RESEARCH PAPER

PVA–Gelatin–Rutin–Glycerol–Borax Hydrogel Composites for Cytotoxicity Evaluation and Potential Wound-Healing Applications

Divya T[1], Nagaraju G[2] and Nandeshwarappa B P[1]*

[1]*Department of Studies in Chemistry, Shivagangothri, Davangere University, Davanagere, Karnataka- 577 007, India.*
[2]*Department of Chemistry, Siddaganga Institute of Technology, Tumakuru 572 103, India.*
**Corresponding author: e-mail: belakatte@gmail.com*

Received: 14th June, 2026; Revised: 20th July 2026; Accepted: 20th August, 2026; Available Online: 05th September 2026

ABSTRACT

Wound healing is a process of formation of new tissue by restoring destroyed or damaged tissue. Unfolding advanced wound healing method has gained significant attention to promote faster and more efficient healing while preventing infection. In the present study, hydrogel polymer composites were drawn up blending polyvinyl alcohol (PVA)/gelatin, Rutin by cross-linking with borax. Thus, prepared polymer composites were characterized for its physiochemical properties, swelling behaviour and biocompatibility. The biological efficacy of the prepared polymer composites is evaluated through antimicrobial activity, cytocompatability and enhanced wound healing under experimental conditions, suggesting the potential of rutin containing PVA –gelatin composite films for wound healing applications

How to cite this article: Divya T, Nagaraju G and Nandeshwarappa BP, PVA–Gelatin–Rutin–Glycerol–Borax Hydrogel Composites for Cytotoxicity Evaluation and Potential Wound-Healing Applications. Int J Drug Deliv Technol. 2026; 16(8): 317-326. DOI: 10.25258/ijddt.16.8.33
Source of support: Nil.
Conflict of interest: None

1. INTRODUCTION:

Wound healing is a complex sequence of biological process which involves haemostasis, inflammation, proliferation and tissue remodelling. Restoration not only require cellular responses but it also need extracellular environment to support tissue regeneration. However, delayed wound healing is a common clinical challenge, particularly in chronic wounds, diabetic and infections. Traditional wound dressing often fails to safeguard, retention of moisture or bioactivity and there is a need for regenerative material that can assist and speed up the healing process. Effective wound care demands materials that can provide a moist environment, prevent microbial infections, promote speedy cell proliferation and support tissue regeneration. In the recent days polymer composites have emerged as viable alternative for wound healing application due to their tailorable physiochemical and biological properties.[1]

Polyvinyl Alcohol has been widely used in biomedical applications in view of its excellent film-forming ability, biocompatibility, non-toxicity and hydrophilicity[2] but they lag in biodegradation. It is hence combined with natural polymers and functional additives. Gelatin is a natural polymer obtained from collagen and is known for its biodegrability and potential to enhance cell adhesion and proliferation[3]. The inclusion of gelatin into the PVA matrix improves the biological properties of the composite, making it more suitable for wound healing application by aiding cellular interactions and tissue regeneration. Rutin is a naturally occurring bioactive flavonoid, it exhibits strong antioxidant, anti-inflammatory and antimicrobial activity[4]. Its integration to the polymer matrix aids in minimising oxidative stress at wound site, inhibiting microbial infection and facilitates improved wound healing. Glycerol is used as plasticizer in PVA-gelatin-rutin polymer system. It improve the elasticity and handling characteristics of the composite film reducing intermolecular forces within the polymer network, thereby increasing elasticity and preventing brittleness, which is essential for comfortable application on wound surface.[5]

In a system based on PVA-gelatin-rutin polymer composite, borax facilitates the formation of three-dimensional network through interactions via reversible bonds with hydroxyl group by hydrogen bond formation. It contributes to development and stabilisation of porous structure. Porosity is essential for effective wound healing, as it supports cell infiltration, nutrient transport and gas exchange. Crosslinking also improves water holding capacity by enabling it to retain moisture which is critical for accelerating tissue generation and improving healing at wound site.

The present study focuses on development of PVA-gelatin based polymer composite integrated with rutin, glycerol and borax for wound healing applications. This combination result in development of multifunctional polymer composite with enhanced physiochemical and biological properties. The rationale behind this formulation lies in combining PVA film forming, structural and mechanical strength, the biological functionality of gelatin, the antioxidant, anti-inflammatory and antibacterial activity of Rutin, elasticity and flexibility

**Author for Correspondence: belakatte@gmail.com*

provided by glycerol and borax induces crosslinking. Collectively these components contribute to the formulation of a stable, biocompatible and functionally active composite making it a suitable candidate for wound healing application.

2. EXPERIMENTAL:

All the chemicals used as received without further purification and were of analytical grade. Rutin, Gelatin, Borax (Mol. Weight = 381.38) and Glycerol were purchased from Merck, Ltd, Mumbai, India. PVA (Mol. Weight ~ 77,000g/mol), from Himedia laboratories, Mumbai, India. Deionized water was used for experimental work and preparation of solutions. Bruker D8 Advance X-ray diffractometer used to analyse powder X-ray diffractograms (XRD). FT-IR was analysed using a Bruker FT-IR-84000s instrument to analyse different functional moity present, based on stretching and bending vibrations from 4000-400 cm^{-1}. The morphological study of prepared polymer composites was recorded by JEOL scanning electron microscopy (SEM), model IT300, Japan. Swelling study indicates the fluid absorption capacity and the maintenance of moist environment which is very important for wound healing. It depends on the hydrophilicity, porosity of polymer composite. Prepared polymer composite is cut into 2x2 cm dimension and weighed (W_o). It was kept in PBS (pH 7.4 which mimics the body fluid) at 37° C, gently blot the surface and weighed (W_t) after a time period of 1, 2, 3…. hours intervals for 12 hours.[6]

$$\text{Swelling Ratio (\%)} = \frac{W_t - W_o}{W_o} X\ 100$$

Where W_o – initial dry weight

W_t – weight at time t

2.1. Biological activity:

2.1.1. Evaluation of anti-microbial activity:

The Brain heart infusion (BHI) broth (HiMedia, Mumbai, India) was prepared for growing the suitable microorganisms aerobically in shaking incubator at 37 °C during 24 hours, before testing. The inoculum suspension was standardize to yield nearly 10^6 colony forming unit (CFU) / ml of bacteria. Polymer composites 1, 2, 3, 4 and 5 solutions were prepared at a 1 mg/ml concentration in water. The *in vitro* antibacterial activity was assessed by well diffusion method [7]. *E. coli* and *B. subtilis* bacteria were employed in the study period. The prepared bacterial inoculums was spread evenly using sterile L- shaped glass rod on to a Muller Hinton agar plate. The wells (9 mm in diameter) were made using sterile cork borer, and then various concentrations of 100 µl of the test samples were added to each well. Then the plates were kept for incubation at 37 °C for 24 hours. After incubation, whereas the zone of inhibition was measured around each well. Ampicillin was used as a reference compound.

The *in vitro* antifungal activity of the prepared polymer composites were assessed by agar well diffusion method [8]. *Aspergillus niger* and *Aspergillus flavus* have been employed in the study period, cultured on a potato dextrose agar (PDA) medium. In a typical procedure, approximately 9 mm in diameter well was made at the centre of the agar medium, which was previously inoculated with the fungi. Using micropipette the test solutions (100 µL at concentrations 1 mg/ml) was dispensed and the plate was incubated at 37 °C for 72 hours. Over this period, the test solution was penetrated and the proliferation of the inoculated fungi was affected. The inhibition zone developed on the plate was measured. Fluconazole was used as a reference compound.

2.1.2. Invitro cytotoxicity by MTT assay:

MTT is a colorimetric assay that assess the reduction of yellow 3-(4, 5 – dimethylthiazol-2-yl)-2.5-diphenyltetrazoliumbromide (MTT) by mitochondrial succinate dehydrogenase [9]. The MTT enters the cells (L929 Mouse fibroblast cells) and passes into the mitochondria where it is reduced to an insoluble purple colours formazan [10]. The cells are then solubilized with isopropanol, and the amount of released formazan is measured spectrophotometrically. Since, the reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

The cells were seeded at the density of $1x10^4$ cells per well in a 96 well flat bottom microplate at 37 °C in 95% humidity with 5% CO_2 throughout the night. Varied concentrations (100, 50, 25, 12.5 6.25 and 3.12 µg/ml) of test samples were added. Continue the incubation of cells for another 24 hours. Rinse the wells twice with PBS. 20µL of the MTT stained solution was introduced to each well and the plate was incubated at 37 °C. After 4 hours, 100 µL of DMSO was incorporated to each well to dissolve the formazan crystals. Measure the optical density at 570 nm using microplate reader[11].

Surviving cells (%) = Mean OD of test compound / Mean OD of Negative control x 100

2.1.3. Wound healing Scratch assay:

To assess the rate of cell migration *invitro* using L929 cell line, Wound scratch assay was commonly used to study the effects of Polymer composites on cell migration and wound closure[12] . The L929 cells were seeded in to the wells of 24 well plate at a density of $2x10^5$ cells per well. Incubate the plate at 37° C in a 5% CO_2 in a humidified incubator for approximately 24 hours or until a confluent cell monolayer is formed. Remove the culture medium from each well using a sterile 100 µL pipette tip, create a straight linear scratch through the centre of the adherent cell layer. Gently wash the wells with PBS to remove any detached cells and debris from the scratch area. Add fresh culture medium containing different concentrations (100, 50, 25, 12.5 6.25 and 3.12 µg/ml) of the test compound to the appropriate wells. Immediately after adding the treatment, capture images of the scratch in each well using

an inverted microscope. This serves as the initial (0 hours) time point, return the plate to the incubator. Continue to capture images of the same scratch areas at 24 hours. The rate of cell migration is quantified by measuring the distance between the two edges of the scratch at various time points and comparing it to the initial width.

The percentage of wound closure or cell migration can be calculated using the following formula

Cell migration (%) = $(S_0-S_t)/S_0$ x 100%

Where: So= The initial distance of the scratch at 0 hour

St= The distance of the scratch at a designated time point

2.2. Preparation of Polymer composite film

Polymer composite was prepared by solvent casting method. Aqueous soluble of PVA (1g) and gelatin (1g) prepared separately in distilled water at 70º C and 40º C respectively, and mixed in a beaker. Then add rutin to the PVA- gelatin mixture with weight % ranging from 0.2, 0.4, 0.6 and 0.8 followed by 1ml of glycerol. Finally, added 0.1 wt% of borax. The above mixtures were heated at 70° C with stirring for 1 hours. The homogenous mixture was casted into Petri dish and allowed to cool at ambient temperature Table 1.

Table1. Polymer composites and their sample code

Sl. No.	Polymer composites	Sample code
1	PVA+ Gelatin+Glycerol+Borax	PA1
2	PVA+ Gelatin+Glycerol+Rutin 0.2 wt %+Borax	PA2
3	PVA+ Gelatin+Glycerol+Rutin 0.4 wt %+Borax	PA3
4	PVA+ Gelatin+Glycerol+Rutin 0.6 wt %+Borax	PA4
5	PVA+ Gelatin+Glycerol+Rutin 0.8 wt %+Borax	PA5

3. RESULTS AND DISCUSSIONS:

FT-IR study helps to know the various functional groups and to examine chemical interaction between polymer, drug and cross linker used in the film. PA1 showed a broad characteristic band (Fig.1) at 3292 cm^{-1} that corresponds to -OH group [13]. 2900cm^{-1} corresponds to C-H stretching and C-H vibrations [14]. Further prominent sharp peak at 1640 cm^{-1} relates to C=C alkene stretching and C=O stretching in amide [15, 16]. Also, stretching frequency at 1547 cm^{-1} relates to N-H bending vibrations of amides [17]. PA2, PA4 and PA5 displays an intense broad peak corresponding to -OH group. 2936 cm^{-1} indicates the presence of asymmetric C-H stretching vibrations of methylene group [18]. 1631 cm^{-1} comparable to carbonyl group and 1021 cm^{-1} intense peak reveal the ether linkages [19]. In plane and out of plane bending vibrations of B-O-H appears in the range of 1190 cm^{-1} and 790 cm^{-1} [20] is appeared in all the composites. Thus FT-IR depicts that there is no structural modifications in the rutin with polymer. The variation in increase in the intensity is just because of the physical interaction between the rutin and the polymer formulation.

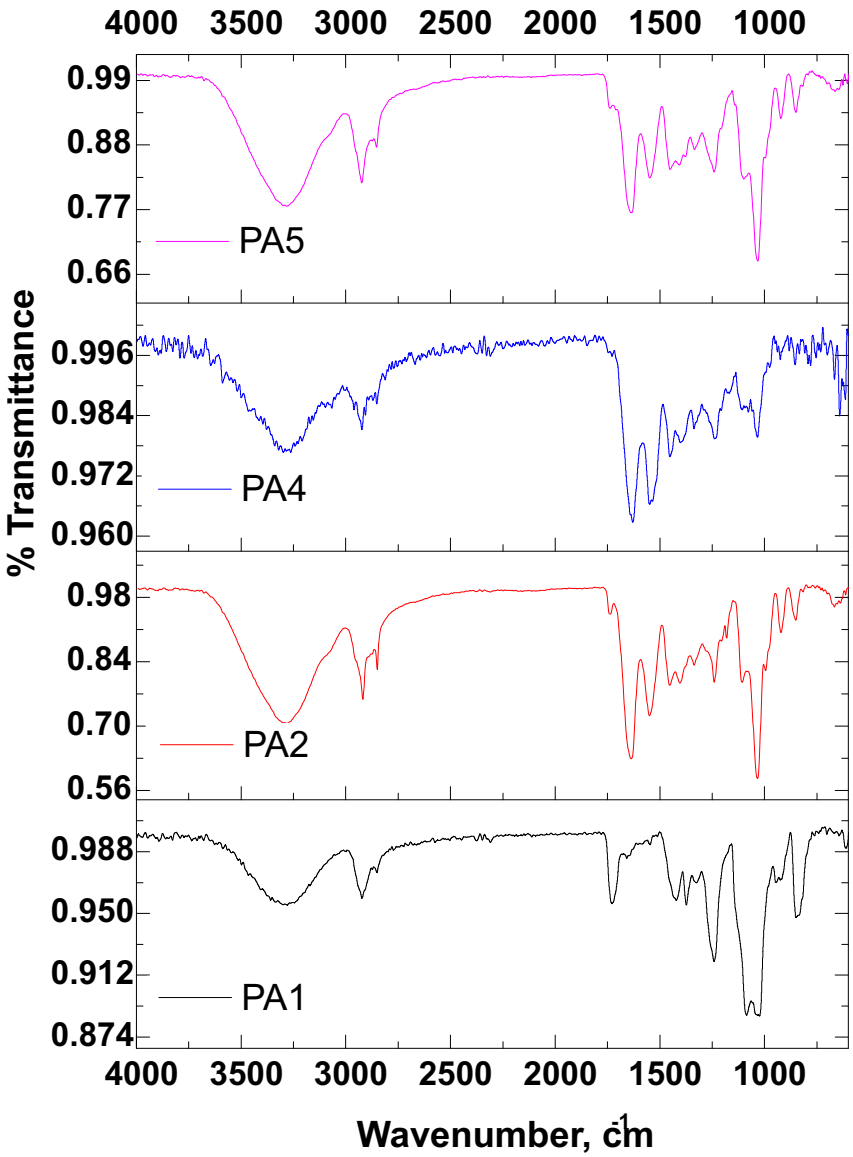


Fig. 1. IR spectra of PVA composites

XRD helps to determine the arrangement of atoms within a crystalline material. A characteristic peak at 19.3º prevailed on XRD spectra (Fig. 2) is characteristic peak of PVA [21]. Retention of same peak in the polymer composite confirms no change in the crystallinity of the PVA by the addition of rutin and other fillers. The highly crystalline rutin display characteristic 2θ peak values at around 10.6°, 15.5°, 17.0°, 20.0°, 23.0° and 25.5° [22]. Which are found to be absent it the XRD spectra of polymer composites (Fig.2) as it only contains a broad peak at 19.3° indicating the loss of crystallinity during the preparation of composites. This indicates the amorphous nature of the prepare polymer composites, the amorphous nature play significant role in the cell adhesion and hence in turn enhances tissue growth [23].

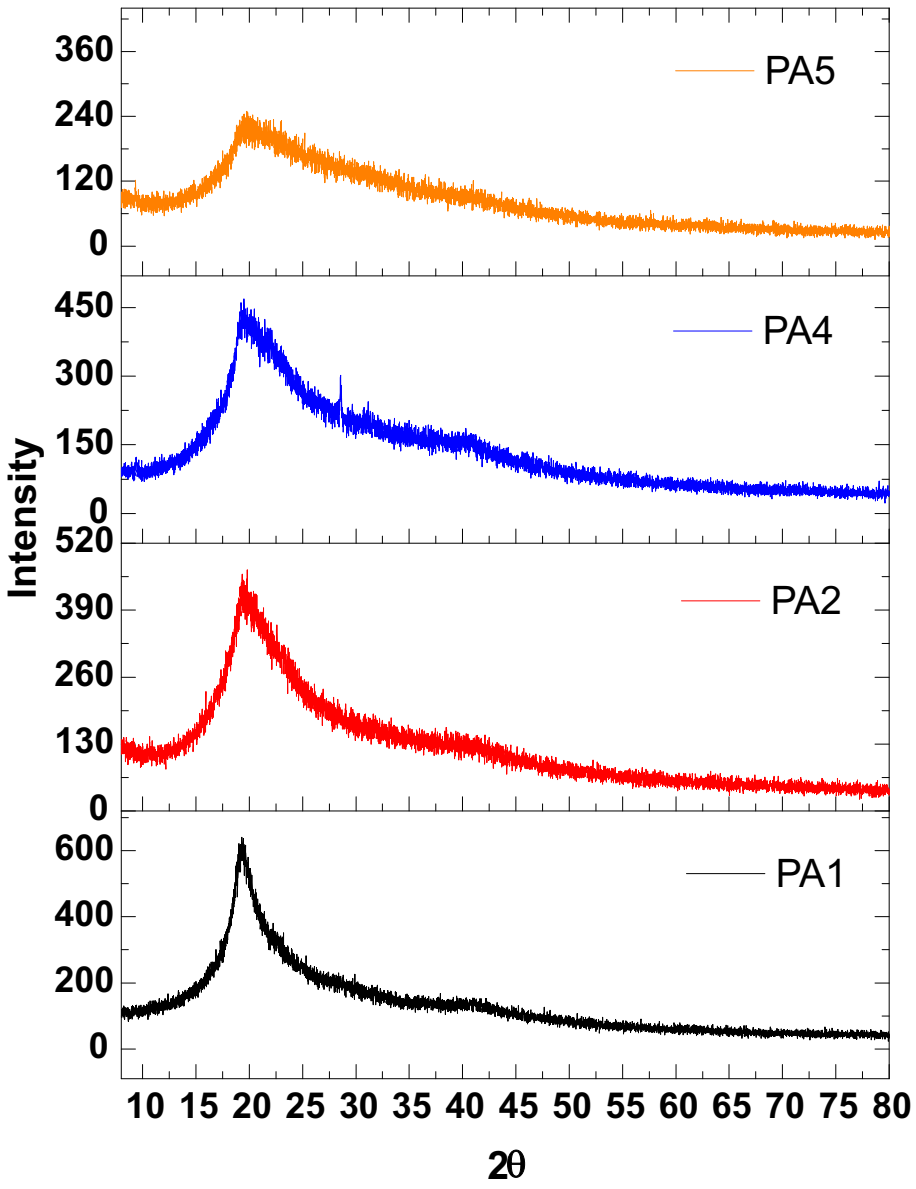


Fig. 2. XRD spectra of PVA composites

The SEM-EDS technique provides the details about porous nature (Fig. 3) and elemental composition Fig. 4 of the prepared polymer composites. The presence of borax along with gelatin creates a highly cross-linked hydrogel composites, such combination will further contribute to the increased porosity [24]. The presence of microspores on the surface of the prepared hydrogel composites as revealed by SEM images. The porous microstructures provide a physical space to migrate fibroblast, continuous oxygen exchange and absorption of excess wound fluids [25]. Hence, maintaining the favourable environment for wound healing.

Initial rapid increase is due to surface adsorption and capillary action. Gradual increase indicates equilibrium swelling 200%-800% is considered for wound healing. From the graph (Fig. 5) the swelling capacity is increased with increase in rutin and Borax content. This enhanced water absorption ability play significant role in exudate management and keeping moist environment around wound, thereby improving wound healing process [26].

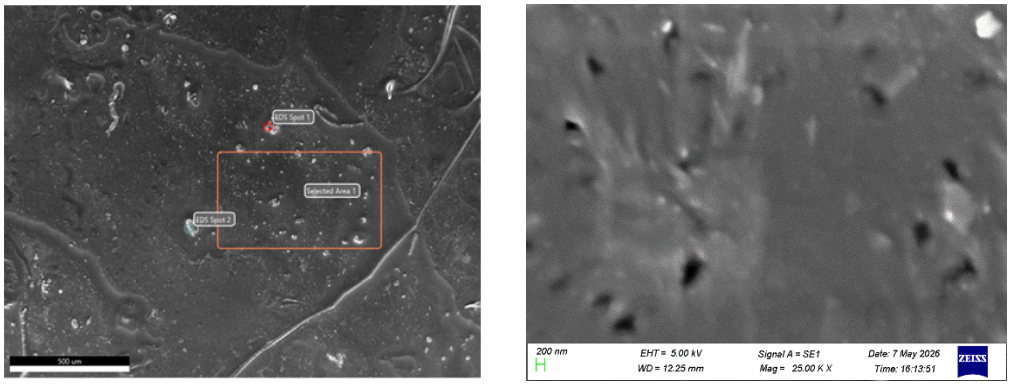


Fig. 3 SEM image of PA2

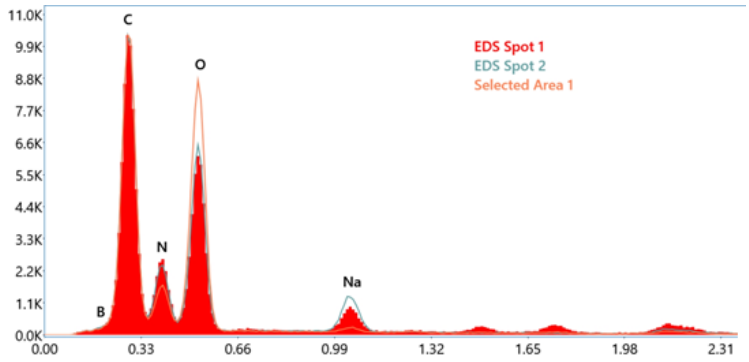


Fig. 4 EDS Spectrum of PA2

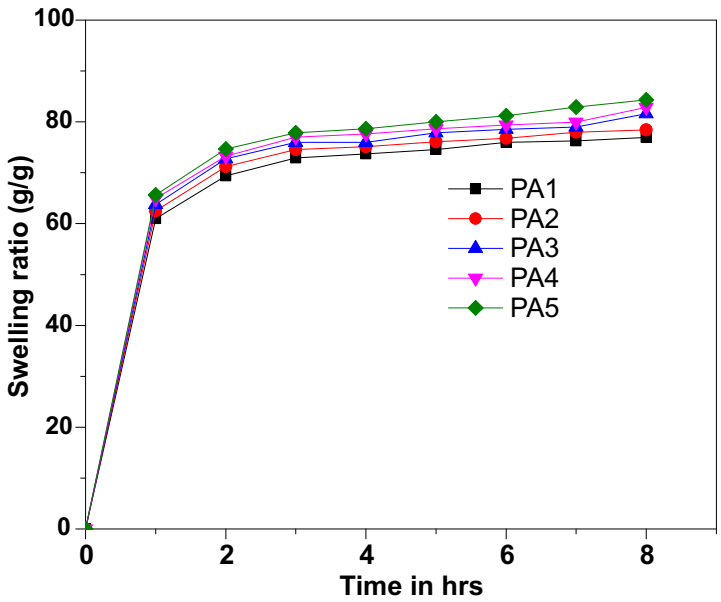


Fig. 5. Swelling Ratio graphs of PA1 to PA5

3.1. Biological Activity

3.1.1. In-vitro Antimicrobial Assay

In the present study, *in vitro* antibacterial activity for the prepared polymer composites was evaluated against one Gram-negative bacteria *E.coli*, and one Gram-positive bacteria *B. subtilis* and *in vitro* antifungal activity by treating two fungi *A. niger,* and *A. flavus*. A significant range of zone of inhibition was observed around individual wells. The zone of inhibition around each well

was measured upon zone size in millimeter (mm). The measured growths of inhibitions against both Gram negative and positive microorganisms are listed in Table 2, and are expressed as the mean of three replicates. The results from the quantitative assay revealed that the tested polymer composites had a good inhibitory activity on bacterial growth.

Table 2: Results of antimicrobial activity of polymer composites

Compound	Zone of inhibition (mm)[a]			
	Bacteria		Fungi	
	E. coli	*B. subtilis*	*A. niger*	*A. flavus*
PA1	10	9	13	10
PA2	11	10	14	11
PA3	12	10	15	12
PA4	14	11	16	13
PA5	15	11	17	14
Ampicillin	27	25	-	-
Fluconazole	-	-	20	20

[a]The results obtained are from the average of the three replicates.

3.1.2. In-vitro cytotoxicity by MTT assay

The cytotoxicity results were shown in (Fig.6) following a 24 hours incubation period. The untreated L929 cells (Positive control) display 100% cell viability. The percentage cell viabilities of different polymer composites are above 70%, which indicate their nontoxic nature [27]. The IC_{50}, is the concentration of polymer composites required to reduce cell viability by 50% is obtained by the dose response sigmoidal curve (Table 3). These concentrations less than IC_{50} values were used for the cell scratch assay. Microscopic images of L929 mouse fibroblast cell lines exposure after 24 hr, with concentrations at 50 µg/mL and 100 µg/mL of polymer composites are shown in Fig. 7. The spindle shaped elongated structure of healthy cells for pristine PVA indicating less cytotoxicity whereas a slightly less elongated or round structures in PVA + 0.2 wt% rutin composite indicate moderate cytotoxicity at 100 µg/mL.

Table 3: IC_{50} calculated for prepared polymer composites

Cell line	PA1	PA2	PA3	PA4	PA5
L929	179.12	141.27	134.36	125.89	119.48

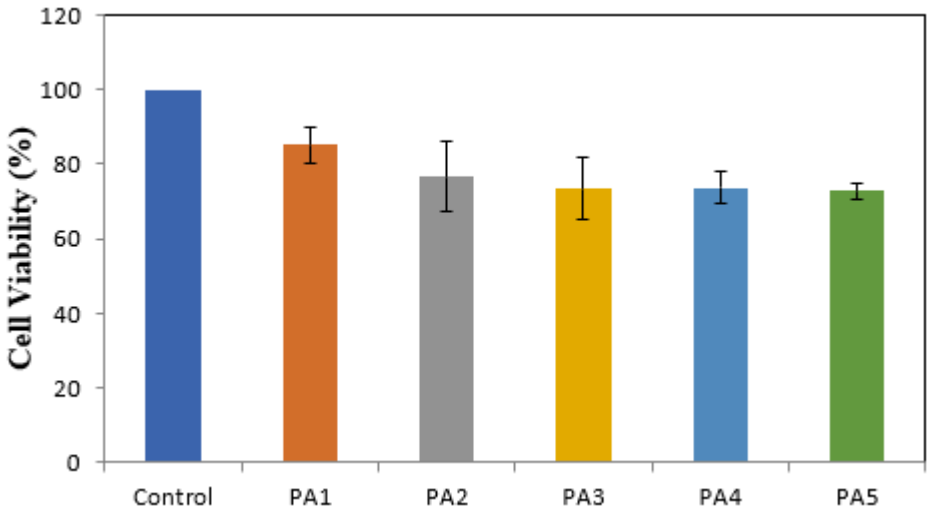


Fig. 6. *In-vitro* cytotoxicity by MTT assay for all prepared polymer composites

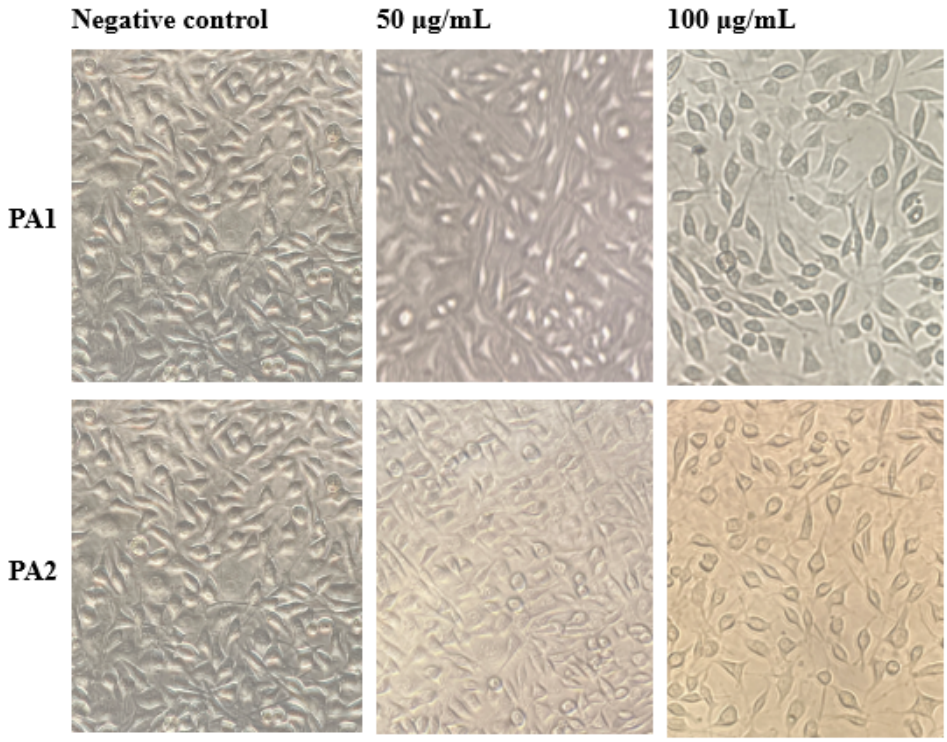


Fig. 7. Microscopic images of L929 mouse fibroblast cell lines exposure after 24 hr, with concentrations at 50 µg/mL and 100 µg/mL of polymer composites.

3.1.3. Wound Healing by Scratch Assay

The cytotoxicity and biocompatibility of the prepared hydrogels for L929 mouse fibroblast cells are assessed by MTT assay and wound scratch study [28]. The scratch assay was used to study *invitro* wound healing dependent on cell migration and in turn helping in regrowth of dermal tissue. The quantitative cell migration was shown in (Fig. 8), wherein, the scratched areas for control, PVA and with various composite of PVA-rutin polymer composites, images took at 0 hours and 24 hours. The cell migration to the scratched area is significantly improved in a time dependent manner for the composites under study and control as well. The cell migration is pronounced for 0.8% of Rutin, the gaps are completely covered after 24 hours, indicating the biocompatibility of prepared hydrogels.

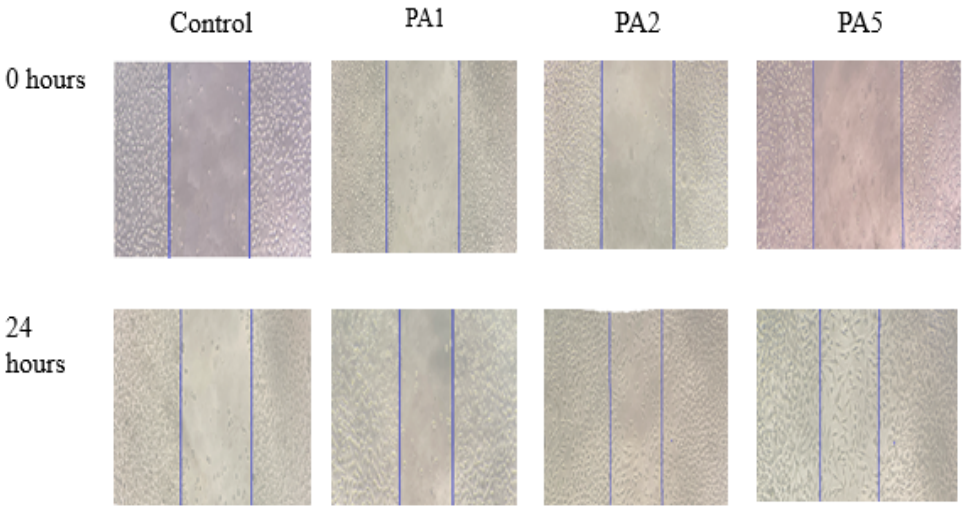


Fig. 8. A representative microscopic image of cell migration by *In-vitro* scratch assay using L929 Mouse fibroblast cells lines at 0 hours and 24 hours incubation at 50 µg/mL of polymer composites.

4. CONCLUSION:

Rutin modified along with boric acid as cross linker, gelatin and glycerol as plasticizer uniformly dispersed on to PVA for the preparation of hydrogels. The prepared hydrogels water retention was assessed by swelling study, displaying the hydrophilic and porous nature of the hydrogels. The incorporation and interaction of PVA with the rutin and other additives are confirmed by Infrared

spectroscopy, XRD, SEM analysis. The hydrogels show concentration dependent antimicrobial activity, the biocompatibility and wound healing assessment for L929 Mouse fibroblast cells lines by MTT Scratch assay display their ability for cytocompatibility with a cell viability more than 70% and a time dependent wound closure by cell migration. Hence, they behave as promising biomaterials for wound healing applications.

ACKNOWLEDGEMENTS

The authors are grateful to A V Kamalamma college for Women and Davangere University for encouraging research activities. They would also like to thank Energy Materials Research Laboratory, Chemistry Research Centre, Siddaganga Institute of Technology, Tumakuru for providing the spectroscopic data.

REFERENCES:

[1] Krishnan Y, Sampath S. Biopolymers and synthetic polymeric materials in wound healing: Cellular mechanisms and therapeutic potential. Discover Materials. 2025;254(5):1-36. doi.org/10.1007/s43939-025-00435-z

[2] Rezvan G, Pircheraghi G, Bagheri R. Curcumin incorporated PVA-borax dual delivery hydrogels as potential wound dressing materials—Correlation between viscoelastic properties and Curcumin release rate. Journal of Applied Polymer Science. 2018;135:1-11. doi.org/10.1002/app.46734

[3] Cao H, Wang J, Hao Z, Zhao D. Gelatin-based Biomaterials and Gelatin as an additive for chronic wound repair. Frontiers in Pharmacology. 2024;15:1-15.doi.org/10.3389/fphar.2024.1398939

[4] Almuhanna Y, Alshalani A, Alsudais H, Alanazi F, Alissa M, Asad M, Joseph B. Antibacterial, antibiofilm, and wound healing activities of Rutin and Quercetin and their interaction with Gentamicin on excision wounds in diabetic mice. Biology (basel). 2024;13(9)676-689.doi.org/10.3390/biology13090676

[5] Lyapin AG, Gromnitskaya EL, Danilov IV, Brazhkin VV. Elastic properties of the hydrogen-bonded liquid and glassy glycerol under high pressure: Comparison with propylene carbonate. RSC Advances.2017;7(53):33278-33284.doi.org/10.1039/c7ra06165j

[6] Rashid N, Khalid SH, Khan IU, Chauhdary Z, Mahmood H, Saleem A, Umair M, Asghar S. Curcumin-loaded bioactive polymer composite film of PVA/Gelatin/Tannic acid downregulates the pro-inflammatory cytokines to expedite healing of full-thickness wounds. ACS Omega. 2023;8:7575-7586.doi.org/10.1021/acsomega.2c07018

[7] Hossain TJ. Methods for screening and evaluation of antimicrobial activity: A review of protocols, advantages, and limitations. European Journal of Microbiology and Immunology. 2024;14(2):1497–115.doi.org/10.1556/1886.2024.00035

[8] Kositchaiyong A, Rosarpitak V, Sombatsompop N. Antifungal properties and material characteristics of pvc and wood/pvc composites doped with carbamate-based fungicides. Polymer Engineering & Science. 2014;54(6):1248-1259.doi.org/10.1002/pen.23672

[9] Li A, Yang J, He Y, Qu C, Miao J**.** Double-Network Pva/Γ-Pga/fucoidan hydrogels with favourable biocompatibility and anti-inflammatory activity for wound healing, carbohydr. Polymer Journal. 2026;385:125403-125416.doi.org/10.1016/j.carbpol.2026.125403

[10] Srikanth M, Khan W, Asmatulu R, Misak H, Yang S, Asmatulu E. In vitro cytotoxicity studies of industrially used common nanomaterials on l929 and 3t3 fibroblast cells. Journal of Biomedical Research & Environmental Sciences. 2020;1(5):192-200.doi.org/10.37871/jbres1143

[11] Benov L**.** Improved formazan dissolution for bacterial MTT Assay. Microbiology Spectrum. 2021;9(3). doi.org/10.1128/spectrum.01637-21

[12] Heydari P, Zargar Kharazi A, Shariati L. Enhanced wound regeneration by PGS/PLA fiber dressing containing platelet-rich plasma: an in vitro study. Scientific Reports. 2024;14:1-13. doi.org/10.1038/s41598-024-62855-w

[13] Narasagoudr S S, Hegde V G, Chougale R B, Masti S P, Vootla S, Malabadi R B. Physico-chemical and functional properties of rutin induced chitosan/poly (vinyl alcohol) bioactive films for food packaging applications. Food Hydrocolloids. 2020;109:106096-106130. doi.org/10.1016/j.foodhyd.2020.106096

[14] Abureesh M A, Oladipo A A, Gazi M, Facile synthesis of glucose-sensitive chitosan–poly(vinyl alcohol) hydrogel: Drug release optimization and swelling properties. International Journal of Biological Macromolecules. 2016;90:75-80. doi.org/10.1016/j.ijbiomac.2015.10.001

[15] Zhao M, Yang Huanhuan, Ran C, Chen N, Liao Z , Hu B B, Ji H, Dong, Juan , Sun, and Jingtao, Chitosan-dialdehyde starch/polyvinyl alcohol double-crosslinked network hydrogel film for degradable food packaging: Addressing mono-crosslinking limitations. Progress in Organic Coatings. 2025;208:109487-109499.doi.org/10.1016/j.porgcoat.2025.109487

[16] Guo C, Jiang X, Guo X, Liu Z, Wang B, Du Y, Tian Z, Wang Z, Ou L. Dual stimulus-responsive renewable nanoadsorbent for selective adsorption of low-density lipoprotein in serum. Regen.

Biomaterials. 2024;11:1-13.doi.org/10.1093/rb/rbae045

[17] Liu KZ, Jackson M, Sowa M G, Ju H, Dixon IMC, Mantsch HH. Modification of the extracellular matrix following myocardial infarction monitored by FTIR spectroscopy. Biochimica et Biophysica Acta (BBA)- Molecular Basis of Disease. 1996;1315:73-77.doi.org/10.1016/0925-4439(95)00118-2

[18] Raj A, Raju K, Varghese HT, Granadeiro CM, Nogueira HIS, Yohannan Panicker C. IR, Raman and SERS spectra of 2-(methoxycarbonylmethylsulfanyl)-3,5-dinitrobenzene carboxylic acid. Journal of Brazilian Chemical Society. 2009;20(3):549-559.doi.org/10.1590/S0103-50532009000300021

[19] Khalafi T, Buazar F, Ghanemi K. Phycosynthesis and Enhanced Photocatalytic Activity of Zinc Oxide Nanoparticles Toward Organosulfur Pollutants. Scientific Reports. 2019;9:1-10.doi.org/10.1038/s41598-019-43368-3

[20] Kiliç M, Karabul Y, Özdemir ZG, Erdönmez S, Bulgurcuoğlu AE, Yeşilkaya SS, Okutan M, İçelli O. Effect of borax additive on the dielectric response of polypyrrole, Bulletin of Materials Science. 2018;41:52-61.doi.org/10.1007/s12034-018-1564-8

[21] Gautam A, Ram S. Synthesis, mechanical and I –V characteristics of Ag–PVA nanocomposite films. Physica Status Solidi. 2009;206:1471-1477.doi.org/10.1002/pssa.200925023

[22] Yusuf H, Meidy Nurintan Savitri O, Primaharinastiti R, Agus Syamsur Rijal M. A lyophilized surfactant-based rutin formulation with improved physical characteristics and dissolution for oral delivery. Saudi Pharmaceutical Journal. 2023;31:1077-1083.doi.org/10.1016/j.jsps.2023.03.018

[23] Yu K, Li Y, Li X, Chen Z, Li F, Zhang S, Zhou X, Zhang K, Lu M, Yang P, Zhang X. Injectable amyloid-structured hydrogel incorporating amorphous calcium phosphate promotes periodontal tissue regeneration by mimicking enamel matrix proteins (EMPs). Biomaterials. 2026;334:124277-124295.doi.org/10.1016/j.biomaterials.2026.124277

[24] Sharma AK, Priya, Kaith BS, Bhagya Shree, Simran, Saiyam. Borax mediated synthesis of a biocompatible self-healing hydrogel using dialdehyde carboxymethyl cellulose-dextrin and gelatin. Reactive and Functional Polymers. 2021;166:104977-104989.doi.org/10.1016/j.reactfunctpolym.2021.104977

[25] Al Saleh N, Wang J, Chen D, Ageely E, Al Bihan S, Abdelghafour MM, Javaid R, Mujeeb A, Al-Kenani NS, Khashab NM. A Smart and Highly Porous Hydrogel for Diabetic Wound Healing. Adv. Mater. Interfaces. 2025;12:1-14.doi.org/10.1002/admi.202500256

[26] Ferraz MP. Wound Dressing Materials: Bridging Material Science and Clinical Practice. Applied Sciences. 2025;15(4):1-29.doi.org/10.3390/app15041725

[27] Avcılar İH, Bakir EP, Bakır Ş, Irtegun Kandemir S. Evaluation of the cytotoxicity of flowable composite resins with different compositions: in vitro study. BMC Oral Health. 2026;26:1-11.doi.org/10.1186/s12903-025-07647-y

[28] Yaghoubi A, Ramazani A, Sillanpaa M, Ghasemzadeh H, Mohammadi E. Biocompatible porous PAM/CNT nanocomposite hydrogel films for sustained drug delivery and cancer therapy. Scientific Reports. 2025;15:1-13.doi.org/10.1038/s41598-025-05473-4