RESEARCH ARTICLE

Bioactive Secondary Metabolites Extracted from Some Species of *Candida* Isolated from Women Infected with Vulvovaginal Candidiasis

Khutam J. Hassan, Ali A. Kasim*

Department of Biology, College of Science, University of Misan, Amarah, Iraq

Received: 10th December, 2022; Revised: 12th January, 2023; Accepted: 14th February, 2023; Available Online: 25th March, 2023

ABSTRACT

Candida spp. are well known for their impact on our life as opportunistic pathogens and can produce secondary metabolites, which are an interesting source of high-value chemicals and are often difficult to synthesize chemically. Some of these metabolites are already being used as active antimicrobials or pharmaceuticals. GC-MS analysis of chemical compounds was conducted in ethyl acetate of five *Candida* species (*C. parapsilosis*, *C. albicans, C. krusei*, *C. glabrata* and *C. kefyr*). The results showed that about 249 chemical compounds were detected from all tested *Candida* species. Fifteen bioactive compounds identified in secondary metabolites of all tested species. Benzene, pentamethyl, n-hexadecanoic acid, 1-docosene and bis(2-ethylhexyl) produced by *C. parapsilosis, C. albicans, C. krusei* and *C. glabrata*. All tested *Candida* except *C. krusei* produced 3buten-1-ol and ethyl acetoacetate ethylene acetal. Benzen, 2-ethenyl-1,3-dimethyl- a compound found in four tested *Candida* except *C. albicans*, benezene, (1-ethyl-1-propenyl)- produced by all species except *C. glabrata*, acetic acid, cyclohexyl ester produced by *C. parapsilosis*, *C. albicans* and *C. glabrata*.

Keywords: *Candida albicans*, *Candida* spp, GC-MS, Secondary metabolites, Vulvovaginal candidiasis.

International Journal of Drug Delivery Technology (2023); DOI: 10.25258/ijddt.13.1.42

How to cite this article: Hassan KJ, Kasim AA. Bioactive Secondary Metabolites Extracted from Some Species of *Candida* Isolated from Women Infected with Vulvovaginal Candidiasis. International Journal of Drug Delivery Technology. 2023;13(1):263-267.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The bioactive compounds are chemical products that often indicate secondary metabolites.[1] Secondary metabolites are high-value compounds naturally isolated from natural resources, such as fungal and bacterial cultures or plant biomass and have a wide range of applications.[2] Fungal secondary metabolites a wide class of specialized tiny molecules that can induce or inhibit the survival and reproduction of the host.[3] Moreover, it represents one of the interesting things in microbial sciences and can be considered a remarkable group of bioactive compounds.[4,5]

Yeasts are single-celled eukaryotic organisms that belong to the kingdom of fungi and division Ascomycota, they have been used for the fermentation of food and drinks since antiquity and are today broadly used for the industrial production of chemicals, antimicrobials, pharmaceuticals, and proteins.[6-8] Yeasts can produce many bioactive molecules such as polypeptides, terpenoids and non-ribosomal peptides.[9,10] It includes diverse important biological roles such as defense against biotic and abiotic conditions, signal transmission, antimicrobials, chemicals and nutrients,[7,11,12] nevertheless, are not shared in the development and growth of the organism (yeasts).[2] 2020 and The yeast infections increased simultaneously with the advancement of medicine and the rising of a community of immunocompromised patients.[13,14] *Candida* species are carried in many locations in the human body such as the vaginal canal, vulva, gastrointestinal tract, groin, anus and oral cavity of healthy people.[15] Most of these endogenous yeasts are opportunistic pathogens, and infections usually arise from an imbalance of normal microflora stimulated by the administration of wide spectrum antibiotics, immunosuppression, and the destruction of protective barriers.[16] Moreover, systemic infections caused by *Candida* spp. increased drastically during the last 15 years, and are an important infection agent due to the appearance of large numbers of invasive *Candida*, decreased natural human immunity, the wide use of antibiotics and other factors.[17,18]

Vulvovaginal candidiasis (VVC) is an extremely common mucosal vaginal infection,[19] characterized by watery secretions, itching and erythema. Outermost, almost 75% of women were infected once in a lifetime.[20,21] Few studies have examined the susceptibility to *Candida* spp. to produce various secondary metabolites.[17,22] Kadhim *et al.*[1] identified 31 bioactive compounds in secondary metabolites of *C. albicans*. Moreover, Hamza *et al.*[11] showed that 39 bioactive compounds

*Author for Correspondence: alimycol@uomisan.edu.iq

Table 1: Some of chemical compounds produced by tested *Candida* species

Chemical compounds	*Formula*	*Molecular Weight*	*RT*	*Area Pct*	*Candida species*				
					C. parapsilosis	*C. albicans*	*C. krusei*	*C. glabrata*	*C. kefyr*
Benzene,1,3-dimethyl	C_8H_{10}	106	6.226	0.8665	+	+	+	+	+
O-Xylene	C_8H_{10}	106	6.499	5.3993	+	+	+	+	+
2-Octanol, 2-methyl-6-methylene	$C_{10}H_{20}O$	156	11.293	0.9156	+	+	+	+	+
Benzene,1,2,4,5-tetramethyl	$C_{10}H_{14}$	134	12.001	1.8528	+	+	+	+	+
Benzen,2-ethyl-1,4-dimethyl	$C_{10}H_{14}$	134	12.302	0.1489	+	+	+	+	+
Naphthalene1,2,3,4 tetrahydro-2-phenyl	$C_{16}H_{16}$	208	13.122	1.2451	+	+	+	+	+
Cyclohexanol,4-(1,1-dimethylethyl)-, acetate, trans	$C_{12}H_{22}O_2$	198	15.017	0.6599	+	+	+	+	+
Butanoic acid,1,1-dimethyl-2-phenylethyl ester	$C_{14}H_{20}O_2$	220	17.34	1.375	+	+	+	+	+
1,2 - Benzenedicaboxylic acid ethyl methyl ester	$C_{11}H_{12}O_4$	208	17.783	0.2804	+	+	+	+	+
2(3H)-Furanone, 5-heptyldihydro-	$C_{10}H_{18}O_2$	170	18.388	0.9535	+	+	+	+	+
Diethyl phthalate	$C_{12}H_{14}O_4$	222	18.779	20.2722	+	+	+	+	+
Octanal,2-(phenylmethylene)-	$C_{15}H_{20}O$	216	20.379	4.3164	+	+	+	+	+
Benzeyl Benzoate	$C_{14}H_{12}O_2$	212	20.556	0.4477	+	+	+	+	+
Octadecanoic acid	$C_{18}H_{36}O_2$	284	24.376	1.106	+	+	+	+	+
Tris(2,4-di-tert-butylphenyl) phosphate	$C_{42}H_{63}O_4P$	662	36.737	2.3387	+	+	+	+	+
Benzene, pentamethyl	$C_{11}H_{16}$	148	13.306	0.2612	+	+	+	+	-
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	22.592	1.6207	+	+	+	+	-
1-Docosene	$C_{12}H_{26}O$	186	26.25	0.2386	+	+	+	+	-
Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	27.503	0.5973	+	+	+	+	-
3-Buten-1-ol	C_4H_8O	72	11.735	0.3605	+	+	-	+	+
Ethyl acetoacetate ethylene acetal	$C_8H_{14}O_4$	174	12.672	0.1479	+	+	-	+	+
Benzen , 2 – ethenyl-1,3-dimethyl-	$C_{10}H_{14}$	134	11.462	0.5593	+	-	+	+	+
Benezene, (1-ethyl-1-propenyl)-	$C_{11}H_{16}$	148	13.867	0.1237	+	+	+	-	+
Acetic acid, cyclohexyl ester	$C_8H_{14}O_2$	142	14.752	2.9283	+	+	-	+	-

+ produce the chemical compound, - don't produce the chemical compound

were detected in the methanolic extract of *C. glabratus* The bioactive metabolites of *Candida* spp. play an important role as an antibacterial, antifungal and pharmaceutical agent.[14] The current study aimed to detect the secondary metabolites produced by five species of *Candida* growing on broth cultures.

MATERIALS AND METHODS

Bioactive compounds of 5 clinical isolates of species of *Candida* spp. (*C. albicans, C. glabrata, C. kefyr* (*Kluyveromyces marxianus*)*, C. krusei* (*Pichia kudriavzevii*) and *C. parapsilosis* isolated from April 2007 to December 2021 from women infected with VVC at the Maysan Maternity Hospital (Maysan, Iraq) were analyzed. Agreements were obtained from the University of Misan, College of Science, Biology departments to carry out this study.

The broth cultures of yeast isolates were prepared in an inoculated flasks (250 mL) containing 200 mL of potato dextrose broth (PDB) with 5 discs (6 mm in diameter) cut from the axenic yeast culture (maintained in potato dextrose agar slants) of each isolate and incubated at 25°C in a shaker for 2 weeks at 150 rpm.[13,23] Yeast cultures were filtered on Whatman no. 1 filter paper and then through a 0.2 µm Millipore filter. The filtrate was extracted in ethyl acetate (1:1 v:v) using a separating funnel, organic layer was collected and placed in petri dishes, incubated at 37ºC to dry. The dry matter was scraped and placed in sterile test tubes. The bioactive compounds of the tested *Candida* species were detected using GC-MS (Mass Spectrometer, Agilent 5977 A. Gas chromatography, Agilent 7890 B, MSD, USA). The chemical compounds were identical to structures of the chemical compounds in the MassHunter\ Library\NIST14.L.

RESULTS AND DISCUSSION

The infection of the vaginal mucosa and/or vulva called VVC caused by *Candida spp.* and represent the second most common reason of vaginal infection after bacterial vaginosis, notably, VVC mostly occurs in women of reproductive age (20–40 years).[11,15] When *Candida* spp penetrates the host mucosa surface, fungal form changes from spores to pseudohyphae, releasing several material as degradative enzymes, antibacterial, pharmaceutical and antifungal.[17]

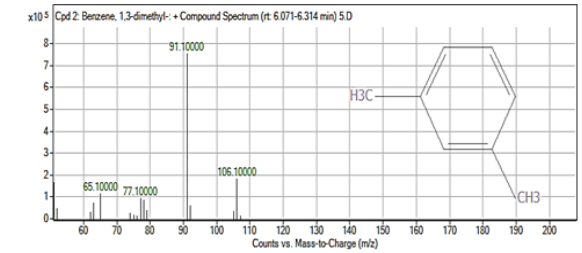

Figure 1: Mass spectrum of benzene 1,3-dimethy.

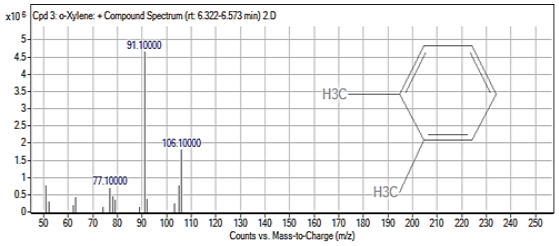

Figure 2: Mass spectrum of O-Xylene

During this study, about 249 chemical compounds were detected from all tested *Candida* species using GC-MS analysis for their ethyl acetate extracts (*C. parapsilosis* 50 compounds, *C. Albicans* 45 compounds*, C. krusei* 51 compounds, *C. glabrata* 51 compounds and 52 compounds of *C.kefyr*). Table 1 showed that 15 bioactive compounds appeared in secondary metabolites of all tested species (Figure 1-15), *C. parapsilosis, C. albicans, C. krusei* and *C. glabrata* shared in the production of 4 compounds which are Benzene, pentamethyl, n-hexadecanoic acid, 1-docosene and bis(2-ethylhexyl). Moreover, all tested *Candida* except *C. krusei* produced two compounds (3buten-1-ol and Ethyl acetoacetate ethylene acetal). Benzen, 2-ethenyl-1,3-dimethyl- compound found in four tested *Candida* except *C. albicans*. Moreover, 4 tested species produced both benezene, (1-ethyl-1-propenyl)-, acetic acid, cyclohexyl ester produced by all 3 species except *C. krusei and C. kefyr* (Table 1). The results revealed that production of the bioactive compounds differed according to the tested species.

The result of this study agreed with some studies which used GC-MS technique for analysis of bioactive materials of some species of *Candida,* notably, Kadhim *et al.*[1] could detect many compounds from secondary metabolites of *C. albicans* which appeared in common with our study, such as 1,4-benzendiol,2,6-bis(1,1-dimethylethyl)-, 2(3H)-furanone,3-butyldihydro-,1,4-benzendiol,2,6-bis(1,1-dimethylethyl)-, 12-methyl-oxa-cyclododecan-2-one, 6-octadecenoic acid, etc. Furthermore, many compounds identified in *C. glabrata* bioactive metabolites such as benzeneacetaldehyde, 1-methyl-4-[nitromethyl]-4- piperidinol and 3-benzylsulfanyl-3-fluoro-2-trifluoromethyl-acrylonitril[12] appeared in secondary metabolites of our *C. glabrata* isolate. Some compounds produced from tested *Candida* spp. as O-xylene, benzene,1,3-dimethyl, ethyl acetoacetate ethylene acetal, diethyl phthalate, bis(2-ethylhexyl) phthalate benzene, 1,2,3,5-tetramethy have been shown to possess combined activities against human

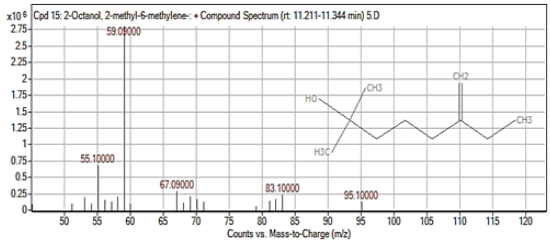

Figure 3: Mass spectrum of 2-Octanol,2-methyl-6-methylene.

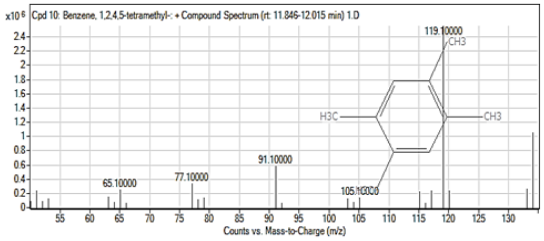

Figure 4: Mass spectrum of benzene,1,2,4,5-tetramethyl

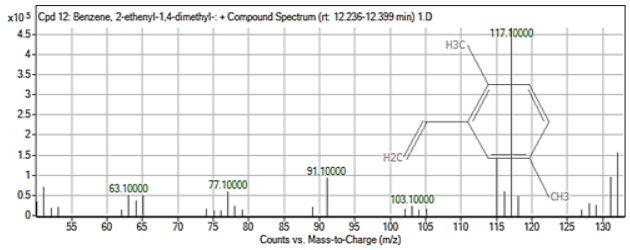

Figure 5: Mass spectrum of benzen,2-ethyl-1,4-dimethyl.

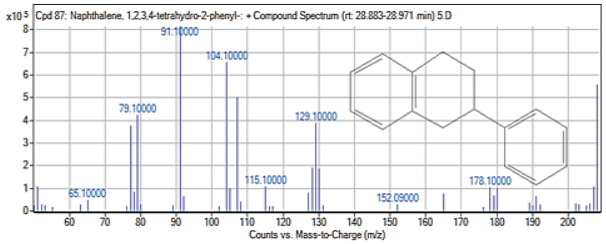

Figure 6: Mass spectrum of naphthalene-1,2,3,4 tetrahydro-2-phenyl.

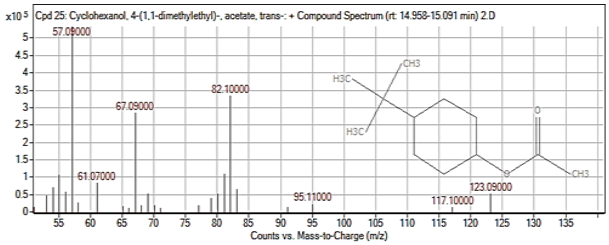

Figure 7: Mass spectrum of cyclohexanol,4-(1,1-dimethylethyl)-, acetate, trans.

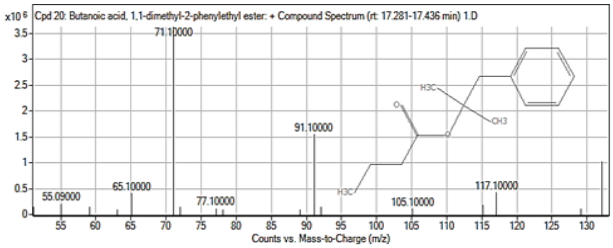

Figure 8: Mass spectrum of butanoic acid, dimethyl-2-phenylethyl ester.

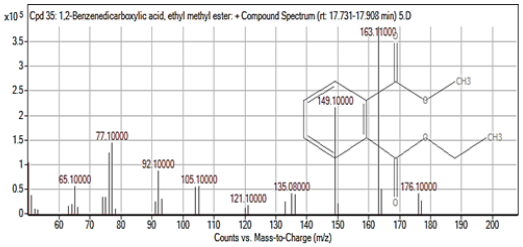

Figure 9: Mass spectrum of 1,2–benzene-dicaboxylic acid ethyl methyl ester.

such as irritation of skin, eyes and respiratory system.[24] Moreover, naphthalene has many effects, including causing damage to red blood cells, liver and nervous system, retinal bleeding and also considered a human carcinogen.[25] Daulton[26] indicated that benzen,4-ethyl-1,2-dimethy causes chronic

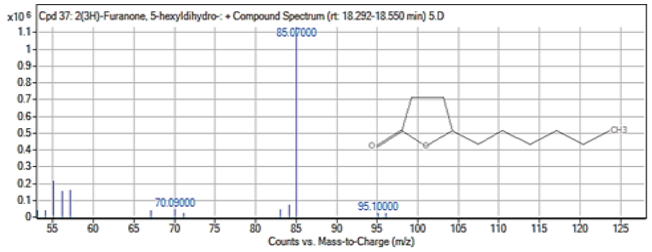

Figure 10: Mass spectrum of 2(3H)-furanone 5-heptyldihydro

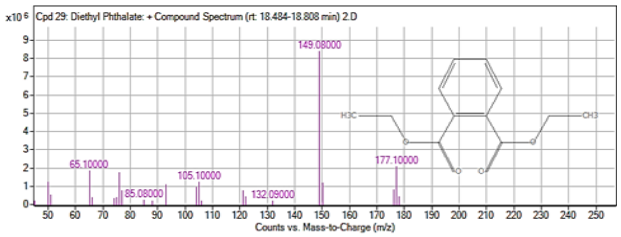

Figure 11: Mass spectrum of diethyl 1,1-phthalate.

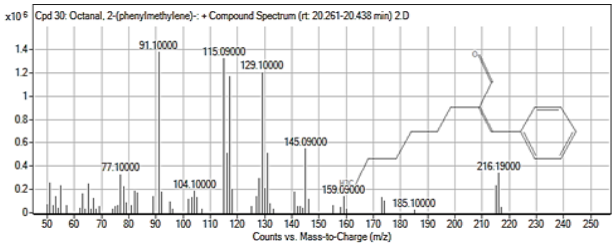

Figure 12: Mass spectrum of octanal,2-(phenylmethylene)

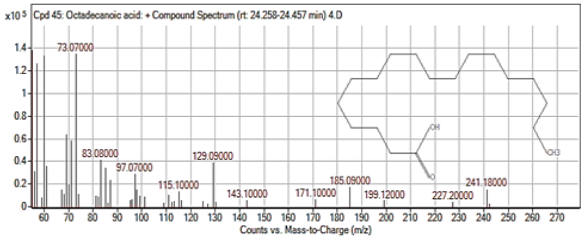

Figure 13: Mass spectrum of benzeyl-benzoate.

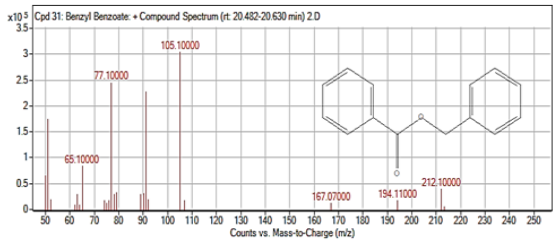

Figure 14: Mass spectrum of octadecanoic acid.

pancreatitis, and pancreatic cancer. The results showed that diethyl phthalate produced from teste species has been effective in male reproductive development by inhibiting the androgen hormone, fetal loss and possesses toxicity against the nervous system.[27] On other hand, n-hexadecanoic acid causes cytotoxicity of cells.[28,29] Some studies revealed that tris(2,4-di-tert-butylphenyl) and butylated hydroxytoluene act as antioxidants.[30,31]

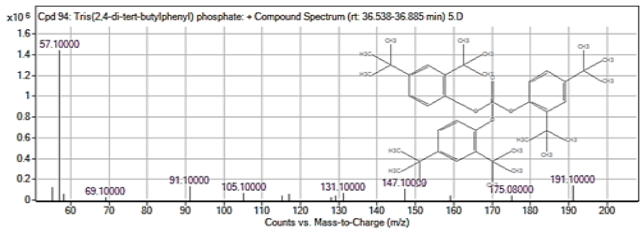

Figure 15: Mass spectrum of Tris(2,4-di-tert-butylphenyl)phosphate.

CONCLUSION

The data of this study provides new scientific information about bioactive compounds that found in secondary metabolites of five species of *Candida*. Moreover, it was found that many compounds appeared in all the filtrates of the tested *Candida* spp., and this result is agreeing with some previous studies.

ACKNOWLEDGMENT

The authors are thankful to the college of Science, University of Misan (Iraq) for providing financial support. We also would also like to thank Asst. Prof. Maitham Dragh (the head of the Biological Department) to provide us with some chemicals.

REFERENCES

1. Kadhim MJ, Mohammed GJ, Hussein H. Analysis of bioactive metabolites from *Candida* albicans using (GC-MS) and evaluation of antibacterial activity. International Journal of Pharmaceutical and Clinical Research. 2016;8(7):655-670.
2. Rahmat E, Kang Y. Yeast metabolic engineering for the production of pharmaceutically important secondary metabolites. Applied Microbiology and Biotechnology. 2020 Jun;104(11):4659-4674.
3. Keller NP. Fungal secondary metabolism: regulation, function and drug discovery. Nature Reviews Microbiology. 2019 Mar;17(3):167-180.
4. Lafta AA, Kasim AA. Effect of Nematode-Trapping Fungi, Trichoderma Harzianumand Pseudomonasfluorescens in Controlling Meloidogyne Spp. Plant Archives. 2019;19(1):1163-1168.
5. Wang G, Kell DB, Borodina I. Harnessing the yeast Saccharomyces cerevisiae for the production of fungal secondary metabolites. Essays in Biochemistry. 2021 Jul 26;65(2):277-291.
6. Smedsgaard J, Nielsen J. Metabolite profiling of fungi and yeast: from phenotype to metabolome by MS and informatics. Journal of experimental botany. 2005 Jan 1;56(410):273-286.
7. Sun L, Kwak S, Jin YS. Vitamin A production by engineered Saccharomyces cerevisiae from xylose via two-phase in situ extraction. ACS synthetic biology. 2019 Aug 2;8(9):2131-2140.
8. Raheem Na, Kasim A. Biodegradation Of Crude Oil By Nematode Trapping Fungi Isolated From Iraq. Poll Res. 2020;39(1):12-18.
9. Hoffman CS, Wood V, Fantes PA. An ancient yeast for young geneticists: a primer on the Schizosaccharomyces pombe model system. Genetics. 2015 Oct 1;201(2):403-23. https://doi.org/10.1534/ genetics.
10. Bond C, Tang Y, Li L. Saccharomyces cerevisiae as a tool for mining, studying and engineering fungal polyketide synthases. Fungal Genetics and Biology. 2016 Apr 1;89:52-61.
11. Papanikolaou S, Kampisopoulou E, Blanchard F, Rondags E, Gardeli C, Koutinas AA, Chevalot I, Aggelis G. Production of secondary metabolites through glycerol fermentation under carbon-excess conditions by the yeasts Yarrowia lipolytica and Rhodosporidium toruloides. European Journal of Lipid Science and Technology. 2017 Sep;119(9):1600507.
12. Hamza LF, Sahi NM, Hameed IH. Analysis of Methanolic extract of Secondary Metabolites Released by *Candida* glabratus using GC-MS and Evaluation of Its Antimicrobial Activity. Indian Journal of Public Health. 2018 Mar 1;9(3).
13. Hameed IH, Al-Rubaye AF, Kadhim MJ. Antimicrobial Activity of Medicinal Plants and Urinary Tract Infections. International Journal of Pharmaceutical and Clinical Research. 2017;9(1):44-50.
14. Alrubayae IM, Al-laaeiby A, Minati MH, ALibraheem SA. Determination of genetic relationships and pathogenicity of oral candidiasis etiological agents in pediatric malignant patients in Basrah Farr A, Effendy I, Frey Tirri B, Hof H, Mayser P, Petricevic L, Ruhnke M, Schaller M, Schaefer AP, Sustr V, Willinger B. Guideline: vulvovaginal candidosis (AWMF 015/072, level S2k). Mycoses. 2021 Jun;64(6):583-602.province, Iraq. Systematic Reviews in Pharmacy. 2020 Dec 1;11(12):180-188.
15. Govind M, Afzal A. *Candida* albicans in periapical infections: A review. EDITORIAL BOARD. 88.
16. Farr A, Effendy I, Frey Tirri B, Hof H, Mayser P, Petricevic L, Ruhnke M, Schaller M, Schaefer AP, Sustr V, Willinger B. Guideline: vulvovaginal candidosis (AWMF 015/072, level S2k). Mycoses. 2021 Jun;64(6):583-602.
17. Kwatra B. Bioactive-Compounds: alternative to control *Candida* spp. Int. J. Sci. Res. Rev. 2019;8:221-3.
18. Trisnadewi NN. Vulvovaginal candidiasis (VVC): A review of the literature. Bali Dermatology and Venereology. 2020;3(1):15-8.
19. Vikrant P, Priya J, Nirichan KB. Plants with anti-*Candida* activity and their mechanism of action: a review. Journal of Environmental Research and Development. 2015 Apr 1;9(4):1189.
20. Gonçalves B, Ferreira C, Alves CT, Henriques M, Azeredo J, Silva S. Vulvovaginal candidiasis: Epidemiology, microbiology and risk factors. Critical reviews in microbiology. 2016 Nov 1;42(6):905-927.
21. Rosati D, Bruno M, Jaeger M, Ten Oever J, Netea MG. Recurrent vulvovaginal candidiasis: an immunological perspective. Microorganisms. 2020 Jan 21;8(2):144.
22. Meenambiga SS, Rajagopal K. Antibiofilm activity and molecular docking studies of bioactive secondary metabolites from endophytic fungus Aspergillus nidulans on oral *Candida* albicans. Journal of Applied Pharmaceutical Science. 2018 Mar 30;8(3):037-45.
23. Kim HJ, Kim JC, Kim BS, Kim HG, Cho KY. Antibiotic and phytotoxic activities of ophiobolins from Helminthosporium species. The Plant Pathology Journal. 1999;15(1):14-20.
24. Guard UC. Chemical hazard response information system (chris)-hazardous chemical data. Commandant Instruction. 1999;16465.
25. Zimmerman HJ. Hepatotoxicity: the adverse effects of drugs and other chemicals on the liver. 1993 Oct;39(10):675-787.
26. Daulton E, Wicaksono AN, Tiele A, Kocher HM, Debernardi S, Crnogorac-Jurcevic T, Covington JA. Volatile organic compounds (VOCs) for the non-invasive detection of pancreatic cancer from urine. Talanta. 2021 Jan 1;221:121604.
27. Frazier LM. Reproductive hazards of the workplace. Van Nostrand Reinhold Company; 1998.
28. Bingham E, Cohrssen B, Powell CH. Toxicoloy Issues, Inorganic Particulates, Dusts, Products of Biological Origin, and Pathogens. Wiley-Interscience; 2001.
29. Luo Y, Rana P, Will Y. Cyclosporine A and palmitic acid treatment synergistically induce cytotoxicity in HepG2 cells. Toxicology and applied pharmacology. 2012 Jun 1;261(2):172-180.
30. Wolf R, Kaul BL. Plastics, additives. Ullmann's Encyclopedia of Industrial Chemistry. 2000 Jun 15.
31. Babu B, Wu JT. Production of natural butylated hydroxytoluene as an antioxidant by freshwater phytoplankton 1. Journal of phycology. 2008 Dec;44(6):1447-1454.