Journal of Intercultural Ethnopharmacology
www.jicep.com
DOI: 10.5455/jice.20160320022623

Short Communication

Antibacterial efficiency of the Sudanese Roselle (*Hibiscus sabdariffa* L.), a famous beverage from Sudanese folk medicine

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Received: February 02, 2016
Accepted: March 01, 2016
Published: March 24, 2016

ABSTRACT

Background: *Hibiscus sabdariffa* L. is a plant native to tropical Africa and intensively cultivated in Sudan. Its calyces are widely consumed with many uses in Sudanese folk medicine. **Materials and Methods:** The dried calyces of *H. sabdariffa* were subjected to soak in 80% v/v methanol to get the methanolic extract, which was tested against five Gram-negative and three Gram-positive referenced bacterial strains using disc diffusion method. Selected bioactive phytochemical compounds were also investigated using qualitative methods. **Results:** The results of the antibacterial test indicate that the methanol extract of *H. sabdariffa* calyces contained effective antibacterial agent(s), revealed a considerable zone of inhibition against all tested Gram-negative and Gram-positive bacteria, and it was a competitor to gentamicin and greatly higher than penicillin which showed weak or no effect. **Conclusion:** The results of current investigation support the folk medicine application of this plant against different microbial ailments and suggest it as a promising source for new antibacterial agents.

KEY WORDS: Antibacterial, disk diffusion, *Hibiscus sabdariffa*, methanol extract, phytochemical

INTRODUCTION

The battle with diseases started since the advent of man on earth and it will continue in an endless warfare. The discovery of antibiotics in the 1950s has turned the result of this war in favor of man, but few years later microbes returned with mutant strains, resistant to almost all inventive antibiotics. This forced scientists searching for new alternatives to be used against these adaptable microorganisms. Stupendous plants were the main renewable source for medications since times immemorial. Yet, many drugs from the modern medicine are originally derived from ancient herbal medicine. Currently, the dramatic increase in resistance of pathogens to current antibiotics leads to the requisite need for new antimicrobial agents [1,2].

Plants, particularly those prescribed against microbial infections since a long time in traditional and folk medicine from different societies could be promising sources for new antimicrobials [3]. In Sudan, the majority of Sudanese people, like many African countries, are still relying on traditional or folk medicine in treatment of diseases which are an integral part of an informal healthcare system, although this popular folk medicine has roots from Islamic and West African medicine [4]. Roselle (*Hibiscus sabdariffa* L) belongs to Malvaceae family, it is an annual tropical small shrub native to Africa and also distributed in Southeast Asia and Central America [5,6], it is Known locally as Karkadeh. The macerate of the red calyces of this plant [Figure 1] is one of the most famous public Sudanese beverages all over this country and all Sudanese people know and use, which are consumed as hot or cold drinks for the treatment of respiratory tract infections, hypertension, colds, and fever. It is also mixed with other plants to treat malaria [7,8].

Internationally, *H. sabdariffa* is well known, many parts of *Hibiscus sabdariffa* is employed and prescribed in traditional medicine in many countries such as African countries, India, Mexico, Brazil, China, and Iran [9]; leaves which eaten as vegetables are diuretic, antiseptic, digestive, purgative, sedative, demulcent, and astringent [10]; calyces are used for treating of hypertension, liver disorders, diuretic, digestive, and sedative [5,11,12]; seeds are rarely mentioned in traditional medicine compared to the other parts of *Hibiscus*, but seeds are roasted and consumed as food, also used traditionally as debility, diuretic, laxative, and tonic [13]. Although Sudan is the biggest African countries cultivated *H. sabdariffa* but scientific studies on this product is not adequate. The red *Hibiscus* calyces were found to have a pigment called anthocyanins; it is also rich in

Figure 1: The dried calyces of *Hibiscus sabdariffa*

polyphenolic compounds such as flavonoids and phenolic acids such as gallic and protocatechuic acid [14]. The objective of this study was to evaluate the antibacterial potential of the calyces of the Sudanese Roselle (*H. sabdariffa*) which intensively used in Sudanese folk medicine.

MATERIALS AND METHODS

Plant Material

Calyces of *H. sabdariffa* were purchased from local herbal markets in Khartoum, Sudan [Figure 1]; Dried calyces were crushed into fine powders, kept dry in a clean, well-tightened glass container until used.

Preparation of Methanol Extract

A total of 100 g of the dried powder of *H. sabdariffa* calyces was weighted and put in a sterile glass container, 500 ml of 80% methanol (to serve as hydroalcoholic solvent) was added gradually and soaked, and then the container was well tightened and kept in the refrigerator to avoid any microbial contamination or fungal growth. The closed container was subjected to frequent shaking (2-3 times a day) and macerated for up to 3 days. Then, the macerate was filtered using Whatman filter papers No.1; the filtrate was put in the incubator and allowed to evaporate at 45°C for up to 10 days to get a semi-solid extract. For antibacterial testing, the extract was reconstituted in absolute methanol to get a working concentration (500 mg/ml) and kept in a closed dark container until used.

Microorganisms

Eight referenced bacterial strains were used in testing the antibacterial activity of the methanol extract of *H. sabdariffa* calyces. These bacteria are representing five Gram-negatives (*Escherichia coli* ATCC 25922, *Salmonella enteric* ATCC 5174, *Klebsiella pneumonia* ATCC 27736, *Proteus vulgaris* ATCC 49132, and *Pseudomonas aeruginosa* ATCC 27853) and three Gram-positives (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 49461, and *Bacillus cereus* ATCC 10876). Bacterial strains were purchased from Watin-Biolife, KSA.

Inoculum Preparation

Bacterial strains were cultured following the manufacturer's instructions. Bacterial cultures were identified microscopically and biochemically, then sub-cultured in sterile bottles containing Nutrient Broth (Scharlab, S.L., and Spain) and incubated overnight at 37°C. Prior of the antibacterial experiment, the working bacterial samples were adjusted to 0.5 McFarland to be equivalent to about 1-2 × 10^8 CFU/ml.

Antibacterial Assay

The antibacterial activity of methanol extract of *H. sabdariffa* calyces was evaluated by disc diffusion method as mentioned in Abdallah and Al-Harbi [15]; 100 µl from each working bacterial cultures were mixed with 20 ml warm autoclaved Mueller-Hinton agar (Watin-Biolife, KSA) in glass bottles size 50 ml, tighten and mixed well and then poured directly into 90 mm sterile plastic disposable plates (Jalil Medicals) and left to solidify at room temperature. 6 mm discs were previously prepared using Whatman No.1 filter paper, sterilized and saturated with 300 mg/ml of the methanol extract of *H. sabdariffa* calyces, the pre-experimental test showed that the blank disk (6 mm) could carry 20 µl of the extract at a concentration of 500 mg/ml, which equivalent to 10 mg/disc. Then, the wet, saturated disks were directly loaded on the cultured Mueller-Hinton Agar plate in aseptic conditions. On the same plate, 6 mm antibiotic discs; penicillin G 10 units and gentamicin 10 µg (Oxoid) were also loaded, which served as positive controls. Cultured plates with extract and antibiotic discs were incubated for about 24 h at 37°C. The mean zone of inhibition of two replicated disks on the same plate was taken in millimeter (mm) using a ruler, 6 mm zone diameter considered as no inhibition.

Preliminary Phytochemical Analysis

The phytochemical constituents of the same methanol extract which used in antibacterial testing were evaluated qualitatively as described elsewhere, for alkaloids (Mayer's test) [16], saponins (Foam test), tannins (Ferric chloride test), anthraquinones (Bornträger test) [17], phenolic compounds, and flavonoids were also investigated [18].

Statistical Analysis

All the statistical analyzes were performed with the SPSS 17.0 (SPSS Inc., Chicago, USA) statistical package, variables considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The current study revealed the considerable antibacterial activity of methanol extract of *H. sabdariffa* calyces against all tested bacteria, particularly the Gram-positives, as shown in Tables 1 and 2, respectively. Disc inhibition zones above 10 mm for the tested crude extract considered a good antibacterial activity (Blank disc diameter 6 mm). Interestingly, the investigated extract was found to inhibit

Table 1: The antibacterial activity of methanol extract of *H. sabdariffa* calyces by disc diffusion method (10 mg/disc) against Gram-positive bacteria

Test	Zone of inhibition (mm)*				
	P. aeruginosa	*E. coli*	*K. pneumonia*	*S. enterica*	*P. vulgaris*
80% v/v methanol extract 10 mg/disc	15.5±0.5	14.5±0.5	17.5±0.5	17.5±1.5	14.5±0.5
Penicillin G 10 units/disc	6.0±0.0	6.0±0.0	7.0±0.0	9.0±0.0	6.5±0.0
Gentamicin 10 μg/disc	20.0±0.0	18.0±0.0	21.0±0.0	12.0±0.0	20.0±0.0

*Inhibition zones are the mean including disc diameter (6 mm)±standard error of means, mm: millimeter, *P. aeruginosa: Pseudomonas aeruginosa* ATCC 27853, *E. coli: Escherichia coli* ATCC 25922, *K. pneumonia: Klebsiella pneumonia* ATCC 27736, *S. enterica: Salmonella enterica* ATCC 5174, *P. vulgaris: Proteus vulgaris* ATCC 49132, *H. sabdariffa: Hibiscus sabdariffa*

Table 2: The antibacterial activity of methanol extract of *H. sabdariffa* calyces by disc diffusion method (10 mg/disc) against Gram-negative bacteria

Test	Mean zone of inhibition (mm)*		
	S. aureus	*S. epidermidis*	*B. cereus*
80% v/v methanol extract 10 mg/disc	18.5±0.5	17.5±1.5	13.5±1.5
Penicillin G 10 units/disc	9.0±0.0	6.0±0.0	7.0±0.0
Gentamicin 10 μg/disc	15.0±0.0	6.5±0.0	10.0±0.0

*Inhibition zones are the mean including disc diameter (6 mm) ±standard error of means, mm: millimeter, *S. aureus: Staphylococcus aureus* ATCC 25923, *S. epidermidis: Staphylococcus epidermidis* ATCC 49461, *B. cereus: Bacillus cereus* ATCC 10876, *H. sabdariffa: Hibiscus sabdariffa*

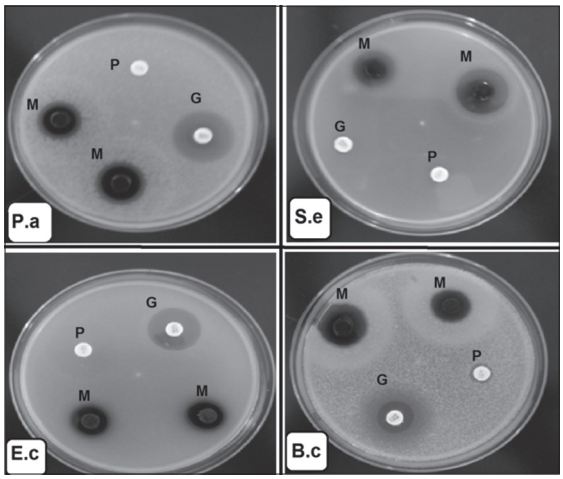

Figure 2: Representative photos showing the antibacterial activity of the methanol extract of *Hibiscus sabdariffa* calyces at concentration 500 mg/ml (10 mg/disc). P: Penicillin G 10 units/disc, G: Gentamicin 10 μg/disc, M: 80% Methanol extract of *Hibiscus sabdariffa* calyces 10 μg/disc, P.a: *Pseudomonas aeruginosa* ATCC 27853, S.e: *Staphylococcus epidermidis* ATCC 49461, E.c: *Escherichia coli* ATCC 25922, B.c: *Bacillus cereus* ATCC 10876

the growth of all tested Gram-positives and Gram-negatives, giving clear, obvious zones by disc diffusion method compared with the antibiotics [Figure 2], being statistically significant ($P < 0.05$). The highest antibacterial activity of *H. sabdariffa* calyces was recorded by *S. aureus* (18.5 ± 0.5 mm), followed by *S. epidermidis* (17.5 ± 1.5 mm), *S. enteric* (17.5 ± 1.5 mm), *K. pneumonia* (17.5 ± 0.5 mm), *P. aeruginosa* (15.5 ± 0.5 mm), *E. coli* (14.5±0.5 mm), *P. vulgaris* (14.5±0.5 mm), and *B. cereus* (13.5 ± 1.5 mm), respectively. Similar several previous studies were reported on the calyces of *H. sabdariffa* using different solvents, concentrations, and bacterial strains (clinical and referenced strains); Borrás-Linares *et al.* [19] published that the ethanol extract from 25 varieties of mexican *H. sabdariffa* calyces was effective against all Gram-negatives (*E. coli* and *S. enteritidis*) and Gram-positives (*S. aureus* and *Micrococcus luteus*), the greater effect was against the Gram-positive bacteria. Its ethanol extract was potent against bacteria isolated from wastewater, particularly *P. aeruginosa* [20]. Aqueous, ethanol, and methanol extracts *H. sabdariffa* calyxes' revealed good antibacterial activity against *Salmonella* cultures [6]. Its 80% methanol extract was also found active against Escherichia coli O157:H7, a major foodborne pathogen [21] and suggested as potential antibacterial in foods [22]. The current investigation and the majority of previous published studies unanimously agreed that *H. sabdariffa* calyces which collected from different localities around the world have effective antibacterial properties; this feature makes it a unique and promising antibacterial plant. It is known that the bioactivity of the extracts from the same plant species may vary according to the plant extraction process which affect greatly by many factors such as type of solvents, method of extraction, temperature (hot or cold liquids), age of the plant and season of harvesting [23], this stability of *H. sabdariffa* calyces as distinguished antibacterial agent is very interesting. Moreover, as represented in Figure 3, the antibacterial activity of the crude methanol extract of *H. sabdariffa* calyces (10 mg/disc) was significantly higher than penicillin G (10 units), and non-significant statistically when compared with the gentamicin (10 μg/disc), meaning that this plant extract is competitor to gentamicin. This is interesting since antibiotics are suffering from deterioration in its effectiveness around the globe [24], *S. aureus* is one of the most prevalent antibiotics resistant pathogens worldwide, particularly the methicillin-resistant *S. aureus* [25]. More antibacterial studies on the effects of the calyces of *H. sabdariffa* on this bacterium in particular are recommended. These considerable antibacterial properties of the methanol extract of *H. sabdariffa* calyces (80% v/v) are attributed to some bioactive phytochemical constituents present in this extract. Table 3 shows that the methanolic extract is rich in phytochemicals such as alkaloids, phenolic compounds, flavonoids, and saponins while tannins and anthraquinones did not detect. This is in agreement with Djeussi *et al.* [26], who found many phytochemicals in the methanol extract of *H. sabdariffa* calyces such as alkaloids, flavonoids, phenols, polyphenols, saponins, triterpenes, and

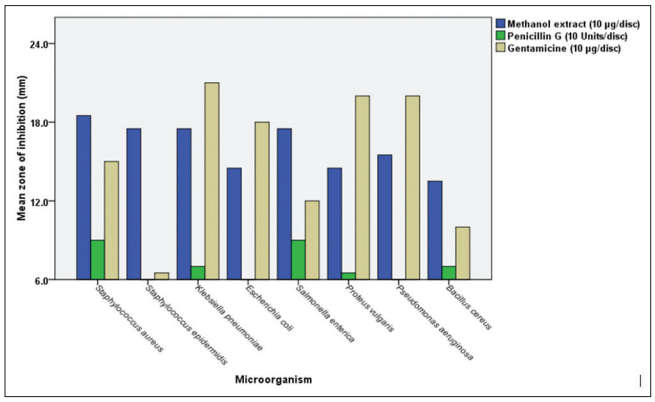

Figure 3: Comparison between the antibacterial effects of methanol extract *Hibiscus sabdariffa* and antibiotics

Table 3: The phytochemical analysis of 80% methanol extract (v/v) of *H. sabdariffa* calyces

Phytochemicals	80% methanol extract (v/v)
Alkaloids	+
Phenolic compounds	+
Flavonoids	+
Tannins	-
Saponins	+
Anthraquinones	-

+: Present, -: Absent, *H. sabdariffa: Hibiscus sabdariffa*

sterols but no anthraquinones, tannins, or anthocyanins. On the other side, Suliman *et al.* [27] figure out many bioactive phytochemicals in the aqueous extract of *H. sabdariffa* calyces such as saponins, phlobatannins, terpenoids, anthraquinones, tannins, steroids, and phenolic compounds. However, the antibacterial agent(s) in the calyces are not determined yet, it was believed that its antibacterial activity could be attributed to anthocyanins [28], but it was found that this antibacterial activity was high with *H. sabdariffa* calyces lacking totally these anthocyanins [6]. Accordingly, more future studies should be conducted to find out and isolate a single compound or group of compounds which could serve as an antibacterial drug. Certainly, additional pharmacological, microbiological, and toxicological studies are recommended.

CONCLUSION

The results of the current study support the widespread use of this popular plant in Sudanese folk medicine, particularly against some illnesses related to microbial infections. The output of this investigation also introduces the calyces of *H. sabdariffa* as one of the most promising sources for new natural and effective antibacterial drugs competitor to antibiotics.

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Source of Support: Nil, Conflict of Interest: None declared.