The effect of aqueous and ethanolic leaves extracts of Ziziphus spina-christi (L.) Desf. var. microphylla against Aspergillus niger and Aspergillus flavus

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INTRODUCTION

In recent years, the world has produced a large number of antibacterial drugs with an aim of eradicating the microbes' strains which were responsible for many infections (Al-Juraifani, 2011). However, these drugs induce mutations in the genetic composition of these microorganisms rendering them resistant to several antibacterial drugs (Cohen, 1992). Furthermore, the side effects associated with the extensive use of the chemical drugs may lead to serious damages in most of the human organ (Divya et al., 2016). Therefore, to solve this limitation of chemical drugs, scientists have shifted their focus towards medicinal plants which are recognized as rich sources of antibacterial drugs and are widely used by various communities for medicinal purposes (Iris et al., 2005 and Demetrio et al., 2015). Z. spina-christi (Family: Rhamnaceae) is a plant that grows wild in Asia and tropical Africa. The plant is originally of the Middle-east south of the Euphrates and spread to Saharan Oases across Africa into the Sahel (Kafamiya et al., 2013). The Genus Zizyphus has a wide range of pharmacological applications. Various Zizyphus species are used frequently as traditional medicine in the middle-east, Africa and some Asian countries for acquiring good health and in treating so many ailments which includes, headache, fever, common cold, asthma, pulmonary ailment, malaria, wounds, burns, stomach discomfort and urinary infections, rheumatics disease from the intestine (Adzu et al., 2003). Besides that, people from various regions believe that the species is used as a source of food (Al- Ghamdi, 2001; El Dakhakhny et al., 2000). Z. spinac-christi has been used in folk medicine as a depurative, demulcent, anodyne, for stomach-ache, toothaches, emollient, astringents, antibacterial, antifungal and as a mouth wash (Moodi et al., 2016; Mohammed et al., 2012). Z. spina-christi was shown to contain betulic and ceanothic acid, three cyclopeptide alkaloids as well as four saponin glycosides (Mahran et al., 1996) and several flavonoids have been isolated from the leaves of Z. spina-christi (Amos et al., 2001).

Over the last decades, the importance of aspergillosis in humans and various animal species has increased. Aspergillus species are found worldwide in humans and in almost all domestic animals, birds and as well as in many wild species, leading to a wide range of disease from...
localized infections to fatal disseminated diseases, as well as allergic responses to inhaled conidia. Some prevalent forms of animal aspergillosis are invasive fatal infections in sea fan corals, stone-brood mummification in honey bees, pulmonary and air sac infection in birds, mycotic abortion and mammary gland infections in cattle, guttural pouch mycoses in horses, Sino-nasal infections in dogs and cats, and invasive pulmonary and cerebral infections in marine mammals and nonhuman primates. This article represents a comprehensive overview of the most common infections reported by Aspergillus species and the corresponding diseases in various types of animals. (Seyedomjotaba et al., 2015; Jensen et al, 2013; Illiam et al., 1983).

The study aimed to investigate the antifungal activity of ethanolic and water extracts of Ziziphus spina-christi var microphylla leaves grown in Sudan on selected clinically fungi.

**MATERIALS AND METHODS**

**Plant material and preparation of extracts**

The fresh leaves of *Z. spina-christi* var *microphylla* was collected from Khartoum State, Sennar State, Blue Nile State and Kordofan State, Sudan. The species identification was done in the field depending on taxonomic keys available in Sudan’s Floras (Gibreel, 2008). The leaves were air-dried at room temperature 37°C in the laboratory of the Department of Silviculture, Faculty of Forestry, and University of Khartoum for 7 days and pounded to fine powder in an electric blender and also mortar.

**Preparation of plant extracts**

**Preparation of aqueous (water) leaves extract**

Fifty grams’ sample of air-dried leaves powder from the *Z. spina-christi* var *microphylla* was transferred into a beaker and 100 ml distill water was added. The solution was kept in rotary shaker for 3 days. The obtained aqueous (supernatant) was filtrated twice with Whatman filter paper and kept to dry for 2 days at room temperature 37°C. The obtained dried filtrate was weighted for the studied species (35 g) and transferred into glass bottles (50 ml) and stored at room temperature, then was diluted to 0.2 mg/ml by dissolving 20 g from extract to 100 ml.

**Preparation of ethanol leaves extract**

The ethanol extract was prepared from the leaves of *Z. spina-christi* var *microphylla* by adopting the extraction method described by Ogbadoyi, et al. (2007) and used by Hameed, et al. (2017). In total, 20 g of previously prepared air-dried leaves powder was taken for each species and transferred into beaker and 100 ml of 100% ethanol was added at ambient temperature (28±2°C). The stock was put in a rotary shaker and extraction was allowed to process for 48 h for full extraction. Then, twice subsequent centrifuged (3500 rpm, 20 min) was made to the samples and finally, the supernatant was harvested. The obtained solvents were then evaporated at room temperature and stored after dilution to 0.2 mg/ml in sterile glass bottles for further in vitro assay.

**Agar diffusion assay**

The extracts were concentrated using rotary evaporator and was further dried individually on Petri dishes. These leaves crude extracts (ethanol and water), were subjected to antifungal activity against two spp of fungi: (Aspergillus *niger* and *Aspergillus flavus*) which were obtained from Microbiology Department, Faculty of Veterinary Medicine, University of Khartoum, Sudan. The antimicrobial activity was carried out using the disc diffusion method. Sabouraud

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**Table 1:** Showing type of human and animal microbial pathogens and diameter of inhibitory zone (cm) obtained by water “aqueous” and ethanolic leaves extracts of *Ziziphus spina-christi* (L.) var. *microphylla*.

<table>
<thead>
<tr>
<th>Name of pathogens</th>
<th>Types of <em>Ziziphus spina-christi</em> (L.) Desf. var. <em>microphylla</em> (Code: ZSmic) leaf extraction, diameter of inhibitory zone (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water (W)</td>
</tr>
<tr>
<td><strong>Aspergillus niger</strong></td>
<td>1.52± (±0.05)</td>
</tr>
<tr>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td><strong>Aspergillus flavus</strong></td>
<td>1.72± (±0.04)</td>
</tr>
<tr>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Pr &gt; F</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>F Value</td>
<td>36.24</td>
</tr>
<tr>
<td>R-Square</td>
<td>0.8880</td>
</tr>
</tbody>
</table>

Means (± Standard deviation) with the same letter along the same columns do not differ significantly at P=0.5 according to Duncans Multiple Test; +ve: sensitive to plants extracts; -ve: not sensitive to plants extracts.
dextrose agar (SDB), (Merck, Germany) was used to prepare the culture medium and autoclaved at 121°C for 15 min. In Sabouraud dextrose agar plates 20 µL of fungi (final concentration) were spread 20 µL each extract were applied in to the plate surfaces. The plates were then incubated at 37°C for 48 h. Microbial growth inhibition was determined as the diameter of the inhibition zones around the discs (cm). The growth inhibition diameter was an average of three measurements taken at three different directions. All tests were performed in triplicate and the data was presented as the mean values with their standard deviation.

RESULTS AND DISCUSSION

It was indicated that, the ethanolic extract (E) and aqueous extract (W) were affected, the diameter of the inhibitory zone in cm in W were ranged between 1.52 cm in A. niger to 1.72 cm in A. flavus while in E extract were ranged from 1.9 cm in Aspergillus niger to 2.00 cm in A. flavus. The results of present study are shown in Table 1 and Figures 1 and 2.

In this study the antifungal activity of Z. spina-christi (L.) Desf. var. microphylla against A. niger and A. flavus were observed in two types of leaves extraction (W and E) and this is in agreement with the findings of Hanan et al. (2017) and Shahat et al. (2009) who mentioned that the water and ethanolic extraction were affected in fungi.

CONCLUSION

In the current investigations, the overall findings from the preliminary antifungal effect of the leaves extracts (ethanol and water) of Z. spina-christi var. microphylla showed the potentials for developing antifungal agents from them against two species of Aspergillus. Both extracts of were affect in the fungi under the current study with different diameters. Alcoholic extraction was affected on Aspergillus spp, this is an agreement with Abd El-Hameed (2017).

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REFERENCES


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