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Research article

In vitro and *In vivo* antimicrobial effect of coconut oil against Methicillin Resistant *Staphylococcus aureus* from wound infections

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Abstract

Methicillin resistant Staphylococcus aureus (MRSA) is associated with a significant rate of skin and other systemic infections throughout the world in both human and animals. This study was conducted to evaluate the in vitro and in vivo antibacterial activity of commercial coconut oil against MRSA. Clinical isolates of MRSA were obtained from AL- sadder Teaching hospital /Basra / Iraq and were identified and confirmed by standard methods. The in vitro antibacterial activity of coconut oil was studied by disc diffusion method which has showed strong suppressive activity on MRSA. Full strength coconut oil exhibited better zone of inhibition around MRSA in comparison to diluted preparations. For in vivo activity, 24 adult rabbits were allocated to test MRSA induced skin infection. Four sets of experimental animals each consisting of 6 rabbits were grouped. Multiple regions in each animal in all groups were inoculated with 0.1 ml of MSRA at a concentration of 1.5 x10^8 cfu/ml. Groups 1, 2, and 3 were additionally treated through injection in the areas of bacterial inoculation with coconut oil, vancomycin antibiotic, and normal saline respectively. Group 4 was left without any additional treatment. Viable bacterial count in the tested skin was measured at two occasions in all animals (24 and 48 hours from the experimental period). Compared to the first 24 hours, the results after 48 hours showed a significant reduction in the viable bacterial count following coconut and vancomycin treatment in comparison to those rabbits treated with normal saline or not treated at all (P-value < 0.05).Coconut oil treatment produced $1\frac{1}{2}$ *folds reduction in the viable bacterial count with mild visible skin reaction which was comparable to the effect of vancomycin.The current study concluded that the in vivo and in vitro results show that concentrated coconut oil is active against MRSA, making it a possible alternative to some of antimicrobial agents to which these bacteria are resistant.*

Keywords: Coconut oil, *Staphylococcus aureus*, MRSA, Rabbits

Introduction

Wound infection in humans represents a potentially serious medical problem that can result in a variety of complications such as bacteremia and remote infection in other systems of the body. *Staphylococcus aureus* is known to be the causative bacteria in the majority of skin infection[1, 2].In addition to skin infection and abscess formation, *Staphylococcus aureus* is the cause of a variety of other diseases such as pneumonia, bone and joint infections, endocarditis and bacteremia[3,4]. During recent years, the frequent use of antibiotics worldwide has resulted in the emergence of more and more bacteria that showed resistant to the currently used antibiotics and chemotherapeutic agents. Methicillin resistant *Staphylococcus aureus* (MRSA) bacteria is a typical example of antibiotic resistant bacteria that impose considerable health challenges in human communities as well as animals including, particularly rabbits[5-8]. The resistance of MRSA to different antibiotics and other therapeutic agents is common such as their

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resistant to the entire class of beta lactam antimicrobial agents in human5. Because of the rapid evolution of multi-drugs resistance of MRSA together with the continuous change in the pattern of MRSA resistance, the need to search for a different new antimicrobial agent becomes indispensable[9]. A wide range of medicinal plant therapies had evolved over the years to treat a variety of health problems in human. The essential oils contained in natural plants remedies have high volatility and lipophilicity that allow them to pass through the membranes of bacterial cells and thus exert their biological effects[10]. Coconut oil is a fatty oil that can be obtained from the white core of the coconut (Cocos nucifera Linn.). Coconut oil, in particular, has been utilized to treat dermal infections due to its antibacterial, antifungal and antiviral capabilities[11-13].The purpose of this study is to explore the in vitro and in vivo effects of crude coconut oil against MRSA wound infection in experimental rabbits.

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Basrah, Iraq, has approved the present study.

Bacterial isolates from wound infection patients admitted to AL Sadder Teaching hospital /Basra -Iraq were obtained. Microbiological identification of these Gram positive and Gram-negative bacterial strains was performed in the microbiology department using standard methods and Vitac. The following bacterial species were selected for the study Methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.Following laboratory cultivation, few single pure colonies of the tested bacteria were separately transferred to sterile normal saline containing test tubes. The turbidity of bacterial suspension in each tube was adjusted to $1.5x10^8$ cfu /ml as compared to McFarland turbidity standard.

Plant oil

Pure commercial crude plant coconut oil was obtained from the local market in Amman, Jordan. Hexane 4o% (w/v) was used as a diluent solution to obtain concentrations of 25%, 50% and 75% of coconut oil as described by Al-Shamma et al[14].

Detection of antibacterial activity of plant coconut oil

Well diffusion method was utilized to detect the inhibitory activity of the crude plant coconut oil. From each of the already prepared bacterial suspension, bacteria were streaked individually over different Muller Hinton agar plates using sterile cotton swabs. Two wells of 6 mm diameter were made on the surface of each agar plate that harbored a single species of the bacteria used in this study. One well was filled with 50 microliters of crude coconut boil, and the second well with 40% hexane solution to act as a control. All plates were inoculated aerobically for 24 hrs. at 37℃. The diameters of inhibition zones around wells were then measured. The same procedure was repeated once more but with the use of 25%, 50% and 75% coconut oil concentrations for those bacterial strains that were inhibited by the crude form of the oil. Tests were performed in triplicate (Valgas et al[15] 2007).

Experimental animals

MRSA was selected for in vivo testing since it had shown the maximum in vitro inhibition by the crude coconut oil. The in vivo antimicrobial activities of coconut oil against MRSA was evaluated by using 24 mature rabbits weighing from 2000-2500 gram. All rabbits were purchased from the local market.The inoculated areas (abdomen and chest) of the tested rabbits were prepared according to the Abu-Al-Basalc[16] method with modification. The process of preparing the inoculated area was proceeded several days before bacterial inoculation. The hair in the inoculated area was initially shortened using a scissor, and then totally removed by

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a depilatory lotion.The experimental rabbits were randomly separated into four groups numbered 1, 2, 3, and 4, each consisting of six rabbits. Seventy per cent Ethanol (Disinfectol®, 102 Chem-Lab NV, Zedelgem, Belgium) was used to disinfect the inoculation areas of each tested rabbits. Following evaporation of ethanol (about five minutes), several areas in each rabbit were inoculated subcutaneously using a tuberculin syringe and a 22 gauge needle with 0.1 ml of MRSA at a concentration of 1.5×10^8 cfu /ml. In addition to MRSA inoculation, same areas of rabbits in group 1 were injected with crude coconut oil, group 2 with vancomycin and group 3 with normal saline only. Group four was left without any additional treatment to MRSA inoculation in order to consider it as a control group.

Bacterial counts

After 24 and 48 hours of bacterial inoculation successively, three rabbits from each group were sacrificed in order to quantify the viable bacterial count in the infected area. The inoculated site was initially disinfected with 70% ethanol before skin and underlying tissue removed and then homogenized in 2ml of normal saline. The process of quantification of the bacterial count was conducted by plating the samples after dilution with nutrient agar in a proportion of 1:10 respectively. The agar plates were incubated at 37 C for 24 hrs., after which bacterial count was estimated depending on the number of colony-forming unit per gram of tissue which was expressed as (cfu g^{-1})[17,18].

Statistical analysis

Data analysis were performed by the statistical software package (SPSS) version 26.0. Differences were considered significant if the P-value is less than 0.05.

Results

Crude coconut oil showed an in-vitro efficient inhibitory effect against Methicillin resistant *Staphylococcus aureus* -MRSA- and to a lesser extent against *Streptococcus faecalis*, but not against the Gram-negative *Escherichia coli*, Pseudomonas *aeruginosa* and *Proteus vulgaris* as shown in table 1.

Table (1): The in vitro sensitivity of tested bacteria to crude coconut oil

Type of bacteria	Sensitivity
MRSA	Sensitivity
Streptococcus faecalis	Sensitive
Escherichia coli	Resistant
Pseudomonas aeruginosa	Resistant
Proteus vulgaris	Resistant

The inhibition zone reported around MRSA with the crude form of coconut oil was 13 mm compared to 4 mm in case of *Streptococcus faecalis* as shown in table 2.

Table (2): The in vitro inhibitory zones of tested bacteria to crude coconut oil

Type of bacteria	Inhibition zone (mm)
MRSA	13
Streptococcus faecalis	4
Escherichia coli	0
Pseudomonas aeruginosa	0
Proteus vulgaris	0

Serial dilution of the crude oil resulted in proportional decrease in its Inhibition zone and hence on its inhibitory effect against MRSA as well as streptococcus *faecalis* as shown in table 3.

Table(3): Effect of serial dilution of coconut oil on MRSA inhibition zone

Oil concentration (%)	Inhibition zone (mm)	
	MRSA	***Streptococcus faecalis***
25	4	0
50	7	1
75	9	2
100	13	4

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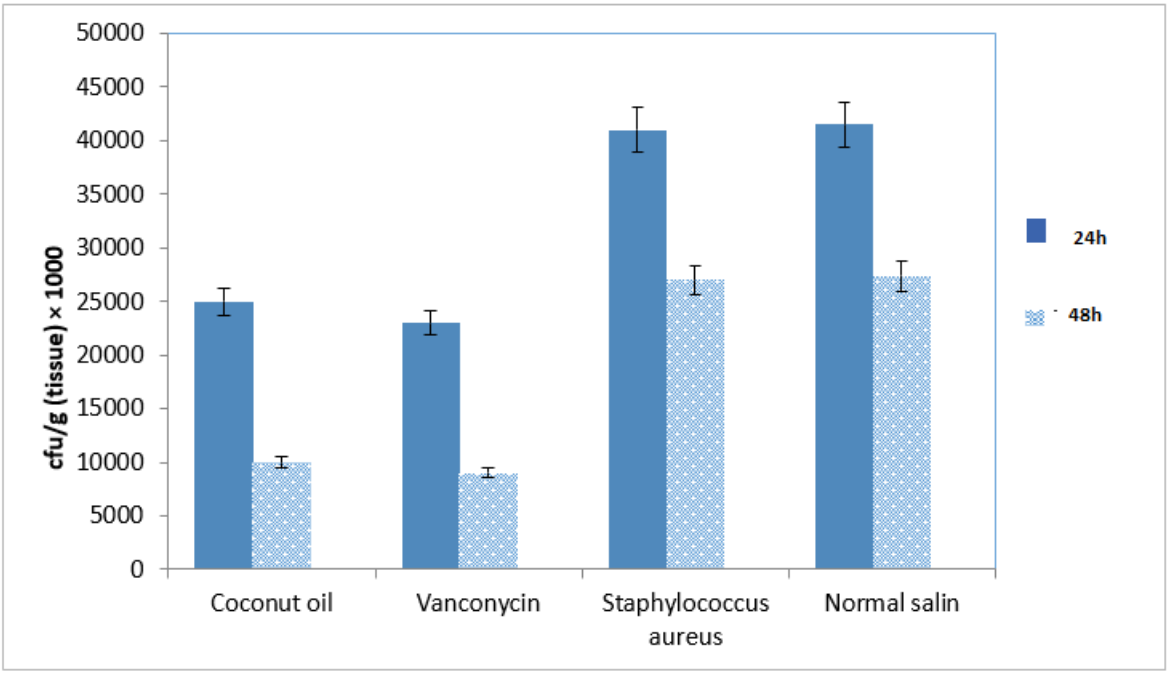
In *vivo* anti-microbial effect

The count of viable bacteria at the inoculated site showed a decline in their number after the use of coconut oil at 25×10^6 and 10×10^6 cfu in 24 hours and 48 hours of inoculation respectively. Vancomycin exhibited a slightly stronger antimicrobial effect compared to coconut oil in which the number of counted viable bacteria reduced to 23×10^6 and 9×10^6 cfu at 24 hours and 48 hours of infection respectively (figure 1).

Figur(1): Count of Methicillin Resistant *Staphylococcus aureus* at the rabbit infected site after 24h and 48h of infection and treatment



The viable bacterial count of *S. aureus* in the skin of infected and treated rabbits with coconut oil, vancomycin treated rabbits, infected untreated rabbits and saline treated rabbits after 24h and 48h of infection and treatment subcutaneously. The data were expressed as means ± SEM in each group, ($P < 0.05$) was a significant difference between coconut oil treated group, vancomycin treated group and untreated group and normal saline treated group.

The bacterial count in the skin of rabbit injected with normal saline was similar to the untreated groups (control group) in which there is no significant difference between them (P: 0.05). Moreover, rabbits in group three and four (normal saline and untreated rabbits) showed a higher concentration of bacteria compared to other groups of rabbit injected with coconut oil and vancomycin. This difference was found to be statistically significant with a P-value of 0.05. This indicates that the coconut oil has antimicrobial effect against methicillin resistant *staphylococcus aureus.*

Skin lesion description

The skin abscesses were observed in all groups of rabbit injected by MRSA after 24 and 48 hours. The visible skin reaction in the group of rabbit injected with coconut oil was found to be less severe compared to the reaction that observed in the skin of the third (normal saline) and the untreated groups of the rabbit. This is evidence that coconut oil had a powerful antimicrobial effect against the inoculated MRSA.

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Discussion

The use of natural plant products to serve as an alternative antibacterial and therapeutic agent is continuously in progress throughout the world. In the case of MRSA, natural plant products are used to control antimicrobial resistance and persistence of these bacteria. This may resolve the problem of failure of different antibiotics to fight bacterial infection[19]. Another advantage of using natural products is the avoidance of toxic effects of many currently used synthetic antibiotics[20]. In the present study, the antibacterial activity of crude commercially available coconut oil sold for edible purposes was tested both in the laboratory and in vivo by inoculation of MRSA into the skin of rabbit.Several studies[21-24] reported that the chemical properties and fatty acid composition of industrialized coconut oil, particularly lauric acid content, shows no significant difference from that of the virgin coconut oil and are according to standard international food protocol (Codex alimentarius).In this study, the in vitro suppressive effect of coconut oil was evident only on Gram positive bacteria; MRSA and to a lesser extent on *Streptococcus faecalis* but not on Gram negative bacteria; *Escherichia coli, Pseudomonas aeruginosa* and *Proteus vulgaris*.To a large extent, Gram negative bacteria show more resistance to plant essential oils compared to Gram positive bacteria[25]. This can be attributed to the difference in the structure and composition of their cell walls. Unlike Gram positive bacteria, the cell wall of Gram-negative bacteria is more complex forming a barrier for plant oil molecules to penetrate through and so preventing the active ingredients of the oils from acting on the bacterial cell wall itself or within the cytoplasm[26].Since MRSA was found in the current study to be the bacteria most intensely inhibited by coconut oil, these bacteria are subjected to in vitro testing through inoculation of 0.ml of bacterial suspension into the skin of different groups of rabbits together with supplemental injection to the same areas of coconut oil in group 1, vancomycin as a positive control in group 2, normal saline as a negative control in group 3, and no additional injection in group 4. The whole oil with full concentration was utilized in the animal testing because with serial dilution, it was found that its antimicrobial activity against MRSA dropped progressively as evident by a decline in the inhibition zone on tested plates with each fractional dilution. This might be attributed to a reduction in the amount of lauric acid, the active ingredient of coconut oil that accompanies serial dilution.Several properties of coconut oil are attributed to the properties of the fatty acid, lauric acid it contains. It is well known that lauric acid accounts for about half of the fatty acids in coconut oil[27]. Several studies have reported the antimicrobial activity of lauric acid both in vitro and in vivo. These studies have shown that lauric acid is very active against several Gram-positive bacteria and some viruses and fungi [28-32]. The antimicrobial activity of lauric acid has been attributed to its ability to disrupt microbial cell wall as well as impeding microbial cell singling and transcription. Due to such multiple modes of action, lauric acid compounds are unique in that development of bacterial resistance to their action is unlikely[33].The two parameters used in this study to determine the suppressive action of coconut oil on MRSA in rabbit were the degree of the local inflammatory reaction, and viable bacterial count at the inoculated skin site after 24 and 48 hours. For animals inoculated with MRSA alone or MRSA and normal saline, moderate or severe degrees of local skin swelling and inflammation in contrast to only a mild degree of skin lesion in case of simultaneous inoculation of both MRSA and coconut oil or MRSA and vancomycin. This finding reflected the therapeutic ability of coconut oil to combat infection triggered by staphylococci. After injection of coconut oil,

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the decline in the viable bacterial count of about $1\frac{1}{2}$ folds after 48 hours) 10×10^6 cfu/g at 48 hours versus 25×10^6 cfu/g at 24 hours) was comparable to vancomycin effect that reported a reduction of bacterial count to 9×10^6 cfu /g at 48 hours from 23×10^6 cfu/g at 24 hours post inoculation. This is the other measurable evidence of the antibacterial activity of coconut oil against MRSA bacteria. The ability of the coconut oil to inhibit the growth of MRSA both in vivo and in vitro in this experiment indicates the presence of an active antibacterial agent in this oil. The mechanisms by which lauric acid specifically disturb *Staphylococcus aureus* metabolisms were studied by several authors[34,35]. Moreover, several in vivo and in vitro studies reported the suppressive activity of lauric acid as such against both methicillin sensitive and methicillin resistant *Staphylococcus aureus*[36,37]. Modification of virgin coconut oil through enzymatic hydrolysis[38], lauric acid monoester formulation[39], or combination with other antimicrobials[40] were other options utilized by researchers to maximize the inhibitory action against *Staphylococcus aureus* bacteria.In conclusion, our in vitro and in vivo studies show that coconut oil in full concentration is active against MRSA making it a possible alternative to some of the antimicrobial agents to which these bacteria are resistant. The viable bacterial counts found in rabbit skin treated with coconut oil was comparable to vancomycin and are significantly lower than those treated with normal saline or not treated at all (P-value < 0.05).

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