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Antioxidant and Antiproliferative Activities of Ethanolic Extracts of *Olax subscorpioidea* Components on MCF-7 Cell Line

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Abstract

Several experiments have explored the chemotherapeutic profiles of certain plant species, and one such shrub is known as *Olax subscorpioidea*, which is widely used in checking almost all types of cancer and many metabolic diseases. Despite their utilisation and control of most cancers, there is not much clinical evidence of the anti-proliferative and anti-cancerous impact capabilities of the plant. This study was aimed to evaluate the epidermal and anti-proliferative effects of the leaves extract of *Olax subsubicorpioida* to the chronic DNA damage of breast cancer, or MCF-7 cells using the comet test. The quantitative phytochemical screening revealed that the highest amounts were alkaloids (23.49 mg/g) and reducing sugar (24.29 mg/g). Mass spectrometry analysis identified Phytol (24.82) and 9-Octadecenoic Acid Methyl Ester (28.47) as possessing the highest values. The DPPH scavenging activity revealed that *O. subscorpioidea* extracts at 100 g/ml exhibited the best activity at 75.1 compared to the control (ascorbic acid) at 74.4. The comet assay showing levels of damaged nuclei in cancer cells revealed *Olax subscorpioidea* extract at 50 g/mL to have a higher increase of 13.5 at 48 h compared to untreated, which has a value of 9.8, and cisplatin, which has a value of 12.8. The ethanolic plant extract of *Olax subscorpioidea* has chemopreventive as well as chemotherapeutic (DNA-damaging) potential due to its high scavenging activity and high DNA damage of cancer cell nuclei. *Olax subscorpioidea* extract can be useful as food or a supplement used in combination with conventional drugs to enhance the treatment of mammary carcinoma.

Keywords: *Olax subscorpioidea*, Anti-tumor, Anti-cancer, Anti-proliferative, and Breast cancer.

1. Introduction

Several chemical and physiological changes within an organism's cells can result in cancer, one of the most deadly diseases (Balali-Mood *et al.,* 2021). One of the disease's hallmarks is the uncontrollable and unabated proliferation of human body cells (Miller & Zachary, 2017). Cancer is ranked the third most common cause of death worldwide, alongside cardiovascular and infectious diseases (Kelloff, 1999). According to estimates, various cancer disorders claim most people's lives worldwide (Organization, 2022; Talukder *et al.,* 2020).

As the most common cancer among women, breast cancer has had an enormous impact on the lives of women and is a major contributor to early mortality, thus constituting a public health threat worldwide (Azubuike *et al.,* 2018; Organisation, 2022; Soliman *et al.,* 2006). It is pertinent to mention that the string of breast cancer cases in Pakistan is the highest in South-Central Asia. In women, breast cancer accounts for 38.5% of all malignancies, and most are at an advanced stage (43.7%) in almost half of all patients (Menhas & Shumaila, 2015). This risk is

mediated by various factors including age and gender, the presence of a breast lump and the genetics of a breast cancer history in the family. Age at menarche and first full-term pregnancy, menopause age, inactivity, radiation exposure, postmenopausal estrogen replacement therapy, and stoutness after menopause are additional risk factors for breast cancer (Łukasiewicz *et al.,* 2021).

Despite its widely recognized extreme lethality, significant progress has been made, both clinically and in the elucidation of the proteomic basis of the diseases (Mesri, 2014). Currently, treatments for breast cancer mainly involve radiation therapy, surgical treatment, and chemotherapy (Shah *et al.,* 2021). The literature demonstrates that hormone therapy, radiation, and chemotherapy can lead to a range of long-term side effects, including fatigue, sleep problems, pain, cognitive dysfunction, decreased bone density, and heart issues (Gegechkori *et al.,* 2017; Society, 2022). Because of potential side effects, the costs of treatments based on radiotherapy and chemotherapy together with the potential serious side effects that also accompany these methods, efforts towards the identification of new effective therapeutic alternatives that can improve the beneficial effects of chemo drugs, while also mitigate their harmful consequences, are still ongoing (organization, 2018; Sharma & Gupta, 2015; V Simoben *et al.,* 2015; Habib *et al.,* 2019).

Presently, various researchers are employing several novel techniques to manage breast cancer, such as oncolytic viruses, proven to combat cancer cells (Nejad *et al.,* 2020). Furthermore, in the quest for other non-hazardous effects of cancer management, cancer research investigations are still underway to find novel therapeutic agents free of the hazardous side effects connected with the current chemotherapeutic medicines (Nejad *et al.,* 2020; Newman & Cragg, 2020).

Natural products from plants therefore still account for over 50% of the anticancer drugs currently in use, including many chemotherapeutics (Laskar *et al.,* 2020). Regardless of the synthesis of many phytochemical constituents for therapeutic strategies, the usage of plant extracts for their clinical and functional characteristics has remained robust (Choudhari *et al.,* 2020). Plant-derived bioactive compounds, such as taxanes, camptothecin, and vinca alkaloids, are a promising source of new compounds for cancer drug development (Shrihastini *et al.,* 2021). Other components of a wide variety of plant extracts include flavinoids, alkaloids, phenolics, tannins, glycosides, and steroids (Patle *et al.,* 2020). In addition to having therapeutic benefits, plant extracts act as an antioxidant, counteracting free radicals that can cause a myriad of health problems in humans. However, it is cost-effective and widely obtainable (Sekhon-Loodu & Rupasinghe, 2019).

Olax subscorpioidea is a shrub or tree *Olax subscorpioidea* is a shrub or tree of the Olacaceae family (Ahmad *et al.,* 2021). Multiple west Africa nations such as Nigeria, Senegal, Zaire, and western Cameroon widely harbor this species (Ayandele & Adebiyi, 2007). Due to its widespread use, the plant is known by numerous household names in Nigeria, including Ifon in Yoruba, Aziza in Igbo, and Gwano kurmi in Hausa (Adeoluwa *et al.,* 2014; Ibrahim *et al.,* 2007). Ethnobotanical research has demonstrated the traditional medicinal uses of some of the plants in controlling diseases like diabetes mellitus, malignant disease, infectious diseases, and psychiatric disorders (Agbabiaka & Adebayo, 2021; Alam *et al.,* 2002; Clarke, 1969; Kazeem *et al.,* 2015; Vongsak *et al.,* 2013).

Hence, the current study is aimed to evaluate the possible cytotoxic activity of *Olax subscorpioidea* leaves against MCF-7 human breast cancer cell line. It also aims to assess the cytotoxicity of its ethanolic extracts on the Michigan Cancer Foundation-7 (MCF-7) cell line using Cell Proliferation Kit II (XTT assay).

2. Materials and Methods

Chemicals

1, 1-diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide, catechin (CA), ascorbic acid (AA), Hydrogen chloride (HCl) and Sulfuric acid (H_2SO_4) were all provided by Sigma-Aldrich. Folin-Ciocalteu's phenol reagent, phosphate-buffered saline (PBS), dimethyl sulfoxide (DMSO), ethanol, methanol, tryptophan, ethidium bromide, and Giemsa dye were all purchased from Katchey Company Limited. Other reagents used in this study were obtained from the Department of Biochemistry, College of Medicine, University of Lagos, and were of analytical grade.

Equipment

For the activity assays: a weighing balance, heat block, spectrophotometer, centrifuge, thermal pH-meter (USA), water bath (Germany), Vortex (Vor-tex-5), hemocytometer, UV-Vis transilluminator, an Eppendorf centrifuge, an inverted micros-cope, a CO_2 incubator, and a plate reader were all used.

Cell lines and cell cultures

MCF-7 cell lines (estrogen receptor positive) were sourced from the Department of Cell Biology and Genetics at the University Of Lagos, Nigeria. This glucose was adjusted to the appropriate concen-tration and cell lines cultured in a 75-mL flask in DMEM medium containing 10% fetal bovine serum (FBS), 100 units/mL of penicillin, and 100 µg/mL of streptomycin. The cultures were maintained at 37°C in a humidified environment supplemented with 5% CO_2.

Sample Collection and Plant materials' Identific-ation

Plant collection: Fresh leaves of *Olax subscor-pioidea* were procured from Ketu Market in Lagos State, Nigeria for this study. Plant samples were identified and authenticated at the Department of Botany, University of Lagos. Once collected, the leaves were kept to air dry in the shade for 14 days. Drying of each sample was carried out then samples were ground to fine powder, weighed and kept in an airtight container until further used.

The powdered leaves were used for extracting by soaking them in 2L of ethanol at room temperature inside airtight glass jars for 72 h. The obtained filtrate was concentrated at 45°C and reduced pressure with the help of a rotary evaporator. The extract was subsequently dried in a Kottermann 2716 oven at 50 °C.

Phytochemical Analysis

Qualitative Tests

The study carried out Phytochemical screening for the presence of alkaloids, tannins, terpenoids, flavonoids, saponins, cardiac glycosides, reducing sugars, and phenolics on the extract of Olax subscorpioidea was carried out using standard procedures (Clarke, 1969; Evans, 1997; Harborne, 1984; Sofowora, 1993).

Test for Alkaloids

Five milliliters (5 mL) of 1% diluted HCl solution was added to the 0.5 g of plant extract and heated in steam bath. After filtration of the solution, a few drops of Dragendorff's reagent were added. A reddish-brown deposit was observed, which is indicative of the presence of alkaloids.

Test for Saponins

For each 10 mL of distilled water, 0.5 grams of plant extract were added and boiled in a water bath and filtered. Then, 5 milliliters of distilled water was added to 10 milliliters of the filtrate and vigorously shaken to determine the presence of a stable, lasting froth. A few drops of olive oil were then added to this froth and the mixture shaken again. This formation of an emulsion indicates saponins.

Testing for Tannins

Ten milliliters of water was added to a test tube containing 0.5 g of the plant extract, and the mixture was boiled for hot water extraction and filtered. A few drops of 0.1% ferric chloride solution were added after filtration, and the mixture was checked for brownish-green or blue-black color appearance.

Testing for Phlobatannins

The 0.5 g of extract was dissolved in 1% dilute hydrochloric acid and heated according to the standard process. A lack of red precipitate signified no presence of phlobatannins.

Test for Anthraquinones

10mL of benzene was added to 0.5 g of the plant extract and filtered. Five mL of 10% ammonia solution were added to the filtrate and mixed. The absence of pink, red, or violet coloring in the lower phase indicated that free anthraquinones were neither detected.

Test for Steroids

Dissolved 0.5 g of extract in 2 mL of acetic acid and placed on ice. Then added 2 milliliters of con-centrated sulfuric acid (H_2SO_4). A color change from violet to blue-green indicated steroids.

Test for Terpenoids

0.5 g of plant extract was added to 2 ml of chloroform and 3 ml of concentrated H2SO4 to form a thin layer. A reddish-brown coloration at the interface indicated the presence of terpenoids.

Test for Flavonoids

0.5g of extract was added to 5 ml dilute ammonia solution and 1 ml of concentrated sulphoric acid (H_2SO_4). Brownish precipitate indicates that flavornoid is present.

Quantitative tests

The levels of various phytochemicals were quantitatively evaluated according to the established methods.

Total Phenolic content:

The total phenolics content was determined using Folin-Ciocalteu reagent with gallic acid as standard (Haile *et al.,* 2016 and Seifu *et al.,* 2017). In this method, 0.5 g of the extracts was mixed with distilled water and 0.25 mL of Folin-Ciocalteu reagent. After dark shaking for five minutes, 1mL of 7.5% Na_2CO_3 was added. It was then permitted to incubate at room temperature for 90 minutes. Absorbance was measured at 760 nm with a reagent blank for calibration. Total phenolic compounds were quantified and expressed as mg of gallic acid equivalents per 100g of sample (mg GAE/100 g).

Total flavonoid compounds

The total flavonoid content was determined using the aluminum chloride method (Chang *et al.,* 2002), with quercetin as the reference standard. The total volume of the aluminum chloride mixture was 4.5mL comprised 0.1mL of 10% aluminum chloride, 0.1mL of 1M potassium acetate, 1.5mL of 80% ethanol, and 2.8 mL of distilled water. To each mixture, 0.5mL of the extract or quercetin standard was added, and distilled water was utilized as a blank control for every assay. Absorbance was measured after incubation for 30 min at room temperature, and the total content was calculated as milligrams of quercetin equivalent per gram of extract.

Total Alkaloid Content

0.5g of the extract was diluted in a 95% acetic acid solution at a 1:95 ratio. The mixture was kept for 24 hours before being strained. After concentration to one-quarter capacity, ammonia water was added drop by drop until alkaloids precipitated. The filtered precipitate was washed with 1% ammonia and dried at 80 °C in an oven until constant weight. Alkaloid concentrations were expressed as percentage of initial weight of extract (Harborne, 1984).

Total Saponin Content

The analysis of saponin was carried out based on the method of Obadoni and Ochuko (Obadoni & Ochuko, 2002), with some minor modifications. 0.5 g of powdered plant material was added to 100 ml of 20% aqueous ethanol, left for 30 minutes on a stirrer, and then heated over a flame for 4 hours at 45 °C. The combined solution was filtered, and the concentrated filtrate was re-extracted with 100 ml of 20% ethanol. The combined extracts were evaporated at 40°C until they reached 40 ml, collected, and transferred into a separator funnel. The purification process was repeated, and 30 ml of n-butanol was added. Fifteen mL of n-butanol extracts was washed two times with 10mL of 5% NaCl solution. The final solution collected was evaporated in a water bath, dried until a constant weight was reached. The saponin content was expressed as a percentage of the total weight.

Gas chromatography mass spectrometry (GC-MS)

GC-MS analysis was conducted according to Ajayi *et al.* (Odo *et al.,* 2017). Gas chromatography-mass spectrometry (GC-MS) was performed on Shimadzu (Japan) GCMS QP 2010 plus instrument, operating in split mode (10:1) and with helium (99.999%) as a carrier gas at 1 mL/min. Ethanolic extracts (8 µL) were injected into the column maintained at 250 °C, starting with the oven temperature at 70 °C for 5 min, which was increased to 280 °C at the rate of 10 °C/min for 6 min. Ion polarity and source temperature were held at 200 °C under isothermal conditions for 5min. The temperatures of the injector and the detector were set to 250 °C, and the mass spectra of the compounds in the samples were obtained using electron ionization (70 eV). The detector was operated in dispensing mode, operating at scan range about 50-600 dalton. ACQ mode data were collected with a scan interval of 0.5 s at 666 with fragment analysis for atomic units (Da) 30 to 350. It took 40 minutes to complete the whole analysis.

Chemotherapeutic effect

Cell Viability Xtt Assay

The cytotoxicity of the plant extracts against the MCF-7 cell line was evaluated using a modified XTT assay (Huyck *et al.,* 2012). Cell viability test was performed using Cell Proliferation Kit II (XTT) according to the instructions of the manufacturer.

Ten thousand to one hundred thousand MCF-7 cells were cultured in a 24-well plate filled with 100 µL of culture medium (DMEM) with the 100 µL of the ethanolic extract from *Olax subscorpioidea* leaves. The plant extract and the cells were incubated at CO_2 (24 and 48 H). Twenty-four hours later, the medium was removed, and the cells were washed with DMEM and phosphate-buffered saline (PBS). 50 µL of XTT test solution, what was mixed with two reagents (50:1 ratio of sodium 3V- (1- (phenyl-aminocarbonyl)-3, 4-tetrazolium)-bis (4-methoxy-6-nitro) benzenesulfonic acid hydrate and N-methyl dibenzopyrazine methyl sulfate) of XTT-labeling reagent (5 mL) and electron coupling reagent (100 µL), was added to each well, according to the supplier's guideline of protocol. After incubation in a 37 °C and 5% CO2 incubator for 3h, using an ELISA reader, ELISA assay was performed to measure the absorbance at 490 nm and 650nm wavelength of both the 24 and 48 hour cultures.

Comet Assay

Comet assay was conducted as described by Singh *et al.* with little modification (Singh et al., 1988). First, the cells were seeded in 6-well tissue culture plates and allowed to attach for 24 hours. Then treating the cells with increasing concentrations of Olax subscorpioidea and Cisplatin (used as a positive control) for an additional 24 hours. Cells were trypsinized after treatment, washed with phosphate-buffered saline (PBS), and re-suspended in an ice-cold solution.

For the assay, 75 µL of low melting point agarose were mixed with 7.5µL of re-suspended cells and carefully pipetted into the wells. The slides were maintained in the dark at 4 °C until the agarose solidified. After 1 hour, the slides were immersed in ice-cold lysis buffer (4 °C), aspirated off the buffer replacing with fresh alkaline solution in equivalent temperature (4 °C) for 30 minutes. Once lysis and DNA unwinding were completed, the slides were transferred to a horizontal electrophoresis tank containing freshly prepared alkaline electrophoresis buffer. Electrophoresis was performed for 20 min at 35 V, 300 mA.

After the electrophoresis, the slides were washed in pre-chilled distilled water for 2 min followed by a second wash. The last wash was aspirated out and replaced with 70% cold ethanol for 5 minutes. Slides were then allowed to air dry and subsequently stained with 100 microliters per well of diluted Vista Green DNA dye and incubated at 37 °C dark for 15 minutes. DNA migration was observed under fluorescent microscope's 10X magnification (Carl Zeiss Apo Tome, Germany). Then, 100 cells from each concentration (50 from one of the duplicate slides and 50 from the other duplicate slide) were randomly selected for analysis. Tail DNA percentage and tail moment were calculated using the TriTek CometScore™ software.

DPPH radical Scavenging Activity Assay

Radical scavenging activity of the extract was measured using the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, according to the method described by Cuendet *et al.* (Burits & Bucar, 2000; Cuendet *et al.,* 1997). An aliquot of 0.5 ml of extract in ethanol (95%) at different concentrations (25, 50, 75, 100µg/ ml) was mixed with 2.0 ml of reagent solution (0.004 g of DPPH in 100 ml ethanol). The control contained only Ascorbic acid (DPPH) solution in place of the sample while ethanol was used as the blank. The mixture was vigorously shaken and left to stand at room temperature. After 30 minutes the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm. The percentage scavenging effects (%) were calculated using the following equation: % inhibition = [(A0 − A1)/A0] x 100, Where A0 is the absorbance of the blank sample and A1 is the absorbance of extract.

Reducing Power Assay

To evaluate the capacity of the extract to reduce Fe+3 to Fe+2 (reducing effect), the method proposed by Oyaizu (Oyaizu, 1986). Various concentrations (20 to 100 g/ml) of the extracts were added to 1.0 ml of deionized water followed by adding 2.5 ml phosphate buffer and 2.5 ml potassium ferricyanide. The mixture was incubated at 50 °C for 20 minutes. After 24h incubation, 2.5 ml trichloroacetic acid was added to the reaction, later the mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of recently prepared solution of ferric chloride. Absorbance was measured at 700 nm, with a control sample that did not contain extract. Standard was ascorbic acid at different concentrations (1 to16 g/mL). Using the

following formula, the percentage increase in reduction power was measured:

% change in reduce power = [(A1/A0) − 1] × 100

Where A1 is the absorbance of the test solution and A0 is the absorbance of the blank. The antiradical activity of the ethanoic extract of *Olax subscorpioidea* means values of IC50 are given as compared with the standard.

Statistical analysis

Mean ± standard deviation (SD) was reported for all analyses, and differences between treated and untreated cells were assessed using one-way Analysis of Variance (ANOVA). Statistical significance was determined when $P < 0.05$ relative to the untreated control cells.

3. Results

Table 1 show the result obtained from the qualitative phytochemical screening of the ethanolic extract of *Olax subscorpioidea* leaves. The phytochemical screening revealed that Saponins, Tannins, Cardiac glycoside, Fehlings, Molisch, Steroids, Terpenoid, Flavonoid and Alkaloid were present while Banfoed, Phlobatanin and Anthran were absent.

Table 1: Qualitative analysis of ethanolic extract of *Olax subscorpioidea* leaves.

S. No	Phytochemical Components	Ethanolic Extract
1.	Saponins	+
2	Tannins	+
3	Cardiac glycoside	+
4	Anthraquinones	+
5	Banfoed	-
6	Polyphenols	+
7	Steroids	+
8	Terpenoid	+
9	Flavonoid	+
10	Phlobatanin	-
11	Reducing sugar	+
12	Alkaloid	+

KEY: (+) = Presence of phytochemical; (-) = Absence of phytochemical.

Table 2 displayed the quantitative phytochemical screening present in the ethanolic extract of *Olax subscorpioidea*. The analysis confirmed the amount of the major phytochemical constituent's. These are: flavonoids (9.36mg/g), Phenol (11.79mg/g), Alkaloid (23.49mg/g), Saponin (17.00 mg/g), Reducing Sugar (24.29 mg/g), and Cardiac Glycoside (15.0 mg/g). From the data, Reducing Sugar was discovered to be present in the highest amount (24.29 mg/g) while flavonoid was indicated to be in the lowest amount (9.36 mg/g).

Table 2: Quantitative analyses of selected phytochemicals present in ethanolic extract of *O. subscorpioidea* leave.

Serial number	Phytochemical mg/100g	Concentration
1	Flavonoid	9.36±0.39
2	Phenol	11.79±0.11
3	Alkaloid	23.49±O.5
4	Saponin	17.00±0.11
5	Cardiac Glycoside	15.04±0.35

Table 3 shows the Gas Chromatogram of *Olax subscopioidea* leave extract. From the result, six compounds were revealed in the *Olax subscrpioidea* extract. Some of these compounds are Hexadecanoic acid ethyl ester, Phytol, methyl 9, 12-Octadecadienoic acid, methyl 9-Octadecenoic acid, ethyl 9, 12, 15-Octadecatrienoic acid, and Squalene. Among these six constituent from the analysis, retention time shows that Squalene has the highest value of 27.978 compared to other bio active compounds. The molecular mass also reveals that 9,12,15-Octadecatrienoic acid ethyl ester has the highest value of 308 compared to other bio active component while the percentage indicated that phytol has the highest percentage of 24.82 compared to Squalene that has the least percentage of 3.38.

Table 3: Gas Chromatography-Mass Spectrometry analyses of ethanolic extract of *O. subscorpioidea* leaves.

Retention Time	Name of the Compound	Percentage (%)	Molecular Mass
16.888	Hexadecanoic acid, ethyl ester	23.29	284
18.508	Phytol	24.82	296
19.217	9,12-Octadecadienoic acid,methyl ester	9.05	294
19.292	-9-Octadecenoic acid methyl ester	28.47	264
19.372	9,12,15-Octadecatrienoic acid ethyl ester	8.64	308
27.978	Squalene	3.38	

Fig. 1 presents the concentration values for the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of the *Olax subscorpioidea* leave extract. Results indicated that. Increase in concentration causes increase of (DPPH) free radical scavenging activity in both the tested *Olax subscorpioidea* extracts and control (Ascorbic acid). However the data revealed that *Olax subscorpioidea* extracts at 100µg/ml exhibited the best activity at 75.1 compared to control (Ascorbic acid) at 74.4.

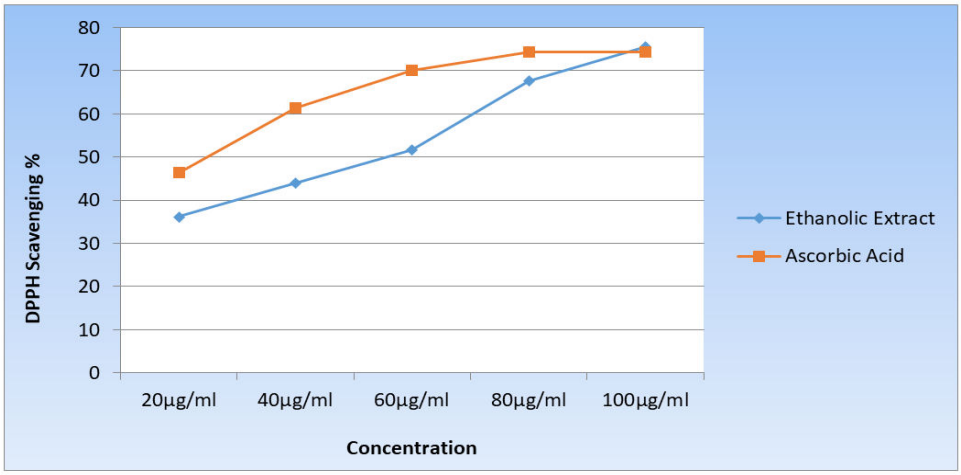


Fig. 1: DPPH radical scavenging activity of the *Olax subscorpioidea* leaves extract.

As can be seen, reducing power of ascorbic acid and *Olax subscorpioidea* extracts increased at concentrations of 20µg/ml and 40µg/ml ($p<0.05$). On the other hand, ethanolic extract of *Olax subscorpioidea* shows a higher reducing power (0.53) than ascorbic acid (0.63) at 100µg/ml concentration (**Fig. 2**).

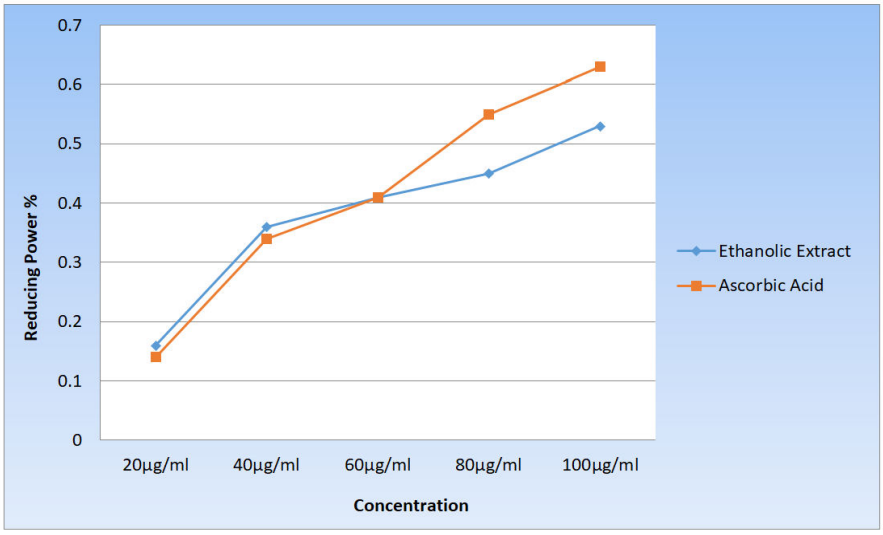


Fig. 2: The reducing power of the plant extracts (as indicated by the absorbance at 700nm).

Fig. 3 presents the cytotoxic effect of ethanolic extract of *O. subscorpioidea* leaves on MCF-7 cells. Cell viability was determined by comparing the survival of cells in the treated MCF-7 cells cultures between 24h and 48h which was normalised to 100 %. The cells were treated with serial dilutions of

O.subscorpioidea extract for 24h and 48 h. Results from this figure indicated increase in cell viability (110-105) at 0.00-625.00 dilutions with a slight decrease at 48hrs. The IC_{50} results reveal the anti-proliferative effect of the 11 extract on cancer cell lines. Sample of 48hrs at 625.00 µg/Ml , 1250 µg/Ml and 2500 µg/Ml showed the most potent among others as it exhibited the lowest IC_{50} (half-maximal inhibitory concentration) for the breast cancer cell lines (MCF 7 = 220 µg/mL) compared to IC_{50} at 24hrs as depicted in **Fig. 3**. However, the IC_{50} of the *O. subscorpioidea* extract at 48hr of 2500 µg/mL was considerably the most effective compared to *O. subscorpioidea* extract at higher IC_{50} and other *O.subscorpioidea* extract at lower IC_{50}.

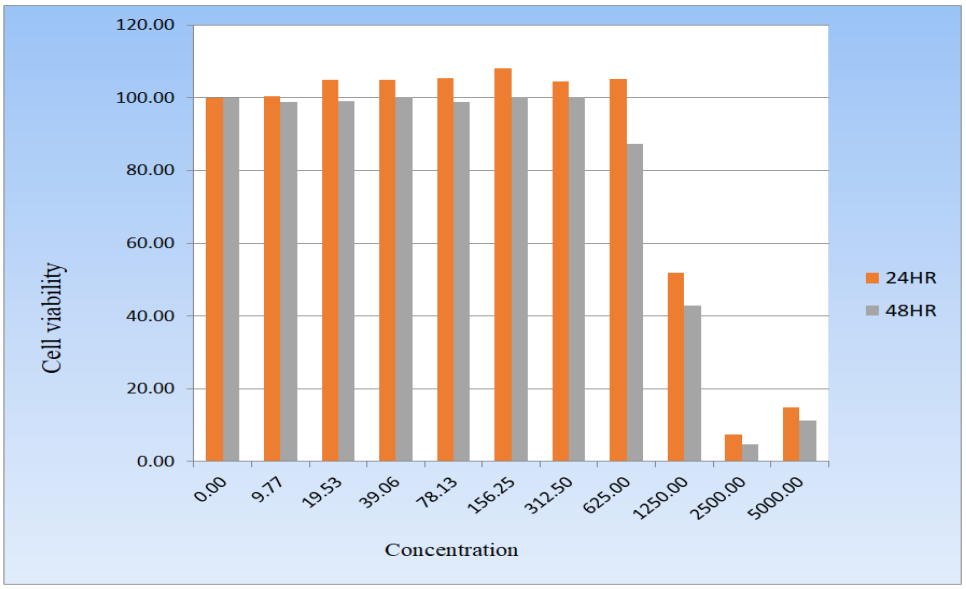


Fig. 3: The cytotoxic effect of ethanolic extract of *Olax subscorpioidea* leaves on MCF-7 cells.

Fig. 4 displayed levels of damaged nuclei in the comet assay. From the figure, the untreated cells shows a higher increase at 48hrs (9.8) compared to the untreated at 24hrs.

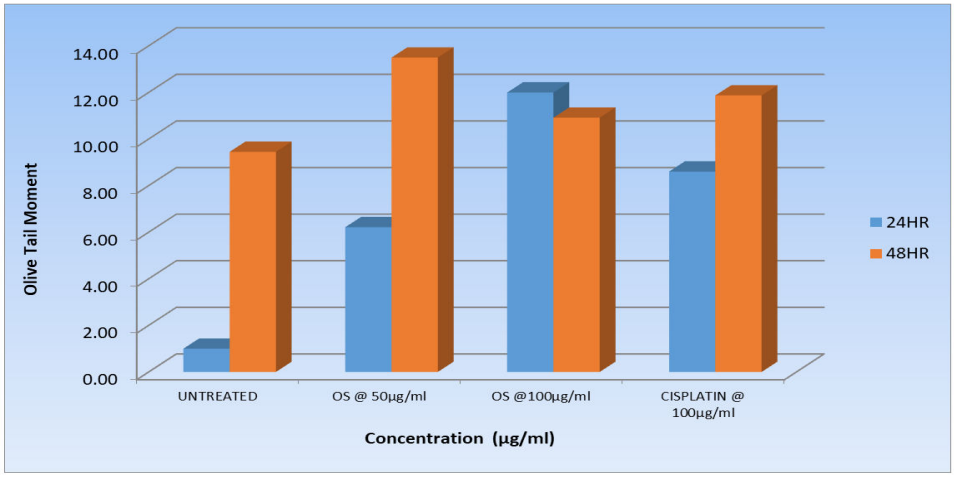


Fig. 4: Analysis of DNA damage as measured by comet assay in MCF-7 cells treated with *O. subscorpioidea*.

The *O. subscorpioidea* extract at 50µg/mL shows a higher increase of 13.5 at 48hrs compared to untreated which has a value of 9.8 and cisplatin a value of 12.8. *O. subscorpioidea* at 100µg/mL also displayed a high increase of 11.9 at 24hours compared to concentrate at 48hours. Cisplatin (standard) at 100µg/mL shows a high increase of 11.9 at 48hours compared to cisplatin at 24hrs. However from the observation, data reveals that the *O. subscorpioidea* extracts induced a higher increase of DNA damage in MCF-7 cells at 48h of treatment compared to other treatments. Moreover in comparison with the standard, there was no significant difference compared to cisplatin which is the control groups ($P > 0.05$).

4. Discussion

The ideal anticancer medicine still eludes scientists despite significant advancements in our understanding of the molecular underpinnings of cancer. Necessitating a persistent and intense search for new molecules that might be helpful in the treatment of cancer, pharmaceutical plants have long been the mainstay of cancer treatment. This is due to their therapeutic properties that have little to no side effects (Cheung-Ong *et al.,* 2013; Singh *et al.,* 1988). In this study, the active compounds, reducing power, cytotoxicity, and DNA damage of ethanolic extracts of *O. subscorpioidea* leaves against Cisplatin in MCF cell lines were investigated. This research was conducted as part of ongoing research into the anticancer potential of *O. subscorpioidea* leaves extract for breast cancer management. By using qualitative phytochemical screening, it was possible to identify secondary bioactive compounds that have been demonstrated to have pharmacological effects on cancer cells, such as saponins, tannins, cardiac glycoside, steroids, terpenoid, flavonoid, and alkaloids. According to earlier studies, *Olax subscorpioidea* contains a number of biologically produced chemicals that have pharmacological effects in the rat model (Banerjee *et al.,* 2013; Liao *et al.,* 2009; Ruiz & Hernández, 2016). In addition, many of these compounds have anti-cancerous properties (Catalano, 2016; Chikara *et al.,* 2018).

Saponins, tannins, cardiac glycoside, steroids, terpenoids, flavonoids, alkaloids, anthraquinones, and polyphenols were some of the phytochemicals identified in this study, as shown in **Table 1**. This is consistent with a previous study by Okoro *et al.* (Greenwell & Rahman, 2015) and Popoola *et al.* (Popoola *et al.,* 2020). Among the very diverse alkaloids, and particularly those of plant origin, numerous studies demonstrate the anti-proliferative and anticancer activity of many of them both in vitro and in vivo against different cancer types (Mondal *et al.,* 2019). Furthermore, the study pointed out that these chemical groups, also known as alkaloids, are found in a wide range of plants and are important for the emergence of new medicines (Carvalho *et al.,* 2020). According to the findings as shown in **Table 2**, alkaloid had the highest concentration of 23.17 in the quantitative study of the phytochemicals found in the ethanolic extract of *Olax subscorpioidea leaves*.

Phytol (24.82) and-9-Octadecenoic acid methyl este (28.47) were found to have the highest values among the primary bio-active components discovered from the results of GC-MS analysis of the ethanolic extract of *Olax subscorpioidea* constituents as indicated in **Table 3**. A component of chlorophyll called phytol is a diterpene alcohol (Taj *et al.,* 2021). It is frequently used as a flavoring agent and an aromatic element in food (Song & Cho, 2015). According to certain publications, phytol promotes apoptosis in human stomach adenocarcinomas, although the molecular mechanisms behind its cytotoxic actions at the time on cancer cells were not known (Obadoni & Ochuko, 2002). On the other hand, earlier investigations also suggested that 9-octadecenoic acid (Z)-methyl ester also contains anti-inflammatory, antiandrogenic, and anemiagenic properties (Singh *et al.,* 2008). The damaging effects of free radicals in dietary and biological systems make radical scavenging activities crucial. Free radicals are a class of organic compounds inevitably generated internally in biological systems or found externally, which are involved in a variety of degenerative diseases such as mutagenesis, carcinogenesis, cardiovascular diseases, and aging (Singh *et al.,* 2008). Free radicals can attack and damage cells, but there are many substances in food, known as antioxidants - such as micronutrients and many phytochemicals - that are able to neutralize them, thus improving the oxidative stability of food, balance pro-oxidants and other oxidative intermediates, and are very important in protecting our cells and body. They can efficiently stabilize or neutralize free radicals without damaging the cell (Chib *et al.,* 2020). The DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging activity results are depicted in **Fig. 1**, the ethanolic extract presented a significant DPPH radical scavenging activity, similar to that of ascorbic acid. This conclusion is consistent with Popoola *et al.* (Popoola *et al.,* 2020).

Numerous studies have revealed that many plant extracts have a powerful lowering ability. Because of this, a compound's reducing capacity may be a key marker of its potential antioxidant action (Oladipupo *et al.,* 2019). **Fig. 2** illustrates this. The reducing power of the extract is demonstrated by contrasting it with a dietary antioxidant such as ascorbic acid. The research showed that *Olax*

subscopioidea ethanolic extract had better reducing power than ascorbic acid. This may be due to the antioxidant and redox characteristics of the *O. subscorpioidea* ethanolic extract. The cytotoxicity of *Olax subscorpioidea* extract against the MCF breast cancer cell line was also investigated in the present study. The compelling basis for the present study is the earlier observations that *Olax subscorpioidea* demonstrated anticarcinogenic activity in diverse in vitro and in vivo studies across various diseases (Agbabiaka & Adebayo, 2021; Cheung-Ong *et al.,* 2013; Popoola *et al.,* 2020). Such effects mostly apply to compounds derived from Olax subscorpioidea which have possible clinical applications. However, the literature surrounding this topic is lacking. The constituents of ethanolic extract of *O. subscorpioidea* leaves were found to play an important role that can be reflected by IC50 of 2500 after 48 hours (**Fig. 3**), which agreed with the results of leaf extract showed a more potent activity.

DNA targeting based anticancer therapies led to the discovery of several anticancer agents, such as cisplatin, doxorubicin, 5-fluorouracil, etoposide, and gemcitabine (Singh *et al.,* 1988). The SCGE assay, also known as the comet assay, was created by Ostling and Johanson in 1984 and later modified by Singh *et al.* (Banerjee *et al.,* 2013). It is a quick, sensitive, and reasonably easy method for detecting DNA damage at the level of individual cells (Liao *et al.,* 2009). This approach produces a picture that resembles a "comet" with a distinct head made of intact DNA and a tail made of broken or damaged fragments of DNA. During electrophoresis, the amount of DNA released from the comet's head depends on the substance being tested for effectiveness (Ruiz & Hernández, 2016). Compared to Cisplatin given up to a dose of 100 mg/kg, as shown in figure 4 after 48 hours, the amount of tail DNA from ethanolic extracts of *Olax subscorpioidea* leaves at the 50 mg/kg dose was significantly greater. These results showed that the leaf extracts of tested plants with significant DNA damage show its motivation to be suited for anticancer agents. These DNA-damaging effects observed as mentioned above, may serve the purpose of further necessitating their incorporation within anticancer treatment protocols. This study shows that this plant leaf extract has antioxidant properties that may be useful in cancer chemotherapy and chemoprevention (Catalano, 2016; Ruiz & Hernández, 2016).

5. Conclusion

Natural products of plant origin have attracted much interest in recent years due to their wide variety of pharmacological effects, with antioxidant and anticancer activities being one of many. Results from the current study clearly showed that the leaves of *O. susscorpioidea* possess potential chemopreventive activity via free radical scavenging and oxidative stress protection, as well as chemotherapeutic activity associated with DNA damage. Perhaps these woven activities will reveal something about some of the traditional guises of these plant extracts as herbal remedies. Furthermore, the study indicates that extract of *O. subscorpioidea* may be a good candidate as either a food or a supplement to improve the effectiveness of treatment with conventional medicines on mammary carcinoma.

6. Author Contributions

The authors were involved in writing, planning, assembling, and editing the final version of the manuscript.

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8. Conflicts of Interest

The authors declare that there are no conflicts of interest to this article.

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