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Review Article

Antimicrobial Activity of Aqueous and Ethanolic Extraction of *Ziziphus spina-christi* (L.) Desf. Var. *Microphylla* Leaves Against Different Pathogens

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Abstract

In this study antimicrobial activity of aqueous and ethanolic extraction of *Ziziphus spina-christi* (L.) Desf. var. *microphylla* leaves was evaluated against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumonia*, *Escherichia coli*, *Salmonella dublin*, Yeast and *Candida albicans* isolated from animals in Laboratory of Microbiology at the Faculty of Veterinary Medicine, University of Khartoum, Sudan. Leaves extracts were prepared at concentration of 20g/100 ml and the agar diffusion method was adopted to assess the antimicrobial activity of the prepared extracts against the isolated microorganisms. The results indicated that, the ethanolic Extract (E) and aqueous extract (W) were affected, the diameter of the inhibitory zone in cm was ranged between 1.60- 2.90 cm in W while in E the zones between 2.20- 3.50 cm.

Keywords: *Ziziphus; Spina-Christi*; *Microphylla*; Ethanolic; Aqueous; Extract; Aureus; Epidermidis; *Klebsiella*; *E Coli*; *Salmonella*; Yeast; Candida

Introduction

The family *Rhamnaceae* comprises 52 genera and 925 species distributed worldwide, particularly across tropical and warm temperate regions. *Ziziyphus spina-christi* (Family: *Rhamnacese*) 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 [1]. The Genus *Zizyphus* has wide ranging pharmacological applications. The genus *Ziziphus* consists of approximately 100-170 species of deciduous or evergreen trees and shrubs known for being drought tolerant and very resistant to heat [2,3]. The species *Ziziphus spina-christi* has been among the key plants of the Middle East and the Sahel region since ancient times. The epithet name '*spina-christi*' derives from the belief that this tree provided the crown of thorns said to have been placed on Jesus' head before he was crucified [2,4,5]. Phylogenetic analysis of chloroplast genomic SSR markers showed that *Z. spina-christi* clustered with *Z. mauritiana*, while *Z. jujuba* clustered with *Z. acidojujuba* [6]. In recent years, a large number of antibacterial drugs were produced in the world with an aim of eradicating the microbes' strains, which were responsible for many infections [7]. However, these drugs induced mutations in the genetic composition of these microorganisms rendering them resistant to several antibacterial drugs [8]. Furthermore, the side effects associated with the extensive use of the chemical drugs may lead to serious damages to many of human organs [9]. 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 [10,11].

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

Collection and preparation of plant samples

Leaves from *Ziziphus spina-christi* (L.) Desf. var. *microphylla* growing in different sites in Sudan (Khartoum State, Sennar State, Blue Nile State and Kordofan State) were collected. The species identification was done in the field depending on taxonomic keys available in Sudan's Floras [12]. The leaves were air-dried at room temperature (37°C) in the laboratory of the Department of Silviculture, Faculty of Forestry, University of Khartoum for 7 days and pounded to fine powder using 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 different *Ziziphus spina-christi* (L.) Desf. 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 was 35 g and transferred into glass bottles (50 ml) and stored at room temperature, then were diluted to 0.2 mg/ml by dissolving 20 g from extract to 100 ml.

Preparation of ethanol leaves extract

The ethanol extracts were prepared from the leaves of *Z. microphylla* species by adopting the extraction method described by [13] [and used by [14]. In total, 20 g of previously prepared air-dried leaves powder was taken for each species and transferred into beaker (250 ml) and 100 ml of 100% ethanol was added at ambient temperature (28 ± 2°C). The stock was put in rotary shaker and extraction was allowed to process for 48 hours for full extraction. Then, twice subsequent centrifuged (3500 rpm, 20 min) was made to

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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 leaf extracts of *Ziziphus spina-christi* var. *microphylla.*

	Methods of *Ziziphus spina-christi* (L.) Desf. var. *microphylla* (Code: ZSmic) leaf extraction, diameter of inhibitory zone (cm)	
Name of Pathogens	Water (W)	Ethanol (E)
Staphylococcus aureus	2.42^{b} (±0.04)+ve	3.00^{b} (±0.07)+ve
Staphylococcus epidermidis	2.20^{c} (±0.16)+ve	3.32^{a} (±0.11)+ve
Klebsiella pneumonia	1.90^{d} (±0.32)+ve	2.90^{b} (±0.14)+ve
Escherichia coli	2.20^{c} (±0.12)+ve	2.24^{c} (±0.21)+ve
Salmonella dublin	1.60^{e} (±0.07)+ve	3.00^{b} (±0.12)+ve
Bacillus sp	2.94^{a} (±0.11)+ve	3.50^{a} (±0.19)+ve
Yeast	2.22^{c} (±0.04)+ve	2.30^{c} (±0.14)+ve
Candida albicans	1.94^{d} (±0.09)+ve	2.32^{c} (±0.11)+ve
Pr > F	<.0001	<.0001
F Value	36.24	57.86
R-Square	0.888	0.9268

Means (±Standard deviation) with the same letter along the same columns do not differ significantly at P=0.5 according to Duncan's Multiple Test; +ve: sensitive to plants extracts; -ve: not sensitive to plants extracts.

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.

Preparation of culturing and identification of bacteria

Enriched media (Blood and MacConkey agars) were prepared in microbiology laboratory at faculty of Veterinary Medicine, University of Khartoum and cultured by swabs collected from diseased animals, incubated for 24-48 hrs at 37°C. Smears were prepared from different colonies and stained with Gram's stain to differentiate between gram positive and gram negative bacteria. Purified colonies were cultured in nutrient agar. All primary and secondary biochemical testes were made according to [15] to detect the genera and species.

Preparation of culturing and identification of *candida* and yeast

The basic culture media used in isolating yeast and *Candida* species are blood agar. Also used Lee's synthetic medium for mycelial development and yeast formation for *Candida albicans* [16,17]. Biochemical tests are also routinely done following the initial phenotypic identification of the cultures on agar media and microscopy. Tests using single enzyme are able to detect the presence or absence of an enzyme or a biochemical reaction within seconds to minutes. These tests are economical, rapid and simple to perform [18]. Various *Candida* species can be detected by observing the changes in the indicator color when the yeast cultures utilize 1% carbohydrates such as glucose, maltose, sucrose, trehalose and raffinose [18].

Agar Diffusion Assay

Antimicrobial activity test (Sensitivity Test): The agar diffusion method was adopted to assess the antibacterial activity of the prepared extracts [14]. The stocked cultures of five bacterial strains *Staphylococcus aureus*, Staph epidermidis, *Klebsiella pneumonia*, *Bacillus cereus*, *Salmonella dublin*, *Escherichia coli* and yeast were grown in nutrient broth medium (Merck, Germany) at 37°C for 22 hours. A lawn culture of studied bacteria was prepared on the wells of 6.0 mm in diameter which were cut in the Muller-Hinton Agar (MHA, Merck) by using a sterile pasture pipette and agar discs were removed. Then sample of equal amounts (0.1 ml) of each extracts were filled into each well at concentration of 0.2 mg/ml and added the tested bacteria using micro pipette. The extracts were allowed to diffuse into the agar matrix for 1 hour before incubating in the upright position at 37°C for 24 hours. The diameter of the zone of inhibition was a measured in millimeter by using transparent ruler to determine the antibacterial activity of the *Ziziphus* extracts.

Results and Discussion

The results of analysis of variance indicated significant difference (Pr=0.0001) in antimicrobial activity of aqueous and ethanol leaves extracts at concentration of 0.2mg/ml of *Ziziphus spina-christi* (L.) Desf. var. *microphylla* against isolated microorganis (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumonia*, *Salmonella dublin*, *Escheichia coli*, *Bacillus* spp, yeast and *Candida albicans*). The highest inhibitory zone in W was 2.94 (±0.11) cm in *Bacillus* spp and the less one was 1.60 cm (±0.07) in *Salmonella dublin* while the highest zone in E was 3.50 cm (±0.19) in *Bacillus* spp and the less one was 2.24 cm (±0.21) in *E. coli*. The results of present study are shown in Table 1, Figure 1.

The potency of the *Ziziphus spina-christi* var. *microphylla* extracts depends on the solvent used. This may be due to the degree of solubility of the bioactive constituents. It has been documented that different solvents have diverse solubility capacities for different phytochemical. Result indicated that the antimicrobial effect of both extracts demonstrated varying levels of activity against Gram-positive bacteria, Gram- negative bacteria and Yeast, but the inhibitory zones of E extract were larger than W extract. Alcoholic extraction was affected on *Candida albicans* and other bacteria in this study, this is an agreement with [19-21].

Conclusion

In the current investigation, the overall findings from the preliminary antimicrobial effect of the leaves extracts (ethanol and water) of *Ziziphus spina-christi* var. *microphylla* against certain type of bacteria, *Candida albicans* and yeast.

Figure 1: Sensitivity tests by used the leaves aqueous "water" (W) and ethanoic (E) extracts of *ziziphus spina-christi* var *microphylla* against some bacterial and yeast spp.

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