



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 2.42mm against *Staphylococcus aureus* in the root extract of 300 mg/kg concentration in the least inhibition of 0.17mm against *E.coli* of root extract of 100mg/kg concentration was also noticed.

Keywords: Anti-microbial; *Hibiscus Sabdarriffa*; Phytochemical; Quantitative; Qualitative

1. 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 [1].

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 [2].

Hibiscus Sabdarriffa is referred to as one of the important medicinal properties. The plants grows as an annual and sometimes bi-annual shrubs with straight branches and ramification.

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 drink is popularly known as "Zobo" in Nigeria [3].

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 [2]. 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

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reported efficiency of the *Hibiscus Sabdarriifa* plant, there have been a limited literature on either the leaves, stem and root are more potent [4].

The aim of the study is to compare the phytochemical antimicrobial properties of the *Hibiscus Sabdarriifa* extract to ascertain which part is more medicinal in value in relation with its phytochemical and its antimicrobial properties [5, 6].

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 [7].

2. Material and method

2.1. Plants materials

The *Hibiscus Sabdarriifa* 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.

2.2. 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^{0c} and residues obtained as crude extract.

2.3. Phytochemical analysis

2.3.1. Test for alkanoids

2ml of the crude extract was poured in a test tube and 2ml 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.

2.3.2. Test for tannins

0.5g of the extract sample was boiled in 20ml 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.

2.3.3. Test for saponins

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

2.3.4. Test for flavonoid

2ml of extract was added to concentrated HCl (few drops) and magnesium ribbon. Pink reddish color indicate presence of flavonoids.

2.3.5. Test for glycosides

2.5ml of 5% sulphuric acid was added to 5ml of the extract in 9 test tube the mixture was heated in boiling water for 15 minutes, then cooled and neutralized with 10% NaOH and 5ml 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

2.3.6. Test for steroids

0.5g of the extract was dissolved in 2ml 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.

2.3.7. Test for phenol

10ml/3 of the extract was added to 10ml/3 of the ferric chloride solution. A deep bluish green solution indicate the presence of phenol.

2.3.8. Test for terpenoids

0.5ml of the extract was evaporated to dryness in a water bath and heated with 3ml 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.

2.4. 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 15min 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 fleur borer. Each of the extract solution were poured into the wells and used for the test. The inoculated plate were incubated for 24hrs at 37^oc and the degree of activity were observed. The presence of positive result were 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.

3. Results

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

Table1: show result of qualitative phytochemical analysis for roots, stem and calyx extract of *Hibiscus Sabdariffa*.

Table 1 Result of qualitative Phytochemical Analysis of Roots, Stem and Calyx extract of *Hibiscus Sabdariffa*

Phytochemical (qualitative)	Root	Stem	Calyx
Alkaloid	++	++	+++
Saponins	+++	++	++
Tannin	++	++	++
Flavonoids	++	+++	++
Glycoside	+	+	++

Where + = sparingly present; ++ = moderately present; +++ = very highly present

Table 2 Result of quantitative phytochemical Analysis of roots, stem and calyx extract of *Hibiscus Sabdariffa*

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 The zone of inhibition in (mm) of the ethanol extract of Roots (AR) of *Hibiscus Sabdarrifa* at concentration of 100 mg/kg

Organisms	Diameter of zone of inhibition (mm)		
	Roots	Stem	Calyx
<i>E.coli</i>	-	2.80	2.94
Pseudomonas Aeruginosa	1.42	2.50	2.60
Staphylococcus Aureus	2.12	Nil	2.41

Table 4 The zone of inhibition in (mm) of the ethanol extract of Roots (AR) of *Hibiscus Sabdarrifa* at concentration of 200 mg/kg

Organisms	Diameter of zone of inhibition (mm)		
	Roots	Stem	Calyx
<i>E.coli</i>	-	2.80	2.94
Pseudomonas Aeruginosa	1.42	2.50	2.60
Staphylococcus Aureus	2.12	Nil	2.41

Table 5 The zone of inhibition in (mm) of the ethanol extract of Roots (AR) of *Hibiscus Sabdarrifa* at concentration of 300 mg/kg

Organisms	Diameter of zone of inhibition (mm)		
	Roots	Stem	Calyx
<i>E.coli</i>	-	2.80	2.94
Pseudomonas Aeruginosa	1.42	2.50	2.60
StaphylococcusAureus	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 Sabdarrifa* namely: the roots, the stem and the calyx.

The qualitative result indicated the presence of alkaloids, tannins, saponins, flavonoids, glycosides, terpenoids, steroids etc. in all the three parts of the *Hibiscus Sabdarrifa*. 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 Table3-5. 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 2.94mm of the calyx extract against the *E.coli*. Again in 100 mg/kg concentration of the extracts, the value of 1.42mm 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 3.05mm with yeast in roots extract of 2.15mm 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*.

4. Conclusion

As presented above, from literature and our preliminary investigation one can attest to the fact that *Hibiscus Sabdariffa* is effective in the treatment of cough, hypertension, fever and so many other ailments. This report also justify the uses of this plant in traditional medicine. Attempt are also ongoing to isolate and identify bio-active potentials of the roots, Stems and calyx of the plant extract. This may lead to the development of further studies

Compliance with ethical standards

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Disclosure of conflict of interest

The authors contributed to the research design, analysis and interpretation of the generated data. The paper have not been sent to any journal publication outlet within Nigeria or any other part of the world. The authors only carried out the research as its contribution to humanity without any financial assistant from company or institution.

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