PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL EFFICACY OF EXTRACTS OF TWO VARIETIES OF *Roselle calyces.*

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ABSTRACT

Ethanol and water extracts from two varieties of Roselle calyces were phytochemically screened and tested for their biological activities against Escherichia coli, staphylococcus aureus and Shingella spp. All fractions were subjected to phytochemical screening and antimicrobial activity. Both the water and ethanol extract were positive for Saponins, Tannins, Flavonoids, Alkaloids, Glycosides and Phenols, and negative for volatile oils and resins. The water extract was active on all the pathogens so also the ethanol extract. This shows that Roselle calyces contain bioactive compounds of medicinal significance. Therefore the traditional use of the plant for the treatment of ailments is justified in this study.

Keywords: *Roselle calyces*, bioactive compounds, phytochemicals and extracts.

INTRODUCTION

Plants do not only provide food for human and animals, but other diverse products such as medicine, building materials, textile, gums, resin, waxes, rubber, perfumes, dyes and tanning materials. Investigation into the chemical and biological activities of plants during the past two centuries have yielded compounds for the development of modern synthetic organic chemistry and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents The African continent is one which is endowed with one of the richest biodiversity in the world as abundance of many plants used as herbs, foods and for therapeutic purpose. Great need arises for the evaluation of the constituents' pharmacological properties and detailed screening of bioactive substance for chemotherapeutic purpose. Furthermore, investigation into the antimicrobial activities of these plants will show that plants are potential sources of synthesis of drugs (Clark, 1996).

Plants are very essential for all living things because plants provide all foods for both human beings and animals, plants also serve as the source of diverse products for our industries e.g. perfumes, dyes, textile fibers, building materials e.t.c. plants provides habitats and shelter for wildlife and birds it also contribute to soil building process. Plants of the coniferous era provided the drugs that is now used in modem medicine. (Mc-Graw Hill,1997).

Medicinal plants represent a rich source from which antimicrobial agents may be obtained. Plants are used medicinally in different countries and are a source of many powerful drugs (Srivastava *et al.,* 1996).

The interest in the scientific investigation of medicinal plants from Nigeria is based on the claims of their effective use for the treatment of many diseases. Therefore research into the effects of these local medicinal plants is expected to enhance the use of these plants against diseases caused by the test pathogens.

Medicinal plants are of great importance to the health of individuals and communities, plants have provided source of inspiration for novel drug compounds.

Modern scientist have made phenomennal step in developing this heritage handed over by our forefathers (Sofowora,1986).
The recent examination by the United Nations Commission of Trade and Development (UNCTAD) indicated that about 33% of drugs produced in the developing countries are obtained from plants (UNCTAD/GATT 1974) and if microbes are added, 60% of medicinal plants are of natural origin (Sofowora,1981). According to Sofowora sources, almost 80% of present day medicines are directly or indirectly derived from plants (Myers, 1982). Surprisingly this large quantity of modern drugs come from less than 15% of plants, which are known to have been investigated pharmacologically out of essential 250,000 to 500,000 species of higher plant growing on earth (Farasworth and Bingel, 1977). However most of these plants used in herbal medicine have not been screened for the antimicrobial activity. The active principles for many drugs found in plants are secondary metabolites (Ghani, 1990; Srivastata,1996). Therefore, basic phytochemical investigation of these extracts for their major phytochemicals is also important. In the present study the water and ethanol extract from two varieties of *Roselle calyces* were screened for phytochemicals constituents and antimicrobial activity against *Escharichia coli, Staphylococcus aureu and Shingella,spp.*
Roselle belongs to the family, *malvaceae* with two main varieties, *Hibiscus sabdariffa* and *Hibiscus altissima. L.*

The common names of *Roselle calyces* include: Rosella (Australian), Meshta (Indians), Bissap (Senegalese), Wonjo (Gambians), Chaye torosh (Iranians), Omo te te (Namibians).

In Nigeria the common names include Zoborodo (Hausa), Isapa (Yoruba) and Mburu ojo (Igbo).

The true *Rosella* plant is an annual or perennial herb or woody-based sub shrub growing at 2-2.5m tall, the leaves are deeply three to five lobed, 8-15cm long in diameter, white to pale yellow with a dark red spot at the base of each petal and have a stout fushy calyx at the base, 1-2cm wide, enlarged to 3-3.5cm. It becomes fleshy and bright red as the fruit matures it takes about six months for the fruit to fully mature. Today *Roselle* is attracting the attention of food and beverages manufacturer and pharmaceutical concerns that felt it may have exploitable possibilities as a natural food product and as a colorant to replace synthetic interest is centered more on the medicinal and food potential if managed a lot of income can be generated from the production as a result of the economic, nutritional medicinal and industrial application attached to the plant (Morton, 1987).

The chemical components present in *Rosella* include pectin, anthocyanin, ascorbic acid, niacin, riboflavin, thiamine and malic acid.

For quite some time *Roselle* has been used locally in the preparation of wine (zobo drink), medicine, soup and other food products. The stems are used in making bags, ropes and bast fibers. The *Roselle calyces* are found to contain antispasmodic, anticarcenogenic and antimicrobial properties. The extracts are found to be antihypertensive and stimulate intestinal paristalsis. Pharmacologist in Senegal recommended *Roselle* extract to lowering blood pressure and in the treatment of Urinary tract infections. The aqueous extract was found to be effective against Ascaris gallinarum in poultry. The coloring matters of the *calyces* are lethal to mycobacterium tuberculosis. Roselle extracts decreases the rate of absorption of alcohols and reduced the effect of alcohols in the system in Africa, the *calyces* infusion, called "sedan tea' is taken to relief coughs. The juice with salt, pepper, and molasses, is taken as a remedy to biliousness

A lotion made from the leave is used on sores and wounds. The seeds are said to be diuretic and tonic in action. In

India, a decoction of the seeds is given to relieve mild cares of dyspepsia *Roselle calyces* extracts is a good medicine that fight constipation.

This paper reports the potentials of *Roselle calyces* for their antimicrobial activity. Also in mind is sourcing of extracts from them much could provide the expected chemotherapy against other human pathogens.

MATERIALS AND METHODS

Sampling

The two varieties of *Roselle calyces* were harvested fresh from a farm in Sangere, Girei local government area of Adamawa state, Nigeria.

Identification was done by the Forestry Department of Federal University of Technology, Yola. The samples were air dried in the laboratory before pounding to fine powder using pestle and mortar and sieve with a sieve of 230-310 mesh and then stored in a clean dry container at ambient temperature.

Extraction

250ml of ethanol was added to 50g of the powder each in a conical flask. The mixture was stirred and covered. It was allow to stand for 48 hours and filtered using whatman No.2 filter paper. The filtrate was then concentrated using rotary evaporator to about 50ml. the procedure was repeated with distilled water using fresh 50g of each of the varieties for each extraction. All extracts were cooled and stored in air tight bottles in a refrigerator at 4^0C prior to use.

Phytochemical Screening

Phytochemical screening for major constituents was undertaken using standard qualitative methods as described by (Fadeyi, et al.,1989), (Odebiyi and Sofowora,1990), (Harbone,1992), (Abulude, et al.,2001) and (Abulude,2007). Saponnins, tannins, flavonoids, Alkaloids, volatile oils, glycosides, phenols and resin tests were conducted in all the fractions.

Test for Saponins

5ml of extract was vigorously shaken with 10ml of water in a test tube. Frothing which persisted was taken as an evidence for the presence of saponins.

Test for Tannins

Extract plus 4ml of water and drops of ferric chloride. Immediate green precipitate was taken as evidence for the presence of tannins.

Test for Flavonoids

Extracts plus small quantity of Magnesium chips plus drops of concentrated hydrochloric acid down the side of test tube. Reddish coloration was taken as evidence for the presence of flavanoids.

Test For Alkaloids

2 ml of the extract plus picric acid. An orange colouration was taken as evidence for the presence of alkaloids.

Test for Volatile oils

Extract was dissolved into 90% ethanol and two drops of ferric chloride were added. Green colorations were taken as an indication for the presence of volatile oils.

Test For Phenols

Equal volume of the extract was added to equal volume of ferric chloride, a deep bluish green solution was taken as a positive test for the presence phenols.

Test for Glycosides

5ml of extract plus 25ml of dilute sulfuric acid were poured into a test tube.the mixture is boiled for 15 minutes, cooled and neutralized with 10% sodium hydroxide and 5 ml of Fehling A and B was added. Brick red precipitate is a positive test for the presence of glycosides

Test For Resins

2 ml of extract plus equal volume of acetic anhydride solution plus two drop of concentrated sulfuric acid. A violet colouration was taken as an indication for the presence of resins.

The results of the phytochemical screening are shown in table 1

Antimicrobial Investigation

Test Culture

The test organisms for the antimicrobial screening were obtained from the stock culture of microbiology unit laboratory of peace hospital Jimeta-Yola, Adamawa State. The organisms were *Escherichia coli*, *staphylococcus aureus* and *shingella spp.*

The isolates were maintained on nutrient agar slant and sub-culture in nutrient broth for incubation at 37^{o}C prior to each antimicrobial testing.

Antimicrobial assay

Evaluation of the antimicrobial activity of the extracts was carried out using the disc diffusion method. Inoculation of the test organisms on nutrient agar prepared plates was achieved by flaming a wire loop on a spirit lamp, cooling the wire loop (air cooling) and fetching the test organisms. The discs were prepared using Whatman filter paper. They are obtained by punching and putting in vials-bottles and sterilizing in an oven at 150^{o}C for 20 minutes.

Prepared discs containing the various fractions were carefully placed on the inoculated plates using a sterilized forceps in each case (Fatope, 1993). The plates were then turned upside-down and inoculated at 37^{o}C for 24 hours in an incubator.

Scoring and Reading

The result was taken by considering the zone of growth and inhibition of the organisms by the test fractions activity and inactivity were observed in accordance with the standard and acceptable method (Mackie and McCartney, 1989). Results are shown in Table 2.

RESULTS AND DISCUSSION

Table 1: Results of Phytochemical analysis of extracts of the two varieties of *Roselle calyces*

Test	Water extracts		Ethanol extracts	
	DR	CW	DR	CW
Saponins	+	+	+	+
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Volatile oils	+	-	+	-
Glycosides	+	+	+	+
Phenols	+	+	+	+
Resins	-	-	-	-

Key: Absent (-), present (+), Dark red variety (DR), cream-white variety (CW).

Table 2: Results of antimicrobial efficacy of the two varieties of *Roselle calyces*

		Zone of inhibition (mm) ± standard error		
Solvent name	**Plant variety**	***E.coli***	***S.aureus***	***Shingella spp***
Water	DR	24.4±0.3	23.1±0.4	20.4±0.3
	CW	25.1±0.2	21.3±0.3	17.3±0.4
Ethanol	DR	20.2±0.3	23.2±0.4	15.2±0.4
	CR	25.3±0.4	21.2±0.2	20.3±0.3
Control	Gentamicin	26.1±0.2	24.2±0.2	28.3±0.3

Key: DR = dark red variety, CW = cream white variety

In table 1, saponnin, tannins, flavonoids, alkaloids, glycosides and phenol were present in the two varieties of the ethanol and water extract. Volatile oil is only present in the dark red variety of the extracts. Resin is totally absent in the two varieties of the ethanol and water extracts

Table 2 shows the zones of inhibition (mm) of the two varieties against the microorganisms. The calyces of both water and ethanol fractions are highly efficacious, covering nearly the entire spectrum of organisms. None of the organism shows resistance against these extracts. However, the dark red variety of ethanol extracts is less effective or has low activity against *shingella spp* while the cream white variety of water extract shows low activity against the *shingella spp*. The cream white variety of both extracts show high activity against *E.Coli.* the dark red variety of both extracts show high activity on S *aureus.*

For the growth of these microorganisms to be inhibited, components of the extracts must have bioactive constituent against them (Sofowora, 1986). These three microbes are causative agent of some infectious diseases (Trease, 1983). Thus, justifying the use of the plant extracts for the treatment of some diseases in local communities in Nigeria.

CONCLUSION

Based on the Phytochemically detected chemical compounds, it can now be concluded that *Roselle calyces* have broad spectrum, inhibiting the growth of the microbes which are known to be involved in causing some diseases in humans. Also, the antimicrobial action of the extracts correlated well with its traditional uses in Nigeria.

The presence of these biologically active compounds: alkaloids, saponins, tannins, glycoside, phenols and flavonoids in *Roselles calyces* can make it to be one of the sources of the drug development of natural origin that can be utilized to serve the needs of human beings.

RECOMMENDATION

Since *Roselle calyces* teas aid in digestion, encourage healthy kidney function and are antibacterial; it should be prepared and sold at outlets and canteens as it is done in the case of sachet pure water to encourage its intake regularly. And these can be exploited with suitable comparative and competitive advantages.

Industries producing and preparing juice and wine to try the magnificent power of *Roselle calyces* extracts in making wine and juice for public consumption and see the rush for the demand.

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