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PARTIAL PURIFICATION AND ANTIOXIDANT ACTIVITY OF THE PARTIALLY PURIFIED *CISSUS MULTISTRIATA* LEAF EXTRACT FRACTIONS

OMALE JAMES [1]*, OKAFOR POLYCARP NNACHETA [2] AND IJEH IFEOMA IRENE[2]

[1]Department of Biochemistry, Kogi State University , Anyigba ,Kogi State ,Nigeria.

[2]Department of Biochemistry, Michael Okpara University of Agriculture, Umudike ,Abia State ,Nigeria.

Corres.auhtor :jamesomale123@yahoo.com,
Tel:+234 08068291727.

ABSTRACT: The fresh leaves and extracts of the plant *Cissus multistriata* are a traditional herbal treatment in Nigeria for diverse ailments including Kwashiorkor, cough and arthritis etc. We previously reported that the crude extract possessed antioxidant capacity. This present study has demonstrated that there are many chemical components responsible for the antioxidant activities of *Cissus multistriata* and not only one component . The phytochemical investigation yielded many unidentified chemical components and no one component seems to be sharply dorminant. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging effect of the fractions were determined spectrophotometrically and indicated activity spread throughout the chromatogram. In this preliminary work, we conclude that the extract from *Cissus multistriata* contains a mixture of many powerful antioxidant compounds and that may be one of the potential mechanism responsible for the claimed diverse therapeutic effects.
Key words: *Cissus multistriata*, DPPH, antioxidant and partial purification

INTRODUCTION

Extraction of bioactive agents from plants is one of the most intensive areas of natural product research today,yet the field is far from being exhausted . Sandberg and Bruhn [1] reported that only about ten percent of all the plants had been investigated in details for bioactive agents. For this resean alone it could be argued that further investigation is worthwhile. One important reason for screening plant for bioactive agents is that by isolating such an agent it is possible to demonstrate that the reported physiological activity of the plant is real. The fact that this activity has been shown to be due to a particular compound also makes detailed pharmacological and other academic studies possible.

The use of traditional medicine is wide spread in Africa and medicinal plants are still a large source of natural antioxidants that might serve as leads for the for the development of novel drug against free radical induced diseases. Medicinal plants are commonly used in treating and preventing specific ailments and diseases and are considered to play a beneficial role in health care [2] . It is well known that free radicals cause cell damage through mechanism of covalent binding and lipid peroxidation with subsequent tissue injury [3]. Antioxidant agents of natural origin have attracted special interest because they can protect human body from free radicals. Antioxidant properties of certain flavonoids from plants origin have already been established [4].

Cissus is a genus of about 350 species of tropical and subtropical ,chiefly woody vines of the grape family (Vitaceae). They are often used as medicinal plants because they contain some bioactive compounds such as vitamins, proteins ,carbohdrates and polyphenols among others. The bioactive compounds are contained in their leaves , stems and roots or bark , which makes these plants to be used medicinally in indegenous system of medicine [5]. *Cissus multistriata* belong to the family vitaceae and is found in places like Togo,River

Niger basin,and yola in Nigeria. It is popularly called *Ojekere* , *Okanogbo,oro-ugunu,mukala* ,*ewe-ebiomo* or e*juri*n in different parts of Nigeria. Traditionally, it is used in the management of Kwashiorkor, infertility in humans , cough ,arthritis and infectious diseases etc. Investigations of various species from the genus , *Cissus rheifolia* contains quinolizidine, alkaloids ,flavonoids, terpenoids [6]. The stem wood of *Cissus palloida* showed presence of stilbene, triterpenoids and steroids [7].

According to reviewed literature, litle or nothing is known about real identity of the chemical components of *Cissus multistriata* that are responsible for its biological activity. This has encouraged us to set out to attempt investigation on the real identity of the components of *Cissus multistriata* growing in Nigeria. In our previous work we screened the plant for various phytochemical and reported the presence of polyphenols [8].The present paper is a preliminary work that attempt to purify the components that are responsible for the antioxidant activity of *Cissus multistriata* leaf extract.

MATERIALS AND METHODS

Plant material

The leaf of *Cissus multistriata* was obtained from Kogi State University staff quarter in Nigeria . The plant material was washed with water to remove dirts and air dried for two weeks. The dried plant material was pulverized using electric blender.

Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl) was purchased from from Sigma chemical company (Sigma Germany), Methanol used was product of BDH and all other chemicals and reagent used were of analytical grade.

Methods

Preparation of plant extract: Cold extraction method was used .A portion (250g) of the powdered leaf sample was weighed out and soaked in a jar containing 1500ml of distilled water for 72 hrs. This was then filtered under vaccum pressure and used for fractionation process.

Fractionation using miniature reverse phase cartridge

The crude leaf extract of *C. multistriata* was fractionated using the miniature reverse phase cartridge supplied by Agilent Technologies ,USA (Accubond SPE ODS-C18 cartridge ,Lot No.A1A70126F). The cartridge was activated with 1ml of methanol twice.This was then washed thoroughly three times with 1ml of water. The sample solution was forced into the cartridge manually using a syringe. The solution was passed through the cartridge under low centrifugation . This was done by placing the cartridge in a test tube so that the outlet of the cartridge is well clear of the test tube bottom and was placed in a centrifuge with a swing- out rotor so that the solution on top of the cartridge was drawn slowly and evenly through the gel packing.

The flow through was collected for antioxidant testing .The cartridge was washed twice each time with 1ml of water . The non-retained fractions were collected for testing. The retained materials were eluted sequentially with 1ml of: 20, 40,60,80,and 100% methanol and each eluate was collected separately for testing for antioxidant activity.

The above procedure was repeated precisely as before but only a small proportion of each eluate (200µl) was used for the radical scavenging activity test. A portion (800µl) of each fraction was left. 40,60,80,and 100% methanol fractions were kept separately and dried down. Each was re-dissolved in 1ml of distilled water and loaded back (sparately) onto a reverse phase cartridge and the resulting fractions were assayed for antioxidant activity.

HPLC fractionation

The fraction with the greatest amount of activity was selected and subjected to HPLC for fractionation.Alltech adsorbosphere C18HS of 5µm particle size and 150 X4.6mm was used. 0.1%TFA in H_2O, and 0.2% formic acid in acetonitrile containing 2mM ammonium acetate were used as buffers A and B respectively. A portion (250µl) of water was injected as blank. A portion of the sample (50µl) in H_2O /CH_3CN/TFA in the ratio 40:10:0.1 was centrifuged and all injected .Sample tube was washed out with 250µl of the same solvent, centrifuged and injected. The chromatogram was 80 minuites long and one minuite fraction was collected.

The antioxidant capacity of the fractions from the miniature reverse phase cartridge and that of the HPLC were determined using the modified method of [9]. The free radical –scavenging activity of all the fractions were measured in terms of radical scavenging ability using stable radical DPPH. Solution of DPPH (0.3mM) in methanol was freshly prepared and 1.0ml of this solution was added to 1.0ml each of all the fractions . Thirty minutes later (after incubation in a dark chamber) the absorbances were measured at 517nm. Lower absorbance of the reaction mixture indicates higher free radical –scavenging activity.The antioxidant capacity was calculated thus: % radical scavenging activity =

$$\frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

TABLE 1: DPPH free radical scavenging activity of fraction from reverse phase cartridge

Fractions /con trol activity	Absorbance at 517nm	% scavenging
First flow- through	1.195	43.15
First wash with H_2O	0.637	69.70
Second wash with H_2O	0.768	63.46
Elution with 20% methanol	0.703	66.56
Elution with 40% methanol	0.789	62.46
Elution with 60% methanol	0.953	54.66
Elution with 80% methanol	1.090	48.14
Elution with 100% methanol	1.096	47.86
Control	2.102	------

The activity lies majorly in the first ,second wash with water and 20% methanol

TABLE 2: Antioxidant capacity of the fractions (200µl) from miniature reverse phase cartridge

Fractions	Absorbance at 517nm	% scavenging activity
First flow- through	2.787	4.23
First wash with H_2O	2.69	7.49
Second wash with H_2O	2.449	14.12
Elution with 20% methanol	2.737	5.95
Elution with 40% methanol	2.349	19.28
Elution with 60% methanol	2.692	7.49
Elution with 80% methanol	2.693	7.46
Elution with 100% methanol	2.720	6.53
Control	2.910	------

TABLE 3: The activity from assay of redissolved fractions

Fractions activity	Absorbance at 517nm	% radical scavenging
Elution with 40% methanol	1.468	47.98
Elution with 60% methanol	1.929	31.64
Elution with 80% methanol	2.415	14.42
Elution with 100% methanol	2.789	1 .17
Control	2.822	-----

TABLE 4: The percentage activity that re-emerged in the second run of the experiment

Methanol fraction	Percentage activities		Total activity	percentage re-emergence
	1st Run	2nd Run		
40%	19.28	47.98	67.20	71.34
60%	7.49	31.64	39.13	80
80%	7.46	14.42	21.88	65
100%	6.53	1.17	7.70	15.19

The highest proportion of activity re-emergence was found in the 60% methanol

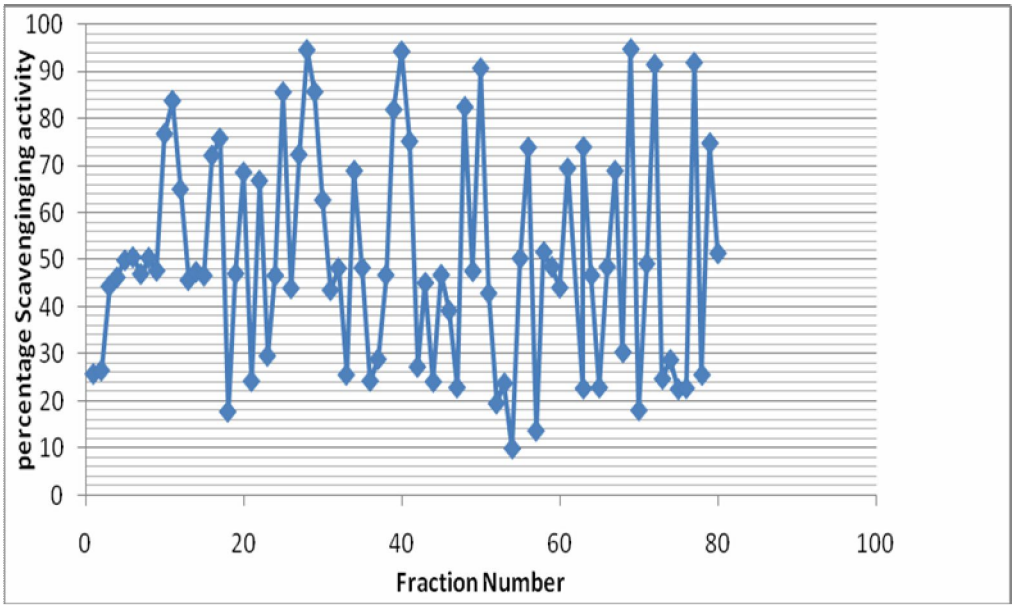

Figure1: Percentage scavenging activities of HPLC fractions

RESULTS AND DISCUSSION

The results of the activity assay of the fractions from the miniature reverse phase cartridge is as presented in table 1. The antioxidant activity lies majorly in the first wash with water fraction (69.70%) and also the eluate of 20% methanol (66.56%). The aim was simply to see if the radical scavenging activity is retained by the reverse phase resin.The bulk of the radical scavenging activity activity was not retained by this resin but appeared in the flow-through and the low organic eluates.Table 2 shows the activity of the fractions when a small portion (200µl) was used for the assay. As it was ,the activity was spread across many fractions and some activities did not stick at all. The highest proportion of activity re-emergence was in the 60% methanol i.e 80% of activity was found in the second run of the experiment. From table 2, activity is high in the second wash with water (14.12%) so also in 40% methanol (19.28%).

From these results the activity eluted across a wide range of acetonitrile concentrations. Figure 1 which is a plot of percentage scavenging activity against fraction number from HPLC does not show a clear peak or peaks of activity.It simply mean that the activity was not the result of one or a few compounds (Fig 1 and 2).There is scavenging activity spread through out the chromatogram. The explanation is that there are many compounds or components that are causing this activity.

The conclusion is that there are many compounds contributing to the antioxidant activity and they vary in hydrophobicity and for that reason the elution profile of activity is broad . Even from a high resolution HPLC column, there was no clear peak of activity but rather small amounts of activity spread across a wide range of eluting conditions. That is, there are many components in the extract that are contributing to the activity , and no one component seems to be dorminant. Much efforts and time is therefore required to pursue vigorously to isolate and characterize the various components responsible for the activity of *Cissus multistriata*. This will help to demonstrate that the reported physiological activity of the plant is real. The mode of action of the whole plant or part producing the biological effect can also be better investigated, if the active principles are characterized.

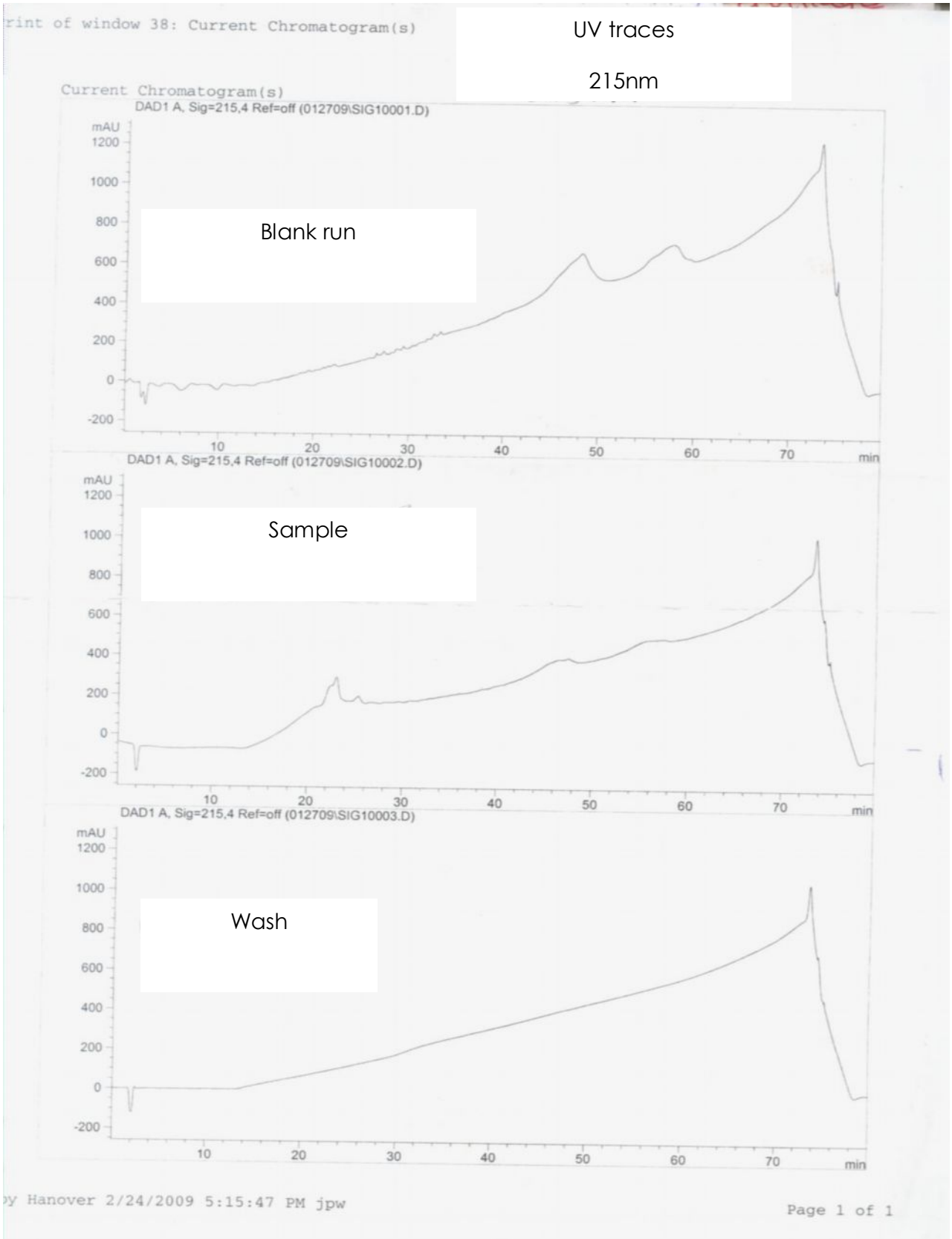

Figure 2: Current Chromatogram for the sample showing broad peaks

REFERENCES

1. Sandberg, S and Bruhn J.G. Screening of plants for biologically active substances in African medicinal plants (Ed Sofowora , E.A) University of Ife ,Nigeria. 1979, p .119.

2.Morton,A.G. History of Botanical Science , Academic press, London. 1981

3.Osawa,T. Kayakishi,S., Namik,M. Antimutagenesis and anticarcinogenesis mechanism. New York , Plenum Ltd .1990, pp 139-153.

4.Di Carlo,G., Mascolo, N., Izzo, A.A., Capasso, F. Flavonoids :Old and new aspects of a class of natural therapeutic drugs.Life Science.1999, 65:337-353.

5.Singh, S.P and Mishra, N . An experimental Study of analgesic activity of *Cissus quandrangularis*.Ind. J. Pharmacol.1984, 167.

6.Saifah,E., Kelly, C.T and Leary,J.D . Constituents of the leaves of *Cissus rheifolia*. J.Natural products .1983, 46-358.

7.Khan,M.A., Nahl, S.G.,Prakash, S. and Zamman,A . Pallidol, a resvertroal climber from *Cissus pallida* .Phytochemistry .1986, 25;1945-1948.

8.Omale ,J.,Okafor, P.N . Comparative antioxidant capacity, membrane stabilization,polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata* .A .J. Biotech. 2008, Vol. 7 (17) 3129-3133.

9.Blois ,M.S. Antioxidant determination by use of stable free radicals. Nature. 1985, 29:1199-1200.

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