Trop J Nat Prod Res, August 2025; 9(8): 4023 - 4032 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

In Vitro Antidiabetic, Anti-Inflammatory, Antioxidant, and Anticoagulatory Effects of *Costus afer* Ker Gawl. Leaf Fractions

Olajide O. Laide*, Kayode A.A Abolanle, Anyasor N. God'swill

Department of Biochemistry, School of Basic Medical Sciences, Benjamin S. Carson (Snr) College of Health and Medical Sciences, Babcock University, Ilishan-Remo Ogun State, Nigeria

ARTICLE INFO

Article history:
Received 23 December 2024
Revised 12 July 2025
Accepted 13 July 2025
Published online 01 September 2025

ABSTRACT

Diabetes mellitus especially type 2 is associated with pro-coagulatory, and oxidative stress states resulting in high levels of pro-inflammatory cytokines. Hyperglycemia increases blood viscosity which triggers coagulation leading to vascular dysfunction. This study explored the *in vitro* antidiabetic, anti-inflammatory, anticoagulation, and antioxidant effects of *Costus afer* Ker Gawl. leaf extract and fractions. Crude ethanol extract of *C. afer* leaves were partitioned using solvent-partitioning technique to obtain hexane, ethyl acetate, butanol, and aqueous fractions respectively, in the order of increasing polarity. The fractions were subjected to *in vitro* assessments for antidiabetic (alpha-amylase and alpha-glucosidase inhibition), anti-inflammatory (erythrocyte stabilization and protein denaturation inhibition), and antioxidant (2,2-diphenyl-1-picrylhydrazyl (DPPH), lipid peroxidation, reducing power activity and NO inhibition) assays. Clotting profiles on blood sample collected from non-diabetic and diabetic human volunteers were also assayed (clotting time, prothrombin time, and activated partial thromboplastin time). A statistical analysis was done using ANOVA which is then followed by Tukey's post-hoc test for comparison and Microsoft Excel 2016 for graphs. *C. afer* leaf fractions showed a significant increase in antidiabetic, anti-inflammatory, and antioxidant activities, and significant prolongation in clotting profiles of healthy and diabetic human volunteers. This study demonstrated that *C. afer* leaf fractions possess antidiabetic, anti-inflammatory, antioxidant, and anticoagulation activities. It is therefore recommended that further research could be done to explore the *in vivo* effects and the bioactive compounds responsible could be identified and isolated for therapeutic purposes

Keywords: Antidiabetic, Anti-inflammatory, Antioxidants, Anticoagulation, *Costus afer*.

Introduction

Diabetes mellitus is a cluster of metabolic disorders that is characterized by insulin resistance, hyperglycemia, and dysfunction of pancreatic β-cells. [1] Diabetic complication especially type-2 diabetes (T2DM) are more severe due to the pivotal role of oxidative stress, chronic inflammation, and coagulation abnormalities.[2] *Costus afer* Ker Gawl. has gained more attention due to its traditional use for various ailments including its therapeutic potential in managing diabetes mellitus. [3, 4] Phytochemicals aid the action of endogenous antioxidants like superoxide dismutase with catalase, prevent lipid peroxidation, and eliminate reactive oxygen species. These protect the β-cells from damage and maintain their ability to secrete insulin, resulting in low blood glucose levels and increased insulin sensitivity. [5,6] Chronic inflammations in type 2 diabetes results in resistance to insulin and endothelial dysfunction, [7] and an increase in fibrinogen levels, thus, decreasing fibrinolytic activity and resulting in coagulation abnormalities.[8] *Costus afer* contains phytochemicals responsible for its anti-inflammatory properties preventing inflammatory complications in diabetic patients. [9]

*Corresponding author. E mail: olajidel@babcock.edu.ng
Tel: +2347033684999

Citation: Olajide LO, Kayode AAA, Anyasor GN. *In Vitro* Antidiabetic, Anti-Inflammatory, Antioxidant, and Anticoagulatory Effects of *Costus afer* Ker Gawl. Leaf Fractions Trop J Nat Prod Res. 2025; 9(8): 4023 – 4032 https://doi.org/10.26538/tjnpr/v9i8.67

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Phytochemicals with the ability to inhibit thromboxane A2 synthesis and reduce platelet surface adhesion molecules were reported to prevent platelet aggregation, prolong clotting time, and also elevate fibrinolytic activity by raising tissue plasminogen activator (tPA) level and reducing plasminogen activator inhibitor-1 (PAI-1) therefore, preventing formation of thrombus, dissolution of formed clots, and prevent complications in T2DM patients.[10]

Materials and Methods

Plant Collection and Authentication

Costus afer Ker Gawl. leaves were procured from farms in Irolu, Ikenne Local Government Area of Ogun State, Southwestern Nigeria in January, 2024. The authentication of the plant sample was done at the Forest Herbarium Ibadan (FHI), Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria with authentication number FHI 113959.

Chemicals and Reagents

Reagents and chemicals utilized were of quality suitable for analysis. Solvents and chemicals were procured from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Diclofenac was sourced from Hovid Pharmaceuticals, Nigeria.

Preparation of Plant Samples

C. afer leaves were firstly cleaned with water, and then dried at 40°C in the oven, followed by pulverization using a warring blender and it was stored in the refrigerator at 4°C till use. One thousand grams (1000 g) of pulverized plant sample was macerated in 95% ethanol using a 1:8 (w/v) ratio and shaken occasionally at room temperature for 48 hours, the suspension was sieved with a fine Muslin cloth, and then a No.1 Whatman filter paper. The crude extract was concentrated using rotatory evaporator at 40°C and dried using hot air oven at the same temperature. Fractionation of the crude extract was done using n-hexane, ethyl acetate, and n-butanol in successive order as the polarity increases. All fractions were stored in the refrigerator at 4°C.

Study Design and Location
The study was done at the Department of Biochemistry, Babcock University, Ilishan-Remo, Ogun State, Nigeria.

Antidiabetic in vitro assays
Inhibition of α-amylase Enzyme
Fractions at varying concentrations (100-500 µg/mL) were combined with 0.5 mg/mL of α-amylase, starch solution at 1% and 100 µL of phosphate buffer 0.2 mM having 6.9 pH were then incorporated. The enzymatic reaction was conducted for 5 minutes at 37°C and subsequently stopped by the introduction of 3, 5-dinitrosalicylic acid (2 mL). The resulting mixture was subjected to heating at 100°C for 15 minutes, followed by dilution with 10 mL distilled water in an ice bath. α-amylase activity was assessed by measuring the color intensity at a wavelength of 540 nm using spectrophotometer.[11,12] The identical procedure was executed for the standard.

$$\% \text{ Inhibitory Activity} = \frac{Ac - As}{Ac} \times 100$$

Ac indicates the absorbance of the control and As indicates absorbance of the sample

Inhibition of α-glucosidases Enzyme
Evaluation of alpha-glucosidases was conducted utilizing the methodology proposed by Vennila & Pavithra.[13] The inhibitory potential was evaluated by incubating 1 mL of a 2% (w/v) starch solution with 0.2 M Tris buffer (pH 8.0) and different concentrations (100 - 500 mg/mL) of the test fractions. The reaction mixture was maintained at 37 °C for 10 minutes. Initiation of the reaction was achieved through the addition of 1 mL of the α-glucosidase enzyme at 1 U/mL, followed by incubation for 40 minutes at 35°C. Subsequently, the reaction was halted by the incorporation of 2 mL 6 N HCl. The colour intensity of the resultant mixture was quantified at a wavelength of 540 nm using a spectrophotometer.
The results were expressed as percentage inhibition, calculated using the appropriate formula:

$$\% \text{ Inhibitory Activity} = \frac{Ac - As}{Ac} \times 100$$

Ac indicates the absorbance of the control and As indicates the absorbance of the sample.

In-vitro anti-inflammatory assays
Erythrocyte Membrane Stabilization Assay
Preparation of Red Blood Cell (RBC) Suspension
Human blood serum was retrieved into tubes containing an anticoagulant and was centrifuged for 10 minutes at 3000 rpm. The resulting supernatant was meticulously decanted, and the packed erythrocytes were scrubbed up to three times with freshly prepared normal saline. The washed red blood cells were then diluted to a 10% v/v concentration using 0.1 M phosphate-buffered saline (PBS) at pH 7.4 to obtain the erythrocyte suspension.[14]

Heat-Induced Hemolysis
Hundred microliter (100 µL) of 10% red blood cell suspension was introduced into 100 µL of each respective fraction. The resultant mixture underwent heating for 30 minutes at 56°C, then centrifugation followed at 2500 rpm for 10 minutes at ambient temperature. The supernatant was harvested, and the absorbance measured at a wavelength of 560 nm. Diclofenac sodium served as the positive control in this experiment. The percentage of membrane stabilization was determined following the method established by [14] utilizing the formula:

$$\% \text{ Inhibition} = 100 - \frac{A1 - A2}{A0} \times 100$$

A1 indicates absorbance of the sample, A2 indicates absorbance of the control and A0 represents absorbance of the positive control.

Protein Denaturation Inhibition
The protein denaturation inhibition was assessed utilizing the method established by.[14]An aliquot of 500 µL of bovine serum 1% albumin was introduced into 100 µL of the plant fraction. The resultant mixture was maintained at ambient temperature for a duration of 10 minutes, subsequently subjected to heating for 20 minutes at 51°C. The resulting solution was left to cool to room temperature, after which optical density measurements were recorded at a wavelength of 660 nm. Diclofenac sodium acted as positive control. This experiment was done in triplicates and protein denaturation percentage inhibition for was calculated using:

$$\% \text{ Inhibition} = 100 - \frac{A1 - A2}{A0} \times 100$$

A1 indicates the optical density of the sample, A2 indicates the absorbance of the product control and A0 represents the optical density of the positive control

In-vitro Antioxidant Activity of the Fractions
The antioxidant activity of the fractions was assessed through the evaluation of the 2,2-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging efficacy, the inhibition of lipid peroxidation through the TBARS assay, the reducing power activity, and the scavenging activity against nitric oxide radicals.

DPPH Radical Scavenging Activity
The DPPH radical scavenging efficacy of the fractions was analyzed using the method delineated by McCune and Johns.[15] A DPPH solution (0.3 mM) was formulated by dissolving 11 mg of DPPH in 100 mL of methanol. The reaction mixture (3.0 mM) comprised 1.0 mL of DPPH in methanol, 1.0 mL of *C. afer* leaf fractions (100, 200, 300, 400, and 500 µg/mL), along with 1.0 mL of methanol. This mixture was subsequently incubated in the dark for 10 minutes, after which measurement of the absorbance at a wavelength of 517 nm was done. The percentage antioxidant potential was calculated using:

$$\% \text{ Antioxidant Potential} = \frac{\text{OD Control} - \text{OD Sample}}{\text{OD Control}} \times 100$$

Where OD control indicates the optical density of the control and OD sample indicate the optical density of the sample.
The IC_{50} values of the samples were calculated, which is the concentration of the fractions needed to scavenge just 50% DPPH radicals.

Inhibition of the lipid peroxidation (LPO) assay
The inhibition of LPO was experimented by the use of egg yolk homogenate as lipid rich media as previously reported.[16] A stock solution was prepared for standard Gallic acid, and test fractions (hexane, ethyl acetate, butanol and aqueous). Egg yolk homogenate at 0.5 mL of 10% v/v was added to varying volumes of 10, 20, 50, and 100 µL of standards and test sample fractions, the volume made up to 1 mL distilled water, 0.05 mL of $FeSO_4$ was added thereafter. The reaction mixture was maintained under incubation conditions for 30 minutes at a temperature of 37°C, after which 1.5 mL of acetic acid was introduced into the reaction mixture, followed by the addition of 0.67% solution of thiobarbituric acid (TBA) at 1.5 mL in a 20% sodium dodecyl sulfate (SDS) solution. The resulting solutions were subjected to thorough mixing using a vortex mixer and subsequently heated to a temperature of 95°C for a period of 60 minutes. Upon cooling, 5 mL of butan-1-ol was incorporated into the mixture, which was then centrifuged at a rotational speed of 3000 rpm for 10 minutes. The absorbance of the organic upper phase was quantified at a wavelength of 532 nm and subsequently converted to percentage inhibition employing the appropriate formula:

Inhibition of lipid peroxidation (%) = $1 - \frac{E}{C} \times 100$

C indicates absorbance of the totally oxidized control and E indicates absorbance taken in the presence of test fraction or the standard.

Reducing Power Assay

The investigation of the reducing power activity was conducted using the method established by [17] albeit with certain modifications. A volume of 0.05 mL fraction in various concentrations was introduced into 0.2 mL of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide solution at 0.2 mL. The resultant mixture was subjected to incubation in a water bath for a duration of 20 minutes at a temperature of 50°C. Subsequently, 0.25 mL of trichloroacetic acid was incorporated into the mixture, which was then centrifuged at 1000 rpm for 10 minutes at ambient temperature. The supernatant (0.5 mL) was combined with 0.5 mL of deionized water and 0.1 mL of a 0.1% $FeCl_3$ solution. The absorbance was measured at a wavelength of 700 nm. The reducing power assay was expressed as mg GAE/L.

Nitric Oxide Radical (NO) Scavenging Assay

Nitric oxide from sodium nitroprusside was generated in an aqueous solution at the physiological pH, which interacts with oxygen to produce nitrite ions, and was measured using the Griess reagent. [18] 3.0 mL of 10 mM sodium nitroprusside in phosphate buffer was added to 2.0 mL of the fractions and reference compound (ascorbic acid) at different concentrations (20 - 100 g/mL). The resulting solutions were subjected to incubation at 25°C for a duration of 60 minutes. An analogous procedure was conducted utilizing methanol as a blank, which functioned as the control. To 5.0 mL of the incubated sample, an equivalent volume of Griess reagent (comprising 1% sulphanilamide, 0.1% naphthyl ethylene diamine dihydrochloride dissolved in 2% H3PO3) was incorporated, and the absorbance of the chromophore (purple azo dye) produced during the diazotization of nitrite ions with sulphanilamide, followed by the subsequent coupling with naphthyl ethylene-diamine dihydrochloride, was quantified at a wavelength of 540 nm. The inhibition percentage (%) of the generated nitrite oxide was determined by contrasting the absorbance values of the control and test preparations.

Blood coagulation profiles on human blood

Blood samples (5 mL) were collected through the help of a trained phlebotomist from 10 healthy adults and 10 newly diagnosed diabetic patients who volunteered (40 years and above) at 'Babcock University Teaching Hospital, (BUTH) Ilishan-Remo, Ogun State, Nigeria'. The volunteers received sufficient information about the study, and their participation was confirmed through a signed consent form. Questionnaires were used to gather biodata and brief medical histories prior to the collection of blood samples. The blood samples collected were subjected to the following assays: Blood clotting time in apparently healthy volunteers according to Wintrobe, [19] Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were measured in both apparently healthy and diabetic patient volunteers using the method described by Brown,[20] as previously reported. [21]

Statistical Analysis

Statistical analysis was done by the use of one-way analysis of variance (ANOVA), Tukey's tests followed for post-hoc test comparison. Both GraphPad Prism® 8.0 and Microsoft excel 2016 were used in graphs and charts construction. A p-value less than 0.05 was statistically considered significant and data were expressed as mean ± SEM in triplicate.

Results and Discussion

In vitro Anti-diabetic Assays

Inhibition of alpha-amylase and alpha-glucosidase by C. afer leaf fractions

Figure 1 shows the percentage inhibition of α-amylase and α-glucosidase by *C. afer* leaf fractions. Ethyl acetate fraction exhibited the strongest inhibitory activity of α-amylase at 16.20 µg/mL when compared to other fractions, followed by hexane fraction (22.72 µg/mL), butanol fraction (26.95 µg/mL) and aqueous fraction (48.71 µg/mL). This pattern was in line with the standard, acarbose having the IC_{50} of 2.59 µg/mL which had the highest α-amylase inhibition activity when compared to the fractions of *C. afer* leaves.

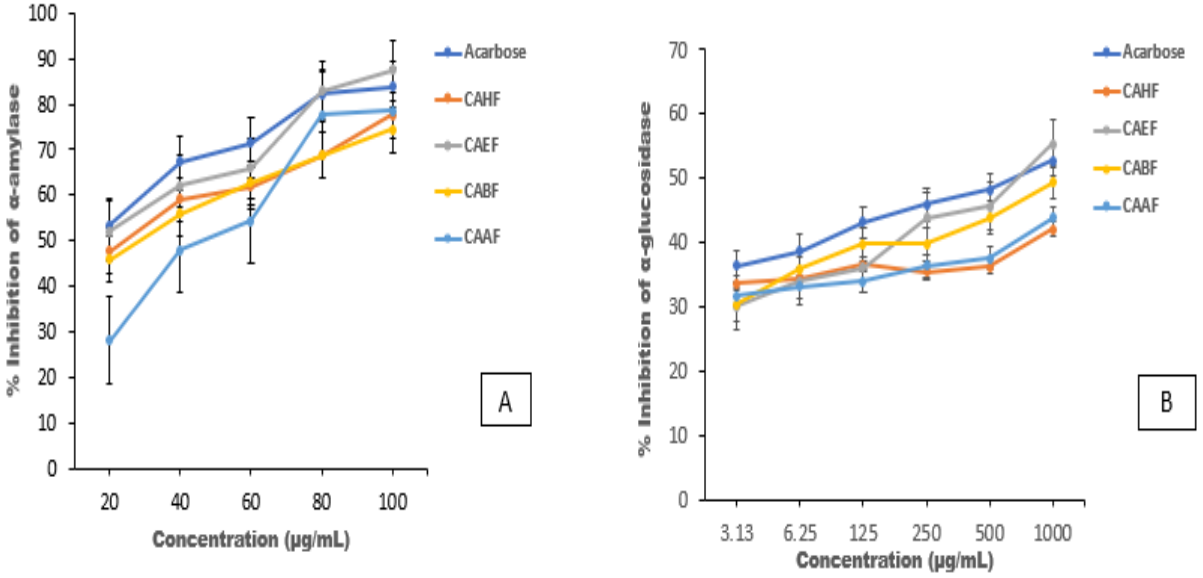


Figure 1: Percentage alpha-amylase and alpha-glucosidase inhibition by *C. afer* leaf fractions

Acarbose- standard,
CAEF- *C. afer* ethyl acetate fraction,
CAHF- *C. afer* hexane fraction,
CABF- *C. afer* butanol fraction,
CAAF- *C. afer* aqueous fraction.

In addition, inhibition of α-glucosidase as expressed in Figure 1B showed that ethyl acetate fraction exhibited the highest enzyme inhibition with the IC_{50} of 0.7154 mg/mL when compared to the other fractions, followed by butanol fraction (1.0157 mg/mL), aqueous

fraction (1.5339 mg/mL), and hexane fraction (2.1252 mg/mL) in a concentration-dependent manner. Orally ingested carbohydrates are metabolized by α-amylase and α-glucosidase to ease absorption; inhibition of these enzyme activities can control the postprandial glucose concentrations and thus minimizing the risk of hyperglycemia. [22] Acarbose has a notable effect on inhibiting the α-glucosidase enzyme, which effectively controls blood sugar levels. [23] This inhibition is considered an effective treatment strategy for carbohydrate metabolic disorders such as diabetes mellitus type II. [24] This study assesses the *in vitro* inhibitory effects of various solvent fractions of *C. afer* leaves on α-amylase and α-glucosidase in a concentration-dependent manner indicating that the ethyl acetate fraction possesses the most significant inhibitory activity, which suggests that the bioactive compounds responsible for this inhibition had more solubility in ethyl acetate compared to other solvents. This inhibitory capacity observed is similar to a previous study indicating that fractions rich in polyphenols and flavonoids demonstrate substantial α-amylase inhibition due to their interaction with the enzyme's active site, [25] which could be a strategy for managing type 2 diabetes mellitus. [26] Studies have indicated that polyphenolic compounds, particularly flavonoids and tannins, contribute to α-glucosidase inhibition by binding to the enzyme's active site and altering its catalytic function. [25, 27] The observed activity aligns with the previous findings that plant extracts with high phenolic content tend to show stronger inhibition against carbohydrate-hydrolyzing enzymes. [28, 29] Therefore, α-amylase and α-glucosidase inhibition by the plant extract could be an essential pharmacological importance in diabetic management. [30]

In-vitro Anti-inflammatory Activity of C. afer Leaf Fractions

The *In-vitro* anti-inflammatory effect of *C. afer* leaf fractions was observed using stabilization of erythrocyte membrane heat-induced hemolysis and protein denaturation inhibition. Each fraction of *C. afer* leaves exhibited erythrocyte membrane stabilization potential in dose-dependent manner (Figure 2A), with aqueous fraction having the highest stabilization at IC_{50} of 19.53 µg/mL, followed by ethyl acetate (40.82 µg/mL), hexane (49.62 µg/mL), and butanol (169.10 µg/mL) fractions. This is similar to the effect of diclofenac sodium (standard drug) but the effect of aqueous fraction is more significantly higher when compared to diclofenac sodium (33.29 µg/mL). However other fractions exhibited significant reduction in erythrocyte membrane stabilization potential when compared to diclofenac sodium.

Lysosomal membrane is structurally similar to erythrocyte membrane and this is a key indicator in anti-inflammation, the ability of the various fractions to stabilize red blood cell membrane could be inferred from lysosomal membrane stabilization which generally explains the *in vitro* anti-inflammatory mechanism of action. [27] During inflammation, lysosomal membrane lysis occurs releasing its enzyme component leading to production of diverse disorders. Finding from this study is similar to the previous reports on anti-inflammatory activity of *S. khasianum* root extract, [31] and *C. afer* leaf ethanol extract. [9] Ability of *C. afer* leaf fractions and the standard (diclofenac sodium) to stabilize the membrane indicate the therapeutic potential to maintain membrane integrity and prevent leakage of the cell components during heat exposure.

The *in vitro* inhibitory activity of *C. afer* leaf fractions on protein denaturation are shown in Figure 2B, each fraction exhibited comparable effect with diclofenac sodium (157.49 µg/mL) which has the highest inhibition, followed by ethyl acetate fraction that showed the highest inhibition at the IC_{50} of 200.66 µg/mL, followed by hexane (350.07 µg/mL), aqueous (355.42 µg/mL), and butanol (395.845 µg/mL) fractions in a concentration-dependent manner.

Protein denaturation is another mechanism used as a marker of inflammation where the secondary and tertiary protein structure are disrupted through the denaturation process and retaining only the primary structure, the covalent bonds are disrupted and amino acid interactions are broken resulting in loss of protein's biological functions. [32] Membrane proteins are essential for maintaining the integrity of cell membranes and play a key role in the regulation of the influx and efflux of sodium and potassium ions thus regulating the volume and water content of the cell which maintains homeostasis. [33] Reports have shown that protein denaturation causes autoantigens during inflammation and these antigens are associated with hypersensitive reactions implicated in various diseases such as diabetes, rheumatic arthritis, and cancer which are inflammatory conditions. [27, 34]

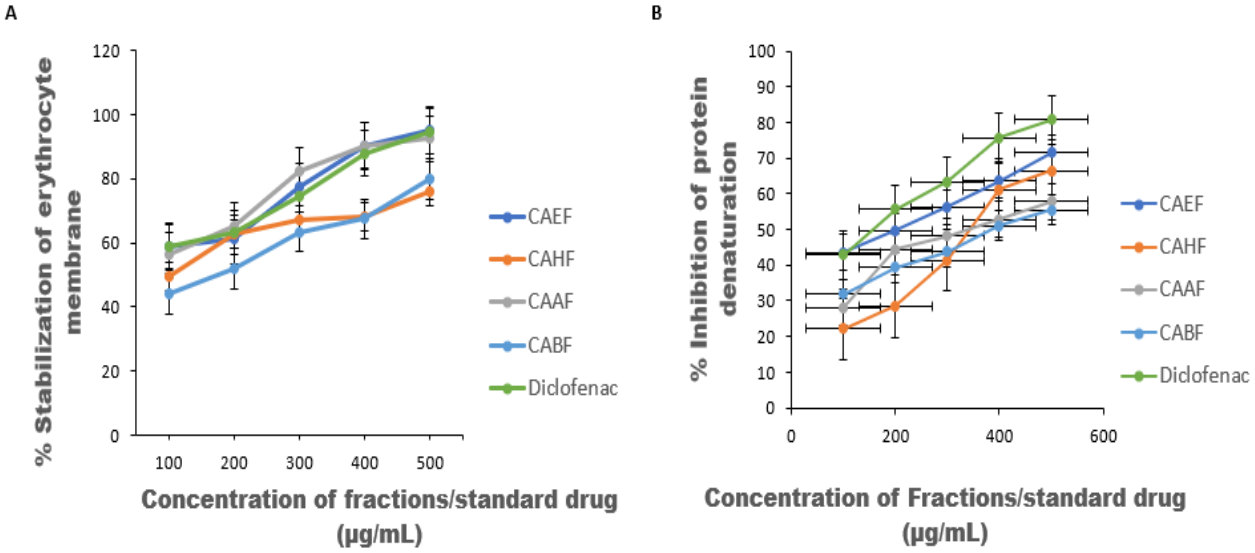


Figure 2: Percentage stabilization of erythrocyte membrane (A) and inhibition of protein denaturation (B) by diclofenac sodium (standard) and fractions of *C. afer* leaf (100-500µg/mL).

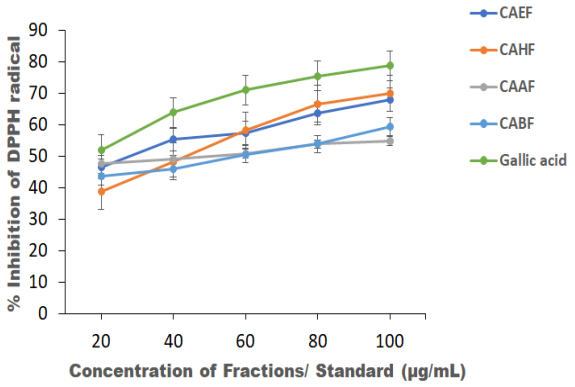
CAEF- *C. afer* leaf ethyl acetate fraction
CAHF- *C. afer* leaf hexane fraction
CAAF- *C. afer* leaf aqueous fraction
CABF- *C. afer* leaf butanol fraction

Therefore, medicinal plants with the ability to prevent protein denaturation are usually identified as possessing anti-inflammatory potential. This study showed that various fractions of *C. afer* leaf possess protein denaturation inhibitory effect with the ethyl acetate fraction possessing the highest inhibitory effect on heat-induced bovine serum albumin denaturation compared with other fractions which is also similar to the high denaturation inhibitory effect that is seen in diclofenac sodium as shown in Figure 2B. This result is similar to the

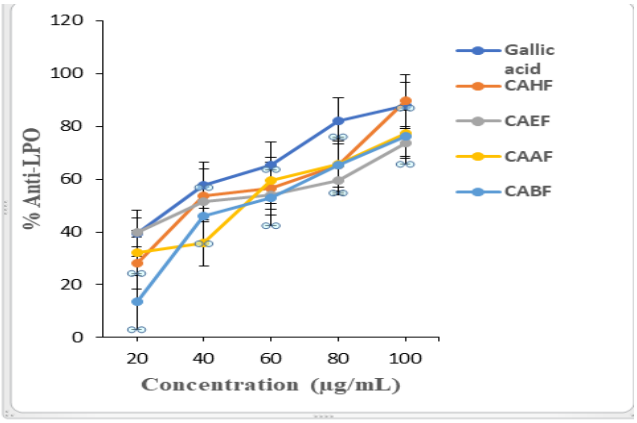
previous report [9] that *C. afer* leaf extract prevents protein denaturation. Therefore, ethyl acetate fraction could be regarded as a promising anti-inflammatory agent capable of inhibiting denaturation of protein and thus maintaining membrane protein integrity.

In-vitro Antioxidant Activity of C. afer Leaf Fractions

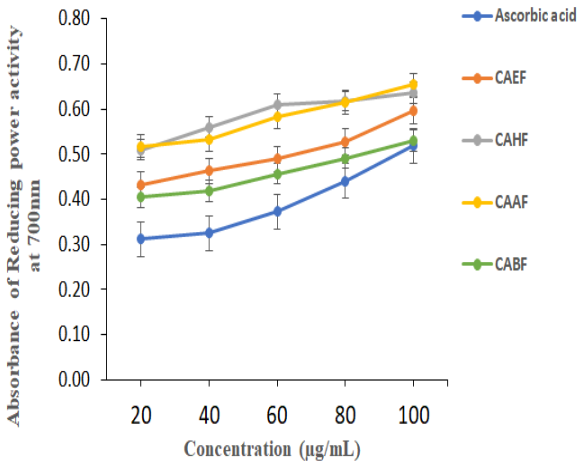
The *in vitro* antioxidant activity of different fractions of *C. afer* leaves are shown in Figure 3. The *C. afer* leaf fractions produced dose-dependent DPPH radical scavenging activity with ethyl acetate fraction exhibiting the highest at IC_{50} 28.22 µg/mL followed by hexane (IC_{50} = 44.14 µg/mL), and aqueous fractions (46.60 µg/mL), while butanol fraction (56.35 µg/mL) exhibited the lowest activity. Gallic acid (standard) showed similar result pattern with IC_{50} of 3.90 µg/mL which had the highest percentage inhibition of DPPH radicals, however, there was significant ($p<0.05$) difference in the DPPH scavenging activities of fractions of *C. afer* leaves and gallic acid.



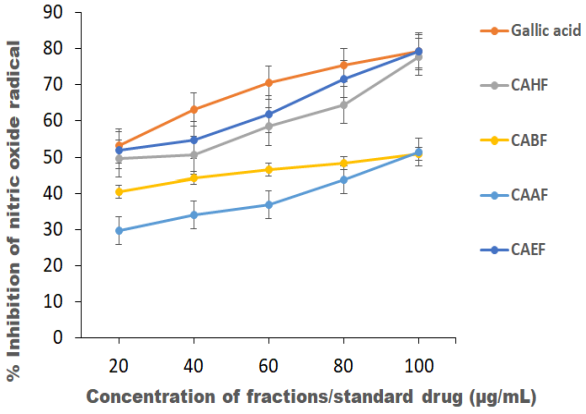
A. DPPH radical scavenging activity of gallic acid (standard) and fractions of *C. afer* leaf



B. Anti-lipid Peroxidation (LPO) Activity of gallic acid (standard) and fractions of *C. afer* leaf



C. Reducing power activity of ascorbic acid (standard) and fractions of *C. afer* leaf



D. Nitric oxide (NO) radical scavenging activity of gallic acid (standard) and fractions of *C. afer* leaf

Figure 3: Antioxidant activity of *C. afer* leaf

Control (PBS) - Phosphate buffer saline, CAAF - *C. afer* aqueous fraction
CAHF - *C. afer* hexane fraction CAEF - *C. afer* ethyl acetate fraction,
CABF - *C. afer* butanol fraction, CAEE - *C. afer* ethanol extract

The results indicated that all *C. afer* fractions exhibited antioxidant activity, ethyl acetate fraction showed the highest DPPH radical scavenging activity. The ethyl acetate fraction demonstrated the strongest lipid peroxidation inhibition at the IC_{50} of 44.98 µg/mL, followed by hexane (47.03 µg/mL), aqueous (IC_{50} = 53.25 µg/mL), and butanol (IC_{50} = 58.76 µg/mL) fractions, all in a concentration-dependent effect as shown in Figure 3B. However, the standard compound, gallic acid exhibited significantly, the greatest inhibitory activity with an IC_{50} of 32.83 µg/mL when compared with fractions of *C. afer* leaves.

Oxidative stress, marked by excess production of reactive oxygen species (ROS) plays a critical role in the aetiology of many chronic diseases, including diabetes, cardiovascular disorders, and neurodegenerative conditions.[35] The ability of plant extracts to neutralize free radicals, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), is widely indicator of their antioxidant potential.[36] These results suggest that the ethyl acetate fraction may contain the highest concentration of bioactive antioxidant compounds including polyphenols, flavonoids, and phenolic acids which have been reported as major contributors to antioxidant activity due to their ability to donate hydrogen atoms or electrons, and neutralize free radicals.[37] Gallic acid, a well-known potent antioxidant, exhibited an IC_{50} significantly lower than any of the *C. afer* fractions. This indicated that while *C. afer* fractions demonstrate

notable antioxidant activity, they are considerably less effective than pure gallic acid.

Lipid peroxidation is a key process in oxidative damage contributing to the pathogenesis of several chronic diseases, such as diabetes, neurodegenerative disorders, and cardiovascular conditions. [38] The ability of antioxidants to prevent lipid peroxidation is important for cellular protection and prevention of diseases. Ethyl acetate fraction demonstrated the most effective inhibition of lipid peroxidation suggesting the presence of bioactive compounds such as flavonoids, polyphenols, and alkaloids [36] responsible for inhibiting lipid peroxidation. These compounds are well reported and documented owing to their antioxidant properties and their ability to neutralize lipid peroxyl radicals, thus preventing oxidative damage'.[39]

Although, gallic acid, exhibited higher inhibition of lipid peroxidation when compared to all *C. afer* fractions which indicated that, while *C. afer* fractions possess antioxidant potential, their potency is lower than that of gallic acid. However, plant extracts often contain multiple bioactive compounds that may act synergistically, providing broader health benefits than a single isolated compound.[40] These findings align with previous research indicating that flavonoids and polyphenols play a vital role in protecting cells against lipid peroxidation [41] and also in another report [42] revealing that methanol extract of *C. afer* possess inhibition of lipid-peroxidation and thus preventing damage to biomolecules. Therefore, the ability of *C. afer* leaf fractions to inhibit lipid peroxidation supports their potential use as natural antioxidants inhibiting oxidative damage-related diseases.

The reducing power assay is a commonly used method for evaluating the electron-donating capacity of bioactive compounds, playing a crucial role in neutralizing free radicals and inhibiting oxidative stress-related damage. The ability of antioxidants to donate electrons correlates with their potential to reduce oxidative stress in biological systems. In this study, Figure 3C shows the reducing power activity of *C. afer* leaf fractions expressed as ascorbic acid equivalent per milligram, aqueous fraction (0.654 ±0.0024 µg/mL, 2745.37 AAE/mg) had the highest reducing power at the highest concentration of 100 µg/mL, followed by hexane fraction (0.636 ±0.0042 µg/mL, 1484.04 AAE/mg), ethyl acetate (0.596 ±0.0022 µg/mL, 2115.74 AAE/mg), and butanol fraction (0.530 ± 0.0061 µg/mL, 1708.33 AAE/mg).

As presented in Figure 3C, the reducing power activity of *C. afer* leaf fractions varied across different solvent extracts, with the aqueous fraction exhibiting the highest reducing power at the highest concentration (100 µg/mL). The observed variation in reducing power among the fractions is likely due to differences in the solubility and concentration of antioxidant compounds in each solvent extract. The aqueous fraction exhibited the highest reducing power, suggesting a high presence of hydrophilic antioxidants, such as phenol and some flavonoids, that contribute to electron donation and free radical neutralization. [43] Ascorbic acids, a well-known standard antioxidant, is used as a reference in reducing power assays due to its strong electron-donating ability.

The findings suggest that *C. afer* leaf fractions possess significant reducing power, with the aqueous fraction exhibiting the highest activity. The ability of these extracts to donate electrons highlights their potential as natural antioxidants, which could be beneficial in combating oxidative stress-related diseases.

Different fractions of *C. afer* leaves exhibited nitric oxide radical scavenging activity with ethyl acetate showing the highest scavenging activity at IC_{50} of 21.21 µg/mL, followed by hexane (IC_{50} = 55.63 µg/mL), butanol (91.18 µg/mL), and aqueous (175.78 µg/mL) fractions, however, the standard (gallic acid) showed significantly higher scavenging activity at IC_{50} = 3.37 µg/mL compared to the *C. afer* leaf fractions in a concentration dependent manner as expressed in Figure 3D. Nitric oxide (NO), a biologically important signaling molecule is involved in various physiological and pathological processes, including inflammation, vasodilation, and immune response. [44] However, high level of nitric oxide production leads to oxidative stress and cellular damage, contributing to various disease conditions. On the other hand, antioxidants are capable of scavenging nitric oxide radicals and help to mitigate these harmful effects, making them valuable in therapeutic applications. [45] As presented in Figure 3D, different solvent fractions of *C. afer* leaf exhibited nitric oxide radical scavenging activity in a concentration-dependent manner. The ethyl acetate fraction had the highest inhibition, suggesting that it contains high concentration of bioactive compounds capable of neutralizing nitric oxide radicals. Ethyl acetate is known to extract polyphenols, flavonoids, and tannins, which have been reported for their strong nitric oxide scavenging abilities. [46] These findings are consistent with previous studies highlighting the role of flavonoids and phenolics in nitric oxide scavenging activity. [47] While all fractions demonstrated antioxidant properties, their effectiveness was significantly lower than that of gallic acid. These findings support the potential of *C. afer* as a natural source of nitric oxide scavengers, which may be beneficial for developing antioxidant-based therapeutics.

Effects of C. afer leaf extract and fractions on the clotting time

Figure 4 presents the blood clotting time of healthy volunteers in the presence of *C. afer* leaf extract and its fractions. All tested fractions significantly ($p < 0.001$) prolonged clotting time at various concentrations compared to both the control (whole blood with phosphate-buffered saline) and normal whole blood alone (WBA). Aqueous fraction exhibited the longest clotting time of 11.86 mins when compared to other fractions, followed by hexane fraction at 11.79 mins, butanol at 9.80 mins and ethyl acetate at 9.09 mins at the lowest concentration 5 mg/kg *b.w.* Crude Extract also showed prolongation at 9.91 mins comparable to the controls PBS at 6.52 mins and WBA at 4.95 mins.

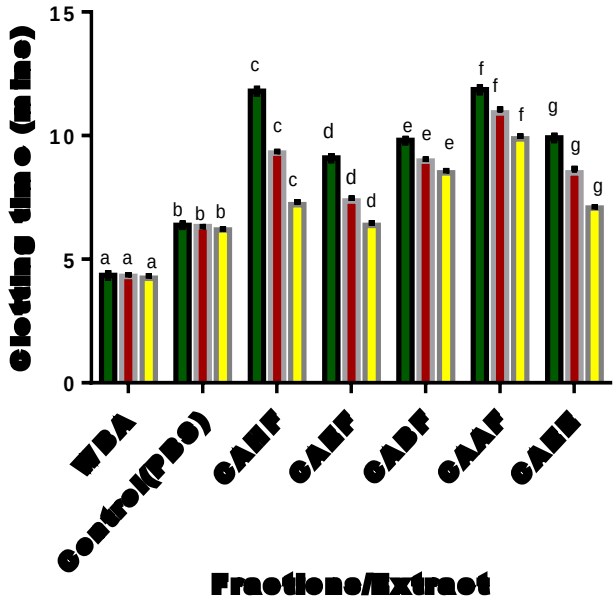


Figure 4: *In vitro* blood clotting time of apparently healthy human volunteers treated with fractions/extract of *C. afer* at 5, 10 and 20 mg/mL

Bars labeled with different letters indicate statistically significant ($p<0.0001$) difference (n=10).
WBA - Whole blood alone
Control (PBS) - Phosphate buffer saline, CAAF - *C. afer* aqueous fraction
CAHF - *C. afer* hexane fraction CAEF - *C. afer* ethyl acetate fraction,
CABF - *C. afer* butanol fraction, CAEE - *C. afer* ethanol extract

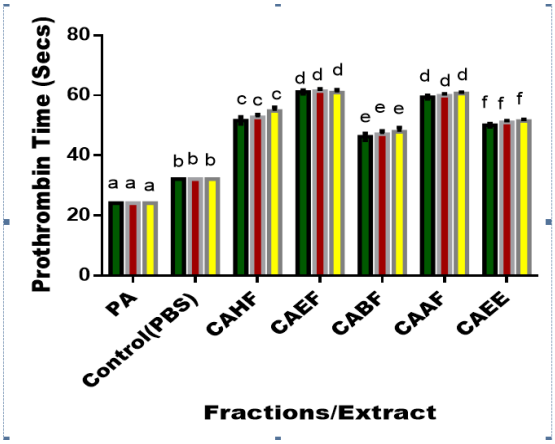
These results showed significant prolongation of clotting time in all fractions and extract at different concentrations as presented in Figure 4, suggesting its crucial role in interfering with the clotting process.

This report is in line with that of [47] where the extract of *C. afer* leaves was reported to cause a dose-dependent increase in blood clotting time. This is also consistent with that of [4] who reported that *C. afer* stem fractions, especially ethyl acetate fraction possess an antithrombotic effect higher than aspirin and other fractions of the extract. These finding is an indication that fractions of *C. afer* leaves play a crucial role in the clotting process, potentially interfering with specific

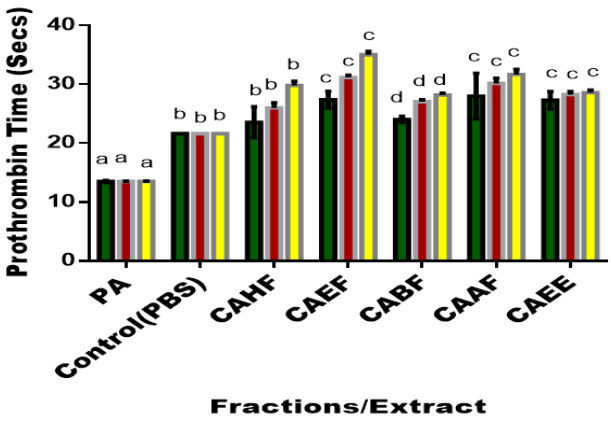
components of the coagulation cascade which may be attributed to the presence of various bioactive compounds in the fractions. Phytochemicals like flavonoids, tannins, and alkaloids have been discovered to possess anticoagulant properties. [48] These compounds can impact platelet function, disrupt clot formation factors, or interfere with fibrin formation, leading to a prolonged clotting time. [49] Furthermore, it is important to mention that the concentrations of the extract and fractions affected the clotting time. The observed dose-response relationship is frequently seen in pharmacological studies, indicating that the effects observed can be attributed to the specific extract fractions under investigation.

Effect of C. afer leaf extract and fractions on the Prothrombin Time (PT) and activated partial thromboplastin time (aPTT)

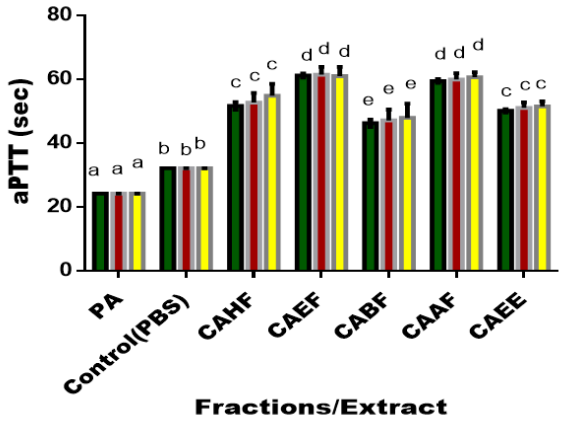
The *in vitro* results of the prothrombin time of the blood samples of apparently healthy volunteers treated with *C. afer* leaf are shown in Figure 5A. All extract/fractions at different concentrations showed significantly ($p<0.0001$) longer prothrombin time when compared to control. Ethyl acetate had the longest prothrombin time at 60.68 secs followed by hexane fraction at 58.29 secs, n-butanol 47.55 secs, while aqueous extract exhibited the shortest PT at 42.95 among the fractions, however, all fractions exhibited significant ($p<0.0001$) increase when compared to controls, plasma alone at 17.15 secs), control PBS (23.35 secs). Furthermore, Figure 5B shows the result of the diabetic volunteer's plasma prothrombin time treated with different concentrations of *C. afer* leaf fractions at 5, 10 and 20 mg/mL. The extract/fractions showed significant ($p<0.0001$) prolongation across the different fractions with ethyl acetate having the longest prothrombin time of 31.18 secs, followed by aqueous at 29.93 secs, butanol and hexane have the lowest prothrombin time of 26.42 secs and 26.45 secs respectively among the fractions, however, ethanol crude extract also add prothrombin time of 28.02 secs.



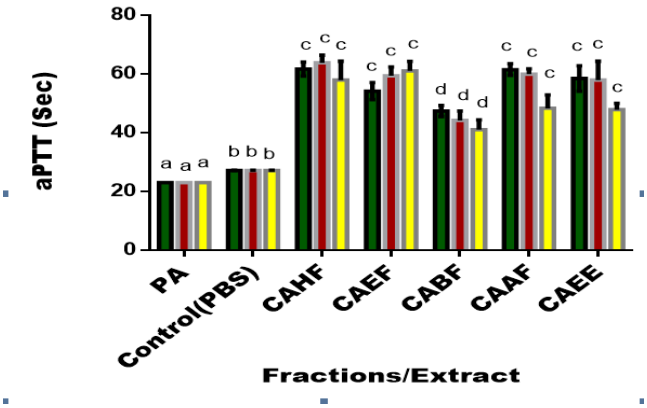
A. Prothrombin time of apparently healthy human volunteers treated with the *C. afer* leaf extract and fractions at 5, 10, 20 mg/mL



B. Prothrombin time of diabetic human volunteers treated with *C afer* leaf extract and fractions at 5, 10 and 20 mg/mL



C. Activated partial thromboplastin time (aPTT) of apparently healthy human plasma treated with *C afer* leaf extract and fractions at 5, 10 and 20 mg/mL



D. Activated partial thromboplastin time (aPTT) of apparently diabetic human plasma treated with *C. afer* leaf extract and fractions at 5. 10 and 20 mg/mL

Figure 5: Prothrombin and activated partial thromboplastin time

Bars labeled with different letters indicate statistically significant ($p<0.0001$) difference (n=10).
Control (PBS) - Phosphate buffer saline, CAAF - *C. afer* aqueous fraction
CAHF - *C. afer* hexane fraction CAEF - *C. afer* ethyl acetate fraction,
CABF - *C. afer* butanol fraction, CAEE - *C. afer* ethanol extract

Figure 5C also showed the effect of *C. afer* leaf fractions on the activated partial thromboplastin time (aPTT) of apparently healthy individuals compared with the control. There was a significant ($p<0.0001$) difference across the fractions when compared with controls. All fractions showed prolonged activated partial thromboplastin time (aPTT) in a dose-dependent manner with ethyl acetate fraction exhibiting the highest aPTT when compared to the control. Butanol fraction exhibited the lowest aPTT when compared with other fractions. However, Figure 5D showed the effect on diabetic individuals (n=10), where there was no significant ($p>0.05$) difference

across the groups, ethyl acetate (58.18 secs), hexane fractions (63.94 secs), aqueous fraction (56.56 secs), butanol fraction (44.19 secs) and ethanol crude extract (54.72 secs). Hexane fraction (63.94 secs) exhibited the highest prolongation of aPTT followed by ethyl acetate fraction (58.18 secs).

Ethyl acetate exhibited the highest PT and aPTT when compared with other fractions; this is similar to the previous report [4] on *C. afer* stem, and also consistent with the result obtained by [47] on the ethanol extract of *C. afer* leaf. As reported in Figures 5A and 5B, the observed prolongation of PT suggests that the extract fractions interfere with prothrombin to thrombin conversion, which is a crucial stage in coagulation cascade. The observed interference may arise from the bioactive compounds found in the extract fractions, which could potentially affect the activity of coagulation factors related to the PT pathway. The observed effect demonstrated the relationship between dosage and response often observed in pharmacological studies, indicating that the extent of PT prolongation varies with the concentration of the extract fractions.

Furthermore, the results in Figure 5C and 5D indicates that the fractions of *C. afer* leaf have a significant effect on the clotting process, as evidenced by the significant difference in aPTT when compared to the control group ($p<0.0001$). In this study, it was found that the duration of aPTT varied based on the concentration of the fractions. Higher concentrations of the fractions were linked to a longer duration of aPTT. Interestingly, the ethyl acetate fraction exhibited the most noteworthy extension of aPTT when compared to control, indicating its potential as a potent modulator of the clotting process. On the other hand, the butanol fraction displayed the shortest aPTT duration among all the fractions, suggesting potential variations in the clotting effects of different elements. The results suggests that the bioactive chemicals present in the *C. afer* leaf fractions may have implications for the clotting process. It is crucial to have a clear understanding of how these fractions impact aPTT in healthy individuals for therapeutic purposes and consider the dosage when using these fractions for therapeutic purposes, as the effects observed are dependent on the dose. Adjusting the concentration of the fractions can optimize the clotting reaction, ensure maximum therapeutic activity and reduce side effects. This suggests that the effect of *C. afer* on PT and aPTT in both diabetic and non-diabetic could be due to the presence of phytochemicals like alkaloids such as ambinine in *C. afer* which are known to possess anticoagulant [47] and antithrombotic activity [4] and tannins known for the inhibition of platelet activation and thrombus formation. [51]

Conclusion

This study demonstrated the pharmacological potential of *C. afer* leaf fractions, providing compelling evidence of their *in vitro* antidiabetic, anti-inflammatory, antioxidant and anticoagulant activities. Among the tested fractions, the ethyl acetate fraction consistently exhibited the most potent bioactivity across multiple assays. These findings highlighted the therapeutic potential of *C. afer* leaf fractions particularly the ethyl acetate fraction as a natural source of multitarget bioactive compounds for managing oxidative stress, inflammation, diabetes, and coagulation disorders. Further research, including compound isolation, mechanistic studies and *in vivo* evaluations are recommended to fully elucidate and validate these pharmacological effects.

Conflict of Interest

The author declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Eguchi N, Vaziri ND, Dafoe DC, Ichii H. The Role of Oxidative Stress in Pancreatic β Cell Dysfunction in Diabetes. Int. J. Mol. Sci. 2021; 22(4):1509.
2. Domingueti CP, Dusse LMS, Carvalho M das G, de Sousa LP, Gomes KB, Fernandes AP. Diabetes mellitus: The linkage between oxidative stress, inflammation, hypercoagulability and vascular complications. J. Diabetes Complicat. 2016; 30(4):738–745.
3. Boison D, Adinortey CA, Babanyinah GK, Quasie O, Agbeko R, Wiabo-Asabil GK, Adinortey MB. *Costus afer*: A Systematic Review of Evidence-Based Data in support of its Medicinal Relevance. Scientifica. 2019; 2019:3732687. doi: 10.1155/2019/3732687. PMID: 32082693; PMCID: PMC7011497.
4. Ogugofor MO, Njoku UO, Njoku OU, Batiha GES. Phytochemical analysis and thrombolytic profiling of *Costus afer* stem fractions. Futur. J. Pharm. Sci. 2022; 8(1).
5. Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Abdull Razis AF, Modu B, Calina D and Sharifi-Rad J. Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. Front. Chem. 2023; 11:1158198. doi: 10.3389/fchem.2023.1158198
6. Muscolo A, Mariateresa O, Torello Giulio, Russo Mariateresa. Oxidative Stress: The Role of Antioxidant Phytochemicals in the Prevention and Treatment of Diseases. Int. J. Mol. Sci. 2024; 25(6):3264.
7. Dubsky M, Veleba J, Sojakova D, Marhefkova N, Fejfarova V, Jude EB. Endothelial Dysfunction in Diabetes Mellitus: New Insights. Int. J. Mol Sci. 2023; 24(13):10705. https://www.mdpi.com/1422-0067/24/13/10705
8. Li X, Weber NC, Cohn DM, Hollmann MW, DeVries JH, Hermanides J, Preckel B. Effects of Hyperglycemia and Diabetes Mellitus on Coagulation and Hemostasis. J. Clin. Med. 2021; 10(11):2419. doi: 10.3390/jcm10112419. PMID: 34072487; PMCID: PMC8199251.
9. Olajide LO, Liasu TA, Shabi DR, Adeoye BO, Olajide OS, Orodele KA, Ogunbiyi BT. Anti-Inflammatory Property of *Costus afer* Ker Gawl Ethanol Leaf Extract in STZ-Induced Diabetic Rats. 2023; Sch. Int. J. Biochem, 6(10): 129-137.
10. Scridon A. Platelets and Their Role in Hemostasis and Thrombosis—From Physiology to Pathophysiology and Therapeutic Implications. Int. J. Mol. Sci. 2022; 23(21):12772. https://mdpi-res.com/d_attachment/ijms/ijms-23-12772/article_deploy/ijms-23-12772.pdf?version=1666520564
11. Sabitha V, Panneerselvam K, Ramachandran S. *In vitro* α–glucosidase and α–amylase enzyme inhibitory effects in aqueous extracts of *Abelmoscus esculentus* (L.) Moench. Asian Pac. J. Trop. Biomed. 2012; 2(1): S162–164.
12. Pant G, Sai K, Babasaheb S, Reddy R, G S. *In vitro* α-amylase and α-glucosidase Inhibitor Activity of *Abutilon indicum* Leaves. Asian J. Pharm. Clin. Res. 2013; 6(9):22-24.
13. Vennila, V., & Pavithra, V. In-vitro alpha Amylase and alpha Glucosidase Inhibitory Activity of Various Solvent Extracts of *Hybanthus Enneaspermus Linn*. 2015.
14. Sakat, S, Juvekar, A, & Gambhire, M. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata Linn*. Int. J. Pharm. Pharm. Sci. 2010; *2*(1), 146–156.
15. McCune LM, Johns T. Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the Indigenous Peoples of the North American boreal forest. J. Ethnopharmacol. 2002; 82(2-3):197–205.
16. Anyasor G, Onajobi F, Osilesi O, Adebawo O. Proximate composition, mineral content and *in vitro* antioxidant activity of leaf and stem of *Costus afer* (Ginger lily). J. Intercult. Ethnopharmacol. 2014;3(3):128.
17. Hue SM, Boyce A, Somasundram C. Antioxidant activity, phenolic and flavonoid contents in the leaves of different varieties of sweet potato (*Ipomoea batatas*). Aust. J. Crop Sci. 2012; 6(3):375–380.
18. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal. Biochem. 1982; 126(1):131–138. Available from: https://www.sciencedirect.com/science/article/pii/000326978290118X
19. Wintrobe, M. W. (1967). Clinical Haematology. 6th edition Philadelphia, Lea and Febiger, 365-367.

20. Brown, B. A. *Haematology*: Principles and Procedures, 5th Ed., Lea and Febiger, Philadelphia, (1988) 195-215.
21. Osoniyi O, Onajobi F. Coagulant and anticoagulant activities in *Jatropha curcas* latex. J. Ethnopharmacol. 2003; 89(1):101–105.
22. Kashtoh H, Baek KH. Recent Updates on Phytoconstituent Alpha-Glucosidase Inhibitors: An Approach towards the Treatment of Type Two Diabetes. Plants (Basel, Switzerland). 2022; 11(20):2722. Available from: https://pubmed.ncbi.nlm.nih.gov/36297746/
23. Akmal M, Wadhwa R. Alpha Glucosidase Inhibitors. PubMed. Treasure Island (FL): StatPearls Publishing. 2020. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557848/
24. Li X, Bai Y, Jin Z, Svensson B. Food-derived non-phenolic α-amylase and α-glucosidase inhibitors for controlling starch digestion rate and guiding diabetes-friendly recipes. LWT. 2022;153:112455.
25. Imieje VO, Onochie CF, Nwaka B, Falodun A. *In vitro* Antioxidant and Antidiabetic Potentials of Various Extracts of the Stem Bark of Cylicodiscus gabunensis (Harms) Mimosaceae, Nigeria. Trop. J. Nat. Prod. Res. 2022; 6(11):1858-1863. http://www.doi.org/10.26538/tjnpr/v6i11.19
26. Rahman MM, Dhar PS, Sumaia, Anika F, Ahmed L, Islam MR, Sultana NA, Cavalu S, Pop O, Rauf A. Exploring the plant-derived bioactive substances as antidiabetic agent: An extensive review. Biomed Pharmacother. 2022; 152:113217. doi:10.1016/j.biopha.2022.113217
27. Aidoo DB, Konja D, Henneh IT, Ekor M. Protective Effect of Bergapten against Human Erythrocyte Hemolysis and Protein Denaturation *In Vitro*. Int. J. Inflam. 2021;2021:1279359. doi:10.1155/2021/1279359
28. Tay YN, Bakar MHA, Azmi MN, Saad NA, Awang K, Litaudon M, Kassim MA. Inhibition of Carbohydrate Hydrolysing Enzymes, Antioxidant Activity and Polyphenolic Content of Beilschmiedia Species Extracts. IOP Conf. Ser. Mater. Sci. 2020; 716(1):012007–012007.
29. Brown A, Anderson D, Racicot K, Pilkenton SJ, Apostolidis E. Evaluation of Phenolic Phytochemical Enriched Commercial Plant Extracts on the *In Vitro* Inhibition of α-Glucosidase. Front. Nutr. 2017;4.
30. Amarowicz R, Pegg RB. Natural antioxidants of plant origin. Adv. Food Nutr. Res. 2019;1–81. https://www.sciencedirect.com/science/article/pii/S1043452619300269
31. Chirumamilla P, Taduri S. Assessment of in vitro anti-inflammatory, antioxidant and antidiabetic activities of Solanum khasianum Clarke. Vegetos. 2022; https://doi.org/10.1007/s42535-022-00410-6
32. Francenia Santos-Sánchez N, Salas-Coronado R, Villanueva-Cañongo C, Hernández-Carlos B. Antioxidant Compounds and Their Antioxidant Mechanism. Antioxidants. Intech Open. 2019; Available from: http://dx.doi.org/10.5772/intechopen.85270
33. Wu D, Saleem M, He T, He G. The Mechanism of Metal Homeostasis in Plants: A New View on the Synergistic Regulation Pathway of Membrane Proteins, Lipids and Metal Ions. Membranes. 2021; 11(12):984. https://doi.org/10.3390/membranes11120984
34. Dharmadeva S, Galgamuwa LS, Prasadinie C, Kumarasinghe N. In vitro anti-inflammatory activity of Ficus racemosa L. bark using albumin denaturation method. Ayu. 2018; 39, 239 - 242.
35. Dash UC, Nitish Kumar Bhol, Swain SK, Rashmi Rekha Samal, Nayak PK, Raina V, Panda SK, Kerry RG, Duttaray AK, Jena AB. Oxidative stress and inflammation in the pathogenesis of neurological disorders: Mechanisms and implications. Acta Pharm. Sin B. 2024; 15(1):15–34. https://www.sciencedirect.com/science/article/pii/S2211383524004040
36. Kumar A P N, Kumar M, Jose A, Tomer V, Oz E, Proestos C, Zeng M, Elobeid TKS, Oz F. Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. Molecules. 2023; 28(2), 887. https://doi.org/10.3390/molecules28020887
37. Kumar N, Goel N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnol. Rep. 2019 Dec 1;24(e00370):e00370.
38. Shabalala SC, Johnson R, Basson AK, Ziqubu K, Hlengwa N, Mthembu SXH, Mabhida SE, Mazibuko-Mbeje SE, Hanser S, Cirilli I, Tiano L, Dludla P V. Detrimental Effects of Lipid Peroxidation in Type 2 Diabetes: Exploring the Neutralizing Influence of Antioxidants. Antioxidants. 2022; 11(10), 2071. https://doi.org/10.3390/antiox11102071
39. Chaudhary P, Pracheta Janmeda, Anca Oana Docea, Balakyz Yeskaliyeva, Faizal A, Babagana Modu, Calina D, Sharifi-Rad J. Oxidative stress, Free Radicals and antioxidants: Potential Crosstalk in the Pathophysiology of Human Diseases. Front. Chem. 2023; 11. https://www.frontiersin.org/journals/chemistry/articles/10.3389/fchem.2023.1158198/full
40. Vaou N, Stavropoulou E, Voidarou C (Chrysa), Tsakris Z, Rozos G, Tsigalou C, Bezirtzoglou E. Interactions between Medical Plant-Derived Bioactive Compounds: Focus on Antimicrobial Combination Effects. Antibiotics. 2022;11(8):1014. https://doi.org/10.3390/antibiotics11081014
41. Rudrapal M, Khairnar SJ, Khan J, Dukhyil AB, Ansari MA, Alomary MN, Alshabrmi FM, Palai S, Deb PK. Devi R. Dietary Polyphenols and Their Role in Oxidative Stress-Induced Human Diseases: Insights Into Protective Effects, Antioxidant Potentials and Mechanism(s) of Action. Front. Pharmacol. 2022; 13(1). https://doi.org/10.3389/fphar.2022.806470
42. Atere TG, Akinloye OA, Ugbaja RN, Ojo DA, Dealtry G. In vitro antioxidant capacity and free radical scavenging evaluation of standardized extract of *Costus afer* leaf. Food Sci Hum Well. 2018; 7(4):266–272. https://doi.org/10.1016/j.fshw.2018.09.004
43. Zahra M, Abrahamse H, George BP. Flavonoids: Antioxidant Powerhouses and Their Role in Nanomedicine. Antioxidants. 2024;13(8):922–922. https://doi.org/10.3390/antiox13080922
44. Andrabi SM, Sharma NS, Karan A, Shahriar SMS, Cordon B, Ma B. Nitric Oxide: Physiological Functions, Delivery, and Biomedical Applications. Adv. Sci. (Weinh.). 2023; 10(30):e2303259. https://doi.org/10.1002/advs.202303259
45. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Aitavilla D, Bitto A. Oxidative Stress: Harms and Benefits for Human Health. Oxid Med Cell Longev. 2017; 2017(8416763):1–13. https://pmc.ncbi.nlm.nih.gov/articles/PMC5551541/
46. Hayat J, Akodad M, Moumen A, Baghour M, Skalli A, Ezrari S, Belmalha S. Phytochemical screening, polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of Marrubium vulgare L. from 2 different localities of Northeast of Morocco. Heliyon. 2020; 6(11):e05609. https://doi.org/10.1016/j.heliyon.2020.e05609
47. Ezejiofor AN, Igweze ZN, Udowelle NA, Orisakwe OE. Histopathological and biochemical assessments of *Costus afer* stem on alloxan-induced diabetic rats. J. Basic Clin. Physiol. Pharmacol. 2017; 28(4):383–391. https://doi.org/10.1515/jbcpp-2016-0039
48. Jaradat N, Hawash M, Dass G. Phytochemical analysis, in-vitro anti-proliferative, anti-oxidant, anti-diabetic, and anti-obesity activities of Rumex rothschildianus Aarons. extracts. BMC Complement. Med. Ther. 2021;21(1).
49. LaPelusa A, Dave HD. Physiology, hemostasis [Internet]. National Library of Medicine. StatPearls Publishing. 2019. Available from: https://www.ncbi.nlm.nih.gov/books/NBK545263/
50. Ambelu YA, Shiferaw MB, Abebe M, Enawgaw B. Prothrombin time, activated partial thromboplastin time and platelet counts of type II diabetes mellitus: a comparative study. J. Diabetes Met. Disord. 2018;17(2):117–121.
51. Zhu L. Tannic Acid Inhibits Protein Disulfide Isomerase, Platelet Activation and Thrombus Formation. Blood. 2018;132(Supplement 1):2418–2418.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)