A survey of the Bio-activity of the Fruit Rind of Kiwano (*Cucumis metuliferus*).

Abstract

Background: Kiwano, commonly called horned melon is a common fruit consumed in the tropics for its nutritional and therapeutic value. This study evaluated the composition of some bioactive compounds in the rind of the fruit as well as its antioxidant and anti-inflammatory effects using in vitro methods.

Methods: The rind of the fruits of kiwano were peeled out, pulverized and extracted with 80% ethanol. The bioactive compounds evaluated were phenol, flavonoid, beta carotene, lycopene and ascorbic acid. The antioxidant properties were evaluated using DPPH, iron reducing power and inhibition of lipid peroxidation assays. Ascorbic acid served as the standard. The anti-inflammatory property was measured in-vitro using anti-proteinase activity, inhibition of albumin denaturation and membrane stabilization assay systems. Aspirin and diclofenac served as reference drugs

Results: Phenol and flavonoids were the bioactive compounds found in higher concentration than the rest with concentrations of 261.73±13.54 and 130.86±9.66 mgGAE/ml of the dry matter respectively. In the antioxidant assay, the highest activity of more than 50% was recorded in DPPH assay. The maximum percentage radical scavenging activity of the extract was 76.74% at 800 µg/ml with a drop in activity at 1000 µg/ml (75.02%) and EC_{50} value of 230 µg/ml. Ascorbic acid exhibited 96.35% scavenging activity of radicals at 1000 µg/ml and EC_{50} value of 260 µg/ml. For the anti-inflammatory assay, the extract exhibited 16.43% anti-proteinase activity, 25.64% inhibition of heat-induced haemolysis activity and 47.14% inhibition of albumin denaturation activity at 1000 µg/ml. More than 50% activity was recorded for the reference drugs.

Conclusion: We therefore conclude that the rind of the fruit of kiwano is rich in biologically active compounds and has antioxidant and anti-inflammatory properties. The rind is a potential source of therapeutic compounds.

Keywords: Antioxidant; anti-inflammatory; bioactive compound; Kiwano; *Cucumis metuliferus*; rind.

Introduction

Medicinal constituents can be found on any part of a plant. Unfortunately, some parts are commonly consumed while others are discarded. These discarded parts form what is regarded as solid food wastes. The rind of a fruit is the most discarded part. This makes it hard to obtain the full nutritional and therapeutic benefits from the fruits. Most fruits are very rich in important bioactive substances and so, removing part of the fruit reduces the health benefits that are contained in them. Apart from loss of some health benefits, fruit rinds also contributes to environmental pollution causing nuisance to the environment. This practice of littering the environment with fruit rinds destroys the aesthetics of the environment. More so, it can breed insect which are disease-causing agents leading to different forms of illnesses in the populace. Nowadays, consumption of fruits have significantly increased due to increase in awareness of the nutritional and therapeutic usage of fruits. This of course will increase production of waste in form of fruit rinds. For effective management and proper utilization, there should be a way of channeling these solid food wastes properly. This could be in form

of subjecting them to various scientific research in order to identify their nutritional and therapeutic properties as well as validate their usage. They can also be useful and recycled in industries such as textile, cosmetics and pharmaceutical, where they could form part of the raw materials in manufacturing processes. Majorly, fruits rinds are used often as animal feeds or utilized as manure to increase fertility of the soil. It has been found out and reported that rinds of some fruits contain higher biologically active constituents and hence better therapeutic effects than their pulps [1]. Different fruit rinds/peels have been reported to possess very important nutrients and phytochemicals [2-4]. Some have equally demonstrated therapeutic abilities and properties [5-7]. Some fruit rinds are also used in traditional medicine for treatment of different disease condition [7]. One of such fruit rind is kiwano rind. From past studies [8], it has been reported that kiwano rind contains nutrients such as carbohydrates, dietary fiber, fats, vitamins and various secondary metabolites which play significant roles in management of diseases.

Kiwano (*Cucumis metuliferus*) is an annual climbing herb that grows naturally in the tropics. It is found in Nigeria, South Africa and other tropical areas. It belongs to Cucurbitaceae family [9]. It is commonly called horned melon. The seeds and the pulp are consumed for nutritional and therapeutic purposes. The seeds are ground to powder and used for treatment of parasitic infections. The pulp has been reported to be rich in various nutrients and phytochemicals [9]. The rind which is mostly removed as waste has been shown to contain various nutrients and phytochemicals making it a potential source of therapeutic compounds. Our previous study on the rind showed that it is rich in carbohydrates, fiber, lipids, vitamins and some important phytochemicals [8]. With these information, it is expedient to explore the rind for possible therapeutic use as this will stimulate interest in its utilization and hence, minimize waste in the environment. Therefore, this study was aimed at evaluating the composition of some vital bioactive compounds and further investigate the antioxidant and anti-inflammatory effects of the rind.

Materials and Methods

Chemicals and Reagents

Ethanol, L-ascorbic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin Ciocalteu phenol reagent were obtained from sigma-Aldrich, Co, Ltd, UK. All other reagents and chemicals were of analytical grade and were supplied by the same company.

Plant material collection and preparation

The fruits of kiwano were bought from local markets in Gboko, Benue State, Nigeria. They were authenticated at the Applied Biology and Biotechnology department of Enugu State University of Science and Technology. The fruits were washed with water to remove dirt and then cut open with knife to remove the pulp and the seeds. The rinds were washed to remove any remaining pulp and then air-dried for 2 weeks. The dried sample was pulverized with grinding machine.

Extraction

The ground powder of the sample material (100 g) was soaked in 250 ml of 80% ethanol for 24 h, stirring intermittently with a spatula. It was filtered using Whatman no 4 filter paper. Then, the filtrate was re-soaked in 200 ml of 80% ethanol for 24 h and filtered. The two filtrates were combined together and concentrated by evaporation in a water bath at 40 °C. The extract was stored in an air-tight container at 4 °C in a refrigerator prior to use.

Bioactive Compound Assay

The composition of total Phenol, flavonoids, beta carotene and lycopene were determined according to the method of Barros et al [10] while Ascorbic acid was determined according to the method of Klein and Perry [11].

Antioxidant Assay

DPPH radical scavenging test

This test was carried out according using the stable DPPH radical according to the method of Ebrahimzadem, et al [12]. The DPPH radicals were exposed to radical scavengers in the extract and the standard and the decrease in absorption was measured spectrophotometrically. 2.7 ml of methanolic solution of 100 µM DPPH was added to 0.3 ml of the extract at different concentrations (0-1000 µg/ml). The mixture was shaken and incubated for 60 min after which the absorbance was read at 517 nm against the blank (DPPH). Ascorbic acid served as the reference antioxidant. The percentage radical scavenging activity (RSA) was calculated thus:

$\%RSA = (ADPPH- As)/ADPPH) \times 100.$

ADPPH is the absorbance of DPPH solution while As is the absorbance of the test solution with the sample. The EC50 (effective concentration of sample at 50% RSA) was calculated from the graph of %RSA against the sample concentration.

Reducing Power Assay

The method of Barros et al [10] was used to measure the reductive ability of the extract in comparison with the standard. The test is based on the ability of the sample to reduce Fe^{2+} to Fe^{3+} and the increase in absorbance measured spectrophotometrically. The extract (2.5 ml; 0-

1000 µg/ml) was mixed with sodium phosphate buffer (2.5 ml, 0.2M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%) and the mixture incubated for 20 min at 50 °C. This was followed by addition of Trichloroacetic acid (2.5 ml, 10%) an centrifugation for 8 min at 1000rpm. 5 ml of the supernatant was mixed with deionized water (5 ml) and ferric chloride (1 ml). The optical density (OD) was read at 700 nm. The $OD_{0.5}$ was extrapolated from the graph of OD against the concentration.

Inhibition of lipid peroxidation assay

This was done according to the method of Barros et al [10] using thiobarbituric acid reactive system and brain of a goat as the source of lipid. The brain was homogenized in an ice cold Tris buffer (20 mM) of pH 7.4. 10% w/v of the homogenate was produced. It was centrifuged for 10 min at 3000 g. The supernatant (0.1 ml) was incubated for 1 hr with the extract (0.3 ml, 0-1000 µg/ml), Ferrossulphate (0.1 ml, 10 µM) and ascorbic acid (0.1 ml, 0.1 mM). 0.5 ml of 28% Trichloroacetic acid was added to stop the reaction which was followed by addition of 0.38 ml of 2% thiobabituric acid (TBA). The mixture was heated for 20 min at 80 °C and then centrifuged for 10 min at 3000 g. The absorbance of the supernatant was measured at 532 nm. The % inhibition ration (IR) was calculated thus:

Inhibition ratio (%) = [(A-B)]/A)×100%

A & B are the absorbance of the control and the reaction mixture respectively. The EC_{50} (extract concentration providing 50% lipid peroxidation inhibition) was extrapolated from the graph of percentage inhibition against the extract concentrations. Ascorbic acid was used as standard.

Anti-inflammatory studies

Anti-proteinase action

This assay was carried out using the method described by Oyedepo et al [13] and Sakat et al [14] with some modifications. To 1 ml of the extract (200-1000 µg/ml) was added trypsin (2 ml, 0.06 mg) and Tris HCL buffer (1ml, 20 mM, pH 7.4). The mixture was incubated for 5 min at 37^0C followed by addition of casein (1 ml, 0.8% w/v). The mixture was incubated for 20 min more and then perchloric acid (2 ml, 70%) was added to stop the reaction. The mixture was centrifuged and absorbance of the supernatant read at 210 nm against blank (buffer). Aspirin was used as the standard. Percent inhibition of proteinase activity was calculated thus:

% inhibition = (Abs control –Abs sample) X 100/ Abs Control

Membrane stabilization assay

Heat induced haemolysis of the red blood cell of albino rat was used to evaluate the membrane stabilization ability of the sample extract according to the method of Sakat et al [14].

Preparation of Red blood cells (RBCs) suspension

Blood sample was collected from healthy albino rat that has not received any non-steroidal anti-inflammatory drugs (NSAIDs) for 2 weeks before the assay. The blood sample was transferred into centrifuge tubes and centrifuged for 10 min at 3000 rpm, washed three times

with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.

Heat induced haemolysis

1 ml of the extract of different concentrations (200, 400, 600, 800 and 1000 µg/ml) were mixed with 1 ml of red blood cell (RBC) suspension in centrifuge tubes. The control tube contains the standard mixed with 1 ml of RBC suspension and normal saline. Aspirin was used as reference drug. The tubes were incubated in a water bath for 30 min at 56 °C, cooled under tap water and then centrifuged for 5 min at 2500 rpm. Thereafter, the absorbance of the supernatant was read at 560 nm. Percent inhibition of haemolysis was calculated thus:

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control.

Inhibition of albumin denaturation

The ability of the extract to inhibit albumin denaturation was evaluated according to the method of Mizushima and Kobayashi [15] and Sakat et al [14]. To 100 µl of the extract was added 500 µl of 1% aqueous solution of bovine albumin and the pH was adjusted using small amount of 1N HCL. The mixture was incubated for 20 min at 37 °C and followed by heating for 20 min at 51 °C. Then, it was allowed to cool to room temperature and the turbidity was read at 660 nm. Diclophenac was used as standard. The percent inhibition of protein denaturation was calculated thus:

% inhibition = (Abs control –Abs Sample) X 100/ Abs control.

Statistical analysis

Data obtained were analyzed using ANOVA and values are expressed as mean ± standard deviation.

Results

Bioactive Compound composition

The composition of the bioactive compounds evaluated are presented in Table 1. The result showed that phenol was the highest in concentration (261.73±13.54 mgGAE/g) while ascorbic acid was the least (0.12±0.03 mgAAE/g).

Table 1: Bioactive compound composition of ethanol extract of kiwano rind.

Bioactive compound	Concentration
Total Phenol (mgGAE/g)	261.73±13.54
Flavonoid (mgGAE/g)	130.86±9.66
Beta Carotene (mg/g)	0.48±0.00
Lycopene (mg/g)	0.22±0.00
Ascorbic Acid (mgAAE/g)	0.12±0.03

Antioxidant Effect

The antioxidant effect of the ethanol extract of kiwano rind was evaluated using three in-vitro tests; DPPH radical scavenging activity, reducing power and inhibition of lipid peroxidation assays.

DPPH radical Scavenging Activity

The extract and the standard displayed a concentration-dependent radical scavenging activity at concentrations of 200, 400, 600, 800 and 1000 µg/ml (Fig. 1). There was rise in antioxidant activity of the extract up to 800 µg/ml which gave the highest activity of 76. 74% and a slight fall in activity at 1000 µg/ml (75.02%). The standard, ascorbic acid gave a higher activity of 96.35% at 1000 µg/ml.

The extract gave a lower EC_{50} of 230 µg/ml while the standard gave EC_{50} value of 260 µg/ml (Table 2).

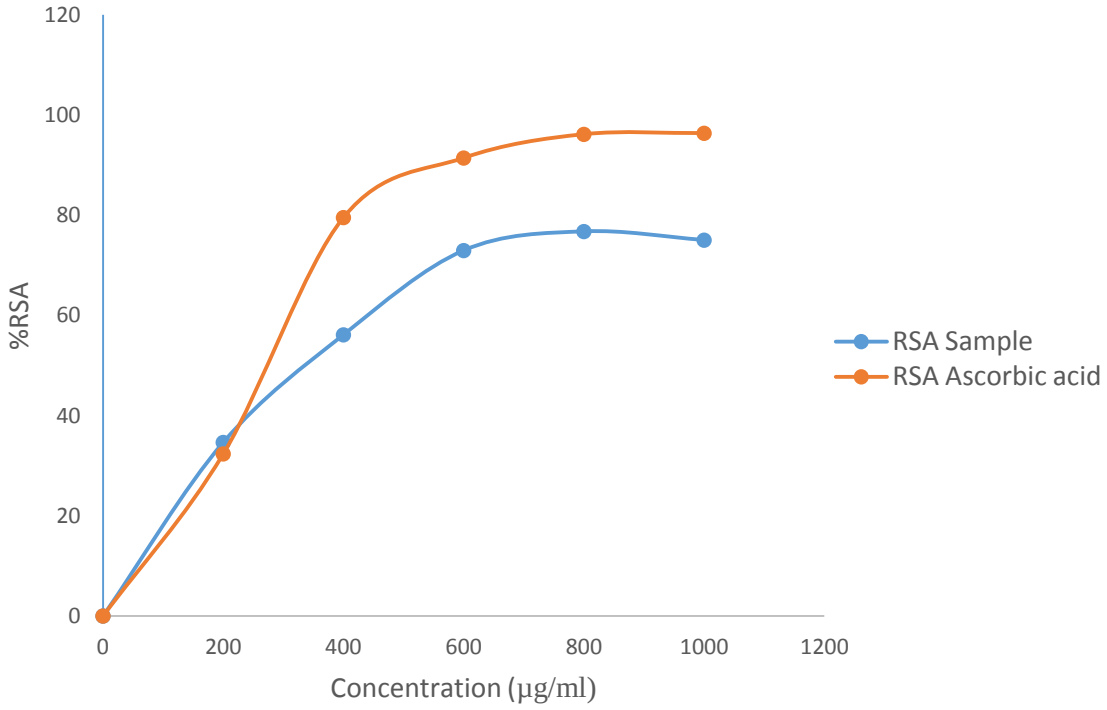

Fig. 1: DPPH radical scavenging Activity of ethanol extract of kiwano rind.

Table 2: The EC_{50} values for DPPH radical scavenging ability of the ethanol extract of kiwano rind and the standard; ascorbic acid.

Extract	EC_{50} (µg/ml)
Kiwano	230
Ascorbic acid	260

Reducing power Activity

The result of the reducing power activity of the extract and the standard is shown in Fig. 2. The optical density increased with the concentration. The standard gave higher absorbance in all the concentration tested. At concentrations of 200, 400, 600, 800 and 1000 µg/ml, the extract gave OD of 0.052, 0.111, 0.211, 0.292 and 0.352 respectively while that of the standard were 0.116, 0.212, 0.721, 0.944 and 1.102 respectively at the same concentrations. However, the effective concentration at $OD_{0.5}$ for the extract (520 µg/ml) was lower than that of the standard (540 µg/ml) (Table 3).

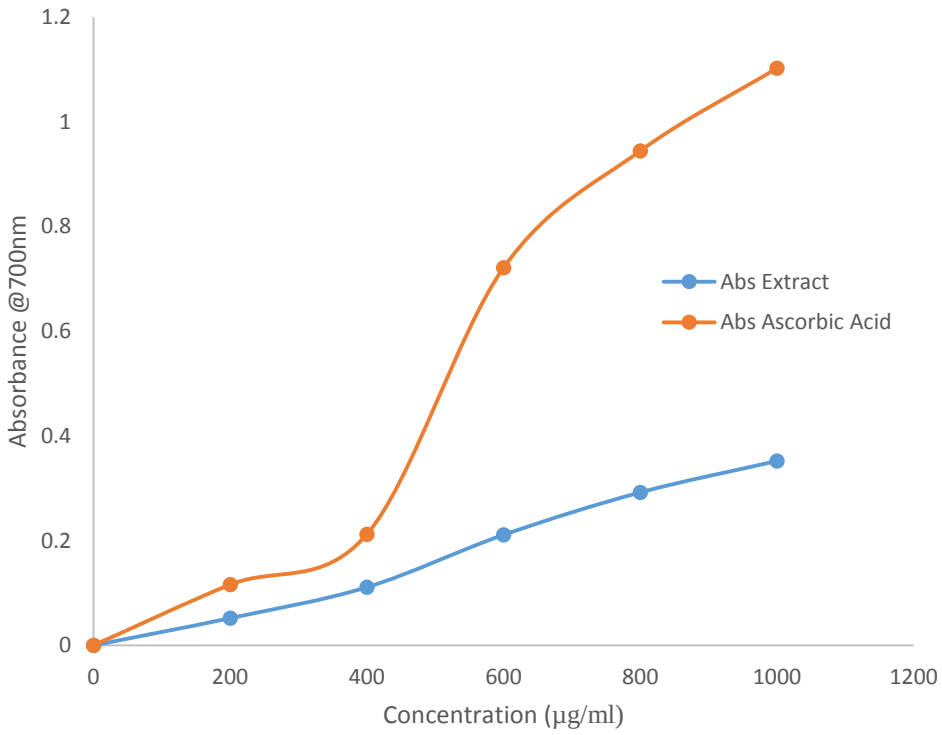

Fig 2: Reducing Power Capacity of ethanol extract of kiwano rind and the standard.

Table 3: The EC_{50} values for reducing power ability of ethanol extract of kiwano rind and the standard

Extract	**EC_{50} (µg/ml)**
Kiwano	520
Ascorbic acid	540

Inhibition of lipid peroxidation

The extract inhibited the process of lipid peroxidation in a concentration dependent manner similar to the standard. The percent inhibition ratios at concentrations of 200, 400, 600, 800 and 1000 µg/ml were 0.95, 2.96, 5.20, 22.93 and 41.49 % respectively for the extract while the standard gave percent inhibition ratios of 4.73, 15.84, 22.70, 40.54 and 53.66% at the same concentrations (Fig. 3). The EC50 of the extract was 770 µg/ml while that of the standard was 625 µg/ml (Table 4).

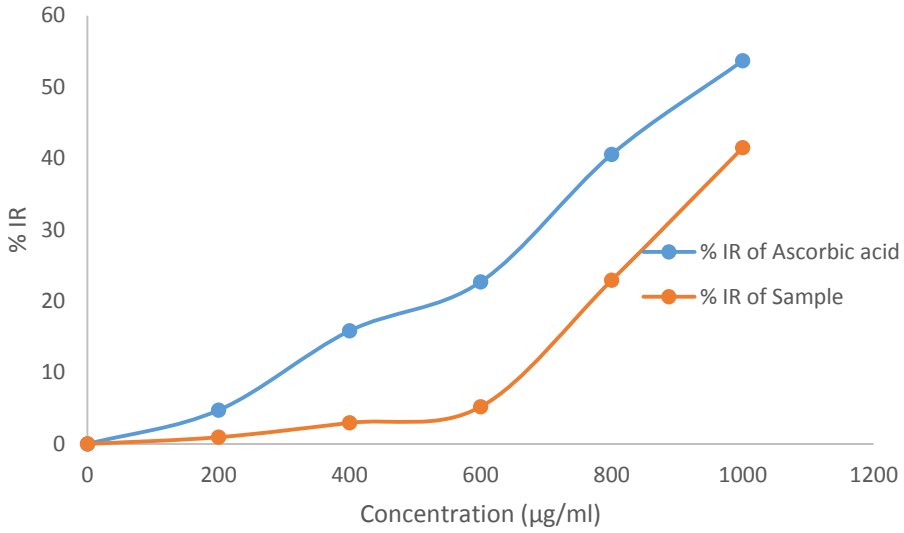

Fig 3: Inhibition of Lipid peroxidation activity of ethanol extract of kiwano rind and the standard.

Table 4: EC_{50} values for inhibition of lipid peroxidation ability of ethanol extract of kiwano and ascorbic acid.

Extract	EC_{50} (µg/ml)
kiwano	770
Ascorbic acid	625

Anti-inflammatory Activity

Anti-Proteinase activity

The results presented in Table 5 shows the ability of the extract to inhibit proteinase activity. Highest activity was observed at 1000 µg/ml (16.42%). Aspirin (standard) was tested at 200 µg/ml and was more efficient with 50.41% activity.

Table 5: Anti-proteinase activity of ethanol extract of kiwano rind and the standard

Sample	Concentration (µg/ml)	% Inhibition
Kiwano	200	2.28
	400	2.76
	600	5.53
	800	11.38
	1000	16.42
Aspirin	200	50.41

Membrane stabilization ability

The ability of the extract to stabilize erythrocyte membrane was evaluated by exposing the RBC to haemolysis through heat induction. The extract inhibited heat-induced haemolysis of the RBC in a concentration-dependent manner exhibiting 25.64% inhibition at 1000 µg/ml. Aspirin exhibited 46.15% inhibition at 200 µg/ml (Table 6).

Table 6: Inhibition of heat-induced haemolysis by ethanol extract of kiwano rind and the standard.

Sample	Concentration (µg/ml)	% Inhibition
Kiwano	200	7.18
	400	10.00
	600	13.08
	800	19.49
	1000	25.64
Aspirin	200	46.15

Inhibition of Albumin Denaturation

Table 7 shows the result of the inhibition of albumin denaturation by the ethanol extract of kiwano rind and that of the standard (diclophenac). The extract exhibited 47.14% inhibition at 1000 µg/ml while the standard exhibited 71.62% at 200 µg/ml.

Table 7: Inhibition of albumin denaturation of ethanol extract of kiwano rind and the standard.

Sample	Concentration (µg/ml)	% Inhibition
Kiwano	200	2.34
	400	6.25
	600	9.12
	800	16.41
	1000	47.14
Diclophenac	200	71.62

Discussion

Kiwano is a well-known fruit consumed for different nutritional and therapeutic purposes. The pulp and seeds have been used to meet various needs. Being high in water content, it is used to quench thirst and as a refreshing fruit. The pulp and the seeds are commonly consumed and with some studies on the nutritional value of the rind, it is necessary to expand studies and research in order to reveal new and more biological and therapeutic potential. In this study, we evaluated the rind to determine the composition of some important bioactive compounds such as phenol, flavonoids, ascorbic acid, beta-carotene and lycopene. These bioactive compounds are known to exhibit a wide spectrum of bioactivities such as antioxidant, anti-inflammatory, anticancer, immunostimulatory and antimicrobial [16]. With

this, we decided to extend or study on the antioxidant and anti-inflammatory properties using in-vitro models.

The result presented in Table 1 shows the composition of the bioactive compounds evaluated. Phenol and flavonoids were found in high concentrations of 261.73±13.54 and 130.86±9.66 mgGAE/g respectively. Phenol and flavonoids are the largest group of phytochemicals and are of great interest in medicine. They account for most of the antioxidant activity in plants. They have the ability to slow or prevent the oxidation of other molecules [17]. Phenols in particular are very important constituent of plant with the ability to scavenge free radicals owing to their methyoxy (–OCH3) and hydoroxyl (–OH) groups substituent [18, 19]. This contributes to their antioxidative action [20]. From reports it's been suggested that they play a role in the prevention of cardiovascular disease and cancer development [21]. In the food industry they play a major role in retarding the oxidative degradation of lipids, and hence, improvement of the quality and the nutritional value of the food [22].

The antioxidant activity of the rind of kiwano was assessed using three in-vitro assay models: DPPH radical scavenging, iron reducing power and inhibition of lipid peroxidation. From the result present in Fig 1. We showed the various activities of the fruit rind at concentrations of 200, 400, 600, 800 and 1000 µg/ml and compared it with that of the standard antioxidant compound; ascorbic acid. The % radical scavenging activity of the extract increased with the concentration and comparable with that of the standard. The extract was more potent with lower EC_{50} value than the standard. This radical scavenging ability could be attributed to the high phenol content of the extract as the radical scavenging activity of plant extracts is dependent on the concentration of polyphenolic compounds in the extracts as reported by Benavente-Garcia et al [23]. The reducing power ability of the extract was also assessed and compared with that of the standard at the same concentration range (Fig 2) and both samples showed a concentration-dependent increase. From the result, it showed that the extract has H+ donating potential and ability to reduce Fe^{3+} to Fe^{2+}. The extract also showed a lower EC_{50} value of 520 µg/ml than the standard with EC_{50} value of 540 µg/ml. This implies that the extract at lower concentration is more potent than the standard. The result of inhibition of lipid peroxidation also showed a concentration dependent increase in activity. However, the standard exhibited more potent inhibition activity with an EC_{50} of 625 µg/ml while the extract gave an EC_{50} of 770 µg/ml.

The anti-inflammatory ability of the extract was evaluated and compared with standard anti-inflammatory drugs; aspirin and diclophenac. These drugs which are generally called non-steroidal anti-inflammatory drugs (NSAIDS) work well in inflammation. They do this by inhibiting the liberation of lysosomes or stabilizing the lysosomal membranes [24]. However, there are associated negative side effects with their usage [25]. This prompts a search for natural anti-inflammatory agents with low to no side effects and low cost. The inhibitory activity against proteinase activity, albumin denaturation and membrane stabilization ability of the extract were studied in-vitro. Proteinases are involved in damage of the tissue in inflammatory reactions particularly in arthritic reactions. Neutrophils are sources of many serine proteinases residing in their lysosomal granules [26]. Protection against the action of these proteinases are provided by proteinase inhibitors. From the result in this study, the extract exhibited a concentration-dependent inhibitory activity against proteinase action up to 16.42% inhibition at 1000 µg/ml (Table 5). This is lower than that of the standard drug; aspirin which gave an inhibition activity of 50.42% at 200 µg/ml. Nevertheless, the extract

could contribute to reduction of inflammatory reactions associated with proteinase actions. The extract was also tested for its ability to prevent protein denaturation. Application of external stress or strong substances can lead to protein denaturation and proteins lose their secondary and tertiary structures. Most inflammatory diseases such as serum sickness, rheumatoid arthritis, systemic lupus erythematosus and glomerulonephritis are normally linked to denaturation of tissue proteins. For instance, the denaturation of tissue proteins results in the production of autoantigens in arthritis [27, 28]. Membrane proteins regulate water/volume content of the cell controlling the movement of ions such as sodium and potassium via ion channels [29]. From the result in this study, it was shown that the ethanol extract of kiwano rind inhibited denaturation of bovine albumin treated with heat in a concentration-dependent manner exhibiting 47.14% maximum activity. The inhibitory effects could be attributed to the enlargement and interplay with membrane proteins which is an indication of anti-rheumatoid properties. Membrane stabilization has to do with the ability to maintain the integrity of bio-membranes against heat and osmotic-induced-lysis [30]. In this study, the ability of the extract to stabilize rat erythrocyte membranes (RBC) exposed to heat was evaluated. The erythrocyte membrane is structurally similar to the lysosomal membrane [31]; therefore, stabilization of the erythrocyte membrane could be associated with the stabilization of the lysosomal membrane [32]. When erythrocyte membrane is exposed to excessive heat, it results in membrane lysis followed by oxidation of its haemoglobin [33]. Stabilization of the erythrocyte membranes brings about the inhibition of the rupture and ensuing liberation of activated neutrophil cytoplasmic components, including bactericidal enzymes and proteases that can aggravate the inflammatory response following extracellular release [34]. The result from this study showed that the ethanol extract kiwano rind protected the erythrocyte membrane against lysis induced by heat with maximum inhibition activity of 25.64%. This ability might have been by increasing the surface area/volume ratio of cells or through its interaction with membrane proteins [35].

Conclusion

Our study demonstrated the biological activity of kiwano fruit rind. From the study, the extract is rich in important phytochemical constituent particularly phenol and flavonoid which have been reported to have various pharmacological effects. These constitutes may be responsible for the bioactivity of the extract as many plants containing these chemical compounds have been reported to possess potent antioxidant and anti- inflammatory properties. Therefore, this study recommends the fruit rind of kiwano as a potential source of agents for formulation of pharmacological products in treatment and management of illnesses associated with oxidative stress and inflammation.

Author Contributions

ONA designed the study, wrote the protocol, wrote the first draft of the manuscript and wrote part of the manuscript, IIU managed the literature searches and contributed to the writing of the manuscript, CKO managed the analyses of the study and performed the statistical analysis. All authors read and approved the publication of the paper.

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DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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