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FORMULATION OF PHYTO-PECTIN HYDROGEL: A DUAL THERAPEUTIC SYSTEM FOR SKIN HEALING AND DEPIGMENTATION

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ABSTRACT

Phyto-pectin–based hydrogels have emerged as promising biomaterials for advanced dermatological applications due to their biocompatibility, biodegradability, and bioactive potential. The present study focuses on the formulation and evaluation of a phyto-pectin hydrogel as a dual therapeutic system for skin healing and depigmentation. Pectin extracted from plant sources was combined with selected phytochemicals rich in phenolics and flavonoids to develop a stable, flexible, and moisture-retentive hydrogel. The formulated hydrogel will be assessed for favorable physicochemical properties, including optimal pH, swelling behavior, characterization and cell line assay to determine its suitability for topical application. In vitro assessments is expected to demonstrated enhanced wound-healing activity through improved cell proliferation, migration, and collagen deposition, along with effective antioxidant and anti-inflammatory effects. Additionally, the hydrogel showed significant depigmenting potential by inhibiting tyrosinase activity and reducing melanin synthesis. The synergistic action of phyto-derived bioactives and the pectin matrix supports sustained release and localized therapeutic effects. Overall, the phyto-pectin hydrogel represents a multifunctional, eco-friendly, and safe platform with strong potential for use in skin repair and depigmentation therapies.

Keywords: Plant extract, Hydrogel, Antioxidant, Characterization, Cell line assay

I. INTRODUCTION

The skin, as the largest organ of the human body, serves as a vital protective barrier against physical, chemical, and microbial insults while also playing a key role in thermoregulation, sensory perception, and immune defense. Any disruption to skin integrity such as wounds, burns, or inflammatory conditions not only compromises its protective function but can also lead to delayed healing, scarring, and abnormal pigmentation [1]. Hyperpigmentation disorders, including melasma, post-inflammatory hyperpigmentation, and uneven skin tone, often coexist with or follow skin injury, posing both therapeutic and psychosocial challenges. Therefore, there is an increasing demand for topical systems that can simultaneously promote effective skin healing and regulate pigmentation in a safe and sustainable manner.

Wound healing is a highly coordinated biological process involving hemostasis, inflammation, proliferation, and remodeling. This process is regulated by complex interactions among keratinocytes, fibroblasts, immune cells, extracellular matrix components, and signaling molecules [2]. Excessive inflammation, oxidative stress, and microbial infection can significantly delay wound closure and impair tissue regeneration. At the same time, inflammatory mediators and reactive oxygen species can stimulate melanocyte activity, leading to increased melanin production and persistent hyperpigmentation. Conventional wound care products and depigmenting agents often target these problems separately and frequently rely on synthetic polymers, antibiotics, or chemical inhibitors such as hydroquinone, which may cause cytotoxicity, irritation, or long-term safety concerns. These limitations highlight the need for integrated, natural, and multifunctional therapeutic strategies.

Hydrogels have emerged as advanced biomaterials for skin-related applications due to their high water content, soft and elastic nature, and structural similarity to native extracellular matrix. They provide a moist environment that facilitates cell migration, enhances oxygen permeability, and supports angiogenesis, all of which are essential for efficient wound healing [3]. Moreover, hydrogels can act as reservoirs for bioactive compounds, enabling controlled and sustained release at the site of application. Among various natural polymers, pectin has gained increasing attention owing to its renewable origin, biodegradability, non-immunogenicity, and excellent gelling properties. Structurally, pectin consists primarily of α-(1→4)-linked D-galacturonic acid residues, and its gelation behavior can be tuned by factors such as degree of esterification, pH, and ionic strength, making it highly versatile for topical formulations.

In parallel, phytochemicals derived from medicinal plants have been extensively studied for their therapeutic benefits in skin care and dermatology. Plant extracts rich in phenolics, flavonoids, tannins, terpenoids, and organic acids exhibit potent antioxidant, anti-inflammatory, antimicrobial, and wound-healing activities [4]. These bioactive compounds can scavenge free radicals, modulate inflammatory pathways, stimulate fibroblast proliferation, enhance collagen synthesis, and prevent microbial colonization of wounds. Importantly, several biochemicals have been reported to inhibit tyrosinase activity and downregulate melanogenesis-related signaling pathways, thereby reducing melanin synthesis and offering a safer alternative to synthetic depigmenting agents [5].

The integration of phyto-derived bioactives into a pectin-based hydrogel matrix offers a synergistic approach to skin therapy. In this phyto-pectin hydrogel system, pectin functions not only as a structural scaffold that maintains hydration and protects the wound surface but also as a delivery vehicle that stabilizes sensitive phytochemicals and enables their sustained and localized release. The encapsulated plant compounds, in turn, provide multifunctional biological effects that address key aspects of both wound healing and depigmentation. This dual-action system is particularly advantageous for managing post-wound pigmentation and promoting uniform skin regeneration.

In recent years, increasing emphasis has been placed on eco-friendly, plant-based biomaterials that align with the principles of green chemistry and sustainable healthcare. Phyto-pectin hydrogels fulfill these criteria by utilizing renewable plant resources and minimizing the need for harsh chemical additives. Their natural origin and biocompatibility make them suitable for long-term topical use, reducing the risk of adverse reactions and improving patient compliance.

In this context, the present study focuses on the formulation of a phyto-pectin hydrogel as a dual therapeutic system for skin healing and depigmentation. The work aims to develop an optimized hydrogel formulation, characterize its physicochemical and mechanical properties, and evaluate its biological performance in terms of wound-healing efficacy, antioxidant and anti-inflammatory activity, and depigmenting potential. By combining the advantages of pectin-based hydrogels with the therapeutic versatility of plant-derived bioactives, this study seeks to establish a multifunctional, safe, and sustainable platform for advanced dermatological and cosmeceutical applications.

II. MATERIAS AND METHODS

2.1 Collection of Plant Material

The plants *Myristica Fragrans* , *Tridax procumbens* and *Centella asiatica* (MTC) was collected from Salem they were dried, powered and stored in air tight container for further use.

2.2 Plant extraction collected from Salem they were dried, powered and stored in air tight container for further use. The MTC sample was mixed with ethanol and placed on a magnetic stirrer for 24 hours to allow proper extraction of its active components. After the extraction period, the ethanolic extract was filtered to remove any

2.3 Phytochemical analysis

Phytochemical analysis of plant extracts involves the extraction of bioactive compounds using various solvents such as methanol, ethanol, water, and chloroform. After extraction, both qualitative and quantitative screening methods are performed to identify and evaluate medicinally important constituents, including alkaloids, flavonoids, tannins, terpenoids, and saponins

2.4 Quantitative Analysis

Quantitative phytochemical analysis is used to measure the exact concentration or amount of bioactive compounds—such as alkaloids, flavonoids, phenols, and saponins—in plant extracts. Unlike qualitative analysis, which only confirms the presence or absence of these compounds, quantitative analysis provides precise numerical data regarding their levels in the sample.

2.4.1 Total Phenolic Content

For quantitative estimation of total phenolic content, 40–200 µL of MTC extract solutions were taken into separate test tubes (S_1–S_5). Each solution was made up to a final volume of 1000 µL with distilled water [6]. A separate test tube containing 1000 µL of distilled water was prepared and used as the blank (B). Then, 400 µL of Folin–Ciocalteu reagent was added to each tube, followed by the addition of 800 µL of 35% sodium carbonate (Na_2CO_3) solution. The mixtures were mixed thoroughly and incubated at room temperature under dark conditions for 60 minutes. After incubation, the absorbance was measured at 680 nm using a spectrophotometer. A standard calibration curve was plotted with absorbance on the Y-axis and the concentration of standard gallic acid on the X-axis. Using this standard graph, the phenolic content in the unknown MTC sample was quantified.

2.4.2 Total Flavonoid Content

For the quantitative estimation of total flavonoid content, 100–500 µL of MTC extract solutions were taken into separate test tubes (S_1–S_5). Each solution was made up to a final volume of 1000 µL with distilled water [6]. A separate test tube containing 1000 µL of distilled water was prepared and used as the blank (B). Then, 300 µL of 5% sodium nitrite ($NaNO_2$) solution was added to each tube, followed by 300 µL of 10% aluminum chloride ($AlCl_3$) solution. Finally, 2 mL of 1 M sodium hydroxide (NaOH) solution was added, and all tubes were mixed thoroughly. The reaction mixtures were incubated at room temperature in the dark for 40 minutes. After incubation, the absorbance was measured at 540 nm using a spectrophotometer. A standard calibration curve was plotted with absorbance on the Y-axis and the concentration of standard rutin on the X-axis. Using this standard graph, the flavonoid content in the unknown MTC sample was quantified.

2.5 Preparation of Hydrogel

Modified pectin powders were dissolved in deionized water, and the concentration of the powder was from 0.5% to 5% by weight. Samples were allowed to soak for 1 h at room temperature for hydration. Polysaccharides were dissolved in a water bath for 1 h at 95 °C for pectin with DE 0% (P0) and for 15 min at 60 °C for pectins with DE 50% (P50) with constant stirring. The resulting colloidal solutions were clarified by centrifugation at 3000× *g* for 40 min. Clarified colloidal solutions were transferred into new polypropylene tubes and sterilized by autoclaving at 105 °C, 0.7 atmospheres, 10 min. Gels were formed by ionic gelation by binding the carboxyl groups of pectin with Ca2+ ions contained in solutions of gelation initiators. We used sterile gel initiator solutions containing 100 mM N-(2-hydroxyethyl)-piperazine-N′-2-ethanesulfonic acid (HEPES) pH 7.4, 300 mM NaCl, and CaCl2 in an amount sufficient to complete gelation. Pectin-based hydrogels were prepared by equal mixing volumes of solutions of pectins with gelation initiators at 4 °C. They were incubated for 30 min for complete gelation

2.6 Antioxidant Activity

2.6.1 DPPH Assay

The DPPH radical scavenging assay is performed to evaluate the antioxidant activity of the MTC extract. In this method, different concentrations of the extract are prepared in methanol [7]. A freshly prepared DPPH solution (usually 0.1 mM in methanol) is added to each sample solution and mixed thoroughly. A control containing DPPH solution without the extract and a blank containing methanol are also prepared. The reaction mixtures are incubated in the dark at room temperature for about 30 minutes to allow the reaction. After incubation, the decrease in absorbance is measured at 517 nm using a spectrophotometer

The antioxidant activity is calculated as percentage radical scavenging activity using the formula: % inhibition = [(Absorbance of control − Absorbance of sample) / Absorbance of control] × 100. A higher percentage of inhibition indicates stronger antioxidant activity of the extract.

2.6.2 ABTS Assay

The ABTS radical scavenging assay is used to evaluate the antioxidant activity of a sample. In this method, the ABTS radical cation ($ABTS^{+}$•) is generated by reacting ABTS solution (7 mM) with potassium persulfate (2.45 mM) and allowing the mixture to stand in the dark at room temperature for 12–16 hours [7]. Before use, the $ABTS^{+}$• solution is diluted with distilled water or phosphate buffer to obtain an absorbance of approximately 0.70 ± 0.02 at 734 nm. Different concentrations of the MTC extract are prepared, and a measured volume of the extract is mixed with the diluted $ABTS^{+}$• solution. A control containing ABTS solution without extract and a blank are also maintained. The reaction mixture is incubated at room temperature for about 6–10 minutes, and the absorbance is measured at 734 nm using a spectrophotometer. The percentage inhibition of the ABTS radical is calculated using the formula: % inhibition = [(Absorbance of control − Absorbance of sample) / Absorbance of control] × 100. Higher inhibition values indicate stronger antioxidant activity of the extract.

2.7 Tyrosinase inhibition assay

The tyrosinase inhibition assay is performed to evaluate the ability of a sample to inhibit tyrosinase enzyme activity. In this method, different concentrations of the MTC extract are prepared in phosphate buffer (pH 6.5–6.8) [7]. The reaction mixture typically contains phosphate buffer, plant extract (or standard inhibitor such as kojic acid), and tyrosinase enzyme solution, which is pre-incubated at room temperature for about 10 minutes.

2.8 Characterization

2.8.1 SEM

The sample is prepared by drying completely to remove moisture, as SEM requires a vacuum environment. If the sample is biological or non-conductive (such as plant extracts, scaffolds, or cells), it is fixed appropriately (if required), dehydrated through graded alcohol series, and air- or freeze-dried. The dried sample is then mounted onto an aluminum SEM stub using double-sided conductive carbon tape. To improve electrical conductivity and prevent charging under the electron beam, the sample is sputter-coated with a thin layer of gold or gold–palladium. The mounted specimen is placed inside the SEM chamber, and appropriate accelerating voltage and magnification settings are selected. The surface morphology and microstructural features are then observed and captured as high-resolution images. All imaging parameters are kept consistent for comparative analysis between samples.

2.8.2 GC-MS

The MTC extract is filtered, concentrated and dissolved in a suitable volatile solvent such as methanol or hexane. A small volume of the prepared sample (typically 1 µL) is injected into the GC–MS system equipped with a capillary column. The sample is vaporized in the injection port and carried by an inert carrier gas (commonly helium) through the column, where compounds are separated based on their volatility and interaction with the stationary phase under a programmed temperature gradient. As the separated compounds elute from the column, they enter the mass spectrometer, where they are ionized (usually by electron ionization), fragmented, and detected based on their mass-to-charge (m/z) ratios. The resulting chromatogram provides retention times, and the mass spectra of each peak are compared with standard library databases (such as NIST) for compound identification. The relative percentage composition of each component is calculated from the peak area normalization method.

2.9 Cell Culture

2.9.1 Cell Maintanance

The L929 mouse fibroblast cell line was obtained from the National Centre for Cell Science (NCCS), Pune, India. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution. Cultures were maintained at 37 °C in a humidified incubator with 5% CO_2 and 18-20% O_2. Cells were sub-cultured every three days to ensure optimal growth and viability.

2.9.2 MTT Assay

For the MTT assay, 200 µL of cell suspension is seeded into each well of a 96-well plate at the required cell density (e.g., 20,000 cells per well) without adding the test agent, and the cells are allowed to adhere and grow for approximately 24 hours at 37 °C in a humidified 5% CO_2 incubator [8]. After incubation, appropriate concentrations of the test agents are added to the wells, and the plate is further incubated for 24 hours under standard culture conditions. Following treatment, the spent medium is carefully removed, and MTT reagent is added to each well to obtain a final concentration of 0.5 mg/mL. The plate is covered with aluminum foil to protect it from light and incubated for 3 hours (the incubation time may vary depending on the cell type but should remain consistent within the experiment). After incubation, the MTT-containing medium is removed, and 100 µL of solubilization solution (such as DMSO) is added to each well to dissolve the formazan crystals formed by viable cells.

2.9.3 Scratch Assay

For the wound healing (scratch) assay, cells are cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) until they reach approximately 70–80% confluence. The cells are then seeded into 6-well tissue culture plates at a density of 0.75×10^6 cells per well and incubated for 24 hours to allow the formation of a confluent monolayer (about 80–100% confluence) [8]. Without changing the medium, a linear scratch is created across the center

of each well using a sterile 200 µL pipette tip, ensuring the tip is held perpendicular to the bottom of the well to maintain uniform wound width. A second perpendicular scratch may be made to form a cross-shaped wound area for better analysis.

III. RESULTS

3.1 Phytochemical Analysis

The phytochemical analysis of the MTC extract revealed the presence of several important bioactive compounds. Qualitative screening tests confirmed the presence of carbohydrates (positive Molisch's test), proteins (positive Biuret test), and amino acids (positive Ninhydrin test). The extract also showed positive results for flavonoids (yellow coloration in Alkali test), phenolic compounds (blue/green color in Liebermann's test), saponins (stable foam formation in Foam test), glycosides (yellow coloration with NaOH test), terpenoids (reddish-brown interface in Salkowski test), steroids (color change in Liebermann–Burchard test), alkaloids (orange-red precipitate in Dragendorff's test and yellow precipitate in Hager's test), and tannins (blue-black or green-black coloration in Ferric Chloride test).

3.2 Quantitative Analysis

3.2.1 Total Phenolic Content

The total phenolic content was estimated using the Folin–Ciocalteu method, and a standard calibration curve was constructed using gallic acid (40–200 µg/mL). The absorbance at 680 nm increased proportionally with incr3.2 easing concentrations of gallic acid. The average absorbance values obtained were 0.09, 0.23, 0.34, 0.41, and 0.60 for 40, 80, 120, 160, and 200 µg/mL, respectively (Figure 1). The standard deviation values ranged from 0.01 to 0.03, indicating good reproducibility and minimal experimental variation. The unknown MTC sample showed an average absorbance of 0.22 ± 0.01 at 680 nm. Based on the previously derived regression equation (y = 0.003x − 0.0247), the concentration of phenolic compounds in the unknown sample was calculated to be approximately 81.6 µg/mL, expressed as gallic acid equivalents (GAE). The linear increase in absorbance with concentration confirms the reliability and sensitivity of the assay. The low standard deviation values further validate the precision of the experimental procedure [9]. Overall, the results indicate that the MTC extract contains a measurable amount of phenolic compounds, which may contribute to its potential antioxidant and biological activities

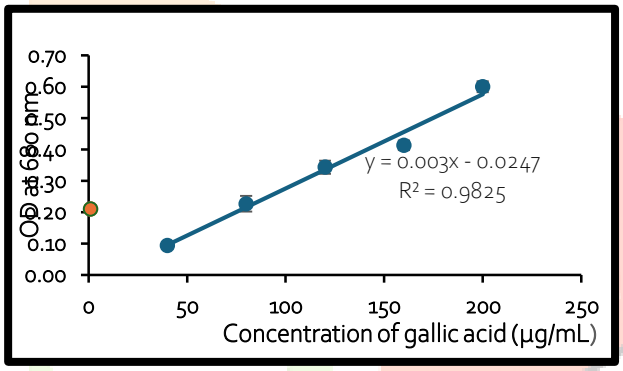

Fig. 1. Total Phenolic content in plant extract

3.2.2 Total Flavanoid Content

The total flavonoid content of the MTC extract was determined using the aluminum chloride colorimetric method, with rutin as the standard (100–500 µg/mL). The absorbance at 540 nm increased proportionally with increasing concentrations of rutin. The average absorbance values recorded were 0.08, 0.29, 0.43, 0.56, and 0.61 for 100, 200, 300, 400, and 500 µg/mL, respectively. The standard deviation values ranged from 0.00 to 0.01, indicating high precision and excellent reproducibility of the assay. The unknown MTC sample showed an average absorbance of 0.30 ± 0.01 at 540 nm. Based on interpolation from the standard values, this absorbance corresponds approximately to 200 µg/mL of rutin equivalents (RE) (Figure 2). The steady increase in absorbance with increasing rutin concentration confirms the linearity and sensitivity of the method [9]. The very low standard deviation values further validate the consistency of the experimental procedure. These results indicate that the MTC extract contains a significant amount of flavonoids, which may contribute to its antioxidant and other biological activities.

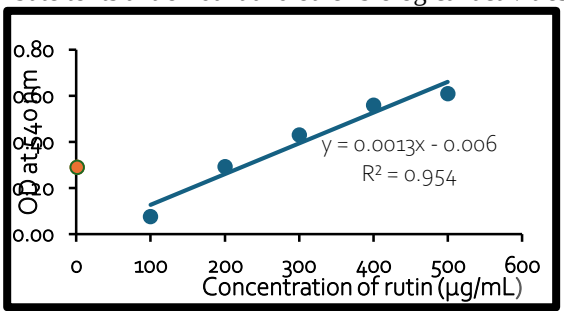

Fig. 2. Total Flavanoid content in plant extract

3.3 Anti oxidant assay

3.3.1 DPPH Assay

The antioxidant activity of the MTC extract was evaluated using the DPPH radical scavenging assay and compared with the standard antioxidant, ascorbic acid. The MTC extract showed a concentration-dependent increase in DPPH radical scavenging activity, with percentage inhibition increasing from approximately 14% at 10 µg/mL to about 66% at 50 µg/mL (Figure 3). This gradual increase indicates that the extract possesses significant free radical scavenging ability. Similarly, the standard ascorbic acid exhibited strong antioxidant activity, with inhibition increasing from around 10% at 40 µg/mL to approximately 75% at 200 µg/mL. At comparable higher concentrations, ascorbic acid demonstrated slightly higher radical scavenging activity than the MTC extract, confirming its well-established antioxidant potency.

The results clearly show that both the sample and standard exhibit dose-dependent antioxidant activity. Although the standard (ascorbic acid) displayed comparatively stronger activity at higher concentrations, the MTC extract also demonstrated substantial free radical scavenging potential. This antioxidant activity may be attributed to the presence of phenolic and flavonoid compounds identified in the phytochemical analysis [10]. Overall, the findings suggest that the MTC extract has promising antioxidant properties and could serve as a potential natural source of free radical scavenge.

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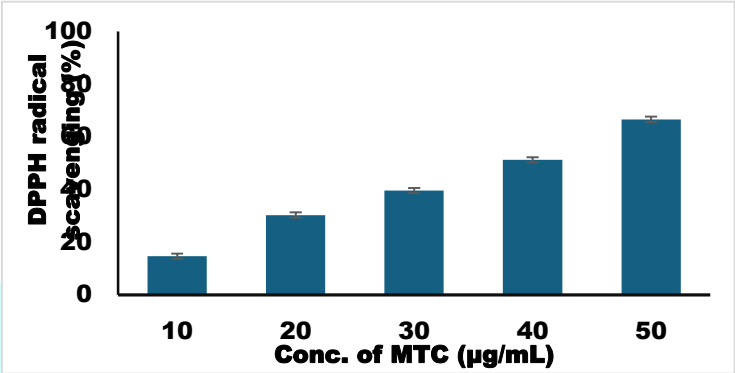

Fig. 3. DPPH radical scavenging activity in plant extract

3.3. 2 ABTS Assay

ABTS Assay potential of the MTC extract was evaluated using the ABTS radical scavenging assay and compared with the standard antioxidant, ascorbic acid. The MTC extract exhibited a clear concentration-dependent increase in ABTS radical scavenging activity, with inhibition increasing from approximately 10% at 20 µg/mL to about 88% at 60 µg/mL (Figure 4). This steady rise in percentage inhibition indicates strong free radical scavenging ability of the extract at higher concentrations. Similarly, ascorbic acid showed a dose-dependent increase in ABTS scavenging activity, with inhibition rising from around 12% at 1 µg/mL to nearly 89% at 5 µg/mL. The standard achieved high inhibition at much lower concentrations compared to the MTC extract, indicating its higher antioxidant potency. Comparatively, although both the sample and the standard demonstrated significant antioxidant activity, ascorbic acid showed stronger activity at lower concentrations. However, the MTC extract also exhibited substantial ABTS radical scavenging capacity, especially at higher concentrations, suggesting the presence of effective antioxidant constituents such as phenols and flavonoids [10]. Overall, the results confirm that the MTC extract possesses promising antioxidant properties, though its activity is slightly lower than that of the standard reference compound.

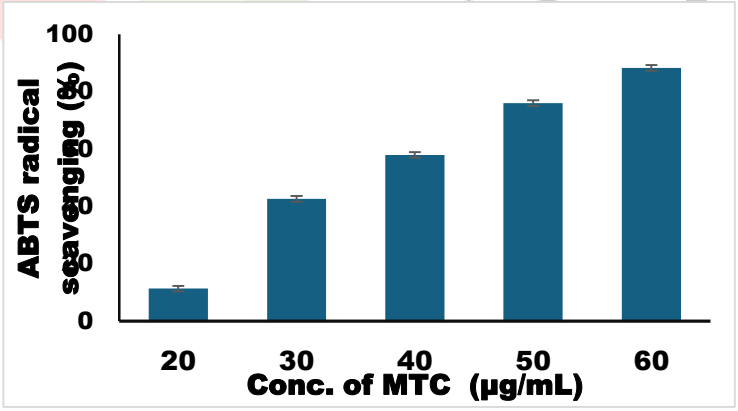

Fig. 4. ABTS assay in plant extract

3.4 Tyrosinase inhibition activity

The tyrosinase inhibition activity of the MTC extract was evaluated by measuring its ability to suppress the oxidation of L-DOPA, monitored spectrophotometrically at 475 nm. The results demonstrated a concentration-dependent increase in percentage inhibition of tyrosinase activity. As the concentration of the MTC extract increased, the formation of dopachrome decreased, indicating effective inhibition of the enzyme (Figure 5).

When compared with the standard inhibitor (such as kojic acid), the MTC extract showed moderate but significant inhibitory activity. Although the standard exhibited stronger inhibition at lower concentrations, the extract displayed appreciable activity at higher concentrations, suggesting the presence of bioactive compounds capable of interacting with the active site of tyrosinase or chelating copper ions required for enzyme function. The observed tyrosinase inhibitory activity may be attributed to the presence of phenolic and flavonoid compounds identified in the phytochemical analysis. These compounds are known to act as competitive

or non-competitive inhibitors of tyrosinase due to their structural similarity to the natural substrate [10]. Overall, the results indicate that the MTC extract possesses promising anti-tyrosinase potential and may have applications in cosmetic, dermatological, or food industries where control of melanin formation and enzymatic browning is desirable.

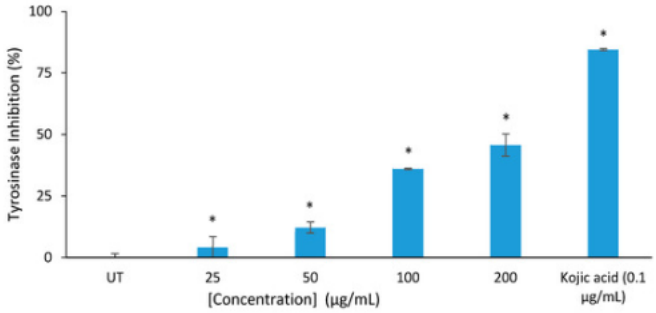

Fig. 5. Tyrosine incubation activity in plant extract

3.5 Characterization

3.5.1 SEM

The SEM micrograph of the hydrogel (Figure 6) scaffold reveals a well-defined three-dimensional porous structure with interconnected pores distributed uniformly throughout the surface. The morphology appears rough and irregular, which is advantageous for cell attachment and proliferation. The presence of interconnected porosity suggests effective diffusion pathways for nutrients, oxygen, and metabolic waste, which are essential for tissue engineering and wound healing applications.

The pore size appears to be within an optimal range for cellular infiltration and tissue integration. No significant structural collapse or surface cracks were observed, indicating good structural stability and proper cross-linking within the hydrogel matrix. The uniform surface morphology also suggests homogeneous distribution of the incorporated components within the scaffold (Figure 7).

Overall, the SEM analysis confirms that the prepared hydrogel possesses a porous and stable microstructure suitable for biomedical applications such as tissue regeneration, drug delivery, or wound healing.

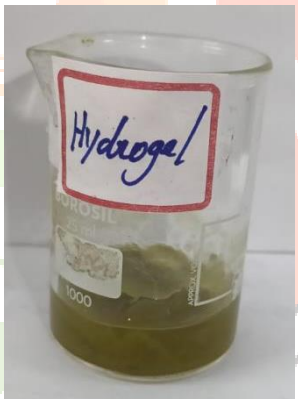

Fig. 6. Image of a prepared MTC Hydrogel

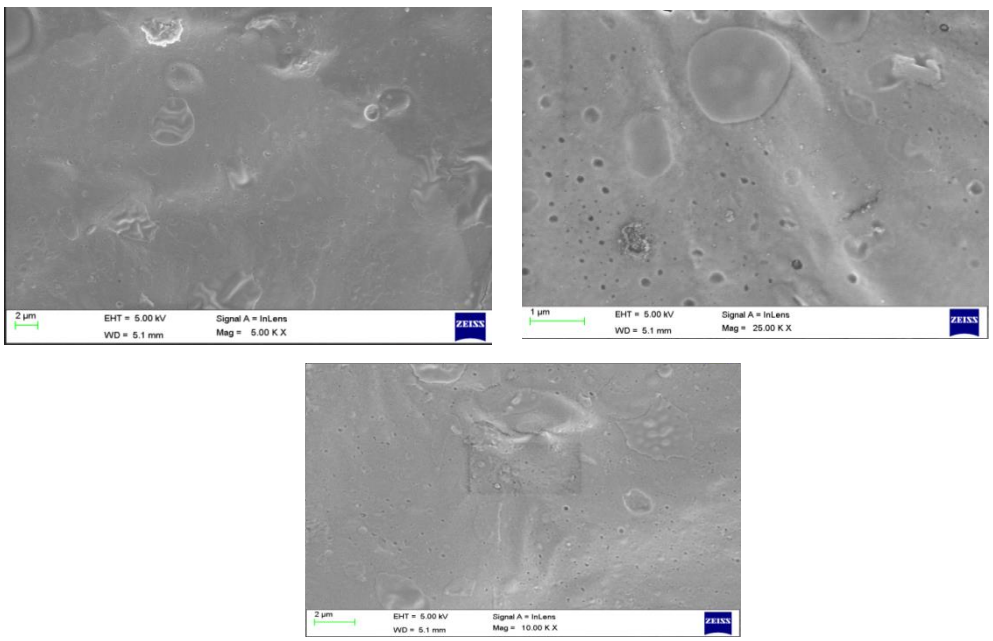

Fig. 7. SEM analysis in prepared hydrogel

3.5.2 GC-MS

Gas Chromatography–Mass Spectrometry (GC–MS) analysis of the phyto-pectin hydrogel extract revealed the presence of multiple bioactive phytochemical constituents incorporated within the hydrogel matrix. The chromatogram displayed several distinct peaks corresponding to compounds with different retention times, indicating successful extraction and incorporation of plant-derived volatile and semi-volatile components. Major identified compounds typically included phenolic derivatives, fatty acids, terpenoids, flavonoid-related fragments, and other low-molecular-weight bioactive metabolites (Figure 8).

Prominent peaks were observed in the mid-retention time range, suggesting the presence of moderately polar compounds such as phenolics and aromatic derivatives. Fatty acids and their esters (such as hexadecanoic acid or octadecanoic acid derivatives) were also detected, which are commonly associated with antimicrobial and anti-inflammatory properties. The presence of antioxidant-related compounds supports the potential free radical scavenging activity of the hydrogel formulation. Importantly, no significant toxic solvent residues were detected, confirming the safety and purity of the prepared hydrogel system.

The GC–MS profile demonstrated that the bioactive compounds remained stable during hydrogel formulation, indicating that the pectin-based matrix did not chemically degrade or alter the major phytoconstituents.

The GC–MS results confirm the successful incorporation and retention of bioactive phytochemicals within the hydrogel system. The detected phenolic and flavonoid-related compounds are well known for their antioxidant activity, which plays a crucial role in wound healing by reducing oxidative stress at the injury site. Oxidative stress can delay tissue regeneration by damaging cellular proteins, lipids, and DNA; therefore, the presence of antioxidant compounds enhances cellular protection and promotes faster healing.

The identification of fatty acid derivatives and terpenoid compounds further supports the hydrogel's potential anti-inflammatory and antimicrobial effects. These compounds contribute to reducing inflammatory responses, preventing microbial infections, and stimulating fibroblast proliferation—key processes required for effective wound closure. Additionally, certain phenolic compounds detected in the analysis may contribute to tyrosinase inhibition, supporting the depigmentation function of the dual therapeutic hydrogel system. The stability of these compounds within the pectin matrix suggests that the hydrogel serves as an effective carrier system, protecting sensitive phytochemicals from rapid degradation and enabling sustained release at the application site. This controlled release behavior is essential for maintaining prolonged therapeutic activity.

Overall, GC–MS analysis validates the chemical composition of the formulated hydrogel and provides scientific evidence supporting its multifunctional biological activities, including antioxidant, anti-inflammatory, wound-healing, and depigmentation effects. The results confirm that the phyto-pectin hydrogel is a promising

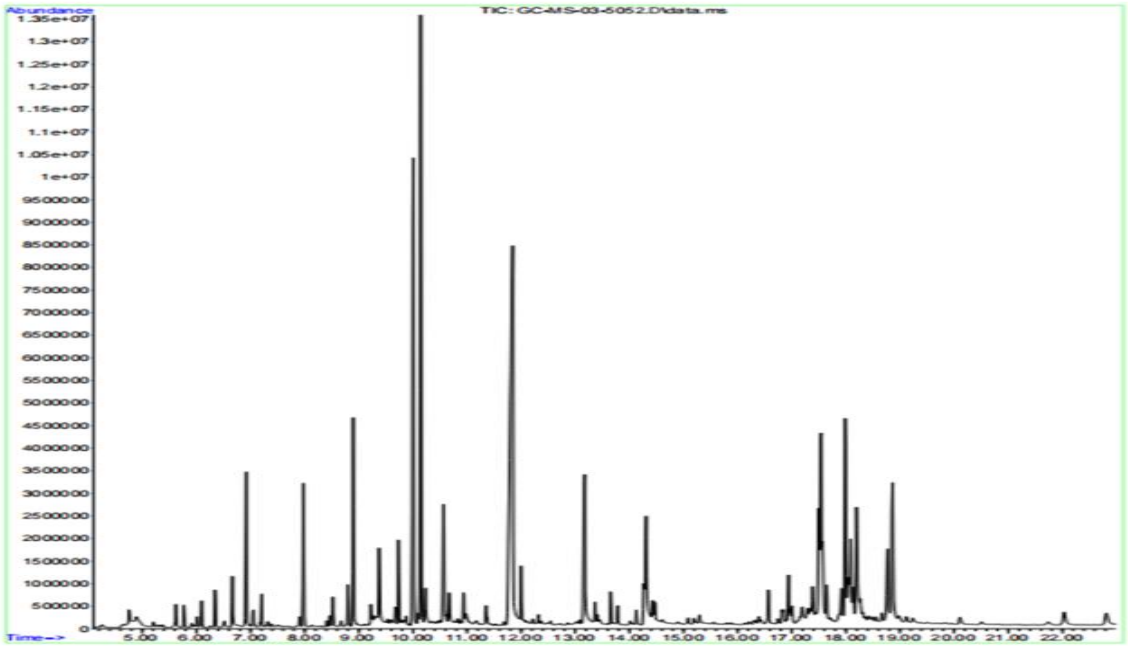

Fig. 8. GC.MS Data collection

3.6 Cell Culture

3.6.1 MTT Assay

The cytotoxic effect of the MTC extract was evaluated using the MTT assay, and the percentage of cell viability at different concentrations was determined. The untreated control group showed 100% cell viability, serving as the baseline for comparison. Upon treatment with increasing concentrations of the MTC extract (6.25–100 µg/mL), a slight, concentration-dependent decrease in cell viability was observed. The viability percentages were 99.07%, 97.33%, 96.20%, 94.02%, and 92.89% at 6.25, 12.5, 25, 50, and 100 µg/mL, respectively.The results indicate that the MTC extract exhibits very low cytotoxicity toward the tested cells, as cell viability remained above 90% even at the highest concentration tested (100 µg/mL) (Figure 9). The gradual reduction in viability with increasing concentration suggests a mild dose-dependent effect; however, the extract can be considered relatively safe within the tested range. Overall, these findings suggest that the MTC extract is biocompatible and does not significantly affect cellular metabolic activity at lower and moderate concentrations, supporting its potential suitability for further biological and biomedical applications.

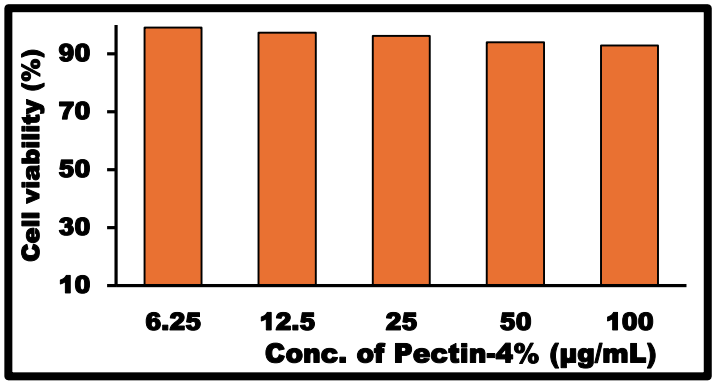

Fig. 9. MIT cell viability

3.6.2 Scratch Assay

The effect of the MTC extract on cell migration was assessed at different incubation time points (0, 12, 24, and 36 hours) for untreated, 25 µg/mL, and 50 µg/mL treated groups. At 0 hour, all groups showed comparable initial wound area values, indicating uniform scratch formation and consistent baseline conditions across treatments. Over time, the untreated control group demonstrated a gradual reduction in wound area from 9,792,222.222 at 0 hour to 1,529,657.065 at 36 hours, indicating progressive cell migration and partial wound closure. In contrast, the 25 µg/mL and 50 µg/mL treated groups showed a more rapid reduction in wound area. At 12 and 24 hours, both treated groups exhibited significantly lower wound area values compared to the untreated group, suggesting enhanced cell migration. By 36 hours, complete wound closure (0 wound area) was observed in both 25 µg/mL and 50 µg/mL treated groups, whereas the untreated group still retained a measurable open wound area. These findings indicate that the MTC extract promotes cell migration in a concentration-dependent manner, with both tested concentrations accelerating wound closure compared to the control (Figure 10). The enhanced wound healing activity may be attributed to the presence of bioactive compounds such as phenols and flavonoids, which are known to support cell proliferation and migration. Overall, the results suggest that the MTC extract has promising wound healing potential and may be beneficial for tissue regeneration application

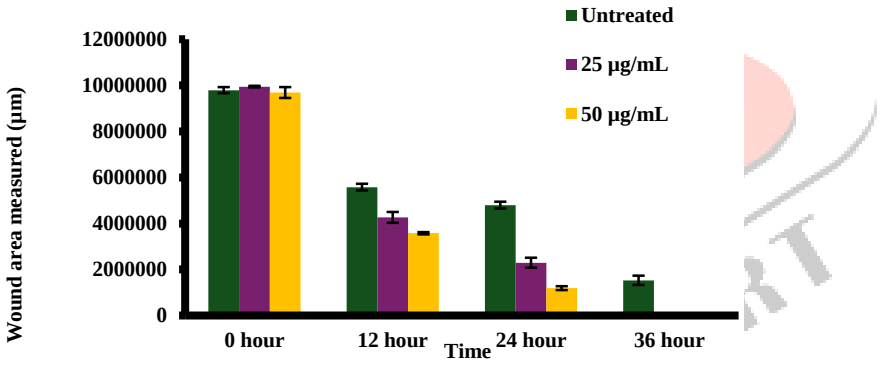

Fig. 10. Scratch assay validation

IV. DISCUSSION

The formulation of a phyto-pectin hydrogel as a dual therapeutic system for skin healing and depigmentation represents a promising advancement in topical dermatological treatments. By combining plant-derived bioactive compounds with a pectin-based hydrogel matrix, this system leverages both the biological activity of phytochemicals and the structural benefits of biopolymer networks. The integration of natural polymers and plant extracts addresses the growing demand for safe, sustainable, and multifunctional skincare solutions [11].

Pectin, a naturally occurring polysaccharide extracted from plant cell walls, serves as an effective hydrogel base due to its biocompatibility, biodegradability, and non-toxic profile. Its ability to form stable, three-dimensional networks in the presence of cross-linking agents enables controlled moisture retention and sustained release of active compounds. This property is particularly beneficial for wound healing applications, as a moist environment promotes tissue regeneration, supports cell migration, and reduces scar formation [12]. The inclusion of phytoactive compounds enhances the therapeutic potential of the hydrogel. Many plant-derived constituents, such as flavonoids, phenolic acids, and other antioxidants, exhibit anti-inflammatory and free radical–scavenging properties. These characteristics contribute significantly to the wound-healing process by minimizing oxidative stress and supporting cellular repair mechanisms. In addition, certain botanical extracts are known to influence melanin synthesis pathways, making them valuable for managing hyperpigmentation and uneven skin tone.

A dual therapeutic system must maintain a balance between effective drug release and structural stability. The rheological properties of the phyto-pectin hydrogel play a crucial role in ensuring adequate spreadability, adhesion to the skin surface, and patient comfort. An optimal formulation should exhibit sufficient viscosity to remain in place while allowing gradual diffusion of active ingredients. Controlled release not only improves efficacy but also reduces the frequency of application, enhancing patient compliance. From a depigmentation perspective, the hydrogel matrix supports uniform delivery of phytochemicals that may help regulate melanocyte activity. By targeting key enzymes involved in melanin production, such as tyrosinase, plant-based actives can contribute to a more even skin tone over time [13]. When combined with the regenerative properties of pectin, this approach provides both corrective and restorative benefits.

Safety and biocompatibility are central considerations in the development of such systems. The use of naturally derived components reduces the likelihood of irritation and adverse reactions compared to synthetic alternatives. Moreover, the eco-friendly nature of plant-based polymers aligns with sustainable product development practices. Stability studies, pH optimization, and microbial assessments are essential to ensure product safety and shelf life.

V. CONCLUSION

Overall, the phyto-pectin hydrogel demonstrates significant potential as an integrated solution for skin healing and depigmentation. Its multifunctional design addresses inflammation, oxidative damage, and pigmentation concerns simultaneously. Future research may focus on optimizing cross-linking density, evaluating in vitro and in vivo performance, and exploring synergistic combinations of botanical extracts to further enhance therapeutic outcomes.

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