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Full Length Research Paper

Antibacterial activity: A comparison of ripe and unripe fruit extracts of *Cissus multistriata* (*Vitaceae*) plant

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The antibacterial activity of methanol extracts of the ripe and unripe fruit of *Cissus multistriata* against *Escherichia coli* (Swine) ISB492, *E. coli* (Swine) ISB440, *Serratia marcescens* FD5/64, *S. marcescens* FD1/62, *Staphylococcus aureus* FD1/62 and *Bacillus cereus* ISB517 were determined using agar ditch diffusion and tube dilution methods. The crude methanol extracts exhibited antibacterial activity against some of the tested bacterial isolates. Both ripe and unripe fruit extracts were inhibitory to *S. marcescens* (FD5/64). The unripe fruit extract also was inhibitory to *S. marcescens* FD1/62 and *S. aureus* FD1/62. The unripe fruit extract exhibited more antibacterial activity than the ripe fruit extract with minimum inhibitory concentration (MIC) of 50 mg/ml. The present findings have added to the fact that *C. multistriata* has some medicinal values which the traditional medical practitioners have been tapping in their treatment of ailments in their localities. Further studies are required to identify the phytochemicals involved and to know the component that is lost during ripening that contributed to loss of some antibacterial activity of the ripe fruit extract of the plant. When these facts are harnessed, it will surely be useful in the development of some new drugs with broad spectrum of antimicrobial activity.

Key words: *Cissus multistriata*, antibacterial activity, ripe and unripe fruit, bacterial isolates, minimum inhibitory concentration (MIC), Kogi State, Nigeria.

INTRODUCTION

The search for agents to cure infectious diseases began long before people were aware of the existence of microbes (Sofowora, 1982). These early attempts used natural substances, usually native plants or their extracts and many of these herbal remedies proved successful (Sofowora, 1982). According to WHO reports, over 80% of the world population depends on traditional medicine for their primary healthcare needs (Duraipandiyan et al., 2006). There is alarming increase in the incidence of new and re-emerging infectious diseases. Hence, there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms, especially due to development of resistance to the antibiotics in current clinical use (Bauer et al., 2003). The screening of plant extracts has been of great interest to scientists in the search for new drugs for greater effective treatment of several diseases (Dimayuga and Garcia, 1991).

Therefore, plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments (Diallo et al., 1999; Rojas et al., 2006; Erdogrul, 2002). The medicinal value of plants lie in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plant

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Figure 1. Photograph of *Cissus multistriata.*

are alkaloids, flavonoids, tannins and phenolic compounds (Edeoga et al., 2005). Many plant parts have antimicrobial properties such as tannins, essential oils and other aromatic compounds (Kumar and Sigh, 1984). In addition, many biological activities and antibacterial effects have been reported for plant tannins and flavonoids (Scalbert, 1991; Chung et al., 1991). *Cissus* is a genus of about 350 species of tropical and subtropical, chiefly woody vines of the grape family –*Vitaceae* (Burkil, 1985). *Cissus multistriata* (Figure 1) is a well known plant to the traditional medicine practitioners in Nigeria (Omale et al., 2009a). It is used as a medicinal plant for the management of diverse ailments in different locations in Nigeria. The Ebiras use the stem prepared in form of decoction, as internal cleanser for new born babies while the Yorubas use the leaves for the treatment of infertility in women and stomach ailment in children.

It is commonly used by the Ibajis in the eastern part of Kogi State for the treatment of malnutrition –kwashiorkor. Other ethno medicinal uses of this plant include its use as cough remedy, fracture healing and management of arthritis (Omale et al., 2009a). The leaf extract has a broad spectrum antimicrobial activity (Omale et al., 2009b). It contains some phytochemical and possesses antioxidant properties (Omale and Okafor, 2008).To the best of our knowledge, there is no record of work on the antibacterial activity of the fruit of *C. multistriata* (Vitaceae) plant. Therefore, the present study was carried out to evaluate the antibacterial potential of the ripe and unripe crude methanol extract of *C. multistriata*.

MATERIALS AND METHODS

Plant material

The plant material used was collected from a local farm located at Idah, Kogi State, Nigeria. It was fully characterized and identified in the Department of Botany, University of Ibadan, Nigeria as *C. multistriata* (Figure 1). The fruit was harvested, rinsed in clean water, and air dried for three weeks until constant weight was

Table 1. Antibacterial activity of methanolic extract of unripe fruit of *C. multistrata* on some clinical isolates.

Bacterial isolates	Mean zone diameter of inhibition (mm)			
	100 mg/ml	50 mg/ml	25 mg/ml	Control (distilled water)
Escherichia coli (ISB 492)	0	0	0	0
Escherichia coli (ISB 440)	0	0	0	0
Serratia marcescens (FDS/ 64)	10	8	2	0
Serratia marcescens (FDI/ 62)	9	5	3	0
Staphylococcus aureus (FDI/62)	7	11	6	0
Bacillus cereus (ISB 517)	0	0	0	0

obtained. It was grounded into powder and stored in a sterile glass bottle in the refrigerator.

Preparation of extracts

A portion (100 g) of the powdered ripe and unripe fruits was weighed into a 1000 ml beaker separately and 500 ml of methanol was added to each. This was stirred every 30 min for 3 h and allowed to stand for 72 h as described by the Association of Analytical Chemist, AOAC (1980). The crude extract was prepared by decanting, followed by filtration through muslin cloth, and further filtered with Whatman No. 1 filter paper (150 mm) to obtain a clear filtrate. The crude filtrates (ripe and unripe extracts) were concentrated by evaporation to semi-solid state on a water bath at 100°C. The green semi-solid extracts obtained were further concentrated using a rotary evaporator .The extracts were reconstituted in their extracting solvent to obtain a stock solution of 200 mg/ml. The stock solutions obtained were then filter-sterilized using Millipore filter (0.45 µm pore size). The sterile extracts were stored in sterile capped bottles.

Test organisms

The test organisms used were kindly supplied by Dr Odugbo from the Bacterial Research Division, National Veterinary Research Institute, Vom, Plateau State, Nigeria. They are *Escherichia coli* (Swine) ISB492, *E. coli* (Swine) ISB440, *Serratia marcescens* FD5/64, *S. marcescens* FD1/62, *Staphylococcus aureus* FD1/62, *Bacillus cereus* ISB517.

Revival of the freeze-dried bacteria cultures

Revival of the freeze-dried cultures of the test organisms were performed following the instructions from the Bacterial Research Division, National Veterinary Research Institute, Vom, Plateau State, Nigeria. The ampoules were opened just above the cotton plug by scoring the glass cutter and broken at the scored mark. Aseptic working conditions and sterility were strictly observed. Each bacterial material was suspended into a test tube containing 3 ml nutrient broth, shaken and then left in the refrigerator for 6 h. The suspension was inoculated on nutrient agar plates. Duplicate plates were made for each organism and then incubated at 37°C for 24 h. Subcultures were made on nutrient agar.

Determination of antibacterial activity of the crude methanol extracts

The antibacterial activity of the crude methanol extracts was tested by first inoculating nutrient agar plates with the test organisms. Duplicate plates were made for each test organism by flooding each plate with 5 to 6 h old revived freeze-dried culture. Thereafter, a sterile cork borer (6 mm diameter) was used to make four ditches on each plate. 0.1 ml of the filter-sterilized extract was dropped into each ditch and was labeled appropriately. Into the fourth ditch was dropped distilled water only to serve as a control. The inoculated plates were left on the lamina flow bench for 1 h to allow the extracts to diffuse into the agar (National Committee for Clinical Laboratory Standard, 1990). Thereafter, the plates were incubated aerobically at 37°C. Zones of inhibition produced after incubation was measured in millimeter.

Determination of minimum inhibitory concentration (MIC) of the extracts on the isolates

A broth dilution method as described by National Committee for Clinical Laboratory Standard (1990) was employed in the determination of MIC. Varying concentrations of the extracts (100, 50 and 25 mg/ml) were prepared. A portion (0.1 ml) of each concentration was added to each 9 ml of nutrient broth containing 0.1 m of standardized test organism of bacteria cells. The bottles were incubated aerobically at 37°C for 24 h. The tube with least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration (MIC).

RESULTS AND DISCUSSION

The antibacterial activities of unripe and ripe fruit extracts on the tested isolates are as presented in Tables 1 and 2, respectively. The *E. coli* strains and *B. cereus* were not susceptible to both the unripe and ripe extracts, of *C. multistriata*. The two strains of *S. marcescens* and *S. aureus* were inhibited by the unripe fruit extract (Table 1) with zones of inhibition ranging from 2 to 10 mm. Only *S. marcescens* (FD5/64) was susceptible to the ripe fruit extract (Table 2). The largest zone of inhibition was observed for unripe fruit extract against *S. marcescens* (FDS/ 64).This antibacterial activity could be attributable to the phytochemicals in the extracts. The polyphenol

Table 2. Antibacterial activity of methanolic extract of ripe fruit of *C. multistriata* on some clinical isolates.

Bacterial isolates	Mean zone diameter of inhibition (mm)			
	100 mg/ml	50 mg/ml	25 mg/ml	Control (distilled water)
Escherichia coli (ISB 492)	0	0	0	0
Escherichia coli (ISB 440)	0	0	0	0
Serratia marcescens (FDS/ 64)	4	2	0	0
Serratia marcescens (FDI/ 62)	0	0	0	0
Staphylococcus aureus (FDI/62)	0	0	0	0
Bacillus cereus (ISB 517)	0	0	0	0

Table 3. Minimum inhibitory concentration (MIC) of the unripe and ripe fruits of *C. multistriata*.

Bacterial isolates	Minimum inhibitory concentration (MIC) (mg/ml)	
	Unripe fruit	Ripe fruit
Escherichia coli (ISB 492)	Nil	Nil
Escherichia coli (ISB 440)	Nil	Nil
Serratia marcescens (FDS/ 64)	50	100
Serratia marcescens (FDI/ 62)	100	Nil
Staphylococcus aureus (FDI/62)	100	Nil
Bacillus cereus (ISB 517)	Nil	Nil

composition of the ripe and unripe fruit of *C. multistriata* (Omale, 2010) and other chemical compositions of the plant (Omale et al., 2009a) have been determined. Both the ripe unripe fruit have been found to contain tannins, flavonoid, phenols and anthocyanins which have been found to exhibit various degrees antimicrobial activities (Rath et al., 2009; Anibijuwon et al., 2010; Pareckh and Chanda, 2007). Similar results have been reported for the leaf of *C. multistriata* when tested on some clinical isolates (Omale et al., 2009b).

In this study, the unripe fruit extract showed more antibacterial activity than the ripe one. This is suggestive that ripening may have transformed certain bioactive components which are responsible for the antibacterial activity of the fruit. The MIC results indicated that unripe fruit extracts of *C. multistriata* appeared more potent with 50, and 100 mg/ml of MIC against *S. marcescens* FD5/64, FD1/62 and *S. aureus* FD1/62 respectively (Table 3). The present findings have added to the fact that *C. multistriata* has some medicinal values which the traditional medical practitioners have been tapping in their treatment of ailments in their localities. From the results obtained in this study further research needs to be carried out to determine the exact phytochemicals (and their nature) involved in the antibacterial activity of the fruit of *C. multistriata*. It will be necessary also to know the component that is lost during ripening, that contributed to loss of some antibacterial activity of the ripe fruit extract of the plant. When these facts are harnessed, it will surely be useful in the development of some new drugs with broad spectrum of antimicrobial activity.

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