UMYU Scientifica, Vol. 4 NO. 4, December 2025, Pp 154 – 164
https://doi.org/10.56919/usci.2544.013
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

Solvent-Dependent Anticoagulant Effects of Combined Garlic (*Allium sativum*) and Roselle (*Hibiscus sabdariffa*) Extracts in Albino Rat Plasma

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ABSTRACT

Cardiovascular diseases, such as coronary heart disease and stroke, are the top killers of people globally, and by 2030, almost 23.6 million people are projected to die from cardiovascular diseases. This study aimed to investigate the anticoagulant potential of a combined *Allium sativum* bulb and Hibiscus sabdariffa using different solvent extracts (Methanol, Hexane, Ethyl acetate, and Butanol). Prothrombin time (PT) and Activated partial thromboplastin time (APTT) were assessed using different concentrations of the combined extract (50mg/ml, 75mg/ml, and 100mg/ml) together with positive control (Enoxaheparin), vehicle control, and normal control. There are significant differences in the combined extract between different solvents ($P<0.05$). At 100mg concentration ethylacetate was found to exibit high prolonged PT and APTT (39.0±2.51s and 48.00±2.4s respectively) followed by methanol (31.0±2.51 and 36.00±6.00s), butanol (24.0±2.00s and 32.60±5.40s) and finally hexane (9.0±2.00s and 0.30±0.07s) respectively compared with vehicle control (0.5% methanol 12.30±0.90s 40.10±2.80s, 0.1% DMSO- hexane fraction for hexane extract 12.20±0.9s 39.70±3.10s, 0.1% DMSO- for ethylacetate, PT 12.30±1.00 APTT 40.30±3.90, 0.5% butanol PT 12.10±1.00s APTT 39.60±3.00s) and normal control (PT 12.60±1.10s APTT 40.80±3.60s) . The ethyl acetate with the longest PT and APTT could be due to the fact that, as a semi-polar solvent, it can extract phytochemicals (both polar and semi-polar), which are known to have anticoagulant properties by inhibiting the synthesis of thromboxane A2, which affects the extrinsic coagulation cascade induced by adenosine diphosphate, arachidonic acid, and collagen. The plant extracts also contain anthocyanins in Roselle and Allicin in garlic (a sulphur-containing compound), thereby enhancing antioxidant and antiplatelet activities. This combined extract has demonstrated potential. However, mechanistic and synergistic interpretations remain speculative and require further biochemical confirmation.

ARTICLE HISTORY

Received 14 July, 2025
Accepted 03 December, 2025
Published 30 December, 2025

KEYWORDS

Anticoagulant activity, phytochemicals, solvents, cardiovascular diseases, combined Garlic and Roselle

INTRODUCTION

The search for safer, more accessible anticoagulant agents has intensified due to the limitations and bleeding risks associated with conventional therapies such as heparin and warfarin. Medicinal plants rich in bioactive compounds have therefore gained renewed scientific attention as potential modulators of hemostasis. Among these, Allium sativum (garlic) and Hibiscus sabdariffa (roselle) are widely used botanicals in African and Asian traditional medicine and are increasingly studied for their potential effects on blood coagulation and cardiovascular risk (Hareera *et al.*, 2022; Kokare *et al.*, 2025). Currently, witnessing an unprecedented pandemic, the coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS Co-V-2), is associated with a significant risk of thromboembolic complications due to a hypercoagulability state of blood, which is called as Covid-19 associated coagulopathy (CAC) (Singhania *et al.*, 2020).

Garlic contains several organosulfur compounds—including allicin, ajoene, and S-allyl cysteine—that have been shown to inhibit platelet aggregation, which affects both the intrinsic and extrinsic coagulation cascades, thereby prolonging clotting times and enhancing fibrinolytic activity (Clark-Montoya *et al.*, 2024). Similarly, Hibiscus sabdariffa is rich in anthocyanins and phenolic acids, which contribute to its antioxidant, anti-inflammatory, and emerging anticoagulant potential

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How to cite: Nasir, M. A., Ladan, M. J., Imam, A. U., & Mohammad, I. S. (2025). Solvent-Dependent Anticoagulant Effects of Combined Garlic (*Allium sativum*) and Roselle (*Hibiscus sabdariffa*) Extracts in Albino Rat Plasma. *UMYU Scientifica*, 4(4), 154 – 164. https://doi.org/10.56919/usci.2544.013

(Belwal *et al.,* 2018). The qualitative phytochemical screening of *Allium sativum* aqueous and ethanol extract indicated the presence of alkaloids, terpenoids, flavonoids, steroids, phenol, Anthraquinones, saponin, tannin and glycoside (Muhammad and Idris 2019). Hibiscus sabdariffa extracts inhibit platelet aggregation and promote vasorelaxation, likely through increased cAMP/cGMP and nitric oxide pathways, supporting cardiovascular benefits beyond anticoagulation (Martins et al., 2023). Both plants' extracts are rich in phenolics and flavonoids, which contribute to their antioxidant and membrane-stabilizing effects (Martins *et al.,* 2023; Maciel *et al*., 2018; Salawu *et al*., 2025; Chongwilaikasem *et al*., 2024). Current research indicates that both Hibiscus sabdariffa and Allium sativum exhibit in vitro anticoagulant and related cardiovascular activities, with extraction method and solvent choice significantly affecting efficacy. While direct studies on their combined anticoagulant effects are lacking, their individual properties and solvent-dependent bioactivity suggest potential for synergistic use in thrombosis prevention and cardiovascular health.

MATERIALS AND METHODS

Reagent

Table A shows the reagents used manufacturer and country where each is made.

Table A: Reagent used

Reagent	Manufacturer
Methanol	OIC Global, Netherlands
n-Hexane	SHELL Plc, United Kingdom
Ethyl acetate	IOL Chemicals and Pharmaceuticals Ltd., Punjab, India
Butanol	OXEA GmbH, Oberhausen, Germany
Distilled water	UDUS Hematology Laboratory, Sokoto, Nigeria
Calcium chloride	Spectrum for Diagnostic Industries, Cairo, Egypt
PT reagent (Spectrum)	Spectrum for Diagnostic Industries, Cairo, Egypt
APTT reagent	Spectrum for Diagnostic Industries, Cairo, Egypt
Sodium citrate	BDH Chemicals Ltd., England

Ethical approval

Ethical approval was obtained from the Ministry of Livestock and Fisheries Development, Sokoto State, with reference no. ML&FD/PLAN197/VOL.I and the approval is valid for 12 months.

Solvent extract preparation (methanol extract)

Fresh leaves of *Hibiscus sabdariffa* and bulbs of *Allium sativum were* cut into pieces and dried at room temperature for 1 week. The dried samples were weighed at equal ratios and soaked in the solvent. 250g of *Hibiscus sabdarifa* leaf *and* 250g bulb of *Allium sativum* were soaked in 2.5L of methanol(w/v) for 72 hours with intermittent stirring. The crude extract was filtered through a Muslin cloth, then through filter paper. The filtrate was concentrated in a rotary evaporator at 40°C under reduced pressure. The sample was placed in an oven dryer overnight at 45°C-60°C. The final residue was weighed, the percentage yield calculated, and the sample stored at 40°C before use.

Activity guided fractionation (Hexane, ethylacetate, and butanol extraction)

80g of the methanol extract was weighed, dissolved in 500ml of distilled water, shaken until completely dissolved, and transferred into a separating funnel. 250 ml of N-hexane was measured and added to the funnel, and the mixture was shaken vigorously. The mixture was allowed to settle, and two layers formed. The upper layer is the hexane extract, and the lower layer is the water filtrate. Both layers were collected in different conical flask .this procedure was repeated three times.

The water filtrate was added back to the separating funnel, and 250ml of distilled water and 250ml of ethyl acetate were added. Shake vigorously, then allow to settle; two layers formed: the upper layer is the ethyl acetate extract, and the lower layer is the water filtrate. The water filtrate and ethyl acetate were collected in separate conical flasks, and the process was repeated three times. The water filtrate was added back, and 250ml of distilled water and saturated butanol were added to the separating funnel, and shaken vigorously. Then allow to settle to form two layers: the upper layer is the butanol extract, and the lower layer is the water filtrate. The extracts were transferred to an oven dryer overnight at 45-60 °C until completely dried. The final solvent % was obtained.

Table B: Percentage Yield of combined *Allium sativum and Hibiscus sabdariffa* extract of different solvents.

S/N	Extract	weight (gram)	% yield
1	Methanol	127.9	25.58
2	Hexane	6.1	7.62
3	Ethylacetate	8.3	10.25
4	Butanol	5.3	6.62

Biochemical tests

The following biochemical tests were used to assess the anticoagulant potential of the combined Garlic and Roselle solvent extract (methanol, hexane, ethyl acetate, and butanol).

Prothrombin test (PT assay) E Kanayake *et al.,* 2008

Fresh blood samples from 6 albino rats (n=6 biological replicate) in four different groups were collected (5ml) in sodium citrate at a ratio of 1:9 (0.5 sodium citrate and 4.5 blood) and centrifuged for 15 minutes at the rate of

3000rpm and platelet poor plasma (PPP) was obtained and stored in a vial tube. 50µL of (PPP) was incubated for 5 min with 50 µL of each plant extract with different concentrations (50, 75 and 100mg/ml) at 37°C. Then, the prothrombin time was directly recorded using a manual stopwatch after the addition of 100 µL PT reagent (Hemostat thromboplastin) (Omar *et al.,* 2017). This procedure was repeated in triplicate (technical replicate) for all solvent extracts (methanol, hexane, ethyl acetate, and butanol), including normal control, vehicle, and positive control.

Activated partial thromboplastin assay (APTT time) Ekanayake *et al.,* 2008

50 µL of (PPP) was incubated for 2 min with 50 µL from each plant aqueous extract with different concentrations (50, 75 and 100mg/ml) at 37°C. After that, 50 µL aPTT reagent (Human, Germany) was added. Subsequently, the sample was incubated for an additional 3 min at 37°C. Then, the aPTT was directly recorded using a manual stopwatch after the addition of 100 µL of calcium chloride solution (Human, Germany) (Omar *et al.,* 2017). This procedure was repeated for all solvent extracts (methanol, hexane, ethyl acetate, and butanol), including normal control, vehicle, and positive control.

RESULTS

Qualitative and quantitative phytochemical content of combined extracts of *Allium sativum and Hibiscus sabdariffa* of methanol, hexane, ethlyacetate and butanol was found in which (+) indicate Trace amount, Moderate (++), Large amount (+++) and Not detected (ND) (Table 1). Quantitatively, Alkaloid was found to be the abundant constituent, accounting for about 7.8%, followed by Volatile oil at 4.8% and flavonoid at 3.12%, respectively (Table 2).

Table 1: Qualitative phytochemical content of combined extracts of *Allium sativum and Hibiscus sabdariffa* of different solvents.

S/N	Parameters	Methanol extract	N-Hexane extract	Ethylacetate extract	Butanol extract
1	Flavonoids	+ +	+	+ + +	+ + +
2	Tannis	++	+	+ +	+
3	Phenols	+ + +	+	+ +	+
4	Glycosides	+	+ +	+ + +	++
5	Alkaloids	+ +	+	+ +	+
6	Steroids	++	+++	+	+
7	Cardiac glycosides	+	ND	+ +	+ +
8	Balsams	+ +	+ + +	+	+
9	Anthraquinones	ND	ND	+	ND
10	Volatile oil	+ +	++	+ +	+

KEY: Trace amount +; Moderate ++; Large amount +++; Not detected ND

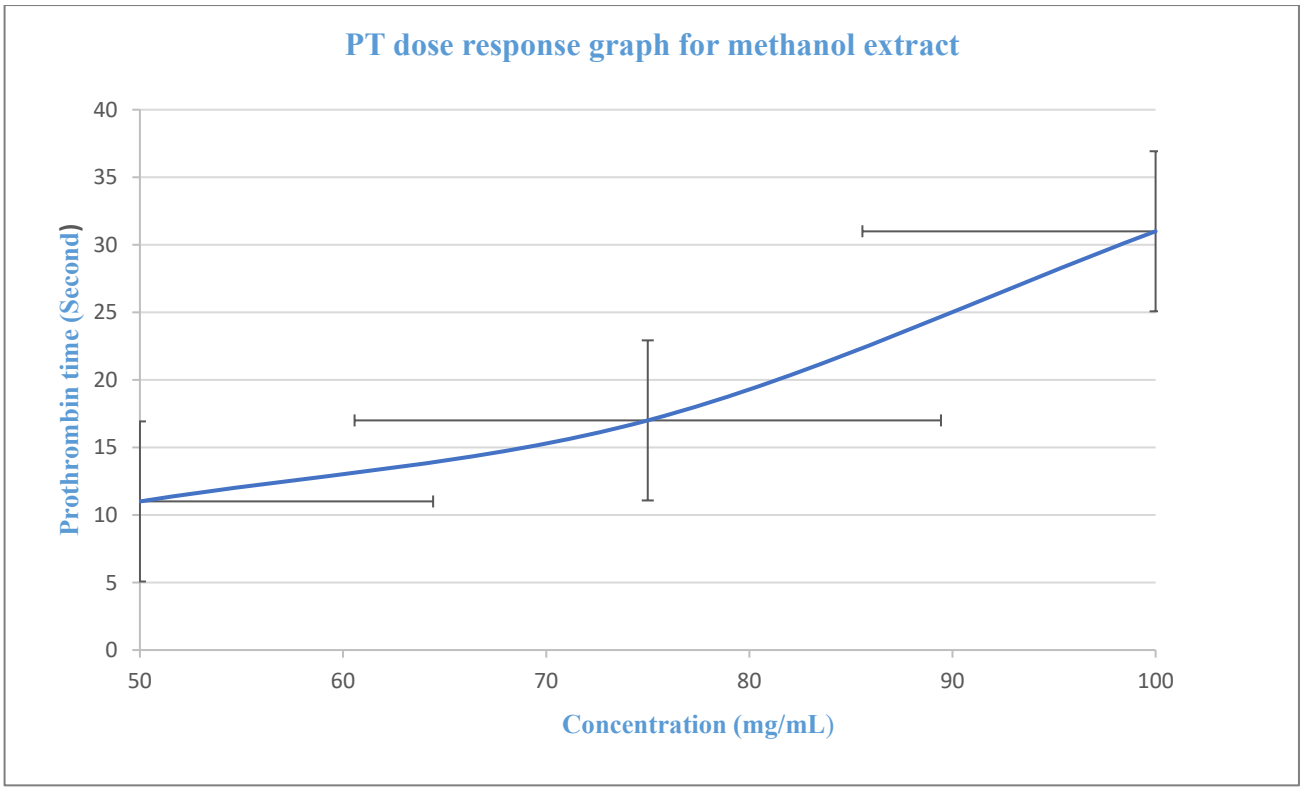

Fig 1. Dose-dependent effect of methanol extract on prothrombin time (PT). PT values measured at 50, 75 and 100mg/mL showed a concentration-dependent increase. Data are presented as mean±SD (n=6). Vertical error bars represent standard deviation. EC_{50} was not calculated due to the limited number of dose levels.

Table 2: Quantitative phytochemical content of combined garlic (*Allium sativum*) and roselle (*Hibiscus sabdariffa*) extract.

S/N	Phytochemical (%)	Quantitative analysis (g/100g)
1	Alkaloids	7.80±0.06
2	Flavonoid	3.12±0.13
3	Glycosides	0.06±0.00
4	Saponin	0.28±0.03
5	Steroids	1.88±0.24
6	Phenols	0.90±0.01
7	Terpenoid	0.05±0.00
8	Volatile oil	4.80±0.01
9	Tannin	0.30±0.01

Each value is expressed as mean±SD(n=3).

Table 3: Prothrombin time (PT) and activated partial thromboplastin time (APTT) of combined *Allium sativum and Hibiscus sabdariffa* methanol and hexane extracts.

	Methanol		Hexane	
Concentration (mg/ml)	**PT (sec)**	**APTT (sec)**	**PT (sec)**	**APTT (sec)**
50	11.00±2.00 ^{a}	34.20±2.40^{a}	6.00±0.50^{a}	16.80±2.40^{a}
75	17.00±2.00 ^{b}	36.60±4.20^{a}	7.00±0.50^{a}	15.60±3.60 ^{a}
100	31.00±2.51^{c}	36.00±6.00^{a}	9.00±2.00^{b}	18.00±4.20^{a}
Vehicle control (0.5% methanol)	12.30±0.90^{a}	40.10±2.80^{b}		
Vehicle control (0.1% DMSO- hexane fraction)			12.20±0.9^{c}	39.70±3.10^{b}
Normal control (normal diet + DH_2O)	12.60±1.10 ^{a}	40.80±3.60^{b}	12.00±1.10^{c}	40.80±3.60^{b}
Positive control Enoxaparin (Sodium 1IU/0.4mL)	10.20±2.08^{a}	43.00±0.24^{c}	10.20±2.08^{b}	43.00±0.24.60^{c}
Final assay concentration 0.50IU/mL				

Each value is expressed as mean±SD (n=6 biological replicates). For each rat sample, assays were performed in triplicate (technical replicates). Data were analyzed using one-way ANOVA followed by Tukey's HSD post hoc test. Means with different superscript letters within the same column were significantly different (P<0.05).

Table 4: Prothrombin time (PT) and activated partial thromboplastin time (APTT) of combined *Allium sativum and Hibiscus sabdariffa* ethylacetate and butanol extracts.

	Ethylacetate		Butanol	
Concentration (mg/ml)	**PT (sec)**	**APTT (sec)**	**PT (sec)**	**APTT (sec)**
50	14.00±3.00^{b}	24.00±3.60^{a}	8.00±1.52^{a}	18.00±3.60^{a}
75	23.00±12.16^{b}	30.00±9.60^{a}	8.00±1.57^{a}	24.00.±4.80^{b}
100	39.00±2.5^{c}	48.00±2.4^{b}	24.00±2.00^{b}	32.60±5.40^{c}
Vehicle control (0.1% DMSO-) for ethylacetate	12.30±1.00^{a}	40.30±3.90^{c}		-
Vehicle control (0.5% butanol)	-		12.10±1.00^{c}	39.60±3.00^{d}
Normal control (diet+ DH_2O)	12.60±1.10^{a}	40.80±3.60^{c}	12.0±1.10^{c}	40.80±3.60^{d}
Positive control Enoxaparin (Sodium 1IU/0.4mL)	10.20±2.08^{a}	43.00±0.24^{d}	10.20±2.08^{c}	43.00±0.24^{e}
Final assay concentration 0.50IU/mL.				

Each value is expressed as mean±SD (n=6 biological replicates). For each rat sample, assays were performed in triplicate (technical replicates). Data were analyzed using one-way ANOVA followed by Tukey's HSD post hoc test. Means with different superscript letters within the same column were significantly different (P<0.05).

The qualitative phytochemical screening of combined garlic and roselle extracts is presented in the table above (Table 2). Quantitatively, Alkaloid was found to be the abundant constituent, accounting for about 7.8%, followed by Volatile oil at 4.8% and flavonoid at 3.12%, respectively.

Prothrombin time (PT) and activated partial thromboplastin time (APTT) of combined *Allium sativum and Hibiscus sabdariffa* methanol, hexane, ethylacetate and butanol extracts (Table 3 and 4).

Final concentration of positive control (enoxaparin) used in 1IU/0.4mL

Enoxaparin sodium (1IU/0.4ML) was used without further dilution. A volume of 50 µL of stock solution (enoxaparin) was directly added to the assay tube (which contained 50 µL plasma + 50 µL positive control + 100 µL calcium chloride), producing a final enoxaparin concentration of 0.50 IU/mL.

Dilution steps

No pre-dilution was performed

50ul of enoxaparin, i.e., stock solution, was pipetted into each assay tube

Final assay volume

50 µL plasma + 50 µL positive control + 100 µL of calcium chloride +50 µL APTT reagent = 250 µL

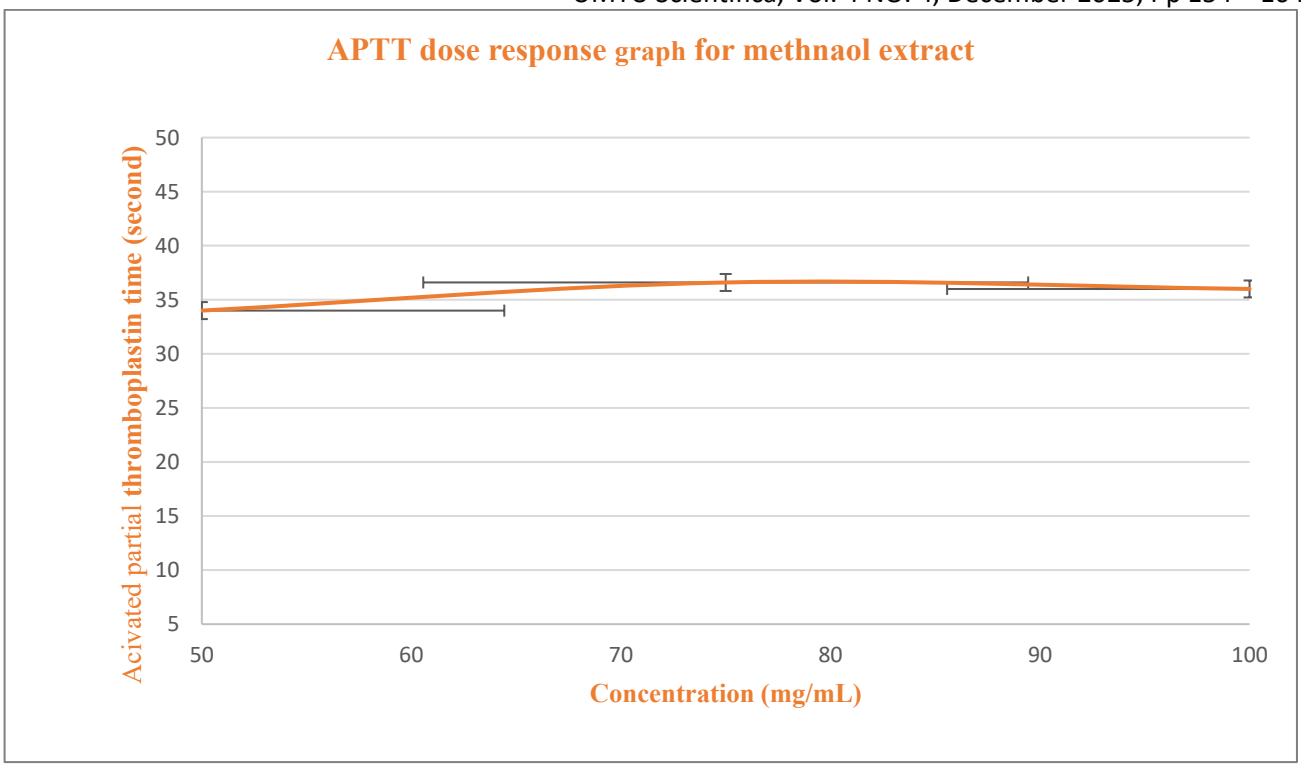

Fig 2. Dose-dependent effect of methanol extract on activated partial thromboplastin time (APTT). APTT values measured at 50, 75, and 100mg/mL showed a slight prolongation. Data are presented as mean±SD (n=6). Vertical error bars represent standard deviation. EC_{50} was not calculated due to the limited number of dose levels.

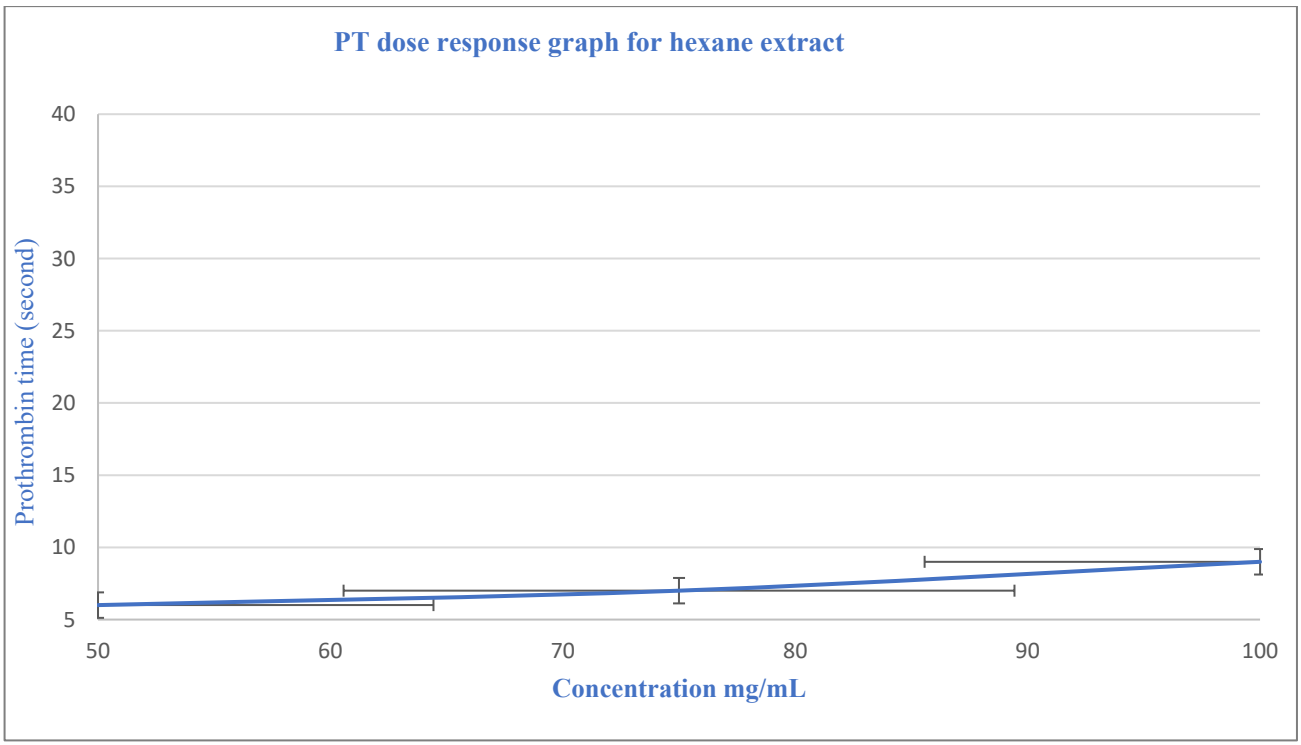

Fig 3. Dose-dependent effect of hexane extract on prothrombin time (PT). PT values measured at 50, 75 and 100mg/mL showed a concentration-dependent decrease. Data are presented as mean±SD (n=6). Vertical error bars represent standard deviation. EC_{50} was not calculated due to the limited number of dose levels.

Final concentration

(2.5IU/mL x 0.050mL) /0.250Ml = 0.50IU/mL.

Assay validation, calibration and consistency of endpoint

PT and APTT assays were validated prior to sample testing. Validation steps included verification of reagent performance against controls, determination of intra-assay and inter-assay precision and assessment of assay linearity across the working range.

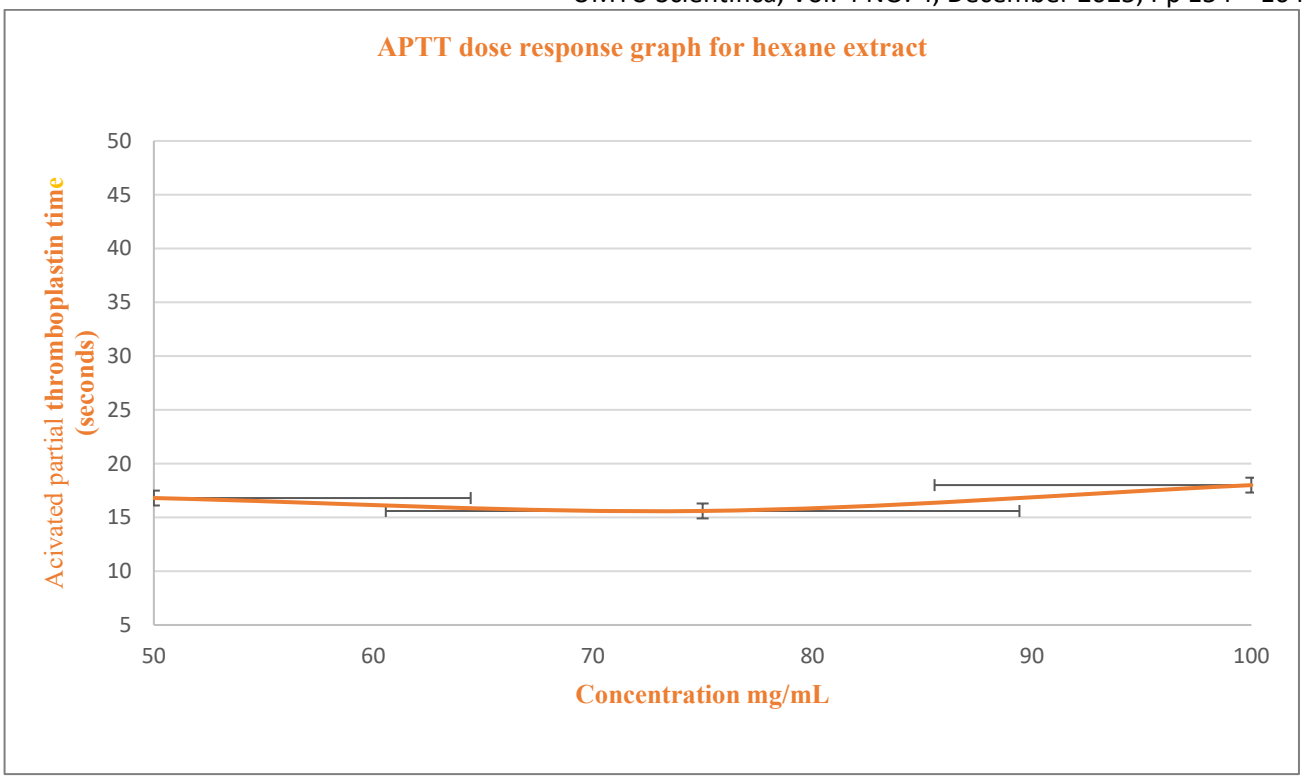

Fig 4. Dose-dependent effect of hexane extract on Activated partial thromboplastin time (APTT). APTT values measured at 50, 75, and 100mg/mL showed a slight prolongation. Data are presented as mean±SD (n=6). Vertical error bars represent standard deviation. EC_{50} was not calculated due to the limited number of dose levels.

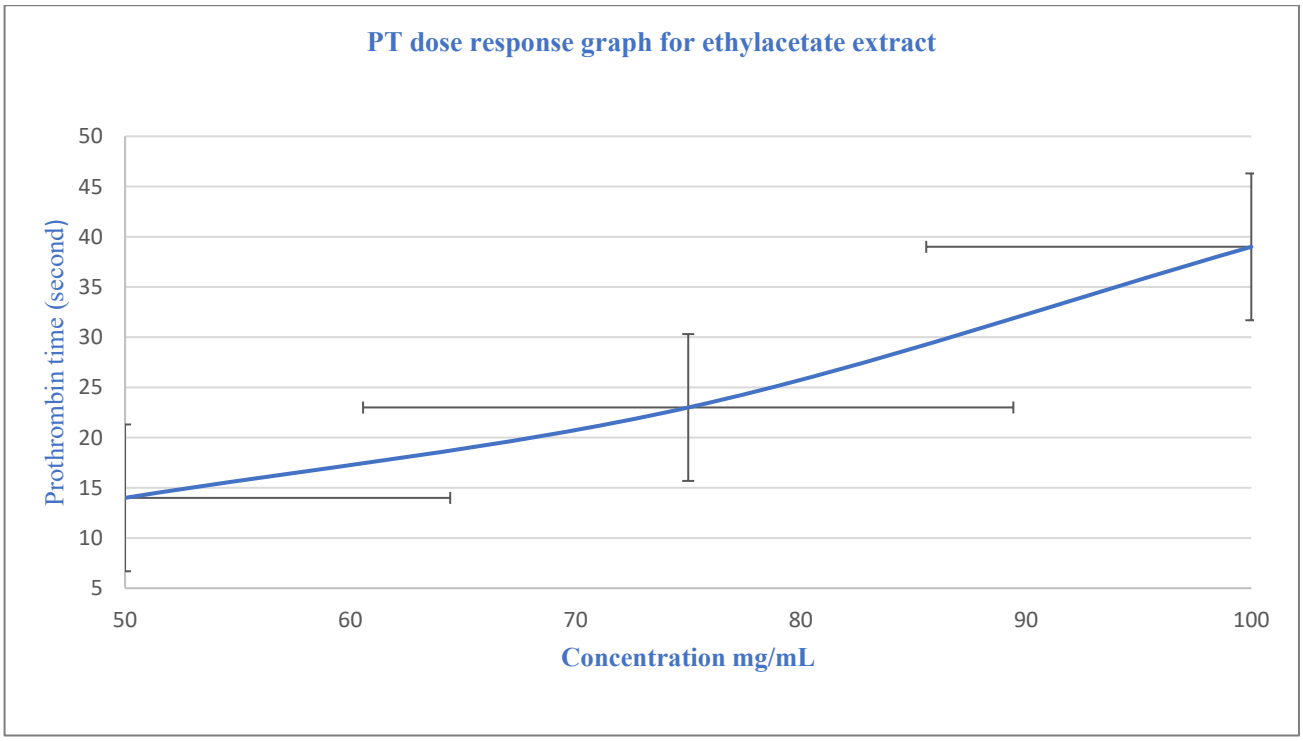

Fig 5. Dose-dependent effect of ethylacetate extract on prothrombin time (PT). PT values increased progressively across the extract concentrations (50, 75 and 100mg/mL), demonstrating a dose-related anticoagulant response. Data are presented as mean±SD (n=6). Vertical error bars represent standard deviation. EC_{50} was not calculated due to the limited number of dose levels.

The clotting endpoint was defined as the first visible fibrin strand using the manual tilt-tube and recorded with a calibrated stopwatch. Once PT and APTT reagents were added, the stopwatch was switched on. As fibrin became visible, the stopwatch was turned off, and the time was recorded. To minimize operator variability, the same trained operator performed all manual determinations. Assay temperature (37 °C), reagent lot numbers, and incubation times were controlled and logged.

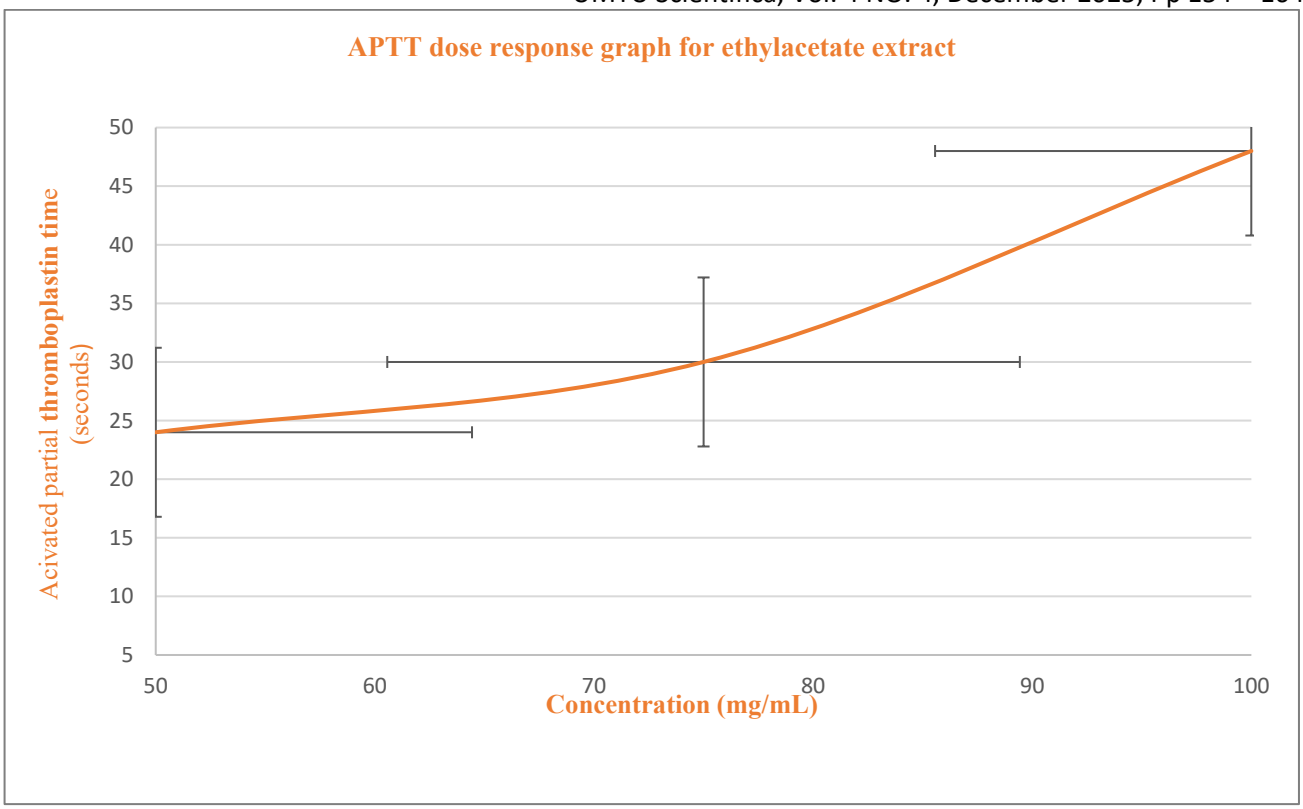

Fig 6. Dose-dependent effect of ethylacetate extract on Activated partial thromboplastin time (APTT). APTT increased markedly with increasing extract concentration (50, 75, and 100mg/mL), indicating a strong dose-dependent anticoagulant response through the intrinsic pathway. Data are presented as mean±SD (n=6). Vertical error bars represent standard deviation. EC_{50} was not calculated due to the limited number of dose levels.

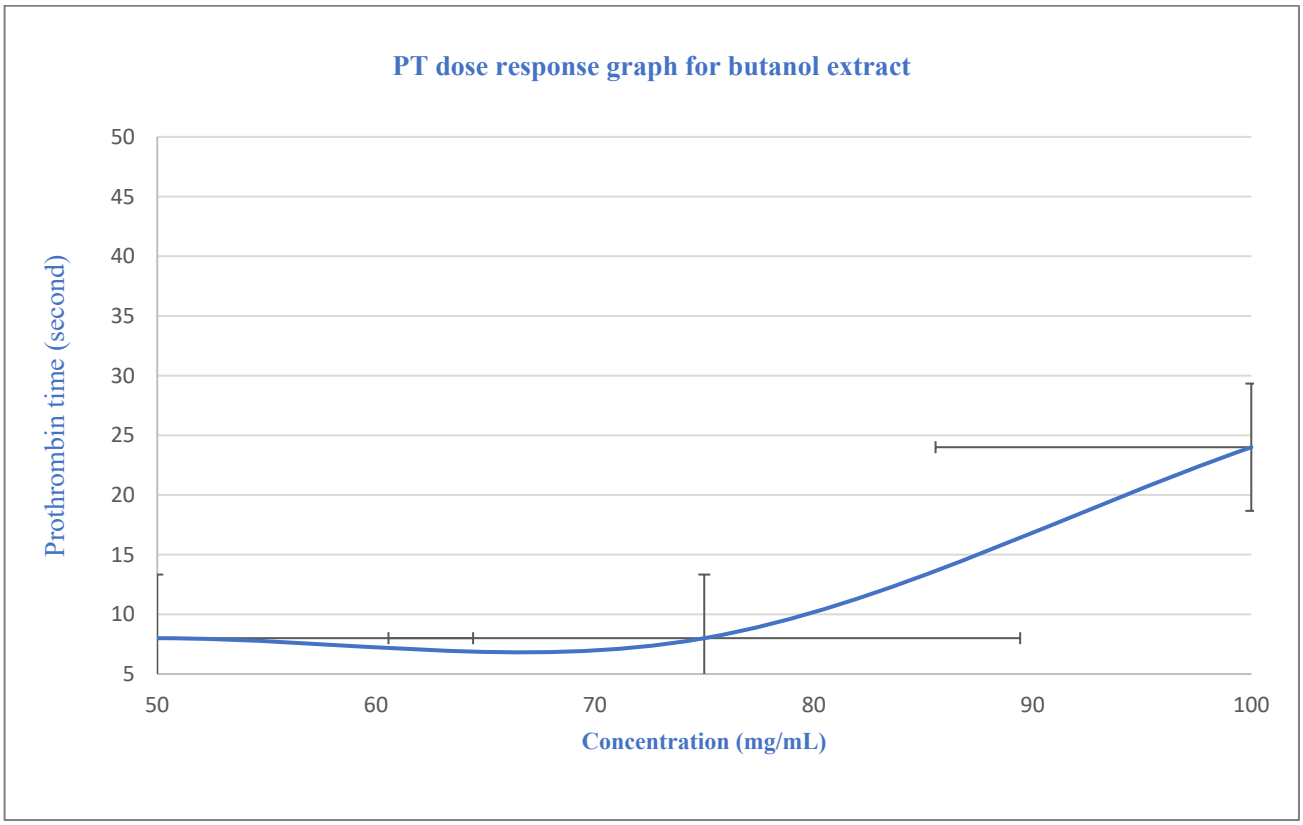

Fig 7. Dose-dependent effect of butanol extract on prothrombin time (PT). PT values measured at 50, 75 and 100mg/mL showed a slight increase through the extrinsic pathway. Data are presented as mean±SD (n=6). Vertical error bars represent standard deviation. EC_{50} was not calculated due to the limited number of dose levels.

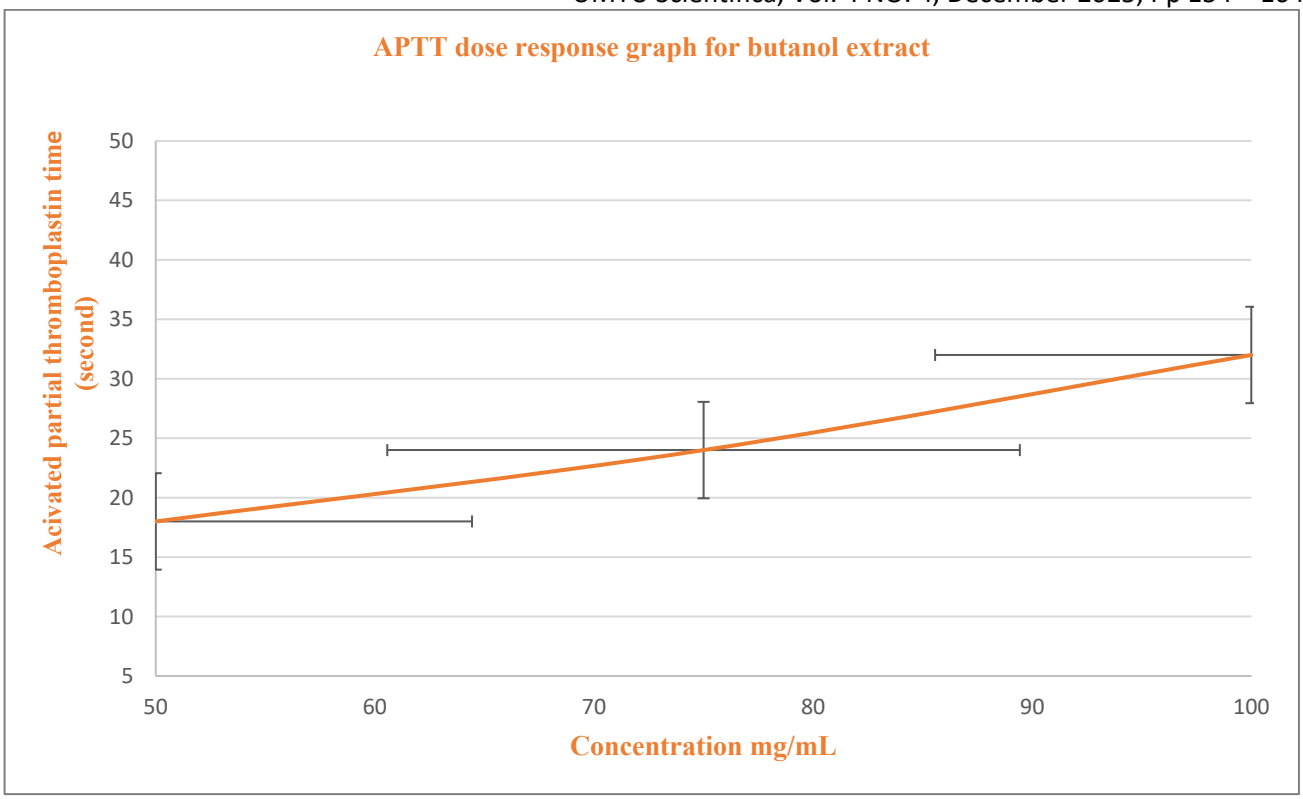

Fig 8. Dose-dependent effect of butanol extract on activated partial thromboplastin time (APTT). Increasing concentrations (50, 75, and 100mg/mL) of the extract produced a measurable prolongation of APTT, indicating an anticoagulant response through the intrinsic pathway. Data are presented as mean±SD (n=6). Vertical error bars represent standard deviation. EC_{50} was not calculated due to the limited number of dose levels.

Table 5: Exact p- value and confidence intervals of methanol and hexane extracts at 95%

	Methanol		Hexane	
Concentrations (mg/mL)	**PT (95% CI)**	**APTT (95% CI)**	**PT (95% CI)**	**APTT (95% CI)**
50	8.90 – 13.10	31.68 – 36.72	5.48-6.52	14.28-19.32
75	14.90 – 19.10	32.19 – 41.01	6.48-7.52	11.82-19.38
100	28.37 – 33.63	29.71 – 42.29	6.90-11.10	13.59-22.41
p-value	**PT**	**APTT**	**PT**	**APTT**
	0.00092	0.203	0.0723	0.506

Table 6: Exact P-value and confidence intervals of ethylacetate and butanol extracts at 95%

	Ethlyacetate		Butanol	
Concentrations (mg/mL)	**PT (95% CI)**	**APTT (95% CI)**	**PT (95% CI)**	**APTT (95% CI)**
50	10.85 – 17.15	20.22 – 27.78	6.1-9.59	14.22-21.78
75	10.23 – 35.77	19.93 – 40.07	6.35-9.65	18.97-29.03
100	36.37 – 41.63	45.48 – 50.52	21.90-26.10	26.94-38.26
P-value	0.00056	0.00102	0.00021	0.0186

Table 7: P- value and confidence intervals of control groups at 95%

			P- Value	
Controls	**PT (95% CI)**	**APTT (95% CI)**	**PT**	**APTT**
Normal	11.45-13.75	37.02-44.58	0.289	0.316
Positive	8.02-12.38	42.75-43.25	0.214	0.301
Methanol vehicle control	11.36-13.24	37.16-43.24	0.001	0.002
Hexane vehicle control	11.26-13.14	36.44-42.96	0.05	0.01
Ethylacetate vehicle control	11.25-13.35	36.20-44.40	0.00102	0.00011
Butanol vehicle control	11.05-13.15	36.45-42.75	0.0186	0.0148

Dose response plot for the major outcomes

Dose response graph of prothrombin time and activated partial thromboplastin time of methanol showed a concentration-dependent increase (Fig 1 and 2) while hexane showed a slight prolongation (Fig 3 and 4). So also ethylacetate showed increased progressively across the extract concentrations (Fig 5 and 6) and finally butanol showed a slight increase through the extrinsic pathway (Fig 7 and 8).

One-way ANOVA revealed highly significant differences in methanol, ethylacetate and butanol P- values PT (P=0.00092, 0.00056 and 0.00021, respectively) when compared with normal and positive control groups (P=0.289 and 0.214, respectively), which displayed significant dose-dependent anticoagulant activity, while hexane did not show any significant difference (P= 0.0723). However, methanol and hexane did not show significant differences in APTT (P=0.203 and P=0.506 respectively) when compared with normal and positive control groups (P=0.316 and 0.310 respectively) while ethylacetate and butanol shows high significant differences (P= 0.00102 and P= 0.00021 respectively) when compared with normal and positive control groups (P=0.316 and 0.310 respectively).

DISCUSSION

The screening of plants for medicinal value has been carried out by numerous researchers using preliminary phytochemical analyses (Mungole and Chatuvechi 2011). The selection of plant parts, such as leaves, that yield the maximum amount of secondary metabolites is the primary or prerequisite step in this investigation. Many researches has been done concerning phytochemicals content of garlic and roselle, in which the results revealed the present of important bioactive compounds, according to Muhammad and Idris (2019), Garlic was found to have the presence of Alkaloid, terpenoids, flavonoids, steroid, phenol, Anthraquinones, saponin, tannin and glycoside in trace amount. Also, roselle was found to have the presence of Alkaloid, Anthocynanis, flavonoids, Quinones phenol, Anthraquinones, saponin, tannin and glycoside in trace amount (Obouayeba *et.,al* 2014). The presence of these active compounds makes these plants have anticoagulant potency. The presence study investigated the phytochemical composition of combined garlic and roselle prepared with different solvents (methanol, hexane, ethyl acetate, and butanol). The results revealed the presence of flavonoids, tannins, saponins, alkaloids, and steroids in large and moderate amounts in the methanol and butanol extracts (Table 1). Previous studies report that methanol is one of the best solvents for recovering antioxidant flavonoids and phenolic compounds from H. sabdariffa calyces and *A. sativum* bulbs (Ameer *et al.,* 2020; Nwachukwu et al., 2022). Because both plants are rich in water-soluble phenolics, their combined methanolic extract is expected to show high phytochemical density. While glycosides are present in trace amount while, glycosides and steroids are present in large and moderate amounts in the hexane extract. Similar observations were made in earlier phytochemical studies on *A. sativum*, where hexane selectively extracted sulphur-containing volatile oils and steroidal compounds (Shelekhova et al., 2021). The lower intensity of polar phytochemicals in hexane seen in this study agrees with established solvent-selection principles. Ethyl acetate produced moderate levels of flavonoids, alkaloids, and glycosides, which is expected given its intermediate polarity. Ethyl acetate is known to selectively extract semi-polar compounds, including organic acids and medium-polarity flavonoids, consistent with studies on H. sabdariffa calyces and *A. sativum* extracts (El-Mahdy *et al.,* 2023).

This result suggests that the polar and mid-polar extracts of the combined garlic and roselle carry a broad spectrum of bioactive compounds. When compared with the result (Garlic only) reported by (Muhammad and Idris 2019) and for roselle (Obouayeba *et.,al* 2014), the current study gives clear evidence that the combined extract shows a prolonged effect compared with a single plant extract which make the extract to have multiple target nodes in the coagulation and thrombus formation pathway. Therefore, the combined extract of garlic and roselle shows anticoagulant potential. These findings demonstrate that the extract interfered with clotting time, but the current study design does not allow a definitive conclusion on the precise mechanism involved.

Blood clotting is an important event during trauma and other vascular injuries, where it plays a pivotal role in stopping bleeding and, consequently, sealing the vascular injury, thus preventing blood loss. Two primary blood clotting pathways are (1) the intrinsic pathway (factors VIII, IX, XI, and XII) and (2) the extrinsic pathway (factor VII). Both pathways induce fibrin clot formation (Alquwaizani *et al.,* 2013). This study was conducted to explore the anticoagulant activity of natural flavonoid compounds and their potential as a safer alternative to prescribed anticoagulant drugs. Aqueous extracts of Hibiscus sabdariffa leaves show significant in vitro anticoagulant and antithrombotic effects at higher concentrations, with minimal cytotoxicity. The anticoagulant effect of the extract increases with concentration and incubation time, suggesting potential for thrombosis management (Jocelyne *et al.,* 2025). Allium sativum, when combined with other plant extracts, inhibits sickle cell polymerisation and improves red blood cell stability, indicating membrane-protective and possibly anticoagulant properties (Salawu *et al.,* 2025). The current study investigated the anticoagulant potential of the combined *Allium sitivum* and *Hibiscus sabdariffa* leaves extract using different solvents (Metanol, hexane, ethylacetate and butanol) with different concentrations (50mg, 75mg and 100mg), in which the result shows in Tables 2 and 3. The anticoagulant activity shows a significant increase (P<0.05) between the normal/control groups and the combined extract at different concentrations. PT and APTT increase progressively as compared with control (PT12.0±10.00s APTT 40.80±3.60s) from 50mg to 100mg of the extract. At 100mg concentration, ethylacetate extract shows the highest prolongation of both PT and APTT (PT 39.0±2.51s APTT 48.00±2.4s), suggesting strong anticoagulant activity followed by methanol (PT 31.0±2.51 APTT 36.00±6.00), butanol (PT 24.0±2.00 APTT 32.60±5.40), and finally hexane (PT 9.0±2.00 APTT 18.00±4.20). The highest PT and APTT by ethylacetate because ethylacetate is a semi-polar solvent which tends to extract both polar and non-polar phytochemicals, which mention earlier, they have anticoagulant property and this makes them inhibit both intrinsic to single extract as reported by Safraz *et al.,*

(2021), while studying garlic extract (PT 15.6±0.60s, APTT 42.5±1.4s) and Hoda *et al.*, (2022) when studying on roselle. (PT 20.35±0.0s APTT 41.10±0.14s). This pattern aligns with known phytochemical profiles from previous studies: garlic is rich in the extrinsic pathway of the coagulation cascade by inhibiting thrombin activity, chelating calcium ion and scavenging ROS. The prolongation of PTT may imply that the intrinsic pathway is affected to some degree; however, this remains speculative, as the present work did not include factor assays, thrombin/Xa inhibition measurements, fibrinogen estimation. Therefore, the observed clotting profile should be broadly interpreted as anticoagulant activity rather than conclusive mechanistic inhibition of any single factor or pathway. Consequently, the results should be described as having anticoagulant potential rather than as a fully characterised mechanistic or synergistic response.

LIMITATIONS

Phytochemical screening is qualitative; the exact concentrations of each compound are unknown. Without quantitative phytochemistry or standardisation, safety/toxicity studies, and reproducibility, safety/toxicity studies are limited. In vitro coagulation assays (PT/APTT) may not reflect in vivo complexity (metabolism, absorption, distribution, coagulation cascade interactions, and endothelial factors). Potential toxicity, interaction with other medications, or bleeding risk are unknown. If the extract inhibits coagulation too much (or affects platelets strongly), the risk of haemorrhage must be evaluated. Current study focuses on PT/APTT only, assays like Platelet aggregation, thrombin time, fibrinogen assays, and thrombin generation. Hemolysis and cytotoxicity were not assessed.

CONCLUSION

Current research indicates that both Hibiscus sabdariffa and Allium sativum exhibit in vitro anticoagulant and related cardiovascular activities, with extraction method and solvent choice significantly affecting efficacy. While direct studies on their combined anticoagulant effects are lacking, their individual properties and solvent-dependent bioactivity suggest a possible interaction between constituents. However, synergy cannot be definitely concluded.

ACKNOWLEDGMENTS

My deepest gratitude goes to Almighty Allah, who has provided all that was needed to complete this research work in particular. My extreme thanks to my supervisors, Prof. M. J Ladan and Dr A.I Umar, for the manner in which they have handled me, and for their guidance, correction, advice, suggestions, and encouragement, which have aided tremendously in the successful completion of this research work. My special thanks to the PG Coordinator, Head of Department, and the entire staff of the Department of Biochemistry and Molecular Biology, Sokoto State University.

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