ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 12, Issue 2, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

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

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Received: 18 August 2018, Revised and Accepted: 12 October 2018

ABSTRACT

Objective: The current study is to evaluate the antimicrobial, antioxidant, anti-inflammatory, and *in vitro* cytotoxicity activities of polyhydroxybutyrate (PHB) and to develop the herbal impregnated PHB cast film for wound healing activities using Albino Wistar rat model.

Methods: PHB produced by *Azotobacter chroococcum* A3 strain was synthesized and characterized (previous study). The PHB was subjected to various biocompatibility studies such as antimicrobial, antioxidant, and anti-inflammatory studies. The PHB was also subjected to cytotoxicity study by (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. PHB films were made using different combinations of plant and algal blends (herbal blends). The herbal blends of PHB films were evaluated for *in vivo* wound healing activity using Albino Wistar rats.

Results: The turmeric impregnated PHB showed the highest result for antimicrobial with *27.25±0.23 mm against skin pathogens* and antioxidant activity with the highest percentage of inhibition of 76%. The result predicts that PHB will not let to any toxic substances rather it acts as a chemoprotective agent followed by the inhibitory concentration value was found to be 1.56 µg/ml for 100 µg. The *in vivo* study showed better wound healing activity for PHB blended with 2% turmeric leaf and rhizome cast film. Whereas the wound healing activity of control and crude PHB was 90.4±0.4 and 91.3±0.56 respectively.

Conclusion: The results from the present study showed that PHB can act as a good candidate for drug carrier and it is biocompatible in living cells.

Keywords: Polyhydroxybutyrate, *Azotobacter chroococcum*, Biocompatibility, (3-[4,5-Dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay, Herbal blends, *In vivo* study.

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i2.29179

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, sling, cardiovascular patches, orthopedic pins, adhesion barriers, guided tissue repair, regeneration 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 tamoxifen (TAM), an anticancer drug and analyzed for the *in vitro* 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 Silbiol or benzoic acid [6]. The PHB was also used in packaging materials in which the antimicrobial activity of PHB/chitosan 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 citreonigrum* 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 tenture, 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-cohydroxyhexanoate) 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 diphenyl tetrazolium 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

Biocompatibility study

The biocompatibility study was done to examine whether the produced PHB was able to compate 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 denaturated 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:

$$\text{Inhibition (\%)}=(A_t-A_c)/A_c\times 100$$

Where, A_c=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 48 h, 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:

$$\text{\% cell viability}=\text{A570 of treated cells/A570 of control cells}\times100\%.$$

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 thermostable herbal product was mixed with PHB in the ratios 58:52 (w/w) and 100:20 (w/w), and solvent cast films were obtained. The plants turmeric and sago and macroalgae *Amphiroa fragilissima* and *Padina tetrastromatica* were used for the formulations. The formulations were used for antimicrobial and antioxidant studies and cast films of PHB with turmeric leaf, turmeric rhizome 1% and turmeric leaf, turmeric rhizome 2%, respectively, were used for wound healing activity study.

Antimicrobial activities of biofilm with PHB against skin pathogens

The antimicrobial activity was performed by Kirby *et al.* [13] method. Using sterile swabs, fresh overnight cultures of various skin pathogen bacterial cultures were swabbed onto Mueller-Hinton agar plates. Using different plant and algal formulation of PHB were impregnated as discs on to the plates. The plates were then incubated at 37°C for 12–24 h. The plates were observed for the zones of inhibition and results were recorded.

2,2-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of various herbs impregnated with PHB

The antioxidant property of the different herbal formulations has been determined by DPPH radical scavenging assay. This is done using the stable free radical DPPH, which get reduced in the presence of an antioxidant compound that, in turn, decreases absorbance ability of DPPH at 517 nm. The higher radical-scavenging activity of the herbal formulations was indicated by a lower absorbance at 517 nm. The scavenging activity of formulation and standard, ascorbic acid on DPPH was expressed as an IC_{50} value. The IC_{50} value was noted. The percentage of radical scavenging activity was calculated using the formula:

$$\text{\% inhibition}=[(AC-AT)/AC]\times100$$

Where AC and AT are the absorbance of blank and extract, respectively [14].

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was estimated according to the procedure stated in Mahendran *et al.* [14]. The equivalent concentration of antioxidant giving an absorbance increase in the FRAP assay is equivalent to the theoretical absorbance value of 1 mol/L concentration of Fe (II) solution. The absorbance was measured at 700 nm with a UV-visible spectrophotometer.

Hydrogen peroxide assay

H_2O_2 scavenging activity by SNP's was used to determine antioxidant ability. 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:

$$\text{\% inhibition}=[1-(\text{Absorbance of extract/Absorbance of control})]\times100$$

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 a 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/PO/EReBi/S/15/CPCSEA, dated: 14.09.2015), with the approval of Institutional Animal Ethics Committee (IAEC) (KSRCT/BT/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 3×3 cm were plastered on the wounded area twice daily. Percentage of wound contraction was calculated as:

Percentage of wound size=Wound area on day X/Wound area on day zero×100. Percentage of wound healing=100−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 IC_{50} 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).

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

PHB: Polyhydroxybutyrate, *A. chroococcum: Azotobacter chroococcum*

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)

PHB: Polyhydroxybutyrate

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 IC_{50} (Fig. 2). Highest IC_{50} 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 IC_{50} 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).

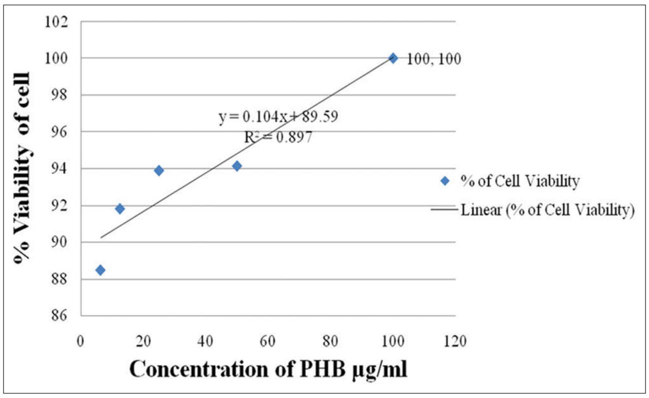

Fig. 1: (3-[4,5-Dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay - cell viability of polyhydroxy butyrate

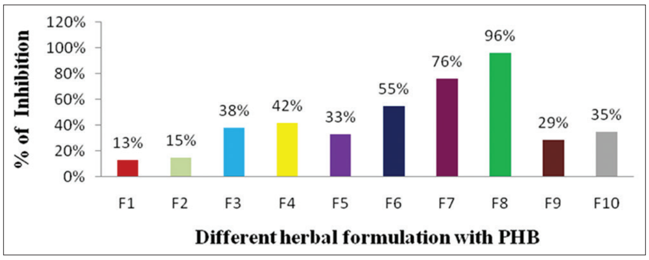

Fig. 2: 2,2-Diphenyl-2-picrylhydrazyl assay of hydrogen free radicals using herbs with polyhydroxybutyrate

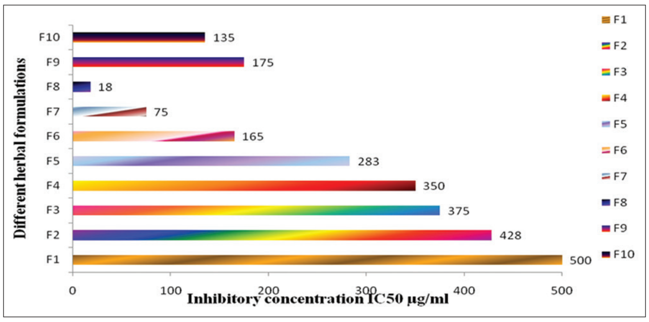

Fig. 3: Analysis of inhibitory concentration of polyhydroxybutyrate film

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

S. No	Zone of inhibition (mm±SD)				
	Formulation	Spp 1	Spp 2	Spp 3	Spp 4
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)	21.85±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	Chloramphenicol (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

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/mm^2, 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, 14^{th,} 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 IC_{50} value in MTT assay was 1.56 µg/ml showed that the livability 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 blendes degradation was carried out by Pachekoski *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*

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	*Amphiroa fragilissima*+PHB (F4)	14.27
5	*Padina tetrastromatica*+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	*Amphiroa fragilissima* (F9)	13.53
10	*Padina tetrastromatica* (F10)	13.87

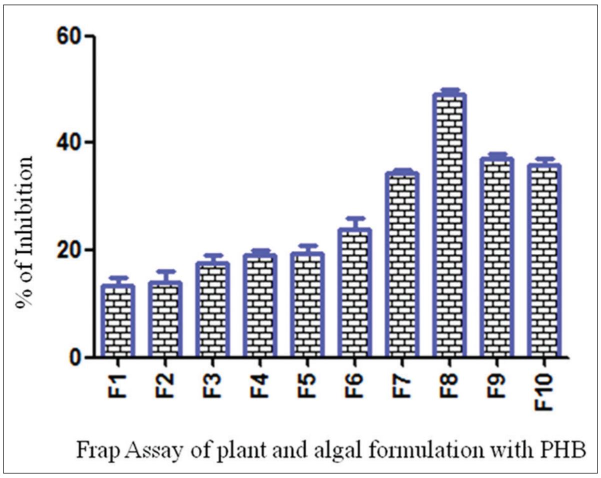

Fig. 4: Ferric reducing antioxidant power assay of the herbal formulation with polyhydroxybutyrate

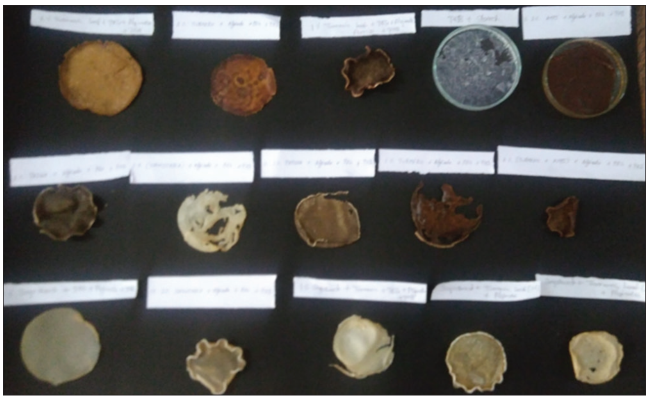

Fig. 5: Biofilm formulation using different herbal resources

Table 5: Mechanical properties of different formulation with PHB

Particulars of cast film	Tensile strength (N/mm²±SD)	Film elongation (%±SD)	Elastic module 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	13.84±0.001	178±0.5
F3 - Sago starch 2%+PHB	0.035±0.003	12.84±0.004	176±0.53
F4 - *Amphiroa fragilissima*+PHB	0.007±0.001	20.4±0.002	289±0.056
F5 - *Padina tetrastromatica*+PHB	0.011±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	0.039±0.04	25.7±0.008	325±0.027
F8 - *Amphiroa 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	8.33±2.2	19±2.8	28.6±0.9	32±0.89	46.2±0.006	68±0.006	79.5±0.07	90.4±0.4
PHB	9.83±1.2	18.6±0.06	32.8±0.29	43.5±0.04	54±0.036	71.9±0.22	80.14±0.52	91.3±0.56
Turmeric leaf 1%+PHB	19±1.25	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±0.83	49.82±0.29	69.8±0.26	83.7±0.08	90.8±0.33	96.5±0.47	98.8±0.21
Turmeric leaf 2%+PHB	22.4±0.84	37.3±0.39	53.92±0.28	76±0.33	84.44±0.52	92.46±0.49	96.58±0.62	100
Turmeric rhizome 2%+PHB	21±0.45	34.7±0.58	52.62±0.07	72±0.09	83.5±0.27	91.2±0.14	97.83±0.84	100

PHB: Polyhydroxybutyrate, SD: Standard deviation

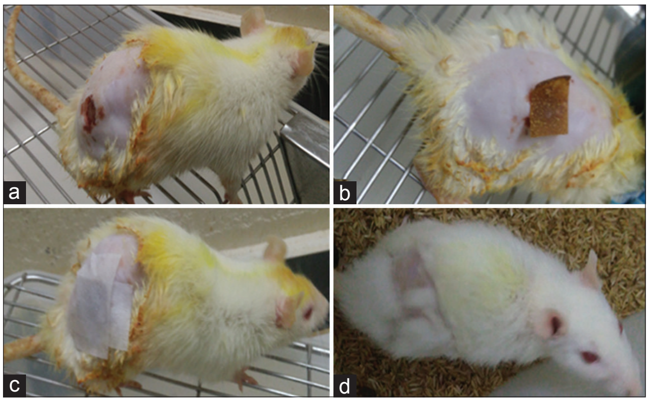

Fig. 6: Wound healing activity of turmeric blended with polyhydroxybutyrate (PHB) on rat model. (a) Incission of wound, (b) PHB with turmeric film, (c) sealed wound (a-c on day 1), and (d) healed wound on day 16

fritschii was 23Mpa with elongation break 5.5 and Young's modulus was 712Mpa [18]. The PHB produced in our study was entirely thin with F7 producing 0.39 Mpa TS and 25.7 elongation break and 325 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 combinational polymer covering the nucleus used in pearl culture could be detrimental for the environment. Tachyplesin, a marine peptide associated with bacterial exopolysaccharides act as a film agent and gave a same response as antibiotics in oyster mortality and nucleus rejection rates. Bacterial exopolysaccharides formed a strong coating on the nucleus and the tachyplesin incorporated into it showed, relatively a good antimicrobial property [20]. The conventional methods for wound healing were using the natural products having anti-inflammatory, antimicrobial, and antioxidant properties such as turmeric, neem, and honey the supportive biomaterials used so far were glucans, dextrans, chitosan, alginate, chondroitin, heparin, etc. [21].

In this study, apart from the regular biomaterials, PHB was used as a supportive material and in combination with plant and algal blends such as turmeric, sago starch, and seaweeds, but PHB alone plays no role in treating the wounds. However, turmeric rhizome with PHB yields very good result in treating the wounds, evaluated in the 10th and 16th days of the experiment. This type of trails was evaluated many times, but a combination of turmeric and PHB was not evaluated so for. A study by Kundu *et al.* [22] for the wound healing potential of turmeric rhizome in rabbits revealed that the contraction of wound area size at 14th day was 4.47% which was much better than the control at 62.5%. Another study with turmeric rhizome extract ointment revealed (RTO 6%) that the wound contraction was 96.99% on the 16th day and 100% on 18th day [15]. In our study, the turmeric rhizome with PHB and turmeric leaf with PHB showed 100% wound contraction on the 16th day, which was similar to the above studies. The antioxidant, antibacterial, and wound healing activity of several herbal plants were studied by Narendhirakannan *et al.* [23]. In their study, the IC_{50} of the antioxidant capacity of turmeric was 57.31 μg/ml and the standard ascorbic acid was 58.82 μg/ml, which was closer to the standard. In our study, the IC_{50} of the standard and turmeric rhizome with PHB was 18 and 75, respectively. The antioxidant capacity of turmeric rhizome with PHB was 76%, and standard was 96% in our study. The antimicrobial activity of turmeric extracts was nearer to the standard drug streptomycin, while in our study the turmeric rhizome with PHB and turmeric leaf with PHB showed promising results against skin pathogens with a maximum zone of inhibition was observed in turmeric rhizome with PHB with 27.55±0.43 against *Streptococcus pyogenes*, while the standard drug chloramphenicol showed 18.32±0.62. The study by

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 95.41±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 curcumin nanofiber mats (Cc/Glt NM) showed statistically significant 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 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 trail 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.

ACKNOWLEDGMENT

The authors are indebted to the Research and Development Centre of Bharathiar University, Coimbatore, Tamil Nadu, India, for the valuable suggestion and support.

AUTHORS' CONTRIBUTION

All the works were performed by Ram Narendran and manuscript was made ready by Ram Narendran, under the 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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