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Farma-Bant: obtaining a wound covering membrane from *Calendula Officinalis, Centella Asiatica* and *Carthamus Tinctorius* plant extract mixtures

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Studies and practical applications related to wound dressings used in wound treatment around the world hold significant importance. These wound dressings cover the wound, protect the skin from external factors, and contribute to the healing process. When examining wound healing practices, it is evident that plants have been used since ancient times. For this purpose, antimicrobial plants have been incorporated into wound dressings in an effort to accelerate healing.

In this study, it was aimed to extract the plant compounds from *Centella Asiatica* (Indian Pennywort), *Carthamus Tinctorius* (Safflower) and *Calendula Officinalis* (Marigold) each possessing different pharmacological properties and to integrate a mixture of these extracts, prepared in specific ratios, into adhesive bandages commonly used for minor cuts and injuries. To investigate the release of the plant extracts from the bandage, time dependent UV-VIS spectroscopic measurements were performed in phosphate-buffered saline (PBS) which simulates body fluid. A swelling test was conducted to evaluate the fluid absorption capacity, and microscopy was used to observe whether the plant extracts were successfully incorporated into the wound dressing material.

Keywords: Marigold, Indian Pennywort, Safflower, wound dressing, antimicrobial

INTRODUCTION

Research and applications related to wound dressings used for wound treatment worldwide hold significant importance. Various types of dressings are used globally for injuries, which covers the wound to protect the skin from external factors and aid healing. The essence of modern wound care is to evaluate the wound correctly, identify the parts and keep the wound moist. At the same time, protecting the wound from bacteria and foreign substances, removing excess leakage from the wound, not harming healthy tissues during wound healing and allowing gas consumption in the wound area are among the features expected from the ideal wound care system [1]. Among the first dressings we use for minor home accidents is the adhesive bandage, an essential component of first aid kits. Additional materials added to the wound dressings used accelerate the healing process of the wound. Recently, modern dressings have incorporated various functional plants to promote faster healing and protect against infections. Antimicrobial plants have been integrated into dressings to expedite healing.

In this study, three high pharmacological plants *Centella Asiatica* (Indian Pennywort), *Carthamus Tinctorius* (Safflower) and *Calendula Officinalis* (Marigold) were highlighted. Research shows that Indian pennywort has significant wound healing properties [2], Safflower is used as a pain reliever and fever reducer [3] and Marigold promotes skin healing [4]. This study aims to extract the essences of these plants and incorporate them into adhesive bandages for small wounds and cuts. The release of plant extracts from the bandages was analyzed using time dependent UV-VIS spectrum measurements in a phosphate buffer saline (PBS) as artificial body fluid. Swelling test was used for the investigation of liquid absorption properties. Microscopy was also used to confirm the incorporation of plant extracts into the wound dressing.

EXPERIMENTAL:

Extraction of Indian Pennywort (Centella Asiatica) plant

10 g of dried *Centella Asiatica* plant is taken and 100 ml of a methanol-water mixture (10:90, v/v) is added. This mixture is left to stay for 1 day. The resulting mixture is then filtered and the solvent in the filtrate is removed using a rotary evaporator. After the evaporation process, the plant extract is obtained [2].

Extraction of Safflower (Carthamus Tinctorius) plant

2 g of safflower is weighed using a precision balance. Then, 10 ml of methanol is added and left to stand for 1 day. The resulting mixture is filtered,

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and the solvent in the filtrate is removed using a rotary evaporator. After the evaporation process, the plant extract is obtained [5].

Extraction of Marigold (Calendula Officinalis) plant

250 mg of *Calendula Officinalis* is weighed using a precision balance. Then, 25 ml of methanol is added to the weighed plant material. The solvent is replaced every 24 hours for 3 days, and all collected solvents are combined at the end of the third day. The resulting mixture is filtered, and the solvent in the filtrate is removed using a rotary evaporator. After the evaporation process, the plant extract is obtained [6].

Integration of the extracts of three different plants into the wound dressing:

Extracts from *Centella Asiatica* (Indian Pennywort), *Carthamus Tinctorius* (Safflower) and *Calendula Officinalis* (Marigold) were each separately weighed to obtain a mass ratio of 5% [7]. Methanol was added to each weighed extract and the mixtures were combined in a beaker. This combined mixture was stirred for 24 h using a magnetic stirrer without applying any heat. The rectangular gauze part in the center of the wound dressing was removed and immersed in the extract-containing solution. The gauze was left in the solution for 24 h and then removed and left to dry at room temperature (Fig. 1).

Fig. 1. Integration of the extracts of three different plants into the wound dressing.

Release of wound dressing containing Centella Asiatica, Carthamus Tinctorius, and Calendula Officinalis extracts in phosphate buffered saline (PBS)

The wound dressing containing the three plant extracts was first weighed. Then, it was placed in 30 ml of phosphate buffered saline (PBS) and kept at a constant temperature of 37°C. A sample was taken at the 96th hour and the final weight of the dressing was measured [8]. Table 1 shows the weight wound dressing before and after 96th hour of immersion in the phosphate buffer solution.

Table 1. Weight of the wound dressing containing three different plant extracts before and after 96th hour of immersion in the phosphate buffer solution.

Weight of wound dressing containing three different plant extracts before placing in phosphate buffer solution	Weight of the wound dressing containing three different plant extracts after 96th hour of placing it in the phosphate buffer solution
0.1 g	0.03 g

To evaluate the release behavior due to degradation, UV spectrophotometric measurements were taken. The plant extract containing wound dressing was kept in 30 ml of PBS at 37°C. Samples were collected at the 48th and 96th h for UV spectrophotometric analysis (Fig. 2).

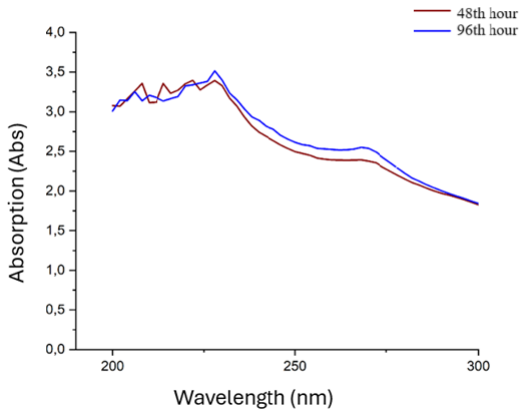

Fig. 2. UV-VIS spectra of the wound dressing coated with plant extracts taken at 48th and 96th hour.

Swelling test

A swelling test was conducted to determine the liquid absorption capacity of the wound dressing coated with three different plant extracts. A 1 cm × 1 cm piece was cut from the wound dressing and placed into phosphate buffer solution (pH=7.4) at 37 °C and kept under stirring with a magnetic stirrer [9]. At specific time intervals, the sample was removed from the phosphate buffer solution, gently blotted with a paper towel to remove excess liquid, and weighed using a precision balance. The swelling ratio of the plant extract containing wound dressing was calculated using the equation below and a time dependent graph was plotted.

$$\text{Swelling ratio} = \frac{W_s - W_d}{W_d}$$

where W_d represents the dry weight of the sample after vacuum oven drying and W_s represents the wet

weight after phosphate buffer solution immersion [9].

The weight of the wound dressing containing these additives were measured by keeping it in a shaking water bath at 37°C in phosphate buffer solution. The measured values are given in Table 2.

Table 2. Swelling weight of wound dressing containing three different plant extracts with respect to time.

Time	Wound dressing with plant extracts
0th hour	53 mg
7th hour	105 mg
24th hour	211 mg
48th hour	325 mg

Microscopic examination of wound dressing with and without plant extract mixture

The wound dressing was examined with and without plant extracts under a microscope at 100, 200 and 400× magnification. Figure 3 gives the appearance of the uncoated and coated form of the wound dressing fabric under the microscope at 100, 200 and 400× magnification.

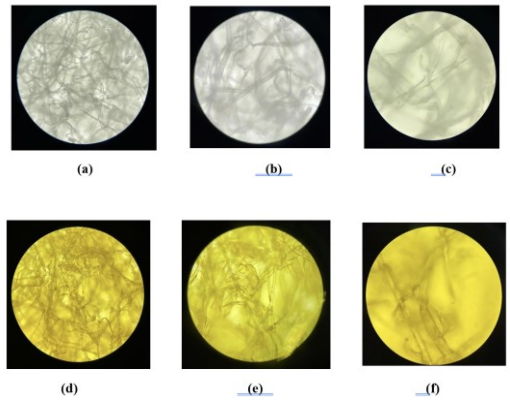

Fig. 3. Photographs of the wound dressing not coated with plant extracts (a) 100×, (b) 200×, (c) 400× magnification and photographs of the wound dressing coated with plant extracts (d)100× (e) 200× (f) 400× magnification.

Materials

In our project, *Centella Asiatica* (Indian Pennywort), *Carthamus Tinctorius* (Safflower) and *Calendula Officinalis* (Marigold) were used and methanol and distilled water were employed to obtain the plant extracts. To observe the release behavior of the plant extracts in the human body, phosphate buffered saline (PBS), a solution that closely resembles human body fluid and is considered a type of artificial body fluid, was used.

In our study, a precision balance, a rotary evaporator (for solvent removal), a Nikon Eclipse E100 microscope (for microscopic analyses), and a Shimadzu UVmini-1240 UV-Vis spectrophotometer (for investigating the release of plant extracts) were used.

RESULTS AND DISCUSSION

In this study, extracts were obtained from *Centella Asiatica* (Indian Pennywort), *Carthamus Tinctorius* (Safflower), and *Calendula Officinalis* (Marigold). The obtained extracts were incorporated into the fabric layer of a wound dressing and the release behavior of the plant extracts from the wound dressing fabric was observed in phosphate buffered saline (PBS), which closely simulates human body fluid. Samples were collected at the 48th and 96th hour from the PBS medium containing the wound dressing fabric and their release profiles were analyzed using a UV-VIS spectrophotometer. A swelling test was also performed to evaluate the fluid absorption capacity of the developed wound dressing. Additionally, microscope analyses at 100×, 200×, and 400× magnifications were conducted to compare wound dressing fabrics with and without plant extracts.

We aimed to utilize the wound-healing properties of Indian Pennywort, the analgesic effect of Safflower, and the skin repairing properties of Marigold. Initially, the plant extracts were prepared based on literature data. The extracts were then mixed in specific ratios and integrated into the wound dressing fabric. The release of these extracts was studied in phosphate buffered saline (PBS) solution, which is considered a close simulation of human body fluid. UV-VIS measurements at the 48th and 96th hour revealed significant changes in absorbance values, indicating the release of plant extracts from the wound dressing. Furthermore, weight measurements before and after immersion in PBS showed a decrease in the weight of the wound dressing fabric by the end of 96 hours, further confirming the release of the plant extracts into the buffer solution.

When the swelling test was applied to the wound dressing containing all three plant extracts, the fluid absorption percentage was found to be 513% at the 48th hour. While wound exudate nourishes the wound, excessive accumulation can interfere with proper observation and healing. Therefore, an ideal wound dressing should provide optimal fluid absorption [9]. According to the literature, an ideal wound dressing should have a fluid absorption capacity between 100% and 900% [10]. The 513% absorption value found in this study falls within this ideal range, indicating that the herbal extract-infused

wound dressing has good fluid absorption performance.

Moreover, microscopy at 100×, 200×, and 400× magnifications confirmed that the plant extracts were thoroughly integrated into the wound dressing fabric, as the coated and uncoated samples showed distinct surface differences.

CONCLUSION

In conclusion, all data obtained in this study demonstrate that the wound dressing fabric was successfully coated with *Centella Asiatica*, *Carthamus Tinctorius* and *Calendula Officinalis* extracts and that these extracts were released into the simulated human body fluid (PBS) over time.

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