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Isolation and Identification of Fungi from Clinical Samples of Diabetic Patients and Studying the Anti-Fungal Activity of Some Natural Oils on Isolated Fungi

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Abstract:

Isolation of fungi was performed from February to July, 2019. One hundred clinical specimens were collected from King Abdullah Hospital (KAH) Bisha, Saudi Arabia. Samples were collected from twenty patients of different ages (30 - 70 years old) ten males and ten females. The samples were collected from patients with the two types of diabetics. Specimens included blood, hair, nail, oral swabs and skin. Specimens were inoculated on Sabourauds Dextrose agar containing chloramphenicol. Thirteen fungal species were isolated and identified. The isolated species were: *Aspergillus flavus, A. niger, A. terrus, A. nidulans, A. fumigatus, Candida albicans, C. krusei, C. parapsilosis, C. Tropicalis, Curvularia lunata, Fusarium solani, Penicillium marneffei* and *Saccharomyces cerevisiae.* Identification of molds was carried out morphologically and microscopically using available methods and books of identification, while identification of yeasts was carried out using API system. *C. albicans* recorded the highest isolated number where 31 colonies were isolated from 18 patients, representing relative density of 22.5%. (R. D.: is the number of a certain fungal species divided by the total number of fungi). Other isolated fungal species recorded relative density less than 16 %. The most common isolated fungus *Candida albicans* was molecularly identified using the 5.8S and flanking ITS regions. The antifungal activity of some natural essential oils (cinnamon, thyme, coconut, almond and clove) was assayed against isolated fungi using disk diffusion method. The used concentration was 5µl / plate. The MIC values were also determined using different oil concentrations (1, 2.5, 5, 10, 20 and 40 µl / disc).

Key words: Antifungal, *C. albicans*, Diabetic, Essential oils, Internal Transcribed Spacer (ITS).

Introduction:

Candida and *Aspergillus* are among the most common fungal species that cause high mortality rate in infected persons (1,2). Antifungal drug resistance and invasive fungal diseases require the development of new antifungal drugs (3). Resistant mycotic species often appear in prolonged use of antifungal agents or after prophylactic treatment (4). *Candida albicans* is the third common pathogen found in bloodstream infections in children (5). *C. albicans* can cause a serious disease candidiasis, which disseminated blood stream with high mortality rate (6). *C. albicans* resistance is due to presence of resistance gene(s) associated with antifungal treatment (7). *Candida* infections increased due to less susceptibility to azole antifungals (8). Fungal infections are often common in individuals of diabetes mellitus (9).

Candida and *Aspergillus* are common fungi in diabetic patients due to increased concentration of salivary glucose which enhance growth of yeast (10). Vaginal candidiasis is common in women with diabetes. *Aspergillus*, the main causes of aspergillosis, was detected in diabetes disease (11).

Non-albicans species prevalence increased during the last decades due to the increase use of azoles (12). Azole resistance can be due to modifications of target enzymes, low access of azole to the target, or both mechanisms (13). Azole resistance is of great importance because azoles like fluconazole are commonly used in the treatment of candidiasis (14). The broad use of fluconazole led to the emergence of resistant species (15). Numerous essential oils were tested for *in-vivo* and *in-vitro* antifungal activity (16). Since ancient times,

folk medicine and agro-food science have benefitted from the use of plant derivatives, such as essential oils, to combat different diseases, as well as to preserve food. In nature, essential oils play a fundamental role in protecting the plant from biotic and abiotic attacks to which it may be subjected. Many researchers have analyzed in detail the modes of action of essential oils and most of their components. The purpose of this brief review is to describe the properties of essential oils, principally as antifungal agents, and their role in blocking cell communication mechanisms, fungal biofilm formation, and mycotoxin production (17). Fungal identification based on DNA is receiving more attention (18). Systems based on DNA use sequences of DNA for the identification and surveys of biodiversity (19). However, worldwide accessible system of identification of fungal organism is needed (20). DNA barcoding is a method used for the identification of species based on DNA sequences (21). This method serves the rapid identification of fungi and other organism at species level. The ITS region is the most widely sequenced region in DNA used for the identification of fungi (22) and has been recommended as the universal fungal barcode sequence (23).

The aim of the study is to isolate and diagnose some fungi from clinical samples of diabetic patients and to study the anti-fungal activity of some natural oils on their effectiveness.

Materials and Methods:

Collection and isolation of fungi from different clinical specimens

Fungi were isolated throughout the period from February to July 2019. These isolates were originated from different categories of twenty patients of different ages (30 to 70 years old) males and females each represented ten cases hospitalized at KA Hospital in Bisha. The origins of the clinical specimens were different. Specimens were blood, oral swabs, hair, nail and skin. Fungal species were cultivated on Sabouraud dextrose agar (SDA) at 25 ºC for 7 days for filamentous fungi.

Identification of molds

Identification of molds was carried out morphologically and microscopically. The identification was done based on culture characteristic such as colony shape, the presence of septa, shape of conidia/spores and colony pigmentation (24). Small fragments of the fungus were removed using inoculating needles and placed on a clean glass slide. The fungus was stained using Lactophenol cotton blue (a fungal stain). The slides were then observed under a light fluorescence microscope. Identification was carried out using two references (25, 26).

Identification of *Candida*

Identification of the isolated yeasts was carried out by using biochemical API *Candida* and API 20C AUX systems (bioMerieux, France). Biochemical characterization for each isolate was performed first using API *Candida* system and then the results were confirmed by using API 20C AUX system (27).

Cultivation of fungal species

Fungal species were cultivated on Sabouraud dextrose agar (SDA) at 25 ºC for 7 days.

Antifungals assays by disk diffusion method

Oils were collected from different local markets. The antifungal activity of five natural essential oils: Thyme (*Thymus vulgaris*; family Lamiaceae.), Coconut (*Cocos nucifera*; family Arecaceae), Clove (*Syzygium aromaticum*; family Myrtaceae), Cinnamon (*Cinnamomum verum*; family Lauraceae) and Almond (*Prunus dulcis*; family Rosaceae), were applied on filter paper (5 µL/disk) disks of 6 mm in diameter separately. 0.3 mg/ mL of a Fluconazole was used as positive control. All plates were incubated at 25 ºC for 24 hours. All the experiments were carried out in triplicate (28).

Estimation of minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (µL/disk) of the tested essential oils were estimated by the method described by Andrews (29). The MIC values were also determined using different oil concentrations (1, 2.5, 5, 10, 20 and 40 µl / disk). The evaluation of the inhibitory effect of different essential oils on Sabouraud medium was employed as culture media for fungi. Test inoculums of 10^3 M spores ml^{-1} was applied. The MIC is defined as the lowest concentration showing no growth.

Molecular identification of *Candida albicans*:

DNA isolation from isolated fungus was performed using as per the SDS extraction method described by Melo et al. (30). Amplification of the ITS gene was performed using the primers ITS1- F (5 1 TCC GTA GGT GAA CCT TGC GG 3 1) and ITS4- R (5 1 TCC TCC GCT TAT TGA TAT GC 3 1) (31). PCR amplifications were carried out in 25 µl reaction mixture containing 2 µl the primers, were also standardized by running at different temperatures. The amplified products along with forward primer were sent for purification and sequencing using automated DNA sequencing. Fungal strains were identified by submitting the ITS

sequences in Westerdijk Fungal Biodiversity Institute database.

Statistical analysis

Statistical analysis was executed using SPSS software version 22. One-way analysis of variance (ANOVA) test was used to study the effect of the applied factors on the studied parameters. Post-hoc Duncan's test was executed to estimate similarities in the parametric variables among the studied groups. Pearson's correlation coefficient was used to correlate the studied parameters to the experimental pH and temperature (32).

Results:

In this study, a total of 13 fungal species were isolated from 100 clinical samples (hair, nail, oral swabs and skin) that were collected from KA hospital in Bisha governorate during the period from February to July, 2019. The data show that twenty patients of diabetics were enrolled in this study.

Male and female patients (ten for each), represented 50 % for males and females. Males' age ranged from 31 to 69 years, while females age ranged from 30 to 70 years.

With respect to the distribution of various clinical samples obtained from patients suspected to have fungal diseases, twenty nine specimens out of hundred were blood specimens, hair and skin, each was represented by twenty one specimens. Oral swabs represented seventeen specimens. The least number of specimens were nail specimens where only twelve specimens were examined.

Thirteen fungal species were isolated including eight molds and five yeasts. The isolated molds were: *Aspergillus flavus, A. niger, A. terrus, A. nidulans, A. fumigatus, Curvularia lunata, Fusarium solani* and *Penicillium marneffei*. Isolated molds recorded 53 colonies with relative density of 38.4 %. While isolated yeasts were: *Candida albicans, C. krusei, C. parapsilosis, C. Tropicalis,* and *Saccharomyces cerevisiae.*Isolated yeasts recorded 85 colonies with relative density of 61.6%, while the isolated molds were 36 colonies with relative density 35.4%.

Candida albicans was the most abundant genus where 31 fungal colonies were isolated from 18 patients representing relative density of 22.5 %; followed by *C. krusei* where 22 colonies were isolated representing 15.9 % relative density recovered from 12 patients. *A. niger* came next in rank where 17 fungal colonies were isolated representing relative density of 12.3 % recovered from 11 patients. *C. tropicalis* recorded 15 fungal colonies representing 10.5 % relative density recovered from 11 patients. Other isolated fungal species recorded colony count from 2 to 12 with relative density of 1.4 to 8.7 % and were recovered from 2 to 10 patients (Table 1).

Table 1. Number of colonies, number of patients isolated from and relative density of isolated fungi

Isolated fungi	Number of isolates	Number of patients isolated from	Relative density (%)
Aspergillus flavus	12	10	8.7
A. niger	17	11	12.3
A. terreus	5	5	3.6
A. nidulans	4	4	2.9
A. fumigatus	8	6	5.8
Candida albicans	31	18	22.5
C. krusei	22	12	15.9
C. parapsilosis	11	5	8.0
C. Tropicalis	15	11	10.9
Curvularia lunata	2	2	1.4
Fusarium solani	3	3	2.2
Penicillium marneffei	2	2	1.4
Saccharomyces cerevisiae	6	6	4.3
Total count	138	---	100

Biochemical differentiation between isolated yeasts was done using API *Candida* and API 20C AUX systems (Tables 2and 3).

Table 2. API *Candida* reading of the isolated yeasts

Test	*Candida albicans*	*C. krusei*	*C. parapsilosis*	*C. Tropicalis*	*Saccharomyces cerevisiae*
Glucose	+	+	+	+	+
Galactose	+	–	+	+	+
Sucrose	+	–	+	+	+
Trehalose	+	–	–	–	+
Raffionse	–	–	–	+	+
β Maltosidase	–	–	–	+	–
α Amylase	–	+	–		+
β-Xylosidase	–	–	–	–	–
β-Glucuronidase	–	–	–	–	–
Urease	–	–	–	–	–
N-Acetyl-β-Glucosaminidase	+	–	–	–	–
β- Galactosidase	–	–	–	–	–

Table 3. API 20C AUX reactions of the isolated yeasts

Test	*Candida albicans*	*C. krusei*	*C. parapsilosis*	*C. Tropicalis*	*Saccharomyces cerevisiae*
Glucose	+	+	+	+	+
Glycerol	–	+	+	+	–
Calcium-2-Keto-gloconate	+	–	–	–	–
L - Arabinose	–	–	–	+	–
D - Xylose	+	–	+	–	–
Adonitol	+	–	–	–	–
Xylitol	+	–	–	–	+
D - Galactose	+	–	+	–	+
Inositol	–	–	–	–	+
D - Sorbitol	+	–	+	–	–
Methyl-αD-Glucopyranoside	+	–	–	–	+
N-Acetyl Glucosamine	+	+	+	–	+
D - Cellobiose	–	–	–	–	–
D - Lactose	–	–	–	–	+
D - Maltose	+	–	–	–	+
D - Sucrose	+	–	–	–	+
D - Trehalose	+	–	–	–	–
D -Melezitose	–	–	–	–	–
D - Raffinose	–	–	–	–	+
Hyphae	+	–	+	+	–

The identification of the commonly isolated fungus *C. albicans* was confirmed via molecular identification (Fig. 1).

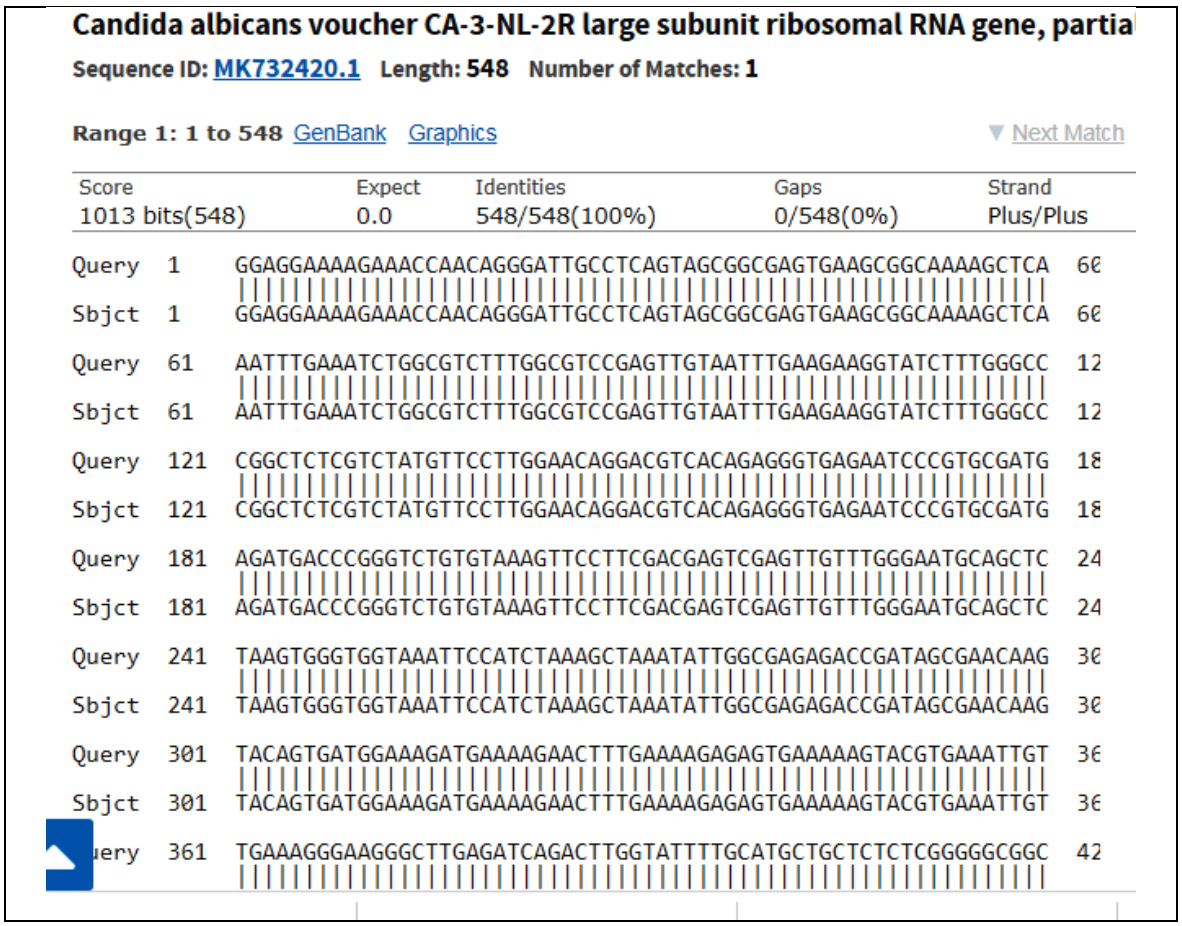

Figure 1. *Candida albicans* voucher CA-3-NL-2R large subunit ribosomal RNA gene, partial sequence

The resulting sequences were then compared with those available in the public databases online of NCBI (National Centre for Biotechnology Information) using BLAST (Basic Local Alignment search Tool) search program (http:// www. nlm. nih. gov/ blast/) (33). The *C. albicans* strain was identified as *Candida albicans* voucher CA-3-NL-2R. Score 989 bits (1096), Expect 0.0, Identities 548/548(100%), Gaps 0/548(0%), strand Plus/Plus.

Table 4.Effect of some essential oils on the isolated fungal species

Treatment	**Diameter of Inhibition zone (mm)**											
	Fluconazole (control)		**Almond (*Prunus dulcis*)**		**Cinnamon (*Cinnamomum verum*)**		**Clove (*Syzygium aromaticum*)**		**Coconut (*Cocos nucifera*)**		**Thyme (*Thymus vulgaris*)**	
	diameter (mm)	R. A. (%)	diameter (mm)	R. A. (%)	diameter (mm)	R. A. (%)	diameter (mm)	R. A. (%)	diameter (mm)	R. A. (%)	diameter (mm)	R. A. (%)
Aspergillus flavus	5	100	3	60	5	100	5	100	2	40	4	80
A. niger van	6	100	3	50	6	100	5	83.3	3	50	5	83.3
A. terreus	7	100	4	57.1	7	100	6	85.7	4	57.1	7	100
A. nidulans	8	100	4	50	8	100	7	87.5	4	50	7	87.5
A. fumigatus	7	100	3	42.9	7	100	7	100	3	42.9	7	100
Candida albicans	7	100	2	28.6	7	100	7	100	2	28.6	6	85.7
C. krusei	8	100	2	25	8	100	7	87.5	2	25	7	87.5
C. parapsilosis	7	100	3	42.9	7	100	6	85.7	3	42.9	7	100
C. Tropicalis	10	100	4	40	10	100	9	90	4	40	8	80
Curvularia lunata	10	100	7	70	10	100	8	80	7	70	8	80
Fusarium solani	11	100	8	72.7	11	100	9	81.8	8	72.7	9	81.8
Penicillium marneffei	8	100	5	62.5	8	100	8	100	5	62.5	7	87.5
Saccharomyces cerevisiae	7	100	5	71.4	7	100	7	100	5	71.4	6	85.7
Mean relative activity (%)		100		51.8		100		90.9		50.2		87.6

R.A.%: percent of relative activity= activity of treatment x 100/activity of control.

Cinnamon oil recorded the highest mean relative activity (100%) among tested oils, followed by clove oil (90.9), thyme oil (87.6%), almond oil (51.8%), coconut oil (50.2%) (Table 4).

Cinnamon achieved MIC values less than or equal to that of fluconazole, while Clove and thyme recorded MIC values equal to or higher than that of control. Clove and thyme recorded similar MIC values against tested fungal species. The MIC values were relatively high or even not detected in case of almond and coconut oils (Table 5).

Table 5. Determination of Minimum Inhibitory Concentration (MIC) of some essential oils

Treatment	Minimum Inhibitory concentration (MIC) (µL/disk)					
	Fluconazole (control)	**Almond (*Prunus dulcis*)**	**Cinnamon (*Cinnamomum verum*)**	**Clove (*Syzygium aromaticum*)**	**Coconut (*Cocos nucifera*)**	**Thyme (*Thymus vulgaris*)**
Aspergillus flavus	5.00 ± 0.44^{A}	Nd^{A}	5.00 ± 0.58^{B}	10.00 ± 0.58^{B}	Nd^{A}	10.00 ± 0.58^{B}
A. niger	5.00 ± 0.33^{A}	Nd^{A}	5.00 ± 0.33^{B}	10.00 ± 1.15^{B}	Nd^{A}	10.00 ± 1.15^{B}
A. terreus	5.00 ± 0.44^{A}	10.00 ± 0.67^{B}	2.50 ± 0.29^{A}	5.00 ± 0.58^{A}	40.00 ± 0.33^{B}	5.00 ± 0.58^{A}
A. nidulans	5.00 ± 0.29^{A}	20.00 ± 0.58^{C}	2.50 ± 0.17^{A}	5.00 ± 1.15^{A}	40.00 ± 1.45^{B}	5.00 ± 1.15^{A}
A. fumigatus	5.00 ± 0.17^{A}	Nd^{A}	5.00 ± 0.17^{B}	10.00 ± 0.58^{B}	Nd^{A}	10.00 ± 0.33^{B}
Candida albicans	5.00 ± 0.44^{A}	Nd^{A}	5.00 ± 0.58^{B}	10.00 ± 0.33^{B}	Nd^{A}	10.00 ± 0.33^{B}
C. krusei	5.00 ± 0.33^{A}	40.00 ± 1.73^{D}	5.00 ± 0.33^{B}	10.00 ± 0.58^{B}	Nd^{A}	10.00 ± 033^{B}
C. parapsilosis	5.00 ± 0.33^{A}	Nd^{A}	5.00 ± 0.88^{B}	10.00 ± 0.33^{B}	Nd^{A}	10.00 ± 0.33^{B}
C. Tropicalis	5.00 ± 0.29^{A}	Nd^{A}	5.00 ± 0.17^{B}	10.00 ± 0.88^{B}	Nd^{A}	10.00 ± 0.88^{B}
Curvularia lunata	5.00 ± 0.88^{A}	10.00 ± 0.33^{B}	2.50 ± 0.17^{A}	5.00 ± 0.58^{A}	40.00 ± 1.14^{B}	5.00 ± 0.58^{A}
Fusarium solani	5.00 ± 0.33^{A}	20.00 ± 0.88^{C}	2.50 ± 0.29^{A}	5.00 ± 0.67^{A}	40.00 ± 1.20^{B}	5.00 ± 0.88^{A}
Penicillium marneffei	5.00 ± 0.58^{A}	20.00 ± 0.67^{C}	5.00 ± 0.29^{B}	5.00 ± 0.33^{A}	Nd^{A}	5.00 ± 0.58^{A}
Saccharomyces cerevisiae	5.00 ± 0.58^{A}	20.00 ± 0.88^{C}	5.00 ± 0.29^{B}	5.00 ± 0.33^{A}	Nd^{A}	5.00 ± 0.58^{A}
Significance level	$F_{12,26}$= 1.14, P>0.05	$F_{12,26}$= 345.78, P<0.000	$F_{12,26}$= 7.73, P<0.000	$F_{12,26}$= 16.31, P<0.000	$F_{12,26}$= 968.14, P<0.000	$F_{12,26}$= 14.24, P<0.000

Discussion:

Thirteen fungal species were isolated including eight molds and five yeasts. The isolated molds were: *Aspergillus flavus, A. niger, A. terrus, A. nidulans, A. fumigatus, Curvularia lunata, Fusarium solani* and *Penicillium marneffei*.

Candida and *Aspergillus* species are the main cause of fungal infection along with other yeasts and filamentous fungi such as: *Aspergillus*, *Fusarium*, *Penicillium*, *Scedosporium*, and Zygomycetes (34,35).

Candida albicans was the most abundant genus where 31 fungal colonies were isolated representing relative density of 22.5 % recovered from 18 patients.

Invasive candidiasis caused by non-albicans sp. is increasing. Non albicans species include *C. auris*, multidrug-resistant yeast causing nosocomial diseases. Moreover, the application of triazole pesticides in agricultural caused emergence of azole-resistant fungi in environmental and clinical isolates (36). The inappropriate use of antimicrobial agents as well as the use of immunosuppressive drugs have contributed to the increase in fungal infections caused by both yeasts and moulds (37).

Candida albicans was identified using molecular techniques. Total genomic DNA was extracted using GeneJet Genomic DNA purification kit (Thermo K0721). Polymerase chain reaction (PCR) amplification analysis of internal transcribed spacer (ITS) regions was done. Comparison of the partial nucleotide sequence of this isolate showed 99 % sequence homology with *C. albicans* (Fig. 1). The *C. albicans* strain was identified as *Candida albicans* voucher CA-3-NL-2R. Score 989 bits (1096), Expect 0.0, Identities 548/548(100%), Gaps 0/548(0%), strand Plus/Plus.

The most widely used molecular targets for identification of yeast are the 28S nuclear ribosomal DNA and internal transcribed spacer region (38).

Cinnamon oil recorded the highest mean relative activity among tested oils, followed by clove oil, thyme oil, almond oil and coconut oil. Cinnamon achieved MIC values less than or equal to that of fluconazole, while Clove and thyme recorded MIC values equal to or higher than that of control. Clove and thyme recorded similar MIC

values against tested fungal species. The MIC values were relatively high or even not detected in case of almond and coconut oils. The presence of different concentrations of different compounds as aldehydes, phenolics, terpenes, and other antimicrobial compounds means that the essential oils show variable inhibitory effect against a diverse range of pathogens (39).

Essential oils can inhibit spore germination, attack fungal cell membrane and disrupt its structure, stop the membrane synthesis, stop cellular respiration causing cell death (40). The efficacy of essential oil of cinnamon and clove was proved against six fungal species: *Aspergillus niger*, *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae*, *Phomopsis viticola* and *Rhizopus stolonifer* (41). Essential oils had a strong antifungal activity against Dermatophytes sp. (42). Carvacrol in clove oil was able to kill *C. albicans* by producing lesions in the plasma membrane as a result the organism dies. Clove oil, coconut oil and almond oil has also showed significant antifungal activity (43). Essential oils can be used as clinically safe and more effective antifungal agents in patients suffering from *Candida* infection. Clove oil showed maximum antifungal activity followed by thyme oil whereas, almond and coconut oils showed weak activity against isolated fungal species (44).

Conclusion:

Identification of molds was carried out morphologically and microscopically, while identification of yeasts was carried out using API system. *C. albicans* records the highest isolated fungal species. *Candida albicans* is molecularly identified using the 5.8S and flanking ITS regions. The antifungal activity of some natural essential oils is assayed. Cinnamon records the best inhibitory effect among tested essential oils, followed by cove and thyme, respectively.

Author's declaration:

- Conflicts of Interest: None.
- I hereby confirm that all the Figures and Tables in the manuscript are mine. Besides, the Figures and images, which are not mine, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Bisha.

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عزل وتشخيص بعض الفطريات من عينات سريرية لمرضى السكري ودراسة الفاعلية المضادة للفطريات لبعض الزيوت الطبيعية على فاعليتها

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الخلاصة:

تم عزل الفطريات من مائة عينة سريرية جمعت للفترة من من فبراير إلى يوليو 2019 من مستشفى الملك عبد الله (KAH) بيشة، المملكة العربية السعودية. والتي تعود الى عشرين مريضاً مصابين بالسكر بنوعيه ومن مختلف الأعمار (30 - 70 سنة) عشرة ذكور وعشر إناث. وشملت العينات الدم والشعر والأظافر ومسحات الفم والجلد. تم تلقيح العينات على أجار Sabourauds Dextrose المحتوي على الكلورامفينيكول. وتم عزل وتشخيص ثلاثة عشر نوعا من الفطريات. الأنواع المعزولة هي: *Aspergillus flavus*، *A. niger*، *A. terrus*، *A. nidulans*، *A. fumigatus*، *Candida albicans*، *C. krusei*، *C. parapsilosis*، *C. Tropicalis*، *Curvularia lunata*، *Fusarium solani*، *Saccharomyces cerevisiae* and *Penicillium marneffei* . تم التعرف على هده الفطريات شكليًا ومجهريًا باستخدام الطرق والمفاتيح التصنيفية المعتمدة ، بينما استخدام نظام API لتشخيص عزلات الخمائر. سجلت *C. albicans* أعلى عدد معزول حيث تم عزل 31 مستعمرة من 18 مريضاً ، اعطت كثافة نسبية 22.5٪. (R.D .: هو عدد أنواع فطرية معينة مقسومًا على العدد الإجمالي للفطريات). سجلت الأنواع الفطرية المعزولة الأخرى كثافة نسبية أقل من 16٪. تم تعرف الفطر الأكثر شيوعًا *Candida albicans* باستخدام S5.8 ومناطق ITS المرافقة. تم تقييم الفعالية المضادة للفطريات لبعض الزيوت العطرية الطبيعية (القرفة والزعتر وجوز الهند واللوز والقرنفل) ضد الفطريات المعزولة باستخدام طريقة الانتشار القرصي. كان التركيز المستخدم 5 ميكرولتر / طبق. تم تحديد قيم MIC أيضًا باستخدام تراكيز مختلفة من الزيت قيد الدراسة (1 ، 2.5 ، 5 ، 10 ، 20 و 40 ميكرولتر / قرص).

الكلمات المفتاحية: عزل ، تعريف، الكانديدا البيكانز، مضاد للفطريات ، مرضى السكري، فاصل نسخ داخلي، زيوت أساسية.