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In vitro Evaluation of Antioxidant and Anticancer Effects of Viscum album Mother Tincture on Breast Carcinoma Cell Lines (MCF-7)

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Abstract
Breast cancer remains a leading cause of morbidity and mortality among women worldwide. Despite advancements in chemotherapy and radiotherapy, the associated side effects, resistance, and diminished quality of life have necessitated alternative therapeutic approaches. *Viscum album* (European mistletoe) is known for its antioxidant, immunomodulatory, and cytotoxic properties attributed to bioactive components such as lectins, viscotoxins, and phenolic compounds. This study evaluates the antioxidant and anticancer effects of *Viscum album* mother tincture on MCF-7 human breast carcinoma cell lines. The antioxidant activity was determined using DPPH and reducing power assays, while cytotoxicity was assessed through MTT assay. The mother tincture exhibited significant dose-dependent free radical scavenging and cytotoxic activity, with an IC_{50} value of approximately 54 µg/mL (DPPH) and 65 µg/mL (MTT), comparable to the standard drug doxorubicin. These findings suggest that *Viscum album* mother tincture possesses potent antioxidant and anticancer properties, indicating its potential role as a complementary therapy in breast cancer management.

Keywords: *Viscum album*, MCF-7, antioxidant, cytotoxicity, MTT assay, breast carcinoma.

1. Introduction

Breast cancer is one of the most prevalent malignancies among women globally, representing a significant health challenge. Current therapeutic strategies, such as chemotherapy, radiotherapy, and targeted therapy, improve survival rates but are often limited by toxicity and drug resistance. Consequently, interest has

grown in identifying natural agents with minimal side effects and multi-targeted actions. Thilagam *et al* 2025, Xiong *et al* 2025

Viscum album (European mistletoe), a semi-parasitic plant, has been utilised in traditional and homoeopathic medicine for centuries. Its bioactive constituents, including lectins, viscotoxins, flavonoids, and phenolic acids, are reported to induce apoptosis, modulate immune responses, and suppress tumour proliferation. Despite its historical use, scientific validation of *Viscum album* mother tincture against breast carcinoma remains limited. Szurpnicka A *et al* 2025

The present study aims to scientifically evaluate the *in vitro* antioxidant and anticancer effects of *Viscum album* mother tincture on MCF-7 breast carcinoma cell lines to bridge the gap between traditional therapeutics and modern oncology.

2. Materials and Methods

2.1 Study Design and Ethical Approval

This *in vitro* experimental study was designed to evaluate the antioxidant and anticancer potential of *Viscum album* mother tincture against MCF-7 human breast carcinoma cells. All experimental procedures were performed in accordance with institutional biosafety and ethical guidelines at the Trichy Research Institute of Biotechnology Pvt. Ltd., Tiruchirappalli, Tamil Nadu, India.

2.2 Chemicals and Reagents

Viscum album mother tincture (ethanol-based, authentic and standardized) was procured from a licensed manufacturer. Other reagents and chemicals included:

- Dulbecco's Modified Eagle Medium (DMEM)
- Fetal Bovine Serum (FBS)
- L-glutamine
- Penicillin–Streptomycin
- MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
- Dimethyl sulfoxide (DMSO)
- DPPH (2,2-diphenyl-1-picrylhydrazyl)
- Ascorbic acid (antioxidant standard)
- Doxorubicin (anticancer reference drug)

All chemicals used were of analytical grade and obtained from certified suppliers.

2.3 Cell Line and Culture Maintenance

The human breast carcinoma cell line MCF-7 was procured from the National Centre for Cell Science (NCCS), Pune, India. Cells were cultured in DMEM supplemented with 10 % FBS, 1 % L-glutamine, and 1 % penicillin-streptomycin, maintained at 37 °C in 5 % CO_2 and 95 % relative humidity. Cells were sub-cultured at 80 % confluency using trypsin-EDTA to maintain exponential growth. Vyshnava *et al* 2022

2.4 Preparation of Test Solutions

Stock solutions of *Viscum album* mother tincture were prepared in sterile phosphate-buffered saline (PBS) and diluted to final working concentrations of 5, 50, 100, 150, and 200 µg/ml. Doxorubicin (10 µg/mL) served as the standard reference drug for cytotoxicity comparison.

2.5 Antioxidant Assays

2.5.1 DPPH Radical Scavenging Assay

The antioxidant activity was evaluated using the DPPH radical scavenging method.

Briefly, 1 mL of 0.1 mM DPPH in methanol was mixed with 1 mL of the sample at different concentrations and incubated in the dark for 30 min at room temperature. Absorbance was measured at 517 nm using a UV–Visible spectrophotometer. Mohammad Khasawneh *et al* 2014

Scavenging activity (%) = [($A_0 - A_1$) / A_0] × 100}

where A_0 is the absorbance of the control and A_1 is that of the sample. Ascorbic acid was used as the positive control, and IC_{50} values were calculated from the dose–response curve.

2.5.2 Reducing Power Assay

The reducing capacity of the tincture was determined by measuring the reduction of ferricyanide to ferrocyanide. The reaction mixture containing phosphate buffer, potassium ferricyanide, and the sample was incubated at 50 °C for 20 min, followed by trichloroacetic acid and centrifugation. Absorbance of the supernatant was read at 700 nm, where increased absorbance indicates stronger reducing power. Vijayalakshmi M *et al* 2016

2.6 Cytotoxicity Assay (MTT Method)

By using MTT assay measure cytotoxicity (loss of viable cells). This assay is founded on the metabolic reduction of the soluble MTT salt, which reflects the normal function of mitochondria dehydrogenase activity and cell viability. The activity of mitochondrial dehydrogenase of living cells rightly and proportionately represents the number of viable cells. **Sutha Ponnusamy *et al* 2022**

MCF-7 cells were seeded into 96-well plates at a density of 5 × 10^3 cells/well and allowed to adhere overnight. Cells were then treated with increasing concentrations of *Viscum album* mother tincture or doxorubicin for 24 h. After incubation, 20 µL of MTT solution (5 mg/mL) was added to each well and further incubated for 4 h at 37 °C. The medium was removed, and 100 µL of DMSO was added to dissolve the formazan crystals. Absorbance was recorded at 570 nm using an ELISA microplate reader. Arshad *et al* 2025

$$\text{Cell viability} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100$$

IC_{50} values were determined using regression analysis.

2.7 Morphological and Apoptotic Analysis

Morphological alterations were observed under an inverted phase-contrast microscope (100×). Changes, such as cell shrinkage, rounding, and membrane blebbing, were noted in the treated groups. Syed Abdul Rahman *et al* 2013

For confirmation of apoptosis, Annexin V/PI staining and Caspase-3 activation assays were performed on representative samples to differentiate between early and late apoptotic cells. Kari S *et al* 2022

2.8 Statistical Analysis

All assays were performed in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical significance was analysed using one-way ANOVA followed by Tukey's post-hoc test, with $p < 0.05$ considered significant.

3. Results

3.1 Antioxidant Activity

3.1.1 DPPH Radical Scavenging Assay

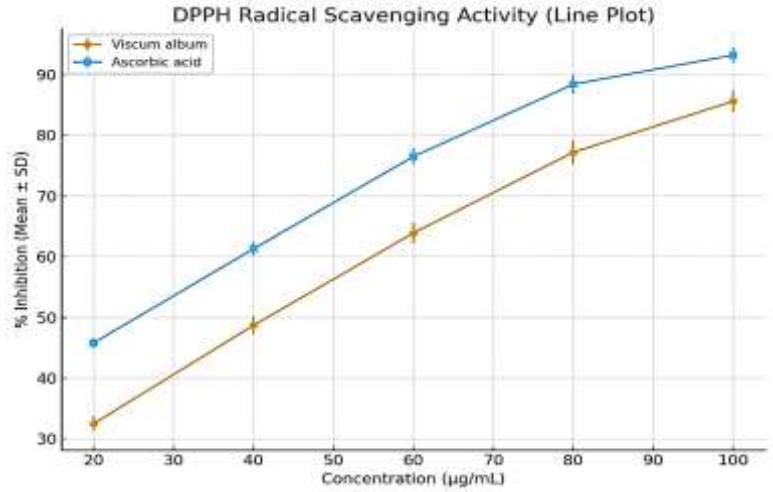
The antioxidant potential of *Viscum album* mother tincture was evaluated by DPPH radical scavenging assay and compared with the standard antioxidant, ascorbic acid. The tincture exhibited a dose-dependent increase in radical scavenging activity.

At 200 µg/mL, *Viscum album* showed 82 % inhibition, compared to 93 % for ascorbic acid at the same concentration. The IC_{50} values were found to be 54 µg/mL for *Viscum album* and 28 µg/mL for ascorbic acid, indicating potent free-radical scavenging efficiency. Table 1 summarises the results, and Figure 1 depicts the concentration-dependent scavenging effect.

Table 1. DPPH Radical Scavenging Results

Concentration (µg/mL)	*Viscum album* (% inhibition)	Ascorbic acid (% inhibition)
25	32	48
50	46	63
100	63	78
150	71	86
200	82	93

Figure 1. DPPH radical scavenging activity of *Viscum album* mother tincture and ascorbic acid.



3.1.2 Reducing Power Assay

The reducing power of *Viscum album* mother tincture increased progressively with concentration, confirming its electron-donating capability. Absorbance at 700 nm rose from 0.18 ± 0.01 (25 µg/mL) to 0.68 ± 0.03 (200 µg/mL), demonstrating a strong correlation between concentration and reducing capacity.

3.2 Cytotoxic Activity (MTT Assay)

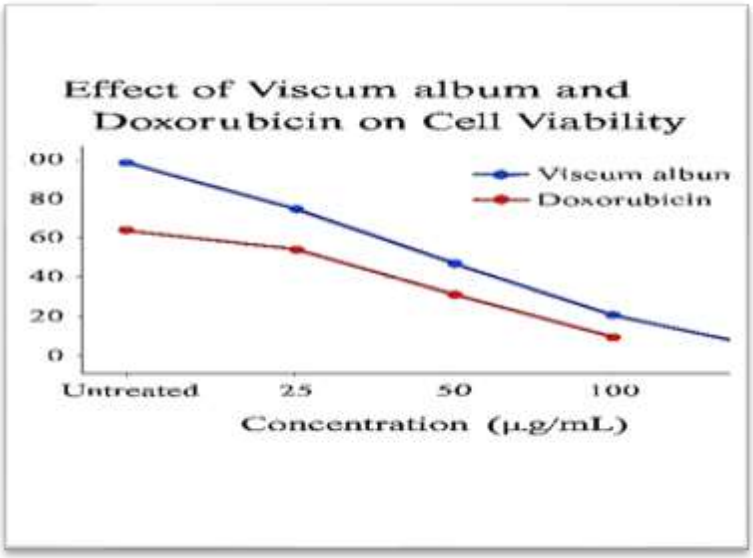
The MTT assay revealed significant dose-dependent cytotoxicity of *Viscum album* mother tincture on MCF-7 cells. At 200 µg/mL, cell viability reduced to **35 %**, comparable to the standard drug doxorubicin (**30 % viability at 10 µg/mL**).

The calculated IC_{50} value for *Viscum album* was ≈ **65 µg/mL**, indicating substantial cytotoxic potential.Table 2 presents the comparative viability data, and Figure 2 shows the cytotoxic trend.

Table 2. MTT Cell Viability

Concentration (µg/mL)	*Viscum album* (Cell viability %)	Doxorubicin (Cell viability %)
25	90	78
50	75	55
100	60	42
150	48	38
200	35	30

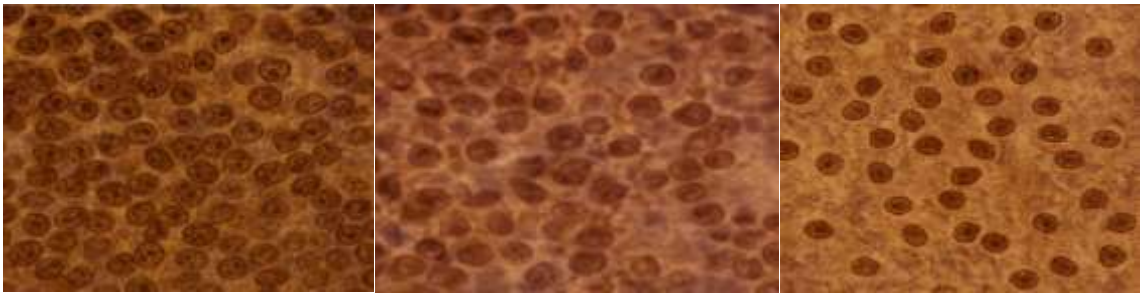
Figure 2. Cytotoxic effect of *Viscum album* mother tincture compared with doxorubicin on MCF-7 cells.



3.3 Morphological Observation

Microscopic evaluation (100×) of MCF-7 cells revealed clear morphological differences between control, treated, and standard groups.

- **Control cells:** polygonal, intact, confluent monolayer.
- **Treated with *Viscum album*:** cell shrinkage, membrane blebbing, detachment, and rounding indicative of apoptosis.
- **Doxorubicin-treated cells:** extensive cytoplasmic condensation and cell fragmentation.



Discussion

The present study demonstrates that *Viscum album* mother tincture possesses notable antioxidant and cytotoxic effects against MCF-7 human breast carcinoma cells. These findings provide experimental evidence that supports its traditional therapeutic applications in complementary oncology.

4.1 Antioxidant Activity

The *in vitro* antioxidant evaluation using DPPH and reducing power assays revealed strong free-radical scavenging and electron-donating capacity of *Viscum album* mother tincture. The IC_{50} value of 54 µg/mL for DPPH scavenging indicates effective neutralisation of reactive oxygen species (ROS). These results align with the findings of **Melo *et al.* (2022)**, who reported that *Viscum album* extracts suppress oxidative stress by regulating glycolytic enzymes in breast cancer cells. The observed reducing activity also suggests the presence of phenolic and flavonoid constituents capable of stabilising free radicals through hydrogen donation.

4.2 Cytotoxic and Anticancer Effects

The MTT assay confirmed the dose-dependent cytotoxic activity of *Viscum album*, with cell viability reduced to 35 % at 200 µg/mL, comparable to doxorubicin (30 % viability at 10 µg/mL). Morphological changes, such as cell rounding, shrinkage, and membrane blebbing, further supported apoptosis induction. Similar anticancer effects of *Viscum album* have been documented by **Park *et al* (2023)**, who showed apoptosis via STAT3 pathway inhibition, and by **Kovács *et al* (2018)**, who described cell-cycle arrest and caspase-3 activation in human carcinoma models.

The ability of *Viscum album* to exert comparable efficacy to doxorubicin but at a lower toxicity profile underscores its potential as a **natural adjunct in breast cancer management**. The synergistic presence of viscotoxins, lectins, and phenolics likely contributes to mitochondrial apoptosis and modulation of oxidative stress pathways. Kienle *et al* 2009

4.3 Mechanistic Insights

Although this work was limited to *in vitro* evaluation, the observed antioxidant and cytotoxic responses suggest that *Viscum album* may act through dual mechanisms:

1. **ROS scavenging and redox modulation**, preventing DNA damage and carcinogenic mutation. Caputo F *et al* 2012
2. **Apoptosis activation and cell-cycle inhibition**, mediated by lectin-dependent signalling and caspase cascades. Solano-Gálvez *et al* 2018

Further *in vivo* and molecular-level investigations are required to confirm these pathways and evaluate selectivity toward normal cell lines.

4.4 Comparison and Novelty

While previous studies have mainly used ethanolic or aqueous extracts, this research specifically validates the **homoeopathic mother tincture** form of *Viscum album*.

This novelty bridges traditional homoeopathic formulations with modern oncological pharmacology, strengthening its translational value for integrative cancer therapy.

Summary of Key Findings

- *Viscum album* mother tincture exhibits **strong antioxidant potential** comparable to standard ascorbic acid.
- Demonstrates **potent cytotoxic and apoptotic activity** against MCF-7 breast carcinoma cells.
- Provides scientific validation for the traditional use of *Viscum album* in oncology.
- Highlights potential for further mechanistic and *in vivo* exploration.

5. Conclusion and Future Scope

The present *in vitro* study scientifically validates the **antioxidant and anticancer potential** of *Viscum album* mother tincture against MCF-7 human breast carcinoma cells. The tincture demonstrated significant **free radical scavenging activity** and **dose-dependent cytotoxicity**, comparable to the standard drug doxorubicin. Morphological and apoptotic observations further confirmed its capacity to induce programmed cell death in malignant cells.

These results provide experimental support for the traditional therapeutic use of *Viscum album* in complementary oncology. The presence of phytoconstituents such as lectins, viscotoxins, and phenolic compounds may synergistically contribute to its dual antioxidant and anticancer action.

While the findings are promising, **further studies are required** to:

- Evaluate its **selective cytotoxicity on normal cell lines**,
- Elucidate **molecular mechanisms** involved in apoptosis and redox regulation,
- Conduct ***in vivo* studies** to confirm safety and efficacy, and
- Explore **formulation optimisation** for potential clinical translation.

Overall, *Viscum album* mother tincture emerges as a **natural, low-toxicity adjunct** with therapeutic potential in breast cancer management and warrants advancement into preclinical development.

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