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Phytochemical and antimicrobial activity of aqueous extract of *Hibiscus sabdariffa*

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Abstract
The calyces of *Hibiscus sabdariffa* are widely taken in the Northern and South-Western part of Nigeria for the treatment of diseases. Aqueous extract of *Hibiscus sabdariffa* was investigated for its phytochemical constituents and antibacterial activity. The extract was found to contain carbohydrates, tannins, saponins, flavonoids and exhibit antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* and other organisms such as *Klebsiella* and *Salmonella species* at different concentrations (10-160mg/ml). The present result support the use of this plant in the treatment of diseases like abscesses, urinary tract infections, coughs and pneumonia in traditional medicine and also provide the basis for isolating antibacterial agents from *Hibiscus sabdariffa*.

Keywords: *Hibiscus sabdariffa*; Antimicrobial activity; Phytochemistry

Introduction

Fruits and vegetable juices of *Hibiscus sabdariffa* and related beverages have formed daily drinks in many countries of the world including Nigeria. They are mainly consumed for their taste and thirst quenching effect. The world yearly production and consumption is estimated to be up to ten billion litres and the demand for such beverages is continuously increasing (McClintock and Tahir, 2007; Tindal, 1968; Eneji, 1992). The Red calyx extracts of *Hibiscus sabdariffa* have been employed traditionally to treat cough, abscesses and urinary tract infections in the North-Eastern Nigeria (personal observation). Juices made from the calyces of *Hibiscus sabdariffa* (Zobo drink) offer some antihypertensive and weight shedding properties (Odigie *et al*., 2003). Morton (1987) confirmed the hypotensive activity of the calyx extracts and reported on the antispasmodic, anthelminthic and anti-bacterial activities as well. Olaleye (2007) also indicated the possibility of isolating antibacterial and anticancer agents from *Hibiscus sabdariffa*. The discovery of the

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attributes of the plant above necessitates the need to evaluate the phytochemical and antimicrobial activity of the plant.

Experimental

Preparation of plant materials: The calyces of *Hibiscus sabdariffa* (Roselle) (Family Malvaceae), bought from Monday Market in Maiduguri Metropolitan Council was used in this project. The plant was identified and authenticated by a Botanist at the Department of Biological Sciences, University of Maiduguri. The fresh plant material was washed and air dried at room temperature, pulverised into powder at the Department of Biochemistry, University of Maiduguri and taken to the Laboratory for extraction, phytochemical and biological analysis.

Source of microorganisms: Pure clinical culture isolates of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella spp* and *Salmonella spp* were obtained from Microbiology Laboratory of the University of Maiduguri Teaching Hospital (UMTH), Maiduguri. These were used for the sensitivity testing of the extracts.

Sterilisation of materials: All materials used in the course of this study such as glassware and culture media were properly washed with detergent and water to remove dirt and contaminants and were dried. These were then sterilised in a portable laboratory autoclave at 121^{o}C for 15 min.

Preparation of culture media: The culture medium used for this study was Nutrient Agar (Oxoid) which was prepared according to the manufacturer's specifications. The medium was sterilised by autoclaving at 121^{o}C for 15 min after which it was allowed to cool and then used in the antimicrobial study.

Preparation of the calyx extract: The pulverised powder of the calyces was extracted at the Department of Chemistry, University of Maiduguri. Hundred (100) grams of the calyces was weighed, soaked in 500 ml of water, boiled to 100 ^{o}C for 24h and then filtered using Whatman's filter paper no.4. The filtrate was then evaporated and dried in an oven to produce the powdered form of the extract, yielding about 4g per 100g of the calyces (4%w/w).

Disc preparation: Whatman filter paper No. 4 was punched into circular discs (each 6mm in diameter), with the aid of an office punch. The discs were then wrapped in aluminium foil and then sterilised in an autoclave at 121 ^{o}C for 15 min. The sterilised discs were soaked with varying concentrations of the plant extracts and were used for sensitivity testing.

Control samples used: The control antibacterial samples used for this study were ofloxacin and ceftaxidine. Distilled water was used as negative control.

Phytochemical analysis: The preliminary phytochemical analysis of the plant extract was carried out according to standard methods of Evans, 1989; Sofowora, 1986. The *Hibiscus sabdariffa* calyx extracts were tested for the presence of Tannins, Saponins, Anthraquinones, Cardiac glycosides, Terpenes, Steroids, Alkaloids and Flavonoids (Table I).

Antibacterial activity assay: The antibacterial activities of the extract were measured using the disc diffusion agar method in an ultraviolet room. Inoculums taken from the pure culture plate of *S. aureus* and *E. coli* with the aid of an inoculating loop were diluted serially using peptone water and the turbidity was compared with McFarland's standard. One millilitre (1ml) of the bacterial inoculum was transferred into pure agar plates with the aid of a new sterile syringe, and the agar plate was slanted to ensure the spread of the inoculum on the entire surface of the agar plate. The excess bacterial inoculum on the plate was poured off to prevent floating of diffused discs on the surface of the agar plate.

The extracts were reconstituted into several concentrations for the sensitivity testing. The discs were soaked with varying concentrations (0.01, 0.02, 0.04, 0.08 and 0.16ml of 1g/ml) of the extract and allowed to diffuse for 1h with the aid of forceps placed on the agar plates at different portions. The discs were properly labelled for easy measurement. The plates were incubated for 24h after which they were examined for zones of inhibition. Readings were measured in millimetres (Tables 2 & 3; Figures 1&2).

Data analysis: The results were analysed using statistical analysis software (SPSS V11); Correlation of Coefficients, Linear Regression and Analysis of Variance (ANOVA).

Results

Phytochemical screening. Phytochemical screening of the extract revealed the presence of carbohydrates, tannins, saponins and flavonoids in *H. sabdariffa* (Table 1).

Antimicrobial activities. The aqueous extracts of *H. sabdariffa* exhibited antibacterial activities (MIC of 1.38, 1.12, 1.29, and 1.02 mg/ml) against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp and *Salmonella* spp respectively. The antibacterial activity of the extract at 160mg compared well with that of ofloxacin and ceftaxidime ($p>0.05$). Ofloxacin (10mg) was less active against the growth of *E. coli* when compared to the extract (Table 2, 3 & 4). The mean zone of inhibition against *S. aureus* and *Klebsiella* in the extract group was significantly different from control groups (Table 5). However, there was no significant difference between the extract and ofloxacin on *E. coli* ($P>0.05$), but there was difference on the same organism due to ceftaxidime. Again, there was no significant difference in the antibacterial action of the extract and ceftaxidime on *Salmonella spp* ($P>0.05$) (Table 5). The coefficient of correlation between concentration of *Hibiscus sabdariffa* and zones of inhibition of bacterial growth showed significant difference ($p<0.05$) from control group in all the organisms tested (one tailed distribution) ($r = 0.73$-0.99).There was no statistical difference in the antibacterial activity of the extract of *H. sabdariffa* against *S. aureus* and *Salmonella spp* when compared to control (two tailed) ($P >0.05$) (Table 6).

Discussion

This study was carried out to investigate the antibacterial activity of the extract of *Hibiscus sabdariffa* against *S. aureus* and *E. coli* and to relate such activities to the possible phytochemical constituents of the plant. Phytochemical study of the extract revealed the presence of relatively low concentration of carbohydrate, moderate concentrations of tannins and flavonoids, and very high concentration of saponins.

Table1: Phytochemical constituents of the aqueous calyx extracts of *Hibiscus sabdariffa*

Chemical components tested	Result
Carbohydrates	+
Tannins	++
Saponins	+++
Anthraquinone derivatives	-
Cardiac glycosides	-
Terpenes and Steroids	-
Alkaloids	-
Flavonoids	++

+ = Present at low conc. ++ = Present at a moderate conc. +++ = Present at a very high conc. - = Negative

Table 2: Antibacterial activities of the aqueous crude extracts of *Hibiscus sabdariffa* calyces

Test sample	Conc. (mg/ml)	Zones of Inhibition (mm)			
		A	B	C	D
Extract	10	10	10	10	12
Extract	20	15	10	12	19
Extract	40	19	12	15	23
Extract	80	23	15	19	26
Extract	160	24	19	22	27
Ofloxacin	10	28	12	26	30
Ceftaxidime	30	26	26	30	28
Water	-	6	6	6	6

A = *S. aureus*, B = *E. coli*, C = *Klebsiella spp*, D = *Salmonella spp*,
P> 0.05 (Not significant) P* > 0.05 (Not significant), P** > 0.05 (Not significant),
* = *Hibiscus sabdariffa* with Ofloxacin, ** = *Hibiscus sabdariffa* with Ofloxacin & Ceftaxidime

Table 3: Conc. and log of conc. of *Hibiscus sabdariffa* with respective zones of inhibition

H. sabdariffa Conc. (mg/ml)	Log of conc.	Zones of inhibition (mm)			
		A	B	C	D
10	1.00	10	10	10	12
20	1.30	15	10	12	19
40	1.60	19	12	15	23
80	1.90	23	15	19	26
160	2.20	24	19	22	27

A = *S. aureus* B = *E. coli* C = *Klebsiella spp* D = *Salmonella spp*

Table 4: Linear regression of log of conc. (Y) against zones of inhibition (X)

	A	B	C	D
Slope	0.08	0.12	0.09	0.07
Y – intercept [(a)] (mg/ml)	0.14	0.05	0.11	0.01
X – intercept (mg/ml)	- 1.77	- 0.43	- 1.12	- 0.11
R	0.98	0.95	0.99	0.96
Anti-log of Y – intercept [(b)] MIC (mg/ml)	1.38	1.12	1.29	1.02

a = minimum inhibitory log of conc., b = antilog of minimum inhibitory log conc. of y – intercept, r = correlation coefficient, A = *Staphylococcus aureus*, B = *Escherichia coli*, C = *Klebsiella spp*, D = *Salmonella spp*

Table 5: Mean zones of inhibition of various bacterial growths due to extracts of *Hibiscus sabdariffa* compared to standard drugs.

Organism	Mean zone of Inhibition (mm)		
	H. sabdariffa extract	Ofloxacin	Ceftaxidime
Staphylococcus aureus	18.2 ± 5.8^{a}	28^{b}	26^{b}
Escherichia coli	13.2 ± 3.8^{a}	12^{a}	26^{b}
Klebsiella spp	15.6 ± 4.9^{a}	26^{b}	30^{b}
Salmonella spp	21.4 ± 6.1^{a}	30^{b}	28^{a}

aa = No significant difference (p>0.05) among means with superscript in the column using one sample student t-test.
a, b = Significant difference (p<0.05) among means with superscripts in the column using one sample student t-test.

Table 6: Coefficient of correlation between concentration of *Hibiscus sabdariffa* and zones of inhibition of various bacterial growths

Organism	R	One-Tailed	Two-Tailed
Staphylococcus aureus	0.85	S	NS
Escherichia coli	0.99	S	S
Klebsiella spp	0.95	S	S
Salmonella spp	0.80	S	NS
All bacteria	0.73	S	S

NS; Not significant (P> 0.05), S; Significant (P< 0.05), r = coefficient of correlation

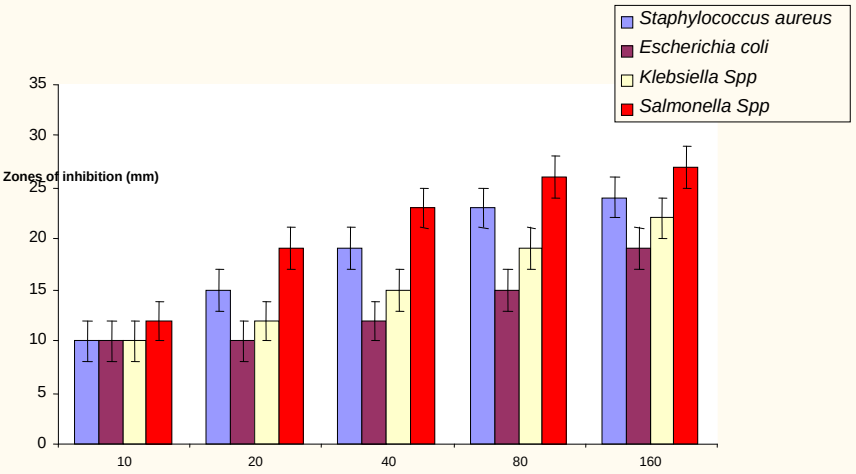

Fig. 1: Zones of inhibitions of different concentrations of *Hibiscus sabdariffa* extract on different bacteria species

Anthraquinone derivatives, cardiac glycosides, terpenes, steroids and alkaloids were absent from the aqueous plant extract used in this study. This agrees with the phytochemical reports of Subramanian and Nairy (1972) and Olaleye and Mary (2007) who found flavonoids, saponins, cardiac glycosides and alkaloid in the calyx and petal extract of *H. sabdariffa*. The results of this study is similar to the results of Hag and Gomes (1974) who reported the presence of carbohydrate, magnesium and potassium in the calyx extract of *H. sabdariffa*. The presence of saponins and flavonoids in the extract might be of advantage in the management of excess cholesterol synthesized *de novo* or exogenous cholesterol (if any) by preventing their excessive intestinal absorption thereby reducing the risk of cardiovascular diseases such as hypertension. This may explain the beneficial use against hypertension (El-Saadany *et al*., 1999; Jonadet *et al*., 1990; Malinow *et al*., 1977; Cheeke, 1971).

The effect of the extract (160mg) against *S. aureus* (24mm) and *E. coli* (19mm) (Table 2 & Figure 1&2) suggests that it may possess therapeutic properties which may be useful in the treatment of gastrointestinal infection, diarrhoea, typhoid fever and skin diseases. The finding correlates well with those of Badreldin *et al* (2007) and Rogger *et al* (1990). There was no statistical difference in the activity of the extract against the various microorganisms studied. Similarly, there was also no difference in the bacterial growth inhibition due to the plant extract and the positive standard (ofloxacin &

ceftaxidime) used in this study ($p>0.05$). The phytochemical constituents of the plants seem to have similar antibacterial activities with ofloxacin and ceftaxidime. This study also agrees with the reports of Wunwisa and Areeya (2005); Mahato *et al* (1988); Olaleye and Mary (2007) in which the plant extracts were found to inhibit the growth of methicillin resistant *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *A. baumannii*.

It is interesting to note that the plant extract was able to inhibit the growth of *E. coli* (19mm) which was less sensitive to ofloxacin (12mm), a standard broad spectrum antibiotic often used in many bacterial diseases. These antibacterial activities may be attributed to the secondary metabolites present in the extract. The minimum inhibitory concentrations (MIC) of the aqueous extract of *Hibiscus sabdariffa* on *S. aureus*, *E. coli*, *Klebsiella* and *Salmonella species* were 1.38, 1.12, 1.29 and 1.02 mg/ml respectively (Table 2, 3 & 4). The result of the MIC obtained from this study agrees with the reports of Olaleye and Mary (2007) in which the range of MIC of some bacteria observed were 0.3-1.30±0.2mg/ml. The linear regression of log concentration against zones of bacteria inhibition shows that correlation coefficient (r) against *S. aureus*, *E. coli*, *Klebsiella* and *Salmonella species* were 0.98, 0.95, 0.99 and 0.96 respectively (Table 6). This indicates that the antibacterial activity of the extracts on the organisms increase with increase in concentration (dose dependent) confirming that the activity was due to the extract.

The antibacterial effect of *H. sabdariffa* was significantly different from those of ofloxacin and ceftaxidime on *S. aureus* and *Klebsiella species* ($p<0.05$). There is no difference in the effect of the aqueous plant extract and ofloxacin on *E. coli* ($p>0.05$), but the effect of ceftaxidime is much higher than the aqueous plant extract on *E. coli* ($p<0.05$). The effect of the aqueous plant extract and ceftaxidime on *Salmonella species* are not significantly different ($p>0.05$), whereas the plant aqueous extract and ofloxacin differs in inhibiting the growth of *Salmonella species* in this study ($p<0.05$) (Table 5). Therefore, the plant extract may be of therapeutic value in the management of infectious disease like typhoid and pneumonia as claimed by traditional healers. Fluoroquinolone (ofloxacin) a potent bactericidal standard agent used in this study against *E. coli* and various species of *Salmonella* and *Neisseria* (Eliopoulos and Eliopoulos, 1993) are equipotent with calyx extract of *H. sabdariffa*.

The correlation of coefficient (r) between concentration of *Hibiscus sabdariffa* and zones of inhibition of various bacteria growth showed significant difference ($p<0.05$) using one tailed student t-Test distribution. However, there was no significant difference against *S. aureus* and *Salmonella species* using two-tailed student t-Test distribution ($p>0.05$).

Conclusion

The phytochemical screening of *Hibiscus sabdariffa* has shown that the plant contains secondary metabolites like carbohydrates, tannins, flavonoids and saponins which are very important pharmacological constituents. Furthermore, the antimicrobial activities recorded against the studied microorganisms have justified the scientific basis for its use in folk medicine in the treatment of infectious diseases. Therefore, further studies are hereby encouraged with the view to isolating and characterizing the specific active components of the plants responsible for its therapeutic effect.

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