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Original Research Article

Evaluation of the Antiproliferative and Free Radical Scavenging Potentials of the Saponin-Containing Chromatography Fractions of *Olax viridis* (Olacaeceae) Root Bark

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Abstract

Olax viridis Oliv. (Olacaceae) is a shrub common in the tropics and grows well in the forest and savannah region. Species of this genus are used traditionally in the treatment of breast cancer and related diseases among others. This study aims to report the antiproliferative and *in vitro* antioxidant activities of butanol fraction of *O. viridis* root bark. Extraction of the pulverized dry bark was done by cold maceration using methanol and was further partitioned successively with N-hexane, dichloromethane, ethyl acetate and n-butanol. Fractionation of butanol fraction was done with vacuum-liquid-chromatography and subfractions monitored with TLC. Phytochemical screening of fraction A-C was carried out using standard phytochemical method while their constituents were evaluated using TLC. Antiproliferative activity was determined by cell viability assay using *Saccharomyces cerevisae* (yeast) as a model organism while the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used for antioxidant evaluation *in-vitro* with ascorbic acid as the reference standard for comparison. All the sub-fractions contain sugar, steroids, triterpenes and saponins. Sub-fractions A, B and C showed constituents (R_f values of 0.23, 0.41, 0.51 and 0.58), (R_f values of 0.46 and 0.63) and (R_f values of 0.44 and 0.62) respectively. The sub-fraction A showed dose-dependent activities for DPPH radical scavenging activity thus A(IC_{50}=1.60mg/ml) while antiproliferative activities of all the sub-fractions were dose dependent A(IC_{50}=8.22mg/ml) >B(IC_{50}=>10 mg/ml). Sub fraction A showed a promising activity, and this preliminary study validated the traditional use of *Olax species* in the treatment of cancer.

Keywords: *Olax viridis*, saponins, anti-proliferative, free radical scavenging, drug discovery and development.

INTRODUCTION

The pathophysiology of cancers has long been linked to the presence of free radicals. Few years ago, cancer was seen to be a disease of Western world, but that is no longer the case. It is now a growing non-communicable disease all over the world. In a 2018 global report, cancer was responsible for 9.6 million deaths translating to nearly 1 in 6 of all global deaths [1].The low and middle-income countries of the world have been reported to account for about 70 % of these deaths [1]. In Africa, cancer is an emerging public health disease of utmost concern. About 715,000 new cancer cases and 542,000 cancer deaths occurred in 2008 in the continent, with these numbers expected to double in the next 20 years [2]. The continue increase in the number of cancer incidence all over the world to a great extent is due to life style and to a lesser extent genetically. Mortality due to this disease is attributed to late diagnosis, ignorance and most of all resistance to the existing drugs or non-responsiveness to treatment. These assertions should awaken researchers in the field of drug discovery and development to dive into studies and discoveries of new drugs or lead compound in other to avert a looming pandemic. Also the amount of money that is spent on cancer chemotherapy is a source of worry to cancer patients, government, agencies, and non-governmental organization. Therefore, any source of effective and cheaper treatment for cancer will be easily accepted by the populace. More so, the serious adverse effect associated with the available cancer chemotherapeutic drugs that worsen the patient's quality of life such as alopecia (loss of hairs), bone marrow depression, immunosuppression,

leucocytopenia etc., are of great concern. Medicinal plants will continue to provide a source for generating novel drug compounds. Plants may become the base for the development of a new medicine or they may be used as phytomedicine for the treatment of diseases [3]. The primary benefit of using plant-derived medicine is that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments. Plant products have always proven to have the potential of providing solution to majority of health related problem of man, and cancer is not an exception. A school of thought believe that the lasting solution to cancer treatment lies within the leaves, stems, trunks, roots, fruits, rhizomes of our God given plants. The extraction of bioactive agent from plants is of the most intensive areas of natural product research today, yet the field is far from being exhausted [4]. Bioactives of natural source are too vast, and in these bioactives lay solutions to man's health challenges. However only few of these plants have been investigated for their physiological activities. Screening of plant bioactive agents prove that the isolates from the plant elicit certain physiological and pharmacological properties, attributed to the plants ethno medicinal uses. *Olax viridis* Oliv. (Olacaeceae Juss), is a plant that has been known to be use by its indigenous populace for several ailment with most of its widely claimed activities due to its antimicrobial properties. In some case liver diseases has been claimed to have been treated with part of this plant. No claim either traditionally or scientifically has been made regarding its anticancer properties; however there is a claim of the use of one of the species of the *Olax* genus specifically, *O. subscorpioidea* root in the management of breast cancer in Abeokuta, Ogun State in Nigeria [5-6]. This specie bears the same native name as *O. viridis* amongst the Igbo ethnic group. Also a report on the traditional use of the alcoholic extract of the aerial parts, a member of the family Olacaeceae (*Jasminum grandiflorum*), traditionally in the treatment of various tumors and cancer, have been documented [7]. Oleic acid isolated from *O. europea* have been demonstrated to possess anticancer activity [8]. A few plant bioactive compounds have found useful and are the main stay in the management of cancer in our contemporary society and more are still in different stages of research of which some are saponins [8-10]. There is a chance that better bio active compounds still exist in plants yet to be investigated for anti-cancer properties.

MATERIALS AND METHODS

Plant Sample Collection and Preparation of Extract

The root of the plant *O. viridis*, was obtained by uprooting and cutting out the root. The roots were collected from farmlands in Nsukka South Eastern Nigeria and authenticated by taxonomist Mr A.O Ozioko of the Bio-resources Conservation and Development Program (BDCP), Nsukka, Nigeria The bark was peeled off the root, washed off of earth material and dried at an ambient temperature. The dried sample was pulverized to fine powdered sample. Extraction was done by cold maceration. A 1000 g of the powdered sample was macerated in methanol (10 L) at room temperature in a glass bottle with intermittent agitation using magnetic stirrer for 72 hours. The mixture was then filtered using the vacuum pump and Buchner funnel. The filtrate was partitioned in a separating funnel using n-hexane, dichloromethane, ethyl acetate, n-butanol and water in their order of polarity to obtain their fractions. The saponin containing n-butanol fraction was used for this study.

Separation of n-butanol Fraction with Vacuum Liquid Chromatography

The n-butanol fraction was subjected to silica gel vacuum liquid chromatography (VLC) to obtain a pure sample. Briefly, a 60-120 mesh size silica gel was packed in a column until it became compact. The n-butanol fraction was adsorbed on small amount of silica gel and added to the column. N-hexane, dichloromethane and methanol were used as a mobile phase, while applying a negative pressure. Five different fractions were eluted; fractions 1,2,3,4 and 5. Fractions 3, 4 and 5 which gave a positive test for saponins were coded sub-fraction A, B and C and were investigated further in this study.

Phytochemical screening

These sub-fractions were screened for phyto-constituents following standard phytochemical screening method [11, 12].

Antiproliferative assay

Anti-proliferative assay was carried out using the *Saccharomyces cerevisiae* (yeast) model [13]. A 20ml sabouraud dextrose broth (SDB) was prepared by dissolving 0.6g of powder nutrient broth in distilled water (20mL) and sterilized in an autoclave at a pressure of 121psi for 15minutes. The broth was cooled and aseptically inoculated with *S. Cerevisiae*. The above mixture was incubated for 24hours at 37^{o}C. This was known as seeded broth. One loop full of the seeded broth was aseptically transferred into 10ml peptone water, incubated for 24hours at a temperature of 37^{o}C and further diluted to a 0.1 Mac Farland standard, this served as the standardized inoculum. A 10mg/ml, 1mg/ml, 0.1mg/ml concentrations each of fractions A-C were prepared aseptically. For each fraction, 1ml each of the above concentrations was transferred into sterilized 2.5ml SDB, which were then inoculated with 0.1ml of the standardized inoculums. A negative control containing the SDB and 0.1ml of the yeast inoculum only was prepared alongside the standard anticancer drug – methotrexate(1000 µg/ml, 100 µg/ml and 10 µg/ml) Each of these were prepared in duplicates and incubated at 37^{o}C for 24hours. The whole procedure above was carried out aseptically to avoid contamination. 0.1ml each of the broth were then stained with 0.1ml of 0.1% methylene blue and were charge under the hemocytometer and viewed under the microscope with ocular lens of ×100 magnification. The

number of viable cells and non-viable cells were determined for each of the concentrations and the percentage death of the cells determined with the following formula;

$$\textbf{Percentage non-viable} = \frac{\text{Total number of non-viable cells}}{\text{Total number of cells}} \times 100$$

Free radical scavenging (Antioxidant) assay

A quantitative diphenyl-1-picrylhydrazyl (DPPH) antioxidant assay was carried out on 2.0, 1.5, 1.0 mg/ml concentrations each of the 3 fractions using methanol as reported [14, 15] with modification. Briefly, the various concentrations were mixed with a freshly prepared methanol solution of (DPPH) in a test tube wrapped with a foil to prevent exposure to light, at a ratio of 1:1 (v/v) and each allowed to stand in the dark for about 30minutes. A solution devoid of the test extracts but containing 2 ml of the DPPH solution and 2 ml of methanol was used as a negative control while ascorbic acid was used as a reference antioxidant agent for comparison. The absorbances were measured at 517 nm the percentage inhibition of the concentrations calculated using the formula as shown below.

$$\textbf{Inhibition of DPPH radical (\%)} = 100x\ \frac{[A_{(negative\ control)} - A_{(sample)}]}{A_{(negative\ control)}}$$

Where: $A_{(negative\ control)}$= Absorbance of the negative control solution (containing all the reagents except the test extract)

$A_{(sample)}$] = Absorbance of the test extract.

The IC_{50} was obtained by extrapolation from the regression curve

Thin Layer Chromatography examination

The three fractions were spotted on a silica gel GF-254nm TLC plate in duplicates and were developed with a mixture butanol, acetic acid and distilled water at a ratio of 4:1:2, as the mobile phase. The plates were allowed to air-dry after development. They were observed in daylight, under UV lamp at λ254nm and λ365nm, and iodine chamber and their retardation factor values noted.

$$\mathbf{R_f} = \frac{\text{distance travelled by components}}{\text{Distance travelled by solvent}}$$

RESULTS AND DISCUSSIONS

The yield of the three sub-fractions A-C were 2.65, 1.61, 3.11 g respectively. The presence of reducing sugar, saponin, steroidal ring and deoxy sugar were noted as phytoconstituents in the n-butanol fraction, while, anthraquinone, alkaloid and phenolic compounds were absent. This result indicates that the butanol extract of *O. viridis* root bark is rich in saponin and is devoid of other phyto-constituents like alkaloids and phenolics among others earlier reported for *Olax viridis* [16]. This is indicative that the chromatography separation step have removed to a greater degree these other constituents not required in this study.

Table-1: TLC profile of the three chromatography fractions from the n-butanol fraction

Chromatography Fraction	No. of component observed	Observed retardation factor	Mobile phase used
A	4	0.23, 0.41, 0.51, 0.58	BAW
B	2	0.46, 0.63	BAW
C	2	0.44, 0.62	BAW

KEY: *BAW → n-butanol: acetic acid: water at a ratio of (4:1:2v/v/v)*

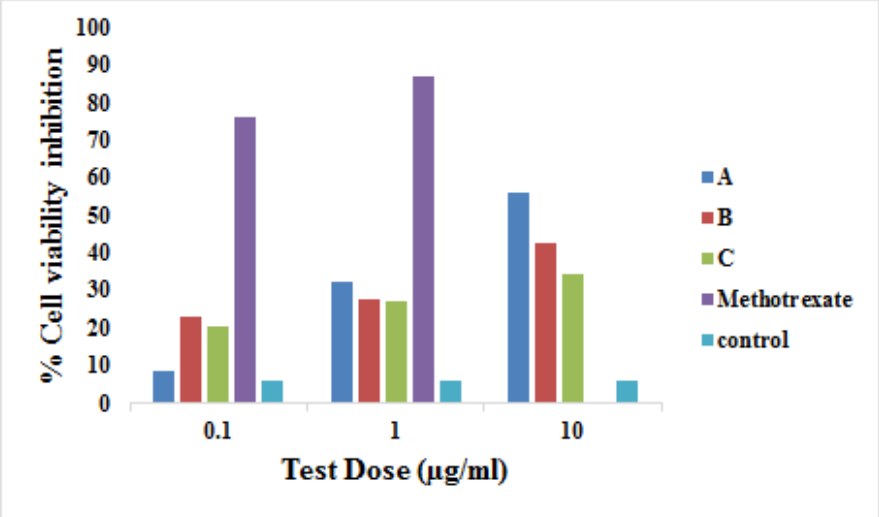

Fig-1: Percentage cell viability inhibition of *S. cerevisae* when treated with fractions A, B, C, methotrexate and negative control at different concentrations

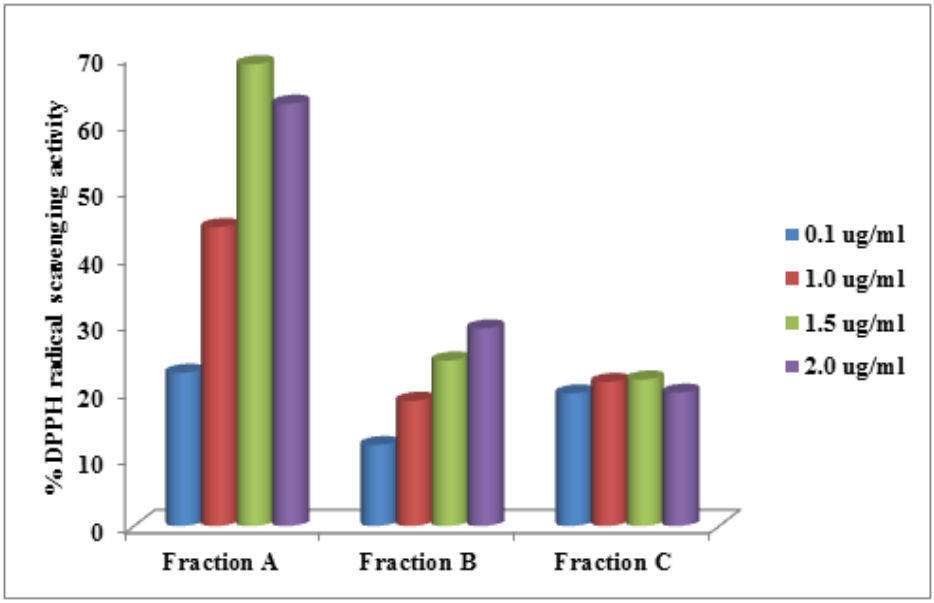

Fig-2: Free radical scavenging activity of chromatography fraction A, B and C

The chromatography sub-fraction A showed four distinct constituents with R_f values 0.23, 0.41, 0.51 and 0.58, while fraction B and C showed two components each with R_f values (0.46 and 0.63) and (0.44 and 0.62) respectively under UV light (see Table 1).

The Anti Proliferative (AP) activity was determined by cell viability method using *Saccharomyces cerevisae* (yeast) as a model organism. The anti-proliferative activities which were expressed in terms of percentage inhibition of viable cell population (Fig. 1) showed a dose dependent reduction in the population of the yeast for each of the sub-fractions. From the study, chromatography sub-fraction A had a better AP activity than others. Although at a lower dose of 0.1 mg/ml, the percentage inhibition viable cells of sub-fraction A was low as shown in the trend of percentage cell non-viability: A (8.4%) <C (20.3%) <B (23.1%), but at a higher concentration of 10 mg/ml, it showed a significant higher activity: A (56.8%) >B (42.5%) >C (34.3%). The standard drug (methotrexate) showed a higher percentage of cell death when compared with the chromatography sub-fractions (Figure 1). The antioxidant activities of the three chromatography sub-fractions were studied by measuring their ability to scavenge DPPH free radicals which was compared with the standard, ascorbic acid. The result of the free radical scavenging activity in Figure 2 showed the % inhibition of the three chromatography sub-fractions at various concentrations. At a 1.5 mg/ml, chromatography sub-fractions A showed a higher activity than other chromatography sub-fraction: A (68.8%) >B (24.6%) >C (21.8%). Although all exhibited a less activity when compared with the standard, chromatography sub-fraction A has a promising result with IC_{50} of 1.16 mg/ml while that of standard is 0.012 mg/ml. The above observation has

shown that chromatography sub-fraction A has a better antiproliferative activity and proton donating ability than chromatography sub-fractions B and C as well and could possibly serve as primary antioxidant. The direct correlation of antioxidant and antiproliferative activities of medicinal plant extracts has been reported [17]. The importance of AP and antioxidant activities in investigation and screening of molecules that can serve as a good lead or drugs for immune related diseases such as cancer, arthritis among others can not be overemphasized [18]. And these sub-fractions have been shown by the phytochemical results to be saponin-rich-fractions. It can be concluded that the antioxidant and antiproliferative activity of these sub-fractions is due to the presence of saponin. Saponin is a diverse group of compounds that are widely distributed in the plant kingdom which are usually characterized by their structure containing a steroidal or triterpenoid aglycone and one or more sugar chains [19] that demonstrate various pharmacological effects against mammalian diseases. Research has shown that a lot of saponins have anticancer activity and displays their effects through various antitumor pathways. A saponin, saikosaponin have been reported to exert its anticancer effect by antiproliferative activity [9]. saponins have also been shown to reduce the effects of radiotherapy and chemotherapy [20].

CONCLUSION

Olax viridis contains many phyto-constituents that possess the potential of proffering solution to man's health challenges. The butanol portion of the methanolic extract of the root bark of *O. viridis* is rich in saponin, and its sub-fractions exhibited a dose dependent anti-proliferative activity. Although all sub-fractions have the same class of phyto-consistuent, sub-fraction A showed a better anti-proliferative and antioxidant activity. This preliminary study also validated the traditional use of *Olax species* in the treatment of cancer. This sub-fraction could be a candidate for new anticancer drugs.

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