

# Antifungal Activity of *Salvia jordanii* Against the Oral Thrush Caused by the Cosmopolitan Yeast *Candida albicans* among Elderly Diabetic Type 2 patients

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The oral thrush caused by the cosmopolitan yeast *Candida albicans* causes pale white cottony patches in the oral regions especially among the Immuno deficient patients. The elderly patients with the diabetes were the main victims of the yeast thrush. The objective of our investigation is to collect the clinical thrush samples from the targeted elderly patients with type 2 diabetes between the age group of 58 to 75 and determine the antifungal activities of *Salvia jordanii* by employing the standard microbiological technique. The results procured were excellent for the antifungal susceptibility analysis of all five types of *S. jordanii* essential oil extracts. The MIC and MFC values of the extracts from the Jordanian, Syrian and Moroccan origin shown the same values, whereas the Libyan and Egyptian extract shown the slightly higher values. The nature blessed essential oil extracts got the potential to combat infections as well as to treat the oral thrush. These findings indicate that *S. jordanii* displays adequate activity against cosmopolitan yeast *C. albicans* in vitro & its merits. Further analysis to treat yeast associated diseases.

## Introduction

The major objective of this work is to determine the antifungal activity of *Salvia jordanii* against the oral thrush caused by the notorious fungal cosmopolitan yeast *Candida albicans* [1,2]. The oral thrush caused by the cosmopolitan yeast *C. albicans* causes pale white cottony patches in the oral regions especially among the Immuno deficient patients [3]. The elderly patients with the diabetes were the main victims of the yeast thrush [3]. The focus of our study is to collect the clinical thrush samples from the targeted elderly patients with type 2 diabetes between the age group of 58 to 75 and determine the antifungal activities of *S. jordanii* by employing the standard microbiological techniques [4-6]. The Mediterranean herb *S. jordanii* is one of the potential medicinal herbs especially against the fungal infections due to its rich source of anti-fungal components present [7-10]. *S. jordanii* was previously described as rosemary but the recent studies based upon its DNA sequencing classified into *Salvia family* [11]. The leaves of *S. jordanii* were collected and dried to extract the essential oil required for this work to check its antifungal activities against the collected specimens [12-14]. The five different samples of the dried leaves of *S. jordanii* were collected

from the grown regions from the local market and were used for our study. The collected *S. jordanii* dried leaves were from the origin of Jordan, Morocco, Libya, Syria and Egypt. The major reason for collecting the sample of dried *S. jordanii* leaves from different origin is to perform the comparative study of the efficacy of its antifungal activity and determine the most effective source. The thrush caused by the fungal cosmopolitan yeast *C. albicans* among elderly type 2 diabetic patients causes numerous notorious issues rather than serious health issues [15-17]. A thick layer over the tongue will be formed. Further when untreated, due the accumulation of the yeast results in the loss of taste senses among the patients. The thrush can result due to the abundant application of antibiotics over the course of life span of the patient. The accumulation of the chemical components present in the synthetic antibiotics suppress the host immune response and make it vulnerable for the complications such as oral thrush especially among the elderly type 2 diabetic patients which makes their life miserable [3]. This study is an attempt to find out the nature blessed remedy to diagnose the complications of the yeast thrush by replacing the chemical constituents [1].

## Experimental

### Materials

*Salvia jordanii* dried leaves, Oral swab sample from a patient, Saraud's Dextrose agar, Rose Bengal agar plates, Dinitrogen and Sodium Sulfate, Gram's stain kit, Sheep's Serum, Whatman No:1 filter paper, Conical flasks, Test tubes, Pasteur pipettes, Laminar Air Flow Chamber, Incubator, Inoculation loop and Bunsen Burner, Cotton plugs, Corn Meal Agar, KOH, Lactophenol, Potato dextrose broth.

### Isolation and Purification of cosmopolitan yeast *C. albicans*

The clinical thrush sample from the suspected patient was collected by employing the oral swab technique and was inoculated on a sterile Rose Bengal agar plate by streak plate method [4,5] and incubated at 37°C for 24 to 48 hrs to observe the yeast oval colonies.

A loopful of colony was taken and Gram's Stain was performed to observe the Gram positive violet colored oval yeast budding cells.

The KOH and lacto phenol test was performed to observe the yeast budding calls under microscope by employing wet mount technique.

The laboratory diagnostic test of the cosmopolitan yeast *C. albicans* was done by performing the confirmatory technique chlamyospore formation test by inoculating the isolated yeast colonies on a corn meal agar and incubated at 37°C for 24-48 hours to observe the chlamyospore formation of the yeast which is an important characteristic of the medically important cosmopolitan yeast *C. albicans* and was also confirmed by the germ tube techniques were the collected sample was inoculated into the sheep's serum in a test tube and incubated for two hours to observe the formation of the characteristic germ tube formation of the yeast.

### Extraction of *Salvia jordanii* essential oil

The five types (Jordanian, Syrian, Egyptian, Libyan and Moroccan) of the dried leaves of *Salvia jordanii* were collected and homogenized into a fine powder using mechanical grinder separately and hydro-distilled. The moisture content of *S. jordanii* was enumerated to be 7.4g/100g on a dry basis and essential oil yield in the raw material was 1.726 mL essential oil/100g of *S. jordanii* dried leaves [4,18]. The extracted essential oil was filtered using the whatman no. 1 filter paper then concentrated with dinitrogen and further dried using anhydrous sodium sulfate. The obtained essential oil was stored at 4°C in amber coloured reagent bottle.

### Determination of the antifungal activity of the *S. jordanii* essential oil extract

The following three standard microbiological techniques were employed methodologies were to determine the anti-fungal activity of testing of *S. jordanii* extract against the

isolated cosmopolitan yeast *C. albicans* clinical specimen collected from the thrush of the type 2 diabetic elderly persons.

### Disc Diffusion Method (Kirby-Bauer)

The disk-diffusion method is the ideal standard microbiological technique to check the anti fungal susceptibility of cosmopolitan yeast *C. albicans* against the *S. jordanii* essential oil extract [4]. This technique is simple and cost effective for the determination of susceptibility testing with a large number of isolates. A standardized antibiotic disc was prepared with *S. jordanii* essential oil extract and incorporated on the Sabaraud's dextrose agar plate inoculated with the test organism. The impregnated inoculated plates were incubated at 37°C for 24 hours to determine the antifungal susceptibility of the yeast against the extract. This method allows the rapid determination of the efficacy of the antimicrobial properties by measuring the diameter of the zone of inhibition that results from diffusion of the agent in to the medium surrounding the disc. The test was repeated for all the types of the extract respectively.

### Minimum Inhibitory Concentration - Tube dilution method (MIC)

Minimum Inhibitory Concentration (MIC) using the standard tube dilution method was performed to all the extracts with different set up respectively to determine the lowest concentration of *S. jordanii* essential oil to inhibit the visible growth of the clinically isolated cosmopolitan yeast *C. albicans* from the thrush of the type 2 diabetic elderly patients and after overnight incubation at 37°C in an incubator, the MIC values were observed with the last tube with turbidity determining the fungistatic effect of the respective extracts [6].

### Minimum Fungicidal Concentration Method (MFC)

The Minimum Fungicidal Concentration (MFC) was performed [6,10] to determine the lowest concentration of all the essential oil extracts respectively required for the fungicidal effect of the clinically isolated cosmopolitan yeast *C. albicans* specimen collected from the thrush of the type 2 diabetic elderly patients by dividing the Sabaraud's dextrose agar into six portions representing each dilution of MIC. The sample from each dilution was inoculated on the each designated portions marked with the respective dilution s for the respective dilutions for all the essential oil MIC dilutions separately. The plates were incubated for 24 hours at 37°C to determine the fungicidal activity.

## Results and discussion

The results procured were satisfactory for the antifungal susceptibility assay of all the five types of *S. jordanii* essential oil extract collected from the different origins against the clinically isolated cosmopolitan yeast *C.*

*albicans* specimens from the oral thrush of the type 2 diabetic elderly patients. The observed results were tabulated and interpreted as shown in **Table 1**.

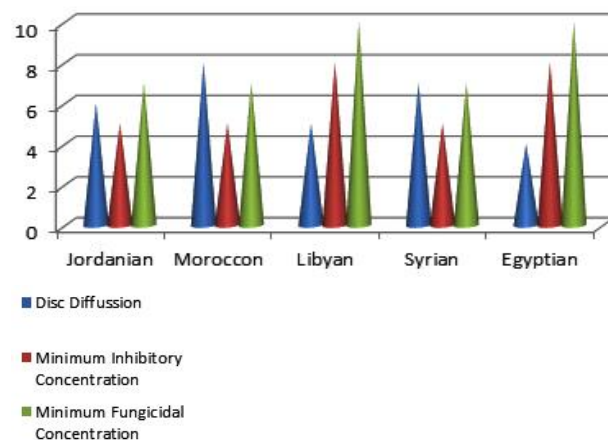
**Table 1.** Comparative Study of Antifungal activities of *S. jordanii* essential oil extract.

Types of essential oil extract	Disc Diffusion	Minimum Inhibitory Concentration	Minimum Fungicidal Concentration
Jordanian	28mm diameter zone Sensitive	2.5 $\mu\text{.mL}^{-1}$ 0.25 – 0.5%	5 $\mu\text{.mL}^{-1}$ 0.5 – 1%
Moroccan	29mm diameter zone Sensitive	2.5 $\mu\text{.mL}^{-1}$ 0.25 – 0.5%	5 $\mu\text{.mL}^{-1}$ 0.5 – 1%
Libyan	23mm diameter zone Sensitive	5 $\mu\text{.mL}^{-1}$ 0.5 – 1%	10 $\mu\text{.mL}^{-1}$ 1%
Syrian	26mm diameter zone Sensitive	2.5 $\mu\text{.mL}^{-1}$ 0.25 – 0.5%	5 $\mu\text{.mL}^{-1}$ 0.5 – 1%
Egyptian	21mm diameter zone Sensitive	5 $\mu\text{.mL}^{-1}$ 0.5 – 1%	10 $\mu\text{.mL}^{-1}$ 1%

The interpretation of the results obtained suggest that the differences in susceptibility to the all the types of *S. jordanii* essential oil extract by the clinically isolated cosmopolitan yeast *C. albicans* from the thrush of the type 2 diabetic elderly patients was minimal and showed almost the same susceptibility results. The isolated yeast shown most susceptible towards the Moroccan origin with 29mm diameter zone sensitivity and less towards the Egyptian with 21mm diameter zone sensitivity. While the extracts from other origin showed moderate sensitivity ranging from 23mm to 28mm diameter zone. Though this comparison is made to distinguish the antifungal activity of the extracts but it is highly inappropriate to figure out to confirm that one type of extract shown better sensitivity towards the isolated yeast as all the types has shown the good anti-fungal susceptibility. The MIC values of the extracts from the Jordanian, Syrian and Moroccan origin showed the same value of 2.5  $\mu\text{.mL}^{-1}$ {0.25 – 0.5%} while the Libyan and Egyptian extract shown the slightly higher values 5  $\mu\text{.mL}^{-1}$ {0.5 – 1%}. The MIC values of the essential oil extract towards the isolated yeast were in line with that of MFC values as the Jordanian, Syrian and Moroccan origin shown the same value of 5  $\mu\text{.mL}^{-1}$ {0.5 – 1%}. While the Libyan and Egyptian extract showed the increased values of 10  $\mu\text{.mL}^{-1}$ {1%}. The interpretation of result values suggests that all the five types of *S. jordanii* essential oil extract has showed almost the same antifungal activities with very minimal difference in the values against the cosmopolitan yeast *C. albicans* specimen isolated from the oral thrush of the type 2 diabetic elderly patients (**Fig. 1**). This study suggests that the *S. jordanii* essential oil extract from any origin has a significant antifungal activities against the fungal yeast such as cosmopolitan yeast *C. albicans* arising due to the abuse of antibiotics. This essential oil extract is not only capable of combating

fungal infections but also it is an antifungal without side effects. These types of essential oil is an alternate towards the synthetic toxic chemical drugs especially for the senior citizens.

**Fig. 1.** Comparative Chart of Antifungal activities of *S. jordanii* essential oil extract.



## Conclusion

The WHO organization was warning with several alerts towards the abuse of antibiotics. Due to the ignorance of the community abundant amounts of antibiotics even without proper prescription from the physicians were consumed. These types of abuse is a serious issue and due to the ignorance these antibiotic substances get accumulated in the human system. The accumulation cross reacts with our routine physiological activities which suppresses our immune system. In the course of time the antibiotic deposits takes control of our body by means of side effects produced like oral thrush especially among the elderly type 2 diabetic patients. The nature blessed essential oil extracts got the potential to combat infections as well as to treat the oral thrush. This study has showed positive satisfactory antibiotic assay results of *S. jordanii* towards the cosmopolitan yeast *C. albicans*. The MIC and MFC values obtained has once again proven as a positive indicator. These findings indicate that *S. jordanii* displayed adequate activity against cosmopolitan yeast *C. albicans* in vitro & its phytochemical components attributes to treat yeast associated diseases.

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## Author's contributions

All authors have made substantial contribution to the work and approved it for publication. No conflict of interest.

## Keywords

Antifungal activity, *Salvia jordanii*, Oral thrush, cosmopolitan yeast *Candida albicans*, Immuno suppressed patient, Type 2 Diabetes.

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