ISSN 2955-1226 (Print)
ISSN 2955-123(Electronic)

Tropical Journal of Phytochemistry & Pharmaceutical Sciences

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

Original Research Article

Inhibitory Effects of n-Hexane Extracts of *Sida acuta* and *Aspilia africana* Leaves on Platelet Aggregation

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ABSRTACT

Platelet hyperactivity significantly contributes to the pathogenesis of arterial thrombosis and atherosclerosis. The primary objective of this investigation was to assess the antiplatelet activity of the n-Hexane leaf extract of *Sida acuta* and *Aspilia africana* leaves using in vitro experiment. Standard analytical procedures were employed using UV-Vis spectrophotometric method. Three concentrations (0.5mg/ml, 2mg/ml and 4mg/ml) of *Sida acuta* and *Aspilia africana* leaf extracts were utilised. Aspirin (2mg/ml) and distilled water serving as positive and negative controls, respectively. Platelet aggregation was induced using 10% w/v Calcium chloride. The findings demonstrated that the n-Hexane leaf extract of *Sida acuta* exhibited a statistically significant antiplatelet activity of 55.00 ± 0.46, 55.45 ± 0.91 and 55.30 ± 0.95 while *Aspilia africana* extract displayed antiplatelet activities of 47.64±0.65, 51.41±1.13, and 51.74±0.29 at concentrations of 0.5mg/ml, 2.0mg/ml, and 4.0mg/ml, respectively, surpassing the antiplatelet activity of Aspirin, which was measured at 39.83%. The findings suggest that the extracts of the leaves of *Sida acuta* and *Aspilia africana* possess significant antiplatelet activity.

Keywords: *Sida acuta*, *Aspilia africana*, platelet, aggregation, Aspirin, Inhibitory

Received 20 September 2023
Revised 13 November 2023
Accepted 30 January 2024
Published online 01 February 2024

Introduction

Stroke claims the lives of around six million individuals globally yearly,[1] while an additional five million individuals suffer from permanent disabilities due to it.[1] The Ministry of Health in Nigeria has acknowledged stroke as a significant health concern, being the primary cause of mortality. It has been determined that stroke-related deaths occur at a rate of one every nine minutes. [2] Stroke, which is sometimes referred to as a cerebrovascular accident, is characterized by the interruption of blood flow to the brain as a result of the rupture or blockage of one or more blood arteries caused by a clot. [3] Thrombus and Embolus are two distinct forms of blood clots that can lead to the occurrence of a stroke. A thrombus refers to the formation of a blood clot within a blood vessel, where it remains localized.

Conversely, an embolus denotes a blood clot that dislodges from its original place and migrates to a different location within the body.[3] The hemostatic plug expands into a thrombus through platelets additions and the integration of a fibrin. This process is typically regulated under normal physiological conditions, and the thrombus finally undergoes fibrinolytic disintegration once its hemostatic role is fulfilled However, in pathological situations such as rupturing of atherosclerotic plaque, previously mentioned vital process of hemostasis undergoes a deviant and intensified transformation, culminating in the formation of a blockage-inducing blood clot known as an occlusive thrombus.

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Citation: Sunday CC, Chimdi EE, Chinyere OH, Olachi OL, Chinweuba NG. Inhibitory Effects of n-Hexane Extracts of *Sida acuta* and *Aspilia africana* Leaves on Platelet Aggregation. Trop J Phytochem Pharm. Sci. 2024; 3(1):131-135. http://www.doi.org/10.26538/tjpps/v3i1.3

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

This detrimental occurrence can ultimately lead to life-threatening conditions like acute myocardial infarction and ischemic stroke.[4] At present, there exist three distinct classifications of pharmaceutical agents that exert an influence on the circulatory system, hence serving as a means of stroke prevention. These categories include anticoagulants, thrombolytics, and antiplatelets, as identified.[5] Platelets, which are little disc-shaped blood cells, are generated within the bone marrow. Thrombocytes, alternatively referred to as platelets, are also recognized by this nomenclature. Platelets undergo aggregation at the site of a wound, so forming a cohesive mass that serves to restrict blood loss, ultimately facilitating the process of wound healing. Platelet aggregation refers to the phenomenon wherein platelets in the bloodstream adhere and aggregate, resulting in their clumping together. Platelet aggregation is a crucial step in the series of physiological processes that culminate in the development of a thrombus or clot, as documented.[5]

Platelets are essential components in the hemostatic process, as they contribute significantly to blood coagulation. The aggregation of platelets represents a critical stage in the production of blood clots, which effectively halt bleeding.[6] Nevertheless, there is a correlation between elevated platelet count, also known as thrombocytosis, and specific pathological states like cancer, persistent infections, and some hematological disorders. The potential consequence of this phenomenon is an elevated propensity for the creation of blood clots[7], which in turn may lead to the occurrence of stroke, also known as a cerebrovascular accident. This is mostly attributed to the obstruction caused by the clot, which restricts the supply of blood to the brain.[8] Antiplatelet medications, also known as platelet aggregation inhibitors, are pharmacological agents that effectively suppress the process of platelet aggregation. As stated, aspirin, which serves as a representative example of nonsteroidal anti-inflammatory medications (NSAIDs), is widely recognized as an effective medication for inhibiting platelet aggregation.[9] The impact of Aspirin on platelet function is to induce a permanent deactivation of the essential platelet enzyme, cyclooxygenase (COX). The reversal of this effect can only be achieved through the production of new platelets.[10] Numerous studies have

demonstrated that platelet aggregation inhibitor medications, including Aspirin and other nonsteroidal anti-inflammatory medicines, have been associated with harmful effects on the gastrointestinal and central nervous systems, such as ulcerations[5] the aforementioned issue prompted researchers to conduct an investigation into potential novel sources of antiplatelet medicines.[11]

Nigeria possesses a diverse array of ethno-pharmacological plants, one of which is the subject of research: *Sida acuta. Sida acuta*, often known as common wireweed as seen in fig. 1, Stubborn grass, Isekotu in Yoruba, Udo in Igbo, Kalkashin kwado in Hausa, is a shrub that is prevalent in many regions of Nigeria.[12] However, it is originally native to Central America and is widely distributed in tropical nations. *S. acuta* exhibits a wide distribution over many soil types, with the exception of clay soils subject to seasonal flooding or those produced from limestone.[13] The plant species exhibits strong competitive behavior when compared to other plants, however, it thrives most effectively in places characterized by tropical or sub-tropical climates that have a clearly defined wet and dry season.[14] In majority of locations, this plant is considered a weed.[14] Traditional herbal practitioners use *sida acuta* for the treatment of various ailments such as malaria, fever, headache, infectious disorders, and rheumatism, among other conditions.[15] Additional ethnobotanical applications of *Sida acuta*, encompass its anti-inflammatory, anticancer, anti-diarrheal, antibacterial, antiulcer, and wound healing attributes.[16,11,17,18,19]

Aspilia Africana commonly called wild sunflower as shown in fig 2, yun yun in Yoruba, mfomfo in Igbo, has been recognized as a potent medicinal plant in the African folk medicine. *Aspilia africana* is classified within the family *Compositae.* The plant in question is a semi-woody herbaceous species that originates from a perennial source and can reach a maximum height of 2 metres. The geographic range of this species encompasses both savannah and forested areas within the wasteland, as well as several places within equatorial Africa. A diverse array of plant constituents, including the root, leaf, stem, or the entire plant, can be employed as herbal treatments, either in the form of desiccated materials or unrefined extracts. Hemostasis is frequently utilized as a means to arrest hemorrhaging, particularly in instances where arterial injury has occurred. Moreover, it promotes rapid healing of wounds and sores, and is employed in the management of cardiovascular disorders. [22] Previous studies have indicated that this particular intervention exhibits the capacity to effectively address ocular opacities, enhance the labour process, and offer therapeutic advantages to those afflicted with anaemia and various gastrointestinal disorders.[23] Aspilia africana has been employed in the treatment of Rheumatism pain, [24] as well as for the mitigation of symptoms arising from bee and scorpion stings. [25] In the context of Kenya, the utilization of this intervention is employed for the purpose of eliminating intestinal parasites. Likewise, within the context of Uganda, it is employed for the therapeutic management of gonorrhoea.[26] .The utilization of root decoction and leaf decoction has been observed in several African nations for treating tuberculosis and febrile headache.[27] The utilisation of *Aspilia Africana* leaves for wound healing, skin disease treatment, and eye infection management has been documented in Nigeria.[28] The study conducted.[28] provided evidence supporting the efficacy of root decoction as a treatment for lumbago and sciatica neuralgia. Additionally[30] indicated that the administration of root decoction contributes to the maintenance of membrane stabilization. The decoctions from *Aspilia africana* are *utilised* as an enema for pregnant women both before and during birth.[31] Additionally, these decoctions are employed as a tonic and are effective in eliminating slimy substances from snails during the process of food preparation.

Nevertheless, a comprehensive assessment of the platelet aggregation activity of these plants has not been conducted. This study aims to assess the percentage of aggregation inhibition by analyzing the changes in absorbance readings of proteins released during platelet aggregation, as well as determining the median concentration effective on the *Sida extract* and *Aspilia Africana* acuta leave extracts on blood platelet aggregation.

Materials and Methods

Collection and identification of plant material

Fresh leaves of *Sida acuta* and *Aspilia africana* were procured from Uturu, in Isiukwuato Local Government Area of Abia State. The leaves were identified and authenticated in the Department of Forestry and Wildlife of Michael Opara University of Agriculture, Umudike, Nigeria in October 2022. Voucher numbers, MOUAU/VPP/046/08/2022 and MOUAU/VPP/048/08/2022 respectively were assigned.

Preparation of extracts

Fresh leaves of *Sida acuta* and *Aspilia africana* were obtained and air dried. They were ground into fine powders, and stored in a sterile and dry container. A total of 30 grammes (30g) of dried leaves from the plants were measured using an electronic weighing balance. Extraction was carried using a Soxhlet Extraction unit, employing n-hexane as the solvent at a temperature of 30˚C. The process of extraction was done severally to obtain the desired extract. The solvent n-Hexane was then evaporated with a water bath till the resulting extract was devoid of any traces of n-hexane. The extracts were labelled and stored in a refrigerator.

Preparation of Test Solutions

A volume of 0.5 mL of the plant extracts were combined with two drops of Tween 80 and thoroughly stirred until homogenized. To obtain a solution of 4mg/ml concentration, a mixture was prepared by adding 10ml of distilled water to a 10ml volume of calcium chloride. A 2 mL aliquot was collected from the initial concentration and subsequently diluted with 2 mL of distilled water, resulting in the concentration of 2 mg/mL. A 0.5 mL aliquot was obtained from the second concentration and subsequently diluted with distilled water to yield the concentration of 0.5 mg/mL.

Figure 1: *Sida acuta* leaf (Wireweed)

Figure 2: *Aspilia africana* leaf (Wild Sunflower)

Preparation of Controls
A 2 mg/mL solution of Aspirin was made by dissolving 300 milligrams of Aspirin in 150 mL of distilled water using a volumetric flask. Distilled water served as negative control for analysis in spectrophotometer with 10ml of it not treated.

Blood collection
A total of five (5) healthy adult individuals who did not exhibit any digestive, coagulation, or cardiovascular issues were chosen as blood donors for this study. The participants reported no prior history of smoking, alcohol drinking, or medication or antioxidant usage for a minimum duration of six months. Additionally, their blood group was confirmed to be O+. The blood was collected at Abia State University Medical Centre. A total volume of 20 mL of blood was obtained from each participant using the venipuncture method with their consent. Subsequently, the collected blood was transferred into separate dry serum vials.

Ethical approval
All subjects gave their informed consent for inclusion before they participated in the study. The research project received ethical approval from the Department of Biochemistry at the Federal University of Technology, Owerri, Nigeria. The approval was granted by the Ethical Committee on Human Research under the reference number FUT/SOBS/BCH/COM.2/013/2022. The study adheres to the principles outlined in the Helsinki Declaration for medical research involving human subjects

Spectrophotometric analysis
A total of six aliquots of platelet suspensions, each measuring 3ml, were placed into individual test tubes. [35] Subsequently, test tubes underwent incubation at a temperature of 37˚C for a duration of 5 minutes. For each solution, a volume of 2ml of prostagladdin E_1 (PGE_1) was added to the three test concentrations, as well as to both Aspirin, which is the positive control and the distilled water which is the negative control with the exception of one solution which remained untreated. The test tubes were incubated at a temperature of 37˚C for 30 minutes. Subsequently, platelet aggregation was stimulated by the addition of 0.25M calcium chloride (0.08ml). The test tubes had an additional incubation period of 30 minutes. Following the induction of platelet aggregation, a volume of 0.5ml blood aliquot was taken from the residual liquid components and subsequently transferred into a separate set of test tubes. To create a 20.5ml solution, 20ml of distilled water was added to each of the liquid components, resulting in dilution. The analysis was conducted using a volume of 4ml for each solution. The samples were put into cuvettes and subsequently positioned within the spectrophotometer. The quantification of aggregated platelets was performed at a wavelength of 810nm.

Mean absorbance reading
The experiment yielded mean absorbance measurements for each concentration tested in every trial. The objective of this experiment was to calculate the mean absorbance values for both the test solutions and the control.[35]

$$\text{Mean Absorbance Reading} = \frac{\text{Sum of absorbance reading of test solution}}{\text{Number of trials performed}}$$

Percentage inhibition of platelet aggregation
The mean absorbance readings were then used to calculate the percent antiplatelet activity with the following formula;

$$\%\ \text{inhibition} = \frac{\text{Absorbance untreated} - \text{Absorbance treated}}{\text{Absorbance untreated}}$$

Results and Discussion

The effect of *Sida acuta* and *Aspilia africana* leaves extracts on platelet aggregation are presented in tables 1 and 2, respectively. The results of the analyses were reported as the mean±standard deviation. one-way analysis of variance (ANOVA) was assessed for the differences among the samples. Statistical significance was established using a predetermined level of significance. With a significance level of $p < 0.05$. The experimental procedure was conducted three times. The results indicate that the percentage antiplatelet aggregation activities at $P < 0.05$ is significantly different. Distilled water, Aspirin, and different concentrations (0.5mg/ml, 2.0mg/ml, and 4.0mg/ml) of *Sida acuta* were 33.94 ± 1.38, 39.83 ± 1.83, 55.00 ± 0.46, 55.45 ± 0.91, and 55.30 ± 0.95, respectively. Similarly, the percentage antiplatelet aggregation activities of distilled water, Aspirin, and different concentrations (0.5mg/ml, 2.0mg/ml, and 4.0mg/ml) of *Aspilia africana* were 40.72 ± 0.57, 24.43 ± 1.35, 47.64 ± 0.65, 51.41 ± 1.13, and 51.74 ± 0.29, respectively. Medicinal plants possess significant potential as sources of primary chemicals that can be further explored and refined for the purpose of developing novel pharmaceuticals.[32] Platelet aggregation, a phenomenon where platelets bind to one another at the site of injury, was not observed using the plant extracts. Numerous invitro studies have been recorded in the European pharmacopoeia (EP) elucidating the antiplatelet aggregation efficacy of these plants. [33] This study aimed to explore the antiplatelet aggregation activity of *Sida acuta* and *Aspilia africana* at different doses, in comparison to Aspirin, which served as the reference drug. The assessment was conducted using whole blood samples obtained from human subjects. The findings demonstrated at statistically significant value ($P \leq 0.05$) the percentage of antiplatelet activity exhibited by the plant samples at concentrations of 0.5mg/ml, 2.0mg/ml, and 4.0mg/ml, surpassing the effects observed with Aspirin and pure water. The identification of various botanical compounds, including alkaloids, sterols, steroids, indoles, and phenols, in the leaf extract of *S. acuta*, as reported.[34]In addition to their pathogenic function, blood platelets play a pivotal part in the etiology of certain cardiovascular conditions, including arterial hypertension, atherosclerosis, and consequent ischemia. The investigation of *Sida acuta* through phytochemical analysis has yielded findings indicating the existence of polar compounds.[34] These chemicals play a significant role in the antiplatelet activity exhibited by the plants.

Table 1: Percentage platelet aggregation activity of *Sida acuta* leaf extract

Concentration	Percentage (%) Antiplatelet activity
Untreated	0.22 ± 0.00
Distilled water	33.94 ± 1.38[a]
Aspirin	39.83 ± 1.83[b]
Sida acuta (0.5 mg/ml)	55.00 ± 0.46[c]
Sida acuta (2.0 mg/ml)	55.45 ± 0.91[d]
Sida acuta (4.0 mg/ml)	55.30 ± 0.95[c]

Values are Mean ± Standard Deviation (SD) for N = 3. Values on the column bearing the different superscripts are significantly different ($P \leq 0.05$) from each other.

Table 2: Percentage platelet aggregation activity of *Aspilia africana* leaf extract

Concentration	Percentage (%) Antiplatelet activity
Distilled water	40.72 ± 0.57[a]
Aspirin	24.43 ± 1.35[b]
Aspilia africana (0.5 mg/ml)	47.64 ± 0.65[c]
Aspilia africana (2.0 mg/ml)	51.41 ± 1.13[d]
Aspilia africana (4.0 mg/ml)	51.74 ± 0.29[d]

Values are Mean ± Standard Deviation (SD) for N = 3. Values on the column bearing the different superscripts are significantly different ($P \leq 0.05$) from each other.

Recent research has identified three potential methods by which the augmentation of calcium levels can have anti-platelet effects such as suppressing platelet granule release, suppressing thromboxane A2 synthesis activation, and GP IIb/IIa receptor activity suppression. [35,36] Aspirin, which serves as a positive control in both experimental procedures, exhibits inhibitory effects on the synthesis of thromboxane A2. According to the findings of this study, it has been shown that the n-Hexane extract derived from the leaves of *Aspilia africana* exhibits superior anti-platelet action compared to aspirin, which was used as the reference medicine, when administered at the levels specified in this particular investigation. Flavonoids are a significant component of *Aspilia africana* and play a substantial role in the pharmacological properties exhibited by *Aspilia africana.*[37] Studies has shown that phytochemical analysis of leaves of *Aspilia africana* revealed alkaloids, tannins, saponins, flavonoids, phenols, and steroids.[38] Several studies have demonstrated that alkaloids have the highest concentration, followed by saponins, flavonoids, and tannins.[38,27] Certain phytochemicals, particularly flavonoids, may have had a role in the observed inhibitory impact of *Aspilia Africana* on platelet aggregation. This observation aligns with prior research that has demonstrated the in vitro and in vivo anti-platelet effects of flavonoids. [39,40,41]The distilled water was employed as a negative control in order to assess whether the solvent had any impact on plant solution having anti-platelet action. In addition, the control which was not treated was employed solely as a reference point to determine the upper limit of protein release by platelets in the absence of any inhibitory factors from test solutions or controls.[42]

Conclusion

This study has demonstrated that n-Hexane extract of *Sida acuta* and *Aspilia African* leaves could inhibit *in vitro* platelet aggregation. Results from this study support the hypothesis that the dietary intake of *Sida acuta* and *Aspilia africana* may be beneficial in normalizing platelet hyper activation in prevention of cardiovascular diseases and are capable of arresting wound bleeding and healing process.

Conflict of Interest

The authors declare no conflict 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.

Acknowledgments

I sincerely thank staff and lecturers of the Department of Biochemistry FUTO, for enabling us to conduct this research in their laboratory and for their tremendous efforts in seeing that this research work was completed

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