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Testing effect some plant extracts on reducing growth of Candida albicans which isolated from vaginal canal of pregnant women

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ABSTRACT— Candida Albicans is considered an opportunistic fungus which affects human as it may cause infections for different parts of the human body, during this study, this type of fungi has been isolated from the genital area for some pregnant women at Gynecology and Obstetrics department / Al Batool teaching hospital / Mosul city. Certain methods have been used to confirm the presence of this fungus; some methods were by inspection through the clinical examination while others were microscopically done. All isolates that have been collected at the mentioned hospital were compared with the standard isolates of the same fungus at the Laboratory department / Mosul Technical Institute. These procedures were done at different periods during 2019 as they were done between January to May inclusively. An antagonist properties for mint and Garlic extracts were examined against the growth of Candida Albicans through the exposure of Candida Albicans culture to saturated tablets with the extracts of both Mint and Garlic with daily monitoring as the results showed that the fungus growth has been ceased completely after 8 days, moreover, during the monitoring phase there was gradual distance between the Fungus and the saturated tablets with extracts which means that the plant extracts obviously affected the fungus growth but with different levels of affection.

KEYWORDS: Dry powder inhaler, Cefdinir, Lung infectious disease, Cystic fibrosis, Wetness Impregnation Method.

1. INTRODUCTION

Fungal infections are caused by microorganisms which invade different parts of human body like lungs, skins, oral cavity, genital area and other parts. Fungi exist usually in air, water and soil, anyhow certain types of fungi are causing infections to the human while others are not. Human body can resist and overcome it. Some fungi which cause diseases are free-living which enter the human body through inhalation, or the spores enter the body through wounds or skin scratches while others are considered part of body normal flora. Recently, fungal infections diseases showed a high prevalence number and cause different symptoms and what complicates the early diagnosis of the infections is the slow mode of growth and replications which makes it is so difficult to be noticed and diagnosed [1]. Most of fungal infections are sensitive to treatment but rarely they lead to death except for immunocompromised patients like HIV patients and other diseases.

Many people think that fungal infections are happening only as local infections like cutaneous impetigo and

foot fungal infections for the athletes but throughout the researches and studies showed that there are certain types of fungi invade the human body and have prominent effects and symptoms, this type of fungal infections inoculate the human body by inhalation as many spores of the fungus enter the body, which are small, air-born and cannot be recognized by naked eye, Moreover, the winds can bea]them for hundreds of kilometers, if the human inhale significant amount of the spores and the airways could not destroy or expelled them can lead to an acute infection to the lungs, and in many cases lead to lung abscess. Rare cases with fungal infections and when the disease progress, bone abscess and gastrointestinal system like liver, appendicitis and other organs could be affected by the infection [2].

Vaginal yeast infections - also known as -``Candida albicans``, one of the most common infections in women which could infect men -as well- by contact, most cases are easy to be managed and treated which exist normally with few amounts in vagina and mouth, when the number of fungi exceed the normal quantities at the vaginal area, the fungal infection flaring up will be initiated, unwanted symptom started to appear like vaginal itching, burning sensation and secretions [3].

There are many anti-fungal / antibiotics are used to be active against these infections which are differ from each other by effect and selectivity. To consider a good antifungal choice for the treatment it should has less or absent side effects with broad antifungal spectrum with high permeability for the tissues. Managing of fungal infections is considered one of the main problems for the time being as the fungi are nuclear microorganism with metabolic and structural properties similar to the hosts which are also nuclear including human so in addition to its ability to destroy the fungi causing infection to the human, it has the ability to destroy as well the host tissues. 10% of women are with Candida Albicans which is similar to the manifestations of other vaginal fungal infections [4].

The study aim to isolate and identify Candida Albicans fungus in pure farms and study the inhibitory effect of plant extracts which are used in the study on fungus growth, in addition to the contribution of plant extracts in finding alternatives methods to treat the microorganisms which became resistant to many anti-fungal medications due to prolonged used of such medications [8].

2. MATERIALS AND METHODS

2.1 Materials

Many Candida Albicans isolates were used in the study, which have been obtained directly from patients who attended Al Batool hospital; the samples were taken from the vaginal secretions or from the thin scales which cover the infected skin.

The following materials were used:
Petri dishes (glass and plastic) with 9 and 6 cm diameter; needle, isolate, forceps, suckers, cups, cotton, Selofan paper, Puncher, growth media (SMASabourud, Maltose agar and Macconckey medium).

2.2 Methods

Culture media used for Candida Albicans growth and isolation:

a) Sabourud Maltose Agar

The culture media is weighted by sensitive weight scale (1.5g, usually the weight is already mentioned on the can), then 1000 ml of distilled water is added to the media. Mixing the contents properly and placed in a water bath for 35-40 minutes until all the materials dissolved and disappeared completely as it will become

transparent [5]. The mixture will be separated into small flasks. 250 ml of antibiotics (Chloramphenicol) is added to the mixture to facilitate pouring the materials in the dishes. These flasks must be sterilized in autoclave for 15-20 minutes then will be ready to be poured in Petri dishes.

b) Corn meal Agar

It consists of 40g of Corn meal, 5 drops of Tweem 80 and 20g of Agar and the media powder will be weighted in sensitive weight scale (usually the net weight is already mentioned on the can), then 1000ml distilled water added. The same method mentioned above for preparation will be used with the presence of the Chloramphenicol antibiotic [6].

2.3 The antibiotics that are added to the culture media

Chloramphenicol: It is added to the culture media before the sterilization step as it will stand with the temperature of the autoclave; it is prepared by dissolving 50mg of the antibiotics in 10ml of Ethyl Alcohol and mixed well then added to 1 liter of culture media when it is hot and then sterilized. Lactophenol dye is added to the fungal sample on the slide to paint the cells and demonstrate them clearly [7].

2.4 Candida Albicans isolation from the patients

Samples have been collected generally from patients suffer from vaginal fungal infection during different periods of time, which has been done through conducting swaps from the infected area and implant it in specific culture media, repetition of implanting processes to other dishes which contain Sabourud media as it is considered a specific media for Candid Albicans growth when the dishes are kept for 72 hours under 37C°. After the mentioned preparations, the dishes are ready to be examined microscopically. By the isolation needle, few amounts from the growth were taken and placed on a slide with the presence of 1 drop of distilled water or Lactophenol dye and then covered with the slide. Finally, the microscopic examination is achieved with different powers [8]. After the confirmation of the presence of the Candida Albicans the reimplantation of the fungus on Corn Meal Agar medium as an additional step to confirm the presence of the needed fungus, the last step of the methods intends to increase the fungal growth on Sabourud and Corn Meal Agar media.

2.5 Plant Samples

The study included both Mint and Garlic which have been obtained from the local markets of Mosul city, the plants were transported to the lab and washed with water till the removal of all sands and impurities, then the plants were dried at room temperature (in shadow), the next step was crushing them with electrical mixer and the powder was preserved in polyethylene plastic bags and special containers (glass and plastic) in the fridge at 5C° until the usage of them in the extractions, in case of cold weather, the powder can be saved at room temperature[6].

2.6 Preparation of plant extracts

The plan extracts were prepared with marinating method, 5 g from each extract included in the study were placed in specific flasks which contain 50ml of sterilized distilled water, the flasks were placed in watery bath with 100 C° for 30 minutes then left outside to be cooled for 4 hours, the next step was to get rid of the solid materials with filtration through the use of filter papers. The filtered solutions were preserved in sterile bottles to be used later.

2.7 The preparation of the tablets saturated with plant extracts

Tablets with 5 cm diameter were designed from the filter papers after sterilizing them, these tablets were dipped in the plant extracts according to the type of the extract for 5 minutes, then were placed in Petri

dishes which contain the fungal growth under study. 5 tablets were distributed for each dish maintaining the same distance from the fungal growth, then they were left at room temperature and were monitored regularly for 10 days and all findings were recorded [9].

2.8 Knowing of the antagonistic efficacy of Garlic and Mint plant extracts on the growth of Candida Albicans fungus and their inhibitory effect for the fungal growth

During this experiment we prepared 30 tablets for both Garlic and Mint extracts, 15 tablets were placed in Mint extract with 100% concentration and the other 15 tablets were for Garlic extract with 100% concentration as well. 3 tablets were placed in each Petri dish for total 5 dishes for each extracts contains a certain amount of mint plant extract and another 15 tablets were placed in Petri dishes contain certain amount of Garlic plant extracts, the tablets were left for 5 minutes to be fully saturated with the plant extract, 10 dishes were prepared for the experiment as 5 dishes for each extract. Bedding method was used to grow the fungus by swap technique then the tablets were distributed on dishes as they were placed on a certain distance from each other, the dishes were preserved at 37C°. monitoring the dishes was done after 4 days from the preservation and the results were recorded [10].

3. RESULT

The results revealed that the obtained fungal isolates during this study represent the Candida Albicans which was proved by different means like the gross fungal prescription or the general appearance for the fungal colonies, which was confirmed through the comparison of the isolates with another identified isolates and we did not depend only on the gross appearance but all isolates were examined microscopically under optical microscope and samples were sent to the specialized people who confirmed the presence of the Candida Albicans fungus. All the collected data were compared grossly and microscopically with previous studies related to Candida Albicans.

Figure 1. explain Candida Albicans in corn mel agar

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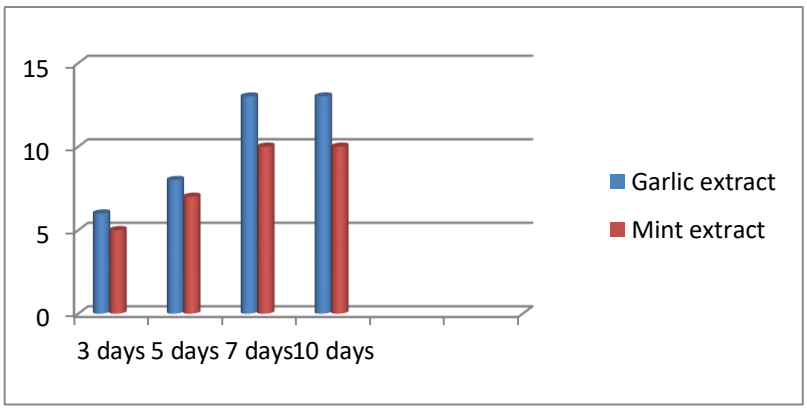

Figure 2. A Figure showing inhibition for two types of medicinal plants used

Table 1. The inhibitory effects of garlic and mint extracts on the isolated vaginal Candida Albicans growth for the pregnant women

Plant extract	Inhibitory diameter measurements			
	3 days	5 days	7 days	10 days
Garlic extract	6 mm	8 mm	13 mm	13 mm
Mint extract	5 mm	7 mm	10 mm	10 mm

4. DISCUSSION

The results showed clear distance between the fungus and the tablets which means a significant inhibitory effect for the plant extracts on Candida Albicans fungus. The fungus did not keep growing toward the tablet direction, the cause for increase the inhibitory effect for the extracts could be due the extracts themselves, it may contain active compounds or being toxic for the growth of some microorganism but some may loose their inhibitory effect during the extraction steps or the preservation techniques used during the extract preserving, the technique used for extract preparation and the concentrations are considered important factors and effective in the inhibitory effect of each extract [11].

5. CONCLUSION

According to the study, both extracts showed inhibitory results which are so close to each, however, the saturated tablet with garlic extract, the distance between it and the fungus was more visible than with the mint extract, which means that if adjusting the concentration, method of preservation and the time period for obtaining the filtrate, garlic extract may give more positive results [12].

Also, this is research according some studying which is refeares to the good inhibitory activity of acetylcholine, which is positive for gram stain, even at low concentrations, is due to the presence of flavonoid glycosides which, when hydrolyzed, give the compounds Ellagic acid, Gallic acid, Myricetin is effective against bacteria that are positive for the dye of a particular gram Staphylococcus aureus [14]. It was also mentioned that two new compounds of Yace leafs, A-Myrtucommulone and B-Myrtucommulone, were observed, as the effectiveness of the Myrtucommulone-A compound was observed against gram positive bacteria [15].

The importance of providing the needed materials for such kinds of studies which represented by the alimentary media firstly and the needed equipment for sterilization and preservation media secondly.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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