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Research Article
In vitro and *in vivo* Antibacterial effect of *Commiphora gileadensis* Methanolic Extract against Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*

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Abstract

Background and Objective: *Commiphora gileadensis* is a plant in the Burseraceae family that grows in the western area of Saudi Arabia. Traditionally, it is used in the treatment of some superficial infections. **Materials and Methods:** The methanolic extract of *Commiphora gileadensis* isolated from its leaves and branches. The *in vitro* study was conducted to determine the effect of this extract on Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* using an agar diffusion and Minimum inhibitory concentration (MIC) methods. The *in vivo* study was conducted through two different methods. The first method, 20 male Balb c^{-1} mice were used for the determination of *Commiphora gileadensis* methanolic extract toxicity (LD_{50}). In the second method, 40 male mice were used and were put into four groups. The first and second groups were injected subcutaneously with 108 CFU of MRSA 1 mL^{-1}, while the third and fourth groups were injected with 108 CFU of *Pseudomonas aeruginosa* 1 mL^{-1}. The comparison between groups was done by using a t-test ($p<0.05$). **Results:** The methanolic extract of *Commiphora gileadensis* had a greater sensitivity zone on MRSA and *Pseudomonas aeruginosa*, 7 and 3 mm respectively. The MIC of the extract was 1/8 and 1/2 for MRSA and *Pseudomonas aeruginosa* respectively. The *in vivo* study showed that the extract was non-toxic, it also showed that the extract decreased the mortality of mice induced by MRSA injection significantly ($p<0.05$) While insignificantly with *Pseudomonas aeruginosa*. **Conclusion:** The total *Commiphora gileadensis* methanolic extract had an antibacterial effect on MRSA and *Pseudomonas aeruginosa*. This extract was non-toxic for the mice.

Key words: *Commiphora gileadensis*, MRSA, *Pseudomonas aeruginosa*, antibacterial, antibiotic

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

During this century, several bacteria enhance resistance against antibiotics available in health markets resulting from the indiscriminate use of these antibiotics[1]. Moreover, most of these antibiotics have unpleasant side effects depending on individuality[2]. Hence, scientists had to look for alternative bactericidal materials. Many local markets have been providing people with a variety of herbal plant mixtures that are used in the treatment of several disorders throughout the ages[3]. *Commiphora gileadensis* is one of those plants are widely known for treating different disorders throughout time[4,5]. *Commiphora gileadensis* belongs to the Burseraceae family that grows in many Arab countries including Saudi Arabia[6,7]. In Saudi Arabia, this plant is found in the Sarawat mountains along the west area of the country. *Commiphora gileadensis* goes with many traditional names in the Arab countries, such as; Balm of Makkah, Basin and Bisham[6]. The balsam of *Commiphora gileadensis* was used as a perfume in Hellenistic and Roman periods[8,9]. Earlier, a few studies used an aqueous extract of this plant as an analgesic, diuretic and antihypertensive agent[10]. To date, many families use this extract as a medicinal compound in the treatment of many symptoms such as pain and fever[11]. A previous study conducted by the same author found that the *Commiphora gileadensis*'s aqueous extract induced an anti-inflammatory effect in rats[11,12]. Moreover, few studies showed that methanolic extract of this plant had an antibacterial activity on different strains of bacteria[13-15]. Al-Sum *et al.*[16] found that an aqueous extract of *Commiphora* inhibit the growth of *Escherichia coli*, *Salmonella typhi* and fungi *Aspergillus niger* and *Penicillium italicum*. In another study, the sap of this plant inhibits the growth of *Pseudomonas aeruginosa* and *Bacillus cereus*[13]. This study aimed at investigating the *in vitro* and *in vivo* antibacterial activity of methanolic extract of *Commiphora gileadensis* against Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

All chemicals were purchased from Sigma Co. Ltd. forty male Balb/c mice were purchased from an animal house in Taif city.

Study area: This study was done in faculty of applied medical sciences, Taif University. It was carried out in January 2020 till March of the same year.

***Commiphora gileadensis* collection:** It was collected from a high mountain area called Alaab valley located in the western area of Saudi Arabia in the south of Al-Madinah Al-Munawara. Both leaves and disgrace branches were collected during February 2020.

Preparation of *Commiphora gileadensis* methanolic extract: Both leaves and disgrace branches were cleaned by water then dried in a hot air oven at 40°C, then the leaves and branches were ground into a fine powder using an electrical blender. The powder was sifted to remove any large residuals. A 10 g of this powder was macerated in 100 mL of absolute methanol in a sterile funnel[1] and left for 24 h. After that, the funnel was vigorously shaken and a sterile filter paper was used to filter this extract. Then the extract was dried in a water bath at 40°C to form a concentrated *Commiphora gileadensis* extract. Finally, the extract was stored at a refrigerator at 4°C for two weeks[6].

Determination of the antibacterial activity of the *Commiphora gileadensis* methanolic extract: The antibacterial activity of *Commiphora gileadensis* methanolic extract against MRSA and *Pseudomonas aeruginosa* was determined by an agar diffusion method[17]. The test was performed in Muller Hinton media for both bacterial species. In eight Muller Hinton agar plates, both MRSA and *Pseudomonas aeruginosa* were applied by the sterile swab. Some antibiotics such as; ciprofloxacin, erythromycin, chloramphenicol and ampicillin discs were applied at the border of the plate. Moreover, two separated discs were immersed separately in methanol and *Commiphora gileadensis* methanolic extract then transferred to the center of the plate under sterilized condition. All six plates were incubated at 37°C for 24 h. Then, the zone of sensitivity for all antibiotics, methanol and the methanolic extract was measured (mm) in all plates. Finally, means mean values of sensitivity zones for each antibiotic, methanol and methanolic extract were calculated[18].

Minimum Inhibitory Concentration (MIC) of *Commiphora gileadensis*: The Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antibacterial agent that inhibits the visible bacterial growth after 24 h of incubation. This method was conducted by a set of six sterilized tubes with 1 mL of distilled water in each tube. A 0.5 mL of methanolic *Commiphora gileadensis* extract was added to the first tube, mixed then a 0.5 mL of this solution was transferred to the second tube. A serial gradient concentration of the

Commiphora gileadensis (1/2, 1/4, 1/8, 1/16, 1/32 and 1/64) was done. The inoculum of 10^4 Colony Forming Unit (CFU)/spots of MRSA was added then to the six tubes. From each tube, a sterile loop was used to inoculate and streak MRSA into six separate blood agar plates. The same method was applied to determine the MIC of *Commiphora gileadensis* methanolic extract against *Pseudomonas aeruginosa*[19]. Finally, all 12 plates were incubated in a 37°C for 24 h. The MIC for *Commiphira gileadensis* methanolic extract appeared in the last plate as it didn't show a visible colony of two bacterial species.

***Commiphora gileadensis* methanolic extract lethal dose (LD_{50}):** Twenty-five male Balb/c mice with 20-25 g average weight were used for determination of *Commiphora gileadensis* methanolic extract lethal dose (LD_{50}). These mice were synchronized for two weeks fed on a normal rodent diet and drank water at 25°C (room temperature) before starting the experiment. Subsequently, the mice were put randomly into five groups (5 mice in each). The first group subcutaneously injected with 100 mg *Commiphora gileadensis* methanolic extract kg^{-1} mouse of body weight, while the second, third, fourth and fifth groups were injected with 1000, 2000, 3000 and 4000 mg kg^{-1} of the extract respectively. After 24 h of being injected the signs of toxicity such as, motor activity, reaction to sound and pinch, salivation around the mouth were recorded. Moreover, the death of any mouse in these five groups was also recorded[6].

Mice and *Commiphora gileadensis* methanolic extract experiment: This experiment was carried out on 40 male Balb/c mice with average weight 22-26 g. These mice were synchronized two weeks before starting the experiment and were fed a normal rodent diet and drank water at 25°1C (room temperature). Subsequently, the mice were put randomly into four groups (10 mice in each). The first group was injected subcutaneously with 10^8 CFU of MRSA/1 mL^{-1} distilled water, while the second group was injected with 10^8 CFU of MRSA/1 mL *Commiphora gileadensis* methanolic extract. As for the third and fourth groups, they were injected with 10^8 CFU of *Pseudomonas aeruginosa*/1 mL^{-1} of distilled water and *Commiphora gileadensis* methanolic respectively. Thereafter the time of the mouse's death after being injected was recorded. Eventually, the death percentage of mice per each group was calculated[20].

Statistical analysis: The SPSS software version 16 (SPSS Inc., Chicago, IL, USA) was used in the performance of the statistical analysis. Data were expressed as mean±standard deviation. The comparison between groups was analyzed by using a t-test and the significance defined as ($p<0.05$).

RESULTS

Findings in Table 1 represent the comparison between *Commiphora gileadensis* methanolic extract antibacterial effect on both MRSA and *Pseudomonas aeruginosa* with some antibiotics (chloramphenicol, ciprofloxacin, erythromycin and ampicillin). The sensitivity zone of *Commiphora gileadensis* methanolic extract on MRSA was 7 mm. On the other hand, the sensitivity zone of the methanolic extract on *Pseudomonas aeruginosa* was 3 mm. Results in Table 2 represents the MIC of *Commiphora gileadensis* methanolic extract on MRSA and *Pseudomonas aeruginosa*. The MIC of the methanolic extract was 1/8 and 1/2 for MRSA and *Pseudomonas aeruginosa*, respectively. Table 3 represents signs of toxicity and the mortality in five groups of mice for 24 h followed the subcutaneous injection of different concentrations of *Commiphora gileadensis* methanolic extract (100, 1000, 2000, 3000 and 4000 mg kg^{-1}). All mice in every five groups didn't show any toxicity sign and still alive. Table 4 represents the *in vivo* study of the methanolic extract of *Commiphora giledensis*. The result depends on the duration of death after the injection of bacterial inoculum of MRSA and *Pseudomonas aeruginosa*. The duration time of death in mice followed the subcutaneous injection of MRSA showed a significant statistical difference between the inoculum dissolved in methanolic extract of *Commiphora gileadensis* and distilled water ($p = 0.049$). The duration time of death followed the injection of *Pseudomonas aeruginosa*

Table 1: Zone of sensitivities in millimeter for some antibiotics, methanol and *Commiphora gileadensis* methanolic extract

	MRSA	*P. aeruginosa*
Antibiotics	Zone radial (mm)	Zone radial (mm)
Chloramphenicol	11	7
Ciprofloxacin	10	5
Erythromycin	9	5
Ampicillin	1	Resistance
Methanol	Resistance	Resistance
C. gileadensis (extract)	7	3

Table 2: The minimum inhibitory concentration of *Commiphora gileadensis* on MRSA and Pseudomonas Aeruginosa

Dilution	1/2	1/4	1/8	1/16	1/32	1/64
MRSA	No visible growth	No visible growth	No visible growth	Visible growth	Visible growth	Visible growth
P. aeruginosa	No visible growth	visible growth	Visible growth	Visible growth	Visible growth	Visible growth

Table 3: Toxicity signs and mortality of different concentration of *Commiphora gileadensis* methanolic extract in five groups of mice

	Five mice groups (mg kg^{-1})				
Methanolic extract concentration	100	1000	2000	3000	4000
Motor activity	Normal	Normal	Normal	Normal	Normal
Reaction to sound	Normal	Normal	Normal	Normal	Normal
Reaction to pinch	Normal	Normal	Normal	Normal	Normal
Salivation around the mouth	Normal	Normal	Normal	Normal	Normal
Death within 24 h	None	None	None	None	None

Table 4: The duration time of death in mice followed the injection of MRSA and *Pseudomonas aeruginosa* in *Commiphora gileadensis* methanolic extract or distilled water

	Death duration (*Commiphora gileadensis*)	Death duration (distill water)	p-value
MRSA	42±12 (40%)	20±8 (100%)	0.049*
Pseudomonas aeruginosa	57±21 (30%)	43±16 (70%)	0.191

*p<0.05

inoculum didn't show a statistical difference but the time of death is larger in inoculum dissolved in *Commiphora gileadensis* than in distilled water.

DISCUSSION

Commiphora gileadensis is one of the traditional plants that grow in the west-southern area in Saudi Arabia. During the long history, this plant was used in teeth cleaning and in the treatment of some diseases and as an antidote for scorpion sting[6]. Abdul-Ghani *et al.*[10] used in their study an aqueous *Commiphora gileadensis* extract as an antihypertensive agent. The study found that an administration of 4 mg kg^{-1} this extract intravenously reduced blood pressure by 20% in rats. Moreover, this dose reduced a heart by 14% in the same rats. They suggested that the hypotensive and bradycardic effect of this extract accomplished by muscarinic cholinergic receptor activation. A previous study found that the administration of 500 mg kg^{-1} of *Commiphora* extract protects stomach mucosal surface against ulcer by increasing mucus production[11]. Previously, the two forms of *Commiphora gileadensis* sap, the transparent sap which presented on the surface of the plant and the milky sap appearing as a viscous solution were used as bactericidal agents. The result showed that the milky sap produced a zone of inhibition about 12 mm while the transparent sap neither[14]. Moreover, a study conducted by Ryan *et al.*[21] found that the zone of inhibition of *Commiphora gileadensis* methanolic extract against *S. aureus* and *Pseudomonas aeruginosa* were about 16 and 8 mm, respectively. The present study showed that the zone of inhibition of the methanolic extract with MRSA and *Pseudomonas aeruginosa* was 7 and 3, respectively. The difference in the results may be due to the difference in the species between two studies because we examined the bactericidal function of the methanolic extract with more resistant bacteria. The previous study which was conducted by Cooper *et al.*[22] found that *Commiphora gileadensis* balsam had prolactin binding monosaccharide that plays as a decoy receptor that binding to *Pseudomonas aeruginosa* lectin blocking them from adhesion to host cells. The MIC for *Commiphora gileadensis* was initially tested by Al-sieni[23] found that the MIC of *Commiphora gileadensis* methanolic extract was greater than ampicillin MIC on some bacteria such as *S. aureus*. The results of the present study with Al-sieni[23] study which showed that the *Commiphora gileadensis* methanolic extract had a MIC greater than ampicillin on both MRSA and *Pseudomonas aeruginosa*. It is near to that of erythromycin. The mode of action of the plant extract proposed by Strobel and his colleague that the active component of *Commiphora gileadensis* may complex with some bacterial cell walls compound destroying the bacterial cell wall or complexed with its chromosomal DNA[24]. An *in vivo* part of this study showed that the *Commiphora gileadensis* methanolic extract is non-toxic and decrease the mortality of MRSA and *Pseudomonas aeruginosa* by nearly 50% in balb c^{-1} mice.

CONCLUSION

We conclude that the total *Commiphora gileadensis* methanolic extract had an antibacterial effect on MRSA and *Pseudomonas aeruginosa*. This extract was non-toxic for the mice in the study and it reduced the mortality rate of mice caused by MRSA and *Pseudomonas aeruginosa* injection. A new study that determines the active component of *Commiphora gileadensis* and compares its effect with the total extract is recommended.

SIGNIFICANCE STATEMENT

This study discovered the antibacterial activity of *Commiphora gileadensis* against the highly antibiotic resisted bacteria (MRSA and *Pseudomonas aeruginosa*) that can be beneficial for the replacement of antibiotics by the extract of *Commiphora gileadensis* in the treatment of a wide range of bacterial infection. This study will help the researchers to uncover the critical areas of medicinal plants that many researchers were not able to explore. Thus, a new theory on medicinal plants may be arrived at.

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