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Investing Chemicals in Oleander Leaf Extract as an Anti-breast Cancer

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Abstract

Cancer is still crucial for the whole world and is a total burden on health; it is the main thing that we should investigate and find new therapeutic ways of treatment. This study investigates Oleander's (Nerium oleander) leaf extract's possible anticancer effects on human breast cancer cells in a test tube. Our study evaluated the cytotoxic effects of Oleander leaf extract on the Michigan Cancer Foundation-7(MCF-7) using the MTT assay. The results were compared with their effects on normal human dermal fibroblasts (HdFn). Preliminary results show differential cytotoxicity, with the extract demonstrating greater toxicity to MCF-7 cells ($IC_{50} = 32.91\mu g\ mL^{-1}$) compared to HdFn cells ($IC_{50} = 129.7\mu g\ mL^{-1}$). This suggests a degree of selectivity in its anticancer activity, a desirable characteristic in cancer therapeutics. The antioxidant potential of the extract was studied using the DPPH assay. An IC50 value less than 50 µg/mL indicates a strong antioxidant activity (The highest activity was documented at a concentration of 12.5 $\mu g\ mL^{-1}$) whereas an IC50 value above 100 $\mu g\ mL^{-1}$indicates weak activity

Key words: MCF-7, anticancer, Oleander, Breast cancer, Breast anticancer, plant extract

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Introduction

Breast cancer ranks as the second leading cause of death among women. It's a multifaceted disease, involving different cell types, and continues to pose a significant challenge to prevention efforts around the globe. Early detection is one of the greatest efficient methods to prevent breast cancer [1,2].
The American Cancer Society forecasts the number of new cancer cases and deaths in the USA each year and compiles the most recent cancer prevalence, survival data, and mortality [3].

According to the presence of estrogen or progesterone receptors and ERBB2 gene amplification, breast cancer can be classified into three main subtypes. The risk profiles and treatment approaches for the three subtypes differ. Tumor subtype, anatomic cancer stage, and patient preferences all influence the best treatment for each patient [3subtypes are determined by the preselection [4,5]. Since the Vedic period, plants for medicinal purposes have been used. Medicinal plants have been used to treat and prevent various diseases and epidemics for a long time. In addition to these, some medicinal plants can be used for coloring, flavoring, and preserving food. It is not just the leaf, stem, and flower; every part of the plant possesses its very own medicinal properties. Secondary metabolites that are present in medicinal plants are the ones that are important in the treatment of many diseases, and the pharmaceutical industry also uses them in its production. Many of the plants have antioxidants, insecticidal, anti-inflammatory, antibiotic, anti-parasitic, and anti-hemolytic

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properties [6,7]. One of the most important examples of these plants is the oleander plant.

Drought tolerance is a feature of oleanders, Mediterranean-bred evergreen plants in the Apocynaceae family. Thevetia peruviana (yellow oleander) and Nerium oleander) There are two common oleanders.

Humans, animals, and certain insects are all poisoned by all parts of the oleander plant. Oleander active compounds have numerous biological activities [8-10]. The plant's poisoning effects caused hemorrhage and severe negative changes in the lung, lesions and infiltration of inflammatory cells into portal spaces with scattered necrosis of hepatocytes in the liver, and cardiac toxicity of the plant in the heart, which caused varying degrees of hemorrhage, myocardial degeneration, and necrosis. In electrocardiograms, it also caused arrhythmia, sinus bradycardia, and a prolonged P-R interval [11]. This plant species also produces secondary metabolites with pharmacological applications, such as alkaloids, flavonoids, and steroids. Among the most important pharmacological activities are antibacterial, larvicidal, anticancer, and antidiabetic properties [12].

1. Methodology

1.1. Preparation of Oleander Leaf Extracts

The leaves of an Oleander plant with a white flower were collected in the Nile district of Babylon Governorate, dried in cleaned room with ventilation of fan for two weeks after that grinding by using electrical grand and dissolved in ethanol at concentrated 95% for 48 hours in container contain ice in refrigerator, extracted using Buchner devices with membrane Filter 0.8 µm pore size, and dried.

1.2. 1,1-diphenyl-2-picrylhydrazyl (DPPH Solution):

It was made by melting 0.01g DPPH Radical in a Methanol-Dimethyl sulfoxide (DMSO) 9:1 (v/v) mixture.

1.3. Ascorbic acid Solution:

It was made by dissolving 0.01g of ascorbic acid in a 9:1 (v/v) mixture of Methanol and DMSO.

1.4. 3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT Assay)

Using the MTT ready-to-use kit, the cytotoxic effect of Multiple concentrations (6.25, 12.5, 25, 50, 100, 200, and 400 g/mL) of Oleander leaf extract was tested:

A- Contents of the Kit

1. A solution of MTT (1mL in 10 vials).
2. 50 mL solubilization solution in two bottles.

2. Results and discussion

2.1. DPPH assay

When the DPPH evaluation is performed, it is the measure of the oleander leaf extract as to its ability to scavenge the free radicals that form via the DPPH is conducted. The outcomes of the analysis are expressed in terms of the percentage of inhibition of free radicals [11, 12].

In the DPPH test, Oleander leaf extract is used to compare the antioxidant activity of a material with that of ascorbic acid (Vitamin C), which is an already known antioxidant material. Ascorbic acid is one of the most studied powerful antioxidants; its strong ability to scavenge the DPPH radical is well documented. Usually, studies on vitamin C serve as a reference in comparing other materials against it. This is because ascorbic acid can easily donate its electrons to reduce DPPH and remove its radical properties, hence changing color from purple to yellow.

Scavenging efficiency of Oleander leaf extract towards DPPH depends on the types of antioxidants present and their levels. Various compounds such as cardiac glycosides, flavonoids, and phenolic acids are reported in Oleander, out of which only a few exhibit antioxidant properties. The DPPH assay of ascorbic acid and Oleander leaf extract was performed under the same conditions. A comparison can be drawn by evaluating the percent scavenging

activity at different concentrations—200, 100, 50, 25, and 12.5 µg/ml—of the extracts, alongside ascorbic acid.

The study tested various concentrations of the extract at levels of 12.5, 25, 50, 100, and 200 µg/ml. The results showed that as the IC50 value decreased, the antioxidant activity of the extract increased. An antioxidant activity level is considered high if it is below 50 µg/mL (with the highest concentration tested being 12.5 µg/mL), while anything above 100 µg/mL indicates weak activity. As shown in Tables 1 and 2.

The graph of the DPPH scavenging activity percent of the Oleander leaf extract Concentration can also be used to determine the antioxidant activity of the extract. The graph shows efficiency decreases above 100µg/ml and increases below 100µg/ml, and the best significant value was recorded at a concentration of 12.5 µg/ml. Notes the steep decrease in absorbance over time, the extract has strong antioxidant activity.

Table 1: Scavenging Activity of DPPH Radical by Oleander Leaves Extract Compared with Ascorbic Acid (Vit C)

Concen.	Oleander leaf extract (OLE) IC50 = 10.30	Ascorbic Acid (Vit C) IC50 = 20.10
	Mean ∓SD	Mean ∓SD
200.00	77.86 ∓0.9976	79.98 ∓2.692
100.00	76.04∓1.782	72.18 ∓3.307
50.00	65.47 ∓2.172	54.48 ∓2.412
25.00	50.54 ∓5.014	40.43 ∓7.081
12.50	33.41∓8.782**	17.63 ∓7.196

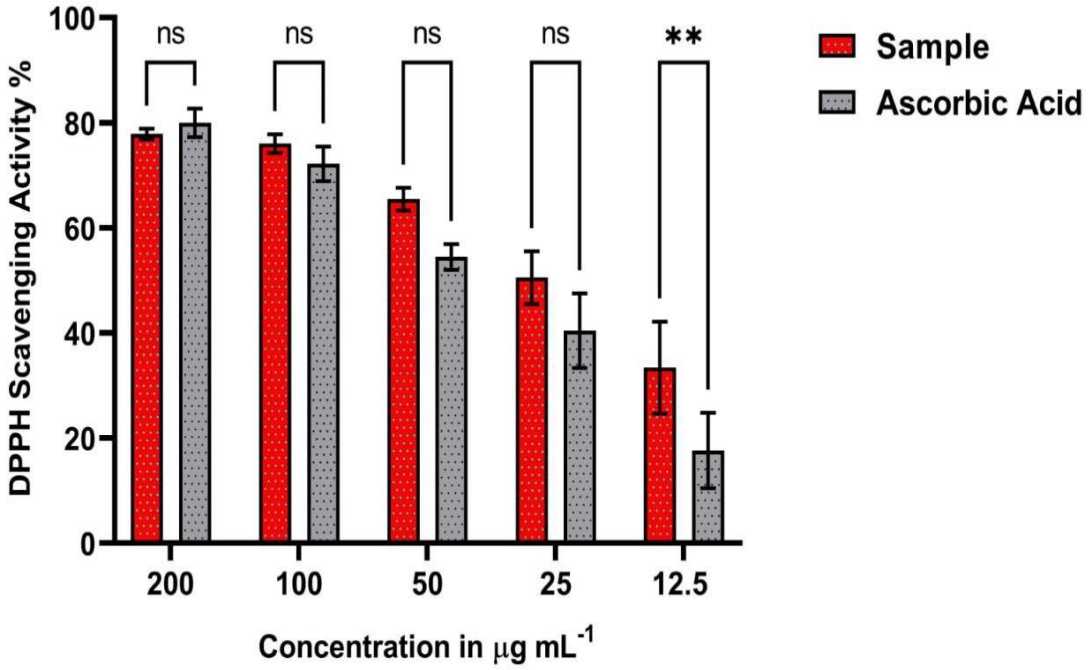

Figure 1: Chart depicting the DPPH antioxidant capabilities at different concentrations, with a comparison between Nerium oleander leaf extract and ascorbic acid.

2.2. MTT assay

The yellow tetrazolium dye is named after the colorimetric reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). It is transformed into a purple product, formazan, through the action of mitochondrial enzymes in living cells. The MTT assay is a commonly used method for assessing cell viability and growth. Based on the absorbance at 570 nm, the level of formazan produced increases in correlation with the number of viable cells. It is commonly used to evaluate the toxicity of various substances, including plant extracts like Nerium oleander leaf [13].

The expression "mean ± SD" denotes the average outcome and the standard deviation, respectively. The average result is known as the mean, while the standard deviation is a measure of how spread out the results are from the mean.

When comparing the effects of Oleander leaf extract on cancerous cells (MCF-7) and normal cells (HdFn), it is clear that the extract specifically targets MCF-7 cells by being more toxic and reducing their survival rates, while having less harmful effects on HdFn cells. This would mean that the extract contains anti-cancer agents. Whereas the degree of selectivity grew with decreasing oleander leaf extract concentrations. Whereas preliminary results show differential cytotoxicity, with the extract demonstrating greater toxicity to MCF-7 cells ($IC_{50} = 32.91 \mu g\ mL^{-1}$) compared to HdFn cells ($IC_{50} = 129.7 \mu g\ mL^{-1}$).

However, those in vitro results do not directly translate into clinical efficacy. More in vivo studies and clinical trials are required to confirm the possible anticancer effects of Oleander leaf extract. The results shown in Table 2 and Figure 2 indicate that the oleander leaf extract possesses high selectivity against breast cancer cell lines, which means it could be a promising treatment if it were employed properly after completing experiments on it according to international drug protocols [14].

Table 2: MTT cytotoxicity assay results (mean∓ sd) for MCF-7 and HdFn cell samples of Oleander leaf extract.

Concen.	Breast cancer cells(MCF-7)	Normal cells(HdFn)
	Mean ∓SD	Mean ∓SD
400.00	31.60 ∓3.64	50.31 ∓4.48
200.00	41.51 ∓4.56	63.50 ∓2.59
100.00	49.00 ∓4.11	74.42 ∓1.52
50.00	61.07 ∓6.48	90.36 ∓1.64
25.00	71.45 ∓2.17	96.95 ∓1.75
12.50	91.51 ∓8.26	93.33 ∓0.87
6.25	95.68 ∓1.79	95.91 ∓0.29

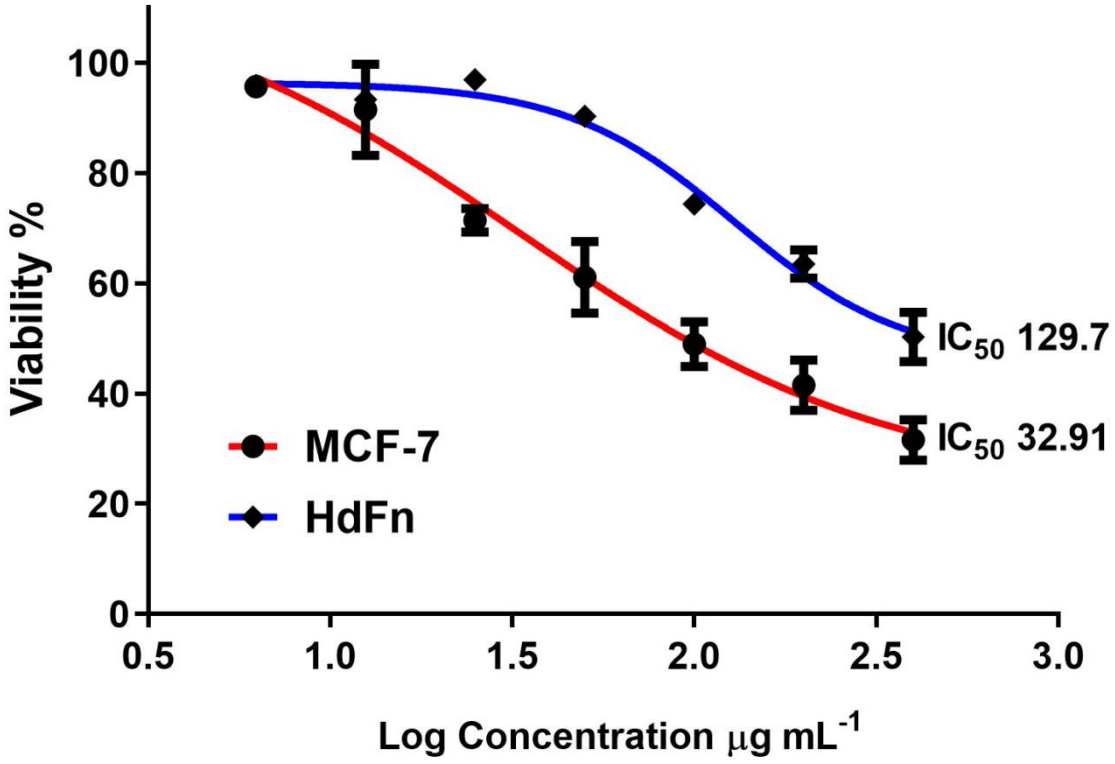

Figure2: The MTT assay was performed in MCF-7 and HdFn cell samples for different concentrations (400, 200, 100, 50, 25, 12.5 and 6.25 $\mu g.ml^{-1}$) of Oleander leaf extract.

Conclusions

The oleander leaf extract showed high efficacy (scavenging free radicals generated by DPPH) in 12.5 µg/ml, which recorded the highest significant value compared with ascorbic acid. Also, the oleander leaf extract showed high selectivity in killing breast cancer cells (MCF-7) compared to normal cells (HdFn), which opens new prospects for breast cancer treatment by plant extracts.

Conflict of interest: NIL

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