The antibacterial effect of some Sudanese plants (Neem, Garad and Sidr)

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ABSTRACT

This study was designed to evaluate the antimicrobial activity of water extract of three species of Ziziphus (Sidr), Azadirachta indica (Neem) and Acacia nilotica var. adstringens (Garad) against five bacterial strains which were known and isolated from diseased animals in Laboratory of Department of Microbiology at the Faculty of Veterinary Medicine, University of Khartoum were as follows: Staphylococcus aureus, Staph. epidermidis, Escherichia coli, Klebsiella pneumonia and Salmonella dublin. Sensitivity tests were made after preparation the aqueous solutions. The results were as follows: the three species of Sidr were affected only in Staph. epidermidis, there is no effect of Neem I all bacterial species but the Garad was affected in all bacteria with high inhibitory zones from 2 cm-4.1cm.

Keywords: Acacia, Azadirachta, Ziziphus, Neem, Garad, Sidr, bacteria, aqueous extract.

1. Introduction

Plants are an important source of potentially useful structures for the development of new chemotherapeutic agents (Nagumanthri et al., 2012). The first step towards this goal is the in vitro antibacterial activity assay (Tona et al., 1998; Nagumanthri et al., 2012). The potential for developing antimicrobials from higher plants appears rewarding, as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu et al., 1999; Nagumanthri et al., 2012). Continued further exploration of plant-derived antimicrobials is needed today (Nagumanthri et al., 2012). Infectious diseases accounts for about half of the death in tropical countries. The use of antibiotics to control it has led 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 (Ali et al., 2001). 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 (Doughari, 2006).

Acacia nilotica (L.) Delile, sub sp. adstringens (Schumach. & Thonn.) named Garad in Sudan is one of the trees distributed in West Africa, from Senegal to Nigeria and widespread in northern parts of tropical Africa (Gibreel, 2015). It occurs in wooded grasslands, savannas and dry scrub forests above the flood plains. Spreading occurs by Cattle eat the mature pods and at least 40% of seed could be transported by water (Fagg, 2001). The seeds of acacia have spasmodic and antiplasmodial activity (Gilani et al., 1999). The roots are used against cancers and/or tumors (of ear, eye, or testicles), tuberculosis and indurations of liver and spleen (Kalaivani et al., 2010b). Leaf Chemopreventive, antimutagenic, antibacterial, anticancer, astringent, antimicrobial activity Tender leaves are used to treat diarrhea, Aphrodisiac, dressing of ulcers, anti-inflammatory and Alzheimer’s diseases (Baravkar et al., 2008). Stem bark Anti bacterial, antioxidant, anti-mutagenic, cytotoxic bark is used as astringent, acrid cooling, styptic, emollient, anthelmintic, aphrodisiac, diuretic, expectorant, emetic, nutritive, in hemorrhage, wound ulcers, leprosy, leucoderma, small pox, skin diseases, biliousness, burning sensation, toothache, dysentery and seminal weakness. The trunk bark is used for cold, bronchitis, diarrhea, dysentery, biliousness, bleeding piles and leucoderma (El-Tahir and Khalid, 1999). Anti hypertensive and antispasmodic, anti-diarrheal, astringent, anti-fertility and against HIV-1 PR. Inhibited HIV-1 induce cytopathogenicity, antiplatelet aggregating activity and anti-oxidant (Cox, 1997).

Azadirachta indica, commonly known as neem, nim tree or Indian lilac Anon (2017) is a tree in the mahogany family Meliaceae. It is one of two species in the genus Azadirachta, and is native to the Indian subcontinent, i.e. India, Nepal, Pakistan, Bangladesh, Sri Lanka, and Maldives. It is typically grown in tropical and semi-tropical regions. Neem trees also grow in islands located in the southern part of Iran. The natural products from Neem show an important role in diseases prevention and treatment through the enhancement of antioxidant activity, inhibition of bacterial growth, and modulation of genetic pathways. Traditional use the products made from
Neem has been used as anthelmintic, antifungal, antibacterial and antiviral (especially in treatment of sweet itch and mud fever in horses. The leaves have also been used to treat skin diseases like eczema, psoriasis (Anna, 2006).

Ziziphus, traditionally called Sidr forest fruits, have numerous proposes; firstly, as medicine (Okoko and Oruambo 2008). It is Fully ripened edible part of Ziziphus has aromatic smell, and it is rich in many nutrients, such as, protein, carbohydrates and fiber (Duke, 1985); also, energy value and vitamin C (Waggas and Al-hasani, 2009b). Some phytochemicals- plant including flavonoids, tannins, lipids, alkaloids, steroids and carbohydrates were extracted from Ziziphus (Shahat et al., 2001). 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 (Shahat et al., 2001). 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 (Talon et al., 2004). 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 (EFSA, 2011). Currently, there is a continuous search for new drugs with reduced levels of toxicity and side effects (AL-Haj et al., 2010 and Ali et al., 2001).

Sidr, Garad and Neem were commonly used in folklore medicine in Sudan for the curing of various human and animal diseases. The study aimed to determine the antibacterial effect of water extract of Azadirachta indica (Neem), Sidr Baldi (Ziziphus spina-christi), Nabak Al Feel (Ziziphus abysinica Hochst.), Nabak Hello (Ziziphus mauritiana Lam.) and Garad Abu Arida (Acacia nilotica subsp. adstringens) against some pathogenic bacterial strains isolated from diseased animals (Staphylococcus aureus, Staph. epidermidis, Klebsiella pneumonia, Escherichia coli, Salmonella dublin).

2. Materials and Methods

2.1. Collection and preparation of plants materials

The plants materials used in this quantitative, comparative and analytic study, conducted during 2018-2019, included Neem (Azadirachta indica) gum collected from trees growing in the roadside near Shambat Campus (15° 39' 33.52” N; 32° 30' 52.52” E); ripe pods of Acacia nilotica subsp. adstringens (Garad Abu Arida or Sunt Abu Arida) collected from trees growing in the Arboretum of the Faculty of Forestry, University of Khartoum (15° 39' 23.48” N; 32° 30’ 54.36” E); leaves of Ziziphus abyssinica (15° 39’ 25.43” N; 32° 31’ 07.64” E) and Ziziphus mauritiana (15° 39' 24.37” N; 32° 31’ 06.34” E) growing in the plant garden of the Faculty of Agriculture, University of Khartoum as well as leaves of Ziziphus spina-christi collected from the Arboretum of the Faculty of Forestry, University of Khartoum (15° 39’ 25.38” N; 32° 30’ 52.30” E). The samples were transferred in clean envelopes to the laboratory of the Department of Silviculture, Faculty of Forestry, University of Khartoum where it’s authenticated, thoroughly washed, sun-dried (7-10 days) and ground into powder using pestle and mortar prior to analysis.

2.2. Extraction process and preparation of stock solution

Extraction process was the same of all samples (leaves, gum, pods free of seeds) and done in the laboratory of the Department of Forest Products and Industries, Faculty of Forestry, University of Khartoum. 20 grams of each sample were weighted, dissolved in 100 ml distilled water in clean conical flask (250ml) and kept 24 hours in shaker at room temperature (37°C). The obtained crude solution was double filtered using Whatman filter paper and dried overnight at room temperature (37°C). The initial stock solution for each sample was prepared by weighting 0.2g (200µg) of the crude extract each dissolved in 10ml of distilled water in glass bottles (30ml) to obtain a concentration of 20mg/ml or 200µg/ml.

2.3. Preparation of Culturing and identification of bacteria

The enriched media (Blood and MacConkey agars) and culture were prepared by different samples collected from diseased animals, incubated for 24- 48 hrs at 37°C. The prepared smears from different colonies were 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 tests were made to detect the genera and species according to Barrow and Feltham (2003).
2.4. Sensitivity test

Sensitivity test was carried out in the laboratory of Microbiology Department, Faculty of Veterinary Medicine, University of Khartoum using well diffusion dilution technique on Mueller-Hinton agar plates adopted by Hiba and Reem (2017). Five bacterial isolates (Staphylococcus aureus, Staph. epidermidis, Klebsiella pneumonia, Escherichia coli, Salmonella dublin) were tested for sensitivity to the prepared plants water extracts. The experiment was in completely randomized design with five replicates was adopted. 0.1 ml of a broth culture of each isolate was spread on each Mueller-Hinton agar plate. Five wells (measuring 8 mm in diameter) were cut out of the Mueller-Hinton agar under aseptic conditions using sterile blue tubes. Each well was filled with 20 µl from each the different water extract (leaves, gum and pods) at concentration of 200µg/ml (0.2g/ml). Plates were refrigerated for 2 hours to allow proper diffusion before incubation in an upright position at 37°C for 24-48 hours. After incubation, the inoculated sensitivity plates were removed from the incubator and under good illumination the inhibition zones diameter (cm) around the wells were measured by using ruler. The test was considered valid only if there were inhibition zones around the positive control. An inhibition zone measuring more than 12 mm was considered sensitive.

2.5. Statistical analysis

SAS statistical analysis software package (Version 9) was used for interpretation of the results. Descriptive statistics was used to get means ± standard error of means; One-Way ANOVA for disc diffusion results was done for comparison between five water plant extracts. Means were separated using Duncan’s method and value of P<0.05 was considered significant.

3. Result

In this study five bacterial species were isolated from clinical materials which were previously collected from infected animals and identified using standard biochemical tests. These were Salmonella Dublin (gram-negatives), Escherichia coli (gram-negatives), Klebsiella pneumonia (gram-negatives), Staphylococcus aureus (gram-positives) and Staphylococcus epidermidis (gram-positives). Statistical analysis (Table 1) indicated that, bacterial strains had significantly different sensitivity response to the different plants water extracts (Table1; Figure 1 and 2). However, water extract of Neem (Azadirachta indica) gum (at 200µg/ml) showed no inhibitory effect to the studied isolated bacteria. In contrary, the water extract of Sunt/Gurad Abu Arida (Acacia nilotica subsp. adstringens) pods (at 200µg/ml) has positive sensitivity effect in all bacteria with significant difference in inhibitory zones that ranged among the strains between the highest 4.1±0.07 cm in Staphylococcus aureus followed by 3.5±0.07cm in Staphylococcus epidermidis and 2.5±0.19 cm in Salmonella dublin, while 2±0.71 and 2±0.071 cm inhibitory zones were recorded in Klebsiella pneumonia and Escherichia coli, respectively. Furthermore, leaves water extract (at 200µg/ml) of Ziziphus species (Z. spina-christi, Z. abyssinica and Z. mauritiana) showed negative effect against Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli and Salmonella dublin, while it was positive with Staphylococcus epidermidis where inhibitory zones of 2.2±0.07 cm (Ziziphus mauritiana), 2±0.07 (Ziziphus spina-christi) and 1.7±0.14 cm (Ziziphus abyssinica) were recorded (Table 1).

<table>
<thead>
<tr>
<th>Type of plant water extract and concentration (µg/ml)</th>
<th>Name of bacterial strains and diameter of inhibitory zone (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>Staphylococcus epidermidis</td>
</tr>
<tr>
<td>Zipinus spina-christi (ZSs) leaves water extract (200 µg/ml)</td>
<td>0⁺ (±0.0) -ve 2⁺ (±0.07) +ve 0⁺ (±0.0) -ve 0⁺ (±0.0) -ve 0⁺ (±0.0) -ve</td>
</tr>
<tr>
<td>Zipinus abyssinica ( Za) leaves water extract (200 µg/ml)</td>
<td>0⁺ (±0.0) -ve 1.7⁺ (±0.14) +ve 0⁺ (±0.0) -ve 0⁺ (±0.0) -ve 0⁺ (±0.0) -ve</td>
</tr>
<tr>
<td>Zipinus mauritiana (Zm) leaves water extract (200 µg/ml)</td>
<td>0⁺ (±0.0) -ve 2.2⁺ (±0.07) +ve 0⁺ (±0.0) -ve 0⁺ (±0.0) -ve 0⁺ (±0.0) -ve</td>
</tr>
<tr>
<td>Acacia nilotica subsp. adstringens ( Ana) pods water extract (200 µg/ml)</td>
<td>4.1⁺ (±0.07) +ve 3.5⁺ (±0.07) +ve 2⁺ (±0.71) +ve 2⁺ (±0.07) +ve 2.5⁺ (±0.19) +ve</td>
</tr>
<tr>
<td>Azadirachta indica (N) gum water extract (200 µg/ml)</td>
<td>0⁺ (±0.0) -ve 0⁺ (±0.0) -ve 0⁺ (±0.0) -ve 0⁺ (±0.0) -ve 0⁺ (±0.0) -ve</td>
</tr>
</tbody>
</table>

Means (±Standard deviation) with the same letter along the same row do not differ significantly at P=0.5 according to Duncan’s Multiple Test; +ve: sensitive to plants extracts; -ve: not sensitive to plants extract.
Figure 1. The diameter of inhibitory zones (cm) of water extracts against isolated bacterial strains.

<table>
<thead>
<tr>
<th>Inhibitory zone (cm)</th>
<th>ZsSw</th>
<th>ZmW</th>
<th>ZaW</th>
<th>NgW</th>
<th>Anaw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella dublin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.5</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>2</td>
<td>2.2</td>
<td>2</td>
<td>0</td>
<td>3.5</td>
</tr>
<tr>
<td>Staphylococcus spina-christi</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.1</td>
</tr>
</tbody>
</table>

Figure 2. Sensitivity testing for different plants water extracts against bacterial strains. Where: Anaw; Acacia nilotica leaves water extract; ZmW: leaves water extract of Ziziphus mauritiana; ZaW: leaves water extract of Ziziphus abyssinica; ZsSw: leaves water extract of Ziziphus spina-christi; NgW: gum water extract of Azadirachta indica.
4. Discussion

In this study the water extract of the pod of *Acacia nilotica* subsp. *adstringens* (Sunt/Gard Abu Arida), gum of *Azadirachta indica* (Neem) and leaves of *Ziziphus spina-christi* (Sidr/Nabak Baldi), *Ziziphus abyssinica* (Nabak Al Feel) and *Ziziphus mauritiana* (Nabak Helo) showed variation, *in vitro*, in antibacterial activity at one concentration against five bacterial strains (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella dublin*, *Klebsiella pneumonia*, *Salmonella dublin*, *Escherichia coli*). In this study, extracts of the leaves of three species of plants were used and the results were as follows: *Acacia nilotica* var adstringens was affected on *Staph aureus* and *E. coli* and this is in agreement with Al-Hafez et al., (2016). In addition, this study showed that *E. coli* was not affected by *Ziziphus spina- Christi* and this is in agreement with Mohammed and Awatif (2018). The positive results obtained by pods water extract (200µg/ml) of *Acacia nilotica* subsp. *adstringens* (Sunt/Gard Abu Arida) against all bacterial strains indicated its potentiality as antibacterial agent. However, Hiba and Reem (2017) reported that, *Acacia nilotica* extract had in vitro, an antibacterial activity against *E. coli* and *Staphylococcus aureus*; and it can be used in treatment of wound infections caused by these organisms. The result was also in agreement with Tahani et al. (2018), who reported that, *Acacia nilotica* subsp. *adstringens* tree was used in the treatment of the respiratory pneumonia which may caused by *Staph aureus* and diarrhea may caused by Enteric bacteria like *E. coli*, *Klebsiella* and *Salmonella* in Sudan. In this study the negative results of antibacterial activity of Neem gum water extract at concentration of 200µg/ml against all bacterial strains studied as well as the negative effect obtained from leaves water extract against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli* and *Salmonella Dublin* at the same concentration did not mean absence of bioactive constituents nor that is the plant inactive. According to Taylor et al. (2001), active compound(s) may be present in insufficient quantities in the extracts to show activity with the concentration employed. More and above, Uwimbabazi et al., (2015) stated that, Neem extracts with low concentrations did not show effect on *Staphylococcus aureus* but by increasing the concentration of extract, effect was obtained as the dilution level decreases.

From this study it may be recommended that further studies should be performed at different concentration and delusions to establish the standard dosage for the water extracts of *Acacia nilotica* pods, Neem gum and *Ziziphus* species leaves. In addition, studies to investigate its toxicity and side effects on human health are recommended. Researchers are needed to study the antimicrobial activity water extracts of the different plants studied against other micro-organisms such as anaerobic bacteria, fungi, and viruses. Conclusion: *Acacia nilotica* subsp. *adstringens* pods water extract at low concentration (200µg/ml) had, *in vitro*, an antibacterial activity against all bacterial strains (*Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli* and *Salmonella Dublin*) and it can be used in treatment of wound infections caused by these organisms in human and/or animals.

Reference


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