INVITRO ANTIMICROBIAL ACTIVITY OF *RHUS LONGIPES* AGAINST SOME HUMAN PATHOGENIC BACTERIA

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Abstract

Medicinal plants have been identified as valuable resources of natural antimicrobial compounds as an alternative that can potentially be effective in the treatment of infections caused by bacteria. This study was aimed to evaluate the in-vitro antibacterial activity of ethanol extract of *Rhus longipes* leaf. Agar well diffusion, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were used to determine the antibacterial activity of *R.longipes* against three bacteria species *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. Result obtained showed that the plant extract inhibited the growth of all the microorganisms used at different concentrations. The zone of inhibition (ZOI) of the plant extract against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* was 25.5mm, 27.5mm and 20.5mm respectively. The experiment confirmed the efficacy of this plant extract as a natural antimicrobial and can serve as a treatment of infectious diseases caused by these organisms.

Keywords: Antimicrobial, Minimum inhibitory concentration, *Rhus longipes*

Introduction

Antimicrobial agents are important in the reduction of global burden of various infectious diseases (Bhatia & Narain, 2010). However, antibiotics resistance among bacterial strains is a serious issue which may be so rapid that the effectiveness of synthesized antibiotics may be lost within a span of 6 years due to genetic changes (Chandra et al., 2017)

Moreover, the rapid emergence of multiple drug resistance strains of pathogens to current antimicrobial agents has generated an urgent intensive need for new antibiotics from medicinal plants. Many medicinal plants have been screened extensively for their antimicrobial potential worldwide (Kaur & Arora 2009; Mothana et al., 2009; Adedapo et al., 2009).

According to World health organization (WHO 2002), it was reported that more than 80% of the world population still depends on the traditional medicines for various ailments. The medicinal properties of plants have been preferred throughout the world, due to their potent pharmacological activities, economic viability and low toxicity when compared with synthetic drugs. Medicinal plants are known to be rich in various bioactive compounds such as tannins, alkaloids, flavonoids and phenolic compounds which have been found to possess antimicrobial properties (Duraipandiyan et al., 2006).

Rhus longipes is one of the commonly used plants in the treatment of malaria, asthma, wounds in Ilaro, Ogun state and other southwest regions of Nigeria. *Rhus longipes* have been reported according to Burkil, (2004) to be used in the treatment malaria and cancer. Due to the scanty in literature of information on the pharmacological and biological activities of the plant, *Rhus longipes* genus has been reported to demonstrate significant pharmacological activities.

Hence, these present studies aims at evaluating the *invitro* antimicrobial activities of ethanol leave extracts of *Rhus longipes* against human pathogenic bacteria.

Materials and Methods

Plant Collection

The fresh leaves of *Rhus longipes* were collected from Oja Odan, Ogun state, Nigeria. The plant was identified and confirmed at the Department of Pure and Applied Botany, Federal university of Agriculture, Abeokuta. Ogun State.

Preparation of plant Extract

Ethanol extraction of the plant was carried using a method of Talent et al., 2015. 180 g of the dried pulverized leaves were macerated into each of two 5 L round bottom flask containing 1.6 L of 95% ethanol solvent, agitating intermittently for 72 hours. The extract was filtered through a Whatman filter paper No. 1 and the filtrate collected was concentrated using rotary evaporator model (RE 300) which ensures evaporation of bulky solution to smaller volume concentrates (semi-solid) at temperature of 40^{o}C. The extract was weighed and transferred to micro tubes and stored in a refrigerator at 4^{o}C until required.

Antimicrobial Assays

Preparation of Extract Concentration

According to Garrod, Lambert & O'Grady (1983), One gram of the extract was dissolved in 5 ml of the extracting solvent (Ethanol) for antimicrobial testing. Three more concentration of each extract was prepared from the above extract using the extracting solvent as diluents; serial dilutions were made from 1000,500, 250,125,62.5,31.25mg/ml respectively. The tubes containing only the diluents served as negative control.

Preparation of Agar-well diffusion

Antimicrobial activity of the leaf extract were tested against three bacteria according to previously reported method by Chariandy et al., 1999.They were *Escherichia coli, Staphylococcus aureus and Pseudomonas aureginosa*. Mueller Hinton agar (an iso-sensite agar) oxoid was prepared by dissolving 38g of MHA into 1000ml of distilled water in an agar bottle(1L) kimax bottle,homogenized and sterilized at 121^{o}C for 15mins in an autoclave. The agar was cooled to 45^{o}C before pouring into sterile surface drying at 45^{o}C in an oven. The leave extract were tested for its antimicrobial action against bacteria using agar-well diffusion method. The Mueller Hinton agar plates were seeded with freshly test – culture strains of 18-24hrs by making a suspension of the test-organism to give a concentration of about 10^{5}cells/ml. Aliquot of 0.1ml of the test organism suspension was inoculated with micropipette with sterile-tips dropped onto the agar surface respectively and with aid of hockey stick (spreader) the bacterial suspension was aseptically spread on the agar surface .The plates were allowed to absorbed the organism suspension at room temperature . A sterile cork-borer of diameter 5mm was punched on the agar surface to make wells, subsequently each well was filled with 100µl of the plant extract. The concentration of the extract employed was 31.25, 62.5, 125, 250, 500 and 1000mg respectively. Control well containing the same volume (100µl) ethanol was made. The plates were incubated at 35^{o}C for 24hours, after 24hours incubation period, the antibiogram plates were observed for zone of inhibition (area of no growth around the wells). Bacterial strains that were resistant to antimicrobial agent grew up to the edge of the well as against the sensitive strains, which are inhibited at a distance from the well. The zone of inhibition around each well was measured using a transparent metric ruler in millimeters (mm).

The Minimum Inhibitory Concentration (MIC)

The method described by El-Mahmood et al., 2010 were used .The following concentrations 1000, 500, 250, 125, 62.5, 31.25 mg/ml were prepared using two-fold serial dilution. The tubes were incubated at 37^{o}C for 24 hrs and were observed for visible growth. The lowest concentration at which no detectable bactericidal growth occurred was considered as minimum inhibitory concentration (MIC).

The Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration of the ethanol extract of *R.longipes* was determined using the method of Mann, Banso and Clifford (2008) by selecting tubes that showed no growth during MIC determination. A loopful was taken from the test tubes and inoculated on sterile Mueller Hinton agar .The plates were incubated at 37^{o}C for 24 hrs. The lowest concentrations of the extracts that showed no colony growth on the solid medium was regarded as minimum bactericidal concentration.

Results and discussion

From table 1 below, the susceptibility of the test organisms to the ethanol extracts on the basis of zone of inhibition varied according to organism. Streptomycin (standard) showed antimicrobial activity against all the test organisms. The extract at 25.5mg/ml showed antimicrobial activity against *Staphylococcus aureus* when compared with the standard at 34mg/ml and the control showed no antimicrobial activity. The extract (27.5mg/ml) showed antimicrobial activity against *Escherichia coli* when compared with the standard (32.00mg/ml) and the control showed no antimicrobial activity. The extract (20.5mg/ml) also showed

antimicrobial activity against *Pseudomonas aureginosa* when compared with the standard (30mg/ml) and the control showed no antimicrobial activity.

It was observed that *R.longipes* showed a zone of inhibition against all bacteria tested. The effectiveness of the extract in tested bacteria strains was determined by measuring the minimum inhibitory concentration (MIC). MIC was performed for all the three organisms and was sensitive to the plant extract in the previous antimicrobial assay by agar well diffusion method. The MIC value for *E.coli* was 125mg/ml, while that of *S.aureus* was 31.25mg/ml and *P.aeroginosa* 62.5mg/ml (Table 2).

	Ethanolic Extract (mg/ml)							
Test Organisms	**1000**	**500**	**250**	**125**	**62.5**	**31.25**	**MIC (mg/ml)**	**MCB (mg/ml)**
Staphylococcus aureus	-	-	-	+	+	+	31.25	250
Escherichia coli	-	-	-	-	+	+	125	125
Pseudomonas aeruginosa	-	-	-	-	+	+	62.5	125

Table 1: Antimicrobial activities of ethanol extracts of *R. longipes* leaf extracts.

Mean zone of inhibition of ethanol extract of *R. longipes* leaf (mm)

Test Organisms	**Ethanol extract**	**Standard (Streptomycin)**	**Control(Ethanol)**
Staphylococcus aureus	25.5	34.0	0.0
Escherichia coli	27.5	32.0	0.0
Pseudomonas aureginosa	20.5	30.0	0.0

Table 2: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of ethanol extract of *R.longipes* leaf extract.

+:Growth –:No Growth

Recently, the need for search of antimicrobial properties has been on the rise due to their potential therapeutic use in the treatment of various chronic and infectious diseases **(**Halliwell, 1995). As a matter of facts, there are different adverse effects encountered with the use of synthetic antibiotics. However, medicinal plants may offer an alternative source for antimicrobial agent with significant activity against pathogenic and infective microorganisms.

The result of antimicrobial activity of ethanol extract of *R.longipes* showed good antimicrobial activity against the three test organisms. *R.longipes* extract was active against *Escherichia coli, Staphylococcus aureus and Pseudomonas aureginosa.*

The extract showed the highest antibacterial activity at 27.5mg/ml and least antibacterial activity at 20.5mg/ml against *Escherichia coli* and *Pseudomonas aureginosa* respectively. This result is in line with the findings of Faparusi et al., (2012) who reported that antibacterial potential of *Brillantaisiapatula* using agar well diffusion method against *Staphylococcus aureus, Escherichia coli, Pseudomonas aureginosa, Enterococcus faecalis and Proteus hauseri* using methanol and ethanol extract.

Ethanol extract demonstrated bactericidal activity against *S. aureus*, *E. coli* and *P. aureginosa* at concentration of 250, 125, 125 mg/ml respectively. The highest MIC value (125mg/ml) was recorded for *E. coli* while the least (MIC) value (31.25mg/ml) was recorded for *S. aureus*.

S. aureus has the highest MBC value at 250mg/ml while *E.coli* and *P. aureginosa* had the least MBC value at 125mg/ml**.** This result is also in line with findings of Faparusi et al., (2012) on antibacterial activities of ethanol and methanol extracts of *Brillantaisiapatula* against similar organisms. The antibacterial activities of the extract could be due to the phytochemical constituents of the plants. *R. longipes* could be a source of potent antibacterial agent for industrial purposes. Further study, however, is still warranted to explore the effectiveness of the extract in inhibiting the growth of viruses, parasites and/or fungi.

Conclusion

In this study, the antimicrobial activities of *R.longipes* were assessed by cold percolation method. The results showed a potential antibacterial effect against bacterial stains tested. Therefore, the leaves of *R.longipes* could be a novel source of antibacterial agent that might have broad spectrum activity. Moreover, other parts of the plants need to be studied to evaluate the studied plant extract as a potential antimicrobial agent.

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