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Phytochemical, proximate and antibacterial properties of *Hibiscus Rosa-Sinensis* L. Leaf

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Abstract

The study was conducted to evaluate the phytochemical, proximate and antibacterial properties of *Hibiscus rosa-sinensis* leaf extracts. The study revealed that the phytochemical constituents were as follows: tannins (+++) combined anthraquinones (+++), cardiac glycosides (+++) while other with less concentration included Alkaloids (+), free Hydroxyanthraquinone were not present. Mean percentage proximate composition of the leaves showed carbohydrate (31.66), lipid (9.60), protein (7.01), fibre (3.99), ash contents (3.07) and morshline (2.63). The leaf extracts also showed composition of mineral elements in mg/g⁻¹ as follows: (Ca = 772.57±0.01, K = 181.00±0.50, P =42.38±0.01, Na =0.33±0.09, Mn =2.40±0.03). Solvent extract of the *Hibiscus* leaves had a higher maximum zones of inhibition with 18.82±0.18 mm than aqueous extract with 14.00±1.05 mm around *Bacillus subtilis*. The least zone of inhibition was 11.00±1.20 mm against *Stapylococcus aureus*. *Hibiscus rosa-sinensis* leaves are recommended for their utilization as herbal medicinal with important antibiotic properties and as highly nutritive vegetable.

Keywords: Phytochemical, proximate, antibacterial properties, *Hibiscus Rosa-Sinensis*

Introduction

Hibiscus rosa-sinensis belongs to the family of *Malvaceae*. The leaves are simple ovate or ovate lancolated with coarsely toothed at the apex. Flowers are pedicillate and 5 petals of red colour (Lalit 2012) [8]. *Hibiscus rosa-sinensis* is planted as an ornamental plant throughout the tropics and sub-tropics. In Akwa Ibom State of Nigeria the young leaves is commonly consumed as vegetable. The leaves also have medicinal value. *Hibiscus rosa-sinensis* has played a prominent role in human health due to the presence of specific biologically active classes of organic compound (Joshi and Dhawan 2005) [6]. Over 50% of all modern clinical drugs used today are of natural product origin (Sumathi and Krishinaveni, 2012) [15]. In traditional setting *Hibiscus* has been reported to have been used as anti-asthmatic agent (Ruban and Gajalakshmi, 2012) [14], analgesic, anti-inflammatory, antipyretic and possess anti-tumor and anti-convulsant properties (Agarwal and Prakash, 2014) [1].

Several studies have revealed presence of antimicrobial properties in flowers of *Hibiscus rosa-sinensis* (Ruban and Gajalakshmi, 2012; Agarwal and Prakash, 2014) [14, 1].

The emergence of antibiotic resistant strains of bacteria and fungi is widespread (Agarwal and Prakash, 2014) [1] and this calls for the search and utilization of alternative source of antimicrobial agents against most human pathogens and microbes with potential of causing diseases in man.

The objective of this study was to determine the phytochemical, nutritional and antibacterial properties of the leaves of *Hibiscus rosa-sinensis*.

Materials and Methods

Hibiscus rosa-sinensis plants were obtained from Botany and Ecological research garden of Department of Botany and Ecological studies in Univerisity of Uyo in Akwa Ibom State, Nigeria. The plants were identified by the taxonomist at the Herbarium Unit of the Department of Botany and Ecological Studies, University of Uyo, where the voucher specimens have been deposited. The leaves were harvested and taken to the pharmacognosy laboratory for the extractions. Matured leaves were selected and washed with distilled water. Then samples of leaves were air dried and ground into powder by using mortar and pestle in the laboratory as described by Mukhtar and Tukur (1999) [9].

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Phytochemical Screening of Crude Extract

The extract were subjected to phytochemical tests to determine the groups of secondary metabolites present in the plants such as alkaloids, tannins, saponins, flavonoids, cardiac glycosides, anthraquinones, phlobatanins, carbohydrates and protein by standard procedures followed by Debela (2002) [4].

Extraction of components of *Hibiscus rosa-sinensis*

- a) Extraction of Aqueous components: The dried powder (10g) of leaves of *Hibiscus rosa-sinensis* was soaked in 100 ml of hot water in a sterile conical flask with continuous shaking on a rotator shaker at 190 rpm for 24 hrs. This was then filtered using muslin cloth and the filtrate was centrifuged at 10000 rpm for 5 mins in an air-tight bottle at 4 °c. The supernatant was collected and concentrated to make the volume of 40 mg L⁻¹. (Ruban and Gajalakshmi, 2012) [14].
- b) Solvent extraction: Ten (10) grams of air-dried powder of leaves of *Hibiscus rosa-sinensis* was placed in 100 ml of methanol in a plugged conical flask. The leaves-methanol solution was then kept in a rotatory shaker at 190 rpm, for 24hrs and filtered using a muslin cloth. It was then centrifuged at 5000 rpm for 10mins and the supernatant was collected. The extract was evaporated using solvent distillation apparatus to concentrate the extract to 40 mg/ml. It was then stored in an air-tight bottle at 4°C for further studies (Ruban and Gojalakashmi, 2012; Agarwal and Prakash, 2014) [14, 1].

Culture Preparation for Antibacterial Assay

The following organisms (*E.coli*, *Staphylococcus aureus* and *Bacillus subtilis*) were cultured and isolated according to methods described by Dubey and Maheswari (2004) [5] using Eosin methylene Blue (EMB) McConkey and nutrient agars respectively at 34±2 °C for 18hrs. The isolates were suspended in 2ml of saline solution (0.85%NaCl). The turbidity was adjusted to 0.5 McFarland standards (180 Cfu in L⁻¹) for subsequent inoculation on the plates (nutrient agar).

Anti-Bacterial Assay

The agar disc diffusion method was adopted to assess the antibacterial activity of both aqueous and organic solvent-extracts of the leaves as described by Agarwal and Prakash (2004) [1]. The concentrated extracts compounds (40mg extracts/ml) were applied in a paper disc of 0.5 mm and allowed to dry. Then the discs were applied to thin layer of Mueller-Hinton agar plates already swabbed with the test bacteria and were incubated at 34°C for 48hrs.

Results

Phytochemical and Mineral Composition

The result of phytochemical analysis of the leaves extracts of *H. rosa-sinensis* L. revealed the presence of varying amount of alkaloids, tannin, saponins, flavonoids, cardiac glycosides, anthraquinones and phlobatanins, as shown in Table 1. The proximate composition of the leaves shows that the carbohydrate (CHO) of the plant is (31.66%). The moisture content is 2.63% and it has a high fibre content of 3.99% while the protein value is 7.01% (Table 2). Table 3 shows the mineral composition of the leaves in mg/g⁻¹. The leaves are riched in calcium (772.57mg/g⁻¹) followed by potassium (181.00 mg/g⁻¹). The sodium content (0.38mg/g⁻¹) is comparably lower than the other minerals. The results show that hot aqueous extraction had maximum zone of inhibition against *Bacillus subtilis*, *Escherichia coli* (*E. coli*) and

Staphylococcus aureus of 14.00±1.05 mm 12.30±0.95 mm and 11.00±1.20 mm respectively while the methanol extract showed zones of inhibitions against *B. subtilis*, *E. coli*, *S. aureus* at 18.82±0.18 mm 17.30±0.51 mm and 15.20±0.90 mm respectively. These results are consistent with the reports of Ruban and Gajalakshmi (2012) [14] and those of Agarwal and Prakash (2014) [1]. The anti-bacterial activity of the extracts of the *Hibiscus* leaves in disc diffusion method are shown in Table 4 below.

Table 1: Phytochemical screening of alcoholic extract of *H. rosa sinensis* L. leaves

Extract Constituents	Concentration
Alkaloids	+
Tannins	+++
Saponins	-
Flavonoids	-
Cardiac Glycosides	-
• Lieberman's	+
• Salkowski	+++
• Keller kiliani	+++
Anthraquinones	-
Free hydroxyanthraquinones	-
Combined Anthraquinones	+++
Phlobatanins	-

Key: +++ = High concentration
 ++ = Moderate concentration
 + = Low concentration
 = Not detected

Table 2: Proximate compositions of leaves of extract of *H. rosa sinensis*

Test	Composition in%
Morsline	2.63
Ash	3.07
Fibre	3.99
Protein	7.01
Lipid	9.60
Carbohydrate	31.66

Table 3: Proximate mineral composition (mg/g⁻¹) of *H. rosa-sinensis* leaves

Mineral Element Tested	Composition in (mg/g ⁻¹)
Fe	31.17 ± 0.21
P	42.38 ± 0.01
Na	0.38 ± 0.09
K	181.00 ± 0.50
Mg	90.33 ± 0.03
Mn	2.40 ± 0.03
Ca	772.57 ± 0.01

Values are means ± standard deviation of 3 replications

Table 4: Antibacterial assay of aqueous and solvent extracts of *H. rosa sinensis* in disc diffusion method (mean ±S.D) millimeter.

Disc diffusion method		Test Organisms
Aqueous extract	Solvent extract	
14.00±1.05	18.82±0.18	<i>B. subtilis</i>
12.30±0.95	17.30±0.51	<i>E. coli</i>
11.00±1.20	15.20±0.90	<i>S. aureus</i>

Discussion

The revealed bioactive chemical compounds from the phytochemical screening of *H. rosa-sinensis* leaf extracts have classified the plant as a medicinal plant. These includes alkaloids, tannins, saponins, flavonoids cardiac glycosides, anthraquinones and phlobatanins. These findings agree with

the report of (Okunade 2002; Krishnaiah *et al.*, 2009 and Anyasor *et al.*, 2010) ^[11, 7, 3]

H. rosa-sinensis is one of the popular plants in Akwa Ibom State and beyond. The leaf extracts are high enough in essential nutrients required for optimal physiological performance and the maintenance of good health. This is in line with the report of (Nayak, *et al.*, 2007) ^[10] that tannins are plant metabolites which are useful in wound healing.

The antibacterial activity of aqueous and solvent extracts of the leaves, *H. rosa-sinensis* observed in this study could be attributed to the presence of some bioactive compounds with antibacterial properties. Agarwal and Prakash (2014) ^[1] reported the presence of some anti-bacterial agents (flavonoids, tannins, alkaloids and terpenoids) in flower extracts of *H. rosa-sinensis*. The bioactive substances in plants which may be produced as secondary metabolites have several biological functions and pharmaceutical properties (Agarwal and Prakash, 2014; Al-Alak, Al-Oqaili, Mohammed *et al.*, 2015) ^[1].

In this study the leaf extracts of *Hibiscus* were used to screen for antibacterial potency against some human pathogens (*E. coli*, *B. subtilis* and *S. aureus*). *E. coli* are normal flora of gastro-intestinal tracts but is responsible for disorder such as diarrhoea. *S. aureus* are normal flora of the human skin but if ingested through contaminated foods may pose health problems such as Staphylococcosis, also *B. subtilis* in food is an indicator of contamination.

The present study showed that *Hibiscus rosa-sinensis* leaves could be utilized medicinally with important antibiotic properties to cure disorder caused by the different species of bacteria (Agarwal and Prakash, 2014) ^[1].

Conclusion

The study revealed that the *Hibiscus rosa-sinensis* plants possess vital properties such as nutritional (with as much as 7.01% of 10g of powder leaf being protein, plus a number of essential mineral elements: Ca, Mg P, K etc). The presence of bioactive photo chemicals such as high contents of tannins and alkaloids which could lend the antibacterial potency to the *Hibiscus* leaves. Therefore, it could be utilized not only for their high nutritional values but also as a medical plant.

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