

Traditional Treatment by Arabic Acacia Seeds Exterminating Microbial Biofilms for Wounds Healing

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Abstract

Microbial biofilms exist in wounds resulting in long-term non-healing infected wounds. Herbal products as ancient traditions treatment and scientists in developing countries using in wounds healing over years. Arabic Acacia present in KSA, it's using in production of some medications. It has gallic-acid, methyl-gallate, quercetin, rutin, myricetin 3-O-(3"-O-galloyl), β -D-rutinoside and catechin. It has a significant antimicrobial agent. Its extract provided fast wounds healing related to antimicrobial activities. The aim was to prove "Traditional Treatment" by Arabic Acacia Seeds exterminate microbial biofilms for wounds healing. Materials and methods were included Arabic Acacia aqueous extract, biofilm isolates included (Staphylococcus sp., Streptococcus sp., Pseudomonas sp., Proteus sp., Escherichia coli, also Klebsiella sp., and Candida sp.) The applied tests were anti-biofilms formation and biofilms elimination, then biofilm estimation by Tissue Culture Plate method (TCP). Results were for estimation of anti-biofilms formation after exposed to Arabic Acacia aqueous extract. It was eliminated by simple Arabic Acacia aqueous extract early by crude dilution in overnight time were Streptococcus sp., and Proteus sp. Then by crude dilution in day time were Escherichia coli, also Klebsiella sp. Staphylococcus sp., Pseudomonas sp., and Candida sp., they had reached the weak point only. Estimation of biofilms elimination after exposed to Arabic Acacia aqueous extract. The effects of all dilutions were faster than anti-biofilms formation. This was giving none for Streptococcus sp., and Proteus sp., by half dilution for overnight. While Streptococcus sp., Proteus sp., Escherichia coli also Klebsiella sp. were resulted none by half dilution for a day. Crude dilution gave none for all biofilms elimination in both times. It was concluded that the Arabic Acacia aqueous extract had microbial anti-biofilms formation. That was by their antimicrobial contents exterminating microbial biofilms for wounds healing. This indicated the using from ancient Arab customs to treat wounds as traditional treatment. It was recommended that for the "Traditional Treatment" of Arabic Acacia extract according healthy doses as simple treatment instead of chemical treatment for wounds healing.

Keywords: microbial biofilms; wounds healing; arabic acacia; antimicrobial activities; "traditional treatment"; aqueous extract

Introduction

Treatment of infection accelerates wounds healing because microbial infection harms the wounds healing and cause delaying [1]. Microbial biofilms exist in wounds had numerous survivals making extremely difficult to eliminate and resulting in long-term non-healing infected wounds [2]. Biofilm creation and exopoly-saccharide (EPS) donate to antimicrobials resistance [3]. Herbal products as ancient traditions and scientists in developing countries using in wounds healing over years. The phyto-medicines for wounds healing are cheap, and purportedly safe [4]. Arabic Acacia present in KSA, it using in production of some medicines. It contains gallic-acid, methyl-gallate, rutin, myricetin, quercetin, myricetin 3-O-(3"-O-galloyl), β -D-

rutinoside and catechin have antimicrobial action [5]. Several species of Acacia have been proven a significant antimicrobials agent [6]. Arabic Acacia using in treat skin deep cuts, foot ulcers or open wounds for traditional in ancient times. It is known that foot ulcers and other wounds are healed by Arabic Acacia powder. According to traditional medical uses that using for treatment skin or topical wounds [7]. Arabic Acacia extract had antimicrobial activity [8]. Arabic Acacia extract provided fast wounds healing, also its powder had action in wounds healing due to antimicrobial activities [9]. Arabic Acacia extract used antimicrobial and wounds healing in albino rats. That was estimated by Pseudomonas aeruginosa living cell. The polyethylene glycol cream had 3% Acacia had antimicrobial

and wounds healing action [5]. Arabic Acacia extract reduced biofilms activity of *Escherichia coli*, *Klebsiella pneumoniae*, *Porteus mirabilis*, also *Pseudomonas aeruginosa* were (62.6, 59.03, 48.9 and 39.2%). The isolates were killed by the Arabic Acacia extract as bactericidal effect over time of (20-24 hours). The Arabic Acacia extract contains high percentage of 3-Cyclohexane-1-Carboxaldehyde, 2,6,6-trimethyl (23.5%); α -Selinene, (15.12%); Oleic Acid (14.52%); Globulol (11.35%). The challenge to improve the production of Arabic Acacia phyto-chemicals considered a very little price [10]. The target was to prove "Traditional Treatment" by Arabic Acacia seeds extract for exterminating microbial biofilms to accelerate the wounds healing.

Materials and Methods

Aqueous Extract: Arabic Acacia seeds was got from native souk. Ranyah, KSA, it was crumpled in sterile pounder mortal. The powder 30 gm were added on 300 ml sterile distill water This was heated moderate (at which degree for how long) in water bath till to less than quarter of its unique volume (should justifiedml from.....ml). The subsequent liquid was filtered, then aqueous extract dilutions were done in (quarter, half and crude) by distilled water [11].

Biofilm Isolates: They were collected from "Private Microbiology Lab.", they were isolated from wounds, included (*Staphylococcus* sp., *Streptococcus* sp., *Pseudomonas* sp., *Proteus* sp., *Escherichia coli*, *Klebsiella* sp., and *Candida* sp.). They were cultured on Mueller-Hinton Agar (Oxoid, Basingstoke, UK) at 37°C for 24 hours. Then they were sub cultured in Peptone Water (Oxoid, Basingstoke, UK). The isolates suspension matched to "McFarland Turbidity Standard" by (0.5 = 106 CFU / mL) [12].

Applied Tests: Anti-biofilms formation: The Microtiter Plate (Falcon, NC, USA) was used. The bacterial isolate suspension 100 μ l were added then aqueous extract dilution 100 μ l in Peptone Water (Oxoid, Basingstoke, UK). Then was incubated for (overnight, and day); (for 18 and 24 hours) at 35 °C [13]. Biofilm's elimination: The Microtiter Plate (Falcon, NC, USA) was used. The biofilm was performed by add 100 μ l isolate suspension in early log phase in Peptone Water (Oxoid, Basingstoke, UK). Then was incubated for 24 hours at 35 °C. The plate was washed 3 times with sterile phosphate buffer saline (PBS pH 7.2). The aqueous extract dilution 100 μ l were added on the preformed biofilm in fresh Peptone Water (Oxoid, Basingstoke,

UK). Then was incubated for (overnight, and day) at 35 °C [14]. Biofilm estimation: Tissue Culture Plate method (TCP) was used. The biofilm remained in plate was fixed by sodium acetate (2%) for 30 minutes and was stained by crystal violet (0.1% w/v) for 30 minutes. Then was washed by deionized water and was reserved for drying. The estimation was by Optical densities (OD) by Micro Enzyme-Linked Immunosorbent Assay auto reader; (OD, wavelength 570 nm) [15].

Data Analysis: The outcomes were conserved by Excel (IBM, NY, USA) [16].

Results and Discussions

Estimation of anti-biofilms formation after exposed to Arabic Acacia aqueous extract: Table 1 existing the effect of the quarter dilution began to become apparent for a daytime. That were affected on *Streptococcus* sp. (0.201 \pm 0.003=Moderate), *Proteus* sp. (0.201 \pm 0.001=Moderate), and *Escherichia coli* (0.221 \pm 0.003=Moderate). This dilution needs long time for anti-biofilms formation effect.

The half dilution affected the biofilm formation during the overnight as moderate. That were for *Staphylococcus* sp. (0.195 \pm 0.003=Moderate), *Streptococcus* sp. (0.182 \pm 0.002=Moderate), *Pseudomonas* sp. (0.231 \pm 0.003 =Moderate), *Proteus* sp. (0.196 \pm 0.002=Moderate), *Escherichia coli* (0.220 \pm 0.002=Moderate), and *Klebsiella* sp. (0.201 \pm 0.001=Moderate). Also, the day time had more impact, as it led to the average for (moderate and weak). They were included effects on *Staphylococcus* sp. (0.156 \pm 0.002=Moderate), *Pseudomonas* sp. (0.188 \pm 0.002=Moderate), *Escherichia coli* (0.170 \pm 0.002=Moderate), and *Candida* sp. (0.254 \pm 0.002=Moderate). Also had weak effect were on *Streptococcus* sp. (0.123 \pm 0.001=Weak), *Proteus* sp. (0.138 \pm 0.002=Weak), and *Klebsiella* sp. (0.136 \pm 0.002=Weak). These dilutions had an effect so clear over anti-biofilms formation but did not lead to complete exterminate microbial biofilms. They need longer time to complete exterminate microbial biofilms [4-10].

The crude dilution had a higher impact, as for overnight time the moderate were for *Staphylococcus* sp. (0.199 \pm 0.001=Moderate), *Pseudomonas* sp. (0.187 \pm 0.003=Moderate), and *Candida* sp. (0.222 \pm 0.004=Moderate). The weak were for *Escherichia coli* (0.144 \pm 0.002=Weak), and *Klebsiella* sp. (0.146 \pm 0.002=Weak). The none were for

Streptococcus sp. ($0.098 \pm 0.002 = \text{None}$), and Proteus sp. ($0.099 \pm 0.001 = \text{None}$). The overnight was led to complete exterminate microbial biofilms for Streptococcus sp., and Proteus sp. that indicator for stronger effect on anti-biofilms formation. As for exposure for a day, it was the strongest in affecting the anti-biofilms formation. The weak were for Staphylococcus sp. ($0.112 \pm 0.004 = \text{Weak}$), Pseudomonas sp. ($0.124 \pm 0.002 = \text{Weak}$), and Candida sp. ($0.147 \pm 0.001 = \text{Weak}$). While none were on Streptococcus sp. ($0.038 \pm 0.002 = \text{None}$), Proteus sp. ($0.032 \pm 0.004 = \text{None}$), Escherichia coli ($0.099 \pm 0.003 = \text{None}$), and Klebsiella sp. ($0.082 \pm 0.002 = \text{None}$). So, this dilution was complete exterminate microbial biofilms in a day time [4-10].

It was clear that the focus had the most effect through the overnight and day, as nearby of them were totally eliminated during the overnight and the rest were eliminated for a day. As for the ideas, they had reached the weak point, which was a reasonable effect, as they need a longer period to be complete exterminate microbial biofilms. It was eliminated by simple Arabic Acacia aqueous extract early by crude dilution in overnight time were Streptococcus sp., and Proteus sp. Then by crude dilution in day time were Escherichia

coli, and Klebsiella sp. On the other side were included Staphylococcus sp., Pseudomonas sp., also Candida sp., they had reached the weak point, they need a longer period to be complete exterminate microbial biofilms [4-10].

The different effect of Arabic Acacia aqueous extract on anti-biofilms formation was found due to the characteristics of biofilm isolates and the presence of resistance genes that preserve them for a long time from Arabic Acacia aqueous extract. This indicated the using is very simple in its preparation and one of the materials that are used from ancient Arab customs to treat wounds as traditional treatment. It may show that biofilm isolates capable of making biofilm reduce the wound healing process. When the biofilm isolates were eliminated by simple Arabic Acacia aqueous extract, it was the main reason for the rapid wounds healing. This method was unique of "Traditional Treatment" for Arab region habitat. This proved the content degree of simple Arabic Acacia aqueous extract that stop the growth of biofilm isolates and thus accelerated the wounds healing process quickly and sterilely by the content of this simple Arabic Acacia aqueous extract. These dilutions antimicrobial contents exterminating microbial biofilms for wounds healing [4-10].

Table 1: Estimation of anti-biofilms formation after exposed to Arabic Acacia aqueous extract.

Item	Arabic Acacia aqueous extract					
	Quarter		Half		Crude	
Dilution	Overnight	Day	Overnight	Day	Overnight	Day
Time	Overnight	Day	Overnight	Day	Overnight	Day
Biofilm isolates	Mean optical densities value = Mean biofilm degree					
Staphylococcus sp.	$0.288 \pm 0.002 = \text{High}$	$0.253 \pm 0.001 = \text{High}$	$0.195 \pm 0.003 = \text{Moderate}$	$0.156 \pm 0.002 = \text{Moderate}$	$0.199 \pm 0.001 = \text{Moderate}$	$0.112 \pm 0.004 = \text{Weak}$
Streptococcus sp.	$0.283 \pm 0.001 = \text{High}$	$0.201 \pm 0.003 = \text{Moderate}$	$0.182 \pm 0.002 = \text{Moderate}$	$0.123 \pm 0.001 = \text{Weak}$	$0.098 \pm 0.002 = \text{None}$	$0.038 \pm 0.002 = \text{None}$
Pseudomonas sp.	$0.299 \pm 0.003 = \text{High}$	$0.256 \pm 0.002 = \text{High}$	$0.231 \pm 0.003 = \text{Moderate}$	$0.188 \pm 0.002 = \text{Moderate}$	$0.187 \pm 0.003 = \text{Moderate}$	$0.124 \pm 0.002 = \text{Weak}$
Proteus sp.	$0.275 \pm 0.001 = \text{High}$	$0.201 \pm 0.001 = \text{Moderate}$	$0.196 \pm 0.002 = \text{Moderate}$	$0.138 \pm 0.002 = \text{Weak}$	$0.099 \pm 0.001 = \text{None}$	$0.032 \pm 0.004 = \text{None}$
Escherichia coli	$0.289 \pm 0.003 = \text{High}$	$0.221 \pm 0.003 = \text{Moderate}$	$0.220 \pm 0.002 = \text{Moderate}$	$0.170 \pm 0.002 = \text{Moderate}$	$0.144 \pm 0.002 = \text{Weak}$	$0.099 \pm 0.003 = \text{None}$
Klebsiella sp.	$0.285 \pm 0.001 = \text{High}$	$0.251 \pm 0.001 = \text{High}$	$0.201 \pm 0.001 = \text{Moderate}$	$0.136 \pm 0.002 = \text{Weak}$	$0.146 \pm 0.002 = \text{Weak}$	$0.082 \pm 0.002 = \text{None}$
Candida sp.	$0.305 \pm 0.003 = \text{High}$	$0.272 \pm 0.002 = \text{High}$	$0.288 \pm 0.002 = \text{High}$	$0.254 \pm 0.002 = \text{Moderate}$	$0.222 \pm 0.004 = \text{Moderate}$	$0.147 \pm 0.001 = \text{Weak}$

Estimation of biofilms elimination after exposed to Arabic Acacia aqueous extract: Table 2 existing all dilutions were faster than anti-biofilms formation. The biofilms elimination was giving none for Streptococcus sp., and Proteus sp., by half dilution for overnight. While Streptococcus sp., Proteus sp., Escherichia coli

and Klebsiella sp. were resulted none by half dilution for a day. Over that the crude dilution give none for all biofilm's elimination in both times. That appeared from the results which reached to half dilution for a day were give none for anti-biofilms formation. Also, crude dilution had all none for both time overnight and day.

So, this test declared and help Arabic Acacia aqueous extract to exterminate microbial biofilms very fast [4-10].

The present of microbial biofilms in wound can be exterminate very easily and very fast by the simple Arabic Acacia aqueous extract. So, the "Traditional Treatment" by the simple extract was very helpful in wounds healing [4-10].

This indicated the using Acacia aqueous extract from ancient Arab customs to treat wounds as traditional

treatment. The biofilm isolates reducing the wounds healing. The biofilm isolates elimination was the main reason for the rapid wounds healing. Arabic Acacia aqueous extract had biofilms elimination of biofilm isolates and accelerated the wounds healing process related to antimicrobial extract contents. These dilutions have antimicrobial contents which exterminated microbial biofilms for wounds healing [4-10].

Table 2: Estimation of biofilms elimination after exposed to Arabic Acacia aqueous extract.

Item	Arabic Acacia aqueous extract					
	Quarter		Half		Crude	
Dilution	Overnight	Day	Overnight	Day	Overnight	Day
Time	Overnight	Day	Overnight	Day	Overnight	Day
Biofilm isolates	Mean optical densities value = Mean biofilm degree					
Staphylococcus sp.	0.188±0.002= Moderate	0.152±0.002= Moderate	0.187±0.001= Moderate	0.199±0.003= Weak	0.095±0.003= None	0.042±0.002= None
Streptococcus sp.	0.176±0.002= Moderate	0.112±0.002= Weak	0.092±0.004= None	0.029±0.001= None	0.044±0.002= None	0.012±0.002= None
Pseudomonas sp.	0.210±0.002= Moderate	0.161±0.001= Moderate	0.161±0.003= Moderate	0.108±0.002= Weak	0.098±0.002= None	0.060±0.002= None
Proteus sp.	0.181±0.003= Moderate	0.122±0.002= Weak	0.090±0.002= None	0.022±0.002= None	0.062±0.002= None	0.029±0.001= None
Escherichia coli	0.213±0.001= Moderate	0.161±0.003= Moderate	0.131±0.001= Weak	0.087±0.001= None	0.073±0.001= None	0.041±0.001= None
Klebsiella sp.	0.198±0.002= Moderate	0.123±0.001= Weak	0.139±0.003= Weak	0.077±0.001= None	0.068±0.002= None	0.048±0.002= None
Candida sp.	0.279±0.001= High	0.233±0.003= Moderate	0.203±0.001= Moderate	0.127±0.001= Weak	0.099±0.001= None	0.067±0.001= None

Conclusion

It was concluded that the Arabic Acacia aqueous extract had microbial anti-biofilms formation. That was by their antimicrobial contents exterminating microbial biofilms for wounds healing. This indicated the using from ancient Arab customs to treat wounds as traditional treatment.

Recommendation

It was recommended that for the "Traditional Treatment" of Arabic Acacia extract according healthy doses as simple treatment instead of chemical treatment for wounds healing.

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Cite this article: Sherifa Mostafa M. Sabra. (2023). "Traditional Treatment" by Arabic Acacia Seeds Exterminating Microbial Biofilms for Wounds Healing. *Clinical Case Reports and Studies*, BRS Publishers. 2(5); DOI: 10.59657/2837-2565.brs.23.035

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Article History: Received: May 08, 2023 | Accepted: May 29, 2023 | Published: June 13, 2023