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Assessment of Haemostatic Activity of Medicinal Plants Using In Vitro Methods: A Concise Review

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Abstract:
Invitro screening of plants for their haemostatic activity is gaining attention due to the concerns related to dietary habits and the ability of the bioactive molecules to develop as effective drugs without undesirable side effects. Studies related to haemostatic activities are reported, mainly designed on the ethnomedical or folkloric information available. Different extractions methodologies, analysis of the phytochemical constituents and biochemical assays used in invitro haemostatic determination were assessed. Conventional extraction methodologies have used in isolation and identification of the plant secondary metabolites. Biochemical tests such as, Platelet Aggregation, Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) Thromboplastin Time (TT), have commonly used to investigate haemostatic potential. The succulent extraction process in isolating secondary metabolites from plant materials could be used as an alternative method of drying plant materials. Use of cheminformatics to identify plants with possible bioactivities and the use of Thin Layer Chromatography as a simple identification method of possible haemostatic compounds is also conversed. A cytotoxicity assay could be valuable to determine the cell viability in presence of the plant extract. A bioassay guided in vitro analysis could be beneficial, as some plant extracts could contain compounds with both pro-coagulation and anticoagulation activities.

Key Word*:haemostatic activity, plant extraction, antiplatelet, anticoagulation*

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Abbreviations*:*
APTT - Activated Partial Thromboplastin Time, ADP - Adenosine diphosphate, $CHCl_4$ – Chloroform, DCM – Dichloromethane, EtOH – Ethanol, EtOAc - Ethyl acetate, FIB - Fibrinogen assay, GC-MS - Gas Chromatography-Mass Spectrometry, HPLC - High Performance Liquid Chromatography, MeOH – Methanol, n-BtOH- n-butanol, PE - Petroleum ether, PT - Prothrombin Time, TT - Thromboplastin Time

I. Introduction

Haemostasis can be identified as the arresting of blood loss from the damaged blood vessels. During the haemostasis, the blood that is in the usual liquid state would be converted to a gel like state, initiating the wound healing process[1]. This is a dynamic process in which clot formation, anticoagulation and clot dissolution are in balance[2]. There are three main steps associated with the haemostasis. Vasoconstriction or the constriction of the blood vessels at the sight of injury is the initial step. Formation of the platelet plug as a temporary blockage (primary haemostasis) and the formation of the fibrin clot (blood coagulation or secondary haemostasis) occurs afterwards[3]. Coagulation of blood is a complex process which is tightly regulated at cellular level[4]. Haemostatic agents can speed up this process by affecting these main steps. Contrast to haemostatic agents, antithrombic agents retard the platelet aggregation whereas anticoagulating agents have the ability to stop coagulation after the initial platelet aggregation step[5].

Platelets (thrombocytes) are the smallest morphotic component in blood. Platelets have an important function in many biological processes including haemostasis, inflammation and thrombosis[6,7]. Dysfunction platelet activity can have an adverse effect in health[8]. Platelet hyperactivity or increase platelet activity can cause coagulation without vascular damage which is known as the vascular thrombosis. Increment of platelet activity has also recognized to be associated with some diseases such as myocardial infarction and cerebral infarction[9,10]. Antiplatelet drugs are used in clinical treatment of coronary syndromes and strokes[6,11,12]. ADP, arachidonic acid, collagen, or thrombin are some commonly known agonists that triggered platelet aggregation[6].

The secondary haemostatic or the final stage of haemostasis is regulated in two separate pathways, the intrinsic pathway and the extrinsic pathway, leading to the production of fibrin clot from fibrinogen. Several enzymes, known as clotting 'Factors' are involved in the plasmatic coagulation. These clotting Factors that are

usually in their inactive precursor state undergo activation and each Factor then catalyses the conversion of next Factor from its inactive form to active form in cascade like fashion[8]. Some of these Factors are common to both intrinsic and extrinsic pathways and formulate the common pathway resulting the final fibrin network[13]. Absence of clotting Factors in blood decrease the coagulation, lengthening the bleeding and some blood disorders such as haemophilia[8].

Many cardiovascular diseases are caused by blood clotting disorders and remain a predominant cause for mortality in modern world. Thrombus formation in vessels is an example for such abnormal coagulation and indicate the importance of antiplatelet drugs in controlling such conditions[11]. It is known that, some drugs used in treatment of anticoagulation therapy have adverse effect on human health[6]. Heparin and warfarin that are used in anticoagulant treatment have demonstrated undesirable side effects such as causing gastrointestinal symptoms, mucosal haemorrhage and bleeding[2,14]. Most of the anticoagulant drugs that are currently used have been chemically synthesised[15]. Because of these challenges, investigation of new antithrombic and anticoagulant drugs are important as a means of producing affordable, less toxic and potent drugs with different mechanism of action[3,11].

In another aspect, rapid haemostatic methods are needed to stop excessive bleeding, especially in surgical settings. Bleeding blood vessels can be sealed by thermal methods such as laser cauterization and electrocauterization or conventional mechanical methods such as adding pressure on the wounded area or using a tourniquet[16]. But, conventional methods could be less effective for complex injuries and thermal methods could create areas with char or necrotic tissues which could create a chance for infection[16]. Topical haemostatic agents seem to be favoured in many situations such as wound dressing and dental surgical situations.

Societies from around the world widely use traditional medicine, as treatments to cure ailments from ancient time[3,17]. These indigenous medicine systems use ample of medicinal plants to make variety of herbal products to use as therapeutics and these herbal therapeutics are generally accepted to be safe without side effects[4]. Especially in developing countries, the continuous use of herbal medicines has driven by the limitations of availability or the high cost of the modern pharmaceutics[5]. According to World Health Organization 65- 80% global population still use herbal medicine for their primary health care[2,18]. Use of plant extract to cure wounds or stop haemorrhage have been a practice in ancient times and still can be seen with native or tribal dwellers[4]. Many plant varieties have been proven to have anti-inflammatory and anti-coagulator activities[19]. It is stated that, natural compounds present in some plant varieties such as *Allium sativum* and *Lycopersicum esculentum* may be beneficial in protection against cardiovascular diseases as a result of inhibiting platelet aggregation[3].

Researchers often use such folklore knowledge as a source to further investigate and to produce reliable scientific information[20]. In present, chemical and biological evaluations are used to screen the potential of the secondary metabolites produced by these plants for their anti-effective nature[3,17]. These studies may lead to identification and isolation of bioactive compounds or such templates that can be utilized to develop and produce effective drugs in treatment of various ailments[2]. This review is focused on the scientific publications in recent past that have conducted on screening plants for their in vitro haemostasis activity and investigating both pro-coagulation and anticoagulation nature. Here, we have explored various methodologies used in plant extractions, phytochemical analysis of the plant extract and assays used in in vitro haemostatic determination.

II. Methodology

Online academic databases were searched using combinations of 'haemostatic, in vitro activity, plant extracts, antiplatelet and anticoagulation' words. Published research papers (with an emphasis on years between 2014 to 2019) were selected based on the relevance to the title of the article. Methods used in extraction of secondary metabolites, phytochemical analysis and various assays conducted to determine the in vitro haemostatic activity of the plant extracts were explored.

III. Result

Results of the literature review are presented under the following topics: 3.1. Extraction of plant metabolites, 3.2. Phytochemical analysis of the plant extracts, 3.3. Preparation of the blood samples, 3.4. In vitro screening methods for platelet aggregation and 3.5. In vitro screening methods for plasmatic coagulation.

3.1. Extraction of plant metabolites

Selection of plant varieties have been done with ethnomedical information gathered. Different plant partshave used for experimentation including aerial parts such as flowers[3,14], petals[7], leaves[5], latex[18], fruits[21], husks[22], seeds[23], roots[11], rhizomes[24] and root bark[6]. Other than plants, mushrooms[25] and seaweeds[26] have also used to collect secondary metabolites. Selected plant materials have been washed with running water to remove dust and debris from the surfaces. Cleaned plant material have dried in shade (or in dark place[6]), cut into small pieces and made in to powder form through mechanical methods like grinding. Drying process has been carried up to two weeks in some cases[5]. Use of industrial oven to dry the plant materials is also reported[15]. In some

studies, washed plant materials have freeze dried and pulverized to obtain the powdered form which had used for extraction process[27].

The powdered plant martials have been subjected to conventional extraction methods such as hot extraction using soxhlet apparatus[11,28] or extracting plant metabolites in to organic, organic/water system or water systems by soaking the plant material in the solvent system[3]. Extractions of secondary metabolites have been carried out for various time duration from an overnight to several days[2,4,5]. Sonication has used to increase the extraction efficiency[15,27]. The organic solvent removal has been achieved by rotary evaporation or using water bath in temperature range from 40–70 ℃[3].

Aqueous extractions have subjected to lyophilisation to obtain the crude secondary metabolites in solid form. Some studies have used liquid-liquid partitioning of the aqueous crude extract to separate them to several fractions ($CHCl_4$, DCM, EtOAc, hexane, n-BtOH, PE and water etc.) and test has been conducted for obtained products from these separated fractions[5,29].

Use of column chromatography (silica and size exclusion) to fractionate the crude extract is also reported[14,27]. High content of sugar and organic acid have been purified using solid phase extractions[21]. Extraction of targeted secondary metabolites such as free flavonoids (isolated using diethyl ether) and heterosidic flavonoids (isolated using ethyl acetate) from the obtained crude extract to carry out haemostatic activity is also reported[10].In a similar fashion, extraction of targeted polysaccharides from *Genipa Americana* has carried out to study the haemostatic activity[30,31]. Evaluation of the crude extract with HPLC has performed to identify the various compounds present in it[19].

Original usage of these plant materials are often in its fresh form to prepare for treatments, without subjecting to any drying process. The succulent extraction method comes from this basis and expresses the concern that drying may diminish the potency of bioactivity of the compounds present in the plants. In other extraction process (hot extraction for prolong period of time) may limit the activity of the plants/compounds involved as the phytochemicals in the plant may degrade to some extent where they might be crucial for the intended activity[32]. In the succulent extraction, plant materials are made in to a paste without subjecting to drying step. The extraction of secondary metabolites would be carried out using this paste.

3.2. Phytochemical analysis of the plant extracts

Phytochemical screenings have been carried out to identify the natural product components present in the plant extracts. Tests for tannins (ferric chloride assay/lead acetate tests), phenolic compounds (Gelatin test), flavonoids (sodium hydroxide method), saponins (emulsifying/frothing test), proteins/amino acids, glycosides (Fehlings solution tes/Kellar Killani Test), terpenes, sterols, sugar, quinones, anthraquinone (Borntrager's test) and alkaloids (Mayer's/Wagner's/Dragendorrf's Test) had conducted with previously published methodologies or modified methodologies with references to previously published literature[3,20,33,34]. The observations have recorded as positive (+) for presence or negative (-) for absence, of the expected results.

The phenolic and flavonoids compounds have quantified as Total Phenolic Content (TPC) using Folin Ciocalteu method and Total Flavonoid Content (TFC) using aluminium chloride assay by spectrophotometrically[11]. The Folin Ciocalteau agent acts as a reducing agent and reacts with polyphenols present and gives a blue coloured complex at the reduced form. The intensity of the colour developed would be proportional to the polyphenols present[5]. Absorbance of the developed colour would be detected using an Ultraviolet Visible (UV-Vis) spectrophotometer. TPC has detected at 492 nm, 760 nm, 765 nm and the TFC has detected at 440 nm, 510 nm in different studies[5,11,13,20]. Differences of maximum absorption could be due to the types of spectrophotometers used. The TPC or TFC have expressed as milligram equivalent of the standard compound used to make the calibration curve. Gallic acid and pyrogallol has used as a standard for phenolic compounds. Rutin and quercetin has used as standards for flavonoids compounds[5,11,13,20].

Other than the above quantitative and qualitative measurements, Ultra Performance or High Performance Liquid Chromatography - Mass Spectrometry, GC-MS and Thin Layer Chromatography (TLC), High Performance Gel Permeation Chromatography (HPGPC), techniques have also used to analyse the phytochemical constituent of the isolated plant extracts[9,11,17,18,27,35].TLC has performed with known anticoagulant or antiplatelet compounds (Warfarin and acetyl salicylic acid) to identified the presence of molecules with similar structure in the plant extract[8]. Selected reagents have used to to identify flavonoids, tannins, coumarins, and triterpenes using TLC as well[36].

3.3. Preparation of the blood samples

Most studies have been conducted using human blood obtained from healthy non-smoking, non-alcoholic volunteers[2,21]. These donors have refrained from taking any medications of anticoagulant, antiplatelet, fibrinolytic or antibiotics duration of a week before the blood was taken to conduct the screening assays[15]. Use of human blood obtained from blood bank[27], bird blood[3], rabbit blood[14] and mice blood[6,11] is also reported in some studies. The blood has been collected to collection tubes containing an anticoagulant solution to prevent

the instant coagulation. Blood collected in to tubes contain anticoagulating reagent has subjected to centrifugation for a determined time and the supernatant plasma has collected and refrigerated[5]. The platelet has been separated from the blood by a series of centrifugation and cell volume has adjusted using automated cell counter[27]. Prepared plasma samples have used within particular day.

Washed Platelets: Washed Platelets has prepared for conducting platelet aggregation assays. Blood has been collected to tubes containing anticoagulation solution (citric acid, trisodium citrate, dextrose) and centrifuges to separate plasma rich platelet (PRP). PRP has again centrifuged to eliminate residual blood cells and to obtain the platelet pallet. The platelet pellet has suspended in wash buffer (pH 6.5) and has centrifuged again and finally suspended in final buffer (pH 7.4) to use in the assays[6,7,12].

A detailed procedure for preparation of Washed Platelets for antiplatelet assay is described as follows. The collected blood has centrifuged to separate the PRP. The PRP has again centrifuged at 120g for 8 minutes to eliminate residual red blood cells and again at 400g for 15 minutes to sediment platelets. The resulted supernatant, Platelet Poor Plasma (PPP) has been collected and suspended in buffer pH 6.5 (NaCl 137 mM, KCl 2.6 mM, $NaHCO_3$ 12 mM, $MgCl_2$ 0.9 mM, $CaCl_2$ 1.3 mM, glucose 5.5 mM, gelatin 0.25%). Again after centrifuging at 400g for 15 minutes, platelet pellet has suspended in final buffer with pH of 7.4 (NaCl 137 mM, KCl 2.6 mM, $MgCl_2$ 0.9 mM, glucose 5.5 mM, gelatin 0.25% Hepes 5 mM, $CaCl_2$ 1.3 mM) for conducting assay[12].

Platelet Poor Plasma (PPP) for anticoagulant assays: Blood has collected to tubes containing anticoagulant, trisodium citrate, and centrifuges at 3000 rpm for 20 minutes to separate the blood cells from PPP[6,12]. The PPP has been used to plasmatic coagulation assays[15].

3.4. In vitro screening methods for platelet aggregation

Platelet aggregation assay has used to monitor the plant extracts effect on primary haemostasis[6]. Different aggregation inducers have used to understand the mechanism of action of the extract[12]. In some studies, IC_{50} values of the different does of the extract is also calculated[10].

The aggregation of platelet has monitored by the change in light transmittance at 950 nm using automated/semi-automated aggregometer[6,11]. For the test; an aliquot of Washed Platelet has been incubated (at 37 ℃ for 30 minutes) and then plant extract has added. After, aggregation has induced by adding platelet aggregation agonists such as collagen, epinephrine, thrombin, ADP, etc. readings have observed through a determined time period[10,12]. The test control would be prepared without adding the plant extract (usually containing the buffer which the extract was dissolved). This extract treated sample would be compared with control sample to observe the effect to get a measure of anti-aggregation or pro-aggregation effect of the used plant extract[6].[6] The platelet aggregation assay and vasodilator-stimulated phosphoprotein (VASP) assay[37] has conducted using whole blood and PRP to monitor the platelet function in presence of plant extract too[9].

3.5. In vitro screening methods for plasmatic coagulation.

The plasmatic coagulation is regulated by two pathways. Intrinsic pathway (contact factors) and extrinsic pathway (tissue factors)[2]. The intrinsic pathway is associated with the coagulating Factors I, II, IX, X, XI, and XII[5]. These Factors also known as fibrinogen, prothrombin, Christmas Factor, Stuart-Prower Factor, plasma thromboplastin, and Hageman Factor, respectively[5]. The common pathway is associated with Factors I, II, V (labile Factor), VIII (antihemophilic Factor) and X[5]. These are activated by serine proteases resulting in the activation of conversion of fibrinogen to fibrin[5]. Plasmatic coagulation assays are used to evaluate the plant extracts' effect on secondary haemostasis[6].

Prothrombin Time (PT) Test: PT is used to screen the effect of plant extract having on extrinsic pathway[5]. This assay is used in detecting the deficiencies in Factors II, V, VII (stable Factor), and X. Deficiency in these Factors indicated by a prolonged PT[3]. Most tests are conducted using commercially available PT reagents following the manufacturers' guidelines. In brief, separated plasma volume will be mixed with an appropriate volume of the plant extract. After incubation (37 ℃) for a predetermined time period (5-10 minutes) thromboplastin reagent which is provided by the assay kit manufacturer would be added to the reaction mixture and the clotting time will be monitored. Prepared plasma alone with the solvent and thromboplastin reagent will be developed as the test control[5].The use of ethylenediaminetetraacetic acid (EDTA) as a negative control in comparison of the plant extract is also reported[38].

A manual method of carrying out the PT test is reported as follows[20]. A portion of isolated plasma has placed in a water bath at 37 ℃ and after incubating for few minutes thromboplastin reagent has added to the plasma. An aliquot of the plant extract prepared in saline has added to this tube containing plasma and thromboplastin reagent and then the tube has gently tilted back and forth for mixing. The time has recorded using a stopwatch till a clot is observed. A similar method is reported in another study with description of preparing the thromboplastin reagent using cow brain tissues[39].

Activated Partial Thromboplastin Time (APTT) Test: APTT test is used to detect the effect of the plant extract having on intrinsic and common coagulation pathways. This test also follows the similar procedure as the PT test with guidelines provided by the commercial assay kit manufactures. The APTT reagent (cephaloplastin[5], kaolin[33]) will be added and then after incubation for few minutes, a solution of $CaCl_2$ will be finally added before taking the coagulation measurements[7,20].

Thromboplastin Time (TT) Test: TT explores the fibrin formation step, which is the final step in coagulation cascade[12]. Prolongation of TT compared with the control indicates the inhibition of thrombin activity or polymerization[14]. Similar procedure has been followed in this test according with the details provided by the assay kit manufactures. After treatment the separated blood plasma with the plant extract and the test samples have been incubated at 37 ℃ and thrombin has been added just before the measurements are taken[7,27].

IV. Discussion

Interest has arisen towards the bioactive natural ingredients to their beneficial health effect[9]. These compounds derived from plant sources have shown to poses antioxidant, anti-inflammatory activities among other beneficial properties. Such bioactivities could be important in controlling the progression of disease associated with oxidative stress such as cancer and cardiovascular diseases[21]. Many natural antioxidants have shown protection for cell component from oxidative stress by preventing free radical formation and have indicated prevention of many pathophysiological processes[27].In this regard, combination of anticoagulant activity with other bioactivities such as antioxidant abilities could be beneficial for human consumption[17]. Information available on such haemostatic plants could be important to the individuals who are taking medications for antiplatelet or anticoagulation therapy. Here, they would be able to adjust their dietary habit accordingly and consume these foods with caution in day today life[40].

Investigations also have executed on the cheminformatics gathered from published literature. Cheminformatics can be introduced as a branch of bioinformatics. Here, information related to anticoagulation compounds and plants have been assembled using scientific forums and databases, opting to the use of native ethnomedical information available. Plant genus or species with possible haemostatic abilities have been narrowed down using previously published literature and with compound structures that have demonstrated anticoagulation activity[40,41]. These information has used to screen haemostatic plant varieties in same genus to obtain successful results[40]. Additionally, a somewhat similar method to the cheminformatics is mentioned on a study conducted on *Satureja thymbra*. A hypothesis seemed to build on that, plant belong to the same genus could show similar biological activities. A Palestinian variety of *Satureja hortensis*, another plant belongs to the same genus, has chosen for the study because of the traditional use of *Satureja thymbra*'s in treatment of cardiovascular and blood clotting ailments[2].

Bioactive compounds present in the plant extract have been identified using Gas Chromatography[15]. Though, accounts of isolation and structure elucidation of the compounds responsible for haemostatic activity is rare. In a study conducted on *Malus pumila* flowers, researchers have successfully complete the isolation and structure elucidation of nine compounds[14]. Out of these compounds, kaempferol-7-O-β-d-glucopyranoside, kaempferol-3-O-α-l-arabinofuranoside, phloridzin, kaempferol, phloretin, β-sitosterol have demonstrated procoagulant activity and compound pyracanthoside has shown to poses anticoagulant activity[14]. These findings imply that, a plant extract could contain mixture of both procoagulant and anticoagulant phytochemicals or one responsible component, emphasizing on the importance of isolation of responsible constituents.[42] Compounds, 2-(2-methoxypropyl)-5-methyl-1,4-benzenediol, thymol and carvacrol have isolated from *Nigella sativa* seed oil have also demonstrated inhibitory activity against platelet aggregation[23].

Tannins and flavonoids are two secondary metabolites that the haemostatic activity can be attributed to[43]. There are reports of the beneficial effect of flavonoid in haemostasis and thrombosis inhibiting the platelet function of adhesion, aggregation and secretion[6]. Different types of flavonoids with antiplatelet activities have been studied and their mechanism of actions have comprehended[44]. Polysaccharide compounds present in plants also might contribute to blood coagulation activities. It is expressed that, tannins (a class of polyphenol) have a high affinity for proteins and therefore, can form strong complex with proteins which can affect the biochemical process as well[13]. Some plants contain high level of tannins as a secondary metabolite. In this situation viability of cells (platelets in antiplatelet studies) in presence of tannins (or the secondary metabolites in the extract)

should be taken in to consideration too[22]. Conducting an assay to determine the effect of crude extract or the cytotoxicity of the extract having on cells is important in this regard[22]. The necessity of removal of tannins from the extract to obtain accurate information arise from this and in some studies tannin removal has been achieved by the use of polyvinylpolypyrrolidone (PVPP) or bovine serum albumin by exploiting their strong hydrogen bonding capabilities[13].

In most cases extracts have shown to give either positive or negative results in a dose dependent manner. Plant extracts could function in different mechanism to produce haemostatic effect. Depending on these interactions observed results of the assays could vary with tests. In a study conducted on *Rhodomyrtus Tomentosa*, the extract has demonstrated anticoagulation activity for PT at a significantly lower extract concentration compared with the doses that exhibited anticoagulation activity for APTT and TT assays[43]. Extracts have shown to demonstrate both pro-coagulation and anticoagulation activities. A study on *Jatropa curcas* latex has illustrate this by giving pro-coagulating and anticoagulating activities for different assays conducted. In this study, the constituents responsible for the opposing activities have been successfully separated by solvent extraction with butanol and ethyl acetate[45].

The Table 1 presents a selection of plant varieties used in screening in vitro haemostatic activities. Details of the plant parts used, extraction medium and in vitro assays conducted to evaluate haemostatic activities are included with references.

Table 1:A selection of plants used in evaluating in vitro haemostatic activities

Plant	Plant part/s used	Extraction medium	Activities tested	Ref.
Acanthus ilicifolius	Roots	Water	PT, APTT	[20]
Acalypha Indica	Leaves	PE,$CHCl_4$,EtOAc,EtOH etc	PT	[34]
Acokanthera oppositifolia	Root, stem, leaves	MeOH or Water	Thrombin assay	[13]
Allium sativum	Bulbs	Water	PT	[46]
Aloe spesiosa	Root, stem, leaves	MeOH or Water	Thrombin assay	[13]
Angelica shikokiana	Aerial part	MeOH	PA, PT, APTT, TT	[42]
Arbutus unedo	Leaves	Acetone/water	PA	[10]
Arnica montana	Flowers	Acetone/water	PA, WBA	[22]
Artemisia argyi	Leaves	Aq. EtOH	PT,TT	[35]
Beta vulgaris	Bulb	Aq. MeOH	PT, APTT	[47]
Calamintha officinalis	Aerial part	PE,CH_2Cl_2,EtOAc, MeOH	PA, PT, APTT, TT, FIB	[12]
Camellia Sinensis	Leaves	Water	PT	[46]
Canna warszewiczii	Rhizome, leaves	Aq. EtOH	PT, APTT	[24]
Cassia petersiana	Roots	MeOH & Water	PT, APTT	[8]
Cassine transvaalensis	Bark	MeOH & Water	PT, APTT	[8]
Catharanthus roseus	Root, stem, leaves	MeOH or Water	Thrombin assay	[13]
Chromolaena odorat	Leaves	MeOH & Water	APTT	[33, 39]
Crassocephalum crepidioide	Leaves	Aq. MeOH	PT, APTT, CT	[29]
Dalbergia melanoxylon	Bark	MeOH & Water	PT, APTT	[8]
Dalbergia sissoo	Bark	Aq. EtOH	PT	[28]
Datura stramonium	Flowers	$CHCl_4$ and MeOH	PT	[3]
Dichrostachys cinerea	Roots	MeOH & Water	PT, APTT	[8]
Elaeagnus rhamnoides	Berries	Aq. MeOH	PT, APTT, TT	[21]
Enteromorpha compressa	Algae	Milli Q Water	PT, APTT	[26]
Ficus auriculata	Leaves	MeOH	PT, APTT	[5]
Ficus benghalensis	Leaves	MeOH	PT, APTT	[5]
Ficus elasticaa,	Leaves	MeOH	PT, APTT	[5]
Ficus palmata	Leaves	MeOH	PT, APTT	[5]
Ficus religiosa	Leaves	MeOH	PT, APTT	[5]
Ficus semicordata	Leaves	MeOH	PT, APTT	[5]
Geastrum fimbriatum	Mushroom	EtOH	PT, APTT	[25]
Genipa americana	Leaves	Aq. MeOH	PA PT, APTT, TT	[30]
Gentiana scabra	Roots	Water & EtOH	PT, APTT, TT	[35]
Gloriosa superba	Root, stem, leaves	MeOH /Water	Thrombin assay	[13]
Gracilaria filiforms	Algae	Milli Q Water	PT, APTT	[26]
Harpagophytum procumbens	Roots	MeOH & Water	PT, APTT	[8]
Humulus lupulus	Spent hops	Acetone/water	PA, VASP	[9]
Jatropha curcas	Latex		PT, APTT	[39, 45]
Jatropha gossypiifolia	Leaves	Water	PT, APTT	[17]

Juglans regia	Root bark	Water	PA PT, APTT, TT, FIB	[6]
Juglans regia	Husks	Acetone/water	PA,WBA	[22]
Kigelia africana	Fruits	MeOH & Water	PT, APTT	[8]
Leonotis leonurus	Root, stem, leaves	MeOH, Water	Thrombin assay	[13]
Lumnitzera racemosa	Leaves	Water	PT, APTT	[20]
Lupinus pilosus	Aerial part	Aq. EtOH	PT, APTT	[2]
Malus pumila	Flowers	EtOH, n-BtOH	PT, APTT, TT, FIB	[14]
Medicago sativa	Leaves	MeOH & Water	PT, APTT	[8]
Mikania cordata	Leaves	EtOH	PT, APTT, TT	[15]
Mikania laevigata	Leaves	EtOH	PT, APTT	[36]
Mikania scandens	Leaves	Succulent	in vivo	[32]
Mundelea sericea	Roots	MeOH & Water	PT, APTT	[8]
Nelumbo nucifera	Leaves	EtOH, MeOH, EtOAc	PT	[38]
Nigella sativa	Seed (oil)	MeOH/Hexane	PA	[23]
Parietaria judica	Aerial part	Aq. EtOH	PT, APTT	[2]
Parinari curatellifolia	Bark	MeOH & Water	PT, APTT	[8]
Paronychia argentea	Aerial part	Aq. EtOH	PT, APTT	[2]
Plinia cauliflora	Leaves	EtOH	PA, PT, APTT	[48]
Panax bipinnatifidus	Root, stem		PT, APTT	[49]
Panax stipuleanatus	Root, stem		PT, APTT	[49]
Rhodomyrtus tomentosa	Leaves	Water	PT, APTT, TT	[43]
Rubia tinctorum	Roots	EtOH /water	PA	[11]
Ruta chalepensis	Aerial part	Aq. EtOH	PT, APTT	[2]
Salix alba	Bark	MeOH & Water	PT, APTT	[8]
Satureja thymbra	Aerial part	Aq. EtOH	PT, APTT	[2]
Solanum aculeastrum	Fruit	MeOH & Water	PT, APTT	[8]
Solanum panduriforme	Fruit	MeOH & Water	PT, APTT	[8]
Sutherlandia frutescens	Root, stem, leaves	MeOH or Water	Thrombin assay	[13]
Syzygium aromaticum	Flower bud	Water	PT	[46]
Taraxacum officinale	Root,Leaves,Petls	MeOH	PA, PT, APTT, TT,	[7,27]
Teucrium creticum	Aerial part	Aq. EtOH	PT, APTT	[2]
Turbinaria conoides	Algae	Milli Q Water	PT, APTT	[26]
Thymbra spicata	Aerial part	Aq. EtOH	PT, APTT	[2]
Thymus atlanticus		Water	PT, APTT	[19]
Thymus satureioides		Water	PT, APTT	[19]
Thymus zygis		Water	PT, APTT	[19]
Typha capensis	Root, stem, leaves	MeOH or Water	Thrombin assay	[13]
Urtica urens	Aerial part	Aq. EtOH	PT, APTT	[2]
Verbascum fruticulosum	Aerial part	Aq. EtOH	PT, APTT	[2]
Vitis vinifera	Seed		PA, PT, APTT	[37]
Zantedeschia aethiopica	Root, stem, leaves	MeOH or Water	Thrombin assay	[13]
Zingiber officinale	Rhizomes	Water	PT	[46]

APTT – Activated Partial Thromboplastin Time, CT-Clotting Time, FIB – Fibrinogen assay, PA –Platelet Aggregation, PT –Prothrombin Time, TT - Thromboplastin Time, WBA – Whole blood anticoagulation

V. Conclusion

Many plant varieties had studied based on the ethnomedical evidences of haemostatic activity of the plants. Conventional extractions procedures have followed to obtain the secondary metabolites and phytochemical screenings have conducted to identify the secondary metabolite constituents. Often, Total Phenolic Content and Total Flavonoid Content have evaluated. Platelet aggregation assay have conducted to evaluate the effect of the plant extract on platelet and plasmatic coagulation of the extract which have evaluated using PT, APTT and TT tests. These biochemical tests have used in predicting the mechanism of action of the plant extract on haemostasis. Studies are rare with isolation of the responsible bioactive compounds or structure elucidation of the responsible bioactive compound/s. A cytotoxicity assay conducted on plant extracts to evaluate the toxic effect could use to eliminate any doubt of the cell viability in presence of the secondary metabolites. A bioassay guided fractionation or a solvent partition of the crude plant extract could be assistance in general identification of the responsible bioactive components.

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Conflict of Interests

Authors of this review article declare no conflict of interests.

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