



Characterization of Bio-active Compounds Essential for Blood Coagulation in the Crude Extracts of *Tradescantia zebrina*, *Tagetes minuta* and *Codiaeum variegatum* Leaves

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Authors' contributions

This work was carried out in collaboration among all authors. Authors GG and OA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OA and BC managed the analyses of the study. Authors GG and BC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Many commercial drugs used for blood clotting are expensive and have associated side-effects. The extracts of *Tagetes minuta*, *Codiaeum variegatum* and *Tradescantia zebrina* are used for blood clotting. These extracts are highly efficient and have no known side-effects. This study aimed at characterizing crude extracts of these plant species used to accelerate blood clotting.

Study Design: An independent measures experimental design was used in the study.

Place and duration of study: The research was conducted between 21st September, 2018 and 21st May, 2019. The study was conducted in Maasai mara university, Kenya and Multimedia university of Kenya.

Methodology: Extracts of these herbs were obtained and analyzed for absorption bands, functional groups, bio-metal concentrations, physical-chemical parameters, phytochemicals and antimicrobial activity. Test for blood clotting factors (calcium and vitamin K) was also conducted.

Results: All extracts had common functional group peaks at 2800-3500 cm⁻¹ (carboxylic OH), 1680

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cm^{-1} (carbonyl), and 1035cm^{-1} ($\text{C-O}_{\text{stretch}}$). The extracts had an average pH of 6.590 ± 0.702 and conductivity of $0.580 \pm 0.079\text{mS}$. The average solubility in distilled water was $16.670 \pm 1.534 \text{g}/100 \text{ml}$ water at 37°C . The extracts were found to be abundant in iron, copper and phytochemicals. All extracts portrayed moderate inhibition to *E. coli* bacteria and *C. albicans* fungi but mild inhibition towards *S. aureus* bacteria. The extracts had trace amounts of Vitamin K and moderate amounts of calcium.

Keywords: Blood clotting; *Tradescantia zebrina*; *Tagetes minuta*; *Codiaeum variegatum*.

1. INTRODUCTION

Blood plays a key role in the metabolism of humans. It is the main mode of transport necessary for other metabolic processes to occur [1]. Blood is lost when its conveyance vessels (arteries, veins and capillaries) are cut. Bleeding is the leading cause of death during accidents [2]. During accidents, blood vessels are cut and blood easily ooze out. The number of fatalities due to accidents (both minor and major) is still high and require more attention. Several precaution measures have been enforced to minimize these fatalities. Nevertheless, it is quite difficult to prevent bleeding after accidents have already occurred. It is therefore pertinent to minimize the bleeding process to ensure minimal blood loss during accident.

Clot formation occurs in veins and arteries with the help of several factors [3,4]. During blood clotting, various factors and enzymes trigger conversion of blood in the affected site to coagulate into a gel [5], preventing more bleeding. Blood clotting has three major steps; vasoconstriction, platelets plug formation and clot formation [6]. Clot formation occur when platelets plugs have been formed [6]. Clot formation is activated by a sequence of events called the coagulation cascade which leads to formation of fibrin [7]. Several plant extracts have been found to inhibit or prolong the blood clotting time [8].

Drugs used for blood clotting include; antiplatelet agents such as aspirin, clopidogrel, dipyridamole and tidopine that work by inhibiting the production of thromboxane [9]. Antiplatelet are not conducive for everyone especially for people with liver or kidney diseases, bleeding disorders and peptic ulcers [10]. Other blood clotting agents are anticoagulants. Several coagulation factors are proteins [11]. These proteins are involved in several stages of blood coagulation process and include thrombin, coagulation factors IX, IXa amongst others [12,13,14,15]. They are synthesized in the liver with the help of vitamin K [16]. Anticoagulants such as warfarin and heparin, slow clot formation by competing

with vitamin K [17]. Anticoagulants such as dabigatran, apixaban and rivaroxaban are simpler to use and less risky compared to warfarin [18].

Some plants contain one or more blood clotting agents in varying concentrations. Some of the blood clotting agents readily found in herbs include calcium, vitamin K as well as other crucial phytochemicals [19]. *Tagetes minuta*, *Tradescantia zebrina* and *Codiaeum variegatum* have successfully been used to treat diseases related with blood disorders. These extracts have traditionally been used for people of varying age with little preparation required to formulate the anti-clotting agents. Crude extracts of these plants were applied on the bleeding part to hasten blood coagulation. There were also few, if any cases of side effects or complications arising from treatment using these extracts. A study was hereby conducted to characterize, analyze and compare chemical compounds present in the extracts of these plants. Elucidation of the exact species and amounts of phytochemicals in the three plants will go a long way in informing further pharmaceutical and pharmacognosy studies. This will help to avoid potential drug toxicities while maximizing on extraction of blood clotting agents from the plants.

2. MATERIALS AND METHODS

2.1 Design of Experiment

An independent measures experimental design was followed for this study. The plant specimen were identified and assigned specimen numbers with the help of a botanist (from Maasai mara university). Purposive sampling was used to collect the plant samples whereby only the fresh leaves were targeted. The leaves were pretreated to obtain crude extracts by maceration method. The extracts were then characterized for physical-chemical parameters, biometal concentrations, absorption bands, functional groups, phytochemicals, antimicrobial activities and presence of blood clotting agents (calcium and Vitamin K). Characterization and analysis were done at Maasai mara university, Kenya

chemistry and biology labs. UV-VIS screening for presence of Vitamin K in the extracts was conducted at Multimedia university, Kenya.

2.2 Methods

2.2.1 Extraction of the sample extracts

The plants species *Tagetes minuta*, *Tradescantia zebrina* and *Codiaem variegatum* were carefully washed with distilled water and then air dried in a cool shade away from direct sunlight for 3 days. The crude extracts of the samples were obtained by squeezing the leaves.

2.2.2 Characterization of extracts

The extracts were characterised for physico-chemicals (pH, conductivity, total solids, volatile solids and solubility) using conventional methods. A pH meter (Hanna G114) and conductivity meter (Jenway 6510) were used for pH and conductivity respectively. Four replicate tests were conducted for each of the tests.

For bio-metal analysis, the extracts were serially diluted 200-folds using 20 ml aliquots distilled water and filtering using Whatman #42 filter paper at each dilution stage. An Atomic Absorption Spectrometer (PG-990) was used. The bio-metals were analyzed after formulation of calibration curve using standard salts prepared for each of the bio-metal analyzed. Table 1 summarizes the conditions used during the bio-metal analysis.

For functional group analysis, the extracts were gradually concentrated by mild warming until all the water was dried. The samples were then cast into pellets using potassium bromide pellet before analyzing for functional groups using IR Spectrometer (Shimadzu 119).

2.2.2.1 Phytochemical screening of the extracts

Test for polyphenols: 3 ml of aqueous ferric chloride solution was added to 10 ml of the sample solutions, shaken and observations made. Formation of green coloration indicated presence of phenols.

Test for flavonoids: Onto the test samples, 2 g of vanillin powder was added and the mixture agitated in an acidic medium.

The procedure was confirmed by adding 3ml of dilute ammonia solution to 2 ml of aqueous filtrate followed by 1 ml of concentrated sulfuric acid. Formation of yellow deposits confirmed presence of flavonoids.

Test for Tannins: About 0.1 g of the dry samples were boiled in 4ml distilled water in a boiling tube then filtered. A few drops of 0.1% ferric chloride solution were then added and observations of change in color to brownish-green made.

Test for saponins: The sample was added to 3 ml distilled water and vigorously agitated until a stable, persistent froth formed. 3 drops of olive oil were then added and shaken vigorously. Presence of emulsion indicated positive results.

Test for terpenoids (Salkowski's test): About 3 ml of the samples were mixed with 1 ml of chloroform and 1ml of concentrated sulfuric acid. Formation of intense red-brown color indicated presence of terpenoids.

Test for alkaloids (Mayer's test): About 3 ml of ammonia solution was added onto the sample followed by 10 ml of chloroform. The mixture was shaken well then filtered. The chloroform layer was then evaporated off and 3 ml of Mayer's solution added to the remaining solution. Formation of a cream precipitate indicated positive test for alkaloids.

Test for steroids: The sample solution was dissolved in 10 ml of chloroform followed by 3 ml of concentrated sulfuric acid. Formation of red precipitates indicated presence of steroids.

2.2.3 Antimicrobial analysis of the extracts

Antimicrobial studies were conducted for both Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli*). *C. albicans* strain was used for antifungal analysis. All aseptic techniques were considered to minimize the contamination rates.

Table 1. AAS conditions used to analyze the bio-metals

Bio-metal	Wavelength	Bandwidth	Lamp current	Flame	Sensitivity
Co	240.7 nm	0.4 nm	5.0 ma	Air/Acetylene	0.05 mg/L
Cu	324.7 nm	0.4 nm	5.0 ma	Air/Acetylene	0.03 mg/L
Fe	248.3 nm	0.2 nm	5.0 ma	Air/Acetylene	0.05 mg/L
Zn	213.9 nm	0.4 nm	4.0 ma	Air/Acetylene	0.01 mg/L

2.2.3.1 Media preparation

28.0 g of Muller-Hinton's agar media was dissolved into 600 ml of sterile distilled water in a media dispensing bottle. The mixture was gradually boiled to completely dissolve the media. Caution was taken not to break the media bottle by loosening the bottle stopper occasionally to avoid pressure build up. The media was then sterilized by autoclaving along with petri-dishes and all apparatus to be used at 121°C and 15 psi pressure for 15 minutes. The media was allowed to cool to 45°C before dispensing in sterile petri dishes. The media plates were allowed to cool, inverted and stored in the refrigerator at 4°C for 24 hours.

2.2.3.2 Antimicrobial tests

Sterile media plates were sub-divided into six equal parts using a marker pen and labelled accordingly. The test microbes (bacteria and fungi) were then spread aseptically on different media plates to prevent cross-contamination. Sterile octo-discs impregnated with different extracts were then placed on the surface of the plates. The plates were then inverted and incubated at 37°C for 24 hours. After the incubation period, the inhibition zones were noted and recorded in millimeters.

2.2.4 Analysis of blood clotting agents

2.2.4.1 Test of calcium contents

5g of the crude extracts were mixed with 50ml of 1M oxalic acid to precipitate out calcium ions from the samples. The residue obtained was then thoroughly filtered using more oxalic acid. The residue was dried and heated gently in a pre-weighed crucible (rinsed with ammonia solution) until a white precipitate was obtained. The mixture was weighed (crucible+residue) and the mass of the residue deducted. The procedure was then repeated three more times and the average value calculated.

2.2.4.2 Test of vitamin K

About 0.1 g of sodium acetate was added onto 10 ml of each of the extract solutions. The mixture was transferred to a separating funnel which had been previously rinsed with acetone. The mixture was shaken vigorously while releasing the gas produced periodically for 1 hour. The upper part of the mixture was transferred into a conical flask. 50ml of dichlorophenolindophenol (DCPIP) was put in a

burette and the solution titrated using DCPIP solution. The end point was denoted by change in colour from pink to green.

2.2.4.3 Screening for vitamin K by UV-VIS spectroscopy (Shimadzu 1800).

5 ml of the samples were added onto 2 ml of 0.2% solution 2,4-dinitrophenyl hydrazine (in hydrochloric acid and absolute ethanol in ratio of 1:5^{v/v}) and thoroughly agitated to mix. The mixture was then heated on water bath to evaporate most of the solvent. 15ml of (ammonia and alcohol in ratio of 1:1) was added to the mixture, shaken gently and left to cool at room temperature. UV absorbance at 635nm wavelength was recorded.

2.3 Data Analysis

Data obtained from analysing pH, temperature, total solids, volatile solids, heavy metals was subjected to statistical analysis. The degree of freedom value was maintained at 8 with 95% confidence level being used for the statistics. The data was analysed using Ms Excel (2016) and Originlab (version 6.5) statistical packages.

3. RESULTS AND DISCUSSION

3.1 Physical-chemical Analysis of the Plant Extracts

All samples were found to be slightly acidic with moderate conductivity values. A large portion of the total solids were found to be volatile. The pH of the extracts was lower than the normal blood pH of 7.3-7.5 [20]. However, the margin between the extracts pH and blood pH was considerable. Blood is an excellent buffer by itself and would quickly normalize the extracts pH when added onto a bleeding vein or artery. Kim et al., [21] reported application of garlic powder on bleeding surfaces as a blood coagulant, whereas garlic has a pH of 6.0 [22]. All extracts had little electrical conductivity in water solution. These findings imply that the extracts had limited salts available. Bowen and Remaley [23] showed that abundance of salts lead to reactions between samples and blood components. Some salts inhibit normal metabolic functions leading to sickness or death. *T. minuta* extracts were found to have the least conductivity values of 0.307±0.002 mS. Szczurko et al., [24] reported low conductivity values in *Ginkgo biloba* water extracts used as blood coagulants. The composition of volatile solids in the total solids was 44% (*T. minuta*), 46% (*T. zebrina*) and 47%

(*C. variagatum*). Jiang et al., [25] showed that a good composition of *Ginkgo biloba* extracts had volatile terpenes which might also be present in the test samples above. Table 2 summarizes the chemical parameters conducted for the three blood clotting plant extracts.

Vishwas et al., [26] reported volatile solids concentrations of 6.3% in *Homonoia riparia* leaves extracts used to prolong blood clotting time. Solubility of blood coagulants is a crucial parameter in elucidating its effects and effectiveness. Generally, blood is composed of several components, ferried by blood plasma which has a lot of water thus soluble in water. It is practically impossible to determine the exact solubility of blood in water due to the numerous components and factors involved. Nevertheless, the test extracts showed varying solubilities in water at 37°C. *C. variagatum* had the largest solubility value of 33.148±0.164 g/100 ml of water at 37°C.

3.2 Bio-metal Concentration of Blood Clotting Plant Extracts

The composition of metals in the extracts is vital in predicting its efficacies and possibly toxicity effects when applied to blood. Some metals are inert in blood, others aid the body when absorbed in the proper channel while others are toxic even at minute concentrations. The concentrations of four bio-metals crucial in blood coagulation in the test extracts are summarized in Table 3.

Iron was found to be the most abundant specie, especially in *T. zebrina* extracts. Greunz et al., [27] reports that abundance of iron during blood coagulation process prolongs prothrombin, thrombin and partial thromboplastin duration, in human plasma in vitro. This effect leads to reduced blood loss as formation of blood clots are prolonged. Ke et al., [28] found out that presence of iron in the coagulants provide optimal conditions for fibrinogen coagulability and fibrin monomer aggregation. However, Ke et al., [28] did not cite the exact concentration of iron needed for these coagulopathies. Rzymiski et al., [29] found out that excessive addition of iron (FeSO_4) in doses above 2-4 mg/ml significantly reduced platelet aggregation in rat blood serum. Copper is involved in synthesis of blood coagulant factor VIII [30]. *T. minuta* extracts were found to have the highest amount of copper ions (78.00 mg/Kg wet extract sample) for this crucial anti-hemophillic factor. Concentrations of copper ions above 300 mg/kg of sample are toxic [31].

Rzymiski et al., [29] found out that in vitro addition of copper ions (CuSO_4) in doses of 300-1000 micrograms/ml elicited an anticoagulant effect, though the thrombin time was not significantly affected. Concentration of zinc in the plant extracts had a large disparity with *T. minuta* having very low levels (15.10 mg/Kg wet sample) whereas both *C. variagatum* (49.88 mg/Kg wet sample) and *T. zebrina* (59.20 mg/Kg wet sample) had considerably higher values. Like iron, addition of zinc ions to blood serum (in this case using rats) was found to increase recalcification, prothrombin and partial thromboplastin times [32]. Doses ranging between 0.3-1.0 mg/ml of zinc ions were used. The thrombin clotting time was not altered even by the highest concentration used (1.0mg/ml). Going by these studies, *T. zebrina* would be best placed as a potential blood coagulant. Cobalt ions do not significantly affect blood clotting process. However, Heemskerk et al., [33] found out that presence of cobalt ions in coagulants alter blood clot retraction. *C. variagatum* extracts were rich in cobalt (77.24mg/Kg of wet sample) thus expected to experience more blood clot retraction processes.

3.3 Functional Group Analysis of Plant Extracts

All spectra of the test samples were found to have similar IR patterns. Presence of carboxylic groups and aromatic compounds as well as conjugation as a result of numerous double bond peaks in the fingerprint regions were observed. From the spectra in Fig. 1, all samples had a broad $\text{O-H}_{\text{RCOOH}}$ peak between 3600-2800 cm^{-1} highlighting carboxylic acids, a sharp peak at 1650-1750 cm^{-1} due to C=O . These postulates were confirmed by C-O-H stretch shallow peaks at around 1030-1050 cm^{-1} . $\text{sp}^3 \text{C-H}$ peaks were observed at around 2900 cm^{-1} , followed by a large rift in the spectra. John et al., [34] reported similar peaks in commercial blood coagulating drugs such as warfarin and heparin. Similar peaks were also observed in garlic, turmeric and *Ginkgo biloba* extracts, all used for blood coagulation purposes [35]. The collated FT-IR spectra of the three plant extracts are demonstrated in Fig. 1.

There were several peaks between 1600 cm^{-1} and 900 cm^{-1} indicating presence of aromatic groups and double bonds. Further peaks at 600 cm^{-1} and 500 cm^{-1} indicated presence of organometalloids and halides present in the plant extracts. Rodríguez-Torres et al., [36] found out these peaks in the IR spectra of heparin drugs.

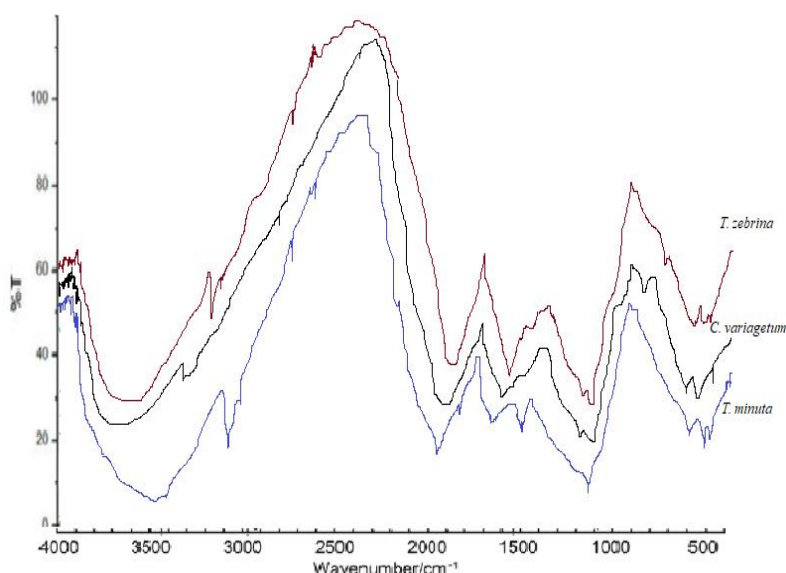


Fig. 1. FT-IR spectra of the blood coagulant plant extracts

Essien et al., [37] observed presence of organohalide peaks in the roots of *Fagara xanthoxyloides* used as blood coagulants.

3.4 Screening of Phytochemicals in the Plant Extracts

Several phytochemical compounds have been observed to aid in blood clotting process. Vishwas et al., [26] showed that presence of alkaloids, glycosides, phenols and flavanoids in *Homonoia riparia lour* leaves extracts increased blood clotting time. The study further found out that extraction using ethanol gave the highest effectiveness. The extracts of *T. zebrina*, *T. minuta* and *C. variagetum* were all found to be abundant in phytochemicals. These findings are summarized in Table 4.

Archana et al., [38] indicated that tannins have the ability to reduce blood clotting at controlled doses. All test extracts were found to test positive for tannins. Green tea, proven to have a lot of tannins and other polyphenols have over time been used to aid in blood clotting processes [39]. This study also showed all three test samples to contain phenolic compounds. Further research by Li et al., [40] has shown that interaction between polyphenols (including tannins and flavonoids) prolongs the interaction between thrombin and these compounds. These effect leads to increased thrombin times for efficient blood coagulants. However, some flavonoids were reported to inhibit thrombin

formation [41]. Only *T. zebrina* sample had steroids and terpenoids. Xavier et al., [42] observed presence of steroids in the extracts of *Homonoia riparia lour* leaves used in blood coagulation. *T. zebrina* and *C. variagetum* extracts tested positive for saponins. Several plant extracts known to increase blood coagulation such as *Ginkoga biloba*, green tea, garlic and turmeric have all been found to have varying concentrations of saponins [43,44]. It is however not clear how saponins affect blood coagulation.

3.5 Antimicrobial Activity of the Plant Extracts

The test extracts were found to exhibit moderate antifungal activity against *C. albicans*. The performance against *E. coli* was moderate while the extracts showed mild suppression of *S. aureus* bacteria. The diameters for the inhibition zones of the plant extracts against the microbes are illustrated in Table 5.

All the plant extracts had close inhibition against the microbes. Effective inhibition against *E. coli* and *C. albicans* by these extracts as observed in Table 5 implies that the extracts are suitable in preventing diseases as a result of these microbes. During bleeding, human blood and its components are exposed to many external pathogens, such as microbes responsible for numerous diseases. The extracts of these three herbs can thus be applied as a remedy to these microbes while still aiding in blood clotting, *in vitro*.

Both calcium and vitamin K are directly involved in formation of the coagulation cascade that leads to fibrin formation [45,46]. Calcium, phospholipids and clotting factors Xa and IXa are involved in activation of prothrombin to thrombin [47,48,49]. Thrombin is then used in activation of fibrinogen to fibrin [50]. Fibrin is the final mesh that prevents bleeding [50]. Calcium is a catalyst in this process and its deficiency in the blood slows down blood coagulation process [51]. The levels of vitamin K in the three extracts were screened by UV-VIS as shown in Fig. 2.

3.6 Calcium and Vitamin K Analysis

Alongside, fibrinogen (found in blood plasma), calcium and vitamin K as well as other compounds are key in blood coagulation process. These factors are required at various points of the process, without which the process is altered. Both calcium and vitamin K are directly involved in formation of the coagulation cascade that leads to fibrin formation [45,46]. Calcium, phospholipids and clotting factors Xa and IXa are involved in activation of prothrombin to thrombin [47,48,49]. Thrombin is then used in activation of fibrinogen to fibrin [50]. Fibrin is the final mesh that prevents bleeding [50]. Calcium is a catalyst in this process and its deficiency in the blood slows down blood coagulation process [51]. The levels of vitamin K in the three extracts were screened by UV-VIS as shown in Fig. 2.

T. zebrina sample was found to have a higher absorbance citing more abundance in the extracts. Abundance of vitamin K is directly

proportional to blood clotting efficacy based on the crucial role this factor plays in the process. Most blood coagulants contain vitamin K in varying amounts. Warfarin drug used in blood coagulation contains loads of vitamin K₁ [52]. Venugopala et al., [53] reported high abundance in vitamin K in coumarin extracts (used in blood coagulation). Venugopala et al., [54] reported that vitamin K conversion cycle was interfered by coumarin extracts leading to hepatic production of partially carboxylated and decarboxylated proteins with procoagulant activity. The vitamin K antagonists also inhibit conversion cycles of other coagulant factors such as anticoagulant proteins C and S [55]. The exact concentrations of vitamin K and calcium in the plant extracts are illustrated in Fig. 3. From the figure, calcium levels were higher than vitamin K levels in all the extracts. Both *T. minuta* and *C. variagatum* had more calcium and vitamin K levels compared to *T. zebrina*.

The order of vitamin K abundance in the extracts was *T. zebrina* (0.34mg/L), *T. minuta* (0.27mg/L) and *C. variagatum* (0.19mg/L). There is little information of the exact amount of vitamin K needed for blood clotting. However, Kaku et al., [56] found out that for optimal blood clotting, about 8.4mg/dL of calcium ions are required. Using these standards, all the extracts qualify as suitable blood clotting agents. It has been noted that in the presence of calcium ions and reduced vitamin K (as vitamin KH₂), carboxylation reactions cause a conformation change in coagulation proteins leading to binding of cofactors on phospholipid surfaces [55]. A fibrin clot is thereafter formed.

Table 2. Physical-chemical parameter of the test extracts

Parameters	Samples (n = 8)		
	<i>T. zebrina</i>	<i>T. minuta</i>	<i>C. variagatum</i>
pH	6.519±0.036	6.573±0.016	5.871±0.000
E. conductivity (mS)	0.587±0.001	0.307±0.002	0.860±0.001
Total solids (g/L)	5.192±2.567	5.268±0.996	5.251±0.957
Volatile solids (g/L)	2.416±0.027	2.358±0.028	2.472±0.020
Solubility (g/100ml water at 37°C)	16.667±0.175	7.870±0.076	33.148±0.164

Table 3. Concentration of biometals in blood coagulating plant extracts

Biometal	Sample concentration (mg/Kg wet sample)		
	<i>T. zebrina</i>	<i>T. minuta</i>	<i>C. variagatum</i>
Iron	180.00	68.40	56.40
Copper	54.00	78.00	46.00
Zinc	59.20	15.10	49.88
Cobalt	43.86	45.38	77.24

Table 4. Phytochemicals present in the test plant extracts

Phytochemicals	Samples		
	<i>T. minuta</i>	<i>T. zebrina</i>	<i>C. variegatum</i>
Tannins	+	+	+
Alkaloids	+	+	-
Steroids	-	+	-
Phenols	+	+	+
Saponins	+	-	+
Flavonoids	+	-	-
Terpenoids	-	+	-

Table 5. Inhibition zone diameters of plant extracts against test microbes

Samples	Inhibition zone diameters (mm)		
	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
<i>T. zebrina</i>	11.50	6.00	12.00
<i>T. minuta</i>	10.50	7.50	13.00
<i>C. variegatum</i>	12.00	6.00	10.50

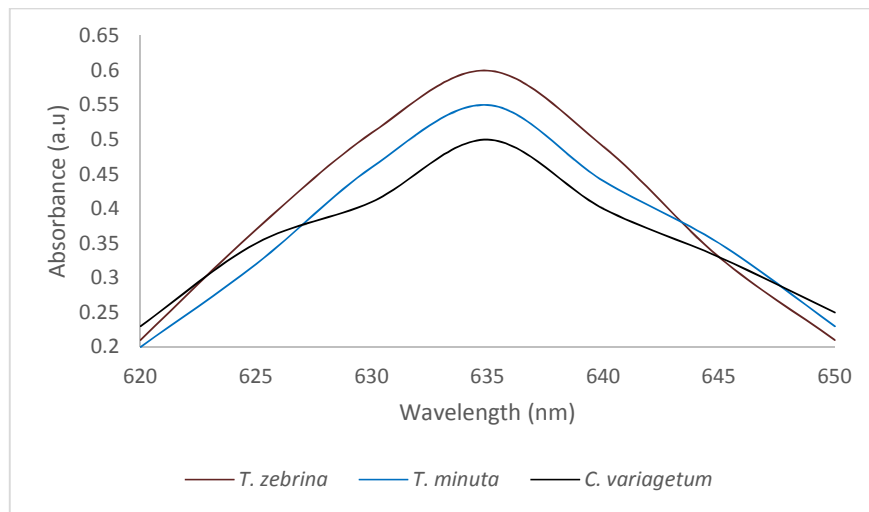


Fig. 2. UV-VIS spectra of vitamin K in plant extracts

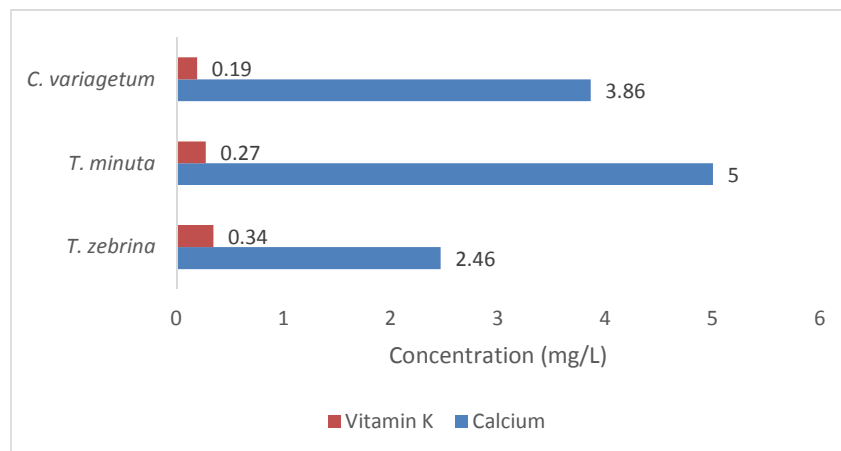


Fig. 3. Analysis of calcium and vitamin K in plant extracts

4. CONCLUSIONS

T. minuta, *T. zebrina* and *C. variagetum* crude extracts were found to have varying amounts of bio-active compounds necessary for blood coagulation. The major bio-active compounds in all the plant extracts were biometals (especially iron, copper and calcium), phytochemicals and vitamin K. The three extracts also exhibited moderate anti-microbial activities against *C. albicans* fungi and *E. coli* bacteria.

DATA AVAILABILITY STATEMENT

All data used in this study is found within the article and the supplementary sheets provided.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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