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Review Article

A Review on Medicinal Plants and Anti-Coagulant Activity

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Abstract

Medicinal plants also known as medicinal herbs which are identified and used from ancient times for treatment of various human diseases, in the addition of primary health care system enlargement and uses of herbal drugs were increased. According to the report of WHO almost 80% of the people serve through medicinal plants and 25% of active pharmaceutical drugs were derived from herbal plants and this study evaluated about anticoagulant medicinal plants. Coagulation mainly involves in the regulated sequence of proteolytic activation and timely haemostasis in an injured vessel, in the non-pathological state exposure of circulating factor VII/VIIa to extravascular expressed tissue factor in such point it brings into the series of steps results in amplification of the initial stimulus leads to the conversion of fibrinogen to fibrin and clot formation. This article provides overviews of interaction of herbal drugs which have been used for treatment of blood coagulation and effect of herbal drugs on anticoagulant therapy in secondary metabolites of medicinal plants among numerous articles tridax procumbent is showed many active constitutions in therapeutic value which are praiseworthy source as an anticoagulant. Active components are extracted by different extraction methods, separation by HPLC and impurity estimation by Lowrey, UV and lipid Methods, further results expression by standard error of mean data by ANOVA and post hoc Dunnett t-test for multiple comparisons. $P<0.05$ was accepted as statistical significant.

Keywords: Medicinal plant; Herbal drugs; Blood coagulation; Haemostasis; Secondary metabolites; Extraction

Introduction

Medicinal plants, also known as medicinal herbs, have been identified and started the practice since ancient dated within various civilizations before the period of modern medicine for treatment of human diseases. According to the Botanical survey of India, the Indian forest having around 8000 species of medicinal and large sources of aromatic plants, which are mainly collected as raw materials for production of drug products, to utilising of these sources of medicinal plants as in healthcare system is an important basic part for India as the rural population depends on traditional remedial methods [1]. Green plants are natural source to produce and store a variety of biochemical products, these plants synthesise thousands of essential chemical compounds including protect against insects, fungi, diseases, and as well as therapies for anticoagulant activity. In the course of last decade, the utilization of traditional medicines has extended globally and was gaining acceptance, this global enlargement has continued to be used in developing countries where conventional medicine was principle in the national care system and also for primary health care of the poor. Hence around 100 plants species are contributed significantly to modern drugs from those medicinal plant species approximately half of the world's 25 bestselling pharmaceutical substances are derived and the importance was now given on the evenness of herbal medicines by testing of biological activity of medicinal plants. On the report of WHO medicinal plants serve the health needs of about 80% of the world's population, especially for millions of people in the extensive rural areas of developing countries [3]. Blood Thinners (Anticoagulants) are chemical substances which prevent coagulation of blood and also known as clotting. This is the process of conversion of liquid blood into the gel form and those anticoagulants are used for the risk of developing blood clots in their body. Medicinal plant

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remedies are used to treat large variety of clotting blood related disorders and mainly clotting of blood may cause severe problems like strokes, ischemic heart disease, heart attack, deep vein thrombosis, pulmonary embolism. Although number of medicinal plant preparations have been reported for variations in clotting time this has primarily by disruption of cascade of coagulation [4]. Coagulation cascade participation was occurred in anticoagulant state which was forcefully encourages in such cases of injured vessel haemostasis and proteolytic activation sequence, those were firstly depends on the coagulation for inciting of non-pathological state in the exposure of circulating factor VII/VIIa to an expressed extra vascular tissue, which results conversion of fibrinogen to fibrin for clot formation by amplification of the initial stimulus. In pathological condition these mechanisms can change normal conditions due to inherited or any acquired effects which lead to thrombosis [5]. Prevention of spontaneous bleeding, rapid healing allowed intricate pathway for activation of the intrinsic pathway. Two paths intrinsic and extrinsic originate separately but both intersect at a particular point which leads to fibrin activation [6].

Haemostasis is the physiological process which stops bleeding at the site of an injury and the blood loss is stopped by formation of haemostatic plug. The endothelium in blood vessels carry an anticoagulant surface that serves to maintain blood in its fluid state, to initiate formation of a blood clot the damaged blood vessel of sub endothelial matrix and which are exposed to the blood, several of these components activate the two-main process of haemostasis and primarily composed of platelets and fibrin. This process is activated within seconds of an injury, there are two main components of haemostasis. Primary haemostasis refers to platelet aggregation and platelet plug formation and secondary haemostasis states to the deposition of insoluble fibrin and this insoluble fibrin is generated by the proteolytic coagulation cascade, insoluble fibrin forms a mesh which serves strengthen and stabilize the blood clot and these two primary and secondary haemostasis occur simultaneously and are mechanically entangled [7].

The intrinsic pathway is under secondary haemostasis and which is longer pathway it begins with the activation of Factor XII (Zymogen, inactivated serine protease) which converts as Factor XIIA (activated serine protease) in the presence of endothelial collagen. when endothelial damage was persisting then endothelial collage was exposed and for activation of factor XI to XIA factor XIIA is required as a catalyst, for factor X into factor Xa, factor IXA serve as a catalyst hence this conversion metabolism was known as cascade. FXa in a complex with its cofactor FVa forms prothrombinase complex that catalyzes the ptothrombin (FII) to thrombin (FIIa). Thrombin acts as a terminal coagulator. When one factor was activated it enters into several other factors for further cascade steps. Movement of further factors down the cascade and that increases the concentration of the blood for example, if the concentration of factor IX is more than that of XI, in this time factor II is activated by any of the intrinsic or extrinsic pathway, this can strengthen the intrinsic pathway by giving positive response to factors V, VII, VIII, IX, XIII which leads to formation of intrinsic Xa. The Subjection of sub endothelial tissue factor which is complexes with activated FVII, recognised as extrinsic Xase, catalyzes the generation of activated FX (FXa). This pathway dependence on tissue-factor which being exposed to the circulation and involves the factor VII and tissue factor for conversion of common pathway to activate factor X, leads to conversion of factor II (Prothrombin) to factor IIa (thrombin) and build up fibrinogen to fibrin. in this cascade model contribution of primary haemostasis, with the initial recruitment of platelets was studied as an independent mechanism [6].

Coagulation happens in three overlying phases: initiation, amplification, and propagation. Exposure of tissue factor in blood by damage or activation of endothelium, tissue factor is a 47kDa and is a cytokine super family, cell-bound transmembrane glycoprotein. It acts a receptor with signal transduction gives in the induction of genes involves in inflammation, embryonic development, apoptosis and cell and this phase is triggered by the release of tissue factor into the blood stream, which cause in the production of a comparatively small amount of thrombin through the extrinsic pathway [8]. The generated amount of thrombin is not sufficient, since many positive feedback loops are present which bind to thrombin with platelets and this thrombin undergo initiation phase further activates factor V and factor VIII while it acts as a cofactor in complex of pro-thrombinase, additionally activates the factor II by FXa by FIXa respectively and the cumulated enzyme complexes prothrombinase and tenase complex on a platelet surface support robust amount of thrombin generation and platelet activation about this continuous generation of thrombin and eventually formation of fibrin which gives an abundantly large clot [5].

The aim of coagulation is formation of fibrin by activating coagulation cascade which leads to the production of thrombin (FIIa), This produced thrombin then converts Fibrinogen (FI) to fibrin and the physiological mechanism of anticoagulation reduces the thrombin production or as reduces the thrombin effect furtherly Anti-thrombin (ATIII) is a primary physiological inhibitor of thrombin and further inhibitors are Heparin Cofactor II (HCII), a1-macroglobulin, a1-antitrypsin and Heparin Cofactor II (HCII). action of AT is primarily inhibits FXa and thrombin and very less foremost inhibits FIXa and FVIIa, then its triggering of prostacyclin production which causes vasodilatation and inhibits platelet aggregation and also has anti-inflammatory effect by inhibiting Fxa and thrombin [9].

Recently described anticoagulant system and is a plasma enzyme which is produced in the liver inhibits FXa in the presence of Protein Z-dependent protease inhibitor and calcium and this increases 100-fold in the presence of PZ. it's a vitamin K-dependent glycoprotein and acts as a cofactor for ZPI. Protein C pathway is a circulating vitamin K dependent serine protease, synthesized in the liver. It is converted to Activated Protein C (APC) by thrombin APC which is a potent anticoagulant, also has anti-inflammatory and anti-apoptotic properties, increases 1000 fold APC production by binding of TM with thrombin. Tissue factor pathway inhibitor is a polypeptide produced by endothelial cells, inhibitor of the tissue factor pathway of coagulation [9].

Anticoagulation in routine homeostatic conditions, thrombus formation and destruction have been maintained by human body, which equilibration is maintained by a complex interaction between vascular endothelium, platelets and the fibrinolytic system. The interaction between the contact activation pathway (intrinsic pathway) and the tissue factor pathway this leads to the formation of X to Xa that is start for the common pathway which converts prothrombin to thrombin as a consequence catalyzes the fibrin formation. The stable clot formation occurred by stabilizing the aggregated plates [10].

Heparin is sulphated polysaccharide which have 3000 to 30,000Da range of molecular weight mean was 15000Da. Hepa-

rin produces major anticoagulant effect by inactivating thrombin and activating factor X (FXa), by an Antithrombin (AT) dependent mechanism. Inhibition of thrombin, heparin should bind to both the coagulation enzyme and antithrombin through a high-affinity pentasaccharide existing on about a third of heparin molecule and the binding of enzyme is not required for inhibition of factor Xa. The bridge between thrombin and antithrombin is fewer than 18 saccharides in heparin molecules which are unable to inhibit thrombin. Inhibition of factor Xa via AT is occurred when very small heparin fragments containing penta saccharide sequence. by inactivating thrombin, heparin prevent fibrin formation and also inhibit-induced activation of platelets of factor V and VIII [11].

Low molecular weight of heparins is derived products from heparin by depolymerisation of chemical and enzymatic interactions, to yield approximately one third of the heparin size. LMWH have a molecular weight mean of 4500 to 5000 Da with a distribution range is 1000 to 10000 Da. comparison of Unfractionated Heparin (UFH) with LMWH, LMWHs have lower the capacity to inactive thrombin after the smaller fragments cannot bind simultaneously to AT and thrombin. Bridging between AT and factor Xa minor demanding for anti-factor Xa activity, the smaller fragments inactive for factor Xa almost as larger molecules. Hence effectively all heparin molecules carry at least 18 saccharide units. and the ratio is 1:1 of UFH has an anti-factor Xa to anti-factor IIa and for commercial LMWHs have angti-factor Xa to anti-factor IIa ratios between 2:1 and 4:1. LMWH have longer plasma half-life compared with UFH and also lower risk of heparin induced thrombocytopenia and osteopenia. Examples of Low molecular weight heparins are parenterally administered drug which include Enoxaparin, Dalteparin and tinzaparin. Enoxaparin has better advantage compare to heparin because of its bioavailability, the molecular weight 4000-5000 daltons and 1mg of enoxaparin is equal to 100 units of anti-Xa activity. It binds and enhance (potentiate) ATIII, a serine protease inhibitor which forms complex that irreversibly inactivates factor Xa and enoxaparin has less activity against factor IIa when comparable to unfractionated heparin [12]. Dalteparin molecular weight is 6000 daltons and most of the material (90%) within the range of 2000-9000.It is more predictable and greater bioavailability and have antithrombic properties, also enhances the inhibition of factor Xa and thrombin which also affects slightly on clotting time, Example is APTT (Activated partial thromboplastin. Tinzaparin molecular weight 6500 g/mol, acts as a potent co-inhibitor of several activated coagulation factor, mainly factor Xa and II a (thrombin) [13].

Plant based medicinal plants are used for treating numerous diseases since earliest times. These medicinal plants are having numerous advantages which are the source for several conventional drugs, in diseases like stroke and ischemia [14]. Medicinal Plants have been used for treatment of human ailment and many disorders since ancient era and it is gradually becoming popular throughout the world. The uses of allopathic medicines are overstated which leads side effects and adverse reaction of drugs for avoiding those reactions, medicinal plants were advisable. Main objective is study of the effect of herbal drugs on anticoagulant therapy in secondary metabolites of medicinal plants which play an important role in world population by participating in medical care of public health and have been source of inspiration for several major pharmaceutical drugs, Many Literatures are showing that *Thymus vulgaris*, *Cyamopsis cassia, Thymus atlanticus*, *Selaginella*, *Terminalia belerica, Tulbaghia violacae, Tridax procumbens, Porana volubilis, panax notoginseng, Petroselinum crispum, Green and Brown algae, Grape seed, Gracilaria debilis, Erigeron Canadensis, Fagonia Arabica, Codium fragile, Cyamopsis tetragonoloba, Bauhinia forficate, Careya arborea, Artemisia dracunculus, Angelica Shikokiana, Syzygium cumini, Melastoma malabathricum, Rhaponticum acaule, Cinnamomum cassia* have showed anti-coagulant properties [2].

***Jatropha Gossypiifolia* L:** It is a medicinal plant belongs to Euphorbiaceae and the leaves of this plant frequently used as an anticoagulant medicine and this activity was evaluated by Prothrombin Time (PT) and which activated Partial Thromboplastin Time (aPTT) tests. Decoction extraction method is one of the liquid-liquid partition polarity analyses by Thin Layer Chromatography (TLC) for extraction of crude leaf and quantification of sugars, phenolic compounds and proteins was analysed by spectrophotometric analysis. In result demonstration aqual leaf extract of vegetal species *Jatropha gossypiifolia* having significant anticoagulant activity by using bioguided fractionation the main fraction is identified for anticoagulant activity, this fraction was proved to be good source of antioxidant activity too, and this is frequently used in modern medicine for treatment of various cardiovascular diseases [15].

Artemisia dracunculus L: It belongs to a family Asteraceae separated the leaves from this plant (*Folium dracunculi*) and dried in a thin layer ventilated plate which has far from direct sunlight, stored the dried leaves in a dark paper bags then powdered the leaves before starting of experiment. powder sample was extracted which yields F1 Fraction and aqueous fraction while evaporating of aqueous fraction F2 fraction will be formed, removed the excess solvent under reduced pressure to evaporate organic fraction Fraction F3. Coumarin compounds were identified and a fraction performance was done by Thin Layer Chromatography (TLC). Qualitative and quantitative analysis of the coumarin derivatives were performed by High Pressure Liquid Chromatography (HPLC). this plant owing to its phytochemical composition which becomes very fascinating in the possible preparation of medicinal products against diseases caused by thromboembolic events. compared of the other phenolic natural products this plant also contains potential therapeutic effect of coumarin derivatives [16].

***Datura Stramonium*:** Datura owned by solanaceae family, mainy anticoagulant drugs show some adverse reactions and some expensive hence, ancient times people explore alternative anticoagulants, which plants are safer source of medicine and datura stramonium flower extracts are used as a standard experimental model for anticoagulant extract. Datura flowers were air dried at room temperature then crushed with followed by dried intervals then this crushed powder was treated with methanol and chloroform for further analysis, the coagulation process mainly occurs by complex interactions of cellular and molecular components-intrinsic and extrinsic pathway found to a balance between procoagulants and anticoagulants by group 4 crude concentration source. The leaf extracted evaluation of anticoagulant in blood sample of normal individuals was measured by principle of prothrombin time [17].

***Artemisia dracunculus*:** The leaf extract of Artemisia dracunculus is potentially useful for decreasing the similarity of coronary diseases in human, also having good experimental studies conducted in rats. Artemisia dracunculus belongs to family is Asteraceae, 100gms of Artemisia leaves were powdered and added 100ml of n-hexane, 600ml of chloroform, and 600ml of methanol in a Soxhlet's apparatus. Performed this extraction

for 8 hours, three separate dry extracts were obtained hexane-chloroform, methanol extract and separated the herbal residue from water after steam distillation which give a vegetable matrix, this herbal residue was split into 4 parts chloroform extract, aqueous extract basified to pH9, aqueous extract basified to pH11 and diethyl ether extract. Essential oil distillation from this plant was analyzed on coumarin presence as well giving organic fraction with water and diethyl ether fraction this identification of coumarin compounds in different extracts and fractions were performed by TLC technique. Qualitative and quantitative analyses of the coumarin derivatives in methanol extract were performed by high pressure liquid chromatography [16].

***Ainsliaea fragrans*:** this is the safe and effective anticoagulant medicinal plant fragrans Coumarin derivatives are main class of C6-C3 plant metabolites which show a various bioactivity, currently available anticoagulants are coumarins such as warfarin, aceoumarol and dicoumarol. Collected whole plant from Shiyan city and the plant were percolated with 95% industrial ethanol at room temperature, further separation with petroleum ether, ethyl alcohol and n-butanol successively by using column chromatography. In vitro anticoagulant activity was performed by using automatic anti coagulative instrument [18].

Medicinal plants exist high in numerous active constitutions of therapeutic value which are used as an admirable source of remedy for treatment of human diseases. the irreversible effect of present-day remedies and improving drug resistance have enlarging our reliance on medicinal plants for a grassy remedy in opposition to the deadly infectious diseases in modern days about 40 percent of people are reporting to use of the herb to treat medical illness. Tridax procumbens commonly known as Ghamra and Coat button is a greatest potent species of wide spread weed. It belongs to Asteraceae family, green perennial with woody base plant, which grows in many tropical countries like America, Africa, Australia and Asia. In India, almost the whole part of the country of India it has found.

Traditionally using Tridax in so many ways like Anti-coagulant, Anemia, Inflammation, vaginitis, gastrointestinal disorders, diarrhea, Stomach pain, cold, hepatopathies, skin infections, mucosal inflammations, dysentery, diarrhea, cataracts, and also been known for its anti-coagulant, insect repellent and anti-fungal activity, alos used as bio-adsorbant for the elimination of hexavalent chromium from industrial waste water act as a plant of industrial significance [22].

Tridax procumbens: it is a common plant native from America, Australia and Asia and which is widely used in the Indian ayurvedic system and ancient medicinal plant product as procoagulant for stop bleeding which enhance wound healing property, various extraction processes will give various activity products like anticoagulant, antioxidant, anti-inflammatory, anticancer, antimicrobial, antihypertensive etc. plant extraction process involves, shade- dried, leaves and stems were collected weighed then blended for homogenization and extraction was carried out by stirring the crushed plant using distilled water on a magnetic stirrer at 500rpm for 24 hrs in room temperature. This extract was flittered using muslin cloth and centrifuges the filtrate at 8000rpm/24min at 40C then supernatant was collected and lyophilized, lyophilized powder was weighed and stored at -200C for further use [9]. Crushed the tridax leaves collected the extracted material in petri dishes air dried the crude extract in different concentrations (0.2, 0.4, 0.6, 0.8 and 1mg) for analysis [20].

Tridax Extraction Methods

Tridax about 30gms were dissolved in 50ml of water and methanol respectively kept on shaker for 48hours, then filters the extracts and lyophilized to get in powder form. Use 1mg/ml of the extract as a stock.

Crushed 100gms and 50gms of plant and leaves respectively then placed in soxhlet apparatus carryout the ethanol as solvent for 12-14 hours. Filter the extract with ethanol then distilled off using the rotary evaporator for removing excess solvent. Different concentrations (100, 200 and 500 µg/ml) were prepared with ethanol as a solvent.

Crushed 25gms of whole plant material/leaves soaked in 25ml, 50ml and 100ml of distilled water respectively for 24 hours. Further filter the extract using muslin cloth.

Final volumes were corrected to viz.25ml, 50ml and 100ml by washing residue with distilled water [21].

Air dried and extracted flower and aerial parts of tridax by maceration for 7 days using 1:1 ratio of methanol and chloroform in room temperature condition. Concentrated solvent was diluted with water and stand for 7 days/40C. Decant the chlorophyll by decantation, extract the aqueous layer with n-hexane followed by ethyl acetate which results organic layer and aqueous layer were concentrated to obtain the parallel extracts. Dried leaves of *T. procumbens* were placed in a Soxhlet apparatus, performed the extraction with 500mL of methanol at 64^0C/24 hours then filter the extraction through a whatmann filter paper 41, and then extracted solution was concentrated to give dryness to the methanol extract. Extracted product was stored at 4^0C for further use [22]. Analysis of crude extracts was done by gas chromatography utilizing mass detector, ultra-one column with cross linked methyl silicon gum. Other some components identified from crude extracts with the chromatogram with a data base and depends of solvent used the extracted product may vary some of the examples were given below table [23].

The potential anticoagulant plant details are listed below in table format [21]. How potentially is the herbal drug working than compared to the established anticoagulant as stated of WHO (World health Organisation) herbal medicine means Finished labelled medicinal products which contains underground parts of plants, active ingredient material and other plant material too (gums, essential oils, fatty oil, resins and juices) with the combination of crude state or as plant preparations. Medicine that contains plant material which is combined with chemically defined active substances, plant isolated material these are not considered as herbal drugs. Combinations of pharmacologically active plant substances are present in herbal medicines, [24] tremendously herbal medicinal products and supplements are

S.No.	Solvent	Plant Material	Type of Product Extracted
1	n-hexane	Flowers, aerial parts	Hydrocarbons and long-chain fatty acids, Neophytadiene and hexadecanoic.
2	Ethyl acetate	Aerial parts	Fatty acids, Carboxylic acids, aromatic compounds, , polysubstituted phenols, thiols and polyaromatic.
3	Water	Aerial part, flowers	i) Complex mixture of sugars: d-sucrose, galactose, fructose, galacturonic acid, glucose, d-annopyranoside, d-alose, xylitol, and arabinopyranose. ii) Other derivatives of pyrimidine, carbazole, indole, and short chain-carboxylic acids.

S.No	Medicinal Plant	Family	Part	Activity
1	*Thymus atlanticus*	Lamiaceae	Aerial part, Flowering period,	Anti-Coagulant, Folk medicine for thrombosis, Cardiovascular diseases, Anti-inflammatory, antifungal, Antioxidant, Antimicrobial activity.
2	*Selaginella* (sanjeevini)	Selaginellaceae	Leaf extract	Anti-Coagulant
3	*Terminalia belerica*	Combratiaceae	Fruits	Anti-Coagulant, Anti thromotic, Thrombolytic activity, Antihelmintic, Astringent, Expectorant and Antiseptic
4	*Tulbaghia violaceae*	Alliaceae	Leaf & Bulb	Anticoagulant, Hypertension, Asthma, Cold, Fever and Paralysis
5	*Tridax procumbens*	Asteraceae	Leaf Extract	Excellent anticoagulant, Antifungal, Antibacterial, Insect repellent, Indiarrhea and Dysnentry
6	*Porana volubilis*	Convolvulaceae	Wired branches	Anticoagulant
7	*Panax notoginseng*	Araliaceae	Root extract	Anti-Coagulant, Folk medicine, cardiovascular diseases and Antioxidant
8	*Petroselinum crispum*	Apiaceae	Leaf extract	Anticoagulant, cardiovascular diseases, diabetes and renal diseases
9	*Leucus indica*	Lamiaceae	Leaf extract	Thrombolytic activity & Anticoagulant property, Wound healer, Vermifuge, and Sedative
	Macroalgae ulvarigida	Ulvaceae	Total part	Anticoagulant, Antioxidant, Antiviral and Antitumor
10	*Jatropha curcas*	Euphorbiaceae	Leaves and roots and Latex	Anticoagulant, for cancer therapy, as diuretic, Haemostatic and Purgative
11	*Jatropha gossypifolia*	Euphorbiaceae	Leaves, stem, roots and latex	Anticoagulant, Antihypertensive, Anti-inflammatory, Analgesic, Antipyretic, Haemostatic, Antianemic
12	*Green and Brown algae*	Phaeophyceae	Complete Part	Anticoagulant
13	*Grape Plant*	Vitacacae	Seed	Anticoagulant, Cardioprotective, Antitumor, Immunomodulator and Antioxidant
14	*Gracilaria debilis*	Gracilariaceae	Complete Part	Anticoagulant, Antitumour, Antioxidant, Anti-inflammatory, Immunomodulatory, Antiviral, Antibacterial and Antilipidemic
15	*Ferulago carduchoram*	Apiacae	Aerial parts	Anticoagulant, sedative digestive
16	*Erigeron canadensis*	Asteraceae	lowering part, flowering part	Anticoagulant, rich antioxidant, Anthelmintic, diuretic and Antidiarrhoeic
17	*Fagonia arabica*	Zygophyllaceae	Small spiny under shrub with more or less prostrate, branches	Anticoagulant, Thrombolytic, Antioxidant, Mollucidal activities, also used in liver empowerment and cancer treatment
18	*Codium fragile*	Codiaceae	Complete Part	Anticoagulant
19	*Cyamopsis tetragonoloba*	Fabaceae	Complete Part	Anticoagulant activity by exhibiting anti-thrombin activity
20	*Bauhinia forficate*	Fabaceae	Complete Part	Anticoagulant and fibrinolytic activity
21	*Careya arborea*	Lecythidaceae	Bark	Anticoagulant
22	*Artemisia dracunculus*	Asteraceae	Leaf extract	Anticoagulant
23	*Angelica shikokiana*	Apiaceae	Aerial parts	Potent anticoagulant and Antiplatelete
24	*Syzygium cumini*	Myrtaceae	Leaves	Anticoagulant, Antioxidant, Hypoglycemic and Anti-inflammatory
25	*Melastoma malabathricum*	Melastomataceae	Leaf extract	Anticoagulant, Diarrhoea, dysentery and wound healing
26	*Rhaponticum acaule*	Asteraceae	Flowering part	Alternative to existing Anticoagulants
27	*Cinnamomum cassia*	Lauraceae	Complete Plant	Strong anti-platelets and Anticoagulant activity

increased from past three decades that has not less than 80% of population relaying on them because of part to health care. Even though these herbal medicine also involving in potential therapies for good efficacy and also many of herbal medicines are established with low contraindications and adverse reactions which are higher safe and rational use, that relevant regulatory authorities put in place for appropriate measures for the protection of public health by ensuring that all herbal medicines are safe and of suitable quality [25].

Current system of medicine come out as the primary choice for the treatment of almost all types of health related issues even though it is mainly based on the target based approach which eventually leads to further future side effects. However, patients with such chronic illnesses example as cancer directly/indirectly undergo combinational therapy that is with or without knowledge of physicians, mainly to potential herb-drug interactions [8].

Nearly one in six adults in the U.S proceed with prescribed drugs which are connected to at least one herbal remedy, although herbal medicines are reasoning to cause fewer side effects and toxic effects than conventional medicine due to the less concentration of active components. Only few countries such as Germany, Sweden, Australia and France implemented herbal remedies. Warfarin a drug derived from the sweet clover plant which breaks the vitamin K-dependent posttranslational modification of coagulation proteins II, VII, IX and X via inhibition of vitamin K epoxide reductase. This drug is adjusted as stated to target prothrombin international normalized ratios and this varies according to the sign of treatment, for some patients on chronic anticoagulant therapy shows completely stable PT INR values with mainly interaction with certain drugs and food [27].

Thousand years ago people have been using traditional medicines hence Ayurveda having strong base in Indian history, so in future opportunities with plant based anticoagulants are play an important role in preventing and treating of so many human diseases, however plants having plenty of medicinal values and for the development of new herbal drugs medicinal plants are act as a potential source. in current century medicinal plants have been considered as a promising future medicine for the

management of human health care [28]. Hence anti coagulants play vital role for the prevention and treatment of the thromboembolic disorders, anti-coagulants heparin, Vitamin-K antagonists and their derivatives play major role in the clinical setting from past five decades. Plants serve as the alternative source for the development of new anti-coagulant drug because of their biological activity, for example the methanolic bark extract of *Careya arborea* shows anticoagulant activitiy when comparing with the standard warfarin [29].

Venous thrombosis is 3% in the general population. In the U.S approximately 100,000-300,000 people die from venous thrombosis every year and number of hospital admissions is increases 500,000 per year. In recent years' thrombosis has been standing up. There are 500,000 deaths per every year due to venous thrombosis in Europe. As per IMS data showing global market sales of anti-coagulant drugs were USD$23.5 billion in 2013, considering for 53.1% of the sales of drugs for cardiovascular diseases and 2.7% of global drug sales. Saturation state was observed in entire global markets of anti-thrombotic drugs are gradually expanded with market sales expected to reach USD$25.9 billion in 2018. Anti-platelet drugs are performed best in globally with reaching $9.5 billion, which are accounting for 40.4% of all anti-coagulant drug sales. Another projection is anti-platelet aggregation drugs will decline to 19.3% in 2018. With the appearance of new direct thrombin inhibitor and direct coagulation factor Xa inhibitors, anti-clotting drugs are face new challenges in future markets. Total global drug sales of direct thrombin inhibitors were USD$2.4 billion in 2013, this is the third largest percentage of anti-thrombotic drugs which are accounting for 10.4% of all anti-thrombotic drug sales. Dabigatran etexilee leads 73.6%, direct coagulation factor Xa inhibitors were USD$2.1 billion in 2013 (9.6% sale), Rivaroxaban accounted for 93.6% of similar drug sales. The sales of direct coagulation factor Xa inhibitor drugs are expected to account for 32.6% of anti-thrombotic drugs sales in 2018 [30].

Herbal medicines are used to treating of variety of diseases; these herbal preparations have been describing to cause variations in clotting time, which is mainly done by disruption of the coagulation cascade. Some anti-coagulant plant sources are as follows: *B. Vulgaris* (B.V) and *T. Polium* (TP) flowers were imported and purchased from herbal marketing company in Iran, and *O. Stamineus* (OS) leaves were obtained from University Putra Malaysia Taman Pertanian University,. (Note: Te voucher specimens were identified by Dr. Mohd Firdaus Ismail of Institute of Biosciences, UPM) [31]. Commercially available total extract of the Dhamasa herb FA was purchased from Innocon Foods (Pune, India) [32. Crasssocephalum crepidioides was locally obtained from farms in Ilisan-remo, Ogun state, South Western Nigeria.M.malabathricum Linn. matured leaves were collected between September and October 2009 from Lebun silicon, University Putra Malaysia, in Serdang, Selangor, Malaysia [33].

Aqueous Extraction: Collect leaves cut into small pieces and dried under sun shade. Complete dried pieces were ground into small pieces and let to be dried under the sunshade. Weigh required amount of powder and soaked in distilled water as 1:10 dilution at room temperature. Stir the mixture by using magnetic stirrer in a conical flask and left it for overnight, then the mixture was filtered by using Whatman No.1 filter paper and collects the supernatant. Use rotary evaporator to evaporate the filtrates under vacuum reduced pressure at 60^0C for produce a thick syrupy crude extract (mass crude extract). Store the crude extract at -20^0C in scott's bottle for further analysis [34].

Methanol Extraction: Leaves were dried in oven at forty degrees and crushed into powder using an electric blender then stored the grounded material in the refrigerator. The ground sample was soaked in 70% methanol using a ratio 1:8 (W/V) for 48 hours at room temperature followed my intermittent shaking. Filter the suspension with a fine muslin cloth followed by Whatman No 1 filter paper. Evaporate the crude extract under reduced pressure by using a rotary evaporator then dried at 400C in oven and stored at 40C until further analysis. The dried extract powder was reconstituted with water and place across to solvent partitioning using Butanol, Ethyl acetate and Hexane sequentially [35].

Steam Distillation method: Suitable distillation method for extraction of volatile components, which can be extracted with steam without using water which are insoluble in water. Boiling points of these components are more than 1000C. In this distillation vapour pressure of each component is same. Sublimation Method: The process of conversion of solid material into steam without melting after application of heat is known as sublimation method. Natural chemicals having sublimation properties can be extracted directly by sublimation method, for example extraction of caffeine from tea and camphor from camphor wood.

Solvent Extraction: Separation of desired natural products from raw material extraction is the first step. Extraction methods are several types like solvent extraction, Pressing, distillation and sublimation method. Among all these solvent extraction is preferable method and widely used technique. In solvent extraction when solvent is penetrates into the solid matrix, solute dissolves in the solvent and this solute is diffused out of the solid matrix and finally the extracted solutes are collected [36].

Isolation and purification of desired plant substances of plant extracts are purified into monomers either by physical and chemical methods classification as follows: 1) Solvent Method (acid and basic solvent method, polarity gradient extraction method) 2) precipitation method (solvent precipitation method, exclusive reagent precipitation method 3) Salting Out Method 4) Dialysis Method 5) Fractional Distillation Method 6) Crystallization Method 7) Classical Chromatographic Methods (adsorption chromatography, gel chromatography exclusion chromatography, molecular sieve chromatography, ion exchange chromatography, macro porous adsorption resin chromatography, partition chromatography 7) New Technologies and Methods (high performance liquid chromatography, droplet counter-current chromatography high speed counter-current chromatography ,high performance capillary electrophoresis, affinity chromatography) [37].

Physicochemical parameters were determined as per the Unani Pharmacopiea of India Those properties are as listed below: [38].

Characterization of purified samples widely used analytical techniques are usually extracts combination of various types of bioactive compounds/phytochemicals with different polarities, different separation techniques such as column chromatography, TLC, sephadex chromatography and HPLC used to obtain pure compounds, this obtained pure compounds are then used for the structure determination and estimation of biological activity. Among these non-chromatography techniques such as immunoassay-this is used for monoclonal antibodies (MAbs), Fourier-Transform Infrared Spectroscopy (FTIR), phytochemical screening assay can also be used to obtain and facilitate the

identification of the bioactive compounds [39]. Fresh leaves of Tridax were taken rinsed with distilled water allowed to air dry by availing the usage of homogenizer, 250gms of the leaves were homogenized and extracted in 500mL distilled water. Centrifuged the homogenate and carefully decanted the supernatant to get an aqueous free extract which are free from suspended particles. The effect of aqueous extract on blood coagulation was explored to verify the claim that extract can be used to manage bleeding. This method was done by examining the graded amounts of the extract on clotting time and heat treated extract on bleeding time this testing was done using duke method with respect to the heat treated extract, Upon completion of this method mean bleeding time was found to be with addition of extract (control) was 1.400.16 minutes and without adding control (extract) was found to be 2.490.02 minutes in consequences a 57% decrease has observed compare to normal bleeding time. Hence it was observed that the Tridax extract promotes blood coagulation and additionally which retain its activity even after heating too [40].

High-performance liquid chromatography technique is extensively used for the separation of natural products, robust and versatile technique. Main choice for fingerprinting study for the quality control of medicinal plants, and also degree of separation mainly determined by the choice of stationary phase and mobile phase. High pressure up to 400 bars is required to elute the analyte through column before they pass through a Diode Array Detector (DAD). It is useful for compounds that cannot be vaporized or that decomposes under high pressure and which provide a good complement to gas chromatography for detection of compounds [41].

Estimation of impurity concentration was estimated by Lowry, UV and lipid method. In this method BSA was used as standard. The protein concentration of TPE was measured by comparing with known BSA concentration, and the statistical analysis tools for data analysis and interpretation of results are expressed as the means ±standard error of mean data were analysed by one way. ANOVA and post hoc Dunnett t-test for multiple comparisons. $P<0.05$ was accepted as statistical significant.

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