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A METHOD FOR INHIBITING CLOT FORMATION

FIELD OF THE INVENTION

The present invention relates generally to a method of retarding, reducing or otherwise inhibiting blood clot formation. More particularly, the present invention retards, reduces or otherwise inhibits blood clot formation by inhibition of serine-protease mediated clotting pathways. The method of the present invention is particularly directed to the use of tannins or their derivatives, homologues or chemical analogues to retard, reduce or otherwise inhibit thrombin-mediation clot formation. The method of the present invention is useful, *inter alia,* in a range of therapeutic and prophylactic applications.

BACKGROUND OF THE INVENTION

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description.

Circulatory disorders caused by blood clots contribute to a high degree of morbidity. Accordingly, there is a need for substantial research and development into the identification of drugs which can prevent the inappropriate formation of blood clots. Despite the presence of some drugs in the market place there is still a need to identify more efficacious drugs for the prevention of blood clotting. There is also a need to understand the blood clotting procedure in more detail in order to enable the rational design of therapeutic agents.

Among the series of events leading to blood coagulation, the formation of the serine-protease thrombin is the most important step since thrombin activates platelets and protein C, converts circulating fibrinogen to fibrin and amplifies its own synthesis by activation of factor V and VIII. The inhibition of thrombin is considered to be of critical importance for control of thrombotic disorders, such as arterial fibrillation, restenosis, arterial thrombosis and myocardial infarction.

The indirect thrombin inhibitors heparin and warfarin are important therapeutic agents in widespread clinic use. However, the limitations of these drugs highlights the importance of developing more effective anticoagulant agents. The direct-acting thrombin inhibitors hirudin, and hirulog have been important and are effective. They can inhibit thrombin bound to clots or extracellular matrices, which are relatively resistant to heparin; they do not require antithrombin III or vitamin K as cofactors, which may lead to a more predictable dose response. Further; they are not inhibited by activated platelets, which release platelet factor 4 and other molecules that neutralized heparin. However, they are currently only used for acute care because of their prohibitive expense.

A safe and effective inhibitor of thrombin would be a useful tool in the treatment of venous thrombosis, arterial fibrillation, restenosis, arterial thrombosis, and in the prevention of myocardial infarction. Because of this, the modulation of thrombin by direct, small molecule inhibitors is a widely sought goal in the development of anticoagulant agents.

The increasing demand for new pharmaceutical agents has led the pharmaceutical industry to consider molecules found in the natural environment. Accordingly, much effort is being spent on screening aquatic environments, riverbeds, coral, plants, microorganisms and higher animals for potentially useful molecules. The screening process is often referred to as "natural product screening".

In work leading up to the present invention the inventors have studied the plant *Geum japonicum* and have identified the presence of tannins which exhibit anticoagulant activities. This has led to the development of a method of reducing retarding or otherwise inhibiting blood clot formation utilising tannins or their derivatives, homologues or chemical analogues.

SUMMARY OF THE INVENTION

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

One aspect of the present invention provides a method of reducing, retarding or otherwise inhibiting blood clot formation in a subject said method comprising administering to said subject an anti-clotting effective amount of a tannin or derivative, homologue or chemical analogue thereof for a time and under conditions sufficient to inhibit blood clot formation.

Another aspect of the present invention provides a method of reducing, retarding or otherwise inhibiting blood clot formation in a subject said method comprising administering to said subject an anti-clotting effective amount of a plant tannin for a time and under conditions sufficient to inhibit blood clot formation.

Yet another aspect of the present invention contemplates a method of reducing, retarding or otherwise inhibiting thrombin functional activity in a subject said method comprising administering to said subject an effective amount of a *Geum japonicum* tannin for a time and under conditions sufficient to inhibit thrombin functional activity.

A further aspect of the present invention provides a method of reducing, retarding or otherwise inhibiting Factor Xa functional activity in a subject said method comprising administering to said subject an effective amount of a *Geum japonicum* tannin for a time and under conditions sufficient to inhibit Factor Xa functional activity.

Still a further aspect of the present invention contemplates a method of inducing or facilitating inhibition of Factor Xa functional activity in a subject said method comprising administering to said subject an effective amount of a *Geum japonicum* tannin or derivative thereof for a time and under conditions sufficient to inhibit factor Xa functional

activity.

The present invention also provides a method of reducing, retarding or otherwise inhibiting blood clot formation in a subject said method comprising administering to said subject an anti-clotting effective amount of a *Geum japonicum* tannin wherein said tannin comprises a substituent on a phenolic moiety which is capable of forming a bond or other association with an amino acid or side chain thereof in the active site of a serine protease.

Another aspect of the present invention contemplates a method of reducing, retarding or otherwise inhibiting blood clot formation in a subject said method comprising administering to said subject an anti-clotting effective amount of penta-*O*-galloyl-*β*-D-glucose for a time and under condition sufficient to inhibit blood clot formation.

Still yet another aspect of the present invention provides a method of reducing, retarding or otherwise inhibiting blood clot formation in a subject said method comprising administering to said subject an anti-clotting effective amount of pedunculagin for a time and under condition sufficient to inhibit blood clot formation.

A further aspect of the present invention provides a method of reducing, retarding or otherwise inhibiting blood clot formation in a subject said method comprising administering to said subject an anti-clotting effective amount of tellimagrandin II for a time and under condition sufficient to inhibit blood clot formation.

Still a further aspect of the present invention provides a method of reducing, retarding or otherwise inhibiting blood clot formation in a subject said method comprising administering to said subject an anti-clotting effective amount of casuariin for a time and under condition sufficient to inhibit blood clot formation.

In yet another further aspect of the present invention provides a method of reducing, retarding or otherwise inhibiting blood clot formation in a subject said method comprising

administering to said subject an anti-clotting effective amount of 5-desgalloylstachyurin for a time and under condition sufficient to inhibit blood clot formation.

Yet another aspect of the present invention contemplates a method for the treatment or prophylaxis of a disease condition involving clotting in a vascular system in a subject such as but not limited to a thrombic disorder or a stroke, said method comprising administering to said subject a blood clotting inhibition effective amount of a tannin.

Another aspect of the present invention provides a method for the treatment or prophylaxis of a disease condition involving clotting in the vascular system in a subject such as but not limited to a thrombic disorder, said method comprising administering to said subject a thrombin inhibitory effective amount of a tannin.

Yet another aspect of the present invention provides a composition for use in reducing, retarding or otherwise inhibiting blood clot formation comprising a tannin as hereinbefore defined and one or more pharmaceutically acceptable carriers and/or diluents. The composition may also comprise two different types of tannins or a tannin and a known anti-clotting compound or molecule.

Still yet another aspect of the present invention is directed to antibodies to the subject tannins and their derivatives, homologues, analogues and mimetics.

The present invention further extends to the use of the subject tannins in the manufacture of a medicament for inducing or facilitating inhibition of blood clotting.

Yet another aspect of the present invention provides an agent useful for inducing or facilitating inhibition of blood clotting comprising a tannin as hereinbefore defined.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graphical representation of the effect of **1** on Prothrombin Time (PT, ○), Thrombin Time (TT, ■), and Activated Partial Thromboplastin Time (APTT, □) clotting assays. Each point is reported as means (seconds) ± SD (n=3).

Figure 2 is a Lineweaver-Burk graphical representation of the effect of **2** at concentrations of 0.18 μM (□), 0.35 μM (○), and control (●) for bovine thrombin.

Figure 3 is a Lineweaver-Burk graphical representation of the effect of **1** at concentrations of 6.6 μM (□), 13.2 μM (■), and control (●) for bovine thrombin.

Figure 4 is a graphical representation of the effect of **1** (○), **2** (●), **4** (△), **6** (□), and **7** (■) on the inhibitory activity of thrombin-induced fibrin-clotting. Points shown are means of triplicate ± standard derivation.

Figure 5 is a Lineweaver-Burk graphical representation of the effect of **1** (1.85 μM, □), **2** (0.45 μM, ○), **4** (0.17 μM, ■), **6** (0.99 μM, △), **7** (1.09 μM, ▲), and control (●) for human factor Xa.

Figure 6 is a schematic representation of the interaction of tannins with thrombin (A) and factor Xa (B). Compound **2** binds only to the active site of thrombin, whereas compounds **1**, **4**, **6**, and **7** bind to both active site and ABE (A). On the other hand, all the compounds bind only to the active site of factor Xa (B).

Figure 7 is a schematic representation of the chemical structure of pedunculagin.

Figure 8 is a schematic representation of the chemical structure of penta-O-galloyl-β-glucoside.

Figure 9 is a schematic representation of the chemical structure of tellimagrandin II.

Figure 10 is a schematic representation of the chemical structure of casuariin.

Figure 11 is a schematic representation of the chemical structure of 5-desgalloylstachyurin.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is predicated in part on the identification of plant derived tannins which reduce, retard or otherwise inhibit serine protease mediated clotting pathways. The identification of these tannins now permits their use as anti-clotting agents for therapy and prophylaxis.

Accordingly, one aspect of the present invention provides a method of reducing, retarding or otherwise inhibiting blood clot formation in a subject said method comprising administering to said subject an anti-clotting effective amount of a tannin or derivative, homologue or chemical analogue thereof for a time and under conditions sufficient to inhibit blood clot formation.

The present invention is exemplified with respect to tannins isolated from the plant *Geum japonicum*. However, the present invention extends to any tannin regardless of its source as well as derivatives, homologues and chemical analogues thereof. For brevity, reference hereinafter to "tannins" includes reference to its derivatives, homologues and chemical analogues.

Accordingly, the present invention provides a method of reducing, retarding or otherwise inhibiting blood clot formation in a subject said method comprising administering to said subject an anti-clotting effective amount of a plant tannin for a time and under conditions sufficient to inhibit blood clot formation.

Preferably said plant is *Geum japonicum*.

According to this preferred embodiment, the present invention provides a method of reducing, retarding or otherwise inhibiting blood clot formation in a subject said method comprising administering to said subject an anti-clotting effective amount of a *Geum japonicum* tannin for a time and under conditions sufficient to inhibit blood clot formation.

Derivatives of tannins include fragments, parts, portions, chemical equivalents, mutants, homologues and analogs. Chemical equivalents of a tannin can act as a functional analogue of the tannin. Chemical equivalents may not necessarily be derived from a tannin but may share certain conformational similarities. Alternatively, chemical equivalents may be specifically designed to mimic certain physiochemical properties of the tannin. Chemical equivalents may be chemically synthesized or may be detected following, for example, natural product screening. Homologues of tannins contemplated herein include, but are not limited to, tannins derived from different species of flora. Derivatives, chemical analogues and homologues may also posses antagonistic or agonistic properties and the use of such molecules are contemplated by the present invention.

The phrase "blood clot formation" is used in its broadest sense to include reference to any one or more steps of the blood clotting cascade such as, but not limited to, activity of the enzymes which function during various steps of the blood clotting cascade. Such enzymes include, for example, serine proteases such as thrombin and Factor Xa. Thrombin, for example, activates platelets and protein C and converts circulating fibrinogen to fibrin.

Reference to "inhibition" of blood clot formation is a reference to complete or partial prevention of blood clot formation. For example, the method of the present invention may completely inhibit thrombin or Factor Xa functional activity or it may merely reduce thrombin or factor Xa activity. Preferably, said blood clot formation is a reference to thrombin or Factor Xa activity.

Accordingly, the present invention provides a method of reducing, retarding or otherwise inhibiting thrombin functional activity in a subject said method comprising administering to said subject an effective amount of a *Geum Japonicum* tannin for a time and under conditions sufficient to inhibit thrombin functional activity.

The present invention also provides a method of reducing, retarding or otherwise inhibiting a Factor Xa functional activity in a subject said method comprising

administering to said subject an effective amount of a *Geum Japonicum* tannin for a time and under conditions sufficient to inhibit Factor Xa functional activity.

Reference to a tannin "reducing, retarding or otherwise inhibiting" blood clot formation, and particularly serine protease activity, should be understood as a reference to inducing or facilitating the inhibition of blood clot formation by both direct and indirect mechanisms. For example, said tannins may interact directly with an enzyme such as thrombin or Factor Xa to prevent catalysis of the conversion of fibrinogen to fibrin or, alternatively, may act indirectly to prevent said catalysis by, for example, interacting with a molecule other than said enzyme wherein said other molecule interacts with and inhibits the activity of the enzyme.

Without intending to limit the present invention to any one theory or mode of action, the inhibition of thrombin and Factor Xa activity or activity of other serine proteases is due in part, to the formation of hydrogen bonds between a hydroxyl group of a phenolic moiety in the tannin to the polar group comprising the active site of thrombin, Factor Xa or other serine protease. This inhibitory activity is particularly efficient where the hydroxyl group is highly acylated by either galloyl or hexahydroxydiphenoyl. In this regard, the inventors have demonstrated that tannins which have been synthesized without the phenyl hydroxyl groups and bear only methylated hydroxyl groups show no measurable inhibition of thrombin activity.

Accordingly, in a preferred embodiment of the present invention there is provided a method of reducing, retarding or otherwise inhibiting blood clot formation in a subject said method comprising administering to said subject an anti-clotting effective amount of a *Geum japonicum* tannin wherein said tannin comprises a substituent on a phenolic moiety which is capable of forming a bond or other association with an amino acid or side chain thereof in the active site of a serine protease.

Preferably, said tannin is penta-*O*-galloyl-β-D-glucose, pedunculagin, tellimagrandin II,

casuariin or 5-desgalloylstachyurin.

According to this preferred embodiment there is provided a method of reducing, retarding or otherwise inhibiting blood clot formation in a subject said method comprising administering to said subject an anti-clotting effective amount of penta-*O*-galloyl-β-D-glucose for a time and under condition sufficient to inhibit blood clot formation.

In another preferred embodiment the present invention provides a method of reducing, retarding or otherwise inhibiting blood clot formation in a subject said method comprising administering to said subject an anti-clotting effective amount of pedunculagin for a time and under condition sufficient to inhibit blood clot formation.

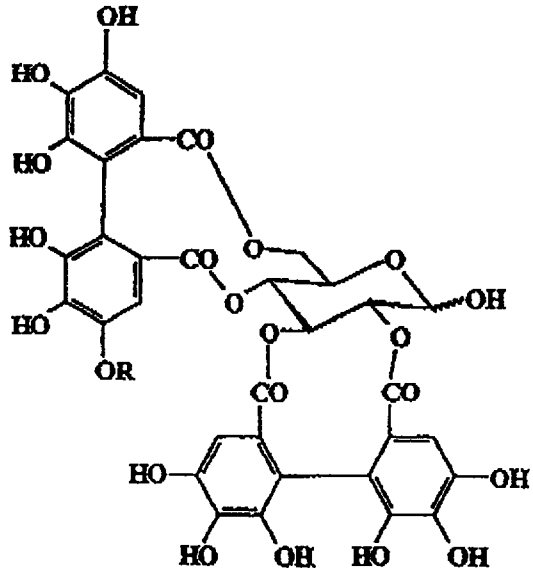
In yet another preferred embodiment the present invention provides a method of reducing, retarding or otherwise inhibiting blood clot formation in a subject said method comprising administering to said subject an anti-clotting effective amount of tellimagrandin II for a time and under condition sufficient to inhibit blood clot formation.

In yet another preferred embodiment the present invention provides a method of reducing, retarding or otherwise inhibiting blood clot formation in a subject said method comprising administering to said subject an anti-clotting effective amount of casuariin for a time and under condition sufficient to inhibit blood clot formation.

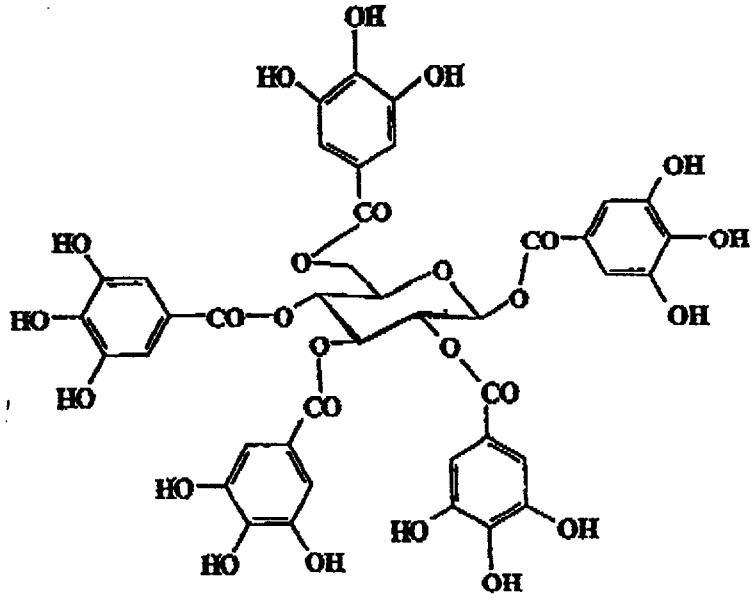
In still yet another preferred embodiment the present invention provides a method of reducing, retarding or otherwise inhibiting blood clot formation in a subject said method comprising administering to said subject an anti-clotting effective amount of 5-desgalloylstachyurin for a time and under condition sufficient to inhibit blood clot formation.

Most preferably said inhibition of blood clot formation is inhibition of thrombin functional activity.

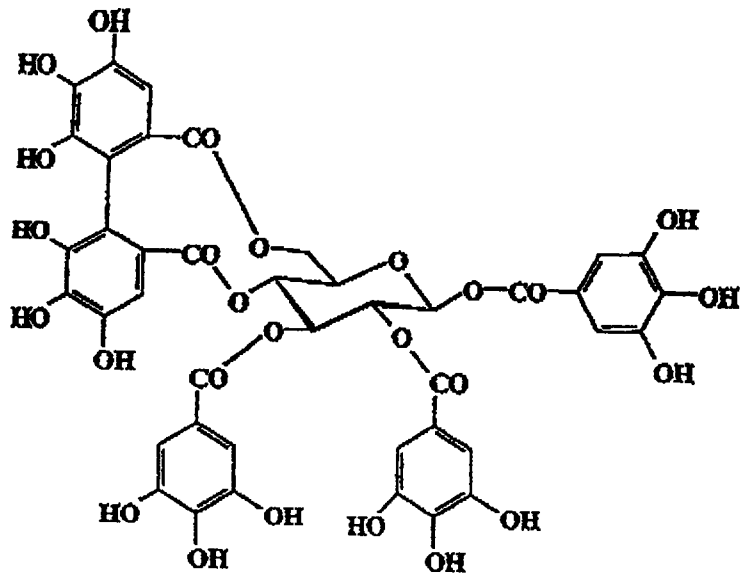
Most preferably said tannins comprise the structures:



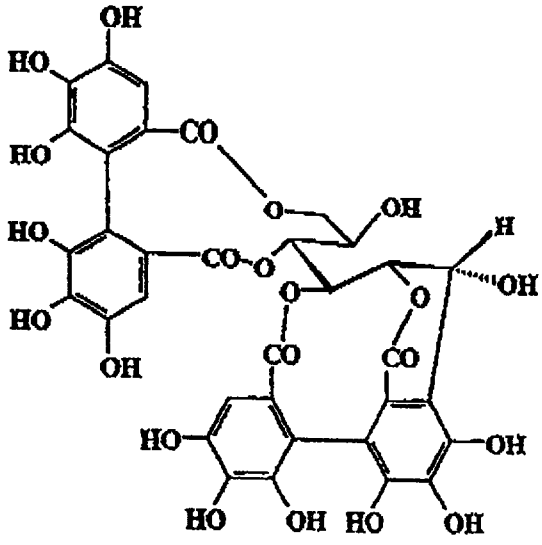
Pedunculagin



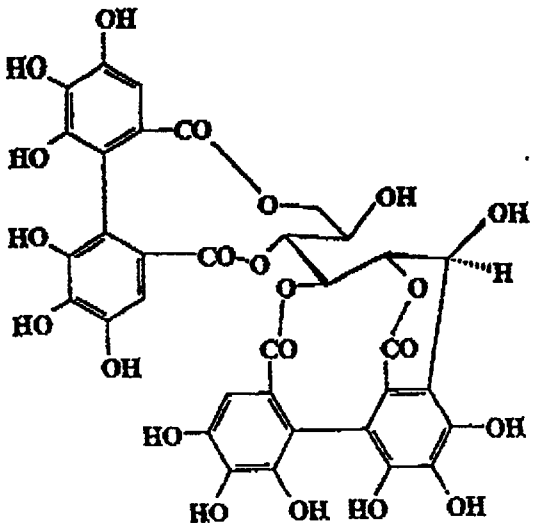
Penta-*O*-galloyl-β-glucoside



Tellimagrandin II



Casuariin



5-Desgalloylstachyurin

The subject of the blood clot inhibition is generally an animal or bird such as but not limited to a human, primate, livestock animal (e.g. sheep, cow, horse, donkey, pig), companion animal (e.g. dog, cat), laboratory test animal (e.g. mouse, rabbit, rat, guinea pig, hamster), captive wild animal (e.g. fox, deer), caged bird (e.g. parrot) and poultry bird (e.g. chicken, duck, pheasant, goose, turkey). Preferably, the subject is a human or primate. Most preferably, the subject is a human.

The method of the present invention is useful in the treatment and prophylaxis of cardiovascular and cerebrovascular diseases such as atherosclerosis, myocardial infarction, stroke and thrombolic disorders such as arterial fibrillation, restenosis, arterial thrombosis and myocardial infarction.

Accordingly, in another aspect there is provided a method for the treatment or prophylaxis of a disease condition involving clotting in a vascular system in a subject such as but not limited to a thrombic disorder or a stroke, said method comprising administering to said subject a blood clotting inhibition effective amount of a tannin.

More particularly, the present invention provides a method for the treatment or prophylaxis of a disease condition involving clotting in the vascular system in a subject such as but not limited to a thrombic disorder, said method comprising administering to said subject a thrombin inhibitory effective amount of a tannin.

Preferably, said tannin is penta-*O*-galloyl-β-D-glucose, pedunculagin, tellimagrandin II, casuariin or 5-desgalloylstachyurin.

In accordance with this method, more than one type of tannin may be administered or the tannin may be co-administered with a known anti-clotting compound or molecule. By "co-administered" is meant simultaneous administration in the same formulation or in two different formulations via the same or different routes or sequential administration by the same or different routes. By "sequential" administration is meant a time difference of

from seconds, minutes, hours or days between the administration of the two types of tannins or the tannin and the known anti-clotting compound or molecule. The tannins or the tannin and known anti-clotting compound or molecule may be administered in any order.

Routes of administration include but are not limited to intravenously, intraperitioneal, subcutaneously, intracranial, intradermal, intramuscular, intraocular, intrathecal, intracerebrally, intranasally, infusion, orally, rectally, *via* iv drip, patch and implant. Intravenous routes are particularly preferred.

The present invention further extends to the use of the subject tannins in the manufacture of a medicament for reducing, retarding or otherwise inhibiting blood clotting.

Preferably inhibition of said blood clotting is inhibition of thrombin activity.

Preferably said tannins are penta-*O*-galloyl-β-D-glucose, pedunculagin, tellimagrandin II, casuariin or 5-desgalloylstachyurin.

Yet another aspect of the present invention provides an agent useful for reducing, retarding or otherwise inhibiting blood clotting comprising a tannin as hereinbefore defined.

Preferably said tannins are penta-*O*-galloyl-β-D-glucose, pedunculagin, tellimagrandin II, casuariin or 5-desgalloylstachyurin.

Most preferably inhibition of said blood clotting is inhibition of thrombin activity.

Another aspect of the present invention provides a composition for use in reducing, retarding or otherwise inhibiting blood clot formation comprising a tannin as hereinbefore defined and one or more pharmaceutically acceptable carriers and/or diluents. The

composition may also comprise two different types of tannins or a tannin and a known anti-clotting compound or molecule.

Preferably said inhibition of blood clotting is inhibition of thrombin activity.

Compositions suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions. They must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof and vegetable oils. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thirmerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by, for example, filter sterilization or sterilization by other appropriate means. Dispersions are also contemplated and these may be prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, a preferred method of preparation includes vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution.

When the active ingredients are suitably protected, they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in

hard or soft shell gelatin capsule, or it may be compressed into tablets. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1ng and 2000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter: A binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such a sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and formulations.

The present invention also extends to forms suitable for topical application such as creams, lotions and gels. In such forms, the anti-clotting peptides may need to be modified to permit penetration of the surface barrier.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion

media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved and (b) the limitations inherent in the art of compounding such an active material for induce or facilitating analgesia in living subjects.

Effective amounts of anti-clotting tannins contemplated by the present invention will vary depending on the severity of the pain and the health and age of the recipient. In general terms, effective amounts may vary from 0.01 ng/kg body weight to about 100 mg/kg body weight. Alternative amounts include for about 0.1 ng/kg body weight about 100 mg/kg body weight or from 1.0 ng/kg body weight to about 80 mg/kg body weight.

Still another aspect of the present invention is directed to antibodies to the subject tannins and their derivatives, homologues, analogues and mimetics.

In a preferred embodiment the present invention is directed to antibodies to the subject tannins for use in modulating blood clot formation.

Preferably said modulation of blood clot formation is modulation of thrombin activity. Said antibody may be monoclonal or polyclonal.

The antibodies of the present invention are particularly useful as therapeutic or diagnostic agents. For example, specific antibodies can be used to screen for tannins using immunoassays or used as antagonists to inhibit tannin activity under certain circumstances such as where temporary clotting inhibition is only required. Techniques for such immunoassays are well known in the art and include, for example, sandwich assays and ELISA. Knowledge of tannin levels may be important for monitoring certain therapeutic protocols.

Antibodies to the peptides (or their derivatives, homologues, analogues or mimetics) of the present invention may be monoclonal or polyclonal. Alternatively, fragments of antibodies may be used such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic antibody" is considered herein to include fragments and hybrids of antibodies.

Further features of the present invention are more fully described in the following examples. It is to be understood, however, that the detailed description is included solely for the purposes of exemplifying the present invention. It should not be understood in any way as a restriction on the broad description of the invention as set out above.

EXAMPLE 1

GENERAL EXPERIMENTAL PROCEDURES

ESI-MS were collected on a PE SCIEX API-300 mass spectrometer. All spectra (^{1}H, ^{13}C, COSY, HMQC and HMBC) were recorded on a Bruker AMX 500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C), and the chemical shifts are reported in parts per million with TMS as an internal standard. TLC was done on HPTLC-Fertigplaten Cellulose F (Merck) plates in the solvent systems: (a) H_2O and (b) 7% aqueous acetic acid.

EXAMPLE 2

MATERIALS AND REAGENTS

The plant of *G. japonicum* was collected from China in Auugst, 1994. It was identified by Dr. Dao-Feng Chen, Department of Pharmacognosy, Shanghai Medical University, China. A voucher specimen is deposited in the Department of Pharmacognosy, Shanghai University of Traditional Chinese Medicine, China.

Heparin sodium salt (from porcine intestinal mucosa, 187 USP units/mg), thromboplastin with calcium reagent (for Prothrombin Time test), thrombin time reagent, activated partial thrombin time reagent, thrombin from bovine plasma (3 NIH units/vial), and fibronogen, fraction I, type IV from bovine plasma were purchased from Sigma Chemical Co. (USA). Human factor Xa was purchased from ICN Bilmedicals Inc. (USA). Substrates S-2238 (H-D-Phe-Pip-Arg-p-nitroanilide) and S-2222 (N-benzoyl-L-isoleucyl-L-arginine-*p*-nitroaniline hydrochloride and its methyl ester) were obtained from Chromogenix Co. (Sweden).

EXAMPLE 3

EXTRACTION AND ISOLATION

Dried whole plant (2.6 kg) were chopped into small pieces and precolated three times with MeOH (20 L) at room temperature, and the extract was evaporated *in vacuo* to yield the MeOH extract (350 g). The MeOH extract was resuspended in distilled water (1 L) and successively partitioned with hexane (2 L x 5), EtoAc (2 L x 5) and n-BuOH (1 L x 5). The n-BuOH soluble fraction was filtered, and the filtrate was evaporated under reduced pressure (50°C) to give a brown residue (73 g). the n-BuOH extract was subjected to Sephadex LH-20 column chromatography and eluted with water with an increasing amount of MeOH, giving nin fractions, Fr. 1-9. Fraction 9 (eluted with 80% MeOH aqueous, 3.3 g) showed potent anticoagulant activity and was further chromatographed over Sephadex LH-20 eluted with the above solvent system to give three fractions, I (0.08g), II

(1.7 g) and III (1.2 g). Fraction II (eluted with 50% MeOH) was chromatographed on Sephadex LH-20 with $CHCL_3$-MeOH (1:1) and MeOH, and then over cellulose column with 3% acetic acid, followed by purification on Bio-Gel P-4 (extra fine, $<45\ \mu M$) column with 0.5% acetic acid to yeild **2** (60.8 mg), **3** (36.2 mg), **5** (9.6 mg), **6** (8.1 mg) and **7** (10.2 mg). Fraction III (eluted with MeOH) was chromatographed over cellulose with MeOH-HOAc-H_2O) (5:5:90) and then over Toyopearl HW-40s with 30% aqueous MeOH to give **1** (120 mg) and **4** (9 mg).

Of these compounds, the assignments of the ^1H or ^{13}C NMR spectral data of the sugar moieties of **6**[2,16] and **7**[6] were different from the data reported in the literatures. The ^1H, and ^{13}C-NMR data of the sugar moiety were reassigned with the help of DEPT, ^1H-^1H COSY, HMQC and HMBC spectra data. The ^{13}C NMR data of the sugar moieties of 6:δ (175 MHz, CD_3COCD_3): 66.1 (C-1), 81.7 (C-2), 73.8 (C-3), 77.0 (C-4), 69.6 (C-6), 68.7 (C-6) and 7:δ (185 MHz, CD_3COCD_3): 68.8 (C-1), 77.5 (C-2), 71.6 (C-3), 78.0 (C-4), 69.2 (C-5), 68.9 (C-6). The ^1H NMR data of 7:δ (500 MHz, DC_3COCD_3):5.64 (1H, d, *J*=4.8 Hz, H-1), 5.45 (1H, , *J*-2.5 Hz, H-3), 5.01 (1D, dd, *J*=2.8, 8.6 Hz, H-4), 4.72 (1H, dd, *J*=2.3, 4.8 Hz, H-2), 4.64 (1H, dd, *J* = 3.2, 12.3 Hz, H-6), 4.12 (1H, dd, *J*=2.5, 8.6 Hz, H-5), 3.83 (1H, d, *J*=11.8 Hz, H-6).

EXAMPLE 4

SYNTHESIS OF PENTA-*O*-BENZOYL-β-D-GLUCOPYRANOSIDE (1A)

A solution of 1 g (5.6 mmol) of D(+)-glucopyranoside, 3.5 g (29.5 mmol) of benzoic acid, 0.33 g (2.7 mmol) of DMAP, 0.41 g (2.7 mmol) of DMAP-HCl, and 3.7 g (17.9 mmol) of DCC in 100 ml of Ch_2Cl_2 was pured under N_2 and then refluxed for 24 h. The reaction mixture was cooled to room temperature and filtered. Solvent was removed, to give a white foam. The crude product was purified by chromatography on silica gel (CH_2Cl_2) to afford 1.5 g of penta=*O*-benzoyl-β-D-glucopyranoside (**1a**). ESI-MS: 723.5 [M + Na]. The ^1H NMR of **1a**:δ (500 MHz, $CDCl_3$):634 (1H, d, *J*=8.4 Hz, Glu H-1) 6.09 (1H, t, *J*=9.4 Hzz, Glu H-3), 5.90 (1H, t, *J*=9.8 Hz, Glu H-4), 5.73 (1H, dd,

J=3.7, 10.2 Hz, Glu H-2), 4.43-4.68 (2H, m, Glu H-5,6), 7.31-8.07 (25H, m, aromatic protons).

EXAMPLE 5

METHYLATION OF 1

An ethanol solution (0.5 mL) of 1 (5 mg) was treated with ethereal diazomethane (1.5 mL) for 1 h at room temperature. The solvent was evaporated off, and the residue was subjected to preparative TLC (CH_2Cl_2-CH_3COCH_3, 7:3, R_f=0.6) to give a pentadecamethylate (**1b**) (2.1 mg). ESI-MS:1173.5 [M + Na]. The ^{1}H NMR of **1b**:δ (500 MHz, CD_3COCD_3):7.35 (2H, s), 7.32 (2H, s), 7.25 (2H, s), 7.25 (2H, s), 7.21 (2H, s), 6.41 (1H, d, J=8.1 Hz, Glu H-1), 6.21 (1H, t, J=9.7 Hz, Glu H-3), 5.90 (1H, t, J=9.7 Hz, Glu H-4), 5.78 (1H, dd, J=8.1, 9.4 Hz, Glu H-2), 4.87 (1H, dd, J=3.1, 12.1 Hz, Glu H-6), 4.74 (1H, m, Glu H-5), 4.44 (1H, dd, J=5.3, 12.1 Hz, Glu H-6), 3.91 (9H, 3 x OCH_3), 3.89 (6H, 2 x OCH_3), 3.88 (3H, OCH_3), 3.83 (6H, 2 x OCH_3), 3.82 (9H, 2 x OCH16 July, 19983), 3.80 (3H, OCH_3), 3.79 (3H, OCH_3), 3.76 (3H, OCH_3), 3.73 (3H, OCH_3) and 3.72 (3H, OCH_3).

EXAMPLE 6

METHYLATION OF 2

An ethanol solution (0.5 mL0 of 1 (6 mg) was treated with ethereal diazomethane (1.5 mL) for 2 h at room temperature. The solvent was evaporated off, and the residue was subjected to preparative TLC (CH_2Cl_2-CH_3COCH_3) to give an isomer mixture of trideca-*O*-methyl-pedunculagin (**2a**) (1.6 mg). ESI-MS: 989.5 [M + Na]. The ^{1}H NMR of **2a**:δ (500 MHz, CD_3COCD_3):6.96 (1H, s), 6.93 (1H, s), 6.89 (1H, s), 6.85 (2H, s), 6.81 (1H, s), 6.62 (1H, s), 6.60 (1H, s), 5.53 (1H, t, J=10.5 Hz, Glu H-3), 5.28 (1H, t, J=9.8 Hz, Glu H-3), 5.25 - 5.08 (4H, m, Glu H-1α, 2α, 2β, 6), 5.02 (1H, t, J=10.1 Hz, Glu H-4), 4.85 (1H, t, J=8.7 Hz, Glu H-4), 4.88 (1H, d, J=6.4 Hz, Glu H-1β), 4.65 (1H, m, Glu H-5), 4.29 (1H, m, Glu H-5), 4.13 (1H, m, Glu H-6), 3.61 - 3.93

(78H, m, 26 x OCH_3).

EXAMPLE 7

BIOLOGICAL ASSAYS

Blood clotting Assays.[9] The effects of these compounds on coagulation of rabbit plasma *in vitro* were measured at 37°C on a fibrometer (BBL) FibroSystem, Becton Dickinson Co., USA) using the prothrombin time (PT) assay. 150 μL of 0.05 M Tris buffer (pH 7.5, containing 0.1 M NaCl). 50 μL of samples solution (compound dissolved in 0.05 M Tris buffer), and 100 μL rabbit plasma were incubated for 2 minutes at 37°C. Clotting was initiated by addition of 100 μL of thromboplastin with calcium reagent (Sigma). Similarly, the thrombin times (TT) were measured by addition of 100 μL bovine thrombin (Sigma. 3-4 NIH units/mL) to a mixture of rabbit plasma (100 μL), 0.05 M Tris buffer (50 μL) and compounds dissolved in Tris buffer (50 μL) which was preincubated for 2 minutes. Activated partial thromboplastin time (APTT) was measured by incubating rabbit plasma (100 μL), 0.05 M Tris buffer (50 μL), compounds dissolved in Tris buffer (50 μL) with APTT reagent (100 μL) for 2 minutes. Clotting was initiated by additing 25 mM $CaCl_2$ (100 μL). Clotting times are based on the average of three separate determinations.

Determination of the inhibitory activity of tannins for thrombin.[10] The activity of thrombin from bovine plasma was measured using S-2238 as substrate on a Ceres 900C Microplate Autoreader (Bio-Tek) Instruments, Inc., USA). The assay mixture of 200 μL comprise 10 μL thrombin (0.19 MIH units/mL) in 0.05 M Tris buffer containing 0.1 M NaCl, pH 7.5, and 10 μL of various concentrations of compounds which was preincubated with thrombin for 2 min at 37°C. The reaction was started by the addition of 20 μL S-2238 (0.66 mM). Activity against the substrate was determined by measurement of the release of *p*-nitroaniline from S-2238 as indicated by the increase in optical density at 405 nm over 5 minutes. The results were expresed as percentage of inhibition of thrombin activity. The kinetics of thrombin from bovine plasma were studied with a range of substrate

concentrations from 0.6 - 1.7 mM.[11] Assays were performed by using a 0.05 M Tris buffer, pH 7.5, containing 0.1 M NaCl and a fixed concentration of thrombin from bovine plasma (0.19 NIH units/mL) at 37°C. The total volume of reaction mixtures was maintained at 200 μL. Reactions were initiated by addition of substrate to the cuvette containing thrombin and compound preincubated for less than 2 minutes. Data from initial rate experiments were used to construct Lineweaver-Burk plots,[12] while the relationship of (substrate concentration)$^{-1}$ versus (initial velocity)$^{-1}$ was analyzed by linear regression. The type of inhibition and K_i value were determined from the plots.[13] Compounds **1a**, **1b** and **2a** were tested in 0.1% DMSO Tris buffer, pH 7.5, containing 0.1 M NaCl, which was used as controls.

Thrombin-induced fibrin-clot assays.[14] All solutions used were prepared with 0.05 M Tris buffer containing 0.1 M NaCl, pH 7.5, except for fibrinogen which was reconstituted in ultrapure water. For the assay, 190 μL of different concentrations of a compound were preincubated for 3 minutes with 10 μL thrombin (0.05 NIH units/mL) at 37°C and then 50 μL fibrinogen (2 mg/mL) were added to initiate enzymatic reaction. The turbidity of clots was measured for 5 minutes at 405 nm and velocities were calculated as milli-optical density units per minutes (mOD/min). Each assay was performed in triplicate. The percentage inhibition of thrombin induced fibrin clotting was calculated according to the formula: Percentage inhibition (%) = (1-velocity of samples/velocity of control) x 100.

Determination of the inhibitory activity of tannins for Factor Xa.[15] The activity of human factor Xa was measured with S-2222 as the substrate. The assay mixtures of 200 μL contained 0.20 mM S-2222 in 0.0 M Tris/HCl buffer containing 0.1 M NaCl, PH 8.3, and various concentrations of tannins. The reaction was started by addition of factor Xa (50 ng/mL). Activity against the substrate was determined by measurement of the release of 4-nitroanilide as indicated by the increase in optical density at 405 nm over 5 min at 37°C. For determination of the K_i, increasing concentrations of S-2222 were added (0.05 mM - 0.20 mM). The IC_{50} value was the concentration inhibiting 50% of the initial activity of enzymes. The data were collected as mean ± SD of three samples.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

EXAMPLE 8

Seven tannins, penta-*O*-galloyl-β-D-glucose (**1**),[1] pedunculagin (**2**),[2] 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose (**3**),[3] tellimagrandin II (**4**),[2] 2,6-di-*O*-galloyl-D-glucose (**5**),[4] casuariin (**6**),[2,5] and 5-desgalloylstachyurin (**7**)[6] were isolated and purified from the methanol extract of the whole plant. Their structures were determined by spectroscopic methods, and by comparison with those data reported in the literature.

EXAMPLE 9

The anticoagulant activity of these seven tannins was determined by measuring their ability to prolong clotting of rabbit plasma. Of these, compound **1** significantly prolonged the clotting times of rabbit plasma over 300 seconds in the prothrombin time (PT), the thrombin time (TT), and the activated partial thromboplastin time (APTT) assays with the concentrations of 0.40, 0.50, and 0.47 mM, respectively (Figure 1). Compounds **2, 4, 6** and **7** also showed significant anticoagulant activity with clotting times over 300 seconds in the PT, TT and APTT assays at a concentration of 1 mM. Compounds **3** and **5** had no detected effect on plasma clotting at concentrations up to 2 mM (Table 1). These results indicate that compounds **1, 2, 4, 6** and **7** inhibited either both intrinsic and extrinsic pathways or the common pathway in the blood coagulation cascade.

EXAMPLE 10

As a first step in characterizing the anticoagulant effects of these tannins, their effect on proteolytic activity of thrombin on synthetic peptide substrate was studied. Compounds **1, 2, 4, 6** and **7** exhibited dose-dependent inhibition of thrombin-catalyzed hydrolysis of the chromogenic substrate, H-D-Phe-Pip-Arg-p-Nitroanilide (S-2238). The IC_{50} values (concentration which inhibits 50% of the enzyme activity) of **1-7** are presented in Table 2. Among them, compound **4** showed the most potent activity with an IC_{50} of 0.070 μM. Compounds **6** and **7** are anomers with a different configuration at the C-1 position of the sugar moiety. The inhibitory activity of compound **7** on thrombin is about 2-fold more potent than that of compound **6**. Under the same experimental conditions, compounds **3** and **5** exhibited no measurable inhibition of thrombin-catalyzed reaction even at a concentration as high as 200 μM.

EXAMPLE 11

The kinetic studes of compounds **1, 2, 4, 6** and **7** were conducted to determine the type of inhibition and the inhibition constants (K_i). Compound **2** competitively inhibited S-2238 hydrolysis by bovine thrombin, with a K_i value of 0.042 μM (Figure 2). On the other hand, compounds **1, 4, 6** and **7** showed mixed noncompetitive inhibition of S-2238 hydrolysis by bovine thrombin with K_i values of 3.9, 0.42, 0.98 and 0.83 μM, respectively (Figure 3, graphs of compounds **4, 6** and **7** not shown). These results indicate that the compound **2** binds to the active site of thrombin, whereas compounds **1, 4, 6** and **7** bind to two different sites, both at the active site of thrombin and a secondary site.

EXAMPLE 12

The key role of thrombin in the blood coagulation cascade is that it catalyzes the conversion of fibrinogen to fibrin clots. Therefore, the effect of these tannins on the proteolytic activity of thormbin on its macromolecular substrate, fibrinogen was examined. The compounds **1, 2, 4, 6** and **7** exhibited dose-dependent inhibition of thrombin-catalyzed conversion of fibrinogen to fibrin clots (Table 2), (Figure 4). All the compounds which exhibited mixed noncompetitive inhibition, compounds **1, 4, 6** and **7**, showed a decrease (2 to 7 fold) in their IC_{50} values of inhibition of fibrin clot formation (Table 2) compared to those of inhibition of S-2238 hydrolysis. This indicate that the second binding site is most probably the anion binding exosite (ABE)[7] of thrombin. On the other hand, the IC_{50} values of compound **2** for hydrolysis of synthetic and macromolecular substrates were comparable (Table 2). This is expected as compound **2** is a competitive inhibitor of thrombin (Figure 2) and it only binds to the active site of thrombin. Among these tannins, compound **4** showed the most potent inhibitory activity on the thrombin-induced fibrin clotting, with an IC_{50} of 0.014 μM.

EXAMPLE 13

To test the specificity of these tannins, their effects on proteolytic activity of factor Xa was determined. As shown in Figure 5, compounds **1, 2, 4, 6** and **7** inhibited factor Xa-catalyzed hydrolysis of synthetic substrate S-2222 (N-benzoyl-L-isoleucyl-L-argininine-*p*-nitroaniline hydrochloride and its methyl ester). The IC_{50} values were 0.17, 0.56, 0.21, 0.72 and 0.28 μM, respectively (Table 3). These values are 1.5 to 3 times less than those for the inhibition of thrombin indicating that only a small preference for thrombin than factor Xa. The kinetics of inhibition of factor Xa for these tannins were also examined. All the five compounds (**1, 2, 4, 6** and **7**) are competitive inhibitors of factor Xa indicating that they bind only to the active site of the enzyme. Compounds **1, 4, 6** and **7**, which apparently bound to ABE and the active site of thrombin, could only bind to the active site of factor Xa, since they are similar in nature. Compound **2**, however, binds to

the active site of both the enzymes. Thus these results can be explained based on the fact that factor Xa, unlike thrombin, does not have ABE (Figure 6). The K_i value of compound **2** for inhibition of factor Xa was 0.99 μM, and about 23-fold more than than of thrombin are about 1.2 - 6.8 times of factor Xa (Tables 2, 3). Compound **4** competitively inhibited factor Xa with the similar K_i values for inhibition of thrombin and factor Xa.

EXAMPLE 14

The formation of tannin-enzyme compexes are usually mediated by hydrogen bonding between phenolic hydroxyl groups and polar groups in the active site of enzyme.[8] In order to determine the role of the hydroxyl groups of phenolic moieties, compound **1a**, with no phenolic hydroxyl groups was synthesized as well as the methylated erivatives, **1b** and **2a**. These three compounds showed no inhibition of thrombin even at concentrations up to 100 μM. These results indicate that the phenolic hydroxyl groups of the tannins play a key role in their inhibitory effect on thrombin.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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Table 1. Effects of 1-7, and Heparin on Prothrombin Time (PT), Thrombin Time (TT) and Activated Partial Thromboplastin Time (APTT) assays *in vitro*[a]

Compound (mM)	PT (s)	TT (s)	APTT (s)
Control	10.2 ± 0.1	11.2 ± 0.3	42.6 ± 4.5
1 (0.5)	>300	>300	>300
(0.25)	13.9 ± 0.8	16.5 ± 4.2	50.6 ± 0.9
2 (1.0)	>300	>300	>300
(0.5)	13.5 ± 0.2	22.7 ± 1.6	43.7 ± 2.3
3 (2.0)	10.0 ± 0.2	9.43 ± 0.1	39.9 ± 4.7
4 (1.0)	>300	>300	>300
(0.5)	11.9 ± 0.1	18.7 ± 0.6	45.7 ± 4.1
5 (2.0)	11.1 ± 0.2	10.7 ± 0.1	40.8 ± 3.2
6 (1.0)	>300	>300	>300
(0.5)	13.6 ± 0.3	12.3 ± 0.3	72.3 ± 8.0
7 (1.0)	>300	>300	>300
(0.5)	13.9 ± 0.9	16.2 ± 0.9	31.6 ± 2.1
heparin,[b] 0.5mg/ml	33.6 ± 0.5	>300	>300
0.25mg/ml	23.7 ± 0.2	>300	>300

[a]PT, TT and APTT were determined in the presence of the indicated concentrations of 1-7 according to the methods described under experimental section. Data are reported as clotting times (seconds) of plasma (Means ± SD. n=3). [b]Heparin sodium salt from porcine intestinal, 187 USP units/mg.

Table 2. Inhibitory Activities of **1-7** on Thrombin-Catalyzed Hydrolysis of S-2238 and Fibrinogen[a]

Compound	IC_{50} (S-2238, μM)	IC_{50} (fibrinogen, μM)	Type of inhibition	K_i (μM)
1	0.11 ± 0.02[b]	0.050 ± 0.006*	MNC[c]	3.9 ± 0.6
2	0.18 ± 0.01	0.15 ± 0.03	CC[d]	0.042 ± 0.004
3	>200	>200	N.D.[e]	N.D.
4	0.070 ± 0.01	0.014 ± 0.001*	MNC	0.42 ± 0.06
5	>200	>200	N.D.	N.D.
6	0.28 ± 0.02	0.059 ± 0.002*	MNC	0.98 ± 0.2
7	0.19 ± 0.02	0.026 ± 0.002**	MNC	0.83 ± 0.2

[a] IC_{50} values were the concentration inhibiting 50% of the initial activities of enzymes, K_i values were determined as reported under experimental section. [b] The data presented are those obtained from the analysis with best fitting; shown as means ± SD (n=3), *$p<0.05$, **$p<0.01$ significantly different from their IC_{50} values for S-2238 substrate, student's test. [c] MNC: mixed noncompetitive. [d] CC: classical competitive. [e] Data not detected.

Table 3. Inhibitory activities of **1, 2, 4, 6 and 7** on Factor Xa-Catalyzed Hydrolysis of S-2222[a]

Compound	IC_{50} (μM)	Type of inhibition	K_i (μM)
1	0.17 ± 0.08	CC[b]	0.57 ± 0.08
2	0.56 ± 0.05	CC	0.99 ± 0.04
4	0.21 ± 0.02	CC	0.44 ± 0.1
6	0.72 ± 0.3	CC	0.45 ± 0.05
7	0.28 ± 0.04	CC	0.69 ± 0.04

[a] K_i values were determined as reported under experimental section. The data presented are shown as means ± SD (n=3). [b] CC:classical competitive.

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CLAIMS

* C L M S *

1. A method of reducing, retarding or otherwise inhibiting blood clot formation in a subject said method comprising administering to said subject an anti-clotting effective amount of a tannin or a derivative, homologue or chemical analogue thereof for a time and under conditions sufficient to inhibit blood clot formation.

2. A method according to claim 1 wherein said blood clot formation is serine protease functional activity.

3. A method according to claim 2 wherein said serine protease is thrombin.

4. A method according to claim 2 wherein said serine protease is Factore Xa.

5. A method according to any one of claims 1 to 4 wherein said tannin is a plant tannin.

6. A method according to claim 5 wherein said plant is *Geum japonicum*.

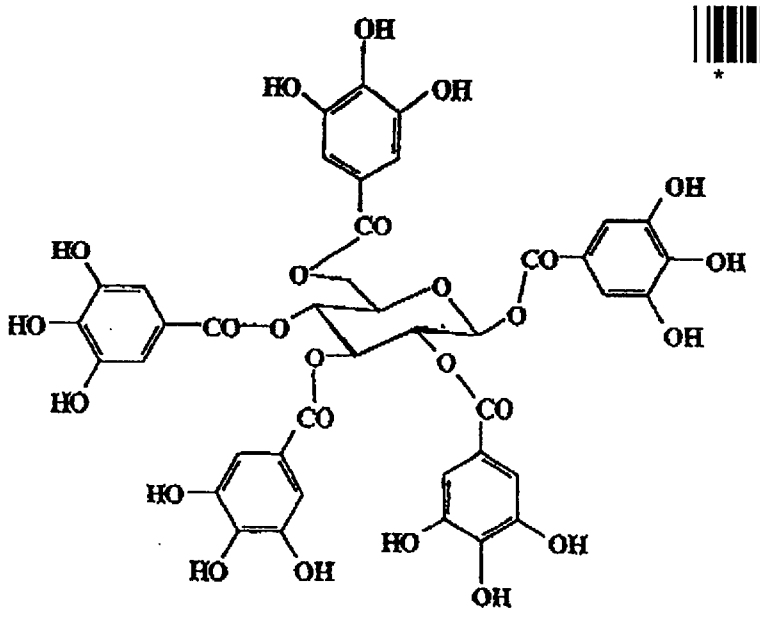
7. A method according to claim 6 wherein said tannin comprises a substituent on a phenolic moiety which is capable of forming a bond or other association with an amino acid or side chain thereof in the active site of a serine protease.

8. A method according to claim 7 wherein said tannin is penta-*O*-galloyl-β-D-glucose.

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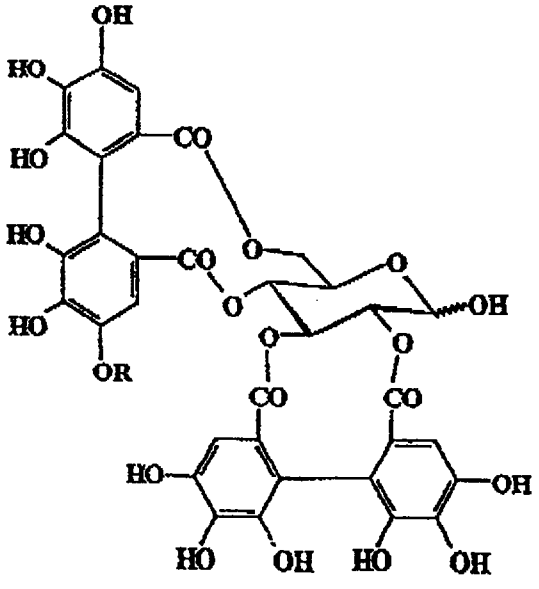
9. A method according to claim 8 wherein said penta-*O*-galloyl-*β*-D-glucose comprises the structure:



* C L M S *

10. A method according to claim 7 wherein said tannin is pedunculagin.

11. A method according to claim 10 wherein said pedunculagin comprises the structure:

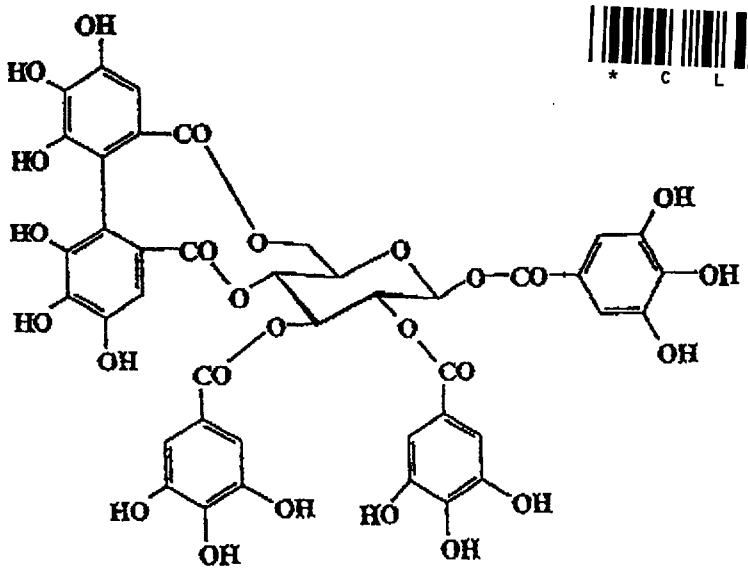


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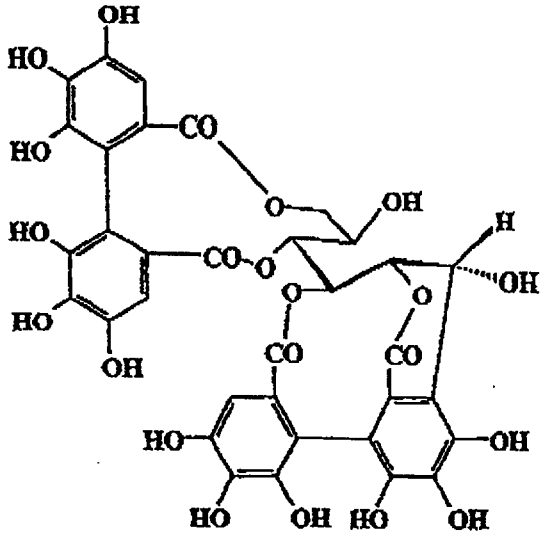
12. A method according to claim 7 wherein said tannin is tellimagrandin II.

13. A method according to claim 12 wherein said tellimagrandin II comprises the structure:



14. A method according to claim 7 wherein said tannin is casuariin.

15. A method according to claim 14 where said casuariin comprises the structure:

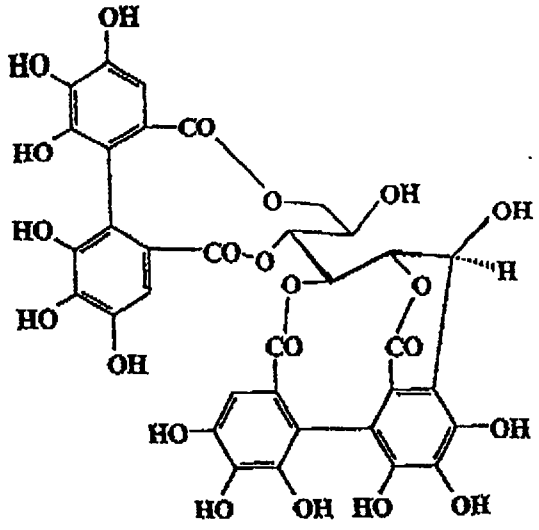


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16. A method according to claim 7 wherein said tannin is 5-desgalloylstachyurin.

17 . A method according to claim 16 wherein said 5-desgalloylstachyurin comprises the structure:



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