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Honey and medicinal plants in the management of certain surgical bovine clow affections

Abstract

Owing to the worldwide spread of bacterial antimicrobial resistance, it is of great concern to search for antimicrobial agents of natural origin. Since the antimicrobial activity of honey was widely documented, the study aimed to evaluate its use comparing with certain medicinal plants extracts as surgical dressing of bovine clow affections. Aqueous extracts of *Thymus vulgaris* (*T. vulgaris*), *Matricaria chamomilla* (*M. chamomilla*) and *Origanum vulgare* (*O. vulgare*) were prepared to be used *in vitro* and *in vivo* studies in this work. Clinical aerobic or anaerobic bacterial strains were isolated from some clinical surgical bovine clow affections, and were used to determine the minimal inhibitory concentrations (MICs) of the prepared extracts against these pathogens.The study concluded that all tested aerobic bacterial strains were inhibited with 10 % of all tested extracts, while the anaerobic strains were inhibited with 10 % of *T. vulgaris* and 15 % of both*O. vulgare* and *M. chamomilla* extracts. According the achieved MICs values, lotions and ointments of the entended medicinal plants were made to be used during the *in vivo* study as follow: 20 lactating dairy cows suffering from different surgical clow affections were classified to equal four groups (A –D) which were managed with surgical dressing and received: honey (A): *T. vulgaris* (B), *M. chamomilla*: (C)and *O. vulgare*: (D) extracts with their MICs. All cows of groups A & B (no = 10)as well as one cow from group (C) showed complete healing by the day 30 while, none of group (D).The study concluded that with the alternative medical trends, application of honey – as it is – in surgical dressing of bovine digital dermatitis or inter digital necrobacillosis is the most effective valuable economic tool among the four materials studied for its superurity and feasibility followed by the use of *T. vulgaris* extract 10% in lotion and ointment preparation.

Keywords: honey, *thymus vulgaris*, *origanum vulgare*, *matricaria chamomilla*, digital dermatitis and interdigital necrobacillosis

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Introduction

By the increase worldwide spread of multidrug resistant pathogens rather than the public health hazard of antibiotic residues in bovine milk and meat; searching for antimicrobial agents other than antibiotic becomes an issue of great interest. Moreover;the ability of bacteria to develop biofilm–associated drug resistance have further increased the number of life threatening bacterial infections in humans1. Honey has recently received attention as a complementary and alternative treatment in modern medicine.[2] and there is attention its use as a topical therapeutic agent for wound infection and becomes part of conventional medicine for wound care.[3] since; it promotes healing process.[4] it contains antibacterial compounds against multidrug–resistant bacterial infections.[5] rather than; prevention biofilm formation; and decrease production of bacterial virulence factors.[6]

Essential oils (EOs) extracted from medicinal plants are considered attractive natural antimicrobial agents.*T. vulgaris* is a medicinal plant which its EO –with the main component thymol– is active against *Salmonella*; *Staphylococcu.*[7–9] *Streptococcus mutans* and *Lactobacillus* species.[10] *T.vulgaris* essential oils are widely used in food preservation mainly meat industry as its antibacterialagents.[11] *M. chamomilla* flower extract contain potential sources of antimicrobial nano molecules and with antimicrobial activities.[12] mainly; against *S. aureus; E. coli.*[13] and *C. albicans.*[5] and reduces bacterial biofilm accumulation.[14] When; it was used as wound dressing loaded with 15% chamomile extract were remarkably capable to heal (99±0.5%) after 14 days post–treatment periods.[15] *O. vulgaris* EO is effective against *Salmonella enterica*; *S. mutans*; molds and yeasts; and mesophilic aerobic bacteria.[7] *Escherichia coli*; *Clostridium perfringens*; and *Salmonella*.[16] where carvacrol and thymol are the major components responsible for the antimicrobial effects of *O. vulgaris* EO.[17] *O. vulgaris* EO has also antifungal activity with minimal fungicidal concentrations 0.05% v/v.[18] The present work aimed to evaluate the use of honey comparing with some medicinal plant extracts in management of certain bovine clow affections.

Materials and methods

Medicinal plant extracts

*T. vulgaris*leaves**;** *O. vulgare* leaves and *M. chamomilla* flowers let to be dried in open air. Each dried plant was soaked in freshly boiled distilled water for 24 hours to collect plant aqueous extract.[19] Different concentrations (2.5% ، 5% ، 10% ، 15%) of each herbal extract were prepared to be standard extract concentrations(SE conc.) used during the work either *in vitro* or *in vivo* procedures.

Bacterial strains

From clinical bovine clow affections (sole ulcer; interdigital dermatitis; interdigital necrobacillosis and digital dermatitis); clinical bacterial strains were isolated as follows; the clows were washed and cleansed to remove mud and manure using sterile distilled water. Gentle curetting of lesion edges with a disinfected curette; then samples were taken aseptically from the affected areas using sterile bacteriological swabs soaked in sterile modified transport broth. Each sample was divided into two brain heart infusion broth tubes.The first portion was incubated aerobically for 24 h to isolate and identificate aerobic bacterial contents.[20] The second was incubated anaerobically for 48 h. for isolation and identification.[21]

MIC determination

MIC of medicinal plant extracts was done to perform the effective solutions and ointments used during the study.The different obtained SE concentrations were used instead of distalled water to prepare blood agar with 10% of citrated sheep blood.[20] and fusobacterium egg yolk agar plates.[21] The isolated aerobic strains were streaked onto blood agar and incubated at 37 °C for 24 h while the anaerobic isolates were sreaked onto fusobacterium egg yolk agar at 37 °C for 48 h.

Preperation of herbal ointments

applicable ointment was achieved by adding the obtained SE conc. as herbal MIC to meltedpetroleum jelly.[19]

Tested animals

20 lactating dairy Holstein cows in a dairy farm at Assiut government aging 4 – 6 years old weighing about 400 – 450 gk. body weight. Cows were suffering lameness with active lesions (digital dermatitis and interdigital necrobacillosis). Animals were maintained under the same management; housing conditions and were fed the same ration.They were randomly devided to four equal groups of 5 cows (group A; B; C and D); where :

a. was managed with honey as it is.

b. was managed with MIC lotion and ointment of *T. vulgaris*.

c. was managed with MIC lotion and ointment *M. chamomilla*.

d. was managed with MIC lotion and ointment *O. vulgare*.

Cows were managed surgically as: application protocol required to wash and cleansed the lesion with a low–pressure water hose.Group (A) treated by honey; after washing and cleaning the lesion; the honey was applied topically; then protected by means of bandage. For other groups(B; C and D); by using calibrated sprayer bottles for spraying the MIC lotions; then the ointment applied topically and protected by means of bandage.On day (0); prior to any treatment; all cows were evaluated for pain; lameness and lesion dimention scores. All cows were treated once daily for 5 consecutive days; 2 days without management; then treated once daily for other 3 days only up to 14 adys. Cows were manged twice weekly for other two weeks. Cows were re–examined on days 14 and 30 for pain; lameness; and lesion scores.[22]

Results

In vitro study: extracts of *T. vulgaris* leaves (B)**;** *O. vulgare* leaves (C) and *M. chamomilla* (D) flowers containing their active principles were tested against the isolated bacterial strains with concentrations (2.5% ‹ 5% ‹ 10% ‹ 15%). All tested aerobic bacterial strains were inhibited with MICs values 10 % of all extracts; while anaerobic ones were inhibited with 10 % of *T. vulgaris* and 15 % of both *O. vulgare* and *M. chamomilla* (Table 1). About the *in vivo* work: according the obtained tested extract MICs; lotion and ointment of *T. vulgaris* were prepared of 10 %; but for *M. chamomilla* and *O. vulgare* were that of 15 % (Table 1). All cows (no = 20) in the four tested groups –before any interference– showed (severe degree of pain and lameness where the lesion dimensions ø were above 2 cm). Table 2 showed all testing score on day 14 & 30; where by the day 30; all managed cows constituted both groups A & B (no = 10) and only one cow belonged to group C showed complete healing with pain and lameness relieved as well as healthy skin (table 2 and fig 1;2;3); while none of group D cured or healed completely (Table 2 & Figure 4).

Table 1 The minimal inhibitory concentration (MIC) of medicinal plant extracts for the isolated micro organisms from clinical cases

Isolated Micro-Organisms		B	C	D
Aerobic	*Staph aureus*	10 %		
	Strept pyogenes			
	Corynebacterium pyogenes			
Anaerobic	*Fusobacterium necrophorum*	10 %	15 %	
	Bacteroides nodosus			
	Peptostreptococcus anaerobs			
	Clostridium sp.			

B: *T. vulgaris*; C: *M. chamomilla*; D: *O. vulgare* extracts.

Table 2 Different treatment managements with medicinal plant extracts and surgical curing scores along the full experimental course

Day 30				Day 14				Day 0				No. of Cows in Each Group	Testing Score	
D	C	B	A	D	C	B	A	D	C	B	A			
2	1	-	-	2	1	-	-	5	5	5	5	5	Severe	Pain
3	3	-	-	3	4	4	3	-	-	-	-		Mild	
-	1	5	5	-	-	1	2	-	-	-	-		Pain relived	
3	1	-	-	5	-	-	-	5	5	5	5		Initial wound ø was more than 2.5 cm	
2	4	-	-	-	3	4	3	-	-	-	-		= 2.5 cm	Lesion dimensions ø
-	-	5	5	-	2	1	2	-	-	-	-		Final wound ø was (>2.5- 0) cm	
3	-	-	-	-	-	-	-	5	5	5	5		Severe	
2	-	-	-	3	4	4	3	-	-	-	-		Moderate	
-	4	-	-	2	1	1	2	-	-	-	-		Mild	Lameness
-	1	5	5	-	-	-	-	-	-	-	-		Lameness relived	

Figure 1 Planter aspect of right hind limb showing digital dermatitis of medial claw (arrows) before and after honey treatment.

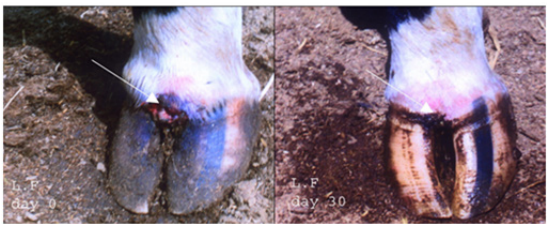

Figure 2 Dorsal aspect of left fore limb showing interdigital necrobacillosis (arrows) before and after *Thymus vulgaris* extract treatment.

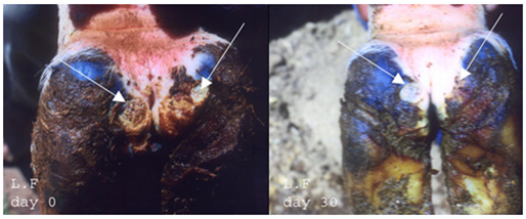

Figure 3 Palmer aspect of left fore limb showing digital dermatitis of medial and lateral claws (arrows) before and after *Matricaria chamomilla* extract treatment.

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Figure 4 Planter aspect of right hind limb showing digital dermatitis of lateral claw (arrows) before and after *Origanum vulgare* extract treatment.

Discussion

Honey dressing is increasingly being used for wound infections with great success because of its multiple benefits over conventional therapy. Thus is due to its antibacterial activity5{either by the high osmotic pressure; low pH acidic environment; low protein content; high carbon to nitrogen ratio; other chemical agents phytochemicals.[23] by the direct action of liberated H2O24; by the synergistic antioxidant compounds flavenoid and polyphenols.[24] or combination of these factors according the floral nectar.[24] Moreover; *in vivo* application it was a favorable for its healing promoting effects.[25] and its immuno–modulating action.[26,27] especially; honey – with proliferation of both B & T lymphocytes – does not help in the growth of yeast and bacteria.[23] rather than its great economic impact. Honey was tested *in vitro* against aerobic bacteria such as; *S. aureus*.[28] MRSA.[29–31] *E. coli*.[32] *S. typhimurium*.[33] *Klebsiella pneumonia*.[34] *Ps. Aerugenosa*35 and anaerobic *Porphyromonas gingivalis*.[36] Also its antifungal activity against *Candida* sp.[37] or *Leptospermum scoparium*.[38] is documented.

According to its *in vivo* studies; honey topical application is recommended in difficult surgical wounds such as burns.[39] chronic leg wounds.[25] venous leg ulcers.[40] or diabetic foot lesions.[41] Carnwath et al (2014) tested *in vitro* the antimicrobial activity of different honey batches against 10 different bacterial species concluding that it may be effective topical treatment up to 16 concentration. Tramuta et al; (2017) recommended manuka honey or honeydew to form honey based membrane as a topical application for wound dressing in veterinary clinical medicine. So; the study aimed to evaluate its use comparing with certain medicinal plants extracts as surgical dressing of bovine clow affections judging by the clinical parametrs (pain testing; lamness and wound contractin dimentions).

In the present study; with the terminal end of the experiment (day 30); both honey and *T. vulgaris* resulted in complete healing process. But honey use was more preferable as cows treated with honey relieved the initial testing scores by the day 14 earlier than those treated with *T. vulgaris*. So; honey is recommended for its superiority as well as its use feasibility since it is used as it is in the dressing instead of lotion and ointment. Honey through different studies has almost equal or slightly superior effects when compared with conventional treatments for acute wounds.[34] especially pan– or multidrug–resistant bacterial infections.[5] Consequently; honey is getting worldwide attention as a topical therapeutic agent for wound infection and potential future candidate for systemic infections.[42]

Thyme essential oil has a significant bacteriostatic activity against the microorganisms9;10 which is more pronounced against the Gram's +ve bacteria.[12] so it is widely used in food preservation mainly meat industry as its antibacterial agents.[11] *M. chamomilla* flower extract containing potential sources of antimicrobial nano molecules.[43] and reduces biofilm accumulation with 1 %.[14] *M. chamomilla* antimicrobial activity is conflicting issue since; good activity was recorded through wound dressing samples loaded with 15% extract were remarkably capable to heal the wounds up to 99 ± 0.5% after 14 days post–treatment periods.[15] On the other hand; during a study.[44] the authors examined the antibacterial effect of several medicinal plants E. oils against 3 Gram's +ve and 3 Gram's –ve concluded no bacterial inhibition activity for *M. chamomilla* against all tested bacterial species. Another study stated that among 7 tested medicinal plant E. oils; *M. chamomilla* showed the least antibacterial effect against the tested bacterial sp.[44] Although; *O. vulgaris* extract exhibit good bactericidal; antibiofilm activity.[17] and rich in small terpenoids and phenolic compounds; which are known to have antimicrobial activities.[7] rather than another study.[18] estimated their antifungal activity in very low concentrations (less than 0.05 %); it resulted delayed healing and persistence of testing scores (pain; lameness and wide wound) in all cows of group (D) during the present study with the least positive effects to be unfavorable not recommendable treatment.

Conclusion

The study concluded that with the alternative medical trends; application of honey – as it is – in surgical dressing of bovine digital dermatitis or interdigital necrobacillosis is the most effective valuable economic tool among the four materials studied for its superiority and feasibility followed by the use of *T. vulgaris* extract 10% in lotion and ointment preparation.

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None.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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