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ORIGINAL ARTICLE

Anticancer Activity of *Opuntia Cochenillifera* Extract Against Triple-Negative Breast Cancer Cells via Apoptosis Induction and Bioinformatics-Based Target Prediction

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Abstract

Background/Aim: Breast cancer remains one of the most commonly diagnosed cancers among women worldwide and is a significant public health concern. Aim of this study was to investigate the *in vitro* anticancer activity of *Opuntia cochenillifera* ethanolic extract (EOC) against triple-negative breast cancer (TNBC) cells and to predict potential molecular targets involved in its mechanism of action through bioinformatics analysis.
Methods: Fresh *O cochenillifera* leaves were extracted by ethanol maceration. Phytochemical constituents were analysed using qualitative screening, thin-layer chromatography (TLC), UV–Vis spectrophotometry and Fourier transform infrared (FTIR) spectroscopy. Cytotoxicity against MDA-MB-231 TNBC cells was determined via MTT assay and morphological assessment. Apoptosis induction was quantified by Annexin V–FITC/PI flow cytometry. Potential molecular targets were identified through integrated bioinformatics platforms, including *STITCH, SEA, SwissTargetPrediction, STRING* and *Cytoscape*, followed by pathway enrichment analysis.
Results: Phytochemical analysis revealed the presence of alkaloids, flavonoids, phenolics, saponins and steroids, with flavonoids as predominant constituents (68 mg/g extract). FTIR spectra confirmed hydroxyl, aliphatic, aromatic and carbonyl functional groups consistent with phenolic and flavonoid structures. EOC exhibited dose-dependent cytotoxicity against MDA-MB-231 cells, with an IC50 of 270.63 µg/mL, accompanied by morphological and flow-cytometric evidence of apoptosis. Network pharmacology analysis identified 34 apoptosis-related target genes, including six hub genes (*CDKN1A/p21, TP53, MAPK1, MAPK3, AKT1* and *FOXO3*), associated with TP53-p21 signalling, MAPK cascades and mitochondrial stress-induced apoptosis pathways.
Conclusion: *O cochenillifera* ethanolic extract demonstrated significant cytotoxic and pro-apoptotic effects in TNBC cells, potentially mediated through TP53-p21 and MAPK pathway modulation. These findings suggest that *O cochenillifera* is a promising candidate for further investigation as a natural adjuvant therapeutic agent for triple-negative breast cancer.

Key words: *Opuntia*; Triple negative breast neoplasms; Apoptosis; Flavonoids; Phytochemicals; Tumour suppressor protein p53; Mitogen-activated protein kinases; Cell cycle proteins.

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Introduction

Breast cancer remains one of the most commonly diagnosed cancers among women worldwide and is a significant public health concern. Critically, the main cause of death among breast cancer patients is not the primary tumour itself but the spread of cancer cells to distant organs such as the lungs, brain, or liver, a process known as metastasis.[1] Metastatic breast cancer accounts for over 90 % of breast cancer-related deaths, with a 5-year survival rate as low as 22 % for affected patients.[2] In 2018, the global cancer burden included an estimated 9.5 million deaths and breast cancer was the leading cause of cancer death among women, with mortality rates continually rising in many parts of the world. Indonesia reflects this global trend, with hundreds of thousands suffering from breast cancer each year.[3, 4] According to the WHO, breast cancer cases and related deaths continue to increase, reflecting both the growing burden of the disease and limitations in current treatment approaches. Despite advances in surgery, radiotherapy and chemotherapy, resistance to conventional treatments remains a major clinical challenge, often contributing to disease relapse and poor outcomes. Chemoresistance develops through genetic mutations, adaptive stress responses and persistent activation of survival pathways, resulting in tumour progression and metastasis.[5, 6]

Among breast cancer subtypes, triple-negative breast cancer (TNBC) is a highly aggressive subtype, marked by poor prognosis and resistance to conventional therapies. One of the major barriers in TNBC treatment is its high rate of chemoresistance and rapid metastasis, which are closely linked to disruptions in cellular redox homeostasis and aberrant reactive oxygen species (ROS)-mediated signalling.[7, 8] TNBC cells adapt to elevated ROS by activating antioxidant defence mechanisms that sustain their growth and resistance.[7, 8] Consequently, targeting ROS-related signalling and modulating redox balance have emerged as promising strategies to limit tumour survival and sensitise TNBC to therapy.[9]

Several *Opuntia* species have demonstrated anticancer effects in various experimental models, including inhibition of cell proliferation, induction of apoptosis and modulation of oxidative stress in colon, liver and breast cancers.[10] Traditionally regarded as an ornamental plant, *Opuntia cochenillifera* contains a rich array of secondary metabolites, including alkaloids, phenolics, tannins, flavonoids and quercetin, all of which have demonstrated potential anticancer activity.[11] However, despite its rich phytochemical composition, the biological activity of *Opuntia cochenillifera*, a species containing abundant flavonoids, phenolics and other bioactive compounds, has not been systematically investigated in triple-negative breast cancer. In particular, the molecular mechanisms underlying its potential anticancer or pro-apoptotic actions remain unclear.

Therefore, this study aimed to evaluate the cytotoxic and apoptosis-inducing effects of the ethanolic extract of *O cochenillifera* on MDA-MB-231 triple-negative breast cancer cells. It was hypothesised that the extract induces apoptosis and cell-cycle arrest by modulating redox-sensitive signalling pathways, as supported by bioinformatics-based identification of its potential molecular targets.

Methods

Plant material authentication and extract preparation

Fresh leaves of *Opuntia cochenillifera* were collected in July 2024 from Gunungpati District, Semarang City, Central Java, Indonesia (approximate coordinates: latitude –7.20°, longitude 110.44°). The plant material was identified and authenticated by a botanist at the Biology Laboratory; Universitas Negeri Semarang and a voucher specimen (No OC-UN-11.2024) was deposited in the herbarium of the same institution for reference. Mature, healthy leaves were selected, washed to remove surface debris, cut into small pieces and air-dried under indirect sunlight for approximately 8 h until constant weight was achieved. A total of 11.13 kg of fresh leaves yielded 0.99 kg of dried *simplicia* (91.10 % reduction by weight). The dried material was ground into fine powder using a mechanical blender. For extraction, 200 g of powdered material was macerated with 800 mL of 96 % ethanol (solid-to-solvent ratio 1:4 w/v) in a sealed Erlenmeyer flask at room temperature (~28 °C) for 24 h with occasional stirring. The mixture was filtered and the residue was re-extracted under identical conditions for two additional 24-hour cycles. All filtrates were combined and concentrated using a rotary vacuum evaporator (*Heidolph*, Germany) at 60 °C until a thick

ethanolic extract was obtained.[12-14] The percentage yield was calculated as 9.76 % (w/w; 96.62 g extract from 990 g dried material). The extract was coded as Batch EOC-07-24, stored in airtight amber vials at 4 °C and protected from light until further use to ensure reproducibility across subsequent analyses.

Phytochemical screening

Phytochemical screening of the *Opuntia cochenillifera* extract was conducted to identify major groups of secondary metabolites, including alkaloids, saponins, flavonoids, tannins and steroids/triterpenoids. Alkaloids were tested by moistening 5 g of *simplicia* powder with 25 % ammonia, extracting with chloroform and detecting coloured precipitates after adding Dragendorff's or Mayer's reagents. Saponins were identified by boiling 5 g of powder in water, filtering and observing stable foam formation after shaking in the presence of HCl. Flavonoids were detected by reacting 5 mL of filtrate with magnesium powder, concentrated HCl and amyl alcohol, producing red, yellow, or orange coloration in the amyl alcohol layer. Tannins were confirmed by boiling 10 g of powder in water, adding ferric chloride and observing a green-violet or black coloration. Steroids and triterpenoids were identified by extracting 5 g of powder with ether, evaporating to dryness and observing characteristic colour changes after adding stearic anhydride and sulfuric acid.[15, 16]

Thin layer chromatography (TLC), UV-Vis spectrophotometry, Fourier transform infrared spectroscopy (FTIR)

Preliminary phytochemical characterisation was performed using TLC, UV-Vis spectrophotometry and FTIR to support the presence of flavonoid-like and related bioactive constituents in the extract. TLC was used to characterise bioactive compounds. Silica gel plates were cut to specific dimensions and the sample was spotted after dilution with methanol. The plates were developed in a saturated chamber with eluent, air-dried and visualised under UV light at 245 nm and 366 nm. The appearance of coloured spots indicated specific metabolites, with particular emphasis on flavonoids.[17, 18] FTIR analysis was performed using KBr pellets to identify functional groups in the extract, covering wavelengths of 4000–500 cm^{-1}, aiding in the chemical characterisation of bioactive compounds.

Cell culture

The human breast cancer cell line MDA-MB-231 (triple-negative breast cancer) was obtained from the American type culture collection (ATCC), USA. The MDA-MB-231 was cultured in RPMI medium supplemented with 10 % foetal bovine serum (FBS), 1 % penicillin-streptomycin and 0.5 % fungizone (*Gibco*). Cultures were maintained in a CO_2 incubator. Cells were passaged and harvested using PBS and trypsin. Experiments used cells between passages 3–10.[19]

Cytotoxicity assay

Cytotoxicity was assessed via the MTT assay. Cells at 80–90 % confluence were harvested, counted and seeded at a density of 5×10^3–1×10^4 cells/well in 96-well plates (100 µL/well). After overnight recovery, cells were treated with serial concentrations of the extract (10–500 µg/mL) and controls. Plates were incubated for 24 h, the media was removed and 100 µL of 0.5 mg/mL MTT reagent was added. After 4 h, the formazan crystals were dissolved in 100 µL DMSO. Absorbance was measured at 595 nm. Data were expressed as mean ± SD of three independent biological replicates, each containing three biological replicates per condition. The half-maximal inhibitory concentration (IC_{50}) value was determined by nonlinear regression using a four-parameter logistic (4PL) model and goodness-of-fit (R^2) was evaluated using Microsoft Excel or equivalent curve-fitting software.[20, 21]

Apoptosis analysis by flow cytometry

Apoptosis was quantified using Annexin V–FITC/Propidium Iodide (PI) staining followed by flow cytometry. After 24 h treatment with various extract concentrations, MDA-MB-231 cells were harvested, washed twice with PBS and resuspended in Annexin V binding buffer. Cells were stained with Annexin V–FITC and PI (*BD Biosciences*, USA) according to the manufacturer's protocol. Samples were analysed using a Sony MA900 flow cytometer (*Sony Biotechnology*, Japan). 10,000–20,000 events per sample were recorded. Compensation was applied using single-stained controls and gating was performed to exclude debris and doublets, followed by quadrant analysis to distinguish viable (Annexin V^-/PI^-), early apoptotic (Annexin V^+/PI^-), late apoptotic (Annexin V^+/PI^+) and necrotic (Annexin V^-/PI^+) populations. Data were processed using FlowJo v10 (*BD Biosciences*, USA) and mean per-

centages of early and late apoptosis were reported from three independent experiments.[22]

Bioinformatics analysis

Active compounds identified via phytochemical screening with anti-breast cancer potential were verified using Dr Duke's and the USDA phytochemical database. Key metabolites (s, saponins, tannins) and their putative targets were identified using *SEA, SwissTargetPrediction* and *STITCH*. Breast cancer-related gene associations were determined via *PubMed* and protein interactions were assessed using *STRING. Cytoscape* and *CytoHubba* were employed to rank hub genes. Survival analysis was performed by dichotomising patient groups based on gene expression levels (high/low), calculating hazard ratios (HR) and visualising results. Relevant signalling pathways were analysed in relation to *Opuntia cochenillifera* and MDA-MB-231 cells.[23, 24]

Statistical analysis

All experiments were conducted *in vitro* using experimental models with 3 biological replications. Data are presented as mean ± standard error of the mean (SEM). Statistical comparisons among treatment groups were performed using one-way ANOVA, followed by Tukey's test for all pairwise comparisons and p-values below 0.05 were considered statistically significant.

Results

TLC is a widely used phytochemical screening technique to detect the presence of secondary metabolite groups within plant extracts. Positive identification of specific secondary metabolites in the *Opuntia cochenillifera* extract (EOC) through TLC provides crucial insights into compounds that may serve as natural adjuvants in breast cancer therapy. In this study, TLC analysis specifically focused on detecting flavonoids using kaempferol as a standard. The TLC results revealed a distinct yellow spot visible under ultraviolet light at 366 nm wavelength, confirming the presence of flavonoids in EOC (Figure 1).

Phytochemical screening of EOC was conducted because the therapeutic potential of medicinal plants is largely determined by their secondary metabolite profiles. Screening identified the presence of alkaloids, flavonoids, phenolics, saponins and steroids in EOC, while terpenoids were absent (Table 1). Among these, flavonoids are particularly noteworthy due to their suspected anticancer properties. The flavonoid class present in EOC includes flavonols such as myricetin and rutin and anthocyanins including pelargonidin, cyanidin, delphinidin, petunidin and malvidin.[25]

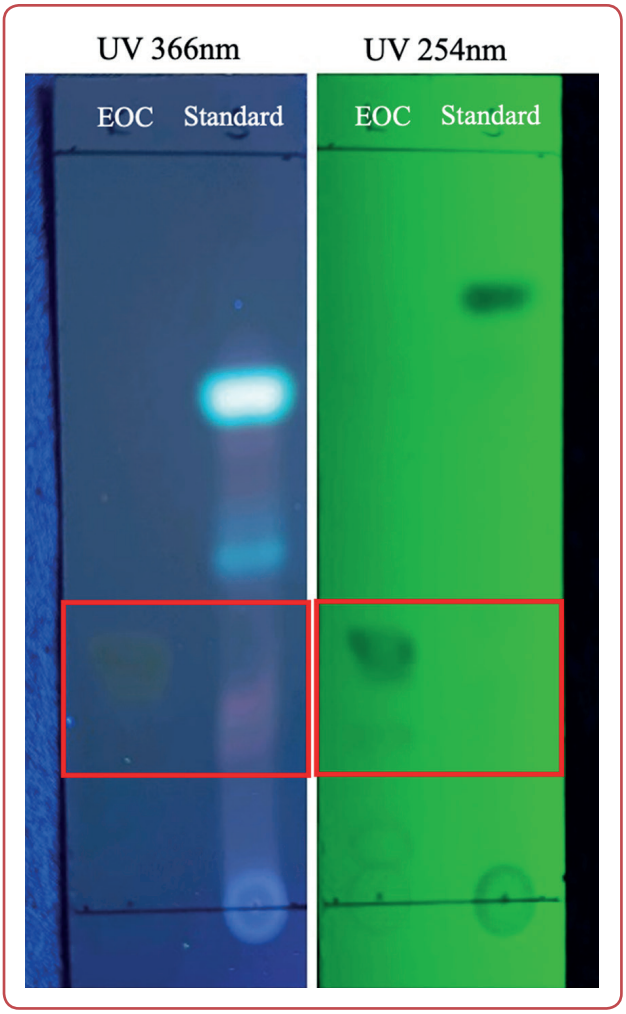

Figure 1: Thin-layer chromatography (TLC) analysis of Opuntia cochenillifera extract (EOC) and the kaempferol standard. The TLC plate was developed to detect flavonoids using kaempferol as a reference compound. Under ultraviolet light at 366 nm, a distinct yellow spot was observed in the EOC lane, indicating the presence of flavonoid compounds

Ultraviolet-visible (UV-Vis) spectrophotometry is an analytical technique commonly used to detect and quantify chemical compounds, including aromatic compounds in medicinal substances, by measuring the absorption of ultraviolet and visible light at specific wavelengths.[26] Each compound produces a characteristic UV-Vis absorption spectrum that can be used for identification and quantification. However, not all compounds

Table 1: Phytochemical screening of Opuntia cochenillifera extract (EOC)

Test parameter	Qualitative result	Detection	Method used
Alkaloids	Reddish-brown precipitate	(+)	Dragendorff and Mayer
Flavonoids	Reddish-orange solution	(+)	Wilstätter
Tannins	Dark brown solution	(+)	Forth
Saponins	Formation of stable foam	(+)	$FeCl_3$
Steroids	Green coloration	(+)	Liebermann–Burchard
Triterpenoids	No red coloration	(–)	Liebermann–Burchard

(+) detected (-) non-detected;

absorb UV light and spectral overlap can sometimes occur, complicating analysis. This method is applicable to both solid samples and substances with high aqueous solubility.[27] Additionally, UV-Vis spectrophotometry is a straightforward and effective approach for assessing the solubility of compounds.[28]

The principle of UV-Vis spectrophotometry involves passing UV and visible light through a sample and measuring the absorbance at different wavelengths. In the present study, UV-Vis spectrophotometry was applied to quantify flavonoid content in the EOC following preliminary detection by TLC. The analysis revealed that 1 g of EOC contained 68 mg of flavonoids at wavelengths from 270 nm to 340 nm, confirming flavonoid-like constituents as the major bioactive compounds in the extract with potential breast cancer inhibitory activity (Figure 2).

FTIR analysis was conducted on EOC to identify functional groups potentially responsible for its anticancer activity. EOC was dissolved in water and partitioned with ethanol-water, then the solvent was evaporated to dryness before FTIR analysis. The FTIR spectra were recorded using a KBr pellet method over the wavelength range of 4000 to 500 cm^{-1}.[29] The analysis revealed the presence of secondary metabolite compounds, primarily

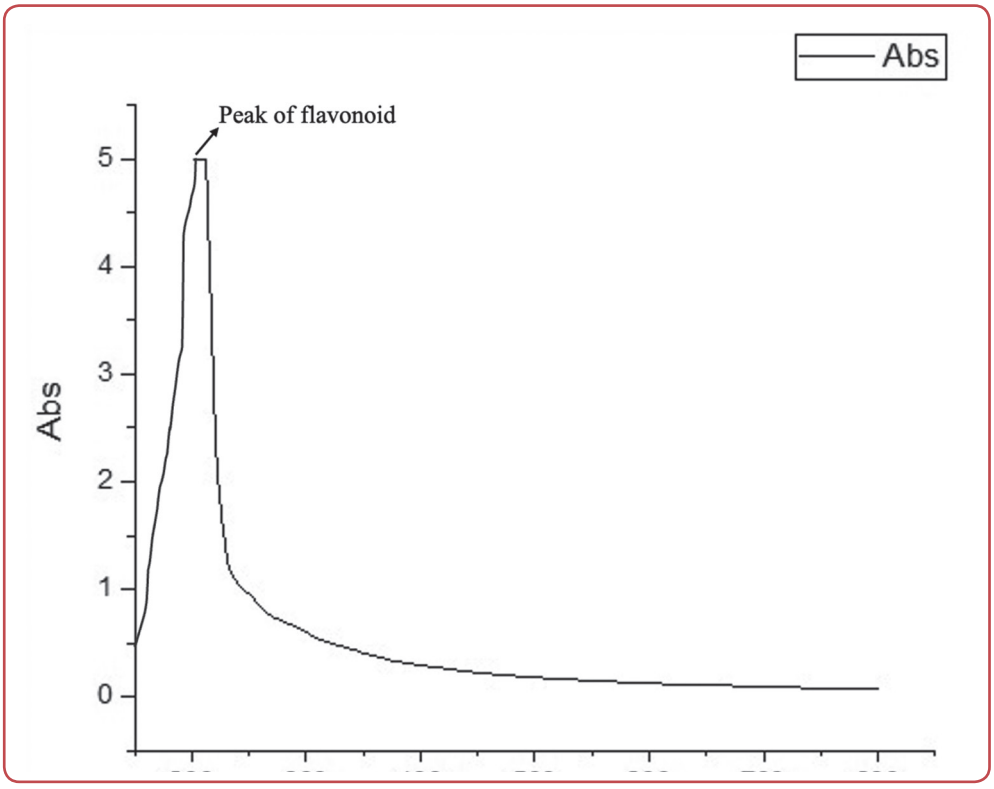

Figure 2: Ultraviolet-visible (UV-Vis) spectrophotometric analysis of flavonoid compounds in Opuntia cochenillifera extract (EOC). The absorbance spectrum displays characteristic peaks corresponding to flavonoid content

flavonoids belonging to the phenolic group (Figure 3).[30] These phenolic flavonoids in *Opuntia cochenillifera* serve as antioxidants, anti-inflammatory agents and immunomodulators.[31] Key absorption peaks included a broad O–H and carboxyl (-COOH) stretching band at 3358 cm^{-1}, indicative of phenolic compounds such as flavonoids. An aliphatic C-H stretching peak was observed at 2978 cm^{-1}. The peak at 1636 cm^{-1} corresponded to C=C double bond stretches, while the 1349 cm^{-1} peak reflected C-H bending vibrations. Peaks between 1044 and 1077 cm^{-1}, within the 1200 to 1000 cm^{-1} region, were attributed to C–O and O–H groups of polysaccharides.[32] Additionally, signals at 999 and 876 cm^{-1} represented C–H bonds characteristic of β-D-glucose. The flavonoid-like constituents identified by FTIR in EOC are hypothesised to contribute to the extract's potential breast anticancer activity.

The MDA-MB-231 breast cancer cell line is a triple-negative subtype characterised by a lack of oestrogen receptor, progesterone receptor and HER2 expression. These cells exhibit an adherent growth pattern on tissue culture surfaces, characterised by an endothelial-like morphology and the formation of spindle-shaped projections that form colonies (Figure 4A). Upon treatment with EOC at concentrations ranging from 10 to 500 µg/mL, notable cytotoxic effects were observed. Specifically, treatment with 25 µg/mL EOC for 24 h significantly reduced MDA-MB-231 cell viability and induced morphological changes characterised by a shift from spindle-shaped to rounded cells, with loss of typical cellular projections, indicating disruption of cell-matrix interactions (Figure 4B). These findings suggest that even at low concentrations, EOC inhibits breast cancer cell growth, while higher concentrations can completely suppress cell proliferation. The cytotoxic activity of EOC against MDA-MB-231 cells was quantitatively assessed using the MTT assay, which measures cell viability based on metabolic activity. The extract demonstrated a dose-dependent decrease in cell viability across treatments, ranging from 10 to 500 µg/mL, after 24 hours of exposure. Viability declined significantly between 10 and 100 µg/mL, with a plateau at higher doses (Figure 4C). The calculated IC_{50} for EOC was 270.63 µg/mL, indicating strong cytotoxic activity, as effective anticancer agents typically exhibit IC_{50} values below 1000 µg/mL. These results are consistent with prior findings where flavonoid-mediated extracts showed IC_{50} values around 104.2 µg/mL against similar cancer models.

A bioinformatics approach was employed to predict proteins involved in apoptosis regulation in breast cancer cells targeted by active compounds from EOC. Target genes of 26 active compounds in EOC were compiled from three comprehensive

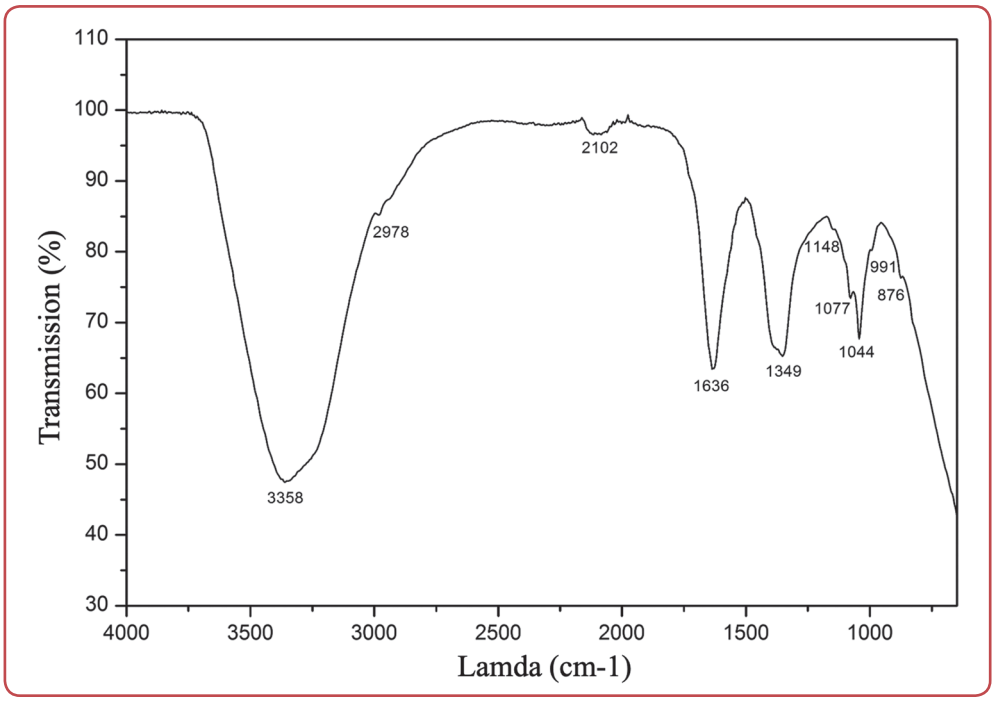

Figure 3: Fourier transform infrared (FTIR) spectrum of Opuntia cochenillifera extract (EOC). The spectrum was recorded using a KBr pellet method over a wavelength range of 4000 to 500 cm^{-1}

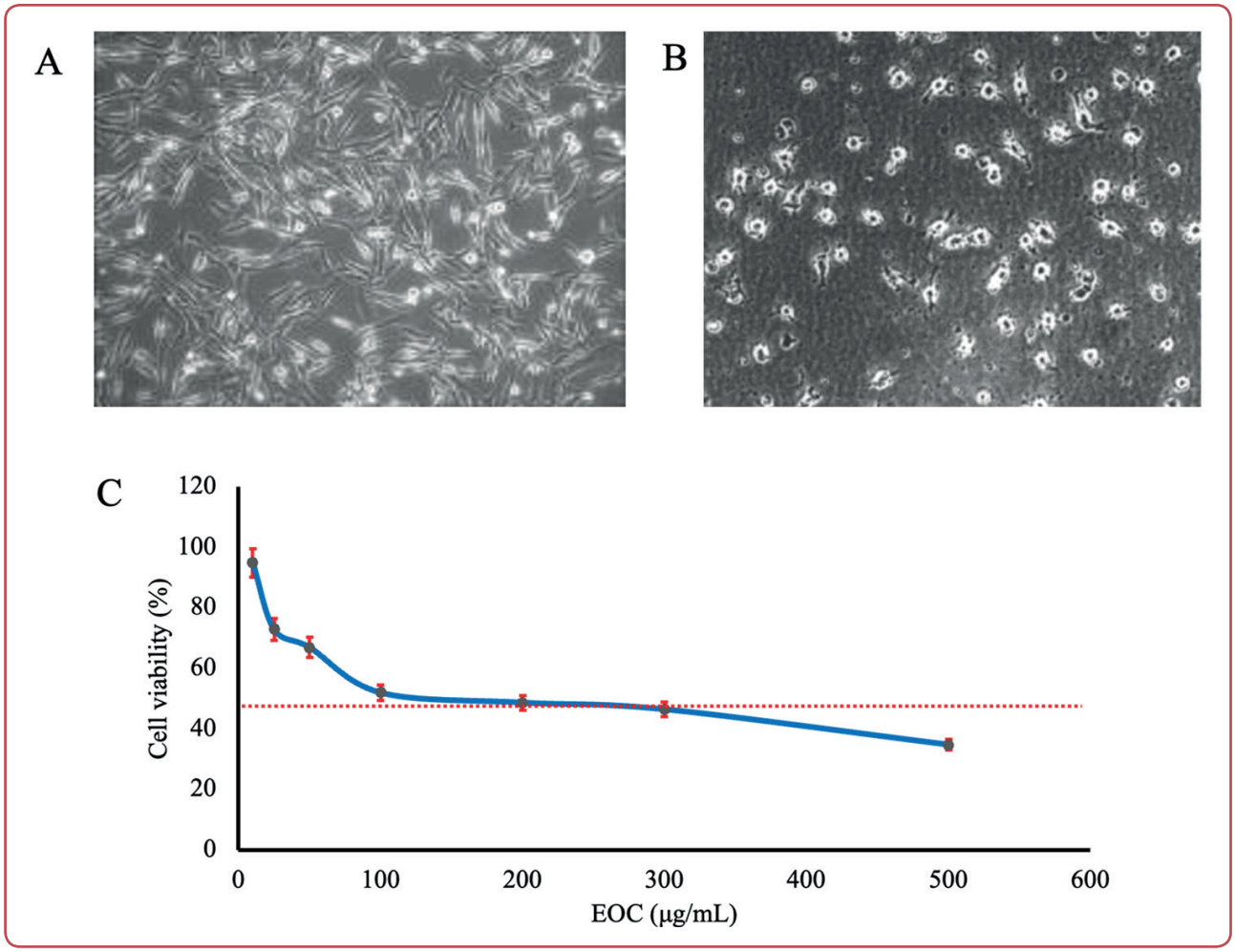

Figure 4: (A) Morphology of MDA-MB-231 breast cancer cells, (B) Morphological changes in MDA-MB-231 cells after 24-hour treatment with 25 µg/mL Opuntia cochenillifera extract (EOC) and (C) Cytotoxic effects of EOC on MDA-MB-231 breast cancer cells. N 3 biological replicates, data represented average ± SD

online databases: *STITCH, SEA* and *SwissTargetPrediction*. A total of 273 genes were identified as potential EOC targets. Separately, 350 genes associated with breast cancer apoptosis were retrieved from NCBI Gene using the keyword "breast cancer apoptosis." Cross-referencing these datasets via Venn diagram analysis revealed 34 overlapping genes linked to both EOC targets and breast cancer apoptosis (Figure 5A).

Potential therapeutic targets of EOC (TTPE) were further analysed for protein-protein interactions using the STRING database, resulting in a network of 37 nodes connected by 150 edges, with an average node degree of 8.11, indicating a robust interaction network among these genes (Figure 5B). Functional gene ontology analysis categorised these 34 genes, showing that TTPE are involved in JUN kinase activity, MAP kinase activity and DNA-dependent protein kinase activity, localised primarily in mitochondria, neoplasms and the cytosol. Biological process analysis highlighted a strong influence of TTPE on cellular aging pathways induced by stress (Figure 5C). These insights guided the focus towards key genes involved in apoptosis regulation in breast cancer. Network analysis using *Cytoscape* identified six hub genes with the highest degree scores: *CDKN1A (p21), TP53, MAPK1, MAPK3, AKT1* and *FOXO3* (Figure 5D). Collectively, these data indicate the promising antitumour potential of *Opuntia cochenillifera* extract as a natural adjuvant therapy for inhibiting proliferation and inducing apoptosis in triple-negative breast cancer cells.

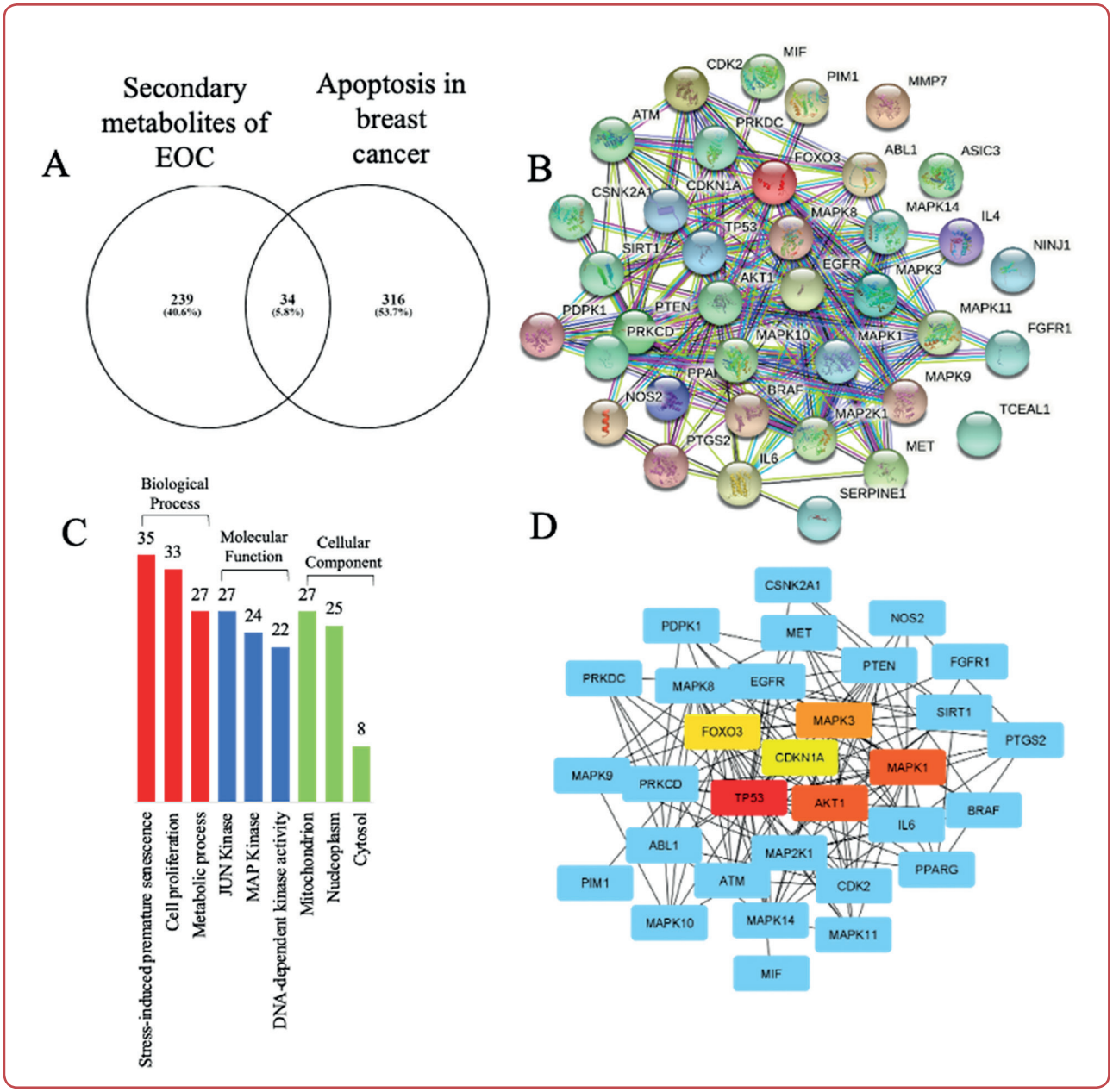

Figure 5: Bioinformatics analysis of Opuntia cochenillifera extract (EOC) active compounds on breast cancer apoptosis pathways. (A) Venn diagram illustrating the overlap of gene targets predicted for 26 active compounds in EOC and genes associated with breast cancer apoptosis. (B) Protein-protein interaction network of the 34 therapeutic target potential genes (TTPE) generated using STRING. (C) Gene ontology enrichment analysis showing the involvement of TTPE in key molecular functions. (D) Cytoscape visualisation highlighting the six hub genes with the highest degree scores

Discussion

Breast cancer remains a formidable global health challenge, ranking as the second leading cause of cancer-related mortality worldwide, with mortality rates continuing to rise largely due to chemoresistance and limited treatment options for aggressive subtypes. TNBC, characterised by the absence of oestrogen receptor, progesterone receptor and HER2 expression, represents one of the most aggressive and difficult-to-treat breast cancer subtypes, necessitating the exploration of alternative therapeutic strategies that can induce apoptosis and suppress tumour proliferation without significant toxicity to normal cells. In alignment with global health, the alternative

therapies that induce apoptosis and suppress tumour proliferation are critically needed. Members of the *Cactaceae* family, including *Opuntia cochenillifera*, have demonstrated therapeutic potential due to their rich secondary metabolite content, including flavonoids, which are known for their anticancer effects.[32] They also have the capacity to reduce tumour resistance to chemotherapeutic agents.[32] The present study investigated the anticancer potential of EOC against MDA-MB-231 triple-negative breast cancer cells through a combination of phytochemical characterisation, cytotoxicity assessment and network pharmacology analysis.

Presented phytochemical screening revealed that EOC contains several classes of bioactive compounds, including alkaloids, flavonoids, phenolics, saponins and steroids, with terpenoids being notably absent. Among these constituents, flavonoids were identified as the predominant bioactive compounds, comprising 68 mg per gram of extract as determined by UV-Vis spectrophotometry. This substantial flavonoid content is particularly noteworthy given the well-documented anticancer properties of this compound class. Flavonoids inhibit cancer cell proliferation by inducing apoptosis without adversely affecting normal cell physiology, primarily through the modulation of apoptotic proteins, including BAX, BCL-2 and caspases,[31] as well as the inhibition of signal transduction pathways involving protein kinases. The FTIR analysis further corroborated these findings, revealing characteristic absorption peaks associated with phenolic hydroxyl groups (3358 cm^{-1}), aromatic C=C bonds (1636 cm^{-1}) and other functional groups typical of flavonoid structures, thereby confirming the chemical identity of these bioactive constituents.

The significant reduction in MDA-MB-231 cell populations following EOC treatment supports the hypothesis that active compounds in *Opuntia cochenillifera* exhibit potent anticancer activity *in vitro*.[29] Secondary metabolites, such as phenolics and flavonoids are recognised for their antioxidant properties and for triggering apoptosis by downregulating anti-apoptotic factors, including Bcl-2 and NF-κB, which are critical transcription factors that promote cancer cell survival and proliferation. Prior research further corroborates that *Opuntia*-derived compounds enhance pro-apoptotic proteins, including p53, caspase-3 and caspase-7, key mediators of programmed cell death.[33]

Among these, p21 and TP53 play central roles in stress-related apoptotic pathways.[31] DNA damage increases ROS, which activate TP53, which in turn regulates downstream genes, such as CDKN1A/p21, during cell replication.[32] Breast cancer cells experiencing p21 activation undergo TP53-dependent cell cycle arrest at the G2 phase following DNA damage.[34, 35] The dependence of TP53-mediated cell cycle arrest on p21 expression, alongside p21's role as a proliferation inhibitor, underscores the gene's importance in TP53-dependent apoptosis induction. These results support a predicted molecular mechanism for EOC-induced apoptosis, involving modulation of p21 and TP53. Subsequently, the binding affinity between EOC active compounds and these key apoptotic proteins was analysed to predict molecular interaction strength. In addition, the MAPK pathway genes (MAPK1 and MAPK3) are crucial regulators of cell proliferation, differentiation and apoptosis, while AKT1 and FOXO3 are key components of the PI3K/AKT survival pathway, frequently dysregulated in breast cancer. The concurrent targeting of these complementary pathways suggests that EOC may overcome the adaptive resistance mechanisms commonly observed in TNBC by simultaneously suppressing survival signals and activating apoptotic programs. These results support a predicted molecular mechanism for EOC-induced apoptosis via modulation of p21 and TP53 proteins. The limitation of this study was the lack of analysis on normal cells. Future work will evaluate selectivity using non-malignant mammary epithelial models. The current findings provide preliminary evidence of cytotoxic activity in TNBC cells. Collectively, these findings highlight *Opuntia cochenillifera* extract as a promising natural therapeutic candidate for adjuvant treatment of TNBC

Conclusion

This study indicates that the EOC contains bioactive compounds, particularly flavonoids, which exhibit strong anticancer activity against MDA-MB-231 cells, a type of TNBC. This activity is mediated through several genes involved in apoptosis and cell cycle arrest, including *TP53, p21/CDKN1A, MAPK1, MAPK3, AKT1* and *FOXO3*. These findings suggest that EOC could be a promising natural candidate for breast cancer therapy.

Ethics

The study was approved by the ethics committee of the Medical Faculty, Universitas Diponegoro, Semarang, Indonesia, under decision No 0014/X/025/Bioethics, dated 30 October 2025.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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Methodology: AA, AAA
Validation: AA, HSA
Formal analysis: AA
Investigation: AA, HSA
Resources: AAA, HSA
Data curation: HSA
Writing - original draft: AA, HSA
Writing - review and editing: AAA
Visualisation: AAA
Supervision: AAA
Project administration: AA.

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