Iranian Journal of Pharmaceutical Sciences
2021: 17 (1): 43-50
www.ijps.ir

Original Article

Antifungal Activity of Topical Microemulsion Containing *Ziziphus spina-christi L.* for the Treatment of Fungal Vaginitis

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Abstract

Vulvovaginal candidiasis (VVC) caused by *Candida albicans* and other *Candida species* is a high-risk event in patients admitted to hospital. *Ziziphus* genus is a rich source of medicinal compounds, effective for the treatment or prevention of some diseases. This study aimed to evaluate the antifungal effect of topical microemulsion containing *Ziziphus spina-Christi L (ZSC)* extracts against *Candida* spp. *Z. Spina-Christi* was collected and extracted in a Soxhlet apparatus using the ethanol and methanol solvents. Microemulsions were formulated by the composition of surfactant (Tween 80) along with cosurfactant (propylene glycol), oil, and water and were characterized regarding their stability. The The *in vitro* antifungal study was performed by measuring and comparing the diameter of zones of inhibition (in mm) against *C. albicans* (ATCC 3153), *C. parapsilosis* (ATCC 2195) and *C. krusei* (ATCC 573) using agar well diffusion test, for the various formulations (without extract and containing ZSC extract). The optimal microemulsion was analyzed for the average diameter of droplets, pH, viscosity and zeta potential. The microemulsion containing Ziziphus *spina-Christi* ethanolic extract had a significant inhibitory effect on the different species of Candida, but the most inhibitory effect was found against *C.* albicans. The result showed that the ethanolic extract in microemulsion was Light green and, average globule size was 2.93 mm, no separation after centrifugation at 3000 rpm for 30 min, pH at 3.8, and viscosity was 8.523cps. The results of this study indicate that the ethanolic extract has interesting antifungal properties and can used for the treatment of fungal infections. More research is required to check this plant performance to treat the patients with Candidiasis.

Keywords: Antifungal activity, *Ziziphus spina-Christi L*, *Candida* spp., Candidiasis, Microemulsion, Extract.

1. Introduction

Vulvovaginal candidiasis (VVC) is a high-risk event in patients admitted to hospital. The vast majority of VVC are caused by *Candida albicans* and other *Candida species*. [1]. People with multiple predisposing factors such as diabetes mellitus, cellular urine catheter,

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Cite this article as: Salimi A, Shirani M, Antifungal Activity of Topical Microemulsion Containing Ziziphus spina-christi L. for the Treatment Fungal Vaginitis, 2021, 17 (1): 43-50.

antimicrobial and corticosteroid users are at risk of fungal infection [2]. Few useful therapeutic options and cross-resistance of pathogens to older drugs (fluconazole and itraconazole) has necessitated the quest for new antifungal drugs from divers sources, especially from medicinal plants [3, 4].

The use of plants or their natural products in the prevention or treatment of disease is based on the experience of the traditional medicine of ethnic societies [5]. Several reports have been published concerning the antimicrobial activity of various plant-derived compounds [6, 7]. The most famous antifungal medicinal plants belong to Liliaceae, Leguminoseae, Rutaceae, Myrtaceae and Lamiaceae families. In the *Rhamnaceae* family, reports on the antifungal activity concern mainly the *Ziziphus* genus [8, 9].

The *Ziziphus* genus is a flowering annual monocot herb with over 40 members. The species may be different in maturing time, color, and taste, but they are close in biochemical and phytochemical contents. The phytochemical components in different species are saponins, sapogenins, sulfuric compounds (thiosulfinates), and flavonoids [10]. The presence of these compounds is responsible for several pharmacological effects like antimicrobial (antibacterial, antifungal, antiviral), antiprotozoal, antioxidant, antitumor, and antithrombotic activities [11].

Microemulsions (MEs) is a clear optical thermodynamic system consisting of lipophilic and hydrophilic phases. The specific characteristics of small droplet size systems are low viscosity and high solubility for lipophilic and amphiphilic drugs.These systems have the ability to release water insoluble drugs by carrying the these in their oil part or on the water-oil interface. This good property allows the use of MEs as biotechnological carriers for large water insoluble molecules [12-14]. Microemulsions have been developed using compounds that are generally safe and used in other vaginal formulations such as gels, creams and spermicides [15-17]. Increased drug solubility, Convenient and easy of manufacturing, good thermodynamic stability are the advantages of microemulsions. These systems also increase the solubility of drugs through the mucous membranes. Today, topical microemulsions are widely used to increase the solubility, stability and release of plant extracts in drug delivery systems [18-20].

Although there are reports regarding the antifungal effects of *Ziziphus spina-Christi L* spp., reviewing the literature show that, so far, no report has been published on the inhibitory activities of topical microemulsion containing *Ziziphus spina-Christi L* against the growth of the *Candida* spp. In view of this, the aim of this study was to evaluate the antifungal activities of topical microemulsion containing *Ziziphus*

spina-Christi L against the *C. albicans, C. krusei*, and *C. parapsilosis.*

2. Materials and Methods

2.1. Materials

The following reagents and chemicals used were supplied by Sigma-Aldrich (Taufirchen, Germany): Clotrimazole, Sabouraud's dextrose broth (SDB), Tween-80 (polyoxyethylene sorbitan Monooleate) and Span-20 (Sorbitan laurate), Propylene glycol, Oleic acid, Transcutol.

2.2. Plant collection and Preparation Extracts

The *Ziziphus spina-Christi L* was collected from southwestern highlands of Iran and identified in Khuzestan Agricultural and Natural Resources Research Center, Ahwaz between March and April, 2018. The collected plant leaves were a shade dried and powdered using an electric blender. Powdered plant was stored in airtight bottles for further use. Forty grams of powdered plant was extracted (ethanolic and methanolic solvent) with the help of Soxhlet apparatus and dried in room temperate. A definite amount of material was dissolved in an appropriate solvent to attain different concentrations.

2.3. Formulation of Microemulsion

Microemulsion system was ready by mixing hydroalcoholic extract with the mixture of 20% Tween-80 (polyoxyethylene Sorbitan monooleate) and 20% Span-20 (Sorbitan laurate) and 10% Propylene glycol as surfactant and co-surfactant, respectively, and then water was added drop by drop into oily phase (Oleic acid) using a magnetic stirring (Table 1). The system was uniform with softly magnetic stirring for 30 min followed by dissolving of proper amount of hydroalcoholic extract under ultrasonication. The final concentrations of hydroalcoholic extracts in the formulations were 4% w/w [21].

2.4. Characterization of Microemulsions

The physical stability of various microemulsions was tested by globule size, pH, and viscosity test.The pH values of microemulsion were determined using a calibrated pH meter (model E520, Switzerland). The viscosity of various microemulsions was measured by using Brookfield Digital Viscometer (DV-II+Pro Brookfield., USA). The globule size of each microemulsions was determined by the Scatter Scope 1 Quidix (South Korea) based on laser light scattering principle [22].

2.5. Globule Size Determination

Globule size was determined using Scatter Scope 1 Quidix (South Korea). To examine the surface charge of microemulsion sample zeta sizer (Malvern, ZEN3600, United Kingdom) device was occupied. Microemulsion was suspended in distilled water (pH=7) at 25°C. The experiments were carried out three times and the average of the measurements was reported as zeta potential [22].

2.6. Preparation of Organisms

In this research *C.krusei* (ATCC 573), *C. albicans* (ATCC 3153) and *C. parapsilosis* (ATCC 2195) were provided from the

mycology center of Tehran University of Medical Sciences. Each strains was cultured in Sabouraud Dextrose Agar (SDA) (Merk, Germany).

2.7. Well Diffusion Assay

To define the effective concentration, inhibition zones of ethanolic and methanolic extracts and microemulsion containing extract of *Z. Spina-Christi* were examined against *C. albicans*, *C. parapsilosis* strain and *C. Cruise* strain by well assay technique. Overnight cultures of *Candida spp* were spread onto Sabouraud's dextrose agar media (Merck, Germany), and various concentrations of the extracts were added to the pits in the culture with 10 mm diameter and incubated at 27°C for 48 h [23, 24]. Clotrimazole was used as a positive control [25].

2.8. Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software version 12.0. The inhibition diameters of extracts were remarked as a mean standard deviation. A value of $P<0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Characterization of Microemulsions

The pH and viscosity of the prepared microemulsions of ethanolic (A) and methanolic (B) extract are given in (Table 2) which were found that the pH of the prepared microemulsions equivalent to the normal vaginal pH.

3.2. Globule Size Determination

The mean particle size of formulation is demonstrated in figure 1. The ethanolic extract microemulsion had the lowest average particle size 2.93 ± 0.2nm with polydispersity index (PI) of 0.348±0.02. PI showed that the formulation has a homogenous size distribution.

3.3. Zeta Potential

The ethanolic extract microemulsion formulation had the zeta potential average (-0.34mv) (Figure 2).

3.4. Antifungal Study

It was observed from the results that the zone of inhibition alcoholic extracts was lower than the microemulsions A and B. It is clearly understood that the microemulsions A showed better inhibition activity against *Candida* species in comparison to alcoholic extracts used in the study. All the microorganisms studied proved sensitive to microemulsions A and B, which showed activity against *Candida* species. The antifungal effect of A and B microemulsions showed a significant difference compared with the alcoholic extract groups ($p<0.001$) (Table 3).

3.5. Discussion

In this study, we investigated the antifungal effects of different extracts microemulsion of *Z. spina-christi* on *Candida spp*., using agar well diffusion test. The antifungal characteristics of the *Ziziphus* genus have been expressed in several studies. Shams- Mohammad G. T et al. (2013) showed that ethanolic extract of *ZSC*

had anti mycological activity against *Candida spp*. [26]. Another study by Mardani et al. proved the antifungal activity of *ZSC* extract against strains of fungi [27]. The results of the study also showed that the methanolic and ethanolic extract of *Z. Spina-Christi* had antifungal activity against *Candida spp*. As observed in Table 2, the ethanolic and methanolic extracts microemulsion were proved to be more fungicidal than ethanolic and methanolic extract against C. *albicans*. Moreover, the ethanolic extract microemulsion exhibited a significant inhibition in fungal growth. Previous studies have revealed positive associations between, the antifungal activities of plant extracts and the type of the solvents that have been used. Some compounds such as saponins and glycosilated flavonoids come into the polar extract while non-polar components, like terpenoids, are present in the non-polar extract [28]. The dominant activity of ethanoic extract attributed to the presence of some phytochemicals such as terpenoids, flavonoids, tannins, saponins, cardiac glycosides and alkaloid [29]. Some of these compounds, especially the terpenes, showed antibacterial, fungicidal, and insecticidal activities [30-33]. Terpenes have been demonstrated to modify cell permeability by penetrating between the fatty acyl chains. Furthermore, terpenes inhibit the respiratory chain of *Candida*, thus suggesting deleterious results on mitochondria [34, 35]. Also, extracts of Z. Spina-christi has already been shown to affect C. albicans biomass by reducing cell dry weight and increasing glucose levels. This mechanism may be due to cell wall damage and subsequent sterolization of the cell wall [36]. Many researchers use topical microemulsions to improve the antifungal activity of drugs as a practical strategy. The presence of surfactant and cosurfactant in the microemulsions enables achievement of high drug loading for lipophilic compounds [37].

Therefore, it can be assumed that the increase in antifungal activity of ethanolic extract, after its entry into the microemulsion, is probably related to the interaction between the lipid components of the system and the fungal cell wall, thereby increasing the contact area and drug access to the microorganism. Make it easier. Finally, we believe that the microemulsion formulation developed in this work may act as a facilitator for the penetration of ethanolic extract through the fungal cell wall.

4. Conclusion

We suggest that the phytochemicals presented by *Z. Spina-Christi* can inhibit fungal growth and hence might be useful for the treatment of fungal infections. It is worth noting that this study was done in vitro and the response in the body might be completely different. Therefore, additional research is also needed to determine the precise in vivo antifungal activity of the plant extracts.

Acknowledgements

This study was financially supported by the Student Research Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Grant number 95s76.

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Tables:

Table 1. Different formulations of ZSC extracts- loaded Microemulsions.

	Formulations	
	A	**B**
Extract	**Ethanolic extract (4mg)**	**Methanolic extract (4mg)**
Oil phase	Oleic acid	Oleic acid
Weight Ratio	Transcutol 10:1	Transcutol 10:1
Surfactant/Cosurfactant Used [S: CoS Ratio]	*Span-Tween* /Propylene Glycol 4:1	*Span-Tween* /Propylene Glycol 4:1
Aqueous phase	Water	Water
Weight Ratio:o/s/w	50:40:10	50:40:10

Table 2. Physico-chemical characterization of ZSC extracts- loaded Microemulsions (w/o).

Parameters	**Formulations**	
	A (Ethanolic extract)	**B (Methanolic extract)**
pH	3.83	3.62
Color	Light green	Light green
Viscosity (cps)	8.523 ± 1.91	8.021 ± 1.98

Table 3. Mean zones of inhibition (mm) of the prepared microemulsion containing *Z. Spina-Christi* against *candida spp*.

Formulations	**Mean zone of inhibition ± SEM (mm)**		
	C.parapsilosis	***C.albicans***	***C. krusei***
Ethanol extract	12±0.015	12±0.895	11±0.021
Methanol extract	10±0.54	10.756	9±0.024
Microemulsions with ethanol extract (A)	27± 0.48	28 ± 0.97	22.45 ± 0.07
Microemulsions with methanol extract (B)	22.00 ± 1.02	25± 0.85	21.00 ± 1.10
Clotrimazole	26.00 ± 0.30	27.00 ± 0.41	25.00 ± 0.05
Placebo microemulsion	00.00	00.00	00.00

SEM: Standard error of the mean
mm: milimetr