IJPSR (2021), Volume 12, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 November 2019; received in revised form, 18 December 2020; accepted, 17 January 2021; published 01 February 2021

IN-VITRO ANTIFUNGAL STUDY OF FILTRATES OBTAINED FROM SOME MARINE FUNGI AGAINST SOME DERMATOPHYTES AND YEASTS

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Keywords:

Aspergillus terreus, Cladosporium cladosporioides, Dermatophytes, Yeasts, Anti-scavenging, Total phenolic content, High-performance liquid chromatography

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ABSTRACT: Many infectious diseases have been treated with natural medicine. Now, fungi metabolites continue to play a major role as a therapeutic medicine in many countries. The present study reveals the potentiality of filtrate of *Aspergillus terreus* and *Cladosporium cladosporioides* as antidermatophitic agents. Molecular identification of isolates fungi using DNA sequencing of EF4 and ITS4primerswere performed. The fungal filtrate of *A. terreus* and *C. cladosporioides* were prepared in Potato dextrose broth and Sabouraud dextrose broth media. Antidermatophitic activity of it was evaluated against *Trichophyton mentagrophytes, Trichophyton verrucosum, Microsporum gallinae, Microsporum gypseum, Microsporum canis, Microsporum floccosum, Candida albicans,* and *Candida tropicalis* using dry weight method. The results show that *E. floccosum* was the most sensitive for Sabouraud dextrose broth filtrate of *A. terreus* while *M. gallinae* was the most sensitive for Potato dextrose broth of *A. terreus*. The potato dextrose broth filtrate of *A. terreus* was further subjected to the determination of the Minimal inhibitory concentration using different concentrations. The minimal inhibitory concentration values of different extracts were found to be different but in the range of (2.5 - 10 mg/ml), and defined the Anti-scavenging activity and Total phenolic contents for both fungal filtrates. Potato dextrose broth filtrate of *A. terreus* has the highest anti scavenging activity (71.5 %) and the highest amount of phenolic content (30.08mg/gdw). In addition, some bioactive compounds from fungal filtrates are separated and estimated by using High-performance liquid chromatography.

INTRODUCTION: Infections brought about by growths or mycoses can be clinically named shallow, profound, and fundamental. Dermatophytes are the most significant micro-organisms, which cause shallow mycosis, and the sores are described by round about attitude, desquamation, alopecia and erythema of the edges [1]. They have the ability to attack keratinized tissue (skin, hair, and nails) of people and different creatures to deliver a disease [2]. Dermatophytosis is known as tinea diseases or ringworm and influence (25%) of the all-inclusive community around the world [3, 4].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(2).928-36

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(2).928-36

These shallow contaminations are caused frequently by a few types of *Candida*, which are the second most various operators of parasitic disease around the world [5]. More than (95%) of every single contagious disease have been related with *Candida albicans*, *Aspergillus treats*, and *Cryptococcus neoformans* [6].

Regular items have assumed a key job in pharma investigate, the same number of drugs are either common items or subordinates thereof, to be sure, it is evaluated that (40%) of all meds is either normal items or their semisynthetic subsidiaries. Previously (twentieth) century, unrefined and semi unadulterated concentrates of plants, creatures, organisms, and minerals spoke to the main prescriptions accessible to treat humans and creatures [7, 8].

The marine condition is incredibly unpredictable and contains an expansive range of contagious assorted variety [9]. Normal items from growths are viewed as a significant hotspot for novel antimicrobial mixes due to their bottomless contagious species assorted variety, their rich optional metabolites, and the upgrades in their hereditary rearing and aging procedures. The antimicrobial exercises of an expanding number of growths living in particular conditions are explored for the disclosure of new mixes, for example, endophytic organisms from wild plants and marine parasites [10]. The new mixes from marine parasites have immediately expanded since (2010) likewise, these organisms have been a significant wellspring of antibacterial, antifungal, cytotoxic, anticancer and antiviral mixes [11].

The generally perceived instances of modernly and therapeutically significant metabolites of parasitic cause emitted by *A. terreus* is lovastatin and cholesterol-bringing medication down to forestall cardiovascular infection [12].

Cladosporium species have been appeared to have the capacity to deliver an assortment of characteristic items, among them the melanin, which are, shades giving the parasitic settlements their common dull hued appearance. Other characteristic items separated from Cladosporium species are bioactive mixes, for example, the antifungal cladosporides 13 and cladosporin which show antifungal movement, antibacterial, insecticidal, phytotoxic, and immunosuppressive properties [14].

The current study was conducted for evaluation of the antidermatophitic activity of Potato dextrose broth (PDB) and Sabouraud dextrose broth (SDB) filtrates of some marine medicinal fungi against some dermatophytes and yeasts.

MATERIALS AND METHODS:

Marine Fungi: In this study, two species of marine fungi were isolated from sediments obtained from the marine coast in Yanbu region (Saudi Arabia). The area of study is located between Latitude (24° 2.742 N), Longitude (38° 6.840 E), and it is characterized by a tropical to subtropical climate. Fungi isolates were primarily purified on Sabouraud dextrose agar (SDA), and culture purity was determined from colony morphology.

Fungal Isolates: Tested dermatophytes species included the following: *T. mentagrophytes*, *T. verrucosum M. gallina*e, *M. gypseum*, *M. canis*, and *E. floccosum* and the yeasts *C. albicans* and *C. tropicalis* were obtained from King Fahed Hospital in Jeddah. They cause infections in human.

Preparation of Fungal Filtrates: Each antagonistic agent tested was cultured in (PDB) or (SDB) at (28 °C) under continuous stirring in shaking incubator (JSR) at (150 rpm) for (7 days). Liquid cultures obtained were filtered through (Whatman No. 1) filter paper and the filtrate was then centrifuged for (10 min) at (10,000 rpm) (Ilettich- MIKRO 22 R). The centrifugation was repeated three times [15]. Supernatant fluids were sterilized by using mill pour filtration (Express™ plus-0.22 μm) and stored at refrigerator temperature (4 °C).

Molecular Identification of Fungal Isolates based on EF4 and ITS4 Primers: The isolates were chosen for further molecular analysis and identification using DNA sequencing of EF4 and ITS4 primers [16].

First, the 2000 bp amplicons were separated on 1% agarose gels to proof the accuracy and specify. Second, the fragments with the molecular sizes represented the EF4 and ITS4primers were purified for sequenced **Fig. 1**.

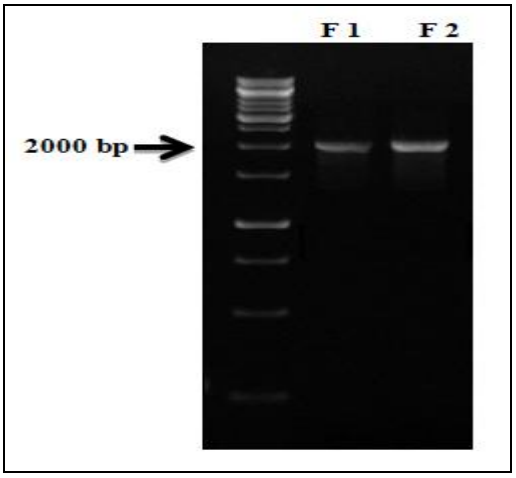

FIG. 1: PCR AMPLIFICATION PRODUCTS OF TWO DIFFERENT FUNGI ISOLATES USING EF4 AND ITS4 PRIMERS. FOLLOWED BY ELECTROPHORESIS ON A 1% AGAROSE GEL. LANE 1: MOLECULAR WEIGHT MARKER 1kb DNA LEADER (BIO-RAD). LANE 2-3: FUNGI ISOLATES REPRESENT FRAGMENTS AT 2000 bp

Antidermatophitic Assay:

Dry Weight of Dermatophytes and Yeasts: To decide the impact of contagious filtrate on the parasitic biomass, different focuses were added to disinfected (SDB) and finished to (100 ml) in a clean funnel-shaped cups (250 ml) ability to get the necessary fixations. Notwithstanding the control test, the cone-shaped cups were immunized by plate (10 mm) of the terminal development of settlements of (10 days) old, hatched at (28 °C) for (seven days) for *M. gallinae*, *M. gypseum*, and *M. canis*, (2 weeks) for *T. mentagrophytes* and *T. verrucosum* and (3 weeks) for *E. floccosum*. Toward the finish of the hatching time frame, dermatophytes were filtrated by utilizing realized weight channel papers, dried medium-term in an electric broiler at (80 °C), at that point, consistent weight was acquired. In the yeast, the funnel-shaped flagons were vaccinated by (1 ml) of *C. albicans* or *C. tropicalis* suspension. After brooding at (28 °C) for (48 h), (1 ml) of the yeast development was moved to rotator containers of known loads, centrifuged at (3500 rpm) (Ilettich-MIKRO 22 R) for (15 min). The supernatant was disposed of, and the pellet was stove-dried medium-term at (80 °C), at that point, steady weight was acquired. The dry weight was resolved as (mg) [17, 18].

Determination of Minimal Inhibitory Concentrations (MICs) of Fungal Filtrate: Sequential weakenings of the most powerful parasitic filtrate (100, 50, 40, 30, 20, 10, 5, 3, 2, 1, 0.5, 0.3, 0.2 and 0.1 mg/ml) were added to disinfected plates containing naturally (SDA) arranged with a standard number of cells for tried growths to decide the negligible inhibitory fixation [19]. The most reduced fixation, which did not demonstrate any noticeable development of micro-organisms was recorded as least inhibitory focus **.**

Biochemical Assay:

Determination of Total Antioxidant Activity by using DPPH Free Radical and Scavenging Activity: The hydrogen atom or electron donation ability of the corresponding extracts was measured from the bleaching of a purple-colored methanol solution of Diphenyl picrylhydrazyl (DPPH) [20].

Determination of Total Phenolic Contents (TPC): The total phenolic content in the extracts, filtrates, and new products was determined by using Folin–Ciocalteu reagent [21].

High-Performance Liquid Chromatography (HPLC): HPLC investigation was done utilizing an Agilent (1260) arrangement. The detachment was done utilizing (C18) section (4.6 mm × 250 mm i.d, 5 µm). The portable stage comprised of (2%) acidic corrosive (An) and acetonitrile (B) at a stream rate (0.8 ml/min). The versatile stage was modified sequentially in a direct angle as pursues: 0 min (85% A); 0–15 min (50% A); 15-17 min (20% A); 17-19 min (85% A) and 19-25 min (85% A). The multi-wavelength indicator was observed at (280 nm). The infusion volume was (20 µl) for every one of the example arrangements. The segment temperature was kept up at (25 °C) [22].

Statistical Analysis: Results are displayed as the mean of three or four repeats ± standard mistake (SE). The measurable examinations were completed utilizing the SPSS program (adaptation 22). Information acquired were broke down measurably to decide the level of centrality utilizing one way (ANOVA) at likelihood level $P \leq 0.05$ levels of significance.

RESULTS:

Molecular Identification of Fungal Isolates based on EF4 and ITS4 Primers: The DNA sequences analyzed using Nucleotide BLAST alignment tools showed that the isolates were identified as *Aspergillus terreus* and *Cladosporium*

endophytica. The EF4 and ITS4 primers sequence of the selected isolates were submitted into the GenBank database under the accession numbers:

1. MK032890 for strain F1 isolated from Yanbu.
2. MK032891 for strain F2 isolated from Yanbu.

Effect of the Fungal Filtrates on Dry Weight of the Dermatophytes and Yeasts:

Filtrates by using Sabouraud Dextrose Broth (SDB): Data in **Table 1, 3** showed that (15 ml) concentration of *A. terrus*filtrate reducing the weight of *E. floccosum*, *M. canis*, and *M. gypseum* by inhibition percentage (98.7, 97.2, and 89.3%) respectively, while the same concentration was inhibited *C. albicans* by (70.2%). As regards (15 ml) of *C. cladosporioides*, filtrate showed the highest inhibition activity against *T. mentagrophytes* (76.3%) followed by *M. canis*, *C. albicans*, and *M. gallinae* were inhibited by (66.6, 66.2, and 60.4%) respectively.

TABLE 1: EFFECT OF DIFFERENT CONCENTRATIONS OF *A. TERREUS* AND *C. CLADOSPORIOIDES* FILTRATES (ML) ON DRY WEIGHT (mg) AND INHIBITION PERCENTAGE (%) OF THE DERMATOPHYTES BY USING (SDB)

Conc.	Fungal filtrates	Dermatophytes											
		M. gallinae		*M. gypseum*		*M. canis*		*T. mentagrophytes*		*T. verrucosum*		*E. floccsum*	
		A	B	A	B	A	B	A	B	A	B	A	B
0.0	*A. terreus*	740±0.57		627±1.00		720±1.15		930±1.52		840±1.15		1023±1.52	
	C. cladosporioides	520±0.57	29.7	247±0.57	60.6	220±1.15	69.4	447±1.52	51.9	407±1.15	51.5	373±1.52	63.5
2.5	*A. terreus*	433±1.52	41.4	417±1.15	33.4	353±1.52	50.9	432±1.15	53.5	627±0.57	25.3	805±0.57	21.3
	C. cladosporioides	447±1.00	39.5	200±0.57	68.1	73±1.15	89.8	430±1.00	53.7	314±0.57	62.6	193±1.15	81.1
5.0	*A. terreus*	328±1.15	55.6	382±0.57	39.0	275±1.00	61.8	407±1.15	56.2	617±57	26.5	775±1.00	24.2
	C. cladosporioides	287±1.15	61.2	147±1.00	76.5	40±1.52	94.4	413±1.15	55.5	220±1.52	73.8	13±1.00	98.7
10.0	*A. terreus*	308±1.00	58.3	367±1.52	41.4	255±1.15	64.5	314±1.52	66.2	607±1.00	27.7	760±1.15	25.7
	C. cladosporioides	140±1.52	81.0	67±1.00	89.3	20±0.00	97.2	207±0.57	77.7	120±1.00	85.7	13±0.57	98.7
15.0	*A. terreus*	293±0.57	60.4	260±1.00	58.5	240±0.57	66.6	220±1.52	76.3	600±1.00	28.5	740±1.52	27.6
	C. cladosporioides	520±0.57	29.7	247±0.57	60.6	220±1.15	69.4	447±1.52	51.9	407±1.15	51.5	373±1.52	63.5
P-value (*A. terrus*)		0.0001*		0.0007*		0.0004*		0.0002*		0.0003*		0.0009*	
P-value (*C. cladosporioides*)		0.0003*		0.0005*		0.0009*		0.0006*		0.0008*		0.0001*	

Each value is the mean of 3 replicates ± SE

* = There is a significant effect of concentrations on tested pathogenic fungi by using One Way ANOVA at $P<0.05$

A: mean ±SE, B: Inhibition percentage

Filtrates by using Potato Dextrose Broth (PDB): Data in **Table 2, 3** showed that *A. terrus* filtrate (15 ml) concentration was complete inhibited the growth of *M. gallinae* (100%) while the inhibition percentages of *M. gypseum*, *E. floccosum* and *C. albicans* were (97.5, 94.4 and 74.5%) respectively. Whereas, *M. gallinae* was the highest sensitivity to *C. cladosporioides* filtrate (75.0%) followed by *M. canis* and *C. albicans* and (69.7 and 68.0%) respectively and finally, *T. verrucosum* was the moderate level of inhibition percentage (59.5%) at the same concentration.

TABLE 2: EFFECT OF DIFFERENT CONCENTRATIONS OF *A. TERREUS* AND *C. CLADOSPORIOIDES* FILTRATES (ml) ON DRY WEIGHT (mg) AND INHIBITION PERCENTAGE (%) OF THE DERMATOPHYTES BY USING (PDB)

Conc.	Fungal filtrates	Dermatophytes											
		M. gallinae		*M. gypseum*		*M. canis*		*T. mentagrophytes*		*T. verrucosum*		*E. floccsum*	
		A	B	A	B	A	B	A	B	A	B	A	B
0.0	*A. terreus*	773±1.52		807±1.15		817±1.52		1000±1.15		923±1.00		1080±0.57	
	C. cladosporioides												
2.5	*A. terreus*	133±0.57	82.7	547±1.15	32.2	333±1.52	59.2	133±0.57	82.7	547±1.15	32.2	333±1.52	59.2
	C. cladosporioides	393±1.52	49.1	407±1.00	49.5	312±0.57	61.8	393±1.52	49.1	407±1.00	49.5	312±0.57	61.8
5.0	*A. terreus*	33±1.00	95.7	327±1.15	59.4	147±1.52	82.0	33±1.00	95.7	327±1.15	59.4	147±1.52	82.0
	C. cladosporioides	377±1.15	51.2	392±152	51.4	282±1.15	65.4	377±1.15	51.2	392±152	51.4	282±1.15	65.4
10.0	*A. terreus*	33±1.15	95.7	174±0.57	78.4	140±1.00	82.8	33±1.15	95.7	174±0.57	78.4	140±1.00	82.8
	C. cladosporioides	360±1.00	53.4	382±0.57	52.6	267±1.00	67.3	360±1.00	53.4	382±0.57	52.6	267±1.00	67.3
15.0	*A. terreus*	0	100	20±0.00	97.5	87±1.00	89.3	0	100	20±0.00	97.5	87±1.00	89.3
	C. cladosporioides	193±0.57	75.0	367±1.15	54.5	247±1.52	69.7	193±0.57	75.0	367±1.15	54.5	247±1.52	69.7
P-value (*A. terrus*)		0.0002*		0.0006*		0.0004*		0.0002*		0.0005*		0.0003*	
P-value (*C. cladosporioides*)		0.0008*		0.0001*		0.0005*		0.0009*		0.0003*		0.0002*	

Each value is the mean of 3 replicates ± SE

* = There is a significant effect of concentrations on tested pathogenic fungi by using One Way ANOVA at $P<0.05$

A: mean ±SE, B: Inhibition percentage

TABLE 3: EFFECT OF DIFFERENT CONCENTRATIONS OF *A. TERREUS* AND *C. CLADOSPORIOIDES* FILTRATES (ml) ON DRY WEIGHT (mg) AND INHIBITION PERCENTAGE (%) OF THE YEASTS BY USING (SDB) AND (PDB)

Yeasts	Concentrations	Fungal filtrates							
		SDB				PDB			
		A. terreus		*C. cladosporioides*		*A. terreus*		*C. cladosporioides*	
		A	B	A	B	A	B	A	B
C. albicans	0.0	175±1.00				200±0.57			
	2.5	92±1.52	47.4	2.5	92±1.52	47.4	2.5	92±1.52	47.4
	5.0	73±0.57	58.2	5.0	73±0.57	58.2	5.0	73±0.57	58.2
	10.0	65±1.00	62.8	10.0	65±1.00	62.8	10.0	65±1.00	62.8
	15.0	52±1.15	70.2	15.0	52±1.15	70.2	15.0	52±1.15	70.2
	P-value	0.0001*		0.0005*		0.0004*	0.0003*	*P*-value	
C. tropicalis	0.0	195±1.15				220±1.52			
	2.5	118±1.15	39.4	125±1.00	35.8	117±1.00	46.8	137±1.52	37.7
	5.0	103±1.00	47.1	110±0.57	43.5	94±0.57	57.2	120±1.15	45.4
	10.0	83±0.57	57.4	85±1.73	56.4	79±1.52	64.0	94±1.00	57.2
	15.0	68±1.52	65.1	77±1.15	60.5	66±1.52	70.0	75±0.57	65.9
	P-value	0.0007*		0.0009*		0.0006*		0.0002*	

Determination of Minimal Inhibitory Concentrations (MICs) of the Most Potent Fungal Filtrate of Marine Fungi: Based on the results that recorded the culture filtrate of *A. terrus* by using (PDB) was the strongest activity against tested fungi and yeasts. Thus, MIC values of filtrate against tested pathogenic fungi were in the range of (2.5 - 10 mg/ml). *M. canis* and *C. tropicalis* inhibited by (10 mg/ml) then *M. gypseum*, *T. verrucosum* and *C. albicans* inhibited by (5 mg/ml) followed by *M. gallinae*, *T. mentagrophytes* and *E. floccosum* inhibited by (2.5 mg/ml) **Table 4**.

TABLE 4: MINIMAL INHIBITORY CONCENTRATIONS (MICs) (mg/ml) of A. TERRUS FILTRATE BY USING (PDB)

Fungal filtrates	Inhibition (%)	TPC (mg/gdw)
C. cladosporioides (PDB)	38 %	10.2
C. cladosporioides (SDB)	36.5 %	1.8
A. terreus (PDB)	71.5 %	30.08
A. terreus (SDB)	55.5 %	17.56

Determination of Total Antioxidant by using DPPH Free Radical Scavenging Activity: *A. terreus* filtrate (PDB) showed the highest antioxidant activity (71.5%) and the lowest value was recorded of the *C. cladosporioides* filtrate (SDB) (36.5%) **Table 5**.

TABLE 5: ANTI-SCAVENGING ACTIVITY AND TOTAL PHENOLIC CONTENTS OF *A. TERRUS* AND *C. CLADOSPORIOIDES*

Fungi	*A. terrus* (PDB)
M. gallinae	2.5
M. gypseum	5
M. canis	10
T. mentagrophytes	2.5
T. verrucosum	5
E. floccosum	2.5
C. albicans	5

Determination of Total Phenolic Contents (TPC): The highest total phenolic content was observed with *A. terreus* filtrate (PDB) (30.08 mg/gdw) and the lowest value was recorded of the *C. cladosporioides* filtrate (SDB) (1.8 mg/gdw) **Table 5**.

High-Performance Liquid Chromatography (HPLC): *A. terreus* filtrate (SDB) had a high content of Syringic Acid, Rutin, Coumaric Acid, Vanillin, Querectin and Cinnamic Acid (84.7, 87.9, 17.2, 14.5, 24.4 and 17.3 mg/100gdw) respectively, were as *A. terreus* filtrate (PDB) had a high content of Gallic Acid, Catechin and Coffeic Acid (116.2, 351.7 and 326.7 mg/100gdw) respectively.

C. cladosporioides filtrate (SDB) had a high content of Gallic acid, Catechin and Coffeic acid and Querectin (4.2, 42.4, 3.4 and 9.6 mg/100gdw) respectively, while *C. cladosporioides* filtrate (PDB) had high content of Cinnamic acid (2.7 mg/100gdw) **Table 6**.

TABLE 6: HPLC OF FUNGAL FILTRATES (mg/100gdw)

Compounds	*A. terreus* (SDB)	*A. terreus* (PDB)	*C. cladosporioides* (SDB)	*C. cladosporioides* (PDB)
Gallic Acid	77.9	116.2	4.2	0
Catechin	259.8	351.7	42.4	0
Caffeic Acid	17.8	326.7	3.4	0
Syringic Acid	84.7	15.1	0	0
Rutin	87.9	59.8	0	0
Coumaric Acid	17.2	16.6	0	0
Vanillin	14.5	0	0	0
Querctin	24.4	0	9.6	0
Cinnamic Acid	17.3	0	1.2	2.7

DISCUSSION: Microorganisms turned into a significant wellspring of pharmacologically dynamic metabolites. These life forms created different sorts of metabolites, which are able to restrain different living beings from contending to the equivalent biological specialty [23]. As indicated by [24] revealed that a significant number of the items right now utilized for human/creature treatment, in creature farming, and in agribusiness are delivered by microbial aging or got from synthetic changes of a microbial item. [25] Announced that anti-infection action from the halophilic/halotolerant parasites has been increasingly successful in low water action or increment in salt focus.

A. terrus filtrate by using (SDB) can reduce the weight of *E. floccosum* with an inhibition percentage (98.7%). The growth inhibition by filtrate against other pathogenic fungi ranged from (97.2 - 65.1%). This result similar with [23] evaluated the antifungal activity of marine fungus *Fusarium* sp; they found the extract of fungus had antifungal activity against tested fungi and yeast (*Candida rugosa, F. oxysporium, S. cerevisiae, Rhizopus oryza* and *A. flavus*), and the best activity was observed against (*R. oryza*) also the same extract able to inhibit the growth of pathogenic bacteria (*B. subtilis, S. aureus, E. coli, P. aurogenosa, K. pneumonia*) and the best activity was observed against (*E. coli*).

Furthermore, [26] reported that the intracellular and extracellular extract of *A. terreus* has abroad spectrum antifungal activity against unicellular (*C. albicans* and *S. cirivisae*) and filamentous fungi (*A. flavus, A. fumigatus, A. niger,* and *P. chrysogenum*). Secondary metabolites represent a large source of compounds endowed with ingenious structures and potent biological activities. [27] Found the bioactive metabolites from solvents, and crude extract of fungal species such as *A. flavus, Geotrichum candidum, Penicillium leuteum, Penicillium granulatum* and *Acremonium* species were screened for its antibacterial activity.

A. terrus filtrate by using (PDB) complete inhibition the growth of *M. gallinae* (100%). Whereas, the filtrate inhibited other fungi ranged from (97.5 - 70.0%). The same result appeared with [28] who discovered that the culture filtrates of *A. niger, A. terreus* and *Aspergillus* sp. were more active against (*P. ultimum*), complete inhibition was induced by the culture filtrates of *Aspergillus* sp, *A. niger,* and *A. terreu* used at 20 %. They were found to be more active at 20% than at 15 or 10%. This alteration is probably induced by enzymes present in the culture filtrate of *A. terreus,* such as cellulases causing the degradation of the cell walls of *P. ultimum* [29].

Likewise, the extract of *Penicillium dipodomyicola* have antifungal activity against (*C. albicans*) and antibacterial activity against (*S. aureus*) [30]. In addition, [31] reported that the filtrate of four species of halophilic fungi *Aspergillus* genus, namely, *A. flavus, A. gracilis, A. penicillioides,* and *A. restrictus* also a yeast namely, *Sterigmatomyces halophilus* have inhibition activity on tested bacteria (*B. subtilis* and *E. coli*). Many *Aspergillus* have been reported to produce antioxidants [32].

Recently [33] announced the expanded in cell reinforcement limit of parasites presented to high salt conditions and more extracellular cancer prevention agent profitability than an intracellular generation. Rough filtrates of *A. flavus* and *A. penicillioides* have indicated high TPC, which surely can be expanded by concentrating the filtrates. Phenolic mixes are seen as related to the life expectancy of growths by their cell reinforcement guard against the pressure of natural variables [34].

Furthermore, [35] showed that the extract of *A. terreus* can inhibit the growth of tested bacteria (*S. pneumonia, E. coli*, *S. aureus*, *P. mirabilis,* and *S. epidermidis*). The study of bioactive secondary metabolites revealed that *A. terreus*as a source for the production of effective metabolites. Many fungi produce biologically active compounds, several of which are toxic to animals or plants [36]. This data agreement with our results, which showed the fungal filtrate of *A. terreus* by using (SDB) and (PDB) more active against dermatophytes and yeasts, they displayed the highest antioxidant activity (55.5 and 71.5%) and total phenolic contents (17.56 and 30.08 mg⁄gdw) respectively.

C. cladosporioides filtrate by using (SDB)was more active against *T. mentagrophytes,* which inhibited by (76.3%). The susceptibility of other fungi to the filtrate was decreased respectively according to the recorded inhibition percentage ranged from (66.6- 27.6%). This result agreement with [37] announced that the concentrates of *C. cladosporioides*, *F. oxysporum*, *F. solani* and *Mycoleptodiscus indicus* demonstrated an expansive antifungal action against (*C. acutatum, C. fragariae* and *C. gloeosporioides*). Among the detaches are the *Cladosporium* species, known to deliver a few optional metabolites, for example, cladosporin, emodin, phytase, taxol and antifouling mixes [38]. Additionally, *Fusarium* species that incorporate a few naturally dynamic metabolites including fusaric corrosive, moniliformin, fumonisins, zearalenon, enniatins and trichothecenes [39].

M. gallinae was the largest sensitivity to *C. cladosporioides* filtrate by using (PDB), which inhibited by (75.0%). The growth inhibition by filtrate against other pathogenic fungi ranged from (69.7 - 48.7%). The same result appeared with [40] demonstrated that the extract of fungi *A. niger*, *C. cladosporioides*, *C. sphaerospermum*, *A. alternate* and *C. lunata* have antifungal activity against (*M. phaesolina* and *F. solani*) also the extracts of *C. cladosporioides* and *C. lunata* reduce the growth of pathogenic bacteria (*S. aureus, B. subtilis*, and *S. typhymurium*).

The secondary metabolites from these fungi can be further grouped as alkaloids, terpenoids, steroids, quinones, saponins, tannins, terpenoids, flavonoids, and phenols [41].

Phenolics are not the only components in the extracts that could possess antioxidant activity [42] also by other components such as Gallic acid, Catechin, Coffeic acid, Syringic acid, Rutin, Coumaric acid, Vanillin, Quercetin and Cinnamic acid that purified from plants extracts by HPLC method with other unknown substances in fungal filtrates in our studying.

MIC values of *A. terrus* filtrate by using (PDB) in the range of (2.5-10 mg/ml). *M. canis* and *C. tropicalis* the most resistant to the filtrate inhibited by (10 mg/ml) whereas *M. gallinae, T. mentagrophytes* and *E. floccosum* the most sensitive to the inhibited by (2.5 mg/ml). This result agreement with [43] demonstrated that the MIC values of the culture filtrate of *Fusarium* sp. range of (0.263- 3.100 mg/ml) against Gram-positive bacteria (*S. aureus, Bacillus anthracis* and *α-hemolytic streptococcus*) and Gram-negative bacteria (*E. coli, P. aeruginosa, Bacillus proteus, Salmonellae enteritis, Eberthella typhi, Shigella dysenteriae,* and *Helicobacter pylori*). The antifungal activity of culture filtrates may be attributed either to the production of antibiotics [44] or to the production of lytic enzymes [45].

In addition, [46] revealed that the MIC values for the fungal extracts of *P. citrinum, F. solani, A. niger*, *A. terrus, A. alternata* against all the tested bacteria Gram-positive (*E. faecalis* and *S. aureus*) and 7 strains were Gram-negative includes (*P. aeruginosa, Serratia marcescens, Shigella flexneri, S. typhi, E. coli, P. mirabilis,* and *K. pneumoniae*) ranged in between (15.6 - 250 µg/well). [47] Concluded that *Aspergillus* genus is a major contributor of antimicrobial compound of fungal origin.

CONCLUSION: This work is a successful attempt of phytochemical characterization and anti-dermatophitic efficiency of *A. terrus* and *C. cladosporioides* screening as fungal filtrate for a variety of biological activities. Furthermore attention should be paid to purification and formulation may be needed to understand the mechanisms through which this effect is exerted.

ACKNOWLEDGEMENT: Thanks to the department of biological science, faculty of science, the university of Jeddah for their cooperation and guidance.

CONFLICTS OF INTEREST: There is no conflict of interest. The authors declare no conflicts of interest. All the experiments were undertaken in this study comply with the current laws of the country where they were performed.

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How to cite this article:
AL-Kattan M, Danial EN, Alshehri WA and Alzumay NA: *In-vitro* antifungal study of filtrates obtained from some marine fungi against some dermatophytes and yeasts. Int J Pharm Sci & Res 2021; 12(2): 928-36. doi: 10.13040/IJPSR.0975-8232.12(2).928-36.