Potential of Ziziphus spina-christi seed ethanolic extract on inhibition of microbial growth

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ABSTRACT

The antibacterial activity of ethanolic extracts of Ziziphus spina-christi leaves were examined using agar disc diffusion method against five bacteria (Streptococcus faecalis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa). These extracts had inhibitory activity at various stages of germination; 1st stage has inhibitory activity of 22 mm against Streptococcus faecalis followed by Staphylococcus aureus with 20 mm and Pseudomonas aeruginosa with 15 mm. 2nd stage has inhibitory activity of 15 mm against Streptococcus faecalis followed by Staphylococcus aureus with 20 mm and Pseudomonas aeruginosa with 15 mm against Streptococcus faecalis followed by Pseudomonas aeruginosa and Staphylococcus aureus with 10 mm. 1st and 2nd stages had activity on Streptococcus faecalis, Staphylococcus aureus and Pseudomonas aeruginosa but no activities on the rest of the microorganisms. 3rd and 4th stages had no inhibitory activities against all the tested microorganisms. The inhibitory activities of the ethanolic extract of Z. spina-christi on the tested microorganisms confirmed the local use of the plant for treatment of some ailments traditionally.

Key words: Antibacterial, ethanolic, inhibitory, extract, ailments.

INTRODUCTION

Medicinal plants are plants that have recognized medicinal uses. They range from plant which is used in the production of mainstream pharmaceutical product to plant used in herbal medicine preparation (Lothtipour et al., 2008). Medicinal plant is defined as any plant which in one or more of its parts contains substance that can be used for therapeutic purpose or as precursors for the synthesis of useful drugs (Sofowora, 2008). Traditional medicine like western or orthodox medicine aims to healing or preventing diseases. In this respect, both medicinal practices have come to have similar objectives, however with the very explicit definition of traditional medicine given by the World Health Organization (WHO, 1991). It has been found to differ in the concept of causes of disease, their approach to healing and methods used when compared to orthodox medicine (Sofowora, 2008).

Herbal medicine is one of the oldest forms of medicinal treatment in human history, and could be considered one of the forerunners of the modern pharmaceutical trade. Medicinal plants can be found growing in numerous setting all over the world. Some medicinal plants are wild crafted, meaning that they are harvested wild by people who are skilled at plant identification. Sometimes plant cannot be cultivated, making wild crafting the only way to obtain them and some people believe that wild plant have more medicinal properties (Serrentino, 1991).

Other medicinal plants may be cultivated; one of the advantages of cultivation is that it allows for greater control over growing conditions. Cultivation also allows for mass production, which makes production of the medicinal plant(s) commercially feasible as they can be processed in large numbers and priced low enough that people will able
they have not been researched to occupy important position in modern medicine. On a global basis, at least 130 drugs single chemical entities extracted from higher plants or modified further synthetically are currently in use, though some of them are now being made synthetically for economic reasons (Adzu, 2002).

*Zizyphus spinosa-christi* 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 (WHO, 1991). It has several physiological and morphological characteristics that assure its ability to adapt to arid environment (Keay et al., 1964). *Z. spinosa-christi* is a powerful medicinal plant. It has been found to have a very powerful antimicrobial agent; people from various regions believe that the species is used as a source of food. The basic component or nutrient content of the species constituted the following: carbohydrate, protein, tannins and sugar (Vankataswamy, 2010).

*Z. spinosa-christi* seed have been used for nutritional and medicinal purpose in many middle-east countries and other parts of the world (Al-Ghamdi, 2001; El Dahakhny et al., 2000). *Z. spinosa-christi* seed is used as a natural food additive and condiment. Also, it has been used for medicinal purposes and as natural remedy in many ancient cultures such as those of Egypt, Greece and Rome (Al-Haider and Aqeel, 1993).

The *Z. spinosa-christi* seeds serve as the medicine for every disease except death (Takruri, 2003). Seeds of this species are frequently used in folk medicine in the middle-east and some Asian countries for acquiring good health and treating of many ailments; including fever common cold, headache, asthma, rheumatic diseases from the intestine (Akhtar and Raffat, 1991; Al-Jassir, 1992, Al-Ghamdi, 2001). *Z. spinosa-christi* seed have very basic nutritious fruit and usually eaten fresh, they are applied on cut and ulcers, they are also used to treat pulmonary ailment, fever and to promote the healing of fresh wounds (Kaaria, 1998). The *Ziziphus species* have received a lot of interest in various scientific researches due to their potential medicinal values. They produce diverse bioactive compounds which are found to possess antimicrobial properties (Abalaka et al., 2010). The leaves are applied locally to sores and the roots are used to cure and prevent skin diseases (Adzu et al., 2001). The leaves of *Z. spinosa-christi* are applied locally to sores and the roots are used to cure and prevent skin diseases (Adzu, 2002). The plant is already used in many part of the world for the skin care. *Z. spinosa-christi* seed has been used traditionally as spice, carminative, condiment aromatic, stimulant, diuretic, stomach liver tonic and digestive, the rate at which the bacterial strains are becoming resistant to the available antibiotic necessitates the demands of a renewed effort to investigate for new antimicrobial agents (Hussaini et al., 2010; Hanafy and Hatem, 1991).

The *Z. spinosa-christi* seed were potentially a good source of antimicrobial compound. The use of these compounds for application in crop production was not as many as in medicinal field. (Hussaini et al., 2010). *Z. spinosa-christi* seed (extract) was found to contain benic acid, cyanoothic acid, cyclopeptide, as well as saponin glycoside, flavonoids, lipid, protein, free sugar and mucilage (Adzu et al., 2003). The plant has been useful as food and medicine and a few have been studied especially African medicinal plant (Lee et al., 2003; Adeboye and Opabode, 2004). They also contain vitamins needed by human body for healthy living (Szeto et al., 2002). *Z. spinosa-christi* is also used for treating fever, malaria, common cold headaches and asthma. The plant is already used in many part of the world for the care of skin, the chemical composition and phytochemicals present in the plant would suggest the ethno botanical pattern of this plant, their antimicrobial and antifungal properties are important in cosmetic application (Hosseinzadeh et al., 2007).

The aim of the present study was to investigate the potential of *Z. spinosa-christi* seeds extracts during different phases of germination on inhibition of bacterial growth.

**MATERIALS AND METHODS**

**Sample collection and preparation**

The fruits of *Z. spinosa-christi* were collected from the forest of Gerei Local Government Area of Adamawa State, Nigeria. The fruits were then washed, air dried at room temperature.

**Germination of seeds**

Seed for the germination were placed on four fold damp filter paper at 25°C and incubation in the dark till the initiation of sprouting after which they were placed at a light intensity of 100 µmol·s⁻¹ and a 14/10 h (day/night) photoperiod till the complete plant with two leaves was obtained. The duration of the germination is shown in Table 1.
Table 1. Duration of Z.spina-christi seed before germination.

<table>
<thead>
<tr>
<th>Germination</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st stage</td>
<td>20</td>
</tr>
<tr>
<td>2nd stage</td>
<td>8</td>
</tr>
<tr>
<td>3rd stage</td>
<td>7</td>
</tr>
<tr>
<td>4th stage</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 2. Different stages and zone of inhibition in millimetre (mm).

<table>
<thead>
<tr>
<th>Micro-organisms</th>
<th>1st stage</th>
<th>2nd stage</th>
<th>3rd stage</th>
<th>4th stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus feacalis</td>
<td>22</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>20</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>15</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Preparation of plant extract

The germinated seeds of Z. spina-christi at different phases of germination were thoroughly washed. Plant materials were shade dried and then ground to fine powder using a blender. Known weight of the dried material of the plant was extracted using soxhlet extractor with 95% ethanol at room temperature for 48 h in order to extract the bioactive compounds (AOAC method, 1980). After filtration, extracts were evaporated under reduced pressure using rotary evaporator. The fractions of extracts were stored at 4°C until use.

Antimicrobial investigation

Clinical isolates of Streptococcus feacalis, Staphylococcus aureus, Pseudomonas aeruginosa Escherichia coli, Klebsiella pneumonia were obtained from the microbiology laboratory of Federal Medical Centre, Yola.

The stocks were maintained on nutrient agar slant and sub-culture in nutrient both for incubation at 37°C prior to each antimicrobial testing. Inoculation of the test organisms on nutrient agar prepared plates was achieved by flaming a wire loop on a spirit lamp, cooling the wire loop (air cooling) and fetching the test organisms. The discs were prepared using No. 1 Whatman filter paper and putting in vials-bottles and sterilizing in an oven at 150°C for 15 min. Prepared discs containing the various extracts were carefully placed on the inoculated plates using a sterilized forceps in each case (Fatope, 1993). The plates were then turned upside-down and inoculate at 37°C for 24 h in an incubator. After incubation, the inoculated plates were observed for zones of inhibition (in mm diameter). The result was taken by considering the zone of growth and inhibition of the organisms by the test fraction (Mackie and McCartney, 1989). Activity and inactivity were observed in accordance with the standard and acceptable method. Results are shown in Table 2.

RESULTS AND DISCUSSION

The result shown in Tables 1 and 2 indicate that the leaf extract exhibited different inhibitory activities on the tested organisms. The ethanol extract on first stage exhibited the highest inhibitory activities against Streptococcus feacalis. While in the second stage it exhibited the lowest inhibitory activity on Staphylococcus aureus. The third and fourth stage shows no any zone of inhibition on the tested organisms. The crude extract of Z.spina-christi showed no effect on Escherichia coli and Klebsiella pneumonia.

The results obtain from the antimicrobial activity showed that the Z.spina-christi extract exhibited different inhibitory activity against the different tested micro-organisms from different stages of germination. The result reveals that the activity of the tested organism is inversely proportional to the increase in the in the amount of the extract or concentration of the extract.

CONCLUSION

Z. spina-christi seed extract during various germination stages possesses potential antimicrobial activity against some microorganisms.

REFERENCES


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