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RESEARCH ARTICLE

WOUND DRESSINGS UPLOADED WITH MYRTLE BERRIES EXTRACT AND *NIGELLA SATIVA* HONEY

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ABSTRACT:
Wound dressings are frequently developed by introducing new products to target different aspects of the wound healing process. Many medicated dressings incorporated with natural extracts and chemicals have been developed. Chronic wounds could be invaded by many bacteria and *Pseudomonas aeruginosa* and *Staphylococcus aureus*are the most common. *S. aureus* and *P. aeruginosa* are usually detected in the higher layer of wounds or in the deepest region of wound bed, respectively. They can express many virulence factors affecting wound healing process and leading to severe infections and antibiotic resistance. Starch based impregnated gauze containing either *N. sativa* honey, myrtle berries hydro-alcoholic extract or a combination were prepared. There efficacy against both *P. aeruginosa* and *S. aureus* isolated from chronic wounds. *N. sativa* honey mixture was the most potent against *P. aeruginosa* with an inhibition zone diameter of 18.1±0.3 mm, while the myrtle berries hydro-alcoholic extract mixture was the most potent against *S. aureus* with an inhibition zone diameter of 18.4±0.5 mm. The prepared impregnated gauzes deliver a moist environment that helps wounds epithelialize more rapidly. In addition, honey and myrtle berries hydro-alcoholic extract provide antibacterial and anti-inflammatory properties that will accelerate the healing process of wounds.
Keywords: *Staphylococcus aureus*; *Pseudomonas aeruginosa*; *N. sativa* honey; myrtle berries hydro-alcoholic extract; starch based impregnated gauzes.

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INTRODUCTION

A wound is defined as a simple or severe break in an anatomical structure such as the skin and can outspread to other tissues[1]. Infection occurs in wounds due to competition with the host natural immune system and causes a delay in wound healing. The most common causes of infection are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, and some *Proteus*, *Clostridium*, and *Coliform* species. The efficacy of topical solutions, creams or ointments for drug delivery to the wound is very low as they rapidly lose their rheological characteristics due to the absorption of fluids[2]. Traditionally, wound dressings are used to protect the wound from contamination[3], but they can be developed to deliver bioactive molecules such as antimicrobial drugs to wound sites. Wound dressings uploaded with natural products, including the β-glucans, aloe, essential oils, honey, cocoa, and oak bark extracts are already being used in wound healing due to their antibacterial activity and wound healing properties[4].

Various parts of Myrtle (*Myrtus communis* L.) such as berries, fruits and leaves have been widely used as traditional medicine for the treatment of several diseases due to their anti-inflammatory, antioxidant and antimicrobial properties[5,6]. Many components have been extracted from this herb and are considered to be the main biologically active components including polyphenols, myrtucommulone, semimyrtucommulone, α-pinene, 1,8-cineole, myrtenyl acetate, limonene, linalool and α-terpinolene[7]. High antibacterial activity of ethanol, methanol, and ethyl acetate berry myrtle extracts was observed when tested against *S. aureus*, *P. aeruginosa* and *Escherichia coli*[8,9]. Some results have indicated that phenolic compounds and tannins greatly contributed to the antibacterial efficacy[10,11]. In folk medicine, a decoction of leaves and fruits is used externally for wound healing[12].

Traditionally, honey has been considered to have therapeutic properties since ancient times[13]. Results of different researches had previously proved the efficacy of honey against different types of microbes depending on many factors such as the type, natural structure of the nectar and the environmental conditions[14]. Bacterial resistance is less likely to develop as a result of treatment with honey because of the composition of honey which contains a number of different components responsible for the antimicrobial efficacy[15]. This includes pH, sugar content, hydrogen peroxide levels and the presence of some phytochemicals, mainly phenolic compounds including phenolic acids and flavonoids[16].

Honey has also been proved to accelerate wound healing by offering antibacterial activity, maintaining a moist wound environment that promotes healing, and providing a protective barrier to prevent infection[17,18]. Many researchers report that honey could be an effective dressing for the treatment of different skin infections resulting from burns and wounds[19,20].

In this study, the anti-bacterial effect of impregnated sterile gauzes containing myrtle berries extract and *Nigella sativa* honey was studied on both *P. aeruginosa* and *S. aureus*.

MATERIALS AND METHOD

1.1: Myrtle extract preparation

Myrtle berries were collected from a mountainous region of Syria. 2 g of dried powders of myrtle berries were extracted by maceration in 100 ml of ethanol 50% for 2 hours[21]. The ethanol was evaporated using a rotary evaporator.

1.2: Starch based gel preparation

A starch based gel containing 20 g of starch, 20 ml of glycerol, and 100 ml of water was prepared first[22]. The solution was gently stirred until starch dissolved. It was then homogenized, heated for about 15 min at 80-85ºC and finally cooled to room temperature.

Three different mixtures were prepared using 10 ml of the starch based gel with 10 ml of *N. sativa* honey (purchased directly from beekeepers), 10 ml of myrtle extract or a combination of them in 1:1 ratio.

N. sativa honey was used in this study as it was found to be more potent on *P. aeruginosa* and *S. aureus* than other types of honey[23,24].

1.3: Impregnated gauze preparation

Standard sterile gauze 3 inch by 3 inch was dipped into different starch based mixtures till saturation and the excess solution was extruded by applying pressure. The hardening of the gel on the gauze was accomplished by refrigeration then the prepared impregnated gauzes were placed in sterile envelopes.

1.4: Antibacterial efficacy

P. aeruginosa and *S. aureus* were isolated from chronic wounds and tested for their antibiotic sensitivity. Antimicrobial activity test was carried out using agar diffusion method on Muller Hinton Agar plates[25]. Bacterial isolates were spread on plates, and then a hole was punched into plates with a diameter of 6 mm. One hundred micro liter of each mixture was introduced into the hole and the plates were incubated for 24 h at 37°C. The average of three cross sectional points of inhibition zone diameter was taken as the inhibition zone.

RESULTS AND DISCUSSION

Application of conventional antibiotics is becoming more difficult due to several problems especially antimicrobial resistance and side effects. This has reinforced the use of natural alternative agents to replace synthetic antimicrobials[26]. Accordingly, extensive research has been carried out in order to assess the antimicrobial activity of the natural extracts and different types of honey which showed the ability to inhibit the growth of various pathogenic microorganisms[27].

The antibiotic sensitivities of both *P. aeruginosa* and *S. aureus* isolated from chronic wounds are presented in Tables 1 and 2. Table 3 shows the results of inhibition zone diameter of different prepared starch based mixtures on under-study microorganisms. Accordingly, the *N. sativa* honey mixture was the most potent against *P. aeruginosa* with an inhibition zone diameter of 18.1±0.3 mm similar to that of imipenem and ceftazidime, while the myrtle berries hydro-alcoholic extract mixture was the most potent against *S. aureus* with an inhibition zone diameter of 18.4±0.5 mm similar to that of tetracycline and chloramphenicol.

The positive and potent effect of myrtle extract on *S. aureus* in this study is consistent with the results obtained by Taheri *et al.* who had previously found that the concentration of 80 mg/ml of myrtle hydro-alcoholic extract showed the greatest effect on the *S. aureus* bacterium with an inhibition zone diameter of 20.4±0.3 mm. Same results were obtained by Salvagnini who studied the effect of the oil and ethanolic extact of myrtle on different strains and reported that the ethanolic extract of myrtle has a positive effect on *S. aureus* with 12 mm inhibition zone[28,29]. Ghlamhsynyan Najjar *et al.* acknowledged that the activity of myrtle extract on *S. aureus* strain is partly due to the stimulation of free radicals[30].

The efficacy of honey against different types of microbes has been previously proved in different researches[23,24,31] and bacterial resistance is less likely to develop as a result of the composition of honey which contains a number of different components[15]. Results of different researchers proved that honey was more potent against *P. aeruginosa* than *S. aureus* which is consistent with our results. Boateng and Nso Diunase found that the zone of inhibition values for *P. aeruginosa* ranged from 26.3±0.6 mm for Manuka honey to 34±2.0 mm for Cameroon standard honey, whilst the zones of inhibition against S. aureus was not more than 18.7 ± 1.2 mm for Manuka honey[32].

As shown in Table 3, the combination between *N. sativa* honey and myrtle berries extract was effective against both *P. aeruginosa* and *S. aureus* with a diameter zone of inhibition of 13.06±0.4 mm and 15.6±0.2 mm, respectively.

It is important to care properly for wound, whether it is a minor cut or a major incision. Dressings are a part of this process and are designed to be in contact with the wound, help in faster re-epithelialization, collagen synthesis and promote angiogenesis[33]. Bioactive wound dressings incorporated with antimicrobials are one of the most important modern wound dressings developed to play an important role in healing process compared with traditional wound dressings used only for covering the wound[34]. Commercially available antimicrobial dressings include honey-impregnated dressings, iodine-impregnated dressings, silver-impregnated dressings and chlorhexidine gauze dressing[35].

Misirlioglu *et al.* used honey-impregnated gauze for the treatment of a split-thickness skin graft donor site. The gauze showed a lower sense of pain and faster epithelialization time than paraffin gauzes and saline-soaked gauzes[36]. In the UK, dressings impregnated with Manuka honey were successfully used in the wound care clinic[37].

Subrahmanyam *et al.* has shown in a randomized clinical study that residual scars decrease in patients treated with honey-impregnated gauze compared with those treated with amniotic membrane[38]. It was also proved that wounds dressed with honey-impregnated gauze showed earlier healing compared with silver sulfadiazene dressing in burn patients[39].

As presented in Figure 1, the prepared impregnated gauzes contain either *N. sativa* honey, myrtle berries hydro-alcoholic extract or a combination. The gauze can be cut to fit around the wound due to their soft elastic properties which provides easy application and removal without any damage. They also deliver active compounds with anti-inflammatory and antimicrobial properties; and play an active role in the wound healing process. Starch based mixtures provide a moist environment in addition to a soothing and cooling effect.

CONCLUSION

Simple woven gauze although commonly used, they are known to be painful to remove, destructive to newly formed granulation tissue and provoke infection by leaving some fibers behind in the wound bed. A wide range of more appropriate dressings ensuring appropriate healing process has been available for a number of years such as medicated dressings. Plant extracts with antimicrobial and healing properties in addition to natural antimicrobial agents that were known to ancient cultures such as silver, honey and iodine are used for the preparation of medicated dressings. Although the perfect dressing is yet to be developed, wound dressings have evolved and further researches are still to be done.

CONFLICT OF INTEREST

"No conflict of interest associated with this work".

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Table 1: Antibiotic sensitivities of *P. aeruginosa* isolate.

Antibiotic name	Inhibition zone diameter (mm)	Result
Levofloxacin	29	Sensitive
Cefipime	26	Sensitive
Ceftazidime	20	Sensitive
Imipenem	20	Sensitive
Gentamycin	15	Intermediate
Doxycycline	15	Intermediate
Ceftriaxone	10	Resistant
Amoxicillin+ Clavulanic acid	No inhibition zone	Resistant

Table 2: Antibiotic sensitivities of *S. aureus* isolate.

Antibiotic name	Inhibition zone diameter (mm)	Result
Imipenem	31	Sensitive
Levofloxacin	30	Sensitive
Erythromycin	23	Sensitive
Meropenem	22	Sensitive
Tetracycline	20	Sensitive
Chloramphenicol	19	Intermediate
Cefotaxime	13	Resistant
Linezolid	11	Resistant
Cefazolin	10	Resistant
Cefaclor	No inhibition zone	Resistant
Ceftriaxone	No inhibition zone	Resistant
Cefdinir	No inhibition zone	Resistant

Table 3: Sensitivity of *P. aeruginosa* and *S. aureus* isolates against different mixtures.

Mixture	Inhibition zone diameter (mm)	
	P. aeruginosa	***S. aureus***
N. sativa honey	18.1±0.3	11.2±0.3
Myrtle extract	15.3±0.2	18.4±0.5
Myrtle extract with honey 1:1	13.6±0.4	15.6±0.2

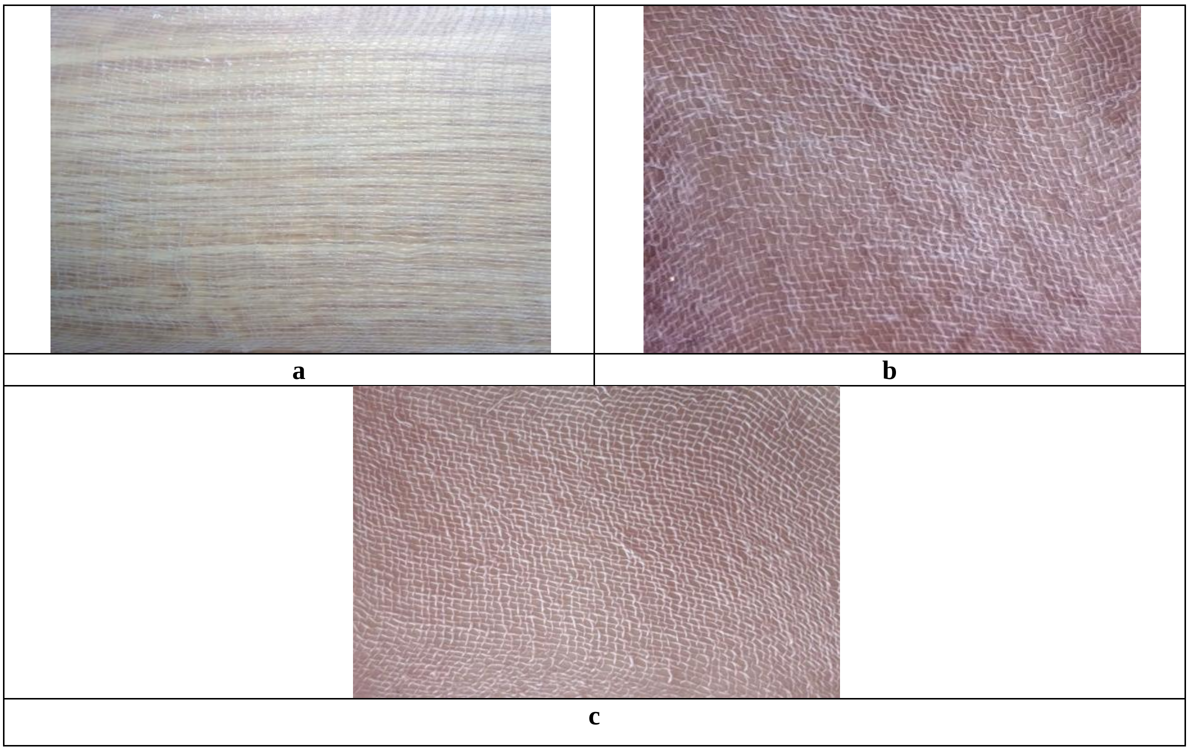

Figure 1: Impregnated gauze.

a. Impregnated gauze with 10 ml *N. sativa* honey. **b.** Impregnated gauze with 10 ml myrtle berries hydro-alcoholic extract. **c.** Impregnated gauze with 10 ml *N. sativa* honey and myrtle berries hydro-alcoholic extract mixture (1:1).