*Original Article*

# Comparison of the Hemostatic Activity of *Quercus persica* Jaub. & Spach. (Oak) With Ferric Sulfate in Bony Crypts

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## Abstract

Effective tissue hemostasis in periapical surgical site is important in the procedures. Plants with large amount of tannins may act as a local hemostatic agent. We aimed to compare the hemostatic effect of the extract of *Quercus persica* with one of the common hemostatic material used in periapical surgery. Six standardized bone holes were prepared in the calvaria of 5 Burgundy rabbits. Two hemostatic medicaments were tested for their hemostatic effect and were compared with control defects: Group 1, cotton pellet soaked in 15.5% ferric sulfate solution; Group 2, cotton pellet soaked in pure ethanolic extract of *Q. persica*. Bleeding score between the groups was compared. The ferric sulfate group exhibited significantly less bleeding than the other 2 groups. *Q. persica* was found to cause more hemostasis than the control group at 4 and 5 minutes but there were no significant differences between normal saline and *Q. persica* extract in bleeding control.

## Keywords

*Quercus persica*, hemostatic activity, Persian medicine

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Persistent apical periodontitis generally is managed by nonsurgical retreatment as the first approach.[1] However, in unsuccessful nonsurgical treatments or impractical cases, periradicular surgery is indicated. The success of endodontic surgical procedures is related to several factors. However, achievement of an effective tissue hemostasis in the surgical site is a key aspect for performance of the procedures. Failure to control bleeding in endodontic surgical procedures results in the reduction of surgical vision quality, increase in surgical time, and increase in blood loss.[2] The sealing ability of retrofilling material can be affected by the presence of bleeding during surgery as well.[3] In addition, postoperative hemorrhage and swelling, the 2 common complications of endodontic surgery, can be prevented by proper hemostasis during surgery.[2] In apical surgery, practitioners need control hemorrhage to facilitate probing of the root-end surface and to provide a clean field for placement and setting of the root-end filling.

Topical hemostatic agents commonly used in apical surgery can be categorized as either non-collagen-based or collagen-based. Vasoconstrictor-impregnated cotton pellets, ferric sulfate, calcium sulfate, gel foam, thrombin, bone wax, and surgicel are common non-collagen-based hemostatic agents. The periapical healing interference and foreign body tissue reaction at the histological level are the main problems of these hemostatic agents, especially if they are left in situ.[4]

Avitene and Instat are 2 popular collagen-based products whose high affinity for wet surfaces and adherence to instruments make them difficult to work with.[5]

Ferric sulfate is one of the most currently used hemostatic agents in apical surgery.[6] Nevertheless, the risk of use of ferric sulfate in contact with important anatomic structures such as maxillary sinus, floor of the nose, or mandibular and mental nerve limits its clinical usage. In addition, failure to adequate curettage and irrigation of the surgical site after ferric sulfate

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application can lead to foreign body reaction, impaired healing, and abscess.[7]

Several studies attempted to explain the procoagulating effect observed with some plant extracts. For example, it was found that the astringent effects of the leaves of *Mangifera indica* were related to their rich tannins. It is important to emphasize that the astringent activity favors vasoconstriction, which is an important factor in hemostasis.[8]

These findings are consistent with Bruneton, who demonstrated that the tannins have a hemostatic and vasoconstrictor effect on the small vessels.[9]

Previous studies on homeostatic properties of some medicinal plants such as *Jatropha multifida*[10] and the bark of *Entada africana*[11] showed that these agents significantly reduced bleeding time. These studies tended to focus on soft tissue surgeries rather than hemostasis in bony crypt. The effect of plants extract as hemostatic agent was confirmed from the results of the study by Dandjesso et al on medicinal plants sold as antihemorrhagics.[8] The study was an in vitro study on 4 medicinal plants during which plasma recalcification time, prothrombin time, and activated partial thromboplastin time were measured for blood samples taken from rabbit. In all of these studies the authors emphasize that the hemostatic activity of these extracts is due to the presence of tannins. Black tea is another example.[12]

Dandjesso et al suggested that the hemostatic effect that was seen in the extracts of 4 species of medicinal plants that they used in their study appears to be related to the effect of certain organic compounds (secondary metabolites), including tannins, on plasma proteins.[8]

With the aim of finding natural sources as hemostatic agents, *Quercus* (oak) species are introduced in traditional medicine as astringent, antiseptic, and hemostatic. Furthermore, the extract of these plants can be used for burns and added to ointments for the healing of cuts.[13,14]

The main species of oak plants in Iran is *Quercus persica* Jaub. & Spach. A large area of forests in the northwest of Iran is covered by different oak species, especially *Q. persica*.[15] The oak fruit is a nut called acorn, which contains 1 or 2 seeds, enclosed in a tough, leathery shell, and borne in a cup-shaped cupule.[16] The leathery shell of oak fruit contains a large amount of tannins (50% to 70%), gallic acid, syringic acid, ellagic acid, sitosterol, amentoflavone, hexamethyl ether, isocryptomerin, methyl betulate, methyloleanate, and hexagalloyl glucose.[17,18]

It is hypothesized that *Q. persica* with large amount of tannins in its different parts could act as a hemostatic agent. Therefore, the objective of this research was to examine the hemostatic effect of *Q. persica* and to compare this activity with ferric sulfate as one of the common hemostatic material used in periapical surgery.

## Materials and Methods

### Collection of Plant

The internal layer of oak fruit (seed hulls) was obtained from a traditional (local) herbal market (*Attari*) in Shiraz. The taxonomic identification of the plant material was performed by a plant taxonomist in the Botany Laboratory and Herbarium of the School of Pharmacy, Shiraz University of Medical Sciences, with voucher number PM777.

### Preparation of Drug

The internal layers of the oak fruit (seed hulls) were separated from the nuts. Then, the collected plant materials were washed, dried, and then crushed with a pestle and mortar. An electric grinder was used to produce a fine powder and passed through a 100-mesh sieve.

Seed hulls was extracted with conventional methods to determine the best formulation based on the total amount of tannins.[19,20]

*Maceration Method.* Twenty-five grams of the powder of the oak seed hulls were poured into an Erlenmeyer flask (250 mL) containing 250 mL of ethanol 70% and was placed for 24 hours at ambient laboratory temperature. Samples were heated for 2 hours on a bain-marie and then filtered. The resulting residue was redissolved in 250 mL of ethanol 70% for 24 hours and the filtering process was repeated. Then the final volume was collected.

*Percolation Method.* In this method, using a percolator, we first removed the separating funnel tap and blocked both sides completely, then one end of the separating funnel was filled with a layer of cotton, and on it, 10 g of oak seed hulls is poured, placed on a sheet of filter paper gently and so that ethanol can be added. The alcoholic extract output was collected in a 250-mL Erlenmeyer flask was covered and the final volume percolation reached 250 mL.

*Soxhelet Method.* First, 10 g of powdered oak seed hulls was poured into a filter paper and was placed into a Soxhelet apparatus. Then 300 mL of ethyl alcohol 96% was poured in the flask and allowed to reach its boiling point. The boiling process was done in 2 phases of 4 hours, with an interval of 24 hours. The alcohol extract was isolated after distillation at low pressure, at a temperature of 45°C and a speed of 60 rpm. In this case the extract was deposited as a layer in the bottom of the container. This precipitate was washed with 100 mL of distilled water and the bath was used to dissolve completely. Then the samples were filtered and the final volume was brought to 100 mL.

### Determining the Total Amount of Tannin

*Tannins Measured (Leuin Shawl's Method).* The reduction properties of tannins are used in permanganate titration with apparent normality. Indigo carmine reagent was used to the determine endpoint.[19]

*Measurement of Reductionable Substances by Potassium Permanganate.* A total of 25 mL of Indigo carmine was poured into a 250-mL Erlenmeyer flask and 10 mL of distilled water was added to it. With shaking 0.1 N potassium permanganate was added drop by drop from a burette until the solution changes color from blue to yellow. Permanganate was used as control sample. The same process is repeated with 10 mL of sample and the volume of potassium permanganate was recorded.

*Measurement of Nonreductionable Substances Other Than Tannins by Potassium Permanganate.* Ten milliliters of distilled water, 5 mL of 3% gelatin, 5 mL of 5% sulfuric acid saturated salt, and 2 g of Kaolinite ($Al_2Si_2O_5(OH)_4$) were mixed in a 250-mL Erlenmeyer flask and after a few minutes the solution was filtered. Then

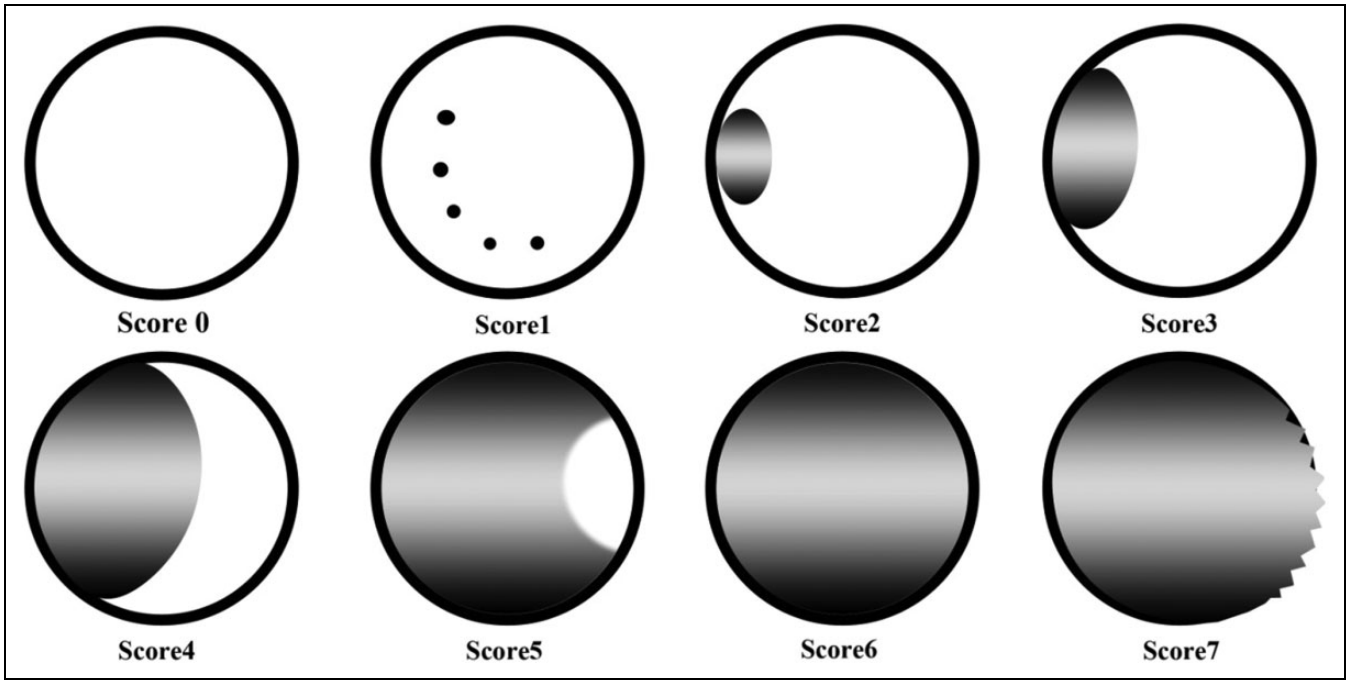


**Figure 1.** Schematic illustrations used for visual assessment of bleeding (scale scores from 0 [*completely dry*] to 7 [*abundant bleeding*]).

10 mL of the filtered mixture with 25 mL Indigo carmine was taken and poured into a 250-mL Erlenmeyer flask and titrated using potassium permanganate. All the aforementioned were repeated for 10 mL samples and extracted and the consumed permanganate recorded.

Using the following formula, the total amount of reductionable substances and reductionable substances other than tannins was calculated; if the results are subtracted the amount of total tannins in the sample is obtained. The total nonreductionable substances other than tannins minus reductionable substances by permanganate is equal to the pure total tannins content.[20]

$$(v_1 - v_0) \times \frac{P}{1000} \times \frac{250}{10} \times \frac{100}{M_0} \times \frac{100}{D}$$

where $v_1 - v_0$ is the difference in the volume of permanganate used for the original and control samples; $P$ is the permanganate quantities in milligrams per milliliter of distilled water; $M_0$ is the sample used in the extraction in grams; and $D$ is the amount of dried sample.

## *In Vivo Study*

We obtained the required approval from the Ethics Committee of Shiraz University of Medical Sciences to perform the study (Study Number 1513).

The study was carried out on five 5-month-old Burgundy rabbits, weighing between 3 and 4.5 kg. Von Arx and colleagues' study protocols and medications were used to perform surgical procedures.[21,22] In each rabbit's calvaria, 6 standardized defects were prepared using a trephine (4 mm outer diameter) in a way that it could not reach the inner cortical layer. Therefore, the thickness of the outer cortical bone layer could determine the depth of the defects. The randomization scheme generated by http://www.randomization.com (seed: 2604) was used to distribute treatments among the defects.

**Table 1.** Results of Extraction of Total Tannins in the Samples.

| Extraction Method | Total Tannin Content (%) |
|---|---|
| Percolation | 9.08% |
| Soxhelet | 17.38% |
| Maceration | 33.65% |

Control group: Mild application of a cotton pellet soaked in normal saline

Group 1: Gentle application of a cotton pellet soaked in 15/5% ferric sulfate solution (Astringedent Ultradent Products Inc, Salt Lake City, UT)

Group 2: Application of a cotton pellet soaked in pure methanol extract of *Q. persica* with the same pressure as previous groups

The competence of hemostasis was evaluated by the operator at 1, 2, 3, 4, and 5 minutes. The medicaments were lightly applied until complete hemostasis was achieved or after 5 minutes. Operators were blinded about sample groups applied to the rats.

Photos were taken after application of the hemostatic agents at each time. The amount of blood per defect was checked on a scale from 0 (*completely dry*) to 7 (*abundant bleeding*) according to the schematic shown in Figure 1. Three evaluators separately examined the photos and assessed the bleeding score per defect. These evaluators were blinded about the sample groups. Median and mean bleeding scores were calculated after the removal of the hemostatic agents at 1, 2, 3, 4, and 5 minutes.

The surgical sites in all groups were gently curetted and irrigated with saline. As complete removal of the medicaments is not practical in common clinical practice, gentle curetting instead of the complete removal of the material would better simulate clinical practice. The flaps were repositioned and sutured with 3-0 Nylon (Supa, Iran).

**Table 2.** Median Bleeding Scores (Mean ± SD) at 1, 2, 3, 4, and 5 Minutes*.

| | 1 Minute | 2 Minutes | 3 Minutes | 4 Minutes | 5 Minutes |
|---|---|---|---|---|---|
| Normal saline | 6.00[a] (4.86 ± 2.54) | 4.00[a] (4.28 ± 2.43) | 4.00[a] (3.86 ± 2.41) | 4.00[a] (4.14 ± 2.67) | 5.00[a] (3.71 ± 3.25) |
| *Quercus persica* | 7.00[a] (6.00 ± 2.45) | 7.00[a] (5.00 ± 2.97) | 4.50[a] (4.00 ± 3.29) | 3.50[a] (3.62 ± 3.20) | 3.00[a] (3.12 ± 3.18) |
| Ferric sulfate | 2.00[b] (2.00 ± 1.52) | 0.00[b] (0.57 ± 1.13) | 0.00[b] | 0.00[b] | 0.00[b] |
| *P* value** | .010 | .009 | .007 | .007 | .024 |

*Different letters in each column show significance between groups (Mann–Whitney *U* test).
**Kruskal–Wallis *H* test.

The rabbits received buprenorphine (% 03 mg/kg sub cut every 12 hours) for 3 days.

### Statistics

Data were reported as median and mean ± SD. Kruskal–Wallis *H* and Mann–Whitney *U* tests were used to compare bleeding scores between the groups. SPSS V.16.0 (Chicago, IL) was used for statistical analysis. $P < .05$ was considered significant.

## Results

### Selected Formulation for Applying in In Vivo Study

The results of the determination of total tannin extraction in different ways are shown in Table 1. The macerated extract with ethanol 70% was found to have the highest amount of tannins (33.65%) for *Q. persica* seed hulls extraction. Therefore, this ethanolic extraction was used in the study.

### In Vivo Study

Table 2 provides the median bleeding scores for each experimental group at different time intervals. It can be seen that the ferric sulfate group reported significantly less bleeding scores than the other 2 groups in all intervals. There were no significant differences between normal saline and *Q. persica* extract in bleeding control at all of these time intervals ($P \leq .05$).

## Discussion

In most cases nonsurgical root canal therapy is a greatly predictable treatment choice; however, surgery may be indicated for well-treated teeth with persistent periradicular disease. Local hemostatic agents are used to reduce surgical time, surgical and postsurgical blood loss, and to minimize postoperative swelling.[2] The use of ferric sulfate as necrotizing material with an extremely low PH (0.21) causes very good homeostasis through rapid intravascular coagulation.[3,23,24] Hemostatic control for 2 minutes in the present study corroborates these earlier findings.

The hypothesis of hemostatic effect of *Q. persica* is based on the astringent properties of tannins,[21] which are predominant components in this species. Therefore, in this study the maceration method was chosen to achieve the highest efficiency (total tannins in the sample: 33.654%) for the extraction of tannins from *Q. persica*.

Although previous studies supported the efficacy of high tannin content plants as hemostatic agents, the findings of the current study do not support the previous research describing the role of herbs with high tannin content on hemostasis. The possible explanation for this result might be the production of nitric oxide in blood platelets by flavonoids, which is one group of the compounds present in this extract. Flavonoids could prevent aggregation of platelets and limit the formation of clots.[25] A reasonable approach to tackle this issue could be to focus on the isolation of pure tannins from seed hulls of *Q. persica* and use them as the hemostatic agent. This rather contradictory result may also be explained by a concentration-related inhibitory effect of ethanol on platelet aggregation and release of thromboxane A2.[26]

## Conclusion

Mean and median scores of bleeding showed that there were not significant differences between the control group and the *Q. persica* extract in hemostasis. Further investigation and experimentation on isolation of pure tannins from seed hulls of *Q. persica* and their use as hemostatic agent could be beneficial.

### Author Contributions

MRN wrote the preliminary draft and contributed in data gathering and first idea of starting this project. FM rewrote the draft and contributed in data gathering. AZ contributed in data gathering and writing the final version of the article. AF and FA contributed in data gathering. PF contributed in the guidance of the project. All authors read and approved the final version of the article.

### Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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### Ethical Approval

This project has ethical approval (No. 1513) from the Ethics Committee of Shiraz University of Medical Sciences.

## References


1. Briggs P, Scott B. Evidence-based dentistry: endodontic failure—how should it be managed? *Br Dent J*. 1997;183(5):159-164.
2. Gutmann JL, Harrison JW. *Surgical Endodontics*. Boston, MA: Blackwell Scientific; 1991.
3. Scarano A, Artese L, Piattelli A, Carinci F, Mancino C, Iezzi G. Hemostasis control in endodontic surgery: a comparative study of calcium sulfate versus gauzes and versus ferric sulfate. *J Endod*. 2012;38:20-23.
4. Witherspoon D, Gutmann J. Haemostasis in periradicular surgery. *Int Endod J*. 1996;29(3):135-149.
5. Takeuchi H, Konaga E, Kashitani M, et al. The usefulness of Avitene for the control of oozing in laparoscopic cholecystectomy. *Surg Gynecol Obstet*. 1993;176:495-496.
6. Jang Y, Kim H, Roh BD, Kim E. Biologic response of local hemostatic agents used in endodontic microsurgery. *Restor Dent Endod*. 2014;39(2):79-88.
7. Larson PO. Topical hemostatic agents for dermatologic surgery. *J Dermatol Surg Oncol*. 1988;14:623-632.
8. Dandjesso C, Klotoa J, Dougnon T, et al. Phytochemistry and hemostatic properties of some medicinal plants sold as antihemorrhagic in Cotonou markets (Benin). *Ind J Sci Tech*. 2012; 5:3105-3109.
9. Bruneton J. Les tannins. In: *Pharmacognosie, Phytochimie, Plantes Médicinales*. 3rd ed. Paris, France: Éditions Tec & Doc et médicales internationales; 1999:369-404.
10. Dougnon TV, Klotoé JR, Sègbo J, et al. Evaluation of the phytochemical and hemostatic potential of *Jatropha multifida* Sap. *Afr J Pharm Pharmacol*. 2012;6:1943-1948.
11. Kerharo J, Adam JG. *La Pharmacopée Sénégalaise Traditionnelle, Plantes Médicinales et Toxiques*. Paris, France Vigot Frères; 1974.
12. Yang CS, Landau JM. Effects of tea consumption on nutrition and health. *J Nutr*. 2000;130:2409-2412.
13. Baytop T. *Therapy With Medicinal Plants in Turkey (Past and Present)*. Istanbul, Turkey: Istanbul University; 1999:312.
14. Bremness L. *Herbs: The Visual Guide to More Than 700 Herb Species From Around the World*. London, England: Dorling Kindersley; 1994.
15. Karimi A, Moradi MT, Saeedi M, Asgari S, Rafieian-Kopaei M. Antiviral activity of *Quercus persica* L.: high efficacy and low toxicity. *Adv Biomed Res*. 2013;2:36.
16. Jalilian Tabar F, Lorestani A, Gholami R, Behzadi A, Fereidoni M. Physical and mechanical properties of oak (*Quercus persica*) fruits. *Agric Eng Int: CIGR J*. 2012;13(4):1-4.
17. Yousef Elahi M, Rouzbehan Y. Characterization of *Quercus persica*, *Quercus infectoria* and *Quercus libani* as ruminant feeds. *Anim Feed Sci Technol*. 2008;140:78-89.
18. Azizi A, Azizi A, Azizi G, Zandi S, Khaghani S. Antioxidant activity of tannins extracted from bark and tan-bark of *Quercus persica* Jaub. & Spach fruit in soybean and sunflower. *Iran J Med Aromatic Plants*. 2011;27:397-406.
19. Masoudi Nejad MR, Rezazade Azary M. Comparison of four methods of tannin extraction from the fruits of oak species in Iran. *Hakim Scientific J*. 2003;6(1):81-91.
20. Nejad MM, Azary MR, Khatiby M. Treatment of wastewater in plating industry by chelate extraction method. *Iran J Environ Health Sci Eng*. 2007;4(1):13-20.
21. Jensen SS, Yazdi P, Hjørting-Hansen E, Bosshardt D, Von Arx T. Haemostatic effect and tissue reactions of methods and agents used for haemorrhage control in apical surgery. *Int Endod J*. 2010;43(1):57-63.
22. Von Arx T, Jensen S, Hänni S, Schenk R. Haemostatic agents used in periradicular surgery: an experimental study of their efficacy and tissue reactions. *Int Endod J*. 2006;39(10):800-808.
23. Jeansonne BG, Boggs WS, Lemon RR. Ferric sulfate hemostasis: effect on osseous wound healing. II. With curettage and irrigation. *J Endod*. 1993;19(4):174-176.
24. Lemon RR, Steele PJ, Jeansonne BG. Ferric sulfate hemostasis: effect on osseous wound healing. I. Left in situ for maximum exposure. *J Endod*. 1993;19(4):170-173.
25. Diallo S. *Etude des propriétés antioxydantes et antiplasmodium des Lannea couramment rencontrés au Mali* [Thèse de Doctorat]. Mali: Université de Bamako; 2005.
26. Mikhailidis D, Jeremy J, Barradas M, Green N, Dandona P. Effect of ethanol on vascular prostacyclin (prostaglandin I2) synthesis, platelet aggregation, and platelet thromboxane release. *Br Med J (Clin Res Ed)*. 1983;287:1495-1498.