

Safe strategies for controlling of *Candida albicans* growth with using plant extracts

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Abstract: Plant extracts are one of methods used since ancient times in alternative medicine and still until recently have been developed by the progress of since and modern technology. The experiment was carried out under laboratory conditions to estimate the antifungal activity of *Lawsonia inermis* which is known as "Henna", *Plectranthus* sp which is known as "Shar" and *Senna italica* which is known as "Eshreq " against *C. albicans*, with using antifungal agent (Fluconazole) as a positive control. Result showed that the extract of Henna leaves is highly effective against the tested *C. albicans*; and the inhibition zone increased with increasing plant extract concentrations where it was 20, 22 and 25mm at 100, 200 and 300 μ L of leaves plant extract respectively. The results showed the same effect for the Eshreq plant extract, but the yeast was resistant to the extract of the root, stalks of the Shar plant. In case of extract *Plectranthus* sp of root, their anticandida activity was undetected but leaves extract showed anticandida growth particularly at low concentrations.

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Key words: Antifungal activity, plant extracts, *Candida albicans*

1. Introduction

The yeast *Candida albicans* is by far the most human pathogenic *Candida* species and cause broad spectrum of diseases including skin, mucosal and systemic infections "candidiasis" (Moran *et al.*, 2012). According to (Gurjeet *et al.*, 2013) the *Candida* species found as normal flora in human beings. Common sites are skin, gastrointestinal tract and female genital tract particularly higher in vagina during pregnancy. *Candida albicans* is the commonest serious fungal pathogen 250.000 and 400.000 deaths per worldwide as well as extensive morbidity of around 100 million episodes of recurrent vaginitis (Kullberg and Arendrup, 2015; Brown *et al.*, 2012). This fungus is classical harmlessly as a commensal in approximately 50% of individuals (Kullberg and Arendrup, 2015), Kept in check by our immune system and protective bacterial micro biome of the gut and other mucosal surface (Brown *et al.*, 2012; Sudbery, 2011). Vulvovaginal candidiasis "VVC" is a disease caused by the abnormal growth of yeast- like fungi in the mucosal of the female genital tract. It afflicts millions of women worldwide annually, causing great discomfort, interfering with sexual and effective relations, and impairing work performance, VVC is classified by " The World Health Organization" as a frequently sexually transmitted disease, and it is considered an important world health issue (Sobel, 2007). The incidence of fungal infections has been increasing over the last decades, being more prevalent in developed countries (Arendrup *et al.*, 2005). An increase incidence of the infections is associated with some predisposing

factors as the use of dentures, xerostomia, mala nutrition, endocrine disorders, increased longevity of people, among other states that diminish the quality of defense of the individual (Rodloff *et al.*, 2011). Oral candidiasis is on the most common clinical features of those patient infected with the human immunodeficiency virus "HIV", This manifestation was seen in up to 90% of individuals infected with "HIV" (Thompson *et al.*, 2010).

Medicinal plants represent rich source of antimicrobial agents (Abdel Ghany 2014; Mahesh and Satish, 2015; Abdel Ghany *et al.*, 2015; Abdel Ghany *et al.*, 2016; ELfify *et al.*, 2016; Abdel Ghany *et al.*, 2017). Some extracts from plants were effective against pathogenic microorganisms because they contain volatile oils, aromatic, alkaloids, flavonoids, turbinones and organic acids, in addition to those used in the installation of many pharmaceutical drugs. Medicinal plants are renewable in nature unlike the synthetic drugs that are obtained from non renewable source of basic raw materials such as fossil sources and petrochemicals (Samanta *et al.*, 2000). Due to all these advantages, plants continue to be major source of new lead compounds. Nowadays, the indiscriminate use of commercial antimicrobial drugs has caused multiple drug resistance in human pathogenic microorganisms (Aliero *et al.*, 2008; Abdel Ghany and Othman 2014). Henna is safe without any health effects (Bele *et al.*, 2010). The side effect of henna mainly includes allergic reactions, but few have been reported. Henna has no genotoxic risk to the consumers Moreover, other studies declared

that henna leaves have anti-microbial and anti-*Candida albicans* effect (Marzin and Kirkland 2004).

Aim of work: The aim of the study is to identify the effect of plant extracts of *Lawsonia inermis* which is known as "henna", *Coleus forskohlii* which is known as "Shar" and *Senna acutifolia* which is known as "Ashraq" or "Ashrag" on the yeast of *C. albicans*, in addition the effect of antifungal (Fluconazole) as a positive control with using paper disc diffusion method against *C. albicans*.

2. Material and Methods

Candidal albicans isolate used

Candidal albicans isolate used in this study was isolated from the vagina from the laboratory of the "Sabia General Hospital, Jazan, KSA..

Plant sample

Three plants were collected from Jazan rejoin ,Saudi Arabia including *Lawsonia inermis*, *Plectranthus sp* and *Senna italica*.

Plant extraction

Fresh leaves (of plants were air-dried at room temperature for three days, ground into powder using an electric grinder. Sample (20 g) of the shade-dried powder of each plant leaves materials was extracted separately with 100 ml methanol. All the extracts were concentrated separately using rotary flash evaporator and preserved at 5°C in an air tight brown bottle until further use.

Antifungal activity of plant extract with using Paper disc diffusion method

The inhibitory effect of methanolic extract of plant samples was tested against *C. albicans* by using the paper disc diffusion method. To determine the anti-candida activity of the crude extract, *Candida albicans* was inoculated with streaking method in petri plates (90 mm) congaing Sabourauds agar medium. Sterilized filter paper discs (Whatman No. 1; 6 mm in diameter) soaked in different beakers containing the dissolved extracts of different concentrations were taken out with sterilized forceps and air-dried and placed on plates. The plates were incubated at 37°C for 24 h for *C. albicans*. After incubation, the inoculated plates were observed for zones of inhibition in millimeter diameter using a transparent ruler. The sensitivity or susceptibility of the test *C. albicans* to the standard drug was tested using Fluconazole. The zones of inhibition were measured and compared with those of the plant extract.

3. Result and Discussion

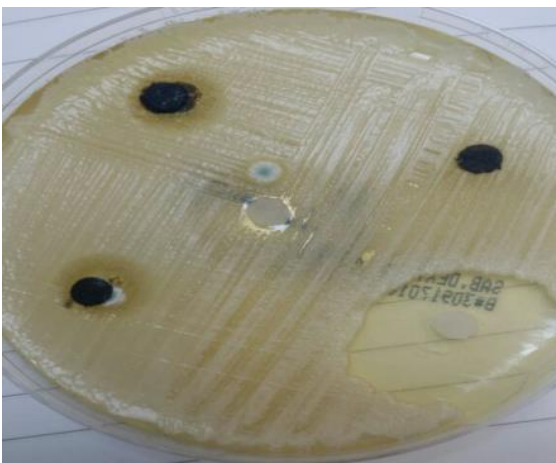
Investigating the antifungal effect of plant extracts involved a comparison of its methanolic extract with commercially available antifungal by

comparing the inhibition zones. In this work, the aim was to discover and develop a good alternative source against *C. albicans* which was free of the disadvantages of synthetic anti-fungal agents. Result showed that the extract of Henna leaves is highly effective against the tested of *C. albicans*; and the inhibition zone increased with increasing plant extract concentrations where it was 20, 22 and 25mm at 100, 200 and 300µL of leaves plant extract respectively (Table 1 and Fig.1). In the current study the commercial antifungal showed a less inhibitory effect than the *Lawsonia inermis* leaf methanolic extract. This is not surprising due to antifungal contents of this plant. *C. albicans* was sensitive to the extract of Henna leaves, where the inhibitory zone is very clear. Recently Nawasrah et al. (2016) reported that Adding Henna to acrylic resin denture could be effective in controlling *C. albicans* proliferation on the denture surface. According to Mohsina et al. (2017) *Lawsonia inermis* is reported to contain numerous active compounds including flavonoids, tannins, phenolic compounds, alkaloids, terpenoids, quinones, coumarins, xanthenes and fatty acids. Also, it has been reported to have analgesic, hypoglycemic, hepatoprotective, immunostimulant, anti-inflammatory, antibacterial, antimicrobial, antifungal, antiviral, antiparasitic, antitrypanosomal, antidermatophytic, antioxidant, antifertility, tuberculostatic and anticancer properties.

At the line the results showed the same effect for the Ashrag plant extract (Fig. 2), but on the other hand the *C. albicans* was resistant to the extract of the root, stalks of the Shar plant compared with antifungal fluconazole, which showed a clear inhibitory zone during the results (Fig.2). The present study was partially agreement with Supreetha et al. (2011) who showed that the ethanolic extract of ginger powder has pronounced inhibitory activities against *Candida albicans*. In case of extract of Shar (*Coleus forskohlii*) root, their anti-candidia activity was undetected may be due type of extracted solvent or plant containing active compounds (Table 1 and Fig.1) but leaves extract showed anticandida growth particularly at low concentrations. Nilani et al. (2006) found that The petroleum ether extract of *Coleus forskohlii* and *C. barbatus* exhibited significant antifungal activity against *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus ruantii*, *Proteus vulgaris* and *Candida albicans*. The extracts of *C. blumei* did not show any significant antifungal activity against the selected organisms. In previous study, anti-candidial activity of *Rhamnus globosa*, *Ocimum basilicum*, *Tecoma stans* and *C. forskohlii* have been studied and the results showed high inhibitory growth in yeast after treatment with *R. globosa* and *O. basilicum* only (Al-Judaibi A. and Al-Yousef, 2014).

Table 1. Inhibition of *C. albicans* growth (mm) after 24 h of incubation with different concentration of methanolic plant extracts

Treatment Concentration	Inhibition zone (mm) of <i>C.albicans</i> growth at different treatment			
	<i>Lawsonia inermis</i>		<i>Plectranthus sp</i>	
	Stems	Leaves	Root	Leaves
100 μ L	0.0	20	0.0	30
200 μ L	0.0	22	0.0	30
300 μ L	0.0	25	0.0	22
Positive control flucazole	30			

**Fig. 1** Inhibition of *C. albicans* growth after 24 h of incubation with different concentration of methanolic plant extracts 1, 100, 2=200, 2=300 μ L, F= flucazole**Fig. 2** Inhibition of *C. albicans* growth after 24 h of incubation with different concentration of methanolic *Senna italic* extracts

Conclusion

Extract of Henna leaves is highly effective against the tested of *C. albicans*. The results showed the same effect for the Eshreq plant extract, but the yeast was resistant to the extract of the root, stalks of the Shar plant. So that, the results indicate the possible

therapeutic uses of the plant extracts and can be a source of natural antifungal in the future.

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