Electronic Journal of University of Aden for Basic and Applied Sciences

EJUA-BA Vol. 5 No. 1 (2024)

https://doi.org/10.47372/ejua-ba.2024.1.330

ISSN: 2708-0684

RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF *TRIBULUS TERRESTRIS* L. AGAINST *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS*

Asma K. A. Bin Obedaan[1], Aya T. S. Farea[1], Bushra B. O. Abd rabbo[1], Fadwa A. A. Albayti[1], Nahwand A. A. Al-Hanaka[1], Omaima A. A. Ba abbad[1], Reem S. M. Awadh[1], Saleh N. H. Ali[1], Nazeeh M. Al-Abd[2], Khalid S. Ali[3], & Othman S. S. Al-Hawshabi[1,*]

[1] Department of Biology, Faculty of Science, University of Aden, Yemen
[2] Department of Microbiology, Faculty of Medicine, University of Aden, Yemen
[3] Department of Chemistry, Faculty of Pharmacy, University of Aden, Yemen

***Corresponding author: Othman S. S. Al-Hawshabi; E-mail: othmanhammod773@yahoo.com**

Received: 23 February 2024 / Accepted: 14 March 2024 / Published online: 31 March 2024

Abstract

The experiment was performed on the plant *Tribulus terrestris* L. which was collected from Khobar region in Al Dhale' Governorate, Republic of Yemen on March. The extracts used (aqueous and methanol) were prepared in Lab of Pharmacognosy, Faculty of Pharmacy – Aden University. The experiment was done on two types of bacteria, one Gram negative (*Escherichia coli*) and one Gram positive (*Staphylococcus aureus*). The antimicrobial part of the experiment was carried out in the Microbiology Lab - University of Science and Technology, Aden, Yemen. The experiment was performed in triplicates, and using three factors (Type of solvent, Time and Concentrations). The results obtained were the following: The best solvent for extracting antibacterial substances was methanol in both bacteria (*E. coli* and *S. aureus*). The concentrations that produced the highest inhibition zones in *E. coli* were 600 and 800 mg/ml using well method, while in *S. aureus* the best concentrations were 400 and 600 mg /ml. The best result was obtained after 72 h using disk method on *S. aureus*, and using well method on *E. coli*. Interaction between concentrations and type of solvent showed that the best result was obtained by methanol extract using disk method at 200 mg/ml against *E. coli*, while the best result against *S. aureus* was obtained by methanol extract using disk method at 800 mg/ml. Interaction between different times and type of solvent indicated that the best result against *E. coli* was observed after 72h by aqueous extract using well method, while the best result against *S. aureus* was observed after 72h by methanol extract using disk method. Interaction between concentrations and different times showed that the best result was obtained after 72h at concentration 800mg/ml using well method against *E. coli*, while the best result against *S. aureus* was after 72h at concentration 400mg/ml using disk method. Interaction between type of solvent, concentrations and different times indicated that the best result in *E. coli* was observed after 72h by methanol extract at 200mg/ml using disk method, while the best result in *S. aureus* was observed after 72h by aqueous extract at 600mg/ml using disk method.

Keywords: Antibacterial activity, Aqueous extract, Methanol extract, *Tribulus*.

1. Introduction

Medicinal plants have been a major source of treatment for human diseases since time immemorial. One fourth of the world population i.e. 1.42 billion people are dependent on traditional medicines, particularly plant drugs for curing ailments [1]. Herbal medicines are promising choice over modern synthetic drugs. They show minimum/no side effects and are considered to be safe. Generally herbal formulations involve the use of fresh or dried plant parts. Correct knowledge of such crude drugs is very important aspect in preparation, safety and efficacy of the herbal product [2]. The use of different parts of the medicinal plant in traditional system to treat various ailments for several centuries. Medicinal plants act as an alternative source for treating several ailments since their usage is increasing day by day [3].

The medicinal value of plants drug is due to the presence of some chemical substance in the plant tissue which produce a definite physiological action on the human body [4]. Hence, preparation and administration of drugs should be done by experts only. Drugs may be obtained from various parts of the plant, several medicinal plants have been tried against pathogenic microorganisms, there is need to explore natural herbs with probable antimicrobial potential against dangerous burn microbes [5].

Approximately 72000 plant species were estimated to have medicinal properties [6]. Yemen has a rich diversity of plants that are being used by local communities for medicinal purposes, 50% of Yemen's population is dependent on traditional medicine for their primary health care, and more than 500 plant species are used in Yemen in the treatment of diseases [7].

Tribulus terrestris is widely distributed in many tropical and moderate areas including the Mediterranean region, and throughout Africa and Asia [8 & 9]. *T. terrestris* exhibited good antibacterial activity against many bacteria and is used in folk medicine as a tonic, aphrodisiac, analgesic, astringent, stomachic, antihypertensive, diuretic, lithotriptic, urinary anti-infective, eye trouble, edema, skin disorders, kidney stones and increasing testosterone through increasing luteinizing hormone [10 - 13].

Most of the plant's parts are used as therapeutic agents, with its leaves used for the treatment of different kinds of wounds, and the fruits applied to treat eye disorders, abdominal disease, and vitiligo [9]. Extracts from the full plant are used as anti-bacterial, anti-virus, anti-inflammation, and immunostimulant [14]. In India, the fruits have been used in the treatment of infertility, and impotence. In Sudan, *T. terrestris* has been used to treat nephritis and other inflammatory disorders [15].

The methanolic extract of *T. terrestris* showed a non-significant effect on *Staphylococcus aureus* and *E. coli* [16]. The whole plant of *T. terrestris* by using methanol extract (200 µg/ml and 400 µg/ml, the results showed the methanol extract at concentrations of 200 and 400, giving inhibition zones 18 mm and 21 mm against *S. aureus* respectively, while against *E. coli* 20 mm and 23 mm respectively [17].

Observed [12] that the methanol extract of *T. terrestris* showed antibacterial activity against *E. coli* and *S. aureus*, producing inhibition zone diameters of 17.3 and 23.2 mm respectively.[18] studied on antimicrobial effect of *an* aqueous extract of *T. terrestris* against some species of bacteria, which showed good antibacterial activity against *E. coli*, with an inhibition zone diameter of 22 mm.

Observed [13] that the Ethanol extract of *T. terrestris* with concentrations of 50 mg/ml and 100 mg/ml, the ethanol extract showed high activity against *S. aureus* with inhibition zone 21.6 mm and 24.2 mm respectively, while the activity against *E. coli* produced inhibition zone 21.3 mm and 24.2 mm respectively. The results showed that the antibacterial activity of this extract was significantly greater than negative control. The total ash value, moisture content, and loss on drying of the fruits of *T. terrestris* were 9.31%, 10.75% and 31.67% respectively, while the extractive values of methanol and water extract were 5.49% and 15.5% respectively [19].

Found [20] that the total ash value and water extractive values of *T. terrestris* were 9% and 134 mg/g respectively. In a study on the antibacterial activity of *T. terrestris*, where the result showed that methanol extract had the highest inhibition zone against *E. coli* and *S. aureus* [15].

[21] study of antimicrobial activity from parts of leaves and fruits of *T. terrestris* against *S. aureus*, which produced inhibition zone diameters 17 mm and 18mm respectively.

The aqueous extract of *T. terrestris* showed no activity against *E. coli*, but showed moderate activity against *S. aureus*, producing inhibition zone diameter 15 mm, compared to 25 mm produced by antibiotic streptomycin [22].

Obtained [23] that the total ash, moisture content and loss on drying of the fruits of *T. terrestris* were 8.34%, 5.0% and 6.26% respectively.

The *T. terrestris* plant from Iran was reported that the methanol extract prepared from different plant parts (stem, leaves and fruit) showed significant antimicrobial activity against *E. coli*, in concentrations of 2 to 4 mg/ml [24].

Observed [11] that methanol and aqueous extracts of fruit of *T. terrestris* plant were tested for their antibacterial activity, where the methanol extract with a concentration of 40 mg/ml showed the best inhibitory effect against *S. aureus*, with an inhibition zone of 26 mm, while the methanol extract showed no inhibitory effect on *E. coli*. The aqueous extract had no activity against both *S . aureus* and *E. coli*. In a study on the antimicrobial activities of ethanol extract of the whole plant of *T. terrestris* against *S. aureus* and *E. coli*, showed inhibition zone diameters of 11.5 mm and 10 mm respectively [25].

Given the immense potential of plants as a source of antimicrobial preparations, the present study was carried out to evaluate the antibacterial activities of aqueous and methanol extracts of the aerial parts of Yemeni *Tribulus terrestris* against one gram-positive bacteria species; *Staphylococcus aureus* and one gram-negative bacteria species; *Escherichia coli*.

2. Materials & Methods

2.1. Plant Material:

2.1.1. Collection of plant materials and Identification:

Aerial parts (leaves, branches and fruits) of *Tribulus terrestris* L. were collected on 29th March 2022 from Khobar region in Al Dhale' Governorate, Republic of Yemen. The plant was identified by Prof. Dr. Othman Al-Hawshabi, Biology Department, Faculty of Science, University of Aden, Yemen.

2.1.2. Preparation of plant materials:

The collected aerial parts of *T. terrestris* were separated from the whole plant. The plant was air-dried thoroughly under a shaded area (at room temperature) for 7-10 days to avoid direct loss of phytoconstituents from sunlight. The air-dried material was powdered using a mechanical blender. It was then homogenized to fine powder and stored in an airtight bottle at room temperature for further analysis.

2.2. The Methods:

2.2.1. Physicochemical Investigations:

Powder of aerial parts of *T. terrestris* was used for determination of physicochemical parameters such as moisture content and total ash values.

2.2.1.1. Determination of Moisture content:

To determine the percentage of water content in sample the water is evaporated under high temperature:

1. Weigh 5 g of whole plant powder immediately and record as wet weight of sample.
2. Dry the sample at temperature not exceeding 135°C using the suitable drying equipment for 1 hour.
3. Allow the sample to cool down in a desiccator.
4. Weigh the cooled sample again without delay and record as the ″ dry weight of sample ″
5. The moisture content was calculated using the following equation:

$$W\% = \frac{A-B}{C} \times 100$$

W%= Percentage of moisture in the sample A= Wet weight of sample with crucible. B= Dry weight of sample with crucible C= Wet weight of sample

2.2.1.2. Determination of Total Ash:

To determine the ash content of sample using muffle furnace and express as the percentage of the dry sample.

1. Place 5 g of whole plant powder, accurately weighted, in a suitable dish (e.g. of silica or platinum), previously ignited, cooled and weighed.
2. Incinerate the material by gradually increasing the heat, not exceeding 450°C for 4 hours in a muffle furnace, until it is white, indicating the absence of carbon.
3. Cool down in a desiccator then weigh the sample.
   - calculate the content in mg of ash per g of air dried material.
4. Total ash content was calculated using the following equation:

$$\text{Ash content } \% = \frac{A-B}{C} \times 100$$

A= Weigh of the sample before igniting with crucible. B = Weight of the sample after igniting with crucible. C = Wet weigh of sample.

2.2.2. Preparation of Water and Methanol extract:

94 g of air-dried and powdered plant materials was weighed and mixed with 940 ml (1:10) of 80% Methanol and 94 g of the powdered plant was mixed with 940 ml of Distilled water in a conical flask. The extracts were prepared by shaking for 24 hours at room temperature. The residue was removed by filtration through Whatman No.1 filter paper. The aqueous and methanol extracts were concentrated and dried by using the oven at 45 °C. the dried crude extracts were allowed to cool and stored at 4 °C until further analysis. The preparation of the methanol and water extracts was carried out in the Lab of Pharmacogancy, Faculty of Pharmacy – Aden University.

2.2.3. Anti-Bacterial Activity:

The experiment was carried out in the Microbiology Lab - University of Science and Technology. Antibacterial activity of aqueous and methanol extract of aerial parts of *T. terrestris* has been determined against two bacterial species; Gram Negative *E. coli* and Gram Positive *S. aureus*, which were clinically isolated samples and were identified by Dr. Nazeh M. Al-Abd Dept. Microbiology, Faculty of Medicine, through the following biochemical tests (Table 1 & 2 & Fig. 1).

Table (1): Biochemical test of *Escherichia coli*

Biochemical tests	*E. coli*
Gram's stain	Gram negative bacilli
Lactose fermentation	+
Indole	+
Methyl red	+
Voges Proskauer	-
Citrate	-
Urease	-
Lysine decarboxylase	+

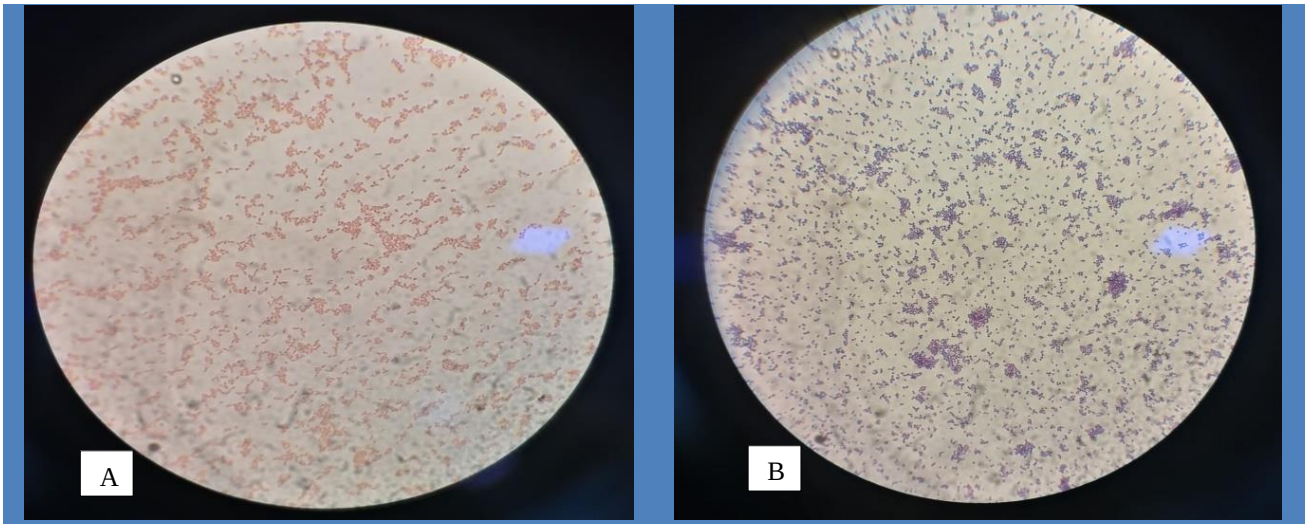

Fig. 1: Gram's Stain A= Escherichia coli **B=** Staphylococcus aureus

Table (2): Biochemical test of *Staphylococcus aureus*

Biochemical tests	*S. aureus*
Gram's stain	Gram positive cocci in clusters
Hemolysis on blood agar	Beta hemolysis
Catalase	+
Coagulase	+
DNAse	+
Mannitol salt agar	+

2.2.3.1. Preparation of Nutrient Agar:

Nutrient agar was prepared according to the manufacturer's instructions on the bottle. 28g of NA was suspended in 1000 ml of distilled water and boiled briefly to dissolve the ingredients, then sterilized by autoclaving at 121 °C for 15 minutes. The agar was allowed to cool, then was poured into sterile petri dishes.

2.2.3.2. Preparation of Mueller Hinton Agar:

The Mueller Hinton agar was prepared according to the manufacturer's instructions on the bottle. 38g of MHA was suspended in 1000 ml of distilled water and boiled briefly to dissolve the ingredients, then sterilized by autoclaving at 121 °C for 15 minutes. The agar was allowed to cool, then was poured into sterile petri dishes.

2.2.3.3. Preparation of inoculum:

Few isolated bacterial colonies were transferred to a tube of sterile normal saline and the turbidity of the bacterial suspension was adjusted to 1.5 × 10^8 CFU/ml by comparison with 0.5 McFarland standard against a sheet of white paper with black stripes. A 0.5 McFarland standard was prepared by mixing 50 µl of 1.175% barium chloride dehydrate ($BaCl_2.2H_2O$) with 9.95 ml of 1% sulfuric acid (H_2SO_4) [26].

2.2.3.4. Antibacterial Assay:

Antibacterial activity of the extracts was tested by using two methods; well diffusion and disk diffusion according to [26 & 27].

In Agar Well Diffusion Assay the Mueller Hinton agar plates were inoculated with respective bacteria (*S. aureus* and *E. coli*) and left to dry for 10 minutes. 5 wells of 5mm diameter were made on each agar plate using sterile pipette tips. The wells were labeled as test wells and control wells. The test wells were filled with 100 µl of different concentrations of the stock solution prepared from the extracts. The concentrations used were (200, 400, 600 and 800 mg/ml). the control wells were filled with distilled water for the plates containing the aqueous extract and methanol for the methanol extracts. The antibiotic Penicillin (1g/ml) was used as a positive control.

In Disk Diffusion Assay disks were cut from Whatman No.1 filter paper using a previously sterilized hole puncher, the paper disks were then also sterilized by oven for 30 minutes. The blank disks were soaked in 100 µl of the different concentrations of the extracts (200, 400, 600 and 800 mg/ml) in sterile petri dishes. MHA plates were inoculated with respective bacteria and left to dry for 10 minutes. Disks containing different concentrations were put on the surface of each agar plate using sterile forceps. A disk of Penicillin (1g/ml) was used as positive control.

The antibacterial activity of the aqueous and methanol extracts of aerial parts of *T. terrestris* was determined after 24- and 72-hours following incubation of plates at 37 °C, by measuring the diameter of the inhibition zone (in mm) around both the wells and disks using a ruler. All experiments were performed at least in triplicates by using three factors (type of solvent, time and concentrations). The assay was repeated twice.

2.3. Statistical Analysis:

Statistical analysis of the obtained results were carried out by using mean values for (loss on drying, total ash and extractive value) and Completely Randomized Design by using computer according to Genstate 5 release 3.2. Differences between means were compared using L.S.D. method at significant level 5% for antibacterial activity of all treatments.

3. Results and Discussion

3.1. Physiochemical parameters:

According to Table 3, the percentage of moisture content value of *Tribulus terrestris* was found to be 8.4%. The determination of the moisture content in drugs is valuable to determine their quality. A high moisture content in pharmaceutical substances is often undesirable and can lead to the growth of different microorganisms like bacteria and fungi which cause the deterioration of drugs [23]. The results were in disagreement with [19 & 20].

The total ash value of the plant was 15.88% according to Table 3. Total ash values are used to estimate the percentage of inorganic salts present in the drug and to determine purity and quality [19]. The results were in disagreement with [19 & 20]. Extractive values for water and methanol extracts were 12.44% and 11.69% respectively according to Table 3. Estimation of extractive values is necessary to determine the amount of active constituents in a given amount of plant material when extracted with solvent, and gives an idea about the nature of the chemical constituents according to their solubility [28]. The water-soluble extractive value indicates the presence of sugar, acids and inorganic compounds, while the alcohol soluble extractive value indicates the presence of polar constituents like phenols, alkaloids, steroids, glycosides and flavonoids [29]. The results for extractive value of water extract were in agreement with [19 & 23], while the results were in disagreement with [19] about methanol extractive value.

Table (3): Physicochemical parameters of aerial parts of *Tribulus terrestris*

Parameters	Percentages %	Yield extractive (gms)
Moisture content	8.4	-
Total ash	15.88	-
Water extractive value	12.44	11.7
Methanol extractive value	11.69	10.99

3.2. Anti-Bacterial activity:

Results in Tables (4-10) illustrated the antibacterial activity of aqueous and methanol extracts of aerial parts of *T. terrestris*. The antibacterial activity was determined by the presence or absence of an inhibition zone around the disks and wells. All the used extracts demonstrated considerable antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*.

3.2.1. Escherichia coli:

Effect of type of solvent shown in (Table 4) demonstrated that aqueous extract by using well method showed a higher activity on *E. coli* than the methanol extract, with the aqueous extract producing inhibition zone diameter 9.44 mm compared to 8.87mm produced by methanol extract, with no significant difference between them, these results were in agreement with [15]. On the other hand, the methanol extract by disk method showed higher activity on *E. coli* with inhibition zone diameter 11.94 mm, compared which produced the aqueous extract with inhibition zone diameter 6.43 mm, with no significant difference between them, these results were in agreement with [25] who studied the same species of plant, but used ethanol extract. Table 4, the results were in agreement with [16] Effect at increasing concentrations observed in Table 5 indicated that both extracts (aqueous and methanol) by well method showed moderate activity on *E. coli*, with the highest activity observed at concentrations 800 and 600 mg/ml, producing inhibition zone diameters 9.75 and 9.63 mm respectively. No significant difference was observed compared with the rest of the concentrations (400 and 200 mg/ml) which produced inhibition zone diameters 9.45 and 7.10 respectively.

Similarly, the results of the disk method obtained from (Table 5) showed that all concentrations had no significant difference between them. The highest activity on *E. coli* was produced at concentrations 200 and 800 mg/ml, with inhibition zone diameters 9.51 and 8.73 mm respectively. Penicillin exhibited higher activity than both extracts (aqueous and methanol) at all concentrations using both well and disk methods (Table 5) with inhibition zone diameters 19 and 21 mm respectively. The positive control penicillin showed increasing inhibition zone significantly compared with all concentrations by using both methods. Effect of time illustrated in (Table 6) indicated that the activity against *E. coli* using both methods (well and disk) after 72h was higher, producing inhibition zone diameter 15.14 and 12.39 mm respectively, with significant increasing compared with activity after 24h.

Interaction between concentration and type of solvent in (Table 7) indicated that both extracts (aqueous and methanol) showed moderate activity against *E. coli* using the well method, with concentrations 600 and 800mg/ml producing inhibition zone with diameters 10 and 9.75 mm respectively by aqueous extract. Also, the methanol extract showed higher activity against *E. coli*, producing inhibition zone diameter 9.75 mm at both concentration (400 and 600 mg/ml), with no significant difference compared with the remaining interactions,

On the other hand, the methanol extract showed stronger inhibition of *E. coli* using the disk method than the aqueous extract. The methanol extract at 200 and 800 mg/ml produced the highest inhibition zone diameters 14.78 and 13.25 mm respectively, with no significant difference compared with the rest of the interactions between concentration and type of solvent. Table 7. Interaction between different times and type of solvent obtained from (Table 8) indicated that both aqueous and methanol extracts by well method showed high activity against *E. coli* after 72h, producing inhibition zone diameters 15.72 and 14.57 mm respectively compared to the activity after 24h, with no significant difference compared with the remaining interactions, these results were in agreement with [17] on *E. coli*, while they were in disagreement with [24] where he used different parts of the plant *T. terrestris* and showed significant antibacterial activity against *E. coli*.

Likewise, (Table 8) demonstrated that the activity against *E. coli* in both extracts (aqueous and methanol) by disk method was also higher after 72 h than at 24 h, with the highest inhibition zone diameter 15.42 mm produced by methanol extract, with no significant difference compared with the remaining interactions between different times and type of solvent. Interaction between concentrations and different times in (Table 9) indicated that activity against *E. coli* was higher after 72h than at 24h using well method, with concentrations 800 and 600mg/ml producing the highest inhibition zone diameters 19.50 and 19.25 mm respectively, with significant increasing compared with rest of the concentrations at 72h as well as all of the concentrations at 24h. No inhibition zone was observed after 24h.

Similarly, (Table 9) demonstrated that the activity against *E. coli* using disk method was also higher after 72h, with highest activity shown at concentration 200 mg/ml producing inhibition zone diameter 15 mm, with significant increasing compared with rest of the concentrations at 72h as well as all of the concentrations at 24h. Interaction between type solvent, concentration and different time obtained from (Table 10) indicated that the highest activity on *E. coli* was produced using well method by aqueous extract at 600 mg/ml after 72h, with inhibition zone diameter 20mm, with no significant difference compared with the rest interactions. On the other hand, the highest activity in disk method was produced by methanol extract at 200 mg/ml after 72h, with inhibition zone diameter 21.50mm with no significant difference compared with the rest interactions.

3.2.2. Staphylococcus aureus:

Effect of type of solvent shown in (Table 4) indicated that aqueous extract by using well method had a higher activity on *S. aureus* with inhibition zone diameter 9.48 mm, compared with the methanol extract which produced inhibition zone diameter 6.02mm, with no significant difference between them. On the other hand, the methanol extract by disk method demonstrated higher activity on *S. aureus* than aqueous extract, with inhibition zone diameter 13.85 mm compared to 10.51 mm produced by aqueous extract, with no significant difference between them. Table 4. Effect at increasing concentrations observed in (Table 5) illustrated that both extracts (aqueous and methanol) by well method showed weak activity against *S. aureus*, with the highest activity observed at concentrations 200 and 400 mg/ml, producing inhibition zone diameters 7.62 and 7.56 mm respectively, with significant increasing compared with the rest of concentrations. On the other hand, the results of the disk method observed at Table 5 indicated that both extracts (aqueous and methanol) showed high activity against *S. aureus*, with the highest activity produced at concentrations 400 and 600 mg/ml, with inhibition zone diameters 13.41and 12.25 mm respectively, with no significant difference compared with the remaining concentrations.

The positive control Penicillin exhibited significant against *S. aureus* result compared with both extracts (aqueous and methanol) at all concentrations using both well and disk methods, with inhibition zone diameter 25.5mm in both methods. Effect of time observed in (Table 6) indicated that the activity against E. coli using both methods (well and disk) after 72h was higher, producing inhibition zone diameters 11.25 and 17.30 mm respectively, with significant increasing compared with activity after 24h. Interaction between concentrations and type of solvent in (Table 7) illustrated that the aqueous extract by the well method showed moderate activity against *Staphylococcus aureus*, with concentrations 400 and 200 mg/ml producing inhibition zone diameters 10 and 9.75mm respectively, with significant increasing compared with rest of the concentrations in aqueous extract as well as all of the concentrations in methanol extract.

On the other hand, the methanol extract by disk method showed the highest activity against *S. aureus* at 800 mg/ml compared with the rest of concentrations in both extracts, producing inhibition zone diameter 16.60 mm, with significant increasing compared with the aqueous extract at 200 and 800 mg/ml, these results were in agreement with [17] on the same test organism. Interaction between different times and type of solvent observed in (Table 8), indicated that aqueous extract by well method showed the highest activity against *S. aureus* after 72h than at 24 h, producing inhibition zone diameter 14.71 mm, with significant increasing compared with aqueous extract at 24h as well as methanol extract at both 72h and 24h.

On the other hand, (Table 8) demonstrated that the activity against *S. aureus* by disk method in both extracts (aqueous and methanol) was higher after 72 h than at 24

h, with the inhibition zone diameters 16.77 and 17.83 mm respectively, with significant increasing compared with the aqueous and methanol extracts after 24h. Interaction between concentrations and different times in (Table 9) showed that the activity against *S. aureus* using well method was the highest after 72h at 200 and 400 mg/ml producing inhibition zone diameters 15.25 and 15.13 mm respectively, with significant increasing compared with the rest of the concentrations at 72h as well as all of the concentrations at 24h.

Furthermore, (Table 9) illustrated that the activity against *S. aureus* using disk method was highest after 72h, with concentration 400mg/ml producing inhibition zone diameter 22.75 mm, with significant increasing compared with concentrations 200 and 800 mg/ml after 72h as well as all concentrations after 24h. Interaction between type solvent, concentration and type different time observed in (Table 10), demonstrated that the highest activity on *S. aureus* using well method was produced by aqueous extract at 400 mg/ml after 72h, with inhibition zone diameter 20mm, with increasing significant difference compared with the remaining interactions. On the other hand, the highest activity using disk method was produced by aqueous extract at 600 and 400 mg/ml after 72h, with inhibition zone diameters 24.85 and 24mm respectively, and by methanol extract at 800, 400 and 200 mg/ml after 72h, with inhibition zone diameters 21.50, 21.50 and 20.50 mm respectively, with increasing significant difference compared with the remaining interactions.

Table (4): Antibacterial activity of aqueous and methanol extracts of aerial part of *Tribulus terrestris* by agar Well diffusion and paper Disk methods

S. No.	Type of solvent	Test Organism			
		Escherichia coli		*Staphylococcus aureus*	
		Diameters of zones of inhibition (in mm)			
		Well	Disk	Well	Disk
1	ME	8.87	11.94	6.02	13.85
2	AE	9.44	6.43	9.48	10.51
L.S.D at 5%		NS	NS	NS	NS

AE = AQUEOUS EXTRACT; **ME** = Methanol EXTRACT; **NS** = NOT SIGNIFICANT; **LSD** = Least Significant Differences at 5% level

Table (5): Antibacterial activity of different concentrations of aqueous and methanol extracts of aerial parts *Tribulus terrestris* by Agar WELL diffusion and paper DISK methods.

S. No.	Concentrations Mg/ml	Test Organism			
		Escherichia coli		*Staphylococcus aureus*	
		Diameters of zones of inhibition (in mm)			
		Well	Disk	Well	Disk
1	Con. Negative	0.00	0.00	0.00	0.00
2	Con. Positive	19	21	25.5	25.5
3	200	7.1	9.51	7.62	10.04
4	400	9.45	8.13	7.56	13.41
5	600	9.63	7.74	3.12	12.25
6	800	9.75	8.73	2.69	11.86
L.S.D at 5%		**2.97**	**6.3**	**2.36**	**4.87**

Control Negative = same solvent; **Control positive** = antibiotic of penicillin; **LSD** = Least Significant Differences at 5% level

Table (6): Antibacterial activity of different times of aqueous and methanol extracts of aerial parts of *Tribulus terrestris* by Agar WELL diffusion and Paper DISK methods

S. No.	Time	Test Organism			
		Escherichia coli		*Staphylococcus aureus*	
		Diameters of zones of inhibition (in mm)			
		Well	Disk	Well	Disk
1	24 h	3.17	5.98	4.25	7.05
2	72 h	15.17	12.39	11.25	17.3
LSD at 5%		**1.66**	**2.82**	**1.01**	**1.36**

Table (7): Antibacterial activity of interaction between concentrations and type of solvent of aqueous and methanol extracts of aerial parts of *Tribulus terrestris* by Agar WELL diffusion and Paper DISK methods

S. No.	Concentrations Mg/ml	Test Organism							
		Escherichia coli				*Staphylococcus aureus*			
		Diameters of zones of inhibition (in mm)							
		Well		Disk		Well		Disk	
		AE	ME	AE	ME	AE	ME	AE	ME
1	Con. Negative	0	0	0	0	0	0	0	0
2	Con. Positive	19	19	21	21	25.5	25.5	25.5	25.5
3	200	8.75	5.45	4.25	14.78	9.75	5.5	6	14.08
4	400	9.16	9.75	5	11.25	10	5.13	12	14.83
5	600	10	9.25	4.11	11.38	6.25	0 *	12.43	12.08
6	800	9.75	9.75	4.21	13.25	5.38	0 *	7.13	16.6
LSD at 5%		NS		NS		3.12		6.6	

AE = AQUEOUS EXTRACT; **ME** = METHANOL EXTRACT; **NS** = NOT SIGNIFICANT;
Control Negative same solvent**; Control Positive** antibiotic Penicillin; **0 *** = contamination

Table (8): Antibacterial activity interaction between different times and types of solvent of aqueous and methanol extracts of aerial parts of Tribulus *terrestris* by agar Well diffusion and paper Disk methods

S. No.	Time	Test Organism							
		Escherichia coli				*Staphylococcus aureus*			
		Well		Disk		Well		Disk	
		AE	ME	AE	ME	AE	ME	AE	ME
1	24h	3.17	3.17	3.5	8.47	4.25	4.25	4.25	9.86
2	72h	15.72	14.57	9.36	15.42	14.71	7.79	16.77	17.83
LSD at 5%		NS		NS		1.5		5.36	

Table (9): Antibacterial activity interaction between concentrations and different times of aqueous and methanol extracts of aerial parts of *Tribulus terrestris* by Agar WELL diffusion and Paper DISK diffusion

S. No.	Concentrations Mg/ml	Test Organism							
		Escherichia coli				*Staphylococcus aureus*			
		Diameters of zones of inhibition (in mm)							
		Well		Disk		Well		Disk	
		24	72	24	72	24	72	24	72
1	Control Negative	0	0	0	0	0	0	0	0
2	Control Positive	19	19	21	21	25.5	25.5	25.5	25.5
3	200	0	14.2	4.03	15	0	15.25	3.83	16.25
4	400	0	18.91	4	12.25	0	15.13	4.08	22.75
5	600	0	19.25	3.13	12.36	0	6.25	3.08	21.42
6	800	0	19.5	3.75	13.71	0	5.38	5.85	17.87
LSD at 5%		3.89		NS		2.77		5.21	

NS = NOT SIGNIFICANT
Control Negative same solvent**; Control Positive** antibiotic Penicillin

Table (10): Antibacterial activity interaction between type of solvent and concentrations and different times of aqueous and methanol extracts of aerial parts of *Tribulus terrestris* by Agar WELL diffusion and Paper DISK diffusion methods

S. No.	Type of solvent	Concentrations Mg/ml	Test Organism							
			Escherichia coli				*Staphylococcus aureus*			
			Diameters of zones of inhibition (in mm)							
			Well		Disk		Well		Disk	
			24	72	24	72	24	72	24	72
1	AE	Control Negative	0	0	0	0	0	0	0	0
2		Control Positive	19	19	21	21	25.50	25.50	25.50	25.50
3		200	0	17.50	0	8.50	0	19.50	0	12
4		400	0	18.31	0	10	0	20	0	24
5		600	0	20	0	8.23	0	12.50	0	24.85
6		800	0	19.5	0	8.43	0	10.75	0	14.25
7	ME	Control Negative	0	0	0	0	0	0	0	0
8		Control Positive	19	19	21	21	25.50	25.50	25.50	25.50
9		200	0	10.90	8.05	21.50	0	11	7.65	20.50
10		400	0	19.50	8	14.50	0	10.25	8.15	21.50
11		600	0	18.50	6.25	16.50	0	0 *	6.15	18
12		800	0	19.50	7.50	19	0	0 *	11.70	21.50
LSD at 5%			NS		NS		3.77		7.12	

NS = NOT SIGNIFICANT
Control Negative same solvent**; Control Positive** antibiotic Penicillin; **0 *** = contamination

3.2.3. Compare between the effect of Well and Disk methods on antibacterial activities:

Table 4 showed that the disk method using the methanol extract had higher activity than the well method against both bacteria (*E. coli* and *S. aureus*), with inhibition zone diameters 11.94 and 13.85 mm respectively. Table 5 demonstrated that the disk method had higher activity on *S. aureus* than the well method, with all concentrations producing inhibition zone diameters 10.04, 13.41, 12.25 and 11.86 mm at concentrations 200,400, 600 and 800 mg/ml respectively. Data obtained from Table 6 indicated that the disk method had higher activity on *S. aureus* than the well method, with inhibition zone diameter 17.30 mm after 72 hours. On the other hand, the well method had higher activity on *E. coli* compared with the disk method, producing inhibition zone diameter 15.14 mm after 72 hours. Table 7 illustrated that the disk method using the methanol extract had higher activity against both bacteria than the well method, with all concentrations.

Data observed in Table 8 showed that the disk method had higher activity against S. aureus using than the well method, with methanol extract producing the highest inhibition zone diameter 17.83 mm after 72 hours. On the other hand, both the disk and well method had similar activity against *E. coli*. Table 9 indicated that the well method had higher activity on *E. coli* than the disk method, with concentrations at 400, 600 and 800 mg/ml , producing inhibition zone diameters 18.91 , 19.25 and 19.50 mm respectively after 72 hours. On the other hand, the disk method had higher activity against *S. aureus* than the well method, with all concentrations after 72 hours. Table 10 indicated that disk method had higher activity on methanol extract against *E. coli* at concentration 200 mg / ml producing inhibition zone 21.50 mm after 72h , all concentrations against *s. aureus* after 72h .

3.2.4. Correlation between time and inhibition zone

The results showed direct and significant correlation between time and inhibition zone in both extracts (aqueous and methanol) against both types of bacteria (*E. coli* and *S. aureus*), where the increase in time was accompanied by an increase in the zone of inhibition diameter (Fig. 2).

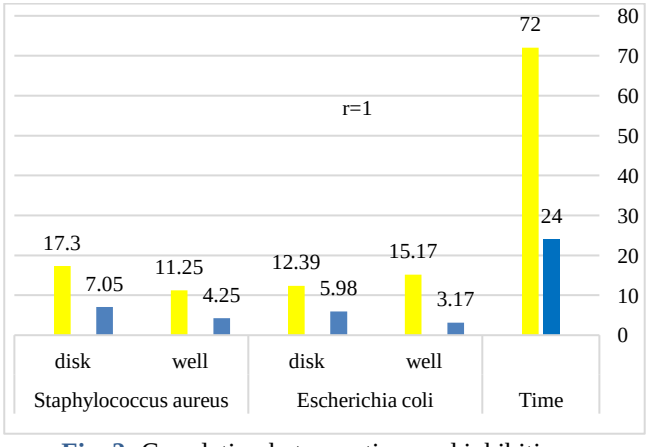

Fig. 2: Correlation between time and inhibition zone

3.2.5. Sensitivity test of studied bacteria (E. coli and S. aureus) against some used antibiotics:

Data obtained from Table 11 illustrates the sensitivity of *E. coli* and *S. aureus* against some antibiotics (Ceftriaxone, Vancomycin, Ofloxacin and Lincomycin). The inhibition zone diameters in *E. coli* were 23, 11, 15 and 10 mm respectively, while they were 14, 15 and 18 mm respectively in *S. aureus*. *S. aureus* was resistant to Lincomycin.

Table (11): Sensitivity test of studied bacteria against some used antibiotics

Antibiotics	Test Organism	
	Escherichia coli	*Staphylococcus aureus*
	Diameters of zones of inhibition (in mm)	
Ceftriaxone (30 mg/disk)	23 mm	14 mm
Vancomycin (30 mg/disk	11 mm	15 mm
Ofloxacin (5 mg/disk)	15 mm	18 mm
Lincomycin (2mg/disk)	10 mm	-

Results obtained from Tables (4 - 10) indicate that *S. aureus* was more susceptible than *E. coli* to both extracts (aqueous and methanol), with wider inhibition zone diameters produced in *S. aureus*. This could be explained by the fact that gram positive bacteria (*S. aureus*) lack an outer membrane (Lipopolysaccharide) which facilitate access of cell-wall active agents to their site of action (the peptidoglycan), while gram negative bacteria (*E. coli*) have the outer LPS membrane that protects them from their surrounding environment, and can selectively keep antibiotic drugs from entering. This made gram negative bacteria more resistant to antibiotics than gram positive ones. [30]. The methanol extract showed higher antibacterial activity compared with the aqueous extract because the methanol had higher concentration of active antibacterial agents. *Tribulus terrestris* contains biologically active substances such as steroids, saponins, flavonoids, alkaloids. This class of compounds has a wide range of biological activates as anti-inflammatory, antimicrobial, antifungal, anticancer and other benefits. Among these compounds, steroidal and triterpenoid saponins have long been known as components of widely used herbal drugs and pharmaceutical preparation [22].

Conclusions

Tribulus terrestris is a medicinal plant widely used in Yemen and other countries to treat various ailments. According to the results obtained from our study, *T. terrestris* has promising potential as a source of antibacterial agents.

Recommindations

- Further study on the studied plant species as well as other species belonging to *Tribulus* is required to detect the activity of plant parts (Branches, leaves and fruits) in order to determine which species and parts are more effective.
- Further study is required to isolate the compounds responsible for the antibacterial activity.

References

[1] S. Kala; M. Johnson; N. Janakiraman; A. Anto Arockiaraj; S. Iyan Raj & B. Dorin. Pharmacognostic and phytochemical studies on some selected ethno-medicinal plants of Tamilnadu, South India. Int. J. Med. Arom. Plants, 1(2): 89-94, 2011.

[2] D. Nivedithadevi & R. Somasundaram. Pharmacognostical and Qualitative Phytochemical Studies on the aerial parts of *Tephrosia purpurea* L. International J. of Research in Biological Sciences, 2(2): 48-53, 2012.

[3] H. Akhtar. Antioxidant potential of dried *Enicostemma littorale*. Pak J. Bio. Sci. 14(20): 956-957, 2011.

[4] J. B. Harborne. Phytochemical Methods. London: Chapman and Hall Ltd; 52-105 p. 1996.

[5] A. C. Farnsworth. The role of Ethnopharmacolgy Drug Development from Plants. England Ciba: John Wiley and Sons; 2-10 p., 1982.

[6] U. Schippmann; D. Leaman & A. B. Cunningham. A Comparison of Cultivation and Wild Collection of Medicinal AND Aromatic Plants Under Sustainability Aspects. Medicinal and Aromatic Plants, 75-95, 2006.

[7] A. S. Al-Dubaie & A. A. Al-Khulaidi. Medicinal and Aromatic Plants of Yemen. Obadi Center for Studies and Publisher, Sana'a, Yemen (in Arabic), 2005.

[8] A. Al Maofari; Z. Mennane; A. Hakiki; M. Mosaddak & S. EL Hajjaji. Chemical Compositions and Antibacterial Activity of Different Extracts of *Tribulus terrestris* Growing in Morocco and Yemen. Der Pharma Chemica. 8(12):14-18, 2016.

[9] T. Sasikala ; R. Prabakaran & S. Sabitha. Antimicrobial Activities of *Tribulus terrestris* L. on Selected Pathogenic Micro-organisms. International Journal of Pharmaceutical and Phytopharmacological Research, 4(3):182-186, 2014.

[10] F. A. AL-Bayati & H. F. AL-Mola. Anti-Bacterial and Anti-Fungal Activities of Different Parts of Tribulus terrestris L. Growing in Iraq. Journal of Zhejiang University Science B. 9(2):154-159, 2008.

[11] L. A. Abdulqwi & S. A. Quadn. Evaluation of Antibacterial and Antioxidant activities of Tribulus terrestris L. Fruits. 14(1): 331-336, 2021.

[12] A. A. Hussain; A. A. Mohammed; H. H. Ibrahim & A. H. Abbas. Study the Biological Activities of Tribulus Terrestris Extracts. World Academy of Science, Engineering and Technology. 33:433- 435, 2009.

[13] S. Soleimanpour; F. S. Sedighinia; A. S. Afshar; R. Zarif & K. Ghazvini. Antibacterial activity of Tribulus terrestris and its synergistic effect with Capsella bursa-pastoris and Glycyrrhiza glabra against oral pathogens: an in-vitro study. 5(3): 210-217, 2015.

[14] A. Sultan & S. L. Rubab, S.L. *Tribulus terrestris*-An Ethnomedical & Phytochemical Review. Archive of Natural and Medicinal Chemistry. (2):1-10, 2017.

[15] W. Zhu; Y. Du; H. Meng; Y. Dong & L. Li. A Review of Traditional Pharmacological Uses, Phytochemistry, and Pharmacological Activities of *Tribulus terrestris*. Chemistry Central Journal. 11(60):1-16, 2017.

[16] B. S. Bazzaz & G. Haririzadeh. Screening of Iranian Plants for Antimicrobial Activity. Pharmaceutical Biology. 41(8):573-583, 2003.

[17] B. Baburao; G. Rajyalakshmi; A. Venkatesham; G. Kiran; A. S. Sunder & B. G. Rao. Anti-Inflammatory and Antimicrobial Activities of Methanolic Extract of *Tribulus terrestris* Linn Plant. International Journal of Chemical Sciences, 7(3):1867-1872, 2009.

[18] M. Hakemi-Vala; M. Makhmor; F. Kobarfar; M. Kamalinejad; M. Heidary & S. Khoshnood. Investigation of Antimicrobial Effect of *Tribulus terrestris* L. Against Some Gram Positive and Negative Bacteria and *Candida spp*. Novelty in Biomedicine. 2(3):85-90, 2014.

[19] S. R. Yanala & D. Sathyanarayana. Comparative standardization and physicochemical evaluation of the fruits of *Tribulus terrestris* L. from different seasonal and geographical sources. Scholars Research Library. 8(10):195-200, 2016.

[20] S. Das; N. Vasudeva & S. Sharma. Pharmacognostical evaluation of *Tribulus terrestris* L. International Journal of Pharmaceutical Sciences and Research, 8(3): 1393 – 1400, 2017.

[21] R. Naz; H. Ayub; S. Nawaz; Z. Ul Islam; T. Yasmin; A. Bano; A. Wakeel; S. Zia & T. H. Roberts. Antimicrobial Activity, Toxicity and Anti-Inflammatory Potential of Methanolic Extracts of Four Ethnomedicinal Plant Species from Punjab, Pakistan. BMC Complementary and Alternative Medicine. 17(302):1-13, 2017.

[22] M. H. Hussein. Study of antibacterial effect of *Tribulus terrestris* extract. International journal of advanced research. 6(1):469-471, 2018.

[23] A. Rauf; M. Waseem & S. Rehman. Physicochemical Standardization and Safety Evaluation of Khar-e-Khasak Khurd (Fruits of *Tribulus terrestris*): A Unani Drug. Journal of Pharmacovigilance and Drug Safety. 16(1):16-21, 2019.

[24] I. B. Semerdjieva & V. D. Zheljazkov. Chemical constituents, biological properties and uses of *Tribulus terrestris*, A review. Natural Product Communications, 1- 26, 2019.

[25] A. Khalid; A. S. Algarni; H. E. Homeida; S. Sultana; S. A. Javed; Z. U. Rehman; H. Abdalla; H. A. Alhazmi; M. Albratty & A. N. Abdalla. Phytochemical, Cytotoxic and Antimicrobial Evaluation of Tribulus terrestris L., Typha domingensis Pers., and Ricinus communis L. Evidence-Based Complementary and Alternative Medicine. 1-11, 2022.

[26] M. Balouiri; M. Sadiki & S. K. Ibnsouda. Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis. 6(2):71-79, 2015.

[27] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Test. Approved Standard M02-A10, 10th ed. CLSI, Wayne, PA, 29:1, 2009.

[28] S. S. Pawar & M. G. Jadhav. Determination of Extractive value and Phytochemical analysis of Bacopa monnieri (L), 2015.

[29] A. K. Meena; K. Sharma; V. Jain; B. Pal; K. Ajit; U. Singh; R. Singh & M. M. Rao. Physicochemical and Preliminary Phytochemical Studies on the Fruit of *Tribulus terrestris* Linn. Research Journal of Science and Technology. 2(2):31-33, 2010.

[30] Z. Breijyeh; B. Jubeh & R. Karaman. Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve it. Molecules. 25(6):1-23, 2020.

Author information

ORCID

Othman S. S. Al-Hawshabi: 0000-0002-9680-0330

مقالة بحثية

النشاط البكتيري لنبات الـقطبة *Tribulus terrestris* L. ضد *Escherichia coli* و *Staphylococcus aureus*

أسماء خالد عاشور بن عبيدان[1]، آية طلال سالم[1]، بشرى بشير عثمان عبدربه[1]، فدوى أحمد علوي البيتي[1]، نهوند عبدالوهاب أحمد[1]، اميمة عبدالكريم أحمد باعباد[1]، ريم سعيد محمد عوض[1]، صالح ناصر حسين علي[1]، نزيه محمد العبد[2]، خالد سعيد علي[3] و عثمان سعد سعيد الحوشبي[1،*]

[1] قسم علوم الحياة، كلية العلوم، جامعة عدن، اليمن
[2] قسم الميكروبيولوجي، كلية الطب، جامعة عدن، اليمن
[3] قسم الكيمياء، كلية الصيدلة، جامعة عدن، اليمن

* الباحث الممثل: عثمان سعد سعيد الحوشبي؛ البريد الالكتروني: othmanhamood773@yahoo.com

استلم في: 23 فبراير 2024 / قبل في: 14 مارس 2024 / نشر في 31 مارس 2024

المُلخّص

أجريت الدراسة على القطبة (الحسك) *Tribulus terrestris* L. من العائلة الرطراطية Zygophyllaceae الذي جمع من محافظة الضالع في منطقة خوبر خلال شهر مارس 2022، وتم عمل الاستخلاص المائي والميثانولي في مختبر قسم العقاقير لكلية الصيدلة – جامعة عدن، و من ثم أجريت الدراسة على نوعين من البكتيريا أحداهما موجبة الجرام *Staphylococcus aurous* والأخرى سالبة الجرام *Escherichia coli*. أجريت التجربة الميكروبية في مختبر الأحياء الدقيقة – جامعة العلوم والتكنولوجيا حيث أجريت تجربة عاملية ذات ثلاثة عوامل (نوع المذيب، التركيز والوقت)، وكررت كل معاملة ثلاث مرات، وكانت النتائج المتحصل عليها كالتالي: أظهر المستخلص لكلا المذيبين أكبر منطقة تثبيط ضد كلا النوعين من البكتيريا و ذلك عند التراكيز العالية 400، 600 و 800 مغ/مل و كانت أفضل النتائج بعد 72 ساعة لكلا النوعين من البكتيريا، إذ أعطت طريقة الأقراص فعالية ضد بكتيريا *Staphylococcus aureus*، بينما كانت طريقة الحفر هي الفعالة ضد بكتيريا *Escherichia coli*. التداخل بين العاملين (التراكيز المستخدمة و نوع المذيب) أظهر أفضل النتائج عند المتخلص الميثانولي باستخدام طريقة الأقراص ضد بكتيريا *Escherichia coli* عند تركيز 200 مغ/مل و 800 مغ/مل ضد بكتيريا *Staphylococcus aureus* . التداخل بين العاملين (الوقت و نوع المذيب) أظهر أفضل النتائج بعد 72 ساعة في المستخلص المائي باستخدام طريقة الحفر ضد بكتيريا *Escherichia coli*، بينما كانت أفضل نتائج ضد بكتيريا *Staphylococcus aureus* بعد 72 ساعة في المستخلص الميثانولي باستخدام طريقة الأقراص. التداخل بين العاملين (التراكيز والوقت) أظهر أفضل النتائج بعد 72 ساعة عند تركيز 800 مغ/مل باستخدام طريقة الحفر ضد بكتيريا *Escherichia coli*، بينما أظهر أفضل النتائج ضد بكتيريا *Staphylococcus aureus* بعد 72 ساعة عند تركيز 400 مغ/مل باستخدام طريقة الأقراص. تشير النتائج المتحصل عليها من التداخل بين الثلاث العوامل (نوع المذيب، التراكيز والوقت) أن المستخلص الميثانولي عند تركيز 200 مغ/مل بعد 72 ساعة باستخدام طريقة الأقراص أعلى قدرة تثبيطية ضد النوع *Escherichia coli*، بينما المستخلص المائي عند تركيز 600 مغ/مل بعد 72 ساعة أظهر أكبر فعالية ضد النوع *Staphylococcus aureus*.

الكلمات المفتاحية: النشاط البكتيري، المستخلص المائي، المستخلص الكحولي، نبات القطبة.

How to cite this article:

A. K. A. Bin Obedaan, A. T. S. Farea, B. B. O. Abd rabbo, F. A. A. Albayti, N. A. A. Al-Hanaka, O. A. A. Ba abbad, R. S. M. Awadh, S. N. H. Ali, N. M. Al-Abd, K. S. Ali, & O. S. S. Al-Hawshabi, "ANTIBACTERIAL ACTIVITY OF TRIBULUS TERRESTRIS L. AGAINST ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS", *Electron. J. Univ. Aden Basic Appl. Sci.*, vol. 5, no. 1, pp. 74-85, March. 2024. DOI: https://doi.org/10.47372/ejua-ba.2024.1.330