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Antimicrobial Effect of Aqueous and Ethanolic Leaves Extracts of *Ziziphus* Species against Animal Bacterial Pathogens

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Abstract

This study was conducted during 2018-2019 to evaluate the antimicrobial activity of aqueous and ethanolic extraction of leaves from three naturalized *Ziziphus* species (*Ziziphus spina-christi* (L.) Desf. var. *spina-christi*, *Ziziphus abyssinica* Hochst and *Ziziphus mauritiana* Lam) against five bacterial strains (*Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumonia*, *Escherichia coli* and *Salmonella dublin*) isolated from diseased 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 antibacterial activity of the prepared extracts against the *Staph aureus, Staph epidermidis, E. coli, Klebsiella pneumonia* and *Salmonella dublin*. The results indicated that, the ethanolic extract (E) was more effective than aqueous extract (W), the diameter of the inhibitory zone in cm in E was ranged between 1.5- 2.3 cm and in W between 2 - 2.2 cm. W extracts of three species of *Ziziphus* were affective only in *Staph epidermidis*. More studies should be done considering other parts of the plants (bark, fruits and roots) and at different concentrations.

Keywords: Bacteria; Blue Nile; Ethanol; Kordofan; *Mauritiana*; Sennar; *Spina-christi*; Water; *Ziziphus abyssinica*

Introduction

Ziziphus traditionally called *Ziziphus* forest fruits have numerous proposes; firstly, as medicine, Okoko and Oruambo (2008) [1] mentioned that the fruits of *Ziziphus* tree are inexpensive and used to development of human fitness and prevention of diseases, to treat pulmonary ailments, fevers, dysentery, and to therapeutic of new wounds [2-4]. As well as, it is used to reduce toothaches pains, astringents and as a mouth wash [5]. Secondly, as food, it is eaten similar to common fruits (fresh or dry), processed as sheet or leather, and crushed to make a light porridge called Nashaor Madeda. Fully ripened edible part of *Ziziphus* has aromatic smell, and it is rich in many nutrients, such as, protein, carbohydrates and fibre (Duke, 1985); also, energy value and vitamin C [6]. As well as, *Ziziphus* fruits contains abundant levels of calcium, potassium, sodium, phosphorous, copper, iron, and zinc [7]. Hot manufacturing has most of the attributes of perfect food protection [8]. The preservation of fruit by jam making is a recognizable procedure carried out on a little quantity by housewives in many countries throughout the world (Abdel-Rahman, 2012).

Some phytochemicals- plant including flavonoids, tannins, lipids, terpenes, alkaloids, steroids and carbohydrates were extracted from *Ziziphus* [9]. These plant extracts and fractions of leaves, fruits and seeds has showed antiviral, antifungal and antibacterial activities and were used in the medicine for the treatment of several diseases including gastrointestinal tract ailments, diabetes and diarrhea [9]. Current antimicrobial therapy for the infectious diseases has certain limitations due to toxicity, side effects and multiple resistances of microorganisms. *Enterobacter* is usually a commensal bacterium, and is a common opportunistic pathogen responsible for urinary and respiratory tract infections and bacteremia [10]. *Escherichia coli* is commonly

found in the lower intestinal tract of healthy animals but there are many types of *E. coli*, a few of which are pathogenic by a variety of infective and toxin-producing mechanisms [11]. Currently, there is a continuous search for new drugs with reduced levels of toxicity and side effects [12,13].

Infectious diseases accounts for about half of the death in tropical countries. The use of antibiotics to control it has Lead to high incidence of side effects, and emergence of resistant bacterial strains. Herbal remedies used in the traditional medicine provide an interesting and still largely unexplored source for the creation and development of potentially new drugs for treatment [13]. The use of medicinal plants, as traditional health remedies have been most popular for 80% of world population in Asia, Latin America and Africa and is reported to have minimal side effects [14]. The *Ziziphus* species (*Rhamnacese*) are commonly used in folklore medicine for the curing of various diseases. They are wide-spread in the Mediterranean Region, Africa, Australia and tropical America. *Z. spina-christi* has been used in folk medicine as a demulcent, depurative, anodyne, emollient, Stomach- ache, for toothaches, astringents and as a mouth washes [9]. This study aimed to evaluate the antimicrobial effect of aqueous and ethanolic extraction of leaves from three Sudanese *Ziziphus* species (*Ziziphus spina-christi* var. *spina-christi, Ziziphus abyssinica* and *Ziziphus mauritiana*) against five pathogenic bacterial strains isolated from diseased animals (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumonia*, *Escherichia coli*, *Salmonella dublin*).

Materials and Methods

Collection and Preparation of Plant Samples

Plants materials used were leaves collected from *Ziziphus spina-christi* var. *spina-christi,* (Sidr Baldi), *Ziziphus abyssinica* (Nabak Al Feel) and *Ziziphus mauritiana* (Al Nabak Al Helo) growing in different sites in Sudan (Khartoum State, Sennar State, Blue Nile State and Kordofan State). The species identification was done in the field depending on taxonomic keys available in Sudan's Floras [15]. 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* species 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 for *Ziziphis spina-christi*, 32 g for *Ziziphus abyssinica* and 36 g for *Ziziphus mauritiana*) 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 different *Ziziphus* species by adopting the extraction method described by Ogbadoyi, et al. (2007) [16] and used by Hameed, et al. (2017) [17]. 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 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 Barrow and Feltham (2003) to detect the genera and species.

Antimicrobial Activity Test (Sensitivity Test)

The agar diffusion method was adopted to assess the antibacterial activity of the prepared extracts [17]. The stocked cultures of five bacterial strains *Staphylococcus aureus, Staphylococcus aureus epidermidis, Klebsiella pneumonia, Salmonella dublin* and *Escheichia coli* 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

The results of present study are shown in Table 1, Figure

1 and Figure 2. 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 the three studied *Ziziphus* species (*Ziziphus mauritiana* and 2 cm in *Ziziphus spina-christi* var. *spina-christi* and Ziziphus *abyssinica*) against bacterial stains tested (*Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumonia, Salmonella dublin* and *Escheichia coli*). At the concentration of 0.2mg/ml, the ethanolic extract of different species of *Ziziphus* was effective and aqueous one. However, the leaves aqueous extract of the three species of *Ziziphus* was inhibited *Staphylococcus epidermidis* with inhibitory zone diameter from 2.2±0.07 cm in *Ziziphus mauritiana* and 2±0.07 cm in both *Ziziphus spina-christi*- var. *spina-christi* and *Ziziphus abyssinica.* The ethanolic extract of *Z. spina-christi* var. *spina-cristi* was positive in all isolated bacteria with different diameter of inhibitory zone between 1.5±0.07 cm in *E. coli* to 2.3±0.14 cm in *Salmonella dublin* while in *Z. abyssinica* the diameter of inhibitory zone was between 1.6±0.07 cm in *Staph aureus,* 1.6±0.07 in *E. coli* and 1.6±0.14 in *Staph epidermidis* to 2.2±0.15 cm in *Salmonella dublin.* Furthermore, the ethanolic extract of *Z. mauritiana* showed diameter of inhibitory zone between 1.5±0.12 cm in both *Staph epidermidis* and *E. coli* to 2.2±0.18 cm in *Salmonella dublin*.

Bacterial strains	***Ziziphus* genotypes and methods of leaf extraction, diameter of inhibitory zone (cm)**					
	Ziziphus spina-christi (L.) Desf. var. *spina-christi* (Code: ZSs)		Ziziphus abyssinica Hochst. (Code: Za)		*Ziziphus mauritiana* Lam. (Code: Zm)	
	Water (W)	**Ethanol (E)**	**Water (W)**	**Ethanol (E)**	**Water (W)**	**Ethanol (E)**
Staphylococcus aureus	0b (±0.0) -ve	1.6bc (±0.07) +ve	0b (±0.0) -ve	1.6c (±0.07) +ve	0b (±0.0) -ve	1.7b (±0.07) +ve
Staphylococcus epidermidis	2a (±0.07) +ve	1.7b (±0.14) +ve	2a (±0.07) +ve	1.6c (±0.14) +ve	2.2a (±0.07) +ve	1.5c (±0.12) +ve
Klebsiella pneumonia	0b (±0.0) -ve	1.7b (±0.1) +ve	0b (±0.0) -ve	1.9b (±0.14) +ve	0b (±0.0) -ve	1.7b (±0.07) +ve
Escherichia coli	0b (±0.0) -ve	1.5d (±0.07) +ve	0b (±0.0) -ve	1.6c (±0.07) +ve	0b (±0.0) -ve	1.5c (±0.12) +ve
Salmonella dublin	0b (±0.0) -ve	2.3a (±0.14) +ve	0b (±0.0) -ve	2.2a (±0.15) +ve	0b (±0.0) -ve	2.2a (±0.18) +ve
Pr> F	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
F Value	4000	40.83	4000	28.4	4840	27.33
R-Square	0.9988	0.8909	0.9988	0.8503	0.999	0.8454

v 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.

Table 1: Showing types of bacterial strains, *Ziziphus* genotypes and methods of leaf extraction, diameter of inhibitory zone (cm).

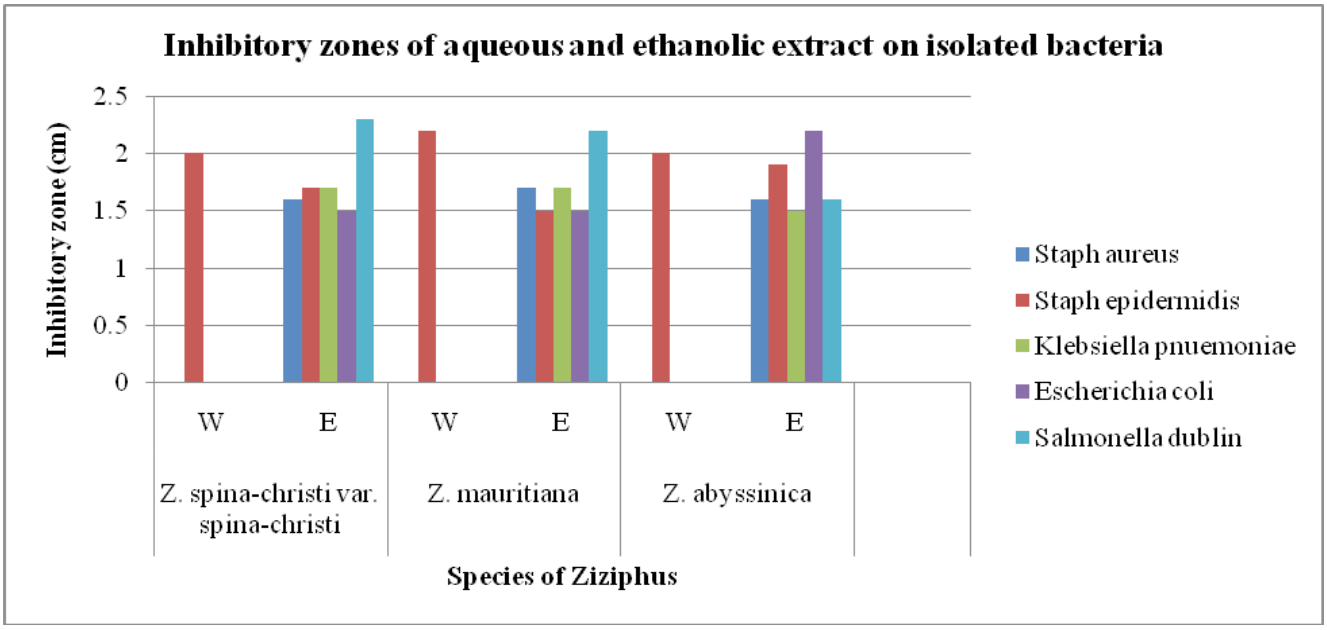

Figure 1: The inhibitory zones (cm) of the water "aqueous" (W) and ethanolic (E) leaves extract of *Ziziphus* species against the five isolated bacteria.

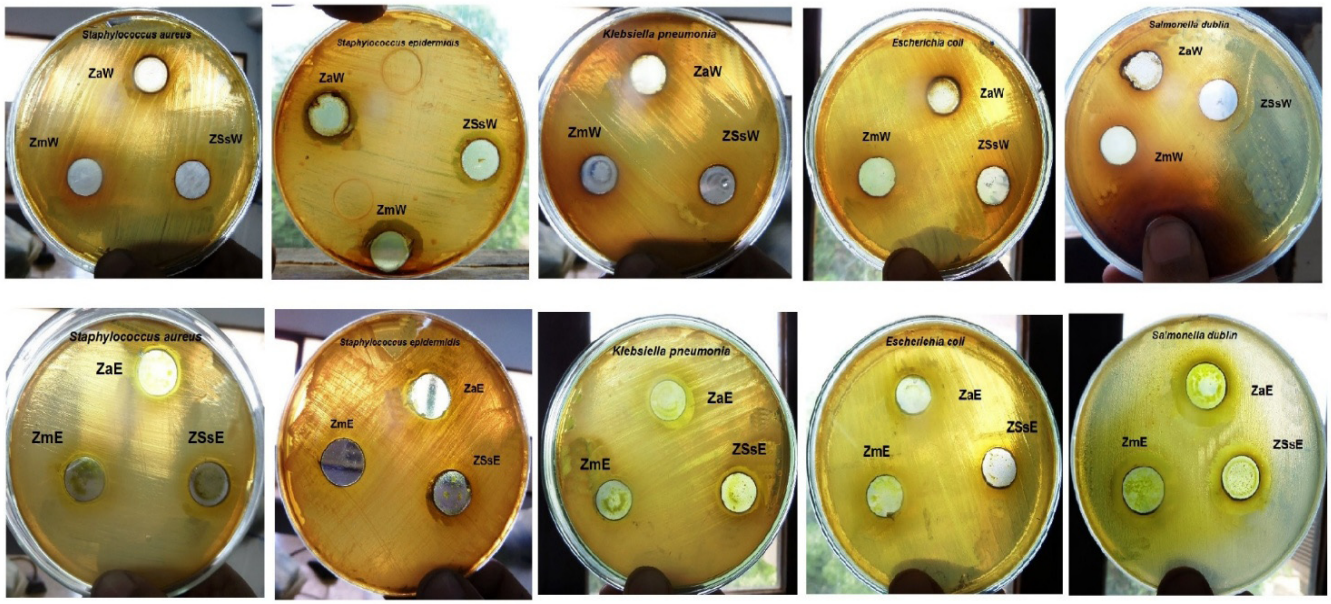

Figure 2: Sensitivity tests by used the leaves aqueous "water" (W) and ethanolic (E) extracts of three species of *Ziziphus* in the Sudan. Where: ZaW: *Ziziphus abyssinica* water extract; ZmW: *Ziziphus mauritiana* water extract; ZSsW: Ziziphus *spina-christi* water extract; ZaE: *Ziziphus abyssinica* ethanolic extract; ZmE: *Ziziphus mauritiana* ethanolic extract; ZSsW: *Ziziphus spina-christi* ethanolic extract.

Discussion

In current world, a microbial infection was one of the major threats to human and animal population. Antimicrobial drugs provide the essential basic for treatment of various microbial infections instead of the elevated genetic inconsistency of some microorganisms enhance them to quickly develop antimicrobial resistance; therefore, there has been a continuing study for new potent antimicrobials drugs [17]. Due to drug resistance nature of microorganisms, there is a need to find out new lead molecules from alternative sources like plants and algae. The present study shows that leaves extracts (aqueous and ethanolic) have inhibitory effect on bacterial growth. The plant extracts show varying degrees of action adjacent to gram-negative bacteria (*Salmonella dublin, klebsiella pneumonia* and *E. coli*), and gram positive bacteria (*Staph aureus* and *Staph epidermidis*). Phytochemical components were extracted from *Ziziphus* leaves to have and inhibition activity against bacteria strains namely alkaloids, saponins, tannins, glycosides, flavonoids and terpinoids [18,19]. In this study W and E extracts of the leaves three species of *Ziziphus* were used to evaluation of the antimicrobial activity. The E extract was affect in *Staph aureus* and this is in agreement with Nisaret, et al. (2010) and Hossein, et al. (2014) [20,21]. The highest effectiveness was demonstrated by the ethanolic extract of sider leaves among the Gram negative bacteria was *Salmonella* followed by *Klebsiella* then *E. coli* and this is in agreement with Sarah and Salah (2016) [22]. In this study the E extract of *Z. mauritiana* and *Z. spina-christi* were affected in *Staph aureus* and *E. coli* and this is in agreement with M. E. Abalaka (2010) [23]. Also *Ziziphus* species were affected in *Staph epidermidis* and this is in agreement with findings of Merve (2016) [24]. The chemical compound detection of *Ziziphus* plant leaves findings showed that aqueous and alcoholic extracts contain effective compounds that have been detected except composite resins in aqueous extract and resins in the alcoholic extract [19].

Conclusion

In the current investigation, the overall findings from the preliminary antibacterial effect of the leaves extracts (ethanol and water) of three types of *Ziziphus* against five species of most pathogenic bacteria in human and animal. The result indicated that the antibacterial effect of ethanol extract was more effective than aqueous. More studies are needed to test different extracts of other parts of these plants as bark, fruits and roots with in different concentrations against Gram-positive bacteria and Gram-negative bacteria.

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