

Full Length Research Paper

Effect of *Cinnamomum zeylanicum* essence and distillate on the clotting time

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A plant called medicinal herb is one in which some special materials are constructed and stored in its configuration which are called effective materials. Active materials are made during a chain of special and complex biochemical processes which are known as second metabolites. The scientific name of Cinnamon is *Cinnamomum zeylanicum* from Lauraceaeis. Physiological effect of Cinnamon is related to essence and tannins. Cinnamon reinforces properties of digestion function and blood stream circulation. Cinnamon has one of the highest antioxidant levels of any species. Also, Cinnamon supports sugar metabolism and helps maintain the healthy blood sugar levels as well as contributes to healthy circulation and heart function. The aim of this study was to determine the effect of the effective materials of Cinnamon herbal plants on blood clotting time. Water and methanol extracts, essence and distillate of Cinnamon were provided by Soxhelt and Clevenger devices after collecting. The test of blood coagulation time determination was studied through tube test method with all of the samples. The results indicate that the blood clotting time significantly decreased in the presence of Cinnamon distillate and the essential oil in comparison with control. But Cinnamon water extracts do not have effect on blood coagulation time. There are significant differences between the water extract of cinnamon and its essence at the level of 5% ($P < 0.05$) as well as between the distillate and the water extract significantly up to 1% ($P < 0.01$). Among the existing data in this study, the distillate and essential oil of Cinnamon have coagulation stronger effects than water and hydro alcoholic extract. These may be particularly useful in situations where the wound is not clotting, which can be due to external factors such as the size of wound, or medical factors like hemophilia.

Key words: Essential oil, cinnamon extracts, distillate, blood coagulation, gas chromatography/mass spectrometry (GC/MS).

INTRODUCTION

A plant called medicinal herb is one in which some special materials are constructed and stored in its configuration which are called effective materials. Active substances of medicinal plants have effective physiological effects on the body (Chevallier, 2000; Zargari, 1996). These plants are used for the treatment of some diseases. Bleeding illness is one of the medical

emergency diseases and if the initial measures for patients with late hemorrhagic cannot be done, complications and even death is inevitable (Yagmur et al, 2005; Salawu et al, 2008; Zhang et al, 2009). Our country, due to climatic variation has varying frequency of medicinal plants. Some of the herbal plant was used as a traditional medicine for bleeding illness and to stop bleeding. Studies

were conducted by using Cinnamon plants for detection of its active ingredients and effect on blood clotting time (Bamosa et al 1997; Van Wyk and Wink, 2004).

The scientific name, Cinnamon (*Cinnamomum zeylanicum*) from Lauraceae, is a small tree of height 5 to 7 m and evergreen in all section, with an aromatic odor and a pleasant Cinnamon smell. Cinnamon contains amidoun, mousilag, tannin, calcium oxalate, glucose, manit, cinnamomin, essential oil and resin. Physiological effect of Cinnamon is related to essence and Tannins (Ebadi, 2006; Chevallier, 2000; Aronson, 2008; Wijesekera, 1978). Cinnamon has reinforcing properties of digestion function and blood stream circulation (Pyo et al., 2002). Due to the presence of tannin, it has the ability to stop diarrhea, improve general body weakness and also its consumption helps stop bleeding (Murray et al., 1998; Izzo and Ernest, 2001). It is anti-parasitic and anti bacterial (Van Wyk and Wink, 2004; Zargari, 1996).

Cinnamon has one of the highest antioxidant levels of any known spice. Many people love the distinctive flavor and aroma of cinnamon and this fragrant spice can do much more than one can imagined. Cinnamon could be described as a natural powerhouse that is filled with antioxidants, anti-inflammatory, and blood sugar-lowering abilities. For instance, cinnamon taken from the inner bark of tropical trees is also a powerful antioxidant. Cinnamon is rich in natural compounds known as polyphenols. These compounds act like insulin within the body and can help regulate blood sugar levels as well as contribute to healthy circulation and heart function (Kannappan et al., 2006).

Two cinnamon bark species (*Cinnamomum zeylanicum* and *Cinnamomum cassis*) are tried and true spice (cinnamon bark). Although there is no abundance of scientific information to support the use of cinnamon for any condition, laboratory studies indicate that cinnamon may be useful in treating diabetes (Type 2) because of cinnamon's blood sugar-lowering effects. Additionally, cinnamon and its compounds may have anti-inflammatory, antibacterial, anti-fungal, and antioxidant properties.

These properties have proven to be effective in treating a variety of conditions, such as, cancer or virus infections. The aim of this research is to study the effect of the effective materials of Cinnamon herbal plants on the time of the blood coagulation.

MATERIALS AND METHODS

This study was conducted in Semnan University of Medical sciences, Faculty of Medicine, Biochemistry department, Semnan, Iran. N-propanole, n-hexane, ethyl acetate, toluene, carbon tetrachloride, cyclohexane, chloroform, methanol, silica gel mesh 60 F -254 materials were prepared from Merk Company, Germany.

Plants collection

Bark of Cinnamon was selected to determine anticoagulant activity.

Cinnamon plant was collected from a store of medicinal plants in Tehran, Iran. Sample was identified and confirmed by an expert of Agriculture Research Center of Semnan Province. The Cinnamon bark was dried in the shade before any experimental use.

Extraction

Aqueous extract was prepared from freeze-dried powder of Cinnamon plant. 200 g of plant sample was mixed with 200^{cc} distilled water for 90 min at a moderate temperature. Hydro alcoholic (methyl alcohol) extract of sample was obtained; 50 g from each was extracted with 500^{cc} methanol of 75% by soxhlet. The aqueous and methyl alcohol extracts were concentrated by rotary devices. Grinding the bark parts of plants (200 g) were subjected to hydro-distillation for 3 h using a clevenger-type apparatus to obtain essential oils and herbal distillate. The oils were concentrated by anhydrous sodium sulfate and stored in tightly closed dark vials at 4°C till analysis. The aqueous, hydro alcoholic extracts, essential oil and distillate were subjected to thin layer chromatography (TLC), gas chromatography with mass spectrometry (GC/MS) to determine the active materials and tube test method to assess the clotting time.

Gas chromatography/ mass spectrometry (GC/MS) and TLC analysis

The essential oil was analyzed on an Agilent Technologies 7890A GC system coupled to a 5975C VLMSD mass spectrometer with an injector 7683B series device. A fused silica capillary column DB-5 (30 μ m, 0.25 mm, film thickness 0.25 μ m) and a flame ionization detector (FID) were used for separation. Helium was used as a carrier gas at a flow rate of 1 ml/min. The oven temperature was programmed at 60°C (4 min), and then rised to 300°C at 4°C/min. The injector and detector temperature were kept at 250 and 300°C, respectively. The mass spectrometer was operated in the electron-impact ionization (EI) mode with 70 ev energy with MS transfer line at temperature of 300°C was used. Ion source and interface temperatures were 200 and 250°C, respectively. The split ratio was 1/50. The percentage compositions were obtained from electronic integration measurements using FID set at 250°C. The column was programmed as follows: 60°C for 2 min and then increased by 4°C/min up to 300°C. Volume of injected samples was 0.5 μ l. Qualitative analysis was based on the comparison of retention times and the computer mass spectra libraries using Wiley 275 GC/MS Library (Wiley, New York) and NIST Libraries. The percentage composition was computed from the GC peak areas measurements. TLC analysis was carried out as follows: the concentrated methanolic and aqueous extracts obtained were spotted on pre coated silica gel plates (Merk) and chromatographed in a saturated chamber containing the N-propanole/H₂O (70:30) solvent mixture. Final chromatography was conducted with this solvent for the 6 to 7 h and in two stages. Visualization of separated bands was carried out under ultra violet (UV) light (254 to 360 nm).

Clotting time test

The first step brought a clotting time (CT) test as a pre-test for a number of healthy cases and the ones without any symptoms subject. CT test of those samples were between 4 to 6 min which were considered as normal. 12 samples were tested. For each individual sample, 14 test tubes were prepared, which Tube No. 13 considered as a normal control and Tube No. 14 for negative control by adding ethylenediaminetetraacetic acid (EDTA) anticoagulation agent. Experiment was referred with different values of extracts or essence and distillate (10, 50 and 100 λ). About

Table 1. The chemical composition of Cinnamon essential oil.

Component	Area	Retention time (RT)	Quality
dl-Limonene or l-Limonene	19.62	6.949	99
Benzene,1-methyl-2-(1-methylethyl)	1.07	6.881	97
1,3,6-Octatriene,	1.61	7.035	97
γ -Terpinene	0.71	7.413	97
Bicyclo[2.2.1]heptan-2-one,	4.38	7.916	95
Benzene,	3.29	9.541	98
Fenchyl acetate	3.01	9.879	97
Benzaldehyde	2.18	10.377	98
Trans-Anethole	64.12	10.829	97

Components were identified on the basis of comparison of their relative retention time and mass spectra with those of standards. The components of the oil were identified by matching their spectra and retention time with those of the Wiley 275 library (Wiley, New York) in the computer library.

About 14cc blood sample was taken from each person, divided into each tube, disabled using stopwatch immediately, and were incubated at the 37°C. After passing for 3 min, all samples were checked at an interval of 1 minute and time of coagulation recorded.

Statistical methods

Both factorial test and analysis of variance (ANOVA) were used in the complete random block design of factorial bilinear difference and with studying samples. Significant difference between samples has been calculated toward the observer in surface ($P > 5\%$).

RESULTS

GC/MS and TLC analysis data

TLC results of each sample in solvent H₂O and n-propanole includes the number of 6 bands in methanol and water extract of Cinnamon (data not shown). All data have been collected by three fold repetitions of tests under the same condition. Essence and distillate of Cinnamon samples have the best result on clotting time in comparison with control group and its water extract. Cinnamon essence was subjected with GC/MS system which showed about nine chemical materials (Figure 1). Table 1 shows chemical compounds of Cinnamon essence analyzed with the GC/MS update library.

Study of active ingredients on clotting time

Cinnamon hydro alcoholic extract did not have any notable effect on clotting time. The average of clotting time in 5 sample showed that distillate and essential oil of Cinnamon significantly decreased clotting time compared with the control group but water extract had no effect on clotting time (Figure 2). All data have been collected by five fold repetitions of tests under the same condition.

DISCUSSION

The aim of this study was to determine the effect of active compounds of Cinnamon plant on blood coagulation time. The data showed that all samples of methyl alcohol extracts do not have any effect on reduction of blood clotting time by tube test method. There are significant differences between the water extract of Cinnamon and its essential oil in the level of 5% ($P < 0.05$), as well as between the distillate and the water extract in the level 1% ($P < 0.01$). Cinnamon essential oil and distillate reduced clotting time in comparison with control but the aqueous extract of Cinnamon has not effect on reducing clotting time (Figure 2). We carried out the same experiment previously about *Urtica dioica* and *Artemisia dracuncululus*, and among the existing data in those studies, the distillate and essential oil of Cinnamon have stronger coagulation effects than Tarragon and Nettle. For this purpose, Cinnamon sample was subjected to GC/MS analysis (Figure 1 and Table 1).

In this assessment, the main components of the oils were trans-anethole (64.12%) and limonene (19.62) which may play a major anticoagulant role, while other components were less than 16%. Since the essential oils are mixtures of several compounds, it is difficult to attribute their biological activity to a particular constituent. Usually, major compounds are the ones responsible for the anticoagulant activity of the essential oils. However, some studies showed that minor components may have a crucial role in the biological activity of the oils (Koroch et al., 2007).

The treatment of internal bleeding is beyond the scope of simple first aid, and should be considered by any first aider to be potentially life threatening. The definitive treatment for internal bleeding is always surgical treatment, and medical advice must be sought urgently for any victim of internal bleeding. In the event of the bleeding being caused by an external source (trauma, penetrating wound), the patient is usually inclined to the

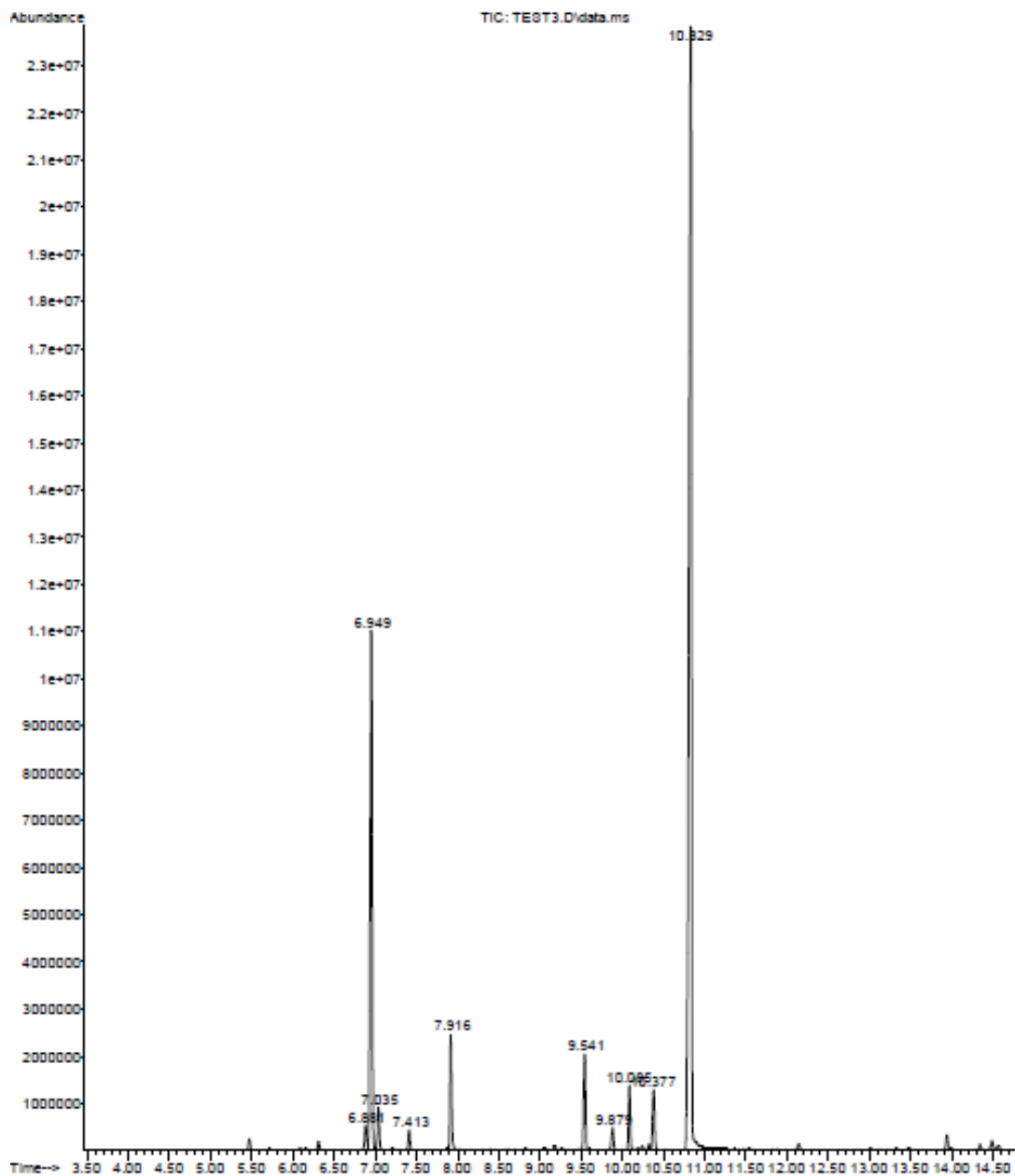


Figure 1. The chemical composition of essential oil isolated from the Cinnamon by hydro-distillation was analyzed by GC/MS, in which a total of 9 components were identified. The major component of this essential oil was: Trans-anethole (64.12%) and dL-limonene or l-limonene (19.62%).

injured side in order to ensure that the 'good' side can continue to function properly without interference from the blood inside the body cavity. These may be particularly useful in situations where the wound is not clotting, which can be due to external factors, such as size of wound, or medical factors; hemophilia.

Emergency bleeding control describes the steps or actions taken to control bleeding from a patient who has suffered a traumatic injury or who has a medical condition which has led to bleeding. In order to manage bleeding effectively, it is important to be able to readily identify

both types of chemical and plants drugs. Further studies are needed to determine the anticoagulant activities of the bioactive compounds responsible for the observed potential value, suggesting that the essential oils of Cinnamon could be a possible source to obtain new and effective herbal medicines to treat bleeding and also the search for novel anticoagulant agents with the potential application of some major or minor constituents alone, mixed of presented essences or in combination with synthetic anticoagulant agent for the treatment and prevention of bleeding. Also, it is necessary carry out

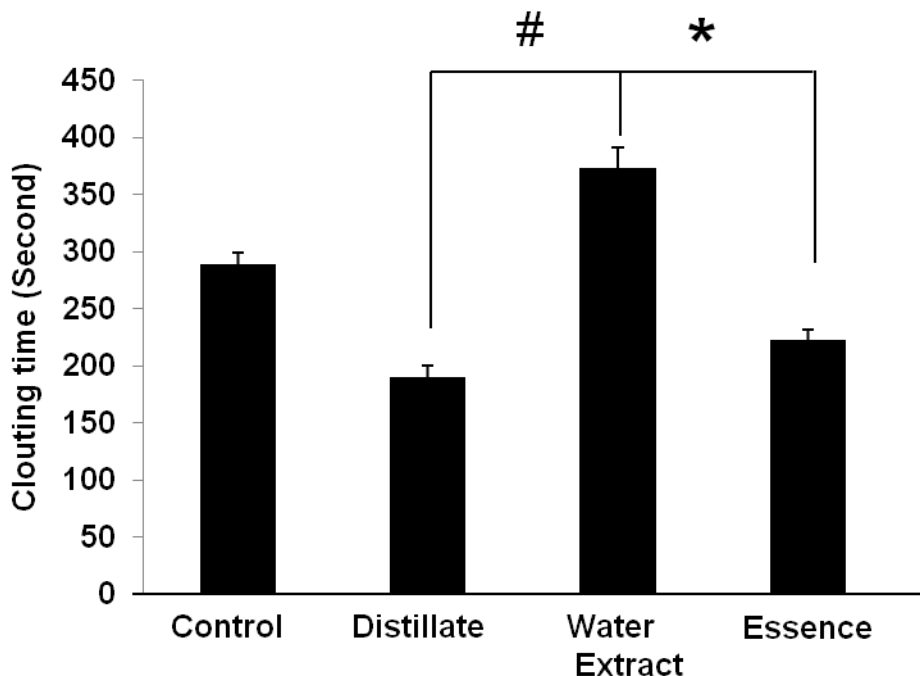


Figure 2. Effect of *Cinnamomum Zeylanicum* chemical material in clotting time. There is significant difference between the water extract of Cinnamon and its essential oil in the level of 5% (* $P < 0.05$), as well as between the distillate and the water extract in the level 1% (# $P < 0.01$). Cinnamon essential oil and distillate reduced clotting time in comparison with control but the aqueous extract of Cinnamon had no effect on reducing clotting time ($P < 0.05$).

more research in future, first to elucidate the mechanisms of Cinnamon essential oils and distillate-induced blood coagulation, and at least make special pad for external bleeding protection in some surgery cases and environmental factors as well as the emergency bleeding situations.

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