeability barrier. Similar effect of the extract was observed on yeast (*C. albicans*). The standard strain was insensitive and more resistance to the extract than clinical isolate. This means that they are different strains and the inhibitory effect of the extract is dose-dependent[14,16]. Such findings need further investigation.

It is worth to mention that *S. aureus* is the causative agent for the majority of primary skin infections such as cellulites, trauma and wound-related infections, especially when associated with co-morbid conditions and/or bacteremia, may lead to severe complications and hospital admission. In some cases they can be a cause of extensive morbidity and mortality[30]. *S. aureus* produces several biologically active products, including hemolysins, nuclease, coagulase, lipase, exotoxins, fibronectin-and collagen-binding proteins and enterotoxins. Immunity to *S. aureus* is primarily via complement-mediated killing by neutrophils and cell-mediated immunity may secondarily contribute to pathogenesis of some lesions[32]. Skin mast cells can detect and be activated by invading bacteria via various receptors. Neutrophil accumulation and bacterial clearance at the sites of subcutaneous *P. aeruginosa* infection are impaired in the absence of mast cells. This indicates that skin mast cells are critically important for the accumulation of neutrophils and the clearance of bacteria at the sites of infection[33].

No previous reports have been published for evaluating the efficiency of petroleum ether extract on the clearance of subcutaneous *S. aureus* infection. The potency of the extract to inhibit the growth of *S. aureus* both *in vitro* and *in vivo* was significantly high, which provides an evidence for the presence of highly active anti-bacterial agents in that extract. Such extract might have had bactericidal effect and/or stimulatory effect on mast cells and recruitment of neutrophils at the site of infection. Therefore, further studies should be followed to isolate pure active anti-microbial agents for testing specific anti-microbial effect. Moreover, histological evaluation of the infected and treated skin biopsies should also be investigated to elucidate the effect of the extract on the inflammatory process.

## CONCLUSION

In a conclusion, the results of this study revealed clear potentiality of *N. sativa* fixed oil as a source for anti-microbial drugs and support its use in folk medicine for the treatment of microbial skin infections.

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