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# Evaluation Study of *Matricaria chamomilla* Extract Toward Pathogenic Isolates of *Candida*

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**Abstract.** Candidiasis is the most common type of yeast infection; the causative agent of this infection is Candida spp. Mostally candida albicans which normally lives on the skin and inside the body cavities such as the mouth, throat, gut, and vagina, without causing infection. *Candida* can cause infections if it grows out of control especially in immunocompromised Patients or if it enters deep into the body for example, the bloodstream or internal organs . Candidiasis can be treated either by topicals or systemic antifungal but some time these drugs are useless because some yeasts aquarid resistance to it also the antifungals have many side effects due to similarity between fungal and human cells so that both eukaryotic cells. This study was designed to isolate and identify pathogenic yeasts that cause oral and vaginal candidiasis . To achieve these goal, 20 swab samples positive were obtained from patients and cultured and identified morphologically and by molecular methods. Fourteen biologically active chemical compounds were identified by GC-MS analysis in a methanol crude extracted from the flowers of the *Matricaria chamomilla* plant. The cytotoxicity assay of *M. chamomile* methanol extract was showed there is no cytotoxic affecting human solution . This extract has a high inhibitory capacity toward *Candida* antifungal (fluconazole, nystatin) showed an inhibitory effect on *Candida* and some *C. albicans* showed resistance to these antifungal used.

**Keywords**: Candidiasis; Matricaria chamomile, GC-MS , antifungal

## INTRODUCTION

Fungal infections are a major problem and have increased the morbidity and mortality of immunocompromised patients. *Candida spp*. They are more interchangeable between fungal pathogens and causing invasive bloodstream infections. The boom of antifungal resistance among *Candida spp*. It makes them harder to treat [1]. The genus of *Candida* is predominantly a harmless symbiont that colonizes human skin and mucosal surfaces, but under conditions of weaked host immune system it turns into dangerous pathogens [2] Candidiasis was variable rely on the site of infection; oral candidiasis is The most common candidiasis [3]. However, presently, a serious medical problem still remains with acute invasive fungal diseases associated with *Candida* and deep-rooted candidiasis of the internal organs, which are characterized by frequent hospital origin and high mortality rates of 30-45% of affected patients [4], [5]. There are 200 species of *Candida* but only 20 species as *Candida albicans*,*C. tropicalis*,*C. glabrata*,*C. parapsilosis* ,*C. krusei* [6]. candidiosis treated either localized antifungal administered or systemically antifungal [7]. There are four group of antifungal drugs were developed, however two obstacles have been emerged during fungal treatment [8]. Firstly, both fungal and human cells are eukaryotes, and secondly, *Candida* strains of antifungal resistance have either been intrinsic or acquired, and this is a major problem in treating candidiasis [9].

Despite the developments of antifungal treatments with enhanced potential effects against *Candida* species, there are still increasing rates of resistance to the current antifungal therapy resulting in their frustration and worsening of clinical outcomes [10]. Therefore, the need to expand new alternative treatments against these fungal infections [11]

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has been highlighted. natural products are preferred as biocompatible and non-toxic drugs without side effects in medicine compared to chemical and synthetic drugs. In recent years, there has been an increasing interest in natural extracts that exhibit biological and medicinal properties that are provided to humans and animals as food ingredients or as specific pharmaceutical preparations. Medicinal plants are considered as the resources of promising drugs for many diseases. However, the biological and pharmacological properties of many plants are still unknown [12].According to the recent evidence, plants serve as natural sources of novel antimicrobial compounds with numerous therapeutic potentials [11]. *Matricaria chamomilla*, one of the well-known medicinal plants in the world, is from the Asteraceae family. It is a traditional medicinal plant that is grown commercially in different parts of Iran, especially in the Kerman and Isfahan provinces. A variety of pharmacological properties of *M. chamomilla* have been known, including antioxidant, antispasmodic, anti-inflammatory, antimicrobial, antiviral, antiseptic, and sedative [13]. The therapeutic activity of *chamomile* belongs to various active substances such as phenols and flavonoids. In addition, previous studies have demonstrated the antimicrobial activity of *chamomile*. The extracts of this plant have been shown to be inhibitory against a wide range of microbial strains, depending on their concentration, test method, and formulation. Bearing in mind that flavonoids are known to be powerful antimicrobial agents [14].

The aim of this study was to isolation and identify the isolated genetically if candida species from candidiasis infection and study the activity of bioactive compounds.

## MATERIALS AND METHODS

### Isolation and Identification

Samples were obtained from 30 patients from the mouth and vagina of patients with oral and vaginal candidiasis. Samples were collected from the Child Specialized Hospital, Al-Faiha General Hospital and some private clinics (Basra Governorate / Iraq) Two swab were gained from oral thrush and vaginal. The samples were transferred to the clinical mycology laboratory. The swabs were culture on SDA and incubat at 37 C° for a period of [2], [3], [4], [5] days for molecular identifficated. Genomic DNA of each strain was extracted using the Presto™ Mini gDNA Yeast Kit (Geneaid) [14]. was used for DNA isolation according to the company instructions. Later, isolated DNA samples were electrophoresed in 0.5% agarose gel stained with ethidium bromide and preserved in the -80 ºC for PCR amplification. The ITS conservative regions of rDNA were amplified using the universal primer sequences of ITS1: F-5-TCC GTA GGT GAA CCT GCG G- 3 and ITS4: R-5-TCC TCC GCT TAT TGA TAT GC-3 [15]. The PCR process was carried out following [15] . The mixture of PCR reaction composed of 5 µl of master mix (Bioneer), 1 µl of forward and reverse primer, 5 µL of genomic DNA (GDNA) and nuclease free water to a total volume of 25 µL. ITS region amplification condition: 5 min at 94 ºC for initial denaturation, followed by 25 cycles including 3 steps of denaturation at 94 C for 30 sec, an annealing step at 56C for 45 sec and an elongation step at 72 C for 1 min, one more cycle at 72 ºC for 7 min as final elongation. PCR products were segregation into agarose gel and fragments size determined by comparison with a 1-kb ladder (Bioneer). Sequencing process was achieved in the Macrogen Inc [16].

### Study Plant

The plant *Matricaria chamomilla* flowers were purchased from local market were collected from Basrah city**.**

### Preparation of Methanol Extract of *M.Chamomilla*

Dry *chamomile* flowers were weighed and grind to powder and a 5% w/v suspension was prepared in a flask by adding methanol cohol. The flask was then placed on a shaker (200 rpm) for 4 h and the temperature was maintained at 37°C. After shaking, the flask was brought to room temperature the suspension was filtered through a series of Whatman filters and finally passed through 0.22 micron filter. The filtered methanol extract was dried at room temperature and stored at −20°C until use [17].

## GC / MS Analysis

The methanol extracts for *M. chamomilla* were analyzed using the GC/MS analysis (Shimadzu GCMS-QP2010 Ultra).

## Cytotoxicity Test

Biocompatibility test was carried out for prepared *M. chamomilla* methanol extract against human fresh blood according to [18] method.

## Test the Effectiveness of *M. Chamomilla* Flower Methanol Extracts and Antifungal on the Isolated Yeasts in the Study

The well diffusion method was to study the sensitivity of antifungals and the sensitivity of isolates for the methanol extract of *M.chamomilla*, where a number of colonies were mixed with 5 ml of physiological solution (normal saline). Sterile cotton scabs were then covered in the fungal suspension and then were plotted on the surface of the wafers and the dishes were left until the mixture was absorbed by the macaroni excavated with 6 mm diameter drills were drilled with a sterile flask and filled with 50 microliters of all concentrations (100. 500 and 1000) mg / ml. After the incubation period, the diameters of the inhibition zones are measured around the drill and the results are recorded [19].

# RESULT AND DISCUSSION

## Morphological and Phenotypical Analysis

Microscopic direct examinations of all oral specimens showed that 60% (18 samples) were yeast infections (oral and vaginal candidiasis) by presence of true and pseudo-hyphae in wet smear with 10% KOH, while only 40% (12 of samples) revealed negative result of direct investigation. However, 66,666% (20 samples) of positive sample swabs were shown growth onto SDA media after incubation interval and the rest were failed to grow.

## Molecular Idefinition for Isolates

All positive isolates in vitro culture (20 isolates) were subjected for molecular identification. The process was completed by amplifying and sequencing the ITS region for sequencing. The results of the ITS region sequences showed that 40% of the isolates were *C. tropicalis*, 35% *C. albicans*, 20% *C. Glabrata* and 5% *C. dubliniensis*. In this study, molecular methods were applied to identify yeast isolates. The morphological methods are time consuming and imprecise compared to the accurate and reliable molecular approach [20]. The plainness and reliability of molecular identification due to the availability of ITS region sequences in the general database is optimal, so the interpretation was based on the molecular method [21]. The molecular results showed the prevalence of *C. tropicalis* , and most studies indicated the prevalence of *C. albicans* over the other species as in the study [22]. Our results may be inconsistent with other studies due to the small sample size.

## GC-MS test for the Methanol Extract of *M. Chamomilla*

A large number of compounds were detected in the GC/MS analysis of the *chamomile* plant .The major components and percentage are summarized in Table 1 *M. chamomilla* contain at least 11 anti-microbial agent . Various pharmacological activities for plants extracts this is consistent with [23] that *chamomile* flowers contain activity compounds , which are due to It has biological activity against pathogens, including *Candida spp.*

**TABLE 1.** The chemical compounds of the methanol extract for flowering of the *M. chamomilla* plant

| NO. | Chemical compounds | Chemical formula | Percentage % | Retention time |
|---|---|---|---|---|
| 1 | 1-(Phenylethynyl)-1-cyclohexanol | C14H16O | 6.123 | 15.539 |
| 2 | 2H-1-Benzopyran-2-one,7-methoxy- | C10H8O3 | 5.123 | 16.049 |
| 3 | Hexadecanoic acid, methyl ester | C17H34O2 | 5.83 | 17.724 |
| 4 | cis-Vaccenic acid | C18H34O2 | 8.232 | 18.505 |
| 5 | 1,6-Dioxaspiro[4.4]non-3-ene, 2-(2,4 hexadiynylidene) | C13H12O2 | 5.83 | 19.411 |
| 6 | 2H-Pyran-3-ol, tetrahydro-2,2,6-trimethyl-6-(4-methyl-3-cyclohexen-1-yl)-, [3S[3.alpha.,6.alpha | C15H26O2 | 38.47 | 20.815 |
| 7 | Benzene, 1,3-dimethyl- | C12H25N3 | 1.3045 | 21.042 |
| 8 | Cyclopentanone, 2-(1-methylheptyl) | C13H24O | 1.1965 | 22.829 |
| 9 | (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one) | C15H10O6 | 22.159 | 23.010 |
| 10 | Phenol, 3-methyl-6-propyl- | C10H14O | 2.411 | 23.665 |
| 11 | 4H_Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-mEthyl | C18H32O16 | 3.321 | 29.875 |

## Cytotoxicity

The cytotoxic activity of methanol extract was assessed by using different concentrations of human blood solution , the result showed no turbidity formation after 15, 30 and 60 min from adding 100µl of flavonide *M. chamomile* extract to all concentrations of human blood solution, indicated no cytotoxic effect of fungal extract so the extract used in the study are safe and can be described as a therapeutic alternative to antifungals with adverse side effects on the host and this is in agreement with [24].

## Antifungal Activity

Based on the results obtained, different concentrations of the flavonide extract extracted from *M. chamomilla* affected the growth of all *Candida* isolates (vaginal and oral) according to the approved dose. The methanol extract showed good efficacy in controlling *Candida*, and this is in agreement with [25]. who concluded that *M. chamomilla* flowers were effective against *Candida* and indicated that *M. chamomilla* could be a promising candidate as an anti-fungal agent and for controlling pathogens responsible for invasive fungal infections. Flavonoids have inhibitory activity for all isolated *Candida* species The largest diameter of the inhibition zone is shown by *C.trobicalis*. As for the antifungal agents used in the current study (fluconazole, nystatin) to compare it with the methanol extract, the effect was variable according to type, and 4 of the isolates belonging to *C. albicanis* were resistant to fluconazole and nystatin and this is in agreement with [26]. The other isolates differed in the diameter of inhibition according to the concentration used and the source from which the sample was taken, whether orally or vaginally. The results also showed that most of the resistant isolates are vaginal as a result of the spread of *Candida* infection in women as a result of hormonal changes in women, pregnancy and taking birth control pills, which weakens the immune status of the woman [27]. Due to the indiscriminate use of antibiotics without consulting a

physician, which encourages *Candida* colonization in the affected area, changes in sensitivity and resistance can also be explained by the yeast's ability to withstand the toxic effects of the antibiotic and its ability to resist the development of resistance mechanisms [28].

Data in Table 2 indicated that, All studied control treatmens superior on inhibition zone parameter of *Candida* isolates compared with the pathogens alone, the highest value was in 1000 ml concentrate of *M. chamomilla* plant and the antifungal properties in all of the *Candida* isolates , the inhibition in extract, Fluconazole and Nystatin in which reached 30.20, 29.16 and 26.66 mm respectively, compared to control 0.00 mm (pathogens alone).

**TABLE 2.** Effectiveness of the methanol extract for flowering of the *M. chamomilla* plant and the antifungal ( fluconazole, nystatin ) properties of the isolated *Candida spp.*

| Sample source | Isolates | Inhibition zone (mm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | M. chamomile extract( µl/l) | | | | Fluconazole( µl/l) | | | Nystatin( µl/l) | | |
| | | control | 100 | 500 | 1000 | 100 | 500 | 1000 | 100 | 500 | 1000 |
| oral | *C. albicans* | 0.00 | 17.10 | 19.16 | 24.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.000 |
| vaginal | *C. albicans* | 0.00 | 18.16 | 20.23 | 26.30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| oral | *C. albicans* | 0.00 | 16.23 | 20.66 | 25.33 | 18.33 | 24.33 | 27.33 | 16.66 | 16.16 | 26.00 |
| vaginal | *C. albicans* | 0.00 | 20.23 | 25.33 | 27.33 | 15.33 | 17.00 | 23.33 | 16.66 | 24.00 | 26.00 |
| oral | *C. glabrata* | 0.00 | 14.13 | 16.26 | 20.66 | 12.33 | 16.16 | 20.33 | 16.66 | 20.66 | 26.66 |
| vaginal | *C. glabrata* | 0.00 | 14.23 | 16.06 | 20.10 | 15.10 | 18.16 | 20.30 | 12.30 | 16.16 | 20.33 |
| oral | *C.tropicalis* | 0.00 | 21.16 | 24.30 | 30.16 | 20.16 | 24.06 | 29.16 | 15.30 | 18.13 | 20.16 |
| vaginal | *C.tropicalis* | 0.00 | 21.30 | 24.23 | 30.20 | 15.26 | 18.30 | 20.16 | 14.26 | 20.13 | 25.26 |
| oral | *C.dublinien sis* | 0.00 | 19.20 | 21.10 | 27.26 | 14.16 | 19.10 | 23.23 | 12.20 | 17.10 | 24.26 |
| L.S.D P≤ 0.05 | | 0.00 | 0.58 | 1.57 | 1.68 | 1.44 | 2.54 | 1.57 | 1.70 | 2.32 | 1.63 |

## CONCLUSIONS

The methanolic extract of *M. chamomilla* flower in this study showed good inhibition activity against the isolated *Candida* species.

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## REFERENCES

1. Panáček A, Kolář M, Večeřová R, Prucek R, Soukupova J, Kryštof V, Hamal P, Zbořil R, Kvítek L (2009) Antifungal activity of silver nanoparticles against Candida spp. Biomaterials 30 (31):6333-6340

2. Guevara-Lora I, Bras G, Karkowska-Kuleta J, González-González M, Ceballos K, Sidlo W, Rapala-Kozik M (2020) Plant-derived substances in the fight against infections caused by Candida species. International journal of molecular sciences 21 (17):6131
3. Khan, M. S. A., Ahmad, I., Aqil, F., Owais, M., Shahid, M., & Musarrat, J. (2010). Virulence and pathogenicity of fungal pathogens with special reference to Candida albicans. In Combating fungal infections (pp. 21-45). Springer, Berlin, Heidelberg.
4. Mamtani S, Aljanabi NM, Rauniyar RPG, Acharya A, Malik BH (2020) Candida endocarditis: a review of the pathogenesis, morphology, risk factors, and management of an emerging and serious condition. Cureus 12 (1)
5. Mesini A, Mikulska M, Giacobbe DR, Del Puente F, Gandolfo N, Codda G, Orsi A, Tassinari F, Beltramini S, Marchese A (2020) Changing epidemiology of candidaemia: Increase in fluconazole-resistant Candida parapsilosis. Mycoses 63 (4):361-368.
6. Kane, J., Summerbell, R C. (1999). Trichophyton, Microsporum, Epidermophyton, and agents of superficial mycoses. In: Manual of Clinical Microbiology, 7th Ed., Murray P R, Baron E J, Pfaller M A, Tenover F C, Yolken R H, eds. Washington DC: ASM Press, 1275–1294.
7. Torres SR, Peixoto CB, Caldas DM, Akiti T, Barreiros MGC, Uzeda Md, Nucci M (2007) prospective randomized trial to reduce oral Candida spp. colonization in patients with hyposalivation. Brazilian Oral Research 21 (2):182-187
8. Ahmad Khan, M. S., Alshehrei, F., Al-Ghamdi, S. B., Bamaga, M. A., Al-Thubiani, A. S., & Alam, M. Z. (2020). Virulence and biofilms as promising targets in developing antipathogenic drugs against candidiasis. Future science OA, 6(2), FSO440
9. Nett, J. E., & Andes, D. R. (2016). Antifungal agents: spectrum of activity, pharmacology, and clinical indications. Infectious Disease Clinics, 30(1), 51-83.
10. Wiederhold, N. P. (2017). Antifungal resistance: Current trends and futurestrategies to combat. Infection and Drug Resistance, 10, 249. https://doi.org/10.2147/IDR
11. Tang, S. S., Prodhan, Z. H., Biswas, S. K., Le, C. F., & Sekaran, S.D. (2018). Antimicrobial peptides from different plant sources:Isolation, characterisation, and purification. Phytochemistry, 154, 94–105. https://doi.org/10.1016/j.phytochem.2018.07.002
12. Matić IZ, Juranić Z, Savikin K, Zdunić G, Nađvinski N, Gođevac D. (2012).Chamomile and marigold tea: chemical characterization and evaluation of anticancer activity; Phytotherapy Research.
13. Razzaghi-Abyaneh, M., Shams-Ghahfarokhi, M., & Rai, M. (2013).Antifungal metabolites from plants. Berlin, Heidelberg: Springer (pp. 27–57). https://doi.org/10.1007/978-3-642-38076-1.
14. Geneaid. PrestoTM Mini gDNA Yeast Kit [Internet]. p.1–7. Available from: http://www.geneaid.com/downloads/genomicdna/ prestotm-mini-gdna-yeast-kit
15. White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications, 18(1), 315-322
16. Mirhendi, H., Makimura, K., Khoramizadeh, M., & Yamaguchi, H. (2006). A one-enzyme PCR-RFLP assay for identification of six medically important Candida species. Nippon Ishinkin Gakkai Zasshi, 47(3), 225-229.
17. Srivastava, J. K., & Gupta, S. (2009). Extraction, characterization, stability and biological activity of flavonoids isolated from chamomile flowers. Molecular and cellular pharmacology, 1(3), 138.
18. Nair M.G., Mishar A.R., Muks M.H., Taf W.H., Kesller J.F., Miller J.R., Zhu P.P., Meinhart J.D. and Lynm D.G.(1989). Faerifungin a new broad spectrum antibiotic from Streptomyces griseous. Autotrophic US,J.Natural Product.; 52:779-809.
19. Perez, C., Pauli, M., & Bazerque, P. (1990). An antibiotic assay by the well agar method. Acta Biol Med Exp, 15, 113-115
20. Samaranayake L.P., Raeside J.M. , Macfarlane T.W., (1984) . Factors affecting the phospholipase activity of Candida species in vitro, J Med Vet Mycol. 22 201–7.
21. Kurtzman C.P., Fell J.W., Boekhout T., Robert V., Robert,v (2011). Methods for isolation, phenotypic characterization andvmaintenance of yeasts, The Yeasts. Elsevier B.V..vp.p. 87–110. Available from:vhttp://dx.doi.org/10.1016/B978-0-444-52149-1.00007-0
22. Alrubayae, I. M., Al-laaeiby, A., Minati, M. H., & ALibraheem, S. A. (2020). Determination of genetic relationships and pathogenicity of oral candidiasis etiological agents in pediatric malignant patients in Basrah Province, Iraq. Systematic Reviews in Pharmacy, 11(12), 180-188.

23. Al-Dabbagh, B., Elhaty, I. A., Elhaw, M., Murali, C., Al Mansoori, A., Awad, B., et al. (2019). Antioxidant and anticancer activities of chamomile ( Matricaria recutita L.). BMC Research Notes, 12 (1), 3. doi: 10.1186/s13104- 018- 3960- y
24. Osman, M. Y., Taie, H. A., Helmy, W. A., & Amer, H. (2016). Screening for antioxidant, antifungal, and antitumor activities of aqueous extracts of chamomile (Matricaria chamomilla). Egyptian Pharmaceutical Journal, 15(2), 55.
25. Seyedjavadi, S. S., Khani, S., Zare Zardini, H., Halabian, R., Goudarzi, M., Khatami, S., & Razzaghi-Abyaneh, M. (2019). Isolation, functional characterization, and biological properties of MCh-AMP1, a novel antifungal peptide from Matricaria chamomilla L. Chemical biology & drug design, 93(5), 949-959
26. AL-Maliki, Rehab. S. and AL-Ani, Zouhair. I. ( 2011). Antifungal resistance of Candida species isolated from Iraqi women infected with vulvovaginal Candidiasis . Qmj Vol. 7 (11) pp: 117-127
27. Kamath, P.; Pais, M., and Nayak, M. 2013. Risk of vaginal candidiasis among pregnant women. Int.J.Curr.Microbiol.App.Sci 2:141-146
28. Denning, D. W. (2003). Echinocandin antifungal drugs. The Lancet, 362(9390), 1142-1151.