DEVELOPMENT AND CHARACTERIZATION OF BIOCOMPATIBLE POLYHYDROXY BUTYRATE IMPREGNATED WITH HERBAL PLANTS AGAINST WOUND HEALING ACTIVITY ON IN VIVO ANIMAL MODEL

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INTRODUCTION

Polyhydroxybutyrate (PHB) is bacterial polymers formed naturally as storage polymers by many microorganisms under unbalanced growth conditions [1]. These are widespread in prokaryotes and enhance the survival during the times of starvation [2]. PHB has widespread attention, that it can be used in a wide range of agricultural, industrial, and various medical applications due to its biodegradability and biocompatibility. In medical field and tissue engineering, PHBs were used to develop devices including sutures, repair devices, repair patches, stents, cardiovascular blends, orthopedic pins, adhesion barrier, regenerative devices, articular cartilage repair devices, nerve guides, tendon repair devices, bone marrow scaffolds, and wound dressings [3]. PHB produced by Bacillus mycoides DFC 1 was incorporated with vanillin and films were produced and subjected to antimicrobial activity against foodborne pathogens and spoilage bacteria and fungi. The minimum concentration of vanillin incorporated PHB films was ≥80 μg/g PHB for bacteria and ≥50 μg/g PHB for fungi [4]. PCL-PHB blend microspheres were prepared by water/oil/water double emulsion solvent evaporation method and were encapsulated with tamofoxen (TAM), an antitumor drug and analyzed for the in vivo drug release study [5]. The study revealed that the TAM was released in a controlled manner for >12 h by influencing the composition of PHB, pH, and drug loaded. PHB produced by Azotobacter chroococcum 23 showed promising results against two Gram-positive (Bacillus cereus and Staphylococcus aureus) and two Gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacterial strains. The antimicrobial materials are PHB and PHB/paper systems including Silibolin or benzoic acid [6]. The PHB was also used in packaging materials in which the antimicrobial activity of PHB/chiotsan films and the quality of white bread packaged with the films was investigated by Kim [7]. In his study, PHB (L) film showed high antimicrobial activity against Fusarium solani KCTC 6636 and Penicillium cinnorenium KCTC 6927. The colony-forming units of microorganisms for white bread packaged with PHB (M), PHB (L), and chitosan film were low during storage. The PHBs were blend with various other compounds for a good texture, support, and degradability. The majorly used substrate was PEG and valeric acid. Many other blends were also used. These blends show good biocompatibility, and hence, these were used in many animal models. PHBs are suitable for scaffolding materials in tissue engineering and are proved by various studies. Shishatskaya and Volova [8] proved that NIH 3T3 fibroblast cells adhere and proliferate on PHA membranes. Mesenchymal stem cells adhere and proliferate on several PHA substrates, with a terpolymer P (HB-co-hydroxyvalerate-co-hydroxyhexanoate) P (HB-co-HV) [9]. PHB matrices have also been tested for hemocompatibility with mammalian blood incubated with polymer films. It was identified that PHB or P (HB-co-HV), in contact with blood did not interfere in platelet responses, nor polymer activates the complement system [10,11]. The present study involves the biocompatibility study of PHB produced by A. chroococcum A3 strain. The various biocompatibility studies include antimicrobial, antioxidant, anti-inflammatory, and antiadherent of bacterial colonies. The cytotoxicity was studied using (3-[4,5-Dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide) (MTT) assay. Various PHB films with different herbal blends were made and were subjected to in vivo wound healing activity using Albino Wistar rats.

METHODS

Bicocompatibility study

The biocompatibility study was done to examine whether the produced PHB was able to compete with the living cells without producing any...
harmful effect. The compatibility study involves anti-inflammatory study and MTT assay using HEK293 fibroblast cell lines.

**Anti-inflammatory study**

From the previous investigation, PHB from *A. chroococcum* A3, A4, and *Bacillus megaterium* was used for the anti-inflammatory study. A 0.5 ml of the PHB extracted from *A. chroococcum* A3, A4, and *B. megaterium* was mixed with and 0.5 ml of 1% of egg albumin solution (phosphate buffered saline). The standard drug diclofenac sodium (5 mg/ml) was mixed in 1.5 ml of phosphate buffered saline (pH6.4) and 0.5 ml of egg albumin solution (1%). The mixtures were incubated at 37°C for 20 min and then denatured at 90°C in a water bath for 2 min. The solution was measured spectrophotometrically at 660 nm. The inhibition of the PHB was calculated using the formula:

\[
\text{Inhibition (\%)} = \frac{(A_0 - A_t)}{A_0} \times 100
\]

Where, \(A_0\) = Absorbance of control, \(A_t\) = Absorbance of test sample.

**MTT assay** [12]

To evaluate the cytotoxicity of PHB, cell viability study was carried out with the conventional MTT - reduction assay with small modifications. HEK 293 fibroblast cells (1×10^5/well) were plated in 96-well plates (Costar Corning, Rochester, NY), and the cells were allowed to attach and were grown for 48h, in 200 ml of Dulbecco’s modified eagle medium with 10% foetal bovine serum. After 48 h incubation, the cell ranges the confluence. Then, cells were incubated with different concentrations of the PHB, namely 6.25, 12.5, 25, 50, and 100 mg/ml (minimum 3 wells were seeded with each concentration). Equal concentrations of ascorbic acid were used as positive control, and the cells were incubated for 48 h followed by addition of MTT (10 ml and 5 mg/ml) and the cells were incubated at 37°C for another 4 h. The viable cell was determined by the absorbance at 570 nm. Inhibitory concentration (IC_{50}) was determined graphically, and blanks were maintained. The effect of the samples on the proliferation of HEK 293 fibroblast cell lines was expressed as the percentage cell viability, using the following formula:

\[
\% \text{cell viability} = \frac{A_{570} \text{ of treated cells}}{A_{570} \text{ of control cells}} \times 100
\]

**PHB-plant and algal cast film preparation**

Plant and algal products were powdered, mixed with water and glycerol in the composition 50:15:35 (w/v/v), respectively. The contents were mixed for 15–30 min in constant stirring speed to obtain a clumsy paste. The paste was transformed into thermoelastic by heating at 100°C in a water bath with continuous stirring for 15 min. The thermoplastic herbal product was mixed with PHB in the ratios 58:52 (w/w) and 50:15:35 (w/v/v), respectively. The contents were mixed in 1.5 ml of phosphate buffered saline without H_2O_2 solution of (0.2 M) was prepared in phosphate buffer (pH 7.4). 1 ml of different concentrations (20, 40, 60, 80, and 100 mg/ml) was added to 0.6 ml solution of 40 mM hydrogen peroxide solution. The absorbance of the mixture was measured at 230 nm using UV-visible spectrophotometer against a blank solution containing phosphate buffer saline without H_2O_2. The ascorbic acid was used as positive control. IC_{50} value was determined by linear regression analysis. The percentage of H_2O_2 scavenging was calculated by the following formula:

\[
\% \text{inhibition} = \left[1 - \frac{\text{Absorbance of extract}}{\text{Absorbance of control}}\right] \times 100
\]

**Mechanical property**

The different herbal blends with PHB films were analyzed for the mechanical property, the tensile strength (TS), percentage elongation at break (% E), and elastic modulus (EM) were measured according to standard method ASTM D882, using Instron 3365 universal testing machine with a load cell of 30 kg. Films were cut in the form of strips with a dimension of 10×70 mm, strips were clamped between two tensile grips, and the initial gauge length was set at 30 mm. The tests took place at room temperature without humidity control. Initial grip separation and crosshead speed were performed at 2 mm/min. TS and EM were expressed in N and MPa, and % E in percentage (%). 10 measurements for each film sample were used for test and values were determined by the mean.

**Wound healing activity of turmeric impregnated with PHB on in vivo model**

Healthy inbred male Wistar albino rats weighing (165–180 g) were obtained from the experimental animal house, KSR College of Technology, Tiruchengode, Tamil Nadu, India, and used for this study. All rats were divided randomly into six groups (n=6 in each). Animal houses were maintained in standard environmental conditions of temperature (22±3°C), humidity (60±5%), and a 12 h light/dark cycle. The animals were fed on standard pellet diet and fresh tap water. All the experimental procedures and protocols used in this study were in accordance with the guidelines of the CPCSEA, New Delhi (1826/P/Re/EB/5/15/CPCSEA, dated: 14.09.2015), with the approval of Institutional Animal Ethics Committee (IAEC) (KSRCT/IT/IAEC/2018/27), of KSR College of Technology, Tiruchengode, Tamil Nadu, India.

**Induction of incision wound model**

The experimental animals were grouped into six containing six animals each and treated as follows:

- **Group I**: Control (untreated).
- **Group II**: PHB biofilm.
- **Group III**: Turmeric leaf 1% with PHB based biofilm.
- **Group IV**: Turmeric rhizome 1% with PHB based biofilm.
- **Group V**: Turmeric leaf 2% combined with PHB based biofilm.
- **Group VI**: Turmeric rhizome 2% combined with PHB based biofilm.
All animals of experimental groups were anesthetized using a low dosage of phenobarbital IP injection in an aseptic condition and observed throughout the study. A circular wound of 2 cm length incision was made through the skin at a distance about 2 cm from the middle on the right side. All the test biofilms of 3 x 3 cm were plastered on the wounded area twice daily. Percentage of wound contraction was calculated as:

\[
\text{Percentage of wound size} = \frac{\text{Wound area on day } X}{\text{Wound area on day zero}} \times 100
\]

Percentage of wound healing was calculated as:

\[
\text{Percentage of wound healing} = 100 - \text{Percentage of wound size}
\]

Percentage of wound contraction was calculated on 2nd, 4th, 8th, 10th, 12th, 14th, and 16th post-wounding days. The statistical analysis was carried out using GraphPad prism software of version 5.0 [15,16].

RESULTS

Biocompatibility study

Anti-inflammatory study

The anti-inflammatory study of PHB produced by *A. chroococcum* A3, A4, and *B. megaterium* was compared with commercial drug and was tabulated (Table 1). PHB from *B. megaterium* showed high activity then followed by *A. chroococcum* A3.

MTT assay

Regardless of the extensive use of PHB, there are no reports to confirm the cytotoxicity effects of PHB. The cytotoxicity of the PHB has been evaluated against HEK 293 cell lines at various concentrations ranging from 6.25 to 100 μg/ml (Fig. 1). The MTT assay was performed in HEK 293 cells. The concentration of MTT was found to be 20 μg/ml. Fig. 1 showed the cytotoxicity activity of PHB with the IC₅₀ value of 1.56 μg/ml.

PHB-plant and algal cast film preparation

The formulations of PHB with various plant and algal blends were tabulated in Table 2.

Antimicrobial activities of biofilm with PHB against skin pathogens

Turmeric rhizome with PHB showed better antimicrobial activities against skin pathogens followed by *P. tetrastromatica* and *A. fragilissima* whereas starch-based biofilms revealed very less significant toward the antimicrobial property. The maximum zone of inhibition was obtained by turmeric rhizome with PHB biofilm as 27.25±0.23 mm against skin pathogens. *P. tetrastromatica* with PHB showed better antimicrobial activities against skin pathogens followed by turmeric leaf and *P. tetrastromatica* whereas starch-based biofilms revealed very less significant toward the antimicrobial property. The PHB showed less antimicrobial activity with 08.65±0.62 mm (Table 3).

Antioxidant capacity of various herbs impregnated with PHB

Turmeric rhizome with PHB showed the highest percentage of inhibition of 76% followed by turmeric leaf. In contrast, starch-based PHB revealed less capturing of hydrogen free radicals. Similar trend was also observed in total antioxidant capacity using ammonium molybdate assay of estimating IC₅₀ (Fig. 2). Highest IC₅₀ indicated that less number of free radial capturing potential like chemical-based biofilm. Turmeric rhizome blended with partially purified PHB was exhibited very less value of IC₅₀ around 75 μg/ml (Fig. 3).

FRAP assay

FRAP assay indicates that increase in absorbance leads to high reducing capacity of free radicals. *A. fragilissima* (F9) showed high percentage of inhibition followed by *P. tetrastromatica* (F10) and turmeric rhizome 1%+PHB (F7) (Fig. 4).

Table 1: Anti-inflammatory study of PHB

| Extract                  | Inhibition (%) |
|--------------------------|----------------|
| PHB from *A. chroococcum* A3 | 45             |
| PHB from *A. chroococcum* A4 | 40             |
| PHB from *B. megaterium*   | 48             |
| Commercial drug           | 58             |

Table 2: Plant and algal formulation with PHB

| S. No | Samples                  |
|-------|--------------------------|
| 1     | PHB (F1)                 |
| 2     | Sago starch 1%+PHB (F2)  |
| 3     | Sago starch 2%+PHB (F3)  |
| 4     | *Amphiroa fragilissima*+PHB (F4) |
| 5     | *Padina tetrastromatica*+PHB (F5) |
| 6     | Turmeric leaf 1%+PHB (F6) |
| 7     | Turmeric rhizome 1%+PHB (F7) |
| 8     | Standard                 |
| 9     | *Amphiroa fragilissima* (F9) |
| 10    | *Padina tetrastromatica* (F10) |

Fig. 1: (3-[4,5-Dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay - cell viability of polyhydroxy butyrate

Fig. 2: 2,2-Diphenyl-2-picrylhydrazyl assay of hydrogen free radicals using herbs with polyhydroxybutyrate

Fig. 3: Analysis of inhibitory concentration of polyhydroxybutyrate film


**Table 3: Antibacterial activities of herbal blended with biofilm against skin pathogens**

| S. No | Formulation                                      | Spp 1 Zone of inhibition (mm±SD) | Spp 2 Zone of inhibition (mm±SD) | Spp 3 Zone of inhibition (mm±SD) | Spp 4 Zone of inhibition (mm±SD) |
|-------|--------------------------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| 1     | Control only PHB (F1)                           | 08.65±0.62                       | 10.82±0.33                       | 9.75±0.03                        | 10.23±0.43                       |
| 2     | Sago starch 1%+PHB (F2)                         | 15.32±0.73                       | 15.02±0.32                       | 14.95±0.63                       | 14.92±0.03                       |
| 3     | Sago starch 2%+PHB (F3)                         | 16.52±0.83                       | 16.68±0.28                       | 16.56±0.38                       | 16.60±0.85                       |
| 4     | PHB+Seaweed 1 (F4)                              | 22.96±0.53                       | 23.06±0.53                       | 22.76±0.53                       | 21.76±0.53                       |
| 5     | PHB+Seaweed 2 (F5)                              | 21.65±0.77                       | 21.73±0.87                       | 21.52±0.83                       | 21.85±0.23                       |
| 6     | PHB+Turmeric leaf powder (F6)                   | 20.75±0.03                       | 23.45±0.37                       | 21.85±0.53                       | 20.42±0.83                       |
| 7     | PHB+Turmeric rhizome powder (F7)                | 26.25±0.33                       | 27.55±0.43                       | 26.95±0.63                       | 26.95±0.63                       |
| 8     | Chlororamphenicol (F8)                          | 23.06±0.62                       | 18.32±0.62                       | 17.12±0.62                       | 25.08±0.62                       |
| 9     | Seaweed 1 (F9)                                  | 22.26±0.62                       | 22.82±0.35                       | 22.16±0.53                       | 21.96±0.13                       |
| 10    | Seaweed 2 (F10)                                 | 21.76±0.63                       | 21.66±0.57                       | 22.11±0.33                       | 22.06±0.64                       |

Spp 1: Klebsiella pneumonia, Spp 2: Streptococcus pyogenes, Spp 3: Enterococcus sp., Spp 4: Corynebacterium spp. SD: Standard deviation

**Table 4: Hydrogen Peroxide scavenging power assay**

| S. No | Samples                              | % Inhibition |
|-------|--------------------------------------|--------------|
| 1     | Control (F1)                         | 08.55        |
| 2     | PHB+Starch (F2)                      | 20.56        |
| 3     | Sago starch 1%+PHB (F3)              | 14.56        |
| 4     | Amphiria fragilissima+PHB (F4)       | 14.27        |
| 5     | Padina tetrastronica+PHB (F5)        | 16.26        |
| 6     | Turmeric leaf 1%+PHB (F6)            | 17.58        |
| 7     | Turmeric rhizome 1%+PHB (F7)         | 21.2         |
| 8     | Ascorbic acid                        | 13.53        |
| 9     | Amphiria fragilissima (F9)           | 13.53        |
| 10    | Padina tetrastronica (F10)           | 13.87        |

**Hydrogen peroxide free radical scavenging assay**

Maximum inhibition was observed at turmeric rhizome with 1% concentration followed by PHB with starch composites. Seaweed with polymer showed moderate inhibitory activity against hydroxyl ions. Turmeric rhizome and leaf exhibited significant activity compared to standard ascorbic acid (Table 4).

**Mechanical property of PHB-plant and algal cast film**

PHB with plants and algal blend films (Fig. 5) was subjected to TS, percentage of elongation and EM are the parameters that characterize the mechanical properties of films. The TS is the measurement of the maximum strength of a film to withstand applied tensile stress. The PHB (F1) is said to be 0.007±0.001 N/mm², whereas the highest TS found in F2, the average result was found in sample F6 and F7. Elongation at break is a measure of the film’s stretchability before breakage. Percent elongation at break was calculated based on the length extended and original length of the films. The percentage elongation was found the maximum in F6 and F7. Young’s modulus was estimated by the rigidity with higher values imply that the films are more rigid. EM was higher in F6 and F7 with 304±0.021 and 325±0.027, respectively (Table 5).

**Wound healing activity of turmeric impregnated with PHB on in vivo model**

The wound healing activity of PHB with herbal films was studied on the incision wound model in rats (Fig. 6). The wound without treatment was used as a control. Percentage of wound contraction was measured on 2nd, 4th, 6th, 8th, 10th, 12th, 14th and 16th days post-wounding days, at the 10th day the healing percentage of control was 46%, and PHB alone was 54%. Turmeric leaf with PHB showed the highest activity with 84%. At day 16, the control cured at 90% and turmeric leaf with PHB and turmeric rhizome with PHB showed 100% healing activity (Table 6). The result shows that PHB along with natural antiseptic gives promising results than crude PHB and control.

**DISCUSSION**

The PHB was subjected to anti-inflammatory and cytotoxicity assay, and the results revealed that it is biocompatible to use in medical applications. The IC₅₀ value in MTT assay was 1.56 µg/ml showed that the viability of the cells was not affected. The percentage of inhibition was nearer to the commercial drug in anti-inflammatory activity, thus, can be used the alternative of the drugs which may have side effects. The PHB produced from A. chroococcum A3 was mixed with different herbal blends and subjected to antimicrobial and antioxidant activities. The TS, elongation break %, and Young’s modulus were examined. The evaluation of PHB and PHB/PP blends degradation was carried out by Pacholski et al. [17] showed that the TS of PHB and PHB/PP blends was 28.5 and 24.5 Mpa with elongation break 2.5 and 1.5 with higher Young’s modulus of 2045 and 1885 Mpa, respectively, degraded after 90 days in soil and reported with decrease in TS with which increases the degrading rate, the TS of PHB produced by cyanobacterium Chlorogloea

**Fig 4: Ferric reducing antioxidant power assay of the herbal formulation with polyhydroxybutyrate**

**Fig 5: Biofilm formulation using different herbal resources**
Table 5: Mechanical properties of different formulation with PHB

| Particulars of cast film | Tensile strength (N/mm²±SD) | Film elongation (%±SD) | Elastic modulus of the film (Mpa±SD) |
|-------------------------|-----------------------------|------------------------|------------------------------------|
| F1 - PHB                | 0.007±0.001                | 20.4±0.002             | 289±0.056                          |
| F2 - Sago starch 1%+PHB | 0.055±0.003                | 13.8±0.001             | 178±0.5                            |
| F3 - Sago starch 2%+PHB | 0.035±0.003                | 12.8±0.004             | 176±0.53                           |
| F4 - Amphireoa fragilissima+PHB | 0.070±0.001 | 20.4±0.002             | 289±0.056                          |
| F5 - Padina tetrastromatica+PHB | 0.110±0.002 | 20.6±0.004             | 256±0.026                          |
| F6 - Turmeric leaf 1%+PHB | 0.033±0.04                 | 25.1±0.006             | 304±0.021                          |
| F7 - Turmeric rhizome 1%+PHB | 0.39±0.04                 | 25.7±0.008             | 325±0.027                          |
| F8 - Amphireoa fragilissima | 0.006±0.004               | 19.8±0.002             | 289±0.056                          |
| F9 - Padina tetrastromatica | 0.011±0.002               | 20.6±0.004             | 256±0.026                          |

PHB: Polyhydroxybutyrate, SD: Standard deviation

Table 6: In vivo model of wound healing effect of various formulation with PHB

| Particulars of cast film | Degree of contraction (%±SD) |
|-------------------------|------------------------------|
|                         | Number of days               |
|                         | 2       | 4       | 6       | 8       | 10      | 12      | 14      | 16      |
| Control                 | 8.3±2.2 | 19±2.8  | 28.6±0.9 | 32±0.89 | 46.2±0.006 | 68±0.006 | 79±0.07 | 90.4±0.4 |
| PHB                     | 9.8±1.2 | 18±0.06 | 32.8±0.29 | 43.5±0.04 | 54±0.003 | 71.9±0.22 | 80±0.02 | 91.3±0.56 |
| Turmeric leaf 1%+PHB    | 19±1.2  | 29.7±0.28 | 46.2±0.06 | 68±0.26 | 81.5±0.07 | 89.4±0.34 | 94.8±0.17 | 98.9±0.01 |
| Turmeric rhizome 1%+PHB | 20.5±0.59 | 30.7±0.63 | 49.8±0.28 | 69.8±0.26 | 83.7±0.08 | 90.8±0.30 | 96.5±0.47 | 98.0±0.21 |
| Turmeric leaf 2%+PHB    | 22±0.04 | 37.3±0.39 | 53.9±0.28 | 76±0.33 | 84.4±0.52 | 92.4±0.49 | 96.8±0.62 | 100      |
| Turmeric rhizome 2%+PHB | 21±0.45 | 34.2±0.58 | 52.6±0.07 | 72±0.09 | 83.5±0.27 | 91.2±0.14 | 97.8±0.84 | 100      |

PHB: Polyhydroxybutyrate, SD: Standard deviation

Fig. 6: Wound healing activity of turmeric blended with polyhydroxybutyrate (PHB) on rat model. (a) Incision of wound, (b) PHB with turmeric film, (c) sealed wound (a-c on day 1), and (d) healed wound on day 16

*hfritschii* was 23Mpa with elongation break 5.5 and Young’s modulus was 71.2Mpa [18]. The PHB produced in our study was entirely thin with F7 producing 0.39 Mpa TS and 25.7 elongation break and 32.5 Mpa Young’s modulus which indicates that the PHB with lesser TS can act as a good biodegradable material.

Turmeric is a very good antiseptic since it was used as a traditional medicine from the ancient days to treat wounds. Since PHB is immunologically inert which will neither promotes or retards the wound healing activity. Turmeric rhizome with PHB showed good antimicrobial and antioxidant activity. PHB which is produced from *A. chroococcum* A3 was used as a carrier in the wound healing activity. PHB acts as a supportive material against wound healing activity, while PHB and turmeric will be a very good therapeutic agent against wound healing activity. The extracts of turmeric leaf and rhizome along with PHB will be a good combination in treating the wounds. Turmeric rhizome with PHB, the in vivo model was evaluated with Wistar rats. The evaluation reports that PHB is a very good supportive material in treating the wounds along with turmeric. Gayathri et al. [19] proved the antiseptic property of turmeric. Antibiotics and petrochemical derived antimicrobial property of turmeric. Antibiotics and petrochemical derived antibiotic resistance and the cost of antibiotics are the main concerns. Turmeric is a traditional medicine for wound healing activity. Turmeric acts as a supportive material against wound healing activity, while PHB and turmeric will be a very good therapeutic agent against wound healing activity. The extracts of turmeric leaf and rhizome along with PHB will be a good combination in treating the wounds. Turmeric rhizome with PHB, the in vivo model was evaluated with Wistar rats. The evaluation reports that PHB is a very good supportive material in treating the wounds along with turmeric. Gayathri et al. [19] proved the antiseptic property of turmeric. Antibiotics and petrochemical derived antibiotic resistance and the cost of antibiotics are the main concerns. Turmeric is a traditional medicine for wound healing activity. Turmeric acts as a supportive material against wound healing activity, while PHB and turmeric will be a very good therapeutic agent against wound healing activity. The extracts of turmeric leaf and rhizome along with PHB will be a good combination in treating the wounds. Turmeric rhizome with PHB, the in vivo model was evaluated with Wistar rats. The evaluation reports that PHB is a very good supportive material in treating the wounds along with turmeric. Gayathri et al. [19] proved the antiseptic property of turmeric. Antibiotics and petrochemical derived antibiotic resistance and the cost of antibiotics are the main concerns.
Narendhirakannan et al. [23] used only the extracts of various herbs, but in our study, the herbs are blended with PHB, and another study by Karri et al. [24] showed that curcumin incorporated in nanohybrid scaffold showed highest wound contraction of 98.1±3.4% at day 15 when compared to control with 44.6±6.3% at day 15. Curcumin-loaded chitosan/gelatin sponge showed the good result of wound healing in rabbits with 95.4±3.62% at day 15, which was higher than the control with 48.80±1.71% on the 15th day [25]. Nanoformulated curcumin with gelatin was evaluated for anti-inflammatory and wound healing in cell lines and animal models [26]. In their study, curcumin was impregnated to nanofibrous mats (NM) with gelatin (Cc/Glt NM). The in vitro study of anti-inflammatory and wound healing was done in HS-27 cell lines. The in vitro study showed the significant anti-inflammatory effect of curcumin. The in vivo study was done in Sprague-Dawley rats, and the nanofiber mats of gelatin showed significantly strong wound healing at day 15 when compared to the control.

Turmeric proved to be effective in healing the wounds and PHB is an inert material, hence, which can be used as scaffold along with turmeric eric an antiseptic which will be a good source of treating wounds for fast recovery; PHB is a biopolymer which will not damage or enhance the cell growth since we have made a trial with in vitro cytotoxicity; and the result reveals that PHB will be a good source of treating many types of wounds. The anti-inflammatory and cytotoxicity assay revealed that PHB does not harm cell growth and proved to be a good candidate for scaffolding activity and can be used in a drug delivery system.

CONCLUSION

Different herbal formulation with PHB was prepared as a biofilm to elucidate the biological activities such as antimicrobial, antioxidant, anti-adherent, and wound healing property. Antioxidant capacity showed the highest inhibition in turmeric rhizome and leaf for >50%. The result indicated that turmeric rhizome coated with PHB showed highly significant in reducing wound activity rather than the other formulation in the rat model.

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AUTHORS’ CONTRIBUTION

All the works were performed by Ram Narendran and manuscript was made under supervision of Dr. S.F. Maleeka Begum. Dr. S.F. Maleeka Begum contributed to manuscript preparation and MTT assay. Rubavathi S contributed to preparation of cast films.

CONFLICTS OF INTEREST

The authors declare that we do not have any conflicts of interest.

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