

A novel chitosan-PEG hydrogel embedded with *in situ* silver nanoparticles of *Clerodendrum glandulosum* Lindl. extract: Evaluation of its *in vivo* diabetic wound healing property using an image-guided machine learning model

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Supplementary file

The code for studying the wound area in the different treatment groups is mentioned below.

```
import cv2
import numpy as np

def sd():

    # Image dimensions
    image_width = 131 # pixels
    image_height = 266 # pixels

    # List of file paths
    image_paths = [
        r"D:\proj\image processing\Animal study pics _ CSE
dept\standard day\Standard _ Day 1.jpg",
        r"D:\proj\image processing\Animal study pics _ CSE
dept\standard day\Standard _ Day 3.jpg",
        r"D:\proj\image processing\Animal study pics _ CSE
dept\standard day\Standard _ Day 5.jpg",
        r"D:\proj\image processing\Animal study pics _ CSE
dept\standard day\Standard _ Day 7.jpg",
        r"D:\proj\image processing\Animal study pics _ CSE
dept\standard day\Standard _ Day 9.jpg",
        r"D:\proj\image processing\Animal study pics _ CSE
dept\standard day\Standard _ Day 11.jpg",
        r"D:\proj\image processing\Animal study pics _ CSE
dept\standard day\Standard _ Day 13.jpg"
    ]

    # Initialize an empty list to store wound areas
    wound_areas = []

    # Initialize a list to store resized images
    resized_images = []

    for image_path in image_paths:
        # Read the image from file
        frame = cv2.imread(image_path)

        # Resize the image to a common height
        ratio = image_height / frame.shape[0]
        resized_frame = cv2.resize(frame, (int(frame.shape[1] * ratio),
image_height))

        # Append the resized image to the list
        resized_images.append(resized_frame)

        # Convert BGR to HSV color scheme
        hsv = cv2.cvtColor(frame, cv2.COLOR_BGR2HSV)

        # Define a narrow range of wound colors in HSV (shades of red)
        lower_red = np.array([175, 120, 105])
        upper_red = np.array([190, 255, 255])
```

```

mask = cv2.inRange(hsv, lower_red, upper_red)

lower_red = np.array([0, 100, 100])
upper_red = np.array([10, 255, 255])

# Threshold the HSV image to get only wound colors
mask = mask + cv2.inRange(hsv, lower_red, upper_red)

square_mask = np.zeros((image_height, image_width),
dtype=np.uint8)
cv2.rectangle(square_mask, (0, 80), (image_width, image_height-
80), 255, -1)

mask = mask & square_mask

# Apply Gaussian blur to the mask for better contour detection
blurred_mask = cv2.GaussianBlur(mask, (5, 5), 0)

# Calculating percentage area
try:
    contours, hierarchy = cv2.findContours(blurred_mask,
cv2.RETR_TREE, cv2.CHAIN_APPROX_SIMPLE)
    cnt = max(contours, key=lambda x: cv2.contourArea(x))

    epsilon = 0.0005 * cv2.arcLength(cnt, True)
    approx = cv2.approxPolyDP(cnt, epsilon, True)

    areacnt = cv2.contourArea(cnt)
    arearatio = (areacnt / (image_width * image_height)) * 100

    boxes = []
    for c in cnt:
        (x, y, w, h) = cv2.boundingRect(c)
        boxes.append([x, y, x + w, y + h])

    boxes = np.asarray(boxes)
    # need an extra "min/max" for contours outside the frame
    left = np.min(boxes[:, 0])
    top = np.min(boxes[:, 1])
    right = np.max(boxes[:, 2])
    bottom = np.max(boxes[:, 3])

    cv2.rectangle(resized_frame, (left, top), (right, bottom),
(255, 0, 0), 2)

    # Append the wound area to the list
    wound_areas.append(arearatio * 0.6615)

except Exception as e:
    # Handle exceptions, if any
    print(f"Error processing image: {e}")
    wound_areas.append(None)

# Print the list of wound areas
for i, area in enumerate(wound_areas):
    print(f"Image {i + 1} - Wound Area: {area} cm squared")

```

```

# Display the resized images with the detected wounds
cv2.imshow('Detected Wounds (Standard Day)',
np.hstack(resized_images))
cv2.waitKey(0)
cv2.destroyAllWindows()

return wound_areas

```

Table S1. The functional groups with their corresponding wavenumbers that are present in CG plant extract, CGH, and BH (results are shown as mean \pm s.d., where n=3).

CG plant extract		CGH		BH	
Wavenumber (cm ⁻¹)	Functional group	Wavenumber (cm ⁻¹)	Functional group	Wavenumber (cm ⁻¹)	Functional group
816	C=C bending of alkene	840	C=C bending of alkene	668	C-Br stretching of the alkene
1035	CO-O-CO stretching of anhydride	1049	CO-O-CO stretching of anhydride	1215	C-O stretching
1361	S=O stretching of sulphonamide	1341	S=O stretching of sulphonamide	1375	O-H bending of phenol
1695	C=C stretching of conjugated alkene	1634	C=C stretching of conjugated alkene	1845	C=O stretching of anhydride
2923	C-H stretching of alkane	2884	C-H stretching of alkane	2350	O=C=O stretching
3252	O-H stretching of alcohol which is intermolecular bonded	3399	O-H stretching of alcohol which is intermolecular bonded	2971	C-H stretching of alkane

Abbreviations: CG – *Cleordendrum glandulosum*; BH – Blank hydrogel; CGH – CG-AgNPs loaded hydrogel

Table S2. The percentage of radical scavenging activity with increasing concentrations of BH and CGH formulations and the standard against DPPH and ABTS free radicals (results are shown as mean \pm s.d., where n=3).

Concentration (μ g/ml)	Percentage of radical scavenging activity			Abbreviations:
	DPPH assay			
	BH	CGH	Standard (ascorbic acid)	
50	0.79 \pm 0.55	1.59 \pm 1.18	9.73 \pm 5.35	BH – Blank hydrogel; CGH – CG-AgNPs loaded hydrogel; DPPH –
100	7.39 \pm 5.35	15.83 \pm 3.87	27.57 \pm 6.00	
200	20.44 \pm 5.11	25.58 \pm 4.02	40.53 \pm 5.53	
400	38.31 \pm 5.51	59.34 \pm 5.94	63.06 \pm 3.31	
800	74.21 \pm 5.05	96.63 \pm 2.89	98.13 \pm 1.71	
	ABTS assay			
50	0.99 \pm 0.81	1.26 \pm 0.94	8.65 \pm 6.93	DPPH –
100	6.50 \pm 3.76	21.79 \pm 6.37	24.66 \pm 5.16	
200	24.28 \pm 4.89	37.28 \pm 6.65	37.31 \pm 5.91	
400	40.92 \pm 6.56	56.02 \pm 5.46	52.59 \pm 6.92	
800	75.91 \pm 5.49	94.33 \pm 5.01	97.08 \pm 2.65	

2,2-diphenyl-1-picrylhydrazyl; ABTS – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid

Table S3. Percentage of wound areas of different treatment groups with respect to the number of days as evaluated by the developed machine learning model using the provided datasets of wound images (results are shown as

Treatment groups	Percentage wound area							
	1 st day	3 rd day	5 th day	7 th day	9 th day	11 th day	13 th day	15 th day
BH	100 ± 0.00	75.9 ± 1.76	74.5 ± 1.87	55.4 ± 2.85	49.7 ± 2.40	48.6 ± 1.50	39.3 ± 3.05	17.4 ± 2.01
CGH	100 ± 0.00	98.9 ± 1.19	97.8 ± 1.40	83.4 ± 2.36	66.3 ± 2.80	55.5 ± 1.92	19.4 ± 2.20	2.5 ± 1.11
Standard	100 ± 0.00	97.2 ± 1.55	94.2 ± 1.65	73.3 ± 1.85	63.5 ± 1.46	42.4 ± 1.56	31.9 ± 1.64	6.6 ± 1.41

mean ± s.d., where n=3).

Abbreviations: BH – Blank hydrogel; CGH – CG-AgNPs loaded hydrogel

Table S4. The connective tissue parameters represented as µg/ 40 mg of wound bed tissue were evaluated through hydroxyproline, hexosamine, and hexuronic acid content on the 7th and 14th day during the treatment with BH, CGH, and standard.

Treatment group	Day 7			Day 14		
	BH	CGH	Standard	BH	CGH	Standard
HPR (µg/ 40 mg of wound tissue)	31.75 ± 3.96	67.61 ± 5.36	61.43 ± 4.81	68.24 ± 17.82	93.39 ± 1.89	119.45 ± 25.26
HXA (µg/ 40 mg of wound tissue)	166.33 ± 14.97	79.83 ± 7.09	82.33 ± 9.07	126.67 ± 2.89	46.67 ± 4.72	44.16 ± 3.61
HUA (µg/ 40 mg of wound tissue)	46.33 ± 7.37	83.66 ± 7.02	75.43 ± 9.16	79.65 ± 12.50	122.17 ± 5.09	77.36 ± 2.52

Abbreviations: BH – Blank hydrogel; CGH – CG-AgNPs loaded hydrogel; HPR – Hydroxyproline; HXA – Hexosamine; HUA – Hexuronic acid

Table S5. The pro-inflammatory (IL-6 and TNF-α) and anti-inflammatory (IL-10) cytokines are represented as pg/ml of the collected blood samples from each of the groups on the 7th and 14th day during the treatment with BH, CGH, and standard.

Treatment group	Day 7			Day 14		
	BH	CGH	Standard	BH	CGH	Standard
IL-6 (pg/ml)	11.86 ± 3.18	5.79 ± 2.84	9.49 ± 1.32	7.32 ± 2.32	1.04 ± 0.37	2.26 ± 0.85
TNF-α (pg/ml)	76.35 ± 6.41	31.01 ± 4.98	42.35 ± 5.41	74.05 ± 6.34	12.61 ± 2.87	21.65 ± 3.71
IL-10 (pg/ml)	8.10 ± 2.77	26.13 ± 5.51	20.16 ± 2.49	9.17 ± 3.00	43.20 ± 4.22	36.47 ± 3.91

Abbreviations: BH – Blank hydrogel; CGH – CG-AgNPs loaded hydrogel; IL-6 – Interleukin-6; IL-10 – Interleukin 10; TNF-α – Tumor Necrosis Factor alpha