Inhibitory Effect of Aqueous Salvia officinalis's leaves in the Growth of Candida albicans from Infected Women with Vaginal Candidiasis

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Abstract

Background: *Salvia officinalis* is a plant belong to Labiatae family .The common name of *Salvia* is sage which mean save. The leaves of *Salvia* have special oil which is effective against filamentous fungi and yeasts such as *Candida albicans* which is the causative agent of vaginal candidiasis in women

Methods. Cultures from 50 swabs of *Candida albicans* isolated from vagina of 70 patient women who complains from vaginal problems, their ages (24-43) years from Central City Hospital during Febreoury 2009 to April 2009 were cultured on Sabouraud Dextrose Agar (SDA) .Nystatin was used as positive reference standard to determine the sensitivity of this fungus . and less this concentration there was no minimum inhibition for the *Candida*

Results: The aqueous extract of *Salvia officinalis* at various concentrations inhibited the growth of *C. Albicans* . This inhibitions reached to a maximum of 100% for extract at 25 mg / ml and the minimum inhibition was 170 mm at 15 mg /ml for the extract . While the minimum and maximum inhibition for nystatin was at 50 µg/ml,and less concentration. There was no minimum inhibition for the *Candida*

Conclutions : The findings provide support for the use of this plant in traditional medicine for fungal infections especially against candidiasis .

Key words : Antifungal activity ,*Candida albicans* ,Nystatin ,*Salvia officinalis*, Vaginal candidiasis

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Introduction

Candida species exist on a worldwide basis of which Candida albicans is the most common apportunistic fungal infection of the candidas [1].

Tissues invasion by *Candida* strains is associated with an ability to produce phospholipases ,enzymes capable of disrupting the phosphate component of fatty acids [2] . About 80-90% of the women suffering from the inflammation of vagina which is caused by different microorganisms especially *Candida albicans* [3] . Discovery of antimicrobial activities of *Salvia officinalis* has one of the longest histories of use of any culinary or medicinal herb [4] . Sage was found to be effective in the management of disease [5] . Modern evidence supports its effects as antibiotic,antispasmodic,oestrogenic,hypoglycemic,and toxic [6] , it is also has antifungal activity against filamentous fungi and yeasts such as *Candida albicans* because of its oil which called volatile oil that has inflammatory activity [7] .

Methods

Culturing of the swabs on agar plates . By using Sabouraud Dextrose Agar (SDA) 65 gm of SDA powder putted in 1000 ml of distilled water in flask , ph fixed at 6.8 ,after sterilization the chloramphenicol antibiotic added in amount 250 mg/L . This media used for isolating and culturing the fungi .After preparation of this media the swabs screened on the plates which contain SDA at sterilized conditions .The plates incubated at 37 °C for 48 hours.

Organisms. Fifty isolated of *Candida albicans* were used in this research isolated from 70 woman infected with vaginal candidiasis during routine diagnostic works in Central City during Febreoury 2009 until April 2009 with ages (24-43) years . These isolates were identified based on colony and microscopic morphology [8] , and formation of germ tube which is specific character for identification of *Candida albicans* by taking 0.5 ml from blood serum of human in test tube and added part of colony of yeast in the test tube by loop and mixed well with the serum ,the test tube incubated for 2 hours at 37° C , then a part of suspension was taken by pasture pipette and spread on slide with spreader and examined under microscope [9].

Preparation of nystatin . Fifty gm of nystatin powder measured and 1000 ml of distilled water added , then sterilized in autoclave . Different concentrations of nystatin solution were done and used [10] .

Preparation of aqueous extract of salvia .

The plant material was collected from plant Laboratory in Department of Biology-College of Science in Baghdad university and identified . The plant part (leaves) were airdried . The dry powder of plant material was in a mount of (100) gm and added to 500 ml of distilled water then boiled for one minute to obtain the aqueous extract . The solution sterilized by passing through 0.22 µm Millipore filters . The distilled water was added at different volumes to make different concentrations from the extract of

salvia [11] ,then will be added to the plates which contains *Candida albicans* .

The minimum inhibition concentration was defined as the lowest concentration that did not yield visual growth. All experiments were performed in triplicate . The statistical analysis which used was Person Correlation Coefficiant, and there were highly significant differences (p _ value less than 0.005).

Results:

Fungi. *Candida albicans* isolates were obtained by making swabs from vaginal discharges of women infected with vaginal candidiasis after culturing of the specimens on (SDA) plates . This isolates were identified based on groups of morphological and biochemical features (8) , the forming of germ tube considard the most important character to identify *Candida albicans* [9] .

Effects of aqueous sage extract of leaves and Nystatine on fungal growth.

The results showed that fungal growth was inhibited by sage extract at many concentrations as compared with control (Table 1) . Minimum inhibition zone for the extract was measured as 170 mm at 15 mg /ml concentration . The growth was completely inhibited in the precence of maximum concentration of the extract at 25 mg /ml . These inhibitions were highly significant as compared with the controls ($p< 0.005$) . While both minimum and maximum inhibition zone for nystatin was 0 mm at 50 µg /ml concentration and less this concentration there no minimum inhibition for this fungus.

Table(1) Effects of Aqueous sage Extract of leaves on the growth of Candida albicans

Concentration of extract mg/ml	Diameter of *Candida* mm
14	Similar to the diameter of control
15	170
16	155
17	135
18	115
19	100
20	86
21	69
22	54
23	46
24	23
25	0.00

P= 0.000
P< 0.005
Correlation= – 0.962
The results are the means of experiment with triplicate*

Discussion

The laboratory culture is considard the most important method in identification the causative agent of vaginal inflammation , it is not only useful in making the right identification but gives us more informations about the organisms which cause the infection and to be a good test in identification of vaginal candidiasis if the culture result was positive [9] . The identification of the causative agent of vaginal inflammation is very important for prescribing better therapy [12,13] .Thirty nine percent of women who complaining from vaginal infection , *Candida* spp. considard the causative agent [14] Fifty isolates of *Candida albicans* were cultured by using medium (SDA) . The obtained results were in agreement with results from other workers of different yeasts

The effects of sage extract on growth pattern of this fungus was subjected . The growth was completely inhibited by the extract at a highest concentration of 25 mg / ml and several reports shown the antifungal properties of sage against fungi [15,16]. This inhibition was highly significant for extract of sage at concentrations from 15 mg /ml to 25 mg /ml as compared with the controls ($p<0.005$) . Growth was also completely inhibited by using nystatin at 50µg/ml concentration and less .There was no minimum inhibition for *Candida* . . *Salvia officinalis* contain 1-2.5% volatile oil (which contain salvene , pinene , camphor , cineole , borneol , 30% thujone , salvene esters and sesquiterpenes) , in addition to saponins , resin , salviatannin and rosmarinic acid which have anti inflammatory activities [7]. The aqueous extract of *Salvia* leaves shown the activity against

filamentous fungi and yeasts such as *Candida albicans* and thus may be useful in the treatment of different kinds of fungi in human .

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