International Journal of Biology Research
www.biologyjournal.in
ISSN: 2455-6548
Received: 22-07-2022, Accepted: 08-08-2022, Published: 24-08-2022
Volume 7, Issue 3, 2022, Page No. 35-42

Evaluation of the efficacy of *Artemisia* extract against pathogenic fungi isolated from coronavirus patients

Saad Hussein Albiaty[1], Hawazin Ahmed Abid[1], Thekra Ahmed Hamada[2]
[1] Department of Biology, College of Sciences, University of Tikrit, Iraq
[2] College of Medicine, University of Tikrit, Iraq

Abstract
Coronavirus 2019 resulted by SARS-CoV-2, which arose in Wuhan City, China and was become a pandemic in the world. This disease lead to acute respiratory infection with a significant rate of intensive care unit (ICU) entry. The infection of bacterial and fungal are pneumonia viral infection complication result to the intense injury of pulmonary tissues, the storm of cytokine and immune-disability result from a virus infection that induced severe respiratory syndrome. *A. absinthium* consider of the most noticeable types of the *Artemisia* genus in the worldwide. In European popular medication, this plant utilized for thousands years for several various diseases. This study aimed attempt to find the medical activities of *Artemisia* extract to inhibit the growing fungi that are taken from covid-19 patients. The results of present study was revealed the antifungals effects appeared toward C. albicans where Nystatin recorded MIC 250 µg/ml and MFC 500µg/ml higher than Amphotericin-B and Clotrimazole. The results of rate diameter colony growth for Aspergillus flavus and A. fumigatus, there were the diameter rate in using nystatin ranged (30-6 mm) and (44.7-6 mm) for each of these fungi respectively at the concentrations (5-2560 µg/ml), this appeared the nystatin higher effect than Amphotericin-B and Clotrimazole**.** Regarding the results of inhibitory for Artemisia plant on molds and yeast. The results were indicated significant differences in diameter rate of the colony growth. Where there were lower diameter growth for 200 mg/ml for each *A. fumigatus* (20 ±0.849) mm, *A. niger* (18±1.061) mm, *A. flavus* (17±1.131) mm. Besides, the concentration 140 mg/ml appeared higher diameter of growth for each *A. fumigatus* (35±0.849) mm, *A. niger* (40±4.17) mm, *A. flavus* (44±39.3) mm ($P<0.05$). In conclusion the prevalence of *C. albicans* in the infected patients with COVID-19 was higher than the molds. Also, the high concentrations of the alcoholic extract of Artemisia gave the highest inhibition zone for yeasts and the lowest growth zones for the studied molds. This indicated the efficiency extract as antifungals

Keywords: *Artemisia*, pathogenic fungi, coronavirus 2019

Introduction
Artemisia is considered the widely spread genera belonging to Kingdom: Plantae Order: Asterales, Family Astraceae there are 500 species or more that grow mostly in the moderate areas of Asia, Europe, and North America (Kshirsagar *et al.*,2021) [1].
In the previous few years, there was increased attention on the chemical and biological effects of *Artemisia absinthium* L. (Ekiert *et al.*, 2021) [2].
Modern pharmacological research have concentrated on *Artemisia absinthium* L. like its effects as antibacterial, antiprotozoal, antifungal, antiulceration, hepato-preservative, anti-inflammation, immuno modula- tion, cytotoxic, pain-reliving, neuro preservative, antidepressant, precognitive, cellular membrane stabilization, neuro-growth and antioxidant impacts (Szopa *et al.*, 2020) [3].
Moreover, *A. absinthium* play role in an significant place in the manufacturing of cosmetics. (Ahamad *et al.*,2019; Hussain *et al.*, 2017).
In European popular medication, this plant utilized for thousands years for several various diseases, in special for parasitic infections and gut disease and fever happened (Amidon *et al.*, 2014) [6].
In the illuminate of the prevalence COVID-19, some plant of Artemisia (Bora and Sharma,2011; Obistioiu *et al.*, 2014) [7, 8], (Ekier *et al.*, 2020).
Different types of the plant *Artemisia* have appeared to inhibit the growth of many cancerous cell involving leukemia, colonic cancer, kidney cell carcinoma and breast cancer cells (Feng *et al.*,2020; Kiani *et al.*,2020) [9, 10].
Hytochemicals and their secondary product, extracts, and fundamental oils obtained from Artemisia have special properties because of their ability to reactive oxygen controlling. In some status they showed powerful activity as

antioxidant and scavenging of the hydroxyl ion and hydrogen peroxide and appear protective impact by enforcement the antioxidant protecting system and decreasing the creation of reactive oxygen (Du *et al.*,2017).
Many phytochemicals separated from different Artemisia types show important antiviral action. Artemisinin has important medicinal to be the therapy promising as antiviral against virus hepatitis B and C, herpes virus (HSV-1, HSV-2), and influenza A virus (Obeid *et al.*,2013; Jang *et al.*,2015) [12, 13].
Considering, their efficient anti-inflammation, immuno regulation, and antiviral characteristics, Artemisinin is pursuing its effectiveness against infection of SARS-CoV-2.

Aim of the present study
The present study aims to reveal the activity of the plant Candida spp. and Aspergillus spp. isolated from respiratory infection in patients in the Intense caring unit (ICU) is repeated

Material and Method
Fungal Investigation
The present study was done at Dept. / collage of /Tikrit university. Samples were collected from patients. The investigation was performed by taking a swap sample and sputum. The specimen was divided into two parts: one part was examined immediately under a microscope for direct examination, the second was usually collected on sterile filter paper in a sterile Petri dish and then transferred to the laboratory for culturing.
Samples were subjected for direct examination by placing the specimen on a clean slide mounted with a drop of 10 % KOH, covered with a coverslip. Then the slide was warmed (but not to boiling) and examined under the microscope for detecting hyphae and spores. (Kwon-Chung and Bennett, 1992).
Samples prepared for culturing proceeded as the followings: Specimens were cultured on sabouraud's dextrose agar (SDA) at pH 6.5 with added chloramphenicol. The plates (triplicate) were incubated at 28±1ºC for 14 days. Plates were examined daily for 7 days.
Vitek 2 Compact utilized for the diagnosis of the isolates of yeast (Identification card: YST ID card Reference number 21343).

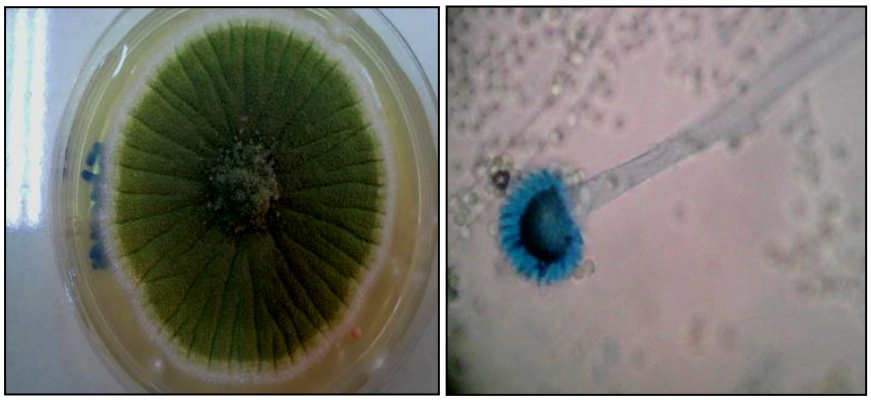
1. Antifungal Agents
Three types of antifungal agent were tested to evaluate the antifungal susceptibility toward fungi genera as illustrated in the table (1).

Table 1: List of antifungal agents that were used in this study

Generic name	Manufacture
Nystatin	Janssen Research Foundation, Belgium
Amphotericin-B	Janssen Research Foundation, Belgium
Clotrimazole	Mission pharmaceuticals limited, India.

Prepared of *Artemisia absinthium* leaves were collected from local markets, and leaves were left to dry in room temperature and dark place. Then were dried grind in an electric grinder and the powder is kept in dry tins till use (Makboul & Backy,1998).
Study the effect of plant extracts on the growth of fungal isolates. 8 gram from plant extract dissolved in 20 ml from sterilized D.W to obtain 400mg/ml as stock solution and the concentrations prepared from stock solution (140, 160, 180, 200) mg/ml. Where 200mg/ml prepared by taking 1 ml from stock and completed to 2ml for obtaining 200mg/ml. Thus, the rest of the concentrations were prepared.
Determination of Inhibitory effectiveness of plant extract on the molds Agar dilution method was used according to (Behbahani *et al.*, 2014) [14].

Results
Diagnosis of mold according to phenotypic and cultivation characteristics
The fungi were diagnosed through growing on Sabouraud dextrose agar for swabs and sputum (100 samples). *Aspergillus flavus* was described morphological characteristics on SDA as green color and edges zone with white. The growth covered the whole plate with incubation of five days (Figure 1A)**.** The examination microscopic of the mycelia appeared no partition conidiophores emerging from thick walls of foot cells. The conidiophore terminus enlarges as globular growth. Conidia grow up by phialides emerging from ends bulge on conidiophores (Figure 1B).



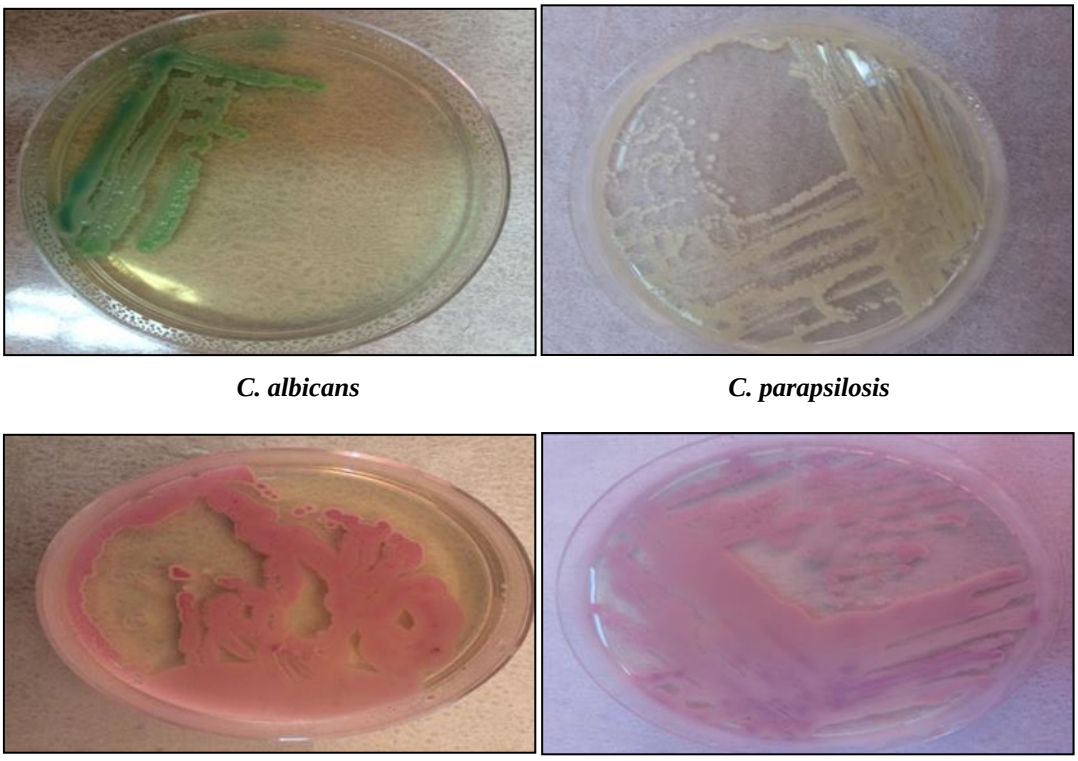
A-Morphology on plate B-Microscopic examination

Fig 1: A-Morphological characteristic of *Aspergillus flavus* and B-Microscopic examination

Aspergillus niger was described morphological characteristics on SDA as a white color colony and turn into black color through the period of time while the side reverse of the colony on the plate appears a yellow color (Figure 4-2 A and B). The examination microscopic appeared the hyphae septate with the wall smooth, the simple of conidiophores. Conidiophores terminus with vesicle, it is spherical and fully coated (beaming) with two chains of sterigmata.

Diagnosis of yeasts by using CHROM agar of *Candida*

The results of isolates appeared with different color changes on CHROM agar. The isolates of green color represent *Candida albicans*, white color isolates was *C. parapsilosis*, pink color was *C. glabrata* and *C. krusei* was purple-cloudy as shown in figure (2).



C. albicans ***C. parapsilosis***

C. glabrata ***C. krusei***

Fig 2: *Candida spp.* on CHROM agar at 37ºC for 48 hrs

CHROM agar is used a wide to identify Candida sp., which is differentiated the colonies with different colors. This agar permits the detection of *Candida albicans, Candida tropicalis, Candida glabrata* and *Candida krusei* (Odds and Bernaerts, 1994; Pfaller *et al.*,1996) [15, 16]. Other researchers have observed of *C. albicans*, it appear with different green (Sullivan and Coleman, 1998). It was necessary to esteem simple, fast, and cost-effective methods such as chromogenic media to detect *Candida* sp. Chromogenic agar considers a modern and more fast method to detect *Candida* because includes enzyme-substrate related to chromogenic components. The color formed as a result of the enzyme that splits the substrate. The yeast species produce different enzymes that result in color diversity, this is beneficial for the detection of yeast (Odds and Bernaerts, 1994).

Diagnosis Yeasts via Vitek2 Compact System

The outcomes diagnosis of the yeast by Vitek 2 compact system it has been diagnosed 70 specimens out of 100 samples that infected with fungi (molds and yeast). It demonstrated most isolates belong to *Candida albicans* with percentage 25.7% (18/70) while other isolates were *C. parapsilosis* with 2.9% (2/70), *C. krusei* 11.43% (8/70), *C. glabrata* 5.7% (4/70) as shown in table (2).

Table 2: Diagnosis of yeasts by Vitek 2 Compact system

Isolate number	VITEK 2 YST card identification (Probability %)	
1	*C.albicans* 90%	Good identification
2	*C.albicans* 91%	Good identification
3	*C.albicans* 91%	Good identification
4	*C.krusei* 89%	Good identification
5	*C.glabrata* 90%	Good identification
6	*C.albicans* 92%	Good identification
7	*C.parapsilosis* 90%	Good identification
8	*C.albicans* 93%	Good identification
9	*C.krusei* 90%	Good identification
10	*C.krusei* 92%	Good identification
11	*C.albicans* 90%	Good identification
12	*C.albicans* 91%	Good identification
13	*C.albicans* 91%	Good identification
14	*C.krusei* 89%	Good identification
15	*C.glabrata* 90%	Good identification
16	*C.albicans* 88%	Good identification
17	*C.parapsilosis* 89%	Good identification
18	*C.albicans* 89%	Good identification
19	*C.krusei* 90%	Good identification
20	*C.krusei* 89%	Good identification
21	*C.albicans* 90%	Good identification
22	*C.albicans* 91%	Good identification
23	*C.albicans* 91%	Good identification
24	*C.albicans* 90%	Good identification
25	*C.albicans* 90%	Good identification
26	*C.albicans* 91%	Good identification
27	*C.albicans* 91%	Good identification
28	*C.albicans* 90%	Good identification
29	*C.glabrata* 90%	Good identification
30	*C.glabrata* 90%	Good identification
31	*C.krusei* 92%	Good identification
32	*C.krusei* 92%	Good identification

Through the results for this study appeared the frequency of *Candida albicans* more than other species, it may be caused to virulence of *C.albicans* and its ability transform to filamentous forms which are an aid to permeate tissue. Hyphae is showed variation responses which appear to assist in penetration. The initiation of colonization is by adhesion on the cell surface that induces adhesion with tissue (Brand *et al.*,2012; Brown *et al.*,2014).*C.albicans* can express other factors that participate in the absorption of iron and zinc, which consider major for virulence (Citiulo *et al.*,2012). Also, its ability of adhesion more than other species This may be due to the presence of special surface receptors (da Silva Dantas *et al.*,2016) [21].

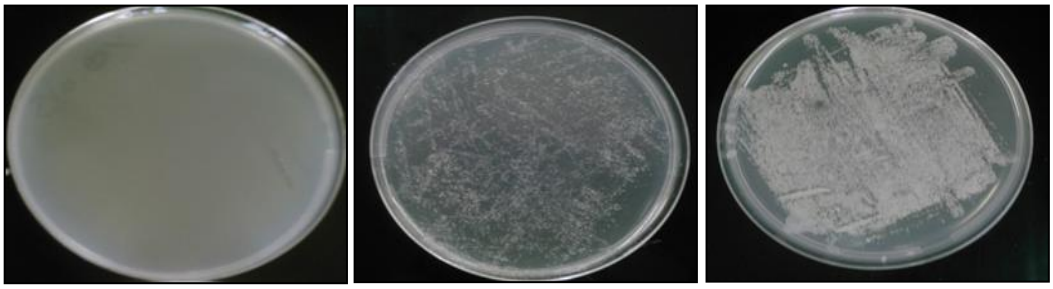
Determination of antifungals effect on some isolates of fungi

Determination inhibition of Antifungals effect on *Candida albicans*, *A. fumigatus* and *A. flavus*

The outcomes appeared MIC (minimum inhibiting concentration) and MFC for antifungal toward *C. albicans* where Nystatin recorded MIC 250 µg/ml and MFC 500µg/ml also, Amphotericin-B recorded MIC15.625 µg/ml and MFC 31.25µg/ml, moreover, Clotrimazole recorded 7.8125µg/ml and 15.625µg/ml as shown in the table (3) and figure (3).

Table 3: MIC and MFC for antifungal against *Candida albicans* by dilution method

Antifungal(µg/ml)	MIC	MFC
Nystatin	250	500
Amphotericin-B	15.625	31.25
Clotrimazole	7.8125	15.625



A **B** **C**

Fig 3: A- MIC, B- MFC and C- Control for antifungal against *Candida albicans* by agar dilution method

The results of antifungals against *Candida albicans* when using the minimum inhibition concentration of them, the highest concentration and the lowest concentration for the antifungals used in the dilution method, in this method showed that nystatin gave the largest inhibitory diameter (30 mm), followed by clotrimazole with an inhibitory diameter (23 mm) as shown in the table (4) and figure (4).

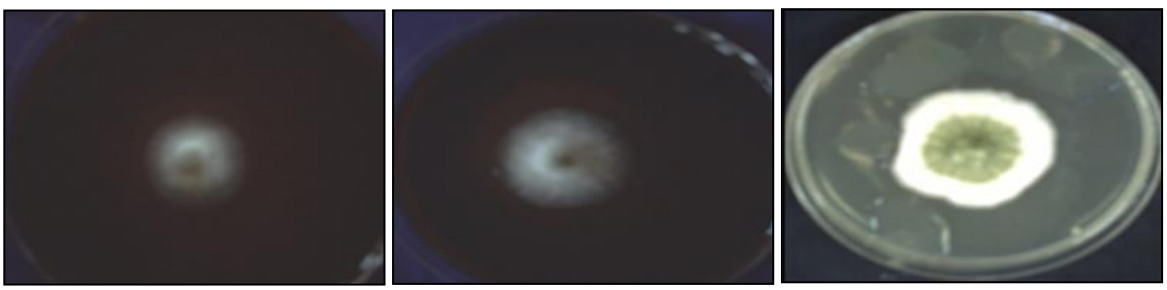
Table 4: The inhibition zone of antifungal against *Candida albicans*

Antifungal (µg/m l)	Inhibition zone at lowest concentration	Inhibition zone at minimum inhibition concentration	Inhibition zone at highest concentration
Nystatin	22	30	33
Clotrimazole	17	23	30
Amphotericin-B	10	12	13

A **B** **C**

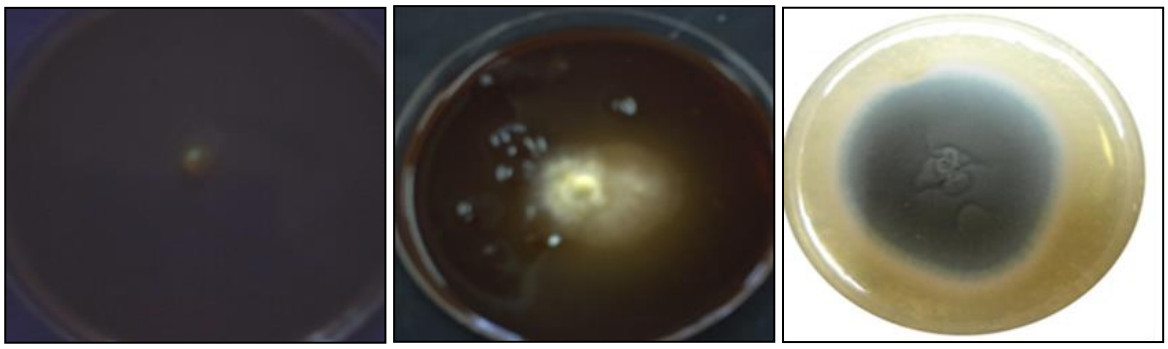
Fig 4: The inhibition zone of antifungal by diffusion method against *Candida albicans* (A-Nystatin, B-Clotrimazole, C- Amphotericin-B)

Regarding, the results of rate diameter colony growth for *Aspergillus flavus* and *A. fumigatus*, there were the rate diameter growth in using nystatin ranged (30-6 mm) and (44.7-6 mm) for each of these fungi respectively at the concentrations (5-2560 µg/ml) with significant differences among concentration in each of fungi compared with control also there were significant differences between these fungi in the concentration that ranged (5-230µg/ml)($P<0.05$) as shown in the figures (5 and 6) and table (5).



A **B** **C**

Fig 5: A- MFC, B- MIC and C- Control for nystatin against *A. flavus*



A **B** **C**

Fig 6: A- MFC, B- MIC and C- Control for nystatin against *A. fumigatus*

Table 5: Effect of nystatin antagonist on the diametric growth of *A. fumigatus* and *A. flavus*

Nystatin	***A. fumigatus***	***A. flavus***
µg/ml	**The rate diameter colony growth (mean ± SE) mm**	**The rate diameter colony (mean ± SE) mm**
Control	30.000 ± 0.000Ac	44.7 ± 0.000Aa
5	30.000 ± 0.000Ac	44.7 ± 0.000Aa
10	24.333 ± 0.667Bc	44.7 ± 0.333Aa
20	16.333 ± 0.333Cb	39.000 ± 0.577Ba
40	12.333 ± 0.333Dc	30.000 ± 0.000Ca
80	10.667 ± 0.333Ec	23.000 ± 0.577Da
160	7.833 ± 0.1668Fc	14.667 ± 0.667Ea
230	7.000 ± 0.000Fb	10.333 ± 0.333Fa
640	6.000 ± 0.000Fa	10.000 ± 0.000Fa
1280	6.000 ± 0.000Fa	9.333 ± 0.333Fa
2560	6.000 ± 0.000Fa	6.000 ± 0.000Ga

Different capital letters mean significant difference ($p<0.05$) to compare between different concentrations of antifungals.
Different small letters mean significant difference ($p<0.05$) for comparison between the fungi.

Besides, the rate diameter colony growth for *Aspergillus flavus* and *A. fumigatus* in using amphotericin B ranged (30-6 mm) and (47-6.7 mm) for each of them respectively at the concentrations (0.1-204.8µg/ml) with significant differences among concentrations(0.4-204.8µg/ml) in each of fungi compared with control also there were significant differences between these fungi in the concentrations (1.6, 3.2 and 6.4µg/ml)($P<0.05$) as shown in the figures (4- 9 and 4-10) and table(4-5)

Study effect inhibitory for *Artemisia* plant on molds

The results in the table (4-6) were indicated significant differences in diameter rate of the colony growth (mm). Where there were lower diameter growth for 200 mg/ml for each *A. fumigatus* (20 ± 0.849), *A. niger* (18≠1.061), *A. flavus* (17≠1.131). Besides, the concentration 140 mg/ml appeared higher diameter of growth for each *A. fumigatus* (35±0.849), *A. niger* (40±4.17), *A. flavus* (44≠39.3) ($P<0.05$). Moreover, the concentration of 180 and 160 mg/ml appeared the highest diameter of growth of each of the molds than the concentration of 200mg /ml and the lower of the diameter of growth than 140mg/ml (Figures 4-11). Regarding the results of the percentage of inhibition of these fungi in the using concentrations as shown in figure (4-10) the percentage for *A. fumigatus* at concentration 200mg/ml (73.3%), 180mg/ml (62.7%), 160mg/ml (60%), and 140mg/ml (53.3%). The percentage for *A. niger* at concentration 200mg/ml (73.1%), 180mg/ml (67.2%), 160mg/ml (58.2%), and 140mg/ml (40.3%). Moreover, The percentage for *A. flavus* at concentration 200mg/ml (72.6%), 180mg/ml (66.1%), 160mg/ml (53.2%), and 140mg/ml (29%). On the other hand, the efficient fungicidal influence was noticed against *A. flavus* and *A. nidulans* at 2.5 µl/ml (lower fungicidal concentration). Whilst the antifungal efficiency against *A. fumigatus* and *A. niger* was observed at 10 µl/ml of the oil concentration (Dehghani Bidgoli, 2021). *Artemisia campestris* essential oil also displayed antifungal effectiveness against *Aspergillus niger*, *Penicillium citrinum*, and *P. viridicatum* (Minimum fungicidal concentration more than 20 µl/ml) (Houicher *et al.*, 2016).

Al-Baldawy *et al.* (2019) studied the effect of *Artemisia herba-alba* and *Thymus vulgaris* against *Aspergillus ochraceus* and *Fusarium graminearum* It was observed inhibition growth rate 35.52, 66.5, 78.50 % for *Aspergillus ochraceus* by using thyme at the concentrations (1, 3, and 5%) respectively. The inhibition growth of *Fusarium graminearum* was 39.5, 68.70, 81.6% using thyme concentrations (1, 3, and 5%) respectively

Table 6: The effect of alcoholic extract of *Artemisia* in the growth of some molds isolated during the study

NO.	Fungus	Diameter rate of the colony growth (mm)± standard error concentrations (mg / ml)				Control	P<0.05
		200mg/ml	180mg/ml	160mg/ml	140mg/ml		
1	*A. fumigatus*	20 ±0.849 a	28±1.77b	30±1.56b	35±0.849c	75±0.0	0.001
2	*A. niger*	18≠1.061 a	22±1.06b	28±1.06c	40±4.17d	67±0.0	0.001
3	*A. flavus*	17 ≠1.131 a	21±1.13b	29±2.26c	44±3.39d	62±0.0	0.0001

Different letters in each row means significance difference (P<0.05).

* Con + represents control Meaning colonies diameters values without treatment with extract

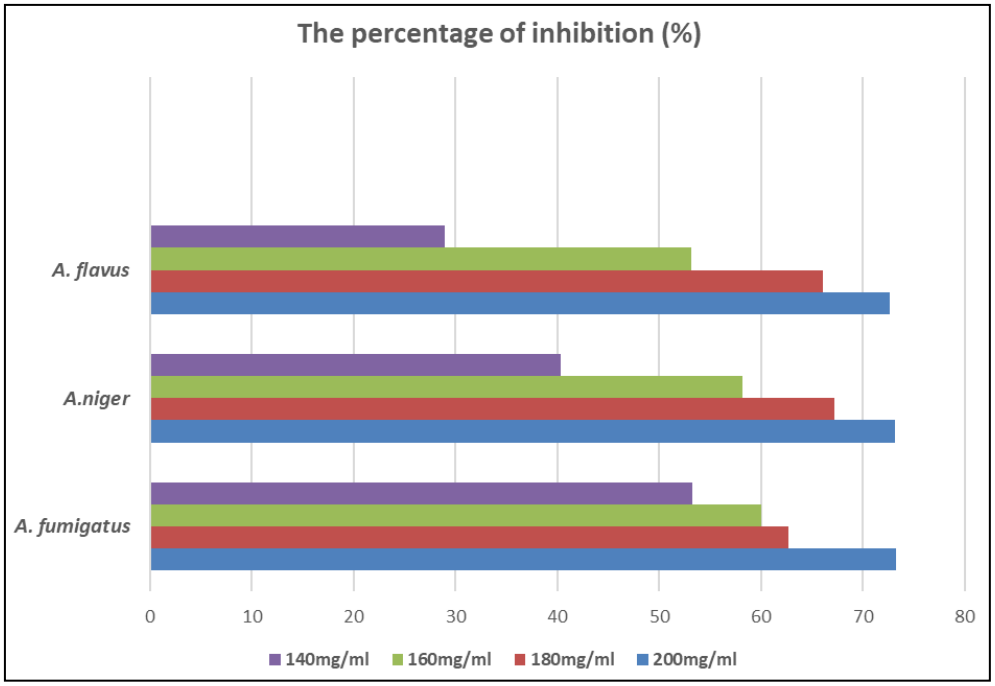


Fig 7: The percentage inhibition of molds isolated for concentrations alcoholic extract of *Artemisia*

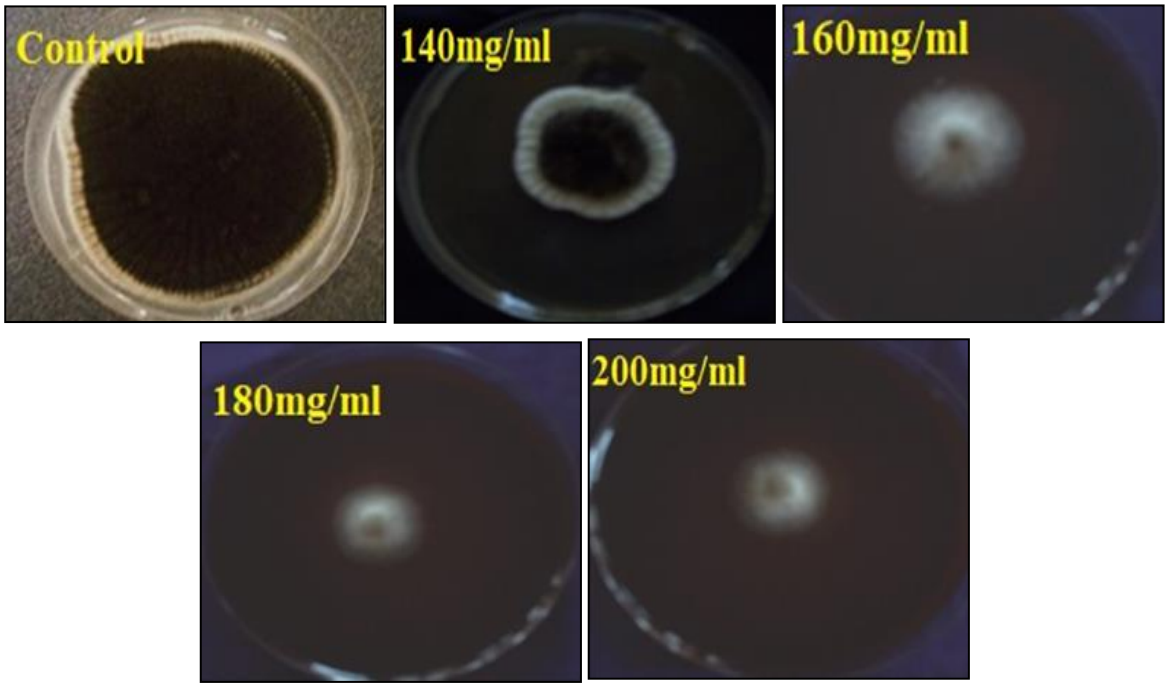


Fig 8: Effect of alcoholic extract for *Artemisia* plant on *Aspergillus niger*

Conclusions

1. The prevalence of *C. albicans* in the infected patients with COVID-19 was higher than the molds, where *C. albicans* had the highest percentage of yeasts spread and *A. fumigatus* mold was higher than the rest of the molds.

2. It was observed the increase of D-dimer and Ferritin in COVID-19 patients.
3. The high concentrations of the alcoholic extract of *Artemisia* gave the highest inhibition zone for yeasts and the lowest growth zones for the studied molds. This indicated the efficiency extract as antifungals.

References

1. Kshirsagar SG, Rao RV. Antiviral and Immunomodulation Effects of Artemisia. *Medicina (Kaunas, Lithuania),*2021:57(3):217.
2. Ekiert H, Świątkowska J, Knut E, Klin P, Rzepiela A, Tomczyk M, et al. Artemisia dracunculus (Tarragon): a review of its traditional uses, phytochemistry and pharmacology. *Frontiers in Pharmacology*, 2021, 567.
3. Szopa A, Pajor J, Klin P, Rzepiela A, Elansary HO, Al-Mana FA, et al.—Importance in the history of medicine, the latest advances in phytochemistry and therapeutical, cosmetological and culinary uses. Plants,2020:9(9):1063.
4. Hussain M, Raja NI, Akram A, Iftikhar A, Ashfaq D, Yasmeen F, et al. A status review on the pharmacological implications of Artemisia absinthium: A critically endangered plant. Asian Pac J Trop Dis,2017:7(3):185-192.
5. Ahamad J. A pharmacognostic review on Artemisia absinthium. International Research Journal of Pharmacy,2019:10(1):25-31.
6. Amidon C, Barnett R, Cathers J, Chambers B, Hamilton L, Kellett A, et al. Artemisia: An Essential Guide from The Herb Society of America. Caroline, A., Thomas, M., Kennel, E., Eds., 2014.
7. Bora KS, Sharma A. The genus Artemisia: a comprehensive review. Pharmaceutical Biology,2011:49(1):101-109.
8. Obistioiu D, Cristina RT, Schmerold I, Chizzola R, Stolze K, Nichita I, et al. Chemical characterization by GC-MS and *in vitro* activity against Candida albicans of volatile fractions prepared from Artemisia dracunculus, Artemisia abrotanum, Artemisia absinthium and Artemisia vulgaris. Chemistry Central Journal,2014:8(1):1-11.
9. Feng X, Cao S, Qiu F, Zhang B. Traditional application and modern pharmacological research of Artemisia annua L. Pharmacology & therapeutics,2020:216:107650.
10. Kiani BH, Kayani WK, Khayam AU, Dilshad E, Ismail H, Mirza B. Artemisinin and its derivatives: A promising cancer therapy. Molecular Biology Reports,2020:47(8):6321-6336.
11. Du L, Chen J, Xing YQ. Eupatilin prevents H2O2-induced oxidative stress and apoptosis in human retinal pigment epithelial cells. Biomedicine & Pharmacotherapy,2017:85:136-140.
12. Obeid S, Alen J, Nguyen VH, Pham VC, Meuleman P, Pannecouque C, et al. Artemisinin analogues as potent inhibitors of *in vitro* hepatitis C virus replication. PLoS One,2013:8(12):e81783.
13. Jang E, Kim BJ, Lee KT, Inn KS, Lee JH. A survey of therapeutic effects of Artemisia capillaris in liver diseases. Evidence-Based Complementary and Alternative Medicine, 2015.
14. Behbahani BA, Yazdi FT, Riazi F, Shahidi F, Noorbakhsh H, Yazdi FT. Antifungal potential of mangrove extracts against Aspergillus flavus and Penicillium italicum. Archives of Advances in Biosciences, 2014, 5(4).
15. Odds FC, Bernaerts RIA. CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important Candida species. Journal of clinical microbiology,1994:32(8):1923-1929.
16. Pfaller MA, Houston A, Coffmann S. Application of CHROMagar Candida for rapid screening of clinical specimens for Candida albicans, Candida tropicalis, Candida krusei, and Candida (Torulopsis) glabrata. Journal of clinical microbiology,1996:34(1):58-61.
17. Sullivan D, Coleman D. Candida dubliniensis: characteristics and identification. Journal of clinical microbiology,1998:36(2):329-334.
18. Brand A. Hyphal growth in human fungal pathogens and its role in virulence. International journal of microbiology, 2012, 517529.
19. Brown AJ, Budge S, Kaloriti D, Tillmann A, Jacobsen MD, Yin Z, et al. Stress adaptation in a pathogenic fungus. The Journal of experimental biology,2014:217(Pt 1):144-155.
20. Citiulo F, Jacobsen ID, Miramón P, Schild L, Brunke S, Zipfel P, et al. Candida albicans scavenges host zinc via Pra1 during endothelial invasion. PLoS Pathog.,2012:8:e1002777.
21. da Silva Dantas A, Lee KK, Raziunaite I, Schaefer K, Wagener J, Yadav B, et al. Cell biology of Candida albicans-host interactions. Current opinion in microbiology,2016:34:111-118.
22. Dehghani Bidgoli R. Chemical composition of essential oil and antifungal activity of *Artemisia persica* Boiss. from Iran. Journal of food science and technology,2021:58(4):1313-1318.
23. Houicher A, Hechachna H, Özogul F. *In vitro* determination of the antifungal activity of Artemisia campestris essential oil from Algeria. International Journal of Food Properties,2016:19(8):1749-1756.
24. Al-Baldawy MSM, Ahed Abd Ali H, Khaeim HM. Antifungal inhibitory activity of Thymus Vulgaris L. and Artemisia Herba-Alba powder and its constituent phytochemicals against Aspergillus Ochraceus and Fusarium Graminearum Growth. Plant Archives, 2019:19(1):801-804.