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Research Article

In vitro the effect of ethanol extracts of *Senna alexandrina* and *Achillea millefolium* on fungi pathogenic to plants and humans

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Abstract
Plant extracts are natural sources of bioactive compounds that could be used for crop protection and preserving human health. The present study aimed to evaluate the impact of ethanolic extracts of *Senna alexandrina* leaves and *Achillea millefolium* flowers on the growth of some filamentous fungi and yeasts isolated from yellow corn and human. Alcoholic extract of *Achillea millefolium* flowers showed significant inhibitory effects against the filamentous fungi: *Aspergillus flavus*, *A. ustus*, *Cladosporium oxysporum*, *Fusarium graminearum* and *Penicillium citrinum*, beside the dermatophytes *Trichophyton mentagrophytes*, *T.rubrum*, *T. schoenleinii*, *T. terrestre* and *T. verrucosum.* The superiority of the *A. millefolium* flower extract over the *Senna alexandrina* leaves extract the growth of most of the tested fungi. There was a direct relationship between the concentrations of the alcoholic extract of *A. millefolium* flowers and the inhibition in halo diameters of *Candida* isolates. High concentrations, including (200, 150, 125, 100) mg/mL inhibited the growth of all *Candida* isolates, while the 25 mg/mL concentration was ineffective against any of the tested isolates. *Candida krusei* was the most sensitive to the alcoholic extract of *Achillea* flowers (inhibition halo=22 mm at a concentration of 200 mg/ mL). *Candida glabrata* was the most resistant. Also direct relationship was recorded between the concentration of the alcoholic extract of *S. alexandrina* leaves and the diameters of the inhibition halos for *Candida* isolates. The high concentrations (200, 150, and 125 mg/mL) inhibited the growth of all the tested isolates. *Candida albicans* (2) was the most sensitive, while *C. glabrata*, was the most resistant. The present results showed the possible use of the two plant extracts as preventing factors for the growth of fungi in plants and humans.

Keywords: *Achillea millefolium, Candida* isolates, Ethanol extracts, Molecular identification, Pathogenic fungi, *Senna alexandrina*

INTRODUCTION

Since ancient times, plants have been a source of numerous significant therapeutic substances for preserving human health. Extracts and effective compounds of plants were used in the past as a traditional folk remedy for about 80% of the people on Earth, in accordance with the WHO (Muthu *et al*., 2006). Over half of all clinical medications used today have natural origins (Baker *et al*., 1995).

Herbal remedies or herbal medicine (phytomedicine) are regarded as the most widely used type of complementary and alternative medicine (Ogbonnia *et al*., 2011). Many medicinal herbs are used to treat many infectious and non-communicable diseases. Many people worldwide are susceptible to fungus-related infections due to the resistance of fungal strains. Antifungal medications are either very costly or have numerous adverse effects. Many therapeutic herbs are a valuable resource for the treatment of fungal diseases, some of them have a wide range of anti-fungal capabilities (Murtaza *et al*. 2015). Compounds of plant origin are generally less expensive and safer to use because they are safer and more available than synthetic ones (Cheesman *et al*., 2017; Chitsazian-Yazdi *et al*., 2015). Iraqi people use many plants to treat traditional infectious diseases (Najla and Awaz, 2009).

Achillea millefolium L. (Yarrow) is a medicinal plant

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that contains approximately 100 active anti-fungal and anti-bacterial compounds (Ghanati *et al*., 2014). It belongs to the Asteraceae family, which has been recorded growing on many continents, such as Europe, Asia, and North America. Many species of Achillea are employed in gardens as decorative plants. The green parts of yarrow possess a lengthy history of use in conventional veterinary herbal therapy (Ludwig, 1996; Hirti ,2000; Jangjoo *et al*., 2023). The essential oils of *A. millefolium* L. have medicinal benefits for their antiseptic and the ability to reduce inflammation (Benedek *et al*., 2007). Yarrow preparation is used in the form of a boiled extract or in the form of juice to treat many ailments such as flatulency, bleeding, bowel inflammation, cramps, loss of appetite, sores, rashes, and treatment of snake bites (Chandler *et al*., 1982). Yarrow is used to treat conventional and folk medicine wounds, digestive problems, respiratory diseases, and skin diseases. Among its other uses is the treatment of some liver diseases and mild analgesics (Applequist *et al*., 2011).

Senna is a broad genus of flowering plants belonging to the Fabaceae family, and the subfamily Caesalpinioideae. There are an estimated number of Senna species) 260 – 350(varying between herbal plants and shrubs. These species are distributed along the tropical, subtropical, and temperate regions (Randell and Barlow, 1998). Senna plants contain many compounds that belong to the anthraquinone group, which are called sennosides, including sennosides A, B, C, and D, as well as naphthalene derivatives, plant sterols, flavonoids, tannins, essential oils etc. (Haiek and Tillett, 2010).

Senna alexandrina has been known as a medicinal plant since 1950 (Duncan, 1957), and is commonly used as a laxative (Kinnunen *et al*., 1993). The leaves and fruits of the plant are used to treat haemorrhoids, skin and respiratory infections, migraine, and epilepsy (Srivastava *et al*., 2006; Ahmad *et al*., 2010).

Some researches indicate that the use of sennosides A (SA) inhibit the growth of a wide numbers of microorganisms, including bacteria (*Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*) and fungi (*Aspergillus flavus*, *A. niger*, *Fusarium moniliforme*, *Rhizoctonia bataticola*, beside *Candida albicans*) (Sharma, 2012; Rizwana *et al*., 2021).

The yeast Candida is the second most infectious pathogen in the world (Whibley and Gaffen, 2015). Candida species can produce biofilms, which enables them to resist the action of antibiotics. Therefore, the mechanism of action of antifungals is often investigated (Bachmann *et al*., 2002). *Of all systemic Candida disorders, C. albicans and C. glabrata account for 65-75%, followed by C. parapsilosis and C. tropicalis* (Brunke and Hube, 2013).

Since the natural products' composition differs depending on the region and the method of extraction, this study aimed to evaluate the impact of ethanolic extracts of *S. alexandrina* leaves and *A. millefolium* flowers on the growth of some filamentous fungi and yeasts isolated from yellow corn and human.

MATERIALS AND METHODS

Isolation of fungi from yellow corn

Thirty seeds of yellow corn were randomly collected from the local markets of Mosul, at the rate of 5 seeds per plate containing Potato Sucrose Agar (PSA(medium after sterilizing them with 0.1% NaOCl solution and three times washing using distilled water, all steps were carried under sterile conditions then incubated for 5-7 days at 28°C (Prakash *et al.,* 2012; and Noman *et al.,* 2018).

Isolation of fungi from human

Skin scrapings were taken from patients with skin infections in the scalp, body, and infected nails (Kidd *et al*., 2016), and cotton swabs were taken from patients with infections in the ears and mouth and between the toes, planted on the Sabouraud Sucrose Agar medium (SSA), incubated at 30□ for 2-4 weeks for dermatophytes, while swab samples were incubated at 37□ for 2 days. Growing samples were kept in slants and identified using their taxonomic keys (Baumgartner *et al*., 1996; Al-Assaf *et al*.,2020).

Diagnosis of fungal isolates

Diagnosis was conducted based on shape, color, diameter, background, phenotypic secretions, microscopic diagnosis of fungal hyphae, reproductive structures, and taxonomic keys (Emmons *et al*., 1977; Leslie *et al*., 1990 ; deHoog and Guarro 1995; Samson *et al*., 2004 ; Pitt and Hocking, 2009 ; Al-Rejaboo *et al*., 2021).*Candida sp.* diagnosis were conducted by the diagnostic culture medium (Chromogenic candida agar), incubating for 24 - 48 hours at 37°C (Pfaller *et al*., 1996; Baumgartner *et al*., 1996).

Collection of plant materials

Yarrow flower and *S. alexandrina* leaves were collected from the Zagros Mountains, in Erbil Governorate/ Kurdistan Region/ northern Iraq, then identification was done according to (Whitson and Burrill, 2002; Lesica, 2002).

Preparation of crude alcoholic extracts

Ten grams of dried powder of each yarrow flower and *S. alexandrina* leaves were immersed in a sufficient amount of absolute ethanol separately, then crushed using a homogenizer, stirred using a magnetic stirrer for 24 hours at10 □ temperature. The mixtures were filtered using multiple layers of gauze and Whatman No. 1 filter paper. The filtrate was subjected to a centrifuge at a speed of 3000 rpm. The filtrates were then left

in a rotary evaporator device to evaporate the solvents. The obtained dry materials were considered crude plant extracts (Iranbakhsh *et al.,* 2020; Al-Rejaboo and Jalaluldeen,2019).

To prepare the 200 mg/mL stock solution, 1 g of each crude extract was dissolved in 5 mL of dimethyl sulfoxide (DMSO). The stock solution was pasteurized for 10 minutes at 62˚C to sterilize it.

Antifungal activity

The concentrations of 5, 10, 15, and 20 mg/mL of alcoholic extracts for each plant were prepared by adding certain quantities of plant extracts to certain quantities of the nutritional medium PDA, then the media was poured into Petri dishes in triplicates of each concentration for each fungal isolate. The plates were inoculated with 6 mm diameters discs of 7 days' age of each certain fungal isolate. Also, the control dishes containing medium without any addition were inoculated in the same way then incubated at 28˚C for 7 days. The results were recorded by calculating the diameters of the growing colonies (Pandey *et al*., 1982; Al-Rejaboo and Al-Obaidy, 2013).

Anti-yeast activity

Assays were carried out in Muller-Hinton agar medium utilizing the Kirby-Bauer disk diffusion method. Two replicates were conducted for each treatment. Each petri dish of the SDA media was inoculated by swabbing 0.1 mL of the specific yeast suspension (prepared from 24-hour incubation colonies at 37˚C and calibrated by comparison with 0.5 McFarland tubes). Six other concentrations were prepared from the stock solution (200 mg/mL) previously prepared for each plant extract (150, 125, 100, 75, 50, 25) mg/ mL.

Then, discs of sterile filter paper measuring 6 mm in diameter were made. Each disc was soaked with 10 µL of one of the seven concentrations prepared previously, discs were sequentially distributed in each pre-inoculated petri dish.

The filter paper discs soaked with 10 µL of DMSO were used as negative control treatments, while standard Nystatin (100 µg) discs were used as positive control treatments. After incubation for 24 hours at 37°C, the findings were recorded by measuring the diameters of the inhibition haloes formed around each disc (Al-Assaf *et al*.,2020; Younes *et al*., 2021).

Identification of fungi by polymerase chain reaction (PCR)

A molecular study was conducted using PCR to confirm the identification of two isolates, one of which was mold and the other was yeast.

DNA extraction from the mold isolate

Geneaid Genomic DNA Mini Kit was used to extract pure genomic DNA.

Preparation of agarose gel and electrophoresis of DNA extracted from the mold sample

DNA was detected on a 2% agarose gel. The electrophoresis was run (10 V/cm, 1.5 h). The strips were photographed under UV light using a gel documentation device and the product of the PCR reaction.

Employing PCR to identify the highly conserved ITS region in mold

DNA concentration was adjusted by dilution with TE buffer. 50 ng/ µL were taken for each sample and the program in Table 1 was used for amplification.

In an 0.2 mL Eppendorf tube from Biolaps (England), the DNA sample, primer (Table 2) (Mhmood *et al*., 2021), and pre-mix ingredients were combined to create the master reaction mixture for PCR. Next, using distilled water to set the reaction volume to 20 µL, the mixture was centrifuged for three to five minutes using a microfuge instrument. Afterward, the tubes were placed into the thermocycler to carry out the multiplex reaction, each reaction utilizing a different program, after that the sample and DNA ladder, manufactured by Biolaps, were loaded into the wells of agarose. After that, the samples underwent (60–70) minutes of electrophoresis. Lastly, a picture of the gel was taken with the gel documentation. Using a DNA clean-up Geneaid kit, the PCR bands were excized from the gel and purified for DNA sequence analysis.

DNA extraction from the yeast isolates

DNA was extracted from the yeast using Geneaid Genomic DNA Mini Kit.

Table 1. PCR program for amplification of the Internal Transcribed Spacer (ITS) region from *Aspergillus flavus*

No.	Stage	Temperature	Time	Cycle number
1.	Initial Denaturation	95	5 min.	1
2.	Denaturation	95	45sec.	
3.	Annealing	55	1 min.	35
4.	Extension	72	1 min.	
5.	Final extension	72	7 min.	1

Table 2. Sequence of primers used to amplify the ITS region in molds

Primer	Sequence 5'-3'
Forward	TGAATCATCGACTCTTTGAACGC
Reverse	TTTCTTTTCCTCCGCTTATTGATAT

Detecting the presence of the highly conserved ITS region in yeast using PCR technique

To get the concentration needed to run PCR reactions (Table 3), the DNA concentration was adjusted by dilution with TE buffer. 50 ng/ µL was taken for each sample.

In a 0.2 mL Eppendorf tube from Biolaps (England), the DNA sample, primer (Table 4) (White *et al*., 1990), and pre-mix ingredients were combined to create the master reaction mixture for PCR.

The tubes were then put into the thermocycler to carry out the multiplex reaction using the designated program after the reaction volume was set to 20 microliters with distilled water. The mixture was then centrifuged using a microfuge equipment for 3-5 minutes.

Agarose gel preparation and DNA electrophoresis

To run and detect DNA, a 2% agarose gel was prepared. The electrophoresis was (10 V/cm, 1.5 h). The strips were photographed using a UV Tran illuminator and also the product of the PCR reaction.

DNA extraction from the gel

Using a Geneaid kit, the PCR bands were removed from the gel for purification and nucleotide sequence analysis.

Statistical analysis

Prism / P<0.0001 one-way ANOVA analysis was used for the statistical analysis.

RESULTS AND DISCUSSION

Isolation and identification of fungi

The filamentous fungi isolated from maize were: *Aspergillus flavus*, *A. niger, A. ustus , Cladosporium oxysporum, Fusarium graminearum,* and *Penicillium citrinum*. Human pathogenic filamentous fungi isolated from the different parts of the body of patients attending Al-jamhouri Hospital in Mosul city and suffering from *Trichophyton* dermatophytes including: *T. mentagrophytes , T.rubrum , T. schoenleinii , T. terrestre* and *T. verrucosum*

The yeasts isolated from some patients suffering from infections in the mouth and between the toes, including *Candida albicans , C. krusei , C. glabrata and C. parapsilosis* .

Molecular identification of *Aspergillus flavus* and *Candida parapsilosis*

To confirm the identification of the genotype and genetic affinity of both *A. flavus* and *C. parapsilosis*, DNA was extracted for both fungi to detect it by PCR method, where it was confirmed that the sample was pure and that the fungus *A. flavus* belonged to molds as shown in Figure (1) where one fluorescent package appeared, and this was evidence that the fungus *A. flavus* was pure.

The purity of the yeast *Candida parapsilosis* was confirmed, and it belongs to the yeasts (Fig. 2). The existence of a single package indicated that the yeast *C. parapsilosis* was pure. The two samples were sequenced and Blast was conducted in NCBI. Results showed that the fungi showed the highest identity with *A. flavus*. The sequence was submitted to, and the strain name Aspergillus flavus M-Z-M1 and the accession number (LC723826.1) were given.

Also, the DNA sequencing of the yeast isolate was carried out and showed the highest identity with *C. parapsilosis* and given the strain name *C. parapsilosis* M-Z-M2 and when submitted to NCBI given the accession number LC723827.1

The origin of *A. flavus* M-Z-M1 strain was shown in Fig. (3). From Fig. 3, the Iraqi isolate *Aspergillus flavus* M-Z-M1 isolated from yellow corn was genetically close to the Chinese isolate and diverged by 0.076 for the same fungus isolated from Nigeria, Poland and Brazil and not similar to the Pakistani isolate.

The origins of *Candida parapsilosis* M-Z-M2 was confirmed by conducting a genealogy search for several neighboring countries, as shown in Fig. (4).

The Iraqi isolate *C. parapsilosis* M-Z-M2 from mouth, which causes stomatitis and periodontitis, is genetically identical to the Argentine isolate, and is genetically close by 2.410 from the Japanese isolate and by 2.911 from the Iranian isolate, and divergent from the Belgium isolate.

Table 3. PCR reaction program for amplifying the ITS region of *Candida parapsilosis*

Co.	Stage	Temperature	Time	Cycle Cumber
1.	Initial denaturation	95	6 min.	1
2.	denaturation	95	45 sec.	
3.	Annealing	58	1 min.	35
4.	Extension	72	1 min.	
5.	Final extension	72	5 min.	1

Table 4. Sequence of primers used in amplifying the ITS region from fungi

Primer	Sequence
Forward (ITS1)	TCCGTAGGTGAACCTGCGG
Revers (ITS4)	TCCTCCGCTTATTGATATGC

Impact of using alcoholic extracts of *Senna alexandrina* leaves and *Achillea millefolium* flowers on isolated filamentous fungi

The results of *A. millefolium* flower alcohol extract on isolated filamentous fungi showed significant inhibitory effects compared to control treatments against each of the following filamentous fungi: *Aspergillus flavus*, *A. ustus* , *Cladosporium oxysporum* , *Fusarium graminearum* and *Penicillium citrinum*, , beside the dermatophytes *Trichophyton mentagrophytes*, *T.rubrum* , *T. schoenleinii*, *T. terrestre* and *T. verrucosum* (Fig. 5).

Figure 5 shows that the higher concentrations caused greater inhibitions, as there was a progressive reduction in the colony diameters, especially at a concentration 20 mg/mL. The growth of the following fungi (*Cladosporium oxysporum*, *Fusarium graminearum*, and *T. verrucosum*) was completely inhibited at the latter concentration, while *Penicillium citrinum* grew to a diameter of 0.7cm, *T. schoenleinii* grew in a diameter of 1.7cm. At the same concentration, the following isolates (*A. flavus, A. ustus, T. terrestre*) grew with colony diameters of (2.2, 2.5, 3) cm, respectively while *T. mentagrophytes* was more resistant to the effect of alcoholic extract, which grew in a diameter of 5.3 cm. Also, the growth of *C. oxysporum* and *T. verrucosum* isolates was completely inhibited at a concentration of 15 mg/mL. The rest of the concentrations also significantly inhibited fungal growth for all fungi used, but the inhibition was less than what was caused by higher concentrations. When comparing the inhibitory effect of *Achillea millefolium* flowers alcoholic extract with the effect of the alcoholic extract *Senna alexandrina* leaves (*Cassia sp.*) at the same concentrations, we notice the superiority of the *A. millefolium* flowers over the Senna leaves in inhibiting the growth of most the tested fungi (Figure 6).

At a 20 mg/mL concentration, *A. ustus* was the only fungus completely inhibited by alcoholic extract of *S. alexandrina* leaves compared to a control (5.7 cm). The inhibitory effect of Senna alcoholic extract on *A. ustus* was less than the inhibitory effect of the *Achillea millefolium* flowers at the rest of the concentrations. The present study noticed a decrease in the diameter of the fungus colony coinciding with an increase in the concentration of the plant extract. The diameters of the colony at concentrations (5, 10, and 15) mg/mL reached (5.6, 3, and 2.9) cm, respectively. This is also the case for the other fungal isolates under study. The growth of all fungal isolates directly decreased proportionally to the increase in concentrations of the plant's alcoholic extract. The highest growth inhibition after *A. ustus* was achieved in *P. citrinum* whose growth diameter was 1.1 cm at a concentration of 20 mg/mL compared to the control treatment (7 cm), followed by *A. flavus* isolate which grew with a diameter of 3.2 cm, compared to the control treatment (7.5 cm). *Fusarium graminearum* diameter was 4.2 cm at the same concentration compared to the control treatment (7 cm). Figure (6) also showed that the dermatophytes *Trichophyton schoenleinii* and *T. terrestre* grew with a diameter of 5 cm compared to the control treatment (7.5 cm) while, *T.rubrum* grew with a diameter 5.1 cm compared with the control treatment (7.5 cm). *Trichophyton mentagrophytes* diameter growth of 6 cm at the same concentration compared to a control treatment (7.5 cm).

The study also compared the inhibition activity of the extracts with antifungal ketoconazole (Figure 7). As a pure compound, we used small concentrations of ketoconazole (0.5, 1, 1.5, and 2 mg/mL). The study noticed a high inhibition in the growth of the tested fungi. The inhibition levels differed significantly from one fungus to

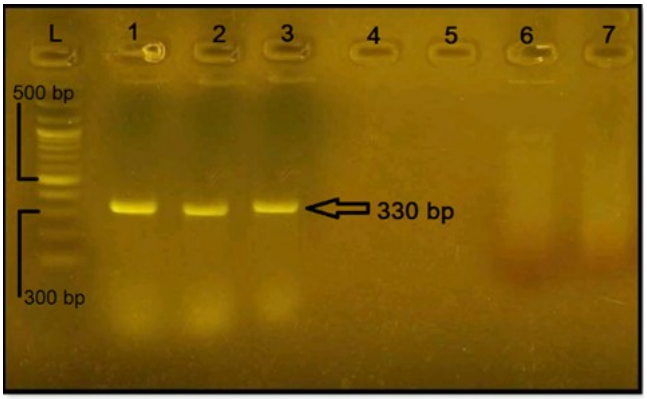

Fig. 1. *PCR reaction product of Aspergillus flavus studied for the ITS region and a reaction product of 330bp, carried over by a 2% agarose gel*

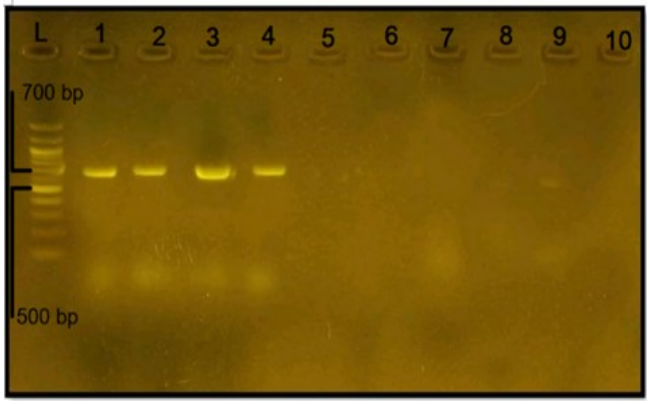

Fig. 2. *Product of the PCR reaction for a sample of the yeast Candida parapsilosis studied in the ITS region, 700bp reaction yield migrated by 2% agarose gel*

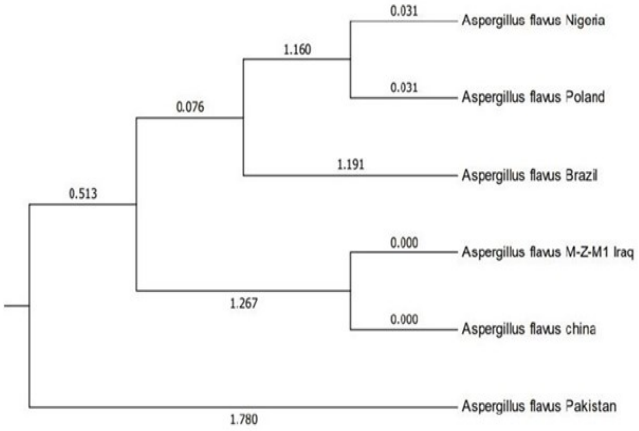

Fig. 3. *Genetic dimension and affinity tree for isolate Aspergillus flavus M-Z-M1 registered with National Center for Biotechnology Information (NCBI)*

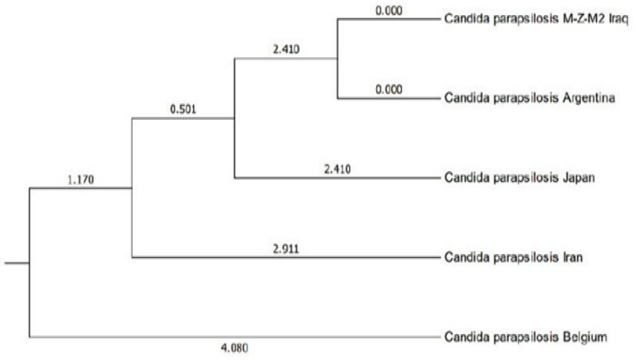

Fig. 4. *Genetic dimension and affinity tree for isolate Candida parapsilosis M-Z-M2 registered in National Center for Biotechnology Information (NCBI)*

another.

The fungi *C. oxysporum*, besides *A. ustus*, *and F. graminearum* were more sensitive to ketoconazole concentrations than the rest of the tested fungi, as they did not grow at all previous concentrations compared to the control treatments (2.2cm, 5.7cm, and 7 cm, respectively).

At 2 milligrams per milliliter of Ketoconzole, *Trichophyton terrestre* grew with a colony diameter 0.4 cm but *T. verrucosum* ,*T. mentagrophytes* and *T.schoenleinii* grew with a colony diameters (0.6, 0.7, and 0.7) cm respectively*. T. rubrum* gave the highest colony diameter (3.5 cm) at the same concentration. Ketoconazole was a growth inhibitor of all the fungi under study, but the fungus *T. rubrum* was one of the most resistant to this antifungal compared to the rest of the tested fungi, despite its inhibition. Thus, this drug is considered broad-spectrum in its inhibition of different fungi. Inhibition is due to the similarity between fungi in the composition of the cell wall, especially the compound ergosterol, which is targeted by the antibiotic to stop lanosterol from being converted to ergosterol in the path of formation of the fungal wall, which prevents its growth and spread and thus reduces or eliminates its pathogenicity.

From the previous results, a gradual reduction in the ability of the fungi colonies under test to grow was noticed, coinciding with an increase in the concentrations of the alcoholic extracts of the two plants. *A. millefolium* alcoholic extract was superior in inhibiting the tested fungi, and greatly increased effectiveness in inhibition compared to the alcoholic extract of the *S. alexandrina* leaves, even though the two extracts gradually inhibited the growth of all fungal colonies. Compared with the control sample, there are highly significant differences in inhibition for all concentrations and all tested fungi. This is evidence of the existence of effective compounds capable of inhibiting the growth of fungi despite their different isolation sources.

Impact of *Senna alexandrina* leaves and *Achillea millefolium* flowers' alcoholic extract on isolated yeasts

Table 5 shows, in general, a direct relationship between the concentrations of the alcoholic *A. millefolium* flower extract and the inhibition halo diameters of the yeast isolates under study. The high concentrations, including (200, 150, 125, and 100) mg/mL prevented every yeast isolation from growing. The inhibition halos' diameters ranged between 9 and 22 mm. The 75 mg/ mL concentration prevented all yeasts from growing except for *C. glabrata*. The diameters of the inhibition halos ranged between 7 and 13 mm. The concentration of 50 mg/mL was only effective in the growth of *C. krusei* and the diameter of the inhibition halo reached 12 mm, while the concentration of 25 mg/mL was not effective against any of the tested isolates. The results of Table 5 also show that *C. krusei* was the most sensitive to the alcoholic extract of *Achillea* flowers, as its growth was affected by all concentrations of the extract (except for the concentration of 25 mg/ mL), and through it, the highest inhibition was achieved (22 mm) at a 200 mg/mL concentration, which exceeded the value of inhibition halo in the positive control treatment (18 mm) and differed significantly from it.

On the contrary, *C. glabrata* was the most resistant, as the lower concentrations of the extract (25, 50, and 75) mg/mL did not show any inhibitory activity, while the high concentrations of the extract (100-200) mg/mL inhibited the growth of this yeast with values ranging between (9 -13) mm, which was less than nystatin treatment (22 mm).

Additional findings indicated that the yarrow flower extract had a moderate effect on *C. albicans* 1,2 and *C. parapsilosis*, as the extract inhibited the growth of these isolates only at higher concentrations (75-200) mg/ mL. In these concentrations, the growth inhibition values did not reach the values achieved when using the antifungal nystatin, except the isolate *C. albicans* 2 .The value of the growth inhibition halo at a 200 mg/mL concentra-

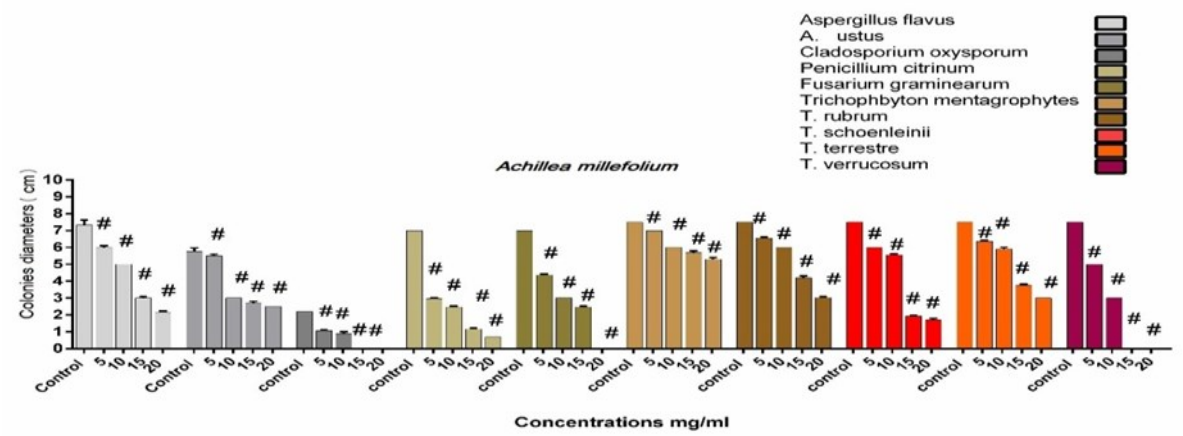

Fig. 5. *Effect of different concentrations of the alcoholic extract of Achillea millefolium flowers on the growth of filamentous fungi; (#) significant inhibitory effect*

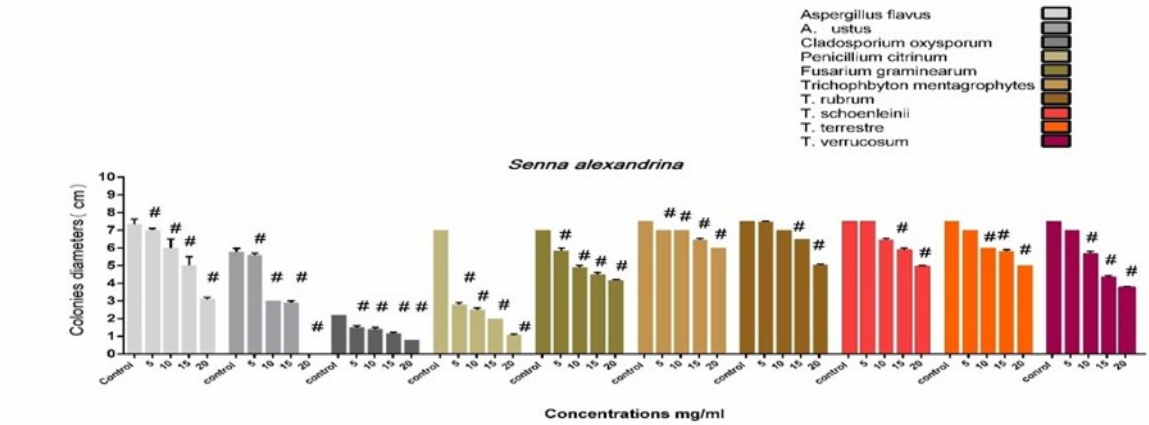

Fig. 6. *Impact of varying concentrations of the alcoholic Senna alexandrina leaves extract on the filamentous fungus under study; (#) significant inhibitory effect*

tion for the last isolate was equal to the halo formed by the antifungal nystatin (20 mm).

Table 6 demonstrates the direct correlation between the alcoholic extract's concentrations of *Senna alexandrina* leaves and the diameters of the inhibition halos for the tested yeast isolates. The high concentrations, including 200, 150, and 125 mg/mL inhibited the development of every isolate examined. The diameters of the inhibition zones ranged between 8 and 18 mm.

All *Candida* isolates were inhibited at a dose of 100 mg/mL except *C. glabrata*, and the diameters of the zone inhibition ranged between (9 and 14) mm. The concentrations (75 and 50) mg/mL did not affect the growth of each of the *C. krusei* and *C. glabrata*, while they inhibited the rest of the species, but the inhibition was less than what was achieved by the rest of the higher concentrations since the inhibition zones varied in size from 7 to 11 mm. The concentration of 25 mg/mL only had an impact on *C. albicans* (2) growth, and the inhibitory zone was only 7 mm in diameter. The growth of *Candida albicans* (2) was inhibited by all concentrations of the extract, so it was considered the most sensitive. In contrast to *C. glabrata*, which was the most resistant. Its growth was affected only in high concentrations of 125, 150, and 200 mg/ mL of the extract. The diameters of the inhibition halos were 8, 10, and 12 mm, respectively.

However, in all treatments, the diameters of the inhibition halos caused by the addition of the standard antifungal (nystatin) were the best. They differed significantly from the best values achieved when adding any concentration of the alcoholic extract. This applies to any of the yeast isolates under study.

All negative control treatments were ineffective against all yeast isolates, which indicates that any inhibitory effect of ethanol extracts of the plant flowers resulted from its active components, and the solvent used in preparing them has no effect. As for the positive control treatments, all *Candida* species showed sensitivity to nystatin (100 µg), which inhibited their growth.

The inhibitory effect of *Achillea millefolium* flower extract in this research can be attributed to its various secondary metabolites like flavonoids, phenolic substances, essential oils, and sesquiterpenes (Fierascu *et al*., 2015; Faiku *et al*., 2018). Khare (2007) indicated that *Achillea millefolium* extract includes salicylic acid, flavonoids, polyacetylenes, triterpenes, coumarins, tannins, alkaloids (achilleine), and volatile oil that contains linalool, camphor, sabinene, chamazulene, and other azulenes. Some of these compounds act as anti-

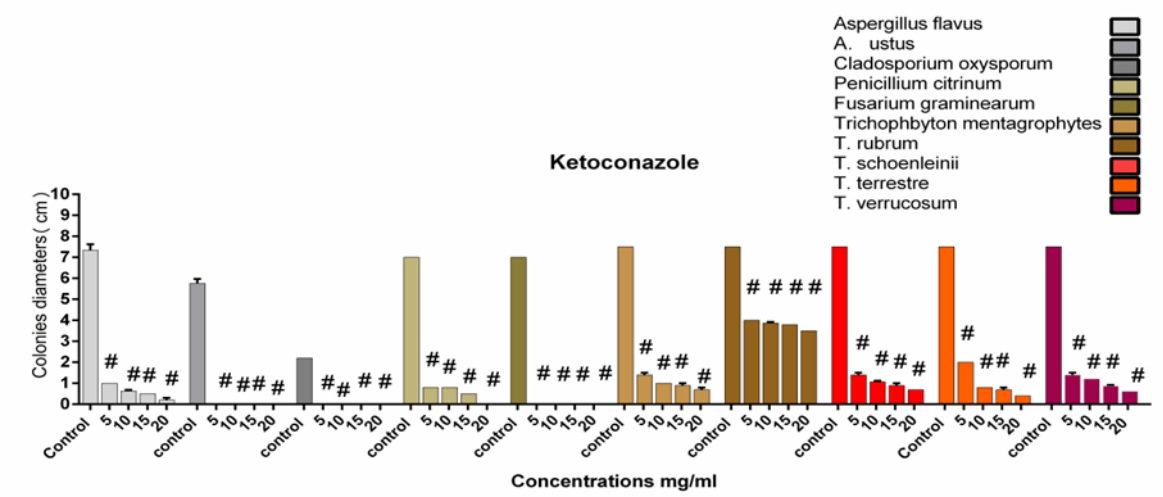

Fig. 7. *Inhibitory effect of different concentrations of antifungal Ketoconazole on the growth of isolated fungal colonies from different sources measured in centimeters*

Table 5. Inhibition zone diameters caused by ethanolic extract of *Achillea millefolium* flowers (mg/ mL) against *Candida* isolates

***Candida* isolates**	**Nystatin (100µg)**	**DMSO**	***Achillea millefolium* flowers extract concentrations**						
			200	**150**	**125**	**100**	**75**	**50**	**25**
C. albicans 1	22 a (0.70)	IN	17 cd (1.41)	14 fg (0.70)	12 hi (0.70)	11 ij (0.00)	7 l (1.41)	IN	IN
C. albicans 2	20 b (0.00)	IN	20 b (1.41)	16 de (1.41)	15 ef (0.70)	13 gh (1.41)	12 hi (0.70)	IN	IN
C. krusei	18 c (1.41)	IN	22 a (0.71)	16 de (0.00)	15 ef (0.71)	14 fg (0.71)	13 gh (0.71)	12 hi (0.71)	IN
C. glabrata	20 b (1.41)	IN	13 gh (0.00)	11 ij (0.71)	9 k (0.00)	9 k (0.70)	IN	IN	IN
C. parapsilosis	23 a (0.70)	IN	13 gh (1.41)	11 ij (0.00)	10 jk (0.70)	10 jk (0.00)	9 k (0.70)	IN	IN

According to Duncan multiple range test, the different letters that follow the inhibition zone diameters indicate significant differences in probability (0.01). Each diameter refers to the mean of two replicates The numbers in brackets refer to the standard deviation values IN= inactive (Inhibition zone diameter= zero)

inflammatories, like salicylic acid and chamazulene. Also the ability of alcoholic *Senna alexandrina* leaf extracts to inhibit the yeasts under study can be explained by the fact that they include a wide range of secondary metabolites such as flavonoids, aromatic compounds, phenols, alcohol (Rizwana *et al*., 2021), beside saponins as well as tannins (Khan and Srivastava, 2009). Tannins bind to protein rich in proline and thus disrupt the process of protein synthesis (Shimada, 2006). Plants produce flavonoids naturally in response to microbial infections, and it is not surprising that these compounds are secreted outside the body of the organism to become effective against a wide range of microorganisms. The effectiveness of saponins against microorganisms is due to their capacity to cause the leaking of microbes' proteins and enzymes (Patra and Saxena, 2009).

The reason for the different sensitivity of yeast isolates to the extract prepared in this research is that each species has unique properties in terms of its possession of virulence factors, and it has different sensitivity patterns to antifungal (Pappas *et al*., 2018), as *Candida* species have diverse pathogenic factors, such as the release of enzymes, cellular adhesion, host defense evasion, and the creation of biofilms (Silva *et al*., 2012). The limited capacity of some extracts to prevent the growth of some yeast isolates, such as *C. glabrata*, may be attributed to these isolates' capacity to create a biofilm, which acts as a barrier that prevents the extract from penetrating the cell membrane, or by binding to

Table 6. Inhibition zone diameters caused by ethanolic extract of *Senna alexandrina* leaves (mg/ mL) against *Candida* isolates

***Candida* isolates**	**Nystatin (100µg)**	**DMSO**	***Senna alexandrina leaves* extract concentrations**						
			200	**150**	**125**	**100**	**75**	**50**	**25**
C. albicans 1	22 a (0.70)	IN	18 c (0.70)	16 de (0.00)	15 ef (0.70)	14 fg (1.41)	11 ij (1.41)	11 ij (0.00)	IN
C. albicans 2	20 b (0.00)	IN	17 cd (1.41)	15 ef (0.00)	14 fg (0.70)	12 hi (0.00)	11 ij (0.70)	9 kl (0.00)	7 m (1.41)
C. krusei	18 c (1.41)	IN	16 de (0.00)	15 ef (0.70)	12 hi (1.41)	9 kl (1.41)	IN	IN	IN
C. glabrata	20 b (1.41)	IN	12 fg (0.70)	10 jk (0.70)	8 lm (1.41)	IN	IN	IN	IN
C. parapsilosis	23 a (0.70)	IN	14 fg (0.00)	13 gh (0.70)	11 ij (0.70)	9 jk (0.00)	9 kl (0.00)	7 m (1.41)	IN

According to Duncan multiple range test, the different letters that follow the inhibition zone diameters indicate significant differences in probability (0.01). Each diameter refers to the mean of two replicates The numbers in brackets refer to the standard deviation values IN= inactive (Inhibition zone diameter= zero)

the active substances of the extract and preventing them from reaching the target locations (Lafleur *et al*., 2006; Nett *et al*., 2007).

Other research results are consistent with what was obtained in this research and related to the effect of the *Achillea millefolium* extract, such as the findings of Ribeiro et al. (2010). Their results showed that the oils of *Achillea millefolium* had a moderated effect on *Candida albicans*, *C. glabrata*, and *C. tropicalis*. The results of Grigore *et al*. (2020) demonstrated that the extract of ethanol of the *Achillea millefolium* did not have an antifungal impact on *Candida albicans* ATCC10231 growth. Also, the results of the current research related to the effect of the alcoholic extract made from senna plant leaves agreed with (Ibrahim and Osman, 1995), who claimed that a senna ethanolic extract had strong antifungal efficacy against dermatophytes, while disagreeing with the results of Rizwana *et al*., (2021), who reported that the human pathogenic Candida species were resistant to all of *Senna alexandrina* leaves extracts.

The values of the inhibition halos were achieved using high concentrations of the plant's alcoholic flower extract against the species of yeasts under test, especially the concentration 200 mg/ mL, which ranged between 13 and 22 mm also, the values of inhibition zones achieved using high concentrations of alcoholic extract of *Senna alexandrina* leaves against *Candida* species under study, especially the concentration 200 mg/ mL which ranged between 12 and 18 mm are close to the values achieved using the antifungal nystatin, which ranged between 18 and 23 mm. This matter enhances the possibility of using *A. millefolium* flowers and *S. alexandrina* leaves as a novel antifungals after conducting more studies related to the detect the pure active substances of these plants, which may be applied in the management of infectious disorders brought on by harmful organisms.

In a related investigation, the alcoholic extract of these two plants was utilized to stop the growth of the fungus *Rhizoctonia solani*, which was isolated from rice plant seeds, which caused its damage and death. Concentrations of 5, 10, 15, and 20 mg/mL of the culture medium were used, and they observed a high inhibition in the growth of the fungus, as it inhibited 100 percent and no growth of the fungus was recorded at all concentrations, starting from the minimum concentration (5 mg/ mL) up to the maximum concentration (20 mg/mL), for both plants. This demonstrates that some substances effectively prevent the growth of this fungus and the other fungi under study in the two extracts. These are good results that indicate the possibility of using the two plants or their waste or extracts to help in sterilizing soil from pathogenic fungi, especially the fungus *Rhizoctonia solani*, which causes the wilting and death of most field crops and other plants that are important economically and globally (Al-Rijabo and Mahmood, 2013).

Conclusion

From the previous results, we note that the alcoholic extracts of the two plants have a clear inhibitory effect on the growth of all the filamentous fungi and yeasts under study due to their effective compounds that may inhibit cell wall formation or prevent the production of compounds important for the activity and vitality of the fungal cell, such as the production of proteins and their vitality or ergosterol, chitin, or beta-glucan, and other important compounds in the maintenance and activity of the fungal cell, especially there is variation in the

proportions of some compounds present in the fungal cell walls, which are more targeted than the rest of the vital compounds of the fungal cell. Antifungals target them due to their difference in composition from the animal cell; thus, the harmful effect is reduced. These antibodies are resistant to animal cells, whether human or animal.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Ahmad, M., Khan, M.A, Zafar, M., Arshad, M., Sultana, S., Abbasi, B.H. & Din, S.U. (2010). Use of chemotaxonomic markers for misidentified medicinal plants used in traditional medicines. *J. Med. Plant. Res.*, 4(13), 1244 1252. doi:10.5897/JMPR10.027
2. Al-Assaf, S. T., Al-Rejaboo, M. A. & AL -Daody, A. C. (2020). Assessing the impact of quercetin isolated from *Ammi majus* seeds upon *Candida* spp. is plants isolated from different sources. *Int. J. Res. Pharm. Sci.*,11(4), 5141-5149. https://doi.org/10.26452/ijrps.v11i4.3118
3. Al-Rejaboo, M. A. & Al-Obaidy, O. M. (2013). Inhibitory effect of alcoholic extract of sage leaves on the growth of pathogenic fungi causing external ear and skin infections. *Journal of Life Sciences*, 7(5), 468-474. DOI: 10.13140/RG.2.2.15258.29121
4. Al-Rejaboo, M. A., Fathi, H. B. & Shahari, N. A. (2021). Using of external secretions of *Trichoderma harzianum* and *T. viridi* as antifungals to different types of pathogenic fungi. *AGBIOL*, 816-839.
5. 5. Al-Rejaboo, M. A. & Jalaluldeen, A. M. (2019). Studying the Airborne Fungi of some rooms in the internal sections of Mosul university campus and the Possibility of using Sage plants to control it. *Journal of Advanced Pharmacy Education & Research*, 9(3), 17-22.
6. 6. Al-Rijabo, M. A. & Mahmood, N. Q. (2013). The Inhibition Effect for Growth of the Fungus Rhizoctonia solani Using Alcoholic Extract for some Plants. *Rafidain Journal of Science*, 24(2), 13-23. DOI: 10.33899/rjs.2013.71018
7. Applequist, W., Moerman, A. & Yarrow, D. (2011). (Achillea millefolium L.): A Neglected Panacea, A Review of Ethnobotany, Bioactivity, and Biomedical Research. *Econ. Bot*., 65: 209-225. DOI: 10.1007/s12231-011-9154-3
8. Bachmann, S. P., VandeWalle, K., Ramage, G., Patterson, T. F., Wickes, B. L. & John R.(2002). In Vitro Activity of Caspofungin against *Candida albicans* Biofilms. *Antimicrobial Agents and Chemotherapy*, 46 (11), 3591-3596. DOI: 10.1128/AAC.46.11.3591-3596.2002
9. Baker, J.T., Borris, R. P. & Carte, B. (1995). Natural product drug discovery and development new perceptive on international collaboration. *J. Nat. Prod.* (58),1325-1325.
10. Baumgartner, C., Freydiere, A. M. & Gille, Y. (1996). Direct identification and recognition of yeast species from clinical material by using Albicans ID and CHROM agar Candida plates. *J. of Clinical microbiology*, 34 (2), 454-456. DOI**:** https://doi.org/10.1128/jcm.34.2.454-456.1996
11. Benedek, B., Koopand, B. & Melzig, M. F.(2007). Achillea millefolium L. s.l. – Is the anti inflammatory activity mediated by protease inhibition*. J. Ethnopharmacol*., 113(2), 312 -17. DOI: 10.1016/j.jep.2007.06.014
12. Brunke, S. & Hube, B. (2013). Two unlike cousins: *Candida albicans* and *C. glabrata* infection strategies. Cellular Microbiology, 15(5), 701–708. doi: 10.1111/cmi.12091
13. Chandler R. F., Hooper S. N. & Harvey M. J. (1982). Ethnobotany and phytochemistry of yarrow Achillea millefolium, Compositae. *Economic Botany*, 36(2), 203-223.
14. Cheesman, M. J., Ilanko A., Blonk B. & Cock I. E. (2017). Developing New Antimicrobial Therapies: Are Synergistic Combinations of Plant Extracts/Compounds with Conventional Antibiotics the Solution?. Pharm. Rev. ,11(22), 57–72. doi: 10.4103/phrev.phrev_21_17
15. Chitsazian-Yazdi, M., Agnolet S., Lorenz S., Schneider B., Es'haghi Z., Kasaian J., Khameneh B. & Iranshahi M. (2015). Foetithiophenes C-F, thiophene derivatives from the roots of Ferula foetida. *Pharm. Biol*.; 53(5), 710–714. DOI: 10.3109/13880209.2014.939765
16. deHoog, G. S. & Guarro, J. (1995). Atlas of clinical fungi. Universitat Rovirai Virgili, Spain, p 720.
17. Duncan A.S. (1957). Standardized Senna as a Laxative in the Puerperium", *British Medical Journal,* 1 (5016), 439–441. DOI: 10.1136/bmj.1.5016.439
18. Emmons, C. W., Binford, C. H., Vtz, J. P. & Chung, K. J. (1977). Medical mycology. 3rd edition, Lea and Febiger, Philadelphia.
19. Faiku, F., Haziri, A., Mehmeti, I., Mehmeti, A. & Hoti, G. (2018). In vitro antibacterial activity of different solvent extracts of *Achillea millefolium* (L.) growing wild in Kosovo. *Fresenius Environ. Bull*., 27(6), 3878–3883.
20. Fierascu, I., Ungureanu, C., Avramescu, S., Fierascu, R., Ortan, A., Soare, L. & Paunescu, A. (2015). In Vitro Antioxidant and Antifungal Properties of *Achillea millefolium* L. *Rom. Biotechnol. Lett.,* 20, 10626–10636.
21. Ghanati, F., Bakhtiarian, S., Parast, B. M. & Behrooz, M. K. (2014). Production of New Active Phytocompounds by *Achillea millefolium* L. after Elicitation with Silver Nanoparticles and Methyl Jasmonate. *Biosciences biotechnology research asia*, 11(2), 391-399. doi: http://dx.doi.org/10.13005/bbra/1287
22. Grigore, A. , Colceru-Mihul , S., Bazdoaca C., Yuksel, R., Ionita, C. & Glava, L. (2020). Antimicrobial Activity of an *Achillea millefolium* L. *Proceedings*, 57(1), 34. https://doi.org/10.3390/proceedings2020057034
23. Haiek, G. & Tillett, S. (2010). El Jardín Medicinal de la Revista El Palo de arco, el Sen, el Yagrumo y la Zarzaparrilla. *Revista Facultad de Farmacia*,73(2), 44-45.
24. Hirti, U. (2000). Phyotherapie bei der KatzEntero. Anwendungsmoglichkeiten und Anwendungshaufigkeiten durch den Besitzer. Dissertation, Vet. Med.Univ. Wien.
25. Ibrahim, D. & Osman, H. (1995). Antimicrobial activity of Cassia alata from Malaysia. *Journal of Ethnopharmacology*, 45, (3), 151-156. https://doi.org/10.1016/0378-8741(94)01200-J
26. Iranbakhsh, A., Asadboland, R., Eslahi, M. & Shirzadian,

S. (2020). Evaluation of Fungicidal Potential of Some Moss Extracts on Phytopathogenic Fungus *Fusarium Solani*. *Anatolian Bryology*, 6(2), 105-111. DOI: 10.26672/anatolianbryology.746243

27. Jangjoo M., Joshaghani A. & Tahernejadgatabi F. (2023). The role of *Achillea millefolium* in traditional medicine: A review of its use in different cultures. *Journal of Multidisciplinary Care*,12(3), 152-156. doi: 10.34172/jmdc.1259
28. Khan, N.A. & A. Srivastava. (2009). Antifungal activity of bioactive triterpenoid saponin from the seeds of Cassia angustifolia. *Nat. Prod. Res.,*23, 1128-1133. DOI:10.1080/14786410802625279
29. Khare, C.P. (2007) Indian Medicinal Plants—An Illustrated Dictionary. First Indian Reprint, Springer (India) Pvt. Ltd., New Delhi, 812p.
30. Kidd, S., Halliday, C., Alexiou, H., & Ellis, D. (2016): Descriptions of Medical Fungi. 3rd Edition. The National Library of Australia Cataloguing-in-Publication entry.
31. Kinnunen, O., Winblad, I., Koistinen, P. & Salokannel, J. (1993). Safety and efficacy of a bulk laxative containing senna versus lactulose in the treatment of chronic constipation in geriatric patients", *Pharmacology* 47 (Suppl 1), 253–5. DOI: 10.1159/000139866
32. Lafleur, M. D., Kumamoto, C. A. & Lewis, K. (2006). *Candida albicans* biofilms produce antifungal-tolerant persister cells. *Antimicrob. Agents. Chemother.*, 50(11) , 3839-3846. DOI: 10.1128/AAC.00684-06
33. Lesica, P. (2002). Flora of Glacier National Park. Oregon State University Press. Corvallis, Oregon.
34. Leslie, J. F., Pearson, A. S., Nelson, P. E. & Toussoun, T. A. (1990). Fusarium spp. From corn, sorghum and soy bean fields in the central and eastern united states. *Phytopathology*, 80 (4), 343-350. https://doi.org/10.1094/Phyto-80-343
35. Ludwig M. (1996): Phytotherapie bei Rind einst und jetzt – alte Indikationen neu bewertet. Dissertation an der Universität für Veterinärmedizin, Wien, Österreich.
36. Mhmood , M., C., Al-Sanjari, M., N. & Khalil, M., I., (2021). Molecular identification of detergents degradation fungi in waste water. Plant Archives , 21, 237-243.
37. Murtaza, G., Mukhtar, M. & Sarfraz, A., (2015). A review: Antifungal potentials of medicinal plants. *J. Bioresource Manage*., 2(2), 23-31. DOI: 10.35691/JBM.5102.0018
38. Muthu, C., Ayyanar, M., Raja, N. & Ignacimuthu, S. (2006). Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. *J Ethnobio Ethnomed.* ;2:43. doi:10.1186/1746-4269-2-43
39. 39. Najla, A.K. & Awaz, F. (2009). Isolation and antibacterial evaluation of plant extracts from some medical plants in Kurdistan region. J. Duhok Univ. 12, (1) (Special Issue) , 250-255.
40. Nett, J. L., Lincoln, K., Marchillo, R., Massey, K., Holoyda, B. H., off, M. V. Handel & Andes, D. (2007). Putative role of β-1, 3 glucans in *Candida albicans* biofilm resistance. *Antimicrob. Agents. Chemother*.51, 510-520.
41. Noman, E. A., Al-Gheethi, A., Rahman, N., Talip, B., Radin, M., Radin M. S. & Kadir, M. (2018). Single Spore Isolation as a Simple and Efficient Technique to obtain fungal pure culture. IOP Conference Series: Earth and Environmental Science.
42. Ogbonnia, S. O., Mbaka, G. O., Anyika, E. N., Emordi, J. E. & Nwakakwa, N. (2011). An evaluation of acute and subchronic toxicities of a Nigerian polyherbal tea remedy. Pakistan *Journal of Nutrition*; 10, 1022-8. pp. 115-127. DOI: 10.3923/pjn.2011.1022.1028
43. Pandey, D. K., Trpathi, N. N., Tripathi, R. D. & Dixit S.N. (1982). Fungitoxic and phytotoxic properties of the essential oil of *H. suaveolens*. Zeit. Pflazenkraneaten and Pflanznschutz. 89, 344-349.
44. Pappas, P. G., Lionakis, M. S., Arendrup, M. C., Ostrosky-Zeichner, L. & Kullberg, B. J. (2018). Invasive candidiasis. *Nat. Rev. Dis. Primers*, 4:18026. DOI: 10.1038/nrdp.2018.26
45. Patra, A.K. & Saxena, J., (2009). The effect and mode of action of saponins on the microbial populations and fermentation in the rumen and ruminant production. *Nutr Res Rev*, 22(2), 204–219. DOI: 10.1017/S0954422409990163
46. Pfaller, M. A., Houston, A. & Coffmann, S. (1996). Application of CHROM agar Candida for rapid screening of clinical specimens for *Candida albicans*, *Candida tropicalis*, *Candida krusei* and *Candida (Torulopsis) glabrata*. *J. of Clinical microbiology*, 34 (1), 58-61. DOI: 10.1128/jcm.34.1.58-61.1996
47. Pitt, J. I. & Hocking, A. D. (2009). Fungi and food spoilage (Vol. 519, p. 388). New York: Springer.
48. Prakash, B., Singh, P., Mishra, P. K. & Dubey, N. K. (2012). Safety assessment of Zanth xylumalatum Roxb. Essential oil, its antifungal, anti-aflatoxin, antioxidant activity and efficacy as antimicrobial in preservation of Piper nigrum L. fruits, International Journal of Food Microbiology, 153(1), 183–191. DOI: 10.1016/j.ijfoodmicro.2011.11.007
49. Randell B. R. & Barlow B. A. (1998). "Senna" In: Alexander S. George (executive editor). Flora of Australia Australian Government Publishing Service: Canberra, Australia, 12, 89-138.
50. Ribeiro, D. I., Alves, M. S., Faria, M. G. I., Svidzinski, T. I. E., Nascimento, I. A., Ferreira, F. B. P., Ferreira, G. A. & Gazim, Z. C. (2010). Determination of antifungal activity of essential oils of Curcuma longa L. (Zingiberaceae) and Achillea millefolium (Asteraceae) grown in the Northwest Paraná. Arquivos de Ciências da Saúde da UNIPAR, 14 (2), 103-109.
51. Rizwana, H., Fatimah , A., Alharbi , R., I., Albasher , G., Moubayed, N.M.S. & Alqusumi R. (2021). Morphology and ultrastructure of some pathogenic fungi altered by leaf extracts of *Senna alexandrina* Mill (2021). *Pak. J. Agri. Sci*., 58(1), 389-408. DOI: 10.21162/PAKJAS/21.9789
52. Samson, R. A., Hoekstra, E. S. & Frisvad, J. C. (2004). Introduction to food-and airborne fungi (No. Ed. 7). Centraalbureau voor Schimmelcultures (CBS).
53. Sharma, R. A. (2012). Antimicrobial Activity of Sennosides from Cassia Pumila Lamk. *J. Med. Plants Res.,* 6 (19): 3591-3595. DOI: 10.5897/JMPR12.335
54. Shimada T. (2006). Salivary proteins as a defense against dietary tannins. *J. Chem. Ecol.*, 32 (6), 1149-1163. DOI: 10.1007/s10886-006-9077-0
55. Silva, S., Negri, M., Henriques, M., Oliveira, R., Williams, D. W. & Azeredo, J. (2012). *Candida glabrata*, *Candida parapsilosis and Candida tropicalis*: Biology, epidemiology, pathogenicity and antifungal resistance. *FEMS Microbiol. Rev*., 36(2), 288–305. https://doi.org/10.1111/j.1574-

6976.2011.00278.x

56. Srivastava, M., Srivastava, S., Khatoon, S., Rawat, A.K.S., Mehrotra, S. & Puhpangadan, P. (2006). Pharmacognostical evaluation of *Cassia angustifolia* seeds. *Pharmaceut. Biol*., 44 (3), 202-207. DOI: 10.1080/13880200600686442
57. Whibley, N & Gaffen, S. L. (2015). Beyond *Candida albicans*: Mechanisms of immunity to non-*albicans Candida* species. *Cytokine*, 76(1), 42-52. DOI: 10.1016/ j.cyto.2015.07.025
58. White T.,J., Bruns, T., Lee, S. & Taylor, J.,W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. San Diego, CA: Academic Press. http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1
59. Whitson, T. & Burrill, L. (2002). Weeds of the West. 9th ed. Western Society of Weed Science in cooperation with the Western United States Land Grant Universities Cooperative Extension Services. Newark, CA.
60. Younes, N. T., Al-Kataan, M. A. & Al-Rejaboo , M. A. (2021). Detection of some virulence factors of fungi caused Otomycosis isolated from some hospitals and clinics in Mosul/Iraq. *Al-Qadisiyah Journal of Pure Science* 26(4), 210–220. DOI: 10.29350/qjps.2021.26.4.1413