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Antibacterial activity, phytochemical screening and cytotoxicity of *Hibiscus sabdariffa* (calyx)

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ABSTRACT

The purpose of the paper was to investigate the *in-vitro* antibacterial activity, preliminary phytochemical screening and cytotoxicity [3-(4,5-dimethyl thiazol-2-yl)-2, 5-diphenyltetrazolium bromide-MTT assay] of methanol extract of *Hibiscus sabdariffa* L. (calyxes). The methanol extract of *H. sabdariffa* was tested against 11 clinical isolates bacteria and 3 standard bacteria, that is: 5 Gram positive bacteria (*Corynebacterium diphtheria, Staphylococcus aureus, Enterococcus faecalis,Listeria monocytogenes* and *Bacillus cereus*), 9 Gram negative bacteria (*Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas aeruginosa* (ATCC 27853), *Serratia* marcescens*, Escherichia coli, Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* and *Klebsiella pneumoniae* (ATCC 70063) using the cup-plate agar diffusion method, preliminary phytochemical screening and cytotoxicity (MTT assay) was also performed with different concentrations (500, 250 and 125 µg/ml) and compared to the reference control Triton-x100. The methanol extract of *H. sabdariffa* (calyxes) exhibited inhibitory effects against most of the tested organisms with the zone of inhibition ranging from 14 to 36 mm in length. The largest inhibition zone was obtained for the Gram-positive against bacteria *L. monocytogenes* (36 mm), *E. faecalis* (33 mm), *B. cereus* (28 mm), *C. diphtheriae* (26 mm) and *S. aureus* (24 mm) while in case of Gram negative highest inhibition was observed against *P. aeruginosa* (ATCC 27853) (28 mm), *K. pneumoniae* (ATCC 70063) (25 mm), *P. aeruginosa* (23 mm), *E. coli* (20 mm), *E. coli* (ATCC 25922) and *P. vulgaris* (19 mm), *K. pneumonia* (18 mm), *S. marcescens* (17 mm) and *P. mirabilis* (14 mm). Preliminary phytochemical screening investigation for the methanolic extracts showed the presences of alkaloids, glycosides, flavonoids, tannins, sterols triterpens and phenols, while saponins was absent. In addition cytotoxicity (MTT-assay) verified the safety of the examined extract with an IC_{50} less than 100 µg/ml. The study concluded that *H. sabdariffa* (calyxes) proved to have potent activities against antibacterial activity *in vitro* with verified safety evidence for use.

Keywords: Antibacterial activity, preliminary phytochemical, cytotoxicity, *Hibiscus sabdariffa*.

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INTRODUCTION

Hibiscus sabdariffa belongs to (family: Malvaceae) has been used in folk medicine as a diuretic, laxative, and treatment for cardiac and nerve diseases, hypertension and cancer (Chewonarin et al., 1999; Akindahunsi and Olaleye, 2004). The heated leaves are applied to cracks in the feet and on boils and ulcers to speed maturation. A lotion made from leaves is used on sores and wounds. In several countries, it is used as a natural medicine for

treating hypertension, pyrexia and liver disorders and microorganism growth limitation (Oboh and Elusiyan, 2004), as well as a diuretic, digestive and sedative (Akindahunsi and Olaleye, 2004). Pharmacological studies of anthocyanins in hibiscus have shown that they have antioxidant activity in patients with atherosclerosis. The plant grows as an annual and sometimes biannual shrub with straight branches and small ramifications, with yields that can reach 0.5 to 2 depending on the variety. Cultivated plants reach between 1 and 3 m in height depending on the location and season of sowing. The crop is susceptible to the attack of various plant pathogens which can infect plants at early development stages, when competition from weeds can also be deleterious. A lot of antimicrobial screening evaluations have been published based on the traditional use of Chinese, African and Asian plant drugs. The studies about the effect of plant extract against different types of bacteria are still one of the most important fields of researches (Ndukwe et al., 2005). *H. sabdariffa* exhibits antihypertensive and cardioprotective effects *in vivo* and supports the public belief that *H. sabdariffa* may be a useful antihypertensive agent (Rota et al., 2004). Administration of a crude extract of *H. sabdariffa* has protected erythrocytes against lipid peroxidation (Suboh et al., 2004). Stems and knowledge on the bioactivities of the genus roots of *Hibiscus taiwanensis* have been used as antiinflammatory, antifungal, antipyretic, and anti-helminthic agents (Wu et al., 2005). Flowers of *Hibiscus tiliaceus* (L.) are widely used for birth control and for treating skin infections (Rosa et al., 2006).

Hibiscus sabdariffa has shown *in vitro* antimicrobial activity against *E. coli* (Fullerton et al., 2011). A recent review stated that specific extracts of *H. sabdariffa* exhibit activities against atherosclerosis, liver disease, cancer, diabetes and other metabolic syndromes (Lin et al., 2011).

The plants are rich in anthocyanins, as well as protocatechuic acid. The dried calyces contain the flavonoidsgossypetin, hibiscetine and sabdaretine. The major pigment, formerly reported as hibiscin, has been identified as daphniphylline. Small amounts of myrtillin (delphinidin 3-monoglucoside), chrysanthenin (cyanidin 3-monoglucoside), and delphinidin are also present. *H. sabdariffa* seeds are a good source of lipid-soluble antioxidants, particularly gamma-tocopherol (Mohamed et al., 2007).

Oboh and Elusiyan (2004) also studied the nutrient composition and antimicrobial activity of sorrel drinks against *P. aeruginosa*, *Lactobacillus* sp., *Bacillus* sp., and *Corynebacterium* and found out that the aqueous extracts of *H. sabdariffa* inhibited the growth of the above mentioned microorganisms. Fullerton et al. (2011) determined the antimicrobial activity of sorrel (*H. sabdariffa*) on *Escherichia coli* O157:H7 isolated from food, veterinary, and clinical samples. Ethanolic extract of *H. sabdariffa* calyces was studied and showed to have antimicrobial agents effective in inhibiting *E. coli* O157:H7. The *H. sabdariffa* plant therefore holds a great promise as an antimicrobial agent. The present study was conducted to investigate the antibacterial activity, preliminary phytochemical screening and cytotoxicity (MTT-assay) of *H. sabdariffa* (calyxes) *in vitro.*

MATERIALS AND METHODS

Plant collection

The *H. sabdariffa* (calyxes) was collected from Alyhya Mole (Khartoum, Jabraa), between January and February 2016. The plant was identified and authenticated at the Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan. Flowers of *H. sabdariffa* were air dried, under the shade and pulverized and stored prior to extraction.

Preparation of crude extracts

Extraction was carried out according to the method described by Harbone (1984). Calyx was separated from the plant in order to prepare extract for screening of the antibacterial activity. Specific weight of sample about 50 g of the powdered sample was successively extracted with methanol (80%) using soxhlet extractor apparatus. Methanol extraction carried out for six-to-eight hours whereas methanol solvent was removed by using rotary evaporator. Extract was allowed to air dryness the percentage yield was calculated. The extract were kept and stored at 4°C until use.

Test microorganisms

The methanol extract of *H. sabdariffa* was tested against 11 clinical isolates bacteria and 3 standard bacteria, that is: 5 Gram positive bacteria (*Corynebacterium diphtheria, Staphylococcus aureus, Enterococcus faecalis, Listeria monocytogenes* and *Bacillus cereus*), 9 Gram negative bacteria (*Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas aeruginosa* (ATCC 27853), *S. marcescens, Escherichia coli, Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* and *Klebsiella pneumoniae* (ATCC 70063) using the cup-plate agar diffusion method. The study was conducted in the microorganisms lab, Department of Microbiology, Faculty of Medical Laboratory Sciences, International University of Africa, Khartoum, Sudan.

Collection of bacterial strains

Various clinical isolates were obtained from Royal Care International Hospital, Khartoum State, Sudan. All bacterial isolates were identified and characterized using standard microbiology technique (Chessbrough, 2006). The bacterial cultures were maintained on nutrient agar (Hi media - India) and incubated at 37°C for 24 h and then stored in refrigerator at 4°C for further testing.

In vitro testing of extracts for antibacterial activity

The cup-plate agar diffusion method as described by **Kavanagh (1972**) was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. Aliquot of 1 ml of the standardized bacterial stock suspension (between 10^8 and 10^9 CFU/ml) was thoroughly mixed with 100 ml of molten sterile Mueller

Hinton agar (HiMedia, India) which was maintained at 45°C. 20 ml aliquots of the inoculated Mueller Hinton agar were distributed into sterile Petri-dish plates. The agar was left to set and in all of these plates 5 cups (8 mm in diameter) were cut using a sterile cork borer and agar discs were removed. Each cups were filled with 0.1 ml of the methanolic extract using an automatic microlitre pipette, and thereafter the extract were allowed to diffuse at room temperature for two hours. The plates were then incubated in an upright position at 37°C for 24 h. Two replicates were carried out for each extract against each of the tested microorganisms. After incubation the diameters of the resultant growth inhibition zones were measured and averaged. The mean values were tabulated.

Standard antibiotic

Gentamicin (30 µg/disc) antibiotic discs used in the present study were procured by Hi media Chemicals Ltd, Mumbai, India and stored at 4°C.

Phytochemical screening of *H. sabdariffa*

In this study the preliminary phytochemical screening was conducted according to Haborne (1984).

Preparation of *H. sabdariffa*

10 mg of the powder of calyx plant were refluxed with 100 ml of methanol 80% for 4 h. The cool solution was filtered and enough methanol 80% was passed through the volume of the filtrate 100 ml. This prepared extract (PE) was used for the various tests.

Test for alkaloids

Aliquot of 7.5 ml of PE was evaporated to dryness on a water bath. Thereafter, 5 ml of HCl (2N) was added and stirred while heating on the water bath for 10 min, then was cooled, was filtered and was divided into two test tubes. To one test tube few drops of Mayer's reagent (Lab tech- Sudan) were added, while to the other tube few drops of Valser's reagent (Lab tech - Sudan) were added. A slight turbidity or heavy precipitate in either of the two test tubes was tanked as presumptive evidence for the presence of alkaloids (Harborne, 1984).

Test for anthraquinone glycosides

10 g of the powdered plant sample were boiled with 10 ml of 0.5N KOH containing 1 ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with10 ml of benzene. 5 ml of the benzene solution was shaked with 3 ml of 10% ammonium hydroxide solution and the two layers were allowed 25 to separate. The presence of Anthraquinones was indicated if the alkaline was found to have assumed pink or red color.

Test for flavonoids

Aliquot of 17.5 ml of the PE was evaporated to dryness on a water bath, was cooled and the residue was defatted with petroleum ether. The defatted residue was dissolved in 30 ml of methanol (80%) and was filtered. The filtrate was used for the following test: To three ml of the filtrate in a test tube was added 1 ml of 1% aluminum chloride (methanolic solution). Formation of yellow color indicated the presence of flavonoids (Harborne, 1984).

Test for tannins

Five ml of saline solution (following n-hexane application on the prepared extract) was treated with few drops of gelatin salt reagent (Lab tech - Sudan). Formation of immediately precipitate was taken as an evidence for the presence of tannin in plant sample. To another portion of this solution, few drops of ferric chloride test reagent were added. The formation of blue, black or green was taken as an evidence for the presence of tannins.

Test for unsaturated sterols and triterpenes

An aliquot of 10 ml of the PE was evaporated to dryness on a water bath and the cooled residue was stirred several times with petroleum ether to remove most of the coloring materials. The residue was then extracted with 20 ml of chloroform. The chloroform solution was dehydrated over sodium sulphate anhydrous. A 5 ml of chloroform solution was mixed with 0.5 ml acetic anhydride followed by two drops of conc. sulphuric acid. The gradual appearance of green, blue pink to purple color was taken an evidence of the presence of sterol (green to blue) and or triterpenes (pink to purple) in the sample (Harborne, 1984).

Test of phenols

Ferric chloride test: Extracts were treated with 3 to 4 drops of 10% ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Test for saponins

1 g of the original dried powdered plant material was placed in a clean test tube. 10 ml of distilled water was added and the tube was stoppered and vigorously shaken for about 30 s. The tube was then allowed to stand and observed for the formation of honeycomb. The appearance of honeycomb, which persisted for least an hour, was taken as an evidence for the presence of saponins.

Cytotoxicity screening

Micro-culture-tetrazolium assay [3-(4,5-dimethyl thiazol-2-yl)-2, 5-diphenyltetrazolium bromide –MTT] was utilized to evaluate the cytotoxicity of the *H. sabdariffa.* This colorimetric assay is based on the capacity of mitochondria-succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate MTT into an insoluble, blue colored-formazan, a product measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells (Patel et al., 2009).

Cell line and culture medium

Baby Hamster Kidney (BHK) cells were cultured in a culturing flask containing a complete medium consisting of 10% fetal bovine serum and 90% minimal essential medium (MEM) and then incubated at 37°C. The cells were sub cultured twice a week.

MTT assay

Serial dilutions of extract were prepared in a 96 well flat bottomed plate (Nalge Nunc, Inter.). The outer walls of the plate were filled with 250 µl of in-complete culture medium except the last row 6

middle wells (B - G), which were used for the negative control receiving 50 µl of culture medium and 2 µl of sterile 0.5% Triton X. Aliquot of 50 µl/wells complete culture medium (CCM) were added and 30 µl more were added to second column wells (B – G) that were used as first extract dilution wells. To the first dilution wells in the row, 500 µg of suspension extract were added to the 80 µl extract were then serially diluted by two-fold dilution from well B3 till B11 by transferring 250 µl to the next well after proper mixing. From the last dilution wells (B-11), 50 µl were discarded. Each compound was tested in triplicate. Cell suspension in a complete culture medium containing 2.5×10^5 /ml was properly mixed, and 150 µl of it were transferred into each well of the plate. The plate was covered and placed in 5% CO_2 incubator at 37°C for three-five days (72 to 120 h). On the third/fifth day, the supernatant was removed from each well without detaching cells. The MTT (a yellow tetrazole) stock (5 mg/ml) was prepared earlier in 100 ml PBS (phosphate buffer saline). MTT suspension was vortexed and kept on magnetic stirrer until all MTT dissolved. The clear suspension was filter sterilized with 0.25 µm Millipore filter and stored at 4 or –20°C until use. MTT was diluted (1:3.5) in a culture medium and brought to room temperature. To each well of the 96 well plates, 50 µl of diluted MTT were added. The plate was incubated further at 37°C for 2 to 3 h in CO_2 incubator. MTT was removed carefully without detaching cells, and 200 µl of dimethylsulfoxide (DMSO) were added to each well. The plate was agitated at room temperature for 15 min then read at 540 nm using micro plate reader. The percentage growth inhibition was calculated using the formula below:

$$\% \text{ cell inhibition} = 100 - \{(Ac-At)/Ac\} \times 100$$

Where, At = Absorbance value of test compound; Ac = Absorbance value of control.

Statistical analysis

All data were presented as means ± S.D. Statistical analysis for all the assays results were done using Microsoft Excel program (2010).

RESULTS AND DISCUSSION

Antibacterial activity of *H. sabdariffa* (calyxes) extract

The results of methanol extract of *H. sabdariffa* (calyxes) exhibited inhibitory effects against most of the tested organisms with the zone of inhibition ranging from (14 to 36 mm) in length. The largest inhibition zone was obtained for the Gram-positive against bacteria *L. monocytogenes* (36 mm), *E. faecalis* (33 mm), *B. cereus* (28 mm), *C. diphtheriae* (26 mm) and *S. aureus* (24 mm) while in case of Gram negative highest inhibition was observed against *P. aeruginosa* (ATCC 27853) (28 mm), *K. pneumoniae* (ATCC 70063) (25 mm), *P. aeruginosa* (23 mm), *E. coli* (20 mm), *E. coli* (ATCC 25922), *P. vulgaris* (19 mm), *K. pneumonia* (18 mm), *S. marcescens* (17 mm) and *P. mirabilis* (14 mm) (Table 1). Therefore these results showed that the extracts tested inhibited the growth of all bacteria though the sensitivities of bacteria varied. The results of the present study were similar to that reported by Abd-Ulgadir et al. (2015) when the calyx methanol extract obtained an intermediate activity against *C. albicans*. Olaleye (2007) also found similar result to ours, where the methanol extract of *H. sabdariffa* inhibited the growth *P. aeruginosa* and *S. aureus* but no inhibitory effect on *Aspergillus niger* was evidenced. Therefore the calyx methanol extract of *H. sabdariffa* exhibited higher activity against the two Gram positive organisms than the two Gram negative organisms and the two fungi tested. This could be due to the fact that the cell wall of Gram positive bacteria is less complex and lack the natural sieve effect against large molecules due to the small pores in their cell envelope. Ewansiha (2014) also evaluated the phytochemical properties and the antimicrobial potency of *H. sabdariffa* (L.) leaf extracts using the cold maceration, agar diffusion and qualitative phytochemical analysis methods respectively. Aqueous and hexane extracts were obtained and were separately tasted against *Klebsiella pneumoniae*, *Salmonella typhi* and *Shigella dysenteriae*. Moreover, Osei-Djarbeng et al. (2014) tested the antimicrobial activities of different solvent extracts of the calyxof *H. sabdariffa*; using the agar well diffusion and the micro-broth dilution methods. *H. sabdariffa* showed the greatest antimicrobial activity with average diameter of zone of inhibition in the range (12 to 19 mm) against the microbes employed in the study. The polar extracts exhibited the greatest antimicrobial activity with MICs of 125 to 250 µg/ml against the bacteria, and 1000 µg/ml against the fungi.

The result of minimum inhibition concentration from Table 2 showed that 6.25 µg/ml was the lowest concentration at which all the tested microorganisms were inhibited. A comparison of observation given in Table 2 showed that the flowers of *H. sabdariffa* dissolved in DMSO inhibited *E. faecalis, L. monocytogenes* and *P. aeruginosa* (ATCC 27853) higher than 30 µg/ml Gentamicin.

Preliminary phytochemical screening of *H. sabdariffa* (calyxes) extract

The screening of plants for medicinal value has been carried out by numerous researchers with the help of preliminary phytochemical analysis (Ram, 2001; Mungole and Chaturvedi, 2011). Phytochemical screening test is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best and judicious use of available natural wealth. A number of medicinal plants have been chemically investigated by several researchers (Ongoka et al., 2006; Lopes-Lutz et al., 2008; Ni et al., 2012). The selection of plant parts such as petals which yields maximum secondary metabolites is the prime or prerequisite step in this investigation. For this, different phytochemicals from petals were extracted and highlighted by different methods; their presence (+) or absence (-) is shown in Table 3.

Table 1. Antibacterial activity of *H. sabdariffa* (calyxes) against the clinical isolates and standard bacteria bacterial.

Tested microorganisms	Mean diameter of growth inhibition zone - MDIZ (mm)
Corynebacterium diphtheria	26
Proteus mirabilis	14
Pseudomonas aeruginosa	23
Staphyococcus aureus	24
Serratia marcescens	17
Enterococcus faecalis	33
Listeria monocytogenes	36
Escherichia coli	20
Escherichia coli (ATCC 25922)	19
Klebsiella pneumoniae (ATCC 70063)	25
Klebsiella pneumonia	18
Pseudomonas aeruginosa (ATCC 27853)	28
Proteus vulgaris	19
Bacillus cereus	28

Key: Interpretation of results: MDIZ (mm): >18 mm: Sensitive, 14 to 18 mm: Intermediate: <14 mm: Resistant. (-): No inhibition; Concentration used 100 mg/ml at 0.1 ml/cup.

Table 2. The antibacterial activity (calyxes) of *H. sabdariffa* and reference antibiotic against the clinical isolates and standard bacteria bacteria.

Tested microorganisms	Concentration (mg/ml)					
	Mean diameter of growth inhibition zone (mm)					
	100	50	25	12.5	6.25	Gentamicin 30 µg/disc
Corynebacterium diphtheriae	26	20	16	12	-	33
Proteus mirabilis	14	12	11	-	-	20
Pseudomonas aeruginosa	23	16	15	12	-	25
Staphyococcus aureus	24	18	15	13	-	30
Serratia marcescens	17	15	14	13	-	25
Enterococcus faecalis	33	20	18	16	-	20
Listeria monocytogenes	36	20	15	-	-	29
Escherichia coli	20	18	16	15	-	22
Escherichia coli (ATCC 25922)	19	16	14	12	-	29
Klebsiella pneumoniae (ATCC 70063)	25	20	18	15	-	30
Klebsiella pneumonia	18	15	14	12	-	23
Pseudomonas aeruginosa (ATCC 27853)	28	20	17	-	-	25
Proteus vulgaris	19	16	15	14	-	28
Bacillus cereus	28	23	21	13	-	30

Table 3. Preliminary phytochemical screening analysis of *H. sabdariffa* extract.

No.	Tested	*H. sabdariffa* extract
1	Alkaloids	+
2	Anthraquinone glycosides	+
3	Flavonoids	+
4	Tannins	+
5	Sterols and triterpens	+
6	Phenols	+
7	Saponins	-

Key: (+) = Present; (-) = Absent.

The Phytochemical analysis of crude methanolic extract of *H. sabdariffa* calyx by the method described earlier and then and then analyzed for phytocompounds like steroids or alkaloids, glycosides, flavonoids, tannins, sterols triterpens, saponins and phenols preliminary analyzed and present in Table 3. Phytochemical investigation for the methanolic extracts showed the presences of alkaloids, glycosides, flavonoids, tannins, sterols triterpens and phenols, while saponins was absenced.

This result was similar to that reported by Obouayeba et al. (2015) who found that the calyces extract of *H.*

Table 4. Cytotoxicity of *H. sabdariffa* extracts on normal cell lines (BHK cell line) as measured by the MTT assay.

No.	Name of extracts	Concentration (µg/ml) Inhibition (%) ± SD			IC_{50} (µg/ml)	IC_{50}
		500	250	125		
1	*H. sabdariffa*	60.06	32.27	-7.03	388.68	> 100
2	Control		96.28			< 30

sabdariffa showed the presence of alkaloids, anthocyanins, flavonoids, polyphenols, saponins and tannins except saponins was absenced. Ewansiha (2014) showed the presence of tannins, flavonoid and phenols in the aqueous extracts while flavonoid and phenols were present in the hexane extracts.

Cytotoxicity assay of *H. sabdariffa* (calyxes) extract

The result of cytotoxicity effects of methanolic extract of *H. sabdariffa* by using MTT-assay in BHK cell line. Table 4 indicated the growth inhibition percentage (%) of BHK cell line *in vitro* by methanolic extract of *H. sabdariffa*at different concentrations from 125 to 500 µg/ml and showed an IC_{50} > 100 (µg/ml) verifying the plant safety. This result was similar to that reported by Olaleye (2007) using brine shrimps lethality assay.

CONCLUSION

H. sabdariffa showed that the various degree of inhibitory activity against the bacteria tested. The obtained results indicated that the*H. sabdariffa*is good antibacterial therapy in traditional medicine in Sudan and the neighboring countries. Further investigations regarding the mode of action and other related pharmacological studies such as *in vivo* investigation, drug formulation and clinical trials are highly recommended.

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REFERENCES

Abd-Ulgadir KS, Suliman SI, Zakria IA, Hassan NA, **2015**. Antimicrobial potential of methanolic extracts of *Hibiscuss abdariffa* and *Ricinus communis*. Adv Med Plant Res, 3(1): 18-22.

Akindahunsi AA, **Olaleye** MT, **2004**. Toxicological investigation of aqueous-methanolic extract of the calyces of *Hibiscus sabdariffa*. J Ethnopharmacol, 89: 161-164.

Chessbrough M, **2006**. District laboratory practice in tropical countries. Part two, 2nd Edn. Cambridge University Press, UK. pp: 123-201.

Chewonarin T, Kinouchi T, Kataoka K, Arimochi H, Kuwahara T, Vinitketkumnuen U, Ohnishi Y, **1999**. Effects of roselle (*Hibiscus sabdariffa* Linn.), a Thai medicinal plant, on the mutagenicity of various known mutagens in *Salmonella typhimurium* and on formation of aberrant crypt foci induced by the colon carcinogens azoxymethane and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine in F344 rats. Food Chem Toxicol, 37(6): 591-601.

Ewansiha JU, **2014**. Evaluation of the antimicrobial activity of roselle (*Hibiscus sabdariffa* L.) leaf extracts and its phytochemical properties. Peak J Med Plant Res, 2(1): 1-5.

Fullerton M, Khatiwada J, Johnson JU, Davis S, Williams L, **2011**. Determination of antimicrobial activity of sorrel (*Hibiscus sabdariffa*) on *Esherichia coli* O157:H7 isolated from food, veterinary, and clinical samples. J Med Food, 14(9): 950–956.

Harbone B, **1984**. Phytochemical methods. 2nd. New York, Champan, Hall, pp. 4-7.

Kavanagh F, **1972**. Analytical Microbiology, Vol II, Academic Press, New York and London, pp. 11.

Lin HH, Chen JH, Wang CJ, **2011**. Chemopreventive properties and molecular mechanisms of the bioactive compounds in *Hibiscus sabdariffa* Linne. Curr Med Chem, 18(8): 1245–1254.

Lopes-Lutz D, Alviano SD, Alviano SC, Kolodziejczyk PP, **2008**. Screening of chemical composition, antimicrobial and antioxidant activities of Artemisia essential oils. Phytochemistry, 69: 1732-1738.

Mohamed R, Fernandez J, Pineda M, Aguilar M, **2007**. *Hibiscus sabdariffa* seed oil is a rich source of gamma-tocopherol. J Food Sci, 72(3): 207-211.

Mungole A, **Chaturvedi** A, **2011**. *Hibiscus sabdariffa* L.: A rich source of secondary metabolites. J Pharm Sci Rev Res, 6(1): 83-87.

Ndukwe KC, Okeke IN, Lamikanra A, Adesina SK, Aboderin O, **2005**. Antibacterial activity of aqueous extracts of selected chewing sticks. J Contemp Dent Pract, 3(6): 86-94.

Ni Q, Xu G, Lu G, Gao Q, Zhou C, Zhang Y, **2012**. Microbial studies of some herbal leaves in Africa. Afr J Biotechnol, 11(14): 3379-3387.

Oboh G, **Elusiyan** CA, **2004**. Nutrient composition and antimicrobial activity of sorrel drinks (soborodo). J Med Food, 7(3): 340-342.

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