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An In Vitro Anticoagulant and Antioxidant Potentials of Methanol Leaf Extracts of *Guiera senagalensis and Ficus platyphylla*

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Abstract
Gueira senegalensis and *Ficus platyphylla* are plants used in traditional medicine, and investigating the anticoagulant and antioxidant properties could be beneficial for various cardiovascular diseases. The current study evaluated the *in vitro* anticoagulant and antioxidant potentials of methanol leaf extracts of *G. senegalensis* and *F. platyphylla* for therapeutic purposes. The methanol leaf extracts of the two plants were prepared using 100 g of already dried leaves in 800 mL of methanol (80%) for 48 hours. The phytochemicals were analysed both quantitatively and qualitatively using standard methods. A prothrombin time (PT) test was used for anticoagulant activity. Antioxidant activity was analysed using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and reducing power, as determined by the Ferric-reducing power assay (FRAP). The antioxidant vitamins A, E and C were determined using spectrophotometric methods. The results showed significant anticoagulant activity in the PT test, suggesting a preferential action toward the extrinsic pathway. The quantitative result revealed the presence of active phytochemicals, including alkaloids, flavonoids, tannins, steroids and terpenoids. The antioxidant vitamins showed the presence of vitamin C in higher amounts and vitamin E in *F. platyphylla* as compared to *G. senegalensis.* In conclusion, *G. senegalensis* and *F. platyphylla* demonstrated anticoagulant activity, indicating their potential as a new source of bioactive molecules for therapeutic purposes, with a particular emphasis on cardiovascular diseases.
Keywords: *Gueira senegalensis* and *Ficus platyphylla*, Anticoagulant activity, Antioxidant activity, phytochemicals and antioxidant vitamins.
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1. Introduction

Herbal medicines play a crucial role within the healthcare system in Africa, especially for its cultural acceptability, availability and cost effectiveness. And more importantly it's compatibility with the human body, and fewer side effects (Verma *et al.,* 2010; Veeresham, 2012). Using plants for medicinal purposes in the prevention and treatment of diseases serves as an alternative source for developing new drugs, including anticoagulant agents, due to the high biological activities of their active compounds (Manicam *et al.,* 2010). There is compelling scientific evidence demonstrating that dietary anticoagulants or phytochemicals with anticoagulant properties can ultimately reduce or eliminate the risks of thromboembolic diseases (Lee *et al.,* 2012). Natural products have been shown to play a significant role in the development of novel drugs for the treatment and prevention of diseases (Gilani and Rahman, 2005). The increasing use of plants for the production of natural products for maintaining human health has been observed over decades and led to increased studies by scientists to discover more medicinal plants, according to the World Health Organization (WHO), medicinal plants could be the best source of a variety of drugs as 80% of individuals in developing countries use traditional medicine, which has compounds derived from medicinal plants (WHO, 2006).

Anticoagulant drugs are needed for both the short-term treatment of arterial and venous thrombotic disorders and the long-term prevention of recurrences (Thaler et al., 2015). Although the main anticoagulants, such as heparins

and their derivatives, have been the major players in acute thrombotic disorders for decades, their efficacy remains undisputed, and life-threatening side effects of these drugs have also been well documented (Kumar *et al.,* 2011). Many restrictions and harmful effects are related to the use of anticoagulants (heparin, warfarin), such as bleeding, which is the most incurable problem associated with this treatment (Evangelista *et al.,* 2012). Increased bruising, red or pink-coloured urine, purple toes, and more blood in a normal menstrual cycle are the side effects of taking an anticoagulant (Kinman, 2021). The research to source new substances with anticoagulant and antithrombotic activities is still relevant (Lapikova *et al.,* 2008). Medicinal plants have historically been the source of anticoagulant and antithrombotic molecules.

Guiera senegalensis (*G. Senegalensis*) is a shrub that grows to about 3 m high. The Hausa people know it as "Sabara", and the plant is commonly used to treat chronic diseases, colds, bronchitis, stomach pain, vomiting, jaundice, kidney stones and infections (Moreira *et al.,* 2023). The decoctions of its leaves are used in the treatment of venereal disease as an antimicrobial agent (Silva *et al.,* 2003). The crushed leaves of *G. senegalensis* mixed with tamarind pulp are used as a laxative and appetiser. The dried, pounded leaves are used by women as a supplement after childbirth to increase milk flow and as a general tonic and blood restorative. A leaf infusion is used to wash newborn babies, and treat respiratory problems, headache and sinusitis (Hinneburg *et al.,* 2006).

Ficus platyphylla (*F. platyphylla*), commonly known as the broad-leafed paperbark Fig or Desert Fig, 'Epo-Obo' in southwest Nigeria and "Gamji" among the tribes of northern Nigeria, is a species of fig tree belonging to the Moraceae family (Cruz *et al.,*2022). The genus *Ficus* is distributed on several continents in tropical and subtropical climates. This tree is native to arid and semi-arid regions of Africa, particularly in the central and northern parts of the continent (Sheidu *et al.,* 2020). It has been reported to have analgesic, anti-inflammatory, and anti-contraceptive activities, and the stem bark is used for the treatment of tuberculosis (Sheidu *et al.,* 2020). It was also reported that the extracts of *F. platyphylla* inhibit gastrointestinal motility (Amos *et al.,* 2002). There is no reported literature on the possible anticoagulant activity of these plants. Therefore, we tested the hypothesis that the methanol leaf extracts of *F. Platyphylla* and *G. Senegalensis* possess anticoagulant and antioxidant activities. The study aimed to investigate the *in vitro* anticoagulant activity of the methanol leaf extracts of *F. Platyphylla* and *G. Senegalensis* using the prothrombin time test (PTT) for therapeutic purposes.

2. Material and Methods

2.1 Material

2.1.1 Plant Collection and Identification

The leaves of *G. Senegalensis and F. platyphylla* were collected from two local government areas of Sokoto State, Nigeria: Wamakko and Sokoto North Local Government. The plant's identification was by a botanist in the Department of Plant Science, Faculty of Chemical and Life Sciences, Usmanu Danfodiyo University, Sokoto and a voucher specimen was deposited at the Herbarium of the Department of Plant Science.

2.1.2 Chemicals and reagents

Ferric chloride, Wagner's reagent, Ammonia, Ethanol, Methanol 99.9% and Lead acetate (P I Park Scientific Ltd. Northampton, UK), Benzene, Chloroform, Sulphuric acid, Sodium hydroxide, Acetone, Hydrochloric acid, Fehling's solution, Baljet's reagent (Loba Chemiepvt., Mumbai, India), Calcium chloride, Sodium chloride (Fisher Scientific Company, USA). 2,2-dipyridyl, potassium ferrocyanide, trichloroacetic acid (TCA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and methanol.

2.1.3 Sample Preparation

The leaf was shade-dried at room temperature for three weeks (21 days) and triturated using a pestle and mortar before being processed into a fine powder with an electric blender. The powdered sample was stored in a sealed bottle and kept away from light and humidity until used for extract preparation.

2.1.4 Extract preparation

One hundred (100g) grams of dried powdered leaves of *G. senegalensis* and *F. platyphylla* were soaked in 800 mL of methanol for 48 hours. The mixture obtained was filtered using muslin cloth, and the solvent was removed at 45°C using a rotary evaporator to give a dark solid extract. The extract obtained was stored in an air-tight bottle and kept at 24°C before further analysis.

2.2 Methods

2.2.1 Blood Collection and Plasma Sample Preparation

The blood samples were collected from healthy volunteer donors (aged 18 – 35 years old) after obtaining their consent. The blood was drawn *via* venipuncture and collected into EDTA-containing tubes (BD Vacutainer, Franklin Lakes, NJ, USA). The pure platelet plasma (PPP) was obtained from the blood plasma pool after centrifugation of the whole blood at 3000 rpm for 15 min at room temperature. The plasma was stored at 20 0C and used for two weeks.

2.2.2 Anticoagulant Activity

2.2.2.1 Prothrombin Time (PT) Test

The action of the crude extracts in the extrinsic pathway was evaluated by the PT test, as described by Gautam *et al.* (2025). The test was carried out, where 0.2 ml of the plasma was mixed with 0.2 ml of methanol extracts of varying concentrations of *G. Senegalensis and F. platyphylla*, and an equal volume of $CaCl_2$ (25 mM) was added in a clean fusion tube and incubated at 37 0C in a water bath as presented in **Table 1** below. For the control group, the extract was replaced with the same volume of saline water (0.9%). The clotting time was recorded using a digital stopwatch by tilting the test tubes every 5 seconds for 5 minutes.

Table 1: Determination of Anticoagulation Activity

Group	Amount of Plasma (ml)	Amount of Extract (µg/µl)	$CaCl_2$ Solution (ml)
Control	0.2	0.2 (0.9% saline water)	-
Group I	0.2	0.2	0.3
Group II	0.2	0.4	0.3
Group III	0.2	0.6	0.3
Group IV	0.2	0.8	0.3
Group V	0.2	1.0	0.3

2.3 Qualitative Phytochemical Analysis

The quantitative analysis for flavonoids, tannins, saponins, alkaloids, glycosides and steroid content was determined as described by the method of Harbone, (1998).

2.3.1 Quantitative Phytochemical Analysis

Alkaloids, Saponins, Glycosides, Flavonoids and tannins were determined using methods reported by Jamuna et al. (2014).

2.4 *In vitro* antioxidant assays

2.4.1 DPPH Radical Scavenging Assay

DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a stable free radical whose spare electron is delocalised over the molecule as a whole, so that the molecule does not dimerise as described by Thaipong *et al.* (2006).

Principle

The principle is based on the reduction of DPPH by an antioxidant (able to donate hydrogen atoms), leading to the change of violet colour to light yellow, and absorbance measured at 517nm.

Procedure

The extracts were prepared at different concentrations of 0.2, 0.4, 0.6, 0.9, and 1.0 mg/mol and adjusted to 4 mL with methanol. A 1 mL DPPH radical (1 Mmol) was added to each tube and incubated for 30 minutes at room temperature; the absorbance was measured at 517nm.

2.4.2 Ferric-reducing power assay (FRAP)

The principle is based on the increase in absorbance of the reaction mixtures due to the formation of a colour complex by the antioxidant compound when it reacts with potassium ferricyanide, trichloroacetic acid and ferric chloride, which was measured at 700 nm, and an increase in absorbance of the reaction mixture indicates the reducing power of the samples Jayaprakasha *et al.* (2001)

Procedure

A volume of 2.5 ml of the various concentrations of the extracts was added to 2.5 ml of potassium ferrocyanide in a test tube. The test tube was incubated at 50°C for 20 minutes. A volume of 2.5 mL of 10 % trichloroacetic acid (TCA) was added to the mixture, which was then centrifuged at 3000 rpm for 20 minutes. 0.5 ml of freshly prepared ferric chloride was added. Then, the absorbance was measured at 700 nm. Ascorbic acid of various concentrations was used as a standard.

2.5 Determination of antioxidant vitamins

2.5.1 Determination of Vitamin A

Procedure

A 0.5 g of the plant's extract was dissolved in 10 ml of distilled water and allowed to stand for 1 hour. The mixture was filtered to remove the residue. 1 mL of the sample extract and 1 mL of methanol were transferred into test tubes, and 2 mL of petroleum ether was added. The solution was mixed and centrifuged at 2000 rpm. 1.0 ml of the supernatant was used to measure the absorption at 450nm against the reagent blank Rutkowski *et al.* (2006).

2.5.2 Determination of Vitamin C

Procedure

A freshly prepared plant extract (1.0 mL) and 1 mL of PR reagent were added to a test tube. The mixture was mixed gently and incubated for 30 minutes at room temperature. The solution was centrifuged at 2000 rpm for 10 minutes, and the absorbance was measured at 700nm. Ascorbic acid was used as a standard Rutkowski *et al.* (1998).

2.5.3 Determination of Vitamin E

Procedure

A plant extract of 0.5 g and 0.5 mL of methanol were added in test tube. The mixture was mixed and incubated for 1 minute at room temperature. A 3.0 mL of xylene was also added into the mixture stirred gently and centrifuged at 2000 rpm. The absorbance was measured at 539nm (Rutkowski *et al.,* 2005).

Statistical analysis

The results were expressed as mean ± SD with n = 3. One-way ANOVA and Student's t-test were performed using GraphPad Prism Version 5.00 for Windows, GraphPad Software, San Diego, California, USA. $P < 0.05$ was considered statistically significant.

3. Results

The anticoagulant activity of the methanol leaf extracts (MLE) of *G. senegalensis* and *F. platyphylla* evaluated by the prothrombin time (PT) assay was comparatively presented in **Figure 1,** the *G. senegalensis* delayed the clotting time in a concentration-dependent pattern, demonstrating its anticoagulant activity via a possible mechanism of inhibiting the extrinsic pathway. Also, a similar mechanism was observed in relation to *F. platyphylla*, where maximum effective PT was achieved but less than that of *G. senegalensis* **Figure 1.** Therefore, the present result suggests a possible similar mechanism in operation by these plants to prolong the clotting time, where the MLE may inhibits the extrinsic pathway, suggesting an effective anticoagulant activity of *G. senegalensis* and *F. platyphylla* (**Figure 1**).

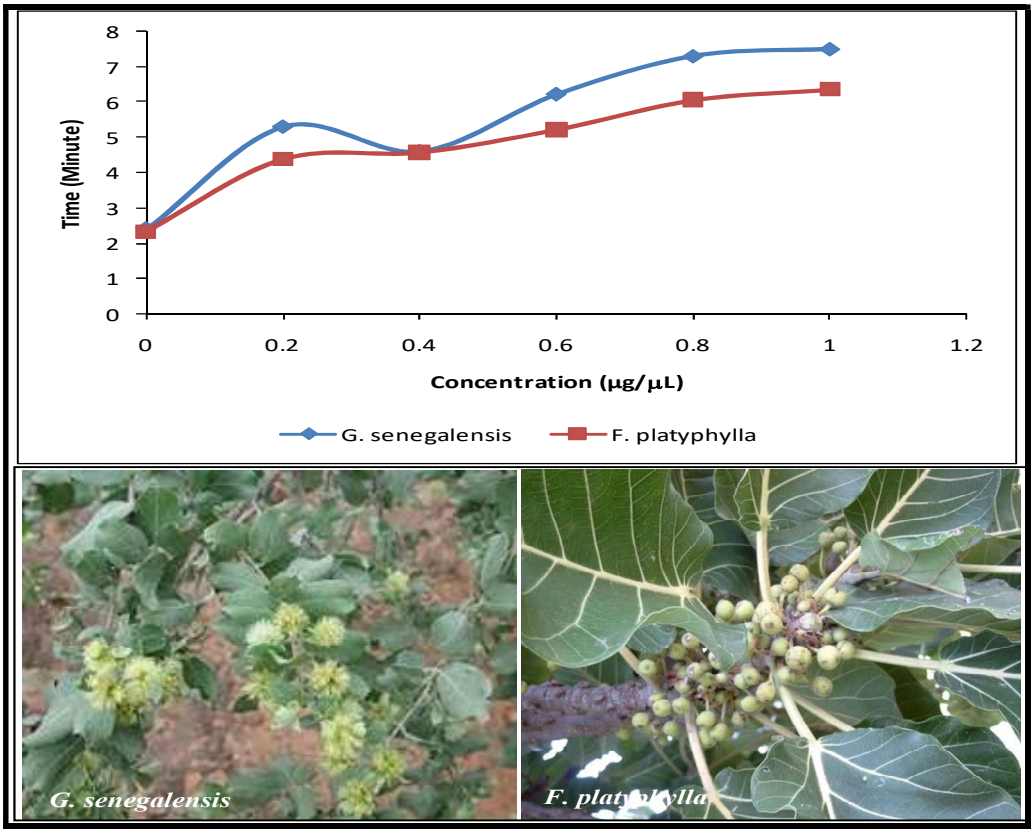

Figure 1: Anticoagulant Activity of Methanol leaf extracts of *G. Senegalensis and F. platyphylla*. Values are mean ± SD with n = 3.

Phytochemical screening performed on the methanol leaf extracts of both *G. senegalensis* and *F. platyphylla*. The result revealed the presence of many active phytochemical compounds; such are tannins, flavonoids, saponins, cardiac glycosides, balsams, glycosides, alkaloids, anthraquinones and volatile oils. However, anthraquinones were not detected in the MLE of *F. platyphylla* and that could be due the method used or they were insignificance to be detected (**Table 2**). The result also indicates variation in phytochemicals such that more alkaloids and flavonoids were found in *F. platyphylla* as compared to tannins, saponins and steroids in *G. senegalensis*.

Table 2: Qualitative Analysis of Bioactive Compounds in Methanol Leaf Extracts of *G. Senegalensis* and *F. platyphylla*

Phytochemicals	*G. senegalensis*	*F. platyphylla*
Saponins	+	+
Alkaloids	+	++
Tannins	++	+
Flavonoids	+	+++
Saponins glycosides	++	+
Steroids	++	+
Cardiac glycosides	++	+
Glycosides	+	+
Anthraquinones	+	ND
Balsams	+++	+++
Volatile oil	+	+

* Keys: + = slightly present, ++ = moderately present, and +++ = highly present and ND = Not detected

In quantitative analysis, the result obtained confirmed the quantitative result that indicated more flavonoids in *F. platyphylla* and more of steroids, tannins, saponins and glycosides content in *G. senegalensis,* respectively has as presented in **Figure 2**.

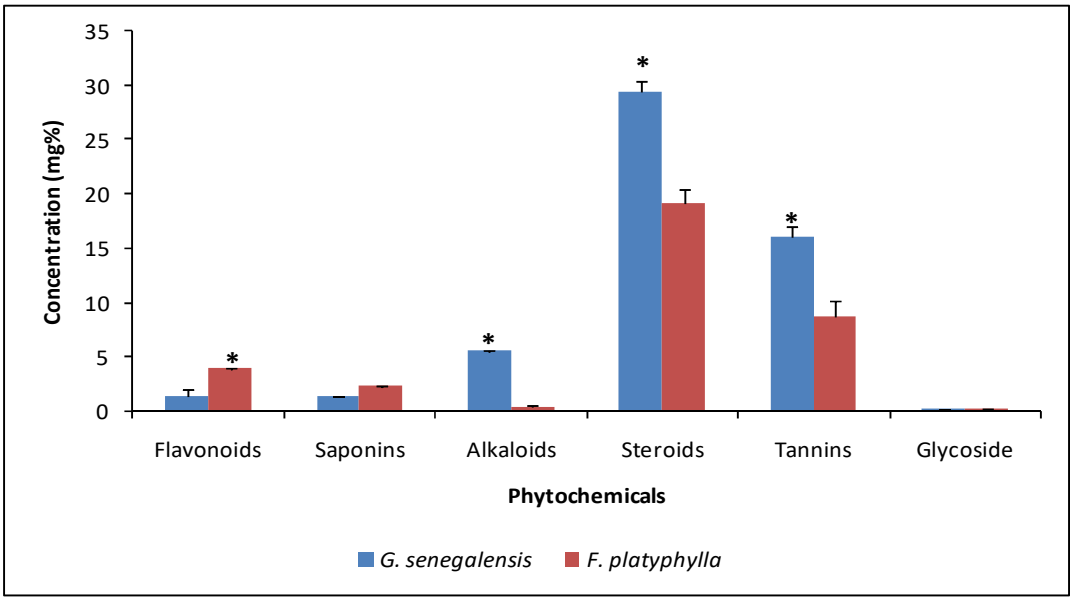

Figure 2: Quantitative Analysis of Bioactive Compounds in Methanol Leaf Extracts of *G. Senegalensis* and *F. platyphylla*. Values are mean ± SD with n = 3.

The antioxidant activity of the methanol leaf extracts was tested using an *in vitro* DPPH and FRAP assays and the result presented in **Figures 3,** revealed that the *G. senegalensis* and *F. platyphylla* recorded good scavenging activity at concentrations of 0.2, 0.4 and 0.6 µg/ml as compared to the standard drug ascorbic acid (**Figure 3A**). Also, the FRAP reducing power of the extracts was tested, the result showed variation between the two plant

extracts, where *G. senegalensis* has more reducing power at 1 µg/ml and *F. platyphylla* at 0.2 µg/ml, respectively (**Figure 3B**), and the control vitamin C showed stronger reducing power, which was attributed to its purity as compared to the crude methanol leaf extracts of the samples.

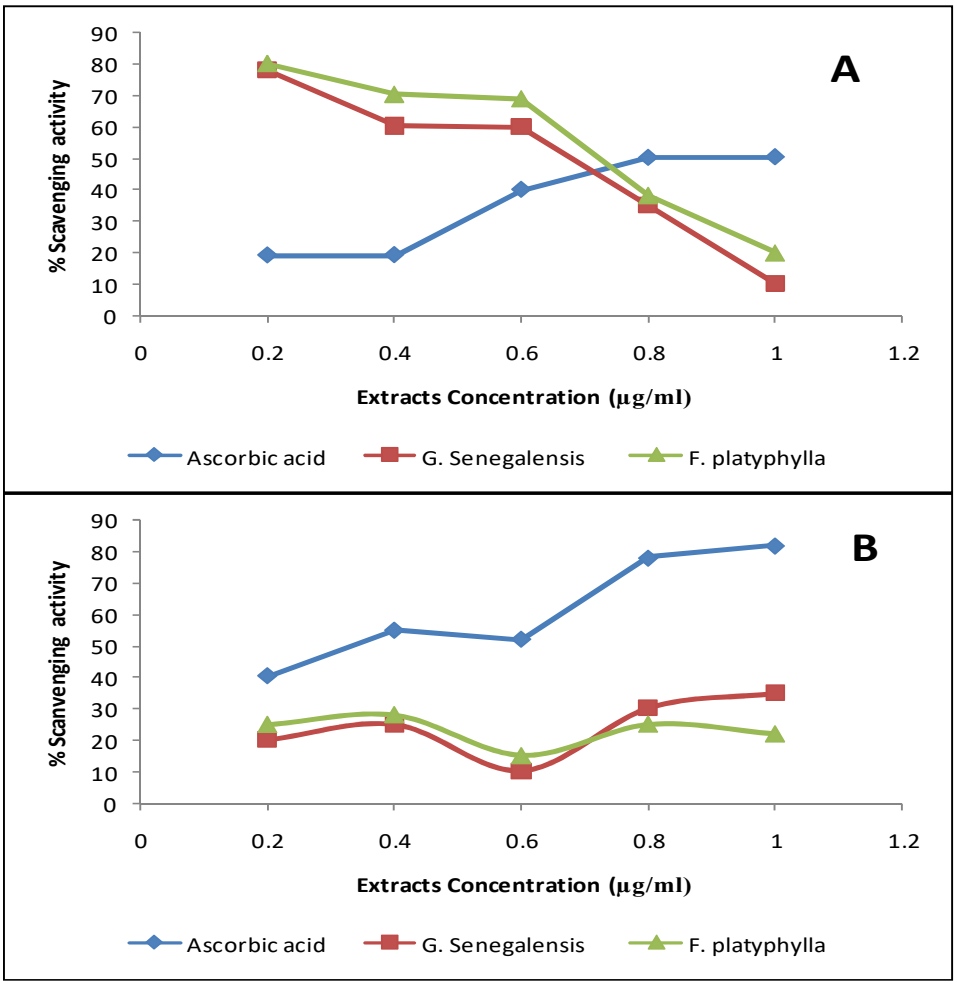

Figure 3: **A**) DPPH radicals scavenging activity and **B**) FRAP reducing power of methanol leaf extracts (MLE) of *G. Senegalensis* and *F. Platyphylla*. The results are expressed as the per cent DPPH scavenging activity and FRAP. The results are expressed as the percentage of reducing activity equivalent to ascorbic acid. Values expressed as mean ± SD with n = 3.

Furthermore, the antioxidants vitamins investigated showed a significant levels of vitamins A, C, and E in *F. Platyphylla*, which further signified its antioxidant properties at lower concentrations and that may be the reasons for its enhance antioxidant activity. The two extracts showed significant levels of Vitamin C contributing to their antioxidant properties (4.4 and 3.5 µg/ml), respectively (**Figure 4**).

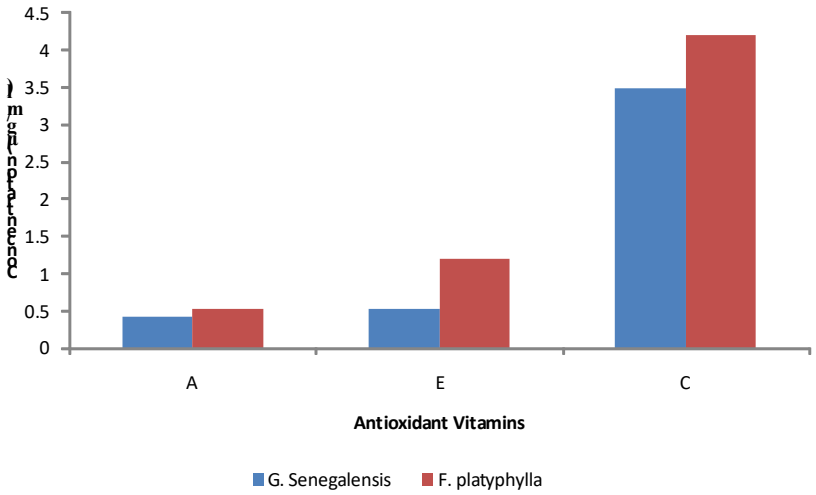

Figure 4: Antioxidant vitamins in aqueous leaf extracts of *G. Senegalensis* and *F. Platyphylla*.

4. Discussion

The process of anticoagulation occurs mainly due to the complex interaction of cellular and molecular components. Initially, clotting involves common pathways, both intrinsic and extrinsic pathways, but it is later found to be due to a balance between procoagulants and anticoagulants. Antithrombotic drugs are important players in the prevention and treatment of thrombotic disorders, and secondary metabolites from plant origin are potential sources of new anticoagulant (Chaves *et al.,* 2010). From the result it was observed that both *G. senegalensis* and *F. platyphylla* have possessed anticoagulant properties by delaying the blood coagulations in a concentration dependent pattern as presented in **Figure 1**. Earlier research have shown that the leaf extract of these plants was used for the treatment of chronic diseases, colds, bronchitis, stomach pain, vomiting, jaundice, kidney stones and infections (Moreira *et al.,* 2023). Similarly, *F. Platyphylla* has shown to possess analgesic, anti-inflammatory and anti-contraceptive activities, and the stem bark is used in tuberculosis treatment (Gautam et al., 2023; Sheidu *et al.,* 2020). The potentials of these plants have been attributed to the active biochemical compounds.

The phytochemical screenings both quantitative and qualitative have shown the present of active secondary metabolites, as presented in **Tables 3 and 4,** which are reported to possess therapeutic properties (Vishnu *et al.,* 2013; Narender *et al.,* 2012). Phytochemicals generally, are linked to important pharmacological activities and play a major role in the anticoagulation process. It has been reported that high content of steroids and tannins were implicated in blood clotting cascades. Different types of tannins, for example, ellagitannins isolated from the butanol fractions of the methanol extract of *G. japonicum,* which include pedunguladin, tellimagradin II, casuariin and 5-desgalloylstachurin, have been shown to prolong prothrombin time (Dong *et al.,* 1998). This observation was further explained by the study of Sajid *et al.* (2017), which implicated the presence of flavonoids, tannins, terpenoids, and saponins to interfered in haemostasis by reducing the time of plasma coagulation as well as blood clotting. Manicam *et al*. (2010) reported the anticoagulant activity of *Mela stoma malabathrum* (Aqueous leaf extract); their study revealed the prolonged coagulation time. Several plant extracts have also been found to exhibit antithrombotic and/anticoagulant activity, such as *Sutherlandia frutescens* leaf extract, *Gloriosa superba*, and *Zantedeschia ethiopica*, which displayed anticoagulant properties (Knee *et al.,* 2008).

Another important properties demonstrated by methanol leaf extracts of *G. senegalensis* and *F. platyphylla* was good antioxidant potential. The DPPH assay demonstrated a radical scavenging activity between 0.2 – 0.6 µg/ml with IC50 of 0.67 for *G. Senegalensis* and *F. platyphylla* above the vitamin C control drug (**Figure 3A**), which aligned with the findings of reported studies that showed plant's capability to neutralise free radicals (El-Beltagi *et al.,* 2020). Also, the FRAP assay further highlighted the plant extract's strong reducing power as shown in **Figure 3B**, indicating its ability to donate electrons and stabilise reactive oxygen species, a key mechanism in antioxidant defence (Kumar *et al.,* 2014). Furthermore, the study has also investigated the antioxidant vitamins in the extracts and showed significant levels of these vitamins as shown in **Figure 4**. These vitamins act as direct

free radical scavengers and play critical roles in regenerating cellular antioxidants such as glutathione (Sies, 2019). These findings underscore the therapeutic relevance of *G. Senegalensis* and *F. platyphylla* as potential anticoagulants and in addressing cardiovascular-related diseases.

5. Conclusions

In conclusion, the results showcase the potentials of *G. Senegalensis* and *F. platyphylla* methanol leaf extracts as anticoagulant and antioxidant agents. And result also showed that both plants have significant beneficial effects in scavenging reactive oxygen species. In addition, absence of any reported toxicity cases against human cells could reinforce their therapeutic potential. Thus, this study has shown the potential of these plants as a new source of bioactive molecules for therapeutic purposes. The research could further recommend more studies on the active phytochemical through isolation and characterisation, molecular docking and in vivo studies using cell lines.

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