European Journal of Medicinal Plants

21(4): 1-6, 2017; Article no.EJMP.31571
ISSN: 2231-0894, NLM ID: 101583475

Anti-bacterial Effect of Ethanolic and Aqueous Extract of *Ptericarpus erinaceous* Stem Bark

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Authors' contributions

This work was carried out in collaboration between two authors. Authors SS and IJB designed the study. While author IJB performed the statistical analysis, wrote the protocol and author SS wrote the first draft of the manuscript. Authors SS and IJB managed the analyses of the study and wrote the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2017/31571
Editor(s):
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(2) Marcello Iriti, Professor, Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
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(3) Ndomou Mathieu, University of Douala, Cameroon.
Complete Peer review History: http://www.sciencedomain.org/review-history/22532

Original Research Article

Received 14th January 2017
Accepted 3rd April 2017
Published 30th December 2017

ABSTRACT

Aim: The phytochemical screenings of aqueous and ethanolic extract of *Ptericarpus erinaceous* stem bark were investigated against three microorganisms.
Study Design: Five groups of experimental animals were used for the study.
Place and Duration of the Study: Department of Microbiology and Biochemistry Laboratories, School of Pure and Applied Sciences, Modibbo Adama University of Technology Yola, Nigeria between April 2016 and September 2016.
Materials and Methods: The phytochemical screening and antibacterial activity of aqueous and ethanolic extracts of *Ptericarpus erinaceous* stem bark were investigated against three microorganisms.
Results: The result of the phytochemical screening of *Ptericarpus erinaceous* revealed the present of alkaloid, tannins, glycosides, terpenes and phenols. Our findings on anti-microbial activity of the plant stem bark extract showed that the aqueous extract has higher activity with a zone of inhibition of 21 mm and 19 mm at higher concentration of 100 mg/ml of the extract for *Escherichia coli* and

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Klebsiella pneumonia respectively when compared to the ethanolic extract of the plant. This perhaps may be due to the presence of bioactive constituent that were present in the aqueous extract but not found in the ethanolic extract. The plant has a minimum inhibitory concentration (MIC) at 50 mg/ml and the minimum bactericidal concentration (MBC) at 100 mg/ml respectively in both *Escherichia coli* and *Klebsiella pneumonia*.

Conclusion: These findings have ascertained that this medicinal plant *Ptericarpus erinaceous* used locally in the northern part of Nigeria by traditional herbalist for the treatment of infections/diseases could be of considerable interest to the development of new drugs such as production of new antibiotics to help in combating the increase in resistance to synthetic drugs by most microorganisms and may serve as a cheaper source of antibiotics.

Keywords: Antibacterial activity; P. erinaceous; E. coli; K. pneumonia; S. aureus.

1. INTRODUCTION

There is an increasing use and popularity for traditional medicine throughout the world. In industrialized countries, adaptation of traditional medicine is termed “Complementary Alternative Medicine (CAM). Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis, and quinine. The World Health Organization estimated that 80 percent of the population in some Asian and African countries depends on traditional medicine for primary health care [1].

Plants represent the principal means of therapy in traditional medicine and the plant kingdom has long served as a prolific source of useful drugs. African indigenous herbal medicines are widely used throughout the African continent, despite the apparent lack of scientific evidence for their quality, safety and efficacy [2]. The chemical constituents in medicinal plants usually explain the rational for the use of the plants in traditional medicine. The trend now is that phytochemists exploit medicinal plants and isolate bioactive compounds from which different analogues are synthesized with the aim of obtaining agents with better actions or even different biological properties. Plant’s active constituents thus serve as templates for future drug developments. *Ptericarpus erinaceus* is a perennial deciduous legume tree of African Savannah and dry forest popularly known as, African rosewood, African kino, or teak (in English).

In traditional medical practice, the leaf infusion is used in Ghana for fever [3]. The bark and resin decoction is an astringent for severe diarrhoea and dysentery. The bark decoction has also been used for the treatment of tumors of the gland, urethral discharges and as restorative. The bark, as a dressing, is used on ringworm of the scalp and chronic ulcers [4]. In Northern Nigeria, the bark is used as an ingredient in a bortifacient prescriptions [4]. Previous studies by Aliyu [5] showed that the ethanolic stem bark extract of the plant possesses significant and dose-dependent analgesic and anti-inflammatory activities in laboratory rats and mice. The ethanolic stem bark extract of the plant has also shown haemostatic activity in another study [6]. Many studies indicated that in higher plants there are many phytochemicals which confer in most instances the pharmacological properties of the plants. The antifungal, antibacterial or antiviral properties is mostly dependent on these plant phytochemicals [7,8,9]. Therefore, the aim of this research was to scientifically assess the basis of the traditional medical practitioner's claimed the use of stem bark of *Ptericarpus erinaceus* in the treatment of bacterial infection.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Collection and identification of the plant

Stem bark of *Ptericarpus erinaceous* were collected from Wuro sembe village of Jalingo Local Government Area of Taraba State,. The plant was identified and authenticated at the department of plant science, School of Pure and Applied Sciences, Modibbo Adama University of Technology Yola.

2.1.2 Chemicals and reagent

All other reagents and solvents used were of analytical grade.

2.2 Methods

2.2.1 Plant extraction

The *Pterocarpus erinaceous* plant part was washed with sterile water and it was allowed to

air dry for 10 days. The sample was grounded into powder using pestle and mortar. The powdered sample, each 20 g of the stem bark was soaked in a flask containing 200 ml of 70% ethanol for 2 days and likewise same was soaked in distilled water and it was filtered using whatmann number 1 filter paper and the filtrates were then kept in a water bath and evaporated. Finally obtained the extracts of both aqueous and ethanol were then used for antimicrobial as well as phytochemical screening.

2.3 Qualitative Phytochemical Analysis

The methods described by Akerele [10] was used to test for the presence of Saponins, tannins, glycosides, flavonoids, steroids alkaloids, terpenes, phenols and glycosides.

2.3.1 Determination of alkaloid

About 2 ml of the stem bark sample was weighed out and macerated with 2 ml of distilled water. 3 ml H_2S0_4 was added, then 3 drops of Meyers reagent was added white coloured precipitate was formed which indicate the presence of alkaloid in the sample [10].

2.3.2 Determination of tannin

To 2 ml of water was added to 5 ml of the extract and drops of ferric chloride. Green precipitate was an indication for the presence of tannins [10].

2.3.3 Determination of glycosides

To 5 ml of the extract was added 25 ml of diluted H_2SO_4 into test tube, boil for 15 minutes, cooled and neutralized with 10% NaOH and 5 ml of Fehling's solution A and B was added; A brick red precipitate was a positive test for the presence of glycosides [10].

2.3.4 Determination of flavonoid

To 5 ml of the extract, a small quantity of magnesium chips and drops of conc. Hydrochloric acid was added down the side of the test tube. A reddish coloration indicates the presence of flavonoids [10].

2.3.5 Determination of saponin

To 2 ml of the extract was vigorously shaken with 5 ml of distilled water in a test tube. Frothing which persisted was taken as an evidence for the presence of saponins [10].

2.3.6 Determination of steroid

About 2 ml of the sample was weighed out and 1 ml chloroform was added and then 1 ml concentrated H_2S0_4 which form two separate layers to indicate presence of steroids in both ethanolic and aqueous extract of the sample [10].

2.3.7 Determination of terpenes

About 2 ml of the sample was weighed out and 1 ml chloroform was added and then 1 ml concentrated H_2S0_4 which form two separate layers to indicate presence of steroids in both ethanolic and aqueous extract of the sample [10].

2.3.8 Determination of phenols

Equal volume of the extract was added to equal volume of ferric chloride, a deep bluish green solution was an indication for the presence of phenols [10].

2.3.9 Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) of the stem bark aqueous extract was determined using agar dilution method of NIPRD [11]. 100 mg, 50 mg, 25 mg, 12.5 mg of the extracts were reconstituted in 2 ml of sterile distilled water and vortexed for homogeneity. (0.5 ml) each of the reconstituted, each extract was added to the test tubes containing nutrient broth (Nutrient Agar) 2 ml of various concentrations of the extracts were added then, a loopful of the test organisms was added into each test tube and it was incubated at 37°C for 24 hrs. The lowest concentration that inhibits the growth of the organism was the MIC.

2.3.10 MBC

The concentration that inhibit the growth of the organism in each test-tube was then sub-cultured into a free antibiotic media (Nutrient Agar) and the concentration that totally eliminate the activities of the organism was therefore referred to as the minimum bactericidal concentration.

3. RESULTS AND DISCUSSION

The results of the various phytochemical screening carried out to determine the presence of those phytochemical in the *Ptericarpus erinaceous* stem bark extract which revealed the presence of Alkaloids, tannins, glycosides, steroids, terpenes and phenols in the aqueous extract while flavonoids and saponnins were absent, and for the ethanolic extract contained all

the constituents with the exception of flavonoids, steroids and terpenes which revealed that the extract is more soluble in aqueous phase as shown in Table 1. Extract of *Ptericarpus erinaceous* stem bark indicated that glycoside was present in both aqueous and ethanolic extracts. According to research carried out by Aboaba and Efuwape [12] reported that glycosides are non-toxic but can get hydrolysed to release phenolics which are toxic to microbial pathogens. Several phenolic compounds like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as pectolytic macerating enzymes used by plant pathogens [13].

Table 1. The results of qualitative phytochemical screening of stem bark of aqueous and ethanolic extract of *Ptericarpus erinaceous*

Phytochemicals	Aqueous extract	Ethanolic extract
Alkaloids	+	+
Tannins	+	+
Glycosides	+	+
Flavanoids	-	-
Saponins	-	+
Steroids	+	-
Terpenes	+	-
Phenols	+	+

Key: Present = +; Absent = -

The results in Tables 2 and 3 shows the antibacterial susceptibility test of the ethanolic extract on the test organism which revealed that *Escherichia coli* and *Klebsiella pneumonia* are susceptible to the extract at various concentrations, in different degree zones of inhibition and the result shown revealed that it was dose dependant because, the higher the concentration the better the zone of inhibition increases while *Staphylococcus aureous* was resistant to the extract at all concentrations.

The antimicrobial properties of substances are desirable tools in the control of infections and in food spoilage [14]. The high level of sensitivity observed in the aqueous extracts towards the bacterial pathogens showed that the active components were soluble in water. This supports the extensive use of *Ptericarpus erinaceous* extract for treatment of ailments by traditional African medical practitioners. Plant based antimicrobials have enormous therapeutic potentials as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials [15].

The results in Tables 2, 3 and 4 of the four different concentrations 100 mg/ml, 50 mg, 25 mg/ml, 12. 5 mg/ml and a control test tubes that contained no extract and at the end of 24 hours when inoculated with 0.5 ml of each concentration into 2 ml of nutrient broth and a loop full of the microorganisms, the tests revealed that at 50 mg/ml and 100 mg/ml concentrations inhibited the growth of the microbes, and therefore since 50 mg/ml was lower and so considered as the minimum concentration at which the activity of the organism was inhibited. So the minimum inhibitory concentration at 0.5 ml of the extract was 50 mg/ml, for both the two microorganism. The result was then sub-cultured and the growth was totally eliminated at 100 mg/ml and was regarded as minimum bactericidal concentration (MBC) for the two organisms that were sensitive to the plant stem bark extract.

A large number of constitutive plant components have been reported have antimicrobial activity. The inhibitory effect of *Ptericarpus erinaceous* plant stem bark extract on some test organisms was investigated and the results obtained in this study revealed that *Ptericarpus erinaceous* has antibacterial activity. In a related development Mara [16] reported that many edible plants extracts such as cranberry, lime and lemon juices have antimicrobial activity. Sekar [17] screened the pharmacological activity of the ethanol and acetone extract of *Phyllanthus amarus,*and *indica Datura metel* for its antimicrobial activity against selected pathogen.

Table 2. Results for the zones of inhibition in (mm) for the antibacterial susceptibility of stem bark of ethanolic extract of *Ptericarpus erinaceous*

Test organism	Normal control	Positive control	Concentrations of ethanolic extract in mg/ml			
			12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
Escherichia coli	-	26 mm	R	15 mm	18 mm	19 mm
Klebsiella pneumonia	-	22 mm	R	14 mm	16 mm	17 mm
Staphylococcus aureus	-	20 mm	R	R	R	R

Key: No zone of inhibition = - ; Resistance = R

Table 3. Results for the zones of inhibition in (mm) for the antibacterial susceptibility of stem bark of aqueous extract of *Ptericarpus erinaceous*

Test organism	Normal control	Positive control	Concentrations of aqueous extract in mg/ml			
			12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
Escherichia coli	-	32 mm	R	17 mm	19 mm	21 mm
Klebsiella pneumonia	-	30 mm	R	15 mm	17 mm	19 mm
Staphylococcus aureus	-	20 mm	R	R	R	R

Key: No zone of inhibition = -; Resistance = R

Table 4. Results for the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the two susceptible organisms

Test organisms	Minimum inhibitory concentration (MIC)	Minimum bactericidal concentration (MBC)
Esxherichia coli	50 mg/ml	100 mg/ml
Klebsilla pneumonia	50 mg/ml	100 mg/ml

Our findings had shown that aqueous extract of *ptericarpus erinaceous* possesses more inhibitory capacity than the ethanollic extract. In the same view it has been demonstrated that the influence of solvent for extraction on the inhibitory capacity of the extract on the test organism has been reported by Al-Bayati and Sulaiman [18]. In a related development, Ada [19] reported that the aqueous lemon leaf extracts against *E. coli* showed a good inhibitory response andn*Klebsiella* the best antimicrobial activity was observed with Eucalyptus leaf ethanol extracts while *Pseudomonas* showed resistance to all the solvents except to Tulsi leaf ethanol extracts.

The active components of these extracts usually interfere with the growth and metabolism of microorganisms in a negative manner and are quantified by determining the minimum inhibitory concentration and the minimum bactericidal activity. Our results had shown that *Ptericarpus erinaceous* stem bark extract has minimum inhibitory concentration (MIC) of 50 mg/ml and the minimum bactericidal concentration (MBC) of 100 mg/ml suggesting that the plant extract has antimicrobial activity comparing the sensitivity of the bacterial strains to the plant extract and to synthetic antibiotics. The result showed that the plant extract can be used as an alternative to the antibiotics as the zones of inhibition shown were very comparable to the positive control and the extract have lesser side effects which are often associated with the use of antibiotics.

4. CONCLUSION

In conclusion plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The result of the phytochemical screening of *Ptericarpus erinaceous* revealed the presence of bioactive constituents. Our findings on anti-microbial activity of the plant stem bark extract showed that the aqueous extract has higher activity with a zone of inhibition of (21 mm) and (19 mm) at higher concentration of 100 mg/ml of the extract for *Escherichia coli* and *Klebsiella pneumonia respectively* when compared to the ethanolic extract of the plant. The *Ptericarpus erinaceous* plant can be used as an alternative source of development of new and cheaper anti microbial drugs.

5. RECOMMENDATION

The results obtained support the fact that further work needs to be done to determine and identify, purify and quantify the antibacterial compound within this plant stem bark extract and also to determine the full spectrum of efficacy.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The paper is a full research paper conducted in the department of Microbiology laboratory at Modibbo Adama University of Technology Yola, Nigeria. All isolates and laboratory chemicals were of grade standards with no violation of any lab ethics.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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