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Cytotoxicity of Moringa leaf powder extracts on mammalian LTK and HEK293 cell lines

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Abstract:

Antimicrobial resistance (AMR) poses a major global health challenge, with projected mortality rates expected to surpass those of cancer by 2050. Moringa oleifera, a nutrient-rich medicinal plant, is traditionally valued for its antimicrobial and therapeutic effects, yet its cytotoxic impact on mammalian cells remains poorly characterised. This study aimed to evaluate the cytotoxicity of M. oleifera leaf extracts on non-cancerous mammalian cells. Leaves were processed via Soxhlet ethanol extraction, dimethyl sulfoxide (DMSO) steeping, and aqueous steeping. LTK fibroblast-like and HEK293 epithelial cell lines were exposed to graded concentrations of extracts. Cell viability was assessed using the MTT assay, and morphological changes were observed under phase-contrast microscopy. Results showed that extraction method and solvent significantly influenced cell responses. Ethanol Soxhlet extracts produced the strongest proliferative effects, enhancing viability up to 278% in HEK293 and 244% in LTK cells relative to controls. Water-steeped extracts supported moderate proliferation at higher concentrations (75 mg/mL), while DMSO-steeped extracts showed lower and more variable activity, particularly in HEK293 cells. Microscopy confirmed normal morphology in untreated controls, whereas water extract treatments at higher doses induced cell rounding, detachment, and reduced confluency. These findings suggest that M. oleifera extracts, particularly ethanol Soxhlet preparations, promote cell viability and exhibit a low cytotoxicity profile in non-cancerous mammalian cell lines. This effect is likely linked to the plant's antioxidant and bioactive constituents. In conclusion, M. oleifera leaf extracts demonstrate potential for safe therapeutic use. Given the plant's established antimicrobial properties, future studies should isolate bioactive compounds, assess selective activity against cancer cells, and optimise extraction methods. Such research could advance the development of safe, plant-derived agents to complement strategies addressing the global AMR crisis.

Keywords: *Cytotoxicity, Cell viability, Fibroblast, Moringa oleifera, Therapeutic potential*

1. Introduction

Antimicrobial resistance (AMR) has become a cause for growing concern among the population of the world. In Europe and the US alone, the World Health Organization (WHO) has estimated that about 50,000 lives are lost due to antimicrobial drug resistance of microorganism. An estimated 700,000 lives are lost worldwide each year and according to WHO [1], this figure is expected to rise to an overwhelming 10 million per year and an overall cost of over $10 trillion per year by 2050 if nothing is done about it. This figure would be greater than death caused by diabetes and cancer combined the two biggest causes of death in the world currently. The World Bank also predicted that an additional 28 million people could be forced into extreme poverty if nothing is done to contain AMR [1].

Antimicrobial resistance is the ability of a microorganism, be it virus, bacteria or a parasite to resist actions of an antimicrobial (antiviral, antibiotic or antimalarial), thereby rendering the drug ineffective, resulting in persistent infection. Antimicrobial treatments have made formerly deadly and life-threatening diseases like tuberculosis and malaria easily treatable, have made surgeries easier, made child birth safe for both baby and mother and also protect cancer patients in chemotherapy. However, since the 1980s, no new class of antibiotics have been discovered. Pharmaceutical companies have chosen to focus on the profitability of the new antibiotics rather than its effectiveness. This has caused a failure in the market and limitation in the development of new drugs in general [2].

In Europe, 1 in 16 people die every year (25,000 in 400,000) due to infection from a resistant bacterial strain [3]. *E. Coli* that is resistant to the antibiotics used for sepsis showed an increase from 8.5% to 11.7% between 2011 and 2015, similarly resistant *K pneumoniae* increased from 12.6% to 18.5% within the same time. These surges in resistance cause an overreliance on carbapenems which are generally considered as the "last resort" antibiotics [4].

In Africa, the dilemma of AMR is poorly understood due to inadequate public health surveillance. Also, factors like inadequate medicine supply, poor distribution system and policies, and inadequate strategies to curb the issues of AMR further amplifies the effect of antimicrobial resistance on the African population [3, 4].

The herbal medicine industry is experiencing rapid growth. In the early 2000s, the United States spent an estimated US$17 billion on traditional herbal medicines. The World Health Organization (WHO) valued the global market for herbal drugs at US$60 billion in 2003. By 2012, the Chinese Traditional Medicine industry alone was valued at US$83 billion, with continued growth expected [5]. Proper integration and regulation of plant-based medicines could make antimicrobials more affordable and accessible, especially in remote areas. However, it's crucial to consider the potential for cytotoxicity, or a plant's toxicity to cells. While a highly cytotoxic plant can pose health risks, damaging organs like the kidneys or heart, it could also be a valuable resource for cancer treatment if it selectively targets cancerous cells without harming healthy ones [5, 6]. The cytotoxic potential of plants has been extensively studied, with many researchers aiming to exploit their properties for treating ailments like cancer. For example, a study by Steenkamp and Gouws [7] tested the cytotoxicity of six South African medicinal plants (*Bidens pilosa, Centella asiatica, Cnicus benedictus, Dicoma capensis, Hypoxis hemerocallidea, and Sutherlandia frutescens*) on human cancer cell lines, including DU-145 prostate and MDA-MB-231 and MCF-7 breast cancer cells. The study found that *Dicoma capensis* exhibited high cytotoxicity. Similarly, Itharat, *et al.* [8] tested three Thai medicinal spices and found that all three showed high cytotoxicity against COR-L23 and MCF-7 cell lines [7, 8].

Moringa oleifera, also known as the horseradish or drumstick tree, is a fast-growing tree native to the sub-Himalayan regions of northern India, but is now widely cultivated in tropical areas globally [9]. It is the most widely grown of the 14 species in the Moringaceae family. *Moringa* is remarkably rich in protein, with 100g of dry matter containing about 29±6g of protein - nearly twice the amount in milk and comparable to that in eggs. It also has more iron (28±6g) and calcium (1924±288mg) than beef and milk, respectively. It has a similar vitamin A content to carrots (15,620±6475 IU) and about seven times more vitamin C than oranges (773±91mg) [10]. An analysis of 100g of fresh M. oleifera leaves revealed high levels of water-soluble vitamins, including thiamine (2.6 mg), riboflavin (20.6 mg), nicotinic acid (8.2 mg), and vitamin C (220mg), as well as fat-soluble vitamins like vitamin A (16.3mg) and vitamin E (113mg) [11]. The leaves are also rich in minerals such as calcium (2003mg), magnesium (368 mg), iron (28.2mg), and potassium (1324mg) [11]. The 27.1g of protein in 100g of edible leaf contains all essential amino acids, including arginine and histidine, which are crucial for infant development and detoxification [11, 12]. These properties make Moringa a vital resource for communities, especially in rural areas, who lack access to conventional medicine and clean water. Given the significant medicinal potential of Moringa oleifera, it is crucial to understand its cytotoxic effects on mammalian cells to pave the way for its wider application in medicine. The primary aim of this study is to assess the cytotoxicity of Moringa leaf powder extracts on mammalian LTK and HEK293 cell lines.

Table 1: Showing compounds isolated from various parts of a Moringa Plant [9, 13, 14]

Part of plant	Compound isolated	References
Leaf	B-sitosterol, Quercetin, Kaemferol, niazirin, niazirinin, niaziminin A, Niaziminin B, lutein, 4-[(4-O-acetyl-α-L--rhamnosyloxy) benzyl] isothiocyanate	[9,13,14]
Stem bark	Epilupeol, β-sitosterol, moringine, moringinine, 4-(alpha-L- rhamnopyranosyloxy) benzyl glucosinolate	[9]
Seed	β-sitosterol, β-sitosterol-3-O-β-D- glucopyranoside, 4-(4'-O-acetyl-α-L- rhamnosyloxy) benzyl isothiocyanate, 4-(α-L- rhamnosyloxy) benzyl isothiocyanate.	[13]

2. Methods

2.1 Soxhlet Extraction

Ethanol-based extraction of *Moringa oleifera* leaf powder was performed using a Soxhlet apparatus. A total of 9.7 grams of powdered material was extracted with 250 mL of 100% ethanol over 6 hours. The temperature was maintained at 100°C for the initial 10 minutes to facilitate solvent reflux and was subsequently reduced to 80°C for the remainder of the extraction process. Following extraction, the ethanol was removed under reduced pressure using a Büchi rotary evaporator. The resulting residue yielded 2.2 grams of crude extract, which was dissolved in 50 mL of 100% dimethyl sulfoxide (DMSO) and filtered using Whatman No. 1 filter paper. This stock extract had a final concentration of 44 mg/mL. For downstream applications, working concentrations of 75%, 50%, 30%, 25%, and 20% (relative to stock) were prepared by diluting the extract in 100% DMSO. Figure 1 shows the diagram of a Soxhlet apparatus consisting of the condenser, thimble and crucible [16].

2.2 Steeping Extraction:

Additional extracts were prepared using a steeping method with either sterile reverse osmosis (R.O.) water or 100% DMSO as the extraction solvent. For the aqueous extract, 6 g of *Moringa* powder was steeped in 80 mL of sterile R.O. water at 70°C for one hour. The extract was

then filtered through Whatman No. 1 filter paper, resulting in a stock solution with a concentration of 75 mg/mL.

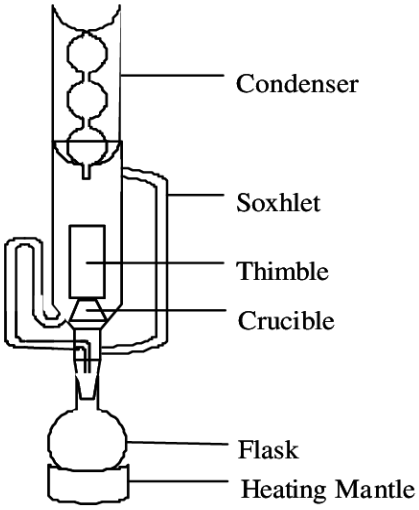

Figure 1: Diagram of a Soxhlet apparatus [16]

In parallel, 2 g of *Moringa* powder was steeped in 20 mL of 100% DMSO under identical conditions to obtain a DMSO-based extract with a stock concentration of 100 mg/mL. The differing solvent volumes were chosen to optimise solubility and filtration efficiency, particularly for the water-based extraction. From both steeped stock solutions, a series of working dilutions (75%, 50%, 30%, 25%, and 20%) were prepared using the same solvent used for extraction to maintain consistency across treatments

Table 2: table showing the initial concentration of undiluted extracts in their respective solvents and extraction methods

Solvent/Extraction Method	Concentration
DMSO/Soxhlet Extraction	44mg/ml
DMSO/Steeping Extraction	100mg/ml
Sterilized R.O Water/ Steeping Extraction	75mg/ml

2.3 Cell Culture

A. LTK and HEK293 cell lines were obtained from in-house stocks and cultured in Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific) supplemented with penicillin and streptomycin. Upon revival, both cell lines were approximately 70–80% confluent. For subculturing, cells were first washed with phosphate-buffered saline (PBS), followed by enzymatic detachment using 2X trypsin for LTK cells and 5X trypsin for HEK293 cells. After incubation, 10 mL of complete medium was added to each flask to neutralise the trypsin. From this, 5 mL of the cell suspension was transferred into new flasks containing an additional 10 mL of fresh medium. This passage resulted in approximately 40% confluency per flask. Cells were maintained in a humidified incubator at 37°C with 5% CO_2 and cultured for an additional 48 hours before experimental use. All procedures were conducted under aseptic conditions in a biosafety cabinet [8].

2.4 Cell viability count

After 48 hours of incubation, cells were again passaged for viability assessment. For each cell line, 100 µL of the cell suspension was mixed 1:1 with 100 µL of 0.4% trypan blue solution. A 100 µL aliquot of the mixture was loaded onto a haemocytometer and examined under a light microscope. Viable (unstained) and non-viable (blue-stained) cells were counted manually. LTK cells showed 87 live and 36 dead cells, while HEK293 cells showed 40 live and 12 dead cells, confirming adequate viability for downstream applications [27].

2.5 Cell Seeding for Cytotoxicity Assay

Freshly passaged LTK and HEK293 cells were seeded into 96-well flat-bottom plates for extract treatment. Each well designated for LTK cells received 180 µL of cell suspension, while HEK293 wells received 230 µL. The total volume per well was standardised to 201 µL using complete medium. A total of six 96-well plates were prepared, with both cell lines seeded in triplicate for each treatment group. Plates were incubated at 37°C with 5% CO_2 for 24 hours to allow cell attachment before treatment [27].

2.6 Treatment Controls

Appropriate vehicle and medium controls were included to account for solvent toxicity and background absorbance. Controls included sterile R.O. water, 70% industrial methylated spirits (IMS), 100% DMSO, and untreated DMEM. For each control, 100 µL was added to designated wells in triplicate. The absorbance value from the DMEM-only control was used as the reference point for calculating relative cell survival, which was set as 100% viability.

2.7 MTT Cytotoxicity Assay

To assess the cytotoxic effects of *Moringa* extracts, a standard MTT assay was performed. Following 24-hour treatment with the respective extract dilutions, 30 µL of MTT reagent (5 mg/mL in PBS) was added to each well (maintaining a 1:10 ratio to the culture volume). Plates were incubated for 3 hours at 37°C under 5% CO_2 to allow formazan crystal formation. After incubation, media and residual extract were aspirated, and 100 µL of DMSO was added to each well to solubilise the formazan. Absorbance was measured at 570 nm using a microplate reader. All treatments were performed in triplicate across three independent experimental replicates [27].

2.8 Statistical Analysis

Data were compiled in Microsoft Excel, and mean absorbance values were calculated for each treatment group. Two-way analysis of variance (ANOVA) was employed to compare treatment effects, with a significance level of $p < 0.05$. Visualisation and statistical analysis were conducted using GraphPad Prism software (v. 10.7). The standard error of the mean (SEM) was calculated and displayed alongside group means.

3. Results

To evaluate the potential cytotoxic effects of the extraction solvents used in the study, LTK and HEK293 cells were exposed to either media alone, sterile R.O. water, 100% DMSO, or 70% isopropyl alcohol (IMS), and cell viability was assessed using trypan blue exclusion. As expected, cells treated with complete media showed the highest viability, with survival rates close to 100% in both LTK and HEK293 cells. Water treatment also resulted in high viability, particularly in LTK cells (~90%), although a decrease was observed in HEK293 cells (~60%).

In contrast, exposure to DMSO or 70% IMS caused a significant reduction in cell viability in both cell lines, confirming their cytotoxic effects. These findings validated the choice of controls, with media and water as positive controls, and DMSO and 70% IMS as cytotoxic conditions for comparison in subsequent assays, as illustrated in Figure 2.

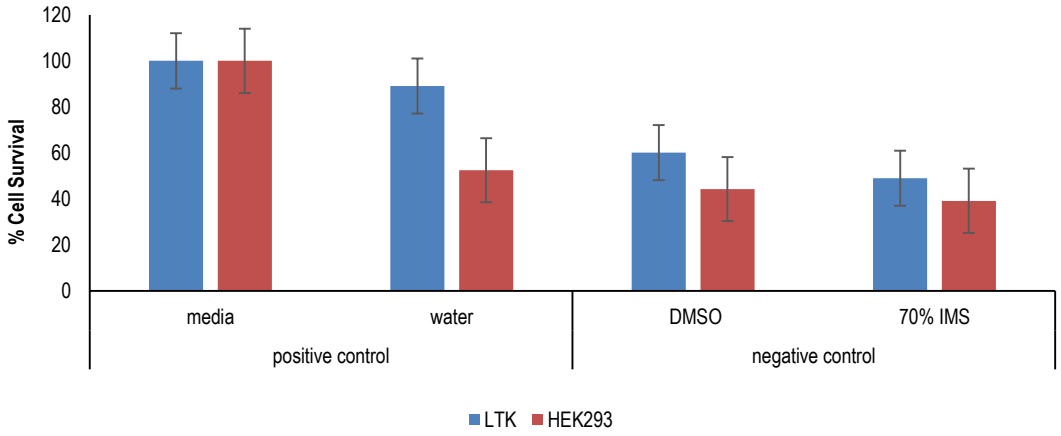

Figure 2: Chart showing the percentage cell survival of HEK293 and LTK cells upon treatment with water, 100% DMSO and 70% Industrial Methylated Spirit (IMS).

The survival of the group treated with only media was assumed to be 100%.

3.1 Cytotoxic effects of Moringa on HEK293T and LTK cells

To assess the cytotoxic potential of Moringa oleifera extracts obtained through different extraction methods, HEK 293 T and LTK cells were treated with serial dilutions of Soxhlet ethanol extract, DMSO steeped extract, and aqueous steeped extract. Cell viability was evaluated using MTT assays after 24 hours, with absorbance measured at 570 nm as a marker of metabolically active cells. The results are shown in Figure 3(a) and Figure 3(b).

In HEK 293T cells shown in Figure 3(a), an apparent dose-dependent increase in absorbance was observed for all extract types. The Soxhlet extract exhibited the highest viability-promoting effect across all concentrations tested, with absorbance values reaching approximately 2.0 at 1 mg/mL. Compared to both DMSO and water-steeped extracts, Soxhlet-treated cells consistently showed significantly higher absorbance at every tested concentration ($p < 0.05$ to $p < 0.0001$), indicating increased metabolic activity and cell viability. While both DMSO and water extracts resulted in modest increases in absorbance with rising concentration, the DMSO-steeped extract consistently produced higher absorbance values than the water extract at equivalent concentrations.

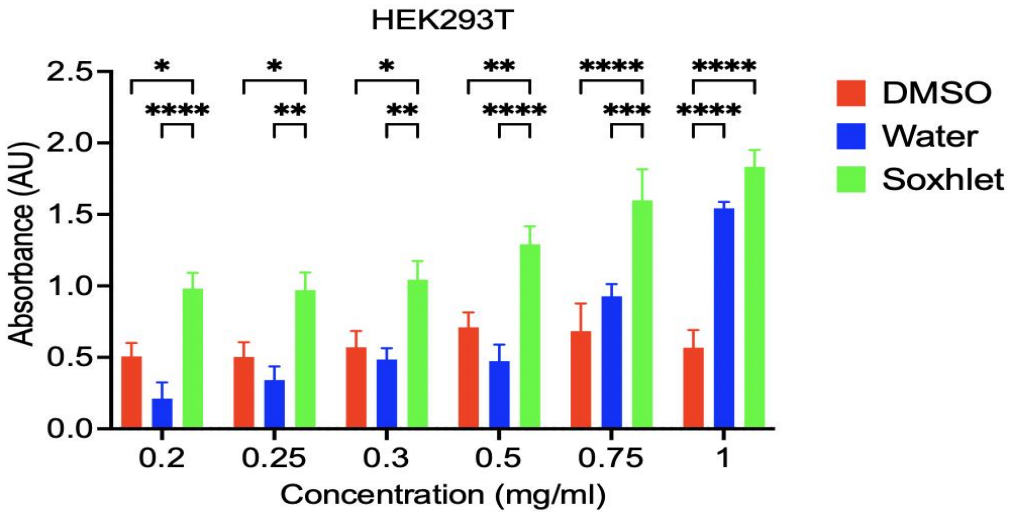

Figure 3(a): Effect of Moringa extracts prepared by different methods on HEK293T cell viability

As shown in Figure 3(b), a similar trend was observed in LTK cells. Soxhlet extracts induced the highest absorbance at all tested concentrations, with values rising from approximately 0.9 at 0.2 mg/mL to about 1.9 at 1 mg/ml. The differences between extraction methods were statistically significant across nearly all concentrations ($p < 0.01$ to $p < 0.0001$), with Soxhlet-treated cells displaying significantly higher viability than cells treated with DMSO or water-steeped extracts. Notably, the water extract demonstrated higher absorbance than the DMSO extract at lower concentrations (0.2–0.3 mg/mL), suggesting a possible cell-type-specific response in LTK cells.

Overall, Figures 3(a and b) illustrates that Soxhlet extraction yielded a more bioactive extract in both HEK 293 T and LTK cell lines, as reflected by higher cell viability across all tested concentrations. These results imply that ethanol Soxhlet extraction may enhance the cytoprotective or proliferative components of *Moringa oleifera* relative to steeping-based extraction methods.

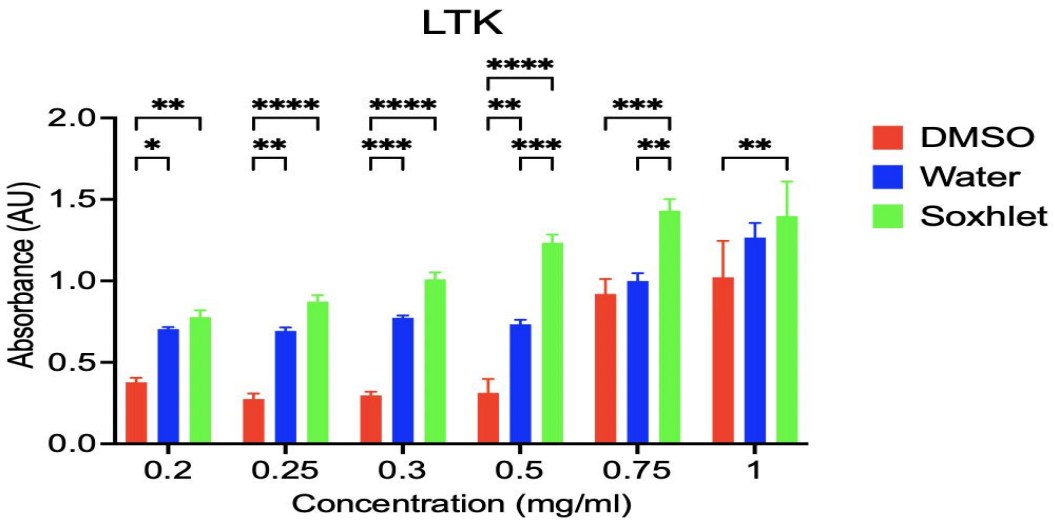

Figure 3(b): Effect of Moringa extracts prepared by different methods on LTK cell viability

HEK293T in Figure 3(a) and LTK in Figure 3(b) cells were treated with increasing concentrations (0.2–1.0 mg/mL) of *Moringa oleifera* extracts prepared via Soxhlet ethanol extraction (green), DMSO steeping (red), or water steeping (blue). Cell viability was assessed using the MTT assay after 24 hours. Absorbance at 570 nm was measured as a proxy for metabolic activity. Data represent mean ± SEM of triplicate experiments. Statistical significance was determined using one-way ANOVA with post-hoc tests comparing extract types at each concentration. $p < 0.05$, $p < 0.01$, $p < 0.001$, $p < 0.0001$.

Phase-contrast microscopy was also used to assess the effect of water-steeped Moringa oleifera extract on LTK cell morphology. Untreated LTK cells (left panel) appeared elongated and spindle-shaped, typical of healthy fibroblast-like morphology, with uniform attachment and even distribution. Upon treatment with the water extract (right panel), LTK cells exhibited signs of cytotoxic stress, including cell rounding, reduced density, and partial detachment from the surface. These morphological changes are consistent with decreased cell viability and support the findings from the MTT and trypan blue assays, as shown in Figure 4.

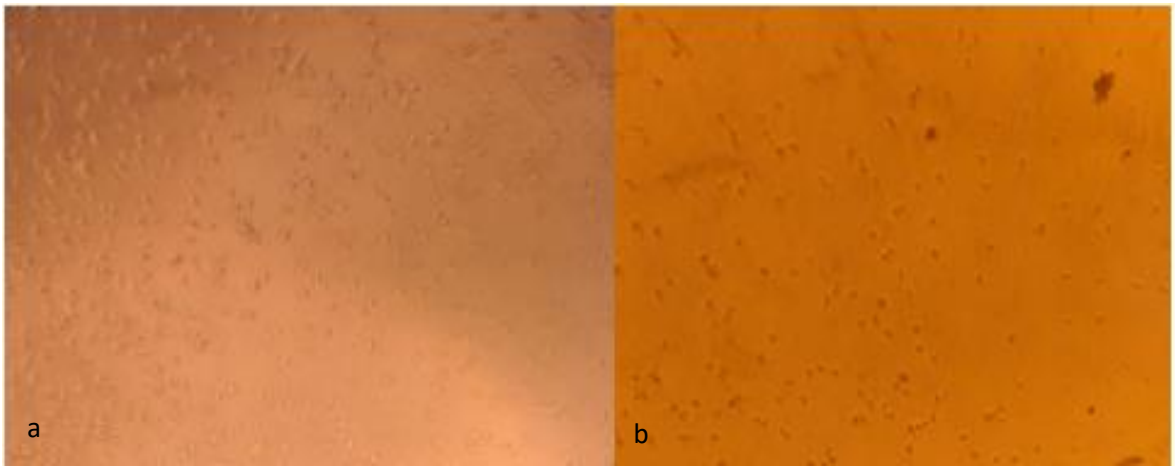

Figure 4: Morphological change in LTK cells after treatment with 15 mg/ml Moringa extract from the steeping done with sterilised R.O. water as a solvent.

To further examine the cytotoxic impact of *Moringa oleifera* extracts, phase-contrast microscopy was used to assess morphological changes in HEK293T cells before and after treatment with the water-steeped extract. Untreated HEK293T cells (5a) exhibited the expected cobblestone morphology, with tightly packed, healthy, and adherent cells. In contrast, cells treated with the water extract (5b) displayed pronounced morphological alterations, including cell rounding, detachment, and reduced confluency, indicative of compromised viability. These observations support the quantitative data from viability assays, confirming that the water-steeped extract exerts a cytotoxic effect on HEK293T cells at the concentrations tested, as shown in Figure 5.

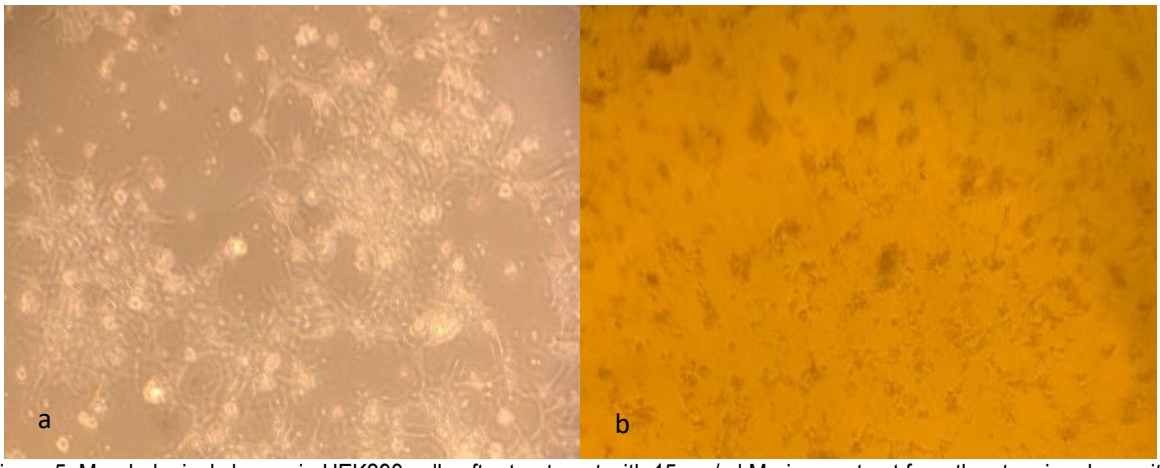

Figure 5: Morphological change in HEK293 cells after treatment with 15 mg/ml Moringa extract from the steeping done with sterilised R.O. water as a solvent.

3.2 Assessment of cytotoxicity via MTT assay

To assess the cytotoxic effects of *Moringa oleifera* extracts prepared with different methods and solvents, LTK and HEK293 cells were treated with various concentrations of each extract and evaluated using the MTT assay. The Soxhlet ethanol extract dissolved in DMSO showed the highest overall metabolic activity in both cell lines. At 44 mg/ml, it yielded mean absorbance values of 1.40 ± 0.28 (LTK) and 1.83 ± 0.24 (HEK293), corresponding to cell viabilities of 243.91% and 278.18%, respectively, compared to media-only controls. Viability exceeded 100% across all tested Soxhlet extract concentrations, indicating notable proliferative or metabolic stimulation.

The water-based steeping extract also demonstrated significant activity, especially in HEK293 cells. At 75 mg/ml, HEK293 viability reached 234.26%, while LTK cells showed 245.74%. However, a gradual decrease in cell viability was observed with lower concentrations, most notably in HEK293 cells. At the lowest concentration (15 mg/ml), HEK293 viability fell to 48.10%, indicating potential dose-dependent sensitivity of this cell line to the water-based extract.

In contrast, the DMSO-prepared steeping extract exhibited more modest cytotoxic effects, with HEK293 cells displaying lower absorbance across all tested concentrations. The highest concentration (100 mg/ml) yielded absorbance values of 0.92 ± 0.14 in LTK and 0.75 ± 0.50 in HEK293, corresponding to viabilities of 192.08% and 114.21%, respectively. Nevertheless, viability progressively declined with decreasing concentrations, especially in HEK293 cells, reaching 61.53% at 20 mg/ml. This contrasts with the LTK cells, which maintained relatively higher viability (>117%) across all concentrations of the DMSO steeped extract.

Overall, Soxhlet extraction produced the most potent extract in terms of increasing cell viability, particularly in HEK293 cells, while the water steeping extract demonstrated dose-dependent effects. The DMSO steeping extract appeared less effective and more variable, notably in HEK293 cells, highlighting the impact of extraction method and solvent on extract bioactivity.

4. Discussion

This study shows that the biological effects of Moringa oleifera leaf extracts on mammalian cells depend heavily on the extraction method and solvent used. Across all tested concentrations, ethanol Soxhlet extraction followed by DMSO solubilisation produced the most potent extract, with HEK293 and LTK cells demonstrating significantly higher viability compared to water or DMSO steeping. These results align with prior research indicating that Soxhlet extraction increases the yield and concentration of phenolics and flavonoids from Moringa [15, 16], likely due to the high-temperature reflux and continuous solvent cycling that improve extraction efficiency. The high cell viability from Soxhlet extracts, especially in HEK293 cells (up to 278% viability at 44 mg/ml), suggests the presence of compounds promoting mitochondrial function and possibly cell growth. This matches studies showing that ethanolic Moringa extracts contain quercetin, kaempferol, and chlorogenic acid, which are known for their antioxidant and protective effects [17, 18]. Such compounds can activate survival pathways and reduce oxidative stress in human cell lines [19,20]. Conversely, DMSO steeping extracts showed lower and less consistent viability, especially in HEK293 cells.

Viability decreased significantly at concentrations below 50 mg/ml, possibly due to extraction of mildly toxic compounds or impurities when DMSO is used at low temperatures, as Fard et al. (2015) observed with reduced viability in human fibroblasts exposed to DMSO-soluble plant fractions. While DMSO is an effective solvent across a broad polarity range, its hygroscopic nature and tendency to extract both beneficial and harmful compounds may explain the biphasic response observed. Water-based steeping extracts were more effective than DMSO extracts but less potent than Soxhlet. They maintained moderate-to-high viability, especially at 75 mg/ml, with 245% and 234% viability in LTK and HEK293 cells, respectively. These effects support findings that water extracts of Moringa leaves contain ascorbic acid, tannins, and water-soluble polyphenols [22], which have antioxidant and anti-apoptotic properties. However, unlike ethanol extracts, water extracts may lack lipophilic phytoconstituents such as sterols and fatty acids [19], which could limit full cytoprotection. Notably, LTK cells showed greater resilience overall, maintaining higher viability across a wider concentration range. This aligns with previous reports suggesting fibroblast-like cell lines are more tolerant to plant extracts than epithelial lines such as HEK293 [23], likely due to differences in antioxidant capacity, uptake, or metabolism.

Overall, these results highlight that the extraction method significantly influences phytochemical efficacy. Soxhlet extraction effectively concentrates bioactive compounds from Moringa, consistent with earlier studies noting its superiority for total phenolic content and antioxidant activity [16]. For applications needing lower toxicity or food-grade solvents, water steeping remains a feasible, though less potent option.

3 Conclusion

The alarming rise of antimicrobial resistance, coupled with a lack of new antimicrobials since the 1980s, highlights the urgent need to explore alternative therapeutic agents. Plants have long been a reliable source of medicinal compounds, known for their anti-inflammatory, antimicrobial, and antimalarial properties. Moringa oleifera, widely consumed for both its nutritional and medicinal benefits, has a well-documented history of traditional use without reported toxicity.

Our study investigated the cytotoxicity of Moringa leaf powder extracts on HEK293 and LTK cell lines. We found that the extracts promoted cell proliferation rather than cytotoxicity, suggesting they are safe for human cells. This proliferative effect was concentration-dependent, decreasing as the extract concentration was reduced and the solvent concentration increased. The statistical significance of these findings confirms that the extracts had a measurable effect on cell viability.

Given the well-documented antimicrobial activity of Moringa [24,25,26] future research should focus on isolating the specific phytochemicals responsible for this activity. Using fewer toxic solvents in future extractions will be crucial for determining the precise mechanism of action, which could pave the way for its use in new medicinal applications.

Limitations and future directions

This study had some limitations. The use of high extraction temperatures (70°C or above) may have degraded some of the phytochemicals. Additionally, preparing samples by percentage dilution was less efficient than fold dilutions. Future studies should consider using low-temperature extraction methods, such as ultrasound-assisted extraction, which is more efficient and environmentally friendly and can yield higher concentrations of phenolic compounds.

Other limitations included the lack of a proper cell growth curve to ensure cells were in their exponential phase and the absence of a detailed phytochemical analysis of the extracts. Future experiments should generate a growth curve to understand cell characteristics better and include phytochemical profiling to identify the specific compounds responsible for the proliferative activity. Furthermore, adding a cancer cell line (e.g., HeLa) to future cytotoxicity tests would provide a more complete understanding of Moringa's therapeutic potential.

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