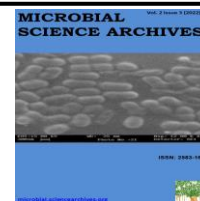




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Research Article



Effect the alcoholic extracts of *laurus nobilis*, *terfezia* and *agaricus bisporus* on the *candida albicans* isolated from newborns and pregnant women in Samarra city

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Abstract

Oral thrush is a visible growth of *Candida albicans* in the mouth that appears in the form of white spots on the tongue, soles of the cheeks, the roof of the mouth and pharynx. It is common for children and women in pregnancy also develop vaginal candida disease, especially in the last trimester of pregnancy, and the infection is transmitted to the infant during childbirth if treatment is not done early. 130 Samples were collected from infants and pregnant women in Samarra and innate truffle and mushroom extracts and laurel leaf extract were used to determine their effect on candida and the possibility of treating it and comparing it with the antibiotic nestatin. All the extracts under study showed a disincentive to the concentration strength used, with moral differences at a probability level of $0.05 > p$ and statistical tables of results worked. 12, 14 mm followed by mushroom mushrooms, which had a slight effect on candida inhibition, where the results of inhibition respectively were 2,5,8,12 mm but less laurel, and the results of inhibition of the muzzle were weak and respectively 2,3,6,7 mm compared to the results of inhibition of laurel and mushrooms with no moral differences at the probability level of $0.05 > p$.

Keywords Bladder *Candida albicans*, Mushroom, Truffle, Laurel

Introduction

Candidiasis is a primary or secondary fungal infection caused by the species *Candidia* spp. This infection may be acute, Acute, Subacute, or Chronic (Asai et al., 2021). The pathogen causes local infection of the mouth, pharynx, skin, vagina, fingers, nails, and trachea, and it can spread within The body and affects the lungs and the gastrointestinal tract, or systemic infections, where the presence of fungi in the blood is considered, a pathological condition known as Fungemia, or it may be endocarditis or meningitis. The clinical appearance of the disease varies according to the place of infection in the body and the age of the person (Pappas et al., 2018).

In recent years, the importance of researching cases of fungal infections and their recurrence has increased. This is due to the increasing number of people infected with diseases that

weaken the immune system, such as diabetes, leukemia, tuberculosis, and AIDS. Acquired Immunodeficiency Syndrome (Prasath et al., 2021). As the infections that affect the body in different areas such as the mouth, genital tract, skin and the genital and vaginal canal was caused by *Candida* and some types of bacteria may participate in the events of infection, but the most common causes are yeasts belonging to the genus *Candida* spp. (Lee et al., 2018). *Candida* is responsible for the oral thrush that affects children, and it is also responsible for Vaginitis, in addition to many skin infections (Kühbacher et al., 2017). *Candida* is also part of the normal flora in the mouth and the digestive system. It is present in small quantities and does not cause any infection when it is present with *Lactobacillus* bacteria that inhibits its growth and adhesion, but in the absence of bacteria it turns into pathogenic organisms capable of causing infection in different areas because it is an opportunistic fungus (Duncan,

2014). The main factor causing candida albicans It is an organism that is well adapted to its human host. However, changes in the host environment can enhance the transition from a symbiont state to a pathogen. This transition is highly dependent on several factors, including cell surface adhesives, proteolytic enzymes, morphological switching and the development of resistance. Medicines in the oral cavity (Talapko et al, 2021).

Several studies have shown that infection rates increase in women during pregnancy, especially during the third trimester of pregnancy, and that this increase is due to many reasons, the most important of which are: the change in the acidity of the vagina PH, the increase in sugar in vaginal secretions, and the increase in the level of estrogen during pregnancy results in More glycogen in the vagina, which provides a source for candida growth and germination (Tariq et al, 2016).

Local innate defenses play a central role in maintaining Candida equilibrium in addition to preventing Candida adhesion to epithelial cells as saliva is enriched with antifungal peptides, which are part of the innate immunity of the host, where the innate immune response is primarily involved in the host mucosal defenses. The primary growth of candida and the prevention of subsequent tissue invasion (Vila et al., 2020). The success of candida in causing infection is due to its possession of virulence factors that enable it to resist the surrounding conditions: it has the ability to withstand high temperatures 37°C, and it has the ability to Dimorphism, its ability to produce lytic enzymes, its ability to adhere to the cell wall and penetrate into host tissues (Montelongo et al, 2019). Diagnosis is the first step in the treatment and prevention of *Candida* infections, as knowing the causative type facilitates the study of virulence factors as well as the use of The appropriate antibiotic, and there are many antibiotics used that have mechanisms against these fungi, such as the antifungal nystatin, which works on the They interact with the plasma membrane and the antagonist works to prevent the formation of the cell well of candida. However, these antagonists have many drawbacks, most of which are very narrow spectrum in the treatment of fungal species, as well as their low permeability in tissues and the proliferation of many isolates resistant to these antibiotics (Brooks et al, 2001).

Medicinal plants and their products have been used as natural healing materials. In recent years, interest in studying medicinal plants and their use in treatment is increasing day by day in different parts of the world in an attempt to find effective alternatives to the used medicines (Khatri et al, 2016). Medicinal plants can provide a wealth of factors. Antimicrobials, which can be used as an alternative source for traditional antibiotics. 25% of the prescribed drugs at present come from plants. It is sufficient that Germany, which is at the forefront of the world producing industrial medicines, consumes annually, as stated in one of its statistics, 8000 kg of dried pepone flowers (Rehman et al, 2015) and among the most important active chemicals in medicinal plants are alkaloids, clocosides and volatile oils, and other reasons that

motivate the use of medicinal plants are their cheapness, availability, possibility of cultivation and their therapeutic benefits. The aim of the study is isolating *Candida albicans* from the mouth of newborns from one day old to one year old and from pregnant women in different stages of pregnancy and knowledge of the relationship between the infection of children and the infection of women in pregnancy and the percentage of infection with the disease and studying the inhibitory effect of extracts of bay leaves (*Laurus nobilis*), (*Agaricus nebularis*) and truffles (*Trfezia*) on *Candida albicans*.

Materials and Methods

The work was carried out in the laboratory of the College of Education - University of Samarra - Department of Life Sciences in the Microbiology Laboratory. The necessary laboratory equipment and tools were used to conduct the study.

100 samples were collected during the period between 1-10-2019 and 1-4-2020 from vaccination centers in the city of Samarra, where 100 oral swabs were taken using a sterilized cotton swab from newborns to one year of age to investigate oral thrush at this stage of life and under the supervision of a specialist doctor. A sample of pregnant women in different months of pregnancy in the form of swab swabs as well as different ages, where the ages of the pregnant women ranged between 17 to 45 years, and in cooperation with the specialized staff in the hospital, to show the relationship of the pregnant mother in transmitting the infection to the child during natural birth. A form was prepared to collect information related to the mother and the child about the type of breastfeeding, childbirth, age of the mother, number of births, housing, and sex to show the relationship of this information to infection. The form is in the appendix.

Direct Microscopic detection using (KOH%10)

After collecting the samples, a swab is taken from the sample on a clean glass slide and a drop of potassium hydroxide solution KOH (10%) is added to it, then it is covered with a cover slide and then it is passed quietly over the flame, after moving it two or three times over the flame while avoiding boiling Because it leads to the crystallization of potassium hydroxide, then the slide is left for 15 minutes, and the glass slides are examined by light microscopy with a small force of 10X and then of a high force of 40X to ensure the presence of spores, yeasts, hyphae or bud cells (Koneman and Roberts, 1985)

Culture of Samples

Oral swabs were transferred directly to the laboratories of the College of Education/University of Samarra and were planted on SDAC with a solid sabroid dextrose medium using the Streaking method. The dishes were incubated in the incubator at a temperature of 37 °C for 48 hours (González et al, 2007) for the purpose of identifying the species causing infection.

Samples diagnosis

After the culture was examined, colonies suspected of being *Candida* were taken Examinations and other confirmatory tests. The shapes and sizes of yeast cells were observed based on taxonomic keys (Morello et al, 2003).

VITEK® System

This test was conducted using the Vitek 2 Compact diagnostic system, which is a fully automated diagnostic system used in the diagnosis of medical bacteria and yeasts, as well as in the antibiotic sensitivity test. Vitek system that is based on a precise set of biochemical reactions and gives accurate results within hours (Kord et al, 2020)

Preparation of extracts

Preparation of plant and fungal extracts

For each of the laurel, truffles, and mushrooms. Dried laurel leaves were obtained from local markets and the leaves were cleaned of dirt and dust, then ground with an electric grinder and kept in a moisture-free atmosphere until the preparation of the extract began. As for truffles, they were obtained fresh as they were collected from the markets of Samarra city. Locally in the gypsum, where it is characterized by a color that tends to black and dust or to reddish and scientifically known as *Terfezia*, this type of truffle spreads in Europe and the deserts of Syria and Iraq (Belfiori et al, 2012).

The truffles were thoroughly cleaned of dust and dirt, then they were cut into light slices to facilitate drying, then they were placed in the electric oven at a temperature of 40°C. After the drying process was completed, they were ground with an electric grinder and stored in a sterile, sealed glass bottle until use, as well as the mushrooms. The type used in the study is of local production and the most common type is *Agaricus bisporus*, also known as white button mushroom (Al-Aroussi and Salem, 2000). The same steps were followed above for truffles. Where it was cleaned, then cut into slices, then dried and ground into a powdered form, and kept ready for use.

Results

The collected 130 samples from vaccination centers, health care and Samarra General Hospital, and I took 100 samples from the mouth of children from the age of one day to a year, and 30 samples from pregnant women in different months of pregnancy to know the relationship between mother and child infection for a period ranging from 1-10-2019 to 1-4-2020. Where samples were taken for children of both sexes, in addition to information regarding the child's age, sequence, mother's age, place of residence, standard of living, type of

natural or short birth, as well as natural or artificial breastfeeding. The samples were examined in three ways. The first method was direct microscopy using 10% KOH. The total number of males was 57 males, the number of infected was 40 males, i.e. (57.15%), and the total number of females was 43 females, the number of infected women was 30 females, at a rate of (42.58%), and the total samples are 70 samples for males and females, as shown in Figure 1.

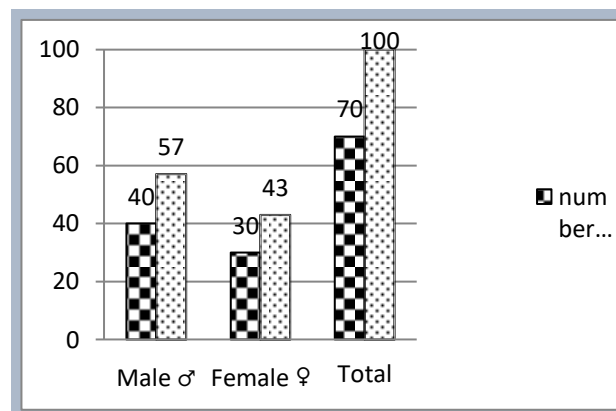


Fig 1. Number of isolates from children and their percentages by sex

All samples diagnosed with direct microscopic examination were cultured after staining, as the results of laboratory culture shown in Table (1) showed the emergence of 50 positive samples, with a percentage of (71.42%), while the number of negative cases with laboratory culture was 20 samples, i.e. (28.5%), the number of positive samples for laboratory culture for males was 29 (41.42%), and the number of negative samples for males was 11 (15.71%), while the number of positive samples for females was 21 (30%), and the number of negative samples for females was 9 (12.85%). That is, the total of the final positive samples for both microscopy and laboratory culture is 50 samples. The results of in vitro culture are more accurate than direct examination.

As for pregnant women, 30 samples were collected for pregnant women, and after conducting the same previous examinations on the samples, the infection appeared in 13 samples, i.e. (43.3%) of the total samples as in Figure (2).

Table (2) shows the inhibitory effect of alcoholic extracts of bay leaf and truffle and mushroom fungal extracts and their comparison with the antibiotic nystatin, as well as the synergistic effect resulting from mixing the extracts with each other. The inhibitory effect of the extracts under study was directly proportional to the increase in concentrations, with significant differences at the level of $0.05 P \leq$ between the mean diameters of inhibition and the concentrations used under study, where the extract of bay leaves showed a clear effect on the inhibition *Candida albicans* where the effect of laurel at the 25% concentration was significantly different from the 50% concentration, while the effect of the laurel at

the 75% concentration was not significantly different from the 100% concentration at a probability level of 0.05. 10, 12, 14 had a lower inhibitory effect than the laurel, where the effect of mushroom was when inhibiting at concentrations 25, 50, 100, 75 mg/ml, respectively, 2, 5, 8, 12 mm, with significant differences between the concentrations at the probability level of 0.05, and the concentration (100%) was the most effective in inhibiting candida, then the diameters of inhibition decreased significantly with truffle extract, where the effect was Truffles at a concentration of 25% and a concentration of 50% did not differ significantly, but differed significantly with a concentration of 75% and 100% mg/ml, and the average diameters of inhibition, respectively, were 2,3,6,7 mm. The apparent growth is compared with the diameters of the colonies growing on the lower medium free of the extract or anti-fungal, which represents the control reading in all the

mm, followed by the alcoholic extract of mushroom, and it extracts under study to observe the effect of the extracts and their antagonistic activity against *Candida albicans*, and the concentration at which the growth of yeast isolates stops represents the minimum inhibitory concentration (MIC). The results also showed that the average treatment (inhibitory effect) of the extracts Under study, the highest percentage in laurel plant was 10.5 mm, then the ratio decreased in mushroom 8.5 mm and became very weak in truffle 4.5 mm, and the average treatment for laurel plant approached the antibiotic nystatin, which was 12.5 mm with no significant differences between them The probability is 0.05, and through the results of the study, bay leaf extract had the best inhibitory effect on the studied candida.

Table 1. Distribution of Candida infections by sex based on direct microscopy test using KOH, double blue staining, and in vitro culture for positive and negative cases

Test sex	Direct (KOH)		Direct Microscopic after staining				Culture results			
	No.	%	No.	%	No.	%	No.	%	No.	%
Male	40	57.15	23	32.85	17	24.28	29	41.42	11	15.71
Female	30	42.85	17	24.29	13	18.57	21	30%	9	12.85
Total	70	100	40	57.15	30	42.85	50	71.42	20	28.5

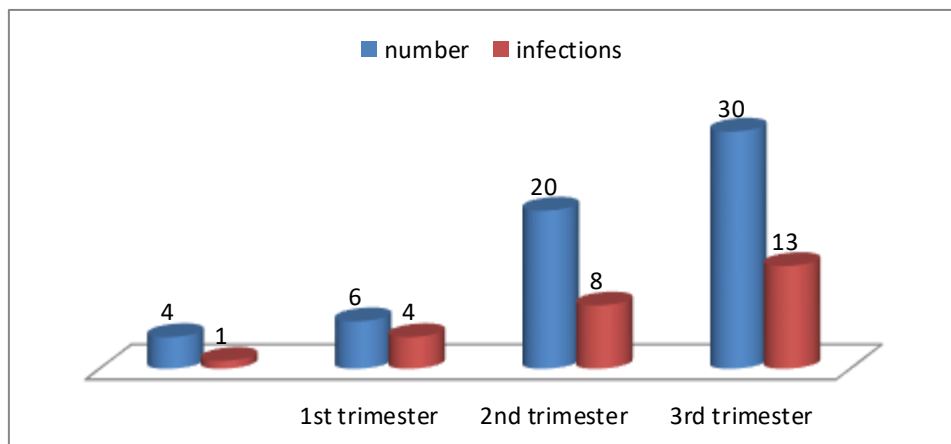


Fig. 2 Distribution of vaginal candidiasis infections in pregnant women according to the months of pregnancy

Discussion

The appearance of some negative results by direct microscopy: the cause may be due to the repeated use of antibiotics by the mother during pregnancy, which causes the lethargy of some candida and as a result its growth is weak and therefore does not appear in the direct microscopy test and appeared when the insulation was implanted on the center of the solid saproid (Truss,1981). The study also

showed that the method of direct examination does not reflect the actual presence of candida yeast in children with symptoms of the disease, with yeast at direct examination. While the ratio changed to 71.42 percent when implanted in the food medium, this indicates that the method of direct examination is inefficient or accurate such as laboratory implantation, and a previous study by Geraldo & witkin (2000) indicated that most cases of candida disease are unable to be detected directly, as the results of the study

showed that there is a close relationship between vaginal candida disease and oral sustenance, The results of most isolations from newborns whose birth was normal showed that the cause of infection was an infection from the mother during childbirth, where the incidence of candida in pregnant women increases more than non-pregnant women (Ibrahim and Yehia, 2017). Also, children whose birth was caesarean section had a lower incidence, but with regard to negative laboratory transplant results, this is due to an error in the method of transmitting the sample or preparing the implant medium, which helps in the growth of the throwing fungi that overwhelm the original sample and its non-appearance, as

well as the method of preserving the sample and not exposing it to contamination is very important in obtaining the results correctly and not showing negative results (Al-Hashimi, 1979) Or it may be due to the fact that the injury may be caused by another pathogen that may be bacterial indistinguishable, especially since the center of the saproid agar, which inhibits bacterial growth as a result of low pH, as well as containing chlorophyncol, which prevents the growth of bacteria (Hamzah, 2020). Or it may be due to the method of implantation and laboratory growth conditions (Collee et al,1996)

Table 2: Effect of laurel extracts, mushrooms, truffles and synergistic effect on the growth of candida albicans mushrooms from the mouth of newborns and pregnant women and compared to nystatin fungal antagonist

Concentration extract	Avarage extract ml	Control	%100	%75	% 50	% 25
<i>Laurus nobilis</i>	10.5 B	0.0	14 aB	12 abB	10 bB	6 cB
Mashroom <i>Agaricus bisporus</i>	6.8 C	0.0	12 aC	8 bC	5 cD	2 dD
truffle <i>Terfezia</i>	4.5 D	0.0	7 aDE	6 aD	3 bE	2 bD
Truffle+ Mushroom	5.3 CD	0.0	8 aD	8 aC	3 bE	2 bD
Truffle + Bay Laurel	4.5 D	0.0	6 aE	5 abD	4 bcDE	3 cCD
Mushroom+ Bay Laurel	9 B	0.0	13 aBC	11 bB	8 cC	4 dC
Truffle+ Mushroom+ Laurel	4.5 D	0.0	6 Ae	5 aD	5 aD	2 bD
Nystatin	12.5 A	0.0	16 aA	14 bA	12 cA	8 dA
Average Concentration	Control	0.0	10.3 a	8.6 b	6.3 c	3.6 d

As for pregnant women, 30 samples were collected for pregnant women, and after the same examinations prior to the samples, the infection appeared in 13 samples, or 43.3 percent of the total samples as in figure (2). The results of the current study corresponded to many previous studies in that candida disease affects pregnant women and increases significantly in the last months of pregnancy as shown in the figure.32 This result was consistent with the conducted by ALSharifi & Ehan (2017), the results of which showed that the highest incidence among pregnant women was in the third trimester of pregnancy at 63.4%, although the lowest infection rate was in the first trimester of pregnancy and 10.5%. This result was also close to Glattheaar (1982) obtained, where he noted in his study that vaginal acidity and hormonal factors change during pregnancy, resulting in more infection of pregnant women, where the vagina becomes less acidic and less able to fight infection.

The high mortality rate of immunodeficiency patients with fungal infections and limited availability of effective and safe factors requires the development of new antifungal treatments to detect such factors. To note the effect of plant extracts and their antifungal activity and how they work synergistically and is this method effective or ineffective, as in table (2) polyphenols, a substance found in plants and effective against pathogenic fungi (Palazzo et al, 2020). We note that there are moral differences between the concentration averages at the probability level of 0.05. The three extracts did not give greater effectiveness and may be due to the difference of active substances in each extract and mixing them together may affect their effectiveness or may give the greater inhibition force in mixing laurel with mushrooms or that synergy can reduce the effectiveness of some substances and their effect as in mixing laurel with truffles and mixing the three extracts with each other and the

results showed that mixing truffles weakens the inhibition effectiveness, As for the effectiveness of laurel, the results of the study matched many studies that have shown the effectiveness of plants in inhibiting fungus growth and reducing dry weight, This is consistent with Peixoto *et al* (2017) as well as the fact that plants produce secondary metabolic substances and oils that act as antivirals, antifungals, antimicrobials (Bigovic *et al*, 2017), as well as containing phenolates, flavonoids, glycosides, dragons, tripons, and alkalids, which have a high capacity to work as a strong fungal antibody that may be associated with the presence of flavonoids known for their strong antifungal and bacteria, flavonoids are due to their ability to interfere with cellular proteins and the formation of a protein complex soluble in extracellular water made up of cell wall and plasma membranes of the microbe after tearing them and the vital effectiveness of secondary metabolic substances is due to morphological and cellular changes in microorganisms.

In addition, phenoli compounds interfere with the synthesis of the cellular wall and membrane and lead to its crash in the killing of mushrooms. 5 and 100, which is in line with what he reached (Waeli, 2014) in his study, where he attributed the effectiveness shown by pomegranate peel extract to containing alkaloids, flavons, titans and resins, while dragons are associated with proteins in the sense that they affect enzymes and change their nature as well as their ability to stimulate phagocytic cells (Amaral, 1998). The chemical composition of the laurel where studies have shown that chemical analysis of the plant contains vitamins such as A. B.C niacin and minerals such as potassium, calcium, iron, manganese, magnesium, zinc, copper, flavonoids (claikosides derivatives) this is consistent with (Palazzo *et al*, 2020) where chemical composition helps provide antibacterial and antifungal properties. In a study (Dammak *et al.*, 2019) it showed that the chemical composition of the laurel sheet tested through a chromatographic mass spectrometer analysis device for a gas whose antioxidant capacity was assessed and the results showed that *L. nobilis* possessed the highest content of phenol (15.15+1.6 4), which is equivalent to galician acid and from the above we conclude that the high content of phenol and chemical composition of laurel germ are important antioxidants and helped in inhibition as well as essential oil ingredients where a study conducted by (Fidan and Hafize, 2019) showed the activity and composition of essential oil for laurel where the oil content reached 0.78% in fruits, 0.80% in twigs and 3.25% in leaves where the highest percentage of leaves and antibacterial properties were examined and the paper showed the highest antifungal activity and bacteria and against almost all microorganisms tested, the presence of Isougenol, a plant-based ingredient found in many essential oils in plants and a natural antibacterial product, has been confirmed by several studies of antifungal activity of eisogenol, consistent with a study of Medeiros *et al.* (2020) He confirmed that eisogenol and hybrid acetamides have an antifungal effect isolated from the mouth, as well as molecular fusion of isogenol, a potential

inhibitor of sterol reductase 14 delta and demethylas enzymes, which is converging.

As for truffle results, which are less effective than laurel and mushrooms, truffles contain valuable nutrients as approximately 5% of dry matter consists of nitrogen and 15% protein, while fats and sugars are about 2% each. Raw nitrogen, dissolved nitrogen, free amino nitrogen and vitamin C while its fat content is very low (Alan & Cook, 1992) and other elements such as phosphorus and magnesium are relatively lacking in these elements (Muhammed, 1995). Many studies pointed out the importance of these therapeutic and nutritional substances and through the results of the study had a weak inhibition effect on candida albicans fungi in a different proportion with increased concentrations with moral differences at a moral level of $0.05 P \leq$ between the rate of inhibition diameters and concentrations used under study, it is worth noting that the effectiveness of some substances and elements is reduced by storage and the storage environment if not ideal, especially truffles, many studies have indicated this especially that truffles are present only in a limited and short period of the year, which forced us to buy truffles before starting the practical laboratory research with a period of drying, grinding and storing, as the practical period lasted approximately four months, so these conditions may have negatively affected the effectiveness of truffles inhibition, or the effectiveness of truffles against fungi may be moderate as the results of the study showed.

These findings were agreed with Khaled *et al* (2014), who noted in his study that all alcoholic extracts of desert truffles had no activity against mushrooms *A. niger*, and also a study carried out by Al-Mazaideh *et al* (2021) where he emphasized the characteristics of therapeutic and pharmaceutical truffles, and indicated that the possible antifungal ingredients in the muzzle were not well studied, We need more intensive study to find out the bios truffles properties against fungi in the future. The results of the study differed with Neggaz *et al* (2014), which referred to the characteristics of bio truffles in inhibiting the growth of both negative and positive bacteria for kram dye and have anti-candida fungus activity. As for the effect of the fungal antifungal nastatin on candid albicans fungi, four concentrations of antibiotic nastatin 25, 50, 75, 100 micrograms/ml and Control were used to control, and the results of the study showed that this antibiotic has an effective effect.

Conclusion

Candida albicans yeast is the dominant type of candida among other types of candida in causing candida vaginal disease in women and oral candida disease in children. Women are more likely to be infected during pregnancy, especially in the last trimester of pregnancy, and may be passed on by mothers to children during childbirth. Candida sensitivity and resistance depend on several factors, including (the concentration of the fungal antibiotic, the surrounding

environment, its location in the body of the organism, as well as the weakening of the immune system of the infected person. The effectiveness of the extracts studied varied in their effect on candida and showed different effectiveness in inhibition by type and concentration of extract.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication

Ethical Considerations

The study was approved by the institutional ethical committee

Funding support

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