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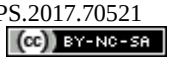
ISSN 2231-3354 

The study of antibacterial activity in enteric pathogens of Roselle (*Hibiscus sabdariffa* Linn.) by broth micro-dilution method

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ABSTRACT

The crude water extract of Roselle (*Hibiscus sabdariffa* Linn.) was determined for its antibacterial activities on 6 bacterial reference strains and 16 isolates of enteric pathogens. Determination of minimum inhibitory concentrations(MICs) by broth microdilution method with final concentration of Roselle extract between 200-3.125 mg/ml showed that Roselle extract could inhibit the growth of bacterial reference strains at MICs 25 mg/ml on *Aeromonas veronii* biogroup *sorbria* ATCC 9071, MICs 50 mg/ml on *Yersinia enterocolitica* ATCC 9610 and MICs 100 mg/ml on *Aeromonas caviae* ATCC 15468, *Aeromonas hydrophila* ATCC 35654, *Vibrio parahaemolyticus* ATCC 17802, *Vibrio vulnificus* ATCC 27562. Roselle extract also exhibited its antibacterial activities on enteric pathogens giving the MICs of 25 mg/ml on *Edwardsiella tarda* and *Yersinia pseudotuberculosis*, the MICs of 50 mg/ml on *Plesiomonas shigelloides*, *Salmonella arizonae*, *Salmonella choleraesuis*, *Salmonella paratyphi A*, *Salmonella* Typhi, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Vibrio cholerae* and *Yersinia enterocolitica*, the MICs of 100 mg/ml on *Aeromonas caviae*, *Aeromonas hydrophila* and *Salmonella enteritidis*. The data indicated that Roselle extract could produce antibacterial activities against enteric pathogens at various concentrations. The extract should be further study to use as antibacterial agent for controlling these organisms.

INTRODUCTION

Emerging foodborne pathogens have become a major public health concern. Infectious diarrhoeal diseases are responsible for considerable morbidity and mortality, especially in developing countries (Thapar and Sanderson, 2004). According to WHO (2009) bulletin, diarrhea diseases account for an estimated annual 1.5 million deaths among children younger than five years old in the world. Antibiotics provide the main therapy for microbial (bacterial and fungal) infections. Although antibiotics play a vital role in the treatment of different diseases, they also have side effects. Altogether, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug-resistant strains of several groups of microorganisms and the spread of the new infections (Abdalla, 2011). Many naturally occurring compounds found in medicinal plants, herbs, and spices have been shown to possess antimicrobial activities against many foodborne pathogens. Use of herbal products as antimicrobial agents may provide the best alternative to the wide and injudicious use of synthetic antibiotics. 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 no adverse side effects, and being easily available at affordable prices. Thus, in light of the evidence of rapid global spread of resistant isolates, the need to find new antimicrobial agents is of paramount importance. Roselle, *Hibiscus sabdariffa* Linn. is a shrub belonging to the family-Malvaceae and thought to be a nature to Asia or Tropical Africa (Mahadevan *et al*., 2009). The crop is widely grown in the tropics for home use. The seeds contain protein (18.8-22.3%), fat (19.1-22.8%) and dietary fiber (39.5-42.6%) (Rao, 1996).

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The leaf is reputed to contain protein, fat, carbohydrate, fiber, ash, calcium, phosphorus, iron, thiamine, β-carotene, riboflavin, niacin and ascorbic acid (Duke, 1979; Prerry, 1980). This medicinal herb commonly uses to make drink and pickle and used in folk medicine in the treatment of hypertension, liver diseases, and fever (Dalziel, 1973: Wang *et al*., 2000; Odigie *et al*. 2003; Akindahunsi *et al.*, 2003). Hibiscus anthocyanins, a group of phenolic natural pigment present in the dried flower of *Hibiscus rosasinensis*, and *H. sabdariffa* have been found to have cardioprotective (Jonadet *et al*., 1990), hypocholesterolemic (Chen *et al*., 2003).

Anthocyanin pigments and other phenolic compounds (Hibiscus protocatechuic acid) also isolated from dried flowers of *H. sabdariffa* demonstrated protective effects against tert-butyl hydroperoxide–induced oxidative damage and hepatotoxicity both in vitro and in vivo (Liu *et al*., 2002). *H. sabdariffa* has been reported to be antiseptic, aphrodisiac, astringent, diuretic, emollient, purgative, sedative, stomachic, and tonic. It is also a folk remedy for abscesses, bilious conditions, cancer, cough, dysuria, and scurvy (Morton *et al*., 1987) and was also found to be anticarcinogenic (Fullerton *et al*., 2008). These findings contribute to support and qualify the importance of screening natural products. Conclusive information is, however, critical with regard to its role as an antimicrobial.

Therefore the objective of this study was to evaluate the antimicrobial activities of Roselle (*H. sabdariffa*) on enteric bacterial pathogens either reference strain and also clinical isolated using the broth micro-dilution method.

MATERIALS AND METHODS

Test microorganisms

The tested organisms were bacterial reference strains included *Aeromonas caviae* ATCC 15468, *Aeromonas hydrophila* ATCC 35654, *Aeromonas veronii* biogroup *sorbria* ATCC 9071, *Vibrio parahaemolyticus* ATCC 17802, *Vibrio vulnificus* ATCC 27562, *Yersinia enterocolitica* ATCC 9610 were given from faculty of Medical Technology, Rangsit University. And also 16 bacteria strains from clinical isolated included *Aeromonas caviae*, *Aeromonas hydrophila*, *Edwardsiella tarda*, *Plesiomonas shigelloides*, *Salmonella arizonae*, *Shigella boydii*, *Salmonella choleraesuis*, *Shigella dysenteriae*, *Salmonella enteritidis*, *Shigella flexneri*, *Salmonella paratyphi*, *Shigella sonnei*, *A*, *Salmonella typhi*, *Vibrio cholerae*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*

Preparation of bacterial culture

Individual colonies isolated from 24 h cultures of tests bacteria were suspended in a sterile 0.85% NaCl solution. The suspension was properly inoculated in Trypyic Soy broth. The suspensions were mixed for 15 sec to ensure homogeneity. The turbidity of microbial suspension was diluted to match the turbidity of a 0.5 McFarland standard by spectrophotometry (OD = 0.08 - 0.1 at 625 nm) corresponding to 1x 10^8 CFU/mL and subsequently suspend 1:100 with Trypyic Soy broth to obtain the bacteria containing 1×10^6 cfu/mL.

Prepare crude water extract from *Hibiscus sabdariffa* Linn.

The plant materials were dried under shade and ground into fine powder using an electric blender. For solvent extraction, 5g of air dried powder was mixed with 500 ml of sterile water in a conical flask, plugged with cotton and then kept for 24h. After 24h, it was filtered through Whatman no. 1 filter paper and centrifuged at 5000 g for 10 min. The filtrates were subject to spray dried until each extract was obtained.

Antibacterial by crude water extraction from *Hibiscus sabdariffa* Linn.

The antimicrobial activities of the crude extracts were assayed against bacteria. A test compound was prepared with the known weight of the crude extract, dissolved in distilled water at an initial concentration of **0.50** g/ml.

The minimum inhibitory concentration (MIC) was determined by using the standard broth micro-dilution method as recommended by the NCCLS and CLSI methodology with a few modifications. The serial two fold dilutions of the crude extracts were made in a concentration which ranged from **200** mg/ml to **3.125** mg/ml.

Test sample solutions (50 µL) and prepared bacterial suspension (50 µL) containing 1×10^6 cfu/mL were added into each well of the 96-well microplate. Each well of the negative control contained 50 µL of microbial suspension and 50 µL of Trypyic Soy broth. The microtiter plates were agitated to mix well and then were incubated at 37 ºC, 16-18 h. All tests were carried out in triplicate. The lowest concentration which showed OD at 620 nm less than $\Re_{control}$+1.5SD consider as no growth was taken as the MIC value.

RESULTS AND DISCUSSION

The antimicrobial activity experiment was conducted using micro-dilution method. The susceptibility testing of crude extract from *H. sabdariffa* showed potent broad spectrum antimicrobial activity which can inhibited six ATCC-registered strains of bacteria, (Table 1). However, the degrees of susceptibility were different depending on pathogen, suggesting that several substances participated in antimicrobial activity.

Among the 6 ATCC-registered strains bacteria used in the present study; the most susceptible microbe were *Yersinia enterocolitica* ATCC 9610 and *Aeromonas veronii biogroup sorbria* ATCC 9071 (MIC = 50 mg/mL) followed by *Vibrio parahaemolyticus* ATCC 17802 and *Vibrio vulnificus* ATCC 27562 (MIC = 100 mg/mL). The less susceptible microbe were *Aeromonas caviae* ATCC 15468 and *Aeromonas hydrophila* ATCC 35654 (MIC = 200 mg/mL).

Table 1: Minimum inhibitory concentration (MIC) of *Hibscus sabdariffa* extracts against standard pathogenic bacteria (ATCC strain).

Organisms tested	Concentration of *Hibscus sabdariffa* extracts (mg/ml)						
	200	100	50	25	12.5	6.25	3.125
Aeromonas caviae ATCC 15468	-	-	+	+	+	+	+
Aeromonas hydrophila ATCC 35654	-	-	+	+	+	+	+
Vibrio parahaemolyticus ATCC 17802	-	-	+	+	+	+	+
Vibrio vulnificus ATCC 27562	-	-	+	+	+	+	+
Yersinia enterocolitica ATCC 9610	-	-	-	+	+	+	+
Aeromonas veronii biogroup *sorbria* ATCC 9071	-	-	-	-	+	+	+

Where (**-**) = growth, (+) = growth.

Table 2: Minimum inhibitory concentration (MIC) of *Hibscus sabdariffa* extracts against clinical isolated bacteria.

Organisms tested	Concentration of *Hibscus sabdariffa* extracts (mg/ml)						
	200	100	50	25	12.5	6.25	3.125
A. caviae	-	-	+	+	+	+	+
A. hydrophila	-	-	+	+	+	+	+
S. Enteritidis	-	-	+	+	+	+	+
P. shigelloides	-	-	+	+	+	+	+
S. Arizonae	-	-	-	+	+	+	+
S. Choleraesuis	-	-	-	+	+	+	+
S. Paratyphi A	-	-	-	+	+	+	+
S. Typhi	-	-	-	+	+	+	+
S. dysenteriae	-	-	-	+	+	+	+
S. boydii	-	-	-	+	+	+	+
S. flexneri	-	-	-	+	+	+	+
S. sonnei	-	-	-	+	+	+	+
V. cholerae	-	-	-	+	+	+	+
Y. enterocolitica	-	-	-	+	+	+	+
E. tarda	-	-	-	-	+	+	+
Y. pseudotuberculosis	-	-	-	-	+	+	+

Where (**-**) = growth, (+) = growth.

In addition, the degrees of inhibition among clinical isolated bacteria were shown in Table 2. The most susceptible microbe were *E. tarda* and *Y. pseudotuberculosis* (MIC = 50 mg/mL) followed by *P. shigelloides*, *S. Arizonae*, *S. Choleraesuis*, *S. paratyphi A*, *S. typhi*, *S. dysenteriae*, *S. boydii*, *S. flexneri*, *S. sonnei*, *V. cholerae*, *Y. enterocolitica* and *A. caviae* showed similar MIC as 50 mg/mL. The less susceptible microbe were *A. hydrophila* and *S. enteritidis* (MIC = 100 mg/mL).

Interestingly, crude extract from *H. sabdariffa* can inhibit ATCC-registered strains bacteria by MIC range from 50-200 mg/mL. However it can inhibit clinical isolated bacteria by MIC range from 25-200 mg/mL. In addition, crude extract also exhibited its antibacterial activities on enteric pathogens giving the lowest MICs of 25 mg/ml on *Edwardsiella tarda* and *Yersinia pseudotuberculosis*. Taken together, crude extract from *H. sabdariffa* showed activity against all strain of tested bacteria used in this study. This finding was correlated with previous work reported that methanol extract from *H. sabdariffa* can inhibit *E. coli* O157:H7 isolated from food, veterinary, and clinical samples (Fullerton *et al*., 2011). Other study was reveal that aqueous-methanolic extract of *H. sabdariffa* exhibited antibacterial activities (MIC 0.30 ± 0.2- 1.30 ± 0.2 mg/ml) against *S. aureus*, *B. stearothermophilus*, *M. luteus*, *Serratia marcescens*, *Clostridium sporogenes*, *E. coli*, *K. pneumoniae*, *B. cereus*, *P. fluorescence* (Olaleye *et al*., 2007).

According to biological activities of *H. sabdariffa*, the antimicrobial activity may be the result of phenolic compounds, including flavonoids. Flavonoids are hydroxylated phenolic substances but occur as a C6–C3 unit linked to an aromatic ring. These compounds have the ability to form a combined complex with bacterial cell walls. Also, with the number of hydroxyl groups present on the phenolic ring. There is increased hydroxylation, and with increased hydroxylation there will be increased antimicrobial activity (Cowan, 1999). Flavonoids are known to be synthesized by plants in response to microbial infection, it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms (Fullerton *et al*., 2011).

In the treatment of infectious diseases, the use of plants is common in traditional medicine. The study showed that the aqueous crude extracts had wide ranges of activity on the selected pathogenic strains. The results indicate that this extracts may contain active components that produced antimicrobial against the selected enteric pathogenic strains. The medicinal plants' value lies in chemical substances that produce a definite physiological action on the pathogens. The biologically active compounds present in plants have the potential for development as medicinal agents.

There is a need for the development of new antimicrobial drugs from medicinal plants to treat infectious diseases; these may be less toxic to humans and have novel mechanisms of action. The plants which showed the highest antibacterial activity can be further subjected to isolation of the therapeutic antimicrobial compounds and evaluated pharmacologically. The study confirmed that *H. sabdariffa* showed maximum inhibitory activity can be used for the treatment of infectious diseases. In the current

study, this finding suggests that crude extracts from *H. sabdariffa* showed a wide spectrum of antimicrobial activity. It is clear that *H. sabdariffa* has antibacterial activities against enteric pathogens. Antibacterial resistance especially among Gram negative bacteria is an important issue that has created a number of problems in treatment of infectious diseases and necessitates the search for alternative drug or natural antibacterial. This aspect of antimicrobial activity will be further investigated to enhance production of secondary metabolites of interest. Therefore, any information and research on *H. sabdariffa* plant is of value.

CONCLUSIONS

Our study confirmed that *H. sabdariffa* was effective at all entero pathogenic bacteria both ATCC-registered strains and clinical sources. This shows that plant extracts possess antimicrobial activity and hold great promise as antimicrobial agents against foodborne pathogens. In addition, micro-dilution method provided quantitative data that reliably predicted the effectiveness of antimicrobial activity of the extracts. Plant extracts may be used as possible sources to obtain new and effective herbal medicines to treat foodborne infections, as they may be an excellent alternative to combat the further spread of multidrug-resistant microorganisms. It is important, however, to determine toxicity of the active constituents, their side effects, and pharmacokinetic properties. Future studies need to address these compounds from various type extractor and also determine their effectiveness on other microbial such as parasite and fungi. Therefore, the use of natural antimicrobials from *H. sabdariffa* and their application are major goals of current research to accomplish environment-friendly technological development.

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REFERENCES

Abdalla EM. Plants: An alternative source for antimicrobials. J App Pharm Sci, 2011; 1:16-20.

Akindahunsi AA, Olaleye MT:Toxicological investigation of aqueous methanolic extract of *Hibscus sabdariffa* L. J Ethnopharmacol 2003;89:161–164.

Chen CC, Hsu JD, Wang SF, Chrang HC, Yang MY, Kao ES, Ho YO, Wang CJ *Hibscus sabdariffa* extract inhibit the development of atherosclerosis in cholesterol-fed rabbits. J Agric Food Chem, 2003; 51: 5472–5477.

Cowan MM Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12:564–582.

Dalziel JM. 1973. The useful plants of west tropical Africa. London: The Crown Agents.

Duke J. A. Ecosystematic data on economic plants, Quart. J. Crude Drug Res, 2011; 17:91-110

Fullerton MSC, Verghese M, Walker LT, Shackelford LA, Cebert E, Boateng J, Ogutu S, Khatiwada J, Guyton M, Jones J, Chawan CB Determination of antioxidant contents in red sorrel and its anticarcinogenic potential in azoxymethane-induced colonic aberrant crypt foci. J Res Phytochem, 2008; 2: 69–76.

Fullerton MSC, Khatiwada J, Johnson JU. Determination of antimicrobial activity of Sorrel (*Hibiscus sabdariffa*) on *Esherichia coli* O157:H7 isolated from food, veterinary and clinical samples J Med Food, 2011; 14: 950-956.

Jonadet M, Bastide J, Bastide P, Boyer B, Carnat AP, Lamaison JL In vitro enzyme inhibitory and in vivo cardio-protective activities of *Hibiscus sabdariffa* L. J Pharmacol Belgium, 1990; 45: 120–124.

Liu C, Wang J, Chu C, Cheng M. Tseng T In vivo protective effect of protocatechuic acid on tert-butyl hydroperoxide-induced rat hepatotoxicity. Food Chem Toxicol, 2002; 40: 635–641.

Mahadevan N., Shivalic Kamboj P. *Hibiscus sabdariffa* Linn – An overview. Natural Product Radiance, 2009; 8: 77-83.

Morton J.F. Roselle. 1998. In: Fruits of warm climate. Dowling, C.F.(Ed.) Media Inc. Greensboro, NCP. pp: 281-286.

Odigie IP, Ettarh RR, Adigun SA Chronic administration of aqueous extract of *Hibscus sabdariffa* attenuates hypertension and reverses cardiac hyperytrophy in 2K-1C hypertensive rats. J Ethnopharmacol, 2003; 86: 181–185.

Olaleye MT. Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus sabdariffa* J Med Plants Res, 2007; 1:009-013.

Prerry LM. 1980. Medicinal plants of east and southeast Asia. Cambridge: MIT Press, Cambridge 1980.

Rao PU. Nutrient composition and biological evaluation Mesta (*Hibiscus sabdariffa*) seeds.Plant Foods Hum Nutr, 1996; 49:27-34.

Thapar N, Sanderson IR. Diarrhoea in children: an interface between developing and developed countries. Lancet 2004; 363: 641-653.

Wang CJ, Wang JM, Lin WL, Chu CY, Chou FP, Tseng TH Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxide–induced hepatic toxicity in rats. Food Chem Toxicol, 2000; 38: 411–416.

World Health Organization. 2009. Why children are still dying and what can be done. WHO Bulletin [ONLINE] Available: http://www.who.int/child_adolescent_health/documents/9789241598415/en/index.html. [Accessed 20 March, 2012]

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