ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology, 3 (1): 41-43, 2004
ISSN 1682-2978

Antimicrobial Activities of *Cajanus cajan, Garcinia kola* and *Xylopia aethiopica* on Pathogenic Microorganisms

G.O. Ezeifeka, M.U. Orji, T.I. Mbata and A.O. Patrick
Department of Applied Microbiology and Brewing Nnamdi Azikiwe University, Awka, Nigeria

Abstract: The antimicrobial effect of the ethanol and aqueous extracts of locally available plants, *Cajanus cajan, Garcinia kola* and *Xylopia aethiopica* on *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans* were evaluated. The agar gel diffusion and paper disk diffusion method were used to determine the inhibitory effects of both the leaves and seeds extract of the test plants on the test microorganisms. The plant extracts produced inhibition zones ranging from 3 to 22 mm against the test microorganisms. The ethanol extracts of the test plants were more effective in producing inhibition zones against the micro-organisms than water extracts. The extracts of *Cajanus cajan* produced wider zones of inhibition against *Candida albicans* than the other plants extracts.

Key words: Antimicrobial activities, *Cajanus cajan, Garcinia kola,Xylopia aethiopica*

INTRODUCTION

The Nigeria climate favours a great array of plant species many of which have varied medicinal and antimicrobial potentials.

Earlier studies have shown that plants that have medicinal and or antimicrobial values have either alkaloid; saponins, steroids, tannins, glucosides and various oils and they tend to be the site for the active medicinal ingredient of such plants. Three compounds may be found in a particular part of the plant or all over its body and they are often localized in glands[1-3] observed that a striking characteristics of plant is that different chemical substance are obtained in members of even the same species in different areas.

A number of plants that have medicinal and or antimicrobial properties in Nigeria have been identified and documented[4,5]. Some of the active ingredients of the extracts of some of these plants have been isolated, tested and documented[6,7].

The development and spread of resistance to the existing antibiotics by microorganisms calls for increased efforts in the development of new antibiotics for treatment of microbial infections and diseases. Although a member of plants with antimicrobial activities have been identified, a great number still remain unidentified.

The purpose of this work was therefore to evaluate the antimicrobial activities of *Cajanus cajan, Garcinia kola* and *Xylopia aethiopica* on *Staphylococcus aureus, Pseudomonas aeruginosa Escherichia coli* and *Candida albicans.*

MATERIALS AND METHODS

Plants extracts: The leaves and seeds of *Cajanus cajan, Garcinia* kola and *Xylopia aethiopica* were collected, sun dried for five days and grinded into powdery form using an electric grinder. The soluble ingredients in each of the grounded plant parts were then extracted by solubilization using ethanol and water as different solvents.

Aqueous extraction: The aqueous extraction of the water-soluble ingredients of the plant parts were carried out using the method as described by Asuzu[2]. 15 g of each of the grounded plant part was extracted by successive soaking for 3 days using 35 ml of distilled water for each sample in separate container. The extracts were filtered using Whatman No 1 filter paper, after which the filtrates were concentrated by evaporation at low temperature of 30°C using water bath. The concentrated extracts were store in the refrigerator until required.

Ethanol extraction: The ethanol extraction of the active ingredient of the plant parts were carried out using the method as described by Harbone[8]. 25 g of each of the grinded plant part was soxhlet extracted using 250 ml of 95% ethanol. The extraction of each of the part lasted for six hour. The volatile oils obtained were concentrated by evaporation using water bath at 100°C for 1 h.

Test organisms: The bacteria and fungi species used in this study were collected from the Medical laboratory unit of Nnamdi Azikiwe University Teaching Hospital Nnewi,

Corresponding Auhtor: T.I. Mbata, Department of Applied Microbiology and Brewing Nnamdi Azikiwe University, Awka, Nigeria

Nigeria. The bacteria were cultured and maintained as described by Cruickshank *et.al.*[9]. The isolates were identified using biochemical method as described by Holt *et al.*[10]. The fungus by the method described by Fawole and Oso[11]. The bacteria species are coagulase positive *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*, while the fungi species is *Candida albicans*.

Antimicrobial test: The antimicrobial tests of the plant extracts were tested on the test microorganisms using the agar-gel diffusion inhibition test and paper disk diffusion inhibition test. In the agar-gel diffusion inhibition test as described by Opara and Anasa[12], 0.2 ml of a 24 h broth culture of each of the test microorganisms was aseptically introduced and evenly spread using bent sterile glass rod on the surface of gelled sterile Mueller-Hinton agar plates. Three wells of about 6.0mm diameter were aseptically punched on each agar plate using a sterile cork borer, allowing at least 30 mm between adjacent wells and between peripheral wells and the edge of the petri dish. Fixed volumes (0.1 ml) of the various plant extract were then introduced into the wells in the plates. A control well was in the center with 0.01ml of the extracting solvent. The plates incubated at 37°C for 24 h for the test bacteria. This same method was used to test for antifungal activity but the fungi were grown on sabourand dextrose agar plates at 28°C for 48 h at room temperature. The plates were duplicated in all the experiments.

In the paper disc diffusion test, sterile paper discs were soaked in the different plant extracts for 2 h. 0.2 ml of a 24 h broth culture of each of the bacteria species were spread on the surface of gelled sterile Mueller-Hinton agar plates. The paper discs containing different extracts were placed at different areas on the surface of each plate. The plates were incubated at 37°C for 24 h. Antimicrobial activity of each extract against the test bacteria was indicated by growth-free "zone of inhibition" near the respective discs. This same method was used to test for antifungal activity but the fungi were grown on sabosurand dextrose agar plates at 28°C for 48 h before the paper disc dipped in the plants extracts were planted on the plates. A disc soaked in the extracting solvent for 24 h was used as the control.

RESULTS

The ethanol extract of all the plants showed various levels of antimicrobial activity when tested by both methods, whereas the aqueous extract showed antimicrobial activity only when tested by the paper disc diffusion method (Table 1-3). Generally, leaf extracts of all the plants had higher antimicrobial activity than the extracts from the seeds. Similarly, the ethanol extracts of the test plants had higher antimicrobial activity than the aqueous extracts.

The ethanol extract of both leave and seeds of *Cajanus cajan* showed the highest antimicrobial activity followed by *Garcinia kola* and *Xilopia aethiopica* in that order when tested by both methods. The aqueous extracts of the leaves and seeds of all the plants had no effect on *Candida albicans*, those of *Xilopia aethiopica* had no effect on all the bacteria tested except *Staphylococcus aureus* and those of the seeds of *Cajanus cajan* and *Garcinia kola* had no effect on *Escherichia coli,* Whereas both seeds and leaf aqueous extracts of *Garcinia kola* had effect on *Pseudomonas aeruginosa*, only the seed extracts of *Cajanus cajan* had effect on these bacteria (Table 3). *Staphylococcus aureus* was inhibited by both aqueous and ethanol extract of both leaves an seeds of all test plants (Table 1 and 3). The antimicrobial activity of the aqueous plants extract on the test organism using Agar-gel diffusion showed no antimicrobial effect on all the microorganisms tested. Higher diameter zones of inhibition was obtained with the paper disc method than with Agar-gel diffusion method for all the microorganisms and for both extraction methods.

DISCUSSION

The result of this study showed that the leaves and seeds extract of the test plants *Cajanus cajan, Garcinia kola* and *Xylopia aethiopia* have good inhibitory effects against *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans.*

The ethanol extracts of the plant parts showed more inhibitory effects than the water extracts. This tends to show that the active ingredients of the plant parts were better extracted with ethanol than water. Akunyili *et al.*[7] observed a similar result when they worked with stem bark of *Kigelia pinnata*. The failure of water to extract maximally the active ingredients in those plant parts may be responsible for the no inhibitory effects the water extracts of the plants had on *Candida albicans* whereas the ethanol extracts of the same plants parts had good inhibitory effects against the same organisms. The plant extracts showed more inhibitory effect against the bacterial species than the fungus *Candida albicans*. The extracts of *Cajanus cajan* showed wider zones of inhibition against *Candida albicans* than other plant extracts.

The two methods used to test the antimicrobial activity of the plant extracts proved to be good but the

Table 1: Antimicrobial activity of the ethanol extracts of the plant parts on the test organisms using paper disc diffusion test

Plant species/part		Zones of inhibition of organisms (mm)			
Cajanus cajan	Plant form	*Staph aureus*	*Pseud. aeruginosa*	*Escherichia coli*	*Candida albicans*
Leaves	crude	15.0	20.1	15.0	22.0
Seed	crude	12.0	14.1	10.2	8.2
Garcinia kola					
Leaves	crude	12.0	18.0	12.0	14.1
Seed	crude	10.1	12.0	10.0	8.0
Xylopia aethiopica	crude				
Leaves	crude	12.0	8.0	10.0	13.2
Seed	crude	10.2	6.0	10.0	10.2

Table 2: Antimicrobial activity of the ethanol extracts of the plant parts on the test organisms using agar-gel diffusion test

Plant species/part		Zones of inhibition of organisms(mm)			
Cajanus cajan	Plant form	*Staph aureus*	*Pseud.aeruginosa*	*Escherichia coil*	*Candida albicans*
Leaves	crude	15.0	18.0	13.0	16.0
Seed	crude	16.0	16.0	14.2	18.2
Garcinia kola					
Leaves	crude	13.0	13.0	12.0	9.0
Seed	crude	10.1	8.0	10.1	8.0
Xylopia aethiopica					
Leaves	crude	11.0	8.0	12.0	8.0
Seed	crude	10.2	5.0	10.2	6.0

Table 3: Antimicrobial activity of the aqueous plants extracts on the test microorganisms using paper disc diffusion test

Plant species/part		Zones of inhibition of organisms (mm)			
Cajanus cajan	Plant form	*Staph. aureus*	*Pseud. aeruginosa*	*Escherichia coli*	*Candida albicans*
leaves	crude	4.0	-	6.0	-
Seed	crude	3.0	6.0	-	-
Garcinia kola					
leaves	crude	4.2	6.0	10.0	-
Seed	crude	10.1	6.2	-	-
Xylopia aethiopia					
Leaves	crude	4.1	-	-	-
Seed	crude	5.0	-	-	-

Key: - =No zone of inhibition.

paper disc diffusion method tends to show wider zones of inhibition than the agar gel diffusion method.

Since the extracts of the plant produced good inhibition zones against the test organisms, it is expected that they could be used to treat infections and diseases caused by these organisms and if the active ingredients of the extracts are isolated and possibly crystallized, therapeutic antibiotics could be produced from these.

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