



Research Article

## Antibacterial activity of fungal extracts against bacterial isolates from Tonsillitis in children

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### Abstract

One hundred samples were collected from children tonsillitis, (1-12) years, in Al-Shirqat General Hospital through the period from October to November 2018. Isolated was divided into two parts according to gram stain (Positive and Negative bacteria). 77 isolates (85.5%) were gram positive and 13 isolates (14.5%) was gram negative bacteria. The sensitivity of the isolates was tested for some antibiotics, and the negative bacteria for the gram stain showed high resistance to the antibiotics compared to the positive bacteria for the gram stain. The inhibitory activity of alcoholic and aqueous for truffle *Terfezia sp.* and *Gonoderma lucidum* was four different concentrations (25%, 50%, 75%, 100%) mg / ml. The results showed that the aqueous extracts against some isolates was inhibition zone diameters between (8-25) mm. While the results of alcoholic truffle extract were not showed any activity against isolates. Also the results of the aqueous extract of the *Gonoderma lucidum* showed the inhibitory activity of some isolates with inhibition zone between (7-30) mm. As for the alcoholic extract of the mushroom, it was not showed any inhibition activity against the bacterial isolates.

**Keywords** Tonsillitis, *Terfezia sp.*, *Gonoderma lucidum*, Antibacterial activity.

### Introduction

Tonsillitis is One of the most common throat diseases in the world. It is one of the diseases that affect the upper respiratory tract and it is more common in children, Infection is more frequent in ages 5-15 years due to weak immune resistance in children (Coutinho et al., 2021). Symptoms of tonsillitis include high temperature, fatigue, headache, swelling, red tonsils, difficulty swallowing, voice change, and snoring (Klaus, 2014). Tonsillitis caused by many bacterial species like *Streptococcus pyogenes*, the main cause of tonsillitis, as it causes 5 - 36% of cases, and many opportunistic pathogen, such as some species of *Staphylococcus spp* (Samarrai et al., 2022; Farooqi et al., 2017). The risk of tonsillitis lies in some of the complications that develop with injury, such as rheumatoid arthritis, abscesses, acute nephritis, and otitis media (Sivapathasundharam and Gururaj, 2020; Memmott et al., 2022). Secondary metabolic compounds produced by fungi

are good and potential treatment sources for producing new antibiotics. As many fungi have shown strong antimicrobial activity against many bacteria that cause disease in humans (Zhang et al., 2014). Because of the resistance of many isolates to antibiotics, an attempt has been made to find alternative methods of treatment, including the use of plant and fungal extracts because they contain effective antibacterial substances that inhibit the effectiveness of bacterial species.

### Materials and Methods

#### Samples collection and identification

One hundred from children with tonsillitis were collected at Al-Shirqat General Hospital for ages 1-12 years for the period from October 2018 to November 2018, Samples were taken from the tonsils using sterile cotton swabs. The isolates were cultured using the culture media, blood agar, mannitol salt

agar and McConkey agar. Depending on their culture and phenotypic properties and based on the applied biochemical tests, Identification was confirmed using the Vitek 2compact system.

### Antibiotic Susceptibility test

This test was conducted according to (Rahim et al., 2013; Kirby et al., 1996). Bacterial isolates were spread the surface of agar Muller Hinton agar using a sterile cotton swab, and put antibiotic discs by 5 discs per dish, and incubated for 24-48 hours, the inhibition zone was measured and compared with the standard tables (CLSI, 2022).

### Preparation of truffle extracts *Terfezia sp.*

#### Aqueous extract

A weight of 50 g of truffles was taken cut into small pieces and soaked in distilled water in a ratio of (1:3) w/v for 24 hours then mixed in the home mixer at the maximum speed for one minute, the mixture was filtered using cheese cloth and discarded in a centrifuge, sterilized the extract by filtering it through a Millipore filter with a diameter of 0.45 nm and kept at -20 ° C until use (Janakat et al., 2004).

#### Methanol extract

The extract was prepared in the same way as above, but soak the truffle with 95% of methyl alcohol at a rate of 1-3 for a period of 24 hours, then mix the mixture with a home mixer and discard it in the centrifuge and evaporate the methyl alcohol in an air stream at a room temperature, completed the original volume of the extract by adding distilled water and sterilized using a Millipore filter 0.45 nm and then kept at -20°C until use (Janakat et al., 2004).

### Preparation of *Gonoderma lucidum* Aqueous extract

Ten gram of mushroom powder was weighed and added 100 ml of distilled water in a conical flask, shaken in a shaker for 24 hours to be mixed well, filtered with a medical gauze and discarded in a centrifuge at 4000 rpm for 10 minutes and filtered with filter paper, Pour the mixture into petri dishes and dry it in the oven at 40 ° C to obtain a dry powder for the extract. Keep in a dry, clean and opaque container until use (Hu et al., 2009).

#### Methanol extract

Extracts was prepared in the same steps as preparing the aqueous extract except replacing distilled water with methanol alcohol at a concentration of 95% (Janakat et al., 2004).

### Effect of antibacterial fungal extracts

The effectiveness of the anti-bacterial fungal extracts causing tonsillitis at concentrations (25%, 50%, 75%, 100%) mg / ml was determined using the method agar well diffusion, after growth of isolates on nutrient broth, it was spread on the surface of dishes containing Muller Hinton Agar medium and then poring using Bork borer with 5 holes per plate with a diameter of 6 mm, the holes were filled with fungal extracts, about 0.2 ml per hole. Then petri dish was incubated at 37°C for 24 hours, and read the diameters of the inhibition zone and compared with the antibiotic inhibiting diameters (Schelz et al., 2006).

### Results and Discussion

#### Isolation

The vast majority of the isolates were spherical bacteria that are gram positive bacteria, while a few were gram negative bacteria. 90% of the samples showed positive growth, while 10% of the samples showed no growth. The Negative growth of these samples may be attributed to the fact that the patient has used antibiotics to treat the inflammation, or perhaps isolated bacteria may need special nutrients and conditions for growth (Masahiro et al., 2005). These samples were distributed among children of both gender (males and females) in different proportions, as 50% of male children and 40% of females. Table 1. showed the number of positive and negative growth samples, and comparison with gender.

Gender	Positive Growth	Negative Growth	Total
Male	50	6	56
Female	40	4	44
Total	90	10	100

**Table 1. The number of positive and negative growth isolates compared to the patient's gender**

The results showed gram positive bacteria was 77 isolates and its percentage was 85.5%, while the number of gram negative bacteria was 13 isolates and by 14.5% as in Table 2.

**Table 2. The percentage of positive and negative isolates of gram stain**

Isolates	Number	Percentages
Gram positive	77	85.5
Gram negative	13	14.5
Total	90	100

### Bacterial isolates percentage

The results showed as in Table 3. that *Staphylococcus aureus* gave the highest level of isolation, they were isolated with 51 (56.7%) followed by *Staphylococcus epidermidis* with 13 isolates (14.5%), then *Staphylococcus haemolyticus*, *Brotedella hinzii*, with 4 isolates (4.5%), then *Serratia*

*marcesceus*, *Staphylococcus xylosus* with 3 isolates (3.3%), *Micrococcus luteus* and *Escherichia coli*, was two isolates (2.2%), whereas, *Staphylococcus vitulinus*, *Rothia dentocariosa*, *Kocuria kristinae*, *Aerococcus viradans*, *Acinetobacter lwoffii*, *Pseudomonas fluorescens*, *Moraxella group*, *Aeromonas salmonicida*, were one isolate (1.1%).

**Table 3. Bacterial species identified from isolates with percentage**

Bacterial isolates	Number	Percentage
<i>Staphylococcus aureus</i>	51	56.7
<i>Staphylococcus epidermidis</i>	13	14.6
<i>Staphylococcus haemolyticus</i>	4	4.5
<i>Brotedella hinzii</i>	4	4.5
<i>Staphylococcus xylosus</i>	3	3.3
<i>Serratia marcesceus</i>	3	3.3
<i>Micrococcus luteus</i>	2	2.2
<i>Escherichia coli</i>	2	2.2
<i>Rothia dentocariosa</i>	1	1.1
<i>Staphylococcus vitulinus</i>	1	1.1
<i>Kocuria kristinae</i>	1	1.1
<i>Aerococcus viradans</i>	1	1.1
<i>Acinetobacter lwoffii</i>	1	1.1
<i>Pseudomonas fluorescens</i>	1	1.1
Moraxella group	1	1.1
<i>Aeromonas salmonicida</i>	1	1.1
Total	90	100%

### The Antibacterial activity of aqueous and methanol extracts of *Terfezia sp* against bacterial isolates

The results in the table showed that the truffle aqueous extract was more effective on bacteria isolates compared with methanol extract which did not give any antibacterial effective four concentrations were used (25%, 50%, 75%, 100%) mg / ml and the aqueous extract showed a different inhibitory efficacy against isolates. It was also found that the inhibitory efficacy of the aqueous extract increased with concentration, as *Staph.aureus* bacteria gave the highest inhibition rate at the concentration 100 mg/ml in diameter 25 mm and the lowest inhibition diameter at concentration 25 mg/ml with a diameter of 12 mm and the lowest inhibition diameter for the bacteria *Staphylococcus vitulinus* at the concentration 100 mg/ml with a diameter of 12 mm as for the rest of the concentrations did not show any inhibition of these isolates, In addition, there are many bacterial species that have not shown any inhibition of all concentrations of aqueous and methanol extracts as in Table 4. and Fig.1,

The activity of the aqueous extract against the gram positive bacteria may be due to the fact that the membrane of these bacteria is more permeable. From the gram negative bacteria

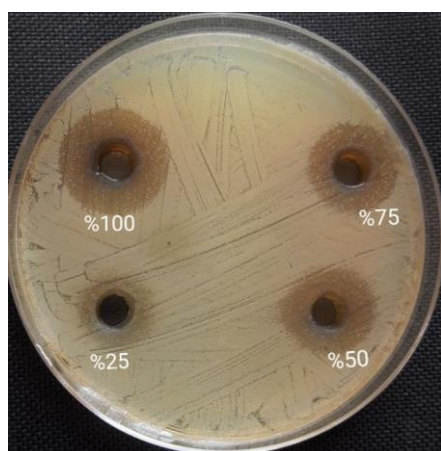
membrane that consists of thick, homogeneous layer of peptidoglycan contains low density fats. While the membrane of gram-negative bacteria. It consists of a peptidoglycan layer covered with an outer membrane that contains fatty sugars and protein and large amounts of phospholipids. Therefore, permeability and interact antimicrobial agents and their crossing over the cell shell are highly effective in gram-positive bacteria, also the solubility efficiency of substances in water is higher than their solubility in alcohol, and thus it is faster to penetrate the bacterial cell wall (Mitra et al., 2021; Qureshi et al., 2022).

Truffles contain antibacterial agents, especially protein. The aqueous extract containing the protein (Darwish et al., 2021; Lee et al., 2020) dissolved in the acid was used against the isolates of *S.aureus*, *P. aeruginosa* and the soluble protein extract showed a minimum inhibition diameter of 50 mg/ ml against these isolates, The results of the current study agreed with (Gouzi et al., 2011; Wu et al., 2022). When they used the aqueous and methanol extract of truffles against *S. aureus* that the concentration of 5% of the aqueous extract prevented the growth of these isolates by 66.4% while the methanol extract did not have any effect against these isolates.

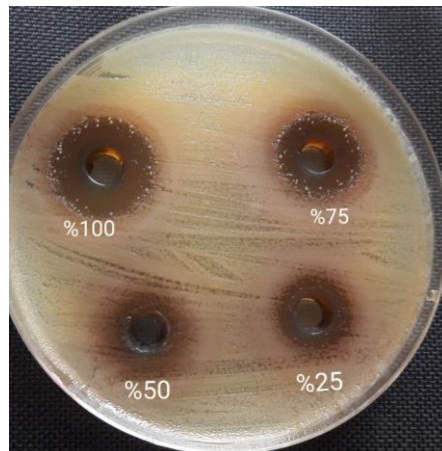
**Table 4. Inhibition zone diameter of the aqueous and methanolic extract of *Terfezia sp* towards bacterial isolates**

Bacterial Isolates	Aqueous extract				Methanol extract			
	Concentrations mg / ml							
	100%	75%	50%	25%	100%	75%	50%	25%
<i>Staphylococcus aureus</i>	25	19	17	12	-	-	-	-
<i>Brotedella hinzii</i>	21	18	14	12	-	-	-	-
<i>Moraxella group</i>	20	18	16	12	-	-	-	-
<i>Acinetobacter lowffii</i>	20	18	15	11	-	-	-	-
<i>Kocuria kristinae</i>	18	15	14	13	-	-	-	-
<i>Aeromonas salmonicida</i>	15	11	8	-	-	-	-	-
<i>Marcescens Serratia</i>	14	13	12	-	-	-	-	-
<i>Pseudomonas fluorescens</i>	11	8	-	-	-	-	-	-
<i>Staphylococcus vitulinus</i>	12	-	-	-	-	-	-	-
<i>Staphylococcus epidermidis</i>	-	-	-	-	-	-	-	-
<i>Staphylococcus xylosus</i>	-	-	-	-	-	-	-	-
<i>Staphylococcus haemolyticus</i>	-	-	-	-	-	-	-	-
<i>Micrococcus luteus</i>	-	-	-	-	-	-	-	-
<i>Rothia dentocariosa</i>	-	-	-	-	-	-	-	-
<i>Escherichia coli</i>	-	-	-	-	-	-	-	-
<i>Aerococcus viradans</i>	-	-	-	-	-	-	-	-

- sign indicates No effect



(a) *Brodetella hinzii*



(b) *Staphylococcus aureus*

**Fig. 1 The inhibitory effect of aqueous extract *Terfezia sp* against some bacterial isolates**

Wu et al. (2022) mentioned that the aqueous truffle extract has an inhibitory effect on the growth of isolates. When it is used, 4% of the aqueous extract inhibits the growth of the *Staph.aureus* isolates by 86.48%, and it also inhibits the growth of *P. aeruginosa* bacteria by 71.11%. Therefore, truffle is a source of natural treatment that can be used for the treatment of infections caused by *Staph.aureus*. This is consistent with the results of our study.

Truffles are a source of therapeutic compounds as they possess both anti-oxidant and anti-inflammatory properties (Janakat et al., 2004; Gouzi et al., 2011; Al-Laith, 2010). Truffles are a good source of protein, carbohydrates, fats and many minerals, such as phosphorus, potassium, calcium, iron, sulfur containing amino acids and fatty acids such as

palmitic, oleic, and linoleic acids, and vitamins (Veeraraghavan et al., 2021; Dingo et al., 2020).

It also contains biologically active compounds such as anthocyanin, oligosaccharides, carotenoids, flavonoids, pheromones and steroids, so it has been used in folk medicine to treat eye diseases, skin, wounds, colds, respiratory infections, and arthritis (El Enshasy et al., 2013; Kaygusuz et al., 2021).

**The Antibacterial activity of aqueous and methanol extracts of *Gonoderma lucidum***

The methanol extract did not have an inhibitory effect on any species of bacterial isolates. But the aqueous extract, was gave inhibitory effect against all bacterial species. Gram



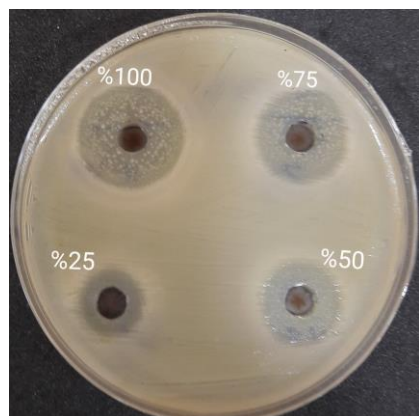
negative bacteria was a higher rate of inhibition than gram-positive bacteria, inhibiting diameters extended from 12-30 mm. As for Gram-positive bacteria, the inhibition rate was in diameters of 7-26 mm. the rate of inhibition was at the highest percentage at concentration 100% and the lowest

diameter of inhibition of isolates was concentration 25%, this concentration was not inhibitory to *M. luteus* and thus the strength of the inhibition was proportional to the increase in concentration as in Table 5. and Fig. 2.

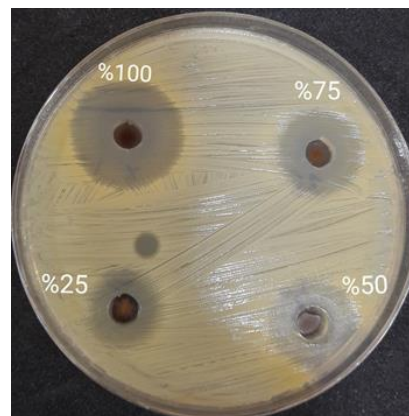
**Table 5. The inhibitory activity of the aqueous and methanol extracts of *G. lucidum* againsts the bacterial isolates**

Bacterial Isolates	Aqueous extract				Methanol extract			
	Concentrations mg / ml							
	100%	75%	50%	25%	100%	75%	50%	25%
<i>Staphylococcus aureus</i>	25	20	19	16	-	-	-	-
<i>Brotedella hinzii</i>	17	15	14	13	-	-	-	-
<i>Moraxella group</i>	25	19	17	13	-	-	-	-
<i>Acinetobacter lowffii</i>	18	16	15	13	-	-	-	-
<i>Kocuria kristinae</i>	26	20	15	11	-	-	-	-
<i>Aeromonas salmonicida</i>	30	25	20	15	-	-	-	-
<i>Marceseus Serratia</i>	18	16	15	13	-	-	-	-
<i>Pseudomonas fluorescens</i>	19	17	15	12	-	-	-	-
<i>Staphylococcus vitulinus</i>	25	16	15	14	-	-	-	-
<i>Staphylococcus epidermidis</i>	24	20	16	12	-	-	-	-
<i>Staphylococcus xylosus</i>	21	19	17	15	-	-	-	-
<i>Staphylococcus haemolyticus</i>	20	14	13	7	-	-	-	-
<i>Micrococcus luteus</i>	19	15	13	-	-	-	-	-
<i>Rothia dentocariosa</i>	22	16	14	10	-	-	-	-
<i>Escherichia coli</i>	19	17	14	13	-	-	-	-
<i>Aerococcus viradans</i>	20	16	12	10	-	-	-	-

- sign indicates No effect



*Staphylococcus aureus* (a)



*Moraxella group* (b)

**Fig. 2 The inhibitory activity of the aqueous extract of *G. lucidum* against some bacterial isolates**

In a study, it was found that the aqueous extract of *G. lucidum* had inhibitory activity against 15 species of bacterial isolates and the highest inhibition action against *M. luteus*.

Our results were consistent with the findings of (Kamble et al., 2011; Ibrahim and Kebede, 2020), as it was found that the aqueous and methanol extracts with a concentration of 100 mg / ml have antibacterial activity, the aqueous extract of the fungus was more effective in bacteria than methanol extract.

### Conclusions

It was found that gram-positive bacteria had a higher percentage than gram-negative bacteria, which necessitates the use of antibiotics that target positive bacteria in particular when infected with tonsillitis in children. The results of our study revealed the high inhibitory activity of the aqueous extract of *Terfezia* sp and *Gonoderma lucidum* on bacterial species under study, and this gives hopeful signs of using this

extract as a new antibiotic against bacteria that have shown broad-spectrum resistance to antibiotics, so it is recommended to detect the effectiveness of these aqueous extracts at the molecular level of bacteria, and study its immunological, physiological and histological effect in rats infected with bacterial species in order to investigate the safety and security of this extract.

### Conflict of Interest

The author hereby declares no conflict of interest.

### Consent for publication

The author declares that the work has consent for publication.

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