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PHYTOCHEMICAL PROFILING AND *IN VITRO* EVALUATION OF *ALOE VERA* FOR ANTIMICROBIAL POTENTIAL AND ANTICANCER ACTIVITY ON MCF 7 BREAST CANCER CELLS

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ABSTRACT

The present study investigates the phytochemical composition and *in vitro* anticancer activity of *Aloe vera* leaf extract against human breast cancer (MCF-7) cell lines. Qualitative screening confirmed the presence of key bioactive constituents such as flavonoids, phenolics, alkaloids, tannins, and saponins, while quantitative analysis revealed a high content of total phenolics (0.498 ± 0.02 OD) and flavonoids (0.412 ± 0.01 OD). The ethanolic extract exhibited significant antimicrobial activity against selected cutaneous pathogens, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, *Trichophyton rubrum*, and *Malassezia furfur*, with zones of inhibition increasing in a dose-dependent manner. The antiproliferative effect assessed by MTT assay demonstrated a dose dependent reduction in cell viability, with maximum inhibition of 76.8% at 500 µg/mL

and an IC_{50} value of approximately 319.8 µg/mL. Morphological evaluation showed distinct apoptotic features including cell shrinkage, nuclear condensation, and membrane blebbing. Apoptosis analysis further confirmed a significant increase in apoptotic cells (up to 68%) at higher concentrations. These findings highlight the potential of *Aloe vera* as a promising natural source of anticancer compounds, warranting further molecular and in vivo investigations.

Keywords: *Aloe vera*, Phytochemical profiling, Antimicrobial, MCF-7 cell lines, Antiproliferative activity, Apoptosis

INTRODUCTION

Cancer remains one of the leading causes of mortality worldwide, with breast cancer being the most commonly diagnosed malignancy among women. In India, the incidence of breast cancer has shown a steady rise over the past decade, posing a major public health concern **[1]**. Despite significant advances in chemotherapy, radiotherapy, and targeted therapies, limitations such as drug resistance, toxicity, and adverse side effects continue to hinder effective treatment outcomes **[2]**. These challenges have prompted increasing interest in the exploration of plant-based bioactive compounds as alternative or complementary therapeutic agents. Medicinal plants have long been recognized as valuable sources of pharmacologically active compounds. Among them, *Aloe vera* (L.) Burm.f., a succulent plant belonging to the family Asphodelaceae, has gained considerable attention due to its diverse therapeutic properties. Traditionally, *Aloe vera* has been used in Ayurveda and other indigenous systems of medicine for its wound healing, anti-inflammatory, antimicrobial, and antioxidant activities **[3]**. The plant is rich in a variety of phytochemicals, including flavonoids, phenolic compounds, anthraquinones, saponins, and polysaccharides, which contribute to its medicinal potential **[4]**.

Systemic infections are a major global health concern due to their ability to spread through the bloodstream and affect multiple organs, often leading to severe morbidity and mortality. These infections are commonly caused by a wide range of pathogenic microorganisms, including bacteria and fungi, particularly in immunocompromised individuals. Among bacterial pathogens, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus pyogenes* are frequently associated with septicemia, urinary tract infections, and respiratory infections. Similarly, fungal pathogens such as *Candida albicans*, *Aspergillus niger*, *Trichophyton rubrum*, and *Malassezia furfur* are known to cause

opportunistic and systemic infections, especially under favorable conditions **[5-6]**. *Aloe vera* extracts exhibit inhibitory effects against various bacterial and fungal pathogens by disrupting cell membranes, inhibiting enzyme activity, and interfering with microbial metabolism **[7-8]**. However, there remains a need to further investigate its effectiveness against microorganisms associated with systemic infections

Recent studies have highlighted the anticancer properties of *Aloe vera*, suggesting its potential role in inhibiting tumor growth and progression. Bioactive constituents such as aloin, aloe-emodin, and acemannan have been reported to exhibit cytotoxic effects against various cancer cell lines by inducing apoptosis and inhibiting cell proliferation **[9]**. Apoptosis, or programmed cell death, is a crucial mechanism in cancer therapy, as it enables the elimination of damaged or abnormal cells without causing inflammation. The induction of apoptosis in cancer cells is considered a key strategy for the development of effective anticancer agents **[10]**. *In vitro* studies using human breast cancer cell lines, particularly MCF-7 cells, provide a reliable model for evaluating the cytotoxic and antiproliferative effects of natural compounds. MCF-7 cells are estrogen receptor-positive and widely used in cancer research due to their well-characterized nature and relevance to human breast cancer **[11]**. Several plant extracts have demonstrated significant inhibitory effects on MCF-7 cell proliferation through mechanisms involving cell cycle arrest and apoptosis induction.

Therefore, the present study aims to evaluate the phytochemical profile of *Aloe vera* leaf extract and to assess its antimicrobial against cutaneous pathogens and anticancer potential against MCF-7 cell lines through *in vitro* assays. The study focuses on determining the cytotoxic effects, analyzing morphological changes associated with apoptosis, and establishing the relationship between phytochemical constituents and their biological activities. These findings may contribute to the development of plant-based therapeutic strategies for breast cancer management and the control of associated skin infections.

MATERIALS AND METHODS

Collection and preparation of plant material

Fresh, healthy leaves of *Aloe vera* were collected from a local cultivated source and authenticated based on standard botanical characteristics. The collected leaves were thoroughly washed with distilled water to remove surface contaminants and shade-dried at room temperature for several days. The

dried material was then ground into a fine powder using a sterile mechanical grinder and stored in an airtight container until further use.

Preparation of plant extract

The powdered leaf material was subjected to solvent extraction using ethanol as the extracting solvent. Approximately 25 g of the powdered sample was soaked in 250 mL of ethanol and kept under continuous agitation for 48 hours at room temperature. The extract was then filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator under reduced pressure. The semi-solid extract obtained was stored at 4°C and used for further analysis.

Preliminary phytochemical screening

The ethanolic extract of *Aloe vera* was subjected to qualitative phytochemical screening to detect the presence of various bioactive constituents. Standard procedures were followed to identify major phytochemical groups such as alkaloids (Mayer's test), flavonoids (alkaline reagent test), phenolic compounds (Ferric chloride test), tannins, saponins (frothing test), and glycosides. The formation of characteristic color changes or precipitates indicated the presence of specific phytochemicals.

Quantitative assessment of phytochemicals

Quantitative estimation of major phytochemical constituents in the ethanolic extract of *Aloe vera* was carried out using standard spectrophotometric methods. All analyses were performed in triplicate, and the results were expressed as mean ± standard deviation.

Estimation of total flavonoid content

The total flavonoid content was determined using the aluminum chloride colorimetric method. Briefly, 0.5 mL of extract at different concentrations (100–500 µg/mL) was mixed with 2 mL of 2% sodium carbonate solution and allowed to stand for 2 minutes at room temperature. Subsequently, 250 µL of 5% sodium nitrite and 150 µL of 10% aluminum chloride were added. After 5 minutes of incubation, 0.5 mL of 1 M sodium hydroxide was added, and the final volume was adjusted to 2.5 mL using distilled water. The mixture was incubated for 10 minutes, and absorbance was measured at 510 nm using a UV Visible spectrophotometer. The flavonoid content was expressed in terms of absorbance values.

Estimation of total phenolic content

Total phenolic content was determined using the Folin Ciocalteu method. In this assay, 0.5 mL of plant extract was mixed with 2 mL of 2% sodium carbonate and incubated

for 2 minutes. Then, 100 µL of 50% Folin Ciocalteu reagent was added, and the mixture was thoroughly mixed. The reaction was allowed to proceed for 30 minutes in the dark at room temperature. The absorbance was measured at 720 nm. Higher absorbance indicates greater phenolic content.

Estimation of tannin content

Tannin content was estimated using the Folin Denis method. Briefly, 0.5 mL of extract was mixed with 1 mL of Folin Denis reagent and 2 mL of sodium carbonate solution. The reaction mixture was incubated for 30 minutes at room temperature, and the absorbance was measured at 700 nm. The intensity of color developed is proportional to the tannin concentration.

Estimation of saponin content

Saponin content was determined using a spectrophotometric method. The extract was mixed with vanillin reagent followed by the addition of sulfuric acid. The reaction mixture was incubated in a water bath at 60°C for 10 minutes and then cooled. The absorbance was recorded at 544 nm. The increase in absorbance indicates higher saponin concentration.

Estimation of alkaloid content

Alkaloid content was estimated using the bromocresol green (BCG) method. The extract was treated with BCG reagent and phosphate buffer, forming a yellow-colored complex. The complex was extracted using chloroform, and the absorbance of the chloroform layer was measured at 470 nm. The intensity of the color is directly proportional to the alkaloid concentration.

Antimicrobial activity

The antimicrobial activity of the ethanolic extract of *Aloe vera* was evaluated using the agar well diffusion method against selected cutaneous pathogens, including *S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa*, *C. albicans*, *A. niger*, *T. rubrum*, and *M. furfur*. Microbial inocula were standardized to 0.5 McFarland and spread on Mueller Hinton agar for bacteria and Sabouraud Dextrose agar for fungi. Wells (6 mm) were loaded with extract concentrations (50–250 mg/mL). Plates were incubated at 37°C (bacteria) and 28°C (fungi). Zones of inhibition were measured in millimeters, and results were expressed as mean ± standard deviation.

Cell line and culture conditions

The human breast cancer cell line (MCF-7) was obtained from a recognized cell repository. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin, and streptomycin. The cell cultures were maintained in a humidified incubator at 37°C

with 5% CO_2. Cells were subcultured regularly to maintain exponential growth conditions.

Preparation of test solutions

The *Aloe vera* extract was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution. Further dilutions were made using culture medium to obtain different concentrations (100, 200, 300, 400, and 500 µg/mL) for experimental analysis. The final concentration of DMSO in all treatments was maintained below 0.1% to avoid cytotoxic effects.

MTT assay for cell viability

The antiproliferative activity of the extract was evaluated using the MTT assay. Briefly, MCF-7 cells were seeded in a 96-well plate at a density of 1×10^4 cells per well and incubated for 24 hours to allow cell attachment. After incubation, the cells were treated with different concentrations of *Aloe vera* extract and incubated for an additional 24 hours. Following treatment, 20 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 hours. The formed formazan crystals were dissolved using DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated as a percentage relative to control cells. Morphological changes associated with apoptosis were observed under an inverted phase-contrast microscope. After treatment with the extract, cells were examined for characteristic features such as cell shrinkage, rounding, membrane blebbing, and detachment from the surface.

Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using standard software, and significance between groups was evaluated using one-way ANOVA.

RESULTS

Phytochemical screening of *aloe vera* extract

Preliminary phytochemical analysis of the ethanolic leaf extract of *Aloe vera* revealed the presence of several biologically active compounds. The qualitative screening confirmed the presence of flavonoids, phenolic compounds, alkaloids, tannins, and saponins, while glycosides were detected in moderate amounts **(Table 1)**. These compounds are known to contribute to antioxidant and anticancer activities, indicating the therapeutic potential of the extract.

Quantitative assessment of phytochemicals

The quantitative estimation of major phytochemical constituents in the ethanolic extract of *Aloe vera* revealed a concentration

dependent increase in bioactive compounds, indicating the richness of the extract in secondary metabolites **(Table 2)**. The total flavonoid content increased progressively with concentration, ranging from 0.142 ± 0.01 to 0.412 ± 0.01 OD at 510 nm. The highest flavonoid content was observed at 500 µg/mL. The content of total phenolic, tannin, saponin and alkaloids also showed a steady increase, ranging from 0.185 ± 0.02 to 0.498 ± 0.02, 0.121 ± 0.01 to 0.365 ± 0.02, 0.098 ± 0.01 to 0.284 ± 0.0, 0.110 ± 0.02 to 0.301 ± 0.02, respectively. The highest level of these photochemical content were observed at 500 µg/mL.

Antimicrobial potential

The ethanolic extract of *Aloe vera* exhibited significant antimicrobial activity against all tested cutaneous pathogens. The zone of inhibition increased progressively with increasing extract concentration, indicating a dose dependent effect **(Figure 1)**. Among the bacterial strains tested, the maximum zone of inhibition was observed in *Staphylococcus aureus* (18.3 ± 0.5 mm) at 250 mg/mL, while the minimum inhibition was recorded in *Pseudomonas aeruginosa* (6.8 ± 0.2 mm) at 50 mg/mL. In the case of fungal strains, *Candida albicans* exhibited the highest inhibition (17.2 ± 0.5 mm) at 250 mg/mL, whereas *Malassezia furfur* showed the lowest activity (6.5 ± 0.2 mm) at 50 mg/mL.

Effect of *Aloe vera* extract on cell viability

The antiproliferative effect of *Aloe vera* extract on MCF-7 cells was evaluated using the MTT assay. The results demonstrated a clear dose dependent decrease in cell viability with increasing concentrations of the extract **(Table 3)**. At the lowest concentration (100 µg/mL), a slight reduction in cell viability was observed, whereas higher concentrations exhibited significant cytotoxic effects. The maximum inhibition of cell viability was recorded at 500 µg/mL, showing approximately 76.8% reduction compared to control cells. The IC_{50} value was estimated to be around 260 µg/mL, indicating moderate cytotoxic potency of the extract against MCF-7 cells.

Morphological changes in MCF-7 cells

Microscopic examination of treated MCF-7 cells revealed distinct morphological alterations when compared to untreated control cells. Control cells appeared healthy, adherent, and displayed a typical epithelial morphology. In contrast, cells treated with *Aloe vera* extract showed progressive changes with increasing concentrations. At moderate concentrations (300 µg/mL), cells exhibited rounding and partial detachment. At higher concentrations (400–500 µg/mL), pronounced

apoptotic features such as cell shrinkage, membrane blebbing, and reduced cell density were observed, indicating induction of programmed cell death.

Apoptotic activity of *Aloe vera* extract

The apoptotic effect of the extract was further assessed based on morphological observations. A significant increase in apoptotic cell population was observed with increasing concentration of the extract. At 500 µg/mL, approximately 65–70% of cells displayed apoptotic characteristics, whereas control cells showed minimal signs of apoptosis **(Figure 2)**.

Table 1: Preliminary phytochemical screening of *Aloe vera* leaf extract

Phytoconstituents	Observation
Alkaloids	Positive
Flavonoids	Positive
Phenolics	Positive
Tannins	Positive
Saponins	Positive
Glycosides	Moderately present

Table 2: Quantitative estimation of phytochemicals in *Aloe vera* extract

Conc. (µg/mL)	Quantitative estimation (OD Value)				
	Flavonoids	Phenols	Tannins	Saponins	Alkaloids
100	0.142 ± 0.01	0.185 ± 0.02	0.121 ± 0.01	0.098 ± 0.01	0.110 ± 0.02
200	0.221 ± 0.02	0.268 ± 0.01	0.198 ± 0.02	0.152 ± 0.01	0.176 ± 0.01
300	0.298 ± 0.01	0.347 ± 0.02	0.256 ± 0.01	0.201 ± 0.02	0.229 ± 0.02
400	0.356 ± 0.02	0.421 ± 0.01	0.312 ± 0.02	0.243 ± 0.01	0.268 ± 0.01
500	0.412 ± 0.01	0.498 ± 0.02	0.365 ± 0.02	0.284 ± 0.01	0.301 ±0.02

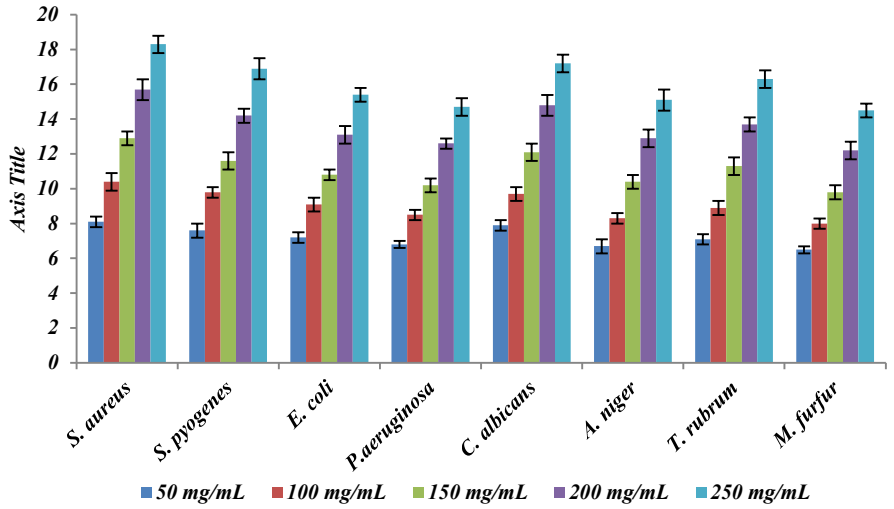


Figure 1: Antimicrobial activity of *Aloe vera* extract against cutaneous pathogens

Table 3: Effect of *Aloe vera* extract on MCF-7 cell viability

Concentration (µg/mL)	Cell viability (%)	Inhibition (%)	IC50
Control	100 ± 0.00	0	319.8 µg/mL
100	85.4 ± 2.1	14.6	
200	69.8 ± 1.8	30.2	
300	52.3 ± 2.4	47.7	
400	40.7 ± 2.0	59.3	
500	20.2 ± 1.6	76.8	

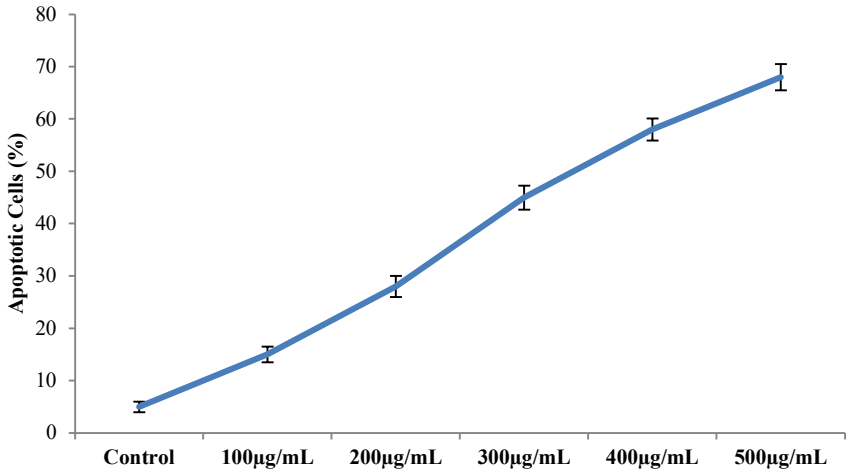


Figure 2: Apoptotic changes in MCF-7 cells treated with *Aloe vera* extract

DISCUSSION

The present study demonstrates that the ethanolic extract of *Aloe vera* possesses significant antiproliferative and apoptosis-inducing activity against MCF-7 breast cancer cells, which can be attributed to its rich phytochemical composition. The qualitative screening confirmed the presence of key bioactive compounds such as flavonoids, phenolics, alkaloids, tannins, and saponins. These findings are consistent with earlier reports indicating that *Aloe vera* contains diverse secondary metabolites with potent biological activities **[3]**. The quantitative analysis further revealed a concentration-dependent increase in flavonoid and phenolic content, suggesting that these compounds play a crucial role in the observed cytotoxic effects. Phenolic compounds are widely recognized for their antioxidant properties, which can reduce oxidative stress and inhibit cancer cell proliferation **[12]**. Similarly, flavonoids are known to modulate key cellular signaling pathways, leading to growth inhibition and apoptosis in cancer cells **[13]**. The presence of tannins and saponins in appreciable amounts may also contribute to the anticancer activity

by interfering with cell membrane integrity and inducing cell death.

The ethanolic extract of *Aloe vera* exhibited significant antimicrobial activity against tested cutaneous pathogens, with inhibition increasing in a concentration dependent manner. Greater susceptibility was observed in Gram positive bacteria compared to Gram negative bacteria, likely due to differences in cell wall structure **[14]**. Notable antifungal activity against *Candida albicans* and *Trichophyton rubrum* supports its traditional use in treating skin infections. The activity is attributed to phytochemicals such as anthraquinones, flavonoids, and saponins, which disrupt microbial membranes and metabolic processes **[4, 7]**. These findings align with previous reports highlighting the broad spectrum antimicrobial potential of *Aloe vera* **[3]**.

The MTT assay results demonstrated a clear dose-dependent reduction in cell viability, with maximum inhibition observed at higher concentrations of the extract. The estimated IC_{50} value indicates moderate cytotoxic potential, which aligns with previous studies on plant-derived extracts exhibiting anticancer properties **[15]**. The decrease in cell viability suggests that the extract effectively suppresses the proliferation of MCF-7 cells, supporting its role as a potential anticancer agent. Morphological observations provided further evidence of apoptosis induction. Treated cells exhibited characteristic apoptotic features such as cell shrinkage, membrane blebbing, and detachment, particularly at higher concentrations. These findings are in agreement with earlier studies reporting that plant extracts can trigger programmed cell death through intrinsic apoptotic pathways **[11]**. The increase in apoptotic cell population with increasing concentration of *Aloe vera* extract indicates its ability to selectively target cancer cells. The induction of apoptosis is a critical mechanism in cancer therapy, as it allows the elimination of abnormal cells without causing inflammation or damage to surrounding tissues. The observed apoptotic activity in this study may be linked to the synergistic effects of multiple phytochemicals present in the extract. Compounds such as anthraquinones and flavonoids in *Aloe vera* have been reported to activate caspase pathways and disrupt mitochondrial function, leading to apoptosis **[16]**.

CONCLUSION

The findings of this study highlight the significant anticancer potential of *Aloe vera* leaf extract against MCF-7 breast cancer cells. The extract exhibited notable antiproliferative activity in a dose-dependent manner and

effectively induced apoptosis, which may be attributed to the presence of bioactive phytochemicals such as flavonoids, phenolics, tannins, and alkaloids. In addition, the extract exhibited notable antimicrobial activity against selected cutaneous pathogens, indicating its broad spectrum therapeutic potential. The combined anticancer and antimicrobial properties emphasize the value of *Aloe vera* as a promising natural agent. Future studies should focus on isolating active compounds, elucidating molecular mechanisms, and conducting in vivo and clinical investigations to validate its efficacy and safety.

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