

## Preparation and analysis of an herbal extract solution having potential restorative effects against radiation dermatitis

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Radiodermatitis refers to skin reactions that occur after radiotherapy in cancer patients, such as redness, scaling, and crusting. The medications used to treat radiodermatitis often contain chemical substances. However, cancer patients, who are already exposed to intense chemical treatments, may find the use of such chemically based drugs burden. For the treatment of wounds resulting from radiotherapy, plant-based substances can be considered as alternatives to chemical-containing products. *Plantago lanceolata* has wound-healing properties and supports cell viability. *Cistus* possesses antimicrobial effects that help prevent infection and support the healing process. *Centella asiatica* is known for its wound-healing and moisturizing properties and it helps prevent deeper fissures caused by dryness. *Aloe vera* hydrates the skin and contributes to wound healing.

In our study, we prepared a plant-based solution in PBS containing *Plantago lanceolata* (allantoin), *Cistus* (*Cistus creticus*; *laden*), *Centella asiatica* (gotu kola) and *Aloe vera* to help the skin wounds that develop in patients receiving radiotherapy heal faster. Allantoin supports wound healing and cell vitality. *Laden* helps prevent infections and supports the healing process. *Centella asiatica* has wound-healing and moisturizing effects, and it also prevents deeper cracks that may form when the wound becomes too dry. *Aloe vera* moisturizes the skin and helps the wounds heal. For the treatment of the skin wounds that appear in cancer patients undergoing radiotherapy, these plant-based ingredients were combined to obtain the solution. This solution was integrated this solution in cotton pads for ease of use. The solution-infused cotton pads were tested using UV/VIS spectrophotometry, microscopy and zetasizer analysis. The obtained results showed that this solution may have the potential to benefit wounds in patients undergoing radiotherapy.

**Keywords:** *Plantago lanceolata* (allantoin), *Cistus* (*Cistus creticus*; *laden*), *Centella asiatica* (gotu kola), *Aloe vera*

### INTRODUCTION

Cancer is a disease known since ancient times and as time has progressed, various treatment methods have been developed. Radiotherapy, one of the oldest known methods and still used today, is carried out by directing radiation to diseased tissue. The radiation affects not only the diseased tissue but also healthy tissue, leading to a condition called radiodermatitis. Radiodermatitis refers to the morphological and functional changes that occur in non-cancerous skin tissue as a result of therapeutic radiation application [1]. It is recommended that patients keep the irradiated area clean and dry, wash it with lukewarm water and pH-appropriate soaps, avoid perfume and alcohol-based solutions, refrain from environments such as saunas and baths and protect themselves from sunlight [2]. Radiation-induced skin damage, a common complication of radiotherapy, occurs in approximately 90% of patients undergoing treatment and negatively affects their quality of life and treatment processes [3]. The chemical substances in the medications used after radiodermatitis may cause cytotoxic effects. One of

the substances used in these drugs, silver sulfadiazine (SSD) has the potential to negatively affect healing by causing toxic effects on keratinocytes and fibroblasts [4]. It also carries the risk of developing contact dermatitis and recurrence in wounds [5]. Patients undergoing radiotherapy use appropriate creams or solutions for radiodermatitis wounds. The fewer chemical substances these wound-covering materials contain, the more beneficial they are for patients. Therefore, herbal-based products can be used. Extracts obtained from the *Cistus* plant have antioxidant properties. This plant is a rich source of polyphenols, including flavonoids and phenolic acids. *Cistus* is valuable for its ability to support the immune system. It also has antiviral and antibacterial properties and helps fight infections [6]. *Centella asiatica* extracts are considered natural remedies that alleviate acne, burns, atopic dermatitis, and wounds [7]. Among the main herbal topical agents investigated for the treatment of radiodermatitis are *Aloe vera*, *Calendula*, and *Centella asiatica* [2]. *Plantago lanceolata* has known pharmacological activities in the literature such as wound healing, irritation relief, moisturizing, removal of necrotic tissue, stimulation

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of cell mitosis; as well as supporting epithelial stimulation, analgesic effects, and keratolytic activity. *Plantago lanceolata* has been used for over 70 years for various therapeutic purposes, especially as a wound healing enhancer in cosmetic and pharmaceutical preparations [8]. *Aloe vera*, due to its soothing and moisturizing properties, is used in skin care products and cosmetics such as lotions, creams, gels, and face masks; in pharmaceuticals, *Aloe vera* extracts are used in the production of ointments, creams, and oral medications for burns, wounds, psoriasis, and gastrointestinal disorders [9].

In our study, a solution prepared using a mixture of *Cistus*, *Centella asiatica*, *Plantago lanceolata*, and *Aloe vera* plant extracts was integrated into cotton pads. It was aimed that the obtained cotton pads containing plant extracts would help in the healing of radiodermatitis wounds occurring after radiotherapy.

## EXPERIMENTAL

### Materials

Methanol, ethanol and distilled water were employed to obtain the plant extracts of *Centella asiatica*, *Plantago lanceolata* and *Cistus*. These substances were combined with the *Aloe vera* gel. 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 was used. A balance, a magnetic stirrer, a rotary evaporator (for solvent removal), a Nikon Eclipse E100 device for microscopic examinations, and a Shimadzu UVmini-1240 model UV-VIS spectrophotometer were used to investigate the release of plant extracts. A Brookhaven Instruments Corporation 90 Plus model Zetasizer device was used for zeta potential, electrophoretic mobility and particle size measurements. UV-VIS and Zetasizer measurements were carried out at 37°C, which is body temperature.

#### Extraction of *Centella asiatica* plant extract:

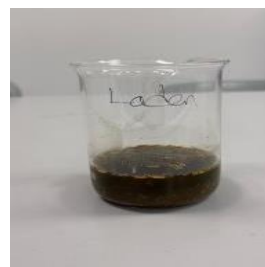
10 g of dried *Centella asiatica* plant were taken and 100 mL of a methanol-water mixture (10:90%, v/v) was added. This mixture was left to stand for one day (see Fig. 1). The mixture was then filtered and the solvent in the filtrate was removed using a rotary evaporator. After the evaporation process, the plant extract was obtained [10].



**Figure 1.** Soaking of *Centella asiatica* plant in a methanol-water mixture.

#### Extraction of *Cistus* plant extract

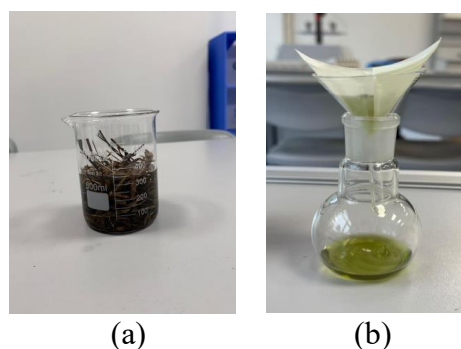
*Cistus* leaves were ground and 20 g of the ground leaves were taken. They were left for 24 h in a 70% aqueous ethanol solution at a solid-to-solvent ratio of 1:20. The obtained mixture was filtered (see Fig. 2) and the ethanol was removed using a rotary evaporator. After the evaporation process, the plant extract was obtained [11].



**Figure 2.** Soaking of the *Cistus* plant in ethanol.

#### Extraction of *Plantago lanceolata* plant extract

30 g of dried and ground *Plantago lanceolata* plant was weighed and kept in 100 mL of ethanol for 6 hours. After the mixture was filtered (see Fig. 3) the ethanol was evaporated under vacuum at 40°C using a rotary evaporator. After the evaporation process, the plant extract was obtained [12].

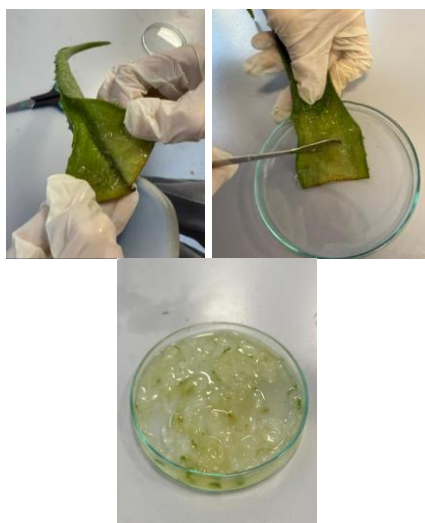


**Figure 3.** (a) Soaking of *Plantago lanceolata* plant in ethanol; (b) Solvent-plant extract mixture obtained after filtration.

#### Extraction of gel from *Aloe vera* plant

The leaves of the obtained *Aloe vera* plant were thoroughly washed and dried. The gel inside the

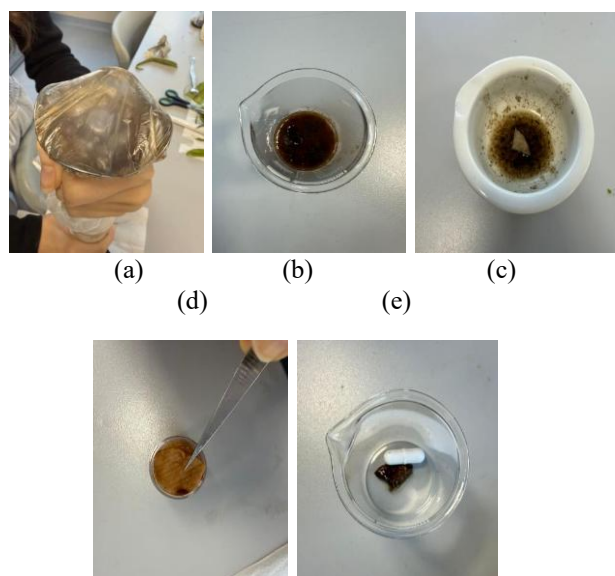
leaves was removed with the help of a spatula and transferred into a Petri dish (see Fig. 4).



**Figure 4.** Extraction of gel from the inner part of *Aloe vera* leaves.

#### Mixing of plant extracts and integration into cotton pads

The extracts of *Centella asiatica*, *Cistus*, *Plantago lanceolata*, and *Aloe vera* were each weighed separately so that their mass ratio was 5%. The obtained mixture was blended using a blender. The mixture containing plant extracts was then added onto cotton pads and left to stand at room temperature for 24 h (see Fig. 5).



**Figure 5.** (a) Mixing of plant extracts in a blender; (b) Mixture of plant extracts; (c) Disk cotton integrated with plant extracts; (d) and (e) Appearance of disk cotton coated with the plant extract solution.

#### Release of cotton pads containing *Centella asiatica*, *Cistus*, *Plantago lanceolata*, and *Aloe vera* extracts in phosphate-buffered saline (PBS)

First, the cotton pads containing the four different plant extracts were weighed. Then, they were left to degrade in 30 mL of phosphate-buffered saline (PBS) at a constant temperature of 37°C (see Fig. 6). To examine the release of the plant extracts integrated into the cotton pads in the PBS solution, samples were taken from the solution at the 24<sup>th</sup>, 48<sup>th</sup> and 72<sup>nd</sup> h (see Fig. 7). The cotton pads containing plant extracts were removed from the phosphate-buffered saline solution and left to stand at room temperature for one day. The weight of the dried cotton pads containing plant extracts was then measured.

**Figure 6.** Mixing of disk cotton containing plant extracts in phosphate buffer solution.



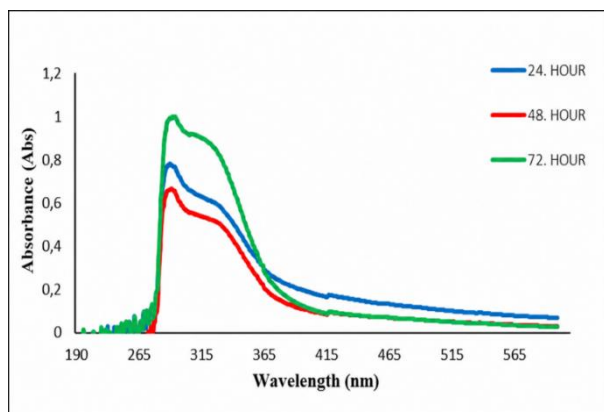
**Figure 7.** (a) 24<sup>th</sup> h of dissolution of disk cotton coated with the plant extract mixture in PBS (b) 48<sup>th</sup> h of dissolution of disk cotton coated with the plant extract mixture in PBS.

To evaluate the degradation-related release of cotton pads containing extracts of *Centella asiatica*, *Cistus*, *Plantago lanceolata*, and *Aloe vera* in phosphate-buffered saline (PBS), UV-VIS spectrophotometer measurements were taken from samples collected at the 24<sup>th</sup>, 48<sup>th</sup> and 72<sup>nd</sup> hour. The measurements were carried out on samples prepared by diluting the phosphate-buffered saline (PBS) solution, in which degradation occurred, with pure water.

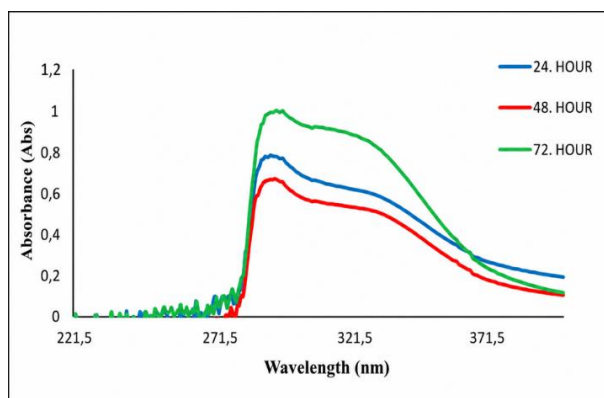
### Release of plant extracts integrated into the cotton pad in phosphate buffer solution (PBS)

*Centella asiatica*, *Cistus*, *Plantago lanceolata*, and *Aloe vera* extract-coated cotton discs were incubated in phosphate-buffered saline (PBS), which closely mimics artificial human body fluid, at 37°C. The initial weight of the cotton disc containing the *Centella asiatica*, *Cistus*, *Plantago lanceolata*, and *Aloe vera* extract mixture was 11.1 g before immersion in PBS, while its weight decreased to 0.15 g after 72 h of incubation.

The release of the cotton pad containing *Centella asiatica*, *Cistus*, *Plantago lanceolata*, and *Aloe vera* extract in phosphate buffer solution (PBS) was analyzed using a UV-VIS spectrophotometer. UV-VIS spectra taken at different wavelengths are given in Figures 8 and 9.



**Figure 8.** Comparative UV-VIS spectra of the cotton pad integrated with plant extracts, taken at 190–600 nm at the 24<sup>th</sup>, 48<sup>th</sup> and 72<sup>nd</sup> h.

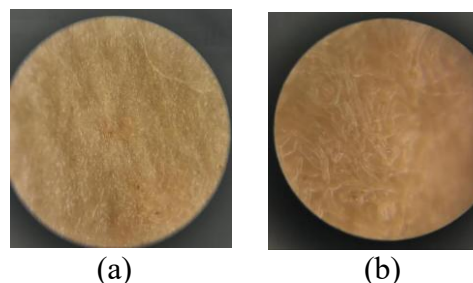


**Figure 9.** Comparative UV-VIS spectra of the cotton pad integrated with plant extracts, taken at 214–465 nm at the 24<sup>th</sup>, 48<sup>th</sup> and 72<sup>nd</sup> h.

In the data obtained from the comparative UV-VIS spectra, the highest peak value was observed at 297 nm with an absorbance value of 1 at the 72<sup>nd</sup> h.

### Examination of the cotton pad containing plant extracts under the microscope:

The cotton pad integrated with *Centella asiatica*, *Cistus*, *Plantago lanceolata*, and *Aloe vera* extracts was examined under the microscope. The magnified images at 40× and 100× under the microscope are given in Figure 10.



**Figure 10.** Photographs of cotton pads coated with plant extracts magnified (a) 40× and (b) 100×.

### Investigation of the stability of the cotton pad containing plant extracts in pbs artificial body fluid

- *Effect of the PBS medium on particle size over time.* The behavior of the cotton pad containing plant extracts in phosphate buffer solution (PBS) was examined at the 24<sup>th</sup>, 48<sup>th</sup> and 72<sup>nd</sup> h, and the particle size values are given in Table 1.

**Table 1.** Particle size of plant extracts in the PBS medium at different times.

| Time(h)          | Particle size (nm) |
|------------------|--------------------|
| 24 <sup>th</sup> | 19.7               |
| 48 <sup>th</sup> | 17.6               |
| 72 <sup>nd</sup> | 20.2               |
| Mean±SD          | 19.2±1.4           |

The particle size of the plant extract particles in the PBS medium was approximately 19 nm, indicating that the particles were very small and may easily pass through the skin.

- *Effect of the PBS medium on zeta potential over time:* The behavior of the cotton pad containing plant extracts in phosphate buffer solution (PBS) was examined at the 24<sup>th</sup>, 48<sup>th</sup> and 72<sup>nd</sup> h, and the zeta potential values are given in Table 2.

**Table 2.** Zeta potential measurement results of plant extracts in the PBS medium at different times.

| Time (h)         | Zeta potential (mV) |
|------------------|---------------------|
| 24 <sup>th</sup> | -1.73               |
| 48 <sup>th</sup> | -3.20               |
| 72 <sup>nd</sup> | -10.03              |
| Mean±SD          | -4.9±4.4            |

The zeta potential values obtained with the release of the plant extracts over time in the PBS medium show that the surface charges of the plant extracts were negative.

## RESULTS AND DISCUSSION

With the developing technology for the treatment of cancer disease, different methods have been developed. Despite these developing methods, radiotherapy, which is one of the old methods that has still not been abandoned, causes wound formation in the patient after treatment. The formation of this condition called radiodermatitis, occurs because the radiation applied in radiotherapy damages healthy cells as well as cancerous cells. Patients use different products such as creams, solutions, lotions and gels on the damaged area of their bodies. These products contain chemical substances, and these chemicals are quite dangerous in cancer treatment. In our study, a cotton pad solution integrated with *Centella asiatica*, *Cistus*, *Plantago lanceolata*, and *Aloe vera* extracts was obtained for patients experiencing radiodermatitis. The cotton pad containing the obtained plant extracts was observed under a microscope at 40x and 100x magnification. In order to examine the release of the cotton pad integrated with *Centella asiatica*, *Cistus*, *Plantago lanceolata*, and *Aloe vera* plant extracts in phosphate buffer solution, which is the closest to human body fluid, samples taken at the 24th, 48th and 72nd hours were analyzed in a UV-VIS spectrophotometer. In addition, the weights of the cotton pad before being placed into the phosphate buffer and at the end of the 72nd h were compared. Furthermore, the particle size and zeta potential of the samples in PBS were measured against time, and the surface charges in this environment were determined.

As a result of the conducted studies, in the observations performed under the microscope on the cotton pad containing plant extracts, the plant extracts were fully integrated into the cotton. When the release in phosphate buffer solution was examined at the 24<sup>th</sup>, 48<sup>th</sup> and 72<sup>nd</sup> h in the UV-VIS spectrophotometer, it was observed that the plant extracts spread in the phosphate buffer over time. In addition, it was revealed that the release reached its peak at the 72<sup>nd</sup> h. When the weight of the cotton pad containing plant extracts was compared before being placed into the phosphate buffer solution and after the 72<sup>nd</sup> hour, it was observed that the weight of the cotton pad decreased at the end of the 72<sup>nd</sup> h. This shows us that the plant extracts spread in the phosphate buffer solution. When the zeta potential and particle size results were examined, it was seen

that the particle size of the plant extract in the PBS environment was small enough to pass through the skin barrier and that it was negatively charged.

When all the obtained data were evaluated, it was concluded that the cotton pad containing plant extracts may help heal radiodermatitis wounds that occur after radiotherapy.

## CONCLUSION

In conclusion, considering all experiments conducted, it was observed that our solution containing *Centella asiatica*, *Cistus*, *Plantago lanceolata*, and *Aloe vera* may have a positive effect on the healing of skin wounds in cancer patients undergoing radiotherapy. In the PBS release study, the release of the proposed solution was found to increase over time and the prepared plant extract particles with an average size of 19 nm were found to have a negative surface charge in solution.

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