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Antifungal effects of the extract of the *Withania somnifera* on *Candida albicans*

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ABSTRACT

Background and aims: *Withania somnifera (W. somnifera),* commonly known as Ashwagandha, is an important medicinal plant that has been used in Ayurvedic and indigenous medicine for over 3,000 years. Candidiasis is one of the most common opportunistic fungal diseases in humans. In fact, the most important fungal disease in women is vaginal candidiasis. This study aimed to investigate the antifungal effects of the extracts of the *W. somnifera* on *Candida albicans* (*C. albicans).*

Methods: In this experimental research, 9 vaginal samples were collected using the sterile swap and Falcon tube by the gynecological specialists. The extracts of the shallot and artichoke were prepared using a rotary device. The inhibitory concentration against *C. albicans* was determined using incubation in media.

Results: The results of this study suggested that the minimum inhibitory concentration (MIC) against *C. albicans*, which is equivalent 50 ppm to 250 ppm has the highest concentration of inhibitor.

Conclusion: The results of this study showed that the antifungal activity of wind cheese against *C. albicans* is good, so it can be used as a drug to treat infections caused by *C. albicans.*

Keywords: Extract plant, *Withania somnifera*, *Candida albicans*, Antifungal effects.

Original article

INTRODUCTION

C. albicans is the major fungal pathogen in humans, particularly in immunocompromised patients. Candidiasis can take many forms, ranging from mucosal candidiasis to disseminated disease, often with multiple organ involvement, depending on the underlying host defect.[1-3]

C. albicans virulence is linked to its ability to transition from yeast-form of filamentous growth. Several lines of evidence support this idea. First, histological analyses of tissues infected with *C. albicans* show the presence of filaments particularly in the deeper regions of the tissue.[4] Second, using *C. albicans* mutants impaired or altered in their ability to filament in mouse models of infections showed a correlation between *C. albicans* filamentous growth and host mortality

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from candidiasis.[5-7] Third, a number of *C. albicans* Eukaryotic cell adhesions and virulence-related gene products are more abundant in the filamentous form of the fungus.[8-10]

W. somnifera L. Dunal, (Solanaceae), commonly known as ashwagandha or winter cherry, is a well-known medicinal plant in Ayurvedic medicine. The principal active compounds include several withanolide-type compounds.[11,12]

"Ashwagandha" is well known for its therapeutic use in the ayurvedic system of traditional medicine. It has been used as an antibacterial, antioxidant, aphrodisiac, liver tonic, and anti-inflammatory agent.[13] Recently, the antimicrobial activity of *W. somnifera* was studied by several authors on control strains (microbial type culture collection).[14,15] It is a reputed health food and herbal tonic and used for cardiovascular diseases in ethanol medicine. It is available for human use either as a single herb or an ingredient of polyhedral or herb mineral formulations. This study aims to investigate the antifungal effects of the extracts of the *W. somnifera* on *C. albicans.*

METHODS

W. somnifera were collected from the Zabol-Iran. After collecting the plants, they are rinsed with water and chopped for the microbial tests. Then they are dried for preparation of the plant extract in the shadow. The taxonomical identification of the plant was confirmed by Dr. Mirtajadini at the Department of Biological Sciences, Shahid Bahonar University of Kerman, Kerman, Iran.

For the extract preparation, 10 g dry powder of the plant was placed in half-liter Erlren Mayer flask containing 100 ml of 96% ethanol and water. The content of flasks was mixed at room temperature for 24 h with the shaker device with 130 rpm speed, and then was filtered using man paper No. 2. The solvent was separated from the extract by the rotary device and by using the vacuum pump (vacuum distillation). The obtained extract was weighted, and then dissolved in DMSO solvent and it was maintained in the refrigerator at 4°C to be used.

After sampling the vaginal using the sterile swap and Falcon tube by the gynecological specialists, *C. albicans* samples have been from patients suspected. Thirty samples were isolated and transferred to the laboratory and cultivated on agar dextrose Sabouraud and broth dextrose saburo according to the manufacturer's instructions. After the growth of each sample, lam was prepared and the candidate samples were identified and re-cultivated according to standard tables.

Colonies of *C. albicans* were prepared in the media of Sabouraud dextrose agar at 37°C in homogenous suspension sterile physiology serum, and the rate of the light passing of the suspension was measured using the spectrophotometry device with 530 nm. The rate of the light passing of 90% is necessary for preparing a suspension with nearly 106 fungi cells per ml. For determining the inhibitory concentration of the extracts, incubation in the media was used (the concentration of 50, 100 and 250 ppm was used). Finally, they were placed in the incubator and the samples were analyzed after 24-48 h.

RESULTS

The results of this study showed that the extract inhibits the growth of *C. albicans* was blowing in soft cheese, so that by increasing the inhibitory concentration was higher, The results showed that the MIC in 50 ppm that a fungus strains inhibited while maximum inhibitory concentration 250 ppm and

two strains of *C. albicans* in the concentration inhibited (Table 1).

Table 1: Minimum inhibitory concentration of plant extract against *Candida albicans* minimum inhibitory concentration (ppm)

C. albicans **isolates**	**Minimum inhibitory concentration**
1	150
2	100
3	150
4	150
5	250
6	50
7	100
8	150
9	250

DISCUSSION

The results of this study represent a good antifungal effect against *C. albicans*. Pnyrbad extract is such that the higher concentration has more inhibitor. The inhibitory concentration differences among samples of *C. albicans* were observed due to differences in antibiotic-resistant infections in them. The results of Bokaeian's study showed that the highest MIC value of extract was found to be 250 ppm against *Klebsiella pneumoniae* (*K. pneumoniae*) and the least MIC values for *K. pneumoniae* was 63 ppm.[16]

Pujavi and Gandhi in their study showed that the crude ethanol root extract of *W. somnifera* created strong antibacterial activity against two tested pathogens, i.e. *S. aureus* and *Salmonella typhi* (*S. typhi*) compared to chloroform root extract while aqueous root extract showed no inhibitory and ethanol extract inhibited the growth of *S. aureus* and *S. typhi* but did not inhibit the growth of *Shigella dysenteriae* (*S. dysenteriae*). The chloroform extract had antibacterial activity against only *S. aureus.* It was observed that none of the test bacterial pathogens were inhibited by aqueous extract applied. None of the extract inhibited the growth of *S. dysenteriae*.[17]

Methanolic and *aqueous* extracts of plants *W. somnifera* and *Aloe Vera* were tested by Srinu for antibacterial activity against *Pseudomonas aeruginosa (P. aeruginosa), Bacillus cereus* (*B. cereus*), *Escherichia coli (E. coli), S. typhi, Staphylococcus aureus* (*S. aureus*) and *K. pneumoneae* and the result show that methanol extracts of plant were exhibited potent antibacterial activity when compared to the aqueous extracts, which are similar to the findings of, who reported that the antibacterial activity of aqueous extract of *W. somnifera* on the tested pathogen is more effective than the methanolic extract. *W. somnifera* plant extract showed more inhibitory activity on gram positive organisms (*S. aureus* and *B. cereus*) when compared to gram negative microorganisms, which is in accordance with the findings.[18,19] Phytochemical screening of methanolic extract of *W. somnifera* showed various bioactive compounds such as tannins', glycosides, steroids and flavonoids.

The study by Singariya showed chloroform extract of leaves *W. somnifera* and *Cenchrus ciliaris* had the highest activity, by *W. somnifera* (IZ- 20.38±0.21 mm) and

(IZ- 20.67±0.24 mm) by *C. ciliaris* against *Bacillus subtilis* (*B. subtilis*) and *P. aeruginosa*, respectively.[20]

Different parts (root, stem, leaf, flower, unripen fruit, ripen fruit and calyx) of *W. somnifera* (RUBL-20668) were evaluated by Singariya for their antimicrobial (antibacterial and antifungal) properties. Maximum extracts were showed highest activity against *P. aeruginosa* and *B. subtilis*. The inhibitory effect is very identical in magnitude and comparable with that of standard antibiotics. Gentamycin, the standard antibacterial drug used was effective in inhibiting these bacteria. The effect on *P. aeruginosa*, *B. subtilis* and *E. aerogens* were comparable to that of gentamycin. Ketoconazole, the standard antifungal used was effective against the fungi (*A. flavus*).[21]

In another study, Singh and Kumar stated that antimicrobial activity of *W. somnifera* L. Dunal (Solanaceae) has been against selected pathogens. Free and bound flavonoids of different parts (root, stem, leaf and fruit) of *W. somnifera* have been studied for their antimicrobial activity using disc diffusion assay against three Gram negative bacteria (*E. coli* MTCC 46, *Proteus mirabilis* (*P. mirabilis*) MTCC 3310 and *P. aeruginosa* MTCC 1934), one Gram positive bacteria (*S. aureus*) MTCC 3160) and three fungi (*C. albicans* MTCC 183, *Aspergillus flavus* MTCC 277 and *Aspergillus niger* MTCC 282). Total activity of bound flavonoid extracts of root was found to be same for *E. coli*, *P. mirabilis*, *S. aureus* and *C. albicans* (153.84 mg/L). The results of the present study reveal that extracts of *W. somnifera* showing great antimicrobial potential against test microorganisms may be exploited for future antimicrobial drugs.[21]

Bisht and Rawat in their research observed that the methanolic leaf extract of *W. somnifera* was very effective in inhibiting the test pathogens, including methicillin resistant S. aureus and Enterococcus spp., with an average zone of inhibition of 20.6 mm and 19.4 mm at 2 mg/ml (100 µl) concentration, respectively.[22]

Pandit evaluated the effect of the methanol extract of *W. somnifera* (MEW) on the growth and virulence properties of Streptococcus mutants and *Streptococcus sobrinus* (*S. sobrinus*) at sub-minimum inhibitory concentration (MIC) levels and identified the main components of MEW. A GC-MS analysis of the main components of MEW was also carried out. MEW showed a broad antibacterial range against oral bacteria (MIC: 0.125-2 mg/mL). At sub-MIC levels, MEW dose-dependently increased doubling times of *S. mutans* and *S. sobrinus* up to 258% and 400%, respectively. Furthermore, MEW inhibited acid production, acid tolerance, and biofilm formation of *S. mutans* and *S. sobrinus* at sub-MIC levels.[23]

Kambiz and Aeolians in their project performed on the in vitro antimicrobial activities of water and methanol extracts from two plants on *Neisseria gonorrhoea* (*N. gonorrhea*) and *C. albicans*, common causes of STIs detected in rural South Africa. Extracts from both species together with aloin pure from *Aloe ferox* (*A. ferox*), were evaluated for activity against six strains of *N. gonorrhoea* and nine strains of *C. albicans*. The extracts showed activity

against *N. gonorrhoea* at concentrations of ranging from 0.5 (methanol extracts from both) to 10 (water extract of *W. somnifera* only) mg/ml, while pure aloin inhibited the growth of both microorganisms. Only the methanol extract of *W. somnifera* was effective against *C. albicans* at a concentration of 20 mg/ml.[24]

IG. Mwitaria in a study investigated that plant extracts have both bactericidal and fungicidal activity. *Warbugia ugandensis* (*W. ugandensis*) is cytotoxic at IC_{50}<50 µg/ml with MIC values of less than 0.78 mg/mL. *Prunus africana* shuts down expression of IL 7 mRNA at 50 µg/ml. *W. somnifera* has the best antimicrobial (1.5625 mg/ml), immune potentiation (2 times IL 7 mRNA expression) and safety level (IC_{50}>200 µg/ml). Extractions from *W. ugandensis* and *W. somnifera* too demonstrated with antimicrobial activity.[25]

Velu and Baskaran in their study reported that the ethanol extract of *W. somnifera* showed more activity against S. aureus in the zone of diameter 20.10±0.17 mm and methanol extract of *W. somnifera* showed more activity against *C. albicans* in the zone of diameter 14.20±0.40 mm compared to other solvent extracts. In this study, ethanol extract in bacteria and methanol extracts in fungus showed a various degree of inhibition to the growth of the tested organism than Ethyl acetate, chloroform, acetone, Petroleum ether, hexane and hot water extract.[26]

The study of Sundaram revealed that higher MIC of *W. somnifera* was obtained for both gram positive bacteria, *S. aureus*, and *B. subtilis* and for both gram negative bacteria *E. coli* and *P. aeruginosa*. Ethyl acetate extract possesses great inhibitory activity for gram positive bacteria, *S. aureus* followed by *B. subtilis*.[27]

CONCLUSION

Regarding these results, it is concluded that the extract of *W. somnifera* had a strong antifungal effect on tested strains. The obtained results suggest that further studies for the isolation and identification of the more active component of extract are required to assess their antifungal activity. For this purpose the degree of toxicity of these extracts should be determined to introduce this plant to the pharmacological industry.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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