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Free radical scavenging and antiproliferative evaluation of the *Olax viridis* Oliv. (Olacaceae) whole root

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Abstract

Olax viridis Oliv (Olacaceae) has numerous ethnomedicinal uses including treatment of breast cancer. The study aimed at evaluating the free radical scavenging and antiproliferative activities of *O. viridis* whole root and the phytoconstituents responsible for this activity. The sample was defatted using n-hexane(NHE), further extracted by cold maceration using absolute methanol to obtain crude extract which was subjected to liquid-liquid partitioning using dichloromethane to afford the dichloromethane fraction (DCM) and aqueous methanol fraction (MEF). Further precipitation of some aqueous methanol fraction (MEF) using acetone yielded saponin-rich fraction (SRF). Furthermore, acid hydrolysis of the SRF gave the sapogenin-rich fraction (ASRF). These extract/fractions: NHE, DCM.,MEF, SRF and ASRF were used for further investigation. Phytochemical screening of fractions was carried out using standard phytochemical methods. 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 while antiproliferative (AP) activity was by cell viability using yeast (*Saccharomyces cerevisae*) as a model organism. *O. viridis* whole root contains carbohydrate, cardiac glycoside, steroids, triterpenes and saponins. The fractions showed dose-dependent activities for DPPH radical scavenging activity with the trend at 1 mg/ml thus: DCM (77.53%) >ASRF(70.60%) >SRF(46.82%) >MEF(33.50%) >NHE(22.83%) and the promising fractions showed median concentration; ASRF(IC_{50}=0.54 mg/ml) >DCM(IC_{50}=0.55 mg/ml). All the fractions showed dose dependent antiproliferative activities ASRF(IC_{50}=3.35 mg/ml) >SRF(IC_{50}=5.10 mg/ml) >MEF(IC_{50}=6.28 mg/ml) >DCM(IC_{50}=7.44 mg/ml). Sapogenin may be responsible for the AP and antioxidant activities as ASRF which contains triterpenidal/steroidal nucleus showed a promising activity compared to other fractions. This study validated the traditional use of Olax *species* in the treatment of cancer.

Keywords: *Olax viridis* whole root; Anti-proliferative; Free radical scavenging; DPPH; Saponin; Drug discovery

1. Introduction

Cancer is the main cause of death worldwide. Cells develop a degree of autonomy from normal cells signals, resulting in uncontrolled growth and proliferation [1]. The damage to cells caused by free radicals, especially the damage to DNA, may play a role in the development of cancer and other health conditions. Each year millions of people globally are diagnosed. The long approved method of cancer treatment are chemotherapy and radiation but unfortunately these are also toxic to the normal cell [2]. This has prompted the quest for natural products as anticancer agent. Through research

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and drug development, a few plant bioactive compounds are 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. [3, 4, 5].

Olax viridis Oliv. Is a member of Olacaceae family (*Jasminum grandiflorum*) and a species of the Genus Olax. Another species of this genus known as *Olax. Subscorpioidea* is used in the traditional management of various tumors and cancer [6, 7]. Chemotaxonomic knowledge has shown that there is a chance that better bioactive compounds which are yet to be investigated for anti-cancer properties may exist in other species of this genus. *Olax viridis* Oliv (Olacaceae) is a shrub commonly found in the tropics. The plant grows well in tropical rain forest and also in Savanna regions. The plant has elliptical green leaves, white waxy flowers and fruits about the size of a pea. Research has shown that it possesses many pharmacological activities [8, 9, 10, 11]. These are due to the presence of bioactive agents such as triterpenoids, steroids, saponins among others which are directly related to their chemical nature. Saponins are a diverse group of compounds which are characterized by their structure containing a triterpene or steroid aglycone and one or more sugar chains. Chen *et al*., [3] have shown that saponins are excellent anticancer agents while Zhao *et al.*, [12]. showed they are good anticancer adjuvants Functionally, the presence, addition or removal of a sugar molecule on a glycoside can either enhance or inhibit an established activity. Thus in addition to investigating the antiproliferative and antioxidant activities of *O. viridis*, the activity of the aglycone moiety was studied.

2. Material and methods

2.1. Plant Sample Collection and Preparation of Extract

The whole root of the plant *O. viridis,* was obtained by uprooting and cutting out the root. It was washed off of earth material and dried at an ambient temperature. The dried sample was pulverized to fine powdered sample. A 1000 g of the powdered sample was defatted with n-hexane. Extraction was done by cold maceration using 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 to obtain crude methanol extract. This crude extract was subjected to vigorous liquid-liquid partitioning in a separating funnel using dichloromethane (DCM) to obtain DCM fraction and aqueous methanol fraction (MEF). A portion of MEF was further subjected to precipitation process for crude saponin (saponin rich fraction, SRF) using acetone while the precipitate was further hydrolysed to obtain the aglycone, sapogenin-rich fractions (ASRF)

2.2. Reagents and apparatus

In the course of the research work, the following analytical grade reagents were used; Methanol, Dichloromethane, Acetone, Ethyl acetate, Butanol, Acetic acid, distilled water, Peptone water, Mayer's reagent, Conc. Sulphuric acid, Fehling's solution, Chloroform, Acetic anhydride, Glacial acetic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) powder.Vacuum pump, Buchner funnel, Filter paper, Desiccators, Prepared Thin Layer Chromatographic Plate (silica gel GF – TLC 254), TLC tank, Iodine tank, Bunsen burner, Microscope, Hemocytometer and Cover Slip, UV- Visible Spectrophotomer, UV- lamp, Water bath.

2.3. Drugs

Positive control drugs used were: Methotrexate and Ascorbic acid

2.4. Phytochemical screening

These fractions were screened for phyto-constituents following standard phytochemical screening method [13].

2.5. DPPH Radical Scavenging Assay

A preliminary quantitative DPPH antioxidant assay was carried out on 1.0 and 0.1 mg/ml concentrations each of all the fractions using methanol as solvent according to Brand-Williams *et al.* [14]. A further two-fold dilutions (1.0000, 0.5000, 0.2500, 0.1250, 0.0625 mg/ml) were made on the fractions that promised a better activity to ascertain their median inhibition concentration (IC_{50}). Briefly, the various concentrations were mixed with a freshly prepared methanol solution of 2,2-diphenyl-1-picrylhydrazyl (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 and the percentage inhibition of the concentrations calculated using the formula as shown below;

$$\% \text{ Inhibition of DPPH activity} = 100[A_{(\text{negative control})} - A_{(\text{sample})}] / A_{(\text{negative control})}$$

Where

$A_{(negative\ control)}$= Absorbance of the negative control solution (containing all the reagents except the test fractions)
$A_{(sample)}$ = Absorbance of the test fractions.

The IC_{50} was obtained by extrapolation from the regression curve of a plot of % Inhibition of DPPH activity against concentration

2.6. Antiproliferative assay (AP)

Anti-proliferative assay was carried out using the *Saccharomyces cerevisiae* (yeast) model [15]. Briefly, a 20ml Sabouraud dextrose broth (SDB) was prepared by dissolving 0.6g of powder nutrient broth in distilled water 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°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°C and further diluted to a 0.1 MacFarland standard, this served as the standardized inoculum. A 10mg/ml, 1mg/ml, 0.1mg/ml concentrations each of n-hexane extract(NHE), DCM, MEF, SRF and ASRF fractions as well as the standard drug 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. Each of these were prepared in duplicates and incubated at 37°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;

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

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

3. Results and discussion

The yield of the fractions, n-hexane extract(NHE), DCM, MEF, SRF and ASRF were 1.8, 1.7, 33.6, 17.0 and 6.3 g respectively. It was observed that the dried whole root of *Olax viridis* showed the presence of four phytoconstituent; carbohydrates, cardiac glycosides, saponin and steroidal/triterpenoidal nucleus while anthraquinone, alkaloid and phenolic compounds were absent. As shown in Table 1, MEF and SRF contain the four phytoconstituents while DCM fraction showed the presence of three phytochemical constituents with the absence of cardiac glycoside. On the other hand, n-hexane extract showed the presence of deoxy sugar and steroidal/triterpenoidal nucleus whereas ASRF result indicated that it is rich in sapogenin (steroidal/triterpenoidal nucleus). Recently, butanol extract of the *O. viridis* root bark has been shown to be rich in saponin [16] while the fruit in addition to the above contains alkaloids and flavonids [10].

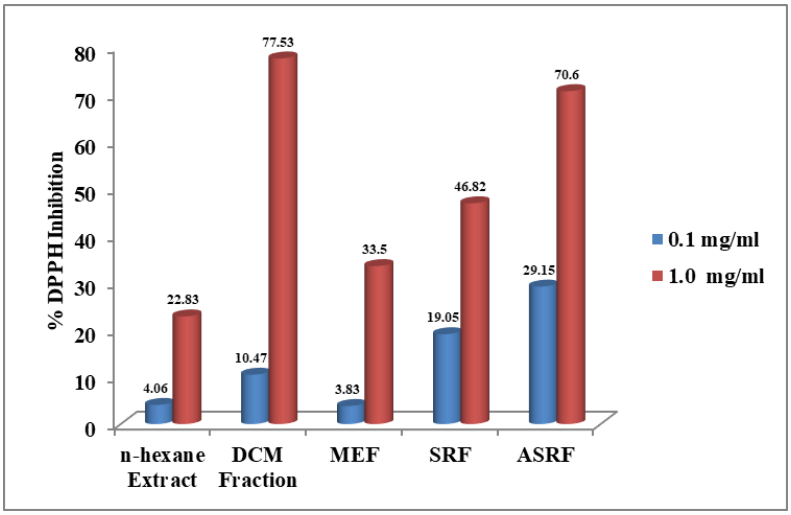

Figure 1 Preliminary free radical scavenging activity of the test fractions

Table 1 Phytoconstituents present in *O. viridis* whole root

S/N	Phytochemical component	Tests	NHE	MEF	DCM	SRF	ASRF
1	**Carbohydrate**	Molisch test	-	+	+	+	-
		Fehling's test	-	+	+	+	-
2	**Steroidal/triterpenoidal nucleus**	Liberman-buchard's test	+	+	+	+	+
		Salkwoski's test	+	+	+	+	+
3	**Cardiac glycoside**	Kedde's test	-	+	-	+	-
		Keller-kiliani test	+	+	+	+	-
	Phenolics	Ferric chloride test	-	-	-	-	-
	Anthraquinone	Borntrager's test	-	-	-	-	-
4	**Saponin**	Frothing test	-	+	+	+	-
		Emulsion test	-	+	+	+	-

Key: (+) = positive; (-) = negative; SRF –*Olax viridis* whole root saponin rich fraction; ASRF – *Olax viridis* whole root sapogenin rich fraction; NHE – *Olax viridis* whole root n-hexane extract; DCM – *Olax viridis* whole root dichloromethane fraction from the defatted methanol extract; MEF – *Olax viridis* whole root aqueous methanol fraction from the defatted methanol extract

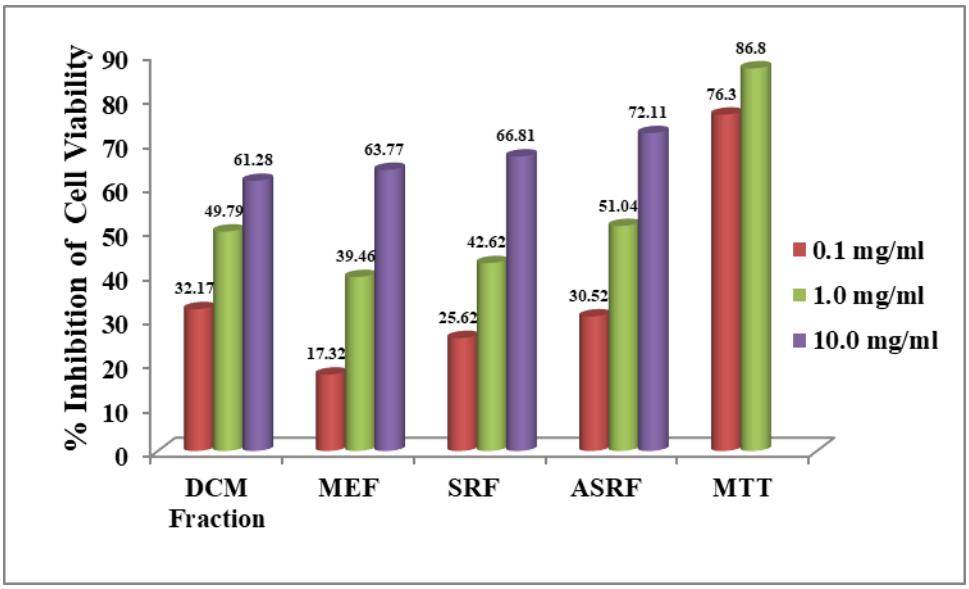

Figure 2 Antiproliferative activities of the test fractions, methotrexate

Table 2 Antioxidant profile of DCM fraction and ASRF

Concentration (mg/ml)	Percentage DPPH inhibition	
	DCM fraction	**ASRF**
1.0000	77.53	72.36
0.5000	55.94	52.92
0.2500	34.62	42.92
0.1250	16.13	35.38
0.0625	6.51	0.85

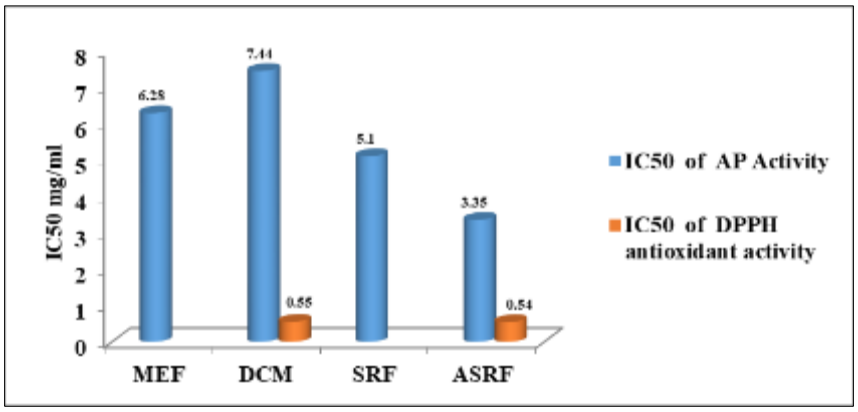

Figure 3 Comparison of the *in vitro* antiproliferative and antioxidant profile of the fractions

The result of the *in vitro* antioxidant assay (DPPH free radical scavenging) of the 5 test fractions as shown in Fig 1 were studied by measuring the ability of the sample to inhibit DPPH scavenging free radicals. The preliminary assay of the fractions at 1.0 mg/ml showed the trend to be DCM(77.53%) >ASRF (70.60%) >SRF (46.82%) >Methanol fraction)MEF) (33.50%) >NHE (22.83%). A further evaluation of fractions with promising activities (ASRF and DCM fraction) showed $IC_{50;}$ ASRF(0.54 mg/ml) >DCM (0.55 mg/ml). The IC_{50} is the concentration that will bring about a 50% reduction in DPPH scavenging activity. The IC_{50} of ASRF is insignificantly higher than that of DCM fraction, and they showed a dose dependent activity.

Cell viability method using yeast (*Saccharomyces cerevisae*) as a model organism was used to ascertain the antiproliferative activities of the fractions. The anti-proliferative activities were expressed in terms of percentage inhibition of viable cell population (Fig. 2) for each of the fractions. The result showed that at 1mg/ml, the trend in AP activities were Methotrexate (86.75%) >ASRF (51.04%) >DCM (49.79%) >SRF (42.48%) > Methanol fraction (39.46%). All the test samples showed a dose dependent reduction in the population of the yeast. The standard drug (methotrexate) showed a higher percentage of cell death when compared with the test samples at 1 mg/ml. On further evaluation, their IC_{50} were obtained and the trend; ASRF (3.35 mg/ml) > SRF (5.10mg/ml) > Methanol fraction (6.28 mg/ml). > DCM (7.44 mg/ml).

The trend shown in the percentage inhibition of DPPH at a lower concentration of 0.1 mg/ml obtained is similar to that observed in the anti-proliferative tests. This direct relationship has since been known to exist between the two activities [16] (Okonkwo *et al*., 2019). The DCM fraction and ASRF exhibited better antioxidant property than other fractions although DCM showed a relatively lower AP activity. Looking at the trend of AP activities and their phytoconstituents it could be deduced that the presence of saponins in the fractions could offer a rationale for the observed AP and free radical scavenging (FRS) activity as saponins have been reported to have antioxidant and anticancer properties [17]. Furthermore on hydrolysis of saponin rich fraction (SRF), it was observed that compared to the SRF and other fractions, ASRF which is devoid of sugar moiety gave a significant activity inferring that the antiproliferative and antioxidant activity of saponin rich fractions could be attributed to the sapogenin moiety. 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 that demonstrate various pharmacological effects against mammalian diseases [18]. Functionally, the presence, addition or removal of a sugar molecule on a glycoside can either enhance or inhibit an established activity, thus looking at the AP and free radical scavenging activities between SRF and ASRF, it could be seen that the presence of a sugar moiety in SRF compared to the sapogenin rich moiety reduced its activities.

4. Conclusion

The saponin-containing fractions SRF and its sapogenin-rich aglycone fraction ASRF exhibited significant antiproliferative and free radical scavenging activity in varying degrees. The sapogenin moiety could be responsible for these activities. Further work is recommended towards the isolation and characterization of the bioactive saponins and the triterpenoid/steroids as leads for the development of drugs for the treatment of cancer and related ailments associated with oxidative stress.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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