International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF THE LEAVES OF *RITCHIEA LONGIPEDICELLATA FAM. CAPPARIDACEAE*

Anowi CF[1], Utoh-Nedosa UA[2], Onyegbule AF[3] and Oche G[4]

[1]Dept of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka
[2]Dept of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka
[3]Dept of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical sciences, Nnamdi Azikiwe University, Awka
[4]Dept of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Awka

***Corresponding author e-mail:** af.onyegbule@gmail.com

ABSTRACT

Ritchiea longipedicellata Gilg had been reported in traditional medicine, to exhibit antimicrobial properties. Therefore, this study is aimed at determining the antibacterial and antifungal activities of Ritchiea longipedicellata Gilg leaves against pathogenic microorganisms by determining the minimal inhibitory concentration and to serve as criteria to recommend the ethno pharmacological uses of the plant. Plant leaves were dried, powdered and extracted by cold maceration with methanol for 24hours. Phytochemical screening was done for alkaloids, saponin, essential oil, phenolic group, steroidal nucleus, simple sugar, starch, cyanogenic glycoside, proteins and flavonoids using standard procedures. Antimicrobial and minimal inhibitory concentration screenings were done using agar diffusion technique. Antibacterial activity test was conducted by screening against seven pathogens comprising both Gram positive and Gram negative bacteria obtained from Pharmaceutical Microbiology laboratory stock. The extracts were screened against 24hour broth culture of bacteria seeded in the nutrient agar at concentrations 200, 100, 50, 25, 12.5 and 6.25 mg/ml in DMSO and incubated at 37^0C, for 24 hours and measuring the inhibition zone diameter - IZD. The same was done for antifungal screening, however, fungi were seeded into a sabouraud dextrose agar and incubated for 72 hours at 25^0C (.*Aspergillus niger* and *Candida albican* were used). The positive controls were ampicillin 20µg/ml and clotrimazole cream 1mg/ml for bacteria and fungi respectively. DMSO was used as negative control. The results of phytochemical screening showed moderate availability of alkaloid, simple sugar and abundance of flavonoids, steroidal nucleus, essential oil, phenolic group, cyanogenic glycoside; absence of starch and protein and doubtful quantity of saponin. Methanolic extract inhibited with minimal inhibitory concentration of 200, 6.25, 200, 12.5, and 12.5 mg/ml against *S. aureus, P. aeruginosa, S. typhi, E. coli, B. subtilis, and Sarcinae lutea* respectively. The extract demonstrated activities against certain bacteria confirming the use of the plant in ethno pharmacology and since the root extract are more often used, it is yet to be confirmed if it has more activity than the leaves against the test organisms. Taking the least IZD of the standard (Ampicillin) as the breaking point, most of the extracts passed the breaking point.

Keywords: Ritchiea longicellata, minimal inhibitory concentration, antimicrobial screening, breaking point and activity.

INTRODUCTION

Over the past decade herbal medicine has become a topic of global importance, making an impact on both world health and international trade. 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. [1] 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 cases) and the cultural, spiritual point of view of the people of the countries. [1] 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. [2] 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. [3]

Traditional medicine use in Nigeria is as old as the people; and has remained relevant among every other types of therapy. At present, WHO has defined traditional medicine as comprising therapeutic practices that have been in existence often for hundreds of years before the development of and spread of modern scientific medicine and are still in use today. [4] The practice of traditional medicine has been noted by WHO in 1991 to vary widely in keeping with the social and cultural heritage of different countries in Africa .The variation is extended to the various regions and group in the countries. In the practice of traditional medicine in Africa much emphasis is placed on supernatural forces so that practitioners are consulted not only for sicknesses but also when any misfortune occur in the families since many of the evil omen are ascribed to supernatural forces. [4] The medications are intended for both internal and external use but none for intravenous administration (besides those applied with scarification). This practice involves several techniques which are mainly diagnosis and treatment. Clearly, it is evident that almost all traditional practice all over the globe indicated herb as an important aspect in the treatment of disease. The importance of plant in the present day method of treatment cannot be over emphasized. In developing countries, thousands of rural communities still depend mainly on folklore medicine to cure diseases. [3]

No surprise that as at today plant still forms one of the major sources of medicines used in clinics, generating about 50% medicinal compounds used by pharmaceutical industry, 25% of prescription drugs are derived from tropical plants three quarter of which from folkloric medicines. [5] Such drugs are *Digitalis* used as important drugs for the management of heart failure from *Digitalis purpurea*, Quinine used for treatment of cerebral malaria from *Cinchona* bark etc. Undoubtedly, a lot of medicine have been isolated from plant that are employed in the health sector today even in the possibility of synthetic chemicals serving as drug, plants still hold many specie. [6] Today focus is changing and people are drifting from the use of conventional therapy to the use of natural product. Based on world Health Organization (WHO) report, some 3.4 billion people in the developing world depend on the plant based traditional medicines. [2]

So also according to WHO, 80% of the world populations rely chiefly on plant based traditional medicines especially for their primary health care needs. About 60million people are estimated to use herbal remedies each year affording cost of about 3.2billion Dollars in USA, $6billion in Europe, more than $2 billion in Germany, over 2.3 billion Dollars in china, $2.1 billion in Japan, and $1-2 billion in Malaysia etc. [5] Though Nigeria Statistics is not documented, it is clear that huge amount of money is being spent on traditional medicine evidenced by ever increasing number of such products and their demands. Among the uses of herbal therapy is in the treatment of infective diseases which form a high percentage of the diseases affecting man all over the world today. The results presently arising from the use of available chemotherapeutic agents are even encouraging factors to the use of herbs. This becomes more serious especially with the claim of benefits of herbal medicines over synthetic counterpart. People seem to have understood and chose to avoid the debilitating side effects that come along with some synthetic chemicals .This coupled with the incidence of resistance to most of the existing chemotherapies by microorganisms, re-establish the strong need for antibiotic from natural

sources. Antibacterial resistance among bacterial pathogens in recent time is a critical area of public health concern. [3] There is need for the development of new antibiotics due to acquired resistance more importantly, from natural sources as this delays resistance. [7] According to Denver Russell plant might prove to be a potentially fruitful source of new antimicrobial agent. Though he indicated toxicity as problem in the use of high plants, all plants might not be toxic plus optimization normally used for every drug developments. Today, there is need to study plants to properly establish those whose efficacy has been a claim (Evans). In this study, focus was on the anti-microbial activity of methanol extract of *Ritchiea Longipedicellata Gilg* leaves using Agar diffusion techniques.

TAXONOMY, DESCRIPTION AND DISTRIBUTION OF PLANT

The plant is of the Kingdom (Plantae), Division (Angiospermae), Class (Dicotyledonae), Subclass (Archichlamydae), Order (Papaverale/Brassicales), Suborder (Capparineae), Family (Capparidaceae), Genus (*Ritchiea),* Species (*Ritchiea longipedicellata Gilg).* 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 odour 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). 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)

ETHNO- BOTANICAL USES OF *RITCHIEA LONGIPEDICELLATA G*

The plant is used in Nigerian local villages (particularly in Ikwo L. G. A. in Ebonyi 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).

Comparing Traditional medicines and Orthodox medicines: [8, 9]

1. The chances of suffering adverse reactions to alternative medical treatments are less when compared to the risks of being harmed by a traditional physician dispensing powerful drugs and performing risky surgeries.
2. Unlike holistic medicine, the risk of being permanently harmed or even killed, by orthodox medicine is very real. Diseases termed "iatrogenic" or doctor caused diseases now exist while adverse drug reactions of orthodox drugs outnumber herb reactions. According to medical authorities iatrogenic disease is responsible for 180,000 or as many as 250,000 deaths annually in the US alone.
3. The effectiveness of orthodox medicine when it comes to the treatment of serious trauma and acute or life threatening disease, is generally accepted as effective.
4. While the efficacy of orthodox medicine when it comes to the treatment of acute infections is also generally accepted, there are difficulties in determining the totality of the impact of such treatments. Although the effectiveness of anti-microbial treatments when it comes to various specific infections is generally accepted, the overall impact of these treatments can only be viewed by adopting a wider perspective which considers the direction of any changes to the general disease resistance and immunity of the community at large.
5. To clearly assess the impact and effectiveness of orthodox medicine it is necessary to examine health statistics which reflect the incidence of various chronic diseases. Cause of death statistics, which are commonly employed to assess trends in public health, are somewhat more difficult to interpret since they are not a direct measure of the incidence of particular diseases.
6. When it comes to the public awareness of the effectiveness of modern health strategies it is indeed interesting to note that the increasing incidence of many disorders is not always apparent from media reports. With asthma for instance, the *breakthroughs in the treatment* of course, correlate with an increasing incidence of this disorder.

7. When it comes to holistic medicine, there is abundant and increasing scientific evidence regarding its effectiveness for the treatment or prevention of various chronic diseases, an example is use of chromium supplements in the treatment of insulin resistant diabetes which have been known for many years, still do not routinely prescribe this mineral for their diabetic patients. Omega-3 fatty acids found in fish oil and flaxseed oil have also been reported to reduce the complications of diabetes as well as the degree of insulin resistance.
8. Perhaps the most impressive evidence regarding the effectiveness of holistic medicine as compared to orthodox medicine relates to the changing treatment strategies for heart disease and cancer, particularly bowel cancer. With both these disorders high tech medical treatments have been found wanting and preventative treatment now revolves around holistic therapies such as diet and nutrition.

In summary, Herbal medicine is cost effective and less expensive than the orthodox medicines.

1. Herbal medicine can be bought without a prescription and are available in any health store.
2. Herbal medicine and remedies are more effective than allopathic medicine for certain ailments.
3. The chemical medicine could have certain negative side effects but many of the herbal medicines and remedies do not have negative side effects. If any, they are softer than orthodox medicine.
4. Herbal medicine can be effectively used for body's natural detoxification process E. g. *Plantago psyllium* seed, rhubarb juice powder, aloe Vera, alfalfa juice, chlorella, carrot concentrate and garlic can be used to cleanse the colon.
5. Herbal medicine which includes herbs such as ginger, capsicum, garlic and motherwort help to control the ailments related to blood circulation and obesity is the cause of many of the health problems. Herbal medicine can help reduce excess weight and regulate appetite.

Challenges of Herbal Medicine: Like all forms of alternative health care, herbal medicine also has few demerits. Here are them.

1. Cure using herbal medicine and supplements would take some time and Herbal medicine contains various ingredients and the patient should be sure that his body agrees with the ingredients and that he is not allergic to it.
2. A point worth mentioning here is, herbal remedies and medicine for certain ailments may have negative side effects. These side effects may not be revealed immediately, but would take months or even years. In the initial stages, if the herbal medicine is not agreeing with you, it is wise to stop using it.
3. Government's regulation herbal medicine industry is not efficient. Hence, there is less or no quality assurance for herbal products.
4. Some plants have been shown to accumulate heavy metals which can cause chronic toxicity so, there is the need to use them in reduced doses e g *Agatea longipedicellata* which is a Nickel hyper accumulator. [10]

MATERIALS AND METHODS

Chemicals and Solvent: The chemicals used for extraction processes include methanol, dimethyl sulphoxide (DMSO), and Nutrient Agar and Sabourand dextrose 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).

Sources of Microorganisms: The microorganisms used were both bacteria and fungi 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*, *Klebsiella species, Escherichia coli*, Bacillus *subtilis*, *Sarcinae lutea*, *Salmonella typhi*) and *Aspergillus Niger Candida albican* were the two fungi used.

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.

Source and Identification of Plant Materials: The fresh leaves of *Ritchiea Longipedicellata* were obtained from Echialike in Ikwo local Government Area, Ebonyi state in November 2010. The plant was identified by Dr C. O. Ezeugwu of the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University. The stalk and other impurities were removed from the leaves. The leaves were air dried in the Pharmacognosy Laboratory and then were pulverized to produce 250g of powdered plant leaf.

Extraction Process: Extraction was done by macerating the 250g of the powdered drug with 500ml of methanol solution for 48hrs. At the end it was strained using white muslin cloth and then filtered using Whatman No 1 filter paper. The filtrate was concentrated using rotary evaporator.

Phytochemical Screening of the Plant: Standard screening tests were carried out on both powdered leave and crude extract for various phytochemical constituents. The procedure used was obtained from Trease and Evans text [6] and Departmental Laboratory Manual. [11]

ANTIMICROBIAL ASSAY

Microorganisms: 24hour Cultures of seven human pathogenic bacteria made up of both gram positive (*S. aureus, S. lutea* and *B. subtilis*) and gram negative (*P. aeruginosa, Klebsiella Spp, E. coli* and *S. typhi*) bacteria were used for the *in-vitro* antibacterial assay. For the antifungal assay, two fungi were utilized for the studies and these were made up of *Aspergillus niger* and *Candida albican*. All microorganisms were obtained from the laboratory stock of the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences Nnamdi Azikiwe University Awka.

Preparation of media: Nutrient broth, nutrient agar, sabouraud dextrose agar (SDA) was used in the assays. Dimethyl sulphoxide (DMSO) was used in solublising the extracts and drugs and as a negative control in the study. The media were prepared by dispersing the weighed amount in water and then were sterilizing them with autoclave. The plates of nutrient agar were poured and allowed to solidify after the appropriate organisms were seeded.

Antimicrobial agents: Ampicillin, 20ug/ml (Me cure Industrial Ltd, Lagos Nigeria.); Clotrimazole cream, 1mg/ml (Drug Field, Nigeria) were included in the study as standard reference drugs.

Antimicrobial activity determination: An overnight broth culture used to obtain 0.5 MacFarlane standard of bacterium was used to seed sterile molten nutrient agar medium maintained at 45°C. Sabouraud dextrose agar plate was similarly seeded with fungi. Seven holes (6mm) respectively, were bored in each of the plates (9cm, diameter) with an aseptic cork borer, when seeded plates had solidified;
200mg/ml,100mg/ml,50mg/ml,25mg/ml12.5mg/ml and 6.25mg/ml of extract were prepared in dimethyl sulphoxide (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 wells were filled with 20µg/ml and 1 mg/ml of ampicillin and clotrimazole cream for bacteria and fungi respectively (also dissolved in DMSO). Diameters of zones of inhibition were determined after incubating plates at 37°C for 24h for the bacteria and at 25°C for 72 hours for fungi respectively. This test was conducted first on the crude extract and then on each of the different fractions and the solvent dimethyl sulphoxide was used as negative control while ampicillin and clotrimazole cream were used as positive control.

RESULTS AND DISCUSSION

The results of the phytochemical screening of the leaves are presented in table 1. The antibacterial properties of extracts at all concentrations in addition to the standard are presented in table 2. The results of phytochemical screening showed moderate presence of alkaloid, simple sugar and abundance of flavonoids, steroidal nucleus, essential oil, phenolic group, cyanogenic glycoside; starch and protein were absent and doubtful quantity of saponin.

The extracts displayed various activities against bacteria inhibiting it at various concentrations ranging from 200 to 6.25 mg/ml. Methanol extract inhibited with minimal inhibitory concentration of 200, 12.5, 200, 12.5, and 12.5 mg/ml against *S. aureus, P. aeruginosa, S. typhi, E. coli*, *B. subtilis, and Sarcinae lutea* respectively. The DMSO used did not show any activity against the bacteria used.

CONCLUSION AND RECOMMENDATION

The results of phytochemical screening showed moderate presence of alkaloid, simple sugar and abundance of flavonoids, steroidal nucleus, essential oil, phenolic group, cyanogenic glycoside; absence of starch and protein but doubtful quantities of saponin in the leaves crude extract and powder screened for secondary metabolites. Some of these active principles (secondary metabolites) have been

reported to have activity against micro-organisms. Flavonoids, phenolics, Alkaloids, triterpenes and essential oils have been shown to have activities. [12] 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. [7, 13] The crude extract yielded enormous quantity of fixed oil. Methanol fraction displayed good activities, inhibiting the growth of *B. subtilis*, P. aeruginosae *and E. coli with* IZD of 9.0, 6.0,.and 6.0mm respectively but with less activity against *S. aureus* and *S. typhi* but lacked activity against *Sarc*inae *lutea* at 200mg/ml concentration. Methanol fraction which is capable of containing non polar to moderately polar ingredient exhibited broad spectrum activity against strain of *P. aeruginosae* (minimal inhibitory concentration <12.5), *E. coli (*minimal inhibitory concentration= 12.5), *B.subtilis* (minimal inhibitory concentration =12.5), *S aureus* (minimal inhibitory concentration=200) mg/ml but had Zero activity against a strain *Sarcinae lutea*. The fact that the extract demonstrated activities against certain bacteria and fungi confirmed the use of the plant in ethno pharmacology. Since the root extract is more often used locally, it is yet to be confirmed if it has more activity than the leaves against the tested organisms. Taking the least IZD of the standard (Ampicillin) as the breaking point inhibition, most of the extracts passed the breaking point. It is recommended that further test be conducted to determine the activity of the root against bacteria and fungi since active principle in the plant is very suggestive of a good antibacterial and antifungal activity. The toxicity of the extracts should be tested in animals to rule out possibilities of poisoning since cyanogenic glycosides and heavy metal accumulation of some plants of the species are suggestive of toxicity.

Table 1: Phytochemical Screening of *Ritchiea longipedicellata Gilg*

2° Metabolites (Plant Leaves)	**Tests/ Observations**	**Inference**
Proteins	Xanthoproteic reaction test (no orange coloration)	-
Alkaloids	Wagner and Hager test (precipitate formation)	++
Cyanidins	Picrate paper (from yellow to brick- red coloration)	+++
Flavonoids	Ammonium test (formation of yellow coloration)	+++
Glycosides	Picric acid test (brick-red coloration)	++
Steroids	Sulfuric acid test (reddish brown interface formation)	+++
Phenolic group	Ferric chloride test (intense coloration)	+++
Starch	Molisch test (no purple interfacial ring formed)	-
Reducing sugar	Benedict's test (rusty brown coloration)	+++
Essential oil	Potassium chromate test (soap formation via frothing)	+++
Saponins	Frothing and Emulsion tests	+

** = not detectable; ± = doubtful; + = low concentration; ++= medium concentration; +++= High concentration*

Table 2: Antibacterial Activity of Methanol Extract.

Methanol fraction	**Inhibition Zone Diameter For Bacteria in Different Concentrations of Extracts (mm)**						
Bacteria Used	200	100	50	25	12.5	6.26 [mg/ml]	Am (20µg)
S. aureus	4.0	-	-	-	-	-	6.0
P. aeruginosa	6.0	5.0	6.0	5.0	5.0	4.0	6.0
Klebsiella	-	-	-	-	-	-	16.0
E. coli	6.0	5.0	4.0	6.0	4.0	-	9.0
B. subtilis	9.0	7.0	7.0	5.0	5.0	-	5.0
Sarcinae lutea	-	-	-	-	-	-	39
S. typhi	4.0	-	-	-	-	-	6.0

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