In Vitro Susceptibility of Diabetic Wound Bacteria to Mixtures of Honey, *Commiphora molmol* and *Nigella sativa*

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Abstract: The search for an ideal dressing for diabetic foot wounds/ulcers is still ongoing. Patients' preferences of such dressing usually relate to their local cultural background. The aim of this study was to evaluate the antibacterial effectiveness of some natural herbal medicines and honey mixtures on clinical isolates from diabetic wounds. Six mixtures consisting of honey, *Commiphora molmol* and *Nigella sativa* at different concentrations have been prepared (NP1 to NP6). All mixtures were effective against the tested bacterial isolates from three different diabetic food wound patients at 50%. NP4 and NP5 were the most effective as antibacterial for the examined seven bacterial isolates among the mixtures as they were able to kill bacteria at 25% after 24 hours of incubation. An over additive action has been noticed between honey and herbal medicines. The effectiveness of mixtures was dose-dependent of *Commiphora molmol*; NP5 contained the highest amount of the later. Further research is needed to elucidate and optimize the effective combination of these natural products in clinical practice.

Keywords: Antibacterial, *commiphora molmol*, *nigella sativa*, diabetes.

INTRODUCTION

Diabetes is a chronic debilitating medical condition which is reaching epidemic proportions in Saudi Arabia. At least one out of five Saudis is affected [1] and self-medication with oral natural preparations and herbs are fairly common across the country as part of complementary and alternative medicine (CAM) [2-4]. Foot disorders are among the most feared complications of diabetes [5] and the ultimate endpoint of diabetic foot ulcer is amputation if not well treated [6]. When amputation happens, it is usually associated with significant mortality [7-9], in addition to immense social, psychological and financial consequences [10, 11].

Medicinal plants are used in developing countries where it is believed to have lesser side effects and lower cost [12]. Moreover, its use depends on many factors including cultural background, education, socioeconomic factors, and nature of health problem and availability of the remedies in the local market.

Honey is one of the most widely used natural products not only in Saudi Arabia but also in all Arabian countries. It has been used since ancient times as a remedy in wound care. Honey has potent antibacterial activity and is effective in preventing and clearing wound infections [13]. Topical honey was shown to be effective in treating postoperative skin wounds in neonates that had failed to respond to antibiotic therapy [14]. It has been demonstrated in many studies that the antibacterial effects of honey are attributed to its high osmolarity, low pH, hydrogen peroxide content, and presence of other uncharacterized compounds [15]. In Saudi Arabia, some herbs, natural products and CAM preparations are occasionally added to honey or used separately by diabetics.

Commiphora Molmol (Myrrh) is one species of the resin-bearing plants which grows across the Red Sea in the area that is now Somalia and Ethiopia, while the collection of the gum resins was initiated in Arabia. Myrrh and many other species of *Commiphora* are becoming recognized to possess significant medicinal properties. Evidence-based researches have reported that these properties are directly attributable to terpenoids (especially furanoses-quiterpenes), the active compounds present in myrrh essential oil. Very recently, current studies have focused on applying clinical trial methodologies to validate its use as an antineoplastic, an antiparasitic agent, and as an adjunct in healing wounds [16, 17]. It was used to treat skin infections and periodontal abscesses [17]. It has also shown antibacterial and antifungal activity against standard pathogenic strains of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* [18].

Nigella sativa (Black seed) has been used for medicinal purposes for centuries either as an herb or pressed into oil. It contains both fixed and essential oils, proteins, alkaloids and saponin [19]. Much of the biological activity of the seeds is attributed to thymoquinone. It has been reported that the later protects against nephrotoxicity and hepatotoxicity induced by either disease or chemicals [20]. Different crude extracts of *Nigella sativa* have shown effectiveness against multi-antibiotic resistance bacterial isolates [21]. Therefore, the aim of the present study is to evaluate the antibacterial effec-

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tiveness of some natural mixtures of honey, Myrrh and black seed against bacteria isolated from diabetic foot ulcers and wounds.

MATERIALS AND METHODS

Natural Preparations (NPs)

Five NPs were prepared by mixing honey (Acacia honey, produced by Langanese Honig, Bargteheide, Germany) to *Commiphora molmol* and *Nigella sativa* (produced by Al Marjal Al Khalejiyah, Qasim, Saudi Arabia) at different concentrations. The sixth NP was honey alone Table **2**. All NPs were prepared freshly and stored in dark at 4 °C. The amounts used were based on authors' experience and local patients' preferences pattern.

Clinical Microbial Isolates

Tissues and deep wound aspirates were collected from three patients with clinically infected diabetic feet admitted to the King Abdulaziz University Hospital (KAUH). All of three patients were not on antimicrobial drugs for at least 3 days before collecting the specimens. The specimens were cultured in the Clinical Microbiology Laboratory at KAUH. A total of seven microorganisms were isolated from the three patients and identified biochemically.

Bacterial Identification

Three microorganisms were isolated from the first patient which were; *Staphylococcus aureus*, an extended spectrum beta-lactamase (ESBL)-producer *Escherichia coli* and *Proteus mirabilis*. Two bacterial strains were isolated from the second patient, namely *Streptococcus pyogenes* and *Morganella morganii*. Methicillin-resistant *Staphylococcus aureus* (MRSA) strain was isolated from the third patient by performing a susceptibility test to Methicillin.

Broth Dilution Method

For each of the six NPs; four sterile test tubes were labeled as following; 100%, 50%, 25% and 12.5%. Using a sterile pipette, 1 ml of each NP was transferred into the 100% test tube (TT). A total of 1 ml of the nutrient broth was transferred into each of other TTs. Then, 1 ml of the tested NP was transferred into the 50% TT. After enough mixing, a total of 1 ml of the mixture was transferred into the next TT. Same procedures were repeated until the 12.5% TT, from which, after well mixing, a total of 1 ml was discarded.

A microbial suspension of 5-6×10^5 CFU (colony-forming unit) was made for each clinical isolate by transferring 1 ml of its 0.5 McFarland suspension into 9.0 ml of nutrient broth, after enough mixing, a total of 10 µl was inoculated into each TT of the different concentrations. A positive control was made by inoculating 10 µl of the microbial suspension into 1 ml of the nutrient broth. A negative control was made by adding 0.5 ml of the nutrient broth to 0.5 ml of the tested NP. For each TT, after well mixing, sub-culture was done immediately (i.e. at 0 hour time) and after 24 hours incubation (at 35 °C) by taking a loopful of the culture medium and inoculating it into a fresh blood agar plate (BAP). All BAPs were incubated at 35 °C aerobically for 24 hours. The minimum bactericidal concentration (MBC) was read as the least concentration showing no growth (on the sub-cultured BAPs) after 24 hours incubation of the TT. All tests were performed in triplicate and were repeated three times to obtain reliable results.

RESULTS

For the microbial isolates of the first patient; the antibacterial effects on *Staphylococcus aureus* showed that NP4 and NP5 were the most effective as their 25% concentration was enough to kill the microorganism after 24 hours incubation. The other NPs were effective at 50% concentration Table **1**. For the ESBL-producer *Escherichia coli*; all of the six NPs showed the same bactericidal effect at 25% concentration which was enough to kill the microorganism after 24 hours. For *Proteus mirabilis*; NP6 (honey alone) was the most effective as the 25% was enough to kill most of the microorganism with few colonies (1 colony) grown after 24 hours incubation. The 50% concentration of each of the NPs was enough to kill the microorganism after 24 hours incubation.

Table 1. The Antibacterial Effectiveness of Natural Preparations (NPs) Against the Clinical Isolates

Concentrations of NPs Number Of Colonies	100%						50%						25%						12.5%					
	NP1	NP2	NP3	NP4	NP5	NP6	NP1	NP2	NP3	NP4	NP5	NP6	NP1	NP2	NP3	NP4	NP5	NP6	NP1	NP2	NP3	NP4	NP5	NP6
S. aureus	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	>100	64	21	NG	NG	89	>100	>100	>100	>100	80	>100
E. coli ESBL	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	>100	>100	>100	>100	>100	>100
Proteus mirabilis	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	19	13	93	60	84	1	>100	>100	>100	55	92	>100
Strep. pyogenes	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	95	>100	51	13	68	NG	>100
Morg. morganii	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	>100	>100	40	80	NG	>100
MRSA	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	>100	>100	5	>100	>100	>100

NG= No growth.

Table 2. Composition of the Six Natural Preparations

	Honey (g)	*Commiphora molmol*	*Nigella sativa*
NP1	100	½ ts	0
NP2	100	½ ts	½ ts
NP3	100	1.0 ts	½ ts
NP4	100	1.5 ts	½ ts
NP5	100	2 ts	½ ts
NP6	100	0	0

Commiphora molmol: 1 ts = 2.50 g
Nigella sativa; 1 ts = 3.42 g

For the clinical isolates of the second patient; the antibacterial effects on *Streptococcus pyogenes* showed that NP5 was the most effective as the 12.5% concentration was enough to kill the microorganism after 24 hours incubation. All of the other NPs were effective at 25% concentration. For *Morganella morganii*; also NP5 was the most effective as the 12.5% concentration was enough to kill the microorganism after 24 hours incubation. All of the other NPs were effective at 25% concentration.

For the MRSA isolate of the third patient, NP3 was the most effective as the 12.5% concentration was enough to kill most of the microorganism with few (5) colonies grown after 24 hours incubation. The NP5 has shown the higher overall antibacterial effectiveness. All inoculated blood agar plates at 0 hour time were positive.

DISCUSSION

With increasing interest in the use of alternative therapies and as the development of antibiotic-resistant bacteria spreads, honey and medicinal plants may receive renewed recognition as wound healers. Both natural products are individually effective against bacteria, although joint use in wounds has not been reported. Isolation of multi-drug resistant strains is a common problem in hospitals and their current prevalence has led to a re-evaluation of the therapeutic use of ancient remedies, including honey and medicinal plants. Honey offers broad-spectrum antimicrobial properties and promotes rapid wound healing [22], but the mechanisms by which these effects are achieved have not been fully elucidated.

In the present study, honey was the most effective NP against *Proteus mirabilis* as the 25% concentration was enough to kill most of the microorganism with only one colony grown after 24 hours incubation. The 50% concentration of each of the six NPs was enough to kill the microorganism after 24 hours incubation.

The results of this study showed that adding honey to *Commiphora molmol* and *Nigella sativa* increases the antibacterial effect against the clinical isolates. It seems that there is an over-additive action between honey and the tested medicinal plants; this action is also called synergism [23]. The exact mechanism of synergy between medicinal plants and honey is unclear. Clinical trials using a mixture of honey and these plants to treat diabetic wounds could further confirm the findings of this work.

The results also showed that the antibacterial effectiveness of the preparation depends on its amount of *Commiphora molmol*. Therefore, the most effective preparation was NP5 followed by NP4. The 12.5% concentration of NP5 was enough to kill *Streptococcus pyogenes* and *Morganella morganii*. The 25% concentration of NP5 was effective in killing *Staphylococcus aureus*, ESBL-producer *Escherichia coli* and MRSA.

These differences in effectiveness may be due to the composition of each natural preparation and also to the susceptibility of each bacterial isolate. Further research studies could elucidate this statement.

Management of the diabetic wounds/ulcers infection should not be limited to local wounds care but may involve other modalities including systemic antibiotics, debridement and revascularization whenever needed. However the topical care of wounds still remains a matter of debate, and an ideal dressing for these wounds has not yet been discovered. In the developing world a reluctance to stay with simple and still effective methods, treatments, and equipment, in order to 'catch up' with the developed world, comes at a cost. For instance, Postmes and Vandeputte [24] suggested that honey could be used instead of high-tech products such as the new recombinant growth factors. Honey and plant extractions used together have been reported to protect against toxicity [25]. As far as we can tell, no literature refers to the cost-effectiveness of honey and herbal medicines in diabetic wound management. This may receive low priority in modern protocols, but should rate highly in resource-based health delivery. Further research is needed to elucidate and optimize the effective combination of these natural products in clinical practice. Neither of these natural products has an adverse effect on tissues, so they can safely be used on wounds and inserted into cavities to clear infection.

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CONFLICT OF INTEREST

None declared

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