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Research article

Anticoagulant effect of *Feijoa sellowiana* extracts generated by different biotechnological techniques

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ABSTRACT

Blood clotting has become one of the most dangerous side effects associated with Corona virus, as well as the high level of cholesterol and triglycerides in the blood. Therefore, it has become necessary to use medicinal plants that are biologically safe and containing anti-clotting compound. *Feijoa sellowiana* represents a prolific source diverse compounds that may have thrombolytic activity. Therefore, the main research point is the production and scaling up of a target contents that have anticoagulants by using biotechnological techniques; calli production, and bioreactors and assessed their activity through *in-vivo* study. Murashige and Skoog (MS) medium enriched with varying concentrations of benzyl adenine (BA) and naphthalene acetic acid (NAA) was used to cultivate calli and cell suspension cultures from *F. sellowiana* seeds. Bioreactors were employed to boost active constituent's production. Moreover, the bioreactor physical factors such as effect of controlled or uncontrolled pH medium were investigated. The leaves of the main plant were extracted by ethanol 70% and polar and non-polar extracts were also prepared. The ethanol extract of calli and cells resulting from bioreactors were also prepared. All prepared extracts were subjected to chemical analysis by HPLC, *in-vitro* antioxidant assays, *in-vivo* anticoagulant activity and histopathological examination. Calli and cell suspension cultures were produced by using MS medium fortified with 1 mg/L BA+ 0.1 mg/L NAA. It was found that culturing of cell cultures in a bioreactor with uncontrolled pH and aeration at the value of 0.5 L/min gave the maximum and economical fresh and dry weights of the plants. After evaluation of all extracts; it was found that the calli ethanol extract for each plant was the highest value of total phenolic and total flavonoid contents either quantitatively or qualitatively. All extracts of Feijoa had antioxidant activity. The IC_{50} of the DPPH of Feijoa calli extract was 13.45 μg/mL, it was also confirmed by FRAP and ABTs values. Feijoa calli extract decreased platelet aggregation by suppression of thrombin, extended aPTT, PT, bleeding and clotting times. It was safer than warfarin medication. From these findings the authors can conclude that Feijoa had highly anticoagulant activity and the calli production achieved the goal of the enhancement of the phenolic constituent and thus their activity.

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1. Introduction

Blood coagulation plays a critical role in thrombosis, cardiovascular disorders (CVD), and vascular damage [1]. Recently, the incidence of thrombosis and stroke increased due to the high level of cholesterol and triglycerides in the blood or it might be accomplished by a disease such as coronavirus disease that occurred in 2019 (COVID-19) [2]. Following COVID-19 infection, the immune and coagulation systems are stimulated. However, more research is still needed to clarify the relationship between COVID-19 infection and coagulation system activation [3].

The intrinsic and extrinsic pathways are the two mechanisms that start the coagulation cascade. The extrinsic pathway is engaged when blood is exposed to tissue components from the surface of extravascular cells, whereas the intrinsic pathway is started by chemicals inside the injured blood artery [4]. Platelets are activated by a number of stimuli, such as collagen, arachidonic acid that lead to Thromboxane B2 (TXB2) formation through Cyclooxygenase 1 (COX-1) and TXA2 synthase pathway (TXAS). TXB2 is one of the most powerful inducer of platelet aggregation, interacts with other platelets, which increase in thrombotic disorders [5].

There are several restrictions and negative effects associated with the use of anticoagulants (heparin, warfarin). Bleeding is the most permanent side effect of this treatment [6]. It has become necessary to use medicinal plants that are biologically safe and contain anti-clotting compounds.

Plant tissue culture technology can be established routinely under sterile conditions from explants, such as leaves, stems, roots, and meristems for both the ways for multiplication and extraction of secondary metabolites [7,8]. The most famous application for the plant tissue culture to produce useful pharmaceutical, is the production of anti-Alzheimer agent huperzine A (HupA) [9].

In-vitro production of secondary metabolite; phenolic acids and flavonoids; by plant cell suspension cultures has been reported from commercial medicinal plants [10].

Plant cell culture in bioreactors [11] has advantages over the whole plants for having sustainable biopharmaceuticals production, such as (a) streamlined purification, particularly for products secreted into the extracellular medium, (b) consistency in product quality and homogeneity achievable under controlled environmental conditions, (c) ease of compliance with cGMP requirements, (d) elimination of the need for cultivation and manipulation of greenhouse or field grown plants which can be quite labor intensive, (e) ability to use inducible promoter systems, (f) reduced potential for endotoxin and mycotoxin contamination derived from the plant and soil source, and (g) minimal cell-to-cell communication that resulted in the possible reduction of systemic post-transcriptional gene silencing (PTGS) and which occurs via plasmodesmata and via the vascular system in whole plants [12]. High volumetric mass transfer coefficients and a homogenous environment can be produced by stirred-tank bioreactors (STB) fitted with the appropriate impellers, that enable consistent control of suspended plant cell growth generation [13].

Feijoa sellowiana Berg. (synonym; *Acca sellowiana*, Common name; pineapple guava, guavasteen pineapple or Feijoa) is related to family Myrtaceae [14]. It was used in gardens as an ornamental plant, due mainly to the exotic beauty of its flowers. Presently, the main interest in this species rests on the edible fruits it produces which, among other components [15]. Traditionally; it was used as antimicrobial [16], antioxidant, anti-cancer, anti-inflammatory, and immunity-stimulating agent [17] and as treatment for goiter [18].

It also considered as a good source of anti-oxidant and anticoagulant agents [19]. Feijoa is very interesting plant with its phenolic and vitamin contents. Feijoa fruits are rich in vitamin C, which is considered as a strong antioxidant agent [20]. Peels extract contains huge amount of vitamin P (P)-active polyphenols, such as catechin, leucoanthocyanins, flavonols, proanthocyanidin and naphthoquinones [21]. The Feijoa leaves contain catechins; i.e. (+)-catechin, (−)-epicatechin, (+)- gallocatechin and (−)-epigallocatechin [19].

The scarcity of *F. sellowiana* plant in Egypt is never sufficient the market demance, so it encourages the team work to use different biotechnological techniques; plant tissue culture and bioreactors for enhancing the production of plant secondary metabolites that have the anticoagulant effect and to verify the claimed traditional usage. This effect was examined *in-vivo* in rat's model. All parameters in the blood serum were measured and the results were confirmed with histopathological examinations.

The objective of this study was to enhance and increase the concentrations of anticoagulants agents including, phenolic and flavonoid contents utilizing biotechnological techniques (calli production and applying of bioreactors). Moreover, Identification of chemical constituents of their extracts followed by biological assessment through *in-vivo* study employing different coagulation tests. Prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), thromboxane B2 (TXB2), cyclooxygenase-1 (COX-1), clotting and bleeding times were evaluated.

2. Material and methods

2.1. Collection and taxonomical characterization of F. sellowiana

F. sellowiana leaves and seeds were collected from Al Zohriya botanical Garden in September 2019. Voucher specimen No. 20190918 was authenticated by Dr. Tereez Labib Consultant of Plant Taxonomy at the Ministry of Agriculture and Director of Orman Botanical Garden, Giza, Egypt.

2.2. Surface sterilization

Surface sterilization was established according to the method by Daud et al. [22]. Briefly, Freshly collected leaves was washed

under slow running tap water (15 min) followed by washing in Tween 80 (1 drop in 200 mL sterile distilled water for 1 min) and then were rinsed with sterile distilled water (SDW, 3 times) in the laminar flow cabinet. Leaves and seeds were dipped separately in 70% alcohol for 30 s and then rinsed twice in the SDW. The plant materials were then sterilized by using a locally available bleach solution Clorox® (5.25% sodium hypochlorite) and $HgCl_2$ (Sigma).

2.3. Callus induction and mass calli production

Sterilized seeds and, young leaves were aseptically cut and cultured into Murashige and Skoog (MS) basal media [23] supplemented with 1 mg/L BA in combinations with different concentrations 1, 3, 5 mg/L NAA. All cultures were incubated under either dark or light conditions (fluorescent lighting lamps 3000 lux for 16/8h per day), for 28 days (4 weeks) at 26 ±1 °C. Each treatment was represented with five replicates for calli production after the choice of the best media. Percentage of calli formation, fresh and dry weights (g/jar) were recorded [24].

2.4. Cell suspensions production

Feijoa cell suspension cultures were established from friable obtained leaves calli cultures according to the described method by Kenneth [25]. A passage of calli (0.5 g fresh weight) were re-cultured into 125-mL Erlenmeyer flasks containing 25 mL of MS liquid culture medium supplemented with 1 mg/L BA+ 0.1 mg/L NAA. The cultures were incubated on a rotary shaker (125 rpm) at 25 ± 1 °C under photoperiod (16-h light/8-h dark; cool white fluorescent tubes; 50 μE/m2/s). Sub-cultured were done at 15-d intervals. The cell cultures were maintained in MS liquid culture medium supplemented with same concentrations of the previous growth regulators, pH 5.7.

The following parameters were recorded after 4,8,12,16,20 and 24 days of cultivation as follow: (a) Cell number was counted during the growth period of cultivation as a growth parameter, (b) Packed cell volume (P.C.V) was determined after 4,8, 12, 16, 20 and 24 days of cultivation [26] .

2.5. Bioreactor studies

A working volume of 1.5–1.7 L was used in the available 2-L turbine stirred tank bioreactor (STB) (B. Braun, Biotech, International, Germany). A sintered steel spurge was used to aerate the culture. The flow rate was configured for the type of the experiment and was kept constant with a mass flow control system until the end of the culture period. Mixing was done with six-bladed turbine impellers (D = 45 mm) and rotation speed was 120 rounds per minute (rpm). Using a thermostatic outlet and a spongy sheet around the jar, the temperature was kept at 26 °C. At a rate of 0.5 L/min, and aeration was changed. Through the experiment, the following variables that affect the mass cell culture production (fresh and dry weights g/run) were examined:(a) the effect of uncontrolled or controlled pH medium at the degree of 5.7 by using either 0.2 N NaOH or 0.2 N HCL through ADI 1030 Bio-controllers (Applikon) equipped with sterilizeable pH–electrode (Ingold) and peristaltic pumps for alkali or acid addition. The rhythm of lighting system was 16/8 h in the light/dark. The light intensity was 1500 Lux from white cooling florescent lamps, (b) the effect of different aeration values 0.5 and 1.0 L/min. The bioreactor was inoculated with one part of suspension culture and five parts of medium, and the cell cultures of each plant were kept at 26 ± 1 °C. The MS-nutrient medium containing cell lines were provided into a glass tank bioreactor under sterilized air condition.

At the end of fifteen days of inoculation, the obtained cells were harvested, lyophilized and weighted as fresh (g/one run) and dry weights. Maybe this study is the first record in the field of controlling of stirred reactor (STB) physical factors, such as controlled or uncontrolled pH medium and different aeration values affecting on *in vitro* cell suspension increasing value and parameters. According to the procedure outlined by Snedecor and Cochran, every experiment was created with a completely random design, and the data obtained was statistically examined using standard error (SE) [27].

2.6. Phytochemical analysis

2.6.1. Preparation of extracts

F. sellowiana dried grind leaves were extracted with 1 L of boiled ethanol (70%), repeated for 3 times until exhaustion and concentrated to dryness under reduced pressure. An aliquot of concentrated ethanol extract (70%, T- Fg) was then suspended in water and the water-soluble portion was partitioned with *n*-hexane to give the non-polar fraction (N-Fg). The remaining aqueous phase were then concentrated to dryness under reduced pressure and lyophilized to give polar fraction (P1- Fg).

The obtained calli as well as the produced tissues by bioreactors were collected individually, grinded, lyophilized, defatted with n-hexane and extracted with ethanol (70%). All extracts of the original plants (T- Fg, P1-Fg and N-Fg), callus (C-Fg) and bioreactor (P2-Fg) ethanol extracts were evaporated under reduced pressure, weighed and used for phytochemical and biological investigations.

2.7. Quantitative estimation of total phenolic & total flavonoids contents

Samples of Feijoa extracts (T- Fg, P1-Fg, C-Fg and P2-Fg) were prepared at the concentration 2 mg/mL in 70% ethanol as stock solution. The total phenolic contents (TPC) and flavonoid contents (TFC) were estimated quantitatively using microplate reader FluoStar Omega concerning pre-established standard calibration curves. Total phenolic, total flavonoid contents were determined

using Folin–Ciocalteau method [28] and aluminium chloride method [29], respectively. Results were expressed as gallic acid (GAE) and rutin (RE) equivalents, respectively.

2.8. Qualitative and quantitative estimation of phenolics by HPLC analysis

High performance liquid chromatography (HPLC) analysis of T-Fg, P1-Fg, C-Fg and P2-Fg extracts were carried out using an Agilent 1260 series (Agilent Technologies, Waldbronn, Germany). The separation was carried out by using Eclipse C18 column (4.6 mm × 250 mm i.d., 5 μm). The mobile phase consisted of (A) water and (B) 0.05% trifluoroacetic acid in acetonitrile with a flow rate 0.9 mL/min. According to the following linear gradient, the mobile phase was sequentially programmed: 0 min (82% A), 0–5 min (80% A), 5–8 min (60 %A), 8–12 min (60 %A), 12–15 min (82% A), and 15–16 min (82% A). At 280 nm, the multi-wavelength detector was observed. Each sample solution was injected in a 5 μL volume. The column was kept at a constant temperature of 40 °C. Based on their UV spectra and retention times, this research allowed for the classification of phenolic chemicals.

2.9. Biological studies

2.9.1. In-vitro antioxidant activity

Antioxidant activity for T-Fg, P1-Fg, C-Fg and P2-Fg extracts were carried out by using three different methods; DPPH, FRAP and ABTS for the confirmation of their activity [30].

2.9.1.1. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. The evaluation of the radical scavenging activity of the samples was carried out by the DPPH (1, 1-diphenyl-2-picrylhydrazyl), according to the method of Boly et al. [31]. The method depends on the reduction in DPPH color intensity which was measured at 540 nm [32,33]. Data were represented as IC_{50}value. The lower IC_{50} values, the greater effectiveness of the antioxidant power of the extract. Samples were analyzed in triplicate.

2.9.1.2. Reducing power (FRAP assay, ferric reducing antioxidant power). The assay was carried out according to the method of Benzie and Strain [34]. A TPTZ reagent (300 mM Acetate Buffer (PH = 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$, in a ratio of 10:1:1 v/v/v, respectively) was freshly prepared. Freshly prepared TPTZ reagent (190 μL) were mixed with 10 μL of the sample in 96 wells plate ($n = 3$) and the reaction was incubated at room temperature. At the end of the incubation period (30 min in dark), the resulted blue colored was measured at 593 nm. Data were represented as means ± SD. The results were recorded using microplate reader FluoStar Omega.

2.9.1.3. 2,2-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay. The assay was carried out according to the method of Arnao et al. [35]. 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay measures the relative ability of antioxidants to scavenge the ABTS generated in an aqueous phase, as compared with a Trolox (water-soluble vitamin E analogue) standard. Briefly; ABTS (192 mg) was dissolved in distilled water and transferred to a volumetric flask (50 mL), then the volume was completed with distilled water. 1 mL of the prepared solution was added to 140 mM potassium persulphate (17 μL) and the mixture was kept for 24 h in the dark. After that, 1 mL of the reaction mixture was completed to 50 mL with methanol to obtain the final ABTS dilution used in the assay. 190 μL freshly prepared ABTS reagent was mixed with the sample (10 μL) in 96 wells plate ($n = 6$), the reaction was incubated for 120 min at room temperature in the dark. The decrease in ABTS color intensity at the end of incubation time was measured at 734 nm [32,33] . **Data are stated as means ± SD according to** equation (1):

$$Percentage\ inhibition = \left(\frac{Average\ absorbance\ of\ blank - average\ absorbance\ of\ the\ test}{Average\ absorbance\ of\ blank}\right) * 100 \quad \text{Eq.(1)}$$

The results of ABTS•+ radical assays were presented asμM TE/mg sample (Trolox equivalent antioxidant capacity (TEAC)) using Trolox as a standard reference. The data were analyzed using *Microsoft Excel* software, *Graph pad Prism version 5* were used to calculate the IC_{50} values [36].

2.9.2. In-vivo anticoagulant experiment

Adult male and female albino Wistar rats weighing150–200 gm were obtained from the animal house at the National Research Centre (Giza, Egypt) and fed by a standard laboratory diet and tap water ad libitum. Experimental animals were housed in an air-conditioned room at 22–25°Cwith a 12 h light/dark cycle. The animal experiments were performed in accordance with recommendations guided by the Care and Use of Laboratory Animals of the National Institutes of Health (NIH No. 85:23 revised 1985) in accordance with the guidelines provided by the CPCSEA and World Medical Association Declaration of Helsinki on Ethical Principles for studies involving experimental animals. This study had approval from the ethics committee of NRC (Egypt) with a certificate number (19,478).

2.9.2.1. Experimental design of acute toxicity study. The control mice group received normal saline (2 mL/kg body weight p.o.), while other groups received 500, 1000 and 2000 and mg/kg of the test extract orally. Immediately after dosing, the animals were observed continuously for the first 4 h for any behavioral changes. They were then kept under observation for 14 days after drug administration to find out the mortality if any. Observations were made twice daily.

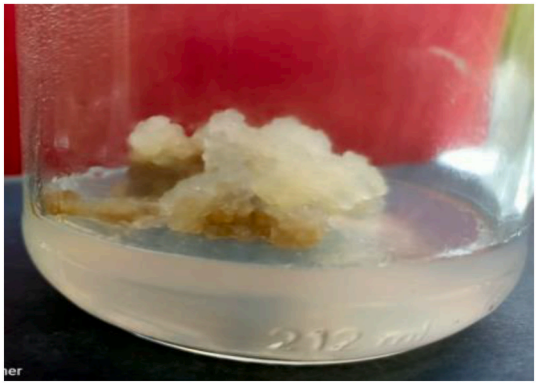

Fig. 1. Calli culture production from *Feijoa sellowiana* leaf explants.

2.9.2.2. Experimental design of the anticoagulant study. After an acclimatization period of one week, male and female albino Wistar rats were randomly assigned into 20 groups (8 rats/group).

Group 1–2 male and female received dis. water and served as normal control. **Group 3–4**: male and female rats received *warfarin sodium* (2 mg/kg, oral.) for 5 days per week according to Liu et al. [5]. **Group 5–6:** male and female rats received bioreactor extracts (P2- Fg, 200 mg/kg, orally) daily for 7 days. **Group 7–8:** male and female rats received calli extract (C-Fg, 200 mg/kg, orally) daily for 7 days. **Group 9–12:** male and female rats received crude extract of the original plant leaves (T-Fg, 100,200 mg/kg, orally) daily for 7 days. **Groups 13–16**: male and female rats received non-polar fraction of the original plant extract (N-Fg 100,200 mg/kg, orally) daily for 7 days. **Groups 17–20:** male and female rats received polar fraction of the original plant extract (P1-Fg, 100,200 mg/kg, orally) daily for 7 days.

At the end of the experimental period, three blood samples were withdrawn from the retro-orbital vein of each animal, under mild anesthesia with chloral hydrate. The first sample was centrifuged at 3000 rpm for 15 min and serum was obtained. Cyclooxygenase 1 (COX1) and Thromboxane B2 (TXB2) were determined from serum using ELIZA kits procured from (Elabscience, USA).

The second sample was collected after 30 min of the last administration into a test tube containing Einethylene diamine tetra acetate (EDTA), Blood samples were analyzed using an automated cell counter (Helena C-2, UK) and accurately programmed for the analysis of red blood cell (RBC) count, hemoglobins (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLC), total white blood cell (WBC) count, Lymphocytes count (LYM), Mid-Cell count (MID) and Granulocytes count (GRN).

The third sample was collected into a test tube containing 3.8% sodium citrate and centrifuged at 1000 rpm for 10 min for measuring of Plasma Coagulation Factors. Prothrombin time (PT), and activated partial thromboplastin time (aPTT) were determined according to the kit instructions.

Bleeding time (BT) and clotting time (CT) were obtained according to reported methods [37]. The tail of the rat was warmed for 1 min in water at 40 °C and then dried. A small cut was made in the tip of the tail. Bleeding time started when the first drop touched the circular filter paper. It was checked at 30 s intervals until bleeding stopped. While, clotting time was calculated after the cut by collecting blood into a standard glass capillary tube by the capillary action. Subsequent to this, the end of capillary tube was broken every 30 s until the clot is formed and the end of capillary tube starts hanging [38].

Data were expressed as mean ± standard error. Statistical analysis was performed by using *Graph Pad Prism 5* Software version 5 (San Diego, CA). The Dunnett multiple comparisons test is conducted after the one-way analysis of variance test (ANOVA). A 0.05 level of probability was utilized as the threshold for the significance in all statistical analyses. Excel software was used to perform regression analysis and create graphics.

2.9.3. 3- Histological analysis

Spleen specimens were sectioned at a thickness of 5 μm, immersed in paraffin, and promptly fixed in 10% formalin at room temperature. Using a light microscope, the slices were stained with haematoxylin and eosin (H&E), to examine the histopathological changes.

3. Results and discussion

Among the different combination of BA and NAA, the calli culture revealed that MS culture medium supplemented with 1 mg/L BA+ 0.1 mg/L NAA was the best medium for calli production from leaf explants (Fig. 1).

In the cell suspension culture study, the maximum cell number 3.80 ($x10^5$) was recorded with *F. sellowiana* after 14 days of cultivation. However, the highest packed cell volume reordered 0.68 after 24 days of cultivation as showed in Figure (2). The most significant and economic period of cell culturing was 14 days of cultivation.

Regarding bioreactor studies, the obtained cell cultures from *F. sellowiana* were optimized. Further, cultured in 2 L stirred tank bioreactor aiming to scaling up of cell cultures. The first experiment pH media was setup as uncontrolled, whereas the second

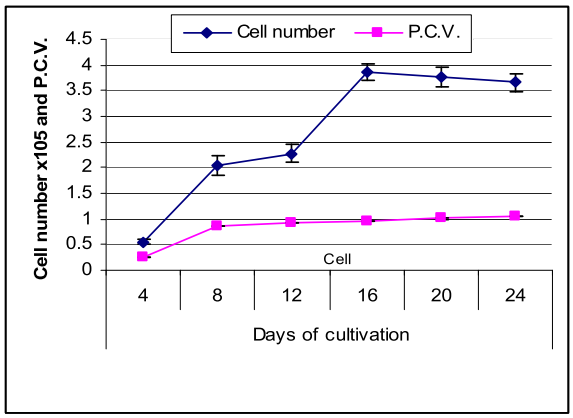

Fig. 2. Cell number x 10 [5] and P.C.V. of *Feijoa sellowiana* during 24 days of cultivation.

Table 1
Total Phenol and total flavonoid contents of *Feijoa sellowiana* extracts.

Samples	Total Phenolic (TP) (μg GAE/mg extract)	Total Flavonoids (TF) (μg RE/mg extract)
T-Fg	291.04	66.16
P1-Fg	315.73	68.79
C-Fg	439.61	132.47
BP2-Fg	277.37	38.32

Table 2
HPLC analysis of *Feijoa sellowiana extracts*.

No	R_t	Compounds	Area%			
			T-Fg	P1-Fg	C-Fg	BP2-Fg
1	2.783	Unknown	10.6468	**12.6758**	3.6005	**53.4211**
2	**3.394**	**Gallic acid**	**14.4494**	**16.0168**	**18.2838**	**13.1059**
3	4.243	Chlorogenic acid	0.0633	0.1343	0.4465	–
4	4.676	Catechin	1.006	0.6981	0.4409	1.1333
5	5.401	Methyl gallate	0.9427	0.1564	0.3960	0.1620
6	5.948	Caffeic acid	**15.4661**	**12.2914**	**16.7143**	4.5874
7	6.641	Syringic acid	2.1234	2.7173	4.0293	0.2717
8	7.128	**Pyrocatechol**	–	–	0.2024	ND
9	7.998	Rutin	0.2336	0.2741	0.5258	0.0442
10	8.834	Ellagic acid	**8.0230**	9.4046	**18.3912**	**18.8141**
11	9.233	Coumaric acid	–	1.7092	3.3729	–
12	9.811	Vanillic acid	0.6570	0.2213	0.8268	–
13	10.200	Ferulic acid	1.3015	1.6303	2.5392	–
14	10.528	Naringenin	1.8302	0.8587	0.7114	–
15	10.731	**Unknown**	–	3.9062	3.2398	ND
16	10.937	**Unknown**	10.4500	6.5326	4.8594	ND
17	11.225	**Unknown**	5.6455	5.0884	3.8465	ND
18	11.402	**Unknown**	**10.2140**	3.0492	2.7481	ND
19	11.568	**Unknown**	0.4200	2.2822	2.493	ND
20	12.334	**Daidzein**	0.3903	0.1733	0.5750	ND
21	12.599	Querectin	0.3219	0.1671	0.2246	0.2325
22	13.855	Cinnamic acid	0.3284	0.4079	0.4210	0.4491
23	**15.554**	**Hesperetin**	0.7558	0.1287	2.7652	2.2001

*ND Not detected.

experiment the pH media were setup at 5.7.

The obtained results from Fig. 2 clearly shows that, the maximum fresh weights and dry weights 260.8 (g/run) and 17.3 were recorded, respectively with *F. sellowiana* with uncontrolled pH media which cultured on MS media fortified with 1 mg/L BA+ 0.1 mg/L NAA. While, the cultured cells in STB with controlled pH media resulted in 181.3 (g/FW/run) and the dry weights were recorded 12.9 (g/DW/run). The effect of different aeration system at the level of 0.5 or 1.0 L/min on mass cell production cultured in STB was

Table 3
Antioxidant activity of the *Feijoa sellowiana* extracts using DPPH, FRAP and ABTS assays.

Antioxidant activity	Free Radical Scavenging activity (DPPH) IC_{50} (ug/mL)	Iron Reducing Power (FRAP)	ABTS
		(μM Trolox equivalent/mg extract)	
T-Fg	26.28	2077.66	652.53
P1-Fg	23.81	2457.11	677.95
C-Fg	13.45	2750.54	1114.97
BP2-Fg	32.06	2319.79	626.86
Trolox	42.42	-	-

assessed. The highest mass cell production 385.25 (g/FW/run) was recorded which aerated with 0.5 L/min. While, it recorded 323.8, (g/FW/run) which aerated with 1 L/min. Whereas dry weights (g/run) recorded 30.5 & 26.7 in STB and aerated with 0.5 and 1.0 L/min, respectively.

It might be the first time for the *in-vitro* cultivation of the Egyptian *F. sellowiana* either by plant tissue culture or through using bioreactors. The results of plant tissue culture was in agreement with the results obtained by Guerra for the plant native to Brazil but with different concentration [39].

3.1. Total phenolic and total flavonoid contents

The amounts of total phenols (TPC) and total flavonoids (TFC) were measured in Feijoa (T- Fg, P1-Fg, C-Fg and P2-Fg) extracts. The determinations were calculated from the linear regression equation of the calibration curve, by using gallic acid and rutin as standards, respectively. The results are showed in Table 1.

The TPC of callus (C-Fg) was the highest 439.61 μg GAE/mg extract followed by the polar extract (P1-Fg, 315.73 μg GAE/mg extract), total ethanol extract and the bioreactor ethanol extracts (T-Fg, BP2-Fg, 291.04 and 277.37 μg GAE/mg extract), respectively. Regarding, the TFC contents, C-Fg was found to be the highest value followed by P1-Fg, T-Fg and BP2-Fg (132.47, 68.79, 66.16 and 38.32 μg RE/mg extract), respectively. The C-Fg had two folded content than the total ethanol extract (T-Fg) of the main plant. The calli extract was the highest content for both TPC and TFC. The results was in agreement with the data obtained by Cecilia and Pasquariello [40,41].

3.2. HPLC analysis

HPLC analysis of *Feijoa sellowiana* extracts (Table 2); revealed the identification of fifteen compounds in T-Fg with caffeic and gallic acids were the majors (15.466%, 14.449%), respectively. There were also five compounds detected but not identified. Sixteen compounds were identified at P1-Fg with gallic and caffeic (16.0168%, 12.2914%) were the major, respectively. Seventeen compounds were identified at callus extract (C-Fg) with ellagic, gallic and caffeic acids were the majors. Ellagic were found in 2.92 folds than T-Fg. While gallic and caffeic were 1.265, 1.08 folds than T-Fg. Pyrocatechol was detected and identified in C-Fg only and there were six compounds detected with different percentage. Ten compounds only identified in BP2-Fg. Ellagic acid were the major identified one, but there was a compound detected with 53.42% detected. The obtained results were similar to those reported by Zhu as gallic acid, ellagic acid, syringic acid and catechin were the main phenolic acid and flavonoid in different reports [19].

3.3. Biological studies

3.3.1. In-vitro antioxidant activity

The plant materials having antioxidant constituents has played an important role in the protection from diseases; such as coronary heart disease and stroke [42]. These constituents help in the maintenance of health by protecting vital cells from the harmful effect of reactive oxygen species (Ros) [43]. Phenolic acids, flavonoids and tannins have strong antioxidant properties as they act as reducing agents, metal chelating agent, hydrogen donators, and singlet oxygen quenchers [44]. *Feijoa* had high contents of phenolic acids and flavonoids. In this study, the antioxidant study was done by three different methods; DPPH, FRAP and ABTS to give wide diverging results (Table 3), as the single method is not adequate for evaluating the activity [45].

The lower IC_{50} value, the higher antioxidant activity and from the data obtained in Table 3. The IC_{50} of the DPPH radical scavenging assay of calli extract (C-Fg) was the 13.45 μg/mL, which was the lowest value, indicates a high antioxidant activity. The reducing power (FRAP) of T- Fg, P1-Fg, C-Fg and P2-Fg were 2077.66, 2457.11, 2750.54 and 2319.79 μM Trolox equivalent/mg extract, thus all extracts showed higher FRAP ability and C- Fg was the highest activity. The antioxidant ability for reducing the ABTS of T- Fg, P1-Fg, C-Fg and P2-Fg were 652.53, 677.95, 1114.97 and 626.86 μM Trolox equivalent/mg extract, respectively. The callus extract showed the highest activity as antioxidant with the different methods. These assays were evaluated before for 12 genotype of Feijoa [41,46] and all studies revealed that *F. sellowiana* leaves and fruits had strong antioxidant activity through either *in-vitro or in-vivo* evaluation.

3.3.2. In-vivo anticoagulant activity

This study might be the first study to demonstrate the significant anticoagulant activity of *F. sellowiana* extracts.

Table 4
Effect of *Feijoa sellowiana* on Hematological Parameters male rats.

	Normal control	Warfarin (2 mg/kg)	Callus *F. sellowiana* (200 mg/kg)	Bioreactor *F. sellowiana* (200 mg/kg)	*F.sellowiana* (100 mg/kg)	*F. sellowiana* (200 mg/kg)	Non polar *F. sellowiana* (100 mg/kg)	Non polar *F. sellowiana* (200 mg/kg)	Polar *F. sellowiana* (100 mg/kg)	Polar *F. sellowiana* (200 mg/kg)
RBC ($\times 10^{12}$/L)	6.23 ± 0.16	6.6 ± 0.25	7.2 ± 0.27[a]	7.02 ± 0.18[a]	6.7 ± 0.33	7.1 ± 0.09[a]	6.1 ± 0.06	7.12 ± 0.09[a]	6.84 ± 0.1	6.6 ± 0.34
Hb (g/dl)	11.4 ± 0.25	11.7 ± 0.34	13.4 ± 0.41[a]	12.16 ± 0.33[a]	12.22 ± 0.45	12.9 ± 0.25[a]	11.24 ± 0.19	12.46 ± 0.7[a]	12 ± 0.04	11.68 ± 0.6
MCV%	53.28 ± 1.8	52.78 ± 1.17	51.46 ± 1.29	51.62 ± 0.8	53.86 ± 1.44	54.56 ± 1.15	52.12 ± 0.12	54.38 ± 2.3	54.34 ± 1	53.16 ± 3.29
MCH (pg)	18.0 ± 0.29	18.12 ± 0.46	18.46 ± 0.73	17.9 ± 0.31	18.22 ± 0.42	18.32 ± 0.28	17.28 ± 0.17	18.28 ± 0.4	18.42 ± 0.26	18.1 ± 0.33
MCHC (g/dl)	33.9 ± 0.7	34.4 ± 0.23	34.64 ± .26	34.7 ± 0.19	33.9 ± 0.38	33.4 ± 0.21	34.06 ± 0.08	32.8 ± 0.97	33.34 ± 0.18	34.1 ± 0.43
Platelets ($\times 10^{9}$/L)	845 ± 15.7	633 ± 16.3[a]	422.2 ± 10[b]	339 ± 8.3[bc]	652.6 ± 15.4 [acd]	516 ± 22.5 [abcd]	625.2 ± 15 [acd]	519.6 ± 35 [abcd]	612.6 ± 16.5[acd]	524.4 ± 9 [abcd]
WBC ($\times 10^{9}$/L)	10.9 ± 0.28	16.6±1[a]	8.32 ± 0.36[b]	7.1 ± 0.45[b]	10.56 ± 1.8[b]	9.42 ± 1[b]	8.58 ± 0.14[b]	8.52 ± 0.25[b]	10.0 ± 1.7[b]	9.32 ± 0.33[b]
LYM ($\times 10^{9}$/L)	5.86 ± 1	9.46 ± 1.45[a]	5.9 ± 0.25[b]	5.6 ± 0.15[b]	6.74 ± 0.16	5.6 ± 0.15 [b]	6.58 ± 0.7	5.84 ± 0.09 [b]	6.74 ± 0.75	5.92 ± 0.76[b]
MID ($\times 10^{9}$/L)	0.46 ± 0.04	0.52 ± 0.07	0.45 ± 0.025	0.58 ± 0.02	0.424 ± 0.006	0.52 ± 0.11	0.42 ± 0.04	0.66 ± 0.12	0.48 ± 0.13	0.38 ± 0.006
GRAN ($\times 10^{9}$/L)	2.32 ± 0.22	4.5 ± 0.63[a]	2.62 ± 0.51[b]	3 ± 0.15[b]	2.74 ± 0.02	2.96 ± 0.18[b]	2.3 ± 0.32	2.78 ± 0.79	1.48 ± 0.10	1.12 ± 0.12[b]

Data are presented as the mean ± S.E. of (n = 8) for each group. Statistical analysis was carried out by one-way analysis of variance followed by Dunnett multiple comparisons test. [a] Statistically significant from control group. [b] Statistically significant from warfarin group at $P < 0.05$.[c] Statistically significant from callus group at $P < 0.05$. [d] Statistically significant from bioreactor group at $P < 0.05$.

Table 5
Effect of *Feijoa sellowiana* on Hematological Parameters female rats.

	Normal control	Warfarin (2 mg/kg)	Callus *F. sellowiana* (200 mg/kg)	Bioreactor *F. sellowiana* (200 mg/kg)	*Total F. sellowiana* (100 mg/kg)	*Total F.sellowiana* (200 mg/kg)	Non polar *F. sellowiana* (100 mg/kg)	Non polar *F. sellowiana* (200 mg/kg)	Polar *F. sellowiana* (100 mg/kg)	Polar *F. sellowiana* (200 mg/kg)
RBC($\times 10^{12}$/L)	6.37 ± 0.17	6.67 ± 0.25	7.558 ± 0.13[a]	7.2 ± 0.18[a]	6.618 ± 0.34	7.26 ± 0.17[a]	6.192 ± 0.04	7.22 ± 0.09[a]	6.9 ± 0.08	6.618 ± 0.34
Hb (g/dl)	11.24 ± 0.09	11.62 ± 0.33	13.42 ± 0.39[a]	12.9 ± 0.27[a]	12.62 ± 0.43	13.1 ± 0.12[a]	11.44 ± 0.22	13.1 ± 0.16[a]	12.64 ± 0.38	12.28 ± 0.63
MCV%	55.08 ± 2.1	53.38 ± 0.95	52.26 ± 0.96	52.56 ± 0.66	54.06 ± 1.46	54.76 ± 1.27	52.32 ± 0.20	55.58 ± 1.96	54.54 ± 0.97	54.12 ± 0.76
MCH (pg)	18.1 ± 0.25	18.2 ± 0.47	18.66 ± 0.62	17.94 ± 0.32	18.3 ± 0.38	18.52 ± 0.48	17.48 ± 0.2	18.52 ± 0.4	18.62 ± 0.26	18.28 ± 0.38
MCHC (g/dl)	34.1 ± 0.6	34.62 ± 0.39	34.66 ± .25	34.84 ± 0.17	34.04 ± 0.34	33.64 ± 0.30	33.86 ± 0.21	33.7 ± 0.2	33.54 ± 0.19	34.3 ± 0.46
Platelets ($\times 10^9$/L)	847 ± 16	630.4 ± 14.4[a]	424 ± 8.6[b]	340.2 ± 8.5[bc]	657.2 ± 13.4 [acd]	512 ± 25.5 [abcd]	627 ± 15.5 [acd]	522 ± 33.5 [abcd]	614 ± 17 [abcd]	530.4 ± 6.8ac[d]
WBC ($\times 10^9$/L)	11.2 ± 0.16	17.06 ± 0.84[a]	8.52 ± 0.20[b]	7.4 ± 0.24[b]	11.14 ± 1.5[b]	10.02 ± 0.66 [b]	8.78 ± 0.05[b]	8.6 ± 0.18[b]	9.48 ± 0.22[b]	10.3 ± 1.6[b]
LYM ($\times 10^9$/L)	5.98 ± 0.99	9.74 ± 1.23[a]	6.08 ± 0.15[b]	5.72 ± 1.3[b]	6.9 ± 0.29	5.78 ± 0.1 [b]	6.6 ± 0.69	6.04 ± 0.95 [b]	6.26 ± 0.36	6.78 ± 0.71[b]
MID ($\times 10^9$/L)	0.48 ± 0.02	0.54 ± 0.06	0.5 ± 0.12	0.4 ± 0.05	0.475 ± 0.04	0.598 ± 0.002	0.43 ± 0.005	0.56 ± 0.1	0.44 ± 0.08	0.68 ± 0.12
GRAN ($\times 10^9$/L)	2.48 ± 0.22	4.7 ± 0.69[a]	1.58 ± 0.08[b]	1.3 ± 0.13[b]	2.76 ± 0.46	3.12 ± 0.19[b]	3.1 ± 0.23	2.8 ± 0.05	2.98 ± 0.76	2.44 ± 0.22[b]

Data are presented as the mean ± S.E. of (n = 8) for each group. Statistical analysis was carried out by one-way analysis of variance followed by Dunnett multiple comparisons test.
[a]Statistically significant from control group.
[b]Statistically significant from warfarin group at $P < 0.05$.
[c]Statistically significant from callus group at $P < 0.05$.
[d]Statistically significant from bioreactor group at $P < 0.05$.

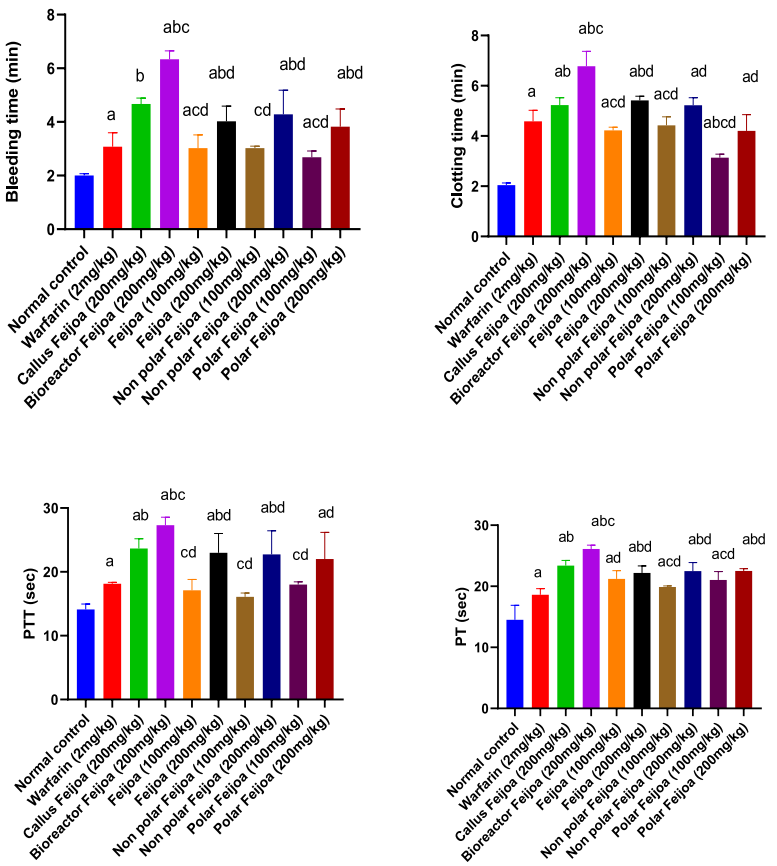

Fig. 3. Effect of *Feijoa sellowiana* on Plasma Coagulation Factors Measurement in male rats
Data are presented as the mean ± S.E. of (n = 8) for each group. Statistical analysis was carried out by one-way analysis of variance followed by Dunnett multiple comparisons test. [a] Statistically significant from control group. [b] Statistically significant from warfarin group at $P < 0.05$. [c] Statistically significant from callus group at $P < 0.05$. [d] Statistically significant from bioreactor group at $P < 0.05$.

3.3.2.1. Acute toxicity of Feijoa sellowiana on mice. Our findings revealed that *Feijoa sellowiana* extracts as treatment at such doses up to 2000 mg/kg did not cause any signs of toxicity or deaths in mice. The LD50 values of all extracts were more than 2 g/kg body wt. which compared with previously reported method [46].

3.3.2.2. Effect of Feijoa sellowiana on hematological parameters in rats. Hematological variables analysis might be used to reveal detrimental effects of exogenous substances, such as plant extracts, on the composition of blood in an animal model [47]. Parameters of red blood cells i.e. Hb, RBC, HCT, MCV, MCH, and MCHC were performed to evaluate the effects of *Feijoa sellowiana* extracts in anemic state.

The total ethanol T-Fg, non-polar N-Fg, polar P1-Fg, calli C-Fg, and bioreactor BP2-Fg extracts intake have not changed: MCV, MCH and MCHC as compared to their normal control groups in male and female rats as shown in Tables (4 & 5). While, RBC and Hb significantly increased as compared to control groups in male and female rats.

Rats given warfarin (2 mg/kg) resulted in thrombocytopenia to be 25% and 25.6% respectively, as compared to control groups in male and female rats. Moreover, administration of calli (C-Fg), bioreactor (BP2-Fg), crude (T-Fg) non-polar (N-Fg) extracts (200 mg/kg) significantly caused thrombocytopenia to 33.4%, 46%, 19%, 17%,16% and 33%, 47%,19%, 18%,17% respectively, as compared to warfarin –treatment groups in male and female rats. While, rats given calli (C-Fg), bioreactor (BP2-Fg) extracts, resulted in a significant thrombocytopenia, as compared to crude, non-polar and polar extracts groups in male and female rats. Moreover, bioreactor showed a significant thrombocytopenia to 19% and 20% respectively, as compared to calli group in male and female rats.

Administration of warfarin (2 mg/kg) was resulted in the presence of lucocytosis, lymphocytosis and granulocytosis as compared to normal control groups in male and female rats. As well as, calli (C-Fg), bioreactor (BP2-Fg), crude (T-Fg), non-polar (N-Fg) and polar (P-Fg) extracts treatment groups showed significant lucocytosis, lymphocytosis and granulocytosis compared to warfarin-treatment groups in male and female rats (Tables 4 and 5).

Following administration of *Feijoa sellowiana* extracts, RBC and Hb levels were significantly increased, specifically at 200 mg/kg

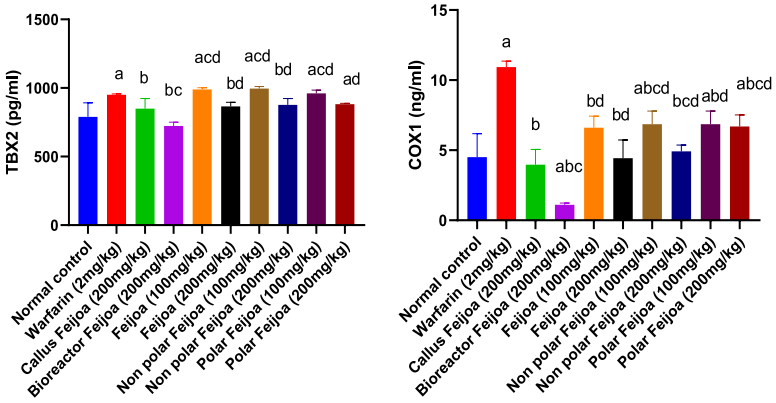

Fig. 4. Effect of *Feijoa sellowiana* on TXB 2 and COX 1 in male rats
Data are presented as the mean ± S.E. of (n = 8) for each group. Statistical analysis was carried out by one-way analysis of variance followed by Dunnett multiple comparisons test. [a] Statistically significant from control group. [b] Statistically significant from warfarin group at $P < 0.05$. [c] Statistically significant from callus group at $P < 0.05$.

calli (C-Fg) and bioreactor (BP2-Fg) extracts. It indicated that *F. sellowiana* plant extracts might contain some active chemical moiety, which can promote the erythropoietin secretion in stem cells. Erythropoietin is a hormone that stimulates the production of red blood cells in the bone marrow [48]. This increase in RBC count improves the oxygen carrying potential of blood; it could be due to the phytochemical constituents that are present in this plant extract. Previous study by Keles et al. [18] demonstrated that higher RBC counts and Hb levels may be connected to the extracts' inclusion of antioxidants such vitamin C, flavonoids, and tannins, and the maintenance of the oxidant/antioxidant balance as a result of the extracts' administration. These phytochemicals may be in charge of lowering lipid peroxidation, which eventually stops RBC hemolysis and increases turnover [18]. Platelet aggregation and the coagulation cascade are the first two phases of hemostasis. Therefore, both platelet and coagulation factors play roles in blood hemostasis. An increase in clotting and bleeding times suggests an inhibition of either platelet aggregation or blood coagulation pathways [49].

3.3.2.3. Effect of F. sellowiana on Plasma Coagulation Factors. Rats treated with warfarin (2 mg/kg) resulted in prolonged bleeding and clotting time by 50% and 126% respectively, as compared to the control group in male rats. Moreover, administration of the calli (C-Fg), bioreactor (BP2-Fg), crude (T-Fg), non-polar (N-Fg) and polar (P-Fg) extracts (200 mg/kg) significantly prolonged bleeding time by 56%,111%, 34%, 43% and 27.3% respectively, as compared to warfarin-treatment group in male rats. Administration of the calli (C-Fg), bioreactor (BP2-Fg), crude (T-Fg), non-polar (N-Fg) extracts (200 mg/kg) significantly prolonged clotting time by 14%, 48%, 19%, 14% respectively, as compared to warfarin-treatment group in male rats.

Furthermore, bioreactor extract (BP2-Fg) significantly prolonged the bleeding and the clotting time by 36% and 30% respectively, as compared to their calli group (C-Fg) in male rats. Administration of warfarin (2 mg/kg) caused prolonged PT and aPTT by 28.4% and 30.4% respectively, as compared to male control group. While, administration of calli (C-Fg), bioreactor (BP2-Fg), crude (T-Fg), non-polar (N-Fg) and polar (P-Fg) extracts (200 mg/kg) significantly prolonged PT by 26%, 40.4%, 19.2%, 21% and 21%, respectively, as compared to warfarin-treatment male group (Fig. 3). Moreover, administration of calli (C-Fg), bioreactor (BP2-Fg), crude (T-Fg) and non-polar (N-Fg) extracts (200 mg/kg) significantly prolonged aPTT by 30.4%, 51%, 27% and 25.3% respectively, as compared to warfarin-treatment male group. Furthermore, administration of bioreactor significantly prolonged PT and aPTT by 12% and 15.4% respectively, as compared to calli group.

Administration of Warfarin (2 mg/kg) significantly prolonged the bleeding and the clotting time by 73% and 118% respectively, as compared to their control groups in female rats. While, administration of the calli (C-Fg), bioreactor (BP2-Fg), crude extract (T-Fg) 200 mg/kg and non-polar (N-Fg) 200 mg/kg significantly prolonged bleeding and clotting time by 45%, 100%,28%,36,20% and 20%,49%,18% and 16% respectively, as compared to warfarin -treatment group in female rats. Furthermore, bioreactor significantly prolonged the bleeding and the clotting time by 33% and 24% respectively, as compared to their callus group in female rats. Moreover, administration of warfarin (2 mg/kg) resulted in prolonged PT and aPTT by 46% and 23% respectively, as compared to control group in female rats. While administration of the calli, bioreactor, crude and non-polar extracts (200 mg/kg) significantly prolonged PT and aPTT by 16%, 30%, 10%, 11%, 10.3% and 25%, 47%, 24%, 22% and 18% respectively, as compared to warfarin-treatment group in female rats (Fig. 5). Moreover, administration of bioreactor significantly prolonged PT and aPTT by 17% and 11% respectively, as compared to calli group.

Coagulation measures like PT and aPTT were utilized to identify the clotting process. The aPTT was used to assess the activity of factors of the intrinsic and common routes, whereas the PT was an efficient assay for assessing the activity of components of the extrinsic coagulation pathway [50]. In the present study, *F.* sellowiana extracts was resulted in a significant increase in bleeding and clotting times as well as prolongation in aPTT and PT assays, with a significant decrease in platelet count when compared to warfarin

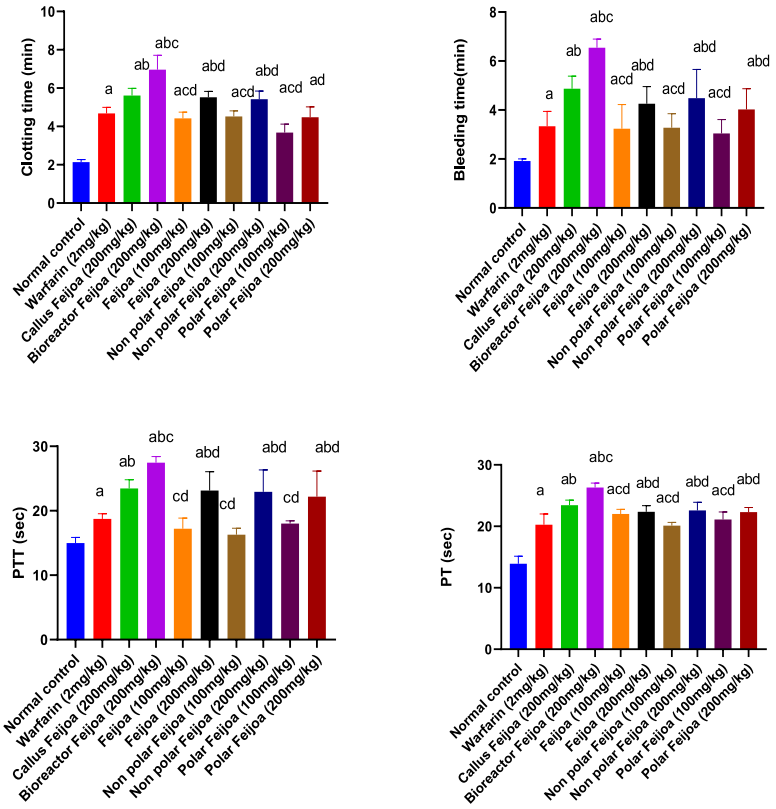

Fig. 5. Effect of *Feijoa sellowiana* on Plasma Coagulation Factors Measurement in female rats. Data are presented as the mean ± S.E. of (n = 8) for each group. Statistical analysis was carried out by one-way analysis of variance followed by Dunnett multiple comparisons test. [a] Statistically significant from control group. [b] Statistically significant from warfarin group at $P < 0.05$. [c] Statistically significant from callus group at $P < 0.05$. [d] Statistically significant from bioreactor group at $P < 0.05$.

group.

Phytochemical analysis of *F. sellowiana* extracts has revealed the presence of flavonoids and coumarins that could prevent platelet functions and protect against cardiovascular diseases. These results were in line with Aniq et al. [51], who showed that *F. Sellowiana* leaf compounds, showed a richness in flavonoids, quercetin, ellagic acid and galic acid compounds [51]. Flavonoids reduced primary haemostasis and various pathways linked to platelet activation and aggregation, according to numerous *in-vitro* and *in-vivo* investigations [52]. According to the above results, we can suggest that the effect of *F. Sellowiana* extracts on primary haemostasis could be linked to the inhibition of platelet aggregation.

3.3.2.4. Effect of F. sellowiana on TXB2 and COX1. Warfarin (2 mg/kg) administration increased TXB2 and COX1 formation in serum by 21% and 142%, respectively, when compared to the control group in male rats. On the other hand, administration of the calli, bioreactor, crude, non-polar and polar extracts (200 mg/kg) significantly inhibited TXB 2 and COX 1 concentrations by 11%, 24%, 9%, 8% and 64%, 90%, 60%, 55% and 39% respectively, as compared to warfarin-treatment group in male rats (Fig. 4). Moreover, administration of bioreactor significantly inhibited TXB 2 and COX 1 concentrations by 15% and 72% respectively, as compared to calli group.

Rats that were given warfarin (2 mg/kg) significantly increased TXB 2 and COX 1 by 22% and 125%, respectively, compared to control female groups. There were significant inhibition of TXB2 and COX1 in groups administered the calli, bioreactor, crude, non-polar and polar extracts (200 mg/kg) by 14.4%, 26%, 25%, 10%, 9% and 57%, 86%, 58%, 54%, 37%, respectively, when compared to warfarin –treatment group of female rats (Fig. 6). Moreover, administration of bioreactor significantly inhibited TXB 2 and COX 1 concentrations by 13% and 67%, respectively, as compared to calli group.

The development of a blood clot begins with platelet aggregation, which can progress to vascular occlusion and ultimately cause thromboembolic diseases like myocardial infarction or stroke [53]. Once attached to the vascular endothelium and activated, the platelets release chemokines and proinflammatory agents [54]. The COX-1 isoenzyme is engaged in the synthesis of prostaglandin that participates in platelet aggregation via the prostaglandin derivative, thromboxane B2 (TXB2). Inhibiting COX-1 prevents the formation of thromboxane B2, which prevents the aggregation of platelets [55]. In the present study, administration of *F. sellowiana* extracts

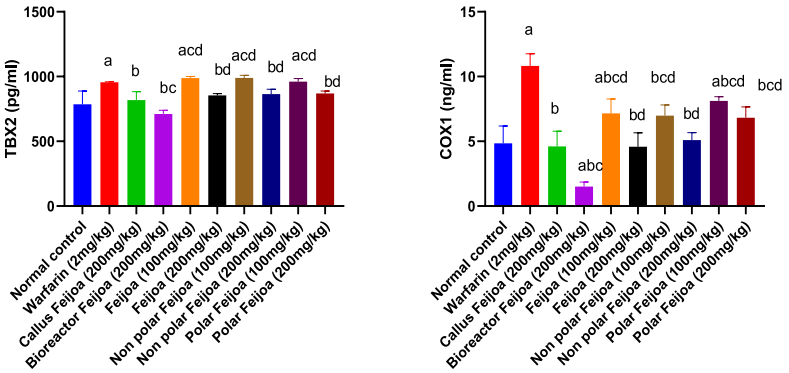

Fig. 6. Effect of *Feijoa sellowiana* on TXB 2 and COX 1 in female rats. Data are presented as the mean ± S.E. of (n = 8) for each group. Statistical analysis was carried out by one-way analysis of variance followed by Dunnett multiple comparisons test. [a] Statistically significant from control group. [b] Statistically significant from warfarin group at $P < 0.05$. [c] Statistically significant from callus group at $P < 0.05$.

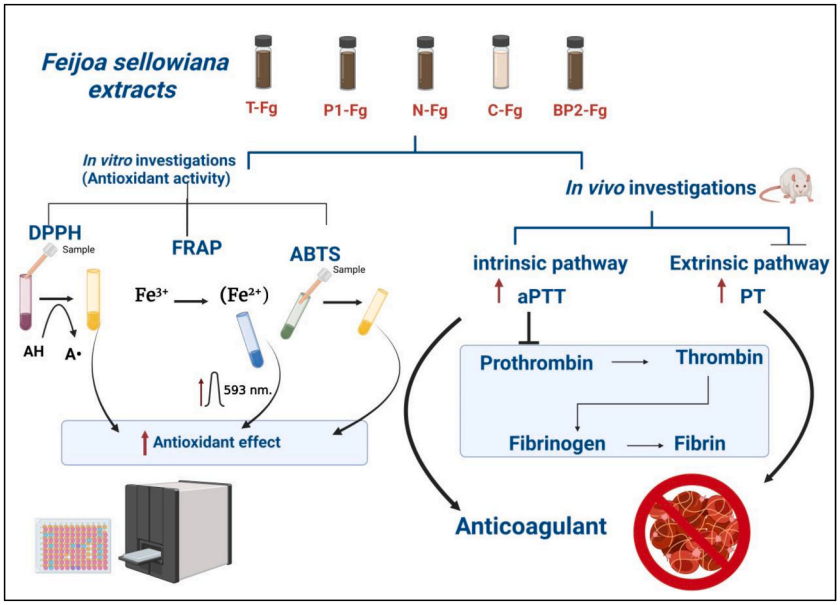

Fig. 7. Mechanism of action of *Feijoa sellowiana* extracts *through in vitro* assessment as antioxidant and *in vivo* investigation as anticoagulant. Created with BioRender.com (*Agreement number:* *BS24PE8WFC*).

significantly decreased COX-1 and TXB2. As mentioned above *F. sellowiana* extracts contain flavonoids and coumarins which were shown to interfere with platelet aggregation by altering the metabolism of arachidonic acid by inhibition of COX-1 and TXB2. Inflammation is a complex process characterized by the contribution of various mediators, including prostaglandins and nitric oxide. Cyclooxygenase is one of the main enzymes involved in the metabolism of arachidonic acid, catalysing the synthesis of prostaglandins and thromboxane. However, several investigations have demonstrated that inflammatory processes are aided by prostanoids produced by COX-1 [56].

The results of the present study demonstrate an anti-inflammatory effect for *F. sellowiana* extracts, possibly acting through COX 1 enzyme inhibition, and further inhibiting the generation of inflammatory mediators. Previous study by Mahmoudi et al., 2021 showed the potential therapeutic use of *F. sellowiana* extracts as a potent anti-inflammatory agent [48]. Additionally, a number of researches have discussed the anti-inflammatory properties and activities of flavonoids. Flavonoids are present in Feijoa, and it's probable that these substances give *Feijoa* its anti-inflammatory properties [57].

The mechanism of actions of *Feijoa sellowiana* extracts (T-Fg, P-Fg, N- Fg, C-Fg and BP2- Fg) as antioxidant (*in-vitro* study) and as anticoagulant *(in-vivo* study) were illustrated in Figure (7).

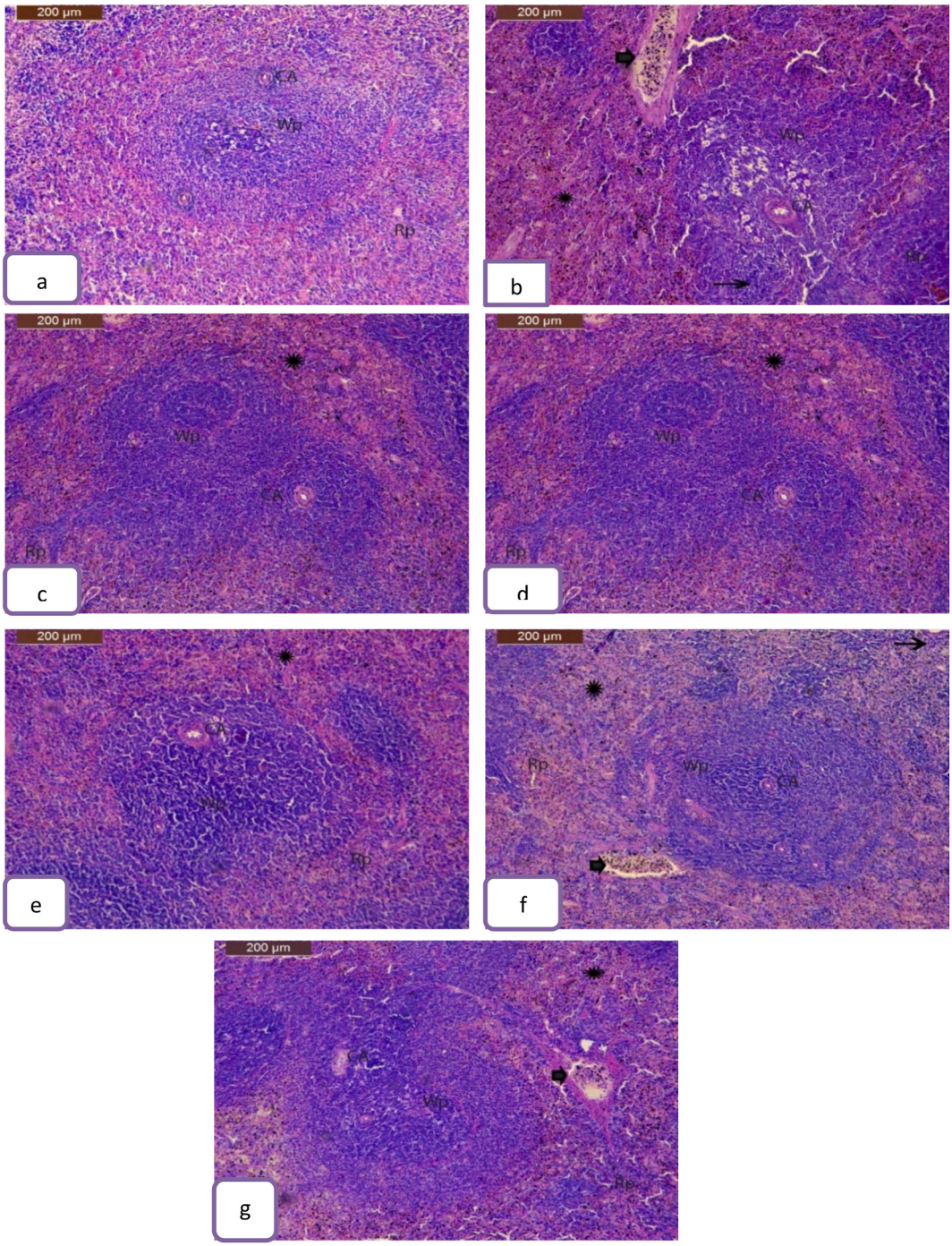

Fig. 8. A photomicrograph of (a) spleen of the control group, (b) spleen of the warfarin group, (c) spleen of the Callus *F. sellowiana* group (d) bioreactor *F. sellowiana* group, (e) *F. sellowiana* group, (f) Non polar *F. sellowiana* group, (g) polar *F. sellowiana* group (H&E X 200).

3.4. Histopathological results

Light microscopic examination of spleen sections stained with **(H&E X 200)** of control group consisted of white pulp **(Wp)**, with its central arteriole **(CA)** and the red pulp **(Rp)** (Fig. 8a).

Warfarin group showed an apparent reduction in the size of white pulp **(Wp)**, loss of distinction between the white and red pulps with dilated central arteriole **(CA)**. Also, red pulp of the spleen revealed loss of architecture **(Rp)** and that the majority of lymphocytes had dark irregular nuclei with heterogenous cytoplasm, and splenic haemorrhages. Also, deposition of hemosiderin pigment in the

splenic parenchyma **(star)**, markedly dilated and congestion blood vessels **(arrowhead)** were noticed (Fig. 8b). Calli *F. sellowiana* (C-Fg) group showed nearly normal splenic structure (white pulp with central arteriole, red pulp had slight degeneration, deposition of hemosiderin pigment in the splenic parenchyma **(star)** (Fig. 8c). In the group treated with Bioreactor *F. sellowiana* extract (BP2- Fg) showed almost nearly normal splenic structure (white pulp with central arteriole, red pulp had slight degeneration, deposition of hemosiderin pigment in the splenic parenchyma (Fig. 8d). In the group treated with total *F. sellowiana* extract (T-Fg) showed nearly normal splenic structure white pulp with central arteriole, red pulp had slight degeneration and deposition of hemosiderin pigment in the splenic parenchyma **(star).** (Fig. 8e). Splenic sections from non polar *F. sellowiana* extract (N- Fg) group showed mild reduction in the size of white pulp **(Wp)** and red pulps **(Rp)** with nearly central arteriole **(CA)**, mild necrosis**(arrow)**, deposition of hemosiderin pigment in the splenic parenchyma, dilated and congestion blood vessels **(arrowhead)** were noticed (Fig. 8f). Polar *F. sellowiana* extract (P-Fg) group showed moderate improvement, nearly normal splenic structure, white pulp with central arteriole, however, red pulp had slight degeneration, deposition of hemosiderin pigment in the splenic parenchyma and mild dilated and congestion blood vessels were noticed (Fig. 8g). From these results, we can concluded that the callus and bioreactor extract maintain the spleen in normal structure.

4. Conclusion

In conclusion, *Feijoa sellowiana* extracts significantly decreased platelet aggregation by suppression of thrombin (46%), extended aPTT (47%), PT (30%), bleeding (111%), and clotting times (48%) as compared to warfarine. These findings will be useful in understanding of the *F. sellowiana's* antithrombotic mechanism, besides; it had a significant antioxidant and anti-inflammatory properties through decreasing TXB 2 and COX 1 contents by 24% and 90%, respectively when compared to warfarine. *F. sellowiana* extracts had high total phenolic constituents especially calli extract which has 1.5 fold more than total ethanol extract. *Feijoa* is recommended for being used as anticoagulant (anticlotting) and the authors recommend starting the clinical trial on large scale.

Author contribution statement

Asmaa A. Amer: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Hussein S Taha: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Rania Elgohary: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Faten M Ibrahim: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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