Alinteri J. of Agr. Sci. (2021) 36(1): 226-233
e-ISSN: 2587-2249
info@alinteridergisi.com

http://dergipark.gov.tr/alinterizbd
http://www.alinteridergisi.com/
DOI:10.47059/alinteri/V36I1/AJAS21033

RESEARCH ARTICLE

The Role of *Hypericum Lydium* Boiss. (Hypericaceae) in Soft Tissue Healing and its Capacity to Prevent Infections after Dental Extraction

Rukiye Boran[1*] • Nurdan Sarac[2] • Tuba Baygar[3] • Aysel Ugur[4]

[1*]*Medical Laboratory Program, Department of Medical Services and Techniques, Vocational School of Health Services, Aksaray University, Aksaray, Turkey. E-mail: rukiyeboran_@hotmail.com*

[2]*Department of Biology, Faculty of Science, Muğla Sıtkı Koçman University, Muğla, Turkey. E-mail: sarac_63@hotmail.com*

[3]*Material Research Laboratory, Research Laboratories Center, Muğla Sıtkı Koçman University, Muğla, Turkey.*

E-mail: tbay48@hotmail.com

[4]*Department of Basic Sciences, Faculty of Dentistry, Gazi University, Ankara, Turkey. E-mail: ayselugur@hotmail.com*

ARTICLE INFO

Article History:
Received: 08.12.2020
Accepted: 02.03.2021
Available Online: 15.05.2021

Keywords:
Hypericum Lydium
Dental Extraction
Wound Healing
Cytotoxicity
Oral Infection

ABSTRACT

The genus *Hypericum* sp. has a number of uses in traditional medicine like curing the burns, ulcers, haemorrhoids and wound healing. The species *Hypericum lydium* Boiss. (Hypericaceae), however, has not been known to have any properties related to the healing of injuries or antimicrobial working against the oral microorganisms. The present study was aimed to evaluate the efficiency of *H. lydium* in soft tissue healing and its capacity to prevent infections after dental extraction. *H. lydium* was extracted with ethanol and the obtained extract was tested for its inhibition ability on extracellular matrix-degrading enzymes; collagenase, hyaluronidase and elastase. To determine the cytotoxicity and wound healing capacity of the extract, *MTT* and *in vitro* scratch wound healing assay were performed using the NIH-3T3 fibroblast cells, respectively. Antimicrobial activity was investigated by microdilution method against oral pathogenic microorganisms. The highest enzyme inhibition activity was determined against elastase (80.27±0.1%). According to the cytotoxic activity results, the IC_{50} value of the *H. lydium* was found to be 82.20±4.05 µg/mL. Scratch wound healing assay of the extract exhibited a significant enhancement at 24 h with a closed wound area when compared with the control. The extract showed potent antimicrobial properties against oral pathogenic microorganisms. The results of the study revealed out that *H. lydium* can be considered as a natural compound for dental industry to improve soft tissue healing and to prevent the possible infections after dental extraction.

Please cite this paper as follows:

Boran, R., Sarac, N., Baygar, T. and Ugur, A. (2021). The Role of Hypericum Lydium Boiss. (Hypericaceae) in Soft Tissue Healing and its Capacity to Prevent Infections after Dental Extraction. *Alinteri Journal of Agriculture Sciences, 36*(1): 226-233. doi: 10.47059/alinteri/V36I1/AJAS21033

Introduction

The genus Hypericum is a common plant all over the world with 484 species belonging to the Hypericaceae family (Crockett and Robson, 2011). The members of the Hypericum have been widely used topically and orally for the improvement of burns and wounds in various countries of cultural medicine (Öztürk et al., 2007). In addition, there are ethnobotanical records of *Hypericum* sp. being used as an antiseptic for burns and wounds (Doğan et al., 2019). *Hypericum* has a large market share due to its medicinal property.

This increased demand has led to an increase in the number of investigations to determine the pharmacological properties of members of this genus, especially for *Hypericum perforatum* (Çırak et al., 2015). However, many studies have also been published which are about the therapeutic benefit of other *Hypericum* species (Dikmen et al. 2011; Boran, 2018).

Hypericum lydium Boiss.is only available in Northern Iraq and Turkey (Çırak, 2006) and it has traditionally been used as an infusion for the treatment of menstrual disorders, indigestion and stomach pains in Turkey (Altundag and Ozturk, 2011). Although some phytochemical studies have

* Corresponding author: rukiyeboran_@hotmail.com

been performed on *H. lydium* (Çırak, 2006; Çırak et al., 2015), little is known about this species and there are only a few studies addressing its pharmacological features (Şerbetçi et al., 2012; Boran and Ugur, 2017; Aygül and Şerbetçi, 2020).

Dental extraction is one of the most frequently used procedures for the treatment of teeth that are severely damaged and non-restorable due to periodontal disease, excessive tooth decay or dental trauma in oral surgery practice (Khanijou et al., 2019). Post-extraction tissue healing is a very complicated process related to a series of biological events, including tissue repair and remodelling in reply to injury. These procedures involve several interactions among the extracellular matrix (ECM), various cytokines, growth factors, multiple cell types and their mediators (Slevin et al., 2002; Yoneda et al., 2014; Haraway, 2017). Damaged and delayed wound healing is a clinical problem that causes pain, alveolar osteitis, infection, or other complications, and it can even affect dental implants and prosthodontics treatment (Afat et al., 2019).

The ECM components, such as hyaluronan (or hyaluronic acid), collagen and elastin, are helpful in the soft tissue healing steps. Hyaluronan is involved in the induction of early granulation tissue formation during wound healing, inhibition of destructive inflammation, and re-epithelialisation and stimulation of angiogenesis (Slevin et al., 2002). Collagen is a key constituent of a wound healing and it is one of the significant proteins of the ECM. When the tissue is injured, the damaged collagen fibrils, along with other proteins, act as a signal to for platelets to form a clot in the wound area. Collagen also interacts with keratinocytes and myofibroblasts in order to interfere with the wound by interacting with the integrins. Elastin, another protein in the ECM, has a number of mechanical and cell interactive properties for wound healing (Haraway, 2017). It has been found that the extreme deterioration of the newly formed ECM is associated with non-healing wounds (Quirk, 2007).

Infection is the most common complication encountered after tooth extraction. A bone infection that may occur after tooth extraction is a very dangerous ailment and if this infection is not treated, there is a possibility that the patient will go into sepsis. The presence of microbial infection is also a problem that slows down the healing process of a wound (Koslovsky et al., 2007). Therefore, the use of antimicrobial agents after extraction is important to prevent contamination in dental surgery.

Although the herb is popular as an effective and natural wound healing agent; the wound healing potential of *H. lydium* and a possible mechanism in healing have not been investigated. Furthermore, the antimicrobial activity of *H. lydium* against oral microorganisms has not yet been characterized. Therefore, this study was planned to evaluate the potential activity of *H. lydium* in soft tissue healing and its role in preventing infections after tooth extraction.

Material and Methods

• *Chemicals*

Clostridium histolyticum collagenase, bovine hyaluronidase, porcine pancreatic elastase, N-(3-[2-Furyl]-acryloyl)-Leu-Gly-Pro-Ala (FALGPA), epigallocatechin gallate (EGCG), sodium hyaluronate, N-Succinyl-Ala-Ala-Ala-p-nitroanilide (SAPNA), Dulbecco's Minimal Essential Medium (DMEM), fetal calf serum (FCS), L-glutamine, trypsin and antibiotic-antimycotic solution were obtained from Sigma-Aldrich Chemicals Company (St. Louis, USA). Other solvents, culture mediums and *p*-dimethyl amino benzaldehyde were provided from Merck Chemical Co. (Darmstadt, Germany).

• *Plant Material and Extraction*

The plant material was collected in the province of Adana (37º26'56''N 35º07'14''W) (Turkey). Botanical identification was carried out and a voucher specimen was deposited in the Department of Biology, Faculty of Sciences, Muğla Sıtkı Koçman University Muğla, Turkey (Voucher No. ARB-H05). The samples were cleaned using sterile distilled water, and then dried at room temperature. Thirty grams powdered material was extracted with ethanol by soxhlet. After cooling, the extract was filtered and the ethanol was evaporated. The residue was kept at -20ºC for further analysis.

• *Inhibition of Matrix Degrading Enzymes*

Collagenase Inhibitory Activity

Twenty-five microliters of extract (in 5% (v/v) dimethyl sulfoxide (DMSO)) and tricine buffer (pH 7.5) (containing 10 mM $CaCl_2$ + 400 mM NaCl) was added to 25 µL collagenase in a 96-well microtiter plate. EGCG was used as reference and DMSO (5% (v/v)) was used as a negative control. The samples were pre-incubated at 37°C for 20 min and 1.6 mM FALGPA was added. Absorbance of samples were measured rapidly at 335 nm and repeated at 2-minute intervals for 20 min (Barrantes and Guinea, 2003).

• *Hyaluronidase Inhibitory Activity*

A 100 µL test sample was added to 7900 U/mL bovine hyaluronidase in a 96-well microtiter plate. Tannic acid and DMSO (5% (v/v)) were used as the reference and negative control, respectively. After 20 min incubation at 37ºC, 100 µL of $CaCl_2$was transferred to the reaction mixture. After incubation for 20 min at 37ºC, sodium hyaluronate was transferred to the solution and again incubated for 40 min at 37ºC. 0.2 M sodium borate solution and 50 µL NaOH (0.2 M) was mixed and, the solution was heated in a boiling water bath for 3-4 min and then cooled to room temperature. Then, *p*-dimethyl amino benzaldehyde solution was transferred to the mixture and again incubated at 37ºC for 20 min. Absorbance of each solution was recorded at 585 nm (Lee et al., 1999).

- ***Elastase Inhibitory Activity***

The assay was performed using a 96-well microtiter plate as previously described (Lee et al., 1999). Microtiter plate containing 200 mM Tris–HCl buffer and sample were inoculated with 25 µL of elastase and pre-incubated at 25°C for 20 min. EGCG and DMSO (5% (v/v)) were used as the reference and negative control, respectively. SAPNA solutions (125 µL) added to the above cited mixture and absorbance was monitored at 410 nm for 20 min. The following formula (1) was used to determine the percentage of inhibition of collagenase, hyaluronidase and elastase:

Inhibition (%) = [(*x* - *y*) – (*z* - *t*)] / (*x* - *y*) x 100 (1)

Where, *x* is the absorbance without the test extract, *y* is the absorbance without the test extract and enzyme, *z* is the absorbance with the test extract, and *t* is the absorbance with the test extract without enzyme.

- ***Cytotoxicity and in Vitro Scratch Wound-healing Assay***

Culture of NIH-3T3 Mouse Embryonic Fibroblasts

The mouse embryonic fibroblast cell line NIH-3T3 (provided from American Type Culture Collection, Manassas, VA, USA), was cultured in DMEM with 10% heat-inactivated FCS and 1% antibiotic-antimycotic solution (1000 U/mL penicillin and 1000 U/mL streptomycin). Then, cells were maintained at 37ºC in 5% CO_2 supplemented humidified atmosphere.

- ***Cytotoxicity***

Before performing the wound-healing assay, cytotoxicity of the *H. lydium* extract was determined against NIH-3T3 fibroblast cells using MTT colorimetric assay (Mosmann 1983). Prior to the *H. lydium* extract treatment, fibroblast cells were cultured as described above, passaged in 96-well plates at plating density of $1x10^4$ cells per well, and incubated at 37°C, 5% CO_2, and 100% relative humidity for 24 h. Later the medium was replaced with the respective medium containing the *H. lydium* extract (at 500, 250, 125, 62.5, 31.25, 15.625 µg/mL concentrations). The plates were incubated at the same conditions for 24 h. Six replicates was maintained and the medium with DMSO at the same concentrations of the sample (without the *H. lydium* extract) served as control. 20 µL of MTT was added to each well after 24 h and incubated at 37°C, 5% CO_2 for additional 3 h. The MTT-containing medium was then poured off and 100 µL of DMSO was used to dissolve the formazan crystals formed in each well. The plates were incubated for 15 min in an orbital shaker and the absorbance values were detected at 540 nm. The % cell inhibition was detected using the following formula (2) and the graph between % cell inhibition and concentration were plotted, from which IC_{50} was calculated:

$$(\%) = [100 \times (Sample_{abs}) / (Control_{abs})] \quad (2)$$

- ***In Vitro Scratch Wound Healing Assay***

The spreading and migration capabilities of NIH-3T3 fibroblasts with the presence of *H. lydium* extract were determined using scratch wound healing assay (Liang et al., 2007). Briefly, fibroblast cells were grown as described above and exponentially growing cells were seeded onto a cell culture plate at a density of 75×10^4cell/plate. After maintaining the plates for 24 h, a linear wound was created in the monolayer with a sterile plastic pipette tip. Any cellular debris was removed by washing the plate with Dulbecco's phosphate buffered saline. The minimum dose lower than the IC_{50} value of the extract, which was determined by pilot studies, was used for *in vitro* scratch wound healing assay to perform the wound healing potential of the *H. lydium*. For this purpose, cells were treated with 10 µg/mL *H. lydium* extract and maintained in culture for a period of 48 h. DMSO was used as a control. Representative images from each cell culture plate of the scratched areas were photographed using a Leica DM IL microscope (Leica Microsystems, Wetzlar, Germany) to estimate the relative cell migration.

- ***Antimicrobial Activity***

S. aureus (ATCC25923), *S. mutans* (ATCC25175), *S. sanguinis* (ATCC10556) and *C. albicans* (ATCC10239) were purchased from American Type Culture Collection. *S. aureus* cultured in Nutrient Broth at 37ºC, *S. mutans* and *S. sanguinis* were cultured in Brain Heart Infusion Broth (BHIB) at 37ºC. *C. albicans* was cultured in Sabouraud Dextrose Broth (SDB) at 30ºC. Broth microdilution method was used to define the minimal inhibitory concentration (MIC) in accordance with the National Committee for Clinical Laboratory Standards (NCCLS), (NCCLS, 1993).

- ***Statistical Analysis***

One-way analysis of variance (ANOVA) followed by Tukey's test was used to define differences between the means. A value of $p \leq 0.05$ was considered as statistically significance.

Result and Discussions

Inhibition of Matrix Degrading Enzymes

In the present study, collagenase, hyaluronidase and elastase enzyme inhibition activities were studied to investigate the inhibitory effects of *H. lydium*on *in vitro* extracellular matrix degrading enzymes (Table 1). At 1 mg/mL, the collagenase, hyaluronidase and elastase enzyme inhibition effects of *H. lydium* were found as 26.3 ± 0.36, 14.2 ± 0.27 and 80.27 ± 0.1%, respectively. EGCG was used as a reference for collagenase and elastase enzyme inhibition tests, while tannic acid was used as a reference for the study of hyaluronidase enzyme inhibition. EGCG was found to have remarkable inhibitory activity on collagenase (35.3 ± 0.24%) and elastase (42.4 ± 0.20%) enzymes at 100 µg/mL concentrations, and tannic acid exhibited hyaluronidase inhibition (57.4 ± 0.51%) at the 1 mg/mL concentration. The study results showed that the extract

of *H. lydium* showed slight inhibitory activity on hyaluronidase enzyme (14.2 ± 0.27%). On the other hand, the extract had a significant inhibitory effect on elastase, with inhibition value of 74.0 ± 0.08% at 100 µg/mL and 80.27 ± 0.1% at 1 mg/mL, while EGCG which was used as a positive control, exhibited the inhibiting activity of 42.4 ± 0.20% at 100 µg/mL concentration.

A tooth extraction often leaves a large soft tissue and bone defect behind. The healing process after extraction is greatly affected by various local and systemic factors. As with all surgical procedures, it is desirable for the wound to heal rapidly as soon as possible without any complications in the wounds related to the tooth extraction (Yoneda et al., 2014). The first target of this study was to determine the potential contribution of the extract of *H. lydium* plant which was used to improve wounds in ethno-botanical recordings, to soft tissue wound healing process after dental extraction.

Table 1. Inhibitory activity of the *H. lydium* ethanol extract to matrix degrading enzymes

Inhibition (% ± S.E.M.)				
Material	**Concentrations**	**Collagenase**	**Hyaluronidase**	**Elastase**
H. lydium	1 mg/mL 100 µg/mL	26.3 ± 0.36[a] 6.2 ± 0.43	14.2 ± 0.27 -[b]	80.27 ± 0.10 74.0 ± 0.08
Epigallocatechin gallate	100 µg/mL	35.3 ± 0.24	NT[c]	42.4 ± 0.20
Tannic acid	1 mg/mL	NT[c]	57.4 ± 0.51	NT[c]

[a]Values expressed are means ± S.D. of three replications ($p \leq 0.05$)
[b]No activity.
[c]Not tested.

Extracellular matrix components play a significant role in the regulation of all stages of tissue repair, cell migration, inflammation, angiogenesis, remodelling, and scar formation (Slevin et al., 2002; Haraway, 2017). Preventing excessive degradation of ECM molecules in tissue is important for wound healing. Thus, components with collagenase, hyaluronidase, and elastase inhibiting properties may provide beneficial results in preventing degradation of the matrix and conferring hyaluronan, collagen and elastin to ECM (Gill and Parks, 2008). In this study, it was observed that *H. lydium* extract had very high elastase inhibition activity (80.27 ± 0.1%). It was also found that the extract showed 26.3 ± 0.36% inhibition of collagenase and a mild inhibitory activity (14.2 ± 0.27%) on the hyaluronidase enzyme (Table 1).

In a similar study, it was reported that *Hypericum hircinum* L. crude extract inhibit half of the collagenase activity at the 156 µg/mL concentration (Mandrone et al., 2015). Similarly, *Hypericum origanifolium* Willd. ethanol extract inhibited the collagenase (79.39%) and elastase (56%) activities at 1 mg/mL (Boran, 2018). In a similar study, *H. perforatum, Hypericum confertum* and *Hypericum calycinum* methanol extracts showed collagenase inhibition activity with IC_{50} values of 61.53, 63.01 and 51.24 µg/mL, respectively; showed elastase inhibition activity with IC_{50} values of 64.76, 61.67 and 55.77µg/mL, respectively. In addition, *H. calycinum* exhibited significant hyaluronidase inhibition (IC_{50} value 22.17 µg/mL), while *H. confertum* and *H. perforatum* showed considerable inhibition (IC_{50} values 36.55 and 30.49 µg / mL) (Ersoy et al., 2019).

Phloroglucinol derivatives, naphthodianthrones, essential oils and various phenolics are thought to be the main bioactive compounds in *Hypericum* extracts, and in various studies, different parts of *H. lydium* have been indicated contain hypericin, pseudohypericin, hyperforin, hyperoside, adhyperforin, quercetin, chlorogenic acid, apigenin-7-O-glucoside, avicularin, amentoflavone, rutin and (+)-catechin (Çırak, 2006; Çırak et al., 2015). The enzyme inhibitory activity may be derived from apigenin, quarcetin, rutin, cathechin and chlorogenic acid etc. which are components of the plant. Apigenin, commonly used as a hyaluronidase inhibitor (Salmen et al., 2005), reported to create a specific inhibition to collagenase while quarcetin, rutin and cathechin inhibited the hyaluronidase reaction (Lee and Kim, 2010; Acton, 2012). Another study reported that catechin contains high inhibitory activity against hyaluronidase, but that chlorogenic acid showed low inhibitory action (Ao, 2009). In the study of Mandrone et al. (2015) chlorogenic acid, flavonol glycosides and 5,7,3′,5′-tetrahydroxyflavanone exhibited high collagenase inhibitory activity.

Cytotoxicity and in Vitro Scratch Wound-healing Assay

Biomaterials that are biologically and toxicologically compatible have a vital role in limiting or preventing irritation or degeneration that may occur in the tissue. Cytotoxicity and cell viability screening assays provide a measure for their clinical usage (Murray et al., 2007). For this reason, the cytotoxicity of *H. lydium* extract on NIH-3T3 fibroblast cells was determined using MTT colorimetric method. The viability of NIH-3T3 cells exposed to *H. lydium* extract was measured and expressed in terms of relative absorbance by comparison with control cells. The IC_{50} value of *H. lydium* extract was found to be 82.20 ± 4.05 µg/mL which might be suggested that the extract has a dose-dependent cytotoxicity on the NIH 3T3 cell line (Figure 1).

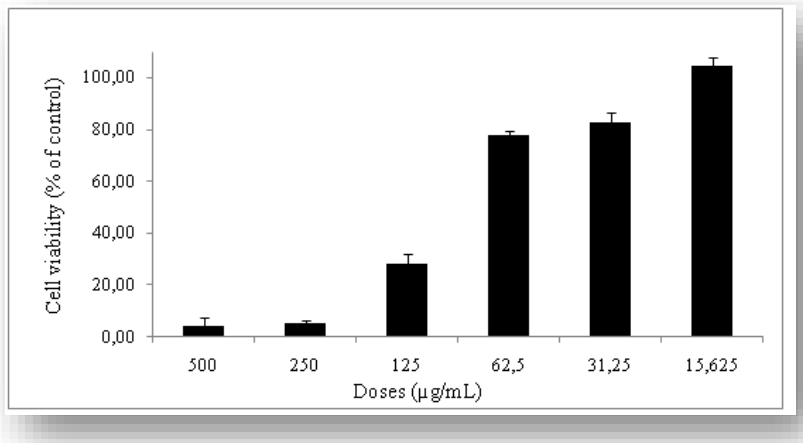

Figure 1. Cytotoxic effects of *H. lydium* extract on NIH-3T3 fibroblasts evaluated by MTT colorimetric assay. Results are presented as viability ratio compared to the control group. Values were expressed as the mean of six replicates

The wound healing ability of *H. lydium* extract was determined by using the scratch wound healing assay by observing the effect of the extract on the NIH-3T3 fibroblasts' spreading and migration capabilities (Figure 2). Fibroblast cells play a remarkable role in the wound healing process due to their migrations, proliferations, contractions and collagen productions. Since they are related to the synthesis of collagen and other adhesion molecules, they play a major role in the proliferative phase of wound healing. At this stage, the fibroblasts migrate to the wound area and then their mitotic activity increases (Diegelman et al., 1987). To establish the effect of *H. lydium* extract on fibroblasts' migration and proliferation, scratch-wound healing assay was applied to NIH-3T3 fibroblasts and images were taken at regular intervals. By 10 µg/mL *H. lydium* extract treatment to cell culture plate, fibroblasts were found to be stimulated of the cell migration after 24 h incubation and the wound gap completely closed after 48 hours (Figure 2).

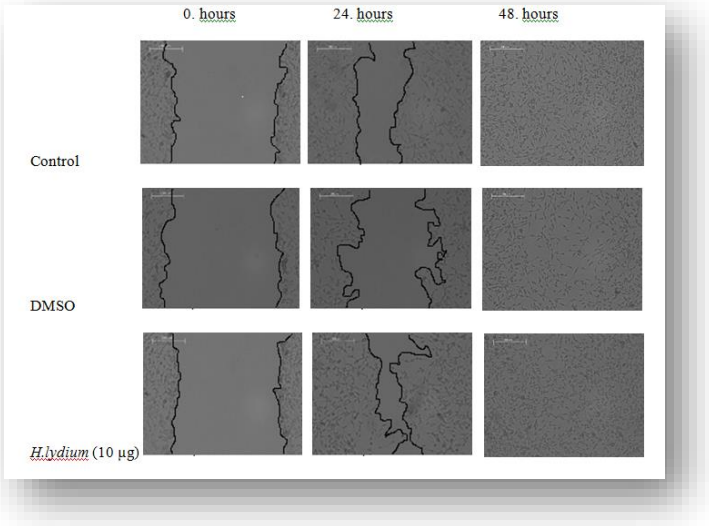

Figure 2. Images of *in vitro* scratch assay for 0., 24. and 48. h after creating the scratch. The bar represents for 200 µm

Dikmen et al. (2011) studied the wound healing effects of *H. perforatum* ssp. *veronense* (Schrank) and *H. perforatum* ssp. *perforatum* on NIH-3T3 fibroblasts. The researchers found that wound healing activity did not appear to be involved in mitotic activities. According to the results of the study, it was stated that the healing effect of the extracts may be due to fibroblast migration and stimulation of collagen production. Similarly, Öztürk et al. (2007) investigated the wound healing effectiveness of *H. perforatum* on chicken embryonic fibroblasts. This effect was reported to be due to an increase in the activation of fibroblast cells and stimulation of collagen production of fibroblasts.

Antimicrobial Activity

Postoperative infection after tooth extraction has been reported to vary between 1.2% and 27% (Afat et al.,2019). As a result of tooth extraction, it is important to prevent

infection in the surgical area. The antimicrobial effect of a drug is important parameter as it creates protect over the injured area to keep it safe from any kind of infections. Figure 3 shows the antimicrobial activity of *H. lydium* ethanol extract against 3 oral pathogenic bacteria and 1 yeast strain. MIC values of extract were found to be1.0, 2.0, 8.0 and 32 mg/mL for *S. sanguinis*, *S. mutans*, *C. albicans* and *S. aureus*, respectively (Figure 3).

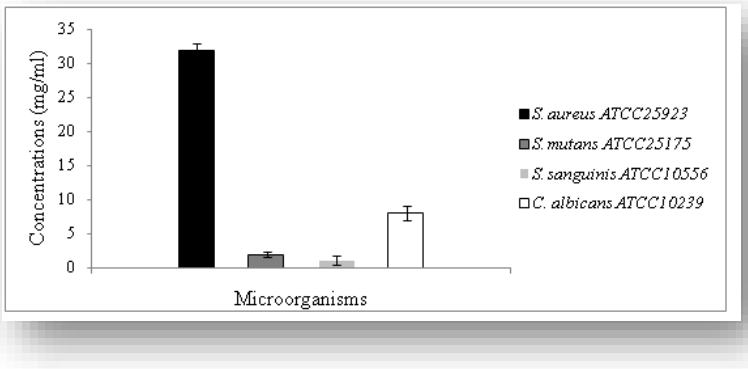

Figure 3. Minimal inhibitory concentration of the *H. lydium* extract on oral microorganisms

Many researchers have studied the antimicrobial effect of extracts or essential oils of *Hypericum* species as the member of the genus have been used antiseptically in a variety of conventional Turkish medicine. These studies have shown that various *Hypericum* species had antimicrobial activity against *S. aureus*, *Streptococcus sobrinus*, *S. mutans*, *Pseudomonas aeruginosa*, *Pseudomonas tolaasii*, *Escherichia coli*, *Micrococcus luteus*, *Bacillus cereus*, *Salmonella enteritidis*, *Sarcinalutea*, *Agrobacterium tumefaciens*, *Proteus mirabilis*, *Enterococcus faecalis*, *Lactobacillus plantarum* and *C. albicans* (Saroglou et al., 2007; Süntaret al., 2016). However, to our knowledge, there are few studies on the antimicrobial effect of *H. lydium* extract or oil, and there is no study on the antimicrobial efficacy of *H. lydium* against oral pathogens. In a study, the ethanol extract of the *H. lydium* inhibited the growth of *E. coli* and *S. aureus* standard strains (Aygül and Şerbetçi, 2020).

Conclusion

Inhibition of ECM degrading enzymes, acceleration of migration and proliferation of fibroblasts and the prevention of microbial infections may be helpful for rapid healing of soft tissue wounds after tooth extraction. In this study, the *in vitro* assays indicated that *H. lydium* extract can promote the wound healing process of soft tissue by accelerating the migration and proliferation of fibroblasts and inhibiting the enzymes related to the wound healing. In addition, antimicrobial potential of *H. lydium* against oral pathogenic microorganisms may play a role for preventing postoperative infection of the wound area. The use of *H. lydium* can be considered as safe, effective, and predictable treatment option for the acceleration of wound healing and prevent of infection in the post extraction site. *H. lydium* might be regarded as a natural remedy to the dental industry to overcome the dental extraction-related problems. This study provides scientific support for the traditional use of *H. lydium* as a wound healing plant.

Compliance with Ethical Standards

a) Authors' Contributions

RB conducted the inhibition of matrix degrading enzymes and antimicrobial activity; wrote the article with the contribution from all co-authors. NS performed the cytotoxic activity. TB performed the *in vitro* scratch wound healing assay. AU supervised all stages of the research. All authors approved the final version of the manuscript.

b) Conflict of Interest

The authors declare no conflict of interest.

c) Statement of Human Rights

This study does not involve human participants.

Acknowledgements

A part of this work was presented at the 7th International Molecular Biology and Biotechnology Congress. The authors would like to thank to Dr. Ceylan for his kind contribution of identifying the plant material.

References

Acton, Q.A. (2012). *Metalloendopeptidases. Advances in Research and Application.* 2012 Edition. Athlanta, Scholary Edt., 1–35.

Afat I.M., Akdoğan E.T., and Gönül O. (2019). Effects of leukocyte- and platelet-rich fibrin alone and combined with hyaluronic acid on early soft tissue healing after surgical extraction of impacted mandibular third molars: A prospective clinical study. *Journal of* Cranio-*Maxillofacial* Surgery, *47*(2), 280-286. https://doi.org/10.1016/j.jcms.2018.11.023.

Ao, C. (2009). *Studies on antioxidant, antibacterial and anti-inflammatory active compounds from medicinal plants.* A dissertation submitted to the United

Graduate School of Agricultural Sciences, Kagoshima University.

Aygül, A., and Şerbetçi, T. (2020). The antibacterial and antivirulent potential of *Hypericum lydium* against *Staphylococcus aureus*: Inhibition of growth, biofilm formation, and hemolytic activity. *European Journal of Integrative Medicine*, 35, 101061.

https://doi.org/10.1016/j.eujim.2020.101061

Altundag, E., and Ozturk, M. (2011). Ethnomedicinal studies on the plant resources of east Anatolia, Turkey. *Procedia - Social and Behavioral Sciences*, 19, 756-777.
https://doi.org/10.1016/j.sbspro.2011.05.195

Barrantes, E., and Guinea, M. (2003). Inhibition of collagenase and metalloproteinases by aloins and aloe gel. *Life Sciences*, 72(7), 843-850.

https://doi.org/10.1016/S0024-3205(02)02308-1

Boran, R. (2018). Investigations of anti-aging potential of Hypericum origanifolium Willd. for skincare formulations. *Industrial Crops and Products*, 118, 290-295.
https://doi.org/10.1016/j.indcrop.2018.03.058

Boran, R., and Ugur, A. (2017). The mutagenic, antimutagenic and antioxidant properties of *Hypericum lydium*. *Pharmaceutical Biology*, 55(1), 402–405.
https://doi.org/10.1080/13880209.2016.1242146

Crockett, S.L., and Robson, N.K.B. (2011). Taxonomy and chemotaxonomy of the genus *Hypericum*. *Medicinal and Aromatic Biotechnology in Plant Science*, 5(1), 1-13.

Çirak, C. (2006). Hypericin in Hypericum lydium Boiss. growing in Turkey. *Biochemical Systematics and Ecology*, 34(12), 897-899.

https://doi.org/10.1016/j.bse.2006.08.002

Çırak, C., Radusiene, J., Ivanauskas, L., Jakstas, V., and Çamaş, N. (2015). Population variability of main secondary metabolites in *Hypericum lydium* Boiss. (Hypericaceae). *Iranian Journal of Pharmaceutical Research*,14(3), 969-678.

Diegelman, R.F., Kim, J.C., Lindblad, W.J., and Cohen, I.K. (1987). Collection of leucocytes, fibroblasts and collagen within an implantable reservoir tube during tissue repair. *Journal of Leukocyte Biology*, 42(6), 667–672. https://doi.org/10.1002/jlb.42.6.667

Dikmen, M., Öztürk, Y., Sagratini, G., Ricciutelli, M., Vittori, S., and Maggi, F. (2011) Evaluation of the wound healing potentials of two subspecies of *Hypericum perforatum* on cultured NIH3T3 fibroblasts. *Phytotherapy Research*, 25(2), 208-214. https://doi.org/10.1002/ptr.3243.

Doğan Ş., Gökalsın B., Şenkardeş İ., Doğan A., and Sesa N.C. (2019). Anti-quorumsensing and anti-biofilm activities of Hypericum perforatum extracts against Pseudomonas aeruginosa. *Journal of Ethnopharmacology*, 235, 293–300.

https://doi.org/10.1016/j.jep.2019.02.020.

Erdogrul, S., Azirak, C., and Tosyali, C. (2004). Antimicrobial activities of Hypericum scabrum L. extracts. *Kahramanmaras Sutcu Imam University Journal of Engineering Sciences*, 7, 38-42.

Gill, S.E., and Parks, W.C. (2008). Metalloproteinases and their inhibitors: regulators of wound healing. *The international journal of biochemistry & cell biology*, 40(6-7), 1334-1347.

https://doi.org/10.1016/j.biocel.2007.10.024

Haraway G.D. (2017). The extracellular matrix in wound healing.

Khanijou, M., Seriwatanachai, D., Boonsiriseth, K., Suphangul, S., Pairuchvej, V., Srisatjaluk, R.L., and Wongsirichat, N. (2019). Bone graft material derived from extracted tooth: A review literature. *Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology*, 31(1), 1-7.

https://doi.org/10.1016/j.ajoms.2018.07.004

Kozlovsky, A., Artzi, Z., Hirshberg, A., Israeli-Tobias, C., and Reich, L. (2007). Effect of local antimicrobial agents on excisional palatal wound healing: a clinical and histomorphometric study in rats. *Journal of clinical periodontology*, 34(2), 164-171.

https://doi.org/10.1111/j.1600-051X.2006.01033.x

Lee, J.H., and Kim, G.H. (2010). Evaluation of antioxidant and inhibitory activities for different subclasses flavonoids on enzymes for rheumatoid arthritis. *Journal of Food Science*, 75(7), 212-217.

https://doi.org/10.1111/j.1750-3841.2010.01755.x.

Lee, K.K., Kim, J.H., Cho, J.J., and Choi, J.D. (1999). Inhibitory effects of 150 plant extracts on elastase activity, and their anti-inflammatory effects. *International journal of cosmetic science*, 21(2), 71-82.

https://doi.org/10.1046/j.1467-2494.1999.181638.x.

Liang C.C., Park A.Y., and Guan, J.L. (2007). In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. *Nature Protocols*, 2(2), 329-333.

https://doi.org/10.1038/nprot.2007.30

Mandrone, M., Lorenzi, B., Venditti, A., Guarcini, L., Bianco, A., Sanna, C., Ballero, M., Poli, F., and Antognon, F. (2015). Antioxidant and anti-collagenase activity of *Hypericum hircinum* L. Industrial Crops and Products, 76, 402-408.

https://doi.org/10.1016/j.indcrop.2015.07.012

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of immunological methods*, 65(1-2), 55-63.

https://doi.org/10.1016/0022-1759(83)90303-4

Murray, P.E., García Godoy, C., and García Godoy, F. (2007). How is the biocompatibilty of dental biomaterials evaluated?. *Medicina Oral, Patología Oral y Cirugía Bucal (Internet)*, 12(3), 258-266.

National Committee for Clinical Laboratory Standards (NCCLS). (1993). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 3rd ed. Approved standard M7-A3*. National Committee for Clinical Laboratory Standards, Villanova, Pa.

Öztürk, N., Korkmaz, S., and Öztürk, Y. (2007). Wound-healing activity of St. John's Wort (Hypericum perforatum L.) on chicken embryonic

fibroblasts. *Journal of ethnopharmacology*, 111(1), 33-39. https://doi.org/10.1016/j.jep.2006.10.029

Quirk S. (2007). *Metalloproteinase inhibitors for wound healing*. US 7,186,693 B2.

Salmen S., Hoechstetter J., Käsbauer C., Paper D.H., Bernhardt G., and Buschauer A. (2005). Sulphated oligosaccharides as inhibitors of hyaluronidases from bovine testis, bee venom and *Streptococcus agalactiae*. *Planta Medica,* 71(8), 727-732.

https://doi.org/10.1055/s-2005-871255

Saroglou, V., Marin, P.D., Rancic, A., Veljic, M., and Skaltsa, H. (2007). Composition and antimicrobial activity of the essential oil of six Hypericum species from Serbia. *Biochemical Systematics and Ecology*, 35(3), 146-152. https://doi.org/10.1016/j.bse.2006.09.009

Şerbetçi, T., Özsoy, N., Demirci, B., Can, A., Kültür, Ş., and Başer, K.C. (2012). Chemical composition of the essential oil and antioxidant activity of methanolic extracts from fruits and flowers of Hypericum lydium Boiss. *Industrial crops and products*, *36*(1), 599-606. https://doi.org/10.1016/j.indcrop.2011.11.002

Slevin, M., Kumar, S., and Gaffney, J. (2002). Angiogenic oligosaccharides of hyaluronan induce multiple signaling pathways affecting vascular endothelial cell mitogenic and wound healing responses. *Journal of biological chemistry*, 277(43), 41046-41059.

https://doi.org/10.1074/jbc.M109443200

Süntar I., Oyardı O., Akkol E.K., and Ozçelik B. (2016). Antimicrobial effect of the extracts from *Hypericum perforatum* against oral bacteria and biofilm formation. *Pharmaceutical Biology*, 54(6), 1065-1070. https://doi.org/10.3109/13880209.2015.1102948.

Yoneda, T., Tomofuji, T., Kawabata, Y., Ekuni, D., Azuma, T., Kataoka, K., and Morita, M. (2014). Application of coenzyme Q10 for accelerating soft tissue wound healing after tooth extraction in rats. *Nutrients*, 6(12), 5756-5769.

https://doi.org/10.3390/nu6125756.