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# *In vitro* anticancer activity of ethanolic extract of *Ipomoea marginata* whole plant andleaves on breast cancer cell lines MDA-MB-231 and MCF-7

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**ABSTRACT**

The morning glory family, Convolvulaceae, is extensively distributed in temperate, tropical, and subtropical areas. About 60 genera and nearly 1,600 species worldwide make up the convolvulaceae, which primarily twines plants or bushes and occasionally has milky sap. The purpose of the current investigation is to evaluate the anticancer potential of human breast adenocarcinoma (MCF-7) and mammary gland carcinoma (MDA: MB-231) cell lines in *Ipomoea marginata*. By using the MTT assay, the effects of ethanolic extract of *I. marginata* leaves and whole plants on the cancer cell lines MDA-MB-231 and MCF-7 were assessed. The results of the study showed that *Ipomoea marginata*'s anticancer profile was efficient against MCF-7 cells, or human breast adenocarcinoma. According to the MTT experiment, whole plant incubation of cancer cell lines decreased the viability of the cancer cells, and the number of dead cells increased considerably with extract concentration. When compared to the leaves of *I. marginata* (130.4864 IC50 g/ml), the entire plant showed higher cytotoxicity (284.8381 IC50 g/ml).

**Key words:** *Ipomoea marginata*, cell lines, anticancer, MTT assay

## 1.0 INTRODUCTION

Since ancient times, medicinal herbs have been used, and today, their usefulness is steadily growing. Herbs have been known and used since ancient times as medical plants, medicinal herbs, or just herbs. Globally, and particularly in developing nations, the use of natural goods, including medicinal plants, has grown in significance in primary healthcare. To discover new drugs or new lead structures for the creation of novel therapeutic agents for the treatment of human diseases like cancer and infectious diseases, numerous pharmacognostical and pharmacological studies are carried out (Chitwood *et al*., 2013). For the treatment of major illnesses like infections, malignancies, and various types of inflammations, huge populations in developing nations still rely on folk medicine.

Traditional medicinal plants have been used in several cancer research investigations to find novel therapeutic agents without the hazardous side effects of conventional chemotherapeutic treatments, and the number of substances classified as clinical phytomedicines has expanded significantly over the past two decades (Rao *et al*., 2004). It has also been stated (Rosangkima and Prasad, 2004) that more than 50% of all modern medications in clinical use are made from natural compounds, many of which have been known to induce apoptosis in a variety of human cancer cells. As a result, there is an urgent need to create drugs that are considerably more effective and less harmful.

Breast cancer is the most lethal type of cancer for women, both in developed and developing nations (WHO, 2010). The study of molecular and cellular causes and the development of therapeutic approaches have made some notable advances in recent years, but the survival rate for breast cancer has not altered considerably (Guarneri and Conte, 2004). Finding new antitumor substances is becoming more and more important because the anticancer medications already available are frequently ineffective in treating cancer in normal cells.

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With more than 500 species, *Ipomoea* is the largest genus in the Convolvulaceae family of flowering plants. The generic name comes from Greek words that indicate "resembling." It alludes to their propensity for twining. Ipomoea is used by people due to its high concentration of therapeutic and hallucinogenic chemicals (Nagendra Prasad *et al*., 2008). The current study aimed to determine whether *Ipomoea marginata* had any anticancer properties in the breast cancer cell lines MDA-MB-231 and MCF-7.

## 2.0. MATERIALS AND METHODS

### 2.1. Collection and identification of the plant material

In December 2017, the experimental plant *Ipomoea marginata* was collected from Karungal in the Kanyakuamri District. The contaminants were eliminated by rinsing in sterile distilled water, and the samples were then authenticated at the Department of Botany, Scott Christian College, Nagercoil. *I. marginata*'s leaves and whole plant were shade-dried, ground, and kept at room temperature until needed. *I. marginata* ethanolic extract was prepared by mixing 50 gm of powder (whole plant and leaves) with 500 ml of ethanol, and then filtering through Whatman filter paper (No. 1) and allowed to evaporate. The dried filtrate was collected, weighed, and kept in a freezer until needed.

### 2.2. MTT assay in MDA-MB-231 and MCF-7 cell lines (Francis and Rita, 1986)

Using DMEM media containing 10% FBS, the monolayer cell culture was trypsinized and the cell density was increased to $1.0x10^5$ cells/ml. A total of 100 µl of the diluted cell suspension (about 10,000 cells/well) was added to each well of a 96-well microtitre plate. After 24 hours, when a partial monolayer had developed, the supernatant was discarded, the monolayer was washed once with medium, and four different concentrations of the *Ipomoea marginata* extract (62.5, 125, 250, and 500 µg/ml), prepared in maintenance media, and were added per well to the partial monolayer in microtitre plates. After that, the plates were incubated for 72 hours at 370°C in a 5% $CO_2$ environment, with microscopic examinations and observations being made every 24 hours. The sample solutions in the wells were removed after 72 hours and replaced with 20 µl of MTT (2 mg/ml) in MEM-PR (MEM without phenol red). The plates underwent a gentle shaking and a 3-hour incubation period at 37˚C with 5% $CO_2$ atmosphere. To dissolve the produced formazan, 50 µl of iso-propanol was added after the supernatant was taken out of the mixture. The plates were then gently agitated. At a wavelength of 540 nm, the absorbance was observed using a microplate reader.

The following formula was used to determine the concentration of drug or test sample required to reduce cell growth by 50% in each cell line. The dose-response curves were used to compute the percentage growth inhibition.

% Growth Viability = (Mean OD of individual test group/Mean OD of control group) X 100

## 3.0 RESULT AND DISCUSSION:

From tables 1 and 2, the percentage of cell viability for four different concentrations of ethanolic extracts of *I.marginata* leaves and whole plant on MDA-MB-231 and MCF-7 cell lines was ascertained. *I. marginata* leaf ethanolic extract yields 39.55% cell viability at 500 µg/ml, 42.29% cell viability at 250 µg/ml, 44.7% cell viability at 125 µg/ml, and 54.22% cell viability at 62.5 µg/ml in MDA-MB-231 cell lines. In MDA-MB-231 cell lines, the ethanolic extract of the whole plant at 500 µg/ml results in cell viability of 39.25%, 250 µg/ml results incell vitality of 42%, 125 µg/ml results in cell viability of 44.52%, and 62.5 µg/ml results in cellviability of 48.19% (Table 1).

The percentage cell viability of the ethanolic extract of *I. marginata* in MCF-7 cell lines was described in Table 2. In the leaves 500 µg/ml results in 42.76% cell viability, 250 µg/ml results 47.09% cell viability, 125 µg/ml results in 49.95% cell viability, and 62.5 µg/ml results in 54.38% cell viability. At 500 µg/ml, the whole plant extract yields 47.22% cell viability, 250 µg/ml yields 49.95% cell viability, in 125 µg/ml of extract yields 53.46% cell viability, whereas in 62.5 µg/ml yields 65.77% cell viability. Different cell lines responded differently

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to the plant extract's activities. This selectivity can result from a tissue-specific response or from the cell line's sensitivity to the extract's active ingredients (Prema *et al*., 2012). In the current study the percentage of cell viability was used to express how the extracts affected the proliferation of MDA-MB-231 and MCF-7 cell lines and found the percentage cell viability of the cell decreased with concentration of the extract. Significant *in vitro* antiproliferative effects of *I. tuba* leaf extract were observed in MCF-7, and the viability of MCF-7 decreased with sample concentration (Venkateswarulu *et al*., 2020). The anticancer activity of different extracts of *I. purpurea* were tested against A-549 (human lung cancer), HepG-2 (human liver cancer), and MDA-MB-231 (human breast cancer) by MTT assay and found that increase in extract concentration till 400 µg/ml decreased the cell viability (Beheshti *et al*., 2021). In the present work also, the viability decreased with the extract concentration in both the parts of *I. marginata*.

**Table 1: Anticancer effect of ethanolic extract of *Ipomoea marginata* in MDA-MB-231 cell lines**

| Concentration (µg/ml) | % cell viability | |
|---|---|---|
| | Leaves | Whole plant |
| 500 | 39.55±0.1 | 39.25±0.01 |
| 250 | 42.29±0.25 | 42±1 |
| 125 | 44.7±0.15 | 44.52±0.1 |
| 62.5 | 54.22±0.11 | 48.19±0.15 |

Values are expressed in mean±SD

**Table 2: Anticancer effect ethanolic extract of *Ipomoea marginata* in MCF-7 cell lines**

| Concentration (µg/ml) | % cell viability | |
|---|---|---|
| | Leaves | Whole plant |
| 500 | 42.76 ±0.09 | 47.22±0.11 |
| 250 | 47.09±0.54 | 49.95±0.22 |
| 125 | 49.95±0.61 | 53.46±0.07 |
| 62.5 | 54.38±0.16 | 65.77±0.15 |

Values are expressed in mean±SD

**Table 3: Cytotoxic studies of leaves and whole plant of *Ipomoea marginata* in MBA-MB-231 and MCF-7 cell lines**

| Sample | MDA-MB-231 $LC_{50}$ µg/ml | MCF-7 $LC_{50}$ µg/ml |
|---|---|---|
| Leaves | 86.78965 | 130.4864 |
| Whole plant | 37.72533 | 284.8381 |

According to table 3, *Ipomoea marginata*'s ethanolic extract produced $LC_{50}$ values of 86.78965 µg/ml for the leaves and 37.72533 µg/ml for the MDA-MB-231 cell lines. The $LC_{50}$ values for the MCF-7 cell lines were 130.4864 µg/ml for leaves and 284.8381 µg/ml for the whole plant (Fig. 1). According to the study, *Ipomoea marginata*'s whole plant had the highest level of activity against MCF-7 (Human, Breast Adeno Carcinoma) cell lines.

Cell viability is frequently assessed using metabolic viability-based assays like MTT. The metabolic viability of cells can be affected by modifications in both mitochondrial composition and metabolism. In order to determine whether *Ipomea marginata* extract may cause cell death in the MCF-7 and MDA-MB-231 breast cancer cell lines, we performed an experiment. Using the MTT cell growth inhibition assay, Meena *et al*., 2013 and Purushoth Prabhu *et al*., 2012 investigated the anticancer efficacy of on the breast cancer MCF-7 cell line and discovered that the maximum% inhibition was at 200 g/ml. By using the MTT assay, Sudhakar Meesala *et al*., 2017 investigated the anticancer effects of leaf extract from *Ipomoea*

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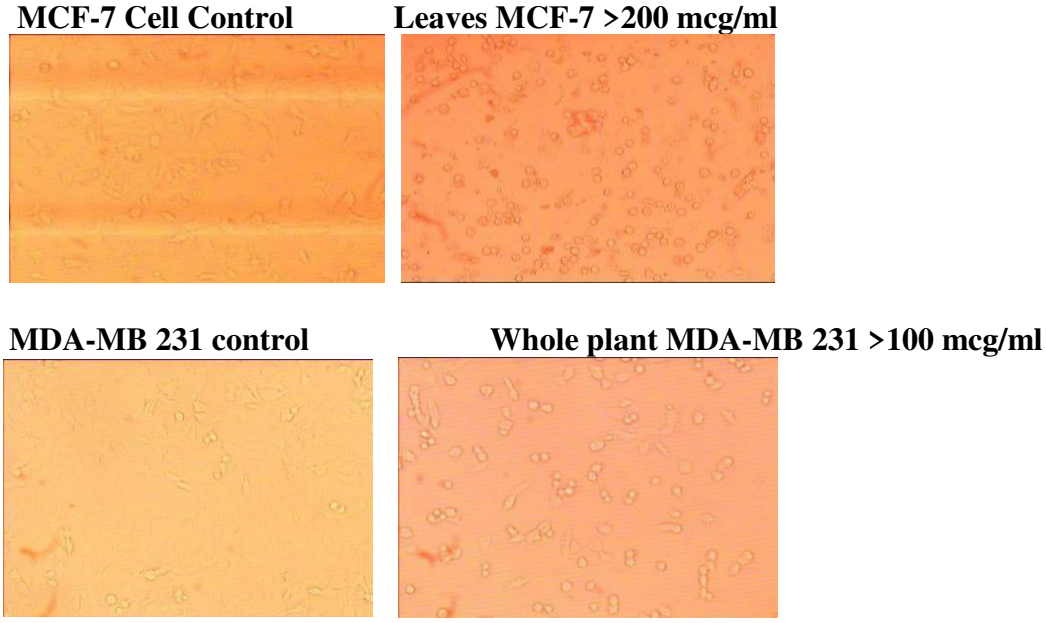
*sepiaria* against the MCF-7 cell line. MCF-7 cell lines were also used in the current investigation to test *Ipomoea marginata* leaf extract's anticancer properties. Through an *in vitro* MTT assay, Grbovic *et al*., 2013 examined the cytotoxic effects of *Origanum vulgqare* on the MDA-MB-231 cell line. In the current investigation, MDA-MB-231 cell lines were also used to assess *Ipomoea marginata*'s anticancer potential. Ghasemzadeh *et al*., 2016 and Graidist *et al*., 2013 used MCF-7 and MDA-MB-231 breast cancer cell lines to test the anticancer activity. Ovgu Isbilen and Ender Volkan 2021 used metastatic breast cancer cells MCF-7 and MDA-MB-231 to study the anticancer activities. *Ipomoea marginata*'s anticancer activity was also assessed in the current investigation using the MCF-7 and MDA-MB-231 cell lines. Mohd Mughees and Saima Wajid determined the cytotoxicity of the extracts from different parts (whole, aerial, and root) of the plant was evaluated against the breast cancer cell lines (MCF-7 and MDA MB-231) by MTT and lactate dehydrogenase (LDH) assays. The presence of flavonoids, anthocynins, and terpenoids are corresponding chemicals for anticancer action against breast cancer cell lines, according to Prema *et al*., 2012 and Ghasemzadeh *et al*., 2016. *Ipomoea marginata*, the plant used in the experiment, also verified the terpenoids and flavonoids in their ethanolic extract of the plant's leaves and overall structure (Sukitha *et al*., 2016). Silva-Correa *et al*., 2020 evaluated the anticancer activity of *Ipomoea batatas* and found that phenolic compounds and anthocyanins were responsible for the anticancer activities. There are no prior reports on the anticancer activity of an ethanolic extract from *I. marginata* employing the MCF-7 and MDA-MB-231 cell lines for the MTT assay and further research on the plant is necessary.

**Fig. 1: Anticancer effect of *Ipomoea marginata* ethanolic extract of leaves and whole plant on MCF-7 and MDA-MB cell lines**




## 4.0. CONCLUSION

The potential cytotoxic activity in breast cancer cell lines is caused by phytochemical components such flavonoids and terpenoids. The presence of flavonoids and terpenoids in *Ipomoea marginata* may contribute to its anticancer action in the breast cancer cell lines MCF-7 and MDA-MB-231. Additional investigation is required to demonstrate this, validate it using different cancer models, and identify the active ingredient.

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