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THE INHIBITORY EFFECT OF TRICHODERMA CRUDE EXTRACT AGAINST SOME HUMAN PATHOGENIC BACTERIA

Ali Shamil Khayat* and Alaa Mohsin Al-Aaraji

Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

Abstract

The study aimed to evaluate the antibacterial activity of *Trichoderma* crude extract against seven human pathogenic bacteria *Escherichia coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*; *Staphylococcus epidermidis* and *Salmonella typhi*. *Trichoderma* isolates were collected from the soil of Baghdad University gardens and they were identified depending of morphological features on plate and microscopic examination. The crude extracts was extracted from *Trichoderma* by ethyl acetate with final yield of 4.3gm, and then Borntrager's test has been used to detect the production of free anthraquinones. Different concentrations (0.5, 1, 2 and 3 mg/ml) of *Trichoderma* crude extract were used against the seven human pathogenic bacteria using agar well diffusion method. The results showed that *Trichoderma* isolate 5 crude extract had high antibacterial activity than *Trichoderma* (10) isolate crude extract, *Staphylococcus aureus* exhibited high sensitive *Trichoderma* isolate 5 and 10 crude extract more than other pathogenic bacteria and the growth zone inhibition increased when concentrations increased, however Escherichia coli, *Acinetobacter baumannii* and *Salmonella typhi* Showed resistant to every concentration of *Trichoderma* isolate (5, 10) crude extract.

Key words: *Trichoderma* spp., anthraquinon, crude extract, bacteria.

Introduction

Trichoderma spp. produce many antibiotics and other chemicals that are kill pathogens or inhibit their growth (Bondaryk *et al*., 2017, Mazu *et al*., 2016). Furthermore, there are several strains of *Trichoderma* genus that have the ability to produce many enzymes such as protease, cellulase, hemicellulase and anthraquinon (Erna *et al*., 2017). The anthraquinon showed a high inhibition activity against both gram positive and gram-negative bacteria that can cause human bacterial infections (Liu *et al*., 2007). Recently, many pathogenic bacteria have resistance to antibiotic and this caused a critical problem of what to think in and beyond biomedical science, and this led researchers looking for new antibiotic agents who can inhibit bacterial growth without harming the host (Mulat *et al*., 2013). The aim this study was to evaluate the antibacterial activity of *Trichoderma* crude extract against *Escherichia coli, Acinetobacter, Acinetobacter baumannii Pseudomonas aeruginosa, proteus mirabilis, Staphylococcus aureus, Staphylococcus epidermidis and Salmonella typhi.*

Author for correspondence : E-mail: alaraji.alaa@sc.uobaghdad.edu.iq

Materials and Methods

Isolation and Identification of *Trichoderma* spp.

Forty soil samples were collected from different locations of Baghdad University gardens at a depth within (5-10 cm) using a metal spatula. The samples were kept in new polythene bags, sealed and transported to the laboratory immediately for the mycological examination. Ten gm of each soil samples were put in a 250ml conical flask containing 100ml sterile distilled water. The flasks were shaken on an electric shaker to get a homogenous suspension and serial dilutions of the soil sample such as (10^{-1}, 10^{-2} and 10^{-3}) were prepared. One ml of (10^{-3}) dilution for each sample was added to petri dish containing potato dextrose agar (PDA) medium (OXOID, England), then it was incubated at 28 ± 1°C for five days. After growing of different colonies on PDA plates, the fungal cultures were then transferred and sub-cultured to have a pure culture. *Trichoderma* isolates were diagnosed depending on the morphology of the colony and microscopic examination (morphological characteristics) according to (Samuels, 2006).

Pathogenic bacteria isolates

All pathogenic bacteria isolated were obtained from central Child Hospital in Baghdad city.

Extraction of antibacterial compounds

In this study, modified Czapek's medium was used to test the inhibitory substances of *Trichoderma* crude extract. *Trichoderma* isolates were grown on PDA for 6 days, then, two block of (10mm) in diameter from *Trichoderma* culture were added to the autoclaved prepared modified Czapek's medium (2g/l Na_2HPO_4, 1.5g/l $MgSO_4.7H_2O$, 7g/l KH_2PO4, 0.2g/l $FeCl_3$, 0.1g/l $ZnSO_4.7H_2O$, 0.1g/l $CaCl_2$, 0.5g/l $NH_4)_2SO_4$, 30g/l Glucose, 150g/l Sugarcane bagasse) in flasks and incubated in the dark for 14 days at 27 ± 2C°with shaking (Al-Rijabo and al-obaidy, 2011). For extraction of antibacterial crude extract from modified Czapek's medium 800ml of ethyl acetate (EtOAc) (HIMEDIA, India) was added and placed on a shaker at 121 rpm (overnight). The extraction was completed with a 24 hour period. Extraction of antibacterial was employed by using rotary evaporator (Gallenhamp, England) at 37°C taking into consideration the boiling point of the solvents (EtOAc, 88°C) (Liu *et al*., 2007).

Detection of *Trichoderma* isolates that produce anthraquinones

Borntrager's test has been used to detect the production of anthraquinones and all *Trichoderma* isolates subjected to this test , this achieved by adding 1ml of ethyl acetate extracts of the *Trichoderma* isolates to 2 ml of 10% ammonia with a good shake, then the presence of a pink, red, or violet color in the ammonia (lower) phase indicated the presence of anthraquinones (Kujur *et al*., 2010).

Estimation of antibacterial activity of *Trichoderma* isolates crude extracts :

In vitro antimicrobial activity of crude extracts of two *Trichoderma*isolates (one produced anthraquinones number 5 and the other non-producedanthraquinones number 10) were studied against seven pathogenic bacteria isolates (*Escherichia coli, Acinetobacter baumannii, pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, Staphylococcus epidermidis and Salmonella typhi*).

Agar Well Diffusion Method

Trichoderma isolates crude extracts (5 and 10) were diluted in 100% dimethyl sulfoxide (Sigma- Aldrich, Germany) to give different concentrations of (0.5, 1, 2, 3mg/ml.Then a well of (5mm) was made in the medium by using sterile cork-borer. 100µl of each concentration of the crude extract was transferred into separate wells. Dimethyl sulfoxide was used as a negative control. Plates are incubated at 28 ± 2°C for 24 hours before determining results. The diameter of the inhibition zone was recorded for each replicate and the average diameter was calculated.

Results and Discussion

From a total of 40 soil samples cultures, we obtained thirteen *Trichoderma* isolates according to morphological identification. The results of Borntrager's Test showed

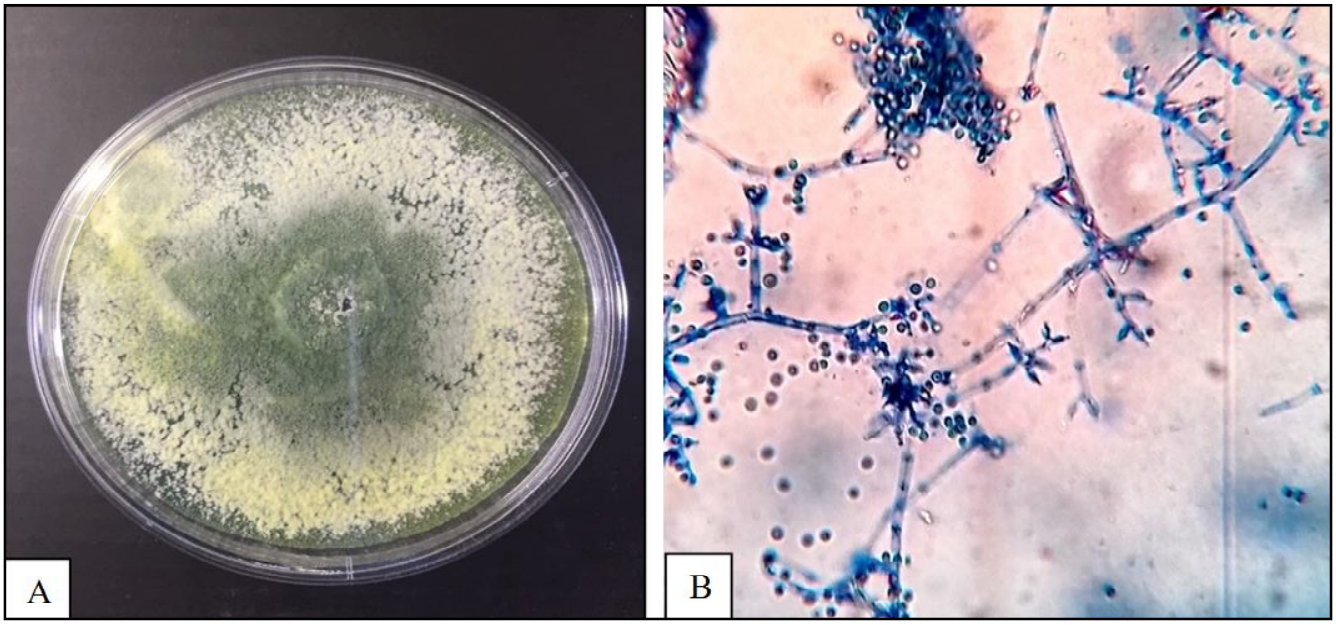

Fig. 1: (A): *Trichoderma* grown on PDA at 25 ± 2°C after 7 days of incubation, **(B):** Microscopic features of *Trichoderma* fungi with lacto phenol cotton blue (40X).

Table 1: Borntrager's test results for *Trichoderma* species.

Trichoderma **isolates**	**Borntrager's Test**
Trichoderma (1)	Negative
Trichoderma (2)	Positive
Trichoderma (3)	Positive
Trichoderma (4)	Negative
Trichoderma (5)	Positive
Trichoderma (6)	Positive
Trichoderma (7)	Negative
Trichoderma (8)	Positive
Trichoderma (9)	Negative
Trichoderma (10)	Negative
Trichoderma (11)	Negative
Trichoderma (12)	Negative
Trichoderma (13)	Negative

that five *Trichoderma* isolates produce anthraquinon, this isolates were (2, 3, 5, 6 and 8) table 1 and the other eight *Trichoderma isolates* (1, 4, 7, 9, 10, 11, 12, 13) did not produce anthraquinons.

Estimation of antibacterial activity of *Trichoderma* crude extract against pathogenic bacteria using agar well diffusion method:

After crude extract concentrated using rotary evaporator, the final quantity of ethyl acetate crude extract of *Trichoderma* isolates (5 and 10) was (3.8 gm) and (3.6 gm) respectively. The results showed that the growth inhibition zone of pathogenic bacteria increased with increasing of *Trichoderma* isolates crude extract concentrations. Crude extract of *Trichoderma* isolate 5 atconcentration (0.5)mg/ml showed high growth inhibition zone against *Staphylococcus aureus* recorded **(**10mm), and this growth zone inhibition increased to (11, 14 and 18 mm) when concentrations increased to (1, 2 and 3mg/ml) respectively (Fig. 5), while growth zone inhibition of *Staphylococcus epiderms* at the concentration (0.5, 1, 2 and 3mg/ml) was (9, 10, 11 and 14) respectively (Fig. 5), growth zone inhibition of *Proteus mirabilis* at the concentration (0.5, 1, 2 and 3mg/ml) was (5, 6, 11 and 11) respectively (Fig. 4.) and growth zone inhibition of *Pseudomonas aeruginosa* at the concentration (0.5, 1, 2 and 3mg/ml) was (0, 5, 7 and 9) respectively (Fig. 4). *Escherichia coli*, *Acinetobacter baumannii* and *Salmonella typhi* have been showed resistant to all concentration of *Trichoderma* (5) crude extract (Fig. 3).

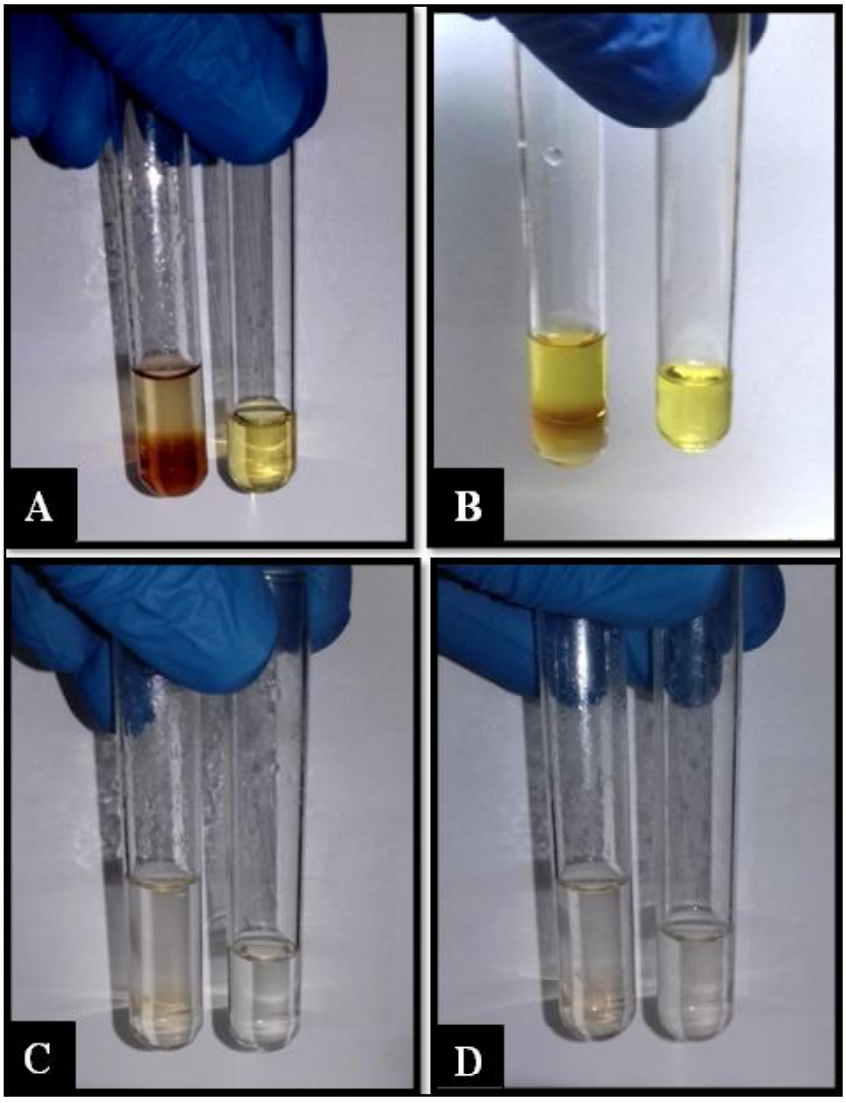

Fig. 2: Borntrager test results for *Trichoderma* isolate crude extracts : **(A)** *Trichoderma (5)*crude extract (positive) **(B)** *Trichoderma (8)*crude extract (Positive) **(C)** *Trichoderma (1)*crude extract (Negative) **(D)** *Trichoderma*

Crude extract of *Trichoderma* isolate 10 atconcentration (0.5, 1, 2 and 3)mg/ml showed high growth inhibition zone against *Staphylococcus aureus* recorded (6, 7, 9 and 12mm) respectively, while growth zone inhibition of *staphylococcus epiderms* at the concentration (0.5, 1, 2 and 3mg/ml) was (5, 5, 7 and 10mm) respectively, But *Proteus mirabilis* growth zone inhibition at the concentration (0.5, 1, 2 and 3mg/ml) was (4, 5, 7 and 8) respectively, and growth zone inhibition of *Pseudomonas aeruginosa* at the concentration (0.5, 1, 2 and 3mg/ml) was (0, 3, 4 and 6) respectively.

Trichoderma isolate 5 crude extract

Table 2: Growth inhibition zone of pathogenic bacteria by *Trichoderma* isolate 5 and 10 crude extract after 24 hour at 37 ±1 ^{0}C and pH 5.5.

Bacteria isolate	***Trichoderma* isolate 5 crude extract concentration**				***Trichoderma* isolate 10 crude extract concentration**			
	0.5mg/ml	**1mg/ml**	**2mg/ml**	**3mg/ml**	**0.5mg/ml**	**1mg/ml**	**2mg/ml**	**3mg/ml**
Escherichia coli	0.00*	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Acinetobacter baumannii	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pseudomonas aeruginosa	0.00	5.00	7.00	9.00	0.00	3.00	4.00	6.00
Proteus mirabilis	5.00	6.00	11.00	11.00	4.00	5.00	7.00	8.00
Staphylococcus aureus	10.00	11.00	14.00	18.00	6.00	7.00	9.00	12.00
Staphylococcus epidermidis	9.00	10.00	11.00	14.00	5.00	5.00	7.00	10.00
Salmonella typhi	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Inhibition zone (mm)

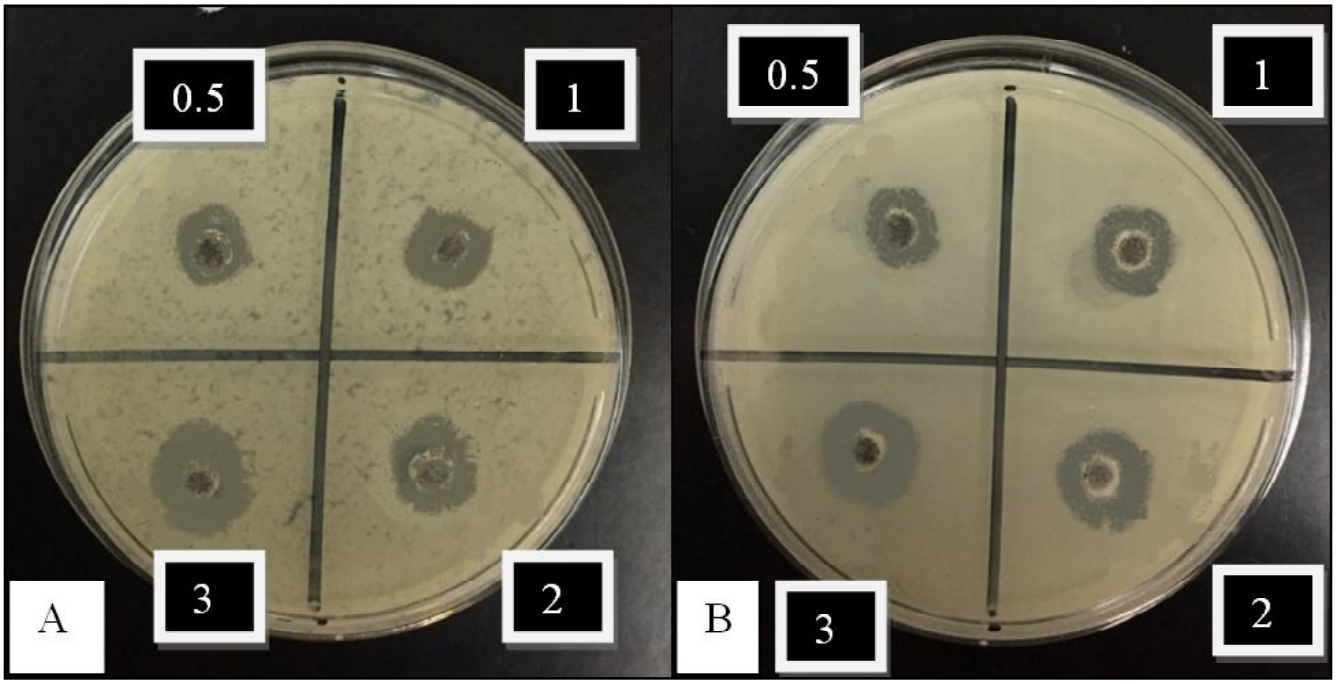

Fig. 4: Antibacterial activity of ethyl acetate crude extracts of *Trichoderma* (5)isolate against **(A)** *Pseudomonas aeruginosa* and **(B)** *Proteus mirabilis* on nutrient agar, after 24 hours at 37 C^o using agar well diffusion method (diameter of the well 5 mm). 0.5mg/ml, 1mg/ml, 2mg/ml, 3mg/ml.

showed high antibacterial than *Trichoderma*isolate 10 crude extract against *Staphylococcus aureus*, *Staphylococcus epiderm, Proteus mirabilis* and *pseudomonas aeruginosa. Staphylococcus aureus* was exhibited high sensitive to crude extract of *Trichoderma* isolates 5 and 10 than all other pathogenic bacteria. *Trichoderma* species produce many antibiotics and other chemicals that are harmful to pathogens and inhibit growth (antibiosis) (Reino *et al.*, 2008). The suggested mechanisms for biocontrol are antibiosis, lysis, competition, and mycoparasitism. These may act alone or in combination. *Trichoderma* species are also effective against various Gram positive and Gram negative bacterial species. They produce among 40 different metabolites of *Trichoderma* and ciprofloxacin and norfloxacin in cultures of *Trichoderma* which are antibacterial in nature (Leelavathi *et al*., 2014).

The antibacterial mechanisms of anthraquinones are diverse, including the simple destabilization of cell wall, alterations of metabolic pathways or DNA inclusions, in a direct or indirect way (via oxidative stress) (Kagan and Flythe, 2014). The efficacy of these mechanisms is related to the molecular properties of the anthraquinone (steric effect, pH, polarity of group substituents). Additionally, the same anthraquinone derivative can have multiple mechanisms of action which makes it difficult for bacteria to develop resistances (Derksen *et al*., 2013).

In this study the results in accordance with (Liu *et al.*, 2007). That the *Trichoderma crud* extracts showed greatest inhibiting activities toward *Staphylococcus*

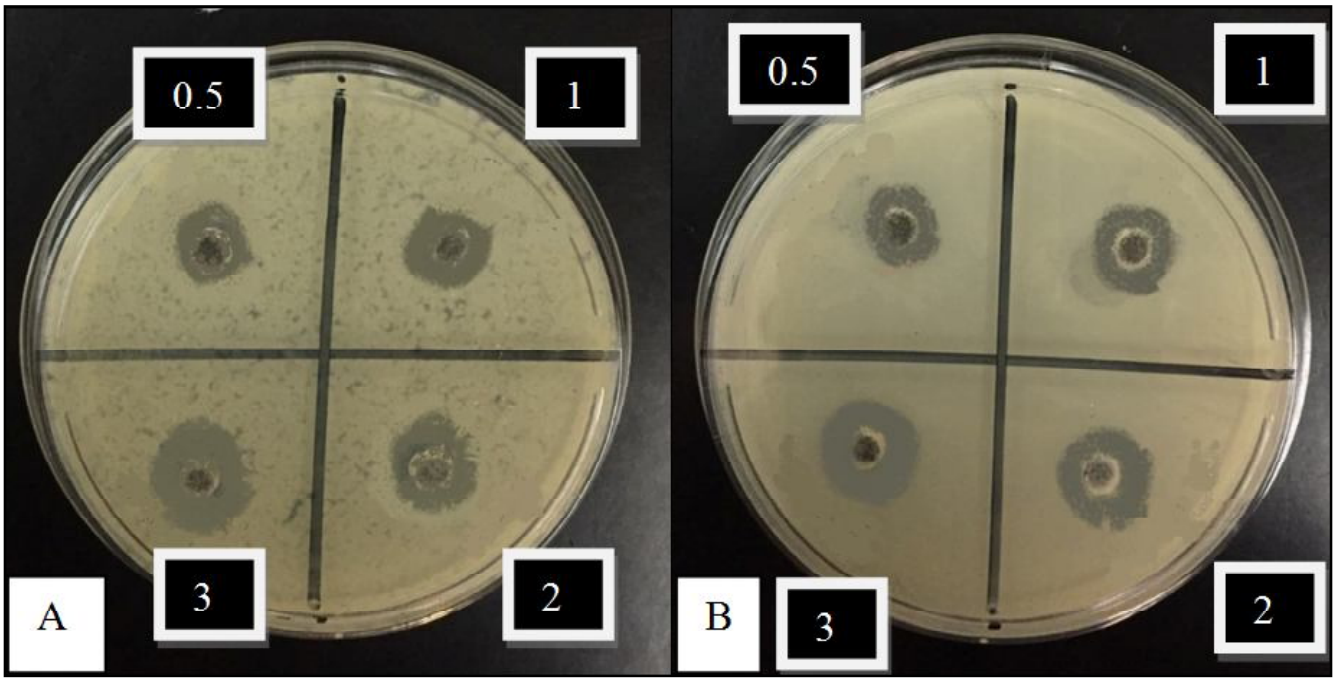

Fig. 5: Antibacterial activity of ethyl acetate crude extracts of *Trichoderma* (5)isolate against **(A)** *Staphylococcus aureus* and **(B)** *Staphylococcus epidermidis* on nutrient agar, after 24 hours at 37 C° using agar well diffusion method (diameter of the well 5 mm). 0.5mg/ml, 1mg/ml, 2mg/ml, 3mg/ml.

aureus. The extracts from *Trichoderma* isolates had different active pharmacological compound which could be responsible for their different antimicrobial activities.

Conclusions

The crude extract concentrations of *Trichoderma* showed a high antibacterial activity against

Staphylococcus aureus and *Staphylococcus epidermidis* was tested in this study and The crude extract of *Trichoderma* (5) showed higher effective than *Trichoderma* (10) because the first one contain anthraquinon compounds as Borntrager test results showed. The extract from *Trichoderma* can be used as an effective treatment to eliminate human pathogenic bacteria rather than the use of chemical antibiotics.

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