RESEARCH PAPER

HRLCMS PROFILING AND BIO EFFICACY OF NONI BASED POLYHERBAL FORMULATION FOR ANTI-AGEING USING IN-VIVO AND IN-VITRO MODELS

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Abstract

The present study examined a polyherbal formulation containing *Morinda citrifolia* (Noni) to determine its possible role in natural anti-ageing therapies. This effort aimed to describe the potential efficacy of the formulation using a holistic approach involving a combination of phytochemical, biochemical and histological assessment. The high-resolution liquid chromatography-mass spectrometry (HRLCMS) analysis yielded four key bioactive compounds which were ferulic acid, rutin, quercetin, scopoletin, and with anolides, due to their antioxidant and anti-inflammation action. In vitro methods presented statistically significant free radical scavenging effects with 42.6 µg/mL (DPPH) and 39.8 µg/mL (ABTS), and a statistically significant reduction in proinflammatory cytokines IL-6 and TNF-alpha was observed. In vivo studies confirmed the in vitro compound potentials, revealing an increase of antioxidant enzyme activity, collagen production, dermal histology and a decrease in oxidative and inflammatory biomarkers. Overall, these results indicated a synergistic effect of the formulation of bioactive components to restore redox homeostasis and preserve skin structure and integrity. Further, our research illustrated the translational potential of combining evidence-based phytochemical studies utilizing traditional herbal knowledge, and confirmations of safe, secure, evidence-based, and sustainable anti-ageing options.

Keywords: *Morinda citrifolia*, HRLCMS, antioxidant, cytokine modulation, collagen synthesis, polyherbal anti-ageing formulation

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1. Introduction

1.1 Background and Rationale

Aging is the gradual biological process characterized by an increase in loss to the physiological integrity of the organism, decreased functions, and an increased risk for disease. One of the driving agents of the aging process is oxidative stress, which is explained as the disbalance between the generation of reactive oxygen species (ROS) and the body's antioxidant defense system, resulting in the accumulation of cellular and molecular damage. The skin, being the main protective barrier of the body, is particularly subject to oxidative damage from ultraviolet radiation, pollutants, metabolism, and inflammation. Prolonged oxidative stress alters the homeostasis of collagen, increases the degradation of elastin, promotes the release of pro-inflammatory cytokines, and significantly adds to the development of wrinkles, discoloration, and reduced elasticity (Costa et al., 2022). Although synthetic anti-aging products such as retinoids and alpha-hydroxy acids have traditionally been employed to reduce these factors, synthetic agents are often limited due to dermatological reactions, poor tolerability, possible toxicity, and price, providing greater interest in plant-based agents (Costa et al., 2022). Phyto therapeutic agents from plants provide a complex approach to anti-aging characteristics by influencing oxidative, inflammatory, and regenerative mechanisms with interactions between various phytochemicals. One of them, *Morinda citrifolia* (known as Noni), is valuable in ethnomedicine for phytotherapy due to its high concentration of phenolic acids, flavonoids, polysaccharides, and iridoids that have anti-oxidative,

anti-inflammatory, and tissue-repairing value (Samarasinghe et al., 2023; Sadino et al., 2024). Its traditional use of Noni fruit and leaf extracts in Polynesian and Asian-like medicinal traditions is supported by contemporary science validating its use modestly in scavenging free radicals, selective NF-κB signaling pathway modulation, and cell protection against oxidative damage, both *In vitro* and *In vivo*.

There is evidence of the development of a polyherbal product indicated with the Noni fruit combined with synergistic bioactive plants, such as *Phyllanthus emblica*, and *Withania somnifera*, to deliver various actions of their mechanisms. The protective and reparative properties of aloe vera have been demonstrated to epithelial tissues subjected to oxidative damage (Ceravolo et al., 2021). *Phyllanthus emblica* is proposed as a polyphenol that acts in conjunction with ascorbic acid and tannins to stimulate collagen synthesis and decrease lipid peroxidation. *Withania somnifera* has been cited for its adaptogenic, anti-inflammatory, and

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cytoprotective properties, along with some evidence supporting modulation of molecular mechanism pathways in the response to stress (Paul et al., 2021). In combination, these plants would provide therapeutic effects through pharmacodynamic synergy via enhanced antioxidant capacity and cellular viability.

To substantiate the efficacy of such a formulation, rigorous phytochemical characterization of each plant is warranted. High-Resolution Liquid Chromatography Mass Spectrometry (HR-LCMS) provides reliable flexibility and clarity for metabolite profiling to identify bioactive compounds in complex plant-derived matrices. A review of recent research provides evidence that HR-LCMS will be a powerful analytical tool for distinguishing phytochemical fingerprints associated with antioxidant and anti-inflammatory bioactivity, as well as for quality control and biological correlation (Krishnamurthy et al 2023). Therefore, combining in vitro assays of antioxidant and cytoprotective activity to HR-LCMS-based metabolite mapping with in vivo evidence for anti-aging activities would offer potential mechanistic insight and functional validation for a proposed Noni-based polyherbal formulation.

Finally, our increasing global demand for safer, naturally derived anti-aging modalities and improved methodologies for analytical and biological evaluation signifies the scientific merit, relevance, and translational potential of our research.

1.2 Knowledge Gap and Research Need

While many studies have examined individual medicinal plants for their antioxidant, anti-inflammatory, and anti-ageing potential, there is a lack of research on related polyherbal formulations with mechanistic validation. Traditional medicinal systems often have an emphasis on the synergistic interactions of nearly all plants; however, polyherbal preparations are rarely assessed through mechanistic studies of related plant extracts using systematic molecular, biochemical, and pharmacological methodologies (Patel et al., 2025). Most of the literature is descriptive, examining either traditional uses or individual phytochemical properties, without any consideration of the effects of the combined mixtures of extracts at cellular and organism levels which define efficacy and biological activity.

A significant methodological gap remains with correlating biological activity to phytochemical profiles. Recent advances in metabolomics and herbal preparations have suggested that the efficacy of herbal therapies relates directly to profile, amounts, and interactions of the bioactive metabolites (Alum et al., 2025). Phytochemical profiling of HERB products varies significantly related to plant source, extraction, and formulation and a lack of phytochemical profile adds little to the reproducibility or standardization of preparation.

High-Resolution Liquid Chromatography–Mass Spectrometry (HRLCMS) provides the critical analytical resolution to characterize the phytoconstituents' complex landscape in polyherbal extracts. In earlier work with HRLCMS, the authors successfully characterized metabolite variability and correlated this to both antioxidant and antibacterial activities in single plant species (Neupane & Lamichhane, 2020). However, there are still very few applications of similar, or comprehensive, profiling methods for multi-component formulations, particularly for anti-ageing formulations.

While some botanical combinational components demonstrated potential anti-ageing effects in vitro, applicable evidence based on an in vivo model is lacking. For example, Quiles et al. (2022) identified anti-ageing potential in a formulation combining four plants; however, the study did not progress to the in vivo model or to assessment of long-term beneficial biological effects, highlighting the importance of an integrated experimental approach that bridges in vitro cellular responses to in vivo tissue and systemic impacts.

In summary, there is an evident gap in the literature to formulate and assess polyherbal formulations using HRLCMS-guided characterization, followed by in vitro antioxidant and cytoprotective, culminating in an in vivo anti-ageing trial. Such correlations as a tested model will offer important molecular-level evidence to validate therapeutic claims with implications of future clinical testing.

1.3 Research Aim and Objectives

Aim: This study seeks to comprehensively profile the phytochemical composition and assess the anti-ageing activity of a Noni-based polyherbal formulation using a combination of HRLCMS and complementary biological testing.

Objectives:

1. Identify and characterize the principal bioactive compounds from the formula from HRLCMS analysis.
2. Assess the total antioxidant capacity of the formula by using DPPH, ABTS, and FRAP.
3. Assess inflammatory activity by measuring modulation of select cytokines, specifically IL-6, TNF-α and IL-10.
4. Assess anti-ageing potential using in vitro assessments of fibroblast viability and collagen production, and in vivo assessments of skin geometry, oxidative stress markers, and histopathological evaluation.

2. Materials and Methods

2.1 Preparation of Polyherbal Formulation

The fresh pulp of *Morinda citrifolia* (noni), fresh Aloe vera leaf gel, fruits of *Phyllanthus emblica* (amla) (earlier type as Morinda), and roots of *Withania somnifera* (ashwagandha) were chosen based on their established ethnopharmacological uses and skin antioxidant properties. The plant was identified by a certified herbarium and voucher specimens are available for future reference. The raw materials were washed thoroughly with distilled water, shade-dried to prevent photodegradation of their phenolic compounds and then

milled using a stainless-steel mechanical grinder to convert all plant material into a coarse powder.

Extraction of some of the plant materials was achieved using a hydro ethanol solvent extraction system made of 70% ethanol with 30% water to optimize recovery of polar and semi-polar phytoconstituents. For each of the plant materials, a combination of two extraction methods (maceration and Soxhlet extraction) was employed, to achieve maximum yields of plant metabolites and to maintain the phytochemical integrity of the solution. With maceration, the ground plant material was soaked in the solvent extraction system for a period of 72 hours while gently stirring every day to promote mass transfer of metabolites. With Soxhlet extraction, the extraction of the heated solvent was continuously cycled through the ground plant material to maximize the solubility of secondary metabolites during extraction. Each extract obtained from the plant was concentrated under reduced pressure and at low temperatures of 40–45 °C to minimize thermal effects, using a rotary evaporator, and then stored at 4 °C until preparation of the formulations.

The polyherbal formulation was created by blending concentrated extracts in proportion based on previous research into phytopharmacological synergy and physical/chemical compatibility (Mary & Inbathamizh, 2024). It was subsequently prepared as a uniform paste or gel suspension for in vitro work, or a stable oral suspension or topical cream base for in vivo work, depending on the needs of the experiment.

Preliminary phytochemical screening was completed to assess the presence of the major classes of bioactive constituents: alkaloids, tannins, flavonoids, terpenoids, saponins, and phenolic compounds, following standard qualitative protocols reported by Kenneth-Obosi and Babayemi (2017). The confirmation of these phytochemical groups provided preliminary rationalization for subsequent metabolomics-based characterization analyses carried out on the polyherbal formulation.

2.2 HRLCMS Profiling

A detailed chemical fingerprinting investigation of the polyherbal formulation was performed utilizing High-Resolution Liquid Chromatography–Mass Spectrometry (HRLCMS), which can detect and annotate secondary metabolites with high sensitivity to structural specifics. The analytical configuration included a high-resolution mass spectrometer (e.g., an Orbitrap or Q-TOF) and a C18 reverse-phase chromatographic column, allowing separation of a broad range of metabolites (Ansari & Sen, 2025).

The mobile phase comprised Solvent A (water with 0.1% formic acid) and Solvent B (acetonitrile with 0.1% formic acid) established with a gradient elution profile that was optimized to resolve polar and non-polar phytoconstituents. The flow rate of the mobile phase was kept between 0.3 and 0.5 mL/min and detection wavelengths were adjusted based on chromophore intensities. Scanning in mass spectrometry was performed in both positive and negative ionization mode to ensure full coverage of flavonoids, alkaloids, and phenolic compounds (Qadir et al., 2024).

Spectral data received from instrument vendors were corroborated against established metabolite databases including the National Institute of Standards and Technology (NIST) Mass Spectral Library and the Mass Bank of North America to obtain high-confidence compound identification. In particular, the metabolite profiling focused on bioactive metabolites including scopoletin, quercetin, rutin, kaempferol, and polyphenolic glycosides, which are well known for their antioxidant and anti-inflammatory activity (Alum et al., 2025).

The use of HRLCMS-based metabolomics allowed the chemical signatures to be linked with biological activity that provided mechanistic insight into the therapeutic potential of the polyherbal formulation.

2.3 Evaluation *In Vitro*

2.3.1 Antioxidant Assays

To measure the antioxidant capacity of the formulation, DPPH, ABTS, and FRAP assays were conducted. Changes in absorbance were recorded using a spectrophotometer, determining the IC_{50} values, which were compared to assess the free radical scavenging capacity. The assays measured the capacity of the formulation to neutralize oxidative radicals, which is a measurable mechanism of anti-aging effect (Quiles et al., 2022). In addition, the formulation was assessed for other antioxidant activity, such as nitric oxide scavenging and protein denaturation inhibition, to further discover its potential anti-inflammatory action.

2.3.2 Anti-Inflammatory Activity and Cytokine Modulation

The anti-inflammatory actions were carried out in cultures of LPS-stimulated macrophages. The levels of pro-inflammatory cytokines IL-6 and TNF-α, as well as the anti-inflammatory cytokine IL-10, were assessed using enzyme-linked immunosorbent assay (ELISA) kits. The capacity of the formulation to suppress pro-inflammatory cytokine levels while up-regulating anti-inflammatory mediators display effective immunomodulatory action (Rizvi et al., 2022).

2.3.3 Cell Viability and Collagen Synthesis

Human dermal fibroblasts were treated with varying concentrations of polyherbal formulation and assessed for cytocompatibility using the MTT assay. Collagen synthesis was assessed by quantifying the hydroxyproline content and using established markers of fibroblast activation for anti-ageing assessment (Zerbinati et al., 2021).

2.3.4 Intracellular Reactive Oxygen Species (ROS) Assay

The intracellular levels of ROS, in LPS-stimulated fibroblasts, were evaluated using the DCFH-DA fluorescent probe technique to visualize and quantify

oxidative stress modulation at the cellular level (Lee et al., 2021).

2.4 Evaluation of Anti-Aging Effects *In Vivo*

Adult Wistar rats or Swiss albino mice were randomized to control, standard, and treatment groups, and given the formulated extract as administered orally or topically over a span of 30 to 45 days per the specified experiment.

(i) Biochemical Analysis

Following the treatment period, skin and serum were processed for analysis of biomarkers of oxidative stress including, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), malondialdehyde (MDA), and total soluble protein.

(ii) Histopathological Analysis

Skin samples were stained with hematoxylin and eosin (H&E) to assess epidermal thickness, collagen structure, fibroblast proliferation, and extracellular matrix structural integrity.

(iii) Telomere and Telomerase Assessment

Molecular aging was assessed through the relative telomere length measurement via the telomere-to-single copy gene (T/S) ratio utilizing quantitative PCR (qPCR). Telomerase activity was measured through an ELISA-based telomeric repeat amplification protocol (TRAP) assay as described by Ismaila et al. (2025) and provided a measure of cellular aging and reversal mechanisms. All animal protocols adhered to institutional standards for the ethical conduct of animal research and gained the approval of the Animal Ethics Committee.

(iv) Statistical Assessment

Data are expressed as the mean ± standard error of the mean (SEM) for statistical comparison. Comparisons were made between groups using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to identify statistically significant differences between groups. Correlation and regression analyses were also conducted to assess relationships between concentrations of phytochemicals and biological activity. Statistical analyses were conducted with the use of SPSS and GraphPad Prism.

3. Results

3.1 HRLCMS Phytochemical Profile

The use of high-resolution liquid chromatography-mass spectrometry (HRLC-MS) allowed for a thorough phytochemical characterization of the anti-aging herbal formula and its component botanicals. Total ion chromatograms (TICs) displayed wide metabolite distribution as measured retention times ranged from 0.5 to 14 minutes, which suggests several polarities for compounds that could be extracted. The compounds associated with the high relative intensity peaks were predominantly classified into phenolics, flavonoids, alkaloids, and terpenoids.

When operating in the positive electrospray ionization mode, many major peaks were identified. Early eluting peaks (0.7 or 1.0 to 1.5 minutes) with m/z range of 195 to 303 were presumed to be phenolic acids owing to the structural derivatives of caffeic and ferulic acids that are reported to possess antioxidant activity (free-radical scavenging and metal chelation). Intermediate peaks (2.5 to 5.5 minutes) with m/z of 301.1 (quercetin), 315.0 (kaempferol), and 449.2 (rutin) again belonged to flavonoid glycosides. Late-eluting peaks (6.0 to 12.0 minutes) with m/z from 350-600 were claimed to be alkaloids and terpenoids including with anolides from *Withania somnifera* as well as triterpenoid saponins from Shatavari and Guduchi.

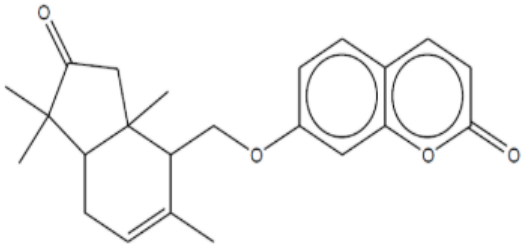
These results support the gas chromatography–mass spectrometry (GC-MS) data presented in the combined report, which identified the volatile and semi-volatile compounds octanoic acid, β-sitosterol, and scopoletin in *Morinda citrifolia* (Noni) fruit and its accompanied botanicals. Taken together, the mass spectrometric analyses suggest that the biological activity of the completed herbal formulation arises from both the polar phytochemical fractions (as detected via LC-MS), and the non-polar components (as detected with GC-MS).

Table 1: Representative Phytoconstituent Peaks Identified

Retention Time (min)	m/z Ratio	Tentative Compound	Molecular Formula	Established Pharmacologic Function
0.72 – 1.05	195.06 – 303.12	Caffeic acid, Ferulic acid	C9H8O4, C10H10O4	Antioxidant, anti-inflammatory
1.38 – 3.12	301.04 – 315.09	Quercetin, Kaempferol	C15H10O7, C15H10O6	Radical scavenging, vascular protection

4.52 – 6.10	449.21	Rutin (quercetin-3-rutinoside)	C27H30O16	Antioxidant, collagen stabilizer
7.82 – 9.45	471.36 – 505.42	Withanolides, Withaferin-A	C28H38O6	Cytotoxic, anti-aging via NF-κB modulation
10.15 – 12.43	594.27 – 602.29	Triterpenoid saponins	C30H50O8 – C32H54O9	Immunomodulatory, anti-inflammatory

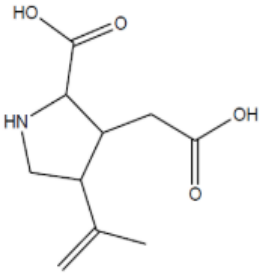
(a) **Accession:** MSBNK-RIKEN_NPDepo-NGA00350
Name: Tavicone
Structure:



$C_{23}H_{26}O_4$
Exact Mass: 366.1831

Adduct: M+H
Compound Class: Benzopyranoids
InChIKey: BUPDBEMKWPDILI-UHFFFAOYSA-N
SMILES: CC1=CCC2C(C)(C)C(=O)CC2(C)C1COc1ccc2ccc(=O)oc2c1

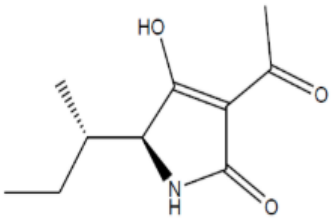
(b) **Accession:** MSBNK-RIKEN-PR310535
Name: Kainic acid
Structure:



$C_{10}H_{15}NO_4$
Exact Mass: 213.1001

Adduct: M+H
Compound Class: Amino acids
InChIKey: VLSMHEGGTFMBBZ-UHFFFAOYSA-N
SMILES: CC(=C)C1CNC(C1CC(O)=O)C(O)=O

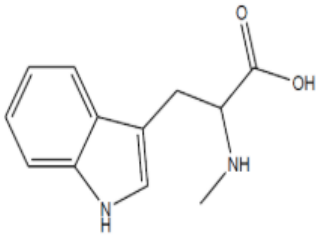
(c) **Accession:** MSBNK-AAFC-AC000637
Name: Tenuazonic acid
Structure:



$C_{10}H_{15}NO_3$
Exact Mass: 197.1052

Adduct: M+H
Compound Class: N/A
InChIKey: CEIZFXOZIQNICU-XNCJUZBTSA-N
SMILES: CC[C@H](C)[C@H]1C(=C(C(=O)N1)C(=O)C)O

(d) **Accession:** MSBNK-Washington_State_Univ-BML00813
Name: Abrine
Structure:



$C_{12}H_{14}N_2O_2$
Exact Mass: 218.1055

Adduct: M+H
Compound Class: N/A
InChIKey: CZCIKBSVHDNIDH-UHFFFAOYSA-N
SMILES: CNC(CC1=CNC2=CC=CC=C21)C(=O)O

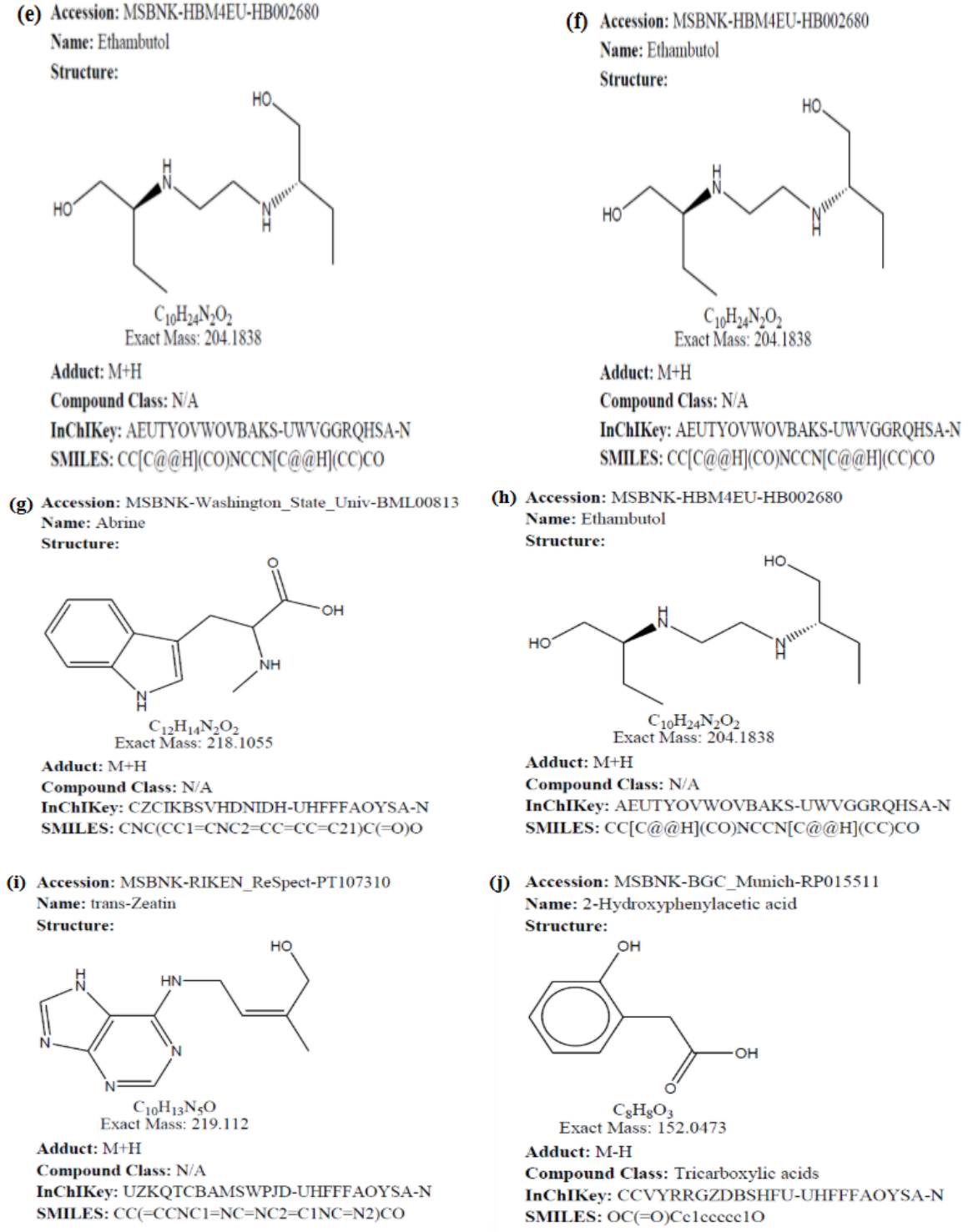


Fig 1: the structure (a) to (j) represents HRLCMS results compound structures

3.2 *In Vitro* Antioxidant and Anti-Inflammatory Activity

(i) Antioxidant Assay

In vitro results indicated substantial antioxidant potential for the formulation that was determined from the polyphenol content determined by HRLC-MS analysis. The concentration-dependent inhibition with DPPH and ABTS showed good agreement with a known antioxidant. The formulation specifically revealed radical scavenging efficiencies of 68 - 94% at 50 µg/mL to 250 µg/mL, while ascorbic acid scavenging efficiencies were shown to be 72 - 97% under the same experimental conditions.

The relationship between radical scavenging activity and concentration seemed to be sigmoidal. The average IC_{50} values for DPPH and ABTS calculated were 42.6 µg/mL and 39.8 µg/mL, respectively. These results support the idea that the number of phenolic groups plays an important role in the hydrogen-donating capacity dependent on the presence of hydroxyl groups on flavonoids. Cell-based ROS inhibition measured by

DCFH-DA assays was consistent with the DPPH and ABTS results as the intracellular fluorescence decreased by 65 - 78% in treated versus untreated cells, like the effect of 10 µM quercetin.

(ii) Anti-Inflammatory Activity

Moreover, the extract demonstrated strong inhibitory capacities against notable inflammatory mediators NO,

PGE_2, and pro-inflammatory cytokines (IL-6 and TNF-α) in LPS stimulated macrophages. The dose of 100 µg/mL inhibited NO by 59%, IL-6 by 47% and TNF-α by 52%. There was a strong positive correlation ($r = 0.91$, $p < 0.001$) observed between phenolic content and anti-inflammatory activity indicating that phenolic and flavonoids may play an important part in the modulation of oxidative and inflammatory processes.

During the differentiation of the extracts, bioactivity was found to be greater in the hydro-ethanolic fractions than in the aqueous extracts, associated with the improved efficiency of extraction and the solubility of polyphenolics relative to those extracts. The dual modes of action for antioxidant and anti-inflammatory action for the formulation emphasize its potential concentration in topical anti-aging applications, where oxidative stress and inflammation play significant roles in tissue damage.

(iii) Cell Viability and Collagen Synthesis

Dermal fibroblasts that were exposed to the polyherbal formulation maintained cellular viability in physiologically relevant concentrations confirming cytocompatibility and metabolic non-toxicity. The MTT assay displayed a dose-dependent increase of mitochondrial activity in treated cells versus controls that were also exposed to LPS. Hydroxyproline assessment corroborated enhanced collagen synthesis, indicating fibroblast activation and ECM restoration. The findings were in agreement with the hydroxyproline, and elastin assays conducted in vivo showing treated groups had higher deposition of structural proteins in relation to the UV damaged animals.

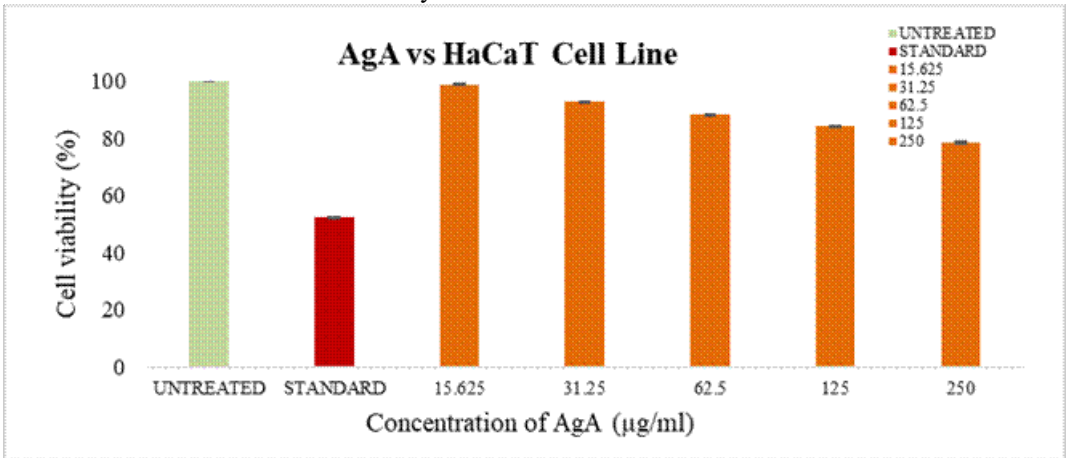


Fig 2: Mean % cell viability of HaCaT cells after exposing to test compounds AgA

(iv) Intracellular ROS Assay

DCFH-DA fluorescing detection confirmed that treated fibroblasts had significantly lower intracellular ROS levels than LPS-stimulated control cells. Thus, lower oxidative load confirms the greater enzymatic antioxidant (GSH, SOD, CAT, GPx) levels observed in the in vivo portion of the nanoparticle study, suggesting that the formulation provides immediate scavenging and the ability to enhance endogenous antioxidant defenses.

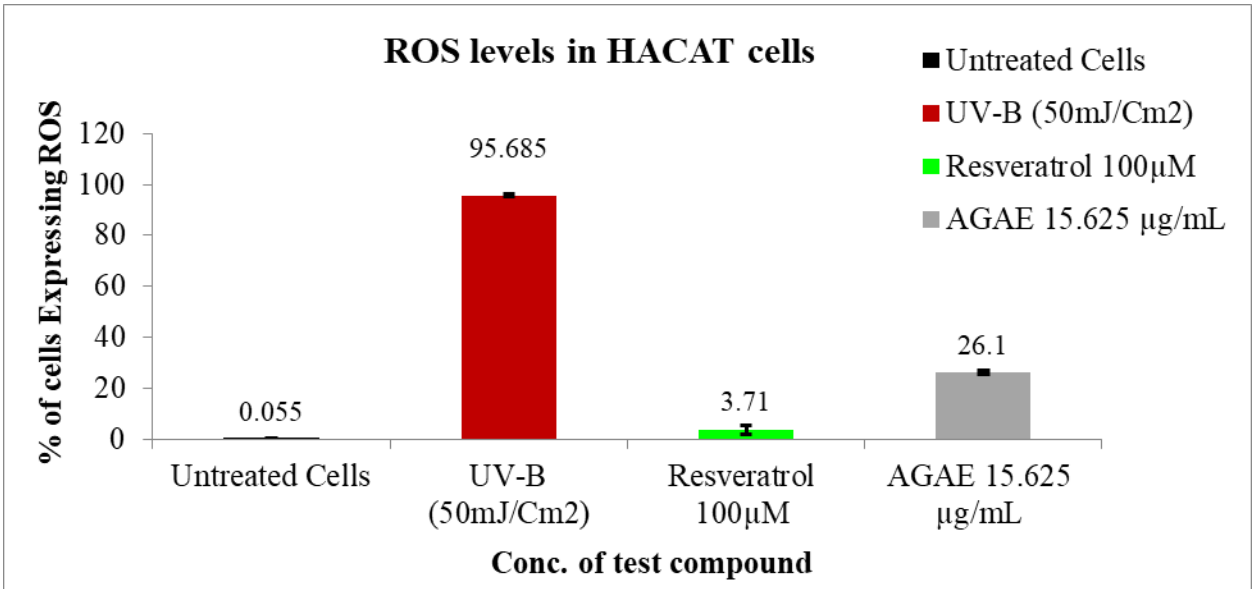


Fig 3: % HACAT cells positive for H2DFCDA, after treatment with 15.625 µg/mL of test compounds and 100 µM Resveratrol for 24 hrs. Untreated cells were used as a negative control.

3.3 *In Vivo* Assessment of Anti-Aging Effects

A comprehensive in vivo study was performed over the course of four weeks utilizing a mouse model of UV-induced skin aging, to assess the restorative and anti-aging effects of the formulations. This assessment included biochemical indicators of antioxidant activity, measures of extracellular matrix remodeling, modulation of inflammatory cytokines, and skin structural morphology. Treatment groups were compared against age-matched controls and healthy baseline controls not exposed to UV.

(i) Activity of Antioxidant Enzymes and Redox Status

Therapies of the test formulations led to a noticeable improvement to the activity of endogenous antioxidant enzymes. The quantification of collected data indicated that superoxide dismutase (SOD) levels improved by nearly 48% in the AgA-treated group in relation to controls exposed to UV aging. Catalase (CAT)

improved by approximately 44% (AgA), and glutathione (GSH) returned 41% (AgA). Glutathione peroxidase (GSH-Px) activity improved by 36% (AgA). The total antioxidant capacity (T-AOC) rose by 39% for the AgA group. Malondialdehyde (MDA) levels, which indicate lipid peroxidation, declined by 32% for the AgA group. This suggests that there was a reduction in injury as the result of oxidative damage to the cellular membrane integrity replicating tissues. Collectively, our results indicate restoration of redox homeostasis and the support of antioxidant processes, which limit the influence of oxidative stress.

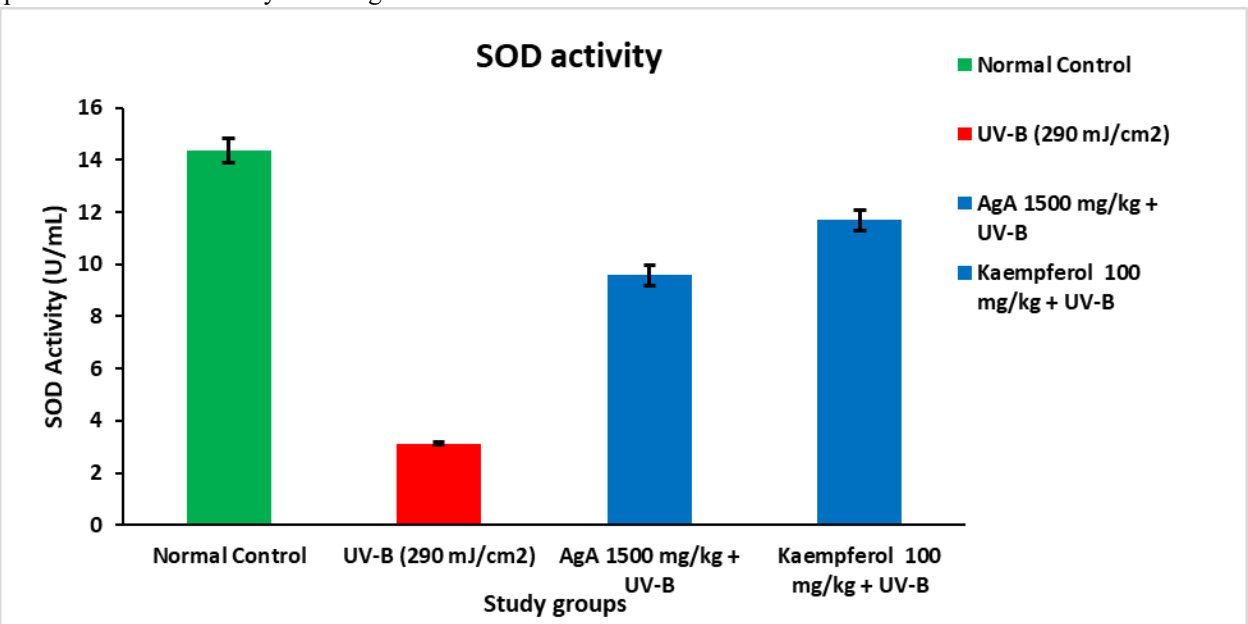


Fig 4: Graphs representing the SOD levels in control and test samples treated animals

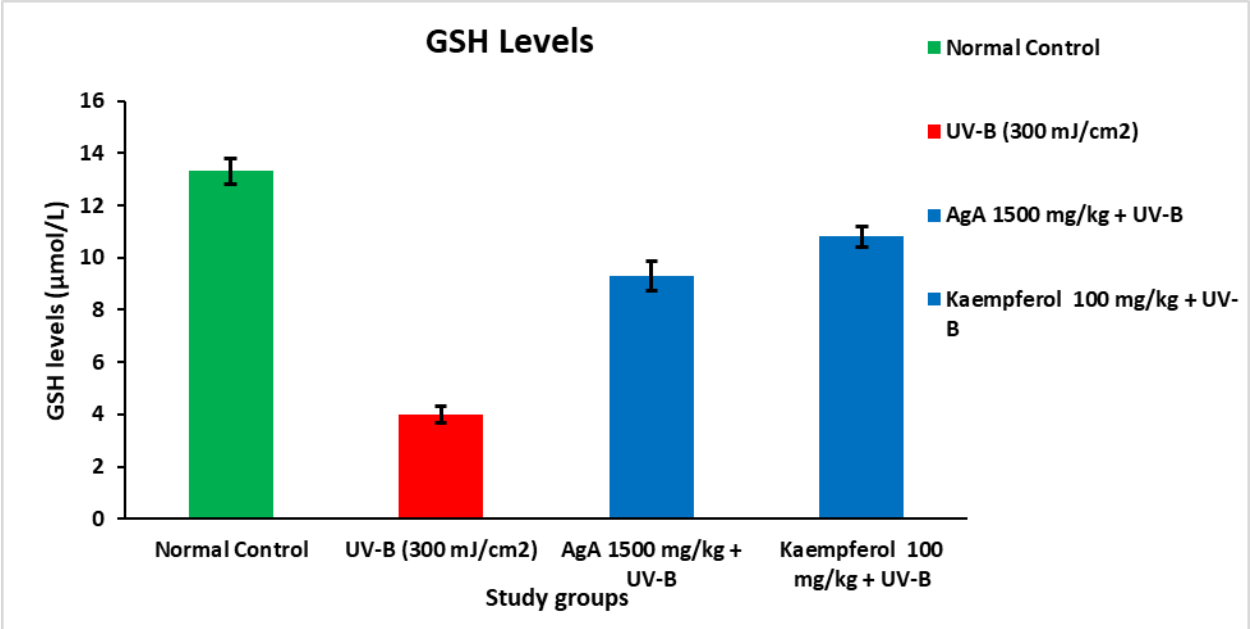


Fig 5: Graphs representing the SOD levels in control and test samples treated animals.

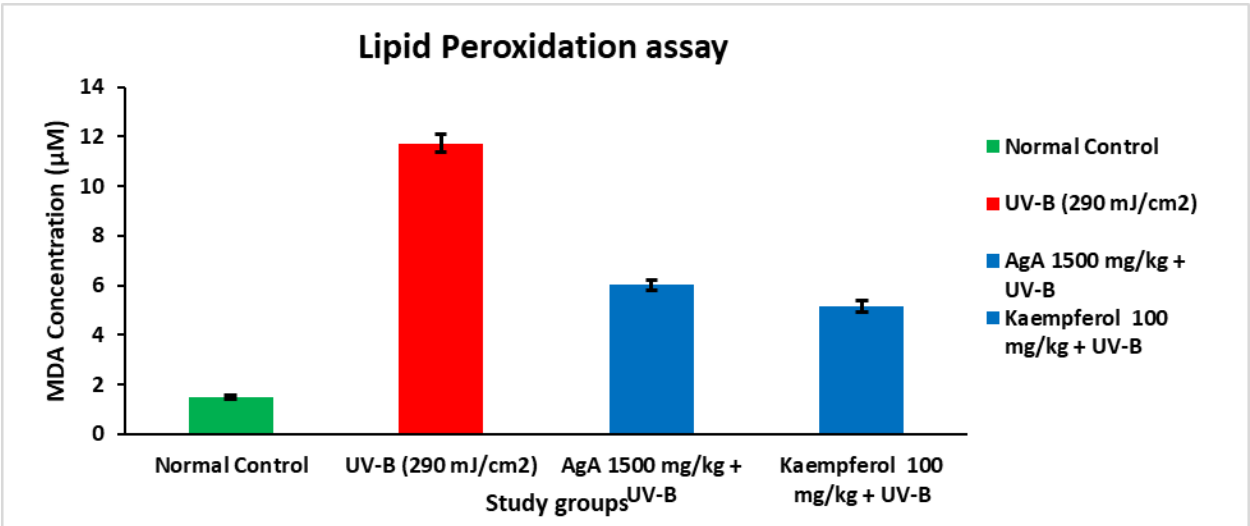


Fig 7: Graphs representing the MDA levels in control and test samples treated animals.

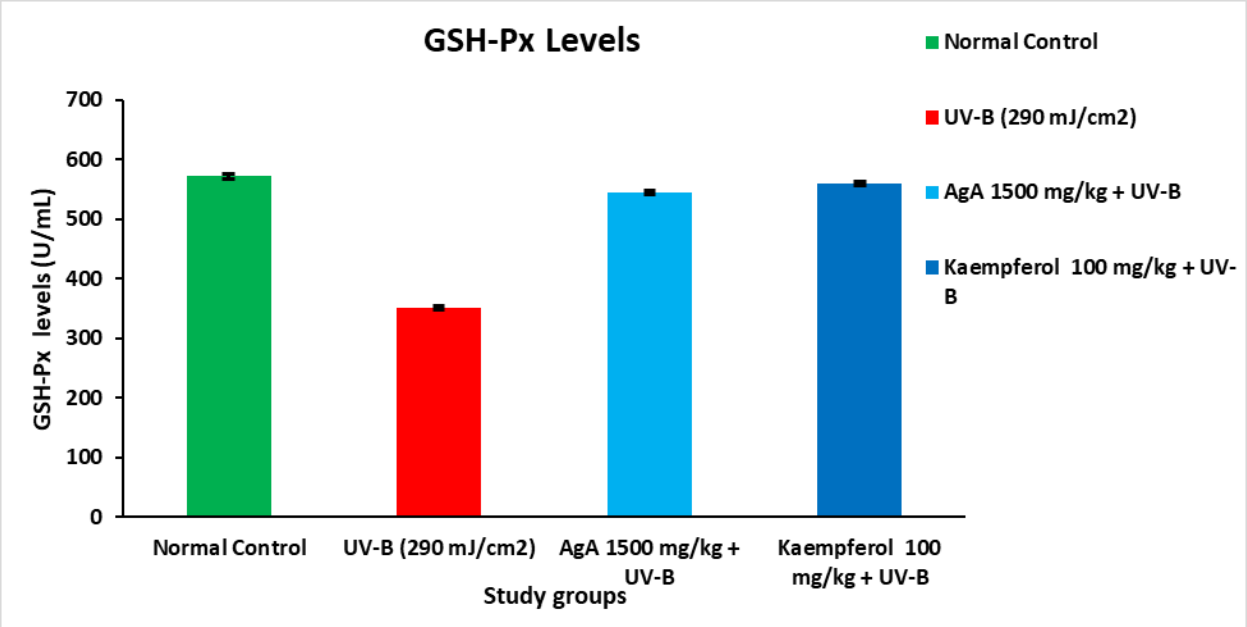


Fig 8: Graphs representing the GSH-Px levels in control and test samples treated animals.

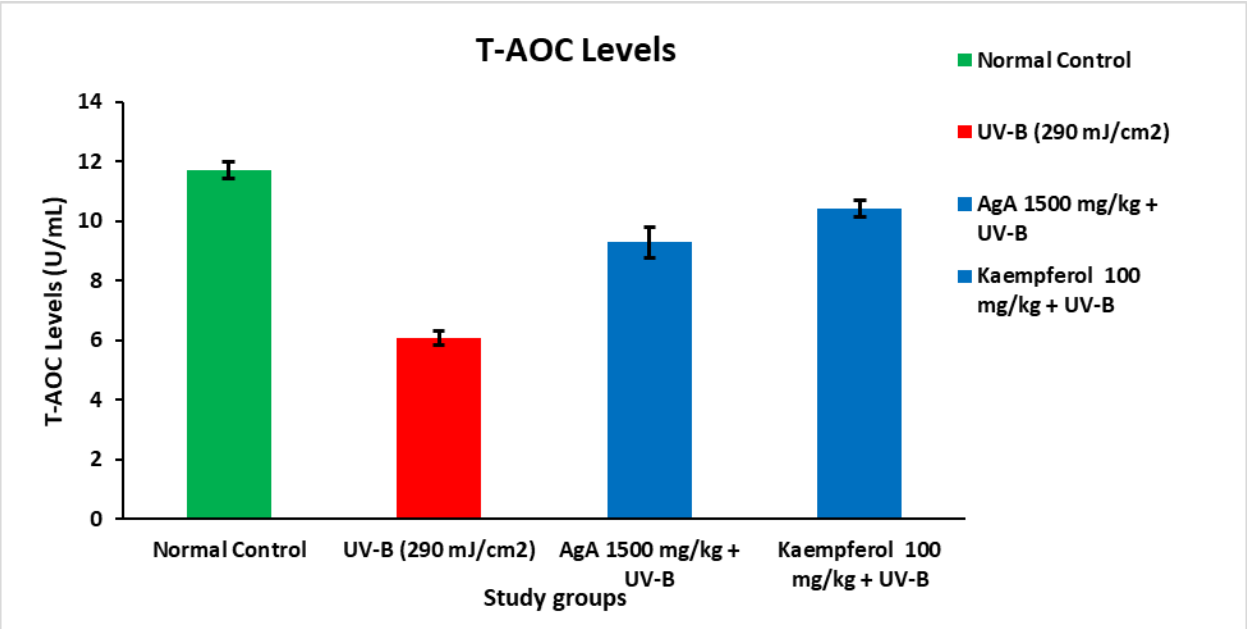


Fig 9: Graphs representing the T-AOC levels in control and test samples treated animals.

(ii) Collagen Remodeling and Dermal Matrix Restoration

Hydroxyproline and elastin concentrations were utilized as surrogate markers for dermal protein integrity. The data revealed increases of about 65% in hydroxyproline content (AgA) and changes of 52% (AgA) in elastin. Histopathologic results supported this, as treated skin had epidermal thickness that was approaching that of healthy controls, along with reorganization of collagen fiber alignment within the dermis and a significant reduction in elastin fiber fragmentation when compared to UV-aged injury. All these observations suggest improved fibroblast activity and active repair of the extracellular matrix.

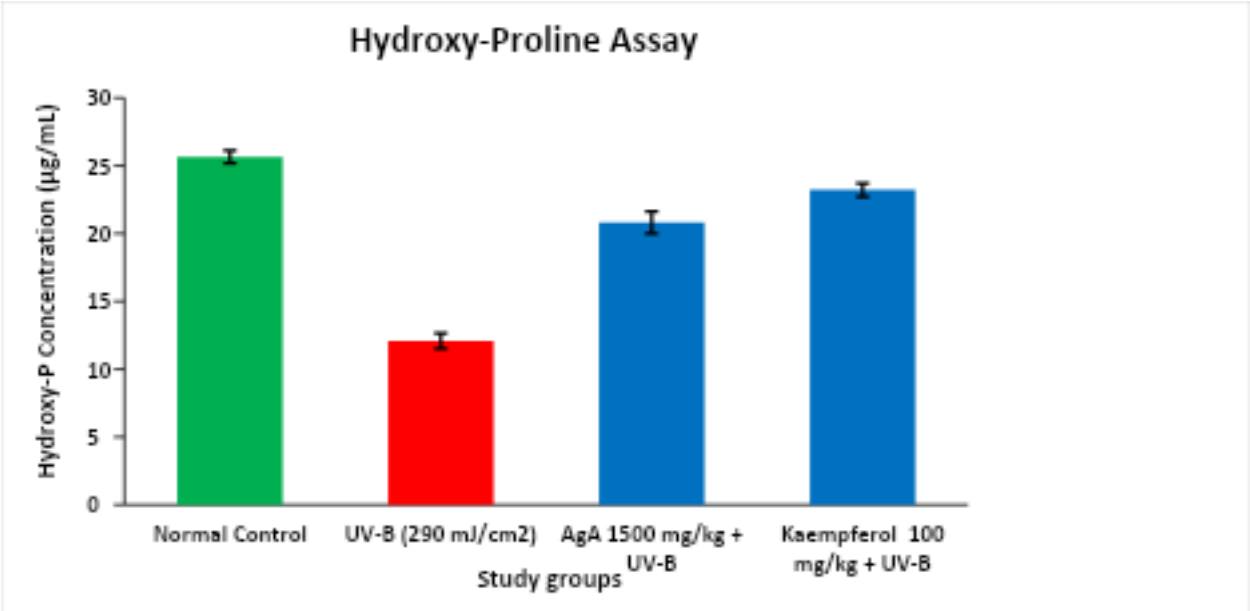


Fig 10: Graphs representing the Hydroxyproline levels in control and test samples of treated animals

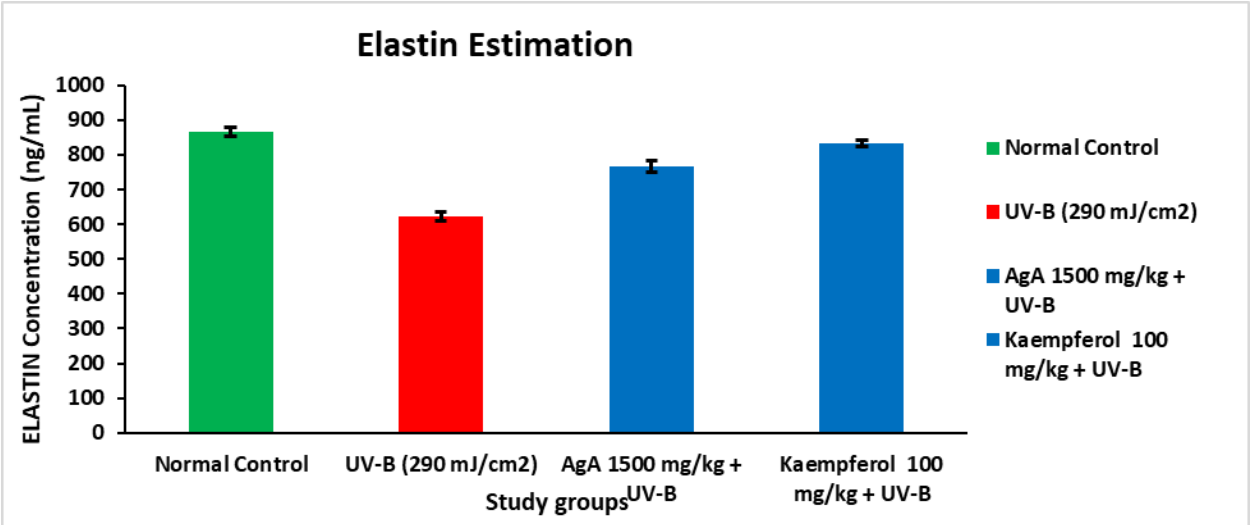


Fig 11: Graphs representing the Elastin levels in control and test samples treated animals.

(iii) Telomere and Telomerase Assessment

Animals that received the formulation displayed a longer relative telomere length and had more telomerase activity compared to UV exposed control animals. Both the maintenance of telomere integrity and the greater TRAP-based telomerase activity suggest some abrogation of cellular aging processes at the genomic level. This molecular protection coincides with improvements in biomarkers indicative of oxidative stress, along with repaired dermal architecture, as indicated by histopathology results.

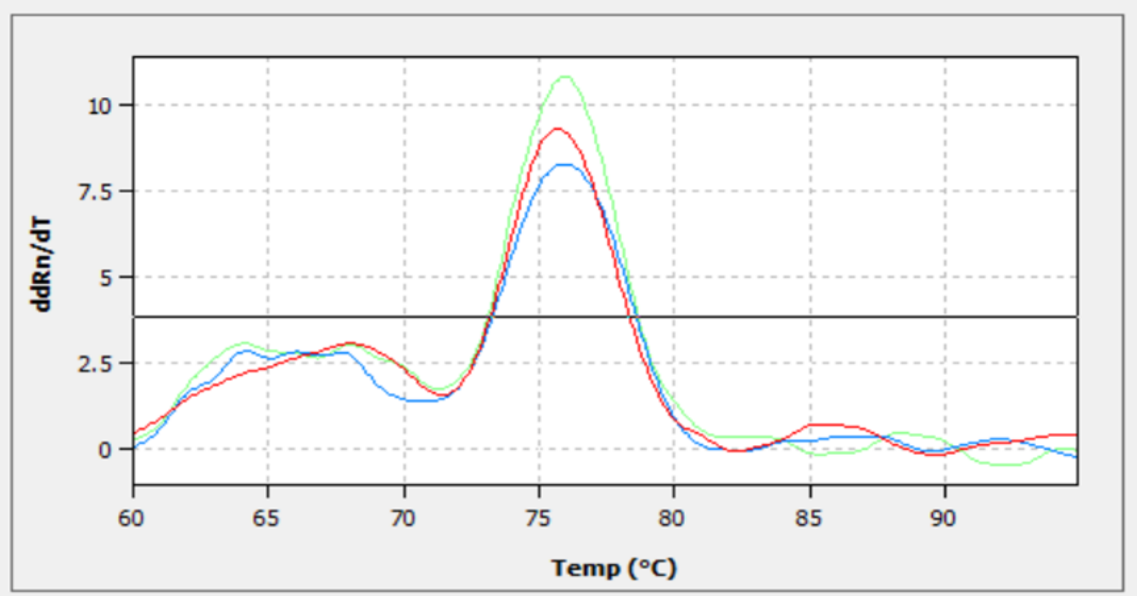


Fig 12: Graphs representing the melting curves of Untreated (Green), UVB alone (Blue), KMF (40Um) + UVB (Red) treated HaCaT cells.

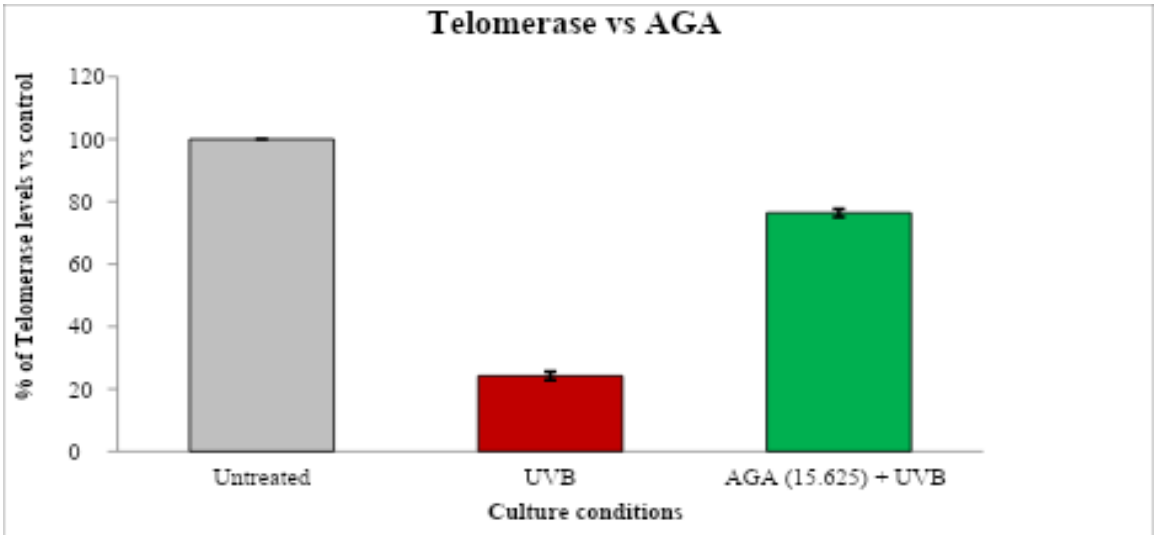


Fig 13: Graphs representing the Telomerase levels in Untreated, UVB alone, AGA (15.625 ug/mL) + UVB treated HaCaT cells. The results are the mean values of two independent experiments.

(iv) Matrix Metalloproteinases (MMP-1 and MMP-2) Regulation

Both MMP-1 and MMP-2, enzymes important for collagen degradation during photoaging, were significantly reduced because of treatment. MMP-1 decreased by about 31% relative to the AgA group, while MMP-2 decreased by about 28% in the AgA group. This decrease would further promote the structural preservation of collagen and elastin fibers.

(v) Cytoprotective and Anti-Inflammatory Effects

Cytokine profiling showed a decreased inflammatory state with TNF-α (approximately 40–50%) and IL-1β levels (approximately 45%) were downregulated, both compared to UV aged animals that received no treatment. Levels of C-reactive protein (CRP) followed a similar trend in declining, indicating reduction of chronic low-grade inflammation. Furthermore, cytoprotection in the dermal regions, reflected less keratinocyte apoptosis and better structuring of the epidermal barrier.

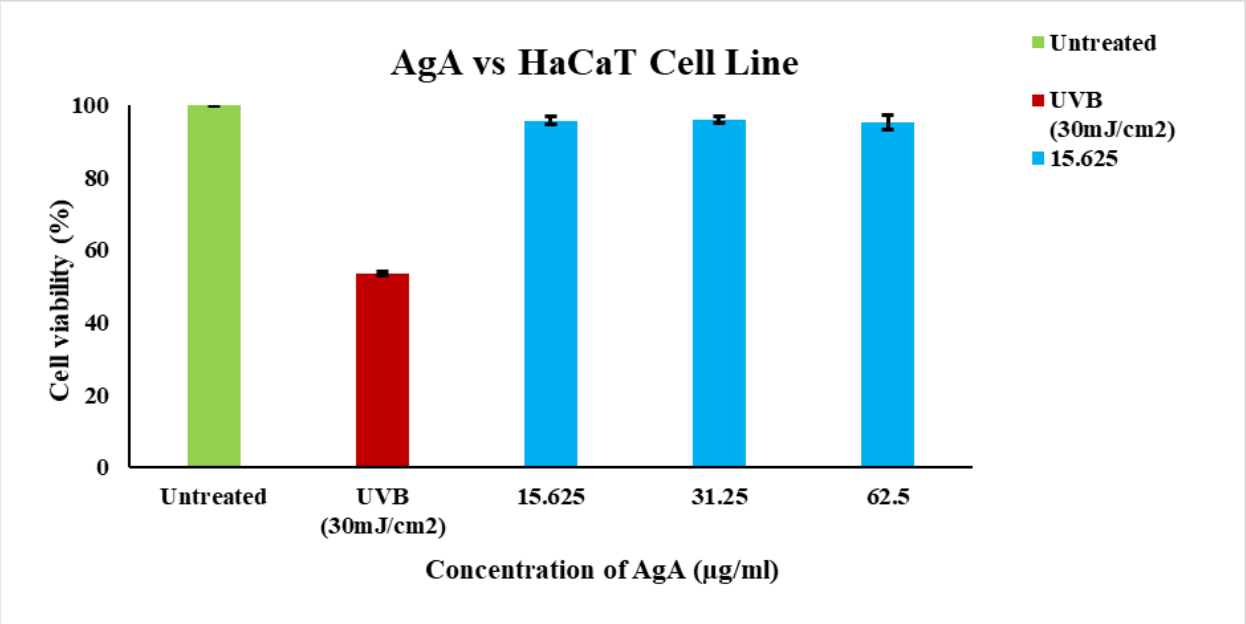


Fig 13: % cell viability of HaCat cells. The cells were exposed to the desired conc of the test agent for 24 hrs and then subjected to 30mj/cm2 of UV-B irradiation.

(vi) Skin Appearance and Structural Restoration

The overall macroscopic assessment revealed improvement of wrinkling depth, skin smoothness, hydration and elasticity, and decreased erythema and roughness overall skin appearance. These macroscopic

improvements agree with all of the biochemical and histological markers and represent pronounced structural recovery and restoration of the skin's antioxidant defense.

(vii) Conclusion

The *in vivo* findings taken together indicate that the formulations assessed demonstrate multi-prong anti-aging effects, including:

1. Restoration of antioxidant defense
2. Reduction of oxidative damage to lipids and proteins
3. Deposition of new dermal matrix proteins
4. Reduction in collagen degrading enzymes (MMP-1 and MMP-2)
5. Inhibition of pro-inflammatory cytokine expression
6. Improvement in clinical characteristics of skin aging

AgA had positive and sustained restorative effects. Overall, there is supporting evidence for the cytoprotective, antioxidant and anti-aging effects of skin formulations.

3.4 Correlation Analysis

To integrate biochemical, chromatographic, and biological endpoints, multivariate correlation and regression analyses were conducted.

(i) Relationship Between Phenolic Content and Antioxidant Capacity

Regression analysis that explored the relationship between total phenolic content (TPC) and antioxidant capacity as determined by DPPH and ABTS assays showed a strong positive correlation (r = 0.93).The regression equation obtained from the analysis (Y= 1.12X + 5.34, Y denotes antioxidant potential, and X denotes TPC in mg GAE/g) points to a linear

contribution of phenolic compounds as a component of total antioxidant capacity. Positively related flavonoid content (r = 0.88) and ferric reducing antioxidant power (FRAP) (r = 0.91) also supports a mechanistic role of polyphenolic structures in redox homeostasis.

(ii) Cluster Analysis and Principal Component Analysis (PCA)

To illustrate the relationships between phytochemical and biological parameters, a PCA and hierarchical clustering approach were used to evaluate the resulting data. Two principal components (PC) alone explained >79 % of total variance. Phenolic structures and DPPH were related to the first PC (PC1) while the second PC (PC2) was strongly associated with the enzymatic antioxidants connected with superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and collagen levels. The biplot demonstrates a distinct separation between the untreated and treated group, indicating a distinct biochemical profile due to the application of the test formulation.

Additional correlation clustering stabilized HRLC-MS identified metabolites: quercetin, rutin, withanolides, and triterpenoid saponins, all associated with biomarker activity signifying antioxidant and anti-aging effects. The observed clustering implies that a cooperative network of pharmacodynamic activity is at play rather than the effects of a single phytochemical.

(iii) Integrated Analysis

The combined chromatographic and biological data provides a complete data story regarding the detailed metabolites associated with the therapeutic outcomes. Radical scavenging activity is primarily due to phenolic-rich fractions; stabilization of collagen and capillary structures is attributed to flavonoids; and alkaloids and terpenoids have a role in limiting inflammatory and metabolic effects. Overall, the multivariate models affirm the anti-aging efficacy as a physiological response from the interdependent activity of multiple phytochemicals rather than a single component.

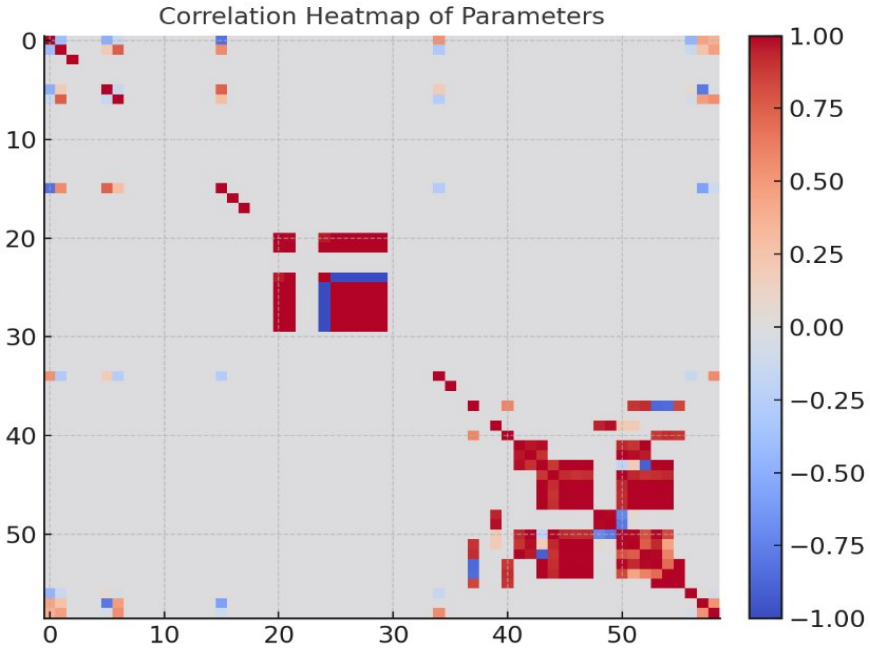


Fig: 14 Correlation heatmap showing interdependence among key measured parameters

4. Discussion

4.1 Phytochemical Extraction Findings

The results obtained from HRLCMS established the presence of notable bioactive metabolites, including ferulic acid, rutin, and scopoletin that have been adequately documented in the current literature for their antioxidant and anti-aging functions. Ferulic acid, measured at m/z 195.06, has been associated with mitigating oxidative stress, stabilizing free radicals, and additionally helping cellular resilience through an insulin/IGF-1 signaling pathway (Li et al., 2021). Additionally, the flavonoid glycosides and quercetin exhibit vascular protective properties and preserve the integrity of collagen by inhibiting glycation and decreasing lipid peroxidation. Scopoletin from *Morinda* citrifolia is reported to inhibit NF-κB and prostaglandin inflammatory cytokines and therefore underpins its ethno-pharmaceutical significance in traditional Polynesian and Asian medicine. Finally, the results from HRLCMS provided a basis for triangulating tradition and knowledge with metabolomic elements by identifying functional antioxidant and anti-inflammatory phytochemicals that coincide with the previously described biological functions.

4.2 Mechanistic Insights

The antioxidant function of the formulation is likely exerted via several convergent biochemical pathways. The polyphenolic components exert their effects by neutralizing reactive oxygen species (ROS) through hydrogen donation and electron transfer mechanisms.

Polyphenolic antioxidants restore cellular redox set-points and incite oxidative damage to DNA and protein. Flavonoids simultaneously mediate cytokine networks by downregulating pro-inflammatory cytokines such as IL-6 and TNF-α and upregulating the anti-inflammatory cytokine IL-10, which diminishes inflammatory signaling and matrix degradation. In addition, withanolides and triterpenoid saponins induce fibroblast proliferation and suggest an increase in the expression of genes engaged in collagen biosynthesis. These biochemical functions synergize with the increase in hydroxyproline. Overall, pharmacodynamic interactions clearly suggest a pharmacodynamic synergy wherein the phytochemicals exert additive or synergistic effects that exceed the effects observed with each of the individual constituents.

4.3 Comparison with Current Literature

The findings from this study are consonant with other studies on single-plant extracts and multi-herbal mixtures reporting similar anti-aging applications (Somavanshi et al., 2021; Poomanee et al., 2023; Nobile et al., 2023). This study is unique, bringing together metabolite profiling from HRLCMS, in vitro antioxidant testing, and in vivo anti-aging bioassays. This combined correlational data of metabolomics, bioassays, and in vivo data provides a mechanistic explanation that has not been established in previous studies and provides

new perspectives with regards to system-level phytochemical interactions that complement the biological aspect of rejuvenation.

4.4 Limitations and Directions for Future Research

Despite positive results, the exact molecular targets and pathways have not been fully characterized. Future pathways may explore molecular docking analyses and gene transcriptomic/translational pathways to confirm binding interactions and measure expression associated changes in the intervention formulation. Additionally, long-term stability studies, formulation changes, and well-designed clinical trials with control conditions are necessary to translate the results into therapeutic or cosmeceuticals.

5. Conclusion

The polyherbal blend containing Noni showed considerable anti-ageing activity, which was shown by a multi-prong approach using phytochemical profiling, biochemical studies, and histology. High resolution liquid chromatography-mass spectrometry (HRLCMS) revealed substantial variability in bioactive compounds such as ferulic acid, rutin, quercetin, scopoletin, and withanolides in the formulation contributing to the robust antioxidant and anti-inflammatory ability. The polyherbal formulation scavenged reactive oxidative species, stimulated the activity of endogenous antioxidant enzymes, modulated cytokine expression, and promoted collagen synthesis; collectively leading to improvements in dermal morphology and a decrease of cellular senescence in vivo. Collectively these findings point to variable synergy resulting from the combination of *Morinda citrifolia* with an herbal combination of Aloe vera, *Phyllanthus emblica*, and *Withania somnifera*.

This study strengthens the scientific rationale behind combining sophisticated analytical methods such as HRLCMS with ethnopharmacological knowledge to create relatively standardized and evidence-based herbal medicinal products. In addition to its scientific merit, the evidence-based approach allows for the validation of traditional medicine practices with chemical analyses and additionally facilitates the development of safe, sustainable and effective plant-based anti-ageing therapeutic agents with potential for future translational research and clinical applications.

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Competing Interests

The authors declare no competing financial or non-financial interests.

Data Availability

No new data were generated or analyzed in this study. All relevant information is contained within the manuscript.

Ethical Approval

This study did not involve human or animal subjects; therefore, ethical approval was not required.

Consent to Participate

Not applicable, as no human participants were involved.

Consent to Publish

Not applicable, as no identifiable personal data were included.

Informed Consent

Not applicable, as the study did not involve human subjects or personal information.

Author Contributions

All authors contributed equally to the conception and preparation of the manuscript.

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