DOI: 10.22037/jds.v38i1.32529

Original Article
ISSN 2645-4351

Asparagus Officinalis: An Herbal Candidate for an Intraoral Healing Mouthwash

Erfan Shamsoddin [a], Davood Bashash [b], Malek Zarei [c], Behzad Hooshmand [d]

[a]Dentist, Tehran, Iran.
[b]Associate Professor, Dept. of Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[c]Assistant Professor, Dept. of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[d]Profwssor, Dept. of Periodontology, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Correspondence to Behzad Houshmand (email: bhoushmand@sbmu.ac.ir).
(*Submitted: 12 September 2020 – Revised version received: 01 February 2021 – Accepted: 021 February 2021– Published online: Winter2021*)

Objectives Asparagus officinalis (A. officinalis) extract has several bioactive ingredients. This study assessed the healing effects of A. officinalis methanolic extract.

Methods In this experimental study, after preparing the methanolic extract of A. officinalis with a concentration of 100µg/mL, its bioactive ingredients were determined using high-performance liquid chromatography (HPLC) and then its cytotoxicity was assessed using the methyl thiazolyl tetrazolium (MTT) assay. Five experimental groups with 25 samples were assessed as follows: (I)human gingival fibroblast(HGFs) cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM), (II) same as group Ibut with 10 µg/mL methanolic extract of A. officinalis, (III) same as group Ibut with 25µg/mL methanolic extract of A. officinalis, (IV) same as group Ibut with 50 µg/mL methanolic extract of A. officinalis, and (V)same as group Ibut with 100µg/mL methanolic extract of A. officinalis. Cell motility in the control group and group Vwas examined quantitatively using the cell scratch assay at 24 h. We used one-way ANOVA and t-test to analyzethe cytotoxicity of A. officinalis extract and the motility of HGFs, respectively.

Results The MTT assay showed no significant difference in cell viability among the experimental groups (P=0.07). A remarkable cellular wound closure equal to 60.85% was noted after 24 h.

Conclusion The methanolic extract of A. officinalis with a concentration of 100 µg/mL showed significant healing effects on an experimental scratch setup of HGFs.

Keywords Wound Healing; Fibroblasts; Asparagus Plant; Herbal Medicine

Introduction

Intra-oral mucosal healing has been one of the most critical issues in dentistry and regenerative medicine. Several systemic diseases and local factors such astrauma, and fungal and viral infections can induce or aggravate intra-oral wounds and ulcers. These factors can exert their adverse effects via several mechanisms. Physical or chemical damage to the epithelial cells, local inflammation induction, bacterial load increase resulting in acute or chronic infections, viral invasion to the epithelial cells, and any change in cell cycle are among the etiologic or exacerbating factors forintra-oral wounds.[1, 2] Surgical wounds are among other types of wounds to be considered. Nowadays, many surgical processes can only be done viaintra-oral incisions which would require further healing in patients. Multiple cell types are involved in the wound healing process, such as fibroblasts that have a moderate proliferative rate necessary for tissue re-organization and regeneration.[3] On a broader perspective, oral ulcers can decrease the quality of life in addition to causing pain and discomfort for the patients. They can impair nutrition, complicate oral hygiene, and impair speech. Malignancies can also cause life-threatening ulcers.[4] Aside from the etiology of oral ulcers, different interventions have been suggested to decrease oral ulcersymptoms such as using immunosuppressive medications, anti-inflammatory drugs, topical anesthetic agents, and analgesics.[5, 6] One major goal of regenerative medicine is to accelerate oral wound healing.

Mouthwashes play an important role in oral hygiene and decreasing oral bacterial load. Herbal mouthwashes have been widely investigated for their antibacterial[7], antiviral[8], and anti-fungal[9] efficacy, and treatment of oral malodor and oral mucositis.[10] Asparagus officinalis (A. officinalis) is among the herbal species with medicinal properties. This plant is a subgroup of Asparagacea genus, which is currently used as an oral supplement worldwide. This plant is rich in vitamins A, B1, B2, C, and E and minerals such asMg2+, Zn2+, P3-, Ca2+, Fe2+, Mn2+, folic acid, asparagusic acid, and steroidal saponins.[11] Considering the biological functions of A. officinalis, it is plausible to assess its healing effects.

The main biological functions of the active ingredients of A. Officinalis are as follows: anti-oxidative activity, immune modulation, fibroblast proliferation, collagen deposition and synthesis, anti-glycation, and induction of angiogenic path ways.[12, 13] There are also several minor ingredients in A. officinalis extract such ascalcium, steroidal saponins, anthocyanins, quercetin, carotenoids, β-carotene, Fe, Mg, Se, and vitamin A[14], which are usually considered as active molecular regulators in homeostasis and wound healing/[15] There is evidence supporting the contribution of these components to proliferative and

resolution phases of wound healing.[11, 16] Quercetin, another compound in A. officinalis, is involved in reduction of inflammation through protein kinase B and microtubule-associated protein kinase pathways.[17] Looking at its bioactive profile, we can suggest A. officinalis as a suitable choice to be investigated with regard to its healing effects. This study assessed the healing effects of A. officinalis methanolic extract.

Methods and Materials

Firstly, the methanolic extract of A. officinalis stem (4-month dried) was prepared using the wetting technique. For this purpose, 20 Lof methanol was usedto wet the stem parts of the plant, leaving them to stir for 3 days for maximum extraction. The obtained extract was then filtrated using filter papers and injected into an industrial spinning evaporator machine (Hiedolf, Germany) at 40℃; afterwards, the solvent was repeatedly added to the plant extract. Finally, 164 gof methanolic extract was obtained.

High-performance liquid chromatography (HPLC) was done to develop a chemical index for the prepared methanolic extract. A Waters HPLC apparatus consisting of a separation module (2695;Waters, USA) and a PDA detector (996;Waters, USA) was used for HPLC analysis. We used auto sampler injector for chromatography. The chromatographic assay was performed on a 15 cm×4.6 mm pre-column, and Eurospher 100-5 C18 analytical column provided by Waters (Sun fire) reversed-phase matrix (3.5 µm) (Waters). Elution was done in a gradient system with methanol as the organic phase (solvent A) and distilled water (solvent B).The flow-rate ($1^{mL}/_{min}$. peaks) was monitored at 195-400 nm wavelength. The injection volume was 20 µL and the temperature was maintained at 25°C.

Next, a cellcytotoxicity test was performed. After determining the highest non-toxic concentration of A. officinalis extract, the cell scratch assay was done using the concentration found in the previous step. Human gingival fibroblasts (HGFs) were purchased (with a cell line number of IBRC C10459) and cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum, 100 µg/mL streptomycin, and 100U/mL penicillin in a humid environment with 5% CO2 at 37℃ for 24 h. According to a previous study on the cytotoxic effects of A. officinalis, we chose the following concentrations to examine the cytotoxicity: 10, 25, 50, and 100µg/mL(18). Our five experimental groups (n=5)were as follows: (I) HGFs cultured in high-glucose Dulbecco's modified Eagle's medium, (II) same as group Ibut with 10 µg/mLmethanolic extract of A. officinalis, (III) same as group Ibut with 25µg/mL methanolic extract of A. officinalis, (IV) same as group I but with 50 µg/mL methanolic extract of A. officinalis, and (V) same as group I but with 100µg/mL methanolic extract of A. officinalis. To do the cytotoxicity test, we performed a methyl thiazolyl tetrazolium (MTT) assay for each concentration of the extract. HGFs were first treated with methanolic extracts for 24 and 48 h; then, 100µL of the MTT solution (5mg/mL) was added to each well of a 96-well plate followed by an incubation for 3 h. After removing the MTT solution from the wells, 100 µL of dimethyl sulfoxide solution was added to each well. After 15min, the plate was transferred to an ELISA-reader and its absorbance was measured at 570nm.The cell viability was determined using the following formula:

$$\text{Cell viability (\%)} = (\text{OD experiment}/\text{OD control}) \times 100$$

OD experiment: optical density of the treatment group
OD control: optical density of the control group

To prepare an appropriate collagen coating for HGFs to attach to, we used the human plasma. A blood sample was taken from a volunteer who verbally consented to participate, sitting still for 30 min in an upright position. In the next step, the blood sample was fractionated (5418R;Eppendorf, Germany) at2200 rpm for 15 min. The supernatant (plasma) was poured into the culture plates; then, 0.05 mL of calcium chloride (CaCl2) was added to each plate as a coagulant with a concentration of 2M (Sigma, Saint Louis, USA).

After preparing the collagen coating, the extra plasma solution in the plates was discarded and the cultures were fixed by 3mL of bovine albumin serum with a concentration of 2 mg/mL at 37℃ for 1 h. Afterwards, we washed the plates with PBS and added 4mL of DMEM to them; the control group was ready as such. The cultured cells in DMEM containing A. officinalis methanolic extract with a concentration of 100µg/mL were washed with tryps in twice and mixed with serum-containing medium, after the cells reached 90%confluence. The floating cells were transferred on the collagen coating using a p200 pipette; next, the plates were gently shaken and incubated for 6 hat 37℃, allowing the HGFs to form a cellular monolayer. A scratch was then created in the cellular monolayer by a p200 pipette tip and the cellular debris was removed by washing the plates with 1mL of DMEM. Next, the media in the plates were replaced with 5mL of DMEM solution containing the methanolic extract of A. officinalis with a concentration of 100µg/mL. The exact place of the cellular scratch was determined by a scratch on the exterior bottom surface of the plates. The outer scratch was placed at the lower border of the substage, and the first cellular image was taken at the initiation time by a phase-contrast microscope. The cellular plates were incubated for 24 hat 37℃; afterwards, the sub stage was adjusted similarly and a second phase-contrast microscope image (Interlabs, Ambala cantt, India)was taken. Finally, HGFs were fixed using 1mL of 2% formalin solution for each plate at 37℃. The extra formalin was washed using 1mL of saline solution and the HGFs were stained with Giemsa staining technique; a final phase-contrast cellular image was taken at the end. All the steps in our study are shown in Figure 1.This study was approved by the Ethics Committee of the Research Institute

of Dental Sciences-Shahid Beheshti University of Medical Sciences with the code number IR.SBMU.RIDS.REC.1396.527 in 2018.

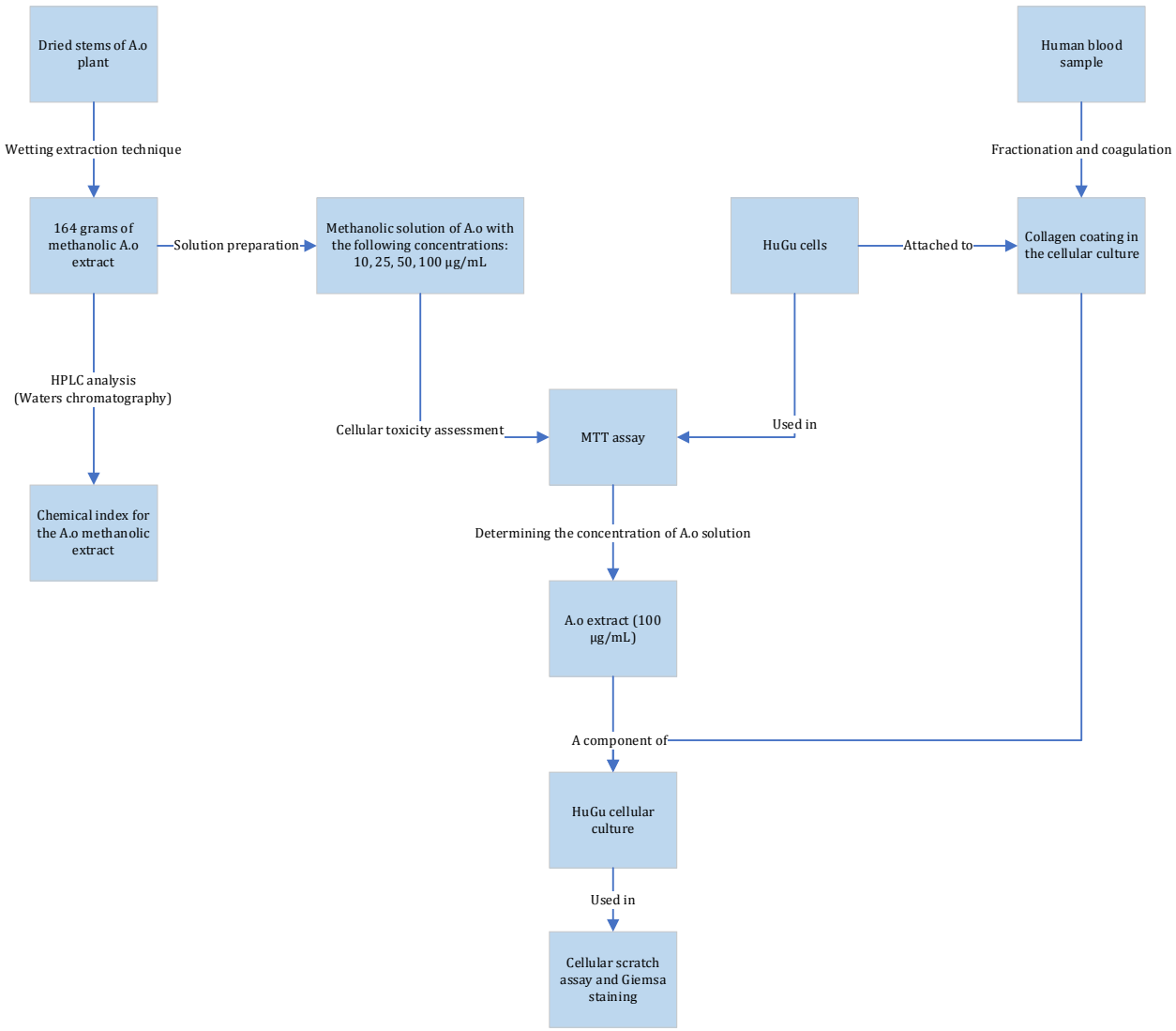

Figure 1-Workflow of the materials used, and methods applied in this study
A. o: Asparagus officinalis

To quantify the cell scratch assay result, we used the following formula[19]:

$$\text{Wound closure (\%)} = \frac{At=0h - At=\Delta h}{At=0h} \times 100$$

At=0h: Surface of the wound immediately after creating the cell scratch

At=Δh: Surface of the wound "h" hours after creating the cell scratch

Data acquisition and integration for the HPLC analysis were performed by Millennium32 software. Based on a previously approved protocol, all the measurements were madeusing "Image J" application (V. 1.52e, Wayne Rasband, USA).[19]

Results

Regarding the results of HPLC analysis, the most abundant and biologically notable components existing in our methanolic A. officinalis extractare demonstrated in Figure 2. Phenolic compounds and especially flavonoids, catechins, vitamins and a range of other bioactive compounds were present in A. officinalis extract. Methanolic extract of A. officinalis showed a high biocompatibility profile even in group five with maximum concentration.

A) 3,4 DHB with a concentration of 71.09 ppm in A. officinalis methanolic extract (100 µg/mL)

B) Catechin with a concentration of 64.57 ppm in A. officinalis methanolic extract (100 µg/mL).

3,4 DHB: 3,4-Dihydroxybenzoic acid; T: time in minutes; C: concentration in ppm

A. o: Asparagus officinalis.

DOI: 10.22037/jds.v38i1.32529
ISSN 2645-4351

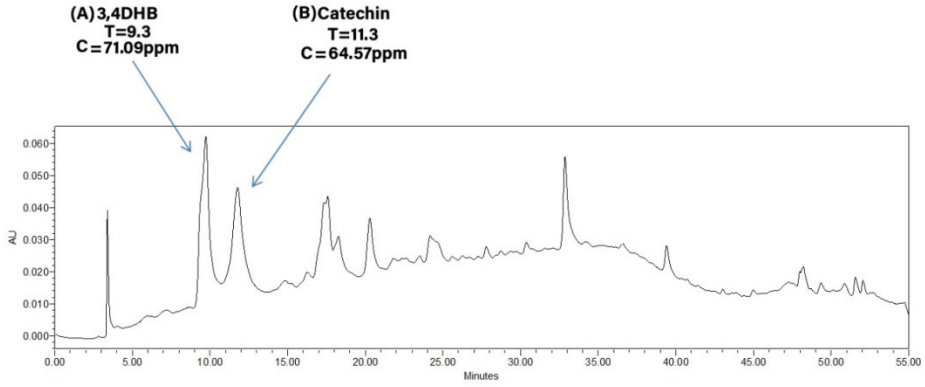

Figure 2- Results of HPLC analysis for the methanolic extractof Asparagus officinalis

According to the results of one-way ANOVA , 24- and 48-hMTT assays fot he cytotoxicity assessment showed no significant difference in cell viability among the experimental groups (P=0.07, Table 1).

Considering the results of the cell scratch assay, the cellular wound closure percentage after 24 h was 60.85%. Paired sample t-test showed a significant difference in wound closure between groups one and five after 24 h(P<0.001). According to the results of t-test, wound closure in the control group was not significant after 24 h(P=0.86). Cell motility is illustrated in Figures 3 and 4.

Table 1- Quantitative results of 24- and 48-hMTT assays

A. officinalis concentration (µg⁄mL)	Relative cell viability after 24 h	Relative cell viability after 48 h
0	1	1
10	1.27	0.84
20	1.46	0.94
50	1.64	0.84
100	1.35	0.96

A. officinalis: Asparagus officinalis.

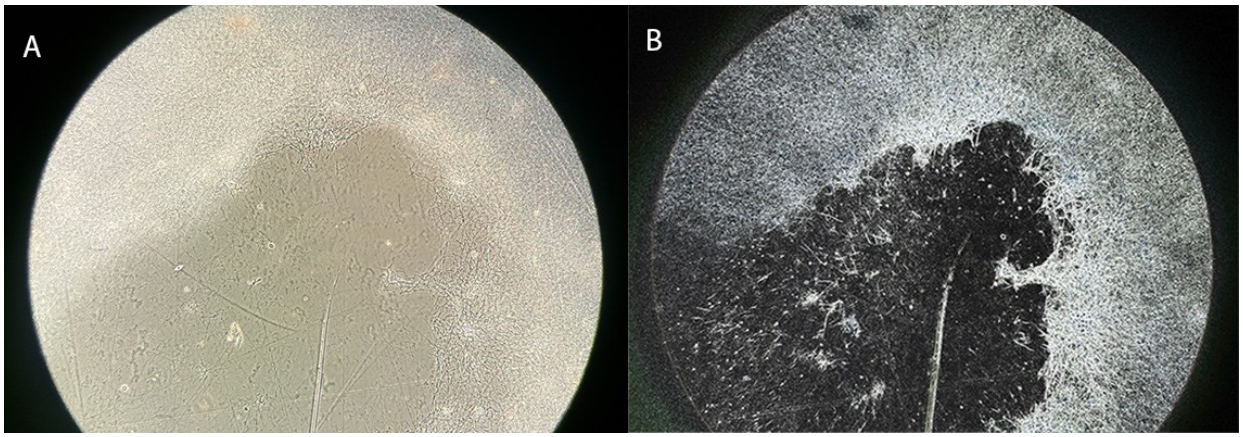

Figure 3- Phase contrast image of cell motility in the control group after 24 hours. HGFswere cultured on a collagen cellular base. Scratch was made by a p200 pipette tip. (A). No cellular nucleus is seen in the phase-contrast original image. (B). Sharpening, edge enhancement, and contrast enhancement weredone using ImageJ software. Cellular motility was observable in this image, but it was not statistically significant.

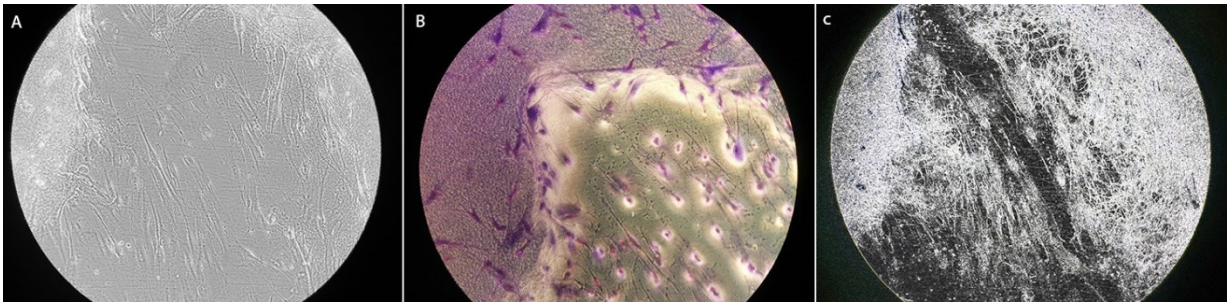

Figure 4- Phase contrast image of cell motility in group five after 24 h. HGFswere cultured on a collagen cellular base. Scratch was made by a p200 pipette tip. (A). Multiple cellular nuclei are seen in the phase-contrast original image. (B). Alizarin red staining was done on HGFsand the cells were fixed using 1mL of 2% formalin solution for each plate at 37℃. (C) Sharpening, edge enhancement, and contrast enhancement weredone using ImageJ software. Cellular motility was notable and statistically significant.

Discussion

In this study, we evaluated the healing effects of methanolic extract of A. officinalis in an experimental setting. As of now, there is no herbal mouthwash with confirmed healing effects in the literature, and considering the trend towards the consumption of herbal products, the need for appropriate herbal products for this purpose is obvious. One of the best candidates is A. officinalis, a species with several bioactive compounds and a history of usage as an oral supplement.[20] The stem part of A. officinalis plant was used to prepare a methanolic extract and investigate its healing effects. By evaluating the most concentrated and most biologically active elements in A. officinalis extract, we were able to further suggest the healing pathways in which this plant may play a role. Based on HPLC analysis, protocatechuicacid (PCA; 3,4-dihydroxybenzoic acid [3,4, DHB]), and epicatechingallate (ECG) were the two main ingredients present in the methanolic extract. These two can be considered as the main active compounds in A. officinalis extract.

Controversial results regarding the cytotoxicity of A. officinalis extract provokes a discussion about dose-dependence and time-dependence of the cytotoxic effects of A. officinalis methanolic extract. Since there was no significant wound closure in the control group after 24 h (P=0.86), the healing can not be merely attributed to the proliferation of HGFs. This finding substantiates the promotive wound closure effects of A. officinalis extract. After doing the cellular tests, a search in Kyoto encyclopedia of genes and genome (KEGG) pathways and investigating the possible cellular pathways responsible for the bioactivities of A. officinalis extract seemed indispensable. A. officinalis might exert its healing effects through several intracellular pathways that inhibit inflammation, induce collagen accumulation and cross-linkage, and enhance angiogenesis(20). Catechins found in officinalis are classified as flavonoids which generally have antioxidant and collagen and extracellular matrix synthetic characteristics A.[21] The catechin in our A. officinalis extract, ECG, induces angiogenesis via the vascular growth factor as well.[22]

PCA is considered as an active component of some traditional Chinese herbal medicines and is present in considerable amounts in A. officinalis extract. Its mechanism of action is mainly based on its antioxidant activity, including inhibition of generation and the scavenging effect of free radicals, metal chelation, and up regulating antioxidant enzymes like glutathione reductase.[23, 24] Other biological aspects seem to affect the activity of inducible isoenzyme of cyclooxygenase and nitric oxide synthase, glucose transporters and inflammatory cytokines. Accordingly, the following cellular mechanisms are regulated in the wound healing process: insulin-like activity, angiogenesis, inhibition of pro-inflammatory cells'motility, and fibroblastic proliferation.[23, 25] These pathways are depicted in more details in the appendices.

Steroidal saponins are minor bioactive components in this plant with antioxidant, anti-hepatotoxicity, antibacterial, anti-cancer, and wound healing effects.[11] A. officinalis is also rich in minerals like iron (Fe), zinc (Zn), magnesium (Mg), and manganese (Mn). Zinc, magnesium, and selenium (Se) have antioxidant activities.[11, 15] To date, no significant healing effects have been reported for herbal mouthwashes in clinical and in-vivo studies; although, some herbal extracts have shown promising results. Nicolas et al. examined the healing effects of Calendula officinalis extract. Based on the results of their study, ethanolic extract of Calendula officinalis can regulate the motility of keratinocytes via activating NF-kB and increasing the level of IL-8 cytokine.[26] These effects are in line and comparable with the healing effects of ECG, PCA, anthocyanin, carotenoids, steroidal saponins, and linoleic acid in A. officinalis extract.

There are two compounds in A. officinalis with angiogenic inductive effects: ECG and quercetin. ECG acts as a VEGF-inductive factor while quercetin acts through PKB and MAPK pathways. In a study by Liu et al, angiogenic characteristics of an herbal mixturenamed NF3 was evaluated; it increased the endothelial cell migration and regulated tubular systems intra- and inter-cellularly.[27] Torabi et al. evaluated the healing effects of Thymus caramanicus extract and essence in Jalas mice. The results showed significant healing effects, which were attributed to carvacrol, the main bioactive compound in Thymus caramanicus.[28] This compound is not present in A. officinalis; whilst, its antioxidant and anti-inflammatory effects are in line with the inhibitory effects of ECG against COX-2 enzyme which leads to a drop in thromboxane A2 and platelet receptor levels in fibrinogen.[21] Nirmala and Karthiyayini aimed to measure the healing effects of alcoholic and aqueous extracts of Achillea millefolium L. in intraoral wounds of Wistar rats and showed a significant increase in wound contraction rate, skin tear resistance, and dry and wet weight of granulation tissue. [29] These effects are similar to the effects of vitamin A and β-carotene on cross-linkage and accumulation of collagen in the resolution phase of wound healing.

Based on our results, the methanolic extract of A. officinalis with a concentration of 100 µg/mL showed significant healing effects on HGFs in an experimental setup. This extract can be introduced as an appropriate candidate to produce a healing mouth wash. There are also some limitations in using A. officinalis products such as the presence of cytotoxic compounds in specific parts of the plant such as the fruits or the seeds. Compounds like protodioscin and methyl protodioscin in the seeds, and steroids and as paragosides in the root section of the plant have shown significant cytotoxic effects. Inhibitory effects on cyclooxygenase-2 enzyme and side effects of this pathway on the cardiovascular system as well as decreasing the prostacyclin production should also be considered while consuming this herbal product.[11]

Conclusion

Overall, the methanolic extract of A. officinalis showed significant healing effects on HGFs. Therefore, we can suggest this herbal species as a suitable candidate to produce a healing mouth wash for intraoral wounds. To further reinforce this statement, further in vivo studies are recommended.

Conflict of Interest

No Conflict of Interest Declared ■

Funding

This studydid not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1 Bjarnsholt T. The role of bacterial biofilms in chronic infections. APMIS Suppl. 2013 May;(136):1-51.
2 Zaleckiene V, Peciuliene V, Brukiene V, Drukteinis S. Traumatic dental injuries: etiology, prevalence and possible outcomes. Stomatologija. 2014;16(1):7-14.
3 Politis C, Schoenaers J, Jacobs R, Agbaje JO. Wound healing problems in the mouth. Front. physiol. 2016;7:507.
4 Porter SR, Mercadente V, Fedele S. Oral manifestations of systemic disease. Bdj Team. 2018;5:18012.
5 Scully C, Felix D. Oral medicine—Update for the dental practitioner Aphthous and other common ulcers. Br Dent J. 2005 Sep;199(5):259-64.
6 Bilodeau EA, Lalla RV. Recurrent oral ulceration: Etiology, classification, management, and diagnostic algorithm. Periodontology 2000. 2019;80(1):49-60.
7 Singla S, Malhotra R, Nd S, Saxena S. Antibacterial efficacy of mouthwash prepared from pomegranate, grape seed and Guava extracts against oral streptococci: An in vivo study. J Clin Pediatr Dent. 2018;42(2):109-13.
8 Bekut M, Brkić S, Kladar N, Dragović G, Gavarić N, Božin B. Potential of selected Lamiaceae plants in anti (retro) viral therapy. Pharmacol Res. 2018;133:301-14.
9 Mardani M, Badiee P, Gharibnavaz M, Jassebi A, Jafarian H, Ghassemi F. Comparison of anti-Candida activities of the ancient plants Lawsonia inermis and Ziziphus spina christi with antifungal drugs in Candida species isolated from oral cavity. J Conserv Dent. 2018;21(4):359- 362.
10 Gomes VTS, Nonato Silva Gomes R, Gomes MS, Joaquim WM, Lago EC, Nicolau RA. Effects of Matricaria recutita (L.) in the treatment of oral mucositis. Sci. World J. 2018; 2018:4392184.
11 Negi JS, Singh P, Joshi GP, Rawat MS, Bisht VK. Chemical constituents of Asparagus. Pharmacogn. Rev. 2010;4(8):215-20.
12 Semaming Y, Pannengpetch P, Chattipakorn SC, Chattipakorn N. Pharmacological properties of protocatechuic acid and its potential roles as complementary medicine.Evid Based Complement Alternat Med. 2015;2015:593902.
13 Kakkar S, Bais S. A review on protocatechuic acid and its pharmacological potential. ISRN Pharmacol. 2014;2014.
14 Al-Snafi AE. The pharmacological importance of Asparagus officinalis-A review. Pharm Biol. 2015;5(2):93-8.
15 Wild T, Rahbarnia A, Kellner M, Sobotka L, Eberlein T. Basics in nutrition and wound healing. Nutrition. 2010;26(9):862-6.
16 Gopalakrishnan A, Ram M, Kumawat S, Tandan S, Kumar D. Quercetin accelerated cutaneous wound healing in rats by increasing levels of VEGF and TGF-β1. Indian J Exp Biol. 2016;54(3):187-95..
17 Ehrlich HP, Tarver H, Hunt TK. Effects of vitamin A and glucocorticoids upon inflammation and collagen synthesis. Ann Surg. 1973;177(2):222.
18 Kato-Noguchi H, Nakamura K, Ohno O, Suenaga K, Okuda N. Asparagus decline: Autotoxicity and autotoxic compounds in asparagus rhizomes. J Plant Physiol. 2017;213:23-29.
19 Grada A, Otero-Vinas M, Prieto-Castrillo F, Obagi Z, Falanga V. Research techniques made simple: analysis of collective cell migration using the wound healing assay. J Invest. Dermatol. 2017;137(2):e11-e6.
20 Lee JW, Lee JH, Yu IH, Gorinstein S, Bae JH, Ku YG. Bioactive compounds, antioxidant and binding activities and spear yield of Asparagus officinalis L. Plant Foods Hum Nutr. 2014;69(2):175-81.
21 Kapoor M, Howard R, Hall I, Appleton I. Effects of epicatechin gallate on wound healing and scar formation in a full thickness incisional wound healing model in rats. Am J Pathol. 2004;165(1):299-307.
22 Zhou YD, Kim YP, Li XC, Baerson SR, Agarwal AK, Hodges TW, et al. Hypoxia-inducible factor-1 activation by (−)-epicatechin gallate: potential adverse effects of cancer chemoprevention with high-dose green tea extracts. J Nat Prod.2004;67(12):2063-9.
23 Kakkar S, Bais S. A review on protocatechuic acid and its pharmacological potential. ISRN Pharmacol. 2014 Mar;2014:952943.
24 Heleno SA, Martins A, Queiroz MJR, Ferreira IC. Bioactivity of phenolic acids: Metabolites versus parent compounds: A review. Food Chem. 2015;173:501-13.
25 Nicolaus C, Junghanns S, Hartmann A, Murillo R, Ganzera M, Merfort I. In vitro studies to evaluate the wound healing properties of Calendula officinalis extracts. J Ethnopharmacol. 2017;196:94-103.
26 Nicolaus C, Junghanns S, Hartmann A, Murillo R, Ganzera M, Merfort I. In vitro studies to evaluate the wound healing properties of Calendula officinalis extracts. JEthnopharmacol.2017;196:94-103.
27 Liu CL, Tam JCW, Sanders AJ, Ko CH, Fung KP, Leung PC, et al. Molecular angiogenic events of a two- herb wound healing formula involving MAPK and A kt signaling pathways in human vascular endothelial cells. Wound Repair Regen. 2013;21(4):579-87.
28 Torabi M, Afzali M, Aminizadeh M, Karimiafshar H. Study on the Effect of Essence and Extract of Thymus caramanicus Jalas on the Process of Oral Wound Healing in Rats. Asian j. biomed. pharm. 2015;5(44):1-5.
29 Nirmala S, Karthiyayini T. Wound healing activity on the leaves of Achillea millefolium L. by excision, incision, and dead space model on adult wistar albino rats. Int Res J Pharm. 2011;2(3):240-5.

How to cite:
Erfan Shamssodin, Davood Bashash, Malek Zarei, Behzad Houshmand. Asparagus Officinalis: An Herbal Candidate for an Intraoral Healing Mouthwa. .J Dent Sch2020;38(1):31-36