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Phytochemical Profile and *In vitro* Anti-Inflammatory Potentials of Aqueous and Ethanol Root Extracts of *Chrysophyllum albidum*

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ABSTRACT: The earth harbours a rich source of medicinal plants which are used in the treatment of various ailments. Aqueous and ethanol extracts of *Chrysophyllum albidum* root were assayed for their phytochemical content and *in vitro* anti-inflammatory activity using standard methods. The total phenolic, flavonoid and proanthocyanidin contents were quantified. *In vitro* anti-inflammatory activity was evaluated using membrane stabilization, proteinase inhibitory activity and protein denaturation assay at various concentrations. The total phenolic content of the aqueous extract was 0.061 ± 0.001 mg gallic acid equivalent/g of extract while ethanolic extract was 0.087 ± 0.001 mg quercetin equivalent/g extract. Total flavonoid content of aqueous extract was 0.131± 0.01 mg quercetin equivalent/g extract while ethanol extract was 0.042 ± 0.001 mg quercetin equivalent/g extract. Proanthocyanidin content of the aqueous extract was 0.769 ± 0.01 mg Ascorbic acid equivalent/g extract while the ethanol extract was 1.615 ± 0.001 mg Ascorbic acid equivalent/g extract. The results showed a dose-dependent significant inhibition in all the *in vitro* anti-inflammatory assays. Ethanol root extract significantly exhibited the best anti-inflammatory capacity in membrane stabilization and proteinase inhibition when compare to the aqueous extract. While the aqueous extract significantly inhibited protein denaturation when compared to the ethanol extract. The study reveals that *Chrysophyllum albidum* possessed a number of bioactive molecules (phenols, flavonoids and proanthocyanidin) and significantly demonstrated anti-inflammatory activity at various concentrations compared with standard drug.

Keywords: *Chrysophyllum albidum*, Phytochemical, Anti-inflammatory activity, *Chrysophyllum albidum*

Introduction

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as the increases in vascular permeability and protein denaturation as well as membrane alteration (Anoop and Bindu, 2015). The events of inflammation that underline these manifestations are induced and regulated by a large number of chemical mediators, including kinins, eicosanoids, complement proteins, histamine and monokines (Arya and Arya, 2011). Although medicinal plants have provided biologically relevant products for centuries, they still serve as a source of new medicines (Fermino *et al.*, 2015). They have roles in the protection of human health and more than 4,000 phytochemicals have been cataloged and are classified by their protective function, physical and chemical characteristics (Meagher *et al*., 1999). Plants still symbolize a huge unexploited reservoir of structurally novel compounds that might serve as a guide for the development of innovative and valuable drugs, with wide margin of safety (Harvey *et al*., 2015). It is quite well recognized that corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) are the mainstream treatments for inflammation (Kidd, 2006). Moreover, NSAIDs have been demonstrated to have significant cardiovascular, renal and gastrointestinal toxicity that make them contraindicated in some cases, hence, discontinued as a result of their adverse effects (Krijthe *et al*., 2014)

Chrysophyllum albidum (*C. albidium*), commonly called white star apple or African star apple, and belonging to the family of Sapotaceae (which has up to 800 species) is a lowland rain forest tree species that grows between

25 to 37 m in height at maturity with a girth varying from 1.5 to 2 m (Ogunleye *et al*., 2020). It is common throughout the tropical Central, East and West Africa regions and other parts of the world (Ogunleye *et al*., 2020). *C. albidium* is an edible tropical fruit known by various tribal names. It is called *Utieagadava* in Urhobo, *agbalumo* in Yoruba, *udara* in Ibo, Efik and Ibibio, *ehya* in Igala, *agwaluma* in Hausa and *azongogwe* or *azonbobwe* in Benin tribes of Nigeria (Emudainohwo *et al.*, 2015). Its scientific classification include; Kingdom: Plantea Division: Angiosperm Class: Eudicots Order: Ericales Family: Sapotaceae Genus: Chrysophyllum Species: *C. albidum* (Emudainohwo *et al.*, 2015). In folklore medicine, *C. albidum* bark is employed for the treatment of yellow fever and malaria (Emudainohwo *et al.*, 2015). The roots, barks and leaves are widely used to treat sprains, bruises and wounds in southern Nigeria (Emudainohwo *et al*., 2015). Other ethnopharmacological uses include; pro-clothing, wound healing, anti-microbial, anti-inflammatory and antiplasmodial activities, and for the treatment of stomach ache and diarrhoea (Ogunleye *et al*., 2020; Adeogun and Kolawole, 2024). There is limited research on the phytochemical and anti-inflammatory properties of the aqueous and ethanol extracts of the root of *C. albidum*. Therefore, this study seeks to evaluate the phytochemical and *in vitro* anti-inflammatory potentials of aqueous and ethanol root extracts of *Chrysophyllum albidum*.

Materials and methods

Collection of plant: *C. albidum* was collected from a private farm in Benin City, Nigeria and authenticated in the Department of Plant Biology and Biotechnology, University of Benin where a voucher specimen number UBH-C362 was deposited.

Preparation of plant material: The roots were washed and dried at room temperature in the laboratory. The sample was pulverized and 250 g (in two portions) were soaked in 2.5 litres of distilled water and 2.5 litres of ethanol separately for 72 hours. The suspension was filtered and the filtrate was concentrated using a rotary evaporator and a freeze drier. The dried extracts were stored in air tight containers until needed for further experimentations.

Determination of total phenolic content: The total phenolic content was determined using the Folin - Ciocalteau method as described by Cicco *et al*., 2009. Concentrations of gallic acid, ranging from 0.2 - 1 mg/mL or extracts (1mg/mL), were prepared in methanol. Then, 4.5 mL of distilled water was added to 0.5 mL of the extract and mixed with 0.5 ml of a ten-fold diluted Folin- Ciocalteau reagent. Five milliliters of 7% sodium carbonate was then added to the tubes and another 2 ml of distilled water was added. The mixture was allowed to stand for 90 min at room temperature and absorbance was read at 760 nm. All determinations were performed in triplicates with gallic acid utilized as the positive control. The total phenolic content was expressed as Gallic Acid Equivalent (GAE).

Determination of total flavonoid content: Total flavonoid content was determined using the method described by Ayoola *et al.*, (2008). Two milliliters mL of 2% $AlCl_3$ in ethanol was added to 2 mL extracts. A concentration of 1 mg/mL of the extract prepared in methanol was used. Similar concentrations of quercetin, the standard control, were used. The absorbance was measured at 420 nm after 1 h incubation at room temperature. The total flavonoid content was expressed as quercetin equivalent (QE).

Determination of proanthocyanidin content: The determination of proanthocyanidin was carried out according to the method of Sun *et al*. (1998). To 0.5 mL of 1.0 mg/mL of each extract was added 1mL of 4 % methanol solution and 0.75 mL of concentrated hydrochloric acid. The mixture was left undisturbed for 15 min and the absorbance was read at 500 nm. The total proanthocyanidin content was expressed as ascorbic acid equivalent (AAE).

***In vitro* Anti-infammatory Assay**

Inhibition of protein denaturation: The anti-inflammatory activity of aqueous and ethanol extracts was evaluated according to the method described by Sakat *et al*. (2010), Saleem *et al*. (2011) and Anigboro *et al*. (2024) with slight modifications. The reaction mixtures contained egg albumin (0.2 mL, 10% v/v freshly prepared with phosphate bufer), phosphate-bufered saline (2.8 mL; pH 6.4), and the various extracts (2.0 mL; 100–1000 µg/ mL) replaced with aspirin/distilled water for standard/negative control, respectively. The mixtures were incubated at 37 °C for 15 min, before heating in a water bath at 70 °C for 5 min to induce denaturation, after which the absorbance readings of the solutions were measured at 660 nm. The percentage inhibition of protein denaturation was calculated using the formula below:

$$\% \text{ Inhibition} = 100 - [(A_1 - A_2)/A_0 * 100]$$

where A_1 is the absorbance of the sample,
A_2 is the absorbance of the product control and
A_0 is the absorbance of the positive control.

Membrane stabilization

Preparation of red blood cells (RBCs) suspension: RBC suspension was prepared according to Sadique *et al.* (1989) and Sakat *et al.* (2010). Blood was collected from a healthy human volunteer into centrifuge tubes. The tubes were centrifuge at 3000 rpm for 10 minutes and washed three times with equal volume of normal saline. The volume of blood was measured and re-constituted as 10% v/v suspension with normal saline.

Heat induced haemolysis: This was done according to the method described by Oyedapo and Famurewa (1995) and Sakat *et al.* (2010). The reaction mixture (2mL) consisted of 1mL test sample of different concentration (100-1000 µg/mL) and 1 mL of 10% RBCs suspension. Normal saline was added to the control tubes in place of the test sample. Aspirin was used as standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30 min. At the end of the incubation the tubes were cooled under running water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatant was be taken at 560 nm. The experiment was performed in triplicates for all the test samples. The percentage inhibition of haemolysis was calculated as follows:

$$\text{Percentage inhibition} = (\text{Abs control} - \text{Abs Sample}) \times 100/\text{Abs control}$$

Hypotonicity – induced haemolysis: This was done according to Oyedapo *et al.* (2004) and Azeem *et al.* (2010). Different concentration of extract (100-1000µg/mL), reference sample, and control were separately mixed with 1 mL phosphate buffer, 2 mL hyposaline and 0.5 mL human red blood cell suspension. Aspirin (1000 µg/mL) was used as a standard drug. All the assay mixtures were incubated at 37°C for 30 min and centrifuged at 3000 rpm. The supernatant was decanted and the haemoglobin content was estimated at 560 nm. The percentage hemolysis was estimated by assuming the haemolysis produced in the control as 100%.

$$\text{Percentage protection} = 100 - (\text{OD sample}/\text{OD control}) \times 100$$

Proteinase inhibitory activity: Inhibition of protein denaturation was evaluated by the method of Sakat *et al.* **(**2010) and Saleem *et al.* **(**2011). Control solution (50 mL): 2 mL of egg albumin, 28 mL of phosphate buffer (pH 6.4) and 20 mL distilled water. Standard drug (50 mL): 2 mL of egg albumin, 28 mL of phosphate buffer and various concentration of standard drug (Aspirin) concentration of 100, 200, 400, 600, 800 and 1000 µg/mL. Test solution (50 mL): 2 mL of egg albumin, 28 mL of phosphate buffer and various concentration of plant extract concentration of 100, 200, 400, 600, 800 and 1000 µg/mL. The samples were incubated at 37°C for 15 min and heated for 5 min at 70°C. After cooling, the absorbance of the above solution was taken at 660 nm and percent inhibition of protein denaturation was calculated using:

$$\% \text{ Inhibition} = (A_t / A_c - 1) \times 100$$

where A_t is the absorbance of the test sample, A_c is the absorbance of the control.

Data analysis: Data analysis was performed using GraphPad Prism version 8.0. The results were presented as mean ± SEM of three determinations. The differences between samples were analyzed by one-way analysis of variance (ANOVA). Duncan's post-hoc test was performed to determine statistical significance at $p<0.05$. Two-way ANOVA was used where applicable.

Results and Discussion

The total phenolic content (Figure 1) showed that the ethanol extract exhibited a higher phenolic content. This indicates a greater extraction efficiency of phenolics using ethanol compared to the aqueous extract. Plants owe their medicinal properties to the presence of bioactive metabolites (Akinpelu *et al*., 2018; Adeogun *et al*., 2019). These values are relatively lower compared to the ethanol root extract of ginger which exhibited 48.56 mg GAE/g in ethanol extract (Adefegha *et al*., 2016), while Ahmed *et al* (2019) reported 48.56 mg GAE/g dry weight in ethanol extract of ginger. Also, turmeric root ethanol extract exhibited phenolic content of 82.47 mg GAE/g dry weight (Adefegha *et al*., 2016). Phenolics exhibit a range of biological activities, including anti-inflammatory, antioxidant, anti-tumour and antidepressant effects (Mamta *et al*., 2013).

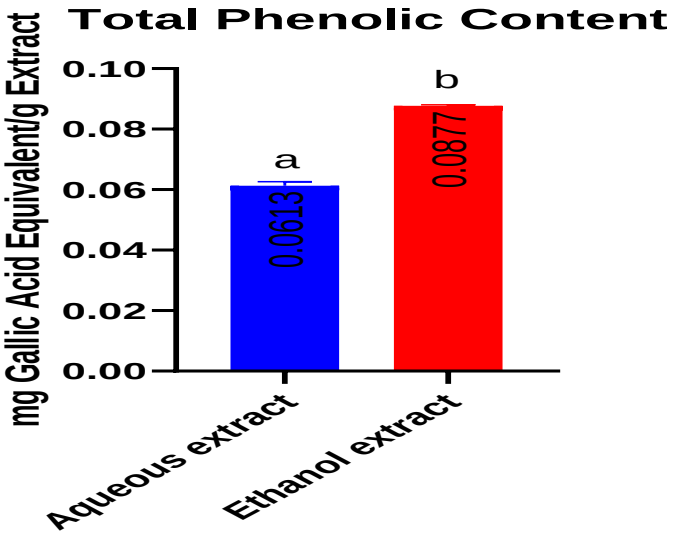

Figure 1: Total phenolic content of aqueous and ethanol extracts of *Chrysophyllum albidum* roots. Each value represented the mean ± SEM of n = 3 determinations. Different lowercase letters represent significant difference between means at $p < 0.05$

The total flavonoid content (Figure 2) showed that aqueous extract exhibited higher flavonoid content. The flavonoid content of the aqueous extract of *C. albidum* roots in this study was higher than the flavonoid content of aqueous turmeric extract (0.006 mg QE/g) reported by Aktar *et al* (2019), while the ethanol extract of turmeric (0.377 mg QE/g) (Ghasemzadeh and Jaafar, 2011) was higher than the flavonoid content of the ethanol extract of *C. albidum* observed in this study.

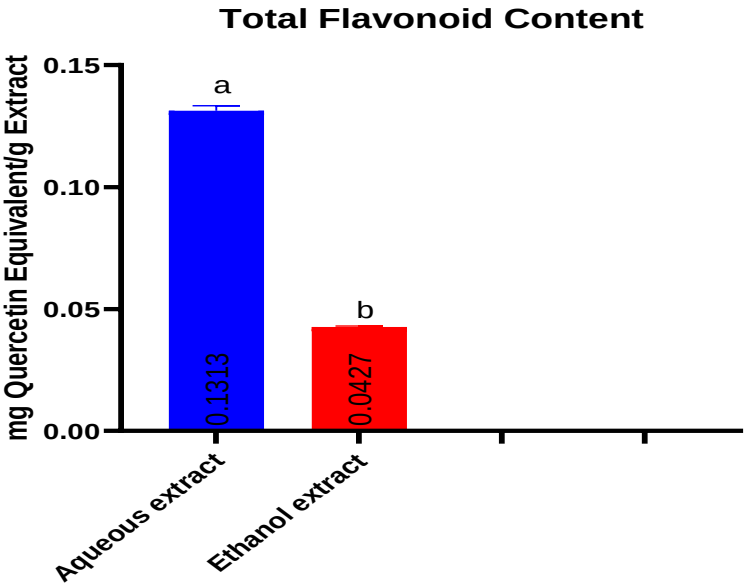

Figure 2: Total flavonoid content of aqueous and ethanol extracts of *Chrysophyllum albidum* roots. Each value represented the mean ± SEM of n = 3 determinations. Different lowercase letters represent significant difference between means at $p < 0.05$

Flavonoids are an important class of natural products. Flavonoids are associated with a broad spectrum of health-promoting effects and are an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is because of their antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme functions (Adetutu *et al*., 2015; Panche *et al*., 2016).

Proanthocyanidin content (Figure 3) showed that the ethanol extract exhibited significantly ($p<0.05$) high proanthocyanidin content compared to the aqueous extract.

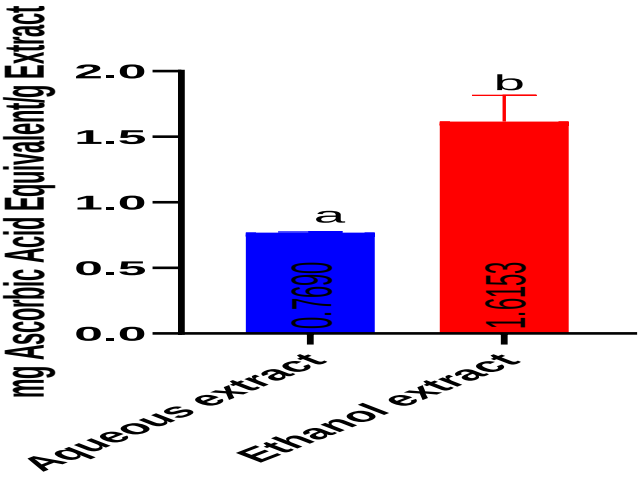

Figure 3: Total proanthocyanidin content of aqueous and ethanol extracts of *Chrysophyllum albidum* roots. Each value represented the mean ± SEM of n = 3 determinations. Different lowercase letters represent significant difference between means at $p < 0.05$.

This suggest that ethanol is a more effective solvent for extracting proanthocyanidin from *C. albidum* roots. This is consistent with studies showing that ethanol is effective solvents for extracting proanthocyanidin (Lishan *et al*., 2024). Proanthocyanidin serves multiple crucial functions including scavenging free radicals thereby mitigating oxidative stress (Yildrim *et al*., 2015; Xie *et al*., 2022).

Figure 4 depicted the inhibition of heat-induced haemolysis on red blood cell membrane, which was dose-dependent for both extracts. From the results, ethanol extract of *C. albidum* was the most effective in stabilizing red blood cell membrane compared to the aqueous extract. Also, Figure 5 revealed the inhibition of hypotonicity-induced haemolysis by the root extracts of *C. albidum* on membrane of human red blood cell. It followed the same pattern as seen in Figure 4. This may be due to the high phenolic and proanthocyanidin content in the ethanol extract compared to the aqueous extract. These two phytochemicals are known for their potent anti-inflammatory activity (Mansoor *et al*., 2016). Erythrocytes have been used as a model by a number of researchers for the study of interaction of drugs with membranes. Membrane stabilizing activity of RBC exhibited by some drugs, serves as a useful *in vitro* method for assessing the anti-inflammatory activity of drug candidates (Aina *et al*. 2013). Also, this result aligns with several reports that ethanol extract of some plant species exhibits significant membrane stabilization activity stronger than their aqueous counterpart (Owolabi *et al*., 2014; Das and Das, 2017; Naz *et al*., 2020).

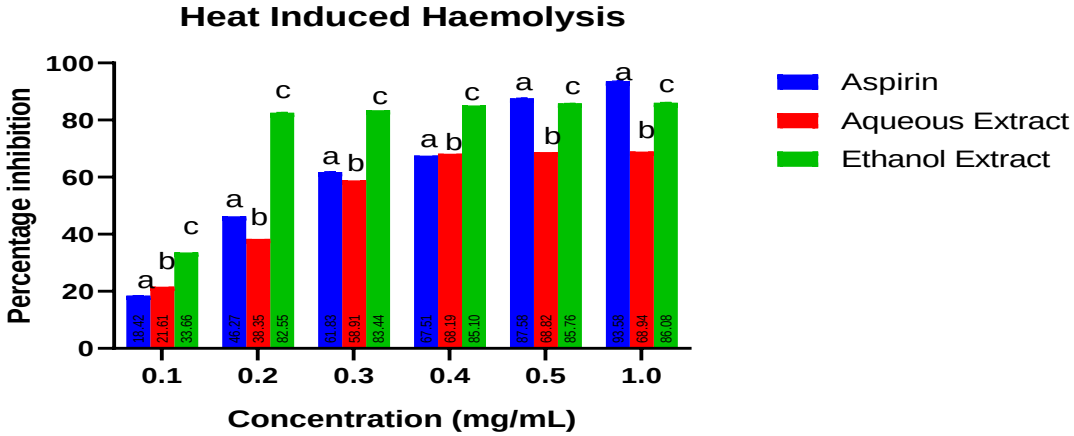

Figure 4: Percentage inhibition of heat-induced haemolysis of aqueous and ethanol extracts of *C. albidum* roots. Each value represented the mean ± SEM of n = 3 determinations. Different lowercase letters represent significant difference between means at $p < 0.05$

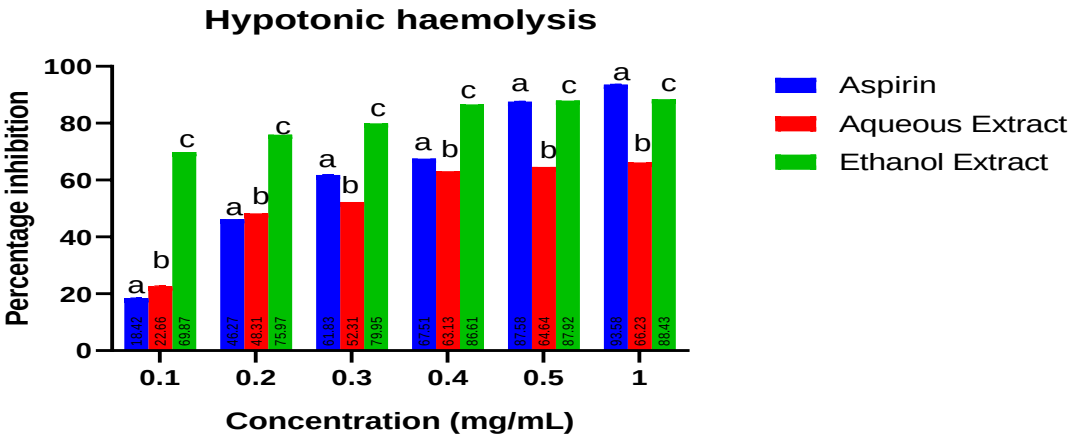

Figure 5: Percentage inhibition of hypotonic-induced haemolysis of aqueous and ethanol extracts of *C. albidum* roots. Each value represented the mean ± SEM of n = 3 determinations. Different lowercase letters represent significant difference between means at $p < 0.05$

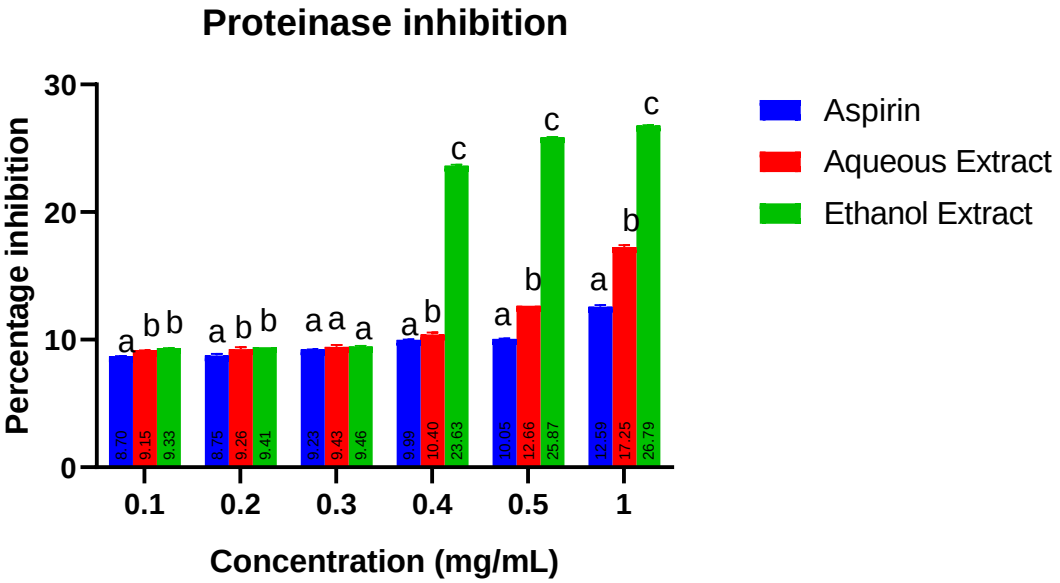

Figure 6: Percentage inhibition of proteinase of aqueous and ethanol extracts of *C. albidum* roots. Each value represented the mean ± SEM of n = 3 determinations. Different lowercase letters represent significant difference between means at $p < 0.05$

From Figure 6, the ethanol extract showed significantly ($p<0.05$) higher proteinase inhibition than the aqueous extract. The ethanol extract contains more flavonoids and proanthocyanidin compare to the aqueous extract. Flavonoids and proanthocyanidin are known as scavengers and inhibitors of lipid peroxidation (Aina *et al.*, 2013). Therefore, the ability of the extract to alleviate oxidative stress could be attributed to the phenolic and proanthocyanidin contents.

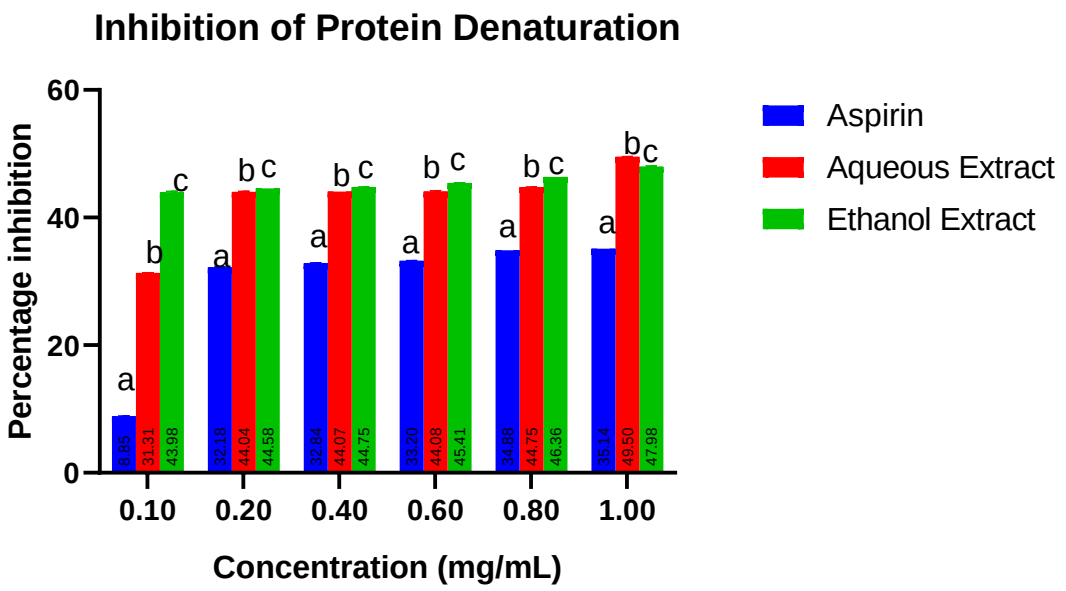

Figure 7: Percentage inhibition of protein denaturation of aqueous and ethanol extracts of *C. albidum* roots. Each value represented the mean ± SEM of n = 3 determinations. Different lowercase letters represent significant difference between means at $p < 0.05$

Furthermore, the aqueous extract exhibited significantly ($p<0.05$) high percentage inhibition of protein denaturation (Figure 7). Percentage inhibition of protein increased with increasing concentration. This may be due the high amount of flavonoid in the aqueous extract. Flavonoid are potent water-soluble free radical scavengers that prevent oxidative cell damage (Panche *et al*., 2016; Adeogun *et al*., 2017; Orumwensodia and Uadia, 2023).

The results from this study revealed the presence of bioactive phytochemicals which may contribute to its medicinal values and thus, its usage as remedy in traditional medicine. Further research is recommended for the isolation of its useful bioactive compounds which can be potential sources of therapeutic agents.

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