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The phytochemical and antimicrobial studies of *Ficus Exasperata* and *Cida Acuta* on *Staphylococcus Aureus* and *Escherichia Coli*

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ABSTRACT

Several herbs known to cure some ailments in Africa have not been scientifically evaluated for their phytochemical and antimicrobial properties, as a panacea for knowing the mechanisms of action of such herbs, in view of the current wave of microbial resistance to drugs. This study aims to screen the extracts of Ficus exasperata and Cida acuta for their effect on S. aureus (gram-positive bacterium) and E. coli (gram-negative bacterium). The crude extracts of F. exasperata and C. acuta were prepared using standard procedures, which involved soaking 20g of the powdered leaves in 80ml of 95% ethanol for 48hrs at room temperature, to allow for maximum extraction of active ingredients, followed by evaporation to retain the crude extract of each of the test plants. Parts of the extracts were screened phytochemically using standard methods, while the remaining parts of the extracts were used for the sensitivity test of the test organisms. The phytochemistry of the plants showed that F. exasperata contained high levels of tannins, flavonoids, and steroids and moderate levels of terpenes and anthraquinones. On the other hand, C. acuta contained high levels of alkaloids and flavonoids with moderate levels of saponins, tannins and cardiac glycosides, indicating that the two plants are of medicinal significance. It is inferred that the high content of alkaloids and moderate levels of saponins and tannins in C. acuta could have been responsible for the susceptibility of E. coli to it (zone of inhibition >21mm) as chloramphenicol (control). The levels of tannins, flavonoids and steroids with low levels of alkaloids make F. exasperata more effective against gram-positive S. aureus than gram-negative E. coli. S. aureus was very sensitive to F. exasperata (zone of inhibition >21) and resisted C. acuta (zone of inhibition<21mm). E. coli was observed to be 100% resistant to F. exasperata. It is conclude that C. acuta and F. exasperata are medicinal herbs of choice against E. coli diarrhoea and S. aureus infection, respectively.

Key words: Medicinal plants, bacterial resistance, alternative antibiotics

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INTRODUCTION

Ficus exasperata and *Cida acuta* are used by the people of Southern Nigeria for the treatment of several ailments. One plant material is used for different treatments within a community. For instance, Obot (1996) (1) states that *Newboildia laevis* (BIGNONIACEAE) is used in Okwa of Cross River State of Nigeria as cough remedy and as an aphrodisiac. Thus, *Ficus exasperata* (MORACEAE) commonly called sandpaper tree and traditionally called "Uknok" by the Ibibio tribe of Nigeria, is used to cure cough when mixed with lime or lemon juice; leaf juice or leaf decoction is used to stop bleeding and nagging stomach pains. Also *Sida acuta* (MALVACEAE) commonly called brown weed or alcali mallow and traditionally called "Akana-anwan idippeke kisoro" by the Ibibio tribe of Nigeria, who use the leaves as an abortifacient and as emolient. They also use the leaves to treat haemorrhoids and impotence, while the extracts are used to relieve hay fever and asthma. The herb is also used as antibacterial, antiprotozoal and antidiuretic.

The literature on the phytochemical and antimicrobial properties of *F. exasperata* and *Cida acuta* is scanty, but they are qualified to be called medicinal plants because the local populations use the plants in various forms to treat some diseases (2). However, a few studies on medicinal plants have pointed to the fact that the therapeutic compounds that are pharmacologically active have alkaloids, cyanogenetic glycosides, phlobotanins, polyphenols, saponins, anthraquinones, etc., and these are known to be present in most medicinal plants (3, 4, 5 and 6).

Recently too, there has been an increasing concern and the need to source for locally available drugs because of unaffordability of conventional chemotherapeutic agents, antimicrobial resistance and clinical cost due to increased poverty in Africa (7). Andy *et al.* (2008) (7) states that the situation has generated a few studies on the phytochemistry and the medicinal potency of some of the medicinal plants known to Africans, including garlic. Besides, the current wave of antimicrobial resistance to chemotherapeutic drugs is a global concern (8), and therefore there is need to search for such plants that would be resistance-free. Also, some plants, e.g., *Ocimum gratissimum* (LABIATEAE) and *Eugenia uniflora* (LINN) have been reported to be rich in volatile oils which contain up to 75% thymol that has antimicrobial effect, and has been popularly used in the treatment of diarrhoea and even ear infection, besides having antimicrobial properties against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Shigella dysenteriae* (9, 10, 11).

This study aims to investigate qualitatively the phytochemical components of the extracts of the plants leaf, and the sensitivities of known pathogenic bacteria, *Staphylococcus aureus* and *Escherichia coli,* to the plants leaf extracts.

MATERIALS AND METHODS

Sources of Test Organisms and Plants

Known cultures of *S. aureus* (WD20) and E. coli (OD15) were obtained from the Department of Medical Microbiology and Parasitology Laboratory of the University of Calabar Teaching Hospital (UCTH), Calabar. The cultures were preserved in agar slants until they were used. The test plants were collected from the botanical garden of the Department of Biological Sciences, Cross River University of Technology, Calabar. They were carried to the herbarium of the Department for identification as *Ficus exasperata* and *Cida acuta.*

Preparation of the Plant Extracts

After the leaves of the plants were air-dried and ground in a mortar (12), the crude extracts of the leaves were prepared using standard procedures (13, 14). This involved soaking 20g of the powdered leaves in 80ml of 95% ethanol for 48hrs at room temperature to allow for maximum extraction of the active ingredients. This was followed by evaporation of the filtrate using a rotary evaporator (STUARC SCIENTIFIC, ENGLAND). The residues were retained as the crude extract of each of the test plants and stored in reagent bottles and maintained in the freezer until it was used.

Phytochemical Screening of Plant Extracts

The qualitative determinations (phytochemical screening) of the plant extracts were done using the methods of (15, 3, 16, 17, and 18).

Preparation of Extract Concentration for the Determination of Zones of Inhibition

The weights of the crude extracts were respectively 0.34g (*F. exasperata*) and 0.52g (*C. acuta*). The complete weight of each extract was resuspended in 1ml of Dimethyl sulfoxide (DMSO) and used for antibacterial susceptibility test by the disk diffusion technique with extracts (19, 20).

Preparation of the Concentration of a Broad-Spectrum Antibiotic, Chloramphenicol used for test as a control

The drug used was chloramphenicol 250mg (Clarion Medicals Ltd, Lagos). Chloramphenicol was selected as a control because it is a drug of choice for gram-negative enteric bacterial pathogens, and S. *aureus*, a gram-positive bacterium (20). The powdered chloramphenicol (250mg) was dissolved in deionized water and DMSO as a solubilizing agent made up to a volume of 25.0ml at room temperature (14). This gave a concentration of 10mg/ml. Further dilutions as with the extracts were made to obtain a solution with a concentration of 1mg/ml. By mixing 1.0ml of the solution with 9.0ml of DMSO, a final concentration of 100µg/ml was obtained.

Sensitive Test

A disc diffusion technique using the Kirby-Bauer method was applied in testing pure cultures of the test organisms for their antimicrobial sensitivities based on zones of inhibition on agar plates (19, 20). In this method, punched circular discs from filter paper (Whatman No.1) that were sterilized in a hot air oven for 1hr. were impregnated with 0.1ml of each of the plant extracts. They were air-dried for a few minutes, and transferred aseptically onto the

surface of previously prepared Mueller-Hinton agar plates. This followed incubation at 37^0C for 24hrs, following which the plates were observed for zones of inhibition measured in millimeter (mm).

RESULTS

Results of the phytochemical screening of the plants are displayed in Table 1.

The table shows that *F. exasperata* contains high levels of tannins, flavonoids and steroids, and moderate levels of terpenes and anthraquinones. Alkaloids and cardiac glycoside levels are low in *F. exasperata*. On the other hand *C. acuta* contains high levels of alkaloids and flavonoids, with moderate levels of saponins, tannins and cardiac glycoside, and low levels of steroids and anthraquinones. Saponins and terpenes were respectively absent in *F. exasperata* and *C. acuta.*

Table 1: **Phytochemical Screening of Plants Used**

Substance	*F. exasperate*	*C. acuta*
Alkaloids	*	***
Saponins	-	**
Tannins	***	**
Flavonoids	***	***
Steroids	***	*
Terpenes	**	-
Anthraquinones	**	*
Cardiac glycosides	*	**

*-= Absence of bioactive substances; *= Low level of bioactive substance; **= Moderate level of bioactive substances; ***= High level of bioactive substances.*

The effect of the extracts of *F. exasperata* and *C. acuta* are shown in Table2. The table shows that *S. aureus* is sensitive to E_1 (zone of inhibition >21mm), but resistant to E2 (zone of inhibition <21). On the other hand, *E. coli* is 100% resistant to E1 but sensitive to E2. However, *S. aureus* is slighty resistant to chloramphenicol, unlike *E. coli* which is sensitive to chloramphenicol (control) (zone of inhibition >21mm).

Table 2: Effect of ethanolic extracts of *Ficus exasperata* (E_1) and *cida acuta* (E_2) on *S. aureus* and *E. coli.*

Plants Sample	Mean Zones of Inhibition (mm)	
	S. aureus	*E. coli*
Ficus exasperata	30	-
Cida acuta	12	24
Chloramphenicol	19	26

The figures represent means of duplicate zones of inhibition obtained from 100µg/ml of plant extracts.
Resistance = zone of inhibition <21mm; susceptibility = zone of inhibition ≥21mm; - = 100% resistant.

DISCUSSION

The presence of bioactive substances in *F. exasperata* and *C. acuta* indicates the medicinal significance of the plants. For instance, there is high content of alkaloids in *C. acuta* and low level in *F. exasperata.* Alkaloids, especially the sanguinarine alkaloids, are known to be antibiotic, while the berberine alkaloids are used to treat diarrhoea and general gastrointestinal complaints (21). It is inferred that the high content of alkaloids and moderate levels of saponins and tannins in *C. acuta* could have been responsible for the susceptibility of the Gram-ve bacteria, *E. coli* to it (zone of inhibition >21mm), just as chloramphenicol (control). It is however not known why it is the reverse with *S. aureus* which resisted *C. acuta* (zone of inhibition <21mm) and chloramphenicol. *S. aureus* was rather very sensitive to *F. exasperata* (zone of inhibition=30mm) while *E. coli* was 100% resistant to *F. exasperata*. This implies that *F. exasperata* is the medicinal plant of choice against *S. curreus* infection, while *C. acuta* is a medicinal plant of choice against gram-ve bacteria, *E. coli.*

Further research appears to be necessary to find out whether other gram-positive bacteria and gram-negative bacteria will respectively be sensitive to *Ficus exasperata* and *Cida acuta.* However, the cell wall structural nature of gram-negative bacteria may be responsible for the observed susceptibility of *E. coli* to *C. acuta.* Carpenter (1968) (22) reports that the cell wall of gram-negative bacteria contains 15-20% polysaccharides and 10-20% lipid, whereas that of gram-positive bacteria contains 35-60% polysaccharides and only 0-2% lipid. The membrane of *E. coli* has been reported to contain 20% lipid. It has also been reported (23, 24) that the polysaccharides and the lipid contents of the

cell wall affect the permeability of some plant active ingredients, e.g. allicin and other garlic constituents (25), and thus the observed susceptibility of *S. aureus* to *F. exasperata.*

E. coli is seen to be 100% resistant to *F. exasperata.* It appears that *E. coli* has used its resistance strategy to "pump" out *F. exasperata* from its cell as reported by Prescott *et al.* (2005) (20), thus rendering *F. exasperata* ineffective.

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