INTRODUCTION

Many Chinese medicinal herbs contain substances that promote hemostasis and when administered orally or externally, can help to support the process through which bleeding stops. The hemostatic components and mechanisms of action in some of these herbs have been investigated using the mouse bleeding model [1-5]. Hemostyptics are specific hemostatic agents that retard or stop bleeding by causing blood vessels to contract or by accelerating blood clotting when externally applied [3,6,7]. The hemostatic activities of herbal hemostyptics are often due to mechanisms such as tannin astringency [8]. Some hemostatic herbs can shorten bleeding and blood coagulation times [9], thus preventing bleeding from fragile capillaries, and they can also inhibit infection and inflammation that leads to vessel leakage and damage. We previously showed that Cat-tail pollen (Pollen Typhae), a traditional Chinese medicinal herb, has hemostytic effects in vitro and in the mouse bleeding model in vivo [10]. That study also showed that the hemostytic properties of externally applied Cat-tail pollen were attributable to the activation of intrinsic coagulation [10]. Acidic polysaccharide in pollen extract directly activates factor XII in the intrinsic coagulation cascade [10]. The present study searched for novel hemostyptics by assessing ability of 114 Chinese herbal extracts to enhance coagulation activity in vitro.

MATERIALS AND METHODS

Materials

Extracts from 114 Chinese medical herbs were supplied by the Cooperative Research Project at the Joint Usage/Research Center (Joint Usage/Research Center for Science-Based Natural Medicine), Institute of Natural Medicine, University of Toyama [Table 1]. Human factor XII was purchased from Hematologic Technologies Inc. (Essex Junction, Vermont, USA). Pooled normal human plasma was obtained from Axis-Shield (Oslo, Norway). The coagulation assay reagent, Thromborel S was purchased from Dade Behring (Marburg, Germany) and Sysmex (Kobe, Japan), respectively.

Preparation of Extract

Extracts were prepared by boiling 45 g of each herb in 900 mL of water for 30 min and then passed through a cotton plug.
Filtrates were lyophilized and dissolved in water to a final concentration of 10 mg/mL.

**Coagulation Assays**

Coagulation was assayed as prothrombin time (PT). Plasma diluted five-fold with isotonic sodium chloride solution (100 μL) was incubated with extracts (50 μL) for 5 min. The PT reagent (50 μL), was added and then coagulation was started by adding 25 mmol/L CaCl₂. The time required to form clots was measured using a KC4A coagulometer (Amelung, Lemgo, Germany). The plasma coagulation time of the control was about 350 s. Extracts that shortened coagulation time were regarded as containing...
active compounds. Extracts that shorted coagulation time to <230 s were selected. However, some herb extracts that were very turbid or contained precipitates were not further investigated.

**Factor XII Activation Assay**

Factor XII activation was assayed [6]. A volume of 70 μL of purified human factor XII (0.14 mg/mL) and 70 μL of extracts (10 mg/mL) were incubated for 5 h and then the mixtures were resolved by SDS-PAGE and stained with silver.

**RESULTS**

We determined the potentiating effect of herbal extracts on the coagulation time of the extrinsic pathway to assess hemostatic activity in vitro. Trace amounts of PT reagent were used to start the reaction. A shortened coagulation time was taken to indicate hemostatic activity. Extracts of Alpinia Rhizome, Areca, Artemisia Leaf, Cassia Bark, Danshen Root, Ephedra Herb, Epimedium Herb, Forsythia Fruit, Great Burdock Achene, Moutan Bark, Perilla Herb, Red Paeony Root, Schizonepeta Spike, Senticosus Rhizome, Sweet Annie,
Uncaria Thorn and Zanthoxyllum Peel shortened coagulation time [Figure 1]. We then assessed the ability of these extracts to activate factor XII. Purified human factor XII was incubated with the extracts for 5 h at 37°C and then the presence of Factor XIIa was assessed. Proteins in the mixture were resolved by SDS-PAGE and stained with silver. The light and heavy chains of factor XII were detected after adding extracts of Cat-tail pollen, Artemisia Leaf and Great Burdock Achene. A slight amount of light chain was generated by extracts of Perilla Herb [Figure 2].

**DISCUSSION**

The hemostatic properties of Cat-tail Pollen have been attributed to the activation of intrinsic coagulation [10,11]. Acidic polysaccharide in extracts of Cat-tail pollen directly activate factor XII in the coagulation cascade [10,11]. However, whether other hemostatic herbs such as Artemisia Leaf can activate intrinsic coagulation has remained unknown and other medicinal herbs might have as yet undiscovered hemostatic activity. Therefore, we screened active substances in 117 extracts of Chinese herbs by measuring the ability to enhance the extrinsic coagulation reaction. Extracts of Alpinia Rhizome, Areca, Artemisia Leaf, Cassia Bark, Danshen Root, Ephedra Herb, Epimedium Herb, Forsythia Fruit, Great Burdock Achene, Moutan Bark, Perilla Herb, Red Paeony Root, Schizonepeta Spike, Senticosus Rhizome, Sweet Annie, Uncaria Thorn and Zanthoxyllum Peel shortened coagulation times. The ability of these extracts to activate factor XII was assessed by incubation for 1 h at 37°C, followed by resolving the reaction products by SDS-PAGE. Figure 2 shows that extracts of Artemisia Leaf and Great Burdock Achene cleaved factor XII to the light and heavy chains of factor XIIa, namely the active form of factor XII. Artemisia Leaf is a known antipyretic, insecticide, diuretic, and hemostatic agent [12]. Kneaded leaves of Artemisia Leaf have been used to treat excoriations or cuts on the skin surface. The hemostatic activity is thought to be a result of tannin astringency because mugwort contain high levels of tannin [8]. The present study found that Artemisia Leaf directly activates Factor XII. Hayakawa et al. reported that extracts of Artemisia Leaf leaves contain sulfated polysaccharide [13], and this probably induced the activation. However, the contribution of factor XII activation to the hemostatic activity of Artemisia Leaf is not clear. Great Burdock Achene has traditionally been used in China as an anti-inflammatory, detoxifying, or diuretic agent, and to dispel pathogenic wind-heat, promote eruption and remove toxic substances such as heavy metals [13]. The major bioactive principles in Great Burdock Achene are phenolic compounds such as lignans that have various biological properties in vitro and in vivo such as anti-cancer, antioxidant, antibacterial, antiviral anti-inflammatory and immunosuppressive activities [14-16]. However, whether Great Burdock Achene has hemostatic activity and effects on blood coagulation had not been reported. The present results showed that extracts of Great Burdock Achene and of Cat-tail pollen have essentially identical ability to activate factor XII. Negatively-charged compounds such as acidic polysaccharides in burdock fruit might contribute to the activation of factor XII. Sweet Annie and Artemisia Leaf both belong to the genus *Artemisia*. Sweet Annie (*Artemisia annua*) is a renowned antimalarial [17] and its effect on hemostasis has already been established in traditional Chinese medicine [18]. Wang et al. screened the hemostatic active fraction of sweet Annie using assays of plasma recalcification times and found an active fraction [19]. However, the mechanism involved in the reduction of coagulation time was not clear. Sweet Annie did not activate factor XII in the present study. Schizonepeta spike (*Schizonepeta tenuifolia*) exerts hemostatic action by promoting coagulation and inhibiting fibrinolysis [20]. Here, Schizonepeta spike shortened PT, but the activity was not dependent on factor XII activation. Extracts of Red Paeony Root also shortened blood coagulation time in the present study, which contradicted the findings of Wang and Ma, who showed that an extract of Red Paeony Root prolonged coagulation time [21]. We did not detect factor XII activation by other herbs that shorten coagulation time. These extracts might activate coagulation factors downstream of the intrinsic coagulation cascade such as factor XI and factor IX or inhibit anticoagulant proteins such as antithrombin. Our screening study showed that some popular Chinese herbs have
potential as hemostatic agents. However, to the best of our knowledge, these herbs have not been used as a treatment to preventing bleeding. The mechanisms through which these herbs shortened blood coagulation time remain also obscure. Nonetheless, these traditional herbs could be applied as a novel clinical approach to the control or prevention of bleeding.

ACKNOWLEDGMENTS

This study was supported by a Grant-in-Aid from the Cooperative Research Project for Joint Usage/Research Center (Joint Usage/Research Center for Science-Based Natural Medicine) Institute of Natural Medicine, University of Toyama in 2010.

REFERENCES

1. Ishida H, Umino T, Tsuji K, Kosuge T. Studies on antihemorrhagic substances in herbs classified as hemostatics in Chinese medicine. VII. On the antihemorrhagic principle in Cirsium japonicum DC. Chem Pharm Bull (Tokyo) 1987;35:861-4.
2. Kosuge T, Ishida H, Satoh T. Studies on antihemorrhagic substances in herbs classified as hemostatics in Chinese medicine. V. On antihemorrhagic principle in Biota orientalis (L.) Endl. Chem Pharm Bull (Tokyo) 1985;33:206-9.
3. Ishida H, Umino T, Tsuji K, Kosuge T. Studies on the antihemorrhagic substances in herbs classified as hemostatics in Chinese medicine. IX. On the antihemorrhagic principles in Typha lactiflora L. Chem Pharm Bull (Tokyo) 1988;36:4414-20.
4. Ishida H, Umino T, Tsuji K, Kosuge T. Studies on the antihemorrhagic substances in herbs classified as hemostatics in Chinese medicine. VIII. On the antihemorrhagic principle in nelumbins receptaculum. Chem Pharm Bull (Tokyo) 1988;36:4585-7.
5. Ishida H, Umino T, Tsuji K, Kosuge T. Studies on the antihemorrhagic substances in herbs classified as hemostatics in traditional Chinese medicine. I. On the antihemorrhagic principles in Sophora japonica L. Chem Pharm Bull (Tokyo) 1989;37:1616-8.
6. Sadiq A, Hayat MQ, Ashraf M. Ethnopharmacology of Artemisia annua L.: A review. In Artemisia Annua-Pharmacology and Biotechnology, Berlin, Heidelberg: Springer; 2014. p. 9-26.
7. Isler SC, Demircan S, Cakarer S, Cebi Z, Keskin C, Soluk M, et al. Effects of folk medicinal plant extract Ankaferd Blood Stopper on early bone healing. J Appl Oral Sci 2010;18:409-14.
8. Kimura Y, Okuda H, Okuda T, Hatano T, Agata I, Arichi S. Studies on the activities of tannins and related compounds from medicinal plants and drugs. VII. Effects of extracts of leaves of Artemisia species, and caffeic acid and chlorogenic acid on lipid metabolic injury in rats fed peroxidized oil. Chem Pharm Bull (Tokyo) 1986;33:2028-34.
9. Cordier W, Steenkamp V. Herbal remedies affecting coagulation: A review. Pharm Biol 2012;50:443-52.
10. Ohkura N, Tamura K, Tanaka A, Matsuda J, Atsumi G. Experimental study on the hemostatic activity of Pollen Typhae: A traditional folk medicine used by external and oral application. Blood Coagul Fibrinolysis 2011;22:631-6.
11. Ohkura N, Tauchi C, Nakayama A, Atsumi G. Pollen Typhae is a rapid hemostytic. Blood Coagul Fibrinolysis 2012;23:254-5.
12. Ferreira JF, Luthria DL, Sasaki T, Heyerick A. Flavonoids from Artemisia annua L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. Molecules 2010 29;15:3135-70.
13. Chan YS, Cheng LN, Wu JH, Chan E, Kwan YY, Lee SM, et al. A review of the pharmacological effects of Arctium lappa (burdock). Inflammopharmacology 2011;19:245-54.
14. Awale S, Lu J, Kalauni SK, Kurashima Y, Tezuka Y, Kadota S, et al. Identification of arctigenin as an antitumor agent having the ability to eliminate the tolerance of cancer cells to nutrient starvation. Cancer Res 2006 66;1761-7.
15. Matsumoto T, Hosono-Nishiyama K, Yamada H. Antiproliferative and apoptotic effects of butyrolactone lignans from Arctium lappa on leukemic cells. Planta Med 2006;72:276-8.
16. Park SY, Hong SS, Han XH, Hwang JS, Lee D, Ro JS, et al. Lignans from Arctium lappa and their inhibition of LPS-induced nitric oxide production. Chem Pharm Bull (Tokyo) 2007;55:150-2.
17. Ziffer H, Higetl RJ, Klaman DL. Artemisinin: An endoperoxidic antimalarial from Artemisia annua L. Fortschr Chem Org Naturst 1997;72:121-214.
18. Feng WY. Preliminary exploration of Artemisia annua L. and its praeparatum. Tibetan Med J 1978;2:62-3.
19. Wang B, Sui J, Yu Z, Zhu L. Screening the hemostatic active fraction of Pollen Typhae. Iran J Pharm Res 2011;10:57-62.
20. Ding AW, Wu H, Kong LD, Wang SL, Gao ZZ, Zhao MX, et al. Effect of an extract of Pollen Typhae on the hemostatc activity of extracts from carbonized Schizonepeta tenuifolia Brig. Zhonggou Zhong Yao Za Zhi 1993;18:598-600, 638.
21. Wang Y, Ma R. Effect of an extract of Paeonia lactiflora on the blood coagulative and fibrinolytic enzymes. Zhong Xi Yi Jie He Za Zhi 1990;10:101-2, 70.