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The Antimicrobial Activity of *Quercus Infectoria* Extracts against Bacteria Isolated from Wounds Infection

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ABSTRACT

Bacteria that cause wound infections were isolated and diagnosed from patients, who were hospitalized at the Martyr Al-Sadr Hospital from October 2019 to April 2020. Antibacterial effect of *Quercus infectoria* extract (aqueous and alcoholic) on the isolated bacteria has been studied. The results showed that the alcoholic extract has a higher antibacterial effect than the aqueous extract against Gram-positive bacteria like *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococuuss saprophyticus* except *Micrococcus roseus*, which the effect of aqueous extract was higher than alcoholic extract, whereas aqueous and alcoholic extracts showed the same effect against *Streptococcus sp.*. The alcoholic extract has higher antibacterial effect than the aqueous extract against Gram-negative bacteria (*Escherichia coli, Pseudomonas sp., Enterococcus faecalis, Shigella dysnteriae, Enterobacter sakazkii, citrobcter freundii and Alcaligenes faecalis*) except *proteus sp.*, which the aqueous extract was higher than alcoholic extract.

KEYWORDS: wound inflammation ; *Q.infectoria*.

الخلاصة

تم عزل البكتريا المسببة لالتهاب الجروح من المرضى الراقدين في مستشفى الشهيد الصدر من تاريخ تشرين الاول ٢٠١٩ الى نيسان ٢٠٢٠ ، تم دراسة تاثير مستخلص العفص (المائي والكحولي) على البكتريا المعزولة, أظهرت النتائج أن المستخلص الكحولي له تأثير مضاد للجراثيم أعلى من المستخلص المائي ضد البكتيريا موجبة للصبغه الجرام مثل *Staphylococuuss aureus* ، *Staphylococuuss epidermidis*, و*Staphylococuuss saprophyticus* ، باستثناء *Micrococcus roseus* حيث كان المستخلص المائي أعلى من المستخلص الكحولي ، بينما اضهرت المستخلصات المائية والكحولية نفس التاثير ضد *streptococcus sp*. . اضهر المستخلص الكحولي تأثيرا مضاد للجراثيم أعلى من المستخلص المائي ضد البكتيريا سالبة للصبغه الجرام (*Escherichia coli, pseudomonas sp.,* *enterococcus faecalis, Shigella dysnteriae, Enterobacter sakazkii, citrobcter freundii and Alcaligenes faecalis*) باستثناء *proteus sp* حيث كان المستخلص المائي أعلى تاثيرا من المستخلص الكحولي.

INTRODUCTION

Due to the essential functional and aesthetic role skin, the treatment of skin wounds is a research priority. When the skin is impaired due to cut or burn, bacteria can immediately penetrate into underlying tissues، which may cause life-threatening infections. Consequently, effective therapies for such pathological conditions are required. Recently, antimicrobial-loaded wound dressings have emerged as a viable alternative to minimize bacterial wound colonization and infection [1].

Medicinal plants have a promising future because there are about half a million plants worldwide and most of them have not yet investigated their medical activities and their medical activities, which may be crucial in the treatment of present or future studies [2], the galls of *Quercus infectoria* have been used for centuries in oriental traditional medicines for treating inflammatory diseases [3].

MATERIALS & METHODOLOGIES

Bacterial isolates

Bacterial isolates were collected from Martyr Al-Sadr Hospital; the bacteria had been isolated from infected wounds and diagnosis with API.

Plant collection

Querus infectoria were obtained from the local markets in Baghdad, the plants were classified by Dr. Zeinab Abd-Aoun Department of biology/ College of Science for Girls / University of Baghdad. The plant gall has been grinded with the electric grinder.

Preparation of the plant extract

The alcoholic extract was prepared by the Soxhlet extraction method. About 100 g of plant powder was packed into a thimble and run in a Soxhlet extractor. It was extracted with 70% ethanol for the period of about 7 hr. or till the solvent in the tube of an extractor became colorless. After that extracts were evaporated from extract in a rotary evaporator to get the syrupy consistency. Then extract was kept in the refrigerator at 4°C until use [4].

The aqueous extract was prepared by dissolving 50 g of plant powder in 500 ml distilled water and put it in a shaking incubator in 70°C for 30 min. After that extracts were evaporated from extract in a rotary evaporator to get the syrupy consistency. Then extract was kept in the refrigerator at 4°C until use [5].

Qualitative detection of antimicrobial activity of plant extract against bacteria

Plant extracts were tested for antimicrobial activity via an agar well diffusion method against different pathogenic microorganisms, *E. coli, P. aeruginosa, Enterococcus faecalis, Proteus spp., Shigella dysenteriae, Enterobacter sakazakii, Citrobacter freundii, and Alcaligenes faecalis* (Gram-negative), *S. aureus, S. Epidermidis, S. Saprophyticus, Micrococcus roseus, and Streptococcus spp*. (Gram-positive). Pure bacterial cultures were subcultured on Mueller-Hinton agar (MHA). Each strain was uniformly swabbed onto the individual plates by using sterile cotton swabs. Wells with a diameter of 8 mm were made on Mueller-Hinton agar plates by using the gel puncture method. A micropipette was used to pour 100µL of each extract solution onto each well on all plates. After incubation at 37°C for 24 h, the zone inhibition diameter was measured in millimeters [6].

RESULTS AND DISCUSSION

The test was done by the Agar well Diffusion technique, the results showed that the *Q. infectoria* extracts (both alcoholic and aqueous) have high antibacterial effect on bacteria, the antibacterial effect of alcoholic and aqueous extracts on Gram-positive bacteria were higher on Gram negative bacteria, as show in Figure 1 and 2.

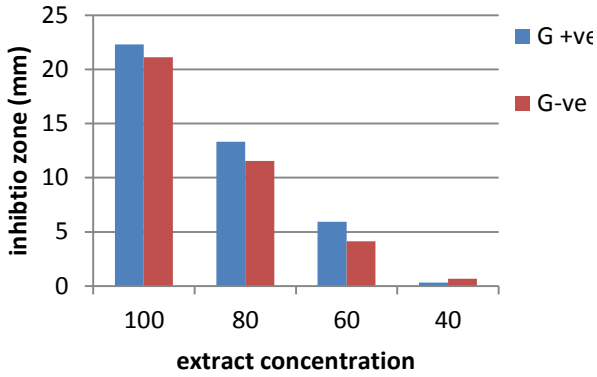

Figure 1. Antibacterial effect of *Q. infectoria* alcoholic extracts on Gram- positive and negative bacteria.

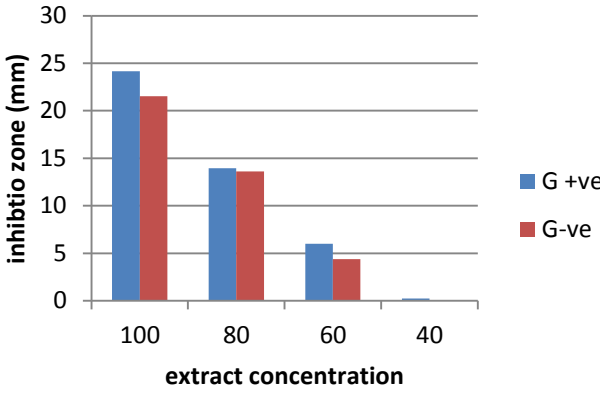

Figure 2. Antibacterial effect of *Q. infectoria* aqueous extracts on Gram- positive and Gram negative bacteria.

The results showed that the alcoholic extract has higher antibacterial effect than the aqueous extract against Gram-positive bacteria like *S. aureus, S. epidermidis, and S. saprophyticus,* except *Micrococcus roseus*, which the aqueous extract was higher than alcoholic extract, whereas aqueous and alcoholic extracts showed the same

effect against *streptococcus sp.*, as shown in Figure 3.

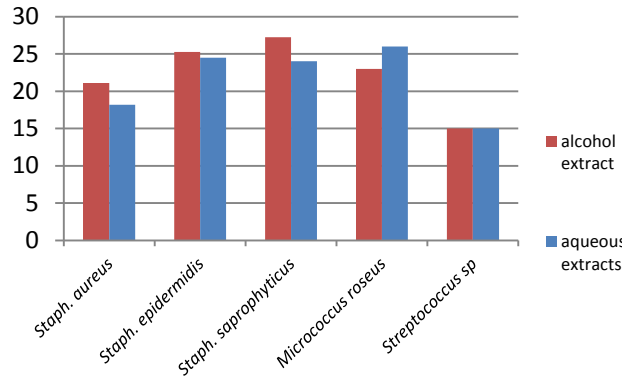

Figure 3. Antibacterial effect of *Q. infectoria* aqueous extract and alcoholic extract on Gram-positive bacteria.

The results showed that the alcoholic extract has higher antibacterial effect than the aqueous extract against Gram-negative bacteria *(E.coli, pseudomonas sp., enterococcus faecalis, Shigella dysnteriae, Enterobacter sakazkii, citrobcter freundii* and *Alcaligenes faecalis*) except *proteus sp*. which the aqueous extract showed higher effect than alcoholic extract.

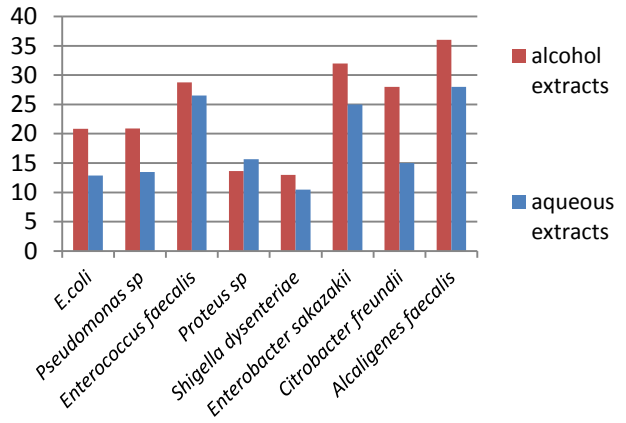

Figure 4. Antibacterial effect of *Q. infectoria* aqueous extract and alcoholic extract on Gram-negative bacteria

The antibacterial activity of alcohol extracts from galls of *Q.Investor* against each bacterial species, which caused wound infections were listed in (Table 1). Alcohol extract displayed strong inhibition activity against Gram-positive [*S. aureus* (21.09 mm), *S. epidermidis* (25.25 mm), *S. saprophyticus* (27.25 mm), *Micrococcus roseus* (23 mm), *Streptococcus sp.* (15 mm)] and against Gram-negative [*E. coli* (20.84 mm), *Pseudomonas sp* (20.91 mm), *Enterococcus faecalis* (28.75 mm), *Proteus sp* (13.66 mm), *Shigella dysenteriae* (13 mm), *Enterobacter sakazakii* (32 mm), *Citrobacter freundii* (28 mm), *Alcaligenes faecalis* (36 mm)].

The antibacterial activity of aqueous extract from galls of *Q.infectoria* against each bacteria species caused wound infection were listed in (Table 2), Gram-positive *S. aureus* (18.19 mm), *S. epidermidis* (24.25 mm), *S. saprophyticus* (24 mm), *Micrococcus roseus* (26 mm), *Streptococcus sp*. (15 mm), and against Gram-negative *E. coli* (12.89 mm), *Pseudomonas sp* (13.5 mm), *Enterococcus faecalis* (26.5 mm), *Proteus sp* (15.66 mm), *Shigella dysenteriae* (10.5 mm), *Enterobacter sakazakii* (25 mm), *Citrobacter freundii* (15 mm), *Alcaligenes faecalis* (28 mm).

Table 1. Antibacterial effect of Q.infectoria alcohol extracts on Gram- positive and Gram negative bacteria

Type of bacteria		**Conc. (mg/ml)**			
		1	**0.8**	**0.6**	**0.4**
G+ve	*Staphylococcus aureus*	21.09	12.66	6.14	0.47
	Staphylococcus epidermidis	25.25	17.75	6	0
	Staphylococcus saprophyticus	27.25	14.75	7.25	0
	Micrococcus roseus	23	9.5	4	0
	Streptococcus sp	15	11	0	0
G-ve	*Escherichia coli*	20.84	11.68	5.05	1.10
	Pseudomonas sp	20.91	10.33	2.5	0.75
	Enterococcus faecalis	28.75	18	6.5	0
	Proteus sp	13.66	7.66	0	0
	Shigella dysenteriae	13	5	0	0
	Enterobacter sakazakii	32	20	13	0
	Citrobacter freundii	28	15	8	0
	Alcaligenes faecalis	36	24	13	0

Table 2. Antibacterial effect of *Q.infectoria* aqueous extracts on Gram positive and Gram-negative bacteria

Type of bacteria		**1**	**0.8**	**0.6**	**0.4**
G+ve	*Staphylococcus aureus*	18.19	10.57	5.14	0.28
	Staphylococcus epidermidis	24.5	16.25	6.25	0
	Staphylococcus saprophyticus	24	15.25	4.5	0
	Micrococcus roseus	26	15	6	0

	Streptococcus sp	15	11	0	0
G-ve	*Escherichia coli*	12.89	5.68	2.63	0
	Pseudomonas sp	13.5	5.91	0.83	0
	Enterococcus faecalis	26.5	14.5	2.75	0
	Proteus sp	15.66	7.66	2.33	0
	Shigella dysenteriae	10.5	2.5	0	0
	Enterobacter sakazakii	25	14	0	0
	Citrobacter freundii	15	0	0	0
	Alcaligenes faecalis	28	16	9	0

DISCUSSION

The results of this study showed that the Gram-positive bacteria were more susceptible to the extracts than Gram-negative bacteria, which *E. coli* exhibited more resistant than *S. aureus* and *B. subtilis* when they were tested with *Q. infectoria* extract. The reason would be that the lipopolysaccharide (LPS) layer of Gram-negative bacteria in the outer membrane has high hydrophobicity and acts as a strong barrier against hydrophobic molecules [7]. The extract can pass through the cell wall of Gram-positive bacteria easier than the Gram-negative bacteria because the cell wall of the Gram-positive consisted of peptidoglycan and lack of outer membrane [8]. The main constituents found in the galls of *Q. infectoria* are tannin (50-70%) and small amounts of free gallic acid and ellagic acid [9-10]. High amounts of tannin present in the galls of *Q. infectoria* implied that tannin is the active compound responsible for the antibacterial activity in this study [8-11]. Tannin is a phenolic compound which is soluble in water, alcohol and acetone, and gives precipitates with protein [7, 11]. The similarity in the antibacterial activity of both the alcohol and aqueous extracts suggests that the extracts may have high total tannin content. Our findings were also supported by other researchers who observed that the extracts of *Q. infectoria* had antibacterial effects on pathogenic bacteria [12, 13, and 14].

CONCLUSIONS

The results obtained from this study revealed that *Quercus infectoria* galls have antimicrobial activity against Gram-positive and Gram-negative bacteria that cause wound infections. Both extracts from the galls inhibited the Gram-positive bacteria more than Gram-negative bacteria. This study corresponds with the results of other researchers who observed that the alcohol extracts of *Q. infectoria* was found to be more active against all bacteria studied during present work

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