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Antibacterial effect of leaf extract of *Ricinus communis*

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ABSTRACT: The antibacterial activity of aqueous, ethanolic and methanolic extracts of *Ricinus communis* were studied by the agar diffusion method. The ethanolic extract produced the greatest antimicrobial activity and the aqueous extract the least. *Staphylococcus aureus* was the most sensitive to the extracts while *Bacteroides fragilis* was the most resistant. The minimum bactericidal concentration of the extracts ranged between 60% (w/v) and 100% (w/v). Thus the extracts of *Ricinus communis* could be useful as a source of antibiotics against aerobic and anaerobic bacteria.

Key Words: Traditional medicine; Medicinal plants; *Ricinus communis*; Antibacterial activities.

Introduction

Utilization of all available local resource is essential in primary health care. In developing countries, traditional medicine and its practitioner are involved; where traditional medicine is patronized by communities, it is necessary to adopt safe and useful traditional practices that would enable its incorporation into the design and implementation of natural health care system. However, that will involve putting traditional medicine on a scientific basis.

Green plants possess the broadest spectrum of synthetic activity and have been the source of many useful compounds (Sofowora, 1986). The use of higher plants and shrubs including some vegetables were originally recognized as antiseptic, for example, thymol, a simple phenol present in essential oil of plants like *Thymus vulgaris* and *Monarda punctata* have both antibacterial and antiviral properties (Ekong *et al.*, 1968; Bedows *et al.*, 1982). *Acalypha indica* has acalyphine used in the treatment of sore gum, it has expectorant and emetic properties (Ekong *et al.*, 1968; Bedows *et al.*, 1982). Many plant from different parts of the world have been known to produce antimicrobial substances (Bhandari, 1959; Sakuma and Tomiyan, 1967; Malcolm and Sofowora, 1969; Bhakumi *et al.*, 1974; Boakye-Yiadom, 1977; Shakarma *et al.*, 1979). Since there are so many naturally occurring substances of plant origin which cover a wider range than synthetic chemical, it is obvious that the plant kingdom offers a better opportunity of providing useful medicinal compounds (Nwaiwu, 1982).

This work reveals the *in vitro* susceptibility pattern of leaf extracts of *Ricinus communis* against some medically important bacteria.

Materials and Methods

Sources of plant materials and bacteria

The leaves of the plant were obtained from The School of Medical Laboratory Technology, National Veterinary Research Institution, Vom, Nigeria. They were identified based on criteria stipulated by international committee for botanical nomenclature (I.C.B.N) as *Ricinus communis*.

The microorganisms used include *Staphylococcus aureus*, *Streptococcus* species *Escherichial coli*, *Bacteroides melaninogenicus*, *Bacteroides fragilis*, *Clostridium perfringens* and *Clostridium chauvoel*. They were obtained from Bacteriology Department, National Veterinary Research Institute, Vom, Plateau State, Nigeria.

Preparation of Plant Extracts

Ten grammes of powdered air-dried leaf samples of *R. communis* was weighed separately into 100ml of 70% ethanol, methanol or distilled water contained in 500ml capacity flasks. The flasks were plugged with cotton wool, wrapped in aluminium foil, shaken vigorously and allowed to stand in the refrigerator for 24 hours. The extracts were filtered through a membrane filter and stored in refrigerator in reagent bottles. (Akinyanju *et al.*, 1986) each extract was tested for growth and or contamination by plating them on nutrient agar 37°C for 24 hours. When no growth was observed in the extract, it was then assessed for antimicrobial activity (Olorundare *et al.*, 1992).

Susceptibility Test

The antibacterial test was performed using the agar diffusion method of Bakyeiyadom (1979). The test organism was inoculated on nutrient agar plates and spread uniformly using a sterile glass spreader. Holes of 5mm diameter were punched on the nutrient agar using a sterile cork borer. The cut agar disks were carefully removed by the use of forceps sterilized by flaming. To each hole was introduced different concentrations of the extracts of the plant material, control experiments with no plant extracts were set up.

The plates were allowed to stand for one hour at room temperature for diffusion of the substrates to proceed before the growth of organism commenced. The plates were finally incubated 37°C for 24 hours.

Determination of Minimum Inhibitory Concentration (MIC) of the Extracts

Various concentrations of the extracts ranging between 20% (w/v) and 100% (w/v) were introduced into different test tubes, each culture of *S. aureus*, *Streptococcus sp.*, *E. coli*, *C. perfringens*, *C. Chauvoei* *B. melaminogenicus* or *B. fragilis* diluted to give a final concentration of 10^6 cells per ml. The tubes were inoculated at 37°C for 24 hours. The least concentration of the plant extracts that did not permit any visible growth of the inoculated test organism in broth culture was taken as the MIC in each case (Irobi, 1992)..

Determination of Minimum Bactericidal Concentration (MBC) of the Extracts

After culturing the test organisms separately in nutrient broth containing various concentrations of the extracts, the broth was inoculated onto freshly prepared agar plates to assay for the bactericidal effect. The culture was incubated at 37°C for 24 hours. The lowest concentration of extract that does not yield any colony growth on the solid medium after the incubation period was taken as MBC (Alade and Irobi, 1993).

Results and Discussion

The results indicate that *S. aureus*, *Streptococcus sp.*, *E. coli*, *C. chauvoei*, *C. perfringens*, *B. melaminogenicus* and *B. fragilis* are susceptible to leaf extracts of *R. communis* (Table 1). The results reveal that an increase in the concentration of the extract brought more activity as shown by the diameter of zone of inhibition.

Table 1: Susceptibility Test of Leaf Extracts of *Ricinus communis* (Paper 3027).

Concentration % (w/v)	Diameter of Zone of Inhibition (mm)																				
	Aqueous Extract							Ethanolic Extract							Methanolic Extract						
	Sa	Ss	Ec	Cc	Cp	Bm	Bf	Sa	Ss	Ec	Cc	Cp	Bm	Bf	Sa	Ss	Ec	Cc	Cp	Bm	Bf
20	3.8	3.6	2.7	3.0	3.2	2.3	2.6	4.5	4.7	3.8	4.2	4.6	3.6	3.9	4.2	4.4	3.6	3.9	4.0	3.3	3.5
40	4.2	4.0	3.2	3.9	3.9	2.5	2.8	5.5	5.3	4.5	4.9	4.7	3.5	3.8	5.2	5.0	4.1	4.7	4.6	3.4	3.7
60	4.8	4.5	3.6	4.5	4.4	2.9	3.0	5.8	5.6	4.8	5.6	5.9	4.0	4.6	5.7	5.4	4.5	5.3	5.4	3.8	4.1
80	5.5	5.4	3.8	4.8	4.8	3.5	3.2	6.7	6.5	4.9	5.8	5.9	4.7	4.9	6.5	6.3	4.7	5.7	5.6	4.4	4.2
100	5.5	5.4	4.8	5.2	5.2	3.5	3.2	6.8	6.5	5.9	6.5	6.3	4.7	4.5	6.6	6.3	5.7	6.1	6.2	4.5	4.3

Sa = *Staphylococcus aureus*

Ss = *Streptococcus species*

Ec = *Escherichia Coli*

Cp = *Clostridium perfringens*

Bm = *Bacteroides melaninogenicus*

Bf = *Bacteroides fragilis*

This observation agrees with the report of Boakye (1979); Kurosaki and Nishi (1983) that higher concentrations of antimicrobial substance showed appreciable growth inhibitions. The results of the MIC of aqueous extracts of *R. communis* ranged between 60% and 80% (w/v). The MIC figures for ethanolic extract ranged between 40 and 70% (w/v). A dose of between 50% (w/v) and 80% (w/v) was observed for aqueous extract against the test organisms (Table 2). Table 3 shows the result of MBC of the leaf extracts within the ranges of 60 and 100% (w/v). Ethanolic extract proved to possess more bactericidal action as indicated by the low value of its MBC (60 to 90% w/v (Table 3). Relatively higher values were obtained in assays with methanolic and aqueous extracts (Table 3). The results indicate that the MBC of the extracts were obtained at higher concentration than in the MIC studies. This observation therefore suggests that the antibacterial substances contained in the extracts were bacteriostatic at lower concentrations while becoming bactericidal at higher concentrations of the extracts.

This investigation suggests that extracts of *R. communis* could serve as a source of antimicrobial agent against some disease causing microorganisms.

Table 2: Minimum Inhibitory Concentration of Leaf Extracts of *Ricinus communis* (% w/v).

Extract	Organism						
	Sa	Ss	Ec	Cc	Cp	Bm	Bf
A _E	60	70	80	60	70	90	80
E _E	40	50	60	30	40	70	70
M _E	50	60	80	50	60	90	80

A_E = Aqueous extract; E_E = Ethanolic extract; M_E = Methanolic extract; Sa = *Staphylococcus aureus*; Ss = *Streptococcus species*; Bf = *Bacteroides fragilis*; Ec = *Escherichia coli*; Cc = *Clostridium chauvoei*; Cp = *Clostridium perfringens*; Bm = *Bacteroides melaninogenicus*

Table 3: Minimum Bactericidal Concentration of leaf Extract of *Ricinus communis* (% w/v)

Extract	Organism						
	Sa	Ss	Ec	Cc	Cp	Bm	Bf
A _E	80	90	90	80	90	100	100
E _E	60	70	80	60	70	90	90
M _E	70	80	100	70	70	100	100

A_E = Aqueous extract; M_E = Methanolic extract; E_E = Ethanolic extract; Sa = *Staphylococcus aureus*; Ss = *Streptococcus species*; Ec = *Escherichia coli*; Cc = *Clostridium chauvoei*; Bm = *Bacteroides melaninogenicus*; Bf = *Bacteroides fragilis*

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