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Evaluation of bioactive compound content, *in-vitro* antioxidant and anti-inflammatory effects of ethanol extract of the seed of *Cucumis metuliferus* fruit

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Abstract

Cucumis metuliferus commonly called Kiwano is a well-known plant in the middle belt area of Nigeria. It is consumed for nutritional and medicinal purposes. This study evaluated the bioactive compound content, *In-vitro* antioxidant and anti-inflammatory properties of ethanol extract of the seed of *Cucumis metuliferus* fruit. The antioxidant properties were evaluated using DPPH (2, 2 diphenyl-1-picrylhydrazyl) radical, iron reducing power and inhibition of lipid peroxidation tests. Ascorbic acid served as standard. The anti-inflammatory activity at concentration of 200-1000 µg/ml was measured *In-vitro* using anti-proteinase activity, membrane stabilization and inhibition of albumin denaturation assay systems. Aspirin and diclofenac were used as reference drugs. The bioactive compounds evaluated were phenol, flavonoid, beta carotene, lycopene and ascorbic acid. From the result, the seed extract contains phenol (149.40±9.93 mgGAE/g), flavonoids (97.84±2.84 mgCE/g), beta carotene (0.110±0.001 mg/g), lycopene (0.085±0.001 mg/g) and ascorbic acid (0.190±0.028 mgAA/g). In the antioxidant assays, the antioxidant activities were dependent on the concentration. At lower concentrations, the seed extract displayed higher activity in all the assays compared with the standard. The DPPH scavenging and lipid inhibition abilities of the seed were more potent with lower IC50 while the reductive potential of the standard was better. From the anti-inflammatory assays, the seed extract exhibited anti-proteinase activity of 0-8.78%, membrane stabilization (inhibition of heat-induced haemolysis) activity of 5.13-20.51% and percent inhibition of albumin denaturation of 0.78-16.15%. These results showed that ethanol extract of *Cucumis metuliferus* seed contains bioactive compounds which are beneficial to health and possess antioxidant and anti-inflammatory abilities.

Keywords: *Cucumis metuliferus*; Seed; Bioactive compound; Antioxidant; Anti-inflammatory; *In-vitro*

1. Introduction

Different parts of plant are used as food for nutritional purposes and are believed to contain different biologically active compounds. They contain nutrients in abundance and are needed daily by man for appropriate biochemical and metabolic processes. Vegetables and fruits are regarded as important sources of many indigestible components (fiber) and phytochemicals that act in synergism contributing to the nutrition and therapeutic benefits of foods either separately, or in combination. Seeds especially are good sources of carbohydrates, protein, minerals and fats; and so have nutritive and calorific values which make them necessary components of diets [1]. More so, the bioactive components of seeds are found useful therapeutically. Bioactive compounds are said to be extra-nutritional components present in low concentrations in foods [2]. They are chiefly in vegetables, fruits and whole grains, and beneficial to

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health in addition to the basic nutritional value. They are molecules with therapeutic potential and influence on calory intake, while reducing oxidative stress, pro-inflammatory state and disorders of metabolism [3].

Oxidative stress is the underlying and root cause of a malfunctioning immune system and invokes several inflammatory responses [4]. It functions significantly in the origin and progression of debilitating illnesses which include cancer, chronic inflammation, diabetes and heart diseases [5]. These diseases can be a consequence of a rise in manifestation of pro-inflammatory factors (eicosanoids and cytokines) which are regulated by oxidation-reduction reactions [6]. However, free radicals are produced from normal cellular metabolic processes. At low or moderate levels, they may be beneficial in defending the body against infections and in the normal functioning of many signaling systems in the cell [7]. But when in excess which results from a disparity between the antioxidant reactions geared towards the entrance of a poisonous substance in the body, it can destroy protein, DNA or fats in the cells, introduce various diseases and increase the inflammatory processes [8, 9]. Inflammation is the reaction elicited by the vascular tissues of the body to injurious stimulant [10]. This can cause pain, a rise in denaturation of protein, permeability of the vascular tissues and alteration of the membrane [11]. Reactive oxygen species functions greatly in inflammation activating production of inflammatory mediators like the leukotrienes, prostaglandins, cytokines (TNFα, IL1β, IL6), chemokines and activation of NF-κB [12]. Leukotrienes and prostaglandins are synthesized through the pro-inflammatory enzymes; lipoxygenase (LOX) and cyclooxygenases (COX) activation respectively. The phospholipase activation produces the substrate of these pro-inflammatory enzymes. These mediators recruit and mobilize greater number of inflammatory cells to the damaged area and produce increased reactive oxygen species [13]. The migration of these cells to the damaged area and subsequent liberation of cytokines plays an important role in the inflammatory response [14]. Maintaining the equilibrium between the harmful and beneficial effects of free radicals is pertinent and this is accomplished by regulation of the oxidative processes [5]. Redox regulation processes protect cells from different stress induced by free radicals and confirms redox stability by taking control of the in-vivo oxidation-reduction state [15]. To manage inflammatory conditions, prescription or over-the-counter drugs such as nonsteroidal anti-inflammatory drugs (NSAID) or corticosteroids pain relieving drugs are prescribed. However, most of these drugs have some short-term or long-term negative side effects such as gastric ulcers leading to bleeding, heart problems and kidney issues. More so, some of these synthetic drugs are costly and may not be affordable for a common man. The high cost coupled with the adverse side effects of synthetic drugs are some of the considerations for an inclination towards medicinal drugs of natural products, which are presumed to be effective in treatment of chronic diseases. Hence, it is a necessity to evaluate further and search for more widely available medicinal plants with less cost and minimal side effects that will help prevent this alarming health problems. Medicinal plants are regarded as essential sources of compounds that could be useful in treatment of diseases arising from oxidative stress and inflammation [16]. Therefore, in pharmaceutical research, identifying biologically active compounds from plants has become an active field [17].

Cucumis metuliferus is of the *Cucurbitaceae* family. It is also called Kiwano. It grows annually, can be grown anywhere and mostly in warm seasons [18]. It is called 'bùuràr zaàki', 'nòònòn-kuùraà''gautar kaji' by the Hausas in Nigeria [1]. It naturally grows in South Africa, Nigeria, Senegal, Namibia, Swaziland and Botswana. It is called horned melon because of the horn-like spines on its fruits. Different parts of the plant are used for different medicinal and culinary purposes. The fruits and seeds are eaten raw as food supplement [19]. In Nigeria, the seeds are ground into powder, turned into an emulsion with water and then taken as worm expeller [19]. Although there has been reported works on the nutritional and phytochemical constituents of *Cucumis metuliferus* seeds [20], the present study was conducted to identify the concentration of some bioactive compounds present and determine the antioxidant and anti-inflammatory properties using various *In-vitro* bioassays.

2. Material and methods

2.1. Collection and identification of Plant Sample

The fruits of *Cucumis metuliferus* were obtained from local market in Benue State, Nigeria and authenticated at the department of Applied Biology and Biotechnology in Enugu State University of Science and Technology, Nigeria.

2.2. Sample preparation and extraction

The fruits were washed thoroughly with portable water to eliminate dirt and then slit open with a knife. The pulp containing the seeds were scooped out and the seeds were then washed until all the succulent pulp attached to them were removed. The seeds were dried under the sun for 5 days. The dried seeds were pulverized into powder by the use of mortar and pestle and then subsequently into fine powder using an electric blender. The powdered sample (100 g) was extracted using 1L of 80% ethanol. The mixture was filtered through a muslin cloth and then with whattman No. 4

filter paper. The filtrate was evaporated at 50°C in a water bath. The dried residue was kept in an air-tight container at 4 °C in a refrigerator prior to analysis.

2.3. Bioactive compound assay

The presence and concentration of the following bioactive compounds were analyzed: total phenol, flavonoids, ascorbic acid, Beta-carotene and lycopene. The assays were done using standard procedures.

2.3.1. Total Phenol

This was evaluated using the method of Barros et al [21]. One milliliter (1 ml) of the extract was combined with 1 ml of Folin and Ciocalteu's phenol reagent. 1 ml of saturated sodium carbonate solution was added to the mixture and made up to 10 ml with distilled water and incubated for 90 min. Afterwards, the absorbance was measured at 725 nm. Gallic acid served as a reference and the total phenol content was expressed as mg gallic acid equivalent (mg GAE) per g of extract.

2.3.2. Flavonoids

The flavonoid content was determined using a slightly modified colorimetry method previously described by Barros et al. [22]. 0.5 ml of diluted sample solution (250 µg/ml), 2 ml of distilled water and 0.15 ml of 5 % $NaNO_2$ solution were mixed together. This was left for 5 min after which 0.15 ml of 10% $AlCl_3$ solution was added. This was left to stand for 6 min and followed by addition of 2 ml of 4% NaOH solution. The mixture was made up to 5 ml with water, mixed thoroughly and left to stand for additional 15 min. The absorbance was measured at 510 nm against water blank and compared to a standard curve of catechin. The analyses were performed in triplicate. The flavonoid content was expressed as mg Catechin equivalent per g of sample (mg CE/g).

2.3.3. Beta carotene and lycopene contents

These were evaluated using the method of Barros et al [21]. 100 mg of the extract was shaken vigorously with 6 ml acetone-hexane mixture in the ratio of 4:6 for one minute and filtered using Whatman No.4 filter paper. The absorbance of the filtrate was measured at 453, 505 and 663 nm respectively. The lycopene and β-carotene content were evaluated thus:

$$\text{Lycopene (mg/100ml)} = -0.0458A663 + 0.372A505 + 0.0806A453$$

$$\beta\text{-carotene (mg/100ml)} = 0.216A663 + -0.304A505 + 0.452A453.$$

2.3.4. Ascorbic Acid

The method of klein and Perry [23] was used in the determination of the ascorbic acid content. Metaphosphoric acid (1%; 10 ml) was used in extracting 20 mg of the sample. This was left to stand for 45 min at 28 °C and then filtered through Whatman No.4 filter paper. The filterate (1 ml) was combined with 2,6-dichlorophenolindophenol sodium salt hydrate (9 ml of 50 µM). After 30 min., the absorbance was read at 515 nm. The ascorbic acid content was calculated from the standard curve of L-ascorbic acid and the result expressed as mg ascorbic acid equivalent per gram (mgAE/g) of the sample.

2.4. Antioxidant assay

2.4.1. DPPH scavenging activity Assay

This was done according to the method of Ebrahimzadem, et al [24]. Free radical scavenging ability of the sample extract was evaluated using the stable 2,2-diphenyl-1-picryl hydrazyl radical (DPPH). The extract (0.3 ml; 0-1000 µg/ml) were combined methanolic solution of DPPH (2.7 ml; 100 µM) in test tubes. The mixture was swirled and incubated for 60 min. The absorbance was read 517 nM. Vitamin C was used as a reference. The % scavenging activity was calculated thus:

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

As = absorbance of the sample test solution;
ADPPH = absorbance of DPPH solution.

The IC50 (sample concentration at 50% radical scavenging activity (RSA) was extrapolated from the graph of %RSA versus the sample concentration.

2.4.2. Reducing Power Ability test

This was evaluated using the method of Barros et al, [21]. The principle is dependent on the increase in the absorbance of the reaction mixture.

The sample 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 of 1%), incubated for 20 min at 50^{o}C. Thereafter, Trichloroacetic acid (2.5 ml; 10%) was added to the mixture and centrifuged for 8 min at 1000rpm. 5 ml of the upper layer was mixed with deionised water (5 ml) with subsequent addition of ferric chloride (1 ml; 0.1%). The absorbance was read at 700 nM. The graph of absorbance versus the extract concentrations was plotted. Vitamin C was used as the reference antioxidant.

2.4.3. Inhibition of Lipid peroxidation Activity Assay

This was done using the method of Barros et al. [21]. The degree of inhibition of lipid peroxidation was determined using homogenate of a goat brain. The brain was cut into pieces and homogenized using mortar and pestle in an ice-cold Tris-HCl buffer (pH 7.4, 20mM) to produce brain homogenate (50% w/v) and centrifuged for 19 min at 3000g. The supernatant (0.1 ml) was incubated with the sample extract (0.2 ml; 0-1000 µg/ml), ferrosulphate (0.1ml; 10uM) and ascorbic acid (0.1ml; 0.1nM) for 1 hr at 37 °C. TCA (0.5 ml; 28%) and TBA (0.38 ml; 2%) were added to stop the reaction. Then the mixture was warmed for 20 min at 80 °C, and centrifuged for 10 min at 3000 rpm. The intensity of the colour of the malondialdehyde (MDA)-TBA complex in the supernatant was read at 532 nM. The inhibition ratio; IR (%) was evaluated thus:

$$\text{Inhibition ratio (\%)} = [(A-B)/A] \times 100\%$$

A and B = the absorbance of the control and the extract solution respectively. IC50 was extrapolated from the graph of IR% versus the extract concentrations. Vitamin C was used as the standard.

2.5. Determination of Anti-inflammatory Activity

2.5.1. Anti-proteinase action

This was done using a modified method of Oyedepo et al. [25] and Sakat et al [26]. The sample extract (1 ml; 200 -1000 µg/ml) was mixed with trypsin (2 ml; 0.06 mg) and Tris HCL buffer (1 ml; 20 mM; pH 7.4). This was incubated for 5 min at 37 °C followed by addition of casein (1 ml; 0.8% w/v) and further incubation for 20 min. Perchloric acid (2 ml; 70%) was added to stop the reaction. This was followed by centrifugation and the absorbance of the supernatant was measured at 210 nm against buffer as blank. The % inhibition of proteinase activity was calculated thus:

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

2.5.2. Membrane stabilization Assay

Stabilization of erythrocyte membrane exposed to heat-induced haemolysis was employed to evaluate ability of the extract to maintain membrane stability.

Preparation of Red Blood cells (RBCs) suspension.

Blood was collected from healthy rat that has not taken any NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) for 2 weeks prior to the experiment and transferred to centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were 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.

Inhibition of Heat induced haemolysis Assay

This was done using the method of Sakat et al [26]. The test sample (1 ml; 200 - 1000 µg/ml) was mixed with RBCs suspension (1 ml; 10%) and saline in test tubes. This was followed by incubation for 30 min at 56ºC in water bath and subsequently cooled under running water. The reaction mixture was centrifuged for 25 min at 2500 rpm and the absorbance of the supernatants was measured at 560 nm. Aspirin was used as the reference drug. The % inhibition of Haemolysis was obtained thus:

Percentage inhibition = (Absorbance of control –Absorbance of sample) X 100/ Absrbance of control

2.5.3. Inhibition of albumin denaturation

This was evaluated using the method of Mizushima et al [27] and Sakat et al [26]. The sample extracts were mixed with aqueous solution of bovine albumin fraction (1%) and the pH adjusted with a little quantity HCL (1N). This was followed by incubation for 20 min at 37 °C and heating to 51 °C for 20 min. The mixture was left to cool and then the turbidity was measured at 660 nm. The % inhibition of protein denaturation was calculated thus:

Percentage inhibition = (Absorbance of control –Absorbance of Sample) X 100/ Absorbance of control

2.6. Statistical analysis

Experimental data was analyzed using ANOVA and values were presented as mean ± standard deviation.

3. Results and discussion

3.1. Bioactive Compound Content

The result of the analysis of the total phenol, flavonoid, ascorbic acid, beta-carotene and lycopene composition of the seed extract is presented in Table 1. The phenol content was expressed in mg gallic acid equivalent per gram of the extract (mgGAE/g). The results showed that the seed extract is very rich in phenol with a total phenol content of 149.40 ± 9.93 mgGAE/g. This is a very high value as Tawaha, et al. [28] suggested that a total phenol content of greater than 20 mg GAE/g dry weight could be regarded as very high. Therefore, it can be deduced that the seed extract of *Cucumis metuliferus* can be a source of phenolic antioxidant as phenols are reported as the most abundant phytoconstituent in plants as antioxidants through its ability to chelate metals efficiently and scavenge radical oxygen species [29].

The flavonoid content was expressed in mg Catechin per gram of dry extract. The flavonoid content was also high with a value of 97.84 ±2.84 mgCE/g. Flavonoids are also antioxidants and are referred to as modifiers; they reinforce the reaction of the body's reactions against allergens, carcinogens and viruses [30].

The concentration of ascorbic acid (0.190 ± 0.028 mgAA/g) and the carotenes (beta-carotene- 0.109 ± 0.000 mg/g and lycopene-0.085 ± 0.001 mg/g respectively) were low. This low value notwithstanding still confirms that the seed extract is a good source of therapeutic compounds as the antioxidant activity of carotenoids has been reported [31]. Ascorbic acid, beta-carotene and lycopene are known to have several health benefits. Ascorbic acid is a known antioxidant that protects the cells from free radical damage and in addition, important for collagen formation [32]. Collagens are necessary for strong bones Carotenoids are efficient singlet oxygen quenchers and powerful scavengers of every other reactive oxygen species [33, 34]. Lycopene in particular is the most efficient singlet oxygen and peroxyl radical scavenger [35].

Table 1 The composition of total phenol, flavonoid, ascorbic acid, beta-carotene and lycopene in ethanol extract of *Cucumis metuliferus* seed

Bioactive constituent	Concentration
Phenol (mgGAE/g)	149.40 ± 9.93
flavonoid (mgCE/g)	97.84 ± 2.84
Ascorbic Acid (mgAA/g)	0.190 ± 0.03
Beta Carotene (mg/g)	0.109 ± 0.00
Lycopene (mg/g)	0.085 ± 0.001

3.2. Antioxidant Activity

In the literature, there are several methods that can be used to evaluate antioxidant ability *In vitro* in that one test is not enough in determining the total antioxidant ability of a sample under study. Different measurements are essential in order to identify and factor in the different antioxidant mechanisms [36, 37]. Thus, the antioxidant activity of the seed

extract was measured by employing three *In-vitro* methods: the DPPH (2,2-diphenyl-1-picrylhydrazyle) free radical scavenging test, reducing power activity and inhibition peroxidation ability methods.

3.2.1. DPPH Radical Scavenging Activity

DPPH radical is an ideal system used generally in determination of the scavenging activity of various natural antioxidants. Evaluation of the antioxidant ability gives an insight on the health benefits and functions of foods and can be established by measuring DPPH radical scavenging ability [38]. In this study, we found a dose-dependent relationship in ability of the ethanol extract of *Cucumis metuliferus* seed to scavenge radicals as shown in Fig. 1 compared with the standard. The extract showed better radical scavenging activity at lower concentration range of 200-600 µg/ml than the standard while at the highest concentration tested, the seed extract exhibited 66.37% activity which is comparable with that of the standard (71.39%). From the result of the IC_{50} (Table 2), the seed also showed better potency with IC_{50} of 180 µg/ml while the standard has IC_{50} of 520 µg/ml. IC_{50} is in inverse proportion to the antioxidant ability of a compound. IC_{50} is the concentration of the extract that is needed to inhibit 50% of DPPH free radical under experimental conditions [39]. The extract that can produce a high percentage DPPH scavenging ability at the least concentration is regarded as a high free radical scavenger. The IC_{50} result revealed that the ethanol seed extract of *Cucumis metuliferus* scavenged 50% DPPH radicals at lower concentration and so has the capacity to scavenge free radicals which could find application as natural antioxidant. This ability may be as a result of the high phenol content of the seed as phenols have been reported to possess the ability to scavenge free radicals through electron donation [40].

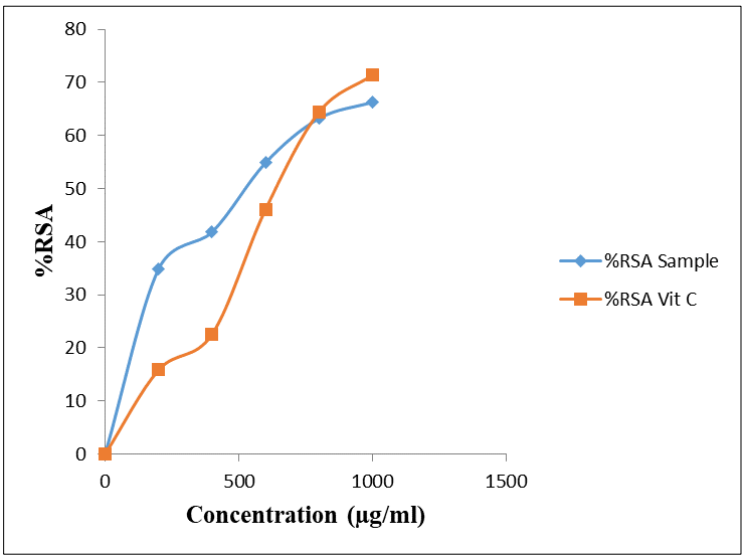

Figure 1 DPPH Radical Scavenging Activity of ethanol Seed extract of *Cucumis metuliferus* in comparison with the standard; ascorbic acid

Table 2 The IC_{50} values of the ethanol extract of *Cucumis metuliferus* seed and the standard; vitamin C

Sample	**IC_{50} (µg/ml)**
Cucumis metuliferus seed	180
Vitamin C	520

3.2.2. Reducing Power Activity

The reducing power capacity of the ethanol extract of *Cucumis metuliferus* seed was evaluated based on its ability to reduce Fe3+ complex to the Fe2+ in relative to that of the standard; ascorbic acid. Each sample was mixed with ferric compounds, the yellow colour of the test solution turned to different shades of grey and blue, and the reducing power was evaluated by measuring absorbance at 700nm. The higher the absorbance, the higher the reducing power capacity. In this study, the plot of optical density against the concentration showed that the reducing power of the sample increased with increasing concentration comparable with that of the standard and the sample exhibited higher reductive ability at lower concentrations which reflected in higher absorbance than the standard (Fig. 2). The reductive

potency is presented in Table 3. *Cucumis metuliferus* seed extract showed greater reductive ability with OD0.5 value of 510 µg/ml while that of the standard was 580 µg/ml. The reducing capacity of compounds may function as a remarkable index of electron donating ability and consequently of their potential antioxidant activity.

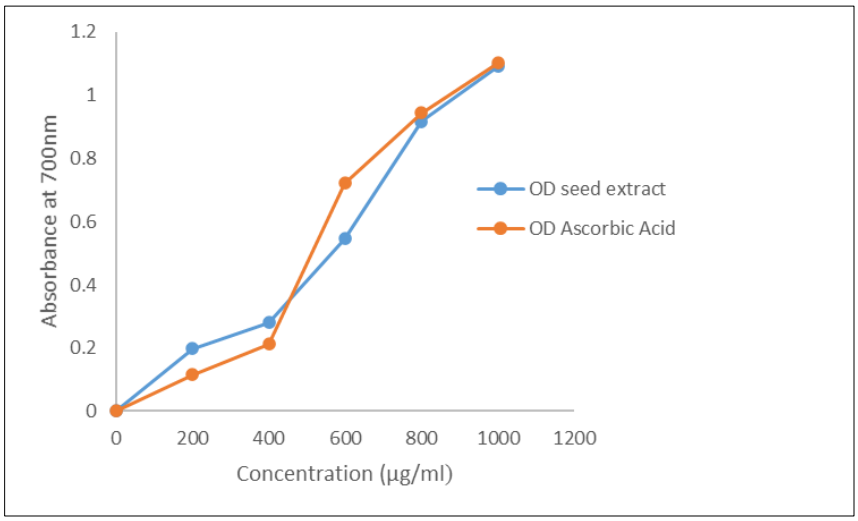

Figure 2 Optical density (OD) of *Cucumis metuliferus* seed extract and the standard against concentration

Table 3 Reductive ability of *Cucumis metuliferus* seed extract and the standard; ascorbic acid.

Sample	**$OD_{0.5}$ (µg/ml)**
Cucumis metuliferus seed	510
Vitamin C	580

3.2.3. Inhibition of lipid Peroxidation

Lipid peroxidation process proceeds through free radical chain reaction and linked with damage of cells in biological membranes. The damage gives rise to illnesses like cardiovascular diseases, diabetes and cancer. A well-known and presently used test system as an indicator for lipid peroxidation products such as malondialdehyde (MDA) is the thiobarbituric acid assay (TBA test) which is relies on the reaction of MDA with TBA producing a red adduct [41].

The extract's ability in comparison with the standard to inhibit the process of lipid peroxidation was measured using brain homogenate as a medium of peroxidation. Incubation of brain or liver homogenates with $FeSO_4$ gives rise to a notable increase in lipid peroxidation. Brain tissue contains unsaturated lipids which are prone to peroxidation when they are exposed to free radicals leading to the production of various compounds. The absorbance of these compounds are measured and hence, the level of peroxidation can be evaluated. If lipid peroxidation is inhibited by a compound, it decreases absorbance in a particular wavelength [42]. Inhibition of lipid peroxidation can take place using natural or synthetic antioxidant substances [43]. In this study, ethanol extract of *Cucumis metuliferus* seed demonstrated dose-dependent inhibitory activity favourably comparable to the standard; ascorbic acid. At 1000 µg/ml, the percentage inhibitory activity of the extract and the standard were 48.69 and 53.66% respectively (Fig. 3). But the extract was more potent with an IC_{50} of 540 µg/ml while that of the standard was 650 µg/ml (Table 4). Therefore, *Cucumis metuliferus* seed, has the ability to inhibit the process of lipid peroxidation which could be associated with its high phenolic content. It was suggested from studies that phenolic compounds have the ability to suppress lipid peroxidation and this correlates to antioxidant activity of plants [44].

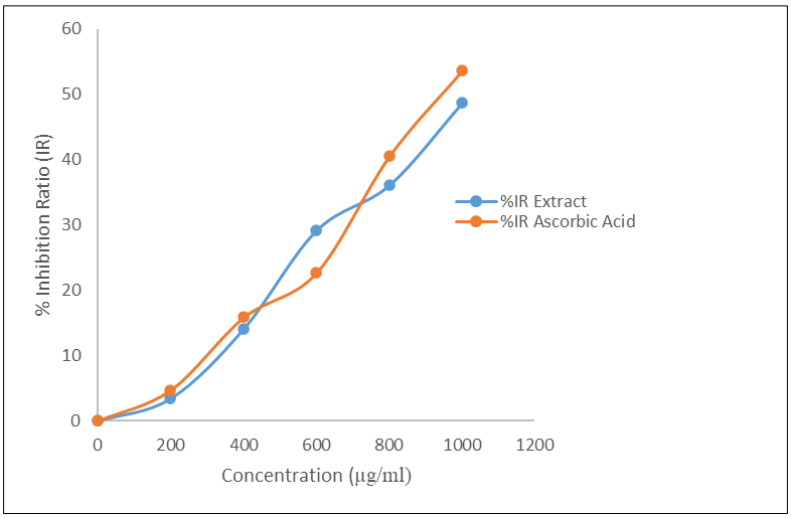

Figure 3 Percentage IR of the ethanol extract of *Cucumis metuliferus* seed and the standard against the concentration

Table 4 IC50 values of the ethanol extract *Cucumis metuliferus* seed and the standard; Ascorbic acid

Sample	IC50 (µg/ml)
Cucumis metuliferus seed	540
Ascorbic acid	650

3.3. Anti-inflammatory Activity

The anti-inflammatory ability of the extract of *Cucumis metuliferus* seed was evaluated using three *In-vitro* anti-inflammatory tests. *In-vitro* studies is useful and help in examining reactions of cells in an excluded environment under the maintenance of conditions of experiment [45], and are expedient in understanding the mode of anti-inflammatory activity of natural medicinal products [46].

3.3.1. Anti-proteinase action

Proteinases are implicated in tissue damage in inflammatory reactions. There is an association between proteinase activities with arthritic reactions. Neutrophils carry many serine proteinases in their lysosomal granules [47]. Protection against the action of these proteinases could be possible by proteinase inhibitors. Ethanol extract of *Cucumis metuliferus* seed exhibited anti-proteinase activity at different concentration tested with maximum percentage inhibition of 8.78% at 1000 µg/ml (Table 5). The standard; aspirin exhibited a better inhibitory activity of 50.41% at 200 µg/ml.

Table 5 Anti-proteinase Activity of ethanol extract of *Cucumis metuliferus* seed and the standard; aspirin.

Sample Conc (µg/ml)	% Inhibition by Seed
200	0
400	2.44
600	6.51
800	7.32
1000	8.78
Aspirin 200	50.41

3.3.2. Membrane stabilization

The ability of the extract to provide membrane stabilization by preventing heat-induced erythrocyte membrane haemolysis was evaluated and compared with the standard drug; aspirin. The extract inhibited the heat-induced haemolysis more effectively than in other anti-inflammatory assays, producing 20.51% inhibition at 1000 µg/ml. The standard however, was more effective with 46.15% inhibition at 200 µg/ml (Table 6).

During inflammation, lysosomes are lysed leading to release of their enzyme component producing numerous health disorders. The NSAIDs bring about beneficial results either through inhibition of the liberation of lysosomes or stabilization of the lysosomal membranes [48]. When RBCs are exposed to damaging substances such as heat or hypotonic medium, it brings about the lysis of the membranes, followed by oxidation and haemolysis of haemoglobin [49]. As the erythrocyte membranes resembles the lysosomal membrane [48], the prevention of heat induced lysis of red blood cell membrane and hence, stabilization of the membrane was used to evaluate anti-inflammatory ability of *Cucumis metuliferus* seed extract. Membrane stabilization is achieved when serum protein and fluids are prevented from leaking into the tissue when there is an increase in membrane permeability arising from inflammatory mediators [50]. From the result, it could be said that the *Cucumis metuliferus* seed prevented the release of inflammatory mediators and lytic enzymes by stabilizing the red blood cell membrane.

Table 6 Inhibition of heat-induced haemolysis Activity of ethanol extract of *Cucumis metuliferus* seed and the standard

Sample Conc (µg/ml)	%Inhibition by seed
200	5.13
400	6.41
600	6.41
800	9.74
1000	20.51
Aspirin 200	46.15

3.3.3. Inhibition of albumin denaturation

Protein denaturation is defined as a change in the folding structure and hence physical properties of proteins which occurs as a result of exposure to either heat, certain chemicals or changes in pH. This results in loss of their biological functions. There is a correlation between protein denaturation and development of inflammatory disorders like rheumatoid arthritis, diabetes and cancer [51]. Therefore, prevention of denaturation of protein may just as well lead to the prevention of inflammatory disorders [52]. In this study, the ethanol extract of *Cucumis metuliferus* seed inhibited protein denaturation in a dose-dependent way and ranged from 0.78-16.15% at concentration range of 200-1000 µg/ml. There was an insignificant activity at 200 µg/ml. The inhibitory effect in comparison with that of the standard; diclophenac is presented in Table 7. The standard exhibited better percentage inhibition of 71.62% at 200 µg/ml. However, the seed extract was able to inhibit the heat induced albumin denaturation but was less active than the standard. And so, loss of biological functions of cells due to denaturation of their protein component could be minimized following the use of *Cucumis metuliferus* seed. Prevention of denaturation of proteins may be contributory factor in action of NSAIDs in treatment of rheumatism [53]**.**

Table 7 Result of the Inhibition of albumin denaturation Activity of ethanol extract of *Cucumis metuliferus* seed and the standard

Sample Conc (µg/ml)	% inhibition by seed
200	0.78
400	3.13
600	8.60
800	10.94
1000	16.15
Diclophena 200	71.62

Our finding from the anti-inflammatory assays is that the ethanol seed extract of *Cucumis metuliferus* exhibited anti-inflammatory activity although not as effective as the standard drugs. This ability could be associated with the high concentration of biologically active compounds in the seed. It has been shown from studies that flavonoids contribute to the anti-inflammatory activities of plants. Therefore, the presence of these compounds in the seed of *Cucumis metuliferus* may be responsible for its anti-inflammatory activity. Our study has shown that the seed of *Cucumis metuliferus* is rich in phenols and flavonoids.

4. Conclusion

This study has shown the various bioactive compounds present in the seed of *Cucumis metuliferus*. From the analysis, the seed is highly rich in phenols and flavonoids. The extract exhibited high antioxidant activity through its ability to scavenge free radicals, inhibit lipid peroxidation and also demonstrated high potent reducing power ability. We also found out that the seed possesses anti-inflammatory property through its ability to prevent albumin denaturation and heat-induced haemolysis of the erythrocyte as well as protection against proteinase activity. These abilities could be attributed to the high phenol and flavonoid content of the seed. However, these bioactive compounds need to be purified in order to obtain increase in activity. Therefore, we conclude that the ethanol extract of *Cucumis metuliferus* seed is rich in antioxidant substances, has antioxidant and anti-inflammatory potentials, and could be used in drug design for treatment of diseases associated with free radicals.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no known competing interests of whatsoever that could influence the work reported in this paper. All authors read, approved and are aware of this submission. We also certify that the paper has not been submitted for consideration or publication elsewhere.

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