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

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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 Malvacaea, 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, razelle, 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, choleretic, febrifugal and hypotensive, decreasing the viscosity of the blood and stimulating intestinal peristalsis. It has antispasmodic, anthelmintic 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, hibiscetine and sabdaretine. The major pigment, formerly reported as hibiscine, has been identified as daphniphylline. Small amounts of myrtillin (delphinidin-3-monoglucoside), chrysanthenin (cyanidin-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 minimum 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. Addditionally, 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 1hour before incubating at 37 ºC 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 ºC 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. sabdarriffa* 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 (plasma) 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].

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)

Organisms	Average Zones of Inhibition (mm)						
	Extracts				Sobolo	Controls	
	P	T	M	Q	S	G	C
Staphylococcus aureus	13±0.5	15±0.5	16±0.5	16±0.6	18±0.5	23±0.5	NA
Bacillus subtilis	14±1	16±0.5	18±1.0	18±0.5	19±0.3	26±1.0	NA
Escherichia coli	11±0.5	12±0.6	13±0.5	13±0.3	15±0.5	24±1.0	NA
Klebsiella pneumonia	09±0.5	10±0.6	11±0.6	11±0.3	12±0.3	23±0.3	NA
Candida albicans	12±1.0	14±1.0	15±0.6	15±0.0	16±0.3	NA	22±0.5

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)

Organism	Average MICs						
	Extracts (µg/ml)				Sobolo (mg/ml)	Controls (µg/ml)	
	P	T	M	Q	S	G	C
Staphylococcus aureus	1000	500	250	250	12.5	7.8	NA
Bacillus subtilis	500	500	250	125	12.5	3.9	NA
Escherichia coli	2000	1000	1000	500	25.0	7.8	NA
Klebsiella pneumoniae	>2000	1000	1000	500	25.0	7.8	NA
Candida albicans	>2000	>2000	1000	1000	100.0	NA	16.0

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

Fraction **S** (sobolo) which is normally taken as a refreshing drink gave a relatively higher activity than all the other fractions (Table 1 and 2). This can be attributed to a possible synergistic activity of the added ingredients. Pineapple, for example, has been found to possess antibacterial activity [17]. Additionally, ginger [18] and honey which are part of the added ingredients have both been reported to possess antibacterial activity against bacteria, including *Staphylococcus aureus*, in carious teeth [19]. This finding is similar to that reported by Obo and Elusiyan [20], that fortification of roselle extract with pineapple juice and lemon grass enhanced inhibition of growth of 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 against the bacteria. As seen from Tables 1 and 2 it gave a superior antibacterial activity than that of the extracts and the refreshing drink. Gentamicin is a 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 extracts and the drink.

Reported phytochemical investigation of the calyx of the plant has shown that it contains various flavonoids and other polyphenolic compounds [22], and flavonoids have been reported to be responsible for antimicrobial activities of many plants against a broad range of microbes [23]. Flavonoids and other hydroxylated phenolic compounds have the ability to form complexes with extracellular and soluble proteins and to complex with bacterial cell wall [24]. This probably accounts for the antibacterial activities of flavonoids. Presence of flavonoids in the plant might have contributed partially or significantly to the observed antimicrobial activities.

4. Conclusion

The calyx of *H. sabdariffa* has exhibited antimicrobial activity against both Gram-positive and Gram-negative bacteria as well as *Candida albicans*. Extracts obtained with polar solvents (methanol and water) showed a relatively better antimicrobial activity than the medium polar (ethyl acetate) and the non-polar (petroleum ether) extracts. The study has shown that the refreshing drink (sobolo) made from the calyx of the plant has an enhanced antimicrobial activity because of the added ingredients. In general Gram-positive bacteria are more susceptible to the antibacterial effects of the plants extracts than the Gram-negatives.

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