

Review Article

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

Salih RRM^{1*}, Gibreel HH², Daldoum MA², Hammad ZM² and Ahmed AI³

¹Department of Clinical Medicine, Faculty of Veterinary Medicine, University of Khartoum, Sudan

²Department of Silviculture, Faculty of Forestry, University of Khartoum, Sudan

³Faculty of Natural Resources, University of Kordofan, Sudan

*Corresponding author: Reem Rabie Mohammed Salih, Department of Clinical Medicine, Faculty of Veterinary Medicine, University of Khartoum, Sudan

Received: November 17, 2020; Accepted: December 28, 2020; Published: January 04, 2021

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 pneumoniae*, *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. *Ziziphus 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 [1]. The Genus *Ziziphus* 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. acidujujuba* [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

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*.

Name of Pathogens	Methods of <i>Ziziphus spina-christi</i> (L.) Desf. var. <i>microphylla</i> (Code: ZSmic) leaf extraction, diameter of inhibitory zone (cm)	
	Water (W)	Ethanol (E)
<i>Staphylococcus aureus</i>	2.42 ^b (±0.04)+ve	3.00 ^b (±0.07)+ve
<i>Staphylococcus epidermidis</i>	2.20 ^c (±0.16)+ve	3.32 ^a (±0.11)+ve
<i>Klebsiella pneumonia</i>	1.90 ^d (±0.32)+ve	2.90 ^b (±0.14)+ve
<i>Escherichia coli</i>	2.20 ^c (±0.12)+ve	2.24 ^c (±0.21)+ve
<i>Salmonella dublin</i>	1.60 ^e (±0.07)+ve	3.00 ^b (±0.12)+ve
<i>Bacillus sp</i>	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
<i>Candida albicans</i>	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 microorganism (*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 ethanolic (E) extracts of *Ziziphus spina-christi* var *microphylla* against some bacterial and yeast spp.

Acknowledgement

Authors are intended to express their thanks to the Ministry of Higher Education and Scientific Research in the Sudan for providing research grant and also the authority of FNC (Forest National Corporation) at Sennar, Blue Nile and Kordofan States for their support and help in filed survey and data collection. A lot of thanks are also extended to the Department of Microbiology at the Faculty of Veterinary Medicine and Department of Silviculture at the Faculty of Forestry, University of Khartoum.

References

- Kafamiya N, Shagal MH, Haruna M. Potential of *Ziziphus spina-christi* seed ethanolic extract on inhibition of microbial growth. *Academia Journal of Biotechnology*. 2013; 1: 053-056.
- Saied AS, Gebauer J, Hammer K, Buerkert A. *Ziziphus spina-christi* (L.) Willd.: a multipurpose fruit tree. *Genetic Resources and Crop Evolution*. 2008; 55: 929-937.
- Stevens PF. *Angiosperm Phylogeny*. 2012.
- Dafni A, Levy S, Lev E. The ethnobotany of Christ's Thorn Jujube (*Ziziphus spina-christi*) in Israel. *Journal of Ethnobiology and Ethnomedicine*. 2005; 1: 8.
- Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A. *Agroforestry Database: a tree reference and selection guide version 4.0*. Kenya: World Agroforestry Centre. 2009.
- Huang Jian, Chen RuiHong, Li XinGang. Comparative analysis of the complete chloroplast genome of four known *Ziziphus* species. *Genes*. 2017; 8: 340.
- Al-Juraifani AA. Antimicrobial activity of some medicinal plants used in Saudi Arabia. *Can J Pure Appl Sci*. 2011; 5: 1509-12.
- Cohen ML. Epidemiology of drug resistance: implications for a post antimicrobial era. *Science*. 1992; 257: 1050-5.
- Divya G, Jyotirmay D, Mukesh K. Phytochemical analysis and antimicrobial activity of some medicinal plants against selected common human pathogenic microorganisms. *Asian Pac J Trop Dis*. 2016; 6: 15-20.
- Iris C, Zampini AM, Vattuone IM. Antibacterial activity of *Zuccagnia punctata* Cav. ethanolic extracts. *J. Ethnopharmacol*. 2005; 102: 450-456.
- Demetrio LVJ, Jeannie IA, Juliana JM, Puzon P, Esperanza CC, Windell L. Antibacterial activities of ethanol extracts of Philippine medicinal plants against multidrug-resistant bacteria. *Asian Pacific Journal of Tropical Biomedicine*. 2015; 5: 532-540.
- Gibreel HH. A Taxonomic Study on Trees and Shrubs of El Nour 15. Natural Forest Reserve, Blue Nile State, Sudan. M.Sc. thesis, University of Khartoum. 2008; 22-27.
- Ogbadoyi EO, Akinsunbo OA, Adama TZ, Okogun JI. *In vivo* trypanocidal activity of *Annona senegalensis* Pers. leaf extract against *Trypanosoma brucei*. *Journal of Ethnopharmacology*. 2007; 112: 85-89.
- Hameed FR, Astabraq AM, Amal AK, Wala'a TY, Bushra QD. Antimicrobial Effect of *Acacia Nilotica* on Some Gram Positive and Gram Negative Bacteria. *Al-Mustansiriyah Journal of Science*. 2017; 28: 2521-3520.
- Barrow GI, Feltham RKA. *Cowan and Steel's manual for identification of medical bacteria*. 3rd (ed) Cambridge press. 2003.
- Jatta B, Gunasekaran M, Mohan N. Influence of cultural conditions on lipase production in *Candida albicans*. *Asian J. Biotechnol*. 2009; 1: 118-123.
- Yang R, Mohan N, Muthukumar C, Thajuddin N, Gunasekaran M. Influence of cultural conditions on glutathione peroxidase synthesis in *Candida albicans*. *Asain J. Biochem*. 2009; 4: 99-105.
- Aslanzadeh J. and Roberts GD. Direct microscopic examination of clinical specimens for the laboratory diagnosis of fungal infections. *Clin. Microbiol. Newsl*. 1991; 13: 185-192.
- Abd El-Hameed Abd El-Samie Ibraheem. Using Natural Polyphenol Extracts of *Sider* (*Zizyphus spina-christi* L.) Leaves and Seeds as Anti-Microbial. *Middle East Journal of Applied Sciences*. 2017; 7: 262-271.
- Abdollah GP, Mahmood B, Majid A. Anti-Candida Activity of Some of the Iranian Medicinal Plants. *Electronic Journal of Biology*. 2009; 5: 85-88.
- Al-Ghamdi MS. The anti-inflammatory, analgesic and antipyretic active of *Nigella sativa*. *J. Ethnopharmacol*. 2001; 76: 45-48.