Effect of raspberry extract on wound healing

Wenjing Lu (陆文静)1, Meng Xu (徐萌)1, Youwei Yuan (袁友卫)1, Xueimei Zhang (张雪梅)2, Jianxin Tan (檀建薪)1, Junping He (何俊萍)1,* and Yiling Tian (田益玲)1,*

1College of Food Science and Technology, Hebei Agricultural University, Baoding, China and 2College of Forestry, Hebei Agricultural University, Baoding, China

*Correspondence to: Dr. Junping He and Yiling Tian, No. 2596 Lekai South Road, Baoding 071000, China. Email: 1176937802@qq.com (Dr. Junping He), tougaotian@126.com (Yiling Tian)

Received 11 January 2021; Revised 7 April 2021; Editorial decision 7 April 2021.

Abstract
The main purpose of this study was to investigate the effect of raspberry extract on wound healing and compare it with that of ellagic acid. The elimination of excess free radicals was the key to preventing wound inflammation; cellular antioxidation activity was evaluated using an oxidative stress damage cell model. Cell proliferation ability was measured using the WST-1 assay, and the migration capacity was determined using the wound scratch assay. A mouse wound model was used to verify the effect of raspberry extract on wound healing. The cellular antioxidant activity of the extract (50.31±3.17 μg/mL) was slightly lower than that of ellagic acid (44.59±2.38 μg/mL). The results of a cell proliferation assay showed that both raspberry extract and ellagic acid at 5 μg/mL could significantly (P<0.01) promote the proliferation of HaCaT cells. After culturing for 24 h and 48 h, the cell healing rates of the extract were (41.11±0.38) per cent and (68.88±2.51) per cent, respectively, whereas the corresponding rates of ellagic acid were (39.01±2.40) per cent and (70.33±0.89) per cent; hence, there were no significant differences between them (P>0.05). The wound areas of mice fed low, medium, and high doses of raspberry extract for 14 days were 1.66, 1.41, and 1.24 mm², respectively, which were significantly lower than that of the blank control group, 2.18 mm² (P<0.05). These findings indicate that raspberry extract and ellagic acid exhibit similar antioxidant capacities and equivalent cell proliferation-promoting capabilities. In the mouse test, raspberry extract effectively promoted a reduction in wound area. This work demonstrates the potential of raspberry extract in wound healing, suggesting a promising application of raspberry resources in the fields of functional foods, cosmetics, and medicine.

Keywords: Raspberry extract; ellagic acid; antioxidant activity; cell proliferation; wound healing.

Introduction

Raspberry (Rubus idaeus L.) is a Rosaceae plant that is both a medicine and food, and is mainly distributed in the temperate regions of the northern hemisphere (Giuffrè et al., 2019). Raspberries contain ellagic acid, salicylic acid, superoxide dismutase, polysaccharides, anthocyanins, flavonoids, vitamins, and other active ingredients. They are of high value as food, as well as in Chinese traditional medicine as intervention treatments for ailments such as diabetes, inflammation, immunomodulatory disorders, and hyperlipidemia (Teng et al., 2017; Baby et al., 2018; Kowalska et al., 2019; Zhao et al., 2020).

Ellagic acid is widely distributed in various fruits and nuts (Vadhanam et al., 2011). The chemical structure of ellagic acid is shown in Figure 1. The ellagic acid content in red raspberry has been reported to be 119.8–323.5 mg/100 g (Bobinaitė et al., 2012). Ellagic acid exhibits strong antioxidant, antibacterial (Bobinaitė et al.,
2013; Bobinaitė et al., 2016; Teng and Chen, 2019), and anticancer effects (Khanduja et al., 1999; Hussein and Khalifa, 2014; Aslan et al., 2020; Duan et al., 2020; Mansouri et al., 2020; Wang et al., 2020). Yang et al. (2019) found that an ointment made of pomegranate seed ellagic acid could promote cell proliferation and heal skin burns and wounds. Ellagic acid at concentrations of 40 per cent and above can enhance the functional effect of pomegranate extract (Jurenka, 2008; Kunle et al., 2012).

As the largest organ system of the human body, the skin protects the body from external stimuli. However, loss of skin integrity caused by injury or disease is common. Studies have shown that a high amount of free radicals surround a wound (Milvy et al., 1973; Kuhn, 2003). The elimination of free radicals can accelerate the disappearance of inflammation and promote wound healing. Epidermal cells play a key role in the process of wound healing, and also affect the speed and quality of the healing (Parnell, 2013). In a previous work study of our laboratory, it was found that raspberry extract contains 539.30 mg/g ellagic acid. In addition to ellagic acid, raspberry extract contains 120.30 mg/g ellagic acid dimer, 98.30 mg/g ellagic acid glucose derivatives, and 242.10 mg/g other unknown compounds. This is somewhat different from the composition of pomegranate extract (Satomi et al., 1993; Romani et al., 2012). Although the biological roles of ellagic acid have been studied, the influence of raspberry extract on wound healing remains to be elucidated.

The main aim of this study was to evaluate the effect of raspberry extract on wound healing. Using ellagic acid as a control, cellular antioxidant activity (CAA), cell proliferation, and migration assays were performed. Finally, verification was done using mice tests.

Materials and Methods

Chemicals and standards
Ellagic acid (high-performance liquid chromatography grade), 2,7’-dichloro-di hydrofluorescein diacetate (DCFH-DA), and 2,2’-azois-2-methyl-propanimidamide dihydrochloride (ABAP) were ordered from Sigma-Aldrich (St. Louis, MO, USA). Raspberry extract containing 539.30 mg/g ellagic acid, 120.30 mg/g ellagic acid dimer, 98.30 mg/g ellagic acid glucose derivative, and 242.10 mg/g other constituents was prepared and characterized in our laboratory. Roswell Park Memorial Institute (RPMI) 1640 medium was obtained from Hyclone Laboratories Inc. (Logan, UT, USA). Dulbecco’s Modified Eagle Medium (DMEM) was obtained from Gibco (Grand Island, NY, USA). Dimethyl sulfoxide (DMSO), pancreatin-EDTA, and phosphate-buffered saline (PBS) were obtained from Solarbio (Beijing, China). Recombinant human epidermal growth factor (rhEGF) was procured from PeproTech (Rocky Hill, NJ, USA). The WST-1 Cell Proliferation and Cytotoxicity Assay Kit was purchased from Beyotime (Shanghai, China). Kunming mice were ordered from SPF Biotechnology Co., Ltd. (Beijing, China).

Cell culture and animal feeding

Human liver cancer SMMC-7721 cells were obtained from the Cell Bank of the Chinese Academy of Sciences (Beijing, China), and human immortalized epidermal HaCaT cells were obtained from Shengbo Biomedical Technology Co., Ltd. (Shanghai, China). Cell culture was conducted in an incubator with a humidified atmosphere of 5 per cent CO₂ at 37 °C. The SMMC-7721 cells were cultured in RPMI 1640 medium containing 10 per cent fetal bovine serum (FBS; ExCell Bio, Shanghai, China), whereas HaCaT cells were maintained in DMEM. Both media contained 1 per cent penicillin and streptomycin mixture.

Twenty-five male mice at 4 weeks of age and weighing 18–20 g were used in this research. The mice were given commercial food and water ad libitum.

Detection of SMMC-7721 cell antioxidation by raspberry extract

SMMC-7721 cells in logarithmic growth phase were collected and adjusted to a concentration of 1×10⁴ cells/mL in RPMI 1640 medium containing 10 per cent FBS. Then, the cells were inoculated in a 96-well plate at 100 μL/well with three replicate wells for each group. After culturing for 24 h, the culture medium was removed, and the cells were washed once with PBS. Sample solution (100 μL) diluted with serum-free culture medium and containing DCFH-DA probe was added to make the final concentration of DCFH-DA 25 μmol/L. After incubation at 37 °C for an additional hour, the culture medium was discarded. The cells were washed 1–2 times with 100 μL PBS (37 °C); then, 100 μL of 20 μmol/L ABAP was added. For the blank and control groups, only fresh medium was added. Absorbance was measured every 5 min for 1 h at 485 nm excitation and 538 nm emission, using a microplate reader. Three wells were used for each measurement.

The area under the curve-fluorescence intensity curve was calculated. The CAA values of different concentrations of ellagic acid were determined according to Equation (1):

$$\text{CAA} = 100 - \frac{\int \text{SA}}{\int \text{CA}} \times 100$$  \hspace{1cm} (1)

where ∫SA (sample area) represents the integrated area under the curve for the sample fluorescence intensity versus time, whereas ∫CA (control area) represents the integrated area from the control curve.

The median effective dose (EC₅₀) was calculated based on the median effect principle of log (fa/fu) and log (concentration), where “fa” is the fraction affected and “fu” is the fraction unaffected by the treatment. The EC₅₀ values were expressed as mean±standard deviation (SD).

Detection of HaCaT cell proliferation induced by raspberry extract

HaCaT cells in the logarithmic phase were adjusted to a concentration of 2×10⁴ cells/mL in DMEM containing 10 per cent FBS. Then, the cells were inoculated in a 96-well plate at 100 μL/well with three replicates for each treatment. After 24 h of incubation, the culture medium was discarded, raspberry extract or ellagic acid-supplemented medium was added, and the cell were cultured for another 24 h. Cell proliferation was determined using the WST-1

Figure 1. Structural formula of ellagic acid.
cell proliferation and cytotoxicity detection kit. The control group was treated without ellagic acid; the blank group was treated only with cell culture medium, and contained no cells. The OD values were measured at 450 nm on a microplate reader, and recorded as OD_{experiment}, OD_{control}, and OD_{blank}. The cell proliferation rate was calculated according to Equation (2):

\[
\text{Cell proliferation rate} (\%) = \frac{\text{OD}_{\text{experiment}} - \text{OD}_{\text{blank}}}{\text{OD}_{\text{control}} - \text{OD}_{\text{blank}}} \times 100\%
\]  

(2)

Detection of HaCaT cell migration induced by raspberry extract
HaCaT cells (6×10^5 cells/mL) were inoculated on a cell culture plate and cultured for 24 h to cover 90 per cent of the well area. Then, a line was drawn with a 200 μL tip along a ruler. The cells were washed 2–3 times with PBS and then cultured with medium containing 5 μg/mL of raspberry extract or ellagic acid. The blank group was not processed. The positive control group was cultured with a medium containing 3 ng/L rhEGF. Images were captured using an inverted microscope at 0, 24, and 48 h. Image J 1.8.0 image processing software (Joonas “Regalis” Rikkonen, Turku, Finland) was used to calculate the area of the irregular figure at the scratch. The scratch closure rate at different time points was calculated according to Equation (3):

\[
\text{Scratch healing rate} (\%) = \frac{S_0 - S_t}{S_0} \times 100\%
\]  

(3)

where \(S_0\) is the scratch area of the cell at 0 h, and \(S_t\) is the scratch area of the cell at \(t\).

Measurement of wound healing area in mice
Mice were adapted in the laboratory for a week before the procedures. Then, the mice were randomly separated into five groups, namely, blank control group, raspberry extract groups (low, medium, and high doses: 20, 40, and 80 mg/kg, respectively), and rhEGF (5 μg/kg) group, each with 5 animals. After intraperitoneal injection of 10 per cent chloral hydrate to anesthetize the mice, the back hair was cut off, and the full-thickness skin was sampled 0.5 cm from the midline of the back of the mice using a 4 mm skin sampler. The mice were fasted for 8 h after the operation, but were allowed to drink water normally. The day of model building was recorded as day 0. After 12 h of modeling, the mice were administered the test sample (0.2 mL/10 g) by oral gavage every day; the control group was administered physiological saline. The wound condition was observed and the remaining area of the wound was calculated.

Statistical analysis
Delimitation of regions of interest and quantification of structures were performed using built-in tools in Image J 1.8.0 image processing software. All the samples were processed and analyzed in triplicate. The values were expressed as means±SD. Statistical analyses were performed using IBM SPSS Statistics version 17.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by a Student’s t-test was applied to determine the statistical significance of differences between data. Differences with \(P<0.05\) were considered statistically significant.

Results and Discussion
Cellular antioxidant activity of raspberry extract
The antioxidant activity of raspberry extract was evaluated using the cellular antioxidant assay (Adom and Liu, 2005; Wolfe and Liu, 2007). In this assay, free radicals induced by ABAP are the main type of free radicals in the human body, resulting in a better simulation of the damage caused by peroxy free radicals (Niki, 1990). The EC50 values were used to determine the antioxidant activity of an extract and ellagic acid was positively associated with the concentration. Both raspberry extract and ellagic acid increased steadily with the incubation time, whereas it gradually decreased with an increase in the concentrations (1–50 μg/mL). This observation is consistent with the results of the study performed by Katsunari et al. (2009) who found that the antioxidant activity of an extract and ellagic acid was positively associated with the concentration. Both raspberry extract and ellagic acid inhibited the oxidation process of 2',7'-dichlorofluorescein diacetate (DCF) effectively (Hanneken et al., 2006; Rüweler et al., 2008), thus reducing the fluorescence intensity and showing good antioxidant capacity. The EC_{50} values were used to determine the antioxidant capacity. The concentration of an antioxidant is inversely

Figure 2.  Kinetic curve of fluorescence intensity versus time. (A) is the experimental group of raspberry extract, and (B) is the experimental group of ellagic acid.
proportional to its antioxidant activity; that is, a small EC₅₀ value indicates a strong antioxidant capacity. The EC₅₀ values of the extract and ellagic acid were calculated based on the regression equation shown in Figure 3 and the dose–antioxidant median effect diagram shown in Figure 4. The EC₅₀ values of raspberry extract and ellagic acid were (50.31±3.17) μg/mL and (44.59±2.38) μg/mL, respectively. The antioxidant activity of raspberry extract was slightly lower than that of ellagic acid. Our previous study showed that ellagic acid accounted for 53 per cent of the raspberry extract contents (539.30 mg/g). The antioxidant activity of the extract was approximately 89 per cent that of ellagic acid, indicating that the other components present in the extract might also exert antioxidant activity (Zheng et al., 2020), but not as strongly as the ellagic acid monomer. This could be plausibly explained by the fact that the ellagic acid dimer and ellagic acid glucose derivatives have large molecular weights and contain various hydroxyl groups, which can easily cause intramolecular or intermolecular hydrogen bond interactions, and further change the conformation and affect the antioxidant activity (Fogliani et al., 2005).

Effect of raspberry extract on HaCaT cell proliferation
The effects of raspberry extract and ellagic acid on the proliferation of epidermal cells during wound healing were compared. Figure 5 shows the cell proliferation rates determined at different concentrations of raspberry extract and ellagic acid. The cell proliferation rate increased with the concentration. When the concentration of the extract was 0.63 μg/mL, the proliferation rate was significantly (P<0.05) higher than that of the control group. Similarly, when the concentration of ellagic acid was 1.25 μg/mL, the cell proliferation rate was significantly higher than that of the control group (P<0.05). These findings indicate that the effect of raspberry extract on cell proliferation was not limited by its ellagic acid content. The other components present in the extract may have also contributed to the cell proliferation. At 5.00 μg/mL, the cell proliferation rate of the two treatment groups was significantly (P<0.01) higher than that at lower concentrations, but there was no significant difference between the groups (P>0.05).

These results are in partial agreement with those of Mottola et al. (2020), who reported that ellagic acid has a protective effect on the proliferation of mammalian cells. In addition, this study demonstrates that there is no obvious relationship between the antioxidant activity of antioxidants and their cell proliferation-promoting ability, which is in contrast to the finding of Mendis et al. (2005). This might be due to the difference in efficiency between the ellagic acid dimer and ellagic acid glucose derivative in raspberry extract, or because the antioxidant activity of the extract and ellagic acid eliminated excessive free radicals that affect cell proliferation. In the process of wound healing, subsequent injury caused by free radicals...
can be reduced to achieve favorable conditions for cell proliferation (Padma et al., 2014; Mehrzadi et al., 2019).

Effect of raspberry extract on HaCaT cell scratch healing

HaCaT cell scratch assays were used to simulate cell growth and phases of wound healing. This involved the intuitive manifestation of cell proliferation and migration. We selected 5 μg/mL of raspberry extract and ellagic acid to perform the treatment, which had extremely significant effects on the proliferation of HaCaT cells compared with the control treatment. Figure 6 shows the proliferation and migration of HaCaT cells cultured at 0, 24, and 48 h in the treatment, blank, and positive control groups. The scratch area gradually decreased the culture time of HaCaT cells. A significant difference was found in the cell scratch areas at the different time periods (P<0.05).

Figure 7 shows that when the cells were cultured for 24 h, the scratch healing rate of the raspberry extract group (Y) was (41.11±1.92) per cent, which was not significantly different from that of the ellagic acid group (B) at (39.01±2.00) per cent (P>0.05). However, compared with that of the blank control group (K) at (20.67±1.16) per cent, a very significant difference was found (P<0.01), indicating that raspberry extract and ellagic acid promoted cell proliferation and migration. This finding is consistent with the results of previous experiments on HaCaT cell proliferation, in which the cells were cultured for 48 h and the scratch healing rates of raspberry extract, ellagic acid, and rhEGF positive control (R) groups were (68.88±1.84) per cent, (70.33±0.67) per cent, and (85.53±0.40) per cent, respectively, which were significantly higher than that of the blank group (34.21±1.30) per cent (P<0.01). During the culture, rhEGF was used as the positive control, and its effect on cells was significantly different from those of the blank, raspberry extract, and ellagic acid (P<0.01). Raspberry extract and ellagic acid promoted cell proliferation and migration, but significant differences existed in terms of their effects in comparison with that of rhEGF. The scratch results of HaCaT cells further confirmed the effect of raspberry extract and ellagic acid on the proliferation of HaCaT cells.

Rens and Merks (2020) found that the interaction between cells and extracellular matrix has a certain impact on cell proliferation.
and migration. Tang et al. (2015) found that ellagic acid can reduce the expression of inflammatory factors such as IL-1β and NLRP3. Therefore, the extract and ellagic acid may activate the expression of some genes directly or indirectly, promote the synthesis of related proteins, and increase cell proliferation efficiency or weaken the inhibitory effect of certain genes on cell proliferation, thereby promoting wound recovery (Jara et al., 2020; Li et al., 2020). Further work is needed to establish the relationship between the antioxidant and cell proliferation-promoting capacity, and to determine the underlying mechanisms for wound healing.

Effect of raspberry extract on wound healing in mice

Skin wound healing is the process of gradual repair of skin tissue over time. After modeling, mice were continuously intervened for 14 days; the wound status of the mice is shown in Figure 8. The mice wounds did not show any adverse conditions such as infection, pus accumulation, redness, or swelling. The skin wounds of raspberry extract gavage mice formed obvious dark-red hard scabs. After 12 days of intervention, the wound area was almost filled and the wounds healed well. After 14 days, the color around the wounds became lighter, and the wounds gradually became normal tissue. However, the wounds of mice in the rhEGF group did not achieve the expected effect. It is speculated that rhEGF entered the mice and

![Image of wound healing in mice](https://academic.oup.com/fqs/article-lookup/doi/10.1093/fqsafe/fyab013/6308357)
was decomposed by the digestive system, weakening the effect on wound healing (Lee, 2002).

Table 1 shows the changes in the wound area of mice. After gavage with raspberry extract for 4 days, the remaining wound area of mice was smaller than that of the blank control group (K). The high-dose raspberry extract group (H) showed the highest effect on wound healing compared with the blank control group. The intervention effect was statistically significant \((P<0.05)\), which suggested that raspberry extract promoted wound healing. Then, as the dose of raspberry extract and intervention time increased, the wound area of mice significantly or extremely significantly decreased compared with that in the blank group. The wound area of the mice treated with rhEGF (R) was smaller than that of the blank control group. After 14 days of intervention, there was a significant difference between the rhEGF and blank control groups \((P<0.05)\), but no significant difference between the rhEGF and raspberry extract \((P>0.05)\).

These results indicated that the proliferative activity of epidermal cells was one of the most important mechanisms that affected wound healing (Shaw and Martin, 2016).

Conclusions
In this study, we compared the effects of raspberry extract and ellagic acid on wound healing in cells. Both promoted cell proliferation effectivity. Our findings show that raspberry extract has great potential as a natural wound-healing product, and can be developed as a low-cost wound treatment.

Author Contributions
Wenjing Lu, Yiling Tian, and Junping He contributed to the conception of the study; Wenjing Lu, Meng Xu, and Youwei Yuan performed the experiment; Wenjing Lu, Yiling Tian, Jianxin Tan, and Xuemei Zhang contributed significantly to analysis and manuscript preparation; Wenjing Lu, Jianxin Tan, and Yiling Tian performed the data analyses and wrote the manuscript; Yiling Tian, Junping He, and Xuemei Zhang helped perform the analysis with constructive discussions.

Conflict of Interest
The authors declare no conflict of interest.

Funding
This study was financially supported by the project grant from red raspberry high-efficiency cultivation technology integration and deep processing product development of Hebei Province, China (No.19226815D). This work was also funded by the Modern Forestry Discipline Group (XK1008601519) and the Food Processing Discipline Group (No.2021-05) of Hebei Agricultural University, China.

References
Adom, K. K., Liu, R. H. (2005). Rapid peroxyl radical scavenging capacity (PSC) assay for assessing both hydrophilic and lipophilic antioxidants. *Journal of Agricultural and Food Chemistry*, 53(17): 6572–6580.

Aslan, A., Gok, O., Beyaz, S., et al. (2020). The preventive effect of ellagic acid on brain damage in rats via regulating of Nrf-2, NF-kB and apoptotic pathway. *Journal of Food Biochemistry*, 44(6): e13217.

Baby, B., Antony, P., Vijayan, R. (2018). Antioxidant and anticancer properties of berries. *Critical Reviews in Food Science and Nutrition*, 58(15): 2491–2507.

Bobinatę, R., Viskelis, P., Bobinas, Č., et al. (2016). Raspberry marc extracts increase antioxidative potential, ellagic acid, ellagitannin and anthocyanin concentrations in fruit purees. *LWT–Food Science and Technology*, 66: 460–467.

Bobinatę, R., Viskelis, P., Šarkinas, A., et al. (2013). Phytochemical composition, antioxidant and antimicrobial properties of raspberry fruit, pulp, and marc extracts. *CyTA–Journal of Food*, 11(4): 334–342.

Bobinatę, R., Viskelis, P., Venskutonis, P. R. (2012). Variation of total phenolics, anthocyanins, ellagic acid and radical scavenging capacity in various raspberry (*Rubus* spp.) cultivars. *Food Chemistry*, 132(3): 1495–1501.

Duan, J., Li, Y. X., Gao, H. H., et al. (2020). Phenolic compound ellagic acid inhibits mitochondrial respiration and tumor growth in lung cancer. *Food & Function*, 11(7): 6332–6339.

Fogliani, B., Raharivelomanana, P., Bianchini, J. P., et al. (2005). Bioactive ellagitannins from Cunonia macropylla, an endemic Cunoniaceae from New Caledonia. *Phytochemistry*, 66(2): 241–247.

Giafre, A. M., Lousady, L., Rizzo, P., et al. (2019). Packaging and storage condition affect the physicochemical properties of red raspberries (*Rubus idaeus* L., cv. Erika). *Food Control*, 97: 105–113.

Hanneken, A., Lin, F. F., Johnson, J., et al. (2006). Flavonoids protect human retinal pigment epithelial cells from oxidative-stress-induced death. *Investigative Ophthalmology & Visual Science*, 47(7): 3164–3177.

Hussein, R. H., Khalfia, F. K. (2014). The protective role of ellagitannins flavonoids pretreatment against *N*-nitrosodiethylamine-induced-hepatocellular carcinoma. *Saudi Journal of Biological Sciences*, 21(6): 589–596.

Jara, C. P., Wang, O., Paulino do Prado, T., et al. (2020). Novel fibrin–fibronection matrix accelerates mice skin wound healing. *Bioactive Materials*, 5(4): 949–962.

Jurenka, J. S. (2008). Therapeutic applications of pomegranate (*Punica granatum* L.): a review. *Alternative Medicine Review*, 13(2): 128–144.

Katsunari, I., Atsuko, T., Keiko, A. (2009). Prevention of peroxynitrite-induced retinal pigment epithelial cells from oxidative-stress-induced death. *In Vitro Cellular & Developmental Biology–Animal*, 45(6): 2491–2507.

Kowalska, K., Olejnik, A., Zielińska-Wasielica, J., et al. (1999). Prevention of N-nitrosodiethylamine-induced lung tumorigenesis by ellagic acid and quercetin in mice. *Food and Chemical Toxicology*, 37(4): 313–318.

Kovalska, K., Olejnik, A., Zielińska-Wasielica, J., et al. (2019). Raspberry (*Rubus idaeus* L.) fruit extract decreases oxidation markers, improves...
