In Vitro Antibacterial activity of Methanolic-aqua extract of Tragia brevipes Leaves

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Abstract

The plant Tragia brevipes is used traditionally as purgative and in the treatment of stomach problems, in the lower eastern part of Kenya. The aim of this research was to evaluate the antibacterial activities of the crude extract of Tragia brevipes leaves. The crude was extracted using methanol and water in the ratio of 9:1. Antibacterial activity was done using well diffusion methods and the data analysed using SPSS software to compare the means and also check whether there was significance between the zones of inhibitions caused by the plant extract and also those caused by the positive control. Tragia brevipes was found to inhibit the growth of Escherichia coli (12.00 ± 0.577), Salmonella sp. (10.33 ± 0.333), Enterobacter aerogenes (9.33 ± 0.333), Bacillus cereus (23.67 ± 0.882), Serratia liquefaciens (5.00 ± 2.646) and Proteus vulgaris (8.67 ± 0.333). The results obtained scientifically justify the traditional use of the plant to treat against enteric microbes such as Salmonella typhi and Enterobacter aerogenes. Tragia brevipes can be used as an antibiotic against all the microorganisms tested.

Key-Words: Antibacterial activity, Tragia brevipes, Medicinal herbs, Well diffusion method

Introduction

Tragia brevipes is armed with needle like elongations on the leaves which the plant uses for protection against its enemies. The plant roots are used as purgative and also given to expectant mothers during labor pain to increase uterus contraction rate. The leaves of the plant are used to treat against rhematism by rubbing them on the knees and other joints [1]. The leaves are also used traditionally to treat against stomach problems.

Since plants cannot avoid the enormous number of potential enemies in their surrounding by simply moving away they must develop other ways to do so. Plants produce a group of compounds known as secondary metabolites to protect themselves against herbivores and pathogenic microbes. Almost every ecosystem contains a wide variety of viruses, fungi, bacteria, mites, nematodes, insects, mammals and other herbivores; hence the need for the plants to develop some defense mechanisms [2].

Secondary metabolites have no direct roles in the processes of photosynthesis, protein synthesis, translocation, nutrient assimilation, differentiation, carbohydrates formation, proteins and lipids, they differ from primary metabolites by the way they are distributed in the plant kingdom. The secondary metabolites unlike primary metabolites which are found throughout the plant kingdom, the secondary metabolites are found in specific species or related plant species only. Secondary metabolites can be grouped into three main categories’ viz. terpenoids, alkaloids and phenolic. Those chemicals produced by the plant and have effect on the growth, health, or behaviors of other organisms are called allelochemics. They can be divided into two, one those that appear to benefit the producer such as insect repellents, growth suppressants on competing species and poisons that protect against feeding organisms and two, those that seem to benefit the recipients [3]. The secondary metabolites are also referred to as phytochemicals. The difference in the type of these important compounds in the plants kingdom is the root for the difference in characteristics in plants of different species. Phytochemicals are non-nutritive plant chemicals that can protect the plant and
also prevent it against diseases [4]. Recent researches have shown that these secondary metabolites can protect humans and animals against pathogens [5]. According to Anthoney [6], the use of medicinal plants is greatly increasing even in the western world with 40% of the population in the developed countries using herbal medicine; however the number of the population using herbal medicine in the developing countries is still high with approximately 90% of the population using herbal medicine for treatment. The high number in the developing countries could be attributed to the lack of enough financial capability to acquire the commercially synthesized drugs which are expensive. There is growing interest in the use of herbal medicine which can be attributed to the believe that green medicine has no side effects and also dependable as compared to expensive synthetic drugs which have been associated with adverse side effects [7].

Secondary metabolites have medicinal value due to the presence of chemical compounds which produce definite physiological actions in the body of humans [8 & 9]. Researches done on plants have shown plants to possess great pharmacological values viz. antifungal activity, antibacterial activity, anticancer activity, antioxidant activity, anti-diabetic activity, hepatoprotective activity, anti-inflammatory activity, haemolytic activity, larvicidal activity, central nervous system activity, pupicidal activity, anthelmintic activity and pain relief activity [10, 11 & 12]. Due to multiple compositions in plants they have been known to possess multiple medicinal values hence making a good source of raw materials for the pharmaceutical industry [13].

The defense mechanisms of plants and the compounds produced to protect themselves from the environment have been studied in depth in the past and studies are still on. These compounds which plants produce have been analysed and found to have great medicinal value. Researches on species of Tragia have shown diverse pharmacological activity against various ailments affecting human beings today. According to Joshi [14], *Tragia involucrata* showed potent cytotoxicity with methanol and hexane showing the highest activity on MCF-7 and KB cell lines. The cytotoxicity of the plant was attributed to the presence of flavonoids in methanolic and chloroform extracts of the plant showing the highest significance as compared to standard ibuprofen. *Tragia plukenetii* a species in the same genus with *Tragia brevipes* was found to have antipyretic, antiasthma, antiplasmodic, diuretic and analgesic activity [17]. According to Farook [18], the plant *Tragia involucrata* was also found to have anti-hyperglycemic and hypolipidemic effect. To the best of our knowledge very little data is found on literature on the medicinal value of the plant *Tragia brevipes*. This study was done to analyse the antibacterial activity *Tragia brevipes* leaves against the selected bacteria.

**Material and Methods**

**Sample Collection and Preparation**

The herb was randomly collected from the natural forest around University of Eastern Africa, Baraton. The plant samples were identified by a taxonomist from Baraton University. The samples were thoroughly mixed and spread to dry at room temperature in the chemistry laboratory for about three weeks. They were then ground into fine powder and put in transparent polythene bags.

**Extraction procedure**

Using electric analytical beam balance 50 grams of the powdered leaves of *Tragia brevipes* was placed in 1000 ml conical flask, methanol and water were then added in the ratio of 9:1 respectively until the leaves were completely submerged in the solvent. The mixture was then agitated for thorough mixing and kept for 24 hours on a shaker for effective extraction of the plant components. The extract was filtered using Butchner funnel; Whatman no.1 filter paper and a vacuum and pressure pump. The filtrate was re-filtered again using the same apparatus. The solvent was evaporated using rotary vacuum evaporator (R-11) with a water bath at 40°C. The extract was brought to dryness using vacuum and pressure pump at room temperature. The residue was then obtained and analysed for antibacterial activity.

**Bioassay Study**

**Preparation of the Extract Concentrations and Antibiotic**

Stock solutions for the extracts were prepared by dissolving 500 mg in 1 ml of dimethylsulfoxide (DMSO). An antibiotic control was made by dissolving 1μg of Augmentin in 1 ml of sterile distilled water. DMSO served as a negative control.

**Preparation of the Bacterial Suspension**

The turbidity of each of the bacterial suspension was prepared to match to a 0.5 McFarland standard, a
procedure similar to that used by Biruhalem [19] and Donay et al., [20] with slight modifications. The McFarland standard was prepared by dissolving 0.05 g of BaCl₂ in 50 ml of water to obtain a 1% solution of Barium chloride (w/v). This was mixed with 99.5 ml of 1% sulphuric acid solution. Three – five identical colonies of each bacterium was taken from a blood agar plate (Himedia) culture and dropped in Mueller Hinton broth (Himedia). The broth culture was incubated at 37°C for 2 - 6 hours until it achieved turbidity similar to the 0.5 McFarland standards. The culture that exceeded the 0.5 McFarland standard were each adjusted with the aid of a UV spectrophotometer to 0.132Å at a wavelength of 600 nm in order to obtain an approximate cell density of 1x10⁸ CFU/ml.

Determination of bioactivity of the Extract
Mueller Hinton agar plates were prepared by the manufacturer’s instruction. 0.1 ml of each of the prepared bacterial suspension for the test was transferred to 3 plates for each organism to give a triplicate for each concentration and organism. Five wells were drilled in each agar plate. Three of the wells were filled with the extract dilution and the other wells were filled with Augmentin and DMSO control respectively. Three plates were made for each bacterial organism and extract giving a triplicate reading for each microorganism and extract. The wells were labeled on the underside of the plate. The plates were incubated at 37°C for between 24 to 48 hours and the zones of inhibition were measured in millimeters with the aid of a ruler.

Results and Discussion

Table 1: Zones of Inhibition (mean± S.E.) of 500 mg/ml of Tragia brevipes Against Selected Microorganisms

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Mean ± S.E.</th>
<th>Penicillin</th>
<th>DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>12.00±0.577</td>
<td>51.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>10.33±0.333</td>
<td>30.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>S. liquefaciens</td>
<td>5.00±2.646</td>
<td>45.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>9.33±0.333</td>
<td>33.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>23.67±0.882</td>
<td>30.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>8.67±0.333</td>
<td>31.00±0.00</td>
<td>0.00±0.00</td>
</tr>
</tbody>
</table>

Table 2: Tukey’s Honestly Significant Difference Among Microorganisms Using 500mg/ml of Tragia brevipes Extract

<table>
<thead>
<tr>
<th>Comparison</th>
<th>P. value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli vs. Salmonella</td>
<td>0.911</td>
<td>NS</td>
</tr>
<tr>
<td>E. coli vs. S. liquefaciens</td>
<td>0.013</td>
<td>S</td>
</tr>
<tr>
<td>E. coli vs. Enterobacter aerogenes</td>
<td>0.620</td>
<td>NS</td>
</tr>
<tr>
<td>E. coli vs. Bacillus cereus</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>E. coli vs. Proteus vulgaris</td>
<td>0.402</td>
<td>NS</td>
</tr>
<tr>
<td>Salmonella sp. Vs S. liquefaciens</td>
<td>0.067</td>
<td>NS</td>
</tr>
<tr>
<td>Salmonella sp. Vs Enterobacter aerogenes</td>
<td>0.989</td>
<td>NS</td>
</tr>
<tr>
<td>Salmonella sp. vs Bacillus cereus</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>Salmonella sp. vs P. vulgaris</td>
<td>0.911</td>
<td>NS</td>
</tr>
<tr>
<td>S. liquefaciens vs. E. aerogenes</td>
<td>0.175</td>
<td>NS</td>
</tr>
<tr>
<td>S. liquefaciens vs. B cereus</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>S. liquefaciens vs. P. vulgaris</td>
<td>0.998</td>
<td>NS</td>
</tr>
<tr>
<td>E. aerogenes vs. B. cereus</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>E. aerogenes vs. P. vulgaris</td>
<td>0.998</td>
<td>NS</td>
</tr>
<tr>
<td>B. cereus vs. P. vulgaris</td>
<td>0.000</td>
<td>S</td>
</tr>
</tbody>
</table>

KEY: S=Significant; NS= Not Significant

From the study (Table 1) the plant extract was found to have antibacterial activity against all of the six bacteria it was tested against. Tragia brevipes was found to inhibit the growth of Escherichia coli (12.00 ± 0.577), Salmonella sp. (10.33 ± 0.333), Enterobacter aerogenes (9.33 ± 0.333), Bacillus cereus (23.67 ± 0.882), Serratia liquefaciens (5.00 ± 2.646) and Proteus vulgaris (8.67 ± 0.333).

Analysis of variance the mean zones of inhibition of 500 mg/ml of Tragia brevipes (Table 2) showed that there was significant difference in the zones of inhibition among the bacterial organisms (p<0.001). Further comparisons showed that the zones of inhibition of E. coli were significantly bigger than that
The extract of Tragia brevipes was found also to be used to treat immunologically compromised panophthalmitis. The plant compounds can inhibit the growth of Bacillus, Enterobacter aerogenes, and especially the bacteria tested against. The plant can be used to treat against all the ailments caused by the bacteria tested against. The results obtained in this work are in conformity with the data recorded by Panda [21], were a similar species Tragia involucrata demonstrated antibacterial activity against Staphylococcus aureus a gram positive bacteria and Escherichia coli a gram negative bacterial. The plant also showed antibacterial activity against all the gram positive and gram negative bacteria used in the study by Panda [21].

The plant Tragia brevipes methanol aqua extract can be a potent antibiotic against all the ailments caused by the bacteria tested against. The plant can be used to treat against Salmonella typhi which causes typhoid fever and also Salmonella paratyphi A, B and C which causes paratyphoid fever. The isolation, purification and the use of the active compounds from the plant can be a great breakthrough in the treatment against this dangerous disease in the world. Salmonellosis can cause relapse, death or cause serious complications that may include gastrointestinal bleeding, typhoid encephalopathy and intestinal perforation to approximately 10% of all the infected patients [22 & 23]. The compounds can also be used as sanitary agents in food handling and in the veterinary industry to treat domestic animals, which are carriers of these pathogens hence preventing infection to human at the human-animal interphase [24].

The extract from the plant Tragia brevipes also was found to inhibit the growth of Bacillus cereus. From these results it is therefore worthy to mention that the plant can be used to treat against all the infections caused by Bacillus cereus and even other species of Bacillus viz self-limited gastroenteritis, posttraumatic wounds, surgical wounds infections, burns, ocular infections such as endophthalmitis, corneal abscess and panophthalmitis [25& 26]. The plant compounds can also be used to treat immunologically compromised patients including AIDS and malignant disease victims [27 & 28]. The extract of the plant was found also to inhibit the growth of Enterobacter aerogenes, hence showing high potency of the plant to treat against this important hospital pathogen which is responsible for nosocomial respiratory tract infections [29 & 30].

plants capability to inhibit the growth of E. coli is a scientific proves that the plant can be used to great extent to treat against enteric infections caused by the bacteria. The plants extract can treat against gastrointestinal diseases, ear infections, urinary tract infections and wounds infections caused by Proteus vulgaris [31 & 32].

Conclusion

The antibacterial activity could be attributed to the phytochemicals present in the plant which according to Anthoney [33], plant leaves were found to contain tannins, saponins, terpenoids, flavonoids, and phenols. According to Ngule [34], the plants infused stem was also found to contain tannins, saponins, phenols, and alkaloids. Similar phytochemicals found in the plant have shown great pharmacological activity in other studies and therefore their presence in the plant greatly gives it its high potency in pharmacology [35 & 36]. The data obtained and recorded in this study is a scientific justification of the plants traditional use. The traditional practice is therefore recommended for the use of the plant in the treatment of all the ailments caused by the bacteria used in this study. More research needs to be done to isolate the plants active compounds, there medicinal value in comparison with other commercial drugs, analyse there effect in the in vivo environment and there mode of action against various microorganisms.

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