Introduction

1.1 Significance and approaches to drug discovery from natural products

From the ancient times natural products have been recognized as a source of medicines worldwide for treating and preventing human diseases [1-5]. They have been derived from various sources including plants, microorganisms, marine organisms, vertebrates and invertebrates [6-8]. Despite major scientific and technological progress in combinatorial chemistry, drugs derived from natural products still make an enormous contribution to drug discovery today, and about half of the pharmaceuticals in use today are derived from natural products [6,9,10]. Natural sources possess chemical diversity; therefore, they serve as an important reservoir of bioactive leads in the development of new drugs by providing novel templates, as well as patterns for structural modifications to produce more potent and safer drugs [4,10,11].

Natural products and their derivatives represent over 60% of all drugs clinically used world-wide whereas natural products from medicinal plants alone contribute to 25% of the total drugs [12-15]. Natural products and related drugs are reportedly used, for example, as antibacterial, anticancer, anticoagulant, antiparasitic and immuno-suppressant agents to treat 87% of all categorized human diseases [4,6,7,12,16]. More than 28% of new chemical entities introduced into the market are derived from natural products [17,18].

More than 100 compounds specifically, anti cancer and anti-infective agents, which are derived from natural products, are undergoing clinical trials at present and at least 100 natural products-derived compounds (primarily plant or microbial sources) are in preclinical development stage [19].

The value of natural products can be assessed using 3 criteria- (i) the rate of introduction of new chemical entities of wide structural diversity, including serving as templates for semi-synthetic and total synthetic modification [6,20], (ii) the number of diseases treated or prevented by these substances [21], and (iii) their frequency of use in the treatment of various diseases [20].

Natural products also serve as pharmacological tools, for example digitoxin from fox glove helped to elucidate the role of the sodium-potassium-ATPase pump in the human body; or morphine isolated from poppy seeds was used to explore the way endogenous opoids affect their receptors. In addition, muscarine, nicotine and tubocurarine helped to identify the different types of acetylcholine receptors [19].

Table1.1 shows some examples of drugs derived from plant sources that are currently used in clinical practice [4,22,23].

Noteworthy, drugs derived from natural sources have different modes of action as well as they are employed to treat different types of disease. Success in natural product research from plant sources demand scareful plant selection, based on several approaches such as, a) chemotaxonomic data; b) information from traditional medicine which is based on i) plants used in an organized traditional medicine system, ii) herbalism, folklore, and shamanism, iii) use of databases; c) field observations; d) random selection by chemical screening and; e) follow-up of biological activity reports.

One principal approach in the isolation of new lead compounds is bioactivity-guided isolation, in which pharmacological or biological assays are used to target the isolation of bioactive constituents [24,25]. Another effective approach is to isolate the bioactive constituents from extracts by metabolic profiling which involves the detailed analysis the chemical composition of an extract by chromatographic-spectroscopic techniques and a subsequent activity evaluation [26].

Table 1.1. Some drugs developed from natural products.

Drug	Medical use	Source	Mechanism of action	Reference
Aspirin	Analgesic,anti-inflammatory, antipyretic	*Salix alba*	Inhibition of COX	[27,28]
Digoxin	For atrial fibrillation and CHF	*Digitalis purpurea*	Inhibition of the Na^+/K^+ATPase membrane	[29]
Quinine	Malaria prophylaxis	*Chincona*	Protein synthesis inhibition	[30]
Reserpine	Hypertension	*Rauwolfia serpentina*	irreversibly blocks the vesicular monoamine transporter (VMAT)	[31,32]
Vincristine	Leukemia	*Catharanthus roseus*	Vincristine binds to the microtubular proteins of the mitotic spindle, leading to crystallization of the microtubule and mitotic arrest or cell death	[33,34]
Taxol	Ovarian cancer	*Taxus brevifolia*	Stabilizes microtubule disaseembly	[35,36]

COX= Cyclooxygenase

1.1.1Bioactivity-guided isolation

Bioassay-guided isolation is a multidisciplinary approach to drug discovery, which involves the evaluation of the biological activity of a crude extract from the natural resource followed by fractionation to isolate the active ingredient (Figure1.1). The individual fractions are then tested for their biological activity or pharmacological property. The active fraction(s) undergo further fractionation, until active fractions or pure compounds are obtained. Different technologies are used in this fractionation process, such as column chromatography, flash chromatography, vacuum liquid chromatography, thin layer chromatography, semi-preparative and preparative HPLC [37,38]. Bioactivity-guided fractionation has resulted in most of the biologically active natural products currently in use [39].

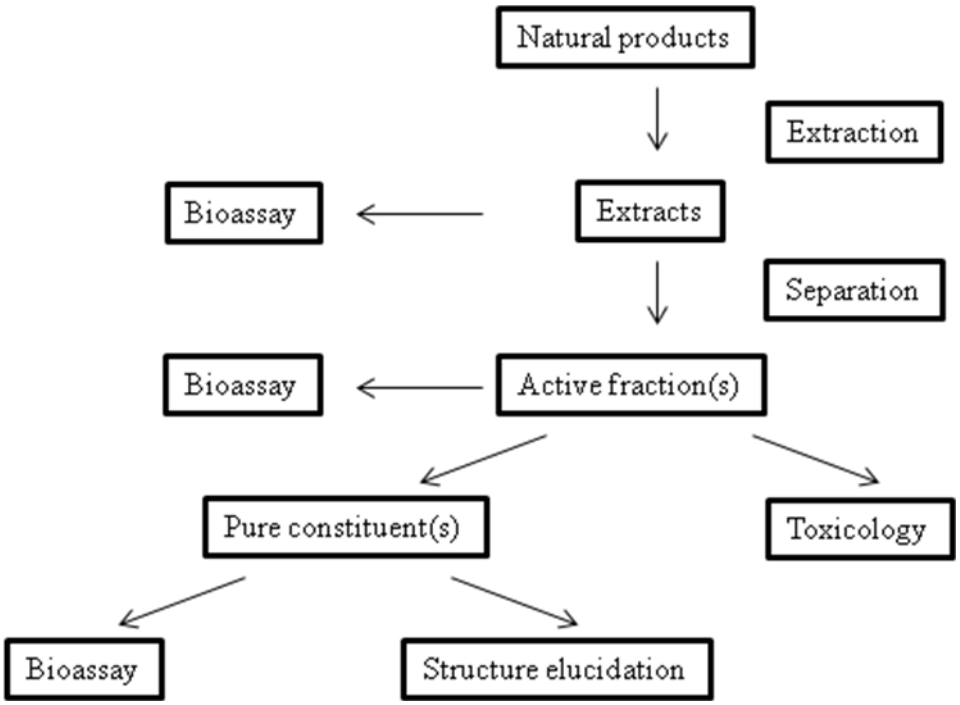

Fig. 1.1 Overview on bioactivity-guided isolation.

1.1.2 Metabolic profiling

Metabolic profiling provides information on absolute or relative quantities of metabolites present in natural source extracts, thus allowing for their detection and isolation [40]. Furthermore, it provides information on the chemical composition of

extracts, with known and unknown spectra, which can lead to the isolation and spectroscopic identification of new bioactive compounds [41]. A metabolic profile of a plant extract can be obtained using techniques such as, liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS) and capillary electrophoresis-mass spectrometry (CE-MS) [26].

1.2 Significance of medicinal plants in drug discovery

Plants have been utilized as medicines for centuries. To date, plants remain as the principal source of new drugs, new lead compounds, and new chemical entities [4,17,42]. Plant-based medicinal systems play a significant role in the healthcare systems of almost all the countries of the world. According to the World Health Organization (WHO), about 65% of the world's population and 80% of developing countries' population depend primarily on about 85% of plant-derived traditional medicines [17,20,43]. Approximately, 80% of 122 pure compounds on the market derived from 94 plant species were originally used for the same or related ethnomedical purposes [4,17]. Plant studies indicate that there are about 250,000 to 350,000 plant species identified so far, and of the about 35,000 have been used for medicinal purpose across the world [44,45].

Some of the examples of plants serve as a source of bioactive compounds for direct use as drugs are- sennosides A&B from *Cassiaa culifolia* and *Cassia angustifolia*, vincristine and vinblastine from *Catharanthus roseus*, digitoxin from *Digitalis purpurea* and ephedrine from *Ephedrasinensis* [25,46]. Medicinal plants also led to the isolation of novel bioactive compounds which in turn serve as lead compounds for semi synthesis of patent able entities with enhanced activity and/or reduced toxicity [46].

These drugs are employed for the treatment of a broad array of diseases. In addition, bioactive compounds obtained from plants also serve as pharmacological tools, for example, lysergic acid diethylamide, mescaline, and yohimbine [25]. Some examples of the plant derived lead compounds are discussed below. These examples show that medicinal plants represent an important source of bioactive lead compounds, possessing chemical diversity and as such providing core scaffolds for the discovery and development of drugs (Table 1.2).

Bronchodialator: Khellin, isolated from *Ammivisnaga* L., led to the development of chromolyn, a bronchodilator [4,47].

Anti-diabetic: Galegine, isolated from *Galega officinalis* L., served as the model substrate for the synthesis of metformin and other bisguanidines used in the treatment of diabetes [4,48].

Analgesic and anticoagulant: Codeine was isolated from *Papa versomniferum* and served as the model substrate for the development of the analgesics, meperidine, pentazocine, and propoxyphene [49,50]. In addition, salicin is a lead compound isolated from the bark of *Salix alba*, that led to the development of a potent pain killer and anticoagulant drug, aspirin [51,52].

Anti-hypertensive: Papaverine obtained from *Papa versomniferum*, formed the basis for the semi- synthetic anti-hypertensive drug, verapamil [25,53].

Anti-malarial: The anti-malarial drug, quinine was isolated from the bark of *Cinchona officinalis*, formed the basis for the synthesis of the anti-malarial drugs, chloroquine and mefloquine. Due to resistance to chloroquine and mefloquine in many tropical regions, Chinese scientists discovered an exciting new anti-malarial lead compound, artemisinin from *Artemisia annua* which had long been used in the treatment of malaria in Traditional Chinese Medicine [4,54]. Based on artemisinin, two promising analogues, OZ277 and an artemisinin dimeric analogue, with better efficacy and utility were synthesized which are now used for the treatment of malaria in many countries [4,55]

Anti-asthmatic: The anti-asthmatic drug, ephedrine, was isolated from *Ephedrasinica* and has formed the basis for the synthesis of anti-asthmatic beta agonists salbutamol and salmetrol [4].

Table 1.2. Lead compounds isolated from medicinal plants

Lead compound	Developed drug	Plant source	Pharmacological uses	References
Khellin	Chromolyn	*Ammivis naga*	Bronchodilator	[47]
Galegine	Metformin	*Galega officinalis*	Anti-diabetic	[48]
Codeine	Meperidine, pentazocine, and propoxyphene	*Papaver somniferu*	Analgesics	[49,50]
Salicin	Aspirin	*Salix alba*	Analgesic and anti- coagulant	[51,52]
Papaverine	Verapamil	*Papaver somniferum*	Anti-hypertensive	[53]
Ephedrine	Salbutamol and salmetrol	*Ephedra sinica*	Anti-asthmatic	[56]
Quinine	Chloroquine and mefloquine	*Cinchona officinalis*	Anti-malarial	[30,57]
Artemisinin	OZ277 and the artemisinin dimeric analogue	*Artemisia annua*	Anti-malarial	[55]

1.3 Cardiovascular diseases and cardiovascular drugs from medicinal plant

1.3.1 Cardiovascular diseases- A major burden of human life

Diseases of the heart and circulatory system (cardiovascular disease or CVD) are the major cause of mortality and morbidity, causing over 17.9 million deaths a year worldwide (WHO, 2017). More than 75% of CVD deaths occur in low-income and middle-income countries and furthermore 80% of all CVD deaths are due to heart attacks and strokes (WHO, 2017). With the turn of the century, cardiovascular diseases (CVDs) have become the leading cause of mortality in India [58-61]. In comparison with the people of European ancestry, CVD affects Indians at least a decade earlier and in their most productive midlife years [62,63]. For example, in Western populations only 23% of CVD deaths occur before the age of 70 years; in India, this number is 52% [64]. In addition, case fatality attributable to CVD in low-income countries, including India, appears to be much higher than in middle- and high-income countries [61,65]. The World Health Organization (WHO) has estimated that, with the current burden of CVD, India would lose $237 billion from the loss of productivity and spending on health care over a 10-year period (2005–2015) (WHO, 2011).Reasons for the high propensity to develop CVD, the high case fatality, and the high premature mortality include biological mechanisms, social determinants, and their interactions. Addressing this significant burden requires an understanding of both the biological and social determinants, and the complex dynamics underlying their interaction, as well.

Although cardiovascular medicine has achieved many breakthroughs, ischemic heart diseases (IHD), such as myocardial infarction (MI), heart failure (HF), thrombosis remain among the most important health challenges worldwide according to a report by WHO in 2007. MI, due to coronary artery disease (CAD), is currently one of the major contributors to the development of HF [58,66] Deep vein thrombosis is a common and important medical problem. Thrombus can spontaneously form in the larger veins of the lower limb, obstructing blood flow from the leg back to the heart. Rudolf Virchow described the classic triad of factors leading to formation of thrombus: abnormal blood flow, abnormal blood vessel wall and abnormal blood coagulation (clotting) function [67-69]. Vascular blockage occurs in the circulatory system as a consequence of blood clots formed due to failure in hemostasis as an imbalance occurs in fibrin formation (coagulation) and fibrin dissolution (fibrinolysis)[70,71]. Accumulation of these fibrin

clot in the blood vessels usually leads to thrombosis, which results in myocardial infarction and other cardiovascular diseases [72]

There are many factors, for example thrombin and plasmin, which contribute in the maintenance of the hemostasis; the former transform fibrinogen to fibrin whereas the latter is mainly responsible for dissolution of fibrin clot [70,71,73]. The formation of a fibrin clot inside the blood vessels owing to uncontrolled hydrolytic action of thrombin on fibrinogen, or overproduction of thrombin leads to a pathological condition known as disseminated intravascular coagulopathy (DIC) [74,75]. Moreover, in hyperfibrinogemia where blood fibrinogen level rises, the risk of myocardial infarction through blood coagulation enhances [76]. There are a number of mechanisms by which higher levels of fibrinogen causes thrombosis, including increased blood viscosity, increased fibre density of the fibrin clot, increased resistance of the fibrin clot to fibrinolysis, and altered mechanical properties of the fibrin clot. Increase of fibrinogen levels seem to be related to age, inflammatory processes, hematocrit, hypertension, glucose intolerance, cigarette smoking, and adiposity [76,77].

Thrombus formation in the “deep veins” of the lower limb is known as deep vein thrombosis, as opposed to superficial thrombophlebitis, which affects the superficial veins [78,79]. The distinction is important, as thrombus in the smaller, more superficial veins does not carry the same risk of propagation or embolism of deep vein thrombosis. Superficial thrombophlebitis is usually treated with anti inflammatory medication and requires no further investigation or treatment [80]. An exception to this is when the vein segment affected approaches a junction with a deep vein. Some practitioners may treat these in the same way as deep vein thrombosis because of the risk of extension into the deep system [78,80]. Although 90% of deep vein thrombosis occurs in the lower limbs, less commonly it does affect other areas of the body, including veins in the arm, head and neck, pelvis and rarely other locations [78-80].

Apart from arm vein thrombus, which is diagnosed similarly to leg thrombus, when other areas are affected the signs may be subtle and will require special tests for confirmation. Acutely, lower limb deep vein thrombosis causes swelling, pain and discoloration. The natural history is either progression or resolution, although in Western medicine the condition is either treated or closely observed. In the long term, it can cause “post-thrombotic syndrome,” which affects 23-60% of individuals with deep

vein thrombosis within 2 years [81,82]. The formation of thrombus damages the vein valves and increases venous collateral circulation, leading to venous hypertension (elevated pressure). Over time, this manifests as swelling, hyper pigmentation, venous ulcers, rash and discomfort. Venous ulcers heal slowly, are difficult to treat, and are a significant cost burden on healthcare systems [82].

The major mortality associated with deep vein thrombosis is when thrombus embolises (travels) towards the heart and lungs, where it lodges in the pulmonary arteries, known as pulmonary embolism [83-85]. It obstructs blood flow from the heart to the lung, which causes impaired blood oxygenation, resulting in symptoms of breathlessness, chest pain and cough. The blood must flow through the pulmonary circulation and in adults there is no bypass route; therefore, severe obstruction will cause hypoxia, (low blood oxygenation) hypotension (low blood pressure) and ultimately cardiac arrest leading to death. A distinction is made between proximal (above-knee) and distal (below-knee) lower limb deep vein thrombosis, because proximal thrombus is much more likely than distal to embolise [85-87]. There is approximately a 20% risk of distal thrombus progressing to involve the proximal veins [88-90] and the 3-month risk of thromboembolism in patients with suspected deep vein thrombosis but negative proximal leg ultrasound is around 1%. [91].

1.3.2 Classification of cardiovascular drugs

There is a range of commercial cardiovascular drugs available to clinically tackle various types CVDs such as tissue plasminogen activator, urokinase, streptokinase, Nattokinase, warfarin, heparin, argatroban, and rivaroxaban. Tissue plasminogen activator, for example urokinase and streptokinase hydrolyze the inactive plasminogen to an active plasmin [92,93] which subsequently degrades fibrin; Nattokinase commercially produced from bacterium *Bacillus natto* is a stong fibri(onogen)lytic serine protease; warfarin is a vitamin K inhibitor; heparin is an indirect thrombin inhibitor; argatroban is a direct thrombin inhibitor; and rivaroxaban exerts its anticoagulant activity by inhibiting Factor Xa [94-101].

Nevertheless, these drugs are associated with major adverse effects such as hemorrhage, recurrence at the site of the residual thrombosis, allergic reactions and vomiting and immunological responses [102-108]. Further, tissue plasminogen activators also have

low specificity towards the fibrin upon intravenous administration [58,102]. The seriousness of the cardiovascular diseases and life threatening adverse effects of the commercially available drugs has emphasized the need for the discovery of some potent anticoagulant components from natural resources which will be efficient, safe, and cost-effective.

Brief descriptions of the major category of cardiovascular drugs are summarized below.

1.3.2.1 Anticoagulant drugs

Anti-coagulants are molecules that prevent the blood from clotting. They inhibit the chemical process of proteolytic formation of the three-dimensional fibrin polymer. These include natural products such as high molecular weight heparin, low molecular weight heparin, coumarins and synthetic compounds, for example argatroban, dabigratran etexilate. Some examples of commercial anticoagulant drugs are listed in Table 1.3.

Table 1.3. Some examples of commercial anticoagulant drugs.

	Anticoagulants	Mechanism of action	Clinical Scenario	References
Heparin	UFH	Antithrombin and anti-Xa activity	DVT, PE, post –MI, UA/NSTEMI, coats stents	[109,110]
	LMWH (enoxaparin, dalteparin)	Mostly anti Xa activity	Similar to UFH, but easier doing, no monitoring and less	[109-111]
Direct thrombin and FXa inhibitors	Bivalirudin	Direct thrombin inhibitor	Used in patients with HIT	[112-114]
	Lepirirudin	Direct thrombin inhibitor	Unstable angina and PTCA for patients with HIT	[115-118]
	Argatroban	Direct thrombin inhibitor	Thrombosis	[100,119-122]
	Dabigatran	Direct thrombin inhibitor	After knee and hip replacement	[122-125]
	Ximelagatran	Binds to thrombin active site	Discontinued	[126-128]
	Rivaroxaban	Direct Xa inhibitor- binds to free and unbound Xa	After knee and hip replacement	[129-132]
	Fondaparinux	Synthetic inhibitor of factor Xa, even more selective than LWMH	Prophylaxis after knee, hip replacement	[133-135]
Vitamin K antagonist	Warfarin	Competitively inhibits vitamin K (II, VII, X protein C, protein S)	VTE prevention, DVT, after prosthetic heart valves, MI	[136-138]

DVT, Deep vein thrombosis; HIT, Heparin-induced thrombocytopenia; LMWH, Low molecular weight heparin; MI, myocardial infarction; PE, Pulmonary embolism; PTCA, Percutaneous transluminal coronaryangioplasty , UA, Unstable angina; NSTEMI, non–ST-segment elevation myocardial infarction; UFH, Unfractionated heparin; VTE, venous thromboembolism

1.3.2.2 Thrombolytic drugs

Thrombolytic drugs are used to dissolve (lyse) blood clots (thrombi). Table1.4 provides some examples of commercial thrombolytic drugs.

1.3.2.3 Antiplatelet drugs

Antiplatelet drug is a generic term, describing agents which decrease platelet aggregation and inhibit thrombus formation. Antiplatelet drugs are most effective for arterial clots that are composed largely of platelets. Table1.5 lists the commercial antiplatelet drugs used to prevent thrombosis associated CVDs.

1.3.3 Cardiovascular drugs from medicinal plants: Special example of Ayurveda

Over the centuries, many plants have been used in the treatment of cardiovascular disease; however, without knowing in detail about their active ingredients and mechanism of action. In recent years, the usage of natural herb as drug has been increasing because of potential beneficial effects of herbs without or less side effects in comparison to chemical/synthetic drugs. In some countries, approximately 80% of the drugs supplied to the pharmaceutical market have natural origin, so that now 90% of people in these countries use herbal medicines [45,139,140].

Herbal drugs constitute a major share of all the officially recognized systems of health in India *viz*. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy, except Allopathy. More than 70% of India's 1.1 billion populations still use these non-allopathic systems of medicine [141-143]. India has 15 Agroclimatic zones and 17000-18000 species of flowering plants of which 6000-7000 are estimated to have medicinal usage in folk and documented systems of medicine, like Ayurveda, Siddha, Unani and Homoeopathy. About 960 species of medicinal plants are estimated to be in trade of which 178 species have annual consumption levels in excess of 100 metric tons. Medicinal plants are not only a major resource base for the traditional medicine & herbal industry but also provide livelihood and health security to a large segment of Indian population (The National Medicinal Plants Board). They have been identified as medicinal plants because of their therapeutic properties contributing to the treatment of various ailments such as cancer, inflammation, diabetes, asthma, digestive problems, cardiac disorders [144-149].

Table 1.4. Some examples of commercial thrombolytic drugs

	Thrombolytic drugs	Mechanism of action	Clinical Scenario	References
Tissue plasminogen activator	Alteplase	More specific for clots because fibrin acts as cofactor for tPA activation of plasminogen	MI, thrombolytics in ACS, PE, administered as IV infusion	[150-152]
	Reteplase	Derivative of tPA with longer half life	Acute MI, pulmonary embolism	[150,153,154]
	Tenecteplase	Derivative of tPA with longer half life	Acute MI	[150,155]
Indirect fibrinolytic enzyme	Streptokinase	When complexed with plasminogen, can convert other plasminogen molecules into plasmin, massive lytic state	Rarely used	[156-159]
	Urokinase	Not specific for fibrin, so produces massive lytic state	Rarely used	[160,161]
Direct fibrinolytic enzymes	Nattokinase	Induces fibrinolysis by direct as well as indirect mode of action		[94,162,163]

ACS, Acute coronary syndrome; MI, Myocardial infarction; PE, Pulmonary embolism; tPA, tissue plasminogen activator; IV, Intravenous

Table 1.5. Some examples of commercial antiplatelet drugs.

	Antiplatelet drugs	Mechanism of action	Clinical Scenario	References
ADP receptor antagonists	Aspirin	Irreversible, non selective COX-1 and 2 inhibitors	Angina, acute MI, TIA, stroke	[28,164,165]
	Dypiridamole	Blocks uptake of adenosine, PDE inhibitors	Prophylaxis	[166-168]
	Clopidogrel	Irreversibly inhibits ADP receptors	Recent MI, unstable angina, recent stroke, PAD, post stenting	[169]
	Prasugrel	Irreversibly inhibits ADP receptors	Prevention of CV, thrombosis, PCI	[170]
	Ticagrelor	Reversibly inhibits ADP receptors	Prevention of CV, thrombosis after MI	[171,172]
GPIIb/IIIa inhibitors	Abciximab	Monoclonal Ab that block GPIIa/IIIb	During PCI	[173-175]
	Eptifibatide	Small molecule that block GPIIa/IIIb	During PCI	[176]
	Tirofiban	Small molecule that block GPIIa/IIIb	During PCI	[177]

Ab, Antibody; ADP, Adenosine diphosphate; COX, Cyclooxygenase; CV, Cardiovascular; GP, Glycoprotein; MI, Myocardial infarction; PAD, peripheral artery disease; PDE, Phosphodiesterase; PCI, coronary intervention; TIA, transient ischemic attack.

Although many plants suitable for commercial developments as therapeutics have already been recognized but they still remain unexplored scientifically. Proper scientific evaluation both pharmacologically and phytochemically are required for the development of safe and potent drugs. Some of the plants used in traditional medicine [147,149,178-181] are mentioned below.

i. Herbs which decrease blood pressure – *Rauwolfia serpentine* (Sarpagandha), *Fumaria indica* (Parpata), *Daucus carota* (Carrot seeds), *Cassia absus* (Chaksu), *Acorus calamus* (Vacha).

ii. Herbs which are Diuretic – *Tribulus terrestris* (Gokshura, Small Caltrops), *Boerha aviadiffusa* (Punarnava, Spreading hogweed), *Phyllanthu sniruri* (Bhumi amalaki), *Tinospora cordifolia* (Guduchi), *Taraxacum officinale* (Dugdha, Dandelion)

iii. Herbs which reduce serum cholesterol – *Commiphora mukul* (Guggulu)

iv. Herbs which act as cardiac tonics - *Terminalia cordifolia* (Arjuna), *Saussurea lappa* (Kushtha), *Sidacordi folia* (Bala), *Digitalis purpurea* (Hatapatri, Foxglove)

v. *Herbs which decrease platelet aggregation - Allium sativum (Rasona, Garlic)*

vi. Herbs which possess antistress/ general tonic properties- *Withania somnifera*(Ashwagandha), *Bacopamon niera* (Brahmi), *Evolvulusal sinoides* (Shankhpushpi)

1.4 Selection of Indian medicinal plants for the current study

In the present study, six medicinal plants (*Centella asiatica, Solanum indicum, Momordica charantia, Hibiscus rosasinensis, Allivum sativum; Leucas indica)* were selected for screening of anticoagulant, fibrinogenolytic, thrombolytic and platelet aggregation inhibition activities. Figure 1.2 depicts the photographs of the selected plants. All 6 plants have been used traditionally as antihypertensive, anti-inflammatory, for treatment of stomach ailments, fever, sinusitis, anti-infectious agents albeit none of them were explored for their anticoagulant activities. The decision to study the selected species was based on the availability of adequate quantity of plant material and has wide traditional uses.

1.4.1 Previous pharmacological and phytochemical evaluation of the selected medicinal plants

A literature search on the selected plants for this study revealed specific information on pharmacological and phytochemical activities (Table 1.6).

Table 1.6. Some pharmacological activities of the selected medicinal plants.

Plant Species	Pharmacological activities	References
Allivum sativum	Anticancer property, Prevention of cardiac ailments and atheroscherosis, antileishmanian, immunomodulatory activity	[182-185]
Centella asiatica	Used for treatment of vericous veins, chronic venous insufficiency, Psoriasis, minor wounds, bronchitis, diarrhoea, dysentery , epilepsy, gastritis, skin diseases, antimicrobial, antiviral, immunomodulatory effects,	[186-189]
Hibiscus rosasinensis	Peptic ulcer treatment, Hypoglycemic effect, antimicrobial, hair growth potential	[190-193]
Leucas indica	Antifungal, antioxidant, prostaglandin inhibitory activities, antimicrobial, antinociceptive, antipyretic, used for treatment of sinusitis	[194-199]
Momordica charantia	antidiabetic, abortifacient, anthelmintic, contraceptive, dysmenorrhea, eczema, emmenagogue, antimalarial, galactagogue, gout, jaundice, abdominal pain, kidney (stone), laxative, leprosy, leucorrhea, piles, pneumonia, psoriasis, purgative, rheumatism, fever and scabies	[199-203]
Solanum indicum	Analgesic, antipyretic, antiinflammotory, CNS depressant activity, antitumor activity, antimicrobial activity	[204-206]

CNS, Central nervous system.

Allivum sativum

Centella asiatica

Hibiscus roasinensis

Leucas indica

Momordica charantia

Solanum indicum

Fig.1.2 Photographs of the selected plants

1.5 Rationale of the project

As already discussed, cardiovascular disease is considered to be one of the life threatening diseases worldwide. The therapeutic agents to prevent and/or treat cardiovascular diseases are associated with adverse pharmacological effects in patients, for example, internal hemorrhage, severe anaphylactic reaction, and lacks specificity. Herbs with antithrombotic activities suggested that harnessing of our own natural resources such as medicinal plants might lead to discovery of novel therapeutic agents to treat thrombosis associated diseases [16,207-211]. Based on the ethnomedical information, chemical diversity, and current literature, Indian medicinal plants may serve as great source of new bioactive compounds. ***Unfortunately, there is no scientific validation to prove the pharmacological effect of the many of the traditionally used medicinal plant(s) in treating cardiovascular disorders.*** Therefore, in the present

investigation, an effort was given to investigate the thrombolytic, anticoagulant, fibrinogenolytic/fibrinolytic and anti-platelet properties of an unexplored plant(s) with an aim to develop a safer herbal drug to treat cardiovascular disorders.

1.6 Aims and objectives of the project

The overall aim of the PhD work is to identify and characterize new or previously uncharacterized anticoagulant, thrombolytic and/or antiplatelet fractions/protein/component(s) from medicinal plants of North-East India that were unexplored till date, and their mechanisms of anticoagulant action. Another important aim was to explore the possibility of development of potential antithrombotic therapeutic agents from medicinal plants that can be used to treat and/or prevent cardiovascular associated disorders such as thrombosis and hyperfibrinogenemia.

Therefore, the objectives of the present study were:

1. Screening of selected medicinal plants of Assam for assessment of anticoagulant property and preparation of active anticoagulant fraction from selected plants
2. Identification of the components present in the active anticoagulant fraction and comparison of anticoagulant, thrombolytic, and antiplatelet properties of active fraction with commercial drugs
3. Elucidation of mechanism of anticoagulant, anti-platelet, and thrombolytic activities of major components of active fractions
4. Assessment of *in vivo* toxicity, antithrombotic, and anticoagulant potential of active fractions and their components in rodent models

Bibliography

[1] Cragg, G. M. and Newman, D. J. Plants as a source of anti-cancer agents. *J Ethnopharmacol*, 100(1-2): 72-79, 2005.

[2] Cragg, G. M. and Newman, D. J. Biodiversity: A continuing source of novel drug leads. *Pure App. Chem*, 77(1): 7-24, 2005.

[3] Cragg, G. and Newman, D. Nature: a vital source of leads for anticancer drug development. *Phytochem rev*, 8(2): 313-331, 2009.

[4] Cragg, G. M. and Newman, D. J. Natural products: a continuing source of novel drug leads. *Biochim Biophys Acta Gen Subj*, 1830(6): 3670-3695, 2013.

[5] Shoeb, M. Anticancer agents from medicinal plants. *Bangladesh J pharmacol*, 1(2): 35-41, 2006.

[6] Newman, D. J. and Cragg, G. M. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod*, 75(3): 311-335, 2012.

[7] Balunas, M. J. and Kinghorn, A. D. Drug discovery from medicinal plants. *Life Sci*, 78(5): 431-441, 2005.

[8] Zhang, A., Sun, H., and Wang, X. Recent advances in natural products from plants for treatment of liver diseases. *Eur J Med Chem*, 63: 570-577, 2013.

[9] Rasoulian, B. and Kheirandish, F. Herbal medicines: from traditional medicine to modern experimental approaches. *Herbal Med J.*, 2(1): 1-2, 2017.

[10] Maiti, B., Nagori, B., and Singh, R. Recent trends in herbal drugs: a review. *Int J Drug Research Technol*, 1(1), 2017.

[11] Mukherjee, P. K., Bahadur, S., Harwansh, R. K., Biswas, S., and Banerjee, S. Paradigm shift in natural product research: traditional medicine inspired approaches. *Phytochem Rev*, 16(5): 803-826, 2017.

[12] Enioutina, E. Y., Salis, E. R., Job, K. M., Gubarev, M. I., Krepkova, L. V., and Sherwin, C. M. Herbal Medicines: challenges in the modern world. Part 5. status and current directions of complementary and alternative herbal medicine worldwide. *Expert Rev Clin Pharmacol*, 10(3): 327-338, 2017.

[13] Gurib-Fakim, A. Traditional roles and future prospects for medicinal plants in health care. *Asian Biotechnol Develop Rev*, 13(3): 77-83, 2011.

[14] Verma, S. and Singh, S. Current and future status of herbal medicines. *Vet. World*, 1(11): 347, 2008.

[15] Li, J. W.-H. and Vederas, J. C. Drug discovery and natural products: end of an era or an endless frontier? *Science*, 325(5937): 161-165, 2009.

[16] Chen, C., Yang, F. Q., Zhang, Q., Wang, F. Q., Hu, Y. J., and Xia, Z. N. Natural products for antithrombosis. *Evid Based Complement Alternat Med*, 2015: 876426, 2015. 10.1155/2015/876426

[17] Newman, D. J. and Cragg, G. M. Natural products as sources of new drugs from 1981 to 2014. *J Nat Prod*, 79(3): 629-661, 2016.

[18] Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E.-M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., and Heiss, E. H. Discovery and resupply of pharmacologically active plant-derived natural products: a review. *Biotechnol Adv*, 33(8): 1582-1614, 2015.

[19] Harvey, A. L. Natural products in drug discovery. *Drug discovery today*, 13(19-20): 894-901, 2008.

[20] Newman, D. J., Cragg, G. M., and Snader, K. M. Natural products as sources of new drugs over the period 1981− 2002. *J Nat Prod*, 66(7): 1022-1037, 2003.

[21] Koehn, F. E. and Carter, G. T. The evolving role of natural products in drug discovery. *Nature Rev Drug Discovery*, 4(3): 206, 2005.

[22] Farnsworth, N. R. The role of ethnopharmacology in drug development. *Bioac compd from plants*, 154: 2-21, 1990.

[23] Balandrin, M., Kinghorn, A., and Farnsworth, N. in *ACS symposium series (USA)*.

[24] Hostettmann, K. and Terreaux, C. Search for new lead compounds from higher plants. *CHIMIA Int J Chem*, 54(11): 652-657, 2000.

[25] Fabricant, D. S. and Farnsworth, N. R. The value of plants used in traditional medicine for drug discovery. *Environ Health perspecT*, 109(Suppl 1): 69, 2001.

[26] Halket, J. M. and Zaikin, V. G. Derivatization in mass spectrometry—5. Specific derivatization of monofunctional compounds. *Eur J Mass Spectrom*, 11(1): 127-160, 2005.

[27] Mahdi, J. G. Medicinal potential of willow: A chemical perspective of aspirin discovery. *J Saudi Chem Soc*, 14(3): 317-322, 2010.

[28] Vane, J. and Botting, R. The mechanism of action of aspirin. *Thromb Res*, 110(5-6): 255-258, 2003.

[29] Katz, A. M. Effects of digitalis on cell biochemistry: sodium pump inhibition. *J Am Coll Cardiol*, 5(5 Supplement 1): 16A-21A, 1985.

[30] Achan, J., Talisuna, A. O., Erhart, A., Yeka, A., Tibenderana, J. K., Baliraine, F. N., Rosenthal, P. J., and D'Alessandro, U. Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. *Malar J*, 10(1): 144, 2011.

[31] Haikerwal, D., Dart, A. M., Little, P. J., and Kaye, D. M. Identification of a novel, inhibitory action of amiodarone on vesicular monoamine transport. *Pharmacol Exp Ther*, 288(2): 834-837, 1999.

[32] Mandela, P., Chandley, M., Xu, Y.-Y., Zhu, M.-Y., and Ordway, G. A. Reserpine-induced reduction in norepinephrine transporter function requires catecholamine storage vesicles. *Neurochem Int*, 56(6-7): 760-767, 2010.

[33] Tyson, G. E. and Bulger, R. E. Vinblastine-induced paracrystals and unusually large microtubules (macrotubules) in rat renal cells. *Z Zellforch Microsk Anat Histochem*, 141(4): 443-458, 1973.

[34] Sisodiya, P. S. Plant derived anticancer agents: a review. *Int J Res Dev Pharm L Sci*, 2(2): 293-308, 2013.

[35] Arnal, I. and Wade, R. H. How does taxol stabilize microtubules? *Curr Bio*, 5(8): 900-908, 1995.

[36] Manfredi, J. J., Parness, J., and Horwitz, S. B. Taxol binds to cellular microtubules. *J Cell Bio*, 94(3): 688-696, 1982.

[37] Eloff, J. N. Quantification the bioactivity of plant extracts during screening and bioassay guided fractionation. *Phytomedicine*, 11(4): 370, 2004.

[38] Hostettmann, K., Wolfender, J.-L., and Rodriguez, S. Rapid detection and subsequent isolation of bioactive constituents of crude plant extracts. *Planta Med*, 63(01): 2-10, 1997.

[39] Pezzuto, J. M. Plant-derived anticancer agents. *Biochem Pharmacol*, 53(2): 121-133, 1997.

[40] Torras- Claveria, L., Berkov, S., Jáuregui, O., Caujapé, J., Viladomat, F., Codina, C., and Bastida, J. Metabolic profiling of bioactive *Pancratium canariense* extracts by GC- MS. *Phytochem Anal: Int J Plant Chem Biochem Tech*, 21(1): 80-88, 2010.

[41] Berkov, S., Bastida, J., Nikolova, M., Viladomat, F., and Codina, C. Rapid TLC/GC- MS identification of acetylcholinesterase inhibitors in alkaloid extracts. *Phytochem anal*, 19(5): 411-419, 2008.

[42] Dias, D. A., Urban, S., and Roessner, U. A historical overview of natural products in drug discovery. *Metabolites*, 2(2): 303-336, 2012.

[43] Farnsworth, N. R. Screening plants for new medicines. *Biodiversity*, 15(3): 81-99, 1988.

[44] Goh, C. S., Junginger, M., Cocchi, M., Marchal, D., Thrän, D., Hennig, C., Heinimö, J., Nikolaisen, L., Schouwenberg, P. P., and Bradley, D. Wood pellet market and trade: a global perspective. *Biofuels Bioprod Bior*, 7(1): 24-42, 2013.

[45] Wijesekera, R. *The Medicinal Plant Industry*. Routledge, 2017.

[46] Lahlou, M. The success of natural products in drug discovery. *Pharmacol Pharm*, 4(3A): 17-31, 2013.

[47] Kennedy, M. and Stock, J. The bronchodilator action of khellin. *Thorax*, 7(1): 43, 1952.

[48] Mooney, M., Fogarty, S., Stevenson, C., Gallagher, A., Palit, P., Hawley, S., Hardie, D., Coxon, G., Waigh, R., and Tate, R. Mechanisms underlying the metabolic actions of galegine that contribute to weight loss in mice. *Br. J. Pharmacol*, 153(8): 1669-1677, 2008.

[49] Hoing, S. and Murray, K. A. An appraisal of codeine as an analgesic: single- dose analysis. *J Clin Pharmacol*, 24(2- 3): 96-102, 1984.

[50] Williams, D. G., Dickenson, A., Fitzgerald, M., and Howard, R. F. Developmental regulation of codeine analgesia in the rat. *Anesthesiology: J Amer Soc Anesthesiology*, 100(1): 92-97, 2004.

[51] Higuchi, S., Tanaka, N., Shioiri, Y., Otomo, S., and Aihara, H. Two modes of analgesic action of aspirin, and the site of analgesic action of salicylic acid. *Int J Tissue React.*, 8(4): 327-331, 1986.

[52] Undas, A., Brummel-Ziedins, K. E., and Mann, K. G. Antithrombotic properties of aspirin and resistance to aspirin: beyond strictly antiplatelet actions. *Blood*, 109(6): 2285-2292, 2007.

[53] ZsotÉr, T. T., Hart, F., and Radde, I. Mechanism of antihypertensive action of prolonged administration of hydrochlorothiazide in rabbit and dog. *Circ Res*, 27(5): 717-725, 1970.

[54] Wongsrichanalai, C., Pickard, A. L., Wernsdorfer, W. H., and Meshnick, S. R. Epidemiology of drug-resistant malaria. *Lancet Infect Dis*, 2(4): 209-218, 2002.

[55] Iribhogbe, O., Agbaje, E., Oreagba, I., Aina, O., Emordi, J., and Nmorsi, O. Artemisinin based combination versus micronutrient combination in malaria therapeutics: A randomized controlled clinical trial. *Am J Med Sci*, 3(2): 27-37, 1926.

[56] Barlow, O. and Frye, J. The antiasthmatic efficiency of epinephrine, ephedrine and atropine: their comparative effects on a series of experimental attacks in a subject with a complex type of asthma. *Arch Intern Med*, 45(4): 538-545, 1930.

[57] Looareesuwan, S., White, N., Karbwang, J., Turner, R., Phillips, R., Kietinun, S., Rackow, C., and Warrell, D. Quinine and severe *falciparum* malaria in late pregnancy. *The Lancet*, 326(8445): 4-8, 1985.

[58] Choi, J. H., Sapkota, K., Park, S. E., Kim, S., and Kim, S. J. Thrombolytic, anticoagulant and antiplatelet activities of codiase, a bi-functional fibrinolytic enzyme from *Codium fragile*. *Biochimie*, 95(6): 1266-1277, 2013.

[59] Kim, D.-W., Sapkota, K., Choi, J.-H., Kim, Y.-S., Kim, S., and Kim, S.-J. Direct acting anti-thrombotic serine protease from brown seaweed *Costaria costata*. *Process Biochem*, 48(2): 340-350, 2013.

[60] Aje, T. O. and Miller, M. Cardiovascular disease: a global problem extending into the developing world. *World J Cardiol*, 1(1): 3, 2009.

[61] Prabhakaran, D., Jeemon, P., and Roy, A. Cardiovascular diseases in India: current epidemiology and future directions. *Circulation*, 133(16): 1605-1620, 2016.

[62] Joshi, P., Islam, S., Pais, P., Reddy, S., Dorairaj, P., Kazmi, K., Pandey, M. R., Haque, S., Mendis, S., and Rangarajan, S. Risk factors for early myocardial infarction in South Asians compared with individuals in other countries. *JAMA*, 297(3): 286-294, 2007.

[63] Karthikeyan, G., Xavier, D., Prabhakaran, D., and Pais, P. Perspectives on the management of coronary artery disease in India. *Heart*, 93(11): 1334-1338, 2007.

[64] Krishna, B. H., Pal, P., Pal, G., Balachander, J., Jayasettiaseelon, E., Sreekanth, Y., Sridhar, M., and Gaur, G. Effect of yoga therapy on heart rate, blood pressure and cardiac autonomic function in heart failure. *J Clin Diagn Res: JCDR*, 8(1): 14, 2014.

[65] Yusuf, S., Rangarajan, S., Teo, K., Islam, S., Li, W., Liu, L., Bo, J., Lou, Q., Lu, F., and Liu, T. Cardiovascular risk and events in 17 low-, middle-, and high-income countries. *New Eng J Med*, 371(9): 818-827, 2014.

[66] Sampson, J. J. and Hutchinson, J. C. Heart failure in myocardial infarction. *Prog Cardiovasc Dis*, 10(1): 1-29, 1967.

[67] Watson, T., Shantsila, E., and Lip, G. Y. Mechanisms of thrombogenesis in atrial fibrillation: Virchow's triad revisited. *The Lancet*, 373(9658): 155-166, 2009.

[68] Bennett, P. C., Silverman, S. H., Gill, P. S., and Lip, G. Y. Peripheral arterial disease and Virchow's triad. *Thromb Haemost*, 102(06): 1032-1040, 2009.

[69] Chung, I. and Lip, G. Y. Virchow's triad revisited: blood constituents. *Pathophysiol Haemos Thromb*, 33(5-6): 449-454, 2003.

[70] Nilsson, I. Coagulation and fibrinolysis. *Scand J Gastroenterol*, 22(sup137): 11-18, 1987.

[71] Heimburger, N. and Trobisch, H. Blood coagulation and fibrinolysis. *Angew Chem Int Ed*, 10(2): 85-97, 1971.

[72] Marder, V. J. and Novokhatny, V. Direct fibrinolytic agents: biochemical attributes, preclinical foundation and clinical potential. *J Thromb Haemost*, 8(3): 433-444, 2010.

[73] Monroe, D. M., Hoffman, M., and Roberts, H. R. Platelets and thrombin generation. *Arterioscler Thromb Vasc Biol*, 22(9): 1381-1389, 2002.

[74] Martin, V. Disseminated intravascular coagulopathy. *Trans Ophthalmol Soc U K*, 98(4): 506-507, 1978.

[75] Dunn, A. L. Disseminated intravascular coagulopathy. In, *Transfusion Medicine and Hemostasis (Second Edition)*, pages 775-780. Elsevier, 2013.

[76] Ariens, R. A. Elevated fibrinogen causes thrombosis. *Blood*, 117(18): 4687-4688, 2011.

[77] Machlus, K. R., Cardenas, J. C., Church, F. C., and Wolberg, A. S. Causal relationship between hyperfibrinogenemia, thrombosis, and resistance to thrombolysis in mice. *Blood*: blood-2010-2011-316885, 2011.

[78] Kyrle, P. A. and Eichinger, S. Deep vein thrombosis. *Lancet*, 365(9465): 1163-1174, 2005.

[79] Lensing, A. W., Prandoni, P., Prins, M. H., and Büller, H. Deep-vein thrombosis. *Lancet*, 353(9151): 479-485, 1999.

[80] Leon, L., Giannoukas, A., Dodd, D., Chan, P., and Labropoulos, N. Clinical significance of superficial vein thrombosis. *Eur J Vasc Endovasc Surg*, 29(1): 10-17, 2005.

[81] Barnes, C., Newall, F., and Monagle, P. Post-thrombotic syndrome. *Arch Dis Child*, 86(3): 212-214, 2002.

[82] Ashrani, A. A. and Heit, J. A. Incidence and cost burden of post-thrombotic syndrome. *J Thromb Thrombolysis*, 28(4): 465, 2009.

[83] Goldhaber, S. Z., Visani, L., and De Rosa, M. Acute pulmonary embolism: clinical outcomes in the International Cooperative Pulmonary Embolism Registry (ICOPER). *Lancet*, 353(9162): 1386-1389, 1999.

[84] Tow, D. E. and Wagner Jr, H. N. Recovery of pulmonary arterial blood flow in patients with pulmonary embolism. *N Engl J Med*, 276(19): 1053-1059, 1967.

[85] Appelman, P., De Jong, T., and Lampmann, L. Deep venous thrombosis of the leg: US findings. *Radiology*, 163(3): 743-746, 1987.

[86] Cronan, J. Venous thromboembolic disease: the role of US. *Radiology*, 186(3): 619-630, 1993.

[87] Konstantinides, S. V., Barco, S., Lankeit, M., and Meyer, G. Management of pulmonary embolism: an update. *J Am Coll Cardiol*, 67(8): 976-990, 2016.

[88] Heit, J. A., Silverstein, M. D., Mohr, D. N., Petterson, T. M., O'fallon, W. M., and Melton, L. J. Risk factors for deep vein thrombosis and pulmonary embolism: a population-based case-control study. *Arch Intern Med*, 160(6): 809-815, 2000.

[89] Vaccaro, J., Cronan, J., and Dorfman, G. Outcome analysis of patients with normal compression US examinations. *Radiology*, 175(3): 645-649, 1990.

[90] Murphy, T. P. and Cronan, J. J. Evolution of deep venous thrombosis: a prospective evaluation with US. *Radiology*, 177(2): 543-548, 1990.

[91] Righini, M. Is it worth diagnosing and treating distal deep vein thrombosis? No. *J Thromb Haem*, 5: 55-59, 2007.

[92] Maizel, A. S. and Bookstein, J. J. Streptokinase, urokinase, and tissue plasminogen activator: pharmacokinetics, relative advantages, and methods for maximizing rates and consistency of lysis. *Cardiovasc Intervent Radiol*, 9(5-6): 236-244, 1986.

[93] Vassalli, J.-D., Sappino, A., and Belin, D. The plasminogen activator/plasmin system. *J Clin Invest*, 88(4): 1067-1072, 1991.

[94] Sumi, H., Hamada, H., Tsushima, H., Mihara, H., and Muraki, H. A novel fibrinolytic enzyme (nattokinase) in the vegetable cheese Natto; a typical and popular soybean food in the Japanese diet. *Experientia*, 43(10): 1110-1111, 1987.

[95] Hirsh, J., Dalen, J., Anderson, D. R., Poller, L., Bussey, H., Ansell, J., and Deykin, D. Oral anticoagulants: mechanism of action, clinical effectiveness, and optimal therapeutic range. *Chest*, 119(1 Suppl): 8S-21S, 2001.

[96] Bhakuni, T., Ali, M. F., Ahmad, I., Bano, S., Ansari, S., and Jairajpuri, M. A. Role of heparin and non heparin binding serpins in coagulation and angiogenesis: A complex interplay. *Arch Biochem Biophys*, 604: 128-142, 2016.

[97] Miller, R., Harvey, W. P., and Finch, C. A. Antagonism of dicumarol by vitamin K preparations. *N Eng J Med*, 242(6): 211-215, 1950.

[98] Ufer, M. Comparative pharmacokinetics of vitamin K antagonists. *Clin Pharmacokinet*, 44(12): 1227-1246, 2005.

[99] Salazar, C. A., Malaga, G., and Malasquez, G. Direct thrombin inhibitors versus vitamin K antagonists or low molecular weight heparins for prevention of venous thromboembolism following total hip or knee replacement. *Cochrane Database Syst Rev*, (4), 2010.

[100] Hursting, M. J., Alford, K. L., Becker, J. P., Brooks, R. L., Joffrion, J. L., Knappenberger, G. D., Kogan, P. W., Kogan, T. P., Mckinney, A. A., and Schwarz, R. P. in *Semin Thromb Hemost.* 503-516 (Copyright© 1997 by Thieme Medical Publishers, Inc.).

[101] Samama, M. M., Martinoli, J.-L., LeFlem, L., Guinet, C., Plu-Bureau, G., Depasse, F., and Perzborn, E. Assessment of laboratory assays to measure rivaroxaban–an oral, direct factor Xa inhibitor. *Thromb Haemost*, 104(04): 815-825, 2010.

[102] Mukhametova, L., Aĭsina, R., Lomakina, G., and Varfolomeev, S. Characterization of urokinase type plasminogen activator modified by phenylglyoxal. *Bioorg Khim*, 28(4): 308-314, 2002.

[103] Hirsh, J. Oral anticoagulant drugs. *N Eng J Med*, 324(26): 1865-1875, 1991.

[104] Bircher, A., Harr, T., Hohenstein, L., and Tsakiris, D. Hypersensitivity reactions to anticoagulant drugs: diagnosis and management options. *Allergy*, 61(12): 1432-1440, 2006.

[105] Zarar, A., Khan, A. A., Adil, M. M., and Qureshi, A. I. Anaphylactic shock associated with intravenous thrombolytics. *Am J Emerg Med*, 32(1): 113. e113-115, 2014.

[106] Warkentin, T. E., Sheppard, J.-A. I., Moore, J. C., Cook, R. J., and Kelton, J. G. Studies of the immune response in heparin-induced thrombocytopenia. *Blood*, 113(20): 4963-4969, 2009.

[107] Ahrens, I., Lip, G. Y., and Peter, K. New oral anticoagulant drugs in cardiovascular disease. *Thromb Haemost*, 104(01): 49-60, 2010.

[108] Di Minno, A., Frigerio, B., Spadarella, G., Ravani, A., Sansaro, D., Amato, M., Kitzmiller, J. P., Pepi, M., Tremoli, E., and Baldassarre, D. Old and new oral anticoagulants: food, herbal medicines and drug interactions. *Blood Rev*, 31(4): 193-203, 2017.

[109] Warkentin, T. E., Levine, M. N., Hirsh, J., Horsewood, P., Roberts, R. S., Gent, M., and Kelton, J. G. Heparin-induced thrombocytopenia in patients treated with low-molecular-weight heparin or unfractionated heparin. *N Eng J Med*, 332(20): 1330-1336, 1995.

[110] Weitz, J. I. Low-molecular-weight heparins. *N Eng J Med*, 337(10): 688-699, 1997.

[111] Hirsh, J. Low molecular weight heparin. *Thromb Haemost*, 69(01): 204-207, 1993.

[112] Stone, G. W., Witzenbichler, B., Guagliumi, G., Peruga, J. Z., Brodie, B. R., Dudek, D., Kornowski, R., Hartmann, F., Gersh, B. J., and Pocock, S. J. Bivalirudin during primary PCI in acute myocardial infarction. *N Eng J Med*, 358(21): 2218-2230, 2008.

[113] Warkentin, T. E., Greinacher, A., Koster, A., and Lincoff, A. M. Treatment and prevention of heparin-induced thrombocytopenia: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest*, 133(6): 340S-380S, 2008.

[114] Gladwell, T. D. Bivalirudin: a direct thrombin inhibitor. *Clin. Ther*, 24(1): 38-58, 2002.

[115] Neuhaus, T., Goetschel, P., Schmugge, M., and Leumann, E. Heparin-induced thrombocytopenia type II on hemodialysis: switch to danaparoid. *Pediatr Nephrol*, 14(8-9): 713-716, 2000.

[116] Petros, S. Lepirudin in the management of patients with heparin-induced thrombocytopenia. *Biologics*, 2(3): 481, 2008.

[117] Greinacher, A., Janssens, U., Berg, G., Böck, M., Kwasny, H., Kemkes-Matthes, B., Eichler, P., Völpel, H., Pötzsch, B., and Luz, M. Lepirudin (recombinant hirudin) for parenteral anticoagulation in patients with heparin-induced thrombocytopenia. *Circulation*, 100(6): 587-593, 1999.

[118] Farner, B., Eichler, P., Kroll, H., and Greinacher, A. A comparison of danaparoid and lepirudin in heparin-induced thrombocytopenia. *Thromb Haemost*, 85(06): 950-957, 2001.

[119] Bambrah, R. K., Pham, D. C., and Rana, F. Argatroban in heparin-induced thrombocytopenia: rationale for use and place in therapy. *Ther Adv Chronic Dis*, 4(6): 302-304, 2013.

[120] Berry, C. N., Girardot, C., Lecoffreo, C., and Lunven, C. Effects of the synthetic thrombin inhibitor argatroban on fibrin-or clot-incorporated thrombin: comparison with heparin and recombinant hirudin. *Thromb Haemost*, 71(03): 381-386, 1994.

[121] Lewis, B., Wallis, D., Berkowitz, S., Matthai, W., Fareed, J., Walenga, J., Bartholomew, J., Sham, R., Lerner, R., and Zeigler, Z. Argatroban anticoagulant therapy in patients with heparin-induced thrombocytopenia. *Circulation*, 103(14): 1838-1843, 2001.

[122] Di Nisio, M., Middeldorp, S., and Büller, H. R. Direct thrombin inhibitors. *N Eng J Med*, 353(10): 1028-1040, 2005.

[123] Van Ryn, J., Stangier, J., Haertter, S., Liesenfeld, K.-H., Wienen, W., Feuring, M., and Clemens, A. Dabigatran etexilate–a novel, reversible, oral direct thrombin inhibitor: interpretation of coagulation assays and reversal of anticoagulant activity. *Thromb Haemost*, 104(06): 1116-1127, 2010.

[124] Sorbera, L., Bozzo, J., and Castaner, J. Dabigatran/dabigatran etexilate. *Drugs Fut*, 30(9): 877, 2005.

[125] Eriksson, B. I., Dahl, O. E., Rosencher, N., Kurth, A. A., van Dijk, C. N., Frostick, S. P., Prins, M. H., Hettiarachchi, R., Hantel, S., and Schnee, J. Dabigatran etexilate versus enoxaparin for prevention of venous thromboembolism after total hip replacement: a randomised, double-blind, non-inferiority trial. *Lancet*, 370(9591): 949-956, 2007.

[126] Albers, G. W., Diener, H.-C., Frison, L., Grind, M., Nevinson, M., Partridge, S., Halperin, J. L., Horrow, J., Olsson, S. B., and Petersen, P. Ximelagatran vs warfarin for stroke prevention in patients with nonvalvular atrial fibrillation: a randomized trial. *JAMA*, 293(6): 690-698, 2005.

[127] Ho, S.-J. and Brighton, T. A. Ximelagatran: direct thrombin inhibitor. *Vasc Health Risk Manag*, 2(1): 49, 2006.

[128] Southworth, H. Predicting potential liver toxicity from phase 2 data: a case study with ximelagatran. *Stat Med*, 33(17): 2914-2923, 2014.

[129] Patel, M. R., Mahaffey, K. W., Garg, J., Pan, G., Singer, D. E., Hacke, W., Breithardt, G., Halperin, J. L., Hankey, G. J., and Piccini, J. P. Rivaroxaban

versus warfarin in nonvalvular atrial fibrillation. *N Eng J Med*, 365(10): 883-891, 2011.

[130] Eriksson, B. I., Borris, L. C., Friedman, R. J., Haas, S., Huisman, M. V., Kakkar, A. K., Bandel, T. J., Beckmann, H., Muehlhofer, E., and Misselwitz, F. Rivaroxaban versus enoxaparin for thromboprophylaxis after hip arthroplasty. *N Eng J Med*, 358(26): 2765-2775, 2008.

[131] Abdulsattar, Y., Bhambri, R., and Nogid, A. Rivaroxaban (xarelto) for the prevention of thromboembolic disease: an inside look at the oral direct factor xa inhibitor. *Pharm Ther*, 34(5): 238, 2009.

[132] Gómez-Outes, A., Terleira-Fernández, A. I., Calvo-Rojas, G., Suárez-Gea, M. L., and Vargas-Castrillón, E. Dabigatran, rivaroxaban, or apixaban versus warfarin in patients with nonvalvular atrial fibrillation: a systematic review and meta-analysis of subgroups. *Thrombosis*, 2013, 2013.

[133] Yusuf, S., Mehta, S. R., Chrolavicius, S., Afzal, R., Pogue, J., Granger, C. B., Budaj, A., Peters, R., Bassand, J.-P., and Wallentin, L. Effects of fondaparinux on mortality and reinfarction in patients with acute ST-segment elevation myocardial infarction: the OASIS-6 randomized trial. *JAMA*, 295(13): 1519-1530, 2006.

[134] Eriksson, B. I., Bauer, K. A., Lassen, M. R., and Turpie, A. G. Fondaparinux compared with enoxaparin for the prevention of venous thromboembolism after hip-fracture surgery. *N Eng J Med*, 345(18): 1298-1304, 2001.

[135] Cafolla, A. and Gentile, G. Anticoagulant therapy with fondaparinux in a liver transplant patient with thrombosis and liver fibrosis: a case report. *Clin Case Rep*, 5(3): 342-345, 2017.

[136] Nagashima, R., O'reilly, R., and Levy, G. Kinetics of pharmacologic effects in man: the anticoagulant action of warfarin. *Clin Pharm Ther*, 10(1): 22-35, 1969.

[137] Ezekowitz, M. D., Bridgers, S. L., James, K. E., Carliner, N. H., Colling, C. L., Gornick, C. C., Krause-Steinrauf, H., Kurtzke, J. F., Nazarian, S. M., and Radford, M. J. Warfarin in the prevention of stroke associated with nonrheumatic atrial fibrillation. *N Eng J of Med*, 327(20): 1406-1412, 1992.

[138] Orkaby, A. R., Ozonoff, A., Reisman, J. I., Miller, D. R., Zhao, S., and Rose, A. J. Continued use of warfarin in veterans with atrial fibrillation after dementia diagnosis. *J Am Geriatr Soc*, 65(2): 249-256, 2017.

[139] Ekor, M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol*, 4: 177, 2014.

[140] Wagner, H., Hikino, H., and Farnsworth, N. R. *Econ Med plant Res,* Academic press, 2012.

[141] Vaidya, A. D. and Devasagayam, T. P. Recent advances in indian herbal drug research guest editor: Thomas Paul Asir Devasagayam current status of herbal drugs in India: An Overview. *J Clinical Biochem Nutri*, 41(1): 1-11, 2007.

[142] Archana, J. S., Paul, R., and Tiwari, A. Indian medicinal plants: A rich source of natural immuno modulator. *Int J Pharm*, 7(2): 198-205, 2011.

[143] Sen, S., Chakraborty, R., and De, B. Challenges and opportunities in the advancement of herbal medicine: India's position and role in a global context. *J Herbal Med*, 1(3-4): 67-75, 2011.

[144] Kumar, S., Chand, G., Sankhyan, P., Chaudhari Manojkumar, V., Gupta, V., Keshari, B. B., Sase, S., Limaye, R., Soni, N., and Gaikwad, S. Herbal folk remedies for curing various ailments in Lug Valley of district Kullu, Himachal Pradesh (NW Himalaya). *Int J Ayurved Herbal Med*, 3(5): 1308-1314, 2013.

[145] Rathi, S., Grover, J., Vikrant, V., and Biswas, N. Prevention of experimental diabetic cataract by Indian Ayurvedic plant extracts. *Phytotherapy Res: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 16(8): 774-777, 2002.

[146] Patel, R. and Kayande, N. Review on: Indian Medicinal plants having anticancer property. *Pharma Tutor*, 4(7): 25-28, 2016.

[147] Kapoor, L. *Handbook of Ayurvedic medicinal plants: Herbal reference library*. Routledge, 2017.

[148] Grover, J., Yadav, S., and Vats, V. Medicinal plants of India with anti-diabetic potential. *J Ethnopharmacol*, 81(1): 81-100, 2002.

[149] Tandon, N. and Yadav, S. S. Contributions of Indian Council of Medical Research (ICMR) in the area of medicinal plants/traditional medicine. *J Ethnopharmacol*, 197: 39-45, 2017.

[150] Collen, D. and Lijnen, H. R. Tissue- type plasminogen activator: a historical perspective and personal account. *J Thromb Haemost*, 2(4): 541-546, 2004.

[151] Hacke, W., Kaste, M., Bluhmki, E., Brozman, M., Dávalos, A., Guidetti, D., Larrue, V., Lees, K. R., Medeghri, Z., and Machnig, T. Thrombolysis with

alteplase 3 to 4.5 hours after acute ischemic stroke. *N Eng J Med*, 359(13): 1317-1329, 2008.

[152] Wahlgren, N., Ahmed, N., Dávalos, A., Ford, G. A., Grond, M., Hacke, W., Hennerici, M. G., Kaste, M., Kuelkens, S., and Larrue, V. Thrombolysis with alteplase for acute ischaemic stroke in the safe implementation of thrombolysis in stroke-monitoring study (SITS-MOST): an observational study. *The Lancet*, 369(9558): 275-282, 2007.

[153] Investigators, Global Use of Strategies to Open Occluded Coronary Arteries (GUSTO). A comparison of reteplase with alteplase for acute myocardial infarction. *N Eng J Med*, 337(16): 1118-1123, 1997.

[154] Çoner, A., Çiçek, D., Balcıoğlu, S., Akıncı, S., and Müderrisoğlu, H. Successful treatment of massive pulmonary embolism with reteplase. *Turk Kardiyol Dern Ars*, 46(2): 143-146, 2018.

[155] Wang, A., Pednekar, N., Lehrer, R., Todo, A., Sahni, R., Marks, S., and Stiefel, M. F. DRAGON score predicts functional outcomes in acute ischemic stroke patients receiving both intravenous tissue plasminogen activator and endovascular therapy. *Surg Neurol Int*, 8, 2017.

[156] Dotter, C. T., Rösch, J., and Seaman, A. J. Selective clot lysis with low-dose streptokinase. *Radiology*, 111(1): 31-37, 1974.

[157] Collen, D. Staphylokinase: a potent, uniquely fibrin-selective thrombolytic agent. *Nature Med*, 4(3): 279-284, 1998.

[158] Young, K.-C., Shi, G.-Y., Wu, D.-H., Chang, L.-C., Chang, B.-I., Ou, C.-P., and Wu, H.-L. Plasminogen activation by streptokinase via a unique mechanism. *J Biol Chem*, 273(5): 3110-3116, 1998.

[159] Collen, D. The plasminogen (fibrinolytic) system. *Thromb Haemost*, 82(02): 259-270, 1999.

[160] Blasi, F., Vassalli, J.-D., and Danø, K. Urokinase-type plasminogen activator: proenzyme, receptor, and inhibitors. *J. Cell Biol*, 104(4): 801-804, 1987.

[161] Duffy, M. Urokinase-type plasminogen activator: a potent marker of metastatic potential in human cancers. *Biochem Soc Trans*, 207-210, 2002

[162] Fujita, M., Hong, K., Ito, Y., Fuji, R., Kariya, K., and Nishimuro, S. Thrombolytic effect of nattokinase on a chemically induced thrombosis model in rat. *Biol Pharm Bull*, 18(10): 1387-1391, 1995.

[163] Kotb, E. Activity assessment of microbial fibrinolytic enzymes. *Appl Microbiol Biotechnol*, 97(15): 6647-6665, 2013.

[164] Roth, G. J., Stanford, N., and Majerus, P. W. Acetylation of prostaglandin synthase by aspirin. *Proc Natl Acad Sci* , 72(8): 3073-3076, 1975.

[165] Michelson, A. D., Cattaneo, M., Eikelboom, J., Gurbel, P., Kottke- Marchant, K., Kunicki, T., Pulcinelli, F., Cerletti, C., Rao, A. Aspirin resistance: position paper of the Working Group on Aspirin Resistance. *J Thromb Haemost*, 3(6): 1309-1311, 2005.

[166] FitzGerald, G. Dipyridamole. *N Eng J Med*, 316(20): 1247-1257, 1987.

[167] Diener, H., Cunha, L., Forbes, C. e., Sivenius, J., Smets, P., and Lowenthal, A. European Stroke Prevention Study 2. Dipyridamole and acetylsalicylic acid in the secondary prevention of stroke1. *J Neurol Sci* , 143(1-2): 1-13, 1996.

[168] Michelson, A. D. Advances in antiplatelet therapy. *ASH Education Program Book*, 2011(1): 62-69, 2011.

[169] Gurbel, P. A. and Bliden, K. P. Durability of platelet inhibition by clopidogrel. *Am J Cardiol*, 91(9): 1123-1125, 2003.

[170] Hagihara, K., Kazui, M., Kurihara, A., Yoshiike, M., Honda, K., Okazaki, O., Farid, N. A., and Ikeda, T. A possible mechanism of the differences in efficiency and variability of active metabolite formation from thienopyridine antiplatelet agents, prasugrel and clopidogrel. *Drug Metab Dispos*, 2009.

[171] Wallentin, L., Becker, R. C., Budaj, A., Cannon, C. P., Emanuelsson, H., Held, C., Horrow, J., Husted, S., James, S., and Katus, H. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Eng J Med*, 361(11): 1045-1057, 2009.

[172] Capodanno, D., Dharmashankar, K., and Angiolillo, D. J. Mechanism of action and clinical development of ticagrelor, a novel platelet ADP P2Y12 receptor antagonist. *Exp Rev Cardiovas Ther*, 8(2): 151-158, 2010.

[173] Mascelli, M. A. and Nakada, M. T. Pharmacological Properties of Abciximab. *Hamostaseologie*, 19(03): 101-107, 1999.

[174] Topol, E. J., Byzova, T. V., and Plow, E. F. Platelet GPIIb-IIIa blockers. *The Lancet*, 353(9148): 227-231, 1999.

[175] Coller, B. S., Anderson, K., and Weisman, H. F. New antiplatelet agents: Platelet GPIIb/llla antagonists. *Thromb Haemost*, 73(01): 302-308, 1995.

[176] Phillips, D. R. and Scarborough, R. M. Clinical pharmacology of eptifibatide. *Am J Cardiol*, 80(4): 11B-20B, 1997.

[177] Moser, M., Bertram, U., Peter, K., Bode, C., and Ruef, J. Abciximab, eptifibatide, and tirofiban exhibit dose-dependent potencies to dissolve platelet aggregates. *J Cardiovas Pharmacol*, 41(4): 586-592, 2003.

[178] Abdul-Ghani, A.-S. and Amin, R. The vascular action of aqueous extracts of *Foeniculum vulgare* leaves. *J Ethnopharmacol*, 24(2-3): 213-218, 1988.

[179] Scartezzini, P. and Speroni, E. Review on some plants of Indian traditional medicine with antioxidant activity. *J Ethnopharmacol*, 71(1-2): 23-43, 2000.

[180] Bhujbal, V. S. R. and Sangoram, A. review of medicinal flowers in India. 2017.

[181] Gogte, V. M. *Ayurvedic pharmacology and therapeutic uses of medicinal plants*. Dravyaganvigyan, I Edn. Mumbai: Bhartiya Vidya Bhavan, 2000.

[182] Lau, B. H., Adetumbi, M. A., and Sanchez, A. *Allium sativum* (garlic) and atherosclerosis: a review. *Nutri Res*, 3(1): 119-128, 1983.

[183] Reuter, H. *Allium sativum* and *allium ursinum*: Part 2 pharmacology and medicinal application. *Phytomedicine*, 2(1): 73-91, 1995.

[184] Thomson, M. and Ali, M. Garlic [*Allium sativum*]: a review of its potential use as an anti-cancer agent. *Curr Cancer drug Targ*, 3(1): 67-81, 2003.

[185] Foroutan-Rad, M., Tappeh, K. H., and Khademvatan, S. Antileishmanial and immunomodulatory activity of *Allium sativum* (Garlic) A Review. *Evid.-Based Complementary Altern. Med*, 22(1): 141-155, 2017.

[186] Babu, T., Kuttan, G., and Padikkala, J. Cytotoxic and anti-tumour properties of certain taxa of *Umbelliferae* with special reference to *Centella asiatica* (L.) Urban. *J Ethnopharmacol*, 48(1): 53-57, 1995.

[187] Shukla, A., Rasik, A., Jain, G., Shankar, R., Kulshrestha, D., and Dhawan, B. *In vitro* and *in vivo* wound healing activity of asiaticoside isolated from *Centella asiatica*. *J Ethnopharmacol*, 65(1): 1-11, 1999.

[188] Gohil, K. J., Patel, J. A., and Gajjar, A. K. Pharmacological review on *Centella asiatica*: a potential herbal cure-all. *Indian J Pharm Sci*, 72(5): 546, 2010.

[189] Arora, R., Kumar, R., Agarwal, A., Reeta, K., and Gupta, Y. Comparison of three different extracts of *Centella asiatica* for anti-amnesic, antioxidant and anticholinergic activities: *in vitro* and *in vivo* study. *Biomed Pharmacother*, 105: 1344-1352, 2018.

[190] Venkatesh, S. and Thilagavathi, J. Anti-diabetic activity of flowers of *Hibiscus rosasinensis*. *Fitoterapia*, 79(2): 79-81, 2008.

[191] Prasanna, R. Evaluation of antioxidant activity of phenol, *Hibiscus rosasinensis*, neem and leaves extract at different infusion times. *Int J Adv Res Innov Ideas*, 281-286, 2017.

[192] Kumar, L., Chakraborthy, G., Singh, V., and Mazumder, A. *Hibiscus rosasinensis*: A review on divine herb. *J Ad Pharm Healthcare Res*, 2(4): 9-18, 2012.

[193] Fernandes, L., Casal, S., Pereira, J. A., Saraiva, J. A., and Ramalhosa, E. Edible flowers: A review of the nutritional, antioxidant, antimicrobial properties and effects on human health. *J Food Comp Anal*, 60: 38-50, 2017.

[194] Ramani, R., Sudini, S., Boddupalli, B. M., and Anisetti, R. N. Antioxidant, free radical scavenging and *in vitro* cytotoxic studies of ethanolic extract of *Leucas indica* var *lavandulifolia* and *Leucas indica* var nagalapuramiana. *Asian Pac J Trop Biomed*, 2(3): S1637-S1642, 2012.

[195] Sarkar, M., Biswas, P., and Samanta, A. *In vivo* anti-inflammatory and *in vitro* antioxidant studies on methanolic and aqueous extract of *Leucas indica* linn. *Asian J Pharma Clin Res*, 6(2): 284-290, 2013.

[196] Maheswaran, R., Sathish, S., and Ignacimuthu, S. Larvicidal activity of *Leucas aspera* (Willd.) against the larvae of *Culex quinquefasciatus* Say. and Aedes aegypti L. *Int J Integr Biol*, 2(3): 214-217, 2008.

[197] Rahman, M., Sadhu, S., and Hasan, C. Preliminary antinociceptive, antioxidant and cytotoxic activities of *Leucas aspera* root. *Fitoterapia*, 78(7-8): 552-555, 2007.

[198] Prajapati, M., Patel, J., Modi, K., and Shah, M. *Leucas aspera*: A review. *Pharmacogn Rev*, 4(7): 85, 2010.

[199] Grover, J. and Yadav, S. Pharmacological actions and potential uses of *Momordica charantia*: a review. *J Ethnopharmacol*, 93(1): 123-132, 2004.

[200] Basch, E., Gabardi, S., and Ulbricht, C. Bitter melon (*Momordica charantia*): a review of efficacy and safety. *Am J Health Syst Pharm*, 60(4): 356-359, 2003.

[201] Raman, A. and Lau, C. Anti-diabetic properties and phytochemistry of *Momordica charantia* L.(Cucurbitaceae). *Phytomedicine*, 2(4): 349-362, 1996.

[202] Ahmed, I., Lakhani, M., Gillett, M., John, A., and Raza, H. Hypotriglyceridemic and hypocholesterolemic effects of anti-diabetic *Momordica charantia* (karela)

fruit extract in streptozotocin-induced diabetic rats. *Diabetes Res Clin Prac*, 51(3): 155-161, 2001.

[203] Roopashree, T., Dang, R., Rani, R. S., and Narendra, C. Antibacterial activity of antipsoriatic herbs: *Cassia tora*, *Momordica charantia* and *Calendula officinalis*. *Int J Appl Res Nat Prod*, 1(3): 20-28, 2008.

[204] Chiang, H.-C., Tseng, T., Wang, C.-J., Chen, C., and Kan, W. Experimental antitumor agents from *Solanum indicum* L. *Anticancer Res*, 11(5): 1911-1917, 1991.

[205] Srividya, A., Arunkumar, A., Cherian, B., Maheshwari, V., Piramanayagam, S., and Senthoorpandi, V. Pharmacognostic, phytochemical and anti-microbial studies of *Solanum indicum* leaves. *Ancient Sci life*, 29(1): 3, 2009.

[206] Deb, P. K., Ghosh, R., Chakraverty, R., Debnath, R., Das, L., and Bhakta, T. Phytochemical and pharmacological evaluation of fruits of *Solanum indicum* Linn. *Int J Pharm Sci Rev Res*, 25(2): 28-32, 2014.

[207] Choi, J.-H., Kim, D.-W., Park, S.-E., Choi, B.-S., Sapkota, K., Kim, S., and Kim, S.-J. Novel thrombolytic protease from edible and medicinal plant *Aster yomena* (Kitam.) Honda with anticoagulant activity: Purification and partial characterization. *J Biosci Bioeng*, 118(4): 372-377, 2014.

[208] Luzak, B., Golanski, J., Przygodzki, T., Boncler, M., Sosnowska, D., Oszmianski, J., Watala, C., and Rozalski, M. Extract from spent hop (*Humulus lupulus* L.) reduces blood platelet aggregation and improves anticoagulant activity of human endothelial cells *in vitro*. *J Funct Foods*, 22: 257-269, 2016.

[209] Olajide, O. A. Investigation of the effects of selected medicinal plants on experimental thrombosis. *Phytotherapy Res*, 13(3): 231-232, 1999.

[210] Memariani, Z., Moeini, R., Hamedi, S. S., Gorji, N., and Mozaffarpur, S. A. Medicinal plants with antithrombotic property in Persian medicine: a mechanistic review. *J Thromb Thrombolysis*, 45(1): 158-179, 2018.

[211] Akram, M. and Rashid, A. Anti-coagulant activity of plants: mini review. *J Thromb Thrombolysis*, 44(3): 406-411, 2017.