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Preliminary Phytochemical Studies and Evaluation of Antimicrobial Property of the Methanol Extract of the Roobark of *Ritchiea longipedicellata* Gilg Family *Capparidaceae*

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Purpose: The root of *Ritchiea longipedicellata* was claimed to have antimicrobial properties. The people of Idemili area in Anambra State of Nigeria use the decoction of it to treat wounds, running stomach, aches and pains. It is because of this background that this investigation was carried out to ascertain the veracity of the claim.

Methodology: The root of *Ritchiea longipedicellata* was collected and dried at ambient temperature. It was pulverized into powder. 500gm of the powdered drug was placed into a 2litre beaker containing 1litre of methanol. It was allowed to stand with occasional shaking for 48hrs. The content was filtered and the filtrate was concentrated using rotary evaporator. The extract contains the following secondary metabolites – alkaloids, flavonoids, terpenoids, saponins and glycosides. Agar diffusion method was used to investigate antimicrobial activity.

Result: The root of *Ritchiea longipedicellata* exhibited antimicrobial property.

Conclusion: The claim of Idemili people of Anambra State Nigeria on the use of *Ritchiea longipedicellata* appears to be obvious in line with the results of the investigation

Keyword: *Ritchiea longipedicellata*, Agar Diffusion

1. Introduction:

Over the past decade herbal medicine has become a topic of global importance, making an impact on both world health and international trade (Sofowura A. 2008). Medicinal plants continue to play central roles in the healthcare system of large proportion of the world's population. This is particularly true in the developing countries, where herbal medicine has a long and

uninterrupted history of use. Recognition and development of medicinal and economic benefits of these plants are on the increase in both developing and industrialized nations (Srinivas *et al*, 2007). Continuous usage of herbal medicine by a large proportion of the population in the developing countries is largely due to the high cost of western pharmaceuticals, health care, adverse effects that follow their use (in some

case) and the cultural and spiritual point of view of the people of the countries (Srinivas *et al.*).

In Western developed countries however, after a downturn in the pace of herbal use in recent decades, the pace is again quickening as scientists realize that the effective life span of any antibiotic is limited (Satyejji and lutfun, 2007). Worldwide spending on finding new anti-infective agents (including vaccines) was expected to increase 60% from the spending levels in 1993. New sources, especially plant sources, are also being investigated. Secondly, the public is becoming increasingly aware of problems with the over-prescription and misuse of traditional antibiotics. In addition, many people are interested in having more autonomy over their medical care. All these makes the knowledge of chemical, biological and therapeutic activities of medicinal plants used as folklore medicine become necessary. (Fagbohun *et al.*, 2010).

Before the era of Louis Pasteur (1822-1895), world renowned chemist and biologist who proved the germ theory of disease, the notion that tiny organisms could kill vastly larger ones (including human) seemed ridiculous to many people. Nowadays, it has been accepted that infectious diseases are the number one causes of death worldwide, accounting for approximately one half of all deaths in tropical countries (Iwu *et al.*, 1999). In fact, there are more patients today in hospitals than there are effective drugs due to the development of resistance to available agents.

The use of plant parts as a source of medicine to treat infectious diseases predates history. Nearly all cultures and civilizations from ancient times to the present day have used herbal medicines (Erdemeier *et al.* 1996; Lino and Deogracious, 2006) to cure infections. The intractable problem of antimicrobial resistance has led to the resurgence of interest in herbal products as sources of novel compounds to fight the ever increasing problems of emergence of newer diseases and preventing the resurgence of older diseases thought to be brought under control. Herbal medicine practice plays an important role in the primary healthcare delivery system in most developing countries including Nigeria. Even the

World Health Organization (WHO, 2002) is actively encouraging national governments of member countries to utilize their traditional systems of medicines with regulations suitable to their national health care systems. The WHO estimates that 80% of the population living in rural areas use or depend on herbal medicine for their health needs (WHO Traditional Medicine Strategy, 2002). However, in spite of the obvious and important contribution the herbal medicine makes to primary health care, it continues to be antagonized by majority of allopathic medical practitioners as it is considered to have no scientific basis. This work is therefore a preliminary work to prove that there is scientific evidence to the use of the root of *Ritchiea longipedicellata* in the treatment of diseases.

One major problem of herbal medicine practice is that there is no official standard and / or local monograph. In Nigeria, the Federal Government has urged the federating states to set up traditional medicine boards to license and regulate the practice of herbal practitioners under the supervision of ministries of health.

Many medicines including reserpine, ergotamine, vincristine, and vinblastine are of herbal origin. About one quarter of the present prescription drugs dispensed by community pharmacies in the United States contain at least one active principle originally derived from plant materials (Farms Worth and Moris, 1976).

2. Taxonomy of the Plant and Its Description

Kingdom	: Plantae
Division	: Angiospermae
Class	: Dicotyledonae
Subclass	: Archichlamydae
Order	: Papaverales/Brassicales
Suborder	: Capparineae
Family	: Capparidaceae
Genus	: <i>Ritchiea</i>
Species	: <i>Ritchiea longipedicellata</i> Gilg.



Fig1: Plant of *Ritchiea longipedicellata*

3. Plant's Description:

The plant is an evergreen climber but when alone it is a self-supporting shrub with compound palmate leaves. The leaves can be collected all-year round as the plant can stand dry season. The roots are tuberous and with strong pungent odours when perceived. Like other capparidaceae, this herb is indigenous to the tropics, found mostly in the lowland area of rain forest, especially beside water body and virgin up-lands. As a shrub it grows to a height of few meter(s) and as climber can grow a considerable length of about 5 meters with several branches (**Local source**).

4. Distribution of *Ritchiea Longipedicellata* Gilg.

The plant *Ritchiea longipedicellata* G. is virtually all over the tropical land of Africa and particularly West Africa. In Nigeria, the plant is found in the south east where the plant is used locally for various indications. The local name springs from the number of leaves (three) present in a leaflet hence it is called Nchi-ato [3-ears] by the Ibo people (Ikwo).

5. Ethnobotanical Uses of *Ritchiea Longipedicellata* G

The plant is used in Nigerian local villages (particularly in Ikwo L. G. A. in Ebonyi State and Idemili in Anambra State) where the root and the leaves are used for treatment of various illness.—small quantity of the root can be chewed (with closed mouth) to relieve pain in the head, cold,

upper respiratory tract infections. Local palm wine extract of the plant is used for the treatment typhoid fever and malaria and general illness that prove resistance to modern therapies (**Local users and traditionalist**).

6. Review of Activities of Some Capparidaceae Plants

The results of screening of Petroleum ether, chloroform, ethanol and water extracts of roots of *Capparis grandiflora* Wall. ex Hook's & Thomson showed inhibitory activities against 24-hour cultures of *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus pumillu*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* but none showed antifungal activity. The phytochemical screening showed the presence of tannins, saponins, sesquiterpenes, alkaloids, and phlobatannins (Karanayil *et al*, 2011).

The *in vitro* antibacterial screening of the extracts of *Boscia angustifolia* roots (family Capparidaceae) showed that the crude water and chloroform extracts possess significant ($P < 0.05$) inhibitory activities against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus pneumonia* with the exception of *S. typhi*. Phytochemistry showed absence of flavonoids, steroids, free anthraquinones balsams and resins (Hassan *et al*, 2006)

Cappari tomentosa used traditionally as spices and for healing a lot of complaints ranging from cough to infertility and impotence reportedly showed no activity against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa*, With no effect on fibroblast growth.

Leaf extract of *Capparis zeylanica* L. (CZ) is locally taken with black pepper powder is taken twice daily for treatment of dysentery. Leaf juice of CZ taken orally with cup of fresh goat milk for curing cough and cold. For the treatment of diabetes, ripe fruits are consumed twice for fortnight. The screening of water extract (200 mg/kg) significantly ($P < 0.01$) reversed yeast-induced fever in rodents. The aqueous extract

from total aerial parts of the plant has been used for its antifungal, anti-inflammatory, antidiabetic, and antihyperlipidemic activities and is among the constituents of polyherbal formulations to treat liver ailments; preliminary phytochemical screening of the leaf extracts show the presence of alkaloids, flavonoids, saponins glycosides, terpenoids, tannins, proteins and carbohydrates . The roots of *C. zeylanica* contain alkaloid, phytosterol, acids and mucilage (Sunil et al, 2011).

Gynandropsis gynandra commonly called spider flower is member of the family capparidaceae. It has demonstrated high activity against both bacteria, fungi and helminthes .It has steroidal nucleus, alkaloid, reducing sugar, and cyanidin (Ajaiyeoba, 2000).

7. Materials and Method

A. Materials

1. Chemicals and Solvent

The chemicals used for this experiment include methanol (Qualichem pvt ltd), dimethyl sulphoxide (DMSO), Nutrient Agar. The reagents used were – concentrated sulfuric acid, naphthol solution in ethanol (Molisch reagents) picric acid, ammonium solution, nitric acid, Aluminum chloride solution, Fehling solution A and B, Wagner’s reagents (iodine and potassium iodide), Hager’s reagent (saturated solution of picric acid).

2. Sources of Microorganisms

The microorganisms used were both bacteria obtained from laboratory stock of the Department of Pharmaceutical Microbiology and Biotechnology Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka. The organisms include bacteria (*Staphylococcus aureus*, *pseudomonas aeruginosae* , *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*

3. Equipment

Weighing Balance [Scout pro u401 made in China], Beakers, measuring cylinder, test tubes, incubators (GentLab UK), autoclave, test tubes, test tube racks, syringes and needle, Pasteur’s

pipette, conical flask, glass rod, inoculation loop, Tripod stand, filter paper (Whatman No 1), Mortar and pestle , water bath, muslin- cloth, reagent bottles, Bunsen burner, and permanent marker.

B. Methods

1. Source and Identification of Plant Materials

The root of *Ritchiea Longipedicellata* was obtained from Echialike in Ikwo local Government Area, Ebonyi state in January 2012. The plant was identified by Mr Ozioko – a Taxonomist of University of Nigeria, Nsukka. The root was air dried in the Pharmacognosy Laboratory and then were pulverized to produce 500g of powder.

2. Extraction Process

Extraction was done with methanol. The 500g of powdered dried root was macerated with one Liter of methanol in a 2liter beaker for two days with occasional agitations. At the end it was strained using white muslin cloth and then filtered using Whatman No 1 filter paper. The process was repeated using the marc. The combined filtrates were concentrated using rotary evaporator under reduced pressure. Aliu AB et al (2008).

Qualitative assay for the presence of secondary plant metabolites were carried out on the methanol extract of the root of *Ritchiea longipedicellata* using the standard procedures (Harborne 1991), (Trease and Evans, 1989).

8. Phytochemical Screening of the Plant

Standard screening tests were carried out on powdered root for various phytochemical constituents. The procedure used was obtained from Evans (2002) and Departmental Laboratory Manual (2009), Awe et al (2003) and Beena et al (2010).

a. Test for Protein

Xanthoproteic reaction test: 5 ml volume of the filtrate obtained from boiling few grams of powdered plant is heated with few drops of concentrated nitric acid; yellow colour that

changes to orange on addition of alkali indicates the presence of protein.

b. Test for Carbohydrates

0.1g of the powdered leave was boiled with 2mL of distilled water and was filtered .To the filtrate, few drops of naphthol solution in ethanol (Molish reagent) were added. Concentrated sulphuric acid was then poured gently down the test tube to form a lower layer. A purple interfacial ring indicates the presence of carbohydrate (starch).

c. Test for Alkaloids

About 5 g of powdered root placed in the test tube and 20ml methanol added to the tube, the mixture was heated in water bath and allowed to boil for two minutes. It was cooled and filtered. 5ml of the filtrate was tested with two drops Wagner's reagent (solution of iodine and potassium iodide).

To another 5mL portion of the extract 2 drops of Hager's reagent (saturated picric acid solution) was added. The presence of precipitate indicates alkaloid.

d. Test for Steroids

About 9ml of ethanol was added to 1g of the extract and refluxed for a few minutes and filtered. The filtrate was concentrated to 2.5ml on a boiling water bath. 5ml of hot distilled water was added to the concentrated solution. The mixture was allowed to stand for 1 hour and the waxy matter was filtered. The filtrate was extracted with 2.5ml of the chloroform using separating funnel. To 0.5ml of the chloroform extract in a test tube, 1ml of concentrated sulfuric acid was added to form a lower layer. A reddish brown interface shows the presence of steroids.

e. Tests for Saponins

About 20ml of water was added to 0.25g of crude extract and boiled gently in a hot water bath for 20 minutes. The mixture was filtered hot and allowed to cool and the filtrate was used for the following tests.

- I. Frothing test: 5ml of filtrate was diluted with 20ml of water and vigorously

shaken. The test tube was observed for the presence of stable foam upon standing.

- II. Emulsion test: To the frothing solution, 2 drops of olive oil was added and the content shaken vigorously and observed for the formation of emulsion.
- III. Fehling's test: To 5ml of the filtrate was added 5ml of Fehling's solutions (equal parts of A and B) and the content was heated in a water bath and a reddish precipitate which turns brick red on further heating with sulphuric acid indicates the presence of saponins (general test for glycosides).

f. Test for Flavonoids

About 10ml of ethylacetate was added to 0.2g of the extract and heated on a water bath for 3 minutes. The mixture was cooled, filtered and used for the following test.

- I. Ammonium test: 4ml of filtrate was shaken with 1ml of dilute ammonium solution. The yellow colour in the ammonical layer indicates the presence of the flavonoids.
- II. Aluminum chloride solution (1% test) another 4 ml portion of the filtrate was shaken with 1ml of 1% aluminum chloride solution. The layers were allowed to separate; a yellow colour in the aluminum chloride indicates the presence of flavonoids.

g. Fixed Oil

Whole extract solution (0.5ml) with two drops of 1M alcoholic $K_2Cr_2O_7$ and 3 drops of phenolphthalein were added in a clean test tube. Soap formation shown by frothing indicated the presence of fixed oil.

h. Phenolic Group

Alcoholic plant extract (0.5ml) was taken in a test tube. Two drops of 1M ferric chloride was added. Appearance of intense color indicated the presence of phenolic groups.

i. Cyanogenetic Glycosides

About 1 g of powdered sample was boiled with distilled water and moist sodium picrate paper held inside the tube with a cork. A colour change from yellow to Brick-red of the picrate paper is positive for cyanogenetic glycosides.

9. Pharmacological Tests

A. Antimicrobial Assay

a. Microorganisms: 24hour Cultures of five human pathogenic bacteria made up of both gram positive (*S. aureus*, and *B.subtilis*) and and gram negative (*P. aeruginosa*, *E. coli* and *S. typhi*) bacteria were used for the *in-vitro* antibacterial assay. All microorganisms were obtained from the laboratory stock of the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences Nnamdi Azikiwe University Awka.

b. Preparation of Media

Nutrient agar was used in the assays. Dimethylsulphoxide (DMSO) was used in solublising the extracts and drugs and as a negative control in the study. The media was prepared by dispersing the weighed amount in water and then sterilized in autoclave. The plates of nutrient agar were poured and allowed to solidify after the appropriate organisms were seeded (Majorie Murphy Cowan 1999).

c. Antimicrobial Agents: Ampicillin, 20ug/ml (Mecure industrial ltd lagos Nigeria.); was used in the study as standard reference drug.

d. Antimicrobial Activity Determination

An overnight broth culture used to obtain 0.5 **Marcfarland** standard of bacterium was used to seed sterile molten nutrient agar medium maintained at 45°C. Seven holes (6mm) respectively, were bored in each of the plates (9cm, diameter) with an aseptic cork borer, when seeded plates had solidified; 400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml of extract were prepared in dimethylsulphoxide (DMSO) by preparing a stock solution and carrying out double fold dilutions on it. And with the aid of a Syringe, the wells were filled with 0.25 ml (5drops) of different dilutions of the extract while the centre well was filled with 20µg/ml of ampicillin (also dissolved in DMSO). Diameters of zones of inhibition were determined after incubating plates at 37°C for 24h for bacteria. This test was conducted first on the crude extract and the solvent dimethylsulphoxide was used as negative control while ampicillin was used as positive control.

10. Results and Analysis

The results of phytochemical screening showed presence of alkaloid, glycosides and flavonoid. Also present were saponins and flavonoids Lather Amit et al (2010).

Table 1: Result of Phytochemical Screening of *Ritchiea longipedicellata* Gilg

Secondary metabolites	Results
Alkaloid	+
Tannin	-
Flavonoids	+
Saponins	+
Steroid	-
Terpenoid	+
Glycoside	+

Key: + = present; - = absent

Table 2: The Results of Antimicrobial Screening

Bacteria	400mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	Amp20ug/ml
<i>Staphiloccocus aureus</i>	8mm	6mm	4mm	2mm	1mm	-	-	6mm
<i>Escherichia coli</i>	-	-	-	-	-	-	-	9mm
<i>Bacillus subtilis</i>	-	-	-	-	-	-	-	5mm
<i>Pseudomonas aeruginosa</i>	6mm	4mm	2mm	1mm		-	-	6mm
<i>Samonela typhi</i>	6mm	4mm	2mm	1mm	-	-	-	6mm

The extracts displayed various activities against bacteria inhibiting it at various concentrations ranging from 400 to 6.25 mg/ml. The inhibition zone of the extract at 400mg/ml, 200mg/ml, 100mg/ml and 50mg/ml are 8mm, 6mm, 4mm and 2mm respectively against *Staphiloccocus aureus*. At 400mg/ml and 100mg/ml the activity of the extract is comparable to the standard antibiotic ampicillin with inhibition zone of 6mm. it has no activity against both *E. coli* and *Bacillus subtilis*. But it is effective against *Pseudomonas aeruginosa* and *Salmonella typhi* with inhibition zones of 6mm, 4mm, and 2mm at concentration of 400mg/ml, 200mg/ml and 100mg/ml respectively. At 400mg/ml the extract is comparable with the standard drug with inhibition zone of 6mm.

11. Discussion, Conclusion and Recommendation

The results of phytochemical screening showed presence of simple sugar and flavonoid, essential oil, phenolic group, glycoside, and saponin in the methanol root extract screened for secondary metabolites. Some of these active principles (secondary metabolites) have been reported to have activity against micro-organisms. Flavonoid, phenolics, Alkloids, triterpenes and essential oils have been shown to have activities (Majorie, 1999). The Presence of alkaloids, cyanogenetic glycosides, steroidal nucleus and reducing sugars, phenolic group and essential oil are normal with the plants of this family capparidaceae (Kjaer and Thomson, 1973; Lakshimi and Chanhan, 1977) *Ajaiyeoba E. O.*, 2000)..

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12. Conclusion

The present study shows that the root of *Ritchiea longipeccellata* has a lot of potential as an anti-microbial agent. These observed activities appear to justify the ethmopharmacological uses of the plant.

13. Recommendation

There is need for further study and characterization of the plant to ascertain the active constituent of the drug for easy design and synthesis.

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