

Phytochemical Screening and Antibacterial Activities Of *HIBISCUS SABDARIFFA* L. Leaf Extracts

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ABSTRACT

*The phytochemical properties and the antibacterial potency of rosselle (*Hibiscus sabdariffa* L) leaf extracts were evaluated using the cold maceration method, agar diffusion method and qualitative phytochemical analysis respectively. The methanolic extract was tested against *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus*. The phytochemical analysis results showed the presence of tannins, flavonoids, saponins and steroids in the methanolic extract of the leaves of *Hibiscus sabdariffa* L. The mean zones of inhibition of the methanolic leaf extracts showed that *Hibiscus sabdariffa* L exhibited more antibacterial activity against *Staphylococcus aureus* compared to other test organisms. Further studies are required to assess the toxicity of the extract.*

INTRODUCTION

Hibiscus sabdariffa is commonly called Roselle. it is a dicot plant of the malvaceae family originating from Africa. The plant grows as an annual and sometimes biannual shrub with straight branches and small ramifications. Cultivated plants reached between 1 to 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. The plant is currently grown in tropical region of India and part of Asia, Australia and America. The vegetable is widely grown and commonly used in the northern part of Nigeria. In Hausa language, the plant is called “yakuwa”. The calyx drink is popularly known as zobo in Nigeria. It is used in folk medicine in the treatment of hypertension¹.

Pharmacological studies of anthocyanins in hibiscus have shown that they have antioxidant activity in patients with atherosclerosis. In several countries, it is used as a natural medicine for treating hypertension, pyrexia, liver disorder and microorganism growth limitation, as well as a digestive and sedative¹.

Traditional medicines has a diverse health practices, approaches, knowledge and beliefs that incorporate plant, animals or mineral-based medicines, spiritual therapies, manual techniques and exercises which are applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness². The increasing widespread use of traditional medicines has prompted the WHO to promote the

integration of traditional medicine into the national health care systems of some countries. Herbal medicines, also called botanical medicines or phyto-medicine refers to the use of herbs, herbal materials, herbal preparations and finished herbal products that contains parts or whole of plants materials, as active ingredient². The plants materials include seeds, berries, roots, leaves, bark or flowers³. Plants are used in the dried form due to differences in water content within different plant tissue before extractions. The studies about the effect of plant extract against different types of bacteria are still one of the most important fields of researches⁴. The extracts thus obtained after extraction may be used as medicinal agents⁵ normally expected to contain phytochemicals. The chemicals are the natural defense system against diseases and pest⁶.

Phytochemicals are bioactive components present in plants. They includes: tannins, alkaloids, saponins, flavonoids and steroids⁷. Fruits that are brightly coloured yellow, orange, red, green; blue and purple generally contains the most phytochemicals and the most nutrients. Phytochemicals are naturally bioactive components found in vegetables which may reduce cancer, strokes, hinder the aging process and antimicrobial properties⁸. They have complimentary and overlapping mechanisms of action in the body including antioxidant effects, modulation of detoxification and enzymes stimulation of immune system, modulation of hormone metabolism, anti-bacteria and antiviral effects⁹.

The antibacterial activities of the plant extract and phytochemical was evaluated with antibiotics susceptible and resistant microorganism.

MATERIALS AND METHOD

Materials

Methanol, dilute sodium hydroxide, chloroform, concentrated sulphuric acid, acetic anhydride, Wagner reagent, 1% lead acetate, 1% hydrochloric acid, glacial acetic, distilled water, normal saline, procaine penicillin (control), nutrient broth, nutrient agar, dilute hydrochloric acid, crystal violet, safrannin, lugol iodine solution.

Collection of Plant Material and Identification

The plant materials *Hibiscus sabdariffa* (Roselle plant) were collected from the central Market in Kaduna, Nigeria. The specimens were identified and authenticated using preserved specimen available in the herbarium of Department of Applied Science, Kaduna Polytechnic.

Preparation of Plant Material

The freshly collected leaves of *Hibiscus sabdariffa* were carefully cleaned and plugged from the stem. The leaves of *Hibiscus sabdariffa* were air-dried in an aerated room for 6 weeks. The dried leaves were pulverized using mortar and pestle into smaller particles and then blended to powder using an electric blender. 32g of the powdered leaves of *Hibiscus sabdariffa* was obtained. 500ml of methanol was measured into the 32g of the powdered leaves and then stored in an air-tight container for 4 days.

Extraction

The powdered leaves (32g) of *Hibiscus sabdariffa* was extracted successfully with

methanol using maceration method. The solvent was removed at reduced pressure to give 5g of the crude extract.

Preliminary Phytochemical Screening

The methanol extract of the leaves was used for the preliminary phytochemical screening procedure for the presence of bioactive ingredients such as tannins, alkaloids, flavonoids, saponins, and steroids¹⁰.

Collection of Test Organisms

The test organisms (*Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*) were collected as clinical isolates from Shehu Kangiwa Hospital in Kaduna Polytechnic. All the test organisms were preserved in a refrigerator at 4°C in nutrient agar slant until required.

Gram Staining

A sterile wire loop was used to pick a small portion of the isolate into a clean grease free slide, it was then emulsified with little drop of distilled water air-dried and heat fixed. The smear was flooded with crystal violet for 1 minute and rinsed off with distilled water, lugols, iodine solution was added for one minute and rinsed off with distilled water. It was decolourized with 95% of the alcohol and then rinsed immediately with distilled water. The smear was finally flooded with safranin for one minute and

Preparation of McFarland Standard

Barium sulphate (1% V/V solution of sulphuric acid was prepared by adding 1ml of concentrated H₂SO₄ into 99ml of distilled water. One percent (1%) weight per volume solution of barium chloride was also prepared by dissolving 0.5g of dehydrated barium chloride solution was combined with 99.4ml of sulphuric acid solution to yield 1.0% v/v barium sulphate suspension. The

washed off with distilled water. The stained slides were observed under oil immersion objective lens (x100)¹¹. The Gram-positive bacteria (*Staphylococcus aureus*) retained purple colour of the primary dye (crystal violet) and the Gram-negative bacteria (*Salmonella typhi* and *Escherichia coli*) retained pink or red colour of the secondary dye (safranin).

Preparation of Overnight Broth Cultures

A loop full of the test organisms from the preserved slant cultures were aseptically introduced into sterile nutrient broth in test-tube each and labeled appropriately, then incubated for 24 hours at temperature of 37°C.

Culture Media Preparation Nutrient agar is a solid media that is used for developing surface-colony of bacteria. It was used for antimicrobial assay which was prepared according to the manufacturer's specification.

The nutrient agar was prepared by dissolving 7g of the agar in 250ml of distilled water contained in 500ml of sterile conical flask. The media was autoclaved at 121°C for 15 minutes. The sterilized media was allowed to cool to temperature of 45°C and then approximately 20ml was poured into each sterile petri dish and allowed to gel¹².

turbid solution formed was transferred into a test tube as the standard for comparison. This matches with 0.5 McFarland standard turbidity to prepare using the test bacterial culture¹².

Standardization of Inoculums

The previously prepared overnight nutrient broth culture of each bacteria isolates was used to prepare inoculate by diluting with

sterile saline solution. 0.1ml of each overnight broth culture of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* was dispensed into the separate test tubes containing the sterile normal saline. The suspension was adjusted to match the 0.5 Mcfarland's standard which has a similar appearance of an overnight broth culture. This serves as the standard inocular which was used for the antibacterial potential testing¹².

Antibacterial Potential

The antibacterial potential of the methanolic extract or susceptibility test was conducted using the well diffusion technique.

24 hours old bacterial cultures were used for well diffusion method. One well of 6mm size was made in the help of sterile cork borer under aseptic condition in laminar air flow chamber. The wells were loaded with different concentrations such as 100mg/ml, 50mg/ml, 25mg/ml of the leaf extracts (1g in 10ml of solvent). The plates were incubated at 37°C for 24 hours. The plates were observed after 24 hours for clearing zone around the well. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well.

RESULTS AND DISCUSSION

Table 1 shows the result of phytochemical screening of methanolic extracts of *Hibiscus sabdariffa L.* (Roseselle) leaves revealing the presence of saponins, steroids, flavonoids and tannins. The antibacterial activity of methanolic extract of *Hibiscus sabdariffa L.* against *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli* is shown in Table 2.

The phytochemical screening of *Hibiscus sabdariffa L.* leaf extracts revealed the presence of saponins, steroids, tannins, and flavonoids and absence of alkaloids. This may be due to environmental or physiological conditions experienced by the plant and also may be due to species difference. The distribution of saponins, flavonoids, tannins and steroids in the extract confirmed its use as an antimicrobial agent. The result of the phytochemical test therefore indicate that *Hibiscus sabdariffa* possessed numerous biologically active compounds, which could serve as potential source of drugs in herbal medicine. The activity against all the test organisms occurred at all concentrations with highest inhibition zone of 18mm at 100mg. According to plant database (2008), phytochemical components have antibacterial properties which were confirmed in this study. The presence of tannins in the plant extract agrees with the report of Evans (1998) that tannins are important in herbal medicine and they are applied to bleeding and wound healing. One phytochemical constituent may contain hundreds of species which are extracted differently based on the polarity of solvent used¹¹. Tannin or flavonoid compound extracted from two different plants or plant part may show different activity on a single test organism.

The tannins in the aqueous extract may not be the same with that extracted in the methanolic extract. The results show that *Hibiscus sabdariffa L.* methanolic extract can be used for therapeutic purpose against the infections caused by the test organisms, especially *Escherichia coli* which had the highest zone of inhibition.

Table 1: Phytochemical screening of methanolic extracts of *Hibiscus sabdariffa* L. (Roseselle) leaves

Phytochemical Constituents	Inference
Flavonoids	[+]
Tannins	[+]
Saponins	[+]
Steroids	[+]
Alkaloids	[-]

+ = presence
- = absence

Table 2: Antibacterial activity of methanolic extract of *Hibiscus sabdariffa* L. against *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli*.

Clinical Bacterial Isolates	Zones of Inhibition (mm) of <i>Hibiscus sabdariffa</i> L. Methanol Leaf Extract at different Concentrations (mg/ml)			
	100	50	25	Control (Procaine Penicillin)
<i>S.typhi</i>	8	5.5	10	40
<i>S. aureus</i>	17.5	6.5	8.5	25
<i>E. coli</i>	18	11	7.5	15

CONCLUSION

The results of phytochemical screening in the present study have shown that roselle (*Hibiscus sabdariffa* L) leaves has therapeutic potentials and contains bio-components whose antibacterial potentials are highly comparable with that of the antibiotic procaine penicillin against the gram negative and gram positive bacteria tested. The activity of the leaf extract may be indicative of the presence of broad spectrum bioactive compounds in the leaf. Therefore, roselle (*Hibiscus sabdariffa* L) could be a promising natural antibacterial agent with potential applications in pharmaceutical industry for controlling infections caused by the organisms used in this study.

REFERENCES

1. Akindahunsi A.A., Olaleye M.T. (2004). Toxicological Investigation of Aqueous-Methanolic Extract of the Leaves of *Hibiscus sabdariffa* L. *Ethnopharmacol.* 89:161-164.
2. Baues, A.W., W.M. Kirby, H. Truck and Shreeies, J.C. (1996). Anragonistic, Susceptibility. Testing by Standardized well diffusion method *Am. J. Clin pathol.* 45: 495-496.
3. Chessebrough M. (2002). Direct Laboratories Pracices in Tropical Countries (2) Press Syndicates of the University of (Cambridge) U.K. pp. 243-244.
4. Coughim, J.B., Debusk. A. (1998). *Phytochemical Methods – A Guide to Modern Techniques of Plant analysis* Chapman and Hall, London pp. 182-190.
5. Ehrlich S.O. (2010). *Herbal Medicine.* University of Maryland Meidial Centre.
6. Eusaniha J.U, Garba S.A., Manwak J.D., Oyewole O.A. (2012). Antimicrobial Activity of *Cymbopogon citratus* (Lemon Grass) and its phytochemcial properties. *Frontiers Sci.* 2(6): 214-220.
7. Evans W.C. (1989): *Solino lars* Pharmacological Company Ltd. London Press pg. 344.
8. Founet L., (2002). *Phytochemcial Screening of Medicinal Plans* Retrieved May 15th, 2015 from www.friedli.com/herbs/phytochem/flavonoid.
9. Krama, S.N., Templaten, S., John, A.O. (2006): “Studies on Bioactive Saponins from Chinese Medicinal plants” *Advances in Experimental Medicine and Biology* 404:371-82 PMID 8957308.
10. Ndukwe K.C., Okeke I.N. Lamikanra A, Adesina SK., Abdoerin O. (2005). Antibacterial Activity of Aqueous extracts of Selected Plant Materials *J. Contemp. Dent. Pract.*, 3(6): 086-094.

11. Silver *et al.*, (1998): Phytochemical Method, A Guide to Modern Techniques of Plant Analysis 1st Edition, Chapman and Hall London, ISBN: 04/2572605 pp. 15.
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12. World Health Organisation (2008). Fact Sheet on Traditional Medicine WHO.
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