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Original Research Article

In-vitro Antioxidant Activity and Cytotoxic Effect of Ethanol Leaf Extract and Fractions of *Olax subscorpioidea* Oliv. (Olacaceae)

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ABSTRACT

The *Olax subscorpioidea (Oliv)* plant belongs to the Olacaceae family. It is a local remedy used in Nigeria to treat a wide range of conditions, including cancer. This study examined the *Olax subscorpioidea* fractions and ethanol leaf extract for phytochemical content, antioxidant activity, and cytotoxic activity. Using the accepted methods, total tannin (TTC), total flavonoid (TFC), and total phenolic (TPC) were assessed. The antioxidant activities were determined through the DPPH, ABTS, and OH methods. The lethality assay for brine shrimp was used to determine the cytotoxic activity. The ethyl acetate fraction (OSEA) had the highest yields of phenolic (148.7 ± 2.41 mg GAE), flavonoid (145.5 ± 9.49 mg/QUE), tannin (35.8 ± 2.15 mg GAE) and total antioxidant capacity (94.7 ± 1.47 mg GAE) as well as the lowest IC_{50} values for DPPH (0.315 ± 0.004 mg/ml), ABTS (0.329 ± 0.0195 mg/ml), and hydroxyl (5.910 ± 0.0244 mg/ml). The high concentration of phenolic compounds in the ethyl acetate fraction of *Olax subscorpioidea* suggests its high scavenging potential compared with other fractions. All the fractions and ethanol extract of *Olax subscorpioidea* were cytotoxic to the brine shrimp at LC_{50} values less than 200 µg/ml. However, the hexane fraction is more cytotoxic at LC_{50} value of 39.254 µg/ml. The cytotoxic activity of *Olax subscorpioidea* is associated with the presence of secondary compounds such as phenolic compounds, tannin, and other compounds present in the plant.

Keywords: *Olax subscorpioidea*, ethyl acetate, antioxidant activity, phenolic, flavonoid, and cytotoxic.

Introduction

Free radicals form and are amassed in humans because of specific physiological processes. Free radicals are molecules that can function independently despite having unpaired electrons in their atomic orbits. These unpaired electrons give them a great deal of instability and reactivity.[1] These unstable free radicals interact with biological macromolecules such as DNA, lipids, and proteins to form stable bonds. Free radicals can harm the human body if they form bonds with these macromolecules and then react with biological elements like DNA or cell membranes. Attacks on these crucial cellular components may therefore result in several pathologic processes, such as cancer, diabetes, cardiovascular disease, and neurodegenerative diseases.[2] By removing free radicals from the cells and preventing damage, antioxidants protect against cellular assaults.[3] Reactive oxygen species (ROS), which are oxidizing agents, are neutralized by antioxidants at the cellular and molecular levels, preventing them from causing oxidative processes.[4] The body's natural antioxidant system, which consists of enzymatic enzymes, eliminates free radicals in small quantities. Likewise, the existence of non-enzymatic antioxidants in living things.[5] As people age, the potency of these natural antioxidants decreases, resulting into a significant production of free radicals. Antioxidant synthetic compounds may therefore be necessary at this later stage of life.[3,6]

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To control oxidative stress, synthetic antioxidant compounds such as tert-butylated, butylated hydroxytoluene (BHT), octyl gallate (OG), propyl gallate (PG), and butylated hydroxyanisole (BHA) are used. However, due to their difficulty of use, high cost, and undesirable side effects, many of these chemicals have not yet been proven to be more effective. It is therefore necessary to use a highly effective natural antioxidant therapy that doesn't have the negative effects of synthetic drugs.[7,8] Finding plant-based compounds with a higher likelihood of providing antioxidant activity against oxidative stress is one strategy. Antioxidants found in medicinal herbs help prevent oxidative stress. They lower the risk of cancer, heart disease, stroke, and other diseases.[9] Phytochemicals in medicinal plants, such as flavonoids, phenols, carotenoids, and vitamin C, are responsible for these health benefits.[10] In nations like Zaire, Senegal, and Nigeria, *Olax subscorpioidea (Oliv)*, is abudant.[11] The plant has been used traditionally in treating various diseases such as inflammation, cancer, infectious diseases, hepatic diseases, asthma, pain, gastrointestinal disorder, and several other diseases. [12] Scientific studies of *Olax subscorpioidea* have confirmed the anti-protease, antimicrobial, anti-depressant, antinociceptive and anti-ulcer properties of this plant.[13,14]
Previous studies on the cytotoxic activity of the leaf and stem of this plant have been done. However, the cytotoxic activities of this plant on various fractions will be worthwhile. This study was designed to evaluate the antioxidant and cytotoxic activity of solvent-partitioned fractions of *Olax subscorpioidea*.

Materials and Methods

Chemicals and Reagents
The solvents and chemicals used were of the highest quality. Ethanol, hexane, ethyl acetate, butanol, Griess reagent, Folin-Ciocalteau phenol reagent, $AlCl_3$, ABTS (2, 2'-azino-bis-(3 ethylbenzothiazoline-6-sulphonic Acid), Na_2CO_3, NaOH, sodium nitrite, quercetin, sulfuric acid, sodium phosphate, ammonium molybdate, trolox standard

solution, molybdenum (VI), methanol, DPPH (2-diphenyl-1-picrylhydrazyl), deoxyribose, EDTA, ascorbic acid, ferric chloride ($FeCl_3$), phosphate buffer saline (PBS), brine shrimp, Dimethyl sulfoxide (DMSO), gallic acid, hydrochloric acid, glacial acetic acid, thymol were used in this study.

Collection of plant samples
Olax subscorpioidea (Oliv) fresh leaves were obtained from the Gambari Forest Reserve in Ibadan, Oyo State, Nigeria, in May 2021. The plant samples were authenticated by Dr Odewo from the Forestry Research Institute of Nigeria (FRIN). In the FRIN herbarium, *Olax subscorpioidea (Oliv)* samples were deposited, and a voucher number (No. FHL 113038) was assigned.

Extraction and differential solvent fractionation process
Fresh plant leaves were air-dried for three weeks in the laboratory and then ground into powder and kept in a container. *Olax subscorpioidea (Oliv)* was defatted using petroleum ether. Following a 72-hour cold maceration process in 30:70 aqueous ethanol. The rotary evaporator was used to concentrate the filtrate to dryness at 50^0 C. Twenty (20 g) grams of the dry ethanol leaf extract of *Olax subscorpioidea* was reconstituted in distilled water before it was sequentially extracted between the solvents into with hexane, ethyl acetate, butanol, and aqueous fraction.[15] The percentage yield of the extract was calculated using the formula.

$$\% \text{ Yield} = \frac{\text{Extract Weight}}{\text{Plant Weight}} \times 100$$

Total phenolic content (TPC) estimation in O. subscorpioidea extracts
According to Hatami et al., the TPC was determined using an oxidizing agent, the Folin-Ciocalteau phenol reagent.[16] Following the addition of the Folin-Ciocalteau phenol reagent (0.2 ml), 0.9 ml of water, and 0.1 ml of sample were added, and the mixture was vortexed. After being stirred for 5 minutes, a 7 per cent (w/v) Na_2CO_3 solution was added. The mixture was left at room temperature for 90 minutes after it was distilled to 2.5 ml. Comparing the absorbance at 750 nm to a negative control group that contained 1 ml of aqueous solution rather than the sample. After establishing a calibration curve, 0.02, 0.04, 0.06, 0.08, and 0.1 mg/ml of Gallic acid concentrations were used to calculate the Gallic acid equivalent (GAE) of the sample. We used distilled water as a control.

Evaluation of total flavonoid content (TFC) of O. subscorpioidea extracts
Standard quercetin concentrations of 0, 1, 2, 3, 4, and 5 mg/ml were used as controls in comparison to the sample extracts. According to Miliauskas et al.[17], the aluminium chloride ($Alcl_3$) colorimetric test method was utilized. 0.1 ml of extract/standard was mixed with 0.4 ml of distilled water. Then 0.1 ml of sodium nitrite at 5 per cent was added. After five minutes, 0.1 ml of $Alcl_3$ (10%) and 0.2 ml of sodium hydroxide were added. The amount was raised to 2.5 ml by using distilled water. Comparing the absorbance at 510 nm to a blank allowed for measurement. According to mg quercetin equivalent per gram of plant extract, the TFC is as follows:

$$X = q \,.\, {}^{v}/_{w}$$

X= Total flavonoid compound concentration in quercetin equivalent
q= concentration of quercetin determined from the standard curve
v= the amount of extract
w= weight of the resulting crude ethanol extract

Determination of tannin content of O. subscorpioidea extracts
To identify tannins, the Folin-Ciocalteau method was applied. 0.5 ml of Folin-Ciocalteau phenol reagent, 7 ml of distilled water, and 1 ml of a 35 per cent Na_2CO_3 solution were added into a 10 ml volumetric flask. Thereafter, 10 ml of distilled water was added to dilute to the required volume. It was set aside for 30 minutes at room temperature after mixing thoroughly. The same procedure was used to create reference standards for gallic acid at concentrations of 20, 40, 60, 80, and 100 g/ml. The absorbance of the test and standard solutions were measured at 725 nm using the UV/visible spectrophotometer. The amount of tannin per gram of extract was given in milligrams of GAE.[18]

Determination of total antioxidant capacity of O. subscorpioidea extracts
This procedure is based on the idea that when an extract forms a complex at an acidic pH, molybdenum (VI) is reduced to molybdenum (V).[19] A reagent solution composed of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate was added to 0.1 ml of the oil extracts or standard solutions of ascorbic acid (20, 40, 60, 80, and 100 g/ml). A water bath set at 95^0C was used to incubate the reaction mixture-containing tubes for 90 minutes and cooled to room temperature. The reaction mixture was then measured in comparison to a blank at 695 nm. Ascorbic acid equivalents were used to measure the extract's antioxidant activity.

Assessment of ABTS radical scavenging activity
The ABTS free radical cation decolourization test was used to assess the extracts' capacity to scavenge free radicals.[20] The ABTS radical cation ($ABTS^+$), was then left at room temperature for 16 hours in the dark and was made by mixing potassium persulfate (140 mM) and the ABTS stock solution (7 mM) in a 2:1 ratio. An ABTS working solution was created by diluting the stock solution with 70% ethanol to an absorbance of 0.75 ± 0.05 before use. 50 µl of various extract/trolox standard concentrations were combined with ABTS working solution (2ml) and assessed after 20 minutes at 734 nm.

Assessment of DPPH radical scavenging activity
The term "DPPH" refers to a stable radical. The extract's scavenging power was assessed using the method of Chandra et al. [21] At 517 nm, the colour changes from deep violet to light yellow. In each test tube, 1 ml of 0.3 mM DPPH in methanol was added to 1 ml of the oil extract or standard (vitamin C) at various doses (5, 2.5, 1.25, 0.625, 0.3125, and 0.156 mg/ml). The mixture was mixed, then allowed to rest in the dark for 30 minutes. Then, in place of the extract, a DPPH control containing just 1 ml of methanol was read against the absorbance at 517 nm.
These numbers were used to calculate the percentage inhibition:

$$\% \, inhibition = (A_{blank} - A_{sample})/A_{blank} \text{ x } 100$$

A sample = Test compound absorbance
A blank = Control reaction absorbance
The sample concentration that provides 50% inhibition (IC_{50}) was determined using a graph showing the percentage of inhibition against extract concentration.

Hydroxyl Radical Scavenging Assay (OH)
The method outlined by Halliwell. [22] was used to evaluate the extract's ability to scavenge OH. The reaction mixture contained 1.0 ml of reagent (0.1mM $FeCl_3.6H_2O$ in 10mM phosphate buffer, pH 7.4, 3.0mM deoxyribose, 2mM H_2O_2, 0.1mM L-ascorbic acid, 0.1mM EDTA) and different concentrations of the fractions. After 60 minutes of incubation at 37°C, 1.0 ml of 1% (w/v) TBA (in 0.25N HCl) and 1.0 ml of 10% (w/v) TCA were added to the mixture. The pink chromogen (malondialdehyde (TBA) adducts) was extracted into 1.0 ml of butan-1-ol, after heating the reaction mixture in a boiling water bath at 100^0 C for 20 minutes. Thereafter, the absorbance was measured at 532 nm against the reagent blank.
The percentage inhibition was calculated using the expression.

$$\% \, inhibition = (A_{control} - A_{sample})/A_{control} \text{ x } 100$$

Cytotoxicity bioassay Test
The stock solution for the samples was created by the method described by Sulaimon et al.[23] To create a stock solution with a concentration of 1 mg/ml, dimethyl sulfoxide (DMSO) was used to lyse ethanol leaf extract and fractions of Olax subscorpioidea. To avoid any potential unintended effects of DMSO toxicity, the final DMSO concentration in the test volume was kept at 2 %. Artificial seawater was used as the

negative control and thymol was used as the reference standard in this test.

Hatching of Brine shrimp cysts

Incubation tanks containing 500 ml of artificial seawater were used to hatch brine shrimp eggs (0.15 g) after 48 hours. Artificial seawater was made using bottled water and two per cent (2 %) of commercial sea salt dissolved in it. The tank was aerated by an air pump, and a light source (1000–4000 lux) was installed inside of it.

Brine shrimp lethality test

In seawater with 2 per cent DMSO (v/v), the extracts' toxicity was tested at various concentrations of 40, 80, 120, 160, and 200 g/ml. There were ten nauplii in each test. Three replicates were used for each concentration. A parallel series of experiments used the standard thymol solution (concentration = 6.25, 12.5, 25, 50, 100 g/ml) and the blank control. Twenty-four hours later, the survivors were counted. The lethal concentration (LC_{50}) was determined with 95 per cent confidence intervals using probit analysis.

Statistical analysis

Microsoft Excel and SPSS 23 software were used to analyze the data and prepare the result tables and figures. The data represents the mean ± S.D (Standard Deviation) of the triplicate experiment.

Results and Discussion

Extraction and per cent yield of Olax subscorpioidea ethanol leaf extract and its fractions

Olax subscorpioidea ethanol leaf extract extraction resulted in a yield of 70 g. With various organic solvents, 20 g of ethanol were polarity separated. The fractions produced various yields which were ranked in the following order. OSB (12.22 g) > OSEA (3.65 g), OSA (3.21 g), and OSH (1.15 g).

Total Phenolic content (TPC)

The extraction of polyphenolic compounds from plant matrices has previously been reported to be possible using semi-polar and polar solvents, with mixtures of aqueous solvents containing ethanol, acetone, methanol, and ethyl acetate being the most effective extraction agents. [24] The redox properties of phenolic compounds, which are crucial for the antioxidant effect, make them very significant.[25] Phenolic compounds' ability to neutralize free radicals has been linked to the presence of hydroxyl groups in these compounds.[26,27] In our study. the TPC value is between 29.8 and 148.7 mg GAE/g extract. According to Table 1, the yield is in the following order OSEA (148.7 mg GAE/g extract), followed by OSB, OSE, OSH, and OSA (90.2, 68.6, 32 .3, and 29.8 mg GAE/g extract). Previous studies have reported the presence of different levels of total phenols in the leaves of *O. subscorpioidea*. In line with Ayoola et al.[28], the ethanol root extract of *Olax subscorpioidea* gave a TPC of 43.9 mg GAE/g. Our study reported different values for the ethanol leaf extract and fractions of *Olax subscorpioidea* and this could be attributed to the extraction solvent, the plant part used and the preparation and extraction method. Topcu et al.[29] reported that the solvent polarity determines the amount of polyphenol content extracted. Phenolic compounds are usually identified by the presence of a phenol group, aromatic ring, and one or more hydroxy groups. They are commonly considered to be beneficial to health by scavenging free radicals.[30]

Total flavonoid content

Flavonoids have a variety of biochemical and antioxidant effects connected to diseases like cancer, atherosclerosis, and Alzheimer's. [31] The hydroxyl groups and their interactions with different parts of the chemical groups gives flavonoids the ability to scavenge free radical. [25] The content of total flavonoids (TFC) in our study is expressed as the number of mg of quercetin equivalents per gram (QUE/g), and it ranges from 22.1 to 145.5 mg QUE/g. The total flavonoid content is high in ethyl acetate fraction corresponding to 145.5 ± 0.52 mg QUE/g extract. Following this are the n-butanol fraction, the ethanol extract, the hexane fraction, and the aqueous fraction (67.5, 60.1, 26.4, and 22.1, respectively, mg QE/g extract) Table 1. The harvest period, environmental conditions, extraction techniques, and solvents are just a few of the variables that affect the amount of flavonoid content.[25]. Flavonoids, the most common polyphenolic compounds, are mainly present in fruits and vegetables.[32]

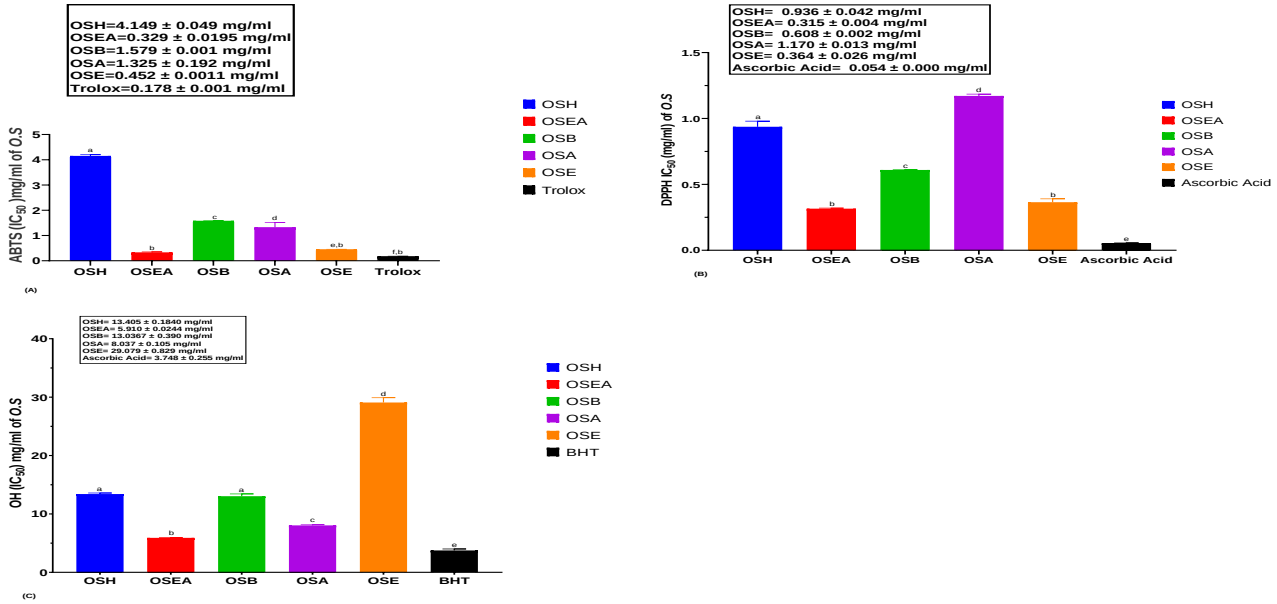

Figure 1: *In-vitro* antioxidant activities of ethanol extract and fractions of *Olax subscorpioidea*.
IC_{50} values in the ABTS radical scavenging activity assay, b. IC_{50} values in DPPH radical scavenging activity assay c. IC_{50} values in OH radical scavenging activity assay. Each value represents a mean ± SD (n = 3). OSH: hexane fraction, OSEA: ethyl acetate, OSB: n-butanol fraction, OSA: residual aqueous fraction, OSE: ethanol extract, ascorbic acid, BHT: Butylated hydroxytoluene, Trolox

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Table 1: Estimation of plant extraction yield, total phenolic contents, flavonoid, tannins, and antioxidant capacity of crude extract of *Olax subscorpioidea* and its fractions

Sample	Yield (g)	Total phenolic contents expressed as gallic acid equivalents (mg GAE/g of extract)	Total flavonoid contents expressed as quercetin equivalents (mg QUE/g of extract)	Total tannin content expressed as gallic acid equivalents (mg GAE/g of extract)	Total antioxidant capacity expressed as ascorbic acid equivalents (mg AAE/g of extract)	Flavonoid ratio
OSE	70	68.6 ± 3.40^{b}	60.1 ± 12.65^{a}	$13.2 \pm 0.30^{b,e}$	41.8 ± 0.52^{b}	87.61
OSH	1.15	32.3 ± 2.41^{a}	$26.4 \pm 6.32^{b,d}$	$5.6 \pm 1.55^{a,e}$	74.6 ± 0.24^{d}	81.73
OSEA	3.65	148.7 ± 2.41^{d}	145.5 ± 9.49^{c}	35.8 ± 2.15^{c}	94.7 ± 1.47^{e}	97.85
OSB	12.22	90.2 ± 1.77^{c}	67.5 ± 7.38^{a}	18.5 ± 0.83^{d}	48.5 ± 1.20^{c}	74.83
OSA	3.21	29.8 ± 0.21^{a}	$22.1 \pm 10.54^{a,d}$	5.2 ± 0.11^{a}	20.7 ± 1.32^{a}	74.16

Table 2: Percentage Mortality of shrimp nauplii after treating with ethanol extract and fractions of *Olax subscorpioidea*

Plant Extracts	Concentration (ppm or µg/ml)	Total Number of Nauplii Exposed	Total Number of Nauplii Responding after 24hrs	Total Number of Nauplii Survivors after 24hrs	% Mortality	LC_{50} (µg/ml)	Toxicity
Control	50	50	5	45	10%	-	-
Standard (Thymol)	6.25	20	12	8	60%		
	12.5	20	15	5	75%		
	25.0	20	17	3	85%	5.653	toxic
	50.0	20	19	1	95%		
	100.0	20	20	0	100%		
N-hexane fraction	40	30	19	11	63.3%		
	80	30	21	9	70%		
	120	30	28	2	93.3%	39.254	mildly toxic
	160	30	29	1	96.6%		
	200	30	30	0	100%		
Ethyl acetate fraction	40	30	11	19	36.6%		
	80	30	18	12	60%		
	120	30	21	9	70%	70.594	mildly toxic
	160	30	24	6	80%		
	200	30	28	2	93.3%		
Butanol fraction	40	30	10	20	33.3%		
	80	30	10	20	33.3%		
	120	30	13	17	43.3%	138.56	mildly toxic

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	160	30	17	13	56.6%		
	200	30	23	7	76.6%		
Aqueous fraction	40	30	8	22	26.6%		
	80	30	11	19	36.6%		
	120	30	16	14	53.3%	120.618	mildly toxic
	160	30	19	11	63.3%		
	200	30	23	7	76.6%		
Ethanol extract	40	30	9	21	30%		
	80	30	11	19	36.6%		mildly toxic
	120	30	18	12	60%	99.038	
	160	30	24	6	80%		
	200	30	27	3	90%		

Total tannin content

Tannins help humans and plants scavenge free radicals, protect them from predators, and control growth.[33] Due to its antioxidant properties, tannin plays an important role in preventing diseases such as cancer, cardiovascular disease, etc. [34] According to this study, OSEA (35.8 ± 2.15) had the highest total tannin content, followed by OSB (18.5 ± 0.83), OSE (18.5 ± 0.83), OSH (5.6 ± 1.55) and OSA (5.2 ± 0.11) (Table 1). The tannin content is represented as mg GAE/g dry sample. An earlier investigation discovered tannins in *Olax subscorpioidea*. According to Gbadamosi et al.[35] root extract of *Olax subscorpioidea* contained 863.33 mg/100g of total tannin.

Total antioxidant capacity (TAC)

Typically, TAC includes antioxidants like ascorbic acids, some phenols, carotenoids, and tocopherol. The analysis revealed a total antioxidant capacity (TAC) value that varied from 20.7 to 94.7 mg/g. In Table 1 the OSH, OSB, OSE, and OSA fractions all have lower TAC contents than the OSEA fraction, which had a value of 94.7 ± 1.47 mg AAE/g dry sample. When compared to other fractions, the OSEA and OSH fractions have more TAC contents, possibly because of the abundance of phenols and flavonoids in these two fractions.[36]

Scavenging activity of ABTS

The IC_{50} values of different fractions and extracts of *O. subscorpioidea* with ABTS scavenging activity are given in Figure 1a. The ABTS scavenging ability of *Olax subscorpioidea* is classified as OSEA > OSE > OSA > OSB > OSH. All fractions showed higher IC_{50} values compared to Trolox (0.178 ± 0.001 mg/ml). The ABTS test helps determine the antioxidant status of phenolic phytochemicals and is considered a better method of evaluating antioxidant activity than the DPPH test. [37] The reaction between potassium persulfate and ABTS yields a blue/green ABTS+ chromophore in the ABTS scavenger assay. The formation of radical cation of ABTS occurs during the oxidation of potassium persulfate with ABTS. The reduction in the presence of hydrogen-donating antioxidants is measured spectrophotometrically at 735 nm. This study found an association between phenol levels and antioxidant activity as measured by the ABTS assay. The OSEA fraction proved to be a better antioxidant than the other fractions due to its high phenol content and lower IC_{50}.

DPPH radical scavenging activity

Figure 1b depicts the IC_{50} value for the DPPH radical scavenging activities which are in the following order: OSEA > OSE > OSB > OSH > OSA. The least IC_{50} values are OSEA and OSE (0.315 and 0.364 mg/ml). All fractions showed higher IC_{50} values compared to ascorbic acid (0.054 ± 0.000 mg/ml). The DPPH has a hydrogen-donating ability. In the presence of an antioxidant, it is decolourized by accepting electrons donated by the antioxidant from a violet-colored DPPH solution and being reduced to a yellow-coloured diphenylpicrylhydrazine. [38] Flavonoids, polyphenols, and tannins are phenolic compounds that possess redox properties and contribute to the free radical scavenging ability of antioxidants.[39] As shown in Table 1, the OSEA fraction has the highest overall phenol content. Concerning the other fractions, its high antioxidant activities were undoubtedly influenced by its high phenol content.

Scavenging activity for hydroxyl radicals (OH)

The free radical produced because of the Fenton reaction caused the inhibition of degradation of 2-deoxyribose which was used to measure the amount of OH scavenging activity. The OH scavenging activity of *Olax. subscorpioidea* is in the following order OSEA > OSA > OSB > OSH > OSE. In this study, OSEA and OSA have the lowest IC_{50} values of 5.910 mg/ml and 8.037 mg/ml, Figure 1c. In lipid peroxidation, hydroxyl radicals are the main reactive oxygen species causing biological damage in the cell. [40] The hydroxyl radical is regarded as a dangerous species because it can harm virtually every biological molecule and contribute to cancer growth, mutation, and cytotoxicity. By scavenging hydroxyl radicals, our study demonstrates that all fractions of *Olax subscorpioidea* prevented the degradation of 2-deoxyribose.

Brine Shrimp lethality Assay

The result of the brine shrimp lethality test on *Olax subscorpioidea* ethanol leaf extract and fractions with their LC_{50} values are shown in Table 2. In this study, ethanol leaf extract and fractions from *Olax subscorpioidea* showed low to moderate cytotoxic activities in brine shrimp ranging from 39.254 µg/ml to 138.56 µg/ml. Based on previous studies, a concentration above 100 µg/ml is considered non-toxic. [41] Thymol is used as a standard for comparison with the ethanol leaf extract and fractions from *Olax subscorpioidea*. All the fractions and ethanol extract showed cytotoxicity at LC_{50} values of less than 200 µg/ml. However, the hexane fraction is more cytotoxic with an LC_{50} value of 39.254 µg/ml. A similar study also found that the stems and leaves of *Olax subscorpioidea* exhibited LC_{50} values of 45.2 and 10.7 µg/ml after 24-hour exposure to brine shrimp.[14] A plant extract is said to be non-toxic when the LC_{50} value is more than 1000 µg/ml and toxic when the LC_{50} value is less than 1000 µg/ml.[42] The degree of lethality of the extract is proportional to the concentration. The extracts have a high LC_{50} compared to the standard compound (thymol).

Conclusion

The phytochemical, antioxidant, and cytotoxic activities of ethanol leaf extract and fractions of *Olax subscorpioidea* were studied. The study shows that OSEA has the highest phenolic compounds and tannin suggesting its high antioxidant capacity. The ethanol leaf extract and fractions of *Olax subscorpioidea* exhibited cytotoxic activity against the brine shrimp and thus considered to possess active compounds. Further studies need to be done to isolate the bioactive compounds which can be developed into useful therapeutic drugs.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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