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Research Article

# GC-MS PROFILING AND IN-VITRO ANTI-BREAST CANCER ACTIVITY OF ABUTILON INDICUM ETHANOLIC FRUIT EXTRACT AGAINST HUMAN BREAST CANCER MDA MBs 231 CELL LINE

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**ABSTRACT**

The breast cancer is among the most prevalent malignancies and the major cause of cancer-related death in women globally. The study of plant-based bioactive compounds has become of much interest over the past few years because of the possible positive medical effects and the comparative treatment of lower toxicity of these compounds. The medicinal plant Abutilon indicum, which is a popular component used in traditional medicine, has been known to have different phytochemicals with pharmacological properties. The objective of this study was to determine the phytochemical composition and in-vitro action of the ethanolic fruit extract of Abutilon indicum on HBC. Phytochemical-profiling of the extract was performed by means of (GC-MS) and determination of bioactive compounds in the extract was done. The MTT assay was used to determine the anticancer activity based on cell viability and cytotoxic effect on the cancer cells. The GC-MS analysis showed the existence of various phytoconstituents that have the potential biological activities. The ethanolic fruit extract had concentration-dependent cell viability, which means that it has a significant cytotoxic and antiprolliferative effect on MDA-MB-231 cells. The above observations show that the Abutilon indicum fruit extract holds a promising anticancer future and that it can be adopted as a potential source of the development of new plant-based therapeutic agents and to overcome breast cancer. There is also need to further research on active compounds so that it can be isolated and further clarify their mechanisms of action.

**Keywords**: Abutilon indicum; GC–MS analysis; Human breast cancer; Anticancer activity; Cytotoxicity; Phytochemical profiling; MDA-MB-231 cell line; Ethanolic fruit extract.

## INTRODUCTION

Traditional medicine has been very crucial in the prevention and treatment of a number of diseases over the thousands of years. The World Health Organization (WHO) defines traditional medicine as the therapeutic practices that were practiced centuries ago and are still common in the present times. Medicinal plants also have a number of bioactive compounds which have therapeutic potential on various diseases such as cancer. [1] Cancer is a multifaceted cohort of diseases, which is denoted by uncontrolled cell proliferation. These cancerous cells develop maladaptive tumors disrupting the normal body operations. Cancer is amongst the causes of death worldwide that is most dominant.[2] Breast cancer is a widespread female-diagnosed cancer and a significant health issue in the world. It is a condition that arises as a

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result of uncontrolled proliferation of abnormal cells in breast tissue to the extent that they form tumors. One of the forms of breast cancer is triple-negative, which is considered extremely aggressive and difficult to cure. MDA-MB-231 cell line is an experimental model that is widely utilized in laboratory research to study triple-negative breast cancer.3 Recent scientific research has revealed that Abutilon indicum has a number of pharmacological activities as being antioxidant, antimicrobial, anti-inflammatory, hepatoprotective and anticancer activity which is aided by the presence of phytochemicals including flavonoids and phenolic compounds.[12,13] The present study aimed at determining the in vitro anticancer expression of ethanolic extract of Abutilon indicum fruit on the human breast cancer cell line MDA-MB-231 using MTT assay.

**Morphological Description 5**

Abutilon indicum is a perennial shrub commonly found in tropical regions. The plant grows up to 2–3 meters in height. The leaves are heart-shaped and covered with fine hairs. The flowers are yellow or orange in color and bloom throughout the year.

**Phytochemical Constituents**

The plant contains various bioactive compounds such as:

- Flavonoids
- Alkaloids
- Phenolic compounds
- Steroids
- Tannins
- Terpenoids

These compounds are responsible for its pharmacological activities including antioxidant and anticancer properties.

## MATERIALS AND METHODS

**Chemicals used**

Ethanol from Northman, Fetal bovine serum and Antibiotic solution, Dimethyl sulfoxide and MTT.

**Plant Collection and Authentication**

The plant material of Abutilon indicum was collected from a local area of Dindigul and authenticated by Dr. Anand, Department of Botany, National College, Tiruchirapalli.

**Preparation of Extract**

The collected plant material was washed, shade-dried, and powdered. The powdered material was subjected to Soxhlet extraction using ethanol as solvent.

**GC-MS Analysis**

The analysis that was employed was the clarus 680 GC using a fused silica column, Packed with Elite - 5MS (5% bi phenyl 95% dimethylpolysiloxane 30mX0.25mm ID X250µm df) and the compounds were run using a constant flow of 3ml/min flow using helium as the carrier gas. The chromatographic run was performed at injector temperature of 280°C and the oven temperature as follows: 50°C (2 min); 10°C min-1 followed, and the temperature held at 300°C and kept at 35 mins. The mass detector conditions were transfer line temperature 250° C: ion source temperature 200°C and ionization mode electronic impact at 70Ev and scan time a=0.30 sec and scan interval=0.1sec scan speed=1666 m/z. The fragnance of 40-600 Da, The spectrum of the compounds were compared with the database of spectrum of known compounds located at the GC -MS NIST.

**Preparation of Test Solution**

The dried extract was dissolved in DMSO to obtain different concentrations for cytotoxicity testing.

**In Vitro Procedure**

**MTT Assay**

**Principle**

The principle of the MTT assay is the ability of dehydrogenase enzymes of the mitochondria existing in living cells to reduce the tetrazolium ring of the pale yellow MTT reagent, which causes the production of dark blue formazan crystals. These formazan crystal are much insoluble in cell membranes hence they build up in metabolically active and viable cells. Crystals of the cells are liberated through solubilization of the cells using detergents (DMSO). The survival of the cells depends on the amount of formazan product produced directly. A multi-well plate reader is capable of measuring the color.

**MATERIALS REQUIRED**

Fetal Bovine Serum, 1X PBS, Dimethyl sulfoxide 5mg/ml, MTT 5 mg/ml, (India) and the DMEM medium, 1X PBS, 96 well tissue culture plate and wash beaker (India).

**PROCEDURE7-10**

**Cell culture**

The purchase of MDA-MB-231 (Human Breast Cancer) Cell line was done through the NCCS, Pune and was cultured in liquid medium (DMEM) containing 100 µg/ml streptomycin, 100 µg/ml penicillin and 10% Fetal Bovine Serum (FBS) and maintained in an atmosphere of 5% CO2 at 37°C.

**MTT assay**

In vitro cytotoxic activity of the test sample (NN26) against MDA-MB-231 breast cancer cells was assessed using MTT assay. In summary, cultured MDA-MB-231 cells were trypsinised, and using a 15 mL centrifuge tube, the cells were harvested. A 96-well tissue

culture plate was then seeded with the cells at a density of 1 × 10 -5 cells/mL (200 µL each well) containing DMEM with the addition of 10% FBS and 1% antibiotic solution. To enable the attachment and growth of cells, the plates were incubated 24-48 hours at 37°C in a humidified incubator with 5% CO2.

The incubation was followed by the removal of the culture medium and the washing of the wells with sterile phosphate-buffered saline (PBS). The cells were subsequently subjected to various concentration of the test sample (NN26) made by serum free D M E M medium. All concentrations were triplicated and the incubation of the treated cells in the humidified incubator under 5% CO 2 at 37°C was repeated after 24 hours.

Afterwards, 10 µL of MTT solution (5 mg/mL) was introduced into each well and the plate was incubated between 2 to 4 hours after which purple formazan crystal was seen under the inverted microscope. The culture medium that was supplemented with MTT (about 220 µL) was then aspirated and the wells rinsed with 1× PBS (200 µL) to clear any remnant of the reagent. Fmazan crystals were dissolved in 100 µL of dimethyl sulfoxide (DMSO) in each of the wells and the plate was swirling with a 5-minute shaker.

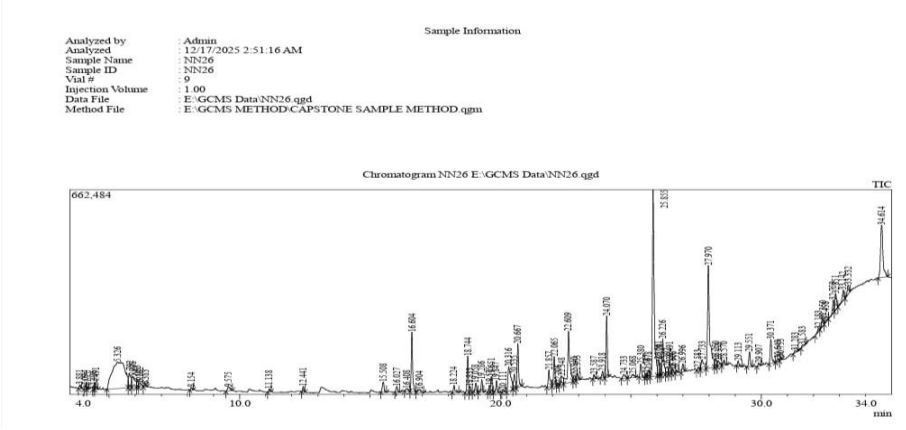
A microplate reader (Thermo Fisher Scientific, USA) was used to measure the absorbance of every well at 570 nm. GraphPad Prism 6.0 software (USA) was used to determine the percentage of cell viability and the IC 50 value of the test sample.

Formula Cell viability % = Test OD/Control OD X 100.

## RESULTS AND DISCUSSION

**Table 1: Phytochemical Analysis**

| S.NO | TEST FOR | TEST | RESULT |
|---|---|---|---|
| 1 | Alkaloids | Hagers test<br>Dragendroff test<br>Mayers test | +<br>+<br>+ |
| 2 | Flavonoids | Ferric chloride test<br>Shinoda test | +<br>+ |
| 3 | Carbohydrates | Benedicts test<br>Fehlings test | +<br>+ |
| 4 | Tannins | Tannis test | + |
| 5 | Saponins | Foam test | + |
| 6 | Proteins | Biuret test | + |
| 7 | Glycosides | Keller killani test | + |
| 8 | Free amino acids | Ninhydrins test | + |
| 9 | Steroids and terpenoids | Terpenoids test<br>Steroids test | -<br>- |




**IN-VITRO RESULT:**

**Standard: Doxorubicin – treated on MDA-MB-231 Cell Line**

**A. OD Value at 570 nm**

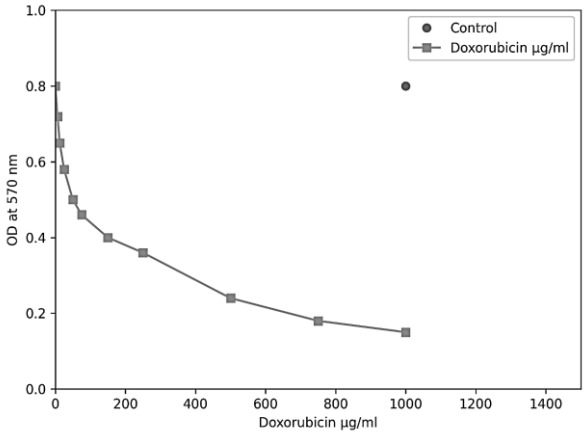



**Figure 1: OD Value at 570 nm**

**B. Standard- Doxorubicin – Cell Viability**

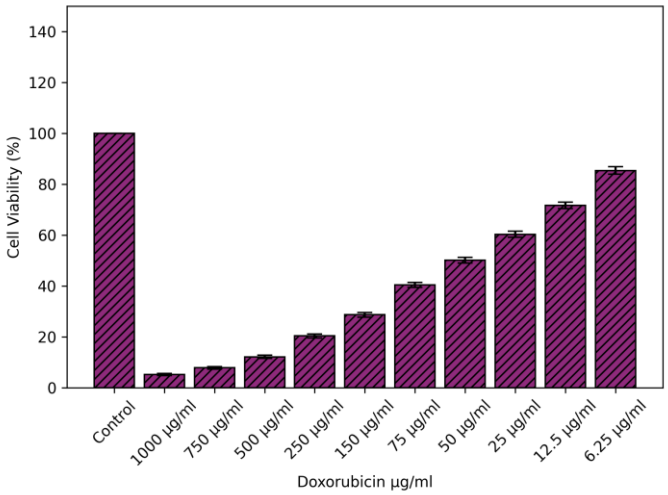


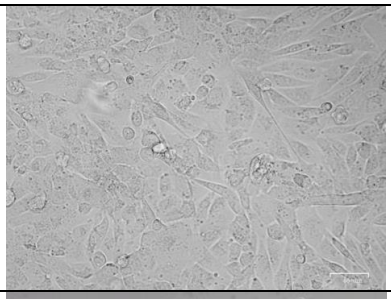
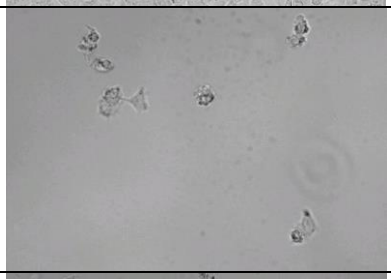
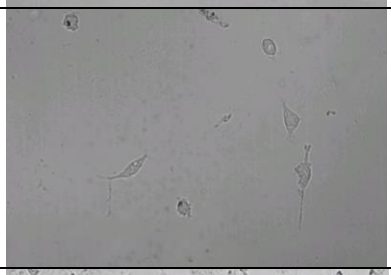
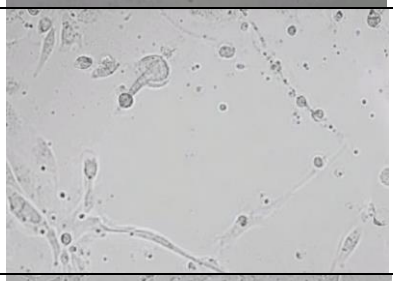
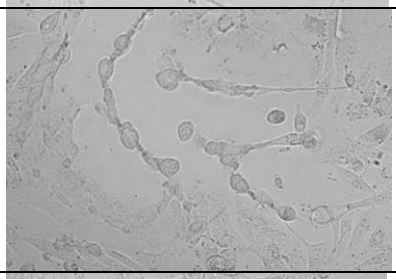
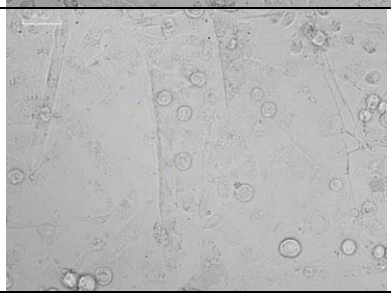

**Figure 2: Standard- Doxorubicin – Cell Viability**

**C. IC50 Value of standard: 8.52 µg/m3**

**Table 2: IC50 Value of standard**

| **log(inhibitor) vs. normalized response** | |
|---|---|
| Best-fit values | |
| LogIC50 | 0.930 |
| **IC50** | **8.52 µg/ml** |
| 95% CI (profile likelihood) | |
| LogIC50 | 0.861 to 0.998 |
| IC50 | 7.27 to 9.95 µg/ml |
| Fit Adequacy | |
| Independent Data Points | 29 |
| Coefficient of Determination ($R^2$) | 0.9624 |
| Total Squared Error | 1246 |
| Standard Error of Estimate | 6.241 |
| Points based on the number | |
| # X | 30 |
| # Y analysed | |

**D. Images of control cells and standard * treated cells.**

| Conc | Image |
|---|---|
| **Control** |  |
| **1000 µg/ml** |  |
| **500 µg/ml** |  |
| **100 µg/ml** |  |
| **75 µg/ml** |  |
| **25 µg/ml** |  |

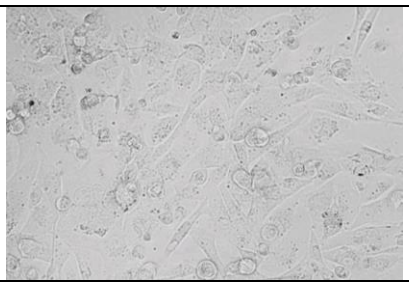
| | |
|---|---|
| **6.25 µg/ml** |  |

**Figure 3: Images of control cells and standard * treated cells**

**Sample – extract treated on MDA-MB-231 Cell Line**

**A. OD Value at 570 nm**

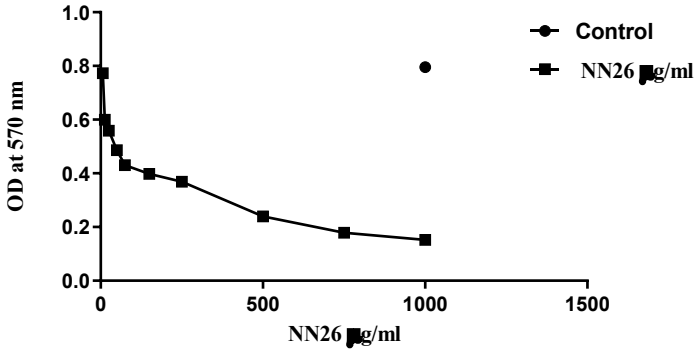



**Figure 4: OD Value at 570 nm**

**B. Sample Cell Viability (%)**

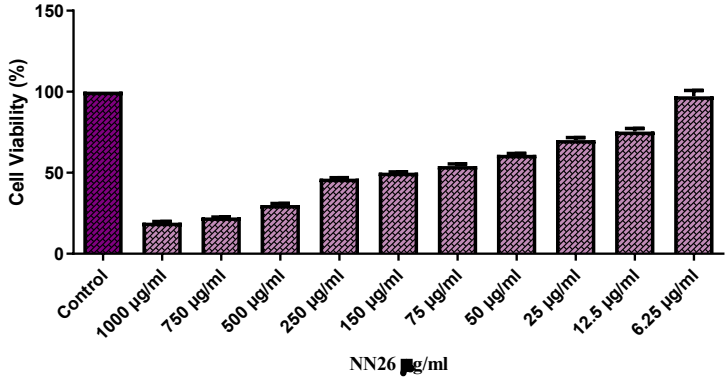



**Figure 5: Sample Cell Viability (%)**

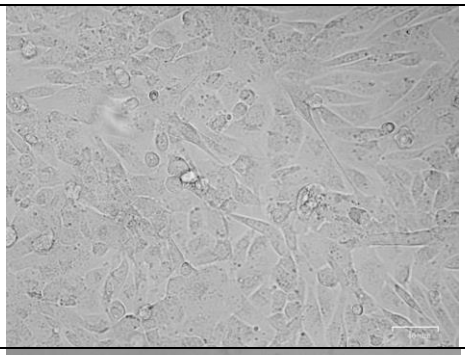
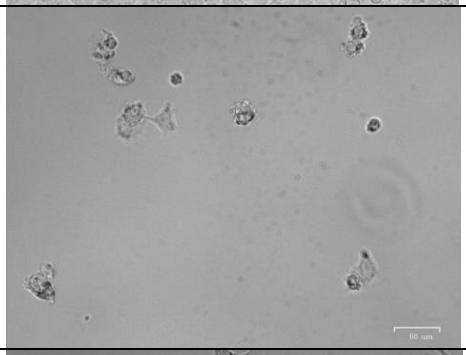
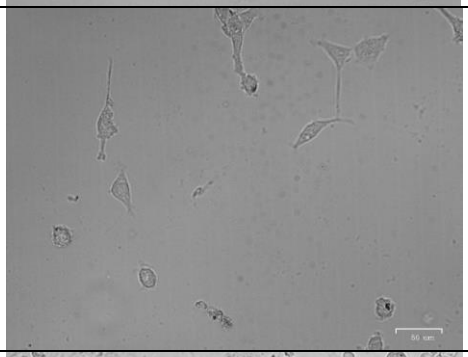
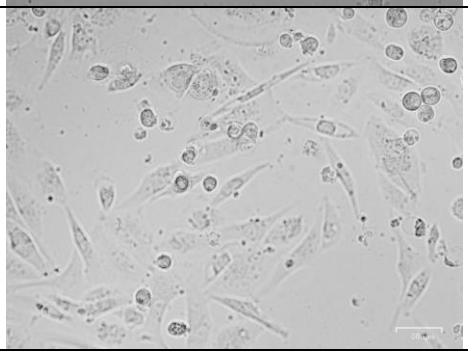
**C. IC50 Value of tested sample: 66.64 µg/ml**

**Table 3: $IC_{50}$ value of the evaluated sample**

| **log(inhibitor) vs. normalized response** | |
|---|---|
| Best-fit values | |
| LogIC50 | 1.824 |
| **IC50** | **66.64** |
| 95% CI (profile likelihood) | |
| LogIC50 | 1.741 to 1.906 |
| IC50 | 55.02 to 80.61 |
| Goodness of Fit | |
| Degrees of Freedom | 29 |
| R squared | 0.9302 |

| | |
|---|---|
| Sum of Squares | 1885 |
| Sy.x | 8.062 |
| Number of points | |
| # X | 30 |
| # Y analyzed | |

**E. Images of control cells and extract treated cells**

| Conc | Image |
|---|---|
| **Control** |  |
| **1000 µg/ml** |  |
| **500 µg/ml** |  |
| **100 µg/ml** |  |

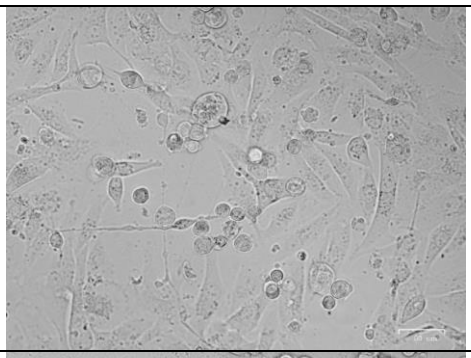
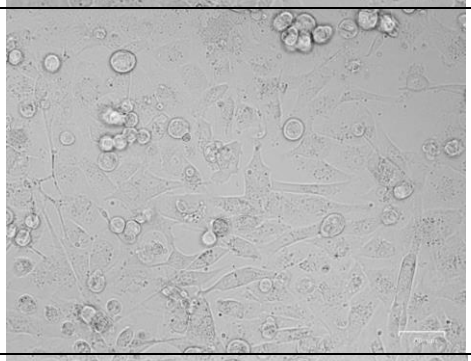
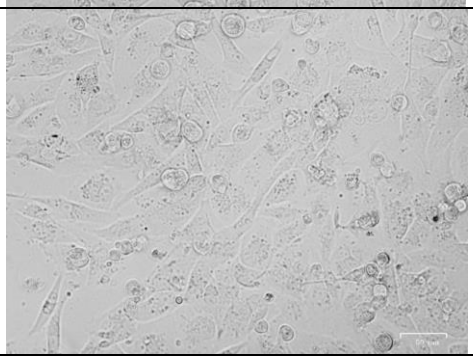
| | | |
|---|---|---|
| **75 µg/ml** |  | |
| **25 µg/ml** |  | |
| **6.25 µg/ml** |  | |

**Figure 6: Images of control cells and extract treated cells**

## DISCUSSION

The current study compared the in vitro anticancer action of ethanolic fruit extract on the human breast cancer cell line MDA-MB-231, which is a well-established TNBC model that is aggressive and metastatic. The results indicated that the ethanolic fruity extract possessed significant cytotoxic and antiprolliferative effects on the MDA-MB-231 cells in a concentration-dependent fashion.

Ethanol has been chosen as a solvent as it is an efficient solvent in extracting polar and moderately non-polar phytochemicals, as well as bioactive, such as phenolics, flavonoids, terpenoids and fatty acid derivatives. These classes of compounds are commonly reported to have anticancer, antioxidant, and pro-apoptotic effects, which can potentially lead to the reported biological activity.

The GC-MS analysis of the ethanol fruit extract showed the existence of a number of bioactive compounds, including phenolic derivatives, fatty acids, esters, and terpenoid-based molecules. A substantial number of these compounds have been reported in the literature to have anticancer effects based on mechanisms such as induction of apoptosis, cell cycle arrest, inhibition of cell proliferation and regulation of oxidative stress signals. The existence of these compounds is a strong indication of the cytotoxic effect of the extract in MDA-MB-231 cells.

The anticancer effect implicated in this research can be explained by the synergistic effect of various phytoconstituents instead of one compound. Multi-target interactions have been shown to improve the therapeutic potential of natural extracts, which is especially privileged to treat complex diseases, including cancer. Phytochemical-based interventions provide alternative therapeutic approaches to MDA-MB-231 cells, which do not have estrogen, progesterone and HER2 receptors.

Moreover, the lowering of cell viability indicates that the ethanolic fruit extract can disrupt major signaling pathways that regulate tumor cell survival and

proliferation. Despite the fact that the underlying molecular processes were not investigated in the current research, other studies have indicated that comparable phytochemicals are able to induce intrinsic-apoptotic pathways and inhibit oncogenic-signaling cascades.

## CONCLUSION

The current research was conducted to test the in-vitro cytotoxic effect of the test sample in human breast cancer cells through the MTT assay of the test sample that is commonly used to determine cell viability and cell cytotoxicity of the test compound against cancer cells. The concept of MTT assay stems on the capacity of the viable cells to lower the yellow tetrazolium salt into purple formazan crystals by the dehydrogenase enzymes in the mitochondrion which is a sign of cell metabolic activity and survival. The findings proved that the test sample had a concentration-related cytotoxic ability on MDA-MB-231 cells. The more the sample was concentrated, the lesser was the cell viability which demonstrated its potential inhibitory impact on the growth of the breast cancer cells. The IC 50 of the tested specimen was determined as 66.64 µg/ml which implied a moderate cytotoxic effect on the MDA-MB-231 breast cancer cell line. The statistical analysis demonstrated that it was well correlated with R 2 of 0.9302, which is a sign of the reliability and consistency of the experimental data. Generally, the results of this research indicate that the test sample has a potential of anticancer activity against the breast cancer cells, which could be explained by the existence of bioactive compounds. Nevertheless, the development of its therapeutic effects and safety in future drug development requires more detailed researches to include its mechanism of action, apoptosis research, and in-vivo research.

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