

Full Length Research Paper

Antimicrobial activity of the aqueous, methanol and chloroform leaf extracts of *Cissus multistriata*

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Antimicrobial activity of aqueous, methanol and chloroform leaf extracts of *Cissus multistriata* were investigated against 8 bacterial and 2 fungal test organisms, using the tube dilution and agar ditch diffusion methods. Aqueous leaf extract had no activity against both the bacterial and fungal test organisms. Both the methanol and chloroform leaf extracts inhibited all the test organisms with chloroform leaf extract showing the highest zone of inhibition against *Escherichia coli* (diameter 25 mm) and least against *Staphylococcus aureus* (diameter 13 mm). The methanol leaf extract was least inhibitory against *Salmonella typhi* (diameter 8 mm) and most inhibitory against *S. aureus* (diameter 15 mm). The methanol leaf extract of *C. multistriata* show more antifungal activity compared with chloroform leaf extract, with *Candida albicans* being more susceptible than *Aspergillus niger* to both methanol and chloroform leaf extracts. The minimum inhibitory concentration (MIC) of methanol leaf extract show least activity against *Yersinia enterocolitica* and *Pseudomonas aeruginosa* (MIC = 100 mg/ml) and higher activity of MIC at 50 mg/ml against the other bacterial test organisms. The chloroform leaf extract MIC of 100 mg/ml had least activity against *Proteus mirabilis* and *P. aeruginosa* and MIC of 20 mg/ml most inhibitory against *E. coli*, *Klebsiella pneumonia* and *S. typhi*. The antimicrobial activity of the heated extracts persisted after exposure to various temperatures between 30°C to 121°C for 15 to 30 min. However, the extract activity decreased as the temperature increased. The killing rate of the MBC of chloroform extract on *E. coli* was 1 cfu/3 min while on *S. typhi* was 1 cfu/3.8 min.

Key words: *Cissus multistriata*, antimicrobial, extract, inhibition, susceptible.

INTRODUCTION

Over the years there has been an incessant and alarming report of drug resistance in medically important strains of susceptible bacteria and fungi (CDC, 1995). This rapid emergence of resistant strains among susceptible pathogens coupled with the fact that many of the present day antibiotics in use are fast losing their potency are some of the reasons for the unabated search for effective and affordable antimicrobial drugs from local medicinal plants which could provide a source of new possible antimicrobial drugs (Egah et al., 1999). It is important that clinicians continue to keep a step ahead of these emerging resistant strains (Okafor, 1987). One of the ways to achieve this objective is through sustained search for new antibiotics and other antimicrobial agents. Consequently,

there has been an upsurge in the interest in herbal remedies in several parts of the world with many being incorporated into orthodox medical practice (Okwuzua, 1973).

As part of the unabated search for antimicrobial drugs and in view of the fact that there are still plants whose medicinal uses have not been ascertained, this study was carried out on *Cissus multistriata* to establish its antimicrobial activity. It is a plant of choice because of its traditional use for treatment of Kwashiorkor, Marasmus and other ailments in children among Ibaji herbal healer in Kogi State, Nigeria.

MATERIALS AND METHODS

Plant source, identification and preparation

C. multistriata plant was collected from Mr. Joshua, a peasant farmer in Idah, Kogi State. The plant was identified by the Forestry Research Institute of Nigeria (FRIN) Jos, Nigeria. The leaves were

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washed and air dried at room temperature $27 \pm 2^\circ\text{C}$ for several days in the Microbiology Laboratory of Kogi State University until the leaves become crispy. They were ground into a fine powder using sterile mortar and pestle. This was stored in air tight glass container protected from direct light and heat until required for analysis.

Test organisms and culture media

The isolates used as test organisms were obtained from the Department of Microbiology, Federal College of Veterinary and Medical Laboratory Technology, Vom, Plateau State. They include *Staphylococcus aureus*, *Escherichia coli*, *Yersinia enterocolitica*, *Proteus mirabilis*, *Shigella sonnei*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Candida albicans* and *Aspergillus niger*. The bacterial isolates were grown at 37°C on nutrient agar (oxoid), confirmed using the standard techniques as described by Cowan and Steel (1965) and maintained on nutrient agar (oxoid) at 4°C while the fungi isolates were cultured and maintained on potato dextrose agar (PDA) to which 0.05% chloramphenicol was added.

Nutrient broth and agar were used as culture media for bacteria while potato dextrose broth and agar were used for fungi. The broths were used for MIC determination while the nutrient agar and PDA were used for the MBC and MFC determination respectively. The media were prepared and sterilized according to the manufacturer's instruction. About 5 ml of the molten agar was poured into 90 mm diameter sterile Petri dishes to give a depth of 4 mm.

Nutrient broth was inoculated with bacterial colonies of the isolates and incubated at 37°C for 18 – 24 h while the fungi isolates were inoculated in potato dextrose broth and incubated for 72 h at room temperature $27 \pm 2^\circ\text{C}$. Vandepitte et al. (1991) method was used to adjust the turbidity.

Crude extract preparation

50 g of the ground leaf powder was weighed and extracted with 95% methanol, chloroform and water respectively, in a ratio of 1:3 of powdered leaves to each solvent. The extraction of the leaves was done by gentle but continuous agitation of each mixture for 3 h using an orbital shaker at 20 rpm. Each mixture was then filtered using dried Whatman filter paper and the filtrate concentrated.

The solvent extract of methanol and chloroform were evaporated to dryness in an evaporating dish placed on water bath at a temperature of 70°C while the aqueous extract was evaporated to dryness in a Gallenkamp oven at temperature of 110°C until a constant weight was obtained.

Each solid extract was reconstituted in their respective solvents to obtain a stock solution of 200 mg/ml. The stock solution obtained was sterilised using a Millipore Membrane Filter (0.45 μm pore diameter).

Determination of antimicrobial activity of the extracts

The antimicrobial activity of the leaf extracts were determined by the agar ditch - diffusion method (Collins and Lyne, 1979; Baron and Finegold, 1999; Conz et al., 1999). Sterile nutrient agar and potato dextrose agar were prepared and aseptically poured into sterile Petri dishes to test antibacterial and antifungal activity respectively. Five to six hours old standardised culture of isolates were used to flood the media surfaces (Onalapo, 1997). A sterile cork borer of 5 mm diameter was used to make ditches on each plate. Then 0.15 ml of the stock extract (equivalent of 30 mg extract) was dropped into each ditch and appropriately labelled. Incubation was made at 37°C for bacterial isolates and 25°C for fungal isolates for 24 h. Zones of inhibition (diameter) produced after incubation were examined and recorded in millimetre. The test

organisms were tested separately.

Determination of minimum inhibitory concentration (MIC)

Using the tube dilution technique 1 g of the extract was dissolved in 5 ml nutrient broth; this gave 200 mg/ml. Thereafter two fold serial dilutions were made from the original stock of 4 ml, using nutrient broth for bacteria and potato dextrose, broth for fungi to achieve the following concentrations: 200, 100, 50 and 25 mg/ml (Egorov, 1985; Scott, 1989; Baron and Finegold, 1990). Having obtained different dilution and concentrations, 0.1 ml of standardized test organisms were inoculated into the dilutions and incubated at 37°C for 24 h for bacterial and 25°C for 7 days for fungal isolates (Tilton and Howard, 1987; National Committee for Clinical Laboratory Standard, 1990 and 2002). Using solvents and test organisms without extract, positive controls were equally set up. The lowest concentration of the extracts that inhibited the growth of the test organism was recorded as the MIC.

Bacterial tubes showing no visible growth from the MIC test were subculture into nutrient agar and incubated at 37°C for 24 h. For fungi, it was subcultured on Potato Dextrose Agar and incubated at 25°C . The lowest concentration of the extracts yielding no growth on subculturing was recorded as the minimum bactericidal concentration (MBC) for bacteria and minimum fungicidal concentration (MFC) for fungi.

Effect of temperature on stability/potency of crude extracts

The crude leaf extracts were heated in a water bath at different temperatures as follows 30, 60, 80 and 100°C for 30 min and in an autoclave at 121°C for 15 min. After cooling, the activity of the heated leaf extract samples were tested against the test organisms.

Determination of killing rate of MBC on most susceptible and least susceptible clinical bacterial isolates

Time-kill studies of the chloroform leaf extract of *C. multistriata* on the most susceptible and least susceptible bacterial isolates used (*E. coli* and *S. typhi* respectively) was carried out. This method involved mixing 0.5 ml of 10^5 cfu/ml of the test organism with 4 ml of MBC of chloroform extract. Then 0.1 ml of the mixture was taken and plated out on sterile nutrient agar at interval of 30 min up till 300 min. The plates were incubated at 37°C for 24 h. The number of surviving bacterial colonies on each plate at the time intervals were counted and recorded against time (Lesly and Maurer, 1974; NCCL, 1999).

RESULTS

The diameters of zones of inhibition of the test organisms using the aqueous, methanolic and chloroform leaf extracts of *C. multistriata* are shown in Table 1. The aqueous leaf extract had no activity against both the bacterial and fungal test organisms. The highest zone of inhibition was observed in the chloroform leaf extract of *C. multistriata* against *E. coli* (diameter 25 mm). The methanol leaf extract was least effective against *S. typhi* (diameter 8 mm) and most effective against *S. aureus* (diameter 15 mm) for bacterial test organisms, while the chloroform leaf extract was least effective against *S. aureus* (diameter 13 mm) and most effective against the *E. coli* (diameter 25

Table 1. Activity of crude extracts from the leaf of *Cissus multistriata* on test organisms.

Test organism	Diameter of zones of inhibition (mm)					
	Extract			Control		
	Aqueous	Methanolic	Chloroform	Aqueous	Methanol	Chloroform
<i>Staphylococcus aureus</i>	0	15	13	0	7	10
<i>Escherichia coli</i>	0	9	25	0	5	12
<i>Yersinia enterocolitica</i>	0	12	20	0	6	8
<i>Proteus mirabilis</i>	0	10	15	0	6	7
<i>Shigella sonnei</i>	0	12	18	0	5	7
<i>Pseudomonas aeruginosa</i>	0	7	16	0	5	6
<i>Klebsiella pneumonia</i>	0	10	21	0	7	10
<i>Salmonella typhi</i>	0	8	10	0	6	6
<i>Candida albicans</i>	0	14	11	0	6	4
<i>Aspergillus niger</i>	0	11	6	0	5	4

Table 2. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of extracts of *Cissus multistriata*.

Test organism	MIC (mg/ml)		MBC & MFC (mg/ml)	
	Methanol	Methanol	Chloroform	Chloroform
<i>Staphylococcus aureus</i>	50	100	100	50
<i>Escherichia coli</i>	50	100	50	25
<i>Yersinia enterocolitica</i>	100	100	50	50
<i>Proteus mirabilis</i>	50	100	100	100
<i>Shigella sonnei</i>	50	50	100	50
<i>Pseudomonas aeruginosa</i>	100	200	100	100
<i>Klebsiella pneumonia</i>	50	200	50	25
<i>Salmonella typhi</i>	50	200	200	25
<i>Candida albicans</i>	50	100	100	100
<i>Aspergillus niger</i>	50	100	200	100

mm). The methanol leaf extract show more antifungal activity compared with the chloroform leaf extract with *C. albicans* being more susceptible than *A. niger* for both methanol and chloroform leaf extracts (Table 1).

Table 2 show the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) values for the methanol and chloroform leaf extracts of *C. multistriata*. The methanol leaf extract showed activity against all the test organisms with least activity against *Y. enterocolitica* and *P. aeruginosa* (MIC = 100 mg/ml) while the other test organisms had higher activity of MIC (50 mg/ml). The chloroform leaf extract was least inhibitory against *P. mirabilis* and *P. aeruginosa* at 100 mg/ml and most inhibitory against *E. coli*, *K. pneumonia* and *S. typhi* at 25 mg/ml. The MIC pattern for *S. aureus*, *E. coli*, *Y. enterocolitica*, and *K. pneumonia* were the same with that of the MBC while the pattern of activity for the other bacterial test organisms were not the same with the MBC (Table 2).

The effect of temperature on the stability and potency of the leaf extract of *C. multistriata* is shown in Table 3.

The antimicrobial activity of the heated extracts persisted after exposure to various temperatures between 30 to 121°C for 15 to 30 min. However, the extract activity decreased as the temperature increased.

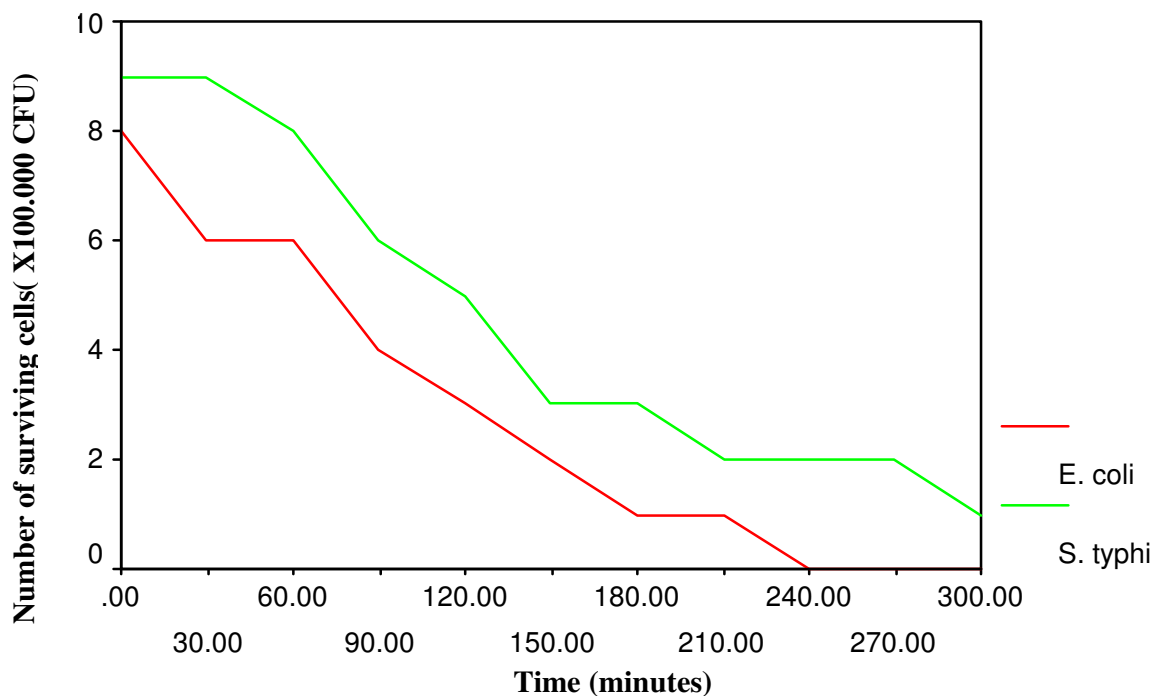
Figure 1 shows the death rate of the most inhibited bacterial test organism *E. coli* and the least inhibited *S. typhi*. There was no surviving cell in the MBC after 240 min exposure for *E. coli* unlike *S. typhi* that still had some surviving cells after 300 min. There was a sharp drop in the number of surviving cells of *E. coli* within 30 min of exposure to MBC of the leaf extract. The sharp drop was also observed between 60 and 90 min of exposure compared to *S. typhi* in which the reduction in number of surviving cells was gradual till 120 min exposure after which a sharp drop was observed, between 120 and 150 min exposure to MBC of the leaf extract.

DISCUSSION

The methanol and chloroform leaf extracts of *C. multistriata* have shown antibacterial and antifungal activity

Table 3. Effect of temperature on the stability/potency of crude extracts of *Cissus multistriata*.

Test organism	Zones of inhibition (mm) of extract heated at different temperatures (°C)				
	30	60	80	100	121
<i>Staphylococcus aureus</i>	13	12	12	10	5
<i>Escherichia coli</i>	15	15	13	12	10
<i>Yersinia enterocolitica</i>	20	18	18	16	15
<i>Proteus mirabilis</i>	16	15	15	14	12
<i>Shigella sonnei</i>	6	6	5	5	4
<i>Pseudomonas aeruginosa</i>	20	20	18	18	15
<i>Klebsiella pneumonia</i>	7	7	6	6	5
<i>Salmonella typhi</i>	22	21	21	19	17
<i>Candida albicans</i>	10	8	8	8	6
<i>Aspergillus niger</i>	12	10	10	10	8

**Figure 1.** Death rate of *E. coli* *S. typhi* in MBC of *C. multistriata* chloroform leaf extract.

against the test organisms. This finding corroborates some investigators report that flowering plants contain antimicrobial substances (Zaria et al., 1995; Ibekwe et al., 2001). The extracts showed varying degrees of antibacterial and antifungal activity. The fact that both the methanol and chloroform extracts inhibited the test organisms differently could imply that the plant contain active principles that can be extracted differently depending on the ability of the extracting solvent (Kafaru, 1996). All the bacterial test organisms are highly susceptible to the methanol and chloroform extract of *C. multistriata*. However, *S. aureus* was observed to be the most inhibited and *S. typhi* and *P. aeruginosa* were the

least inhibited by methanol extract while *E. coli* was observed the most inhibited and *S. aureus* least inhibited by chloroform leaf extract of *C. multistriata*.

With the appreciable level of inhibition exhibited by the leaf extract of *C. multistriata* against the test organisms, it is obvious that the plant is a potential source of novel antimicrobial drugs. The susceptibility of both gram positive and gram negative bacteria to the extract show its potential of broad-spectrum activity (Adebayo et al., 2001). It should be noted that herbal healers in Ibaji, Kogi State, Nigeria use the leaf in treating kwashiorkor and other ailments in children.

The least activity of the extracts against *P. aeruginosa*

and *S. typhi*, both gram negative bacteria was not unexpected, since resistance of gram negative bacteria to most antibacterial agents is well known (Irvin et al., 1981). *E. coli* which is the most inhibited of the test organisms has been implicated in the aetiology of many ailments (Adeyemo et al., 1994; Nedolisa, 1998; Ebie et al., 2001).

Temperature treatment had effect on the leaf extract activity of *C. multistriata*. Generally, the potency of the extracts reduced with increased temperature. The implication of this is that the extract should be properly stored for maximum efficiency. However, the antimicrobial activity persisted even after exposure to heat at 121°C for 15 min. It is worthy to note that heat treatment increased the activity of the extracts against the least inhibited bacterial test organisms, *P. aeruginosa* and *S. typhi*. The death rate of the most inhibited test organism *E. coli* was high with no surviving cells observed after 240 min exposure to MBC of the leaf extract while it was gradual for the least inhibited bacterial test organism, *S. typhi*, which still had some surviving cells after 300 min exposure to MBC of the leaf extract.

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