

*Research Paper***Assessment of Bioactivity and Membrane Stabilizing Potential of *Cissus multistriata* Fruit Extracts*****Omale James¹, Okafor Polycarp Nnacheta² And Ijeh Ifeoma Irene²**¹Pharmaceutical Biology and Toxicology Research Unit, Department of Biochemistry, Kogi State University, Anyigba, Nigeria.²Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

ABSTRACT: In this study, comparative evaluation of cytotoxicity and membrane stabilizing capacity of the ripe and unripe fruit extracts of *Cissus multistriata* was carried out. Medicinal plants constitute an important component of flora and are diverse in Nigeria. The pharmacological evaluation of substances from plants is an established method for the identification of lead compounds which lead to the development of novel and safe medicinal agents. Alcoholic extract of *Cissus multistriata* fruit was screened for cytotoxicity using brine shrimp lethality test. The ripe fruit extract was far less toxic ($LC_{50} = 2845.125 \mu\text{g/ml}$) when both fruits were compared to the reference standard, potassium dichromate ($LC_{50} = 180.147 \mu\text{g/ml}$) used. The membrane stabilizing activity of the extracts compare favorably with that of Indomethacin, a standard anti-inflammatory drug used with the unripe fruit extract being the highest. This study has demonstrated *Cissus multistriata* fruit extract is relatively safe for the ethno medicinal and other uses to which it is put.

KEYWORDS: Cytotoxicity, *Cissus multistriata*, membrane stabilizing and potassium dichromate.

Introduction

Traditional medicine can be defined as the total combination of knowledge and practice, whether explicable or not, used in diagnosing, preventing, or eliminating physical, mental and social disorders. This may rely exclusively on past experience or observation handed down from generation to generation while bearing in mind the original concept of nature which includes the material world, the sociological environment whether living or dead and the metaphysical forces of the universe (Sofowora, 1986; Berdy, 1982). Disease treatment and prevention in Nigeria were for many years handled solely by traditional healers (herbalists) who inherited the act from their forefathers. The traditional medical practitioners make use of largely plant parts (Alake and Irobi, 1992); Ebi and Ofoefule, 1997).

The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin. Most developing

countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant material or in the refined form of crude extracts, mixtures, etc. Recent estimates suggest that several thousands of plants have been known with medicinal applications in various cultures (Farnsworth and Soejarto, 1991). Some of these plants have been subjected to the isolation of the active ingredients (chemical compounds) and their subsequent modification. A large portion of such medicinal compounds have been discovered with the aid of ethno-botanical knowledge of their traditional uses.

In Nigeria, *Cissus multistriata* is widely used in the management of diverse diseases such as kwashiorkor, marasmus, arthritis, infertility, stomach disturbances in children as well as sure remedy for cough. Its application to fracture site have provided healing as claimed by herbal medicine practitioners. The various parts of the plant are put to use traditionally to remedy one ailment or the other including the fruit. The fruit also serve as attractant to fish and as such it is special hook bait in Ibaji, Kogi State. The medicinal properties of this plant have been extensively claimed, hence the need for biosafety assessment of the parts utilized.

In order to study the toxicity of this medicinal plant fruit we performed brine shrimp lethality bioassay which is based on the ability to kill laboratory cultured brine shrimp

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(*Artemia nauplii*). The brine shrimp assay was proposed by Michael *et al.*, (1956) and later developed by Vanhaecke *et al.*, (1981); Sleet and Brendel, (1983). The assay is considered a useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, pesticides and cytotoxicity testing of dental materials (Harwing and Scott, 1971; McLaughlin, *et al.*, 1991; Martinez *et al.*, 1988; Barahona and Sanchez-Furtun, 1999; and Pelka *et al.*, 2000).

The brine shrimp assay is very useful tool for isolation of bioactive compounds from plant extracts (Sam, 1993). The method is attractive because it is very simple, inexpensive and low toxin amounts are sufficient to perform the test in micro- well scale. In this paper, we report the cytotoxicity and membrane stabilizing potential of *Cissus multistriata* fruit extract and the aim is to assess the biosafety of the plant as it is extensively used in some Nigerian localities as food and drug.

Materials and Methods

Plant material

The fruit of *Cissus multistriata* was obtained from Ekwunali-Igboigbo, Unale, Kogi State, Nigeria. The plant material was washed with water to remove dirt and oven dried at 45°C. The dried plant material was pulverized using electric blender. Weighed portion of the sample was subjected to cold alcoholic extraction.

Preparation of plant extracts

Cold extraction method was employed. Portions (20g) of the powdered samples were weighed into a conical flask. Pure methanol (150ml) was added and left for 72 hours. The mixture was filtered and the filtrate was concentrated using rotary evaporator and the concentrate was subjected to activity studies.

Assay of membrane stabilizing activity

The method of Sadique *et al.*, (1989) as modified by Oyedapo and Famurewa, (1995) and Oyedapo *et al.*, (2004) was employed in the membrane stabilizing activity assay. The assay mixture consisted hypo saline (2ml), 1ml of 0.15M Sodium phosphate buffer at pH 7.4. Varying volumes of drug (2mg/ml) (0.0-1.0ml) and 2% (v/v) erythrocyte suspension in isosaline (0.5ml) were made up with isosaline to give a total assay volume of 4.5ml. The control was prepared as above except the drug was omitted, while drug control (4.5ml) lacked erythrocyte suspension. The reaction mixtures were incubated at 56°C

for 30 minutes. The tubes were cooled under running water followed by centrifugation at 5000rpm. The supernatants were collected followed by reading of the absorbance at 560nm. The percentage membrane stability was estimated using the expression: Percentage membrane stability =

$$\frac{100 - (\text{Drug test value} - \text{Drug control value})}{\text{Control value}} \times 100$$

Cytotoxicity bioassay

Modified method of Solis *et al.*, (1992) was used to determine the inhibitory activity of extract on *Artemia salina* in vial bottles. A portion (50µl) of the crude methanol extract (125-1000µg/ml) solution in 0.25% Tween 80 - artificial sea water was added into each well (vial bottles) containing 10 newly hatched brine-shrimps in 50 µl artificial sea water, then incubated at room temperature for 24 hours. All samples were repeated in 2 wells to make the overall tested organism of 20 for each. The living brine shrimps were counted under a hand magnifying lens. Same procedure was followed using Potassium dichromate as the reference standard. Plot of percentage lethality versus log concentration, substituted Y=50 in the resulted linear equation to obtain the X value. The antilog X was then the LC₅₀ (concentration of 50% lethality) value (Ballantyne *et al.*, 1995).

Results and Discussion

The results of membrane stabilizing activity of the extracts are as presented in Table 1. The results showed that the extracts are highly potent on human erythrocyte adequately protecting it against heat and hypotonic induced lyses. The protecting activities were comparable to that of the standard anti-inflammatory drug (Indomethacin) used. The unripe fruit extract protects the erythrocyte membrane even more than the reference standard. This is a highly promising drug candidate. The high membrane stabilizing activity of the unripe fruit extract of *Cissus multistriata* could be attributable to polyphenols (Omale and Okafor, 2008). Earlier reports have shown that various herbal drugs are capable of stabilizing the red blood cell membrane and exert anti-inflammatory activity (Sadique *et al.*, 1989; Olugbenga *et al.*, 2005).

The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxicity and anti-tumor properties (McLaughlin *et al.*, 1993). In this paper, the brine shrimp lethality of

extracts of ripe and unripe fruit of *Cissus multistriata*, a medicinal plant used in Nigerian traditional medicine to brine shrimp was determined following the modified method of Solis *et al.*,(1992).

The LC₅₀ values of the brine shrimps obtained for the extracts of the medicinal plant and that of the positive control, Potassium dichromate, are given in Table 2. Alcoholic extract of *Cissus multistriata* ripe fruit showed

less or mild toxicity (LC₅₀ = 2827.579µg/ml). The degree of lethality was found to be in a way concentration dependent, therefore prolonged usage and at a higher dosage of this plant extract may pose negative health implications. The mild brine shrimp inhibition indicates low or moderate toxicity of the plant and could correlate with its utilization for different purposes without repulsion by the users.

Table 1. Membrane stabilizing activity of crude alcoholic extract of *C.multistriata* fruit on human RBC subjected to heat and hypotonic stresses

Plant part/standard	Percentage membrane stabilization
Ripe fruit	43.43±0.69
Unripe fruit	66.52±0.99
Indomethacin	59.70±1.13

Values are the mean ± S.E.M of three determinations.

Table 2. Inhibitory effects of *C. multistriata* fruit extracts on brine shrimps

Plant part/standard	concentration (µg/ml)	log concentration	% lethality	LC ₅₀ (µg/ml)
Unripe fruit	1000	3.00000	55	499.938 ^a
	500	2.69897	50	
	250	2.39784	45	
	125	2.09691	40	
Ripe fruit	1000	3.00000	45	2827.579 ^b
	500	2.69897	35	
	250	2.39784	30	
	125	2.09691	30	
Potassium	1000	3.00000	100	44.20 ^c
Dichromate	500	2.69897	80	
	250	2.39784	70	
	125	2.09691	70	

a. Linear equation: $y=16.61 X +5.171$

b. Linear equation: $y=16.61 X -7.328$

c. Linear equation: $y=33.219X -4.658$

Conclusion

Brine shrimp lethality assay is very useful and inexpensive way of assessing the bioactivity of plant extracts. Even though very useful, it is not adequate regarding the elucidation of the mechanisms of action of plant extract and their components. Further work is still needed for the pharmacological activity. Mean while this work has indicated that the plant is relatively safe for the purposes utilized and a promising drug candidate for the treatment of inflammation.

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