Comparative antimicrobial activities of different solvent extracts and a refreshing drink (Sobolo) made from Hibiscus sabdariffa Linn.


Abstract
The calyx of Hibiscus sabdariffa is used in making refreshing drinks in many parts of Africa including Ghana. The aim of the current study is to find out if a refreshing drink prepared from the calyx (known as sobolo) has antimicrobial activity and also to compare the antimicrobial activities of different solvent extracts of the calyx; using the agar well diffusion and the micro-broth dilution methods.

Sobolo showed the greatest antimicrobial activity with average diameter of zone of inhibition in the range 12–19 mm against the microbes employed in the study. The polar extracts exhibited the greatest antimicrobial activity with MICs of 125–250 µg/ml against the bacteria, and 1000 µg/ml against the fungi. The medium polar solvent extract showed average activity and the non-polar one, the least activity.

The study has shown that sobolo has antimicrobial activity, and that polar solvents are more effective in the extraction of the antimicrobial principles in the plant.

Keywords: Sobolo, Hibiscus sabdariffa, refreshing drink, antimicrobial activity.

1. Introduction
In many developing countries greater number of the population employ folk medicine for the treatment of diseases and infections of common occurrence [1]. Many traditional healers have claimed that medicinal plants are more effective, cheaper and more organic than modern medicine. It is believed that inhabitants of rural communities where plants are the main source of medication, and those who take in a lot of vegetables, fruits, seeds, plant juices and other products from plants have a reduced risk of getting infectious diseases from resistant pathogens. This is probably due to the fact that these plants and/or their products contain numerous compounds that may have antimicrobial activities; perhaps acting as prophylaxis for those who use these plants. It is possible that many medicinal plants used as condiments, spices or for culinary and other purposes may still exert their medicinal activities when used for these non-medicinal purposes. One such plant worth investigating is Hibiscus sabdariffa. H. sabdariffa Linn belongs to the plant family Malvaceae, the same family to which popular plants such as Cola nitida (cola) and Theobroma cacao (cocoa) belong. The genus Hibiscus has more than forty species; the species sabdariffa being one of the most common ones, perhaps because of its numerous therapeutic claims in many parts of the world.

H. sabdariffa is an important annual or perennial erect, mostly branched, shrub that is grown successfully in tropical and sub-tropical climates [2]. It takes about five months from planting to harvesting. The plant is widely cultivated for commercial purposes in the Tropical and Sub-tropical regions for its fibre and edible calyx; the most important part being the fleshy calyx (sepals) that surrounds the fruit (capsules). Additionally, it is grown for culinary and ornamental purposes in much of the tropical world.

The plant is known by different names in different parts of the world. These include roselle, razzelle, sorrel (red sorrel, Jamaican sorrel, Indian sorrel, Guinea sorrel) sour-sour, and Queensland jelly plant [3]. According to Kays [4] the Japanese call it rohzelu; and the Hindus, lal-ambari, patwa or laalambaar; it is also called sabdriqa or lalambari in Urdu. The Yorubas of southwestern Nigeria call it 'Isakpa' [5].

Roselle (H. sabdariffa) has many traditional and medicinal uses around the world. In Chinese traditional medicine and also in Senegal it is used in the management of hypertension, as well
as pyrexia and liver diseases [6-7]. Its sepal extract has been used as a valuable treatment option against leukemia [8]. Infusions of the leaves and calyces are employed as diuretic, cholereic, febrifugal and hypotensive, decreasing the viscosity of the blood and stimulating intestinal peristalsis. It has antispasmodic, anthemiltic and antibacterial activities as well. Roselle extract is claimed to decrease the rate of absorption of alcohol and so lessen its effect on the system [9]; thus in Guatemala, roselle (ade) is a favourite remedy for the after-effects of drunkenness.

*H. sabdariffa* is rich in anthocyanins and protocatechuic acid. The dried calyces contain the flavonoids gossypetin, hibiscetin and sabdaretine. The major pigment, formerly identified as daphniphylline. Small amounts of myrtillin (delphinidin-3-monoglucoside), and delphinidin are also present. Roselle seeds are a good source of lipid-soluble antioxidants, particularly γ-tocopherol [10].

The demand for plant-based therapeutics is increasing in both developing and developed countries because of growing recognition that they are natural products, non-narcotic, and easily biodegradable, producing minimal environmental hazards, having minimal adverse effects, and being easily available at affordable prices [11].

In our search to investigate edible plants for potential antimicrobial activities, the calyx of *H. sabdariffa*, which is commonly used to make fruit drink in many African countries, came to our attention. Though some antimicrobial activity has been done on the plant [12], the present study aims at finding out if the refreshing drink (called *sobolo* in Ghana) prepared from the calyx of *H. sabdariffa* has antimicrobial activity. Additionally, the type of solvent (namely petroleum ether, ethyl acetate, methanol and water) that is best able to extract the antimicrobial principle from the plant is to be investigated. Furthermore, it also seeks to investigate how the antimicrobial activity of the refreshing drink compares with that of the different solvent extracts.

### 2. Materials and Method

#### 2.1 Plant Material

Dried calyx of *H. sabdariffa* was purchased from the Kumasi Central Market in the Ashanti Region of Ghana. It was identified by experts at the Faculty of Agriculture at the Kwame Nkrumah University of Science and Technology.

#### 2.2 Extraction of Plant Material

Extraction of the plant material was done using four different solvents namely petroleum ether, ethyl acetate, methanol and water. The plant material was divided into four 20g portions and each extracted with 60 ml of one of the mentioned solvent using the cold maceration method. Each extract was filtered through a plug of cotton wool and then Whatman’s Number 10 filter paper. The four samples were each dried and labelled appropriately. The petroleum ether extract was labelled *P*, ethyl acetate extract *T*, methanol extracts *M* and the water (aqueous) extract, *Q*.

#### 2.3 Preparation of Sobolo

One of the recipes used in preparing *sobolo* (a refreshing drink made from the dried calyx of *H. sabdariffa*) in the local area was employed for a fifth sample preparation. Here the calyx was boiled, and extracts of ginger rhizome and pineapple fruit added. Additionally, honey was added to make it sweet to taste. A fifty (50) ml of this preparation contained 10 g of the calyx, 0.5 g of ginger, 2 g of pineapple extract and 2 g of honey. This preparation was also labelled S.

### 2.4 Micro-organisms Used

The micro-organisms employed in the study consisted of both Gram-positive and Gram-negative bacteria and a clinical isolate of the yeast-like fungus *Candida albicans*. The Gram-positives were *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (NCTC 10073) and the Gram-negatives included *Klebsiella pneumonia* (ATCC 70063) and *Escherichia coli* (ATCC 25922).

#### 2.5 Antimicrobial Assay

The agar well diffusion method [13] was used in the assay. Wells were punched on the surface of Nutrient agar plates seeded with 100 µl of an overnight broth suspension of bacteria containing 10^6 CFU/ml of organism. Different plates were prepared for each organism. Cork borer number three of diameter 6 mm were used to punch holes on the agar. One hundred microlitres (100 µl) each of the plant extracts at a concentration of 5 mg/ml, and the prepared drink (sobolo) were introduced into the wells. The negative control used was sterile water which was employed to reconstitute the extracts, and the positive controls were 100 µg/ml Gentamicin (G) against the bacteria and 100 µg/ml Clotrimazole (C) against the *C. albicans*, the fungus. The plates were allowed to sit on the laboratory bench for at least 1 hour before incubating at 37 °C for 24 h. The antimicrobial activity was evaluated by taking measurements of the diameters of the zones of inhibition against the test microbes.

#### 2.6 Determination of Minimum Inhibitory Concentration

The Micro-broth dilution method using 96-well plates [14] which is suitable for determination of Minimum Inhibitory Concentration (MIC) was used to determine the MICs of the samples. Each sample was serially diluted two-fold with Nutrient broth to give a dilution range of 2000 µg/ml to approximately 2 µg/ml in 96 well-plates. One hundred microlitres (100 µl) of overnight broth culture of organisms containing 10^5 CFU/ml was added to each well, and incubated at 37 °C for 18 h.

#### 2.7 Statistical Analysis

The experiments were run in triplicates; and the results were expressed as Mean ± SD (standard deviation) data using Microsoft Excel (Windows 2007).

### 3. Results and Discussion

The results obtained from the study indicates that all the different solvent fractions of *H. sabdariffa* exhibited antimicrobial activity against the selected Gram negative and Gram positive bacteria as well as the yeast-like fungi *C. albicans*. The highest activity was exhibited by the refreshing drink, *sobolo* (S), followed by the water or aqueous (Q) and the methanol (M) extracts (Table 1); and the least activity was given by the petroleum ether extract (*P*). The polar solvents seem to be relatively better at extracting the antimicrobial principles from the plant. The antimicrobial activity is more prominent against the Gram positive organisms (*S. aureus* and *B. subtilis*) than the Gram negatives (*E. coli* and *K. pneumonia*). It is generally known that most chemicals used against microbes exert superior activity against the Gram positives as a result of differences in the cell wall structures between Gram-negative and Gram-
positive bacteria. The cell wall of the Gram-positives is comparatively thicker, continuous and comprises peptidoglycan which may covalently be attached to other cell polymers such as teichoic acids, polysaccharides and peptidoglycolipids. The Gram-negatives have cellular compartment or periplasmic space which is a region between the outer surface of the inner membrane and the inner surface of the outer membrane containing hydrolytic enzymes and binding proteins [15]. The presence of enzymes in the periplasmic space (which is absent in Gram-positive bacteria) is capable of disintegrating molecules introduced from outside the cell. Furthermore, the porin (membrane proteins) in the Gram-negatives provides a channel that limits the passage of hydrophilic compounds across the outer membrane; it therefore serves as a barrier to external molecules [15-16].

In the study, sterile water was used to reconstitute the extracts; aeruginosa [21]. Clotrimazole which was used as control against the micro-organisms such as Bacillus sp and Pseudomonas aeruginosa.

In the study, sterile water was used to reconstitute the extracts; it was therefore employed as the negative control in the experiments. This did not exhibit any activity (results not shown) indicating that the observed antimicrobial activity came from the extracts alone; it was not augmented in any way by the reconstituting solvent. Gentamycin was the positive control used in comparison to the activities of the extracts and the drink. Gentamycin was the standard broad spectrum antibiotic used clinically in the treatment of many diseases caused by both Gram-negative and Gram-positive pathogens [21]. Clotrimazole which was used as control against the C. albicans also showed a better antimicrobial activity than the

Table 1: Antimicrobial activity of different solvent extracts (P, T, M, and Q) of H. sabdariffa at a concentration of 5 mg/ml; 200 mg of crude calyx in one ml of water for sobolo (S); and 100 µg/ml of the controls Gentamicin and Clotrimazole. (n = 3)

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Average Zones of Inhibition (mm)</th>
<th>Sobolo</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P (µg/ml)</td>
<td>T</td>
<td>M</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>13±0.5</td>
<td>15±0.5</td>
<td>16±0.5</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>14±1</td>
<td>16±0.5</td>
<td>18±1.0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>11±0.5</td>
<td>12±0.6</td>
<td>13±0.5</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>09±0.5</td>
<td>10±0.6</td>
<td>11±0.6</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>12±1.0</td>
<td>14±1.0</td>
<td>15±0.6</td>
</tr>
</tbody>
</table>

Key: P – Petroleum ether extract; T – Ethyl acetate extract; M – Methanol extract; Q – Aqueous extract; S – Sobolo Drink; G – Gentamicin; C – Clotrimazole NA – Not applicable

Table 2: Minimum Inhibitory Concentrations (MICs) of the extracts of H. sabdariffa, the refreshing drink (sobolo) and the controls. (n = 3)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Average MICs (µg/ml)</th>
<th>Sobolo (mg/ml)</th>
<th>Controls (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>T</td>
<td>M</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1000</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>500</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>2000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>&gt;2000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>&gt;2000</td>
<td>&gt;2000</td>
<td>1000</td>
</tr>
</tbody>
</table>

Key: As under Table 1. NB; Triplicate experiments gave the same results (MICs).
5. References


