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The Antifungal Activity of Apple Cider Vinegar Against Dermatophytes and Its Effects on Human Keratinocytes

Hyeyoung Kim[1], HyunChan Song[2], Doyeon Kim[1], Minji Lee[2], Yeo-Jin Kim[1]* and Ki-Young Kim[2]

[1]Graduate School of Business Administration, Sungkyul University, 53 Sungkyuldaehak-ro, Manan-gu, Anyang-si, Gyeonggi-do - 14097, Republic of Korea

[2]Graduate School of Biotechnology, Kyung Hee University, Seocheon, Giheung, Yongin, Gyeonggi-do - 446701, Republic of Korea

Abstract

Background: Dermatophytes are a widespread fungal infection with growing concerns of antifungal resistance. Apple Cider Vinegar (ACV) is a traditional remedy known for its antimicrobial properties, but its efficacy against specific dermatophytes and its safety on host cells are not yet well-characterised. **Methods:** In this study, the *in vitro* antifungal activity of ACV was evaluated against six clinically relevant dermatophyte species by measuring the Minimum Inhibitory Concentration (MIC). The lytic mechanism was investigated by measuring the release of intracellular components. Additionally, the effects of ACV on human keratinocytes (HaCaT) were evaluated by measuring mitochondrial membrane potential (ΔΨm) and lipid peroxidation. **Results:** ACV exhibited potent antifungal activity, with MICs ranging from 1.56% to 3.12% for five of six strains. ACV induced fungal cell lysis in a concentration-dependent manner. In HaCaT cells, 10% ACV induced significant mitochondrial depolarisation and increased oxidative stress. In contrast, 1% ACV induced potential mitochondrial hyperpolarisation without causing significant damage, suggesting a protective effect. **Conclusion:** This study demonstrates that ACV has a dual effect in a concentration-dependent manner. ACV is an effective antifungal agent against the most common dermatophytes, acting by disrupting cell integrity, but at high concentrations, it can be cytotoxic to human keratinocytes. These findings suggest that ACV has a therapeutic effect and support its potential as a topical antifungal treatment, but need optimising concentrations to balance efficacy and safety.

Major Findings: Apple Cider Vinegar (ACV) effectively inhibits the growth of most major dermatophytes at low concentrations (1.56% - 3.12%). Results highlight ACV's potential as a topical treatment.

Keywords: Apple Cider Vinegar, Antifungal Activity, Cytotoxicity, Dermatophytes, Therapeutic Window

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1. Introduction

Dermatophytes are a group of pathogenic fungi that cause common infections of the skin, hair, and nails[1,2]. These infections, known as dermatophytosis or tinea, are a global health problem, affecting nearly a quarter of the world's population[3,4]. Major species, such as *Trichophyton rubrum*, *Trichophyton interdigitale*, *Microsporum gypseum*, *Epidermophyton floccosum*, *Trichophyton tonsurans*, and *Trichophyton mentagrophytes*, pose significant clinical challenges[5,6]. Although these infections are not life-threatening, they can be persistent and negatively affect a person's quality of life[7,8]. Standard treatment involves topical or oral antifungal medications[9]. However, these treatments have several drawbacks, including long treatment times, potential side effects, and the rise of antifungal resistance[9-12]. As the number of resistant

*Author for correspondence

fungi increases, the need to explore new alternative treatments is growing[13,14]. Apple cider vinegar (ACV) is made from fermented apple juice and has long been used in traditional medicine[15,16]. Natural fermentation for ACV creates a rich chemical composition that gives medicinal properties, with acetic acid being the main active ingredient[17,18]. Acetic acid creates an acidic environment that makes it difficult for many pathogenic microorganisms to survive[19,20]. Many studies have shown that ACV also possesses antibacterial properties other than acetic acids, allowing it to be effective against a wide range of microorganisms[21-23]. ACV's mechanism of action is believed to involve disrupting fungal cell membranes and inhibiting essential enzymes required for fungal survival[20,24]. While ACV is widely used to treat skin conditions, detailed scientific studies on its effectiveness against the specific dermatophytes causing infections in patients are lacking[22,25]. Because effective, alternative treatments are needed and ACV is known for its ability to control microorganisms, this study was designed to carefully test its antifungal activity. The primary objective of this study was to determine the *in vitro* effects of ACV against the major dermatophytes *T. rubrum*, *T. interdigitale*, *M. gypseum*, *E. floccosum*, *T. tonsurans*, and *T. mentagrophytes*. This study also aims to provide scientific evidence supporting the potential use of ACV as a topical treatment and to offer an inexpensive and readily available alternative for treating these common fungal infections.

2. Materials and Methods

2.1 Materials

Organic apples (Daegu, South Korea), commercial baker's yeast (*Saccharomyces cerevisiae*), and a raw apple cider vinegar starter culture containing active *Acetobacter* species were used[18]. All water was deionised and sterilised before use.

2.2 Preparation of Apple Cider Vinegar (ACV)

ACV was produced through a standardised two-step fermentation process[17,26]. Washed and pitted apples were ground into pulp, then mixed with equal parts of sterilised water (1:1 w/w) to produce apple juice. First, the juice was inoculated with *S. cerevisiae* (2 g/L) and alcoholic fermentation was performed at 25°C for 2 weeks with daily stirring. The vessel was covered with sterilised cheesecloth. Next, the liquid was filtered from the solids, inoculated with the original ACV starter culture (10% v/v), and acetic acid fermentation was performed at 28°C for 4–6 weeks[27]. The final unfiltered ACV original culture was decanted and stored at 4°C until needed for the experiment.

2.3 Dermatophyte Strains and Culture Conditions

The six dermatophyte strains used in this study were *Trichophyton rubrum, Trichophyton interdigitale, Microsporumgypseum, Epidermophyton floccosum, Trichophyton tonsurans*, and *Trichophyton mentagrophytes*. All fungal strains were cultured on Sabouraud Dextrose Agar (SDA) at 28°C[1]. For experiments, fungal spores were harvested from 7- to 10-day-old cultures by soaking the agar surface in sterile phosphate-buffered saline (PBS) containing 0.05% Tween 80. The resulting suspension was filtered through sterile gauze to remove mycelial fragments, and the spore concentration was adjusted using a hemacytometer[28].

2.4 Determination of Minimum Inhibitory Concentration (MIC)

The MIC value of the prepared ACV against each dermatophyte was determined using the broth microdilution method, following the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) document M38-A2[29,30]. The assay was performed in a sterile 96-well microplate. ACV was serially diluted twofold from 0.78% to 50% in RPMI 1640 medium. An equal volume of standardised fungal inoculum (1 x 10^4 spore/mL) was added to each well containing the ACV dilutions. Plates were incubated at 28°C for 4–7 days until visible growth was observed in the positive control wells. The MIC was defined as the lowest concentration of ACV that visually and completely inhibited fungal growth. Experiments were performed in triplicate, and the average value was reported.

2.5 pH Measurement of Culture Medium

To determine the effect of ACV on the acidity of the culture medium, the prepared ACV was serially diluted in RPMI 1640 medium to achieve the final

concentrations used in the antifungal assay (0%, 3.13%, 6.25%, 12.50%, 25%, and 50%). After thoroughly mixing the solutions, the pH of each sample was immediately measured at room temperature using a calibrated digital pH meter[31]. All measurements were performed in triplicate, and the average value was reported.

2.6 Fungal Cell Lysis Assay

The integrity of the fungal cell membrane after ACV treatment was assessed by measuring the release of intracellular substances that absorb light at a wavelength of 260 nm[32,33]. A representative dermatophyte species, *T. rubrum*, was selected, since it is the most common dermatophyte among the strains used in the study[4]. Fungal suspension was prepared and adjusted to a concentration of 1 x 10^6spore/mL in RPMI 1640 medium. These suspensions were then treated with indicated concentrations of ACV (0%, 1.5%, 3%, 6%, 12.5%, 25%, and 50%) and incubated at 28°C for 24 hours. After incubation, the samples were centrifuged at 5,000 x g for 10 minutes to pellet the fungal cells. The supernatant from each sample was carefully collected and transferred to a UV-transparent 96-well plate. The absorbance of the supernatant was measured at a wavelength of 260nm using a microplate spectrophotometer. Experiments were performed in triplicate, and the average value was reported.

2.7 Cell Line

The human keratinocyte cell line, HaCaT, was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin[34]. The cells were cultured in a humidified incubator at 37°C with 5% CO_2.

2.8 MTT

Cytotoxicity of ACV against HaCaT cells was measured using the MTT assay[35]. HaCaT cells were seeded at 10^3 cells per well and cultured in 96-well plates for 24 hours before treatment. Each culture well was treated with various concentrations of ACV in serum-free medium. After culturing for another 24 hours, MTT was added to form formazan. The formazan was eluted with DMSO and incubated in an orbital shaker protected from light. Absorbance was measured at an optical density of 550 nm, and the percentage of viable cells was calculated using the following equation:

$$Percentage\ of\ viable\ cells = 100 \times \frac{reading\ of\ sample}{reading\ of\ control}(\%)$$

Experiments were performed in triplicate, and the average value was reported.

2.9 Analysis of Mitochondrial Membrane Potential (ΔΨm)

To evaluate the effect of ACV on mitochondrial integrity, the mitochondrial membrane potential (ΔΨm) of HaCaT cells was measured using the lipophilic cationic dye JC-1 (Thermo, USA)[36-38].

For the assay, HaCaT cells were seeded at a density of 1 x 10^5 cells per well in six-well black-walled, clear-bottom plates and cultured for 24 hours to allow cell attachment. The culture medium was then replaced with fresh medium containing various concentrations of prepared ACV, and the cells were cultured for an additional 24 hours. Untreated cells served as a negative control. After the 24-hour ACV treatment, the medium was removed, and the cells were washed twice with phosphate-buffered saline (PBS). The cells were then incubated with 10 µM JC-1 staining solution in DMEM for 30 minutes at 37°C in the dark. After incubation, the staining solution was removed, and the cells were washed again with PBS. Fluorescence intensity was measured immediately using a fluorescence microplate reader. Experiments were performed in triplicate, and the average value was reported.

2.10 TBARS Assay

The extent of lipid peroxidation in HaCaT cells after ACV treatment was determined by measuring the concentration of malondialdehyde (MDA), a major byproduct of lipid peroxidation, using the thiobarbituric acid reactive substances (TBARS) assay[39,40].

HaCaT cells were seeded in 6-well plates at a density of 2 x 10^5 cells per well and incubated for 24 hours. The cells were then treated with various concentrations of ACV for 24 hours. After treatment, the cells were washed with ice-cold PBS, scraped, and centrifuged. The resulting cell pellet was lysed on ice with RIPA buffer, centrifuged at 14,000 x g for 15 minutes at 4°C, and the supernatant was collected. The total protein concentration of the lysates was normalised using a BCA protein assay kit[41].

For the reaction, 100 µL of cell lysate was mixed with 500 µL of TBARS reagent (0.375% w/v thiobarbituric acid and 15% w/v trichloroacetic acid in 0.25 M HCl). The mixture was heated in a 95°C water bath for 60 minutes. After heating, the reaction was stopped by placing the sample on ice for 10 minutes. The samples were then centrifuged at 10,000 × g for 10 minutes to pellet the precipitate.

The clear supernatant was transferred to a 96-well plate, and the absorbance of the pink-colored MDA-TBA adduct was measured at 532 nm using a microplate spectrophotometer. The concentration of MDA was calculated using a standard curve generated with known concentrations of an MDA standard. The final level of lipid peroxidation was expressed as nanomoles of MDA per milligram of protein[42]. Experiments were performed in triplicate, and the average value was reported.

2.11 Statistical Analysis

All experiments were performed at least three times and data were presented as the ± mean standard deviation (S.D). Statistical analysis was performed using Microsoft Excel 2021 (Microsoft, USA) and Statistical Package for the Social Sciences (SPSS) 28.0 (IBM Corp, USA). All graphs except microscopic images were analysed by Microsoft Excel 2021. Statistical significance was defined at a *p*-value less than 0.05.

3. Results and Discussion

3.1 Antifungal Activity of ACV Against Dermatophytes

The *in vitro* antifungal efficacy of prepared ACV was determined after obtaining the ACV by assessing the MIC against six common dermatophyte species (Table 1). Results indicated that ACV possessed antifungal properties against all strains tested, but the level of activity varied among species.ACV exhibited the lowest MIC of 1.56% against *T. interdigitale*, *E. floccosum*, and *T. tonsurans*. Slightly higher concentrations were required to inhibit the growth of *T. rubrum* and *M. gypseum*, both of which had MIC values of 3.12%.In contrast, *T. mentagrophytes* showed a higher tolerance to ACV treatment, with an MIC of 50%. This suggests that *T. mentagrophytes* is significantly less susceptible to the antifungal component of ACV than the other dermatophytes tested.

MIC assay results clearly demonstrated that ACV possessed significant antifungal activity against

	MIC
T. rubrum	3.12%
T. interdigitale	1.56%
M. gypseum	3.12%
E. floccosum	1.56%
T. tonsurans	1.56%
T. mentagrophytes	50%

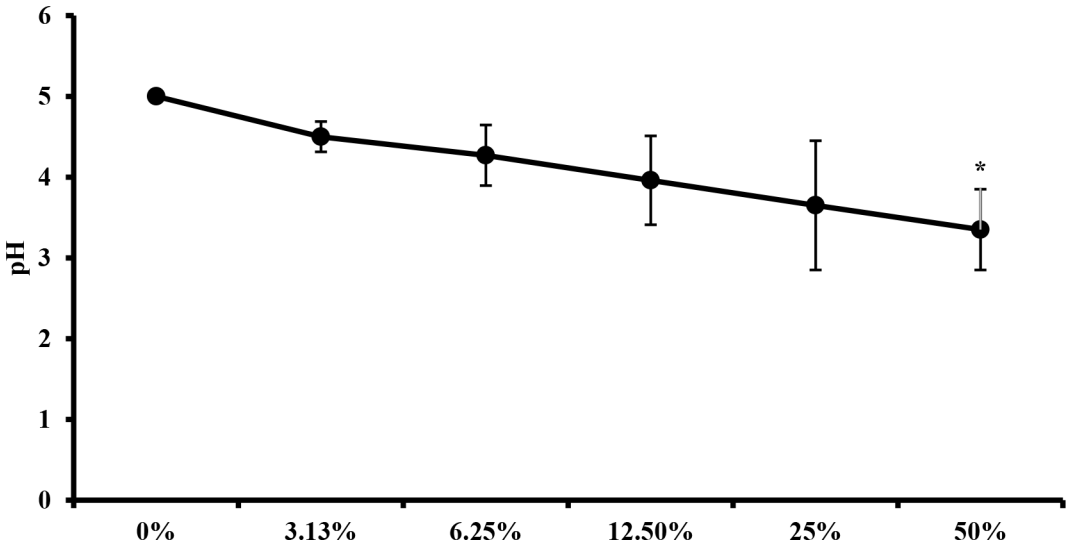


Figure 1. Effect of ACV on the pH of the culture medium. (*:*p*-value < 0.05; ***: *p*-value < 0.001).

dermatophyte species tested. We confirmed that even a relatively low concentration was sufficient to inhibit the growth of the pathogens tested. These potent activities provide strong scientific evidence for ACV's potential as a topical antifungal agent[43]. However, a notable exception is *T. mentagrophytes*, which exhibited a very high MIC value of 50%. This suggests that this species possesses a unique tolerance mechanism not found in other species[44].

3.2 ACV Induces Acidification of the Culture Medium

To investigate the contribution of acidity to the overall antifungal effect, the pH of the culture medium was measured after adding various concentrations of ACV (Figure 1). The baseline pH of the standard culture medium without ACV was 5.0. The addition of ACV resulted in a significant and concentration-dependent acidification of the medium. Even at the lowest ACV concentration of 3.13%, the pH decreased to 4.5. As the ACV concentration increased, the pH steadily decreased, reaching 3.96 at 12.50% ACV and reaching a highly acidic pH of 3.35 at the highest concentration of 50%. This finding confirms that one of the primary effects of applying ACV is to create a low-pH environment, a condition hostile to fungal growth, which significantly influences the observed antifungal activity of ACV. We confirmed that ACV creates a highly acidic environment in a concentration-dependent manner, likely linked to direct cell membrane disruption[45]. This low pH is hostile to fungi, inhibiting key enzymes and metabolic pathways and exacerbating cell damage. This compromises cell integrity and ultimately leads to cell death[46,47].

3.3 ACV Induces Lysis of Fungal Cells

To investigate the mechanism of the observed antifungal activity, the effect of ACV on the cell integrity of representative dermatophytes, *T. rubrum*, was evaluated (Figure 2). Cell lysis was quantified by measuring the release of intracellular components, such as nucleic acids, measurable at 260 nm.

The results show a clear concentration-dependent relationship between ACV exposure and cell lysis. At low concentrations (0–1.5%), ACV caused minimal cell damage, with approximately 1.1% of lysed cells, similar to the untreated control group. A steady, dose-dependent increase in cell lysis was observed as the ACV concentration increased from 3% to 25%. The percentage of lysed cells increased from 4.02% to 14.14% within this range. Most importantly, cell integrity was compromised at the highest concentration tested. Cell lysis increased dramatically to 67.12% at 50% ACV treatment. The cell lysis assay revealed a concentration-dependent release of intracellular substances, suggesting that ACV's primary mechanism of action likely involves disruption of the fungal cell membrane or cell wall[20,48].

3.4 Cytotoxicity of ACV on HaCaTCells

To determine the cytotoxicity of ACV on human keratinocytes, the viability of HaCaT cells was

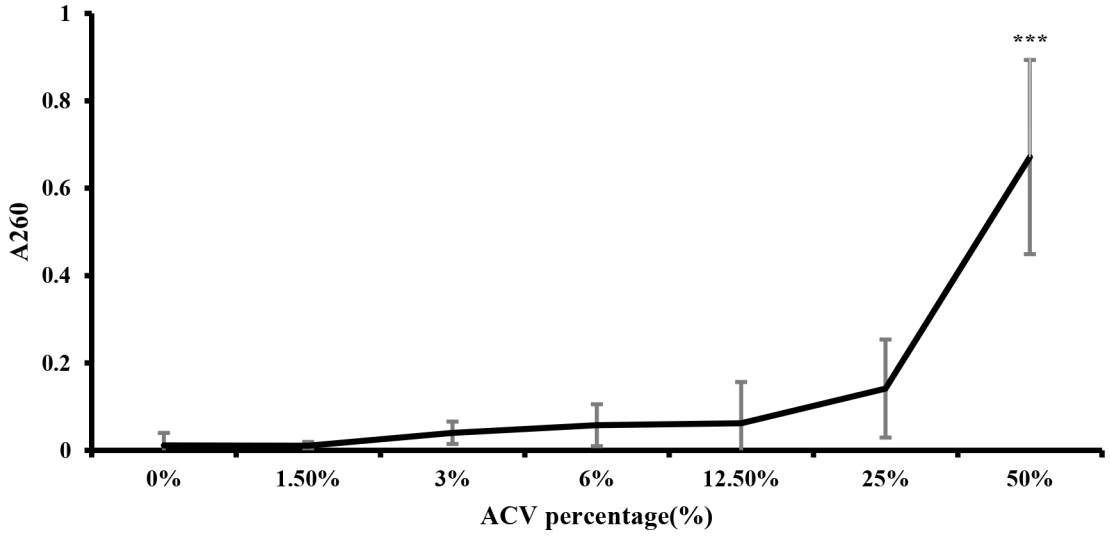


Figure 2. ACV induced leakage of 260nm absorbing substances in *T. rubrum*. (*:*p*-value < 0.05; ***: *p*-value < 0.001).

determined using the MTT assay after 24 hours of exposure to various ACV concentrations (Figure 3). The results showed a marked concentration-dependent decrease in cell viability. The half-maximal inhibitory concentration (IC_{50}) was 1.65%. At concentrations significantly higher than the IC_{50}, ACV induced cytotoxicity. Specifically, exposure to 25% and 50% ACV resulted in a near-absence of metabolic activity, and the percentage of viable cells decreased to less than 1% compared to the untreated control. While demonstrating antifungal efficacy is important, the host cell safety of ACV is important to clinical viability[49]. Here, IC_{50} for HaCaT cells was determined to be 1.65%. This value is highly significant because it closely aligns with the MIC values required to inhibit the most common dermatophytes (1.56-3.12%). However, it must be noted that *in vitro* experiments, cells are much more vulnerable to the chemicals compared to those in vivo. Since the values of MIC and IC_{50} were not significantly different, a strategic approach must be considered usingliposomesor nanoparticles to control dermatophytes with less cytotoxicity towards keratocytes.

3.5 Effect of ACV on Mitochondrial Membrane Potential (ΔΨm) in HaCaT Cells

The effect of apple cider vinegar (ACV) on mitochondrial membrane potential (ΔΨm) in HaCaT cells was evaluated using the JC-1 assay (Figure 4), and the image was taken (Figure 4a). Changes in ΔΨm were quantified by calculating the ratio of red to green fluorescence intensities, where an increase in the ratio indicates mitochondrial depolarisation (Figure 4b). The untreated control group exhibited a baseline Red/Green fluorescence ratio of 5.86. Interestingly, cells treated with a low concentration of 1% ACV showed a significant increase in the Green/Red ratio to 15.56. In contrast, treatment with a high concentration of 10% ACV resulted in a decrease in the Green/Red fluorescence ratio to3.36.The results of this study demonstrate a complex, concentration-dependent effect of ACV on ΔΨm. ΔΨm is essential for ATP production and is a key checkpoint in the intrinsic pathway of apoptosis[50,51]. Loss of this potential is often an early and irreversible step toward programmed cell death, and restoration of this potential indicates that the cells have recovered or are in better condition[52]. We confirmed that a high concentration of 10% ACV significantly impaired mitochondrial health. A significant decrease in the JC-1 Red/Green fluorescence ratio indicates less mitochondrial membrane depolarisation compared to the control group[53]. Conversely, a low concentration of 1% ACV led to mitochondrial hyperpolarisation, observed as a significant increase in the JC-1 Red/Green fluorescence ratio. The interpretation of mitochondrial hyperpolarisation is not straightforward. While ΔΨm is crucial for cellular energetics, hyperpolarisation has also been linked to increased production of reactive oxygen species (ROS) and can be an early event in the

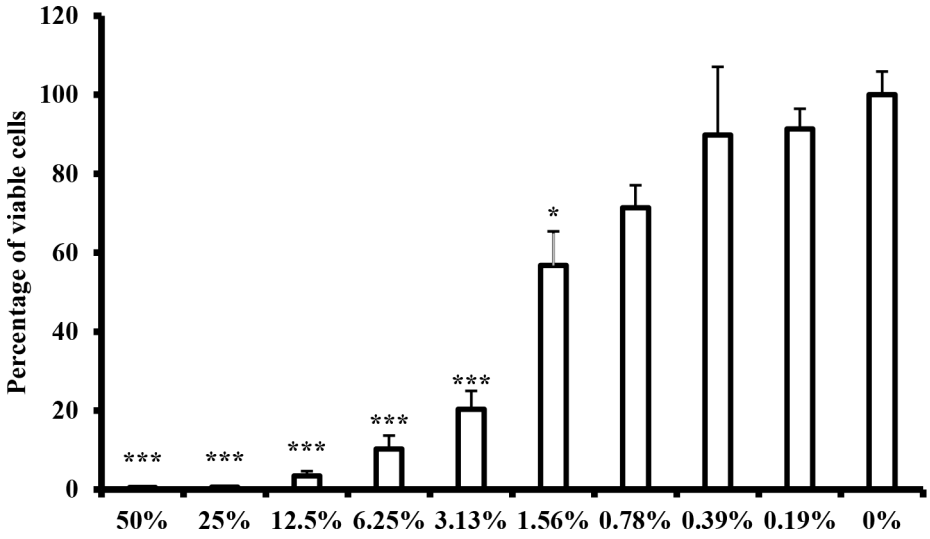
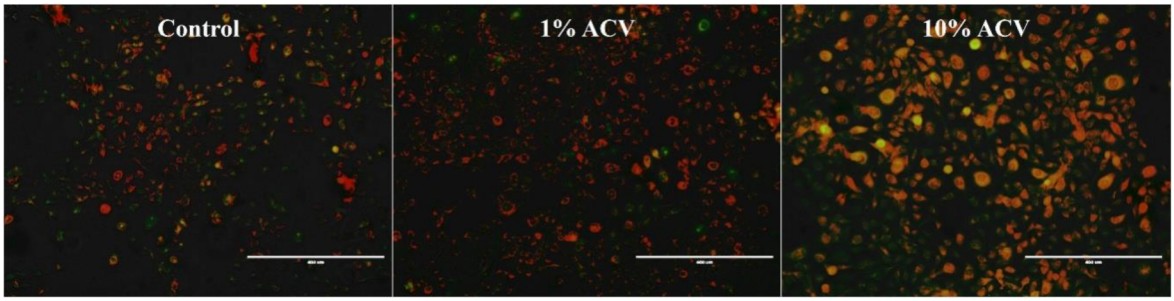
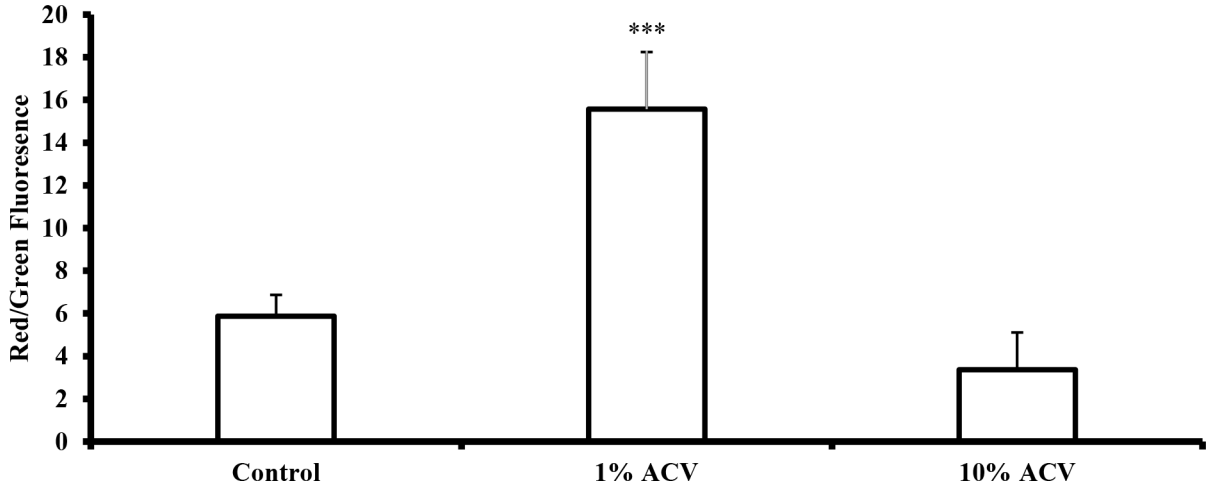


Figure 3. Cytotoxicity of ACV on HaCaT cells. (*:*p*-value < 0.05; ***: *p*-value < 0.001).

(a)



(b)



(a). Fluorescence image of JC-1-treated cells with or without ACV.
(b). Calculated value of Red/Green fluorescence of each group.

Figure 4. ACV induced mitochondrial membrane potential at low concentration. (*:*p*-value < 0.05; ***: *p*-value < 0.001).

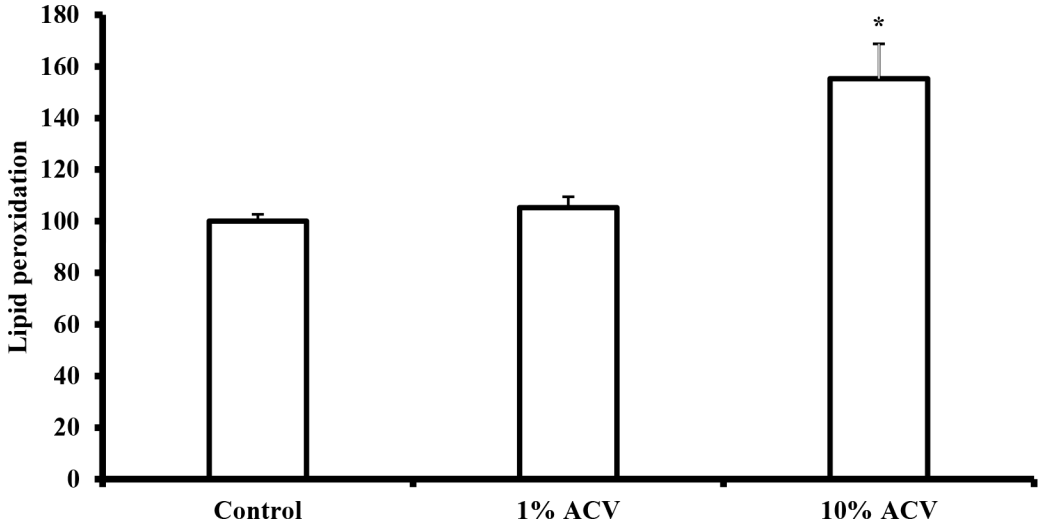


Figure 5. ACV did not affect lipid peroxidation in HaCaT cells.

apoptotic cascade in some contexts[54]. Therefore, the hyperpolarisation induced by 1% ACV could represent a compensatory response to mild stress. However, further studies are necessary to determine whether this state is beneficial for long-term cell survival or serves as an early trigger for a cell death pathway.

3.6 Effect of ACV on Lipid Peroxidation in HaCaT Cells

To determine whether ACV induces oxidative stress, the lipid peroxidation levels were measured by quantifying malondialdehyde (MDA) levels using the TBARS assay (Figure 5). Results are expressed as a percentage compared to the untreated control. Treatment with 1% ACV for 24 hours resulted in a slight increase in lipid peroxidation, with MDA levels reaching 106% of the control level. While 1% ACV did increase MDA levels, the increase was not significant. A significantly more pronounced effect was observed in cells treated with 10% ACV. In this group, lipid peroxidation was significantly increased, with MDA levels increasing approximately 158% compared to the control group. Significant increase in MDA levels at 10% ACV is a key indicator of oxidative stress[42,55]. Interestingly, the effects of lower concentrations of 1% ACV were not different from the control group.

4. Discussion

In conclusion, this study provides scientific evidence supporting the antifungal effect of ACV against several major dermatophytes via cell membrane disruption. Furthermore, it demonstrates that the effects of ACV on human keratinocytes are highly concentration-dependent. Future research should focus on validating these findings in a 3D skin model and optimising ACV formulations to maximise antifungal efficacy within a safe and non-cytotoxic range for therapeutic applications[56].

5. References

1. Weitzman I, Summerbell RC. The dermatophytes. **Clin Microbiol Rev**. 1995; 8(2):240–59. https://doi.org/10.1128/CMR.8.2.240 PMid:7621400 PMCid:PMC172857.
2. Havlickova B, Czaika VA, Friedrich M. Epidemiological trends in skin mycoses worldwide. Mycoses. 2008; 51(Suppl 4):2–15. https://doi.org/10.1111/j.1439-0507.2008.01606.x PMid:18783559.
3. Ameen M. Epidemiology of superficial fungal infections. Clin Dermatol. 2010; 28(2):197–201. https://doi.org/10.1016/j.clindermatol.2009.12.005 PMid:20347663.
4. White TC, Findley K, Dawson TL Jr, Odds FC, Cannon RD. Fungi on the skin: dermatophytes and malassezia. Cold Spring Harb Perspect Med. 2014; 4(8):a019802. https://doi.org/10.1101/cshperspect.a019802 PMid:25085959 PMCid: PMC4109575.
5. Graser Y, Scott J, Summerbell R. The new species concept in dermatophytes—a polyphasic approach. Mycopathologia. 2008; 166(5-6):239–249. https://doi.org/10.1007/s11046-008-9099-y PMid:18478366.
6. De Hoog GS, Dukik K, Monod M, Packeu A, Stubbe D, Hendrickx M, *et al*. Toward a novel multilocus phylogenetic taxonomy for the dermatophytes. Mycopathologia. 2017; 182(1-2):5–31. https://doi.org/10.1007/s11046-016-0073-9 PMid:27783317 PMCid: PMC5283515.
7. Eisman S, Sinclair R. Fungal nail infection: diagnosis and management. BMJ. 2014; 348:g3301. https://doi.org/10.1136/bmj.g1800 PMid:24661991.
8. Drake LA, Scher RK, Smith EB, Faich GA, Smith SL, Lubeck DP, *et al*. Effect of onychomycosis on quality of life. J Am Acad Dermatol. 1998; 38(5 Pt 1):702–704. https://doi.org/10.1016/S0190-9622(98)70199-9 PMid:9591814.
9. Gupta AK, Cooper EA. Update in antifungal therapy of dermatophytosis. Mycopathologia. 2008; 166(5-6):353–367. https://doi.org/10.1007/s11046-008-9109-0 PMid:18478357.
10. Martinez-Rossi NM, Peres NTA, Rossi A. Antifungal resistance mechanisms in dermatophytes. Mycopathologia. 2017; 182(1-2):129–141. https://doi.org/10.1007/s11046-008-9110-7 PMid:18478356.
11. Saunte DML, Pereiro-Ferreirós M, Rodríguez-Cerdeira C, Sergeev AY, Arabatzis M, Prohić A, *et al*. Emerging antifungal treatment failure of dermatophytosis in Europe: take care or it may become endemic. J Eur Acad Dermatol Venereol. 2021; 35(7):1582–1586. https://doi.org/10.1111/jdv.17241 PMid:33768571.
12. Singh A, Masih A, Khurana A, Meena S, Singh PK, Gupta M, *et al*. High terbinafine resistance in *Trichophyton interdigitale* isolates in Delhi, India, harbouring mutations in the squalene epoxidase gene. Mycoses. 2018; 61(7):477–484. https://doi.org/10.1111/myc.12772 PMid:29577447.
13. Gold JA, Lipner SR. The rise of antifungal-resistant dermatophyte infections: what dermatologists need to know. Cutis. 2023; 111(5):E1–2. https://doi.org/10.12788/cutis.1211 PMid:40591859 PMCid: PMC12221198.
14. World Health Organization. WHO fungal priority pathogens list to guide research, development and public health action. editor. World Health Organization. 2022.

15. Johnston CS, Gaas CA. Vinegar: medicinal uses and antiglycemic effect. MedGenMed. 2006; 8(2):61. https://doi.org/10.1016/b978-0-12-374228-5.00022-6 PMid:20477039.
16. Samad A, Azlan A, Ismail A. Therapeutic effects of vinegar: a review. Curr Opin Food Sci. 2016; 8:56–61. https://doi.org/10.1016/j.cofs.2016.03.001
17. Budak NH, Aykin E, Seydim AC, Greene AK, Guzel-Seydim ZB. Functional properties of vinegar. J Food Sci. 2014; 79(5):R757–R764. https://doi.org/10.1111/1750-3841.12434 PMid:24811350.
18. Ho CW, Lazim MA, Fazry S, Zaki UKHH, Lim SJ. Varieties, production, composition and health benefits of vinegars: a review. **Food Chem**. 2017; 221:1621–1630. https://doi.org/10.1016/j.foodchem.2016.10.128 PMid:27979138.
19. Rutala WA, Barbee SL, Aguiar NC, Sobsey MD, Weber DJ. Antimicrobial activity of home disinfectants and natural products against potential human pathogens. **Infect Control Hosp Epidemiol**. 2000; 21(1):33–38. https://doi.org/10.1086/501694 PMid:10656352.
20. Lastauskienė E, Zinkevičienė A, Girkontaitė I, Kaunietis A, Kvedarienė V. Formic acid and acetic acid induce a programmed cell death in pathogenic *Candida* species. *Curr Microbiol*. 2014; 69(3):303–310. https://doi.org/10.1007/s00284-014-0585-9 PMid:24752490.
21. Yagnik D, Serafin V, Shah AJ. Antimicrobial activity of apple cider vinegar against *Escherichia coli, Staphylococcus aureus* and *Candida albicans*; downregulating cytokine and microbial protein expression. Sci Rep. 2018; 8(1):1732. https://doi.org/10.1038/s41598-017-18618-x PMid:29379012 PMCid: PMC5788933.
22. Mota AC, de Castro RD, de Araújo Oliveira J, de Oliveira Lima E. Antifungal activity of apple cider vinegar on *Candida* species involved in denture stomatitis. J Prosthodont. 2015; 24(4):296–302. https://doi.org/10.1111/jopr.12207 PMid:25219289.
23. Yagnik D, Ward M, Shah AJ. Antibacterial apple cider vinegar eradicates methicillin resistant *Staphylococcus aureus* and resistant *Escherichia coli*. Sci Rep. 2021; 11(1):1854. https://doi.org/10.1038/s41598-020-78407-x PMid:33473148 PMCid: PMC7817673.
24. Kang HC, Park YH, Go SJ. Growth inhibition of a phytopathogenic fungus, *Colletotrichum* species by acetic acid. Microbiol Res. 2003; 158(4):321–326. https://doi.org/10.1078/0944-5013-00211 PMid:14717453.
25. Elhage KG, St. Claire K, Daveluy S. Acetic acid and the skin: a review of vinegar in dermatology. Int J Dermatol. 2022; 61(7):804–811. https://doi.org/10.1111/ijd.15804 PMid:34350993.
26. Joshi VK, Panesar PS, Rana NS, Kaur S. Science and technology of vinegar production - a review. In: Singh RS, editor. Beverages: Nutraceuticals and Health Benefits. Daya Publishing House; 2009. pp. 22–63. https://doi.org/10.1016/b978-0-12-800850-8.00001-6
27. Zeng C, Yuan J, Mu Y, Zhang H, Song J, Kang S. The physicochemical components, amino acids, organic acids, phenolic compounds and antioxidant capacity of apple ciders: according to geographical origin and altitude of Fuji apple. Microchem J. 2024; 201:110571. https://doi.org/10.1016/j.microc.2024.110571
28. Espinel-Ingroff A, Fothergill A. Preparation of conidial inocula of filamentous fungi. In: Espinel-Ingroff A, editor. Antifungal Susceptibility Testing. Humana Press; 2003. pp. 23–28.
29. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard—second edition. M38-A2. editor: Clinical and Laboratory Standards Institute. 2008.
30. Santos DA, Hamdan JS. Evaluation of the *in vitro* antifungal activity of the essential oil of *Cymbopogon citratus* (DC) Stapf against *Trichophyton mentagrophytes* isolates. Braz J Microbiol. 2007; 38:327–331.
31. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983; 65(1-2):55–63. https://doi.org/10.1016/0022-1759(83)90303-4 PMid:6606682.
32. Paul S, Dubey RC, Maheswari DK, Kang SC. *Trachyspermum ammi* (L.) fruit essential oil influencing on membrane permeability and surface characteristics in inhibiting food-borne pathogens. Food Control. 2011; 22(5):725–731. https://doi.org/10.1016/j.foodcont.2010.11.003
33. Chaves SR, Rego A, Martins VM, Santos-Pereira C, Sousa MJ, Côrte-Real M. Regulation of cell death induced by acetic acid in yeasts. Front Cell Dev Biol. 2021; 9:642375. https://doi.org/10.3389/fcell.2021.642375 PMid:34249904 PMCid: PMC8264433.
34. Boukamp P, Petrussevska RT, Breitkreutz D, Hornung J, Markham A, Fusenig NE. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J Cell Biol. 1988; 106(3):761–771. https://doi.org/10.1083/jcb.106.3.761 PMid:2450098 PMCid: PMC2115116.
35. Van de Loosdrecht AA, Beelen RH, Ossenkoppele GJ, Broekhoven MG, Langenhuijsen MM. A tetrazolium-based colorimetric mtt assay to quantitate human monocyte mediated cytotoxicity against leukemic cells from cell lines and patients with acute myeloid leukemia. J Immunol Methods. 1994; 174(1-2):311–320. https://doi.org/10.1016/0022-1759(94)90034-5 PMid:8083535.
36. Smiley ST, Reers M, Mottola-Hartshorn C, Lin M, Chen A, Smith TW, *et al*. Intracellular heterogeneity in mitochondrial membrane potentials revealed by a J-aggregate-forming lipophilic cation, JC-1. Proceedings of the National Academy Of Sciences. 1991; 88(9):3671–3675. https://doi.org/10.1073/pnas.88.9.3671 PMid:2023917 PMCid: PMC51514.
37. Reers M, Smith TW, Chen LB. J-aggregate formation of a carbocyanine as a quantitative fluorescent indicator of

membrane potential. Biochemistry. 1991; 30(18):4480–4486. https://doi.org/10.1021/bi00232a015 PMid:2021638.
38. Sivandzade F, Bhalerao A, Cucullo L. Analysis of the mitochondrial membrane potential using the cationic JC-1 dye as a sensitive fluorescent probe. Bio-protocol. 2019; 9(1):e3128. https://doi.org/10.21769/bioprotoc.3128 PMid:30687773 PMCid: PMC6343665.
39. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95(2):351–358. https://doi.org/10.1016/0003-2697(79)90738-3 PMid:36810.
40. Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med. 1990; 9(6):515–540. https://doi.org/10.1016/0891-5849(90)90131-2 PMid:2079232.
41. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, *et al.* Measurement of protein using bicinchoninic acid. Anal Biochem. 1985; 150(1):76–85. https://doi.org/10.1016/0003-2697(85)90442-7 PMid:3843705.
42. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. **Oxid Med Cell Longev**. 2014; 2014:360438. https://doi.org/10.1155/2014/360438 PMid:24999379 PMCid: PMC4066722.
43. Ghannoum MA, Rice LB. Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. Clin Microbiol Rev. 1999; 12(4):501–517. https://doi.org/10.1128/cmr.12.4.501 PMid:10515900 PMCid: PMC88922.
44. Niewerth M, Korting HC. Management of dermatophytoses and dermatomycoses. In: Heitman J, Steinbach WJ, Mitchell AP, editors. Fungal Pathogenesis: Principles and Clinical Applications. ASM Press; 2014. pp. 417–445.
45. Stratford M, Anslow PA. Evidence that sorbic acid does not inhibit yeasts by simply lowering intracellular pH. Yeast. 1998; 14(1):43–53. https://doi.org/10.1046/j.1472-765x.1998.00424.x PMid:9812395.
46. Holyoak CD, Stratford M, McMullin Z, Cole MB, Crimmins K, Brown AJ, *et al.* Activity of the plasma membrane H+-ATPase and optimal glycolytic flux are required for rapid adaptation and growth of *Saccharomyces cerevisiae* in the presence of the weak-acid preservative sorbic acid. Appl Environ Microbiol. 1996; 62(9):3158–3164. https://doi.org/10.1128/aem.62.9.3158-3164.1996 PMid:8795204 PMCid: PMC168110.
47. Ullah A, Orij R, Brul S, Smits GJ. Quantitative analysis of the modes of action of weak organic acids in *Saccharomyces cerevisiae*. Applied and Environmental Microbiology. 2012; 78(23):8377–8387. https://doi.org/10.1128/aem.02126-12 PMid:23001666 PMCid: PMC3497387.
48. Lambert RJ, Stratford M. Weak-acid preservatives: modelling microbial inhibition and predicting minimum inhibitory concentrations. Int J Food Microbiol. 1999; 48(2):157–164. https://doi.org/10.1046/j.13652672.1999.00646.x PMid:10030018.
49. Prowting J, Bem J, Coecke S. *In vitro* safety and toxicity testing. In: Chackalamannil S, Rotella D, Ward S, editors. Comprehensive Medicinal Chemistry III. Elsevier; 2016. pp. 529–556.
50. Ly JD, Grubb DR, Lawen A. The mitochondrial membrane potential (Δψm) in apoptosis; an update. Apoptosis. 2003; 8(2):115–128. https://doi.org/10.1023/a:1022945107762 PMid:12766472.
51. Green DR, Kroemer G. The pathophysiology of mitochondrial cell death. Science. 2004; 305(5684):626–629. https://doi.org/10.1126/science.1099320 PMid:15286356.
52. Perry SW, Norman JP, Barbieri J, Brown EB, Gelbard HA. Mitochondrial membrane potential probes and the proton gradient: a practical usage guide. BioTechniques. 2011; 50(2):98–115. https://doi.org/10.2144/000113610 PMid:21486251 PMCid: PMC3115691.
53. Cardenas M. Integration of pro-apoptosis and pro-survival signalling pathways: a useful approach to in silico biomedical research. Journal of Computer Science and Systems Biology. 2013;06(04). https://doi.org/10.4172/jcsb.1000118
54. Ristow M, Zarse K. How increased oxidative stress promotes longevity and metabolic health: the concept of mitochondrial hormesis (mitohormesis). Exp Gerontol. 2010; 45(6):410–418. https://doi.org/10.1016/j.exger.2010.03.014 PMid:20350594.
55. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. Clin Chem. 2006; 52(4):601–623. https://doi.org/10.1373/clinchem.2005.061408 PMid:16484333.
56. Bojar RA. Studying the human skin microbiome using 3D *in vitro* skin models. Appl In Vitro Toxicology. 2015; 1(2):165–171. https://doi.org/10.1089/aivt.2015.0002

Author Contribution

Conceptualization -H.K. and H.S.; methodology - H.K. and H.S.; software -D.K. and M.L.; validation -H.K. and H.S.;formal analysis -H.K. and H.S.; investigation -H.K. and H.S.; resources -D.K. and M.L.; data curation -H.K. and H.S.; writing—original draft preparation -H.K. and H.S.; writing—review and editing -Y.K. and K.K.; visualization -D.K. and M.L.;supervision -Y.K. and K.K. All authors have read and agreed to the published version of the manuscript.