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Production of Antibacterial Compounds from *Aspergillus terreus* Against MDR *Acinetobacter baumannii* Using Co-culture

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ABSTRACT

Acinetobacter baumannii is a bacterial pathogen, associated with hospital and community acquired infections. It is one of the most common, opportunistic, and serious multidrug resistant bacteria (MDR). It is considered a global problem. The use of natural compounds such as fungal metabolites as an alternative to antibiotics is being explored to overcome the issue of antimicrobial resistance. Among fifty fungal spp. isolated from different sources, only *Aspergillus terreus* showed antibacterial effect against *A. baumannii.* Co-culture is applied to produce bioactive compounds in higher amounts and/or to induce the production of new secondary metabolites. Several solvents and chromatographic techniques were used to isolate, fractionate, and purify *Aspergillus terreus* /*Acinetobacter baumannii* co-culture secondary metabolites. Ethyl acetate extract was the most potent extract against MDR *A. baumannii*, in comparison to chloroform and methanol extracts. Characterization and structure elucidation of the purified compound isolated from ethyl acetate extract were performed using LC-MS, ^{1}H NMR and IR. The compound was identified as terreusinone A with a minimum inhibitory concentration of 160 $\mu g.\mu l^{-1}$ against MDR *A. baumannii.* This is the first record to determine the antibacterial effect of terreusinone A particularly against MDR *A. baumannii*.

Keywords: Terreusinone A, *Aspergillus terreus*, *Acinetobacter baumannii* co-culture, and antibacterial activity.

INTRODUCTION:

*A. baumannii*is one of most the virulent pathogens causing infections in the critical care units in Egypt (See *et al.,* 2013) and worldwide *A. baumannii* commonly causes nosocomial infections, predominantly the aspiration pneumonia, catheter-associated blood stream infection, catheter-associated urinary tract infections and soft tissue infections (Wong *et al.,* 2017). The previous authors concluded that the community-acquired infections of *Acinetobacter* spp. have increased over time. Disease spread of *Acinetobacter* spp. is assisted by host immunity evasion, pathogen resistance to dehydration, and antimicrobial agents. Multidrug resistant (MDR) and extensively drug resistant (XDR) bacteria showed high resistance to all available antibiotics, commonly used in the treating infections caused by *A. baumannii*, which significantly increase mortality (Kengkla *et al.,* 2018). In 2017, the World Health Organization (WHO) reported that, *A. baumannii* is one of the most virulent pathogens, requiring urgent control, so seeking new antimicrobial drugs is very important. The important sources of natural bioactive compounds are microorganisms, which have enormous potential for the exploration of

new compounds for the pharmaceutical applications (Demian 1999; Keller *et al.,* 2005; Porras & Bayman, 2011; Qadri *et al.,* 2013). During the fermentation, a wide range of secondary metabolites are produced by filamentous fungi including: antibiotics, antitumor compounds, antiviral, anti-parasitic agents, immune-suppressants, and the toxins (Ranadive *et al.,* 2013; Nigam & Singh, 2014). Fungal bioactive compounds are therefore more beneficial for human compared to any other natural products (Gulwani *et al.,* 2014). For example, secondary metabolites of the *Chaetomium globosum* act as anti-cancer agents (Moubasher & Hamed, 2015). Additionally, secondary metabolites of *Aspergillus terreus* act as anti-cancer, antibiotic and anti-inflammatory agents (Boruta & Bizukojc, 2017).

Microbial co-cultivation, which is known as mixed fermentation, involves the cultivation of two or more microorganisms in the same environment (Lami *et al.,* 2019; Chasoya *et al.,* 2021). This method induces the production of active metabolites by metabolite precursor formed enzymes or by inducing epigenetic modifications in the producer strain to form these metabolites (Wakefield *et al.,* 2017). Co-cultures of *A. terreus/ C. globosum* stimulated lovastatin production (Boruta *et al.,* 2019; González *et al.,* 2020). Whereas, production of enzymes was induced in *A. terreus / A. niger* co-culture (Rehman *et al.,* 2014). It was concluded that many compounds such as butyro-lactones, terrain, orsellinic acid, and anthranilic acid were induced in *A. terreus / Bacillus subtilis* or *Bacillus cereus* co-cultures (Chen *et al.,* 2015). These compounds were not detected in axenic fungal culture (González *et al.,* 2020). This study aimed to induce fungal secondary metabolites using co-culture techniques and to detect their effect against *A. baumannii.*

MATERIALS AND METHODS:

Isolation and identification of bacteria

Isolation and identification of bacteria from clinical specimens (sputum, blood and wounds) were carried out in the microbiology laboratory of the Clinical Pathology Department, Faculty of Medicine, Cairo University, Egypt. Collection and handling of the clinical specimens were performed by experienced nurses. Bacterial identification was performed according to morphological characteristics, response to Gram stain, and biochemical reactions. Also, enzymatic reactions using VITK MS automated mass spectrometry microbial identification system (Biomerieux) that uses Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) technology were also used in the identification. Antibiotic susceptibility test of the clinical isolates was done according to Clinical Laboratory Standard Institute guidelines (Weinstein, 2020) in the same department.

Isolation of fungi

Fungi were isolated from soil and air, by using the methods of Moubasher *et al.* (2022) on Czapek's-Dox agar w/v (3% sucrose; 0.1% KH_2PO_4; 0.05% $MgSO_4.7H_2O$; 0.05% KCl; 0.001% $FeSO_4.7H_2O$ 0.02%; NaNO3; 1.5% agar and 0.005 % Rose Bengal as bacteriostatic agent). Fungi collected from jam were isolated on the same medium containing 6% w/v sucrose instead of 3% w/v. The inoculated plates were incubated at 28°C for 5 days.

Antibacterial activity of the isolated fungi

Fifty fungi species were isolated from different sources were tested to determine their inhibitory effect against the selected bacterium. One ml of 0.5 McFarland standard (MCF) (Gayathiri *et al.,* 2018) of bacterial suspension was spreaded on plates containing Mueller Hinton agar w/v (0.2% beef extract; 1.75% casein hydrolysate; 0.15% starch; 1.7 % agar), Czapek's-Dox agar, malt extract agar w/v (2% malt extract; 2% glucose; 0.1% peptone; 2% agar) or sucrose peptone agar w/v (2% sucrose; 0.5% peptone; 0.05% KH_2PO4; 0.025% $MgSO_4.7H_2O$; 1.5% agar, pH was adjusted at 7.2 - 7.4 by adding known volume of 40% NaOH). Disc from fungal growth margin (1cm diameter) was transferred into a central bore of each plate. The Petri dishes were incubated at 38°C for 24-72 hrs. At the end of the incubation time, the most potent fungus against the multi-drug resistant bacterium was determined. The potent fungus was kindly identified at Assiut University Mycological Center, Egypt (AUMC).

Induction of the secondary metabolites production using co-culture

Five discs (1cm diameter each) were cut from the growth margin of the potent fungus and transferred into 4L Erlenmeyer flask containing 1L sucrose peptone broth (supplemented with 4 % sucrose). Flasks were incubated at 28°C for 3 days at standing and

shaking (150 rpm) states. At the end of the incubation time, each flask was inoculated with 1ml of 0.5 McFarland standard (MCF) suspension of the selected bacterium. The co-cultured flasks were incubated at 38°C for 10 days at standing and shaking states.

Separate flasks containing either fungus or bacterium isolates were used as controls. At the end of incubation period, filtration of the growth culture of each flask was done by using filter paper Whatmann No.1 to remove fungal mycelia. Then the filtrate centrifuged at 10,000 *g* for 10 min. at 4°C. Partitioning of the supernatant was carried out successively by chloroform, ethyl acetate and methanol. Drying of the extracts was performed using a rotary evaporator at 50 °C and then the extracts were weighed separately. One milligram of each extract was dissolved in 200 µl of its solvent to give a final concentration of 5µg/µl. Bacterial susceptibility to the different types of fungal extracts was carried out using filter paper disc diffusion method (Balouiri *et al.,* 2016). Discs impregnated with 50 µl of each crude extract, which was allowed to dry. This disc was placed on the growth medium previously inoculated with the selected bacterium isolates to assess antibacterial effect of extracts. After incubation at 38°C for 24 hrs the diameter of inhibition zones around the discs was measured to detect the most efficient extract.

Separation, purification and identification of the most potent compound

The most efficient extract was fractionated by silica gel column chromatography. Silica gel 60 P (*Carl Roth GmbH+ Co. KG – Schoemperlenstr- Germany*) was used as stationary phase. Silica gel (100 gm) was suspended in a known volume of solvent mixture (75 ml chloroform + 25 ml ethyl acetate) to pack column with dimensions 10 cm diameter and 100 cm height. In the first column a mixture of chloroform ($CHCl_3$): ethyl acetate (EtOAc) (2: 1 v/v) was the mobile phase with increasing gradient of ethyl acetate up to 100% (v/v) then used a mixture of ethyl acetate-methanol, 1: 12 (v/v) with increasing gradient of polarity by increasing methanol (Moubasher & Hamed, 2015). The collected fractions were categorized into groups by using pre-coated thin layer chromatography (TLC) (*Fluka* 60778254 nm). Mixture of $CHCl_3$: EtOAc (1:1 v: v) was used as developing system. Antibacterial activity of each group was tested. The most potent group was subjected to further purification using the second column of silica gel chromatography. Mixture of $CHCl_3$-EtOAc, 2: 1 (v/v) was used as the developing system with increasing ethyl acetate gradient up to 100% (v/v) then used ethyl acetate-methanol mixture, 2: 1(v/v) with the increasing gradient of polarity by increasing methanol of polarity. Sub-fractions were collected from the second column were running on pre-coated TLC, with using $CHCl_3$-EtOAc, 1:1 (v/v) as developing system. According to their polarities these sub-fractions were categorized into groups by using (*Fluka* 60778254 nm). Further purification of the most effective group, against the selected bacteria, was performed by using preparative TLC silica gel 60 P (*Carl Roth GmbH+ Co. KG – Schoemperlenstr- Germany*). Mixture of $CHCl_3$-EtOAc, 6:1 (v/v) was used as developing solvent. The observed fractions were scraped off from TLC surface and dissolved in 1 ml of EtOAc to determine their antibacterial effect against the 3 isolates of *A. baumannii*. Checking the purification of the fraction which showed highest antibacterial effect against the tested isolates, was done by using pre-coated TLC and $CHCl_3$ as developing system. The purified compound which showed highest antibacterial effect was characterized by subjecting to IR, LC-MS and ^{1}H NMR spectroscopic studies. LC-MS was done by Waters Acquity QSM column reverse phase C-18 using A, B, C, D solvents which were Water + 0.1% formic acid (A); methanol + 0.1% formic acid (B); methanol 90% + Water 10% (C), and acetonitrile (D), with running time 32 min and flow rate 45 min in gradient from 100% to 10%. Column average temperature was 34.2°C. 1HNMR was done by BRUKER 400 MHz using $CDCl_3$ as a solvent, at Center for Drug Discovery Research and Development, Ein Shams University. Finally, IR was done by JASCO 460 plus with wavelength (400-4000) nm and frequency (50-60) Hz at Micro Analytical Center, Cairo University.

Determination of minimum inhibitory concentration (MIC)

Different concentrations (192,160, 128, 96 $\mu g.\mu l^{-1}$) of the most potent purified compound were prepared. The minimum inhibitory concentration for the purified compound was executed by the paper disc-diffusion method (Booq *et al.,* 2021). One ml of 0.5 McFarland

standard suspension of *A. baumannii* was spread on plates containing Mueller Hinton by using sterile cotton swap. Discs impregnated with the different concentrations of the purified compound (50µl) were placed on the inoculated plates and incubated at 38 °C. Inhibition zone diameters were measured after incubation for 24 hrs.

RESULTS:

Identification and antibiotics susceptibility test of the selected bacterium

The clinically isolated bacterium was identified as *Acinetobacter baumannii.* The isolates showed resistance to carbapenem group antibiotics (meropenem and imipenem).

Determination of the highly potent fungus against MDR *A. baumannii* isolates

It was found that only one fungal species, among 50 spp. showed inhibitory effect against the three isolates of *A. baumannii* (**Fig. 1**). The fungus isolated from jam was the potent species and identified as *Aspergillus terreus* (AUMC 14766).

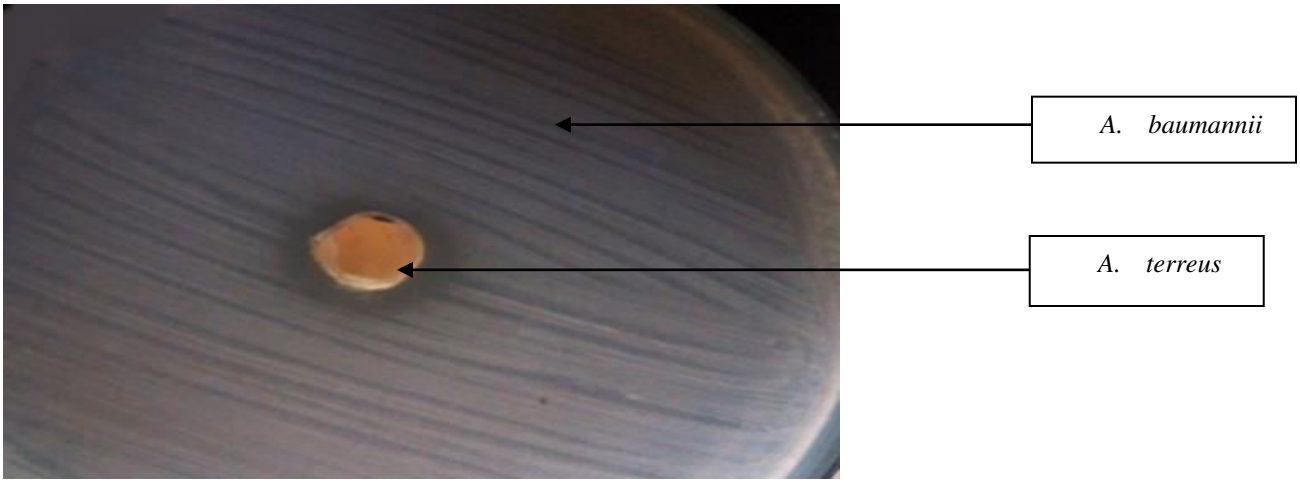

Fig. 1: *A. terreus* against *A. baumannii*

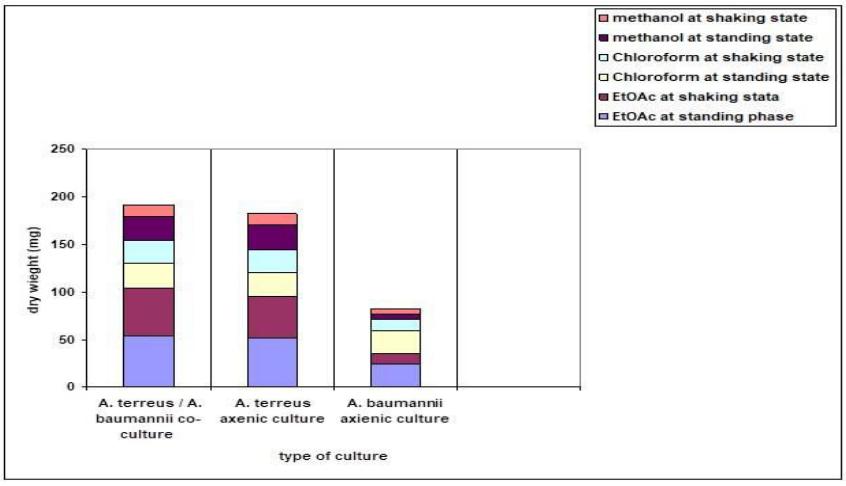

Fig. 2: Dry weight (mg/l) of ethyl acetate (EtOAc), chloroform ($CHCl_3$) and methanol (MeOH) extracts of co- and monocultures filtrates of *A. terreus* & *A. baumannii* that were grown under shaking and standing condition.

Susceptibility of *A. baumannii* to *A. terreus* and *A. baumannii* co-culture and monocultures secondary metabolites

The suitable medium for co-culture of *A. terreus* / *A. baumannii* was sucrose peptone broth with 4% sucrose. Under high osmotic pressure, greater amounts of secondary metabolites were induced in co-culture of *A. terreus* / *A. baumannii* than in each monoculture of both organisms, particularly in ethyl acetate extract (**Fig. 2**). Also, the induction of secondary metabolites

in standing condition was highly stimulated in comparison shaking state. Moreover, secondary metabolites production in axenic culture of *A. terreus* was more than in axenic culture of *A. baumannii*.

Determination of the highly potent extract against *A. baumannii* isolates

The most potent extract was ethyl acetate extract of *A. terreus* /*A. baumannii* co-culture against the three isolates of MDR *A. baumannii* (**Table 1**), followed by chloroform extract of co-culture. However, all extracts of both *A. terreus* and *A. baumannii* monocultures as well as methanol extract of co-culture were ineffective against the selected isolates. The effect of the co-culture extracts on the 3 isolates was nearly similar (**Table 1**)

Table 1: Antibacterial activity of co- culture, monocultures of *A. terreus* and *A. baumannii* extracts.

Isolate number	$CHCl_3$			EtOAc			MeOH		
	1	2	3	1	2	3	1	2	3
IZD* (mm) Isolate No. 1	8 ± 0.8	-	-	23± 0.8	-	-	-	-	-
IZD* (mm) Isolate No. 2	10± 0	-	-	22± 0.8	-	-	-	-	-
IZD* (mm) Isolate No. 3	10 ±0.9	-	-	23± 0.8	-	-	-	-	-

IZD* (inhibition zone diameter), (1) Co- culture of *A. terreus*/ *A. baumannii*, (2) monoculture of *A. terreus*, (3) monoculture of *A. baumannii.*

Fractionation of ethyl acetate extract

Twenty eight fractions were collected from co-culture ethyl acetate extract by the first column. According to polarities, fractions were categorized into 10 groups by using pre-coated thin layer chromatography (TLC). Group (2) showed inhibitory effect against the three isolates of MDR *A. baumannii* with 20 mm inhibition zone diameters (**Fig. 3**). After further purification of this group by the second column, eleven sub-fractions were collected, and categorized into 7 groups, according to their polarities, on pre-coated TLC. Only groups (3) and (4) showed inhibitory effect against *A. baumannii* isolates.

By using agar disc-diffusion method, group (3) showed higher antibacterial effect (23 mm inhibition zone diameter) more than group (4) which showed 19 mm inhibition zone diameter. Further purification of group (3) was executed by the preparative TLC. Only sub-fraction (2) out of (6) sub-fractions exhibited inhibition effect against the MDR isolates of *A. baumannii*.

Structure elucidation and characterization of the purified compound

After complete purification of sub-fraction 2 by using pre-coated TLC its structure elucidation and characterization were performed. LC-MS technique was carried out by Waters Acquity QSM column reverse phase C-18.

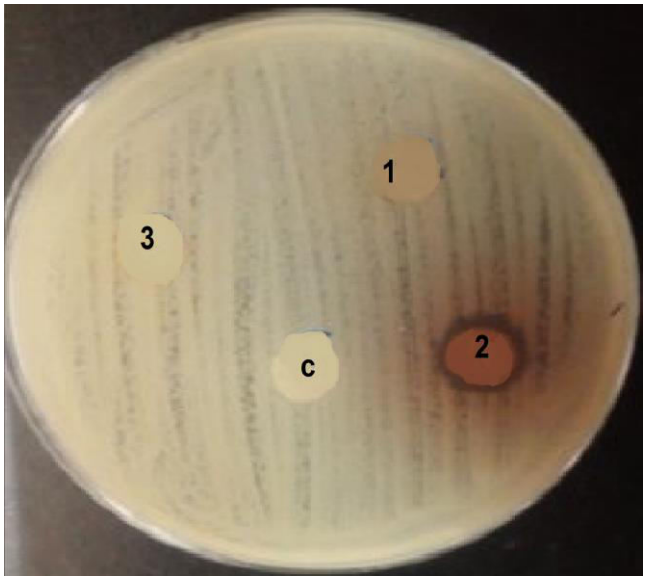

Fig. 3: Bactericidal activity of groups No.1, 2, and 3 which were separated from first Column against *A. baumannii* and C was ethyl acetate disc as a control

The pure compound showed main base peak at $[M^+]$ = 359.^{1}HNMR in CD_3Cl CH_2O (3.3 ppm), CH_2N (2.1 ppm), C_2H_2O (1.4 ppm), 6 CH_2 (1.3ppm), CH_3 (1.1 ppm), CH_2 (1.9 ppm), CH (1.98 ppm), CH (0.2 ppm). IR was done by JASCO I460 plus (**Fig. 4**). In which at (34440 cm^{-1}) NH, (2956 cm^{-1}) COOH,(2854 cm^{-1}) CHO,(1739 cm^{-1}) ester,(1462 cm^{-1}) C=O,(1261 cm^{-1}) C-OH,(1097 cm^{-1}) C-OH,(1029 cm^{-1}) C-OH. Depending on the elucidation of the purified antibacterial compound and by the help of the published data, the

purified compound was identified as terreusinone A with molecular formula $C_{20}H_{26}N_2O_4$ and molecular weight 358. The chemical structure of the identified compound was illustrated as shown in **Fig. 5**.

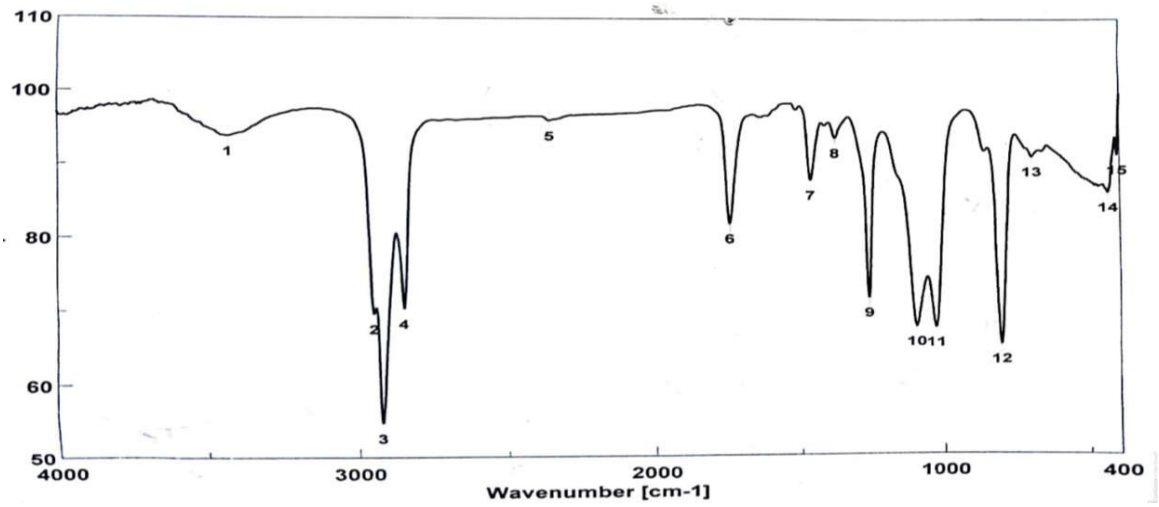

Fig. 4: IR by JASCO I460 plus of purified compound.

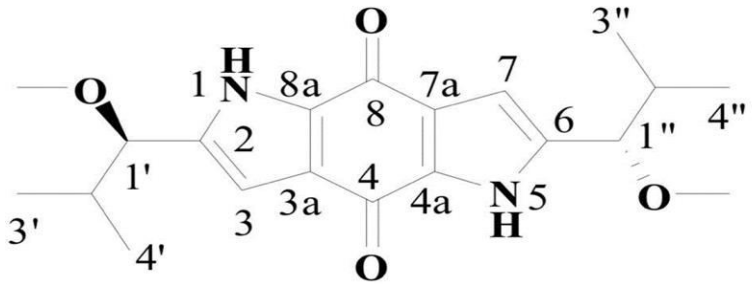

Fig. 5: Chemical structure of terreusinone.

Determination of the minimum inhibitory concentration (MIC) of terreusinone A

Different concentrations of terreusinone A (192,160, 128, 96 µg /µl) showed different effects against the isolates of *A. baumannii.* (**Fig. 6**). Clear zones appeared at two concentrations of terreusinone A (160 and 192 $\mu g.\mu l^{-1}$), whereas the lower concentrations were ineffective. MIC of terreusinone A was 160 $\mu g.\mu l^{-1}$.

DISCUSSION:

Inappropriate antimicrobial treatment and intensive care unit (ICU) stay led to the emerging of antibiotic resistant bacteria more rapidly among pathogens (Provenzani *et al.,* 2020; Vivi *et al.,* 2022). The World Health Organization (WHO) reported that carbapenem resistant *A. baumannii* is one of the most important MDR bacteria associated with hospital acquired infections (Provenzani *et al.,* 2020). Many factors are responsible for emerging of bacterial carbapenem resistance such as carbapenemase enzymes, the combined activity of ESBL and efflux pumps, or porin mutations (Shahen *et al*., 2019; El-kholy *et al.,* 2021).

The same authors observed high prevalence rate of carbapenem resistant *A. baumannii* in Egypt. High prevalence rates of *A. baumannii* occurred also in Mediterranean area, Southern Europe, Middle East and North Africa. While, low prevalence was recorded in Oceania, Western Europe, the Nordic region, and part of the central Europe (Ma & McClean, 2021). *A. baumannii* has a tolerance system for environmental changes by the biofilm formation, which provides protecttion from environmental stresses such as disinfection regimes, desiccation and host immune responses (Upmanyu *et al.,* 2022).

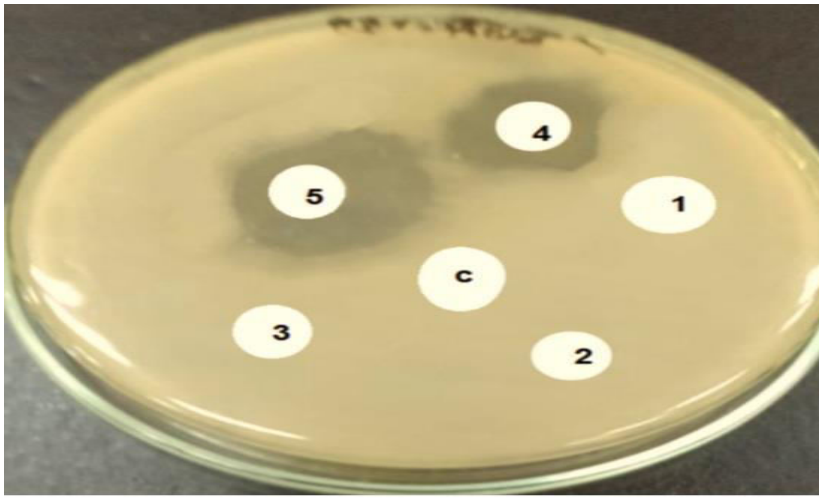

Fig. 6: Effect of different concentrations of terreusinone A on *A. baumannii*. Discs (1, 2) conc. 96 µg.µl^{-1}, disc (3) conc. 128 µg.µl^{-1}, disc (4) conc. 160 µg.µl^{-1}, disc (5) 192 µg.µl^{-1} disc, and (C) ethyl acetate as a control.

The high level of intrinsic and acquired mechanisms of multidrug resistant bacteria makes their treatment difficult. Therefore, it is important to focus on bioactive compounds such as fungal secondary metabolites, to find a suitable and effective alternative treatment instead of carbapenem, the commonly used antibiotic in treating *Acinetobacter* infection (Tiwari *et al.,* 2015). It was reported that *A. terreus* was the only species among 50 spp. which showed antimicrobial activity against *A. baumannii* in the current study. This finding is in harmony with Barakat and Gohar, (2012) that showed that *A. terreus* among 26 fungal isolates had highest antibacterial activity. It was found that incubation conditions can affect the growth of microorganisms and their metabolites (Atalla *et al.,* 2008; Mathan *et al.,* 2013). The results of this experiment and other reports revealed that the growing of *A. terreus* was significantly affected by the type of growth medium. In the current study, the most suitable media for the growth of *A. terreus* and *A. baumannii* were malt extract agar (Zain *et al.,* 2009) and Mueller Hinton agar (Aryal, 2022) respectively. However, for co-culture, sucrose peptone broth was the best. Temperature is an important factor regulating microbial growth and activity (Mathan *et al*., 2013). From this experiment the selected fungus can grow at 28°C and 38°C, which is on line with the study of Atalla *et al.* (2008); Mathan *et al.* (2013) reported that *A. terreus* can grow in a wide range of temperature from 25°C to 45°C, but the optimum temperature is 27°C. Pietikäinen *et al.,* (2005) concluded that fungal growth rates had an optimum temperature ranged from 25 to 30°C. The activity of *A. baumannii* decreased at 25°C and increased by increasing temperature (Monem *et al.,* 2020). In our study incubation temperature at 38°C was suitable for *A. terreus / A. baumannii* co-culture. Both growth and yield of secondary metabolites were higher at standing condition than shaking condition. This finding is in harmony with that of Atalla *et al.* (2008) who suggested that agitation may fragment mycelia to cells that need some time to repair this damage, which will lead to decrease in secondary metabolites yield. In this work *A. terreus* grew well in sucrose peptone medium with pH 7.2 -7.4 that was confirmed by Pang *et al.* (2020). In our study *A. terreus* was isolated from jam and grew under high osmotic stress. (Atalla *et al.,* 2008; Mathan *et al.,* 2013) concluded that *Aspergillus* spp. can grow at broad range of osmotic pressure. Under osmotic stress *A. terreus* can grow and produce important pharmaceutical compounds with anti-alzahimer's disease activity (Ghoran & Kijjoa, 2021), in addition to other secondary metabolites such as gluconic acid (Dowdell *et al.,* 2010). There are 165 compounds produced by *A. terreus* that was reported in the Antibase 2014 data base of secondary metabolites (Boruta *et al*., 2015; Jawaid *et al.,* 2019). It was found that some of these compounds have antimicrobial activity (Al-Fakih & Almaqtri, 2019). Many bioactive compounds having industrial and medical applications are induced by several biosynthetic gene clusters (BGCs) in microorganisms. However, most BGCs are not expressed

and remain inactive under normal *in vitro* conditions, which prevent metabolic potential from being exploited. So, it is important to apply new techniques to stimulate and induce the production of certain metabolites which activate these biosynthetic pathways. Co-culture is an important way to elicit cryptic compounds. Wakefield, (2017); Chasoy *et al.* (2021) concluded that co-culture assay is an efficient way to stimulate the metabolic potential of fungi for production of new therapeutic agents as well as increasing their concentrations in response to quorum sensing molecules, that could control emerging of health issues (Sharma & Jangid, 2017).

In Boruta *et al.* (2019) reported that *A. terreus* / *Chaetomium globosum* co-culture elicited lovastatin like compound that is monacolin J acid to produce. Also, in the co-culture of *A. terreus*/ *Paecilomyces lilacinus*, production of new compound terreins was stimulated (Lei *et al.,* 2020). Boruta *et al.* (2020) found that the production of lovastatine, butyrolactone I, geodin and asterric acid was induced in co-culture of *A. terreus* / *Penicillium rubens* and *Mucor racemosus.* From this study, crude ethyl acetate extract of *A. terreus* / MDR *A. baumannii* co-culture was the most active extract against MDR *A. baumannii* when compared with methanol and chloroform crude extracts. In *A. terreus* monoculture, ethyl acetate (Choudhary *et al.,* 2004; Silva *et al.,* 2017) and methanol (Mohammed *et al*., 2016) crude extracts had bactericidal and antitumor effects, while in this experiment chloroform, ethyl acetate and methanol extracts of monocultures had ineffective against MDR *A. baumannii*. This may be referred to inability of *A. terreus* and *A. baumannii* in monocultures to produce bioactive compounds in sufficient concentrations to control bacterial growth. From our observations, the pure compound exhibited lower toxicity effect than crude extracts against *A. baumannii.* This may be referred to the presence of other compounds in addition to terreusinone A in the crude extract, which aids in increasing antibacterial activity through synergism. The most potent compound was identified as terreusinone A, after subjecting to structure elucidation and characterization. Also Lee *et al.* (2004) isolated this compound from *A. terreus.* Terreusinone A is a yellow pigment and it belongs to the dipyrrolobenzoquinone derivatives. Nguyen *et al.* (2013) concluded that dipyrroloquinone alkaloids are sources of new anti-tumor and dermatological drugs. Terreusinone A acts as a potent UV-A protectant (Lee *et al.,* 2004; Vala *et al.,* 2019). It showed cytotoxic effect against cancer cells (Wei *et al.,* 2015). However, according to our available knowledge terreusinone A antibacterial activity has not yet been reported.

CONCLUSION:

A. terreus exerted antibacterial effect against *A. baumannii*. Ethyl acetate crude extract of *A. terreus* / *A. baumannii* co-culture was effective against *A. baumannii*. Terreusinone A was separated from EtOAc fraction, after subjecting to several purification steps, in addition to structure elucidation and characterization processes. This is the first report that indicates antibacterial activity of terreusinone A particularly against *A. baumannii in vitro*. Terreusinone A may act as a promising drug candidate for the treatment of *A. baumannii* infections after exposing to several clinical trials.

Ethical approval

This work was performed in line with the principle of the Declaration of Helsinki. Approval was granted by Ethics Committee of Kasr Al-Ainy Medical School 2020/22383.

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CONFLICTS OF INTEREST:

The authors declare that there is no conflict of interest

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