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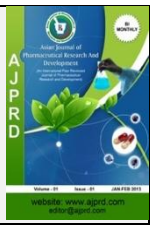

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Review Article

Review: Phytochemical of Some Plants with Anticoagulant

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A B S T R A C T

Background: Anticoagulants are drugs used to treat hypertension and cardiovascular disease.This review article aims to discuss several types of natural plants with anticoagulant activity.

Materials and Methods: The method used is the study of relevant literature(national and international journals) which is accessed through online sites such as Google Scholar, Research Gate, Science Direct, Springer Link, and NCBI, published in the last 10 years (2011-2021) with the keyword "herbal plant" or "medicinal plant" and "anticoagulant" and "cardiovascular".

Results: Based on in vitro and in vivo tests, some plants such as Allium sativum L., Averrhoa bilimbi, Persea americana Mill., Tridax procumbens L., Carica papaya L., Rhizophora sp, Syzygiumcumini L., Piper batle L., Erigeron canadensis L., Ainsliaea fragrans, Averrhoa bilimbi, Carica papaya L., are types of plants with anticoagulant activity.

Conclusion: Herb can be used as alternative sources of new anticoagulant agents cause of their biological activity. The use of medicinal plants with therapeutic effects and minimal side effects can be the best choice for the prevention and treatment of various diseases including cardiovascular disorders.

Keywords: Anticoagulants, Herbs, Phytochemicals

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INTRODUCTION

Cardiovascular diseases such as hypertension, heart attacks, strokes,etc. cause many deaths in several countries in the world, especially in developed countries. Every year, many people die caused this disease field [(1)]. Drugs with antithrombotic, anticoagulant and antiplatelet activity were used in its treatment such as heparin[(2)] and warfarin[(3)].

Anticoagulant drugs consisting of heparin, vitamin K antagonists, and their derivatives are drugs that were commonly used to treat cardiovascular disease, especially heparin. Heparin is the most popular cardiovascular drug was commonly used as an anticoagulant and exhibits strong anticoagulant activity. However, heparin has side effects, such as bleeding and has the potential to carry the risk of viral contaminants of animal origin [(4)]. Therefore, it is important to find new sources of anticoagulants which more effective and safer to use as antiplatelet agents in patients with cardiovascular disease. Continuous research to find more effective and efficient anticoagulant drugs without any side effects has been carried out by many researchers around the world, and plant-derived ingredients are a better source and deserve to be researched.

Herbal plants can be used as alternative sources of new anticoagulant agents caused of their biological activity. The use of medicinal plants with therapeutic effects and minimal side effects can be the best choice for the prevention and treatment of various diseases including cardiovascular disorders. Several studies have shown that consuming plants that contain anticoagulant substances or phytochemicals

with anticoagulant properties can reduce or eliminate the risk of cardiovascular disease. This review discusses natural plants used as anticoagulants, which aims to provide information about some plants around us as sources of anticoagulants.

METHODS AND DATA COLLECTION

In compiling this review article, the technique used is the literature study method in the form of national and international journals published in the last 10 years (2011-2021). In making a review of this article, we used data searches on online media with the keywords "herbal plant" or "medicinal plant" and "anticoagulant". ScienceDirect (112), Researchgate (59), and Google Scholar (162). The articles used are articles published in English and Indonesian language.

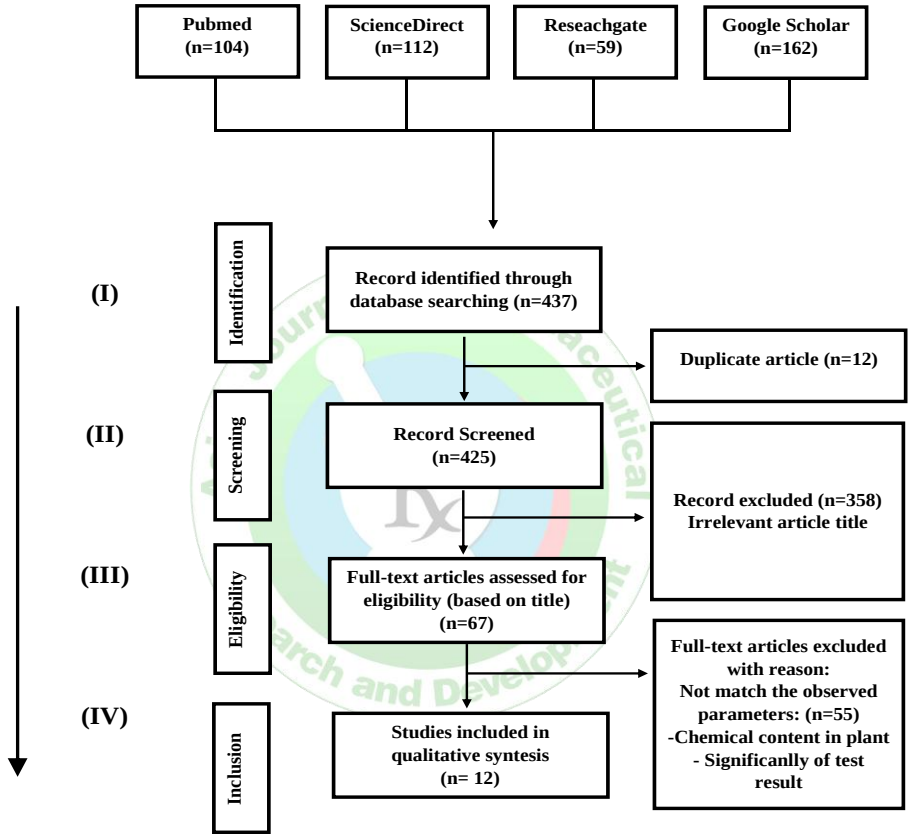

Figure 1: Flow chart of search and selected article.

RESULTS AND DISCUSSION

Literature Review

From the twelve articles selected, six were tested in vitro and six were tested in vivo. The samples used in this article are extracts from several herbal plants with anticoagulant activity with various doses was used. In vivo tests were carried out with Wistar rats (Rattus norvegicus), mice (Mus musculus L.) and rabbits. For the in vitro test using human blood samples, rabbits and also rats or mice.

In Vitro Test

In vitro testing is by using the main cells and tissues isolated from an organism for preclinical testing[5]. In the anticoagulant activities testing, an in vitro test was used using a blood sample, then the blood clotting time was measured. Some herbal plants that have been tested in vitro are presented in table 1.

Table 1: Herbal Plants with Anticoagulant Activity Tested In Vitro

Herbal plant	Part of plant used	Extraction Method and Solvent	Compounds obtained	Test method	Method	Test result	Ref
Allium sativum L.	Bulb	Garlic extract	Flavonoids	Lee-White and blood smear	Blood samples from three donors were 3 mL each. Treatment group: 1 mL blood + 100 µl garlic extract 1 mL blood + 100 µl garlic extract + 1 mg EDTA Negative control: 1 mL blood and 100 µl ethanol 96% Positive control: 1 mL blood and 1 mg EDTA	There was no blood clot in the treatment group	(6)
Averrhoa bilimbi	Leaf and bark	maceration, Ethanol	Flavonoids and alkaloid	aPTT and PT	Treatment group: PT test (2 mg/mL extract ethanol *A. bilimbi* + 100 µl platelet poor plasma (PPP) + 200 µl reagent PT), aPTT test (2 mg/mL extract ethanol *A. bilimbi* + 100 µl platelet poor plasma (PPP) + 200 µl regen aPTT) Negative control: aquades Positive control: heparin 1 mg/ml	↗Coagulation 1.47 times longer (17.1 seconds) ↗Coagulation 1.24 times longer (39.67 seconds)	[12,13]
Persea americana Mill.	Leaf	Maserasionon, Ethanol	Alkaloid	CT and Lee-White	The test was carried out using the Lee-White method Treatment group: avocado leaf extract with concentrations of 0.1, 0.5 and 1 mg/ml, respectively. added to 1 ml of blood sample. Negative control: ethanol Positive control: heparin (100 IU/ml)	↗increase the maximum coagulation time significantly p<0,05 at a concentration of 0.1, 0.5 and 1 mg/ml.	[15]
Tridax procumbens L.	Leaf	Fractionation,acetone	Flavonoids	A PTT	The test sample was used normal human blood. Treatment group: 5, 10, 20, 40, 60, 80 lee 100 µg/mL polysaccharide sulfate isolated from *T. procumbens* Negative control:physiological saline solution Positive control: heparin	↗prolong the aPTT time (113 seconds) four times longer than the negative control and significantly similar effect with heparin (p<0.05)	[16]
Carica papaya L.	Leaf	Maceration, Ethanol	Alkaloids	CT, PT, and APTT	Treatment group: 100 µL blood plasma + 100, 300, or 600 gr test sample + 100 L regen aPTT. Negative control:distilled water 1 ml/kg Positive control: 1 mg rivaroxaban	Significantly prolong CT, PT and APTT (p≤0.01)	[24]
Rhizophora sp	Leaf	Maserasionon, Ethanol	Flavonoids and coumarin	Lee-White	Blood samples were taken from 5 people each 5 ml. Treatment group: 120 µL of Rhizophora sp extract + 1, 2, 3 ml blood sample. Negative control: blood sample 1 ml Positive control: blood sample 1 ml + EDTA	There was no blood clot in the treatment of 120 L of Rhizophora sp extract + 1 ml of blood sample. (active as an anticoagulant)	[27]

Allium sativum L.

Garlic (*Allium sativum* L.) contains essential oils, allicin compounds, alliin and other organosulfur compounds such as ajoene which act as anticoagulants. Garlic belongs to the *Amaryllidaceae* family. The part used is the tuber. Garlic also contains phenolic compounds such as antioxileets [(6)], calcium which can prevent hypertension, ajoene compounds that can decreaseblood cholesterol levels [(7)], anticoagulants [(8)]and other compounds such as protein, sulfur and saltivin. Ajoene can prolong blood clotting time because it has anticoagulation properties. Thus, it can directly reduce the risk of stroke and cardiovascular disease. Garlic is also useful for reducing blockages in the arteries of the heart thereby minimizing the occurrence of heart attacks [(7)]. *Allium sativum* L. extract has an anticoagulant activity that can prevent blood clots (coagulation) by increasing blood clotting time. There was no blood clotting in the first (1 mL blood + 100 µl garlic extract) and second (1 mL blood + 100 µl garlic extract + 1 mg EDTA) treatment samples after being tested for 24 hours[(9)]

Averrhoa bilimbi

Averrhoa bilimbi belongs to the *Oxalidaceae* family. This plant is native in Indonesia and Malaysia andis spread in several countries and become an exotic plant such as in

Argentina, Australia, Brazil, India, Thailand, Venezuela, etc. The part of the plant used is the bark. The chemical content of this plant is alkaloids, phenols, Flavonoidss, glycosides, triterpenes and saponins [10,11].

The ethanol extract of *Averrhoa bilimbi* in the Prothrombin Time (PT) test showed that the coagulation time was 17.10 seconds or 1.47 times longer than the negative control. Meanwhile, the positive control time (heparin 1 mg/mL) was 58.10 seconds or 5 times longer than the negative control. The Activated Partial Thromboplastin Time (aPTT) test showed an extension of the coagulation time which was 39.67 seconds or 1.24 times longer than the negative control, while the positive control had a coagulation time of 110.67 seconds or 3.45 times longer than the positive control. The results showed that the ethanolic extract of A. bilimbi leaves had anticoagulant activity. anticoagulant activity, it is characterized by its ability to prolong blood coagulation time in both the extrinsic (PT) and intrinsic (aPTT) pathways [12,13].

ersea americana Mill.

Persea americana Mill. (Avocados) belong to the *Lauraceae*family. The leaves of this plant contain saponins, tannins, Flavonoidss, alkaloids and polysaccharides. Phytochemical compounds belonging to the alkaloid group can be used as anticoagulation. At least 33 types of alkaloid compounds contained in avocado leaves include theophylline, caffeine, and atropine [14]. The addition alkaloid extract of avocado leaf with concentrations of 0.1 mg/ml, 0.5 mg/ml and 1 mg/ml could significantly inhibit coagulation (in vitro) ($P < 0.05$). In the Clotting Time (CT) test, the average total alkaloid concentration of avocado leaf extract showed a longer coagulation time when compared to the negative control. Based on the test results showed that the total alkaloids extracted from the leaves of P. americana Mill. can prolong the coagulation time significantly ($P < 0.05$) concerning the inhibition of coagulation [15]. Alkaloid compounds work by inhibiting or prolonging the activity of thrombin and factor Xa and inhibiting the polymerization of fibrin into thrombin, alkaloids also prolong the CT process in the blood.

Tridax procumbens L.

Tridax procumbens L. belongs to the *Asteraceae* family. Part of the plant used is the leaf. *T. procumbens* L. was commonly known as a nuisance plant. In India, this plant was used as traditional medicine for treating dysentery, antibacterial, antifungal, and diarrhea. This plant contains sulfated polysaccharides. Aqueous extracts of *T. procumbens* have been investigated in lowering high arterial blood pressure. Sulfated polysaccharides derived from *T. procumbens* exhibit excellent anticoagulant activity and are useful for anticoagulant therapy.A higher concentration of sulfated polysaccharides was required to achieve a significant difference ($p<0.05$) and to have the same effect as heparin in the aPTT test.[16].

Carica papaya L.

Carica papaya L. (Papaya) belongs to the *Caricaceae* family. These plants are commonly grown in tropical areas like Indonesia.The leaves, fruit, seeds, and sap of papaya were commonly used as traditional medicine. Papaya has pharmacological effects such as antimicrobial and anti-inflammatory[17], anticancer[18], decrease blood sugar and fat levels[19], analgesic[20], and antioxileet [21]. Based on the literature, papaya leaves contain alkaloids, Flavonoidss, phenols, phenolic acids, choline, carbohydrates, vitamin C and vitamin E [22]. Papaya alkaloids have an anticoagulant activity that can prolong CT, PT, and aPTT times compared to controls. The total alkaloid content in papaya leaves is between 1.6% [23] and 1.8% [24]. Papaya leaf total alkaloids have antiplatelet, anticoagulant, and thrombolytic activity by increasing the percentage of inhibition of platelet aggregation, significantly prolonging CT, PT, and aPTT and increasing the percentage of thrombolytic [24].PT increased significantly ($p\leq0.01$) at a concentration of 100 gr and ($p\leq0.001$) at a concentration of 300 and 600 gr, respectively.the effect of adding sample is similar to 1 mg of rivaroxaban.

Rhizophora sp.

Mangroves (*Rhizophora sp*) have anticoagulant activity. This plant contains Flavonoids and coumarin compounds which play a role in preventing blood clots and can be used as drug preparations for cardiovascular disease [25]. Human blood samples were taken from 5 volunteers, each as much as 5 ml/person. The addition of 120 µL of *Rhizophora sp* extract into 1 ml of the blood sample, can be used as an anticoagulant. If the sample volume is more than 1 ml then coagulation will occur, this is presumably due to the ability of Rhizophora sp. in inhibiting blood coagulation depending on the number of blood samples used [26]. Coumarin compounds and their derivatives have activity as anticoagulants by blocking the activation of vitamin K so that the -carboxylation process of coagulation factors II, VII, XI, X is disrupted [27].

In Vivo Test

In vivo testing is presented in Table 2.

Table 2: Herbal Plants with Anticoagulant Activity Tested In Vivo

Herbal plant	Part of plant used	Extraction Method and Solvent	Compounds obtained	Experimental Animals	Test method	Method	Test result	Ref
Syzygium cumini L.	Leaf	Percolation, Methanol	Flavonoidss, Phenols, Terpenoids, Tannins and Alkaloids	Rabbit	TT, PT, and aPTT	Two groups of rabbits (groups 2 and 3) were given orally extracts of the leaves of Syzygium cumini L. at doses of 150 mg/kg and 500 mg/kg/day. Negative control: group 1 administration of distillate water Positive control: groups 4 and 5 with aspirin and warfarin	↗Thrombin was very significant (p≤0.005) compared to control ↗Bleeding timewas very significant (p≤0.005) compared to control ↗aPTTwas very significant (p≤0.005) compared to control	[29]
Erigeron canadensis L.	Flower	Reflux, Hexane	Polyphenol-polysaccharide	Mice	aPTT	Male wistar rat weighing between 290-330 gr. Treatment group: 50 mg of extract dissolved in 1 ml of distilled water, adjusted to pH 7.2 with 0.1 MNaOH solution, then added to the rat blood circulation system with a polyethylene tube. Negative control: physiological saline solution Positive control: heparin	↗prolong blood clotting time 3 times more than the control after 40 minutes the sample was injected into mice.	[30]
Ainsliaea fragrans	Whole part of plant	Percolation, Ethanol	Coumarin	Wistar Rat	aPTT, TT and PT	Rats were grouped into 8 groups, with seven rats per group. Each compound tested was dissolved in sodium carboxymethyl cellulose and given to the animals by gavage for three days at a dose of 1 mg/kg BW rats. Negative control: normal saline Positive control: warfarin (0.2 mg/kg)	↗significantly increased PT and TT (P < 0.01) on the 7-hydroxycoumarin derivative ↗increased TT significantly (P < 0.01) with the use of the compound nodakenin on the third day after administration.	[31]
Averrhoa	Leaf and	Maceration,	Flavonoidss	Mice	Bleeding time and	Male mice aged 8-12 weeks with a weight	↗bleeding time from 55.1 seconds to 1	[32]

bilimbi	bark	Ethanol			coagulation time	range 17-40 g. Two groups of mice (6 mice/group) were given ethanol extract of belimbing wuluh leaves with a concentration of 200mg/KgBW and 400mg/KgBW, respectively. All treatments were given a concentration of 0.5 ml. Negative control: aquades *ad libitum* Positive control: Asetosal (0,27 mg/26 gr BW)	second at a concentration of 400mg/KgBW, significantly different ($p<0.005$) against negative control. ↗coagulation time from 42.5 seconds to 75 seconds at a concentration of 400 mg/KgBW, significantly different ($p<0.05$) with negative control and a concentration of 200 mg/KgBW.	
Carica papaya L.	Fruit latex	The latex was drying at 40 C for 14 hrs. The latex was grinded into powder.	Alkaloids and Flavonoidss	Rabbit	PT, aPTT and blood clotting time.	New Zealand White Rabbits (male/female) weighing between 1.3-2.4 kg, grouped into 6 groups (5 rabbits/group). Treatment group: Papaya latex is given orally at doses of 4, 8, and 12 mg/kg. Negative control:distilled water 1 ml/kg Positive control: aspirin 2 mg/kg and heparin 1.5 mg/kg.	↗blood coagulation time was very significant ($p\leq0.0001$) against the control. There was no significant difference between the treatments, heparin, and aspirin.	[33]
Piper batle L.	Leaf	Maceration, Methanol	Flavonoids and alkaloid	Mice	Bleeding time	Five groups of mice (5 mice per group) were treated with 10%, 20% and 40% of betel leaf ethanol extract. Negative control: sterile distilled water Positive control: epinephrine	↗prolong blood clotting time 3 times more than Bleeding time was significantly different ($p<0.05$) compared to control	[35]

Syzygiumcumini L.

Syzygiumcumini belongs to the *Myrtaceae* family. This plant is spread throughout the South Asian continent including Indonesia, India, Sri Lanka and Pakistan. Part of the plant used is the leaf. The chemical constituents contain Flavonoidss (catechins, ferulic acid, and rutin) and essential oils (limonene) [28]. Fresh leaf extract of *S. cumini* was tested in vivo. A total of 5 groups of albino mice consisting of 10 mice each group were administered orally with *S. cumini* leaf extract. The first group was a negative control, the second and third groups were given *S. cumini* leaf extract at different doses, namely 15 mg/kg BW and 500 mg/kg BW. Meanwhile, groups 4 and 5 were positive controls with the addition of aspirin and warfarin. The

results showed a significant increase in the number of red blood cells, hemoglobin, hematocrit and platelets with values of 1.4.103 /cm, 2.2 g/dl, 6%, and 248.2 × 103/cm, respectively. Meanwhile, thrombin and CT prolongation were also very significant (p≤0.005), depending on the dose given when compared to controls. Meanwhile, the levels of platelet aggregation and fibrinogen decreased at high doses [29].

Erigeron canadensis L.

The effect of using *E. canadensis* extract in prolonging blood clotting time (CT) was tested in vivo on Wistar rats weighing 290-330 gr. A total of 50 mg of *E. canadensis* extract was dissolved in 1 ml of water, then injected directly into the heart through the arteries of the head. Two drops of blood were collected at 5-minute intervals to measure clotting time. The strongest anticoagulant effect was observed after 40 min of injection, with clotting time nearly 3 times longer than control measurements. After almost 60 minutes it still remains high until then slowly drops to a control time of 120 minutes. The plant preparation of E. canadensis was effective in vivo as an anticoagulant at a dose of 50 mg in 1 ml, and this effect was neutralized by protamine sulfate, injected into the rat blood circulation system. The mechanism of this reaction is similar to when using heparin [30].

Ainsliaea fragrans

Ainsliaea fragrans belongs to the *Asteraceae* family. This plant is used as a medicine to treat chronic cervicitis. The parts used are the whole plant. Coumarin compounds and their derivatives isolated from plants were tested in vivo to determine their effect on blood coagulation. The coumarin derivatives isolated from this plant are natural 4-hydroxycoumarin enantiomers (1), polycyclic pyrano[3-2c] carbon skeleton (2) and 7-hydroxycoumarin derivative (3) along with five other compounds. In vivo coagulation studies were carried out to measure anticoagulant activity using Wistar rats for each compound. To evaluate the anticoagulant activity in vivo, namely aPTT, PT, and TT were determined on the third and fifth days. Changes in aPTT, PT, and TT values were found on the fifth day. The compound 7-hydroxycoumarin showed anticoagulant activity in Wistar rats at a level of 1 mg/kg by prolonging PT and TT time in observed mice. The results showed that 7-hydroxycoumarin had potent anticoagulant activity to prevent blood clots [31].

Averrhoa bilimbi

The ethanolic extract of *A. bilimbi* leaves was given to the mice group at a dose of 200 mg/kg BW and 400 mg/kg BW orally for 7 consecutive daysbecause the increase in coagulation time in mice occurred on the seventh day. The tail bleeding method was used to measure the bleeding time and the capillary tube method was used to measure the coagulation time. In the bleeding time test, the results showed that the 400mg/KgBW extract group was the best administration level value with a significant value of p>0.005. This shows that 400mg/KgBW extract is more effective in prolonging bleeding time compared to 200mg/KgBW extract. In the blood coagulation time test, the results showed that there was no significant difference between the 400 mg/KgBW extract and the positive control, but there was a significant difference with the 200 mg/KgBW extract. This shows that the 400mg/KgBW extract was more effective in prolonging the coagulation time compared to the 200mg/KgBW extract [32].

Carica papaya L.

In in vivo testing, the part of the papaya plant used is the fruit to extract the sap. The activity of papaya latex showed a significant difference in increasing the bleeding time tested in vivo, the results were almost comparable to aspirin administration.

A total of 5 ml of healthy rabbit blood samples were taken through the marginal vein in the ear, mixed with 3.2% sodium citrate, centrifuged at 5000 rpm for 10 minutes, and the supernatant was immediately taken and put into a tube using a pipette and stored at 2-8° C. Then the sample will be used for PT and aPTT tests. The PT test was carried out using a standard PT assay kit. A total of 100 L of blood plasma was mixed with 100, 300, or 600 papaya latex and incubated for 2 minutes at 37°C. The time for clot formation was determined using a stopwatch. Determination was also carried out using plasma and 1 mg rivaroxaban as a control. As a result, the initial PT and APTT tests of blood samples before treatment were 10.79 seconds and 41.43 seconds, respectively. The addition of papaya latex as much as 100-600 g/100 L, can significantly increase the PT value (p≤0.01-0.001) and aPTT value (p≤0.0001).

The length of the coagulation time depends on the concentration of the addition of papaya latex. For the PT test, papaya latex had an effect similar to 1 mg of rivaroxaban. As for the aPTT test, the effect looks like heparin. Papaya sap was given orally with doses of 4, 8, and 12 mg/kg BW rabbits. The mechanism of action of this papaya latex in inhibiting blood clotting cannot be explained in detail from the results of this study. but it is suspected that because of the presence of constituent preparations such as alkaloids, Flavonoidss, glycosides, terpenes, and many other secondary metabolites can exhibit anticoagulant and antiplatelet effects. The effects of these plant constituents can carry out one or more inhibitions such as the formation

of a platelet plug (platelet activation and aggregation), conversion of serine proteases (zymogens) to active forms, active forms of clotting factors (such as Xa and thrombin), the release of calcium from the cytosol or increase in natural anticoagulant effects such as fibrinolysis inhibitors that can activate thrombin, AT III, and protein C. The results show that the sap from the unripe fruit of C. papaya L. has anticoagulant and antiplatelet properties which are useful as adjunctive therapy in the treatment of blood clotting disorders [33].

Piper batle L.

Piper batle L. (Betel) belongs to the *Piperaceae* family, part of the plant used is the leaf. This plant contains essential oils, alkaloids, amides, Flavonoidss, and lignans(34). Flavonoids compounds isolated from the Piper betle plant exhibited various biological properties including anti-coagulant. In vivo tests have been carried out to determine the effect of adding ethanol extract given to mice to determine the bleeding time. The results showed that 10%, 20%, and 40% concentration of betel leaf ethanol extract could prolong bleeding time with the average bleeding cessation time being 92.60 seconds, 104.80 seconds, and 124.60 seconds, respectively. The results of this study indicate that several compounds in betel leaf function as hemostatic agents. Flavonoids compounds can prolong bleeding time by means of vasoconstriction in blood vessels [35].

CONCLUSION

Some plants are commonly used to treat various diseases, including cardiovascular. However, the use of various types of plants as medicine has not been evaluated scientifically regarding the safety and efficacy as well as the dosage limits for their use. Based on the results of the review, it was found that there are many types of plants that contain certain chemical compounds that can be used as natural ingredients for cardiovascular drugs, especially anticoagulant drugs. This review article explores the various types of herbal plants found around the world that require more exploitation. Based on the results of several plant reviews, Carica papaya is a type of plant that shows the best anticoagulant activity compared to other plant species that have been reviewed. Carica papaya leaves in vitro had significantly different anticoagulant activity ($P \leq 0.01$) and had the same activity as the positive control. The papaya latex in vivo test result showed a significant difference ($P \leq 0.0001$) and had the same anticoagulant activity as positive controls. This indicates that Carica papaya has the potential to be used as a source of anticoagulants, so further research is needed.

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