Proteomic and transcriptomic analyses to explain the pleiotropic effects of Ankaferd blood stopper

Cem Simsek¹, Sebnem Selek², Meltem Koca¹ and Ibrahim Celal Haznedaroğlu³

Abstract
Ankaferd blood stopper is a standardized mixture of the plants *Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum* and *Urtica dioica* and has been used as a topical hemostatic agent and with its clinical application established in randomized controlled trials and case reports. Ankaferd has been successfully used in gastrointestinal endobronchial mucosal and cutaneous bleedings and also in abdominal, thoracic, dental and oropharyngeal, and pelvic surgeries. Ankaferd’s hemostatic action is thought to form a protein complex with coagulation factors that facilitate adhesion of blood components. Besides its hemostatic action, Ankaferd has demonstrated pleiotropic effects, including anti-neoplastic and anti-microbial activities and tissue-healing properties; the underlying mechanisms for these have not been well studied. Ankaferd’s individual components were determined by proteomic and chemical analyses. Ankaferd also augments transcription of some transcription factors which is shown with transcriptomic analysis. The independent effects of these ingredients and augmented transcription factors are not known precisely. Here, we review what is known of Ankaferd blood stopper components from chemical, proteomic, and transcriptomic analyses and propose that individual components can explain some pleiotropic effects of Ankaferd. Certainly more research is needed focusing on individual ingredients of Ankaferd to elucidate their precise and effects.

Keywords
Ankaferd blood stopper, hemostasis, proteomic analysis, genomic analysis, pleiotropic effect

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Introduction and effects of Ankaferd
Ankaferd is a novel hemostatic agent that was formulated from a traditional extract used in Anatolia and is a standardized mixture of the plants *Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum* and *Urtica dioica*. Ankaferd’s hemostatic action and clinical application has been established in randomized controlled trials and case reports. However, Ankaferd has pleiotropic effects including anti-neoplastic, anti-microbial, anti-mutagenic, and antioxidant as well as tissue-healing properties, although the underlying mechanisms have not been well studied. Here, we review the literature on Ankaferd to clarify the mechanistic basis of this pleiotropism. The procoagulant function of Ankaferd blood stopper (ABS) has been extensively discussed elsewhere.

Ankaferd has been successfully used as a topical hemostatic agent, for example, in the treatment of digestive tract ulcers as well as various types of bleeding including variceal/non-variceal, gastrointestinal, endobronchial, mucosal (including in patients with hemorrhagic diathesis), and cutaneous. Ankaferd has also been employed in abdominal, thoracic, dental and oropharyngeal, and pelvic surgeries (Table 1). The precise mechanism of Ankaferd’s
### Table 1. Clinical trials on Ankareferd's hemostatic action.

| Author            | Year | Number of population | Study design                    | Diagnosis of patients | Clinical settings | Conclusions | Perspectives |
|-------------------|------|----------------------|---------------------------------|-----------------------|-------------------|-------------|--------------|
| Yaman et al.      | 2012 | 30                   | Randomized                      | Carious primary molar teeth of 6–9 years old children | ABS was compared with formocresol for 3, 6, and 12 months for pain, swelling, mobility, resorption, furcation, and paresthesia bone destruction | ABS was as effective as formocresol as a pulp dressing of primary molar teeth | ABS appears to be an alternative pulpectomy agent |
| Iynen et al.      | 2011 | 90                   | Prospective                     | <18-year-old patients who needed adenoidectomy | The study assessed the clinical effect of ABS on hemostasis in adenoidectomy and post-adenoidectomy patients | ABS reduces the duration and blood loss of an adenoidectomy and increases postoperative quality of life | ABS is an effective agent to control bleeding in cases that could not be managed by vasoconstrictor agents |
| Teker et al.      | 2009 | 49                   | Prospective, randomized, controlled, non-blinded, clinical trial | Patients with anterior epistaxis | The efficacy of ABS as a hemostatic agent compared to hemostasis by phenylephrine was observed | ABS is effective, safe, quick, and easy alternative to the phenylephrine in patients with anterior epistaxis | ABS is an effective agent to control bleeding in cases that could not be managed by vasoconstrictor agents |
| Uzun et al.       | 2013 | 20                   | Retrospective study             | Patients with hemoptysis who needed bronchoscopic procedures | The study was done to evaluate the hemostatic efficacy of endobronchial application of ABS solution in patients with hemoptysis | Bleeding could be controlled with ABS a few seconds after instillation in the hemorrhagic focus in 23 interventions, but it was ineffective in 2 cases. | Bronchoscopic application of ABS may be an alternative supportive treatment in cases of uncontrolled hemoptysis. |
| Guler et al.      | 2011 | 61                   | Prospective, randomized, controlled trial | Patients with benign euthyroid multinodular goiter who needed total thyroidectomy | The study was designed prospectively to compare ABS and HCT groups in terms of operation time, postoperative drainage, duration of postoperative stay, and complications | The use of ABS is more effective than HCT to control hemorrhage following total thyroidectomy | The usage of ABS might be more effective in preventing hematoma by stopping ooze-type bleeding without causing an increase in hemostasis-related complications |
| Pamuk et al.      | 2015 | 15                   | Prospective, randomized clinical study | Patients with chronic periodontitis | Following the initial periodontal therapy, patients were randomly assigned to two treatments in contralateral areas of the dentition: ACB + ABS or ACB alone and the procedures were applied | ABS enhances the soft tissue healing during the periodontal defect fill by the ACB by stimulating angiogenesis and vascular endothelial cell function, prevents GR and thereby increases the clinical attachment gain | The results indicate that (1) both treatment modalities resulted in statistically significant clinical improvements compared with baseline, (2) ABS may improve the regenerative process and cause less GR, and (3) ABS may lead to an increase in levels of the VEGF in the healing stage of periodontal surgery |
| Istanbulluoglu et al. | 2013 | 90                   | Prospective, randomized clinical study | Patients who undergone PCNL because of renal and/or upper ureter stones | 45 of the patients underwent tubeless PCNL with the use of ABS as a hemostatic agent, whereas the remaining ones underwent tubeless PCNL without ABS and the procedures were applied | ABS is an efficient and reliable hemostatic agent in tubeless PCNL | ABS is a safe and reliable method in tubeless or totally tubeless PCNL interventions leading to expectations that these procedures might find widespread use among endourologists |
| Amer et al.       | 2013 | 205                  | Prospective, randomized clinical study | Patients with a single tooth to be extracted that can be removed with forceps without the need for mucoperiosteal flap and/or dental elevators were included in this study | Patients were selected so that 80 patients have INR values of 1–2, whereas the remaining patients have the INR values ranging from 2 to 3 and the procedures were applied | ABS is an effective hemostatic agent comparable to tranexamic acid in controlling post-extraction bleeding in AOT patients of INR values ≤3 with no evidence support the superiority of tranexamic acid over ABS | For patients with INR values >2 ABS can represent itself as a sufficient local hemostatic agent that is comparable with tranexamic acid |
| Author          | Year | Number of population | Study design                        | Diagnosis of patients                                                                                                                                                                                                 | Clinical settings                                                                                                                                                                                                 | Conclusions                                                                                                                                                                                                 | Perspectives                                                                                                                                                                                                 |
|-----------------|------|----------------------|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Atalay et al.   | 2015 | 50                   | Randomized, prospective clinical study | CABG patients who medicated with clopidogrel and ASA prior to CABG surgery                                                                                                                                          | 25 CABG patients received a high-dose clopidogrel (600 mg) and 300 mg ASA have been included into the study (ABC group). 25 patients have also been included into the study for comparison and the procedures were applied. | Local use of ABC decrease the bleeding from the mediastinum after CABG                                                                                                                                                       | ABC seems to be effective agent to inhibit blood loss after CABG without any complication and provides a significant reduction of bleeding in patients medicated with high-dose clopidogrel |
| Yasar et al.    | 2009 | 60                   | Prospective, non-randomized, non-blinded observational study | Patients who were subjected to the intended procedure if having an upper airway obstruction due to adenoid tissue                                                                                                                                                           | Each child was assigned either to the ABS or the SS group in order of appearance on the surgical waiting list.                                                                                                                                                   | A statistically significantly shorter duration of bleeding and a statistically lower number of packs are required to achieve ABS tamponade-induced hemostasis during adenoidectomy as compared to saline soaked gauze sponge application.                           | ABS aids in the control of intraoperative bleeding and reduces the number of packs required to achieve hemostasis, so that it can be recommended for tamponades performed during pediatric adenoidectomies |
| Teker et al.    | 2009 | 47                   | Prospective, non-randomized, non-blinded study | Patients with chronic tonsillitis, tonsillar hypertrophy, and obstructive sleep apnea syndrome                                                                                                                              | Patients with bleeding disorders, aspirin use within 2 weeks prior to surgery, tonsillar abscess history, acute tonsillitis within 4 weeks prior to surgery, tonsillectomy due to malignity suspicion, and children with systemic diseases were excluded and the procedures were applied. | ABS reduces intraoperative hemorrhage and operation time                                                                                                                                                                                                                     | It is a safe, efficient, and easy to use hemostatic agent with no side effects and it is recommended to use ABS during routine tonsillectomy for healthy children                                                 |
| Akpinar et al.  | 2015 | 50                   | Double-blind, placebo-controlled, randomized clinical trial | Patients with unstable angina unsuitable for percutaneous coronary intervention who were scheduled for urgent or acute CABG                                                                                     | Twenty-five emergency CABG patients premedicated with clopidogrel and ASA were included in the study (Group 1). An additional 25 patients who were premedicated with the same antplatelet agents were selected as a control group (Group 2). | The use of local ABS reduces bleeding, transfusion requirements of packed red blood cells, platelets, and total blood units in patients premedicated with clopidogrel and ASA undergoing emergency CABG. | This study demonstrated that a significant reduction in bleeding and requirement for transfusion can be achieved with the use of ABS in emergency CABG patients premedicated with a high dose of clopidogrel |
| Eyi Yapar et al.| 2012 | 40                   | Prospective, randomized clinical study | Pregnant women with a term singleton fetus in a vertex position who required a mediolateral episiotomy                                                                                                                     | The patients were randomly assigned to two approaches (20 to ABS, 20 to SS).                                                                                                                                                                                        | Application of 4 mL of ABS instead of SS lessened bleeding                                                                                                                                                                 | The study revealed a positive effect of the topical application of ABS tested for bleeding reduction                                                                                                             |
| Atay et al.     | 2013 | 20                   | Prospective, randomized clinical study | Patients with oral mucositis of grade 3–4 according to the WHO classification                                                                                                                                              | After patients developed oral mucositis they used only ABS, and age and gender of patients, type of the underlying malignant disease and used chemotherapeutic drugs, frequency, amount and duration of ABS use and healing time of oral mucositis were recorded. | The healing duration of oral mucositis was shorter with the topical ABS application. And also the hemorrhages from oral mucositis lesions were recovered within 2 days with ABS. | ABS is an effective agent in the chemotherapy-related severe oral mucositis treatment of the patients with hematological malignancies. ABS shortens the healing time with acceptable side effects |
|                | 2008 | 23                   | Clinical trial                        | Dental treatments with bleeding such as periodontitis, tooth removal, etc.                                                                                                                                               | Use of ABS in dental procedures was observed both by physical examination and laboratory tests.                                                                                                                                                                       | Laboratory findings were not affected by ABS application. No GIS side effects were observed                                                                                                                                 | ABS is safe for oral-topical use                                                                                                                                                                             |

ACB: autogenous cortical bone graft, ABS: Ankaferd blood stopper; AOT: Oral anticoagulant therapy; CABG: coronary artery bypass grafting; ASA: acetylsalicylic acid; INR: international normalized ratio; GIS: gastrointestinal system; GR: gingival recession; HCT: hemostasis by conventional technique; PCNL: percutaneous nephrolithotomy; SS: saline solution; VEGF: vascular endothelial growth factor.
hemostatic action is not known, but it is thought to form a protein complex with intrinsic coagulation factors that facilitate erythrocyte and thrombocyte adhesion. The hemostatic mechanism of Ankaferd will not be further addressed here; instead, we refer readers to a recent review of this subject.2

Ankaferd has long been known to promote the repair of bone, periodontal, muscle, skin, gastrointestinal, and oropharyngeal tissues and urogenital mucosae after surgery or injury, although some contradictory findings have been reported.27–30

The antimicrobial activity of Ankaferd is thought to derive from its oxygen-enhancing capacity through erythrocyte aggregation. The antimicrobial spectrum is broad and includes microorganisms such as methicillin-resistant Staphylococcus aureus, Klebsiella, Acinetobacter baumannii, Mycobacterium tuberculosis, and Candida albicans.31,32 Ankaferd has also been used to treat hydatid cysts via injection.33,34

Less is known about the anti-neoplastic activity of Ankaferd, although it was shown to inhibit cell proliferation, promote apoptosis, and prevent blast cell transformation of B-cell chronic lymphocytic leukemia cells in vitro.35 It has also demonstrated toxicity toward multiple myeloma and plasmacytoma cells both in vitro and in vivo, while decreasing M protein production.36,37 Other solid malignancies such as sarcoma and colon cancer are also targets of the anti-neoplastic action of Ankaferd.

Method

The constituents of Ankaferd have been identified by proteomic and transcriptomic analyses.38,39 Proteins were searched in the Uniprot Protein Knowledge Database by accession number, whereas genes were searched in the GeneCards database. Both proteins and genes were searched in National Library for Health and Institute for Scientific Information databases. References and related literature were reviewed by three authors. Based on this information, we hypothesized that the constituents of Ankaferd contribute to each of its three major pleiotropic actions, which are discussed below.

Antimicrobial effects

Ankaferd has antibacterial, germicidal, and antymycobacterial effects. In vitro studies have shown that ABS is effective against both Gram-positive and Gram-negative bacteria;40 foodborne pathogens such as Escherichia coli and Salmonella typhimurium;41 resistant nosocomial pathogens such as Pseudomonas, Klebsiella, Acinetobacter, Enterococcus, and Staphylococcus species;42 Echinococcus granulosus;33 and resistant Tuberculosis strains.

The antimicrobial effects of Ankaferd may be attributable to its chemical components. A time-of-flight mass spectrometry analysis of ABS revealed the presence of the antioxidants tocotrienol, tryptophan, thymol, lycophene, enoxolone, tomatine, tertiary butylhydroquinone, vitamin E derivatives, and galangin.43

Anti-neoplastic effects

Another property of Ankaferd is the capacity to inhibit neoplasia. ABS has demonstrated cytotoxicity against human erythrocytes at some concentrations, as well as against tumoral cells in hematologic malignancies such as multiple myeloma, chronic myelogenous leukemia, and lymphoma.35,37,44 Possible related ingredients of Ankaferd are CREBZF, PIAS-2, HNF-4a, ME-1, P18INK4C, and Midkine and addressed below in alphabetical order.

In transcriptome analyses, ABS has been found to increase the expression of cyclic AMP response element-binding protein (CREB)/ATF BZIP transcription factor (CREBZF), a member of the mammalian CREB family of transcription factors; this increased the level of the anti-neoplastic protein p53, thereby enhancing gene transcription.45 CREBZF also regulates the unfolded protein response to protect against excessive protein synthesis during endoplasmic reticulum stress.46

Hepatocyte nuclear factor (HNF)-4a is a nuclear receptor found not only in the liver but also in other tissues, which is involved in embryonic development, cellular differentiation, and hepatocyte-specific protein synthesis. Recent studies have shown that HNF-4a also has anti-neoplastic activity, with its inhibition leading to tumor growth.47 HNF-4a is a component of ABS extract and may be partly responsible for its anti-tumorigenic effects.

Malic enzyme (ME)-1 is an intracellular cytosolic protein and a component of ABS that converts malic acid to pyruvic acid, yielding nicotinamide adenine dinucleotide phosphate (NAPDH). ME-1 plays an important role in cancer metabolism, since NADPH is required for anaerobic respiration; indeed, ME-1 level is upregulated in some cancers.48,49 However, the role of ME-1 in ABS remains unclear given that it remains outside of cells, and its in vivo function has not been investigated.

Midkine is a heparin-binding protein that is involved in cellular growth, survival, migration, and differentiation. Midkine was found to suppress vascular endothelial growth factor A, which plays a key role in tumoral angiogenesis; its inhibition may thus inhibit tumor growth.50,51 Although a more likely role for midkine is the promotion of tissue healing (discussed below), we speculate that it also has antitumoral effects.

Protein inhibitor of activated signal transducer and activator of transcription (PIAS)-2 is another component of ABS extract. This protein belongs to PIAS family, whose members suppress the activity of STAT proteins,52 which are critical components of the Janus kinase (JAK) cascade that acts downstream of many growth factor receptors. JAK-STAT signaling is a major pathway involved in human carcinogenesis.53 Thus, the anti-neoplastic action of ABS may be exerted via inhibition of STAT protein via PIAS-2.
Table 2. Hypothesized mechanisms of individual components’ contributions on Ankaferd’s pleiotropic effects.

| Pleiotropic effect | Component | Relevance |
|-------------------|-----------|-----------|
| Anti-neoplastic effects | CREBZF | Increases p53 transcription |
| | PIAS2 | Inhibits activity of STAT proteins |
| | HNF-4a | Its inhibition promotes tumor growth |
| | ME-1 | It converts malic acid to pyruvic acid resulting in production of NADPH |
| | P18INK4C | Inhibits CDKs |
| | Midkine | Downregulates VEGF-A |
| Tissue-healing effects | Dynactin | Has a filamentous structure and potential to integrate with other structures in vivo |
| | Egr-1 | Has an important role in cell proliferation and differentiation |
| | Midkine | Promotes endothelial cellular proliferation and angiogenesis |
| | C-myc | Plays a key role in progression of cell cycle, cellular growth, cellular transformation |
| | NF-1 | Inhibits RAS and regulates growth and differentiation of keratinocytes and its increased expression is shown in healing tissues in human epidermis |
| | Twinfilin | Interacts with extracellular actin to promote protein scaffold for tissue healing |
| | YY1 | Promotes cellular differentiation, proliferation, and growth, also is shown to protect from apoptosis |
| Antimicrobial effects | Tocotrienols vitamin E family | Have distinct antioxidant effects |
| | Galangin | The topoisomerase IV enzyme may therefore be implicated in the antibacterial mechanism of action of galangin |
| | Apigenin | Has an antioxidant, anticarcinogenic, and spasmolytic activities and can reduce high blood pressure |
| | Tertiary butylhydroquinone (TBHQ) | Prompts loss of staphylococcal membrane integrity |
| | BHT (butylated hydroxytoluene) | Has an antioxidant effect |

The cyclin-dependent kinase (CDK) inhibitor P18INK4C\(^ {54} \) inhibits tumorigenesis, and its deficiency promotes tumor growth.\(^ {55,56} \) CDKs are serine/threonine kinases that regulate the cell cycle and thus play a vital role in human cancers. P18INK4C may participate in the tumor-suppressor activity of ABS by inhibiting CDKs.

**Tissue-healing effects**

In addition to being a hemostatic agent, ABS has an established role in promoting tissue repair after radiation damage and colitis;\(^ {57} \) bone, cartilage, muscle, and tendon remodeling;\(^ {58,59} \) and repair of dermal and epidermal tissue;\(^ {29} \) gastrointestinal mucosa, and full-thickness injuries in the abdominal viscera.\(^ {9,60} \) We hypothesize that Dynactin, Egr-1, Midkine, NF-1, Twinfilin, V-myc, and Yin Yang 1 can contribute to this effect thus mentioned in following paragraphs with possible mechanisms.

Dynactin along with its partner motor protein dynein is an integral component of cytoskeletal machinery that controls organelle movement during cell division. Dynactin has a central alpha helix with adjacent peptides that interact with dynein and other proteins. Dynactin is an ABS constituent; given its filamentous structure and capacity for integrating with other structures in vivo, it may contribute to the stimulatory effect of ABS on tissue healing.\(^ {61,62} \) Inflammation is an integral aspect of tissue repair. Early growth factor (Egr)-1 is a transcription factor and ubiquitous growth factor present in ABS that has an important role in cell proliferation and differentiation. Increasing Egr-1 transcriptional activity may promote tissue regeneration.\(^ {63,64} \)

Midkine, the heparin-binding protein described above, is known to stimulate the growth of and provide protection to tissue,\(^ {65} \) but also regulates immunity and inflammation. Midkine promotes endothelial cell proliferation and angiogenesis and has been implicated in the pathogenesis of various diseases.\(^ {66} \)

Neurofibromin (NF)-1 is a tumor suppressor whose mutation is linked to neurofibromatosis type 1 and juvenile myelomonocytic leukemia. It is a Ras GTPase-activating protein that inhibits Ras and regulates the growth and differentiation of keratinocytes; it is overexpressed in the human epidermis during tissue repair.\(^ {67,68} \)

Twinfilin is an actin monomer-binding protein that stabilizes and facilitates the function of actin fibrils.\(^ {69} \) It is highly conserved between yeasts, humans, and other mammals. Twinfilin in ABS may interact with extracellular actin to form a protein scaffold that facilitates tissue repair.\(^ {70} \)

V-myc avian myelocytomatosis viral oncogene homolog (c-myc) and its product nuclear c-Myc phosphoprotein play a key role in cell cycle progression and cell growth and transformation. Although c-myc is a known oncogenic protein, its
transcription is upregulated during wound healing; as such, it may be another ABS component that contributes to tissue repair.

Yin Yang 1 is a ubiquitous and highly conserved transcription factor enriched in ABS that is known to promote cell differentiation, proliferation, and growth, especially in the central nervous system, and protect against apoptosis.

**Conclusion**

The aim of this review is to constitute a framework for future research about pleiotropic effects of ABS. It is composed of hypotheses of Ankaferd ingredients’ individual contributions to pleiotropic effects. There are some limitations of this review. The hypotheses are constructed regarding the molecules’ actions in literature. First, these effects may not be reproduced in vivo and within the environment that the drug acts. Discrete studies on each ingredient is needed to confirm isolated effects of these molecules. Second, there may be some alternative explanations for these effects including contribution of other molecules or divergent effects of known molecules. Third, the process for each pleiotropic action needs to be explained more precisely with more studies to enable better assumptions and a more straightforward research tract about underlying molecular processes.

Ankaferd is an effective hemostatic agent that has been shown to be effective in suppressing gastrointestinal, dental, urologic, oropharyngeal, thoracic, and dural bleeding in clinical trials. Animal studies and case reports have demonstrated the pleiotropic effects of Ankaferd that may have therapeutic benefits, although there have been no controlled clinical trials evaluating this in humans. We hypothesized that specific factors in ABS contribute to this pleiotropism (Table 2). Additional studies investigating these individual components can broaden the therapeutic potential and applicability of ABS.

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**References**

1. Ugur A, Sarac N, Cankal DA, et al. The antioxidant and antimutagenic activities of Ankaferd blood stopper, a natural hemostatic agent used in dentistry. *Turkish J Med Sci* 2016; 46: 657–663.
2. Beyazit Y, Kurt M, Kekilli M, et al. Evaluation of hemostatic effects of Ankaferd as an alternative medicine. *Altern Med Rev* 2010; 15: 329–336.
3. Ibis M, Kurt M, Oral IK, et al. Successful management of bleeding due to solitary rectal ulcer via topical application of Ankaferd blood stopper. *J Altern Complement Med* 2008; 14: 1073–1074.
4. Tuncer I, Doganay L and Ozturk O. Instant control of fundal variceal bleeding with a folkloric medicinal plant extract: Ankaferd blood stopper. *Gastrointest Endosc* 2010; 71: 873–875.
5. Turhan N, Kurt M, Shorbaji A, et al. Topical Ankaferd blood stopper administration to bleeding gastrointestinal carcinomas decreases tumor vascularization. *Am J Gastroenterol* 2009; 104: 2874–2877.
6. Uzun O, Erkan L and Haznedaroglu IC. Effective management of hemoptysis via endobronchial application of Ankaferd hemostat. *Archiv Bronconeumol* 2014; 50: 407–409.
7. Leblebisatan G, Bay A, Karakus SC, et al. Topical Ankaferd hemostat application for the management of oral cavity bleedings in children with hemorrhagic diathesis. *Blood Coagul Fibrin* 2012; 23: 494–497.
8. Turgut M, Tutkun F, Celebi N, et al. Topical Ankaferd blood stopper in the management of critical bleedings due to hemorrhagic diathesis. *Int J Hematol Oncol* 2011; 21: 160–165.
9. Atay MH, Arslan NA, Akitimur S, et al. Safety and efficacy of Ankaferd hemostat (ABS) in the chemotherapy-induced oral mucositis. *Int J Hematol Oncol* 2015; 25: 166–171.
10. Al B, Yidirim C, Cavdar M, et al. Effectiveness of Ankaferd blood stopper in the topical control of active bleeding due to cutaneous-subcutaneous incisions. *Saudi Med J* 2009; 30: 1520–1525.
11. Ergenoglu MU, Yerebakan H and Kucukaksa DS. A new practical alternative for the control of sternal bleeding during cardiac surgery: Ankaferd blood stopper. *Heart Surg Forum* 2010; 13: E379–E380.
12. Kurunlu SF, Sari H and Ozturk VO. Investigation of hemostatic effects of Ankaferd blood stopper during periodontal surgery on antithrombotic conditioned rats. *J Anim Vet Adv* 2013; 12: 547–549.
13. Karaman K, Bostanci EB, Ercan M, et al. Topical Ankaferd application to presacral bleeding due to total mesorectal excision in rectal carcinoma. *J Invest Surg* 2010; 23: 175.
14. Hur M, Akgul T, Ayyildiz A, et al. Hemostasis in retropubic radical prostatectomy with Ankaferd bloodstopper (R): a case report. *Kaohsiung J Med Sci* 2009; 25: 445–447.
15. Yaman E, Görken F, Pinar Erdem A, et al. Effects of folk medicinal plant extract Ankaferd Blood stopper® in vital primary molar pulpotomy. *Eur Arch Paediatr Dentist* 2012; 13: 197–202.
16. Iynen I, Bozkus F, San I, et al. The hemostatic efficacy of Ankaferd blood stopper in adenoidectomy. *Int J Pediatr Otorhinolaryngol* 2011; 75: 1292–1295.
17. Teker AM, Korkut AY, Gedikli O, et al. Prospective, controlled clinical trial of Ankaferd blood stopper in children undergoing tonsillectomy. *Int J Pediatr Otorhinolaryngol* 2009; 73: 1742–1745.
18. Guler M, Maralcan G, Kul S, et al. The efficacy of Ankaferd blood stopper for the management of bleeding following total thyroidectomy. *J Invest Surg* 2011; 24: 205–210.
19. Akpinar MB, Atalay A, Atalay H, et al. Ankaferd blood stopper decreases postoperative bleeding and number of transfusions in patients treated with clopidogrel: a double-blind, placebo-controlled, randomized clinical trial. *Heart Surg Forum* 2015; 18: E118–E123.

20. Pamuk F, Cetinkaya BO, Keles GC, et al. Ankaferd blood stopper enhances healing after osseous grafting in patients with intrabony periodontal defects. *J Periodontal Res* 2016; 51: 540–547.

21. Istanbulluoglu MO, Kaynar M, Cicek T, et al. A new hemostatic agent (Ankaferd Blood Stopper (R)) in tubeless percutaneous nephrolithotomy: a prospective randomized study. *J Enduraol* 2013; 27: 1126–1130.

22. Amer MZ, Mourad SI, Salem AS, et al. Correlation between international normalized ratio values and sufficiency of two different local hemostatic measures in anticoagulated patients. *Eur J Dent* 2014; 8: 475–480.

23. Atalay H, Atalay H and Dogan Omer FA. Local use of Ankaferd blood clotter in emergent beating heart coronary artery bypass grafting. *Open Cardiovasc Med J* 2015; 9: 18–25.

24. Yasar H and Ozkul H. Haemostatic effect of Ankaferd blood stopper (R) seen during adenoidectomy. *Afr J Tradit Complement Med* 2011; 8: 444–446.

25. Eyi Yapar EG, Engin Ustun Y and Kaba MML. Ankaferd blood stopper in episiotomy repair. *Clin Exp Obstet Gynecol* 2013; 40: 141–143.

26. Eshik M. Safety and efficacy of Ankaferd blood stopper on oral and periodontal surgery. In: 34th Turkish national hematology conference, Turkish Association of Hematology, Izmir, Turkey, 8-11 November 2008.

27. Isler SC, Demircan S, Cakarer S, et al. Effects of folk medicinal plant extract Ankaferd Blood Stopper (R) on early bone healing. *J Appl Oral Sci* 2010; 18: 409–414.

28. Kocak E, Akbal E, Tas A, et al. Anti-inflammatory efficiency of Ankaferd blood stopper in experimental distal colitis model. *Saud J Gastroenterol* 2013; 19: 126–130.

29. Akalin C, Kuru S, Barlas AM, et al. Beneficial effects of Ankaferd blood stopper on dural wound healing: an experimental study. *Int Wound J* 2014; 11: 64–68.

30. Cancan G, Teksoz S, Aytaç E, et al. Effects of Ankaferd on anastomotic healing of colon. *J Invest Surg* 2014; 27: 1–6.

31. Ciftci S, Keskin F, Ozcan SK, et al. In vitro antifungal activity of Ankaferd blood stopper against Candida albicans. *Curr Therap Res: Clin Exp* 2011; 72: 120–126.

32. Fisgin NT, Cayci YT, Coban AY, et al. Antimicrobial activity of plant extract Ankaferd blood stopper (R). *Fitoterapia* 2009; 80: 48–50.

33. Metin B, Yilmaz N, Beyhan YE, et al. In vitro efficacy of the Ankaferd galenic hemostatic extract as a germicidal agent. *Ir J Parasitology* 2016; 11: 406–410.

34. Deveci A, Coban AY, Tanriverdi Cayci Y, et al. In vitro effect of Ankaferd blood stopper (R), a plant extract against mycobacterium tuberculosis isolates. *Mikrobiyal Balten* 2013; 47: 71–78.

35. Akalin I, Okur FV, Haznedaroglu IC, et al. Acute in vitro effects of ABS (Ankaferd Hemostat) on the lymphoid neoplastic cells (B-CLL and RAJI Tumor Cell Lines). *Int J Hematol Oncol* 2014; 24: 253–259.

36. Albayrak M, Aksu S, Celebi H, et al. Striking promotion of the in vitro myeloma monoclonal immunoglobulin aggregation by Ankaferd hemostat. *Int J Hematol Oncol* 2012; 22: 15–22.

37. Avcu F, Guner M, Misirci M, et al. Evaluation of anti-neoplastic effects of a new hemostatic agent Ankaferd blood stopper on myeloma cell line and plasmacytoma development in Balb/c mice: results of the first in vitro and in vivo study. *Blood* 2014; 124: 5728–5728.

38. Ozel Demiralp D, Haznedaroglu IC and Akar N. Functional proteomic analysis of Ankaferd(R) Blood Stopper. *Turk J Hematol* 2010; 27: 70–77.

39. Yilmaz E, Guleç Ş, Torun D, et al. The effects of Ankaferd® blood stopper on transcription factors in HUVEC and the erythrocyte protein profile. *Turk J Hematol* 2011; 28: 276–285.

40. Akkok N, Akcelik M, Haznedaroglu IC, et al. In vitro anti-bacterial activities of Ankaferd medicinal plant extract. *Turkiye Klinikleri J Med Sci* 2009; 29: 410–415.

41. Koluman A, Akar N and Haznedaroglu IC. Antibacterial activities of Ankaferd hemostat (ABS) on “Shiga toxin producing Escherichia coli” and other pathogens significant in Foodborne diseases. *Turk J Haematol* 2017; 34(1): 93–98.

42. Saribas Z, Sener B, Haznedaroglu IC, et al. Antimicrobial activity of Ankaferd blood stopper AR (R) against nosocomial bacterial pathogens. *Central Eur J Med* 2010; 5: 198–202.

43. Koluman A, Akar N, Malkan UY, et al. Qualitative/chemical analyses of Ankaferd hemostat and its antioxidant content in synthetic gastric fluids. *Biomed Res Int* 2016; 2016: 8957820.

44. Mihmanli A, Ulker Z, Alpsoy L, et al. Evaluation of cytotoxicity of a new hemostatic agent Ankaferd blood stopper (R) using different assays. *Hum Exp Toxicol* 2012; 31: 780–787.

45. Lopez-Mateo I, Villaronga MA, Llanos S, et al. The transcription factor CREBFZ is a novel positive regulator of p53. *Cell Cycle* 2012; 11: 3887–3895.

46. Zhang R, Rapin N, Ying Z, et al. Zhang/fe/CREB-ZF—a potential regulator of the unfolded protein response. *PLoS ONE* 2013; 8: e77256.

47. Walesky C and Apte U. Role of hepatocyte nuclear factor 4alpha (HNF4alpha) in cell proliferation and cancer. *Gene Expr* 2015; 16: 101–108.

48. Zheng FJ, Ye HB, Wu MS, et al. Repressing malic enzyme 1 redirects glucose metabolism, unbalances the redox state, and attenuates migratory and invasive abilities in nasopharyngeal carcinoma cell lines. *Chin J Cancer* 2012; 31: 519–531.

49. Wen D, Liu D, Tang J, et al. Malic enzyme 1 induces epithelial-mesenchymal transition and indicates poor prognosis in hepatocellular carcinoma. *Tumour Biol* 2015; 36: 6211–6221.

50. van der Horst EH, Frank BT, Chinn L, et al. The growth factor Midkine antagonizes VEGF signaling in vitro and in vivo. *PLoS ONE* 2008; 10: 340–343.

51. Huang H, Shen J, Min L, et al. Inhibitory effect of midkine-binding peptide on tumor proliferation and migration. *International Journal of Clinical and Experimental Pathology* 2015; 8: 5387–5394.

52. Zheng Y, Zhang LP, Jia XQ, et al. Interaction of protein inhibitor of activated STAT 2 (PIAS2) with receptor of activated C kinase 1, RACK1. *Febs Lett* 2012; 586: 122–126.

53. O’Shea JJ, Schwartz DM, Villarino AV, et al. The JAK-STAT pathway: impact on human disease and therapeutic intervention. *Annu Rev Med* 2015; 66: 311–328.
54. Eguchi T, Itadani H, Shimomura T, et al. Expression levels of p18(INK4C) modify the cellular efficacy of cyclin-dependent kinase inhibitors via regulation of McI-1 expression in tumor cell lines. Mol Cancer Therap 2009; 8: 1460–1472.

55. Matsuzaki Y, Takaoka Y, Hitomi T, et al. Activation of protein kinase C promotes human cancer cell growth through downregulation of p18(INK4c). Oncogene 2004; 23: 5409–5414.

56. Gagrica S, Brookes S, Anderton E, et al. Contrasting behavior of the p18(INK4c) and p16(INK4a) tumor suppressors in both replicative and oncogene-induced senescence. Cancer Res 2012; 72: 165–175.

57. Ozaslan E, Purnak T, Yildiz A, et al. The effect of Ankaferd blood stopper on severe radiation colitis. Endoscopy 2009; 41: E321–E322.

58. Uygun M, Yavuz OY, Uras I, et al. Effect of Ankaferd blood stopper on muscle healing. Clin Invest Med 2016; 39: S187–S191.

59. Evren C, Ugur MB, Yildirim B, et al. Unpredicted effects of Ankaferd (R) on cartilage tissue. Int J Clin Exp Med 2015; 8: 922–927.

60. Ozturk O, Koklu S, Basar O, et al. Severe radiation esophagitis successfully treated with Ankaferd hemostat. Gastrointest Endosc 2015; 81: 1048–1049.

61. Yeh TY, Kowalska AK, Scipioni BR, et al. Dynactin helps target Polo-like kinase 1 to kinetochores via its lefthanded beta-helical p27 subunit. EMBO J 2013; 32: 1023–1035.

62. Urminavicis L, Zhang K, Diamant AG, et al. The structure of the dynactin complex and its interaction with dynnein. Science 2015; 347: 1441–1446.

63. Sassa Y, Hata Y, Murata T, et al. Functional role of Egr-1 mediating VEGF-induced tissue factor expression in the retinal capillary endothelium. Graefes Arch Clin Exp Ophthalmol 2002; 240: 1003–1010.

64. Cho SJ, Kang MJ, Homer RJ, et al. Role of early growth response-1 (Egr-1) in interleukin-13-induced inflammation and remodeling. J Biol Chem 2006; 281: 8161–8168.

65. Kadomatsu K, Kishida S and Tsubota S. The heparin-binding growth factor midkine: the biological activities and candidate receptors. J Biochem 2013; 153: 511–521.

66. Muramatsu T and Kadomatsu K. Midkine: an emerging target of drug development for treatment of multiple diseases. Br J Pharmacol 2014; 171: 811–813.

67. Koivunen J, Karvonen SL, Yla-Outinen H, et al. NF1 tumor suppressor in epidermal wound healing with special focus on wound healing in patients with type 1 neurofibromatosis. Arch Dermatol Res 2005; 296: 547–554.

68. Atit RP, Crowe MJ, Greenhalgh DG, et al. The NF1 tumor suppressor regulates mouse skin wound healing, fibroblast proliferation, and collagen deposited by fibroblasts. J Invest Dermatol 1999; 112: 835–842.

69. Palmgren S, Vartiainen M and Lappalainen P. Twinfilin, a molecular mailman for actin monomers. J Cell Science 2002; 115: 881–886.

70. Sudakov NP, Klimenkov IV, Byvaltsev VA, et al. Extracellular actin in health and disease. Biochemistry 2017; 82: 1–12.

71. Shi Y, Shu B, Yang R, et al. Wnt and Notch signaling pathway involved in wound healing by targeting c-Myc and Hes1 separately. Stem Cell Res Ther 2015; 6: 120.

72. Ye Y and Casaccia-Bonnefil P. The Yin and Yang of YY1 in the nervous system. J Neurochem 2008; 106: 1493–1502.