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Anticancer, antioxidant, and antibacterial activity of the medicinal orchid *Dienia ophrydis* (J. Koenig) Seidenf.

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ABSTRACT

The terrestrial orchid *Dienia ophrydis* is valued for its therapeutic properties in the traditional folk medicine of China, Taiwan and Vietnam. The study aims to identify the potential biological activities from the whole-plant extract of *D. ophrydis*. Three solvents such as hexane, chloroform, and aqueous-ethanol were used for extraction. The anticancer, antioxidant, and antibacterial properties of these extracts were evaluated. The chloroform and aqueous-ethanol extracts were effective in inhibiting the cancer cell growth with IC_{50} values of 58.2 µg/mL and 43.08 µg/mL, respectively in MCF-7 cell line. The DPPH assay showed a maximum percentage of inhibition in aqueous-ethanol extract with 90.64%. The maximum zone of inhibition against *Staphylococcus aureus* and *Escherichia coli* was 13.10±0.35 mm and 14.05±0.85 mm. The findings support the pharmacological benefits of *D. ophrydis* and highlight its potential as a source of novel therapeutic agents.

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INTRODUCTION

Cancer is a highly complex and heterogeneous group of diseases that continues to threaten human health. Effective therapeutic strategies remain elusive due to the unique assemblage of mutations in each cancer. A therapeutic response that is effective for one patient may be ineffective for another, due to their distinct genetic and molecular alterations. Plants inherit the capacity to synthesize numerous chemical compounds, and some have been utilized as sources for chemotherapeutic drugs. Commercially available anti-cancer drugs such as paclitaxel, docetaxel, cabazitaxel, vincristine, teniposide, and camptothecin were derived from medicinal plants such as *Taxus brevifolia*, *Catharanthus roseus*, *Podophyllum hexandrum*, and *Camptotheca acuminata*, respectively (Jadhav *et al.*, 2025; Jîjie *et al.*, 2025; Khan *et al.*, 2025; Sun & Li, 2025). Orchidaceae is one of the largest plant families, but it is less explored for its promising role in pharmacological effects. The ability of orchid extracts to treat a variety of malignancies is reported for the cerebrum, cervix, lungs, stomach, and mammary glands (Śliwiński *et al.*, 2022). Bioactive compounds like bletilols, bulbocodioidins, dendrobine, pholidonone, phenanthrenes, and vicenin II are isolated from orchids for their anticancer properties (Kang *et al.*, 2019; Luo *et al.*, 2019; Wang *et al.*, 2019; Nonpanya *et al.*, 2020).

Beyond their anti-cancer potential, orchids have gained considerable attention for their antioxidant and antibacterial properties, reflecting the diverse phytochemicals they produce as secondary metabolites. Antioxidants play a crucial role in neutralizing reactive oxygen species. The oxidative stress is considered a central contributor to several chronic and degenerative diseases (Jomova *et al.*, 2023). Moreover, the rise of carbapenem-resistant gram-negative bacteria is becoming a global health menace, with resistance to even last-resort antibiotics leaving limited options for clinicians (Jean *et al.*, 2022). This alarms the urgent need for the search of plants with medicinal properties as they serve as a valuable tool for the discovery of novel bioactive compounds.

D. ophrydis is a terrestrial orchid, distributed in South Asia, New Guinea, Philippines, and Northern Australia. It primarily grows in wet tropical biome and known by its vernacular name common snout orchid (Pedersen *et al.*, 2022). *D. ophrydis*

is a traditionally used for the treatment of inflammation (Ou *et al.*, 2003). The pharmacological importance of the genus *Dienia* has not yet been reported. The present work focuses on investigating the anticancer, antioxidant, and antibacterial efficiency of *D. ophrydis*. The study aims to add scientific evidence for the selected species, which is used in folk medicine.

MATERIAL AND METHODS

Plant Material and Extraction

The plant sample *Dienia ophrydis* were collected from Ponmudi hills, Thiruvananthapuram District, Kerala, India. An herbarium was deposited with the accession No. 178163 at Madras Herbarium, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu, India. The collected plant sample was shade-dried, pulverized, and filtered through a 40-mesh sieve. The powdered sample was placed in the ultrasonic-assisted extraction process described by Jadhav *et al.* (2009). Three solvents such as hexane, chloroform and aqueous-ethanol (70% ethanol and 30% distilled water) were used as the extracting solvent with a plant material-to-solvent ratio of 1:10 (m/v). Once the extraction process reached the time course, the samples were filtered and dried using a rotatory evaporator under reduced pressure at 35 °C.

Cell Culture

MCF-7 breast cancer cells were procured from the National Centre for Cell Science (NCCS), Pune, India. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum, 25 µg/mL amphotericin B, 10 mg/L streptomycin, and 100 U/L penicillin at 37 °C in a 5% CO_2 humidified environment. The cell suspension was placed in a 25 cm^2 T flask and the medium was changed every two days. The cells were subjected to starvation after 75% confluence to induce quiescence. The number of viable cells was counted under the inverted microscope using Neubauer's counting chamber after staining it with Trypan blue dye.

Cytotoxicity Assay

The viability of cancer cells after the treatment of plant extracts was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte-trazolium bromide) assay. MCF-7 breast cancer cells (5,000 cell/well) were seeded on a 96-well micro-culture plate and incubated overnight at 37 °C in a CO_2 incubator for cell attachment. 50 mg/mL concentration of plant extract was prepared as stock solution. Different concentration (20, 40, 60, 80 and 100 µg/mL) of test sample was added into the culture medium along with positive (Doxorubicin) and negative control (DMSO). The culture plate was incubated overnight in a CO_2 incubator at 37 °C and 5% CO_2.The treated cells were examined using an inverted microscope.

MTT powder in phosphate buffer saline (pH 7.2) at a concentration of 5 mg/10 mL was used to prepare the MTT reagent. Media was removed and the 96-well plates were filled with 25 µL of MTT reagent followed by incubation for 4 h at 37 °C n dark conditions. The purple formazan crystals were dissolved by adding 100 µL DMSO in each cell. The optical density of the tested sample was measured using a microplate reader (iMark™ microplate reader, Bio-Rad, USA) at the absorbance at 570 nm.

AO/EtBr Staining

Fifty thousand cells per well were seeded on 24-well plates and incubated for 24 h at 37 °C to verify the apoptotic activity of MCF-7 cells. The cells were stained with Acridine orange and Ethidium bromide in a 1:1 ratio. The stain interacts with nucleic acid to form color variations such as green, greenish yellow, and orange. The stained cells were visualized under a fluorescent microscope (Bio Rad, USA) at 20x magnification under a 450-490 UV filter.

Radical Scavenging Assay

Determination of antioxidant activity by 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) was evaluated by the methodology of Shimada *et al.* (1992). The plant samples and ascorbic acid standard were taken at various doses (5, 10, 20, 40, 60, 80, and 100 µg/mL). 0.1 mM of DPPH solution was mixed with the sample solutions and incubated in the dark for 30 min in the presence of Tris-HCl buffer (50 mM, pH 7.4). The absorbance was noted at 517 nm, and the percentage of inhibition was calculated by (Absorbance of control- Absorbance of test solution)/Absorbance of control X 100.

Bacterial Strains

The antibacterial properties of the whole plant extract of *D. ophrydis* were evaluated with the Gram-positive Methicillin-resistant *Staphylococcus aureus* (MTCC 737) and Gram-negative *Escherichia coli* (MTCC 739) bacteria. The bacterial strains were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh-160036, India.

Agar Well Diffusion Method

Mueller-Hinton agar medium (Hi-media) was prepared as per the manufacturer's protocol, and 40 mL of the medium was poured into petri plates. The inoculum was aseptically dispersed on agar plates, and 6 mm well were bored using a sterile cork borer. 100 µL of the test extracts dissolved in DMSO was added to each well. Co-trimoxazole (10 µg/mL) and DMSO (100%) were served as positive and negative controls, respectively. The culture plates were incubated at 37 °C for 24 hours. The antibacterial activity of the plant extract was determined by measuring the diameter of the zone of inhibition against the bacterial growth.

RESULTS

Cytotoxicity Assessment

The anticancer potential of *D. ophrydis* was evaluated using hexane, chloroform and aqueous-ethanol extracts against breast

cancer cell line (MCF-7 cells). The independent ability of each extract to inhibit cancer cell growth was examined to identify the intrinsic anticancer activity of *D. ophrydis* and to establish its baseline efficacy. The cytotoxic therapies reduce tumour growth primarily by exposing cancer cells to direct killing which favours the survival of drug-resistant clones (Škarková *et al.*, 2024). This direct lethal effect was evident in doxorubicin with IC_{50} value of 0.32µg/mL. The plant extract displayed an anticancer potential with the IC_{50} value of 43.8 µg/mL in aqueous-ethanol extract followed by chloroform extract with 58.2 µg/mL. The hexane extract showed lowest activity against MCF-7 cell line. The decrease in cell viability with increasing extract concentration suggests a dose-dependent cytotoxic effect of the plant extract (Figure 1). The hexane, chloroform, and aqueous-ethanol extract reduced cell growth by 7.14%, 12.64%, and 15.57% at 20 µg/mL concentration. The cell death reached 66.27% in aqueous-ethanol extract at 60 µg/mL concentration, followed by 51.69% and 29.34% in chloroform and hexane extract. At 80 µg/mL, the aqueous-ethanol extracts inhibited cancer cell growth by 87.68%, suggesting selective cytotoxicity toward cancer cells with comparatively lower toxicity to normal cells. The results shows that aqueous-ethanol extract exhibited the highest anti-cancer activity among the tested samples.

Apoptosis Assessment

The nuclear morphology of apoptotic cells can be visualized by using Acridine Orange and Ethidium Bromide (AO/EtBr) dual staining. The acridine orange dye stains all the cells, and ethidium bromide stains the damaged cell membrane. The results showed uniformly green-colored viable cells in the untreated group, whereas cells treated with the plant extract exhibited viable cells progressing towards orange color indicating the early apoptotic stage (Figure 2). The cells treated with plant extract entered early apoptotic phase, reflecting a process of regulated cell death. This finding demonstrates that potential of *D. ophrydis* to induce the apoptosis in breast cancer cells.

Antioxidant Assay

The antioxidant activity of *D. ophrydis* was assessed at different concentrations (5-100 µg/mL) using ascorbic acid as the reference standard (Figure 3). The highest percentage of inhibition was observed in aqueous-ethanol extract with 90.64%, and hexane extract showed the lowest percentage of inhibition at 28.60%. The radical scavenging activity increased in a concentration-dependent manner in the tested sample. The IC_{50} value determines the ability of plant extract to inhibit the free radical formation and the optimum concentration to decrease by 50%. Hexane (126 µg/mL) and chloroform extract (58 µg/mL) showed increased IC_{50} value which indicate the comparatively lower antioxidant activity. The standard ascorbic acid showed an IC_{50} value of 12 µg/mL, while the plant extract was able to reduce the free radicals by 50% at 22 µg/mL in aqueous-ethanol exhibiting better radical scavenging activity.

Antibacterial Activity

The antibacterial activity against *S. aureus* and *E. coli* was evaluated using the well diffusion method. The zone of inhibition produced by hexane extract was 8.04±0.52 mm against *S. aureus* and 6.86±0.93 mm against *E. coli*. Meanwhile, chloroform extract exhibited 12.13±0.64 mm and 11.87±0.57 mm zones of inhibition against *S. aureus* and *E. coli* (Table 1). The highest zone of inhibition was observed in the aqueous-ethanol extract

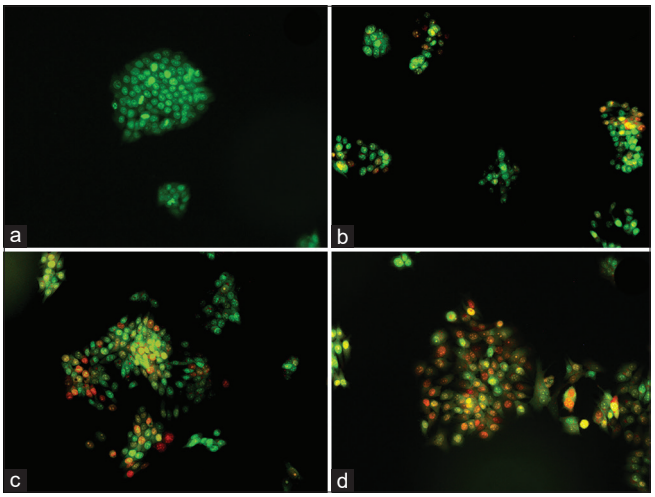

Figure 2: Apoptotic activity (AO/EtBr staining) on MCF-7 breast cancer cells. a) untreated control cells, b) hexane extract, c) chloroform extract and d) aqueous-ethanol extract

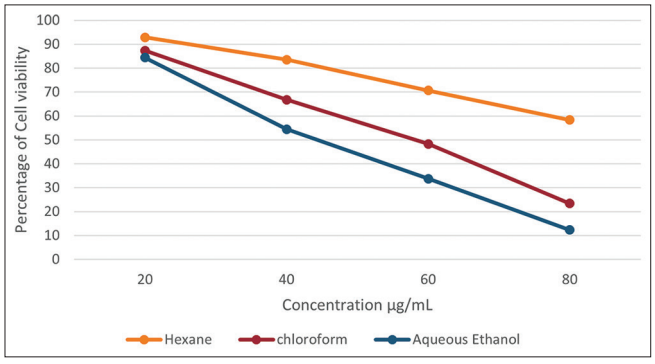

Figure 1: Cytotoxic activity (MTT-assay): The results are expressed as mean with standard deviation (Mean±SD), (n=3). One-way ANOVA was used to analyse the significant differences in test samples. The significant value was set at p<0.05

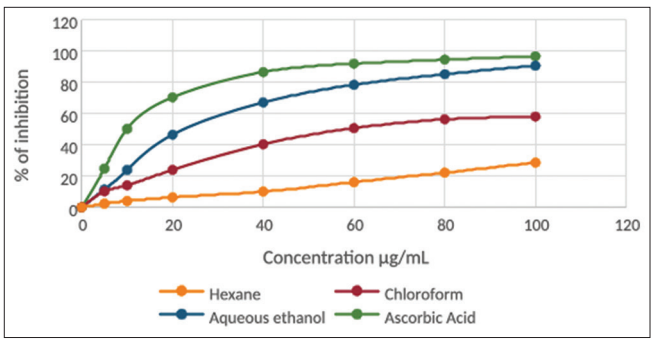

Figure 3: DPPH radical scavenging activity of leaf extracts of *Dienia ophrydis*. Mean value±Standard deviation, n=3 (p<0.05, Two-way ANNOVA)

Table 1: Zone of inhibition (mm) of *D. ophrydis* against pathogenic bacteria

Bacterial strain	Zone of inhibition of Co-trimoxazole (mm)	Zone of inhibition in Hexane extract (mm)	Zone of inhibition in Aqueous-ethanol extract (mm)	Zone of inhibition in Chloroform extract (mm)
Staphylococcus aureus	15.04±0.11	8.04±0.52	13.10±0.35	12.13±0.64
Escherichia coli	18.12±0.96	6.86±0.93	14.05±0.85	11.87±0.57

with 13.10±0.35 mm against S. *aureus* and 14.05±0.85 mm against *E. coli*. The results indicate that the aqueous-ethanol extract exhibited more resistance to bacterial strains when compared to the other two extracts (Figure 4).

DISCUSSION

The non-enzymatic antioxidants synthesized by orchids play a significant role in preventing oxidative damage. The antioxidant effects were previously studied in *Anacamptis papilionacea* (L.) R.M.Bateman, Pridgeon & M.W.Chase, *Ansellia africana* Lindl., *Cyrtorchis arcuata* (Lindl.) Schltr., *Dendrobium amoenum* Wall. ex Lindl., *Dendrobium jenkinsii* Wall. ex Lindl., *Eulophia nuda* Lindl., *Eulophia petersii* (Rchb.f.) Rchb.f., *Prosthechea michuacana* (Lex.) W.E.Higgins, and *Polystachya pubescens* (Lindl.) Rchb.f. (Perez Gutierrez *et al.*, 2010; Chinsamy *et al.*, 2014; Basılı *et al.*, 2025; Borah *et al.*, 2025; Padhee *et al.*, 2025; Paudel *et al.*, 2025). *D. ophrydis* is a terrestrial orchid from South Asia and recognized for therapeutic value in traditional medicine (Teoh, 2016; Margońska *et al.*, 2025). It is essential to validate the therapeutic efficiency of the orchids reported in folk medicine for effective usage. The present study indicates the anticancer, antioxidant, and antibacterial potential of *D. ophrydis*. This orchid exhibits a strong antioxidant activity (IC_{50} value at 22 µg/mL) than other orchids (*Aerides Odorata* Lour. - 132.24 µg/mL, *Bulbophyllum lilacinum* Ridl. - 136.70 µg/mL, and *Dendrobium tortile* Lindl. - 138.59 µg/mL) as reported by Rahman and Huda (2021).

Plants are the natural storehouses of bioactive compounds. *Fagonia indica* Burm.f. and *Phaleria macrocarpa* (Scheff.) Boerl. are some of the medicinal plants used in the traditional medicinal system for the treatment of cancer (Khan *et al.*, 2019). Identifying plants for bioactivity is vital for discovering new anticancer agents that may reduce the side effect of synthetic drugs. The anti-cancer potential of the *D. ophrydis* can be attributed to the bioactive compounds present in it such as Stigmasterol, Tremulone, 17-Pentatriacontene, 2,4-Di-tert-butylphenol and 12-O-Acetylingol 8-tiglate (unpublished data). In addition to its anticancer property, the plant extract also demonstrated the potential benefits of antibacterial activity. Diseases such as sepsis, bacteremia, cellulitis, bacterial meningitis, gonorrhea and syphilis represent some of the serious threats from bacterial infections. The constant use of antibiotics led to the emergence of drug-resistant bacteria and the urgent need to explore new alternatives. *E. coli* and *S. aureus* (MRSA) were selected for the antibacterial assay due to their leading cause of nosocomial and community-based infections. The significance of this study highlights the multifaceted health benefits of *D. ophrydis* and supports its therapeutic potential as reported in traditional medicine.

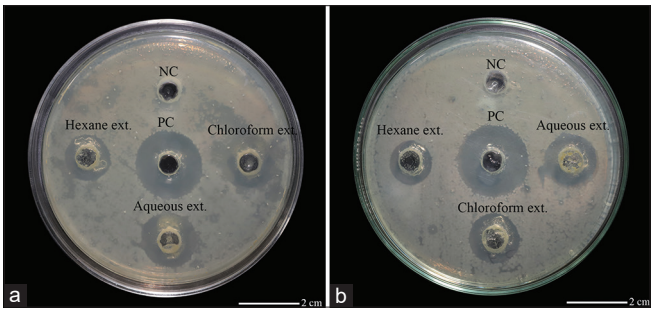

Figure 4: Antibacterial activity of *Dienia ophrydis* against a) *Staphylococcus aureus* and b) *Escherichia coli*

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