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IN-VITRO AND *IN-VIVO* STUDIES OF PHB CAST FILMS BLENDED WITH VARIOUS HERBS AND ITS BIOCOMPATIBLE CHARACTERIZATION

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Keywords:

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ABSTRACT: The present study deals with the PHB made cast film with different plant and algal formulations. Its biocompatibility was tested *in-vitro* and *in-vivo*. The anti-inflammatory activity was higher in F4 and F5, close to the standard Diclofenac. The F7 and F8 exhibited moderate cytotoxicity, and formulation F2 exhibited low cytotoxicity. The maximum zone of inhibition was exhibited in F5 followed by F4 against skin pathogens. The overall results of antioxidant activity indicated that F4, F5, and F8 have good antioxidant properties. Percentage (%) of wound contraction at the 10^{th} day of control was 16.9% and PHB alone was 21.3%. F5 and F3 showed higher activity with 51.7 and 49.7%, respectively. The self-healing potential was found to be 35.6%, but PHB was able to cure additional 5% (40.5) on 16^{th} day. F5, F3, and F4 showed high activity of 81.5, 80.1, and 79.9, respectively. The *in-vitro* and *in-vivo* studies showed that the *Centella asiatica* and *Padina tetrastromatica* with PHB has an effective activity towards antimicrobial, antioxidant, and wound healing activity. In order to identify the positive correlation between *in-vitro* and *in-vivo* study a multiple regression was performed. Data of the experiments were fitted to the model and supported by the multiple correlation coefficients (R^2) and the correlation of determination (adjusted R^2). The model was fit and statistically significant with the p-value (< 0.01) and the indication of positive regression between *in-vitro* and *in-vivo*. Thus *in-vitro* and *in-vivo* studies using *Centella asiatica* and *Padina tetrastromatica* showed positive and significant correlation.

INTRODUCTION: Poly hydroxybutyric acid (PHB), a storage compound widespread in prokaryotes enhances the survival of the cells in times of starvation. PHBs are accumulated under conditions of unbalanced growth in the form of insoluble inclusions (granules) and can amount up to 90% of the cellular dry matter.

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PHB is considered environmentally friendly materials because of their synthesis from renewable resources and their biodegradability [1]. PHB can be produced by certain microorganisms like (*Cupriavidus necator*, *Methylobacterium rhodesianum*, or *Bacillus megaterium*), which can be grown under physiological stress conditions with a limited number of nutrients.

They are mostly water-insoluble in nature and relatively resistant to hydrolytic degradation. They are oxygen permeable and biocompatible in nature. PHB being intracellular, isolation and purification are major steps in the downstream process of the polymer.

Extraction of PHB using organic solvents such as chloroform, dichloromethane methylene chloride and dichloroethane were the most commonly used organic solvents [2]. The PHB has a molecular weight ranging from 50-1600 kDa. It has various structures with a C=O stretch of the ester group and -CH stretch present in the molecular chain. These structures can be identified by FTIR. While visualizing under UV, it has a peak at 235 nm [3].

Azotobacter accumulates a large amount of intracellular insoluble energy storage materials PHB in a minimal salt medium supplemented with excess glucose and poor O_2 and nutrient supply. In most of the studies, PHB production from *Azotobacter* was carried out by the batch method [4]. PHB has a broad application in the development of implanted medical devices for craniomaxillofacial, dental, orthopaedic, hernioplastic and skin surgery. It is a system of sustained drug delivery on the basis of PHB films. Investigations were carried for improving the bioavailability of materials using various biodegradable polymers like PEG, PLGA, and PCL. Biopolymer is exploited for its excellent aqueous solubility, flexible polymer chain, and low toxicity [5]. The innovation of medical devices on the basis of biopolymers by encapsulating of various drugs has wide prospects insignificance of these new devices with pharmacological activity in medicine.

The novel systems for sustained delivery of antproliferative drug dipyridamole (DP) and anti-inflammatory drug indomethacin has been developed. The development of antimicrobial packaging films is required to extend the shelf life of foods and to reduce the health risks caused by foodborne pathogens. Biodegradable active packaging films made with PLA and PHB blends containing D-limonene, a natural terpene, were shown to be transparent and flexible with enhanced oxygen barrier and water-resistant properties. The antimicrobial efficacy of the vanillin precoated PHB film was investigated against food-contaminating bacterial and fungal pathogens. A wound is an injury or damage on the surface of the skin by means of any physical, chemical, mechanical and thermal damages. It is the disruption and impairment of the integrity of anatomical tissues that accidentally occurred by exposure to any external factor [6].

Studied that the modern bandage material made of electrospun biopolymers contain various active compounds that are beneficial to the healing of wounds [7]. Should appear for the next sentence as 7proved that NIH 3T3 fibroblast cells adhere and proliferate on PHA membranes. Mesenchymal stem cells adhere and proliferate on several PHA substrates, with a terpolymer PHB co-hydroxyvalerate cohydroxyhexanoate. PHB matrices have also been tested for hemocompatibility with mammalian blood incubated with polymer films. Thus with all these positive and affordable characteristics, the present study deals with the production of PHB from *Azotobacter* and the PHB was made to cast film with herbals and its biocompatibility was tested *in-vitro* and *in-vivo* to be acted as a good drug carrier.

MATERIALS AND METHODS:

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. A small amount of the paste was taken and mixed with PHB in the ratios 58:52 (w/w) to which the formulations were made with different plant and algal products. The formulations were used for antimicrobial and antioxidant studies. The remaining prepared 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 [8]. The formulations were used for the *in-vitro* studies. The cast films of PHB were used for wound healing activity study.

***In-vitro* Studies using PHB with Plant and Algal Formulation:** The antimicrobial activity was performed by Kirby WMM *et al.,* 1966 [9]. The antioxidant property of the different plant and algal formulations was estimated according to the procedure stated by Mahendran *et al.,* [10] using DPPH radical scavenging assay, FRAP assay, Hydrogen peroxide scavenging assay, SOD assay, CUPRAC assay and ammonium molybdate assay. Anti-inflammatory study of PHB using egg albumin and diclofenac was performed. MTT assay was carried out by Mosmann *et al.,* [11].

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 X 70 mm, strips were clamped between two tensile grips, and the initial guage 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 (%). Ten measurements for each film sample were used for tests, and values were determined by the mean.

Wound Healing Activity of PHB Cast Film Impregnated with Various Herbs on *in-vivo* Model: The experiments were performed as in Farahpour *et al.,* [12]. 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 the Institutional Animal Ethics Committee (IAEC) (KSRCT/BT/ IAEC/2018/27) of KSR College of Technology, Tiruchengode, Tamil Nadu.

Induction of Excision Wound Model: The experimental animals were grouped into six. Each group has six animals each, and the experimental procedure was carried out by the following design.

Group I: Control (Untreated),

Group II: PHB biofilm,

Group III: *Centella asiatica* 2% + PHB based biofilm,

Group IV: *Centella asiatica* 2% + PEG + PHB based biofilm,

Group V: *Centella asiatica* 2% and *Padina tetrastromatica* + PHB based biofilm,

Group VI: *Centella asiatica* 2% and *Amphiroa fragilissima* + PHB based biofilm,

Group VII: Padina 2% + Alginate +PEG + PHB based biofilm,

Group VIII: Amphiroa 2% + Alginate +PEG + PHB based biofilm,

Group IX: *Padina tetrastromatica* 2% and *Amphiroa fragilissima* 2% + PHB based biofilm,

Group X: *Padina tetrastromatica* 2% and *Amphiroa fragilissima* 2% + Alginate + PHB based biofilm,

Group XI: *Padina tetrastromatica* 2% and *Amphiroa fragilissima* 2% + PEG + PHB based biofilm.

Statistical Analysis: To determine the correlation between *in-vitro* and *in-vivo* effect of various formulations with PHB a multiple regression were performed.

RESULTS:

PHB Plant and Algal Cast Film Preparation: The PHB formulation was prepared with different concentrations of *Centella asiatica*, marine seaweeds blended with PHB. Different formulations were made with various shapes of mold like a circle, triangle, *etc*., based on the property and utilization of the film and further, subjected to biomedical properties. The formulations of PHB with various plant and algal blends are presented in **Table 1**.

TABLE 1: PLANT AND ALGAL FORMULATION WITH PHB

S. no.	Formulations
1	PHB **(F1)**
2	*Centella asiatica* 2% + PHB (F2)
3	*Centella asiatica* 2% + PEG + PHB (F3)
4	*Centella asiatica* 2% and *Padina tetrastromatica* + PHB (F4)
5	*Centella asiatica* 2% and *Amphiroa fragilissima* + PHB (F5)
6	Padina 2% + alginate +PEG + PHB (F6)
7	Amphiroa 2% + alginate +PEG + PHB (F7)
8	*Padina tetrastromatica* 2% and *Amphiroa fragilissima* 2% + PHB (F8)
9	Standard - Chloramphenicol, Ascorbic acid, Diclofenac (F9)
10	*Padina tetrastromatica* 2% and *Amphiroa fragilissima* 2%+ Alginate + PHB (F10)
11	*Padina tetrastromatica* 2% and *Amphiroa fragilissima* 2%+ PEG + PHB (F11)

Mechanical Property of PHB Plant and Algal Cast Film: The PHB (F1) had 0.005 ± 0.001 N/mm^2, whereas the highest tensile strength was found in F4, F5, and F10 with 0.85, 0.82, and 0.79 N/mm^2 respectively. Percent elongation at break was calculated based on the length extended and original length of the films. The percentage of elongation was found maximum in F4 and F5 also with Young's modulus with 395 ± 0.036 and 388 ± 0.026, respectively **Table 2**.

TABLE 2: MECHANICAL PROPERTIES OF DIFFERENT FORMULATION WITH PHB

Particulars of cast film	Tensile strength (N/mm^2 ± SD)	Film elongation (% ± SD)	Elastic module of the film (Mpa ± SD)
F1-PHB	0.005 ± 0.001	22.6 ± 0.002	294 ± 0.036
Centella asiatica 2% + PHB (F2)	0.59 ± 0.003	22.84 ± 0.001	320 ± 0.5
Centella asiatica 2% + PEG + PHB (F3)	0.72 ± 0.003	24.84 ± 0.004	335± 0.53
Centella asiatica 2% and *Padina tetrastromatica* + PHB (F4)	0.85 ± 0.005	27.4 ± 0.002	395 ± 0.036
Centella asiatica 2% and *Amphiroa fragilissima* + PHB (F5)	0.82± 0.002	26.6± 0.004	388± 0.026
Padina 2% + alginate +PEG + PHB (F6)	0.78 ± 0.04	25.3 ± 0.007	322 ± 0.032
Amphiroa 2% + alginate +PEG + PHB (F7)	0.65 ± 0.04	24.7 ± 0.008	370 ± 0.027
Padina tetrastromatica 2% and *Amphiroa fragilissima* 2% + PHB (F8)	0.62± 0.004	23.8± 0.002	286 ± 0.056
Padina tetrastromatica 2% and *Amphiroa fragilissima* 2%+ Alginate + PHB (F9)	0.71 ± 0.006	24.6 ± 0.004	295 ± 0.086
Padina tetrastromatica 2% and *Amphiroa fragilissima* 2%+ PEG + PHB (F10)	0.79 ± 0.04	23.3 ± 0.007	272 ± 0.032

Antibacterial Activities of Plant and Algal Formulation with PHB against Skin Pathogens the Antibacterial Property of PHB Blended Formulation was Carried out against Clinical Pathogens: (*Klebsiella pneumonia* 5546, *Streptococcus pyogenes* 5280, *Enterococcus* sp. 5335, *Corynebacterium* sp. 2640)**.** *Centella asiatica* 2% and *Amphiroa fragilissima* + PHB (F5) showed maximum zone of inhibition *against clinical* test strains followed by *Centella asiatica* 2% and *Padina tetrastromatica* + PHB (F4) whereas other formulations revealed less activity towards the antibacterial property. The PHB showed less antimicrobial activity with 07.09 ± 0.25 mm against *Klebsiella pneumonia* **Table 3**. The mean average zone of inhibition of standard drug Chloramphenicol against selected pathogens was 24.17 ± 0.42 mm.

TABLE 3: ANTIBACTERIAL ACTIVITIES OF HERBAL FORMULATIONS WITH PHB AGAINST SKIN PATHOGENS

S. no.	Zone of Inhibition (mm ± SD)				
	Formulation	Spp1	Spp2	Spp3	Spp4
1	Control only PHB (F1)	07.09 ± 0.25	9.11 ± 0.33	9.27 ± 0.13	10.15 ± 0.72
2	*Centella asiatica* 2% + PHB (F2)	19.14 ± 0.23	21.05 ± 0.76	22.89 ± 0.27	19.02 ± 0.03
3	*Centella asiatica* 2% + PEG + PHB (F3)	18.79 ± 0.44	19.27 ± 0.07	19.09 ± 0.28	20.60 ± 0.49
4	*Centella asiatica* 2% and *Padina tetrastromatica* + PHB (F4)	21.23 ± 0.28	20.79 ± 0.05	19.96 ± 0.28	19.80 ± 0.03
5	*Centella asiatica* 2% and *Amphiroa fragilissima* + PHB (F5)	22.92 ± 0.66	23.11 ± 0.26	21.39 ± 0.83	20.78 ± 0.23
6	Padina 2% + alginate + PEG + PHB (F6)	18.25 ± 0.03	18.12 ± 0.37	17.89 ± 0.53	18.57 ± 0.93
7	Amphiroa 2% + alginate +PEG + PHB (F7)	18.56 ± 0.22	18.81 ± 0.43	19.04 ± 0.63	19.20 ± 0.63
8	*Padina tetrastromatica* 2% and *Amphiroa fragilissima* 2% + PHB (F8)	19.24 ± 0.26	18.62 ± 0.35	20.19 ± 0.53	21.35 ± 0.13
9	Standard - Chloramphenicol, (F9)	23.07 ± 0.62	22.93 ± 0.51	23.43 ± 0.62	24.17 ± 0.42
10	*Padina tetrastromatica* 2% and *Amphiroa fragilissima* 2% + Alginate + PHB (F10)	18.12 ± 0.63	17.94 ± 0.29	18.28 ± 0.44	18.32 ± 0.54
11	*Padina tetrastromatica* 2% and *Amphiroa fragilissima* 2% + PEG + PHB (F11)	15.24 ± 0.26	15.62 ± 0.35	17.19 ± 0.53	17.35 ± 0.13

Spp 1-*Klebsiella pneumonia* 5546, spp 2-*Streptococcus pyogenes* 5280, spp 3-*Enterococcus* sp. 5335, spp 4 -*Corynebacterium* spp. 2640

Anti-inflammatory Study: The anti-inflammatory study of PHB formulations of different plants and algae were compared with the commercial drug (Diclofenac). The results indicate that the formulations F2 and F3 showed higher activity of 54 and 52%, respectively, while the standard drug diclofenac showed 58% inhibition of albumin protein denaturation **Table 4**.

MTT Assay: The cytotoxicity of the PHB had been evaluated against HEK 293 cell lines at various concentrations ranging from 6.25-200 µg/ml **Fig. 1**.

The result indicated that standard drug poses IC_{50} at 24.45 µg/ml whereas formulation F9 and F10 exhibited moderate activity at above 65 µg/ml. Further, the PHB formulated with *Centella asiatica* 2% and *Padina tetrastromatica* + PHB (F4) showed activity at 120 µg/ml and PHB alone (F1) had higher IC_{50} above 200 µg/ml which implies that even higher concentration of PHB has no cytotoxic effect. The cell viable percentage also increased at minimal concentrations of PHB, such as 6.25, 12.5 µg/ml.

The results suggested that PHB can be formulated along with herbal ingredients to improve the stability and quality of biological products.

TABLE 4: ANTI-INFLAMMATORY STUDY OF PHB

S. no.	Formulations	Inhibition (%)
1	PHB (F1)	45%
2	*Centella asiatica* 2% + PHB (F2)	54%
3	*Centella asiatica* 2% + PEG + PHB (F3)	52%
4	*Centella asiatica* 2% and *Padina tetrastromatica* + PHB (F4)	49%
5	*Centella asiatica* 2% and *Amphiroa fragilissima* + PHB (F5)	50%
6	Padina 2% + alginate + PEG + PHB (F6)	49%
7	Amphiroa 2% + alginate + PEG + PHB (F7)	41%
8	*Padina tetrastromatica* 2% and *Amphiroa fragilissima* 2% + PHB (F8)	43%
9	Standard - Diclofenac (F9)	58%
10	*Padina tetrastromatica* 2% and *Amphiroa fragilissima* 2% + Alginate + PHB (F10)	47%
11	*Padina tetrastromatica* 2% and *Amphiroa fragilissima* 2% + PEG + PHB (F11)	39

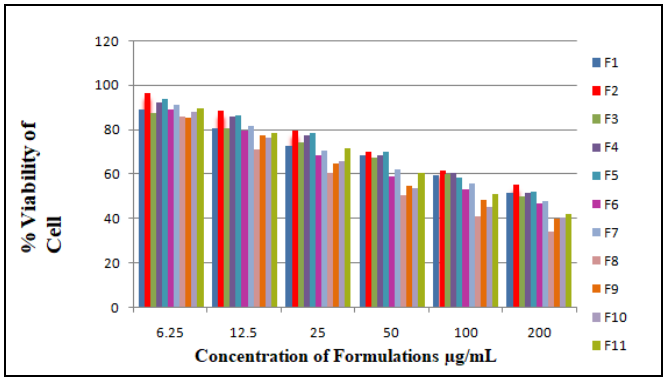

FIG. 1: MTT ASSAY OF DIFFERENT FORMULATIONS OF PHB

Antioxidant Capacity of Various Herbs Impregnated with PHB: Antioxidant activity of different formulations was studied to screen the biological potential of biofilms using the DPPH assay, FRAP assay, SOD assay and CUPRAC assay **Fig. 2**. In DPPH assay *Centella asiatica* 2% and *Amphiroa fragilissima* + PHB (F5) showed the highest percentage of inhibition followed by *Padina tetrastromatica* 2% and *Amphiroa fragilissima* 2% + PHB (F8). FRAP assay indicates that the higher the absorbance higher, the reducing capacity of free radicals. PHB formulated with *Padina tetrastromatica* 2% and *Amphiroa fragilissima* 2% + PHB (F8) showed higher activity followed by F5.

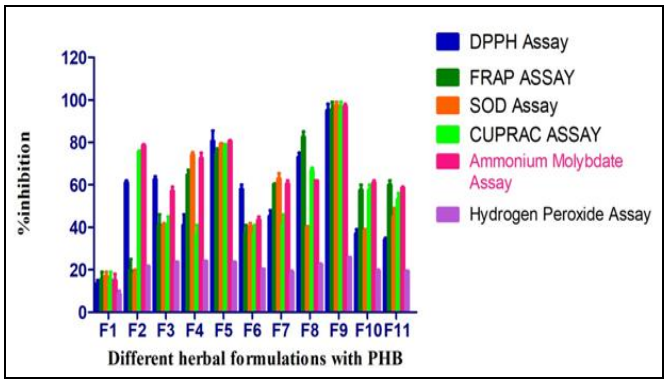

FIG. 2: ANTIOXIDANT PROPERTIES OF DIFFERENT HERBAL FORMULATIONS WITH PHB

Superoxide dismutase assay was performed to understand the production capacity of antioxidant or defense enzymes of herbal formulations. The result indicates that F5 formulation showed a better reduction or highest production of defense

enzymes, nearly 79.5% followed by the formulation F4. Reducing the power of cupric ion directly influences the neutralizing capacity of bioactive substances present in the different formulations. Remaining all other formulation showed the moderate potential of free radical capturing power. The result revealed that F5 formulation showed the highest potential of reducing the capacity of 79% followed by formulation F2.

Ammonium Molybdate assay revealed the F5 formulation of *Centella asiatica* 2%, and *Amphiroa fragilissima* + PHB exhibited 81% inhibition followed by F4 and F2. Standard Ascorbic acid showed a better reducing capacity of more than 96%. Maximum inhibitory concentration was observed H_2O_2 activity with F4 followed by F5, F8 and F2. Ascorbic acid exhibited that moderate potential of H_2O_2 activity of 25.78%. The overall results indicated that F5, F4, and F8 have good antioxidant properties.

Wound Healing Activity of PHB Cast Films on *In-vivo* Model: The natural or self-healing potential was considered as control. Percentage (%) of wound contraction was measured on the 4^{th}, 8^{th}, 10^{th}, $12^{th,}$ and 16^{th}-day post wounding days; at the 10^{th} day, the healing % of control was 16.9%, and PHB alone was 21.3%. *Centella asiatica* 2% and *Amphiroa fragilissima* + PHB with PHB (F5) and *Centella asiatica* 2% + PEG + PHB (F3) showed higher activity with 51.7 and 49.7% respectively. The control cured the wound to 35.6%, and PHB alone cured to 40.5% on the 16^{th} day. F5, F3, and F4 showed high activity of 81.5, 80.1, and 79.9, respectively **Table 5**.

TABLE 5: *IN-VIVO* MODEL OF THE WOUND HEALING EFFECT OF VARIOUS FORMULATIONS WITH PHB

Particulars of cast film	Degree of contraction (% ± SD)				
	No. of days				
	4	8	10	12	16
Control	4.1 ± 1.8^{a}	12.8 ± 0.77^{a}	16.9 ± 0.05^{a}	25.4 ± 0.09^{a}	35.6 ±0.7^{a}
PHB	4.6 ± 0.6^{ab}	13.4 ± 0.09^{b}	21.3 ± 0.92^{b}	27.5 ± 0.03^{b}	40.5 ± 0.37^{b}
Centella asiatica 2% + PHB	17.6 ± 0.02^{c}	37.5 ± 0.60^{fg}	45.5 ± 0.04^{de}	60.4 ± 0.42^{fg}	78.9 ± 0.06^{g}
Centella asiatica 2% + PEG + PHB	22.8 ± 0.56^{i}	39.8 ± 0.63^{hi}	49.7 ± 0.06^{gh}	63.9 ± 0.60^{i}	80.1 ± 0.18^{hi}
Centella asiatica 2% and *Padina tetrastromatica* + PHB	21.3 ± 0.93^{h}	36.7 ± 0.33^{f}	45.45 ± 0.52^{cd}	62.64 ± 0.45^{h}	79.9 ± 0.14^{h}
Centella asiatica 2% and *Amphiroa fragilissima* + PHB	23.2 ± 0.59^{ij}	42.5 ± 0.05^{j}	51.7 ± 0.78^{j}	64.2 ± 0.22^{ij}	81.5 ± 0.12^{j}
Padina 2% + alginate +PEG + PHB	18.4 ± 0.64^{de}	31.5 ± 0.08^{c}	46.7 ± 0.46^{fg}	57.5 ± 0.85^{d}	74.5 ± 0.24^{f}
Amphiroa 2% + alginate +PEG + PHB	18.1 ± 0.58^{cd}	32.9 ± 0.12^{d}	49.7 ± 0.58^{hi}	59.9 ± 0.02^{ef}	72.1 ± 0.37^{cd}
Padina tetrastromatica 2% and *Amphiroa fragilissima* 2% + PHB	18.7 ± 0.37^{ef}	33.5 ± 0.02^{e}	44.3 ± 0.42^{c}	55.7 ± 0.39^{c}	71.5 ± 0.34^{c}
Padina tetrastromatica 2% and *Amphiroa fragilissima* 2%+ Alginate + PHB	19.7 ± 0.66^{fg}	38.9 ± 0.14^{gh}	46.1 ± 0.39^{ef}	58.23 ± 0.64^{de}	73.28 ± 0.42^{e}
Padina tetrastromatica 2% and *Amphiroa fragilissima* 2%+ PEG + PHB	19.7 ± 0.66^{j}	38.9 ± 0.14^{j}	46.1 ± 0.39^{j}	58.23 ± 0.64^{j}	70.28 ± 0.42^{j}

All values are expressed in as Mean ± SD (n=6), superscript in each row indicates a significant difference ($P<0.05$) when compared to control

Multiple Regressions of *In-vitro* and *In-vivo* Studies: The correlation between two variables and the relationship between the two tests was analyzed using multiple regression analysis. The *in-vitro* and *in-vivo* studies showed that the formulations with PHB have an effective activity towards anti-microbial, antioxidant, and wound healing activity. In order to identify the positive and perfect correlation between *in-vitro* and *in-vivo* study, a multiple regression was performed. The multiple regressions reveal a significant correlation with the p-value less than 0.5 between *in-vitro* and *in-vivo* studies **Table 6**. Data of the experiments were fitted to the model and supported by the multiple correlation coefficients (R^2) and the correlation of determination (adjusted R^2). The R^2 of 0.997 is in reasonable agreement with the adjusted R2 of 0.996 ± 0.004, with the residual value of 0.433 **Table 7**.

The model was fit and statistically significant with the p-value less than 0.01 **Table 8** and the indication of positive regression between in vitro and *in-vivo*. The association between *in-vitro* and *in-vivo* studies shows that the antimicrobial and

antioxidant studies have significant effects on wound healing activity. The regression coefficient has a significant effect on *in-vitro* studies, and the lack of fit was not significant.

TABLE 6: MULTIPLE REGRESSIONS ASSOCIATION BETWEEN *IN-VITRO* AND *IN-VIVO* STUDIES

Model	Coefficients value	T	Sig.
(Constant)	2.429E-5	-0.028	0.978
Antimicrobial	-0.671	-7.432	0.244
Ammonium molybdate	0.018	1.585	0.129
Hydrogen peroxide	-0.628	-3.452	0.311
SOD	-0.626	-1.094E6	0.0001
DPPH	-0.626	-1.094E6	0.0001
FRAP	1.410	100.832	0.0001

TABLE 7: SUMMARY OF MULTIPLE REGRESSION ANALYSIS OF *IN-VITRO* AND *IN-VIVO* STUDIES

Model	R	R. square	Adjusted R. square	Std. error of the estimate
	0.989[a]	0.997	0.996	0.004

TABLE 8: ANOVA OF MULTIPLE REGRESSIONS OF *IN-VITRO* AND *IN-VIVO* STUDIES

Model	Sum of squares	Df	Mean square	F	Sig.
Regression	9.235	6	4.617	3.12	Significant
residual	0.433	53	0.213	5E5	
total	8.802	59			

DISCUSSION: Poly B-hydroxybutyric acid (PHB) an intracellular microbial thermoplastic produced widely and accumulated in many bacteria. In certain applications, PHB can directly replace non biodegradable polymers. Hence, use of PHB primarily as polymer blends are, therefore, becoming quite important; such blends greatly increases the spectrum of possible utility.

In this current investigation, apart from the regular biomaterials, PHB was used as a supportive material and in combination with plant and algal blends such as *Centella asiatica*, alginate and Seaweeds. Wound healing is a sequence of events that is initiated by the stimulus of injury to the tissues. A positive stimulus may result from the release of some factors by the wounding of tissues. It has been noted that plants have high cicatrizing and vulnerary properties. Wound healing materials should have good antibacterial activity, and impairment at the cellular level may be prevented by different antioxidants, which reduce damage caused by free radicals [13]. Active components of turmeric have an important role in the treatment and / or have a supporting role in various inflammatory conditions including arthritis, bronchitis, fever, diarrhea, and relieve pain, anti-cancer effects, would healing effect [14]. The statistical analysis between *in-vitro* and *in-vivo* studies revealed that there is a positive correlation between them. The multiple regressions reveal a significant correlation with the p-value of less than 0.5 between *in-vitro* and *in-vivo* studies with antimicrobial activity was significant with a p-value of 0.244 and antioxidant activity such as DPPH, FRAP and SOD were highly significant with p-value of 0.0001. This implies that the herbal formulations having good antimicrobial and anti-oxidant activity that enhances the wound healing activity. Thus, PHB impregnated with different herbal formulations has been an efficient candidate in wound healing.

The study by Narendhirakannan [15] showed that the turmeric extracts had good antioxidant and antimicrobial activity. The turmeric extract also had wound healing activity of about 60% on the 9th day when compared with the control (18%). From his study, he suggests that the constituents like triterpenoids, alkaloids, and flavonoids of the turmeric are known to promote the wound healing process mainly due to their antioxidant and antimicrobial property, which may be responsible for wound reduction and increased formation of epithelialisation. Thus in our study, it was revealed that the *in-vitro* and *in-vivo* studies using *Centella asiatica* 2% and *Padina tetrachromatic* with PHB had a positive correlation between them.

The conventional method used for wound healing was using natural products having anti-inflammatory, antimicrobial, and antioxidant properties such as turmeric, Neem, Honey, *etc*. The supportive biomaterials used so far were glucans, dextrans, chitosan, alginate, chondroitin, Heparin, *etc.* [16]. In the present study, the PHB was used as the supportive biomaterial for sustained drug release. PHB fibers have excellent long-term stability and are highly recommended for the skin covering, wound healing, and supportive therapy of bacterial and fungal infection [17].

The herbs proved to be effective in wound healing, and PHB showed as an inert material hence which can be used as a scaffold along with herbals and algal formulations having a better antioxidant

property for fast recovery; PHB is a biopolymer which will not hurt or enhance the cell-mediated growth since we have made a trial with testing our PHB in *in-vitro* analysis on cytotoxicity, the result showed PHB has no impact on cell growth.

CONCLUSION: Different herbal formulations with PHB were prepared as a cast film to elucidate the biological activities such as antimicrobial, antioxidant, and wound healing property. The correlation of *in-vitro* and *in-vivo* was analyzed and showed that there is a positive correlation between them. The results of the present study indicated that the cast film of PHB has effective antimicrobial activity and anti-inflammatory activity, which implies that it has a good wound healing property.

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REFERENCES:

1. Pfeiffer D and Jendrossek D: Pham is the physiological activator of poly (3-hydroxybutyrate) (PHB) synthase (phac1) in *Ralstonia eutropha*. Applied and Environmental Microbiology 2014; 80: 555-63.
2. Verlinden RA, Hill DJ, Kenward MA, Williams CD and Radecka I: Bacterial synthesis of biodegradable polyhydroxyalkanoates. Journal of Applied Microbiology 2007; 102(6): 1437-49.
3. Bonartsev A, Yakovlev S, Boskhomdzhiev A, Zharkova I, Bagrov D, Myshkina V and Zhuikov V: The terpolymer produced by *Azotobacter chroococcum* 7B: effect of surface properties on cell attachment. PLOS One 2013; 8: 57200.
4. Chee JY, Yoga SS, Lau NS, Ling SC, Abed RM and Sudesh K: Bacterially produced polyhydroxyalkanoate (PHA): converting renewable resources into bioplastics. Current Research Technology and Education Topics in Applied Microbiology and Micro Biote 2010; 2: 1395-04.
5. Bose S, Sarkar N and Banerjee D: Effects of PCL, PEG and PLGA. Chem 2018; 8: 110-20.
6. Payam Z, Rezaeian I, Ranaei-Siadat SO, Jafari SH and Supaphol P: A review on wound dressings with an emphasis on electrospun nanofibrous polymeric bandages. Polymers for Advanced Technologies 2010; 21: 77-95.
7. Shishatskaya EI and Volova TG: A comparative investigation of biodegradable polyhydroxyalkanoate films as matrices for *in-vitro* cell cultures. Journal of Materials Science Materials in Medicine 2004; 15: 915-23.
8. Narendran RR, Begum SFM and Rubavathi S: Development and characterization of biocompatible polyhydroxy butyrate impregnated with herbal plants against wound healing activity on *in-vivo* animal model. Asian J of Pharma and Clinical Res 2019; 12: 196-01.
9. Kirby WMM, Bauer AW, Sherris JC and Turck M: Antibiotic susceptibility testing by a standardized single disk method. Americ J of Clini Patho 1966; 45(4): 493-96.
10. Mahendran G, Manoj M, Prasad KR and Bai VN: Antioxidants, anti-proliferative, anti-inflammatory, anti-diabetic and anti-microbial effects of isolated compounds from *Swertia corymbosa* (Grieb.) wight ex cb clark an *in-vitro* approach. Food Sci and Hum Welln 2015; 4: 169-79.
11. Mosmann T: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J of Immunological Methods 1983; 65(1-2): 55-63.
12. Farahpour MR, Emami P and Ghayour SJ: *In-vitro* antioxidant properties and wound healing activity of hydroethanolic turmeric rhizome extract (Zingiberaceae). International J of Phar and Pharmac Scie 2014; 6: 474-78.
13. Niranjan R, Kaushik M, Selvi RT, Prakash J, Venkataprasanna KS, Prema D, Pannerselvam B and Venkatasubbu GD: PVA/SA/TiO2-CUR patch for enhanced wound healing application: *in-vitro* and *in-vivo* analysis. Int J of Biol Macro-molecules 2019; 138: 704-17.
14. Jagetia GC and Rajanikant GK: Acceleration of wound repair by Curcumin in the excision wound of mice exposed to different doses of fractionated γ radiation. International Wound Journal 2012; 9: 76-92.
15. Narendhirakannan RT, Nirmala JG, Caroline A, Lincy S, Saj M and Durai D: Evaluation of antibacterial, antioxidant and wound healing properties of seven traditional medicinal plants from India in experimental animals. Asian Pacific J of Tropic Biom 2012; 2: 1245-53.
16. Mogosanu GD and Grumezescu AM: Natural and synthetic polymers for wounds and burns dressing. International Journal of Pharmaceutics 2014; 463: 127-36.
17. Kundrat V, Matouskova P and Marova I: Facile preparation of porous microfiber from poly-3-(r)-hydroxybutyrate and its application. Mat Bas 2019; 13: 1-14.

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