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Research Article

Formulation and Quality Evaluation of Films Prepared from Ethanolic Extract of *Carica Papaya* – Gelatin Composite for Wound Healing Treatment: *In-Vitro* Studies

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ABSTRACT

The objective of this research paper was to evaluate wound healing films prepared from a composition of gelatin- *Carica papaya* extract using methods for wound healing purposes. Five films (F1-F5) were prepared from *Carica papaya* extract (100–500 mg) and gelatin (1000 mg). The casting technique was used to formulate the films. The films were evaluated for pH value, thickness, water absorption capacity, folding endurance, tensile strength and extract release properties. Increasing the extract of *Carica papaya* in the formulated film caused significant increases ($p \leq 0.05$) in most evaluated parameters (Film thickness, water absorption capacity, folding endurance, tensile strength and extract release). However, increasing the extract of *Carica papaya* caused significant decreases ($p \leq 0.05$) in the pH values of the films. Therefore, these results revealed that *Carica papaya* /Gelatin films could be a promising pharmaceutical candidate for wound healing treatment.

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INTRODUCTION

Drug delivery is well recognized as the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals [1-3]. The selection of a suitable biopolymer matrix for wound healing purposes should be of prime properties in preparation of wound dressings [4]. Also it was reported that the ideal wound dressing should have good mechanical strength, hydrophilicity, antibacterial property and biocompatibility [5, 6]. Biodegradable polymers such as gelatin have been used for this purposes due to their pharmaceutical properties such as targeting of specific sites, degradation within an acceptable period and release of the drug in a predetermined manner [7-11].

Carica papaya is considered one of the most popular plants in the world. Many parts of *Carica papaya* have been utilized as ingredients in folk medicine. The leaf of *Carica papaya* was investigated for many pharmaceutical activities such wound healing [12-15], antifungal and antibacterial [16], Biochemical, hematological and toxicological effect [17], diabetes treatment [18] and as skin anti-aging effects [19]. Several *in-vivo* and *in-vitro* studies have conducted on medicinal properties of the extracts of different parts of papaya including anti-inflammatory [20], anti-plasmodial [21], anti-dengue [22], antioxidant [23-27] and anti- Alzheimer's disease [28]. Therefore, this study was aimed at formulation and quality evaluation of films prepared from *Carica* papaya – gelatin composite for wound healing application.

MATERIALS AND METHODS

Collection of Papaya Leaves and Preparation of the Extract

Papaya leaves were collected from the farm located at Negri Simbilan, Selangor, Malaysia (Asia Fruits Sdn. Bhd) (Photo 1). The leaves were washed, spread on a clean table and left to dry at room temperature. Gelatin type B was purchased from Sigma Co., Malaysia.

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Photo 1: Collection of Fresh Papaya Leaves from the Farm with the assistance of the Farmer

Preparation of the Films

Carica papaya leaves were collected and extracted using ethanolic aqueous method (20% ethanol: 80% water). The dry leaves were placed in an oven set at 45°C and left for 7 days to fully dry. The dry leaves were broken by hands into small pieces and blended in a Waring blender set at high speed for 20 min. A modified method of aqueous extraction [29] was conducted using ethanol and water in a ratio of 20:80, respectively. 20 g of the dry blended leaves were placed in a 1000 mL flask and the mixture of ethanol in water was added in the ratio of 1:20 (blended leaves: a mixture of ethanol in water, respectively). The flask was covered with aluminum foil and left till the next day. The mixture was filtered through filter paper no.1 using a funnel. The filtrate was collected and rotor vaporized to remove the water. The extract was then freeze - dried using freeze dryer model (Scanvac Coolsafe, RZ 2.5, Germany) and the extract in the form of a powder was obtained. The extract was placed in a clean dry bottle and covered using paraffin lamination.

Preparation of the Wound Healing Films

The solvent casting technique described by Tanwar 2005 [30] was used to prepare the wound healing films according to the protocol in Table 1.

Table 1: Composition of Different Wound Healing Films

Film No.	Film code	Gelatin (mg)	*Carica papaya* (mg)
1	F1	1000	100
2	F2	1000	200
3	F3	1000	300
4	F4	1000	400
5	F5	1000	500

The extract of *Carica papaya* and the bovine skin gelatin were mixed thoroughly individually using deionized water. Polyethylene glycol (PEG) was added (0.2 ml) as a plasticizer to all film formulations. Filtration under vacuum technique was used to remove any entrapped air bubbles. The mixtures of the solutions were then individually cast on Petri dishes and then dried at room temperature in a sterilized environment to form the films.

pH Measurement

The samples of the films were prepared individually using distilled water in a ratio of 1:10 (film sample: distilled water) according to the standard method. The mixtures were blended individually at low speed using a Waring blender. The pH meter (Toledo 320 pH meter) was standardized first using two buffer solutions in pH 4.0 and pH 7.0 before use. The pH values of the films were read from the instrument in triplicates.

Film Thickness Measurement

The thickness of the films was conducted using a micrometer (Mitutoyo, Kanagawa, Japan). The instrument was calibrated to zero point. The thickness values of each film was taken with the smallest possible unit measurement count of 0.01 mm. The thickness of the individual films was measured with the micrometer screw gauge at five different locations (both center and four areas around the edges). The mean thickness of each film was calculated.

Water Absorption Capacity (WAC)

The films were placed individually in a Petri dish contains 15 ml of distilled water. The weight of each film was observed periodically at the first hour, second hour, third hour, and 24th hour. The film is placed in new fresh water every time after the weight is taken. The water absorption capacity of the film was calculated using the following equation:

$$\text{WAC}\ (\%) = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Folding Endurance

The film was repeatedly folded manually at the same place till it breaks or folded up to 300 times according to the method of Hima Bindu et al. [31]. The folding endurance of the films was individually evaluated to determine the flexibility of each film.

Tensile Strength

The tensile strength of the films was obtained using texture analyzer TAXT2i (Stable Micro Systems, Surrey, UK) according to the method described by Dhanikula and Panchagnula [32] with a pre speed of 1. 2 mm/s.

Extract Release Studies

Extract release studies were conducted in the films in order to estimate the time taken to release the total cumulative *Carica papaya* extract from the films using a diffusion membrane for this purpose. The films were individually placed in a beaker containing 100 ml distilled water. The measurement was done every 10 minutes intervals by withdrawing 5 ml of the sample from the beaker. The film is placed in new fresh water every time after the weight is taken. The concentration of *Carica papaya* in the samples was estimated using UV/Visible spectrophotometer at wavelength value of 260 nm. for a total of time of 90 min. Triplicates of reading were recorded and statistically analyzed.

Statistical Analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) test. Minitab statistical package version 17 (Minitab Inc., PA, USA) was used in the analysis of the obtained data with a statistical significance of 0.05 level. The results are expressed as mean ± SD.

RESULTS

The pH values of the films are shown in Table 2. Gradual decreases in the values with increasing the extracts of *Carica papaya* were observed. The lowest pH value 5.2±0.2 was obtained in the film formulated with 500:1000 *Carica papaya*: gelatin, respectively. A significant difference ($p \leq 0.05$) in pH value was observed between the films when *carica papaya* was added in the formulation in the concentration of 300 – 500 mg to 1000 mg of the gelatin. Increasing the addition of *Carica papaya* from 100 to 500 mg caused a decrease in the pH value from 6.5±0.2 to 5.1. The thickness of the films is shown in Table 2. The films had thickness values from 19.6±0.2 to 56.8±0.3 µm. There were significant ($p \leq 0.05$) differences in the values of the thickness of the films. The addition of 100 mg *Carica papaya* extract resulted in a thickness value of 19.6±0.2 µm and increased significantly ($p \leq 0.05$) to values of 26.3±0.1, 34.7±0.2, 46.7±0.5 and 56.8± 0.3 µm in the films formulated with added *Carica papaya* extract in the concentrations of 200, 300, 400 and 500, respectively. It was observed that there was about 37.2 µm an increase in the thickness of the films when *Carica papaya* concentration increased in the formulation from 100 mg to 500 mg. Table 2 shows the folding endurance values of the films. The folding endurance values were found to vary between 239.4±0.2 to 266.6±0.2. The results revealed that increasing *carica papaya* concentration in the formulation leads to increases in the values of the folding endurance. This finding is in good agreement with the finding of Nguyen et al. [33]. The tensile strength values of the films is shown in Fig. 1. Significant differences ($p \leq 0.05$) in the values of the tensile strength were observed. Increasing the concentration of the *carica papaya* extract increased the tensile strength. The lowest tensile strength was 326.2±0.2 (N/cm^2) in film 1 (F1) which formulated with 100:1000 *carica papaya*: gelatin, respectively and significantly ($p \leq 0.05$) jumped to the value of 536.8±0.3 (N/cm^2) in the film 5 that formulated with 500:1000 *carica papaya*: gelatin, followed by a value of 499.5±0.5 (N/cm^2) in film 4 (F4) which formulated with 400:1000 *carica papaya*: gelatin, respectively. The cumulative percentages of the extract release of the films are presented in Table 3.

Table 2: pH, thickness, folding endurance, water absorption capacity and tensile strength parameters of the films

Film No.	Film Code	pH Value	Thikness (µm)	Folding Endorance (mean + SD)	Water Absoption Capacity %
1	F1	6.5± 0.2^a	19.6± 0.2^a	239.4± 0.2^a	766.4± 0.2^a
2	F2	6.2± 0.3^a	26.3± 0.1^b	246.5± 0.4^a	826.3± 0.3^b
3	F3	5.5± 0.4^b	34.7± 0.2^c	249.2± 0.5^b	858.7± 0.4^c
4	F4	5.4± 0.5^b	46.7± 0.5^d	248.3± 0.1^b	976.7± 0.5^d
5	F5	5.1± 0.3^b	56.8± 0.3^e	266.6± 0.2^c	996.8± 0.3^e

Readings were means of triplicate measurements

Means with the same superscript letter within the column are not significantly different at $p \leq 0.5$

F1-F5: Films formulated from *carica papaya* extract & bovine skin gelatin

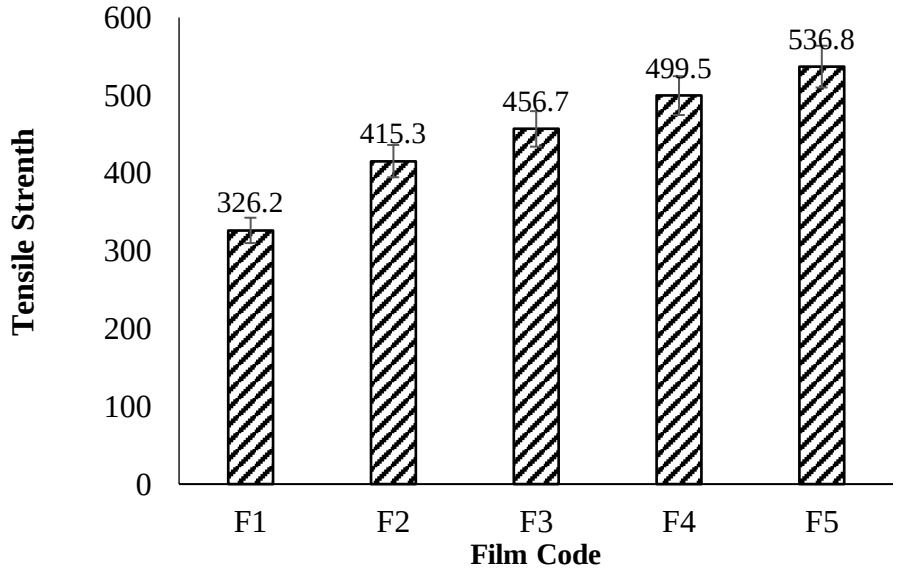

Figure 1: The Tensile Strength Values of the Films

Table 3: Cumulative Percentage of Extract Release From Different Composed Films

Time (min)	Extract Release (%) (mean + SD)				
	F1	F2	F3	F4	F5
10	11.4± 0.2[a]	10.2± 0.2[a]	8.4± 0.2[b]	6.5± 0.2[c]	5.2± 0.2[d]
20	16.4± 0.2[a]	12.4± 0.2[b]	9.1± 0.2[c]	8.3± 0.2[d]	7.2± 0.2[e]
30	31.3± 0.5[a]	19.3± 0.5[b]	15.3± 0.5[c]	12.3± 0.5[d]	10.3± 0.5[e]
40	52.7± 0.4[a]	44.7± 0.3[b]	51.7± 0.5[c]	48.7± 0.1[d]	36.7± 0.4[e]
50	75.7± 0.5[a]	55.7± 0.5[b]	46.7± 0.5[c]	55.7± 0.5[b]	53.7± 0.5[d]
60	82.8± 0.3[a]	62.8± 0.3[b]	57.8± 0.3[c]	61.8± 0.3[b]	59.8± 0.3[d]
70	89.3±0.1[a]	77.3±0.1[b]	66.3±0.1[c]	67.3±0.1[c]	59.9±0.1[d]
80	92.1±0.3[a]	88.1±0.3[b]	72.1±0.3[c]	69.1±0.3[d]	61.1±0.3[e]
90	98.4±0.2[a]	89.4±0.2[b]	83.4±0.2[c]	75.4±0.2[d]	71.4±0.2[e]

Means with the same superscript letter within a row were not significantly different at $p \leq 0.5$
Readings were means of triplicate measurements
F1-F5: Films formulated from *carica papaya* extract & bovine skin gelatin

This study was conducted to find the time taken by the films to release their complete *Carica papaya* extracts using *in vitro* diffusion studies. The values of extract release were significantly different ($p<0.05$). At the end of the time (90 min), the maximum percentage of extract release was 98.4% which was observed in the film coded F1 followed by 89.4%, 83.4, 75.4, and 71.4 which were obtained in film coded F2, F3, F4 and F5, respectively. This result indicated that the films will not interfere in extract release when applied to the wound [34].

DISCUSSION

The pH values play an important factor in wound healing due to their influence on fibroblast activity, the act ivity of matrix metalloproteinases, immunological responses, microbial proliferation and keratinocyte proliferation [35]. The bacterial infection is considered a common problem in all types of wounds which could disturb epithelialization, lengthen inflammation and finally delay wound healing [36]. The pH change has been reported to influence the performance of antimicrobials which is directly related to wound healing progress. Based on that the pH value has a role to play in both treatment and the healing of acute and chronic wounds. Earlier, Gethin and Cowman [37] investigated the effect of pH level on the healing of 20 types of wounds and reported that over two weeks the wounds that have a pH of 7.6 revealed a 30% reduction in the size of the wounds. They also found that there is a

relationship between wound contraction and pH value that is as the pH value increased the contraction in wounds decreased. This statement is in good agreement with those of Schneider et. al [38]. According to Roberts et al. [39] both acute and chronic wounds with pH closer to neutral have demonstrated higher rates of healing as compared to wounds with alkaline pH value. It was also observed that a 90-fold higher in *Enterobacter* growth in an acidic milieu with a pH level of 5.5 as compared with an alkaline milieu with a pH value of 7.8. The values of the thickness of the films could have a great influence on both the time required to absorb the drug and amount of the drug available to the wound. According to Lee et al. [40] an excessively high values of water absorption with wound dressing when topical applied to the wound may give a rise to dry the surface of the wound due to excessive loss of fluid as water vapor. Thus, increasing the addition of *Carica papaya* in the films might enhance high fluid absorption capacity from wounds and consider as important characteristics of the films used for wound topical application [41, 42]. It was reported that when drug transport in the skin, several factors influence its process parameters such as drug partitioning into the stratum corneum, dissolution and release of drug from the formulation, drug diffusion across the stratum corneum which mainly by intercellular lipids and drug partitioning from the stratum corneum into viable epidermis layers and diffusion across the viable epidermis layers into the dermis and drug absorption by capillary vessels to achieve systemic circulation [39]. Folding endurance characteristics of a film together with tensile strength are related to the flexibility of the film and hence represents its physical stability during application [43]. The folding endurance also could indicate the product safety and transport without breakage of the product [44] and also indicate the plasticity of the films [45]. Increasing the values of the cumulative extract release indicated that the films will not interfere when applied to wounds.

CONCLUSION

In this research bovine gelatin - *Carica papaya* ethanolic extract was used to formulate new films for wound healing treatment. Increasing *Carica papaya* extract in the formulation improved the quality of the films. The films had excellent pH, thickness, water absorption, folding endurance, tensile strength and reliable extract release properties which could determine their application efficacy. *In-vivo* studies using these films to determine their anti-bacterial, anti-inflammatory and their activity on wound healing are highly recommended.

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