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PHYTOCHEMICAL ANALYSIS AND INVITRO HEMOSTATIC ACTIVITY OF *MIMOSA PUDICA*, *HEMIGRAPHIS COLORATA* AND *CHROMOLAENA ODORATA* LEAF EXTRACTS

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ABSTRACT

Blood coagulation is an important mechanism in human body. Various plants have components which help in the formation of blood clot. The phytochemicals present in the extracts of *Mimosa pudica*, *Chromolaena odoratum* and *Hemigraphis colorata* are analyzed and evaluated the effect of these extract on blood clotting in vitro. Various phytochemical compounds like tannins, saponins, alkaloids, emodins, proteins, carbohydrate, terpenoids, glycosides, flavonoids, coumarins and phenols were found in the leaf extracts of the plant. The blood clotting time was evaluated using the Prothrombin Test (PT). The blood clotting times of all extracts in PT test were more than those of the control. But when compared between extracts, the extract of *Chromolaena odorata* shows blood coagulation within 2 minutes while the extracts of *Mimosa pudica* and *Hemigraphis colorata* do not show blood coagulation within 2 minutes. Various plants have phytochemical compounds which help in initiating the blood coagulation. Different plants like *Mimosa pudica*, *Chromolaena odoratum* and *Hemigraphis colorata* have phytochemical components like tannins and flavanoids which trigger blood coagulation. Among the three plants evaluated for invitro hemostatic effect, the blood clotting times of all extracts in Prothrombin test were more than those of the control. But when compared between extracts, the extract of *Chromolaena odorata* shows blood coagulation within 2 minutes while the extracts of *Mimosa pudica* and *Hemigraphis colorata* do not show blood coagulation within 2 minutes. In vitro negative results in this study might be due to an insufficient amount of calcium or active compound to induce blood coagulation. Therefore, the amount of calcium and active compound in the extracts should be further investigated.

Keywords: Prothrombin, Hemostatic Effect, *Mimosa pudica*, Polyphenolic Compounds

INTRODUCTION

The term "herbs" refers to plants or parts of them, including grasses, flowers, berries, seeds, leaves, nuts, stems, stalks and roots, which are used for their therapeutic and health- enhancing properties (Zheng and Wang, 2001; Liu and Ng, 2000; Lust, 2014). Generations of skilled herbal practitioners, researchers and scholars have refined and tested the vast science of herbology, producing thousands of plant-based remedies that are safe and effective (Jahan, 2016; Hefferon, 2012; Ross, 2013). The proper and judicious use of herbs is often successful in the treatment of illness when other, more conventional medicines and methods fail (Borgerson, 2005).

Herbs can be used to cleanse the bowels, open congested sinuses, help mend broken bones, stimulate the brain, increase libido, ease pain, aid digestion, blood coagulation and a thousand other purposes (Balch, 2002; Arvigo and Balick, 1993; Waltz, 2004). Topically, herbs can repair damaged skin, soothe a wound, improve complexion, heal bruises and relieve aching muscles (Debjit Bhowmik *et al.*, 2009; Jacknin, 2001; Bell, 2012). Herbs demonstrate great versatility for the treatment of a broad variety of health needs (Gigliotti *et al.*, 2004; Redgwell and Fischer, 2005). Plant medicines are the most widely used medicines in the world today (Farnsworth, 1988; Bailey and Day, 1989; Fabricant and Farnsworth, 2001). An estimated 80% of the world's population employs herbs as primary medicines (Mukherjee and Wahile, 2006; Folashade *et al.*, 2012; Mahady, 2001; Taylor *et al.*, 2001).

Many plants have components which can initiate blood coagulation (Seong and Matzinger, 2004; Goker *et al.*, 2008; Smith *et al.*, 2006). Blood clotting or coagulation is a chemical reaction that leads to a fibrin clot (Smith *et al.*, 2006; Davie and Fujikawa, 1975; Davie *et al.*, 1991). Coagulation is the actual

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formation of a blood clot. It results from a chemical “cascade” which begins with the prothrombin activators released by platelets. These activators activate prothrombin to thrombin. Thrombin acts on fibrinogen and breaks it into monomers which re-polymerize into insoluble fibrin. The fibrin forms threads which knit the platelets and other cells into the clot (Smith *et al.*, 2006; Davie and Fujikawa, 1975; Davie *et al.*, 1991). The component in extracts of some plant leaves helps in coagulation of blood (Goker *et al.*, 2008; Smith *et al.*, 2006).

The objectives of this study are to screen the phytochemicals present in the extracts of *Mimosa pudica*, *Chromola odoratum* and *Hemigraphis colorata* and analyze the effect of these extract on blood clotting in vitro. Given lacking qualitative and quantitative data on hemostatic activity of various herbs in Kerala, objective of this study were to screens the clotting time in various blood group sample and record the effect on clotting time.

Review of Literature

Blood is a specialized fluid or connective tissue that contains cells suspended in fluid matrix (Kimsey and Spielman, 1990; Ng *et al.*, 2005; Hall, 2015). Blood contains plasma proteins, water other important elements such as erythrocytes, leukocytes and thrombocytes. Plasma proteins include albumins, globulins and fibrinogens. Fibrinogen is a blood clotting protein (Kimsey and Spielman, 1990; Ng *et al.*, 2005; Hall, 2015; Balazs *et al.*, 1991).

Blood clotting or coagulation is a chemical reaction that leads to a fibrin clot. Coagulation is the actual formation of a blood clot (Ng *et al.*, 2005; Hall, 2015). It results from a chemical “cascade” which begins with the prothrombin activators released by platelets. These activators activate prothrombin to thrombin. Thrombin acts on fibrinogen and breaks it into monomers which re-polymerize into insoluble fibrin. The fibrin forms threads which knit the platelets and other cells into the clot (Kimsey and Spielman, 1990; Ng *et al.*, 2005; Hall, 2015; Balazs *et al.*, 1991). Hemostatic agents cause hemostasis, a process by which the body spontaneously stops bleeding and maintains blood in the fluid state within the vascular compartment (Rosado *et al.*, 2009).

Clotting is a function of plasma. It depends upon the orderly interaction of a group of plasma proteins (which are sequentially activated following vascular injury) with some phospholipid (from either damaged tissue or platelets) and some Ca⁺⁺. The final stages include the formation of thrombin, which then converts soluble plasma protein fibrinogen to insoluble fibrin (Kimsey and Spielman, 1990; Ng *et al.*, 2005; Hall, 2015; Balazs *et al.*, 1991).

Another factor converts the fibrin into a cross-linked polymer which stabilizes the platelet plug and traps RBCs in the meshwork to form the actual blood clot. Depending on the type of vascular damage or abnormality, clotting can be initiated and proceed according to two different cascading pathways: the intrinsic (initiated by contact with and abnormal/foreign surface) or the extrinsic (initiated by exposure to tissue factors) (Kimsey and Spielman, 1990; Ng *et al.*, 2005; Hall, 2015; Balazs *et al.*, 1991). Herbal medicines are gaining a great importance in recent times (Kamboj, 2000; Koehn and Carter, 2005; Sen *et al.*, 2011).

Medicinal plants are a source of great economic value in the world (Farnsworth, 1988; Balick and Mendelsohn, 1992). Nature has gifted us a very rich botanical wealth and a large number of diverse types plants grow in different parts of the world (Kamboj, 2000; Koehn and Carter, 2005; Sen *et al.*, 2011). Thousands of species are known to have medicinal values and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times (Farnsworth and Soejarto, 1991; Srivastava *et al.*, 1996).

Herbal medicines involve the use of plants for medicinal purposes. The term “Herb” includes leaves, stems, flowers, fruits, seeds, roots, rhizomes and bark. There is currently a large and ever expanding global population base that prefers the use of natural products in various treatments and prevention of medical problems because it is proved that the herbal plants have a rich resource of medicinal properties (Zheng and Wang, 2001; Liu and Ng, 2000; Lust, 2014).

The chemical substances of the medicinal plants which have the capacity of exerting a physiologic action on the human body were the primary features (Haslam, 1996; Lewis and Elvin-Lewis, 2003). The

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bioactive compounds of plants such as alkaloids, flavonoids, tannins, phenolic compounds etc were considered to be very important (Kumaran and Karunakaran, 2007; Stintzing and Carle, 2004).

Tannins (commonly referred to as tannic acid) are water-soluble polyphenols that are present in many plant foods (Bravo, 1998; Chung *et al.*, 1998a; Reed, 1995; Chung *et al.*, 1998b). They have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals (Serrano *et al.*, 2009; Mangan, 1988; Kumar and Vaithyanathan, 1990; Mueller-Harvey, 2006). Therefore, foods rich in tannins are considered to be of low nutritional value.

However, recent findings indicate that the major effect of tannins was not due to their inhibition on food consumption or digestion but rather the decreased efficiency in converting the absorbed nutrients to new body substances (Serrano *et al.*, 2009; Mangan, 1988; Kumar and Vaithyanathan, 1990). Tannins have also been reported to exert other physiological effects, such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immune responses (Chung *et al.*, 1998a; Zhang *et al.*, 2007; Hole *et al.*, 2009). The dosage and kind of tannins are critical to these effects (Chung *et al.*, 1998a).

Flavonoids are polyphenolic compounds present in all foods of plant origin (Balasundram *et al.*, 2006; Zheng and Wang, 2001; Kris-Etherton *et al.*, 2002). Flavonoids have inhibitory effects on the functions of platelets and leukocytes (Middleton, 1998; Nijveldt *et al.*, 2001; Middleton and Kandaswami, 1992). They also protect endothelial cells, and counterbalance the interactions between the blood stream and vascular wall, which may lead to thrombosis. The latter effect is mediated through the effect of flavonoids on human monocyte tissue factor, which itself may trigger blood coagulation (Lale *et al.*, 1996; Visioli and Galli, 1998).

Terpenoids (also called “isoprenoids”) are secondary metabolites occurring in most organisms, particularly plants (Verpoorte, 2000; Singer *et al.*, 2003; Hadacek, 2002; Chen *et al.*, 2011). More than 40 000 individual terpenoids are known to exist in nature with new compounds being discovered every year (Verpoorte, 2000; Singer *et al.*, 2003; Thoppil and Bishayee, 2011).

A large number of terpenoids exhibit cytotoxicity against a variety of tumor cells and cancer preventive as well as anticancer efficacy in preclinical animal models (Bishayee *et al.*, 2011; Thoppil and Bishayee, 2011; Paduch *et al.*, 2007).

Coumarin is a fragrant organic chemical compound in the benzopyrone chemical class, which is a colorless crystalline substance in its standard state (Raj *et al.*, 2015; Kim *et al.*, 2015). It is a natural substance found in many plants. Coumarin was first synthesized in 1868. It is used in the pharmaceutical industry as a precursor reagent in the synthesis of a number of synthetic anticoagulant pharmaceuticals similar to dicoumarol, the notable ones being warfarin (brand name Coumadin) and some even more potent rodenticides that work by the same anticoagulant mechanism (Raj *et al.*, 2015; Kim *et al.*, 2015).

Alkaloids are group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties (Zollinger, 2003; Gordaliza *et al.*, 2000).

Some synthetic compounds of similar structure are also termed alkaloids. Alkaloids have a wide range of pharmacological activities including antimalarial, antiasthma, anticancer, cholinomimetic, vasodilatory, antiarrhythmic, analgesic, antibacterial, and antihyperglycemic activities (Singh *et al.*, 2010; Phillipson and Anderson, 1989; Colombo and Bosisio, 1996).

The *Mimosa pudica*, invites attention of the researchers worldwide for its medicinal activities like antidiabetic, antitoxin, antihepatotoxin, antioxidant, wound healing and effects (Joseph *et al.*, 2013; Azmi *et al.*, 2011; Purkayastha *et al.*, 2016).

It is also known as chuimui (Chauhan *et al.*, 2009) or lajwanti in Hindi or touch me not in English because of its property to droop or collapse when touched and opens after some time (Vinutha *et al.*, 2007; Singh and Singh, 2009). It is an annual or perennial herb belonging to family Mimosaceae. *Mimosa pudica* was first described by Carl Linnaeus in species *Plantarum* in 1753 (Fondeville *et al.*, 1966; Bum *et al.*, 2004; Muhammad *et al.*, 2015). It is usually a short prickly plant with its branches growing close to

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the ground. Its maximum growth height is about 0.5 m and spreads up to 0.3 m. The stem of mimosa is erect, slender, prickly and well branched. Leaves are bipinnate (Saraswat *et al.*, 2012), fern like and pale green in colour with a character of closing when it is disturbed. These are quadri-pinnate, often reddish, leaflets 15-25 pairs, acute, brisky, usually 9-12 mm long and 1.5 mm wide. Flowers of the plant are axillary in position and lilac pink in colour usually occurring in globose heads (Pandey *et al.*, 2005; Saraswat *et al.*, 2012; Muhammad *et al.*, 2015).

Calyxes are companulate and petals are crenate towards the base. Fruits of the plant are pods, pods 1.5 to 2.5 cm long, falcate and closely prickly on sutures (Saraswat *et al.*, 2012). *Mimosa pudica* is well known for its rapid plant movement.

In the evening the leaflets will fold together and the whole leaf droops downward. It then re-opens at sunrise. This type of motion is termed as nyctinastic movement (Sibaoka, 1969; Jaffe, 1973; Fondeville *et al.*, 1966; Ueda and Yamamura, 2000; Volkov *et al.*, 2010). The foliage closes during darkness and reopens in light. The leaves are drooping because of stimulus, in conditions such as touching, warming or shaking. The stimulus can be transmitted to neighboring leaves. These types of movements are termed as seismonastic movements. This is due to loss of turgor pressure (Ueda and Yamamura, 2000; Volkov *et al.*, 2010).

All the five parts of the plant leaves, flowers, stems, roots and fruits are used as medicines in the traditional healthcare systems (Samy *et al.*, 1998; Gandhiraja *et al.*, 2009; Joseph *et al.*, 2013; Singh and Singh, 2009).

The leaf extract of the plant shows the presence of terpenoids, flavonoids, glycosides, alkaloids, quinines, phenols, tannins, saponins and coumarins (Gandhiraja *et al.*, 2009; Joseph *et al.*, 2013; Singh and Singh, 2009). The content of it has the capacity for the arresting of bleeding and it fastens the healing of wounds. The methanolic extract of shoot and root of the plant showed very good wound healing activity (Kannan *et al.*, 2009; Kokane *et al.*, 2009).

Hemigraphis colorota an important plant adapted to India is a versatile low creeping perennial herb that reaches a height of 15-30 cm (Subramoniam *et al.*, 2001; Anitha *et al.*, 2012; Silja *et al.*, 2008). The plant is known by several names such as Aluminium plant, Cemetery plant, Metal leaf, Red flame Ivy, Waffle plant, Java Ivy etc.

In Kerala, the plant is popular in the name ‘murikootti’ or ‘muriampacha’ because of its incredible potency to heal wounds (Priya, 2012; Subramoniam *et al.*, 2001). The leaf has metallic purple lustre on the upper surface and a solid dark purple on the ventral sides. The leaves are opposite, ovate to cordate, serrate-crenate, about 2-8 cm long and 4-6 cm wide, bearing well defined veins. It blooms irregularly throughout yearly in the tropics.

Flowers are small, 1 to 1.5 cm diameter, five lobed, bell shaped with imbricate bracts. These are white in colour with faint purple marks and appear within the terminal 2 to 10 cm long spikes. Capsules are small, slender, oval, linear and light green in colour. Seeds are small, flat and white in colour (Gamble *et al.*, 1921; Narasimhan *et al.*, 1997).

In folklore, the juice of the leaf is applied directly on fresh wounds to stop bleeding (Silja *et al.*, 2008). The phytoconstituents of *Hemigraphis colorota* are phenols, saponins, flavonoids, terpenoids (Sheu *et al.*, 2012), coumarins, carbohydrates, carboxylic acid, xanthoproteins, tannins, proteins, alkaloids, steroids and sterol (Saravanan *et al.*, 2010).

These phytochemicals provide curative property. The crude leaf paste provides excision wound healing (Bhargavi *et al.*, 2011; Pawar and Toppo, 2012).

Chromolaena odorata is an erect shrub of about 3 m height (Te Beest *et al.*, 2009; Obute and Adubor, 2007; McFadyen and Skarratt, 1996; Goodall and Erasmus, 1996). The leaves are alternate and the fruits are one seeded. Its stem branch freely, with lateral branches developing in pairs from the auxiliary buds. The older stems are brown and woody near the base; tips and young shoots are green and succulent. The root system is fibrous and does not penetrate beyond 20-30 cm.

The flower heads are borne in terminal corymbs of 20 to 60 heads on all stems and branches (Te Beest *et al.*, 2009; Obute and Adubor, 2007; McFadyen and Skarratt, 1996). The flowers are white or pale bluish

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lilac in colour. It is included in the family Asteraceae. It is a native of Central and South America which has spread throughout the tropical and subtropical areas of the world. It is used as medicinal as well as ornamental plant (Te Beest *et al.*, 2009; Obute and Adubor, 2007; McFadyen and Skarratt, 1996; Goodall and Erasmus, 1996).

The extract of the leaves contains phytochemically active compounds such as phenols, terpenoids, alkaloids and flavonoids (Akinmoladun *et al.*, 2007; Richter *et al.*, 2003; Triratana *et al.*, 1991). The aqueous and decoction of the leaves is a good ailment for the treatment of soft wounds and skin infections (Phan *et al.*, 1996).

The juice of the crushed leaves used in cuts to arrest bleeding (Biswal *et al.*, 1997). The macerated leaves are usually used for the relief of inflammation in the swollen parts of the body amongst the rural population of the in Nigeria (Owolabi *et al.*, 2010). The *Chromolaena odorata* leaf extract inhibits the growth of some bacteria and enhances haemostasis and blood coagulation (Triratana *et al.*, 1991; Sukanya *et al.*, 2009).

Prothrombin time (PT) is a blood test that measures how long it takes blood to clot. A prothrombin time test can be used to check for bleeding problems.

PT is also used to check whether medicine to prevent blood clots is working (Kheirabadi *et al.*, 2007; Dobrovolskaia *et al.*, 2009).

A PT test may also be called an INR test. INR (international normalized ratio) stands for a way of standardizing the results of prothrombin time tests, no matter the testing method. The Prothrombin Time (PT) evaluates the extrinsic pathway of blood coagulation.

The normal range for PT test is 11-14 seconds. Prothrombin time is an important test because it checks to see if five different blood clotting factors (factors I, II, V, VII, and X) are present. The prothrombin time is made longer by; Blood-thinning medicine, such as warfarin, Low levels of blood clotting factors, A change in the activity of any of the clotting factors. The absence of any of the clotting factors, other substances, called inhibitors that affect the clotting factors, An increase in the use of the clotting factors (Joy *et al.*, 2011; Massicotte *et al.*, 1995).

MATERIALS AND METHODS

Sample Selection

Mimosa Pudica

Mimosa pudica was first described by Carl Linnaeus in species *Plantarum* in 1753. All the five parts of the plant leaves, flowers, stems, roots and fruits are used as medicines in the traditional healthcare systems (Joseph *et al.*, 2013; Azmi *et al.*, 2011; Purkayastha *et al.*, 2016).

The leaf extract of the plant shows the presence of terpenoids, flavonoids, glycosides, alkaloids, quinines, phenols, tannins, saponins and coumarins (Gandhiraja *et al.*, 2009). It is used in coagulating blood, healing wounds, sexual weakness. In folklore medicine it is used in arresting bleeding and in skin diseases.

The content of it has the capacity for the arresting of bleeding and it fastens the healing of wounds. The methanolic extract of shoot and root of the plant showed very good wound healing activity (Kannan *et al.*, 2009; Joseph *et al.*, 2013; Azmi *et al.*, 2011).

Hemigraphis Colorota

Hemigraphis colorota is used in folklore; the juice of the leaf is applied directly on fresh wounds to stop bleeding (Silja *et al.*, 2008; Subramoniam *et al.*, 2001; Anitha *et al.*, 2012). The phyto-constituents of *Hemigraphis colorota* are phenols, saponins, flavonoids, terpenoids (Sheu *et al.*, 2012), coumarins, carbohydrates, carboxylic acid, xanthoproteins, tannins, proteins, alkaloids, steroids and sterol (Saravanan *et al.*, 2010). The crude leaf paste provides excision wound healing (Bhargavi *et al.*, 2011; Pawar and Toppo, 2012; Subramoniam *et al.*, 2001; Anitha *et al.*, 2012).

Chromolaena Odorata

The extract of the leaves of *Chromolaena odorata* contains phytochemically active compounds such as phenols, terpenoids, alkaloids and flavonoids (Richter *et al.*, 2003; Triratana *et al.*, 1991). The juice of the

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crushed leaves used in cuts to arrest bleeding (Biswal *et al.*, 1997). The *Chromolaena odorata* leaf extract inhibits the growth of some bacteria and enhances haemostasis and blood coagulation (Triratana *et al.*, 1991).

Sample Collection

The fresh leaves of *Mimosa pudica* and *Chromolaena odorata* are collected from Poomthoppuward, Ambalappuzha taluk of Alappuzha district in the month of January 2016. The fresh leaves of *Hemigraphis colorota* is collected from the botanical garden of Mar. Augusthinose College, Ramapuram of Kottayam district in the month of January 2016.

Preparation of Plant Extract

Leaves of *Mimosa pudica*, *Chromolaena odorata* and *Hemigraphis colorota* are washed and shade dried separately. The dried leaves were ground into fine powder and extraction was carried out using Soxhlet apparatus. The extractions of powdered leaves of plants (40 grams) were performed separately using methanol solvent. The powdered material was placed in Soxhlet apparatus and 200 ml of methanol was allowed to run continuously through it over a heater for 4 hours. The extract of the plants are collected and stored separately in containers at room temperature. High purity grade of all chemicals and reagents; calcium chloride 0.025 M, Liqueceilin-E (Tulip Diagnostics Pvt Ltd., Goa, India), chemicals for phytochemical analysis.

Phytochemical Screening

Methanolic extracts were used to carry out phytochemical studies. The extracts were filtered using Whatmann No.1 filter paper. The filtrate of the plant extracts is collected and stored in separate containers in room temperature.

Test for Tannins

Two ml of plant extract was taken in a test tube and two ml of water and few drops of five percentage ferric chloride was added and observed for brownish green or a blue-black colouration indicate the presence of tannins.

Test for Flavonoids

Five ml of dilute ammonia solution were added to a portion of the plant extract followed by addition of concentrated H₂SO₄. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Test for Terpenoids

Five ml of each extract was mixed in two ml of chloroform, and concentrated H₂SO₄ (three ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for Saponins

Ten ml of the extract was mixed with five ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with three drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for Phlobatannins

Deposition of a red precipitate when an extract of each plant sample was boiled with one percentage aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Test for Carbohydrate

Two ml of plant extract was taken in a test tube and ten ml of water, two drops of twenty percentage ethanolic α naphthol and two ml of conc. H₂SO₄ were added. Formation of reddish violet ring at the junction shows the presence of carbohydrates.

Test for Glycosides

Two ml of plant extract was taken in a test tube and two ml of chloroform and two ml of acetic acid were added. Formation of violet to blue to green coloration shows the presence of glycosides.

Test for Coumarins

Two ml of extract was taken in a test tube and three ml of ten percentage NaOH was added. Formation of yellow color gives positive result to coumarins.

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Test for Alkaloids

Two ml of plant extract was taken in a test tube and few drops of hager's reagent were added. Yellow precipitate shows positive result for alkaloids.

Test for Proteins

One ml of plant extract was mixed with one ml of conc. H_2SO_4 in a test tube. Formation of white precipitate indicate the presence of proteins.

Test for Phenols

The extract (5mg) was dissolved in distilled water and 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenol.

Test for Emodins

Two ml of plant extract was taken in a test tube and two ml of NH_4OH and three ml of benzene were added. Formation of red color indicates the presence of emodins.

Test for Anthraquinones

Three ml of plant extract was taken in a test tube and three ml of benzene and five ml of ten percentage NH_3 were added. Formation of pink, violet or red coloration in ammonical layer detect the presence of anthraquinones.

Test for Anthocyanins

Two ml of plant extract was taken in a test tube and two ml of 2N HCl and NH_3 were added. Formation of pinkish red to bluish violet coloration indicates the presence of anthocyanins.

Test for Leucoanthocyanins

Five ml of isoamyl alcohol taken in a test tube and five ml of plant extract was added. Turn organic layer into red detects the presence of leucoanthocyanins.

Blood Coagulation Study

Blood samples were collected from healthy volunteers of different blood group, using a disposable polypropylene syringe, and then anti-coagulated using 3.8% tri-sodium citrate in a polypropylene container (9 parts of blood to 1 part of tri-sodium citrate solution). It was immediately centrifuged at 4000 g for 15 min, and plasma was separated and pooled.

The freshly prepared plasma was stored at 4°C until its use. In a test tube 0.1 ml test plasma and Liquiceilin-E were added and shaken briefly to mix the reagent and plasma. The tube was placed at 37°C for 20 min for incubation. After the incubation, 0.1 ml pre-warmed calcium chloride solution was forcibly added into the mixture of plasma and reagent. To this, one ml of methanol extract of three plants are added and kept at 37°C.

A stopwatch was started to record the coagulation time in seconds. The tube was shaken to mix the contents and it was stopped as soon as the clot formation began. The steps were repeated three times for each sample, and average of the test value was noted.

Normal saline was used in place of the extracts for the control. Effect of methanol extracts of leaves of *Mimosa pudica*, *Hemigraphis colorata*, *Chromolaena odorata* on Prothrombin time (PT) of normal human plasma shows in table 1, 2 and 3 respectively.

Samples were collected from different places of Kottayam district of Kerala state, India. The samples were collected from a local farm as well as college herbal garden and were sliced into smaller pieces and transported to the laboratory.

Samples were washed thoroughly with distilled water to remove the adhering dirt and soil particles. They were later finely chopped into pieces using surgical blades. All the samples are then oven dried (KOA4, KEMI lab equipments, Ernakulam, India) at 60°C for 24 h. The dried samples were powdered using a waring blender (Magic V2, Preethi Kitchen Appliances Pvt Ltd, Chennai, India) and stored in air-tight polyethylene bottles until further analysis.

Statistical Analysis

The survey results were analyzed and descriptive statistics were done using SPSS 12.0 (SPSS Inc., an IBM Company, Chicago, USA) and graphs were generated using Sigma Plot 7 (Systat Software Inc., Chicago, USA).



Figure 1: Various Plants Selected for Invitro Hemostatic Study, *Mimosa Pudica* Whole Plant (Top Left); *Mimosa Pudica* Flower (Top Right); Dried Seeds of *Mimosa Pudica* (Middle Left) and (Middle Right); *Hemigraphis Colorota* (Bottom Left); *Chromolaena Odorata* (Bottom Right), Photo Courtesy: Wikipedia

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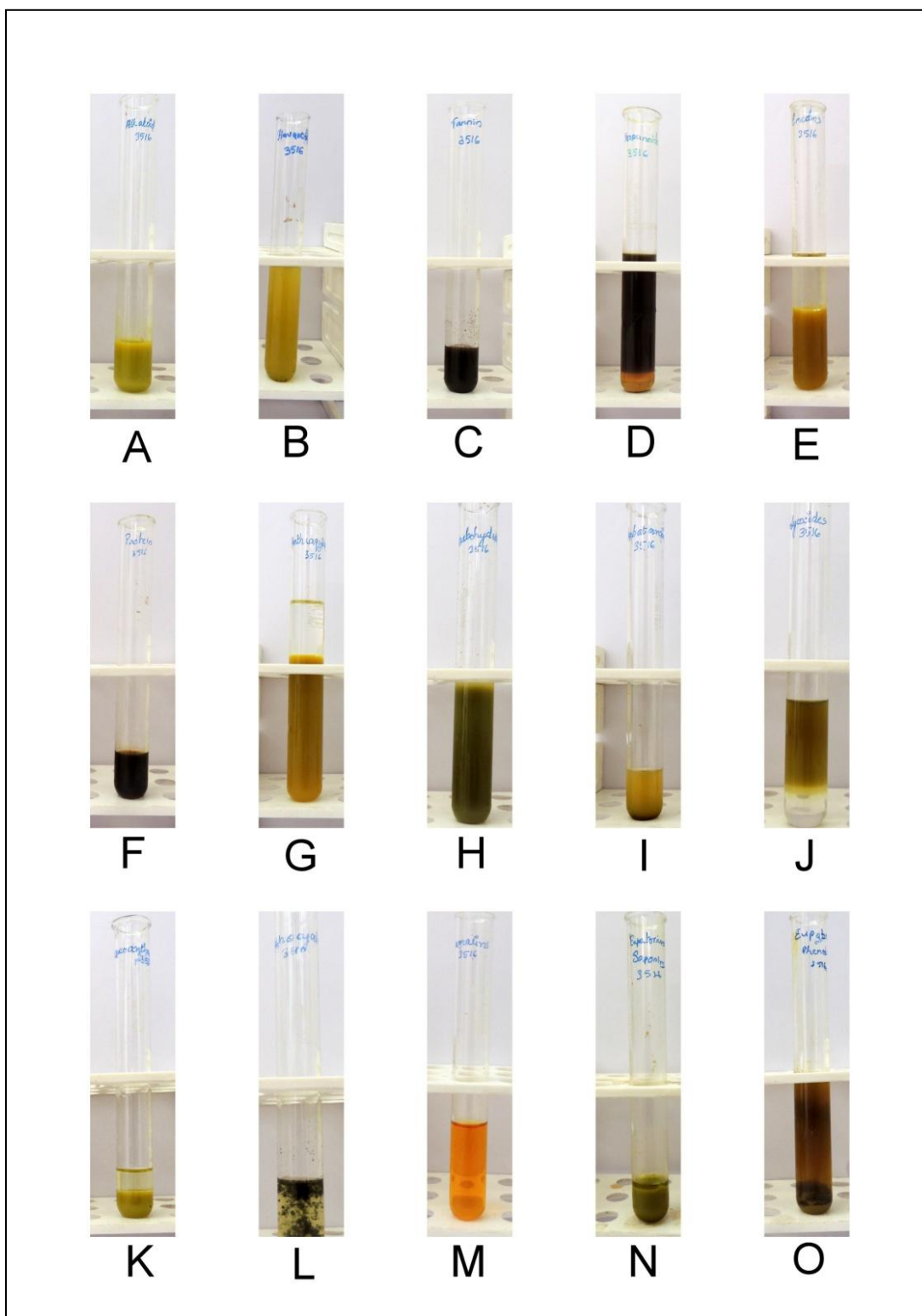


Figure 2: Results of Phytochemical Screening; *Chromolaena Odorata*, A. Alkaloids, B. Anthocyanins, C. Anthraquinones, D. Carbohydrate, E. Coumarins, F. Emodins, G. Flavanoids, H. Glycosides, I. Leucoanthocyanins, J. Phenols, K. Phlobatannins, L. Proteins, M. Saponins, N. Tannins, O. Terpenoids

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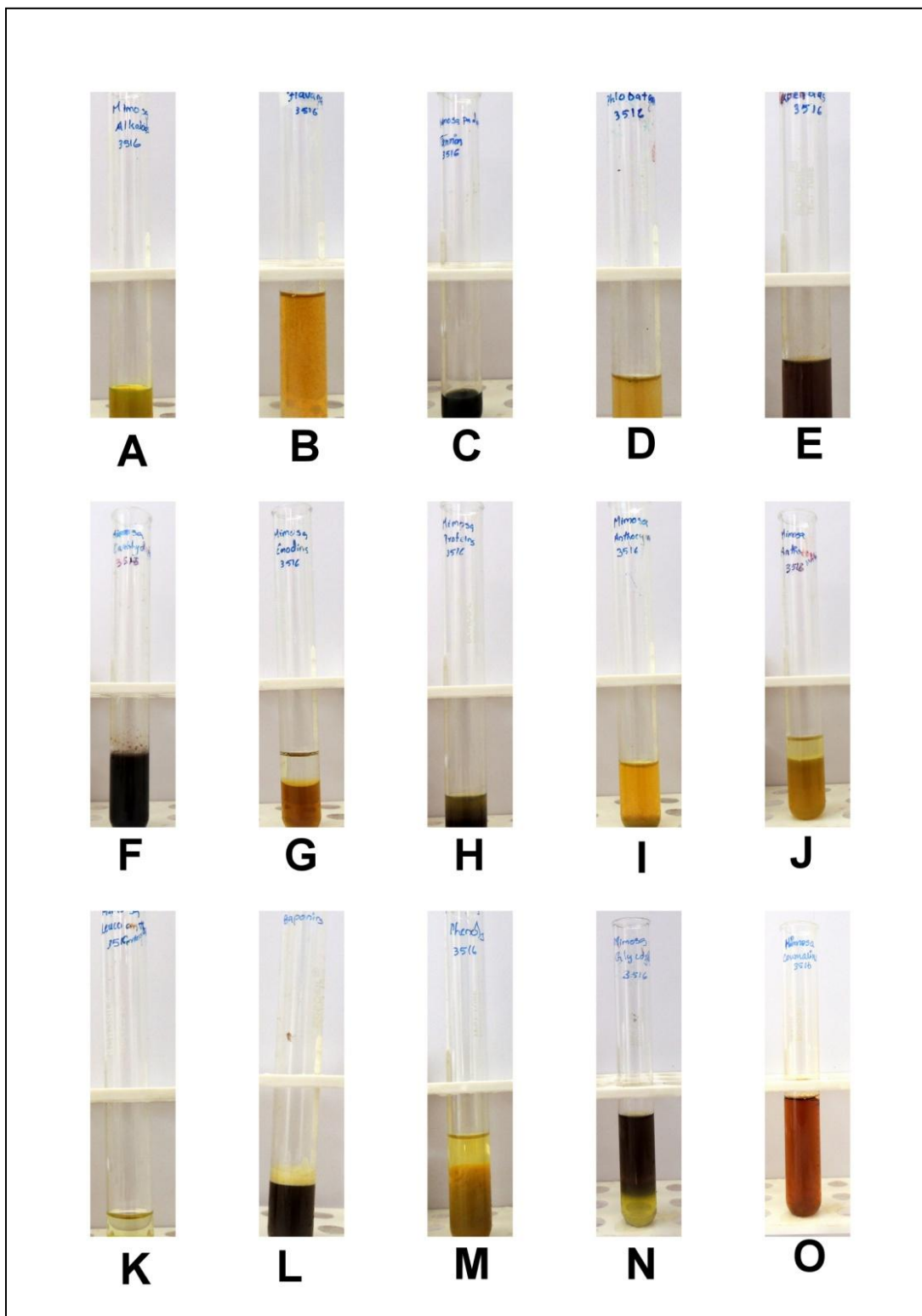


Figure 3: Results of Phytochemical Screening; *Mimosa Pudica*, A. Alkaloids, B. Anthocyanins, C. Anthraquinones, D. Carbohydrate, E. Coumarins, F. Emodins, G. Flavanoids, H. Glycosides, I. Leucoanthocyanins, J. Phenols, K. Phlobatannins, L. Proteins, M. Saponins, N. Tannins, O. Terpenoids

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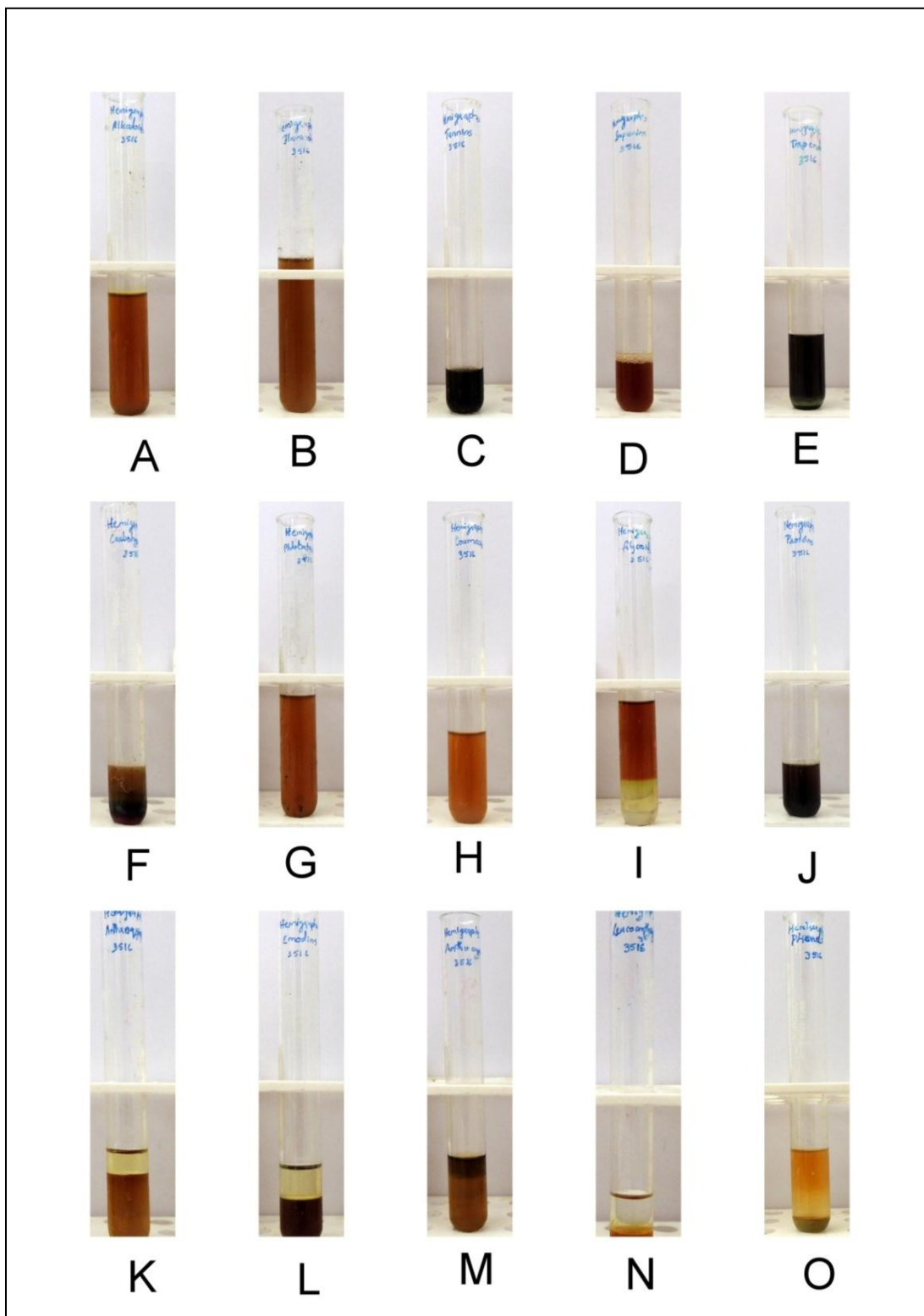


Figure 4: Results of Phytochemical Screening; *Hemigraphis Colorota*, A. Alkaloids, B. Anthocyanins, C. Anthraquinones, D. Carbohydrate, E. Coumarins, F. Emodins, G. Flavanoids, H. Glycosides, I. Leucoanthocyanins, J. Phenols, K. Phlobatannins, L. Proteins, M. Saponins, N. Tannins, O. Terpenoids

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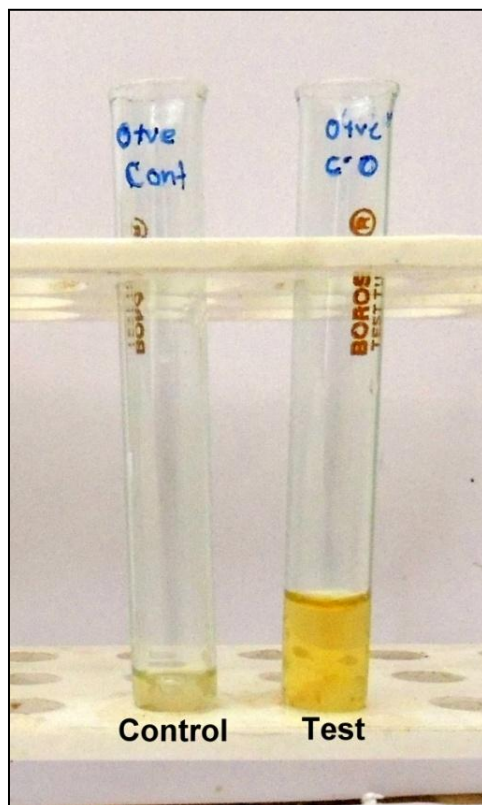


Figure 5: Result of Prothrombin Test for *Chromolaena Odorata*

RESULTS AND DISCUSSION

Phytochemical screening of the methanolic extract of selected herbal plants revealed the presence of compounds like Tannins, saponins, alkaloids, emodins, proteins, carbohydrate, terpenoids, glycosides, flavonoids, coumarins and phenols. Eleven kinds of chemical constituents including flavanoids, terpenoids, tannin, coumarins, proteins, emodins, carbohydrate, glycosides, saponins, phenol and alkaloids were isolated from the leaf extract of *Hemigraphis colorota*. *Mimosa pudica* contains Tannins, saponins, alkaloids, terpenoids, phenols, glycosides, flavonoids and coumarins in their leaf extract. Tannins, phenols, alkaloids, terpenoids, coumarins and flavonoids were identified from the leaf extract of *Chromolaena odorata*.

The experiment to analyze the effect of extract on blood clotting in vitro showed that, the blood clotting times of all extracts in PT test were more than those of the control. But when compared between extracts, the extract of *Chromolaena odorata* shows blood coagulation within 2 minutes while the extracts of *Mimosa pudica* and *Hemigraphis colorota* do not show blood coagulation within 2 minutes (Table 2, 3).

Our experiment on blood coagulation indicated no coagulation in PT, in extracts of *Mimosa pudica*, *Hemigraphis colorota* within 2 minutes and the extract of *Chromolaena odorata* shows blood coagulation within 2 minutes. It was likely that prolongation of PT occurred from the interference of clotting system such as plasma pH or some chemical compounds in the extracts (Wongkrajang *et al.*, 1994). *Mimosa pudica*, *Chromolaena odorata* and *Hemigraphis colorota* have compounds like tannins, saponins, alkaloids, emodins, proteins, carbohydrate, terpenoids, glycosides, flavonoids, coumarins and phenols. Flavanoids and tannins were found to be blood coagulants and triggers and accelerate blood coagulation. Besides these active compounds, calcium is also an important clotting agent (Wongkrajang *et al.*, 1990).

In vitro negative results in this study might be due to an insufficient amount of calcium or active compound to induce blood coagulation. Therefore, the amount of calcium and active compound in the extracts should be further investigated.

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Table 1: Details of Phytochemical Analysis of *Chromolena Odoratum*, *Mimosa Pudica* and *Hemigraphis Colorota*

| Sl. No | Phytoconstituents | <i>Chromolaena Odorota</i> | <i>Mimosa Pudica</i> | <i>Hemigraphis Colorota</i> |
|--------|-------------------|----------------------------|----------------------|-----------------------------|
| 1 | Flavanoids | + | + | + |
| 2 | Terpenoids | + | + | + |
| 3 | Tannins | + | + | + |
| 4 | Coumarins | + | + | + |
| 5 | Proteins | - | - | + |
| 6 | Emodins | - | - | + |
| 7 | Anthraquinone | - | - | - |
| 8 | Anthocyanine | - | - | - |
| 9 | Leucoanthocyanine | - | - | - |
| 10 | Phlobatannins | - | - | - |
| 11 | Carbohydrate | - | - | + |
| 12 | Glycosides | - | + | + |
| 13 | Alkaloids | + | + | + |
| 14 | Saponins | - | + | + |
| 15 | Phenol | + | + | + |

+ indicate presence
 - indicate absence

Table 2: Effect of *Mimosa Pudica* Leaves Extract on Blood Coagulation by PT

| Trail | PT Time for Negative Control (Minutes) | PT Time for Sample | | | | | | | | |
|---------|--|--------------------|---|---|----|----------------|----------------|----------------|-----------------|---|
| | | A | B | O | AB | A ⁻ | B ⁻ | O ⁻ | AB ⁻ | |
| 1 | 0.15 ± 0.01 | X | X | X | X | X | X | X | X | X |
| 2 | 0.14 ± 0.01 | X | X | X | X | X | X | X | X | X |
| 3 | 0.16 ± 0.01 | X | X | X | X | X | X | X | X | X |
| Average | 0.15 ± 0.01 | X | X | X | X | X | X | X | X | X |

X refers to non-clotted blood within 2 min

Table 3: Effect of *Hemigraphis Colorota* Leaves Extract on Blood Coagulation by PT

| Trail | PT time for Negative Control (Minutes) | PT time for Sample | | | | | | | | |
|---------|--|--------------------|---|---|----|----------------|----------------|----------------|-----------------|---|
| | | A | B | O | AB | A ⁻ | B ⁻ | O ⁻ | AB ⁻ | |
| 1 | 0.15 ± 0.01 | X | X | X | X | X | X | X | X | X |
| 2 | 0.16 ± 0.01 | X | X | X | X | X | X | X | X | X |
| 3 | 0.14 ± 0.01 | X | X | X | X | X | X | X | X | X |
| Average | 0.15 ± 0.01 | X | X | X | X | X | X | X | X | X |

X refers to non-clotted blood within 2 min

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Table 4: Effect of *Chromolaena Odorata* Leaves Extracts on Blood Coagulation by PT

| Trail | PT Time for Negative Control (Minutes) | PT Time for Sample | | | | | | | |
|---------|--|--------------------|-------------|-------------|-------------|----------------|----------------|----------------|-----------------|
| | | A | B | O | AB | A ⁻ | B ⁻ | O ⁻ | AB ⁻ |
| 1 | 0.15 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 |
| 2 | 0.16 ± 0.01 | 1.25 ± 0.01 | 1.25 ± 0.01 | 1.25 ± 0.01 | 1.25 ± 0.01 | 1.25 ± 0.01 | 1.25 ± 0.01 | 1.25 ± 0.01 | 1.25 ± 0.01 |
| 3 | 0.14 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 |
| Average | 0.15 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 | 1.24 ± 0.01 |

Numbers represent means ± one standard error (SE) of the mean

Conclusion

Among the three plants evaluated for in vitro hemostatic effect, the blood clotting times of all extracts in Prothrombin test were more than those of the control. But when compared between extracts, the extract of *Chromolaena odorata* shows blood coagulation within 2 minutes while the extracts of *Mimosa pudica* and *Hemigraphis colorata* do not show blood coagulation within 2 minutes. In vitro negative results in this study might be due to an insufficient amount of calcium or active compound to induce blood coagulation. Therefore, the amount of calcium and active compound in the extracts should be further investigated.

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