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

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ABSTRACT

Ritchiea longipedicellata had been reported to exhibit antimicrobial properties. Therefore, this study is aimed at determining the antimicrobial activities of Ritchiea longipedicellata; family caparidaceae leaves against microorganisms and to serve as criteria to recommend the Ethnopharmacological uses of the plant. The plant leaves were dried, powdered and extracted by cold maceration with Ethylacetate for 24h. Phytochemical screening was done for alkaloids, saponin, essential oil, phenolic group, steroidal nucleus, simple sugar, starch, cyanogenic glycoside, proteins and flavonoid using standard procedures. Antimicrobial 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 extract was screened against 24h 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 37ċ, for 24 h and measuring the inhibition zone diameter - IZD. The same was done for antifungal however; fungi were seeded into a sabouraud dextrose agar and incubated for 72 h at 25^{o}C. *Aspergillus niger* and *Candida albican* were used. The positive control was 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 flavonoid, steroidal nucleus, essential oil, phenolic group, cyanogenic glycoside; absence of starch and protein and doubtful quantity of saponin. The extract displayed various activities against bacteria inhibiting it at various concentrations ranging from 200 to 6.25 mg/mL. Ethylacetate extract inhibited all the bacteria in a most appreciable extent. The extract demonstrated activities against certain bacteria and fungi (to some extent) confirming the use of the plant in ethno pharmacology. Taking the least IZD of the standard (Ampicillin) as the breaking point, the extract passed the breaking point.

Key words: Ritchiea longicellata**,** phytochemical, 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 [31]. 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 [31].

In Western developed countries however, after a downturn in the pace herbal use in recent decades, the pace is again quickening as scientists realize that the effective life span of any antibiotic is limited [28]. 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 [12].

Traditional medicine use in Nigeria is as old as the people; and has remained relevant among every other types of therapy. Presently, 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. 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. 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 [12]. 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 [18]. 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 species. 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 [28].

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 [18].

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 anti-infective from natural sources. Antibacterial resistance among bacterial pathogens in recent time is a critical area of public health concern [12]. There is need for the development of new antibiotics due to acquired resistance more importantly, from natural sources as this delays resistance [1].

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.

MATERIALS AND METHOD

Chemicals and Solvent: The chemicals used for extraction processes include, ethyl acetate, dimethyl sophoxide (DMSO), Nutient 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. Ezugwu of the Department of Pharmacognosy and traditional medicine Nnamdi Azikiwe University Awka. 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 ethyl acetate 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 Treas and Evans (2002).

ANTIMICROBIAL ASSAY

Microorganisms: 24h 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. Dimethylsulphoxide (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 (Mecure indusrial ltd Lagos Nigeria.); Clotimazole 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 **Marcfarland** 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 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 the dilution 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). Diameter of zone of inhibition was determined after incubating plates at 37°C for 24h for

the bacteria and at 25°C for 72 h for fungi respectively and the solvent dimethylsulphoxide was used as negative control while ampicillin and clotrimazole cream were used as positive control.

RESULTS

The results of phytochemical screening showed moderate presence of alkaloid, simple sugar and abundance of flavonoid, steroidal nucleus, essential oil, phenolic group, cyanogenic glycoside; starch and protein were absent and doubtful quantity of saponin. The extract displayed various activities against bacteria inhibiting it at various concentrations ranging from 200 to 6.25 mg/ml. Ethylacetate fraction inhibited all the bacteria in a most appreciable manner. The DMSO used did not show any activity against the bacteria used. Example is the activity of ethyl acetate fraction against *Sarcinae lutea see* tables below.

DISCUSSION

The results of phytochemical screening showed moderate presence of alkaloid, simple sugar and abundance of flavonoid, 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. Flavonoid, phenolics, Alkloids, triterpenes and essential oils have been shown to have activities [23]. 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 [2] .The crude extract yielded enormous **quantity of fixed oil.** Ethylacetate extract showed appreciable activity against the entire organism it was screened against (exhibiting broad spectrum activity in nut shell). At 200mg/ml, it displayed activities of 9, 6, 7, 8, 17, and 4 mm against *S.aureus, P. aeruginosae, E. coli, B. subtilis, sarcinae lutea* and *salmonella typhi* respectively. This shows that activity of the plant leaves concentrates between moderately non-polar to polar components of the plant.

CONCLUSION

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, the extracts passed the breaking point as can rightly be seen. 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 extract 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: 2° Metabolites in the Plants its presence in leave

Metabolites	Status
Protein	-
Alkaloids	++
Cyanidins	+++
Flavonoids	+++
Glycosides	++
Steroids	+++
Phenolic group	+++
Starch	-
Reducing sugar	+++
Essential oil	+++
Saponins	±

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

Table 2: Antibacterial Activity of Ethylacetate extract

Bacteria	Inhibition Zone Diameter For Bacteria in Different concentrations (µg/ml) of Extracts [in mm]						
	200	100	50	25	12.5	6.25	Std(20)
S. aureus	9.0	-	-	-	-	-	6.0
p. aeruginosa	6.0	5.0	5.0	4.0	-	-	6.0
Klebsiella	-	-	-	-	-	-	16.0
E. Coli	7.0	6.0	6.0	6.0	5.0	-	9.0
B. subtilis	8.0	6.0	5.0	5.0	4.0	-	5.0
Sarcinaae lutea	17.0	14.0	11.0	9.0	5.0	4.0	39.0
S.typhi	4.0	-	-	-	-	-	6.0

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