Some Herbal Medicinal Plants Activity against Candida spp which Resistance to Antifungal Drugs

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Abstract

Some medicinal plant that used as antibacterial in Iraq has been experimented as antifungal, five of these plants include Zingiber officinale, Salvia officinalis, Origanum vulgare, Glycyrrhiza glabra, Punica granatum were used against four species of candida spp. C. paracitucus, C. albicans, C.tropicus, C.kruzi, which are resistance to antifungal drugs, 80% methanol was used to extract these plants, 5 mg/ml of each extracts used in nutrient broth for 4 days to evaluated anti-candida activity. The Results show that plants extracts causes complete inhibition, decreased in candida growth, don’t effect on activated candida growth, Zingiber officinale causes complete inhibition to candida spp except C. albicans, Salvia officinalis was decreased in all candida spp, Origanum vulgare, effect on C. albicans and C. kruzi but it don’t effect on other species. Glycyrrhiza glabra decreased all candida spp. Punica granatum decreased growth of C. albicans and C. kruzi only. 

Keywords: Candida spp , plant extract, 80% methanol.

Introduction

Medicine plant has been used in different application especially to treated different pathogenesis of microorganisms in the world, in Iraq some of these organisms have resistance to wide spectrum of drugs thus it causes different infection and complication, the pathogenic include yeast such as candida is resistance to several drugs like cycloheximide, fluconazole, nystatin and gresofulvin, this resistance was increased in recent years because of several mechanisms development like genetic changes in some genes via mutation that causes changes in some enzyme pathway which leads to lesion in enzyme pathway or in multidrug protein transport which responsible of drug transport in side cell such as CDR1, CDR2 and CaMDR1 which have been played role in fluconazole resistance (Albertson et al., 1996).

Studies are dealing with medicinal plants mechanism of action on microorganism suggested that these mechanisms may be affected on cell membrane which causes increased membrane preimpility associated with loss ions and reduction in membrane potentiality (Di Pasqua et al., 2006; Turina et al., 2006)

The disruption of the cell membrane causes defect in biological activity like energy conversion processes, nutrient processing, synthesis of structural macromolecules, and secretion of many growth regulators (Oussalah et al., 2006).

Turina et al., (2006) affirm the effectiveness of specific ions on plasma membrane has effective on the different process such as protons motive force, intracellular ATP content and overall activity of microbial cells like turgor pressure, solutes transport and metabolism regulation process.

Essential oils of many plants are composed of terpenes, terpenoids and other aromatic, aliphatic constituents can be penetrate and damage fungal cell wall and cytoplasmic membranes, permeable them and finally disrupted mitochondrial membranes, Changes in electron flow through the electron transport system in mitochondria lead to lipids, proteins and nucleic acid damage (Arnal-Schnebelen et al., 2004). The essential oil can hasse the depolarization of the mitochondrial membranes and decreased membrane potential, also affect Ca²⁺ and other ion channels, lowering pH and effect on the proton pump, ATP pool, changes in the fluidity of membranes resulted into the radicals leakage, cytochrome C, calcium ions and proteins. Thus, permeabilization of outer and inner mitochondrial membranes leads to cell death by apoptosis and necrosis (Yoon et al., 2000).

Present study was suggested for using common plants that used in different medical application in Iraq to treated candida spp infection which is resistance to antifungal drugs, also these plants extracts don’t have harmful side effect if it used under Specialists. Review of literature improved its ability to treated different infections such as pathogenic bacteria, fungal and viral because it contain phytochemicals compounds that have different mechanism to decreased infection or killed microorganism cells. Plants extract which used in present study choosing according to its ability to treated infection and low side effects and no cytotoxicity of its, also methods of extraction improved its ability to extract most of phytochemicals compounds from part of plants which used in present study. (Al-Saadi et al., 2012, Al-Terehi et al., 2012)

Materials and methods

I- Pathogenesis Candida spp : candida spp was isolation from (vaginal, mouth and urine then it diagnosis using macroscopic, microscopic and biochemical tests.

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2- Antifungal susceptibility: these tests performed using Nystatin, Terbinafine, Griseofulvin and Fluconazole using disc diffusion method.

3- Plants extract: The plants extracts of plant was prepared according to the method of Sato et al. (1990) with some modification. Specific weight of the plant and it is mixed with the average 1 gm. to 3 ml of the solvent solution (20 %methanol: 80 % distilled water), the mixture is uniformed by electric blender for 30 minutes in room temperature. The solution is filtered by using gauze fabric for getting transudate solution. It is derided using incubator at 50°C for 24 hours.

4- Plant extract aliquot, it prepares in concentration (0.1 g/ml) then it sterilized using mellipor paper 0.2 mm.

5- Anti-candida activity: this was performed using 50 mg/ml of plant extract, growth evaluation by optical density of growth culture according to Jabor et al (2013)

RESULTS
The results show that these extracts have anti-candida activity, these activity was differences between Candida types and types of plant extract as show in table(1) and figure (1)

Table (1) show plants extract activity against candida spp. Zingiber officinale was more effect than others plant extract it causes completed inhibition to C. paracitucus, C.tropicus, C.kruzi. Other plant extracts cause decreased in growth but these decreasing was Disparity from low to semi-complete inhibition.

In another hand some plants extract causes activate candida growth, Punica granutum with C. paracitucus and C.tropicus, and Origanum vulgare with C.tropicus. Other plants extract don’t effect on Candida spp. growth Origanum vulgare with C. paracitucus as show in table (1).

Significance at (p > 0.05) was show in complete inhibition of growth in C. paracitucus, C.tropicus by Zingiber officinale and C.kruzi by glycyrrhiza glabra and Punica granatum

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>C. paracitucus</th>
<th>C. albicans</th>
<th>C. tropiclus</th>
<th>C. kruzi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>1.02±0.012</td>
<td>0.545±0.012</td>
<td>0.560±0.056</td>
<td>0.806±0.096</td>
</tr>
<tr>
<td>Zingiber officinale</td>
<td>0*</td>
<td>0.433±0.227</td>
<td>0*</td>
<td>0*</td>
</tr>
<tr>
<td>Salvia officinalis</td>
<td>0.157±0.157</td>
<td>0.355±0.355</td>
<td>0.433±0.433</td>
<td>0.283±0.283</td>
</tr>
<tr>
<td>Origanum vulgare</td>
<td>1.01±0.26</td>
<td>0</td>
<td>1.02±0.487</td>
<td>0.30±0.153</td>
</tr>
<tr>
<td>glycyrrhiza glabra</td>
<td>0.50±0.176</td>
<td>0.153±0.153</td>
<td>0.176±0.121</td>
<td>0.07±0.072*</td>
</tr>
<tr>
<td>Punica granutum</td>
<td>1.187±0.11</td>
<td>0.230±0.230</td>
<td>0.580±0.253</td>
<td>0.243±0.243*</td>
</tr>
</tbody>
</table>

Figure (1) Effect of plants extract types on Candida spp. A, Candida paraciticus; B, C. albicans; C, C. tropiclus ; E, C. kruzi; 1, positive control ; 2, Punica granutum; 3, Salvia officinalis; 4, Zingiber officinale; 5, Origanum vulgare; 6, glycyrrhiza glabra.
Discussion
Results of present study show that plants extract have ability to decreased candida spp growth, complet inhibition, don’t effect and activate candida growth, as show in table (1) Zingiber was most plant effect on candida spp it improved its ability against different pathogenic microorganism, Supreetha et al (2011) used ethanol to extract of ginger against candida albicans in vitro using disc diffusion methods at 24 and 48 hours they found that this extract have antifungal activity at 24 hours. Also the previous study recorded that ginger have different phytochemicals compounds which have antifungal antibacterial activity because it effect on different factors such as pH, nutrient ion, osmosis pressure and microenvironment of microorganisms, this compound may be causes disrupted of cellular activity of microorganism that lead to decrease of growth or killing it by oxidative dames (Hayes et al 2013).

Origanum vulgare also inhibited C. spp growth in different level, it causes completed inhibition of C. albicans, Vale-Silva and others (2011) clarified the mechanism of action of this inhibition using flow cytometry to tested different parts of Origanum vulgare phytochemicals compounds effect on cell membrane by direct effect on cell membrane lipids, thus it consider as fungicide for treated superficial infection. Essential oil of Origanum vulgare have significant inhibitory effect on C. albicans and C. kruzi using solid media and disc diffusion method (Souza et al., 2007).

Liquors show decreased candida growth the review of literature recorded inhibition activity of different extract of licorice root against pathogenic bacteria and fungi Khanuja, K. (2006), this activity may be its phytochemical compound that interacted with microorganisms Al-Saadi et al (2012). Salvia officinalis oil was inhibitor pathogenesis candida spp using 15.6, 3.9, 31.3, 31.3 and 1.9 µg/m of its oil in Iran (Badiee et al., 2012).

In Turkey Dulger and Haciorulu (2008) used ethanol extract of sage also have anti-candida ability, sage extract contain from α-pinene, β-pinene, β-thujone, camphor, carvacrol, linalyl acetate, sabinyl acetate and 1,8-cineole these extracts may be have anti-microbial activity which were improved by previous study.

Most of research using oil as antifungal thus methanol–water extract was used in present study, results improved efficacy against candida spp.

Pomegranate has been used as anti-bacterial and anti-fungal activity, other study used different parts of Pomegranate in extract using methanol, ethanol and acetone to extract phytochemicals, (Dahham et al 2010) used it as antifungal against Aspergillus niger growth.

Ahmed and Beg (2001) reported that a most plants extracts methods including ethanol extracts of pomegranate showed antifungal activity against Candida albicans. In vitro studies have revealed that the extract of pomegranate inhibited the growth of oral bacteria and candida species. The present study also improved that used plant extract may be causes activated microorganisms as show in table (1) Origanum vulgare was activate C. paraciticus and C. tropicus also Punica granatum activate C. paraccicus, this may be because these extract have phytochemicals can activate cells nutrition, proliferation or activate some enzymatic pathway thus use herbal medicine must be in carful uses to avoid side effects.

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