



Comparative Phytochemical and Antimicrobial Analysis of Roots, Stems and Calyx of
Hibiscus sabdarriffa Extract

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ABSTRACT

Qualitative and quantitative anti-microbial and phytochemical activities of *Hibiscus* (roots, stem and calyx) extract was carried out. All the three extract of *Hibiscus* root, stem and calyx showed presence of alkaloid, saponins, tannin, flavonoids, glycoside, terpenoids, phenols and steroids. The saponins and flavonoids showed very high presence in the qualitative analysis of the root and stem. Which also justified its high value of 4.21 ± 0.31 in the quantitative analysis of root as well as glycoside of stem with 4.78 ± 0.21 . The antimicrobial activities exhibited significant activities against *E.coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* with higher zone of inhibition of 2.42 mm against *Staphylococcus aureus* in the root extract of 300 mg/kg concentration in the least inhibition of 0.17 mm against *E.coli* of root extract of 100 mg/kg concentration was also noticed.

Keywords: Anti-microbial, Phytochemical activities, *Hibiscus*, Alkaloid, Saponins

INTRODUCTION

Medicinal plants have been discovered and used in traditional medicine practice since pre-historic times. Medicinal herbs are considered as a rich source of ingredients which can be used in drug development, some plants are considered as an important sources of nutrition and as a result they are recommended for their therapeutic functions some of these plants include ginger, green tea, walnuts, *Aloe vera*, pepper and turmeric etc. Some plants and their derivatives are considered as important sources of active ingredients which are used in aspirin and toothpaste etc. Medicinal plant can also be used in natural dyes, pest control, food, perfume and so on. In many countries different kinds of medicinal plants/herbs are used to keep out flies, mice, and flea away from homes and offices. *Hibiscus Sabdarriffa* is referred to as one of the important medicinal properties. The plants grow as annual and sometimes bi-annual shrubs with straight branches and ramification [1].

The plant is currently grown in tropical region of India, and parts of Asia, Australia, and America. The vegetable is widely grown and commonly used in the Northern part of Nigeria. In Hausa language is called "Yakuwa" the calyx

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drink is popularly known as "Zobo" in Nigeria.

It is used in folk medicine in the treatment of hypertension. The plant also have delight taste and also a good sources of nutrients, vitamins and minerals. There are also various reported on poly-herbal formulation that contain *Hibiscus* as a major constituents. The roots orally used stomachic and externally as an emollient and leaves eaten as vegetable after cooking. They are manually used in diuretic, anti-septic, sedative purgative and tonic. Its decoction is useful for cough. The infusion of the red petals is used as a refrigerant drink in fever. *Hibiscus* has demonstrated to have a wide range of anti-microbial activity against bacteria strain. It is also used as antidotes to poisonous chemicals (acid, alkali and pesticides), different part of this plant have been commended as a remedy of various ailment. However, despite reported efficiency of the *Hibiscus sabdarrifa* plant, there have been a limited literature on either the leaves, stem and root are more potent [2].

The aim of the study is to compare the phytochemical antimicrobial properties of the *Hibiscus sabdarrifa* extract to ascertain which part is more medicinal in value in relation with its phytochemical and its antimicrobial properties.

The phytochemical are non-nutritive plant chemical that have protective or disease preventive properties. They are non-essential nutrient meaning that they are not required by human body for sustaining life. The phytochemical are component such as tannins, alkaloids, saponins, flavonoids and steroids. The extract obtained was subjected to both qualitative and quantitative phytochemical analysis using standard procedure according to AOAC 2011 version [3].

MATERIALS AND METHODS

Plants materials

The *Hibiscus sabdarrifa* plants collected in Naze, Owerri North, L.G.A of Imo State, Nigeria and identification at biological science department of Federal university of technology, Owerri.

Sample preparation

The roots, stem and calyx were separated into different portion labelled as AR (Roots), AS (Stem), AC (Calyx) respectively. Each portion of the *Hibiscus* as labelled were dried, grounded and extracted using ethanol as solvent. The percolates were evaporated using rotary evaporator at 40°C and residues obtained as crude extract.

Phytochemical analysis

Test for alkaloids: 2 ml of the crude extract was poured in a test tube and 2 ml of 1% HCl were added and filtered. 5 drops of Meyer reagents were added to the filtrate. A precipitate was formed which indicated the presence of alkaloids.

Test for tannins: 0.5 g of the extract sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green coloration.

Test for saponins: 5 ml of the extract solution was placed in a test tube and 5 ml water was added and shake vigorously. The foam formation observed indicated the presence of the saponins.

Test for flavonoid: 2 ml of extract was added to concentrated HCL (few drops) and magnesium ribbon. Pink reddish color indicate presence of flavonoids [4].

Test for glycosides: 2.5 ml of 5% sulphuric acid was added to 5 ml of the extract in 9 test tube the mixture was heated in boiling water for 15 minutes, then cooled and neutralized with 10% NASH and 5 ml Fehling's solution was added, the mixture heated. A reddish precipitate was observed indicating the presence glycoside. The same procedure

was applied to all the three samples of roots, stem and calyx respectively

Test for steroids: 0.5 g of the extract was dissolved in 2 ml chloroform. Sulphuric acid was carefully added to form a lower layer. Where a reddish brown color at the inter-phase was observed which indicate the present of steroidal ring.

Test for phenol: 10 m³ of the extract was added to 10 m³ of the ferric chloride solution. A deep bluish green solution indicate the presence of phenol [5].

Test for terpenoids: 0.5 ml of the extract was evaporated to dryness in a water bath and heated with 3 ml concentrated sulphuric acid for 10 minutes. A dark reddish color indicates the presence of terpenoids.

The qualitative phytochemical process was carried with the same procedure for the three extract of root, stem and calyx.

Antimicrobial studies

The antimicrobial studies for the three extract roots, stem and calyx was carried out using agar diffusion method. In this process the bacteria (*E.coli*, *Pseudomonas aeruginosa*, *staphylococcus aureus*) were suspended into petri-dishes containing nutrient agar and mixed thoroughly to ensure uniformity. This was allowed to stand for about 15 min so that the nutrient agar got solidified. Three petri dishes for each extract making a total of nine (9) petri dishes were punched on the nutrient agar using fleer borer. Each of the extract solution were poured into the wells and used for the test. The inoculated plate was incubated for 24 hrs at 37°C and the degree of activity were observed. The presence of positive result was established by the length of the zone of inhibition. The internal diameter (in cm³) of the zone of inhibition is an indication of the degree of activity [6].

RESULTS AND DISCUSSION

The result obtained after carrying out phytochemical and antioxidant analysis for the three different part of the *Hibiscus sabdarriffa* namely root, stem and calyx shown in the below Table 1 and 2.

Table 1: Shows result of qualitative phytochemical analysis for roots, stem and calyx extract of *Hibiscus sabdarriffa*.

Phytochemical (Qualitative)	Root	Stem	Calyx
Alkaloid	++	++	+++
Saponins	+++	++	++
Tannin	++	++	++
Flavonoids	++	+++	++
Glycoside	+	+	++
Where +: Sparingly present; ++: Moderately present; +++: Very highly present			

Table 2: Shows result of quantitative phytochemical analysis of roots, stem and calyx extract of *Hibiscus sabdarriffa*.

Phytochemical (Quantitative)	Root	Stem	Calyx
Alkaloids	1.5 ± 0.73	2.2 ± 0.42	2.5 ± 0.75
Saponins	1.2 ± 0.31	1.4 ± 0.29	1.3 ± 0.11
Tannin	0.11 ± 0.21	0.12 ± 0.21	1.0 ± 0.13

Flavonoid	1.10 ± 0.31	0.13 ± 0.34	1.11 ± 0.24
Glycoside	3.21 ± 0.41	2.78 ± 0.21	4.20 ± 0.34

The zone of inhibition in (mm) of the extract of root, stem and calyx of *Hibiscus sabdariffa* on the three different types of bacteria with increase in concentration of 100-300 mg/kg of the extracts was investigated and the result shown in Table 3-5.

Table 3: Shows the zone of inhibition in (mm) of the ethanol extract of Roots (AR) of *Hibiscus sabdariffa* at concentration of 100 mg/kg.

Organisms	Diameter of zone of inhibition (mm)		
	Roots	Stem	Calyx
<i>E.coli</i>	-	2.8	2.94
<i>Pseudomonas aeruginosa</i>	1.42	2.5	2.6
<i>Staphylococcus aureus</i>	2.12	Nil	2.41

Table 4: Shows the zone of inhibition in (mm) of the ethanol extract of Roots (AR) of *Hibiscus sabdariffa* at concentration of 200 mg/kg.

Organisms	Diameter of zone of inhibition (mm)		
	Roots	Stem	Calyx
<i>E.coli</i>	-	2.8	2.94
<i>Pseudomonas aeruginosa</i>	1.42	2.5	2.6
<i>Staphylococcus aureus</i>	2.12	Nil	2.41

Table 5: Shows the zone of inhibition in (mm) of the ethanol extract of Roots (AR) of *Hibiscus sabdariffa* at concentration of 300 mg/kg.

Organisms	Diameter of zone of inhibition (mm)		
	Roots	Stem	Calyx
<i>E.coli</i>	-	2.8	2.94
<i>Pseudomonas aeruginosa</i>	1.42	2.5	2.6
<i>Staphylococcus aureus</i>	2.12	Nil	2.41

The result in Table 1 and 2 shows the qualitative and quantitative analysis of phytochemical compound of the three different portions of *Hibiscus sabdariffa* namely: The roots, the stem and the calyx [7].

The qualitative result indicated the presence of alkaloids, tannins, saponins, flavonoids, glycosides, terpenoids, steroids etc. in all the three parts of the *Hibiscus sabdariffa*. The higher concentration of saponins was noticed in roots of the plant with the other stem and calyx showing moderate and sparingly presence of Phytochemical Compound.

The quantitative result in Table 2 shows value of alkaloids in the calyx with 2.5 ± 0.95 and with lower value of 0.11 ± 0.2 of tannins in the roots while higher value of 4.20 ± 0.34 of calyx was recorded for glycosides with least value of 0.13 ± 0.34 for flavonoids in stem.

The presence of these bio-active compound in crude extract is known to justify the antimicrobial activity against

disease causing micro-organism (pathogens). The alkaloids are known to have both anti-bacteria and anti-fungal properties. While tannin and flavonoids (7) have been reported to have anti-bacterial activity with inflammatory properties of tannin been reported (8). Saponins are been used in the production of detergent as a foaming agent and in intracellular histo chemistry staining to allow antibody access to intra cellular proteins. It also serves an antioxidants, anti-cancer and anti-inflammatory. The steroids are used as contraceptive and precursors for sex hormones while glycoside are cardio tonic.

The result showing the effect of bioactivities of the roots, stem and calyx of the *Hibiscus* were shown in Table 3-5. From the result obtained, it shows that the extract inhibited the growth of bacteria in all the extract with the highest zone of inhibition at 2.94 mm of the calyx extract against the *E.coli*. Again in 100 mg/kg concentration of the extracts, the value of 1.42 mm was noticed in *Pseudomonas aeruginosa* of roots. However, the zone of inhibition increase against all the bacterial zone of inhibition as the concentration of the extract increases. This clearly shown in Table 4 and Table 5 with concentrated of extract of 200 mg/kg and 300 mg/kg respectively.

In the Table 4, the highest zone of inhibition was reported against calyx extract with 3.05 mm with yeast in roots extract of 2.15 mm of zone of inhibition against *Staphylococcus aureus*.

In 300 mg/kg concentration of Table 5. It shows higher values for all the zones of inhibition for the three bacteria namely *E.coli*, *pseudomonas aeruginosa* and *staphylococcus*.

CONCLUSION

The results obtained indicate that *H. sabdariffa* L. is rich in natural antioxidant compounds, antimicrobial agent, and anti-swarming factor. Taken together, these biological properties may allow the tested extracts from *H. sabdariffa* to be considered as having the potential to be candidate in the design of new therapeutic strategies for microbial infections, and may lead to the development of novel bioactive molecules for industrial needs. Further studies, such as the use of other isolation and purification techniques of the active compounds and *in vivo* studies, would help to elucidate the mode of action of the observed beneficial effects of the aqueous and methanolic extracts.

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