

In vitro Investigation of the Effects of *Hylotelephium* and *Typha*
Plant Extracts on Anti-Inflammatory and Wound Healing

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Kardzhali, Bulgaria
16.09.2020

HYLOTELEPHIUM

- treatment of skin diseases
- anti-inflammatory, keratolytic and analgesic activity¹
- anti-oxidant activity²
- moderate bactericidal activity against *P. aeruginosa*, *S. typhimurium*, *S. aureus*, and *B. subtilis*²

Hylotelephium Leaves

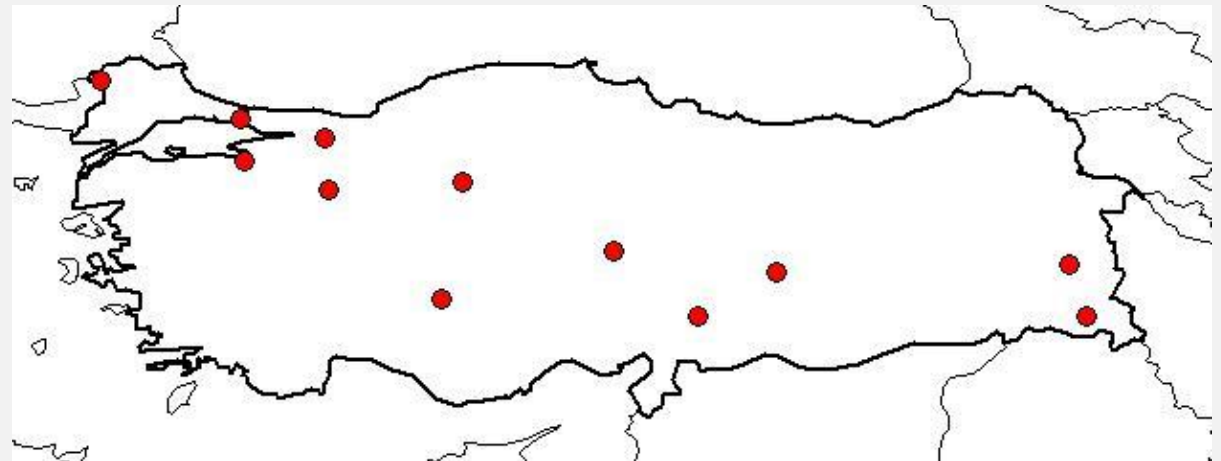
- band-aid
- The epidermis of the fresh leaves is peeled and placed directly on the wound.
- The fresh juice are usually applied topically to wounds.³
- A large amount of evidence indicating the efficacy of this treatment has been registered at the Emergency Unit of the Torre Galli Hospital in Florence.⁴





TYPHA

- Cat tail
- Medical use in Turkey, India and China
- Treatment of bleeding, wounds and stomach pain.^{5,6}



A large, vibrant bouquet of pink flowers, possibly gerberas, is positioned in the top right corner of the slide, partially overlapping the white text box.

AIM

- *Hylotelephium* and *Typha* species are known for their widespread use in the treatment of wounds.
- This study aimed to investigate the total phenolic and flavonoid content, antioxidant capacities, wound healing-inducing and anti-inflammatory activity of these two species.
- Also, all tests will be compared with titrated extract of *Centella asiatica*.

AIM

- Titrated extract of *Centella asiatica* is the main component of Madecassol, a Bayer-produced wound-healing ointment.
- *C. asiatica* (also known as Gotu Kola) has been used in folk medicine for small wounds, burns and eczema hundreds of years commonly in Southeast Asian countries.⁷
- Asiaticoside⁷

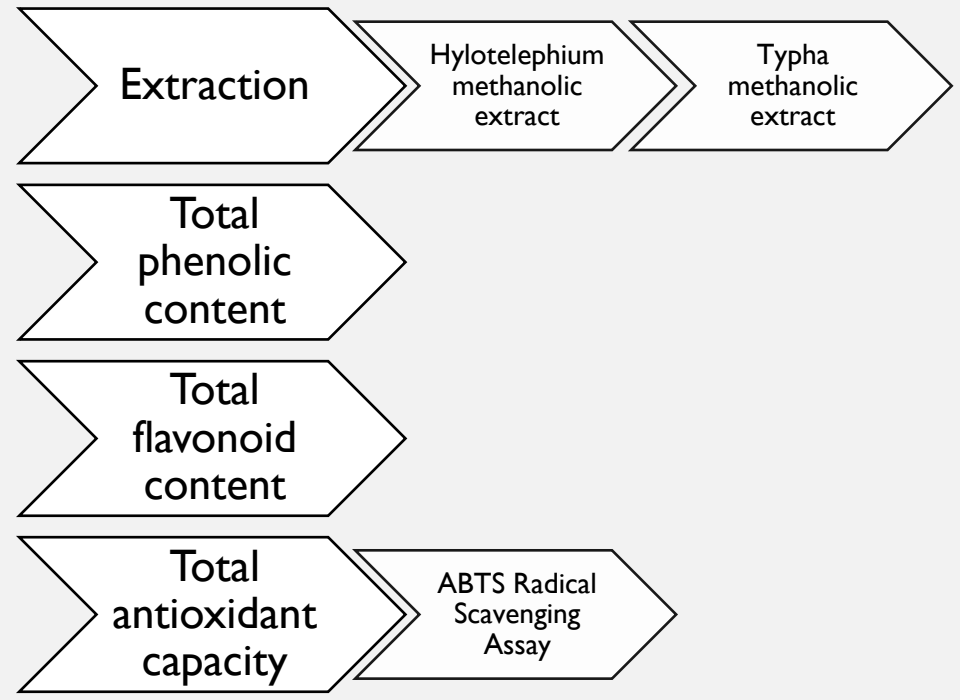




Typhaceae
Typha latifolia L.
Breitblättriger Rohrkolben fast Kosmopolit

Botanical Gardens, Kiel, Germany
09.07.2022

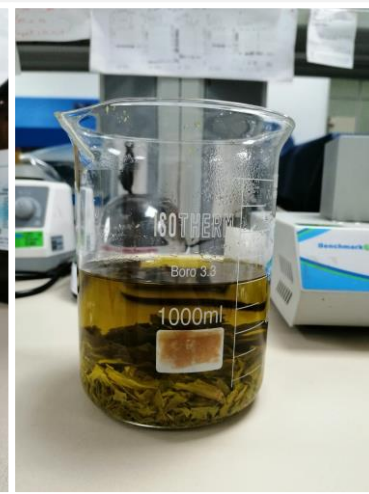
MATERIAL & METHODS





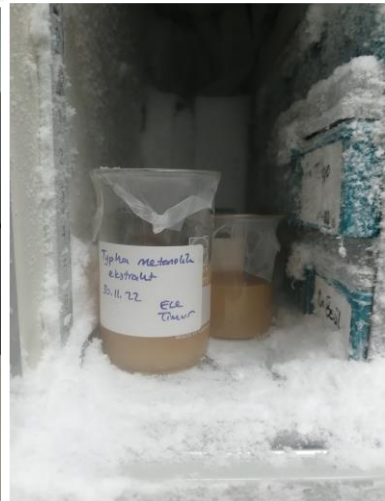
MATERIAL & METHODS

- In September 2022, plants were harvested from Büyükçekmece.
- *Hylotelephium spectabile* was identified by Bezmialem Vakf University Faculty of Pharmacy, Department of Pharmaceutical Botany faculty member Assoc. Dr. Ece Sevgi.



MATERIAL & METHODS

- *Typha domingensis* plants, which grows naturally in the region, were obtained from the Köyceğiz Palm Center in November 2022. (Türkiye Sulak Alan Bitkileri ve Bitki Örtüsü- Ege Üniversitesi Fen Fakültesi Yayınları)



IN VITRO CELL CULTURE EXPERIMENTS

Cell viability test
(MTT assay)

Wound healing test
(scratch assay)

Anti-inflammatory activity

ImageJ software

Cell viability test
(MTT assay)

Measuring TNF- α levels with ELISA kit

HaCaT

human epidermal keratinocyte cell line
MTT assay, scratch assay

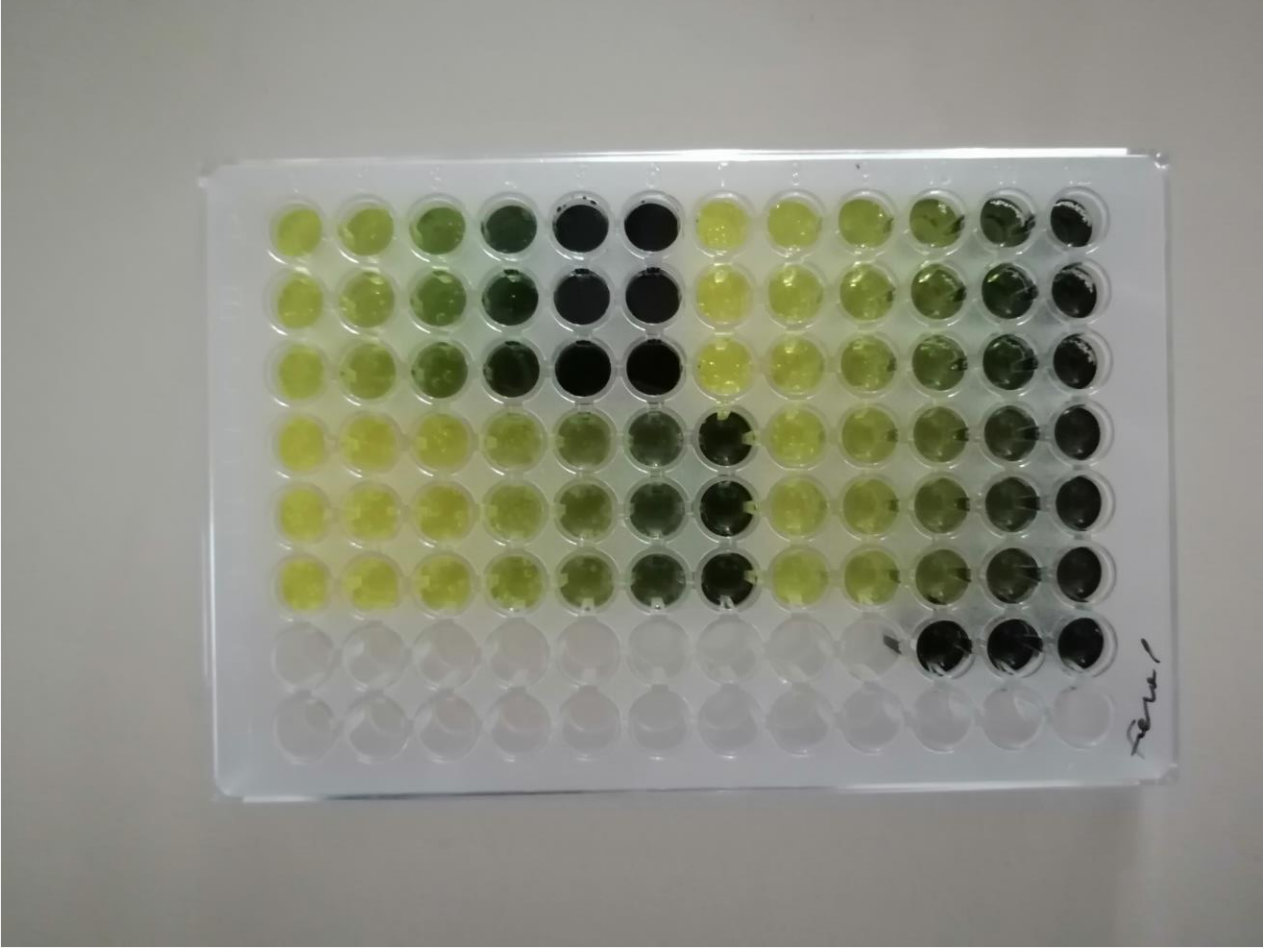
CCD-1072

human skin fibroblast cells
MTT assay, scratch assay

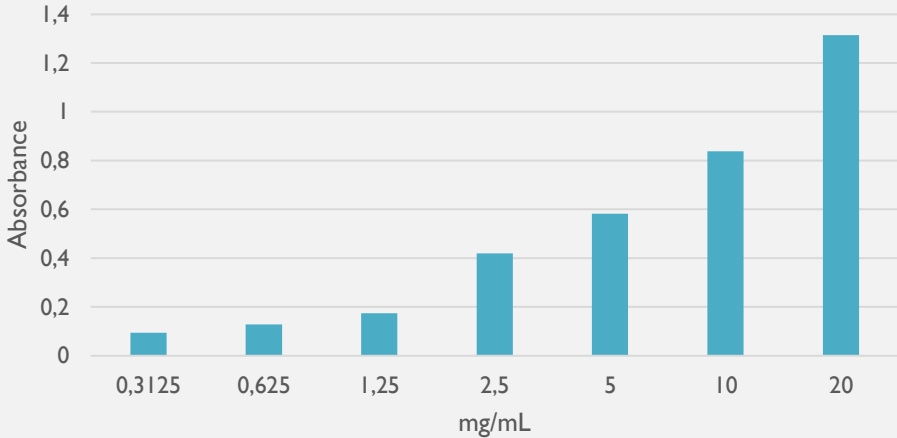
RAW264.7

macrophage cell line
MTT assay, determination of anti-inflammatory activity

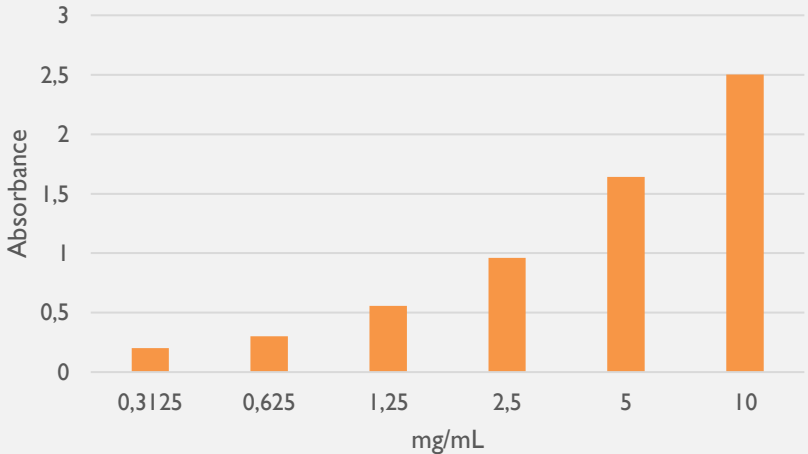
TOTAL PHENOLIC CONTENT



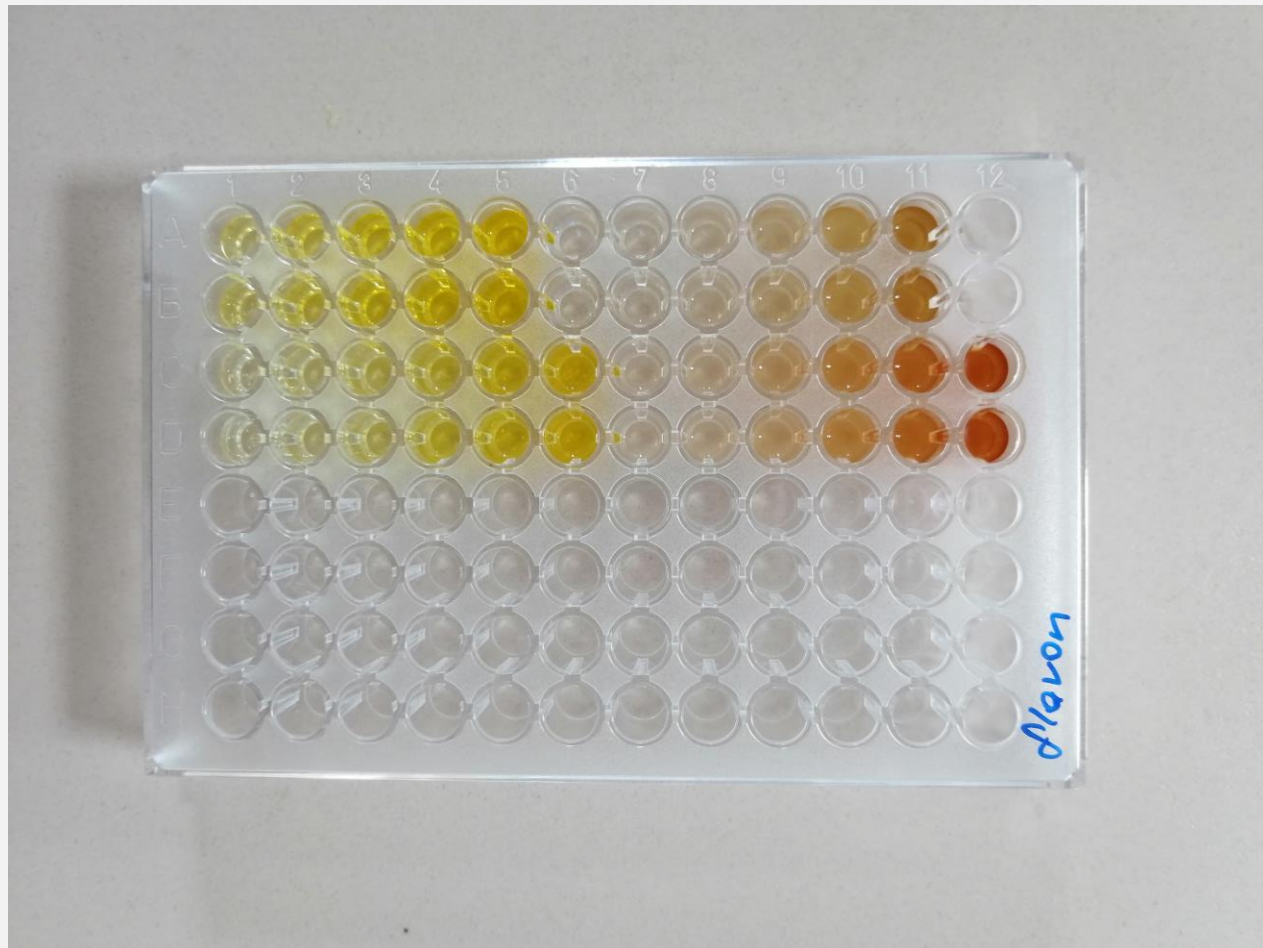
Hylotelephium spectabile



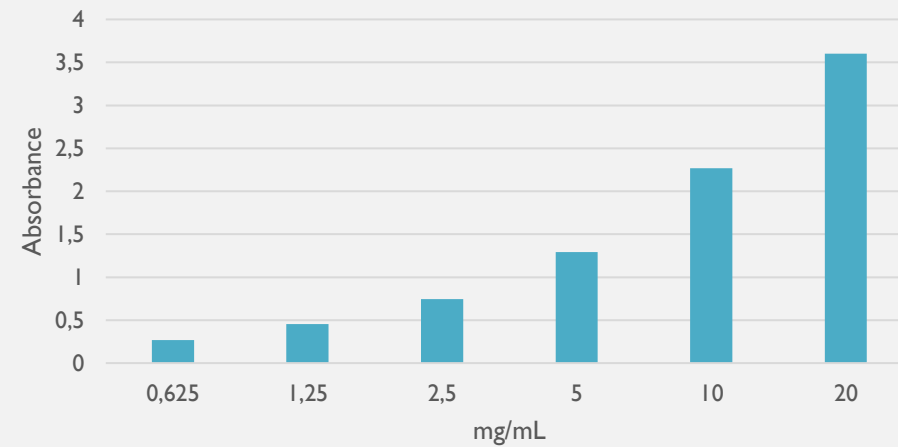
Typha domingensis



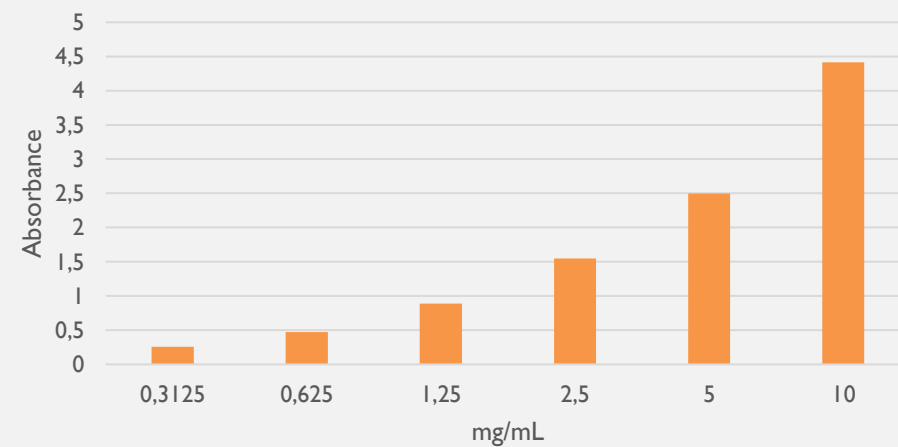
TOTAL FLAVONOID CONTENT



Hylothelephium spectabile



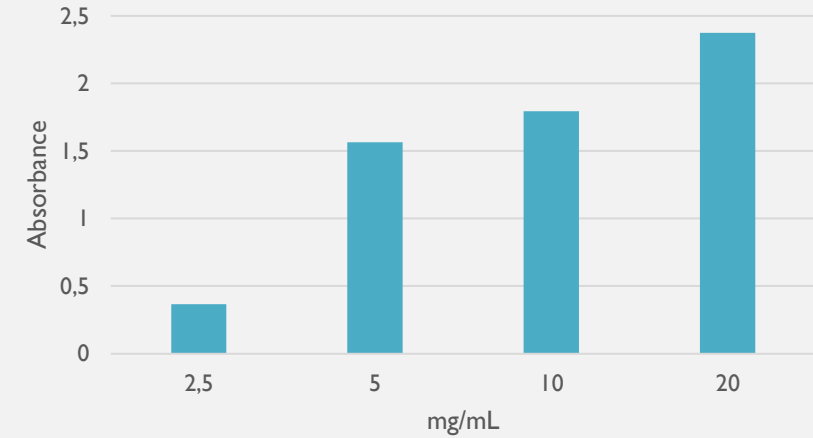
Typha domingensis



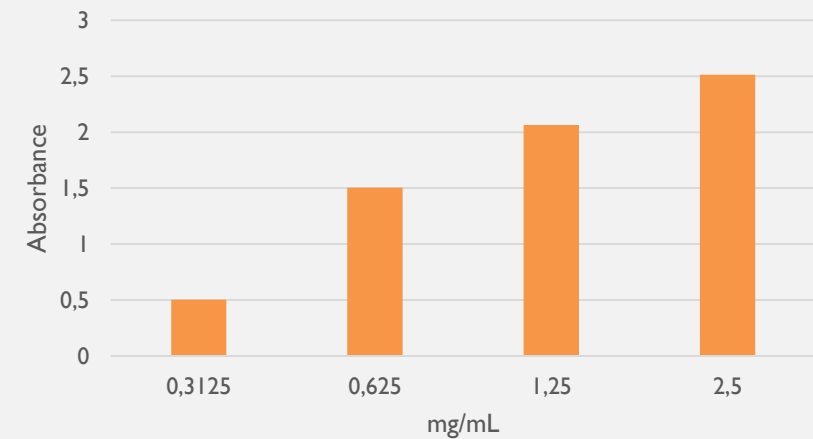
TOTAL ANTIOXIDANT CAPACITY

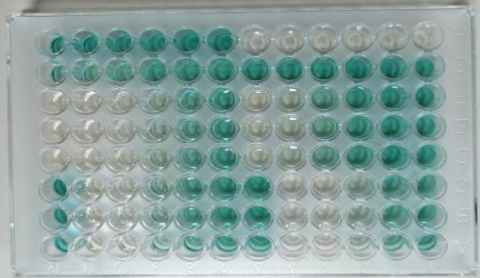
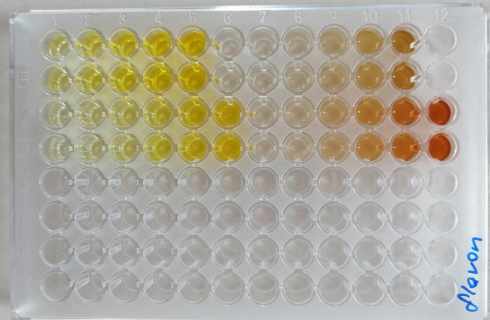
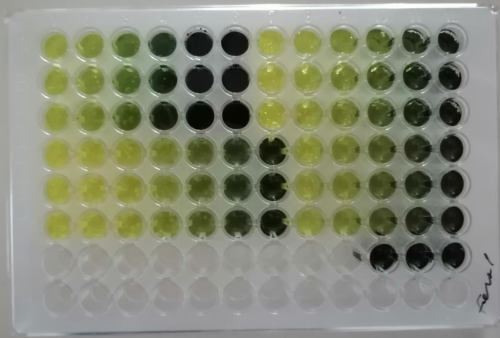


Hylotelephium spectabile



Typha domingensis



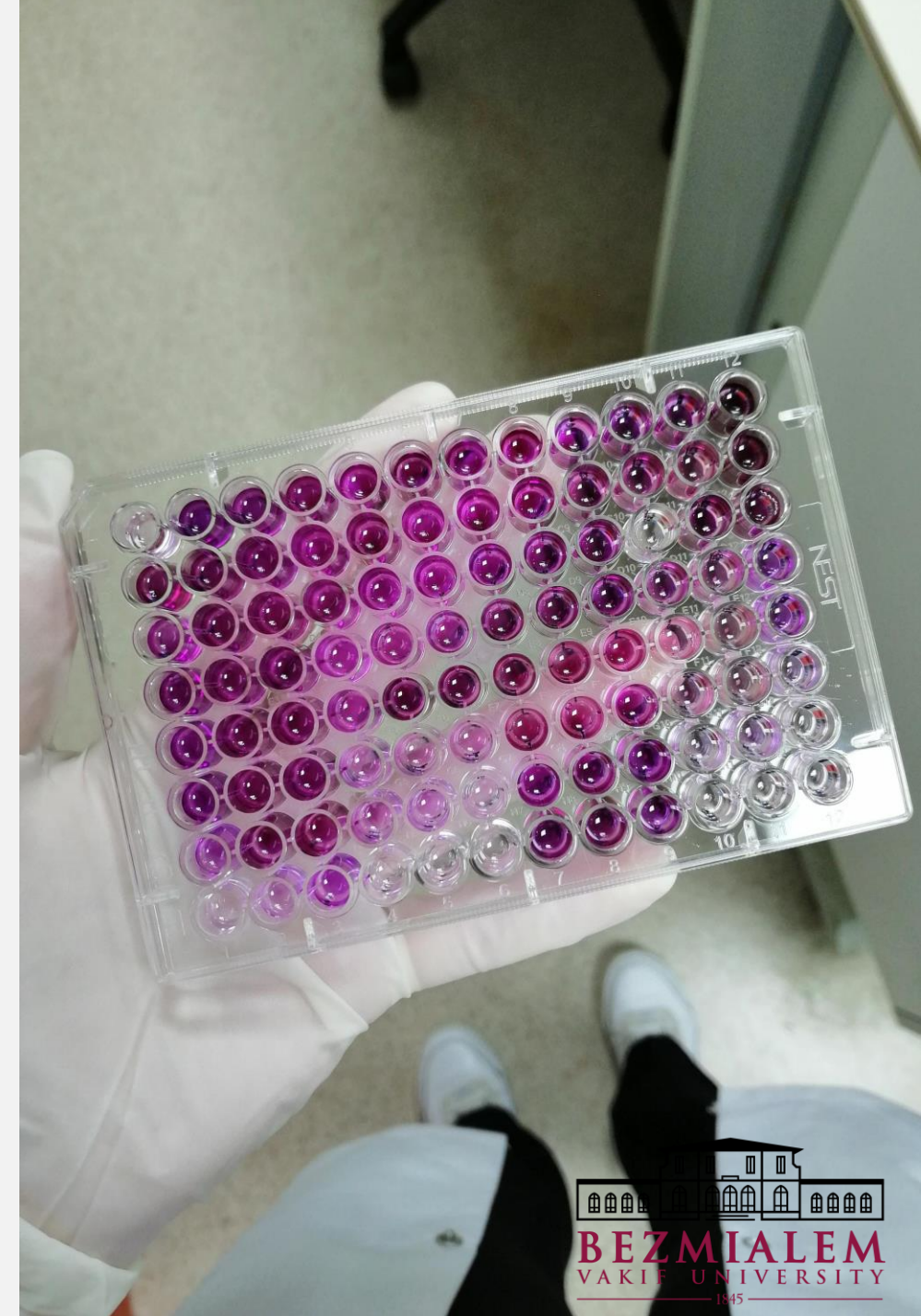
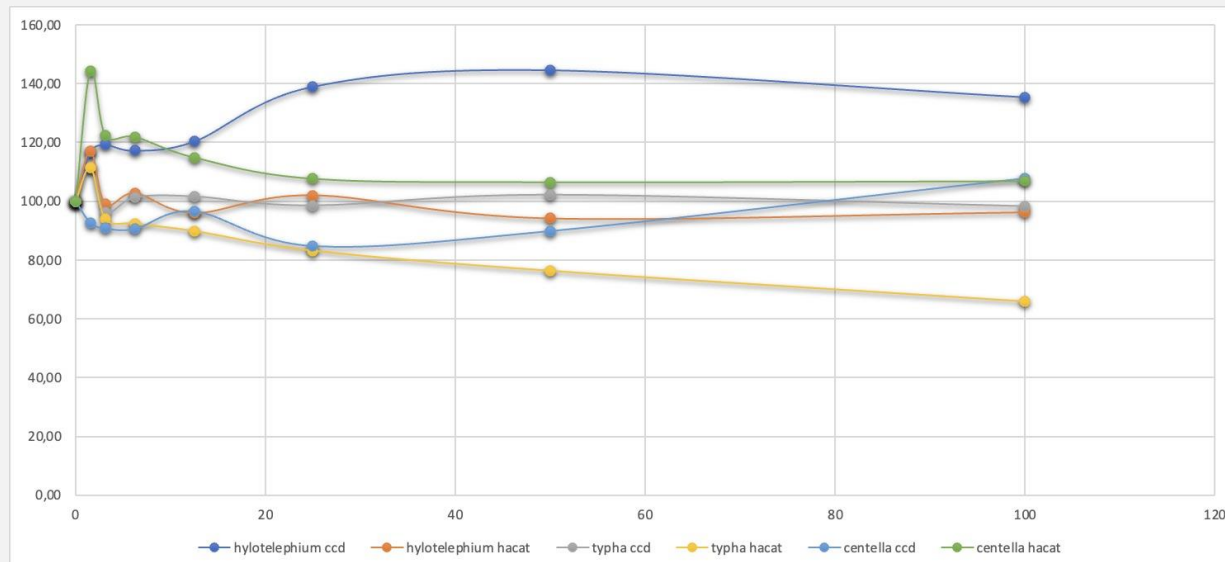


RESULTS

	Hylothelephium spectabile methanolic extract (HSME)	Typha domingensis methanolic extract (TDME)
Total phenolic content	9,8 mgGallicacid Eq/g	36,8 mgGallicacid Eq/g
Total flavonoid content	6,3 mgQueEq/g	13,8 mgQueEq/g
Antioxidant capacity	312,9 ascorbicacidEq/g	521,4 ascorbicacidEq/g

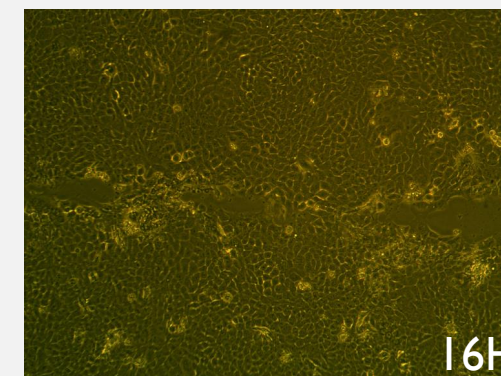
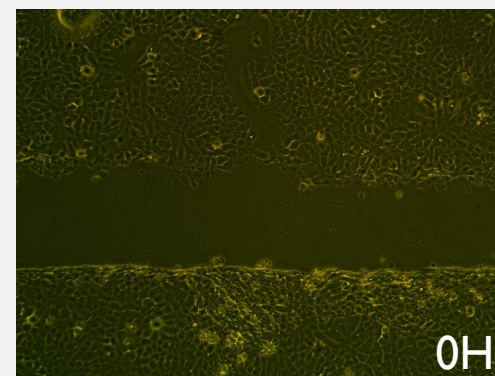
RESULTS

- It was observed that the cell viability was above 80% for each extract under 100 $\mu\text{g/ml}$.
- The maximum non-toxic dose for extracts was determined as 200 $\mu\text{g/ml}$, according to the MTT results.



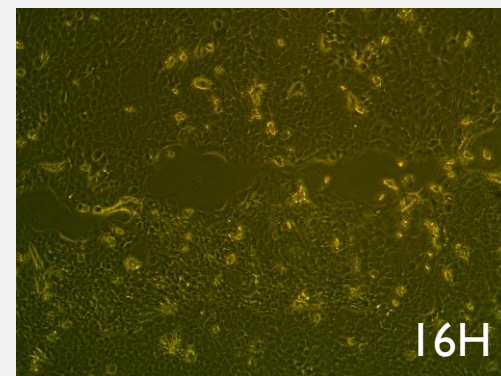
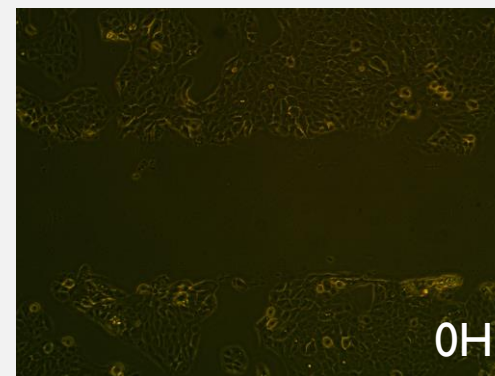
Hylotelephium spectabile methanolic extract – HACAT cell line

	Control	10 ug/mL	25 ug/mL	50 ug/mL	100 ug/mL	200 ug/mL
Percent area closure after 16 hours	74,98	71,15	42,90	54,36	41,86	82,36
Percent area closure after 24 hours	69,85	72,15	78,12	79,25	64,12	93,42



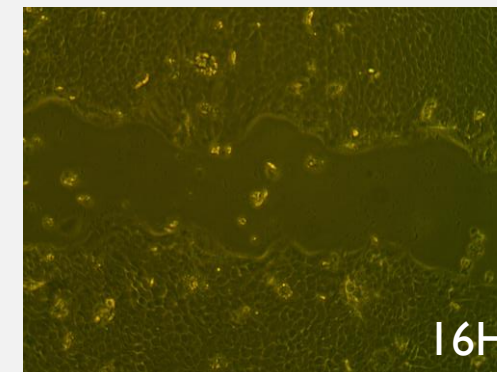
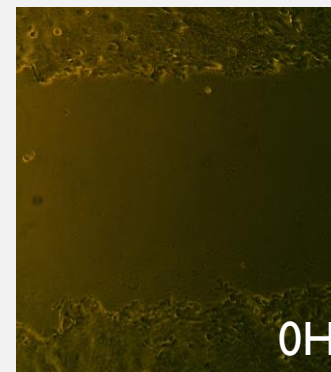
Typha domingensis methanolic extract – HACAT cell line

	Control	10 ug/mL	25 ug/mL	50 ug/mL	100 ug/mL	200 ug/mL
Percent area closure after 16 hours	73,63	85,28		79,03	68,10	44,73
Percent area closure after 24 hours	83,13	87,77		91,45	77,12	59,67

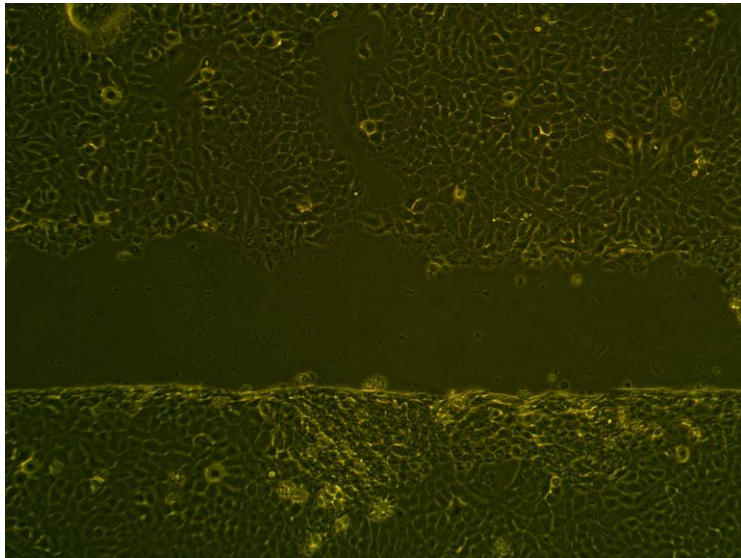


Titrated extract of *Centella asiatica* – HACAT cell line

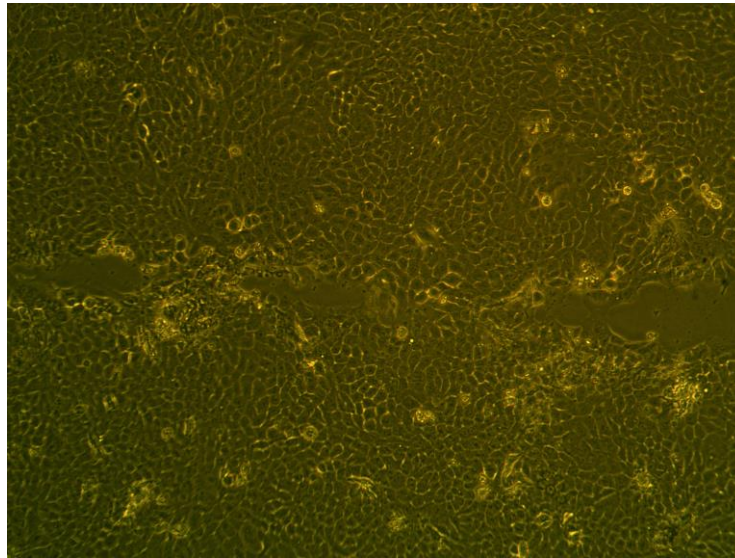
	Control	10 ug/mL	25 ug/mL	50 ug/mL	100 ug/mL	200 ug/mL
Percent area closure after 16 hours	51,36	63,99	64,56	57,51	64,09	46,27
Percent area closure after 24 hours	53,20	77,13	73,50	73,20	70,74	27,74



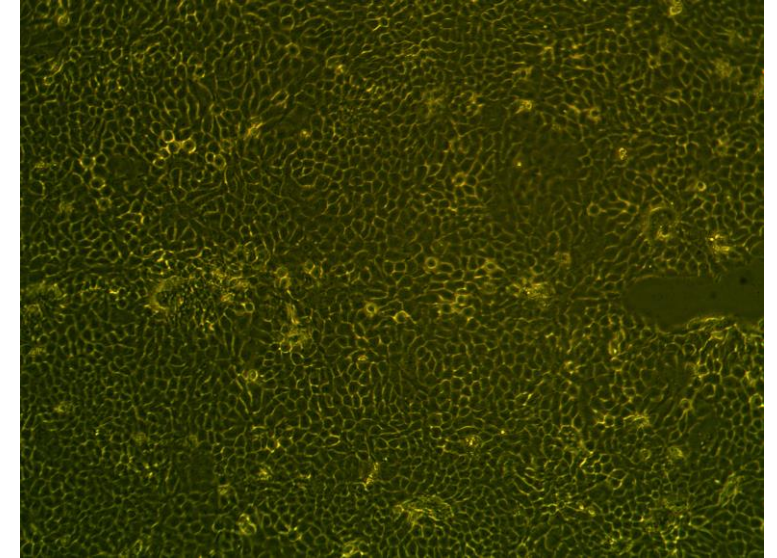
Hylotelephium spectabile methanolic
extract 200 ug/ml
HACAT cell line



0H



16H

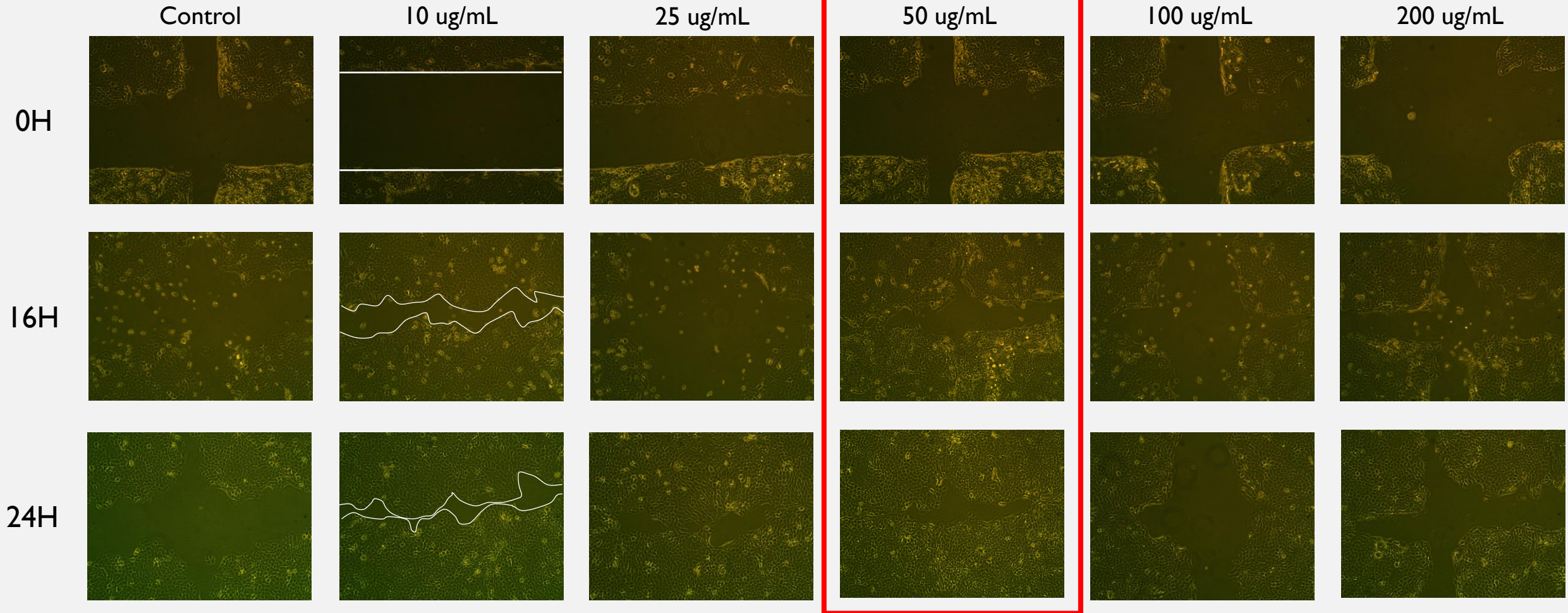


24H

It was observed that 82,3% and 93,4% of the wound area was closed after 16 and 24 hours for 200 ug/ml HSME.

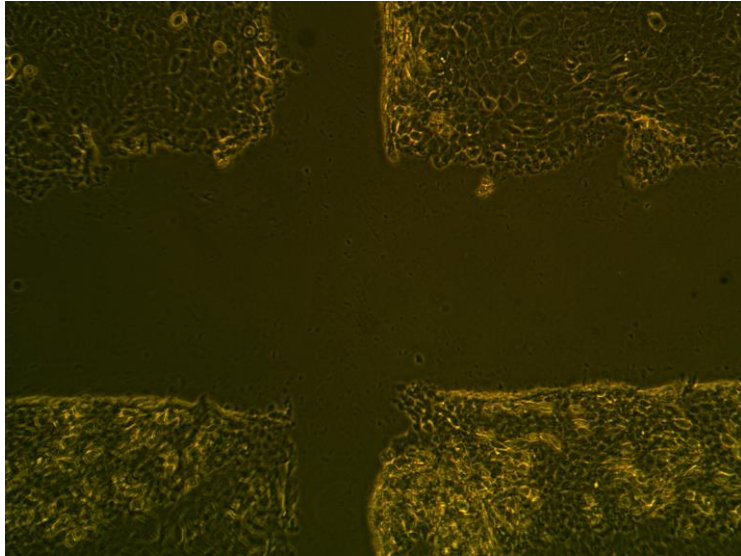
WOUND HEALING ASSAY CCD-I072 CELL LINE

Typha domingensis methanolic extract(TDME)

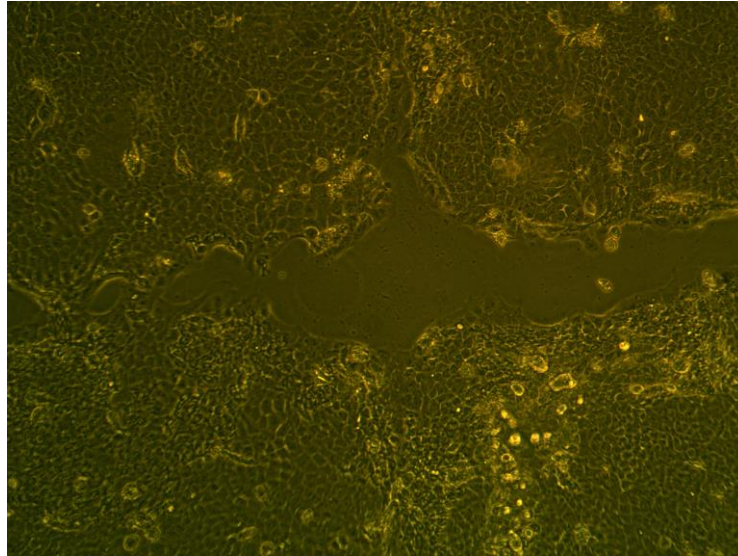


	Control	10 ug/mL	25 ug/mL	50 ug/mL	100 ug/mL	200 ug/mL
Percent area closure after 16 hours	73,63	85,28		79,03	68,10	44,73
Percent area closure after 24 hours	83,13	87,77		91,45	77,12	59,67

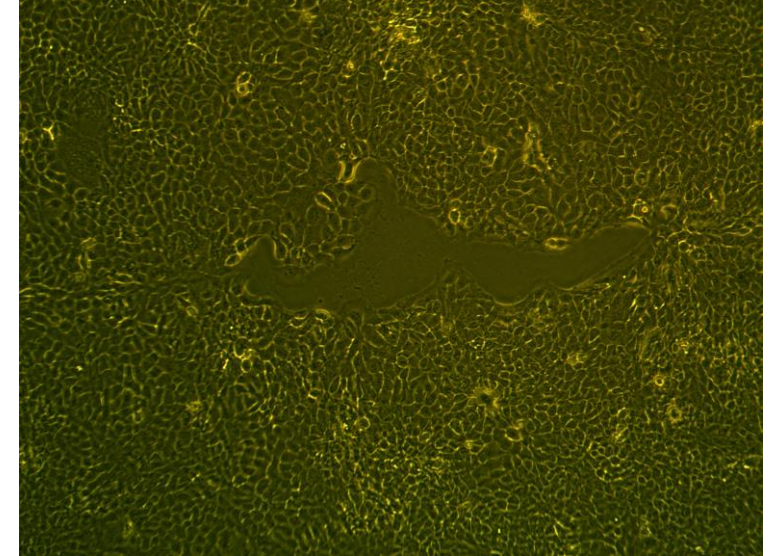
Typha domingensis methanolic extract 50 ug/mL
HACAT cell line



0H



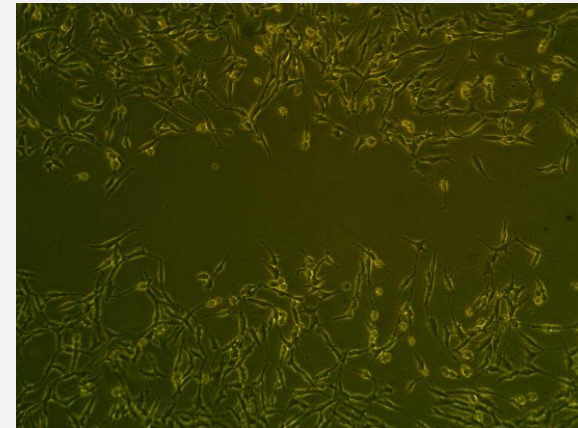
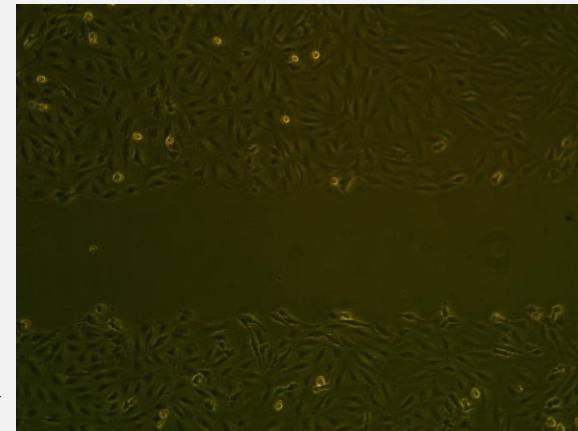
16H



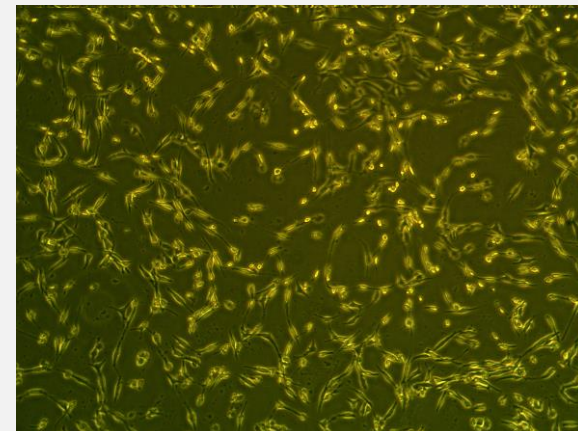
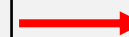
24H

It was observed that 79,03% and 91,45% of the wound area was closed after 16 and 24 hours for 50 ug/ml TDME.

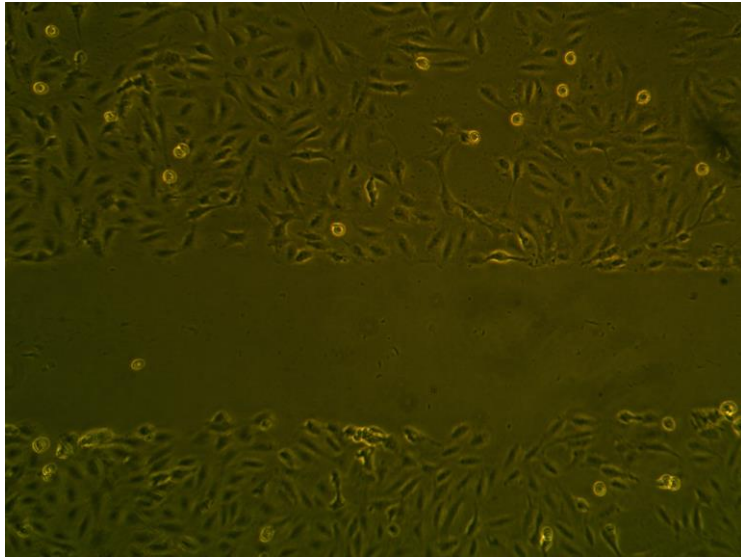
Typha domingensis methanolic extract – CCD-1072 cell line						
	Control	10 ug/mL	25 ug/mL	50 ug/mL	100 ug/mL	200 ug/mL
Percent area closure after 16 hours	22,29	55,24	47,84	79,53	43,27	20,51
Percent area closure after 24 hours	35,89	78,53	64,07	82,14	61,37	55,65



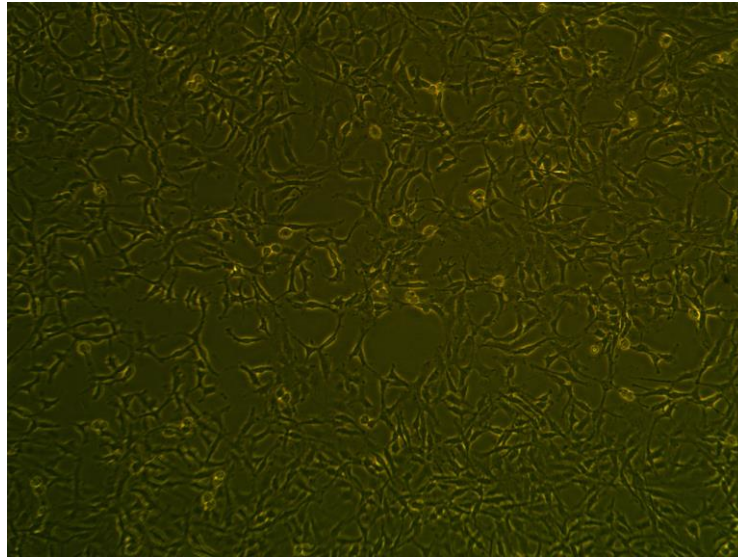
Titrated extract of Centella asiatica – CCD-1072 cell line					
	Control	10 ug/mL	50 ug/mL	100 ug/mL	200 ug/mL
Percent area closure after 16 hours	58,23		94	40,73	-3,04
Percent area closure after 24 hours	61,89		95,54	91,59	-39,10



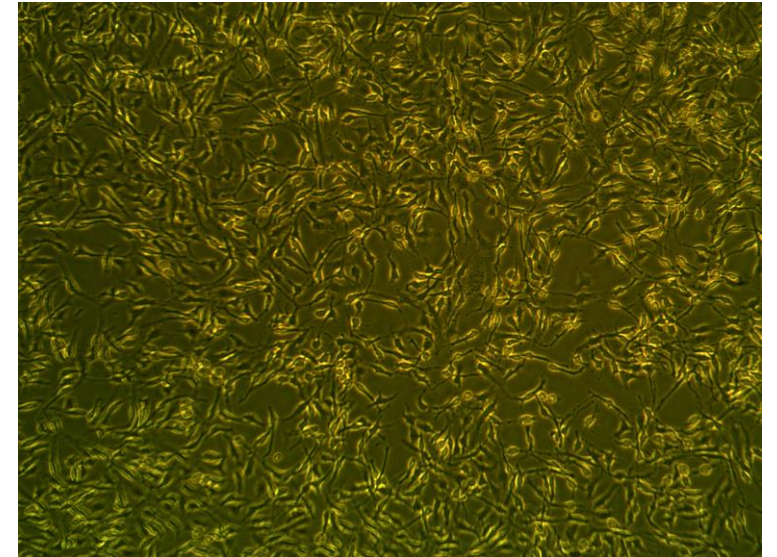
Titrated *Centella asiatica* extract 50 ug/ml
CCD cell line



0H



16H



24H

It was observed that 94% and 95,5% of the wound area was closed after 16 and 24 hours for 50 ug/ml TECA.



Istanbul, Turkey
07.01.2023

CONCLUSION

- TDME was found to have higher phenolic and flavonoid content and antioxidant capacity.
- The findings of this study provide significant evidence for the presence of wound-healing properties in both HSME and TDME.
- The wound healing effect was found comparable to TECA.

DISCUSSION

- It was found that TECA was more effective in promoting the migration and proliferation of fibroblast cells, while TDME was more effective for keratinocytes.
- Since wound healing is a complex process that involves many cell types and signaling pathways, future research can investigate the potential benefits of using a combination.



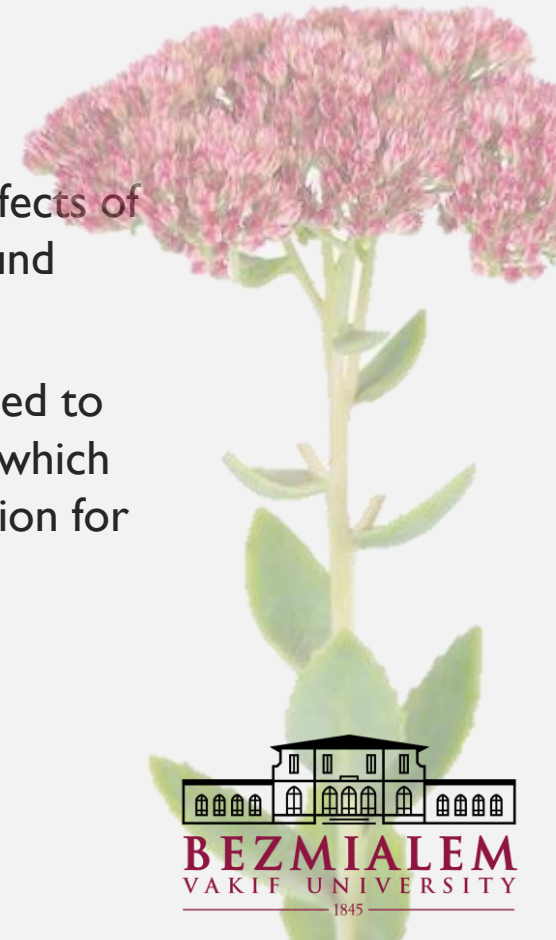
LIMITATIONS & STRENGTH

Limitations

- *in vitro* study
- investigation of the active compounds was not performed, due to financial limitations

Strength

- The first study reporting the effects of *Hylotelephium spectabile* on wound healing.
- These resilient plants can be used to create new synthetic formulas, which might be a more affordable option for treating wounds.



ACKNOWLEDGEMENTS

- Fatmanur Babalı Balıbey
- Zeynep Özman
- Mehmet Timur Başarıcı

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Thank you for listening