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Original Research Article

Anticancer Activity of Ethyl Acetate Fraction and Ethanol Leaf Extract of *Olax subscorpioidea* against DMBA-Induced Female Rats

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ABSTRACT

Breast cancer continues to be a major contributor to cancer-related deaths in developing nations. *Olax subscorpioidea* is used in Nigerian traditional medicine as a treatment for cancer. The study examined the effects of *Olax subscorpioidea's* ethyl acetate fraction (OSEA) and ethanol leaf extract (OSE) on 7,12-Dimethylbenz(*α*)anthracene (DMBA)-induced breast cancer in female Sprague-Dawley rats. The anticancer, antioxidant and anti-inflammatory activities of the extracts were evaluated using established procedures. The study involved 40 female Sprague-Dawley rats with an average weight of 110 ± 20 g. The rats were given a dose of 80 mg/kg of DMBA to stimulate proliferation. Subsequently, OSEA, OSE (250 mg/kg BW), and tamoxifen (6.6 mg/kg BW) were administered. The trial spanned a duration of 22 weeks. The study evaluated the impact of the treatment on various aspects such as body weight, organ weight, liver and kidney function, oxidative stress indicators, oestrogen levels, Interleukin 6 (IL-6), Cancer antigen 153 (CA-153), and mammary tissue histology. It was found that body weight, Superoxide dismutase (SOD), Reduced glutathione (GSH), liver enzymes, and renal function increased significantly with OSEA and OSE therapy. The levels of oestrogen, IL-6, CA-153, and Malondialdehyde (MDA) decreased significantly. The histological study revealed that OSEA and OSE had a positive impact on acini normalisation and the inhibition of breast ductal cell growth. The study found that OSEA and OSE demonstrated promising effects against cancer, as well as antioxidant and anti-inflammatory properties, in rats with DMBA-induced breast cancer. The results offer scientific support for the traditional use of *Olax subscorpioidea* as a potential natural remedy for breast cancer.

***Keywords*:** Antioxidant, anticancer, antiinflammation, oestrogen, 7, 12-Dimethylbenz(*α*)anthracene, *Olax subscorpioidea*.

Introduction

Cancer is a devastating disease that impacts people of all ages and is unfortunately the leading cause of death worldwide.[1] In 2020, there were over 2.3 million cases of breast cancer and 685,000 lives lost. It is worth noting that breast cancer is highly prevalent and ranks as one of the top causes of mortality among women globally.[2] Countries that have undergone transitions tend to have higher rates of breast cancer compared to developing nations. Women in emerging nations experienced a 17% higher breast cancer mortality rate compared to women in transitional countries.[2] Breast cancer mortality and incidence rates are significantly elevated in underdeveloped countries.[3] It is the most common form of cancer among Nigerian women.[4] Genetics, hormones, reproductive, and environmental factors are just a few of the variables that affect breast cancer development. The presence of oestrogen in the body can lead to an increase in breast epithelial cell proliferation, survival, DNA damage, and oxidative stress, ultimately contributing to the development of breast cancer.[5]

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Oxidative stress can cause cell damage, trigger inflammation, and contribute to the development of cancer.[6] There is evidence suggesting that exposure to environmental pollutants, such as PAHs, can potentially elevate the risk of breast cancer. This is thought to occur through mechanisms involving oxidative stress and the activation of oestrogen receptors.[7] 7, 12-Dimethylbenz(α)anthracene (DMBA), a polycyclic aromatic hydrocarbon, is commonly employed to induce breast cancer in rats. The DNA is damaged by DMBA-3,4-diol-1,2-epoxide through the addition of adenine and guanine residues because of liver and breast tissue metabolism.[8] Breast cancer can be treated through various methods, such as targeted therapy, surgery, chemotherapy, radiation, and hormone therapy. These medicines come with challenges such as resistance, recurrence, high cost, toxicity, and adverse effects. It is crucial to identify alternative or complementary therapies that offer improved efficacy, safety, and cost-effectiveness.[9] Medicinal plant alkaloids, flavonoids, terpenoids, phenolics, and saponins exhibit a variety of biological effects. Some examples are anticancer, antioxidant, anti-inflammatory, and immunomodulatory.[10] There is a significant body of research supporting the preventive effects of medicinal herbs and their derivatives on breast cancer.[11] A recent study highlights the significant impact of curcumin, a phenolic component of turmeric (*Curcuma longa*), on inhibiting breast cancer cell invasion, metastasis, and proliferation. It achieves this by modulating key signalling pathways such as NF-κB, PI3K/Akt, MAPK, and nt/*β*-catenin.[12] Vinblastine and vincristine are alkaloids found from the *Madagascar periwinkle* (*Catharanthus roseus*). They disrupt the movement of microtubules, which stops cells from growing and killing themselves. These properties make them highly effective chemotherapies for breast cancer.[13] There are several Nigerian medicinal plants that have been found to treat various diseases,

including cancer.[14] It is worth mentioning that only a select few of these plants have demonstrated anticancer properties.[15] Shrubs and small trees, such as *Olax subscorpioidea* (Oliv) a member of Olacaceae, thrive in the tropical regions of Africa. Some common names for this plant are sandpaper shrub, African olive, akata (Yoruba), ububa (Igbo), and kada (Hausa). The stem bark, leaves, and roots of *Olax subscorpioidea* are used in traditional Nigerian medicine to address a range of health concerns, including cancer, malaria, diabetes, ulcers, inflammation, pain, and infections. The phytochemical analysis of *Olax subscorpioidea* revealed the presence of terpenoids, flavonoids, alkaloids, tannins, saponins, and steroids. In pharmacological research, it was found that *Olax subscorpioidea* fractions and extracts exhibited analgesic, anti-inflammatory, antibacterial, antidiabetic, antimalarial, and antioxidant properties.[16] Unfortunately, there is still a limited understanding of the breast cancer anticancer mechanisms of *Olax subscorpioidea*. Thus, the study evaluated the anticancer properties of the ethyl acetate fraction and ethanol leaf extract of *Olax subscorpioidea* can help fight cancer. The antioxidant status, Cancer antigen 153 (CA 15-3), oestrogen, interleukin 6 (IL-6), and mammary gland histology were assessed in rats with DMBA-induced breast cancer.

Materials and Methods

The materials utilised in this study comprises of 7, 12-Dimethylbenz(*α*)anthracene (DMBA) (Sigma), superoxide dismutase (SOD), malonaldehyde (MDA), reduced glutathione (GSH), and Randox kits for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), Creatinine, and Urea. Elisa kits for cancer antigen (CA-153) (Sunlong, China), elabscience Quickey Pro Rat E2 (Estradiol), and elabscience Rat interleukin 6 (IL-6).

Collection and identification of plant samples
The collection and identification of *Olax subscorpioidea* (Oliv) leaves follows a similar procedure as described in previous work.[17]

Extraction and differential solvent fractionation process
The extraction and differential solvent fractionation process follows a similar procedure as described in our previous work.[17]

Experimental animals
The study utilised a sample of 40 adult female Sprague Dawley rats averaging 110 ± 20 g and an age range of 6–8 weeks. The Covenant University animal handling facility received the animals from the University of Lagos, Department of Physiology, Idi-Ara Lagos central animal house. The animals were given a two-week period to acclimatise before the trial. The rats were provided with rodent pellets for their diet and were consistently given access to water throughout the duration of the study. The experiment was conducted in a controlled laboratory setting, ensuring regulated air, 12:12 h light/dark cycles, 22 ± 30ºC ambient temperature, and 50 ± 10 % humidity.

Clearance for ethics
The criteria set forth by the National institute of health (NIH) office of animal care and use (2016) were adhered to for animal care and experimentation. Prior to commencing the animal research, the Covenant University Health Research Ethics Committee granted approval and assigned a protocol number (CHREC/162/2022).

Tumour induction and dosage
The approach described by Zingue *et al*.[18] resulted in the development of mammary tumours. The control group was given olive oil alone, while the experimental group received 80 mg/kg DMBA dissolved in 1 ml of olive oil orally. The dose of tamoxifen was 6.6 mg/kg.[19] The *Olax subscorpioidea* ethanol leaf extract and ethyl acetate fraction were given at a dosage of 250 mg/kg.[20] Weight measurements and palpations of the rats were conducted on a weekly basis during the trial to monitor the progression of tumours. The experiment meticulously documented the deceased rats and euthanized the remaining rats.

Experimental design
Animals were grouped into five groups. Each group contained eight rats each. The groupings were I, II, III, IV, and V. The experiment lasted for 22 weeks. The control group (Group I) received oral administration of olive oil three times a week for the duration of the study. The rats in Group II were administered 80 mg/kg DMBA in olive oil and provided with regular feed throughout the study. Group III (tamoxifen-treated group) rats received a dose of 80 mg/kg DMBA and were administered 6.6 mg/kg of tamoxifen orally. Group IV (OSEA-treated) rats were given 250 mg/kg of OSEA orally thrice a week after 16 weeks of DMBA induction. Group V following a 16-week period of DMBA induction, the rats were administered OSE orally thrice weekly at a dosage of 250 mg/kg.

Liver and Blood Collection
The rats were deprived of food and euthanized in a humane manner following the experiment. The blood samples for biochemical analysis were collected from rats through the retro-orbital venous plexus into lithium-heparinized tubes. The plasma was spun at a speed of 3000 rpm for a duration of 15 mins and stored at a temperature of -20ºC until it was ready for analysis. The liver was subsequently removed, cleansed in a 0.9% normal saline solution, dried, and weighed.[21] The liver was thinly sliced using a sterile scalpel blade and homogenised in a 1:10 w/v phosphate buffered saline solution at pH 7.0 for biochemical analysis. The supernatants were stored at -18ºC for analysis after centrifuging the homogenate at 5000 rpm for 10 mins.

Analysis of histopathology in mammary tissue
The mammary tissues of the rats were preserved in 10 % neutral buffered formalin for 24 hrs after sacrifice. The tissues underwent a thorough preparation process, including washing, dehydration in alcohol, and clearing in xylene, before being embedded in paraffin. 2-3 µm sections cut by the rotary microtome were stained with haematoxylin and eosin.[22]

Estimating Liver Marker Enzymes and Kidney Functions
Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, and urea levels of the kidney were tested using the Randox diagnostic kit and following the manufacturer's instructions.

Oxidative Stress Test
Evaluating Lipid Peroxidation
The technique used by Ohkawa *et al*.[23] effectively measured lipid peroxidation. Trichloracetic acid (15%), thiobarbituric acid (0.375%), and hydrochloric acid (0.25 mol/L) were mixed in 50 ml of distilled water to make the stock solution. The sample was mixed with the stock solution and then heated in a boiling water bath for 30 mins. The clear supernatant of the sample was obtained by chilling it and then subjecting it to centrifugation at 2000 rpm for 15 mins. The absorbance at 535 nm was measured against a blank.

Assessing superoxide dismutase (SOD) concentration
The Markland technique has been modified to measure superoxide dismutase activity using pyrogallol oxidation.[24] The sample was mixed with 9.0 mL of clean water for a duration of 10 mins. The mixture was placed in a centrifuge tube along with 10 mL of distilled water. The supernatant was obtained by centrifuging the mixture for 15 mins at 4,000 rpm. 2.35 mL of Solution A (a Tris-HCl buffer solution with EDTA at pH 8.2) and 2.00 mL of distilled water was added to 10 mL test tube to obtain blank autoxidation rate. After adding 0.15 mL of Solution B (4.5 mmol/L pyrogallol in HCl), the test tube was vortexed. A sample was collected from the test tube following a 325 nm absorbance. Another aliquot was collected after 1 min. The comparison of the absorbance of the two aliquots revealed the occurrence of pyrogallol autoxidation. The 325-nm absorbance value should be 0.060. The sample test adhered to the specified procedure. A 200 µL sample was added before applying Solution B. The sample solution was diluted by 50% to reduce autoxidation.

Estimating reduced glutathione (GSH) levels

GSH was measured using the method described by Prins & Loose.[25] 0.5 ml of tissue homogenate was mixed with 4 ml of 0.08 N H_2SO_4 in a test tube. The protein precipitation was carried out using a 0.5-ml tungestate solution, allowing it to sit at room temperature for 10 mins. The liquid vigorously shook for 5 mins. The stopper was removed, and the solution was allowed to sit for a few mins to prevent the supernatant fluid from crusting during centrifugation. The suspension was subjected to centrifugation for 20 mins at 860 G. A volume of 2 mL of clear extract was carefully transferred into a 2.5 mL tris buffer, along with 0.2 mL of 5,5-dithio-bis-2-nitrobenzoic acid (DNTB). For the reagent blank, 2 mL of distilled water was used instead of tissue homogenate. After 30–60 secs, the colour developed, and the optical density was measured at 412 nm.

Assay for interleukin 6 (IL-6) and Estradiol

The Elabscience (USA) Quickey Pro Rat E2 (Estradiol) enzyme-linked immunosorbent experiment (ELISA) kit (Cat No. E-OSEL-R0001) was used to measure oestrogen levels. The Rat IL-6 (Interleukin 6) ELISA kit (Cat No. E-EL-R0015) from Elabscience (USA) was used to measure IL-6. The assays were performed according to the manufacturer's instructions.

Assay for cancer antigen-153 (CA153) Serum Tumour Maker

The levels of the rat mammary cancer marker (CA-153) were measured using an ELISA kit from Sunlong in China. The Elisa kit accurately measures the levels of CA-153 in various biological samples, such as rat serum, plasma, culture media, and other fluids. The assay was conducted in accordance with the instructions given by the manufacturer.

Statistical analysis

SPSS 23 (IBM Corporation, Armonk, NY, USA) was used for conducting one-way ANOVA and Duncan's test for post-hoc multiple comparisons. All the data were determined to have statistical significance at a p-value ≤ 0.05. The data presents the average ± standard deviation of the experiment in triplicate.

Results and Discussion

In Sprague-Dawley rats, the chemo preventive effects of OSEA and OSE on DMBA-induced breast cancer were investigated. From a chemical standpoint, DMBA is associated with breast cancer in female rats. DMBA, an aromatic pollutant, has been linked to the development of cancer. It has been known to contribute to the development of mammary gland tumours in rats. The development of DMBA-induced ductal breast cancer in rats is hormone-dependent, like most human breast cancers.[26] This study found that OSEA and OSE were effective in reducing DMBA-induced mammary cancer. Weight loss is a common occurrence in individuals with cancer due to physiological abnormalities.[27] Table 1 shows noticeable differences in weight among all the experimental groups. The rats in the DMBA control group weighed significantly less compared to those in the normal control group. Body weight in rats treated with OSEA and OSE (250 mg/kg bwt) showed signs of improvement, suggesting a potential reduction in tumours. There is evidence to suggest that OSEA and OSE extract have the potential to inhibit tumour growth *in vivo*. Previous studies have indicated that DMBA has a significant impact on reducing rat weight.[28] The liver and kidney weights of the DMBA-control and normal groups showed no significant differences. Indicators of liver health include aspartate, alanine, and alkaline phosphatases. Rats with induced tumours exhibited elevated levels of AST, ALT, and ALP.[29] There may be an increase in ALT, ALP, and AST levels if liver cytoplasm enters the bloodstream, indicating potential tissue damage.[27] The activity of ALP has various effects on liver cell functions, including membrane permeability, metabolite transport, protein synthesis, and glycogen metabolism.[29] The AST, ALT, and ALP activity in the normal control group was significantly lower compared to the DMBA control group as shown in Table 2. The OSEA and OSE treatments showed a reduction in AST, ALT, and ALP levels, indicating the stabilisation of the plasma membrane.

Konan et al.[30] found that the leaf extract of *Olax subscorpioidea* provided protection to the livers of rats exposed to CCl_4. The function of the kidney is to produce essential substances and eliminate waste products from the body. Drug excretion and metabolism can be delayed in individuals with renal impairment, which can lead to an increased risk of toxicity.[31] Elevated levels of creatinine and urea were observed in the DMBA control group, indicating nephrotoxicity. The levels of creatine and urea in the DMBA control group were significantly reduced following the OSEA and OSE treatments. OSEA and OSE effectively protected rodent kidney from DMBA induced injury. Free radicals from DMBA's oxidative metabolism bind to nucleophilic sites on macromolecules in cells, which starts the cancer process. Free radicals and biochemical metabolic processes are the main causes of cancer. Antioxidants are primarily responsible for combating reactive oxygen species (ROS).[32] SOD is essential in combating oxidative stress. Extensive research has been conducted on the pharmacological and physiological applications of superoxide dismutase (SOD).[33]

Table 1: Effect of *Olax subscorpioidea* on body weight (g), kidney weight (g), and liver weight (g)

Groups (g)	Normal Control	DMBA Control	DMBA + Taximofen	DMBA + (OSEA)	DMBA + (OSE)
Body weight	196.3 ± 1.52^{e}	$167.67.0 \pm 3.05^{a}$	189.0 ± 2.00^{d}	175.3 ± 0.57^{b}	182.3 ± 2.30^{c}
Kidney weight	$1.30 \pm 0.06^{b,c}$	$1.10 \pm 0.00^{a,b}$	1.40 ± 0.10^{c}	1.40 ± 0.06^{c}	$1.03 \pm 0.07^{a,b}$
Liver weight	6.37 ± 0.15^{a}	6.43 ± 0.18^{a}	6.80 ± 0.61^{a}	5.93 ± 0.90^{a}	6.97 ± 0.84^{a}

Data are represented as mean ± S.D of triplicates. Mean with different superscript (a-e) are significantly different at ($p < 0.05$)

Table 2: Effect of *Olax subscorpioidea* on liver marker enzymes and kidney function

Groups	Normal Control	DMBA Control	DMBA + Taximofen	DMBA + (OSEA)	DMBA + (OSE)
AST (U/l)	15.53 ± 2.00^{a}	21.60 ± 1.52^{c}	18.40 ± 1.63^{b}	18.16 ± 0.72^{b}	18.07 ± 0.13^{b}
ALT (U/l)	18.15 ± 0.75^{a}	50.45 ± 0.19^{e}	38.056 ± 0.18^{d}	34.36 ± 0.39^{b}	31.67 ± 0.00^{c}
ALP (U/l)	56.12 ± 2.33^{a}	77.72 ± 2.25^{d}	$72.08 \pm 3.10^{c,d}$	$66.24 \pm 1.04^{b,c}$	63.14 ± 2.89^{b}
Creatinine (µmol/l)	53.33 ± 7.88^{a}	120.43 ± 1.48^{d}	$69.68 \pm 5.16^{a,b}$	$88.60 \pm 11.91^{b,c}$	94.62 ± 3.94^{c}
Urea (µmol/l)	3.43 ± 0.21^{a}	24.82 ± 7.61^{c}	6.52 ± 0.69^{b}	7.23 ± 1.00^{b}	5.88 ± 0.54^{b}

Data are represented as mean ± S.D of triplicates. Mean with different superscript (a-d) are significantly different at ($p < 0.05$)

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Table 3: Effect of *Olax subscorpioidea* on oxidative stress parameters in experimental rat groups

Groups	Normal Control	DMBA Control	DMBA + Taximofen	DMBA + (OSEA)	DMBA + (OSE)
SOD (U/ml)	378.35 ± 10.64^{c}	227.61 ± 7.54^{a}	333.58 ± 9.03^{b}	329.58 ± 5.22^{b}	329.58 ± 7.19^{b}
GSH (mg/g wet tissue)	0.02 ± 0.001^{d}	0.009 ± 0.001^{a}	$0.012 \pm 0.000^{b,c}$	$0.011 \pm 0.000^{a,b}$	0.014 ± 0.001^{c}
MDA (nmol/g wet tissue)	0.23 ± 0.002^{a}	0.32 ± 0.004^{c}	0.27 ± 0.000^{b}	0.27 ± 0.001^{b}	0.27 ± 0.002^{b}

Data are represented as mean ± S.D of triplicates. Mean with different superscript (a-d) are significantly different at ($p < 0.05$)

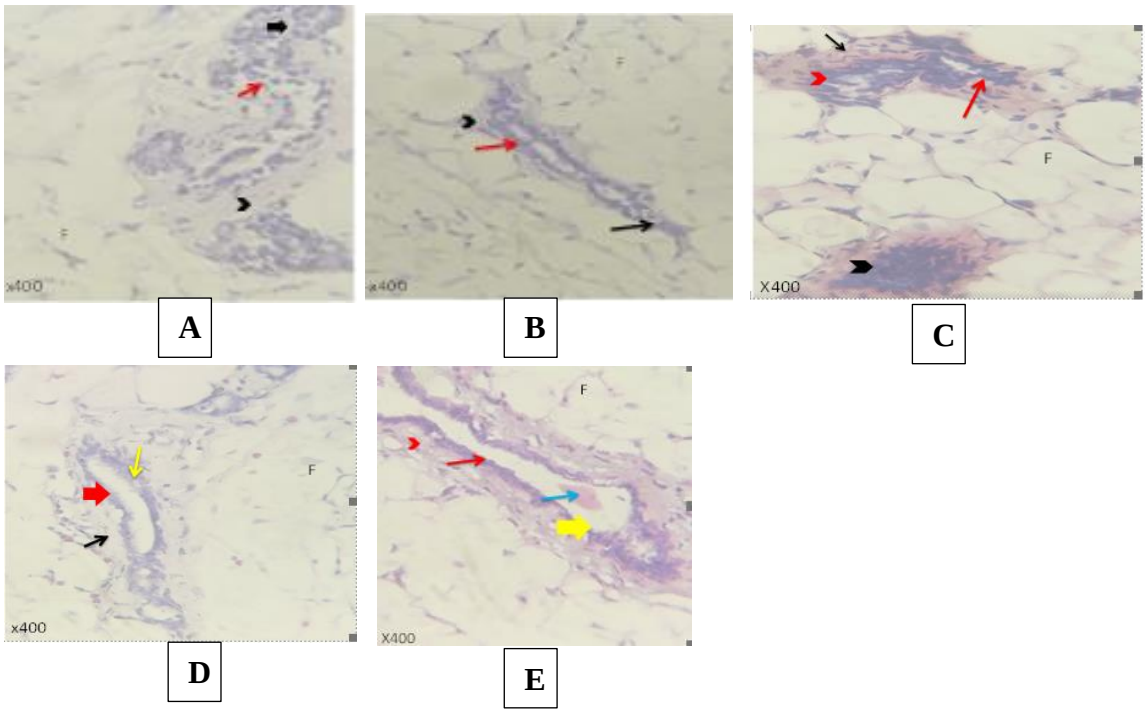

Figure 1: Histopathology section of mammary tissue (A) Normal group, (B) DMBA group, (C) Tamoxifen treated group, (D) OSEA treated group and (E) OSE treated group

Superoxide dismutase is responsible for removing oxygen. The levels of Cu-, Zn-, and Mn-SOD were decreased in cancer cells.[33] The initial ROS defence in humans is SOD. The process of SOD involves the conversion of free radicals and anions into hydrogen peroxide and oxygen. The data in Table 3 clearly shows a noticeable decrease in total SOD activity in the DMBA group when compared to the normal group ($P < 0.05$). The activity of SOD was found to increase significantly in the treated groups. It is possible that OSE and OSEA have the potential to restore SOD activity. This result is consistent with the findings of Krishnamoorthy and Sankaran[34] discovered a decrease in hepatic SOD activity in rats treated with DMBA. The study highlights the antioxidant properties of *Olax subscorpioidea* leaf extract, suggesting its potential to prevent the formation of free radicals that can lead to breast cancer. The MDA levels showed a significant increase ($P < 0.05$) in the DMBA group when compared to the normal and treated groups. The increase significantly dropped in the treated rats. The measurement of lipid peroxidation was conducted using MDA. MDA is formed through the peroxidation of polyunsaturated fatty acids. There is an increased risk of cancer associated with MDA. The MDA level is a measure of cellular oxidative stress. In a study conducted by Jelic *et al.*[35] it was observed that there was a significant increase in MDA levels following the progression of breast cancer from stage III to stage IV. The level of reduced glutathione (GSH) to oxidised glutathione (GSSG) was significantly lower in the DMBA control group compared to the normal and treatment groups. The levels of GSH were increased by OSEA and OSE, which act as non-enzymatic secondary defence mechanisms against reactive oxygen species. This helps to protect cells from oxidative damage. Aquilano *et al.*[36] state that GSH serves as a crucial hydrophilic antioxidant, safeguarding cells against both external and endogenous toxins. GSH has a significant impact on the process of ageing as well as inflammation, infections, and various diseases. The risk of cancer and oxidative stress in animals are both increased by DMBA. The findings of our study align with recent research.[34] The DMBA-control breast tissue revealed the presence of proliferative infiltrating hyperchromatic ductal cells and a compromised basement membrane. The DMBA-induced breast tissue photomicrographs showed that OSEA and OSE were able to restore the acini and basement membrane to their normal state. Figure 1a-e showcases tissue restoration of OSEA and OSE tissues, with the presence of new epithelial cells and fully formed acini. Figure 2 shows that the DMBA control group had significantly higher oestrogen levels compared to the normal control and treatment groups. OSEA and OSE have a significant impact on reducing oestrogen levels. Akhouri *et al.*[31] suggest a potential link between oestrogen and progesterone and the development of breast cancer. Oestrogens can lead to an increase in ROS, potentially causing oxidative damage.[37] In line with the findings of Mvondo *et al.*[37] this study also observed an increase in mammary gland estradiol levels in female rats after exposure to DMBA. The presence of breast tumours leads to an elevation in estradiol levels, which can be attributed to the invasion of cancer cells and the growth of lobules. Cell development is dependent on the presence of estrogens.[37] Figure 3 demonstrates a notable rise in interleukin-6 levels in the DMBA-control group when compared to the normal group ($P < 0.05$). The high increase in the DMBA control group was significantly reduced by the OSEA and OSE extracts. Pro-inflammatory IL-6 is produced by malignant cells and their neighbouring cells in the tumour microcirculation.[38,39] Interleukin-6 (IL-6) promotes cancer cell growth and development. The results of our study provide confirmation of the correlation between elevated IL-6 levels in the blood of cancer patients and the development of tumours. CA15-3 levels have been found to be a reliable indicator of breast

cancer prognosis. This marker is highly indicative of patient disease progression, regression, or stability.[40] Figure 4 shows that the DMBA control group had noticeably higher CA15-3 levels compared to the normal control group. CA-153 levels showed a significant reduction in the treated groups. Indicators of tumours in the patient's blood or urine are generated by cancerous cells. CA15-3-40 is used as a biomarker for breast cancer and for evaluating therapeutic effectiveness.[41] MUC-1, also known as Mucin1, plays a crucial role in the regulation of CA15-3 synthesis. MUC-1 is a transmembrane protein that is predominantly present in epithelial cells.

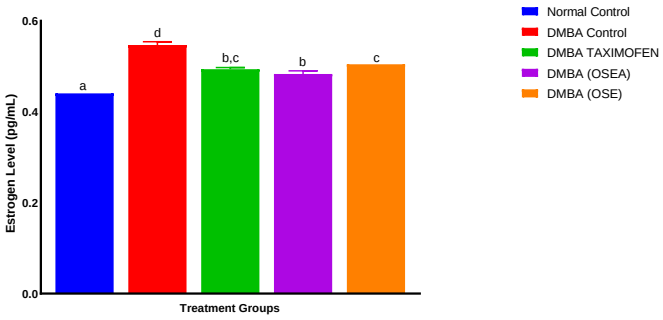

Figure 2: Effect of OSEA and OSE on the mammary gland level of estrogen in DMBA induced mammary rats

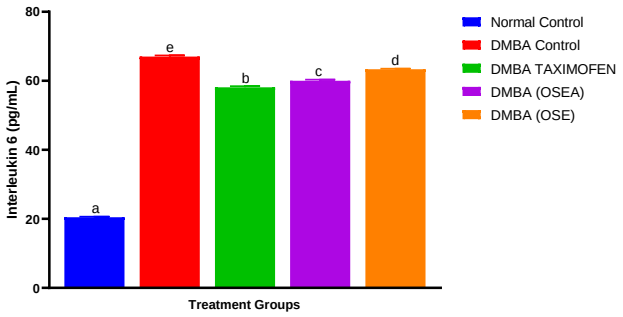

Figure 3: Effect of OSEA and OSE on the mammary gland expression of Interleukin 6 activity in DMBA induced mammary rats

Conclusion

In this investigation, it was found that OSEA and OSE exhibited anti-proliferative, antioxidant, and anti-inflammatory properties. This research provides support for the use of *Olax subscorpioidea* in traditional cancer treatment. There is potential for this plant material to be used in the treatment of breast cancer.

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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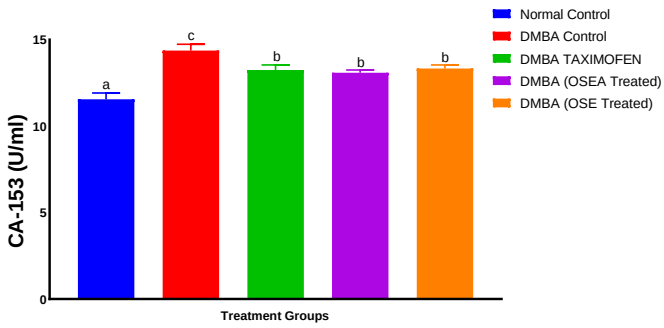

Figure 4: Effect of OSEA and OSE on mammary carcinoma marker (CA-153) in DMBA induced mammary rats

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