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Antifungal effects of alcoholic extracts for plant belong to Brassicaceae family against *Candida albicans* isolated from patients.

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ABSTRACT

The results of the inference detection of alcohol extractor for 8 plants from Brassicaceae family which were: (*Cardaria draba*, *Erucaria cakiloidea*, *Euclium bonmelleria*, *Hirschfeldia incanna*, *Leptaleum filifolium*, *Neotorularia troulosa*, *Rapistrum rugosum*, *Sisymbrium irio*) showed presence of several chemical compounds, including alkaloids, and all species were positive except *E. bonmelleria* and *R. rugosum* that appeared negative at the time of meyer's detection. Phenols compounds, Tannins, Falvonoides, Glycosides, Saponin and Portions were also detected in all species under study, while the result was negative when testing Terpenes in all types under study. The results of the injection of alcohol extract for the 8 species showed 4 concentrations (25,50,75 and 100%) The efficiency of concentration 100% and its ability to inhibit the growth of *C.albicans* yeast was higher than the other concentrations.

Introduction

In recent years, interest in the applications of traditional medicine has increased all over the world, due to the low risks and cost of these medicines, and their efficiency in treating many diseases [1]. Most plants are an important food source, in addition to their high nutritional value because they contain The source of life energy from carbohydrates, proteins and fats [2], it has a medical benefit Therapeutic, as it has a role in treating many pathological conditions [3]. The Brassicaceae (Cruciferae), or mustard family, is one of the largest angiosperm families, can be recognized easily by its floral and fruiting characters. It is cosmopolitan but chiefly distributed in the temperate and Mediterranean region, it represented by 338 genera and 3709 specie [4].

Most of the *Brassicaceae* species are important vegetables consumed worldwide due to their particular essence, aroma, and flavor, but mainly for their broadly recognized functional properties [5]. These properties are directly related with their phytochemical composition and represent the most striking features of this botanical family. The phytochemicals in *Brassicaceae* are classified into several kinds of micronutrients (amino acids, minerals, and vitamins), macronutrients (high content

of proteins and dietary fiber, low in carbohydrates), and secondary metabolites [6].

One of the main groups of microorganisms that can be found in the normal oral flora is the genus *Candida*, which is composed of dimorphic commensal yeast. Although *Candida* species are mainly nonpathogenic, when an imbalance in the oral microbiome.

occurs, they are the main pathogens responsible for the occurrence of fungal infections in the oral cavity [7]. *C. albicans* has the potential for coexistence and pathogenicity. This fungus can have yeast growth, true teliospore, biofilm, and false teliospore, and it is thus called polymorphic fungus which is an important pathogenic factor. Furthermore, the ability of fungi to bind and invade host body cells, secrete hydrolyzing enzymes, contact sensing and thigmotropism, and phenotypic switching are the features of *C. albicans* in pathogenic potential [8].

The current study aims to reveal the possibility of using extracts of some plants of *Brassicaceae* family to inhibit the growth of *C. albicans* fungi.

Material and Methods

1. Plants samples collection

Plant samples were collected from three Governors in northern Iraq (Salah al-Din, Erbil, Sulaimaniyah) belonging to four provinces (E: Erbil district SU: Suleimanya district, G: ghurfa district, LJ: Lower Jazira district) Figure 1. During several field tours for The Period of March-May for the years 2018-2019. It

was classified based on Iraqi, Turkish and Iranian botanical encyclopedias [9,10]. Then it was washed with distilled water to remove impurities and dust stuck in it, dried for 15 days away from sunlight, in a well-ventilated room, milled with electric mill and placed in dark pipes and left until the tests were carried out.



Fig .1: The map of Iraq and the provinces from which the plant samples were collected

2. Preparation of alcoholic plant extract

Alcoholic extract was prepared with a weight of 20 g of vegetable powder in 100 ml of methanol alcohol, stirring for 24 hours, then filtered and concentrated using a rotary evaporator [11]. The active compounds were detected in the extract of the plants under study, which included : Alkaloids that estimation by using the method described in method [12], The method described in [13] followed to detected phenolic compounds, To detect flavonoids, the method was adopted in [14]. To detect Turbines the method described in [15] it used. for detect glycoside method in [13] it used , saponin estimated according to the method shown in [16]. For total proteins the method in [17] it used

3. Collection samples of *C. albicans*

Oral samples in duplicates were collected from Salah al-Din General Hospital and clinics of different ages, and from both sexes from February 1 - 30 on July 2019 . All samples were processed by Gram staining, 10% KOH mount, culture. The yeast species were isolated repeatedly in pure culture from two consecutive early mornings expectorate sputum samples [18]. The samples were subjected to microscopy using Gram staining and KOH mount. India ink preparation was done only when capsulated budding yeast cells were seen on Gram staining. Specimens were inoculated in duplicate on Sabouraud's Dextrose Agar (SDA) with chloramphenicol (16mg/ml) and incubated at 25°C and at 37°C. The cultures were examined on alternate days for growth, for 14 days before discarding them

as negative, Macroscopically fungal growth was identified by rate of growth, colony morphology, texture and surface pigmentation and the diagnosis was confirmed using Vitek compact2 system diagnosis , according to the manufacturer's instructions. *Candida albicans* was identified by germ tube test, chlamyospore formation on cornmeal agar, chrom candida agar. also the diagnosis was confirmed using Vitek compact2 system diagnosis [19].

4. Testing the effectiveness of plants extracts

Four concentrations of alcoholic extract of different plants from Brassicaceae family were prepared 25, 50, 75 and 100% . [20] method was followed to test the effect of different concentrations of extract on the growth of *Candida albicans* yeast as it was poured 25 ml of nutrient agar into each plate. The inoculum was fed by diffusing (0.1) ml with a sterile spreader from the yeast culture containing (1.5×10^{10}) cells / ml, compared with a standard solution of turbidity constant, then the dishes were left to dry at room temperature. A drill with a diameter of 6 mm was made in the culture medium with a sterile cork borer. Then an amount 0.2 ml of the prepared graduated concentrations of the fungal extract was added using a sterile fine pipette. Each treatment was repeated three times, after which the dishes were incubated at a temperature of 37 C° for a period of 48 hours in the incubator. The efficacy of each concentration of the extract was determined by measuring the diameter of the inhibition zone, noting that the zone of inhibition is the region free of yeast growth, and the results

were read by measuring the areas of inhibition around the disc [21].

5. Statistical analysis

All analyzes were performed in triplicate. The Least Significant Difference (LSD) between the treatments was calculated at a probability level of $P < 0.05$ was considered significant.

Result and Dissection

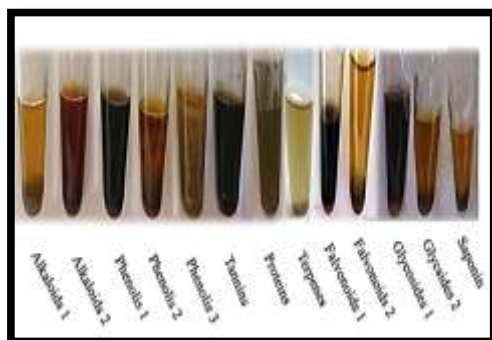
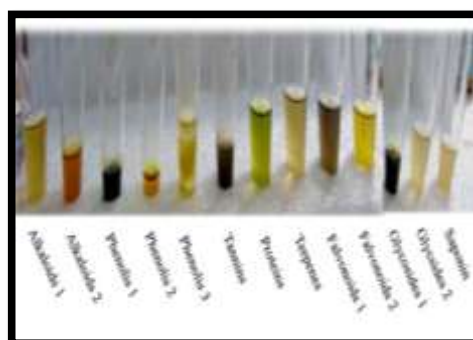
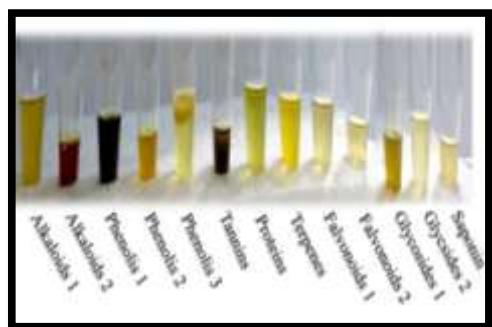
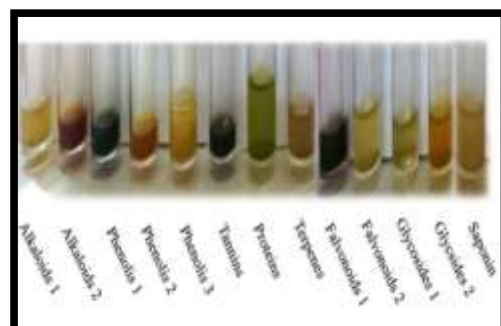
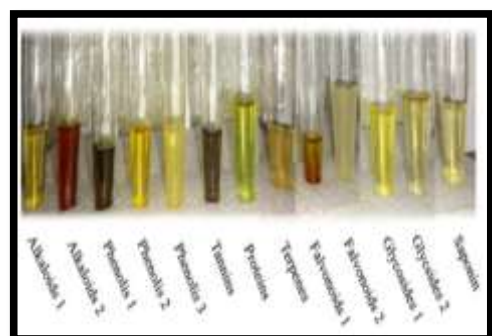
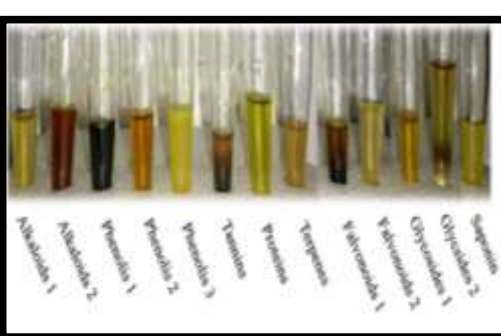
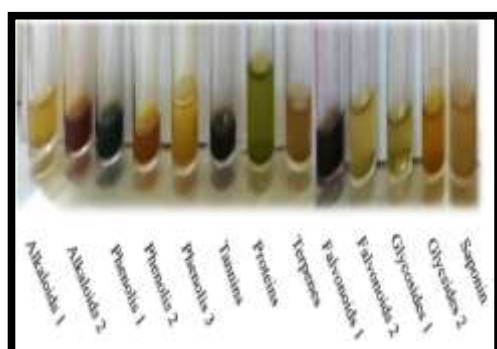
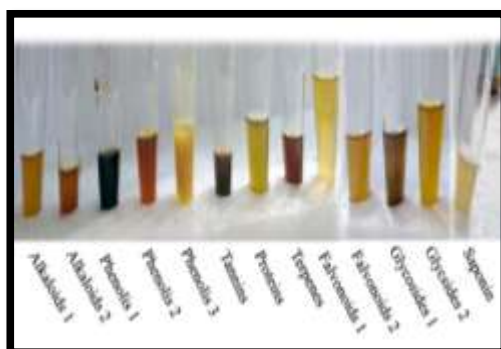
The results of the inference detection of alcohol extractor for 8 plant species which were: (*Cardaria draba*, *Erucaria cakiloidea*, *Euclium bonmelleria*, *Hirschfeldia incanna*, *Leptaleum filifolium*, *Neotorularia troulosa*, *Rapistrum rugosum*, *Sisymbrium irio*). The results of the inference detection of the alcohol extract of the 8 species under study showed the presence of several chemical compounds, including alkaloids, and all species were positive except *E. bonmelleria* and *R. rugosum* that appeared negative at the time of meyer's detection. Phenols compounds, Tannins, Falvonoide,

Glycosides, Saponin and Portions were also detected in all species under study, while the result was negative when testing Terpenes in all types under study. Table 1 and Plate 1.

For years, the *Brassicaceae* plants have been a fascinating research topic, due to their chemical composition characterized by rich in bioactive compounds. The implementation of extracts of these vegetables, causes various beneficial effects of high biological value in the treatment of diseases, owing to their bioactive properties (anti-obesity, anticancer, antimicrobial, antioxidant, hepatoprotective, cardioprotective, gastroprotective, anti-inflammatory, antianemic, and immunomodulator). Therefore, this review summarizes the chemical composition, describes the bioactive compounds isolated in the plant extracts, and highlights diverse biological activities, mainly the antimicrobial and antioxidant capacity [22].

Table 1: Phytochemical studies in alcohol extracts of family Brassicaceae

Chemical compounds Taxon	Alkaloids		Phenols			Tannins	Falvonoide		Glycoside		Saponin	Protines
	1	2	1	2	3		1	2	1	2		
<i>C. draba</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>E. cakiloidea</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>E. bonmelleria</i>	–	+	+	+	+	+	+	+	+	+	+	+
<i>H. incanna</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>L. filifolium</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>N. troulosa</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>R. rugosum</i>	–	+	+	+	+	+	+	+	+	+	+	+
<i>S. irio</i>	+	+	+	+	+	+	+	+	+	+	+	+

*C. draba**E. cakiloidea**E. bonmelleria**H. incanna**L. filifolium**N. troulosa**S. irio**R. rugosum***Plate 1. Specific chemical compounds of the alcohol extract**

The results of the injection of alcohol extract for the 8 species showed 4 concentrations (25, 50, 75 and 100%) The efficiency of concentration 100% and its ability to inhibit the growth of *C.albicans* yeasts was higher than the other concentrations and for all

species studied while the focus was 75% in third place while the concentration was 50% second while the inhibition effect of concentration did not show 25% for the growth of mushrooms studied and for all plant species and showed the presence of moral

differences in the three concentrations as in table 3 and plate 3. These results are consistent with several studies, including [23], which indicated that ethanol alcohol extract has an anti-inflammatory effect against a number of pathogenic microbes, including *C. albicans* that ethanol alcohol extract has a inhibitory effect and that there is an exogressive relationship between the effectiveness of inhibition of bleach with increased concentration the more

concentration of the extract the more concentration of inhibition, and that the inhibitory effect varies from to type.

[24], showed that the antifungal activity of some Brassicaceae plants were more than the antibacterial activity through the efficiency of the ethanolic extract in inhibiting the growth of some pathogenic fungi and yeasts. GAE/ml.

Table 2. The effect of the alcohol extract of the plant studied on *C. albicans* yeast by measuring the inhibition zone

Concentration Taxon	25%	50%	75%	100%
<i>C. draba</i>	4	16	0	18
<i>E. cakiloidea</i>	0	14	9	20
<i>E. bonmelleria</i>	0	24	12	29
<i>H. incanna</i>	10	25	10	30
<i>L. filifolium</i>	0	22	15	31
<i>N. troulosa</i>	0	35	20	40
<i>R. rugosum</i>	0	35	15	40
<i>S. irio</i>	0	32	13	38
LSD at 0.05 %	0.00 NS	5.78	4.17	5.31

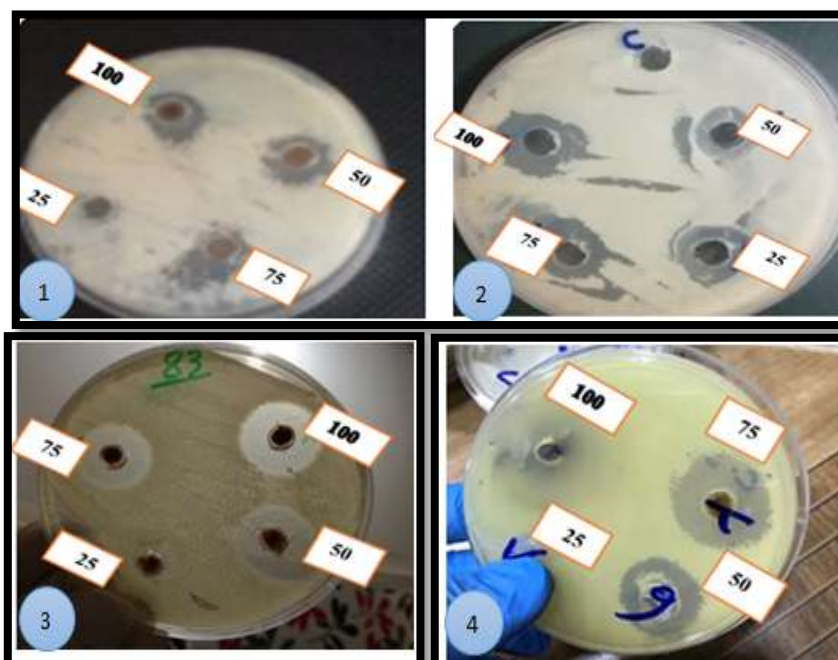


Plate 2. The effect alcoholic extract on *C. albicans* growth
1. *E. bonmelleria* 2. *H. incanna* 3. *L. filifolium* 4. *N. troulosa*

References

- [1] Jamzad, Z. (2013). A survey of Lamiaceae in the flora of Iran. Rostaniha, 14(1), 59-67.
- [2] Khasawneh, F. E.; Sample, E. C. and Kamprath, E. J. (1980). The Role of Phosphorus in Agriculture, Print American Crop and Soil Science Society of America Inc. Madison, Wisconsin, USA.
- [3] Aiyelaagbe, O. O., and Osamudiamen, P. M. (2009). Phytochemical screening for active compounds in Mangifera indica leaves from Ibadan, Oyo State. Plant Sci Res, 2(1), 11-13.
- [4] Warwick, S. I., Francis, A., and Al-Shehbaz, I. A. (2006). Brassicaceae: species checklist and database on CD-Rom. Plant Systematics and Evolution, 259(2), 249-258.
- [5] Aires, A. (2015). Brassica composition and food processing. In Processing and Impact on Active components in Food (pp. 17-25). Academic Press.
- [6] Ramirez, D., Abellán-Victorio, A., Beretta, V., Camargo, A., and Moreno, D. A. (2020). Functional ingredients from Brassicaceae species: Overview and

- perspectives. *International Journal of Molecular Sciences*, 21(1998), 1–21.
- [7] Baumgardner, D.J.(2019). Oral fungal microbiota: to thrush and beyond. *Journal of Patient-Centered Research and Reviews*, 6(4), 252.
- [8] Naglik, J. R., Richardson, J. P., and Moyes, D. L. (2014). *Candida albicans* pathogenicity and epithelial immunity. *PLoS pathogens*, 10(8), e1004257.
- [9] Townsend, C. C. and Guset, E.(1980). *Flora of Iraq vol.4 part 1*. Ministry of agriculture.
- [10] Rechinger, K.H.(1972). *Flora Iranica*. 98:1-67. Akadimische Druck-u. Verlagsanstalt. Graz-Austria.
- [11] Banson, A. and Adeyemo, S. (2006). Phytochemical screening and antimicrobial assessment of *Abutilon mauritanum*, *Bacopa monnifera* and *Datura stramonium*. *Biokemistri* 1. 8(1): 39-44.
- [12] Siddiqui, A. A., and Ali, M. (1997). *Practical pharmaceutical chemistry*. CBS Publishers and Distributors.
- [3] Harborne, J.B.(1973). *Textbook of phytochemical methods*, 1st Edn, Chapman and Hall Ltd. London. pp, 110-113.
- [14] Harborn, J.B. (1984). *Phytochemical Methods* Second edition. Chapman, Hall. New York. USA.
- [15] AL-Khazaraji, S.M.(1991). *Bio pharmacological Study of Artemisia herb*. Ms.c. Thesis, College of pharmacy, Baghdad university. Iraq.
- [16] Gibbs, R. D. (1974). *Chemotaxonomy of flowering plants: four volumes*. McGill-Queen's Press-MQUP.
- [17] Shihata, I. M. (1951). *A pharmacological study of Anagallis arvensis*. MD Vet (Doctoral dissertation, Thesis, Cairo University. 10).
- [18] Tripathi, I. P., and Mishra, C. (2015). Phytochemical screening of some medicinal plants of Chitrakoot region. *Indian Journal of Applied Research*, 5(12), 56-60.
- [19] Koneman E and Roberts GD.(1985) *Practical Laboratory Mycology*. 3rd. Williams and Wilkins, Baltimore, London.
- [20] Jagdish C. (2009) *Textbook of medical mycology*. New Delhi, India: Meheta.
- [21] Perez C, Paul M, Bazerque P. (1990) An antibiotic assay by the agar well diffusion method. *Acta Biologica et Medicinæ Experimentalis*. 15, 113-115.
- [22] Amade P, Mallea M, Bouaicha N. (1994) Isolation, structural identification and biological activity of two metabolites produced by *Penicillium olsoni* Bainier and Sartory. *Journal of Antibiotics*. 47,201-207.
- [23] Favela-González, K.M., Hernández-Almanza, A.Y. and De la Fuente-Salcido, N.M., 92020). The value of bioactive compounds of cruciferous vegetables (Brassica) as antimicrobials and antioxidants: A review. *Journal of Food Biochemistry*, 44(10), p.e13414.
- [24] Fagade, O. E., and Oyelade, A. A. (2009). A comparative study of the antibacterial activities of some wood-decay fungi to synthetic antibiotic discs. *Electron J Environ Agric Food Chem*, 8, 184-188.
- [25] Aydin, S. (2020). Total phenolic content, antioxidant, antibacterial and antifungal activities, FT-IR analyses of *Brassica oleracea* L. var. *acephala* and *Ornithogalum umbellatum* L. *Genetika*, 52(1), 229-244.

فعالية المضادة للفطريات للمستخلص الكحولي لنبات Brassicaceae ضد فطر

Candida albicans المعزولة من المرضى

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الملخص

أظهرت نتائج الكشف للمستخلص الكحولي لثمانية نباتات من عائلة الكرنب وهي: *Cardaria draba*, *Erucaria cakiloidea*, *Euclium bonmelleria*, *Hirschfeldia incanna*, *Leptaleum filifolium*, *Neotorularia troulosa*, *Rapistrum rugosum*, *Sisymbrium irio* ظهور عدة مركبات كيميائية المركبات ، بما في ذلك القلويدات ، وجميع الأنواع كانت موجبة باستثناء *E. bonmelleria* و *R. rugosum* التي ظهرت سلبية عند كاشف ماير. كما تم الكشف عن مركبات الفينول والتانينات والفلافونويدات والجليكوسيدات والسابونين والأجزاء في جميع الأنواع قيد الدراسة ، بينما كانت النتيجة سلبية عند اختبار التربينات في جميع الأنواع قيد الدراسة. أظهرت نتائج حقن المستخلص الكحولي للأنواع الثمانية 4 تراكيز (25، 50، 75 و 100٪) كانت كفاءة تركيز 100٪ وقدرته على تثبيط نمو خميرة *C.albicans* أعلى من التراكيز الأخرى.