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Research Article

Synergistic effect of mountain honey and *Argemone mexicana* plant on bacteria associated with wound infections

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Abstract: Forty five wound specimens were collected from patients suffering from wound infections and taken from various hospitals in Ibb city, Yemen. The study was to determine synergic antibacterial activity of between mountain honey and *Argemone mexicana* plant. Isolation, identification of bacterial isolates and antibiotic sensitivity test were done. Agar-disc and agar-well diffusion method were carried to determine antibacterial activity of honey, *Argemone mexicana* plant and a mixture of them against bacterial isolates. Out of 45 specimens, 29 (64.4%) gave positive cultures. *Staphylococcus aureus* was the predominant bacterial pathogens with percentage (72.4%) followed by *Pseudomonas aeruginosa* (17.2%) and *Staphylococcus epidermidis* (10.4%). The highest concentration (70%) of honey was more effective than other concentrations and crude milky sap of *A.mexicana* plant had higher antibacterial activity against pathogenic bacteria than any milky sap dilutions. The mixture of honey and *A.mexicana* plant gave an excellent inhibitor effect against bacterial growth. Mixture of honey (70%) and crude milky sap of *A.mexicana* revealed higher antibacterial activity against pathogenic bacteria comparing with honey and plant crude extract alone.
Keywords: *Staphylococcus spp*; *Pseudomonas aeruginosa; Argemone mexicana*; honey; wound infection

INTRODUCTION

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. However, as resistant pathogens develop and spread, the effectiveness of the antibiotics is diminished. This type of bacterial resistance to the antimicrobial agents poses a very serious threat to public health and all kinds of antibiotics, including the major last-resort drugs, as the frequencies of resistance are increased worldwide [1]. The increase in consumer use of complementary medicines has prompted an increasing interest in traditional and nonconventional medical treatments. One treatment that has received much interest is honey. Honey has a long tradition of use within various medical systems and over the past decade several research groups have focused their attention to this product. Whereas honey has a number of uses therapeutically and as a food preservation agent, it is most well known for its beneficial actions within the wound environment. Honey maintains a moist wound environment that promotes healing, and its high viscosity helps to provide a protective barrier to prevent infection. In addition, the mild acidity and low-level hydrogen peroxide release assists both tissue repair and contributes to the antibacterial activity of honey. This antibacterial activity is a major factor in promoting wound healing where infection is present [2]. There are many reports of bactericidal as well as bacteriostatic activity of honey and the antibacterial properties of honey may be particularly useful against bacteria, which have developed resistance to many antibiotics [3].

Medicinal plants are important to the health of many peoples in developing countries. According to World Health Organization (WHO), approximately 80% of people in developing countries still rely on traditional medicine for their primary health care needs. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmological activities, have been extensively investigated as a source of medicinal agents. The plant *Argemone mexicana* belongs to family Papaveraceae is commonly known as Mexican poppy or prickly poppy is used as medicinal herbs [4]. It is a prickly, glabrous, branching herb with yellow juice and showy yellow flowers [5].

The aim of this work included detection of antibacterial activity of honey and *Argemone mexicana* plant against bacteria associated with wound infection, also it focused on the synergic effect between honey and plant extract.

MATERIALS AND METHODS

Bacteriological study

A total of 45 specimens were collected from patients suffering from wound infections and taken from various hospitals in Ibb city, Yemen. By using sterilized cotton swabs, they were placed on blood agar and MacConkey agar, and then incubated aerobically at $37C^{o}$ for 18 hr. For the isolation and identification of isolates, each specimen was identified depending on the morphology, cultural characteristics and biochemical

reaction [6]. The antibiotic susceptibility patterns of all isolated were determined by the modified Kirby-Bauer disk diffusion technique [7]. The diameter of the zone of inhibition produced by each antibiotic disc was measured and recorded, and the isolates were classified as "resistant" or "sensitive" based on the standard interpretative according to CLSI (formerly NCCLS) guidelines.

Evaluation of activity:

Honey:

Mountain honey used in this study was diluted with sterile distilled water with the following concentrations; 10, 20, 40, 50 and 70% (v/v). Agar-Well Diffusion (100µ for each well) was applied to determine the antibacterial activity [8].

***Argemone mexicana* plant**

Milky sap extract was obtained from cutting the stem of the plant and collected in sterile container by using sterile syringe. Agar-Disc Diffusion method and serial twofold dilutions of the crude extract (100 µl per disc) by using sterile distilled water to evaluate the antibacterial activity [9].

Mixture of honey and *A.maxicana* plant

The two methods that mentioned above (Agar-well diffusion and Agar-disc diffusion method) were used to determine antibacterial activity of this mixture. Crude milky sap and (70%) concentration of honey were mixed well in equal volumes, and then the two methods were applied against pathogenic bacteria.

RESULTS and DISCUSSION

Out of A total of 45 wound specimens, 29 specimens (46.4%) yielded positive culture. The bacterial types were distributed into 21 isolates with a ratio (72.4%) belonged to *Staphylococcus aureus*, 5 isolates (17.2%) belonged to *Pseudomonas aeruginosa* and 3 isolates (10.4%) belonged to *Staphylococcus epidermidis*. Exposed subcutaneous tissue provides a favorable medium for microorganisms to contaminate and colonize. The conditions become optimal for microbial growth if the involved tissue is devitalized and/or the host immunity is compromised [10]. Wound infection may be accidental or postoperative and many organisms can cause sepsis. The source of infection may be exogenous (from environment) or endogenous (from commensal of body). *Staphylococcus aureus* is the most frequently isolated wound pathogen [11]. This bacterium possesses large number of toxins and enzymes which contribute to the ability of the organism to overcome the body's defense and to invade survive and produce disease in the host. It's an opportunistic pathogen in that it causes infection most commonly at sites of lowered host resistance, e.g. damaged skin [12].

Staphylococcus aureus and *Pseudomonas aeruginosa* are among the most common organisms isolated from both acute and chronic wounds of various etiologies. Their prevalence has been demonstrated in surgical site infections as well as in the military setting where they have been attributed to causing infections of combat related injuries such as penetrating trauma and burn wounds [13].

The results of antibiotic sensitivity test were shown in Tables (1,2). Multiple antibiotic resistances among bacterial isolates were observed and this may be belonged to the intrinsic or acquired resistance, so antibiotic resistance is becoming an extremely serious public health problem [14], emphasizing the need of using antibacterial agent other than antibiotics.

Table 1: Percentages of resistance and susceptibility of Bacterial isolates for antibiotics

Antibiotic	*Staphylococcus aureus*		*Staphylococcus epidermidis*	
	R%	S%	R%	S%
Ceftriaxone	77.7	22.3	63	37
Cefazolin	40	60	60	40
Sparfloxacin	30	70	53	47
Vancomycin	30	70	56	44
Ciprofloxacin	20	80	60	40
Roxithromyein	60	40	50	50
Cefadroxil	50	50	21	79
Erythromycin	60	40	30	70

Table 2: Percentages of resistance and susceptibility of bacterial isolates for antibiotics.

Antibiotic	*Pseudomonas aeruginosa*	
	R%	S%
Ciprofloxacin	40	60
Cefotaxime	100	0
Gentamycin	40	60
Amikacin	20	80
Vancomycin	80	20
Ofloxacin	40	60

Evaluation of antibacterial activity of honey

The inhibition zone diameters (IZD) of mountain honey concentration (10-80%) were determined for *Staph. aureus*, *Staph. epidermidis* and *P. aeruginosa* by agar-well diffusion test (Figure 1). The highest inhibition zone (20 mm) was recorded against *Staph. aureus* at the concentrations of 70, 50, 40% while the mean of inhibition zone diameters (MIZ) for these concentrations were 13.6, 12.2, 7.9 respectively. The effect of honey was lowered at conc. 20% and absence at 10%. The higher IZD against *Staph. epidermidis* were (20, 20, 15, 8 mm) for conc. 70, 50, 40. 20% and the MIZ were 15.6, 10.6, 8.3 and 6 mm for the above conc.70-20%. The lowest IZD was detected for mountain honey against *P. aeruginosa* (11, 12, 14, 15 mm) at concentration ranging from 20-70%. The highest concentration of honey (70%) was revealed the best inhibition effect on the growth of pathogenic bacteria comparing with the concentrations (20, 40, and 50 %), while the concentration (10%) had no effect on any of the pathogenic bacteria, so if honey is diluted, the acidity of honey may not be an effective inhibitor of many species of bacteria (Table 3). In our study, montain honey was tested for its antimicrobial activity on *Staph. aureus*, *Staph. epidermidis* and *P. aeruginosa*. The present study showed varying degree of in vitro growth inhibition activity of mountain honey against the tested organisms. The two species of *Staphylococcus* were very similar in their susceptibility to honey and more susceptible than *P. aeruginosa*. The antimicrobial role of honey is attributed to its high osmolarity, acidity (low pH) and content of hydrogen peroxide and non-peroxide components, i.e., the presence of phytochemical components like methylglyoxal [15]. Honey is an effective broad-spectrum antibacterial agent that has no adverse effects on wound tissues, therapeutic use of honey is often referred to as "alternative" medicine [16].

Evaluation of antibacterial activity of *Argemone mexicana*

The antibacterial activity of the *Argemone mexicana* plant was studied against the bacterial isolates *Staph. aureus*, *Staph. epidermidis* and *Pseudomonas aeruginosa* using agar disc diffusion method (Figure 2). Table (4) illustrates the broad spectrum antimicrobial results of plant against the clinical isolates. The results confirmed the efficiency of crude milky sap to inhibit growth of pathogenic bacteria and the antibacterial activity of crude milky sap was decreased while the dilution was increased. Higher IZD for *Staph. aureus* were (25, 20, 20, 19, 15, 7 mm) for crude extract and dilutions (1:2, 1:4, 1:8, 1:16, 1:32); while for *Staph. epidermidis were* (25, 20, 20, 17, 8, 7 mm). The antibacterial activity against *P. aeruginosa* was lowered comparing with Staphylococcus species, higher IZD were (15, 10 mm) for the crude extract and dilution 1:2, and the other dilutions had no effect on these isolates. *Argemone mexicana* plant confirmed that it has antibacterial activity against Gram +ve and Gram –ve bacteria. Sanguinarine, the main *Argemone* alkaloid, has a strong bacteriocidal effect on Gram-positive bacteria. The antimicrobial and physiological effects of sanguinarine suggest that it may confer protection against diverse pathogens [17]. *Argemone mexicana* is widely well known around the world for its medicinal property to treat several diseases [18].

Table 3: Effect of honey against bacteria evaluated by determination of inhibition zone diameter.

Honey concentration / Bacterial types	10%	20%	40%	50%	70%
	M I Z mm (IZD)mm	M I Z mm (IZD)mm	M I Z mm (IZD)mm	M I Z mm (IZD)mm	M I Z mm (IZD)mm
Staphylococcus aureus	NE	6(11)	7.9 (20)	12.2(20)	13.6(20)
Staphylococcus epidermidis	NE	6(8)	8.3(15)	10.6(20)	15.6(20)
Pseudomonas eeruginosa	NE	6.2(11)	7.6(12)	9(14)	10.8(15)

M I Z : Mean of inhibition zone diameters. IZD : Inhibition zone diameter., mm : millimeter, NE : Not effective

Table 4: Effect of *Argmone maxicana* plant against bacteria evaluated by determination of inhibition zone diameter.

Milky sap dilution / Bacterial Types	Crude	1:2	1:4	1:8	1:16	1:32
	M I Z mm (IZD)mm	M I Z mm (IZD)mm	M I Z mm (IZD)mm	M I Z mm (IZD)mm	M I Z mm (IZD)mm	M I Z mm (IZD)mm
Staphylococcus aureus	11.5(25)	8.6(20)	7.4(20)	6.4(19)	5.6(15)	5.1(7)
Staphylococcus epidermidis	21.6(25)	18.3(20)	17(20)	10.3(17)	6.6 (8)	5.6(7)
Pseudomonas eeruginosa	9(15)	6.8(10)	NE	NE	NE	NE

M I Z : Mean of inhibition zone diameters. IZD : Inhibition zone diameter., mm : millimeter, NE : Not effective

Table 5: Effect of mixture (honey and *A. maxicana* plant) against bacteria evaluated by determination of inhibition zone diameter.

Bacterial types	Disc method	Hole method
	M I Z mm (IZD)mm	M I Z mm (IZD)mm
Staphylococcus aureus	14.1(25)	11.2(20)
Staphylococcus epidermidis	20.6(25)	11(12)
Pseudomonas aruginosa	12.2(16)	9.8(12)

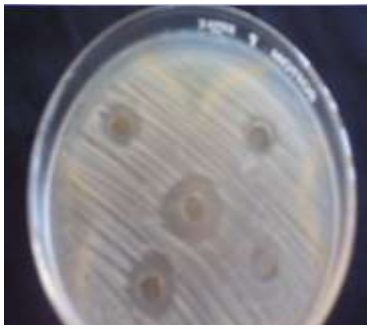

Figure-1 Effect of various concentrations of honey on *Staph. aureus.*

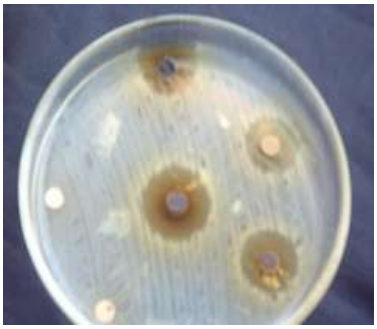

Figure-2 Effect of various dilution of *Argemone maxicana* on *Staph. aureus.*

Figure-3 Effect of mixture of honey and *Argemone maxicana* against *Staph. aureus.*

Synergic effect of Honey and *Argemone mexicana*

Mixture of honey and crude milky sap revealed antibacterial activity against bacterial isolates and the agar-disc diffusion method gave the best results comparing with agar-well diffusion method, (Table 5). The antibacterial activity of mixture of honey and milky sap of *Argemone mexicana* was higher than the activity of honey and milky sap separately (Figure 3). The mean of inhibition zone diameters (MIZ) by using agar-disc method of mixture for the bacterial isolates *Staph. aureus, Staph. epidermidis* and *P. aeroginosa* were (14.1, 20.6, 12.2) mm respectively, while MIZ of the same isolates were (13.6, 15.6, 10.8)mm for honey with conc.70% and (11.5, 21.6, 9) mm for crude milky sap. We observed that there was synergic effect between honey and crude extract of plant according to increase in the diameters of inhibition zone. Agar-disc diffusion method revealed better results than agar-well diffusion method, this may be due to the highest diffusion of the mixture when using disc on the surface of agar comparing with well. In this study, mean of inhibition zones of mixture of mountain honey and *Argemone mexicana* against all tested pathogens was greater than those of the two substances alone. The synergic antimicrobial effect of *Argemone mexicana* extract and honey mixture against all pathogenic bacteria was found to be greater than other antimicrobial agents. That may be the reason why the local society or community widely uses this plant and mountain honey to treat different pathogenic bacterial infections associated with skin. This study was relatively resembled with other researches like [15].

CONCLUSIONS

The results confirmed the efficiency of honey and *Argemone mexicana* to inhibit the growth of pathogenic bacteria. Synergic effect of mixture of honey conc.70% and crude milky sap of *A. mexicana* revealed higher antibacterial activity comparing with honey and *Argemone mexicana* alone.

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