

Full Length Research Paper

Cytotoxicity and antibacterial activity of Methanolic extract of *Hibiscus sabdariffa*

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The use of medicinal plants as raw materials in the production of drugs is again gaining popularity. *Hibiscus sabdariffa* is widely taken in the South-western part of Nigeria for the treatment of various diseases. Aqueous-methanolic extract of *H. sabdariffa* was investigated for its phytochemical constituents, antimicrobial activity and cytotoxicity using brine shrimps lethality assay. The extract was found to contain cardiac glycosides, flavonoids, saponins and alkaloids. It exhibited antibacterial activities (MIC 0.30 ± 0.2 - 1.30 ± 0.2 mg/ml) against *Staphylococcus aureus*, *Bacillus stearothermo-philus*, *Micrococcus luteus*, *Serratia marseilles*, *Clostridium sporogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Pseudomonas fluorescens*. It was also found to be potent against brine shrimps with LC₅₀ value of 55.1 ppm. The present results support the use of this plant in the treatment of diseases like abscesses, bilious conditions, cancer and coughs in traditional medicine and also suggest the possibility of isolating antibacterial and anticancer agents from *Hibiscus sabdariffa*.

Key words: *Hibiscus sabdariffa*; antimicrobial activity; brine shrimps lethality assay.

INTRODUCTION

Hibiscus sabdariffa L (HS) (*Malvaceae*), a common local drink popularly known as *zobo* in Nigeria and medicinal herb, is used in folk medicine in the treatment of hypertension (Wang et al., 2000; Odigie et al., 2003; Olaleye and Akindahunsi, 2005). *Hibiscus* anthocyanins, a group of phenolic natural pigments present in the dried flower of *Hibiscus sabdariffa* and *Hibiscus rosasinensis*, have been found to have cardioprotective (Jonadet, 1990), hypocholesterolemic (Chen et al., 2003); antioxidative and hepatoprotective (Wang et al., 2000) effects in animals. Aqueous extract of *H. sabdariffa* enhances cardiac Na⁺-K⁺-ATPase and Ca²⁺-Mg²⁺-ATPase activities (Olatunji et al., 2000). Anthocyanin pigments and other phenolic compounds (*Hibiscus* protocatechuic acid), also isolated from dried flowers of *H. sabdariffa*, demonstrated protective effect against tert-butyl hydroperoxide (t-BHP)-induced oxidative damage and hepatotoxicity both *in vitro* and *in vivo* (Wang et al., 2000; Liu, 2002).

The aqueous extract was found to be effective against *Ascaris galliavium* in poultry. Also the colouring matter of the calyces is said to be lethal to *Mycobacterium tuberculosis*. In India, a decoction of the seeds is given to relieve dysuria and many cases of dyspepsia and debility. *H. sabdariffa* has been reported to be antiseptic, aphrodisiac, astringent cholagogue, demulcant, digestive, diure-

tic, emollient, purgative, refrigerant resolvent, sedative, stomachic and tonic. It is also a folk remedy for abscesses, bilious conditions, cancer, cough, dysuria, scurvy and stangury and cancer (Morton, 1987). However, there is dearth of literature supporting its uses in the treatment of cancer, abscesses, bilious conditions and cough, which are caused mainly by microbial infections. In light of this, this work is therefore designed to evaluate cytotoxicity (using brine shrimps lethality test) and antimicrobial activity of its aqueous-methanolic extract.

MATERIALS AND METHODS

Plant materials

Red calyces of *H. sabdariffa* were collected fresh in November 2005 from a farm on the Federal Polytechnic Staff Quarter, Ado Ekiti, Ekiti State of Nigeria. Mr. S. T. Arannilewa of the Department of Biology, Federal University of Technology, Akure, Nigeria, authenticated voucher samples were deposited in the herbarium (Code number OA 001) of Biochemistry Department, Federal University of Technology, Akure, Nigeria.

Preparation of the extract

Hibiscus calyces were air-dried for 21 days at room temperature. The air-dried samples were ground to a mesh size of 1 mm. A 350

g sample of the powdered materials was soaked in 1000 ml of a mixture of methanol and water (4:1) for 96 h. This was filtered and concentrated to a small volume to remove the entire methanol using rotary evaporator. The small volume was later freeze-dried. The gummy extract was kept in the freezer at 4 C for further studies.

Phytochemical screening

The methanolic extract was screened for the presence of alkaloids, saponins, tannins, anthraquinones, cardiac glycosides, flavonoids and phlobatannins according to the methods described by Sofowora (1993).

Antibacterial activities

Antibacterial activity was studied by a disc -diffusion method. Each of the inocula (test organisms) (1 ml) was poured into sterile Petri dish. A medium (about 40 C) was poured into each of the Petri dishes (20 ml). The medium was left to stand for 5 min to allow it to set. Holes were bored on the media with the aid of a sterile cork borer of 10 mm diameter. The holes were marked, then different concentrations (20, 40, 60, 80, mg) of the plant extract were pipetted into the hole using sterile syringes. Plates were then incubated at 37 C for 24 h. The sensitivities of the test organisms to the plant extracts were indicated by clear zone of inhibition around the holes containing the plant extracts and the diameter of the clear zone was taken as an index of the degree of sensitivity.

Minimum inhibitory concentration

The minimum inhibitory concentrations of the plant extracts against the sensitive organisms were determined using the agar disc method. Serial dilutions of the plant extracts were prepared to obtain 20, 10, 5, 2.5, 1.25, 0.625 and 0.313 mg/ml. Each of the inocula (1 ml) was poured into each Petri-dish and the agar was later poured and allowed to set. Wells were bored using the sterile 3 mm cork borer. Serial dilutions of the extracts were added into the marked wells. The plates were incubated at 37 C for 24 h. The growth was observed to determine the sensitivity of each organism using clear zones of no microbial growth. The least concentration of the plant extract that had inhibitory effect was taken as the minimum inhibitory concentration (MIC) of that plant extract against such organisms.

Antibiotic assay

The effect of antibiotics on the test organism was obtained using the same procedure as that of the antibacterial susceptibility test, but instead of plant extract, antibiotics were introduced into bored holes. The zone of inhibition was measured and recorded. The antibiotic used was streptomycin (1 mg/ml).

Brine shrimp lethality test of crude extracts

Seawater was put in a soap case (partitioned into dark and light area). Brine shrimp eggs were added to the dark side and covered. The set up was left in a well lit place for 48 h. The hatched eggs, which swarm to the lit side, were used for the bioassay. 20 mg of each of the extracts was dissolved in 2 ml of sea water. From this solution, 500, 50 and 5 µl were transferred into vials and made up to 5 ml. The corresponding concentrations were 1000, 100 and 10 µg/ml, respectively. Ten (10) brine shrimps (nauplii) were transferred into each of these vials using Pasteur pipette. Replicates of each of the dose levels were prepared, using seawater as control. Number of survivors, deaths, and nauplii with sluggish movement were recorded, 24 h later. Data were processed (Using probit analy-

Table 1. Phytochemical constituents of aqueous-methanolic extract of calyces of *Hibiscus sabdariffa*.

Metabolite	<i>Hibiscus sabdariffa</i>
Saponins	+
Tannins	-
Phlobatannins	-
Anthraquinones	-
Flavonoids	+
Alkanoids	+
Cardiac glycosides	
Cardenolides	+
Steroidal ring	+
Deoxy sugar	+

+, Present; -, Absent.

sis) to estimate LC₅₀ values at 95% confidence interval for statistically significant comparisons of potencies; LC₅₀ less than 100 ppm was considered as potent (Gupta et al., 1996).

RESULTS

Phytochemical screening

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

Antimicrobial activities

The extracts of *H. sabdariffa* exhibited antibacterial activities (MIC 0.30 ± 0.2- 1.30 ± 0.2 mg/ml) against *Staphylococcus aureus*, *Bacillus stearothermophilus*, *Micrococcus luteus*, *Serratia marsecences*, *Clostridium sporogens*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Pseudomonas fluorescense*. The antibacterial activity compares well with that of Streptomycin except that the Streptomycin was not able to inhibit the growth of *E. coli*.

Brine shrimp lethality

Extract of (*H. sabdariffa*) were found to be potent against brine shrimps with LC₅₀ value of 55.1 ppm. The brine shrimps lethality was found to be concentration-dependent.

DISCUSSION

Phytochemical screening

Cardiac glycosides are cardioactive compounds belonging to triterpenoids class of compounds. Their inherent activity resides in the aglycone portions of their sugar attachment. Their clinical effects in cases of congestive

Table 2. Antimicrobial activities of the aqueous-methanolic extract of calyces of *Hibiscus sabdariffa*.

Micro-organism	Zone of Inhibition (mm)		MIC (mg/ml)
	Hs (20 mg/l)	Sp (1 mg/l)	Hs
Bacteria			
<i>Staphylococcus aureus</i> (NCIB 8588)	24±0.3	21±0.3	0.30±0.2
<i>Bacillus stearothermophilus</i> (NCIB 8222)	18±0.2	23±0.2	0.63±0.2
<i>Micrococcus luteus</i> (NCIB 196)	22±0.2	25±0.2	0.30±0.2
<i>Serratia marsecences</i> (NCIB 1377)	18±0.4	20±0.4	0.30±0.4
<i>Pseudomonas aeruginosa</i> (NCIB 950)	10±0.4	21±0.4	1.30±0.2
<i>Clostridium sporogenes</i> (NCIB 532)	20±0.4	25±0.2	1.30±0.2
<i>Escherichia coli</i> (NCIB 86)	40±0.2	0.0	1.30±0.2
<i>Klebseilla pneumoniae</i> (NCIB 418)	40±0.2	10±0.2	0.30±0.2
<i>Bacillus cereus</i> (NCIB 6349)	28±0.2	28±0.2	0.30±0.2
Fungus			
<i>Candida albicans</i> (LIO)	0	0	

Values are mean ± SD; n = 3, MIC, minimum inhibitory concentration; LIO, locally isolated organism; NCIB, collection of industrial Bacteria; O, resistant; Hs, *Hibiscus sabdariffa*; Sp, Streptomycin.

heart failure are to increase the force of myocardial contraction (Brian et al., 1985). They exert their hypotensive effect by inhibiting Na⁺ - K⁺ ATPase.

They also act directly on the smooth muscle of the vascular system. They exert a number of effects on neural tissue and thus indirectly influence the mechanical and electrical activities of the heart and modify vascular resistance and capacitance. The presence of cardiac glycosides in both plants thus gives credence to their popular use in the treatment of hypertension. Saponins are glycosides of both triterpenes and steroids having hypotensive and cardiac depressant properties. Saponins bind to cholesterol to form insoluble complexes; dietary saponins in the gut of monogastric combine with endogenous cholesterol excreted via the bile. This prevents cholesterol reabsorption and results in a reduction of serum cholesterol (Cheeke, 1971). Saponins have been found to be potentially useful for the treatment of hypercholesterolemia which suggests that saponins might be acting by interfering with intestinal absorption of cholesterol (Malinow et al., 1977a and 1977b). The presence of saponins in these plants may account for proper management of excess cholesterol synthesised *de novo* or exogenous cholesterol (if any) by preventing the excessive intestinal absorption of this compound and thus reduce the risk of cardiovascular diseases such as hypertension and hence may be responsible for their hypotensive properties (el-Sandany et al., 1991). Alkaloids, comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents (Chabner and Horwit, 1990; Noble 1990). Alkaloids also interfere with cell division, hence the presence of alkaloids in *H. sabdariffa* could account for the antimicrobial and brine shrimps lethality recorded in this study (Table 2). This is in agreement with the findings of Che-wonarin et al. (1999) that isolated an alkaloid from *H. sabdariffa* and demonstrated

its ability to prevent mutagenesis. Flavonoids are a group of phyto-chemicals found in varying amounts in foods and medicinal plants which have been shown to exert potent anti-oxidant activity against the superoxide radical. Its consumption has been documented not to be associated with coronary heart disease mortality (Hertog et al., 1993). This may be as a result of its antioxidant activity and subsequent inhibitions of LDL oxidation known to have been attributed to the dietary and supplemental intake of flavonoids and other micronutrients. Epidemiologic studies indicate an inverse relationship between intake of dietary flavonoids and coronary atherosclerotic disease (Knekt et al., 1996). The presence of flavonoids in this plant may give support to their therapeutic effects especially in the treatment of hypertension. Jonadet et al. (1990) has also reported *in vivo* cardioprotective activities of *H. sabdariffa*. Hibiscus protocatechuic acid (PCA), a phenolic compound found in the dried flower of *H. sabdariffa*, was demonstrated to have an antioxidant effect *in vitro* and *in vivo* and an anti-tumour property (Tseng et al., 1997). The presence of these metabolites probably explains the various uses of this plant in traditional medicine.

Antimicrobial activity

Antibacterial effects of this plant extracts against *E. coli*, *Pseudomonas aeruginosa* and *S. aureus* suggest that they may possess remarkable therapeutic action in the treatment of gastrointestinal infection and diarrhoea in man and skin diseases (Rogger et al., 1990). However, the plant extract was unable to inhibit *Candida albicans* which implies that they could not be used to treat fungal diseases. The high potency of *H. sabdariffa* against these bacteria gives scientific basis for its uses in folk medicine

Table 3. Effects of aqueous-methanolic extracts of calyces of *Hibiscus sabdariffa* on brine shrimps.

Conc. (µg/ml)	No. of subject	No. of Living	No of death	LD ₅₀ (ppm)
1000	10	1.0	9.0	55.7±4.02
100	10	5.0	5.0	
	10	6.7	3.0	

Values are mean ± SD of 3 replicates.

in the treatment of abscesses, bilious conditions, cancer, cough, dysuria, scurvy and stangury and cancer (Morton, 1987). It is interesting to note that the plant extract was able to inhibit the growth of *E. coli* which was not sensitive to *Stepromycin*, a standard broad spectrum antibiotic. These antibacterial activities are likely due to the presence of the secondary metabolites present in the extract.

Brine shrimp lethality

Brine shrimp lethality is a general bioassay, which is indicative of cytotoxicity, antibacterial activities, pesticidal effects and various pharmacologic actions (MacLaughlin et al., 1991). The results indicate the ability of the plant extract to kill cancer cells in cell cultures, kill pests, and exert a wide range of pharmacologic effects (MacLaughlin et al., 1991). The earlier report of Mooton (1987) that the extract could be used to treat cancer is also supported by the present findings. It has been observed that LD₅₀ values for general cytotoxicities are about one-tenth LD₅₀ values in the brine shrimp test (MacLaughlin et al., 1991). The LD₅₀ value of the extract was 55.71 which is less than 100 ppm, which indicates that the extract have high pharmacologic actions (Gupta et al., 1996). Table 3 The presence of saponins, alkaloids and cardiac glycosides may be responsible for the observed brine shrimps lethality activities of the extracts. The brine shrimps lethality further supports the antibacterial activities of *H. sabdariffa* on some pathogenic organisms observed in this study. The extract has been demonstrated in experimental animals to be safe at a dosage of 250 mg/Kg daily for a period of up to 10 days (Akindahunsi and Olaleye, 2003).

Conclusion

This work has revealed further potentials of this plant in the area of pharmacology as anticancer and antimicrobial agent. As a result of the high LD₅₀ value in brine shrimps lethality and high antimicrobial activity, the extract of *H. sabdariffa* would be considered a safe anticancer and antimicrobial agent (Tables 2 and 3).

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