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PURIFICATION AND CHARACTERIZATION OF PECTIN FROM LEMON (*CITRUS LIMON*) AND APPLICATION IT AS ANTI CANDIDIAL INFECTIONS AGENT

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Abstract
The pectin was extracted from lemon peels with hydrochloric acid. The isolated lectin was a light yellow tint after extraction. The content of pectin increased with temperature increasing up to 80°C, and a pH of 1 in the extraction medium produced the highest pectin output. *C. albicans*, *C. tropicalis* and *Candida glabrata* were the predominated species in the urine samples from hospitalized patients. Clotrimazole and miconazole, two routinely prescribed antifungal medications, showed a moderate rate of sensitivity. While employing extracted pectin as fresh material, certain isolates of *Candida albicans*, *C. tropicalis* and *Candida glabrata*, in 100 g/disc revealed a good level of anti-candidial activity that was higher than the utilized antibiotics. It is recommended to encourage using of pectin as an alternate treatment for nosocomial and community-based *Candida* infections.

Keywords: *Citrus limon*, pectin, *Candida* infections

Introduction

Pectin is a very complex polysaccharide that is utilized extensively as functional ingredients in the food and pharmaceutical sectors. Pectin is one of the various polysaccharides that are isolated from plant sources (1,2). Pectin is a gelling and thickening ingredient used in food technology (2).

Pectin is a naturally occurring substance that is present in all higher plants' cell walls. Pectin was used for long time with the ability to produce gels, thicken liquids, and stabilize solids in different fields, including the cosmetic industries and clinical and environmental applications. Detoxification, lowering blood sugar, and an anti-diarrheal action are some of its medical uses (2,3). Pectin can be employed to improve the nutritional compositions' abilities to function, such as their capability to absorb water and oil, gel, thicken, etc (4,5). In addition to the most popular commercial sources of pectin, such as citrus peel and apple pomace, Additionally, novel sources such as sugar beets and sunflower heads were researched. (6,7).

An oval, Gram-positive, budding yeast cell called Candida is a polymorphic fungus that also generates pseudohyphae in tissues and exudates (8). Although C. albicans is the species most usually implicated in human infections, besides to other species of Candida (9). However, Candida species, particularly in hospitals) and in immunocompromised patients, can also function as an opportunistic pathogen, which

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can express severe infections involving major body organs (10). Most healthy persons have commensals of Candida on their skin, mucosa, or gastrointestinal tracts (11,12).
The primary pathogen, *Candida albicans*, is isolated in 50–70% of the cases stated above. *Candida albicans*' effectiveness in colonizing and infecting humans depends on a variety of methods, a few of which could be attributed to its morphological, biochemical, and genetic adaptability (13). To effectively treat and manage candidiasis, *Candida* must be quickly, accurately and reliably identified down to the species level. The main goal of that experiment using of lemon peels as a source to extract of pectin, determine its yield, characterize the extracted material, and test the material's ability to act as an anti-candida complex polysaccharide in hospitalized patients with UTI infections.

Materials and Methods

Sample Preparation

The fresh lemons was collected from neighborhood markets. The peels were removed after cutting the samples into four pieces. In order to eliminate the glycosides, which give peels their bitter taste, as well as the remnants of the pesticide spray, Further cutting into tiny pieces allowed for easy drying of the peels. They were then mixed and stored in a polythene bag for later use after being air dried for 24 hours.

Pectin Extraction

The extraction was carried out by adapting the procedure that was described by (14). Three ml of hydrochloric acid and small grams of the peel powder were combined with 300 ml of distilled water to form PH equal to 1. The sample was heated to 80°C in a water bath for one to two hours. After cooling, filter cheese cloth was used to remove the formed cake and the produced extract precipitated with ethanol at an equal amount of ethanol while thoroughly stirring, then the pectin to float to the top by being left unattended for 30 minutes. Centrifugation was used to separate the formed pectin gel from the ethanol and water. After being refined, the recovered pectin was pressed on a nylon towel after being rinsed in 200 ml of acetone. The cleaned pectin was drained and pressed to remove any leftover solvent and water. The resultant pectin was measured, minced, and left to dry in the open air. Finally, a pestle and mortar were used to further crush the dry pectin into smaller bits. Equation following was used to compute the pectin content. P x Bi x100 = Ypec (%)
Where: P: the weight in grams of extracted pectin while Bi: the initial amount of lemin, and y pec (%) is the extracted pectin yield expressed as a percentage (%).

Characterization of Extracted Pectin

1-Color: This test was carried out by watching how the created gel looked.
2. Cold and hot water solubility: Distilled water was used to combine the pectin samples individually. The heating at 80°C for 15 minutes after giving the mixture a thorough shake to create a solution.
3- Comparable weight

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One hundred ml of distilled water, 1 gram of NaCl, 1 gram of pectin powder, and a few drops of phenol red indicator were combined. With 0.1 M NaOH, the solution was titrated until the endpoint reached a pink tint. The equivalent weight was calculated using the formula below: 100% ml of alkali x sample's weight (in grams) x N of alkalis. (15).

4- Calculating the PH:

To find the ideal pH for pectin extraction, hydrochloric acid was added to pectin extract, resulting in a pH range of 1-2.5.

Isolation and identification of *Candida* spp.

From hospitalized patients, 25 urine samples in total were collected. The spacemens were grown on Sabouraud Dextrose Agar for the isolation. Until fungal growth was seen, the plates were incubated at 37 °C for 5 days and looked at every day. According to (9) microscopic and macroscopic investigation allowed for the identification of fungal components.The growing isolates were diagnosed with an automated technique (Vitek-2 Compact).

Antifungal activity of antibiotics and pectin

This investigation, pure pectin and two available antifungals (clotrimazole and 10 g/disc) and (miconazole and 10 g/disc) were utilized. The fungal isolates were cultured and incubated on SDA at 37 °C for 24 hours prior to the creation of a yeast suspension in distilled water that was equal to 1/2 McFarland. The yeast broth was spreader on SDA plate, and the formed discs coated with clotrimazole, miconazole, and pure pectin were scattered across the plate in three different quantities (25-50-100 g/disc), then incubated for 48 hours at 37 °C (15). An inhibitory zones were measured and noted.

Results and Discussion

Pectin extraction

Lemon peel pectin has a 16.12% yield when it was hydrochloric acid extracted it.Absolute ethanol was used to pre-treat the peel for 30 minutes. Because ethanol peel pre-treatment inactivates enzymes without causing soluble pectin fractions to leach and enhances purity by removing ethanol-soluble components, including sugars (14). These findings showed that hydrochloric acid could be used to successfully extract solutes for the manufacture of pectin, offering potential advantages for commercial pectin extraction from both an economic and an environmental standpoint (16).

Characterization of pectin

The isolated lectin was a light yellow tint after extraction. The content of pectin increased with temperature increasing up to 80°C, and a pH of 1 in the extraction medium produced the highest pectin output. Additionally, it was discovered that utilizing ethanol as a solvent seems to be more effective. According to the table, The lemon equivalent weight was discovered to bee 422 mg/ml, with a yield of 15.7% as in table(1).

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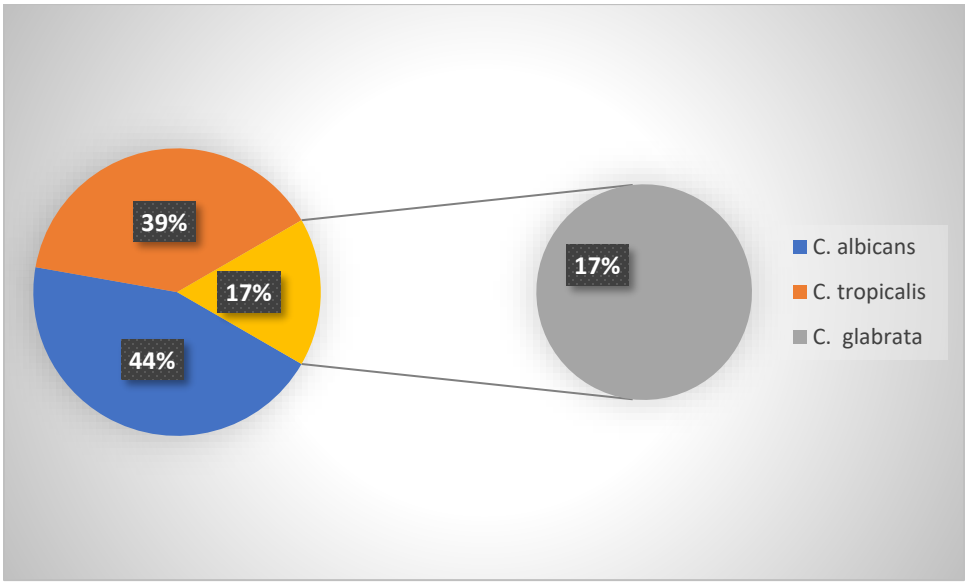
Table(1): Parameters of lectin extraction in different pH values of hydrochloric acid

hydrochloric acid	color	Solubility in hot water	EW (%)	Yield (%)
pH 1	Pale yellow	Full	422	15.7
pH 1.5	Pale yellow	Full	421	13.6
pH 2	Pale yellow	Full	418	9.5
pH 2.5	Pale yellow	Full	413	7.4

Pectins are typically light in color, hence the type of fruit may have played a role in the tangerine's color disparity (17). According to (18) Banana peel pectins had a higher degree of lightness than pectins derived from citrus peel and apple pomace, and they also claimed that pectins from these sources had a higher degree of redness and yellowness than pectins from banana peel. This would be as a result of the various colorants included in the raw ingredients. Because neutral sugar glycosidic bonds are more sensitive to pH than the bond between two galacturonic acids, low pH indicates increased acid concentration (acidity), which leads to the breakdown of the neutral sugar side chains. Increasing the extraction temperature at low pH also increases the yield of pectin((19). In contrast,(20) about 912.17 and 455.1 grams, respectively, of pectin were extracted using HCl and citric acids. More pectin was obtained from pectin extraction when it was done on a dry basis compared to when it was done on a wet basis (21).

Isolation and identification of *Candida* spp.

The findings revealed that 18 different isolates belonged to the genus Candida. According to figure(2) *C. albicans* (8 isolates, 44%), *C. tropicalis* (7 isolates, 39%), and *Candida glabrata* (3 isolates, 17%) were the three species that predominated in the urine samples.



Figure(2): percent and numbers of *Candida* spp. isolated from urine samples

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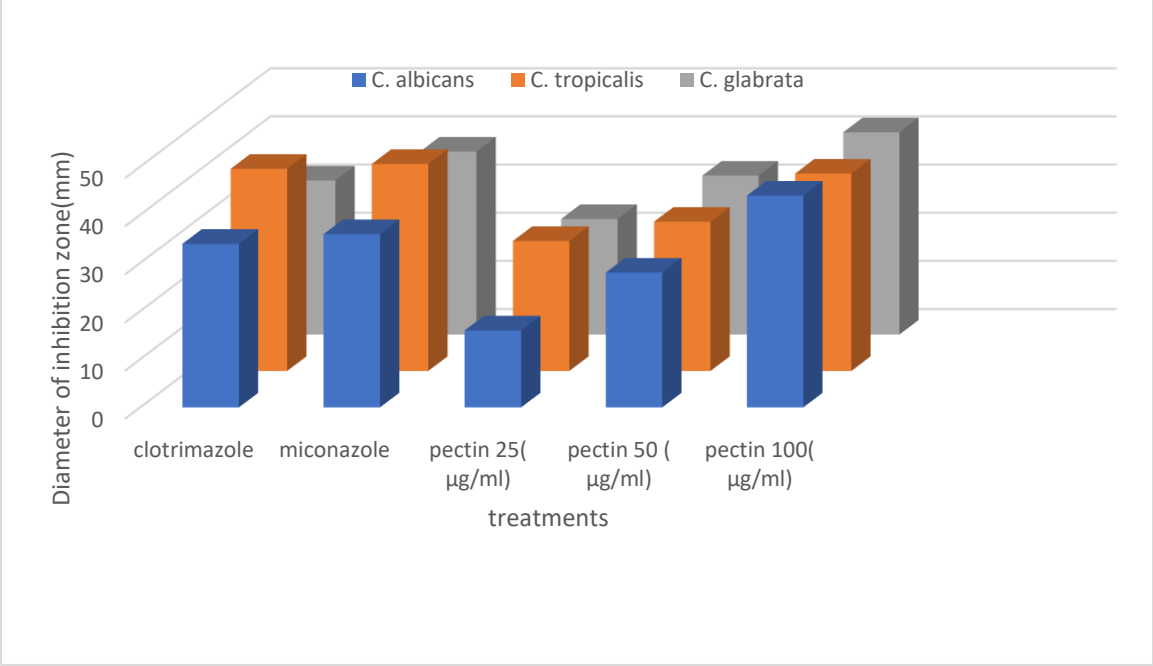
There are asymptomatic and symptomatic variants of candiduria. The vast majority of patients with Candida excretion in their urine don't show any symptoms. However, symptomatic candiduria is a sign of renal candidiasis, pyelonephritis, cystitis, epididymo-morchitis, and prostatitis (15).
According to previously reported findings, *C. glabrata*, *C. parapsilosis* and *C. tropicalis* appeared with high ratio (22). In another research by (15) Despite a rise in UTI cases caused by species other than *C.albicans*, *C.albicans* continues to be the most common cause of fungus-related UTI.

Antifungal activity of antibiotics and pectin

Clotrimazole and miconazole, two routinely prescribed antifungal medications, showed a moderate rate of sensitivity. While employing extracted pectin as fresh material, certain isolates of *Candida albicans*, *Candida glabrata*, and *C. tropicalis* in 100 g/disc shown a good level of anti-candidial activity that was higher than the utilized antibiotics *Aspergillus* species, *Cryptococcus neoformans, Histoplasma capsulatum, Pseudallescheria boydii, Trichosporon* species, *Candida* species, and Gram-positive bacteria are only a few of the many species that miconazole is active against. Resistance to miconazole is rare (16,23).



Figure(3):Antifungal activity of extracted pectin and antifungals drugs towards Candida spp.

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