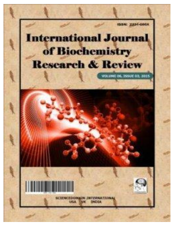

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Phytochemical Composition and *in vitro* Antioxidant Activity of Golden Melon (*Cucumis melo* L.*)* Seeds for Functional Food Application

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Authors' contributions

This work was carried out in collaboration between all authors. Author IPO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author AAO managed the literature searches and performed seed production and extraction. Authors JAB and OFO managed the experimental analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Golden melon (*Cucumis melo)* is an annual herbaceous plant belonging to the family of *Cucurbitaceae (Cucurbit*). This study was carried out to evaluate the phytochemical composition and *in vitro* antioxidant activity of golden melon seed extract.
Place and Duration of Study: The study was carried out between a period of July and August 2017 at Baking Milling Division, Federal Institute of Industrial Research Oshodi Nigeria.
Methodology: The crude methanolic extracts of the seed were tested for phytochemical and antioxidant activities according to standard analytical procedure. The antioxidant potential of the seed extracts was examined using different assays by determining total phenolic content, total flavonoid content, total antioxidant capacity. The free radical scavenging activities of the extract

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such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, *in vitro* lipid peroxidation, and nitric oxide (NO) scavenging assay were determined spectrophotometrically.

Results: The phytochemical screening of the seed extracts revealed the presence of some secondary metabolites such as alkaloids, phenolic, steroids, flavonoids, terpenoids and cardiac glycosides. The total phenolic content of extract was found to be 29.39 mg/100 g while the amount of total flavonoid content was 20.67 mg/100 g. Scavenging ability was observed to increase in proportion to concentration for all the scavenging assays and at the highest concentration of 100 µg/ml. Total antioxidant capacity assay showed 19.44 mg per 100 g. This high scavenging ability in the seed extracts may be attributed to the presence of phenolic and flavonoids compounds in the extract. The DPPH free radical scavenging activity of 100µg/ml *Cucumis melo* extract was 75.20% ± 0.72 while the reference standard (Ascorbic acid) was 83.24% ±0.31. Lipid peroxidation inhibition ability of 100 µg/ml *Cucumis melo* extract was 87.18% ± 0.16 while the standard (ascorbic acid) was 94.96% ± 0.16 at the same concentration. Results obtained from this study showed that the nitric oxide scavenging ability of the extract was 80.50%±0.63 while the standard antioxidant was 85.94% ± 0.54.

Conclusion: In all the assays, *Cucumis melo* extract showed maximum percentage of antioxidant potentials at 100 µg/ml. Additionally, golden melon seed possess appreciable amount of phenols and high antioxidant properties which could be explored and incorporated in functional food applications particularly in baked products.

Keywords: Golden melon seed; phytochemical composition; antioxidant activity; free radical scavenging activity.

1. INTRODUCTION

Fruits and vegetables are vital components of human diet and their consumption has been shown to confer a great deal of nutritional and health benefits on humans including preventing and reducing the risk of certain chronic degenerative diseases [1]. They contain biological active components known as phytochemicals that promote health through the prevention of specific degenerative diseases such as cardiovascular diseases, diabetes, obesity, cancers and gastrointestinal tract disorders. Golden melon *(Cucumis melo)* is an annual, drooping herbaceous plant that is distinguished by a short angular-stem woody rootstock with bristly hairs large belonging to the family of *Cucurbitaceae (Cucurbit),* one of the most genetically diverse groups of food plants that are drought-tolerant [2]. It originated from Europe and Africa before spreading to other parts of the world. It is a bright-yellow melon with a pale green to white inner flesh which is succulent and juicy grown in the Sahel and Sudan savanna of Nigeria. Nutritionally, golden melon is a great constituent of essential minerals and nutrients such as vitamin C, pantothenic acid, calcium, zinc, vitamin B6, fibre, magnesium, iron, potassium, vitamin A and omega-3 & 6 [3].

In Nigeria, there are various types of fruits and vegetables commonly consumed according to their geographical locations and seasons. Among these fruits are citrus, oranges, mangoes, watermelon, apple, cabbage, carrot, cucumbers, pumpkin, grapes, plantain /bananas among many others. However, only the fleshy parts of these fruits and vegetables are eaten leaving the peels, rinds and/or the seeds as waste materials. There are numbers of studies that have been done by researchers exploring the potentials source of antioxidant in plant waste materials. Fruits have high vitamin, mineral, fibre, phytochemical and antioxidant contents in their pulps, seeds and rinds and *Cucumis melo* is no exception.

Reports showed that besides dietary sources, antioxidant can be gained from food processing industries especially agricultural by products [4]. These respective by products are peel, rind, seed, mill wastes and trimming wastes. Reports showed that these by products are promising sources of important bioactive substances called phytochemicals (carotenoids, phenolics, and flavonoids), antioxidants vitamins that possess beneficial physiological properties [5,6]. Previous studies have revealed that the seeds are concentrated sources of many health-benefiting vitamins, minerals, antioxidants, and essential amino acids such as tryptophan, and glutamate [7]. These phytochemicals and antioxidants can be used or incorporated into foods as ingredients for functional foods in order to promote health and prevent the risk of some degenerative diseases. In recent times, there has been an increasing consumer interests in functional foods as a result of their nutraceutical and health benefits. Functional foods are foods that provide additional health benefits beyond the basic nutritional functions.

The use of fruits and vegetables and their by-products as ingredient in food processing as well as products requiring hydration, viscosity development, and freshness preservation, such as baked foods has been well documented [8,9,10,11,12]. Bakery products are ready- to- eat foods that are known for their better shelf life, taste, affordability and nutritional value. They include breads, biscuits, cookies, muffins, cakes, pastries etc. They provide ideal matrix by which functionality in terms of bioactive components can be transferred to the consumer in without altering their organoleptic properties and acceptance of the food product [12]. Except for these reported studies, there is a dearth of information on the phytochemical composition and antioxidant activity of *Cucurmis melo L* seeds. This study, therefore, investigates the phytochemical composition and in vitro antioxidant activity of golden melon (*Cucurmis melo* L*)* seed.

2. MATERIALS AND METHODS

2.1 Sourcing and Preparation of Golden melon (*Cucumis melo*) Seeds

Golden melon *(Cucumis melo L*) fruits were obtained from local market, Lagos state, Nigeria. The seeds were removed, washed and dried in a cabinet dryer at 50°C for 4 hours before being milled. The dried seed was milled into flour which was packaged in a polyethylene for further analyses.

The seed extract was prepared according to the decoction method described by [8]. Milled *Cucurmis melo L* seeds were transferred into a round bottom flask and submerged in methanol in ratio 1:10 (w/v). The flask was stoppered and left to stand for 72 hours. The extract was then filtered using Whatman No 1 filter paper. The filtrate was concentrated using a rotary evaporator at 40°C. The resultant residue were weighed and stored at 4°C.

2.2 Phytochemical Screening of Crude Extract

The crude methanolic extracts of the seeds were tested for the presence of alkaloids, steroids, tannins, saponins and glycosides according to the methods described by [13,14,15,16,17]. The qualitative results are expressed as (+) for the presence and (_) for the absence of phytochemicals.

2.2.1 Test for flavonoids

The presence of flavonoids in the plant samples was determined as follows: (a) Five milliliters of dilute ammonia solution was added to 5 ml of the extract solutions followed by the addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids. (b) Few drops of 1% Aluminum solution were added to 2 ml of each extract. A yellow colouration was observed indicating the presence of flavonoids. (c) Five (5) ml of the extract solution was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4 ml of filtrate was shaken with 1 ml dilute ammonia solution. A yellow colouration is an indication of flavonoids presence.

2.2.2 Test for terpenoids (Salkowski test)

A 5 ml sample of the extract was mixed in 2 ml of chloroform, and 3 ml concentrated H_2SO_4 was carefully added along the sides of the test tube to form a layer. The formation of a reddish brown colouration at the interface indicated the presence of terpenoids.

2.2.3 Test for steroids

A 5 ml sample of the extract was added to 2 ml acetic anhydride and 2 ml H_2SO_4. The colour changed from violet to blue or green indicated the presence of steroids.

2.2.4 Test for alkaloid

A 5 mg sample of the extract dissolved in 3 ml of acidified ethanol was warmed slightly and then filtered. Few drops of Mayer's reagent and 1 ml of Dragendroff's reagent were added to 1 ml of the filtrate and turbidity was observed.

2.2.5 Test for tannins

A few drops of 0.1% ferric chloride was added to the extract solutions and observed brownish-green or a blue-black colouration, which signified the presence of tannins.

2.2.6 Test for saponins

Five milliliters of the extract solution was shaken vigorously for a stable persistent froth. The frothing was mixed with olive oil and shaken vigorously. The formation of emulsion indicated the presence of saponins in the samples.

2.2.7 Anthraquinone glycoside (Borntrager's test)

To the extract solution (1 ml), 5% H_2SO_4 (1 ml) was added. The mixture was boiled in a water bath and then filtered. Filtrate was then shaken with equal volume of chloroform and kept to stand for 5 min. Then lower layer of chloroform was shaken with half of its volume with dilute ammonia. The formation of rose pink to red color of the ammonical layer gives indication of anthraquinone glycosides [18].

2.7.8 Cardiac glycoside (Keller-Killiani test)

Extract (0.5 g) was shaken with distilled water (5 ml). To this, glacial acetic acid (2 ml) containing a few drops of ferric chloride was added, followed byH_2SO_4 (1 ml) along the side of the test tube. The formation of brown ring at the interface gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring [19].

2.3 Antioxidant Activity Assay

2.3.1 DPPH radical scavenging activity assay

The free radical scavenging activity of the extract was determined spectrophotometrically. It was based on the scavenging of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical which was estimated according to the procedure described by [20,21]. An aliquot of 0.5 ml of sample extract in ethanol (95%) at different concentrations (20, 40, 60, 80, 100µg/ ml) was mixed with 2.0 ml of reagent solution (0.004 g of DPPH in 100 ml methanol). The control contained only DPPH solution in place of the sample while methanol was used as the blank. The mixture was vigorously shaken and left to stand at room temperature. After 30 minutes the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm on a UV- visible spectrophotometer (Milton Roy Spectronic 601, USA). The scavenging effect was calculated using the expression:

$$\% \text{ inhibition} = [A_0\text{-}A_1] / A_0 \times 100$$

Where,

A_0 is the absorption of the blank sample and
A_1 is the absorption of the extract
Ascorbic acid was used as standard.

2.3.2 Nitric oxide scavenging activity assay

The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing nitric ions (NO•). Under aerobic condition, NO• reacts with oxygen to produce stable products (nitrate and nitrite), which can be determined using Griess reagent. The absorbance of the chromophore that formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with Naphthylethylenediamine dihydrochloride can be immediately read at 550 nm.

A 4 ml sample of flour extract or standard solution of different concentrations (20, 40, 60, 80, 100 µg/ml) were taken in different test tubes and 1 ml of Sodium nitroprusside (5 Mm in phosphate buffered saline) solution was added into the test tubes. They were incubated for 2 hours at 30°C to complete the reaction. A 2 ml sample was withdrawn from the mixture and mixed with 1.2 ml of Griess reagent (1% Sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H_3PO4). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthylethylene diamine was measured at 550 nm on a UV- visible spectrophotometer (Milton Roy Spectronic 601, USA) as modified by [22]. Ascorbic acid was used as standard and inhibition of nitric oxide radical was calculated using the expression:

$$(\%) \text{ inhibition activity} = [(A_0 - A_1)/A_0] \times 100.$$

Where,

A_0 is the absorbance of the Control and
A_1 is the absorbance of the extract or standard.

2.3.3 Lipid peroxidation assay

Lipid peroxidation was induced by Fe2+−ascorbate system in liver homogenate and estimated as thiobarbituric acid reactive substances (TBARS) by the method as described by [23,24]. In this assay, the end product of lipid peroxidation using liver homogenate as lipid- rich media [25] was quantified by determining the formed adduct of malonyldialdehyde (MDA) reaction with thiobarbituric acid (TBA) under acidic condition. The pink colored product was measured at 532 nm on a UV- visible spectrophotometer (Milton Roy Spectronic 601, USA). Into 0.5 ml of a 0.1 mg/ml liver homogenate was added 0.1 ml of varying concentrations of the extract (20, 40, 60, 80, 100 μg/ml) in a test tube followed by the addition of 1 ml distilled water. Then 50 μl of $FeS0_4$ (0.07 M) was added to the reaction mixture. The reaction mixture was vortexed and allowed to stand for 30 minutes at ambient temperature after which 1.5ml of 20% (v/v) acetic acid and 1.5 ml of 0.8% (w/v) thiobarbituric acid in 1.1% (w/v) sodium dodecyl sulphate were added. The resulting mixture was then incubated in a water bath at 95°C for 1 hr. After cooling, four milliliter of butan-1-ol was added to each tube, shaken vigorously and centrifuged at 3000 rpm for 10 min. the absorbance of the organic upper layer was measured at 532nm. The TBARS values were calculated using the extinction coefficient 1.56 ×10^{-5} M/cm.

Inhibition of lipid peroxidation (%) by the extract was calculated using the formula

$$(1-E/C) \times 100$$

Where,

C= absorbance value of the fully oxidized control
E= absorbance in the presence of extract as (A_{532} + TBA) – (A_{532} –TBA)

2.3.4 Reducing power assay

The ability of methanolic extracts to reduce iron (III) to iron (II) was assessed by the method of [26]. The dried extract (20–100 μg) in 1 ml of the corresponding solvent was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide ($K_3Fe(CN)_6$; 10 g/l), and then the mixture was incubated at 50°C for 30 min. After incubation, 2.5 ml of TCA (100 g/l) was added and the mixture was centrifuged at 1650 rpm for 10 min. Finally, 2.5 ml of the supernatant solution were mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (1 g/l) and the absorbance was measured at 700 nm on a UV- visible spectrophotometer (Milton Roy Spectronic 601, USA). High absorbance indicates high reducing power.

2.4 Estimation of Total Phenolic Content

The amount of total phenol content was determined by Folin-Ciocalteau colorimetric method [27] using gallic acid as a standard following the method as described by [28]. A 0.5 ml sample of extract and 0.1 ml of Folin-Ciocalteu reagent (0.5 N) were mixed and incubated at room temperature for 15 minutes. After this, 2.5 ml sodium carbonate solution (7.5% w/v) was added and further incubated for 30 minutes at room temperature. The absorbance of the solution was measured on a UV- visible spectrophotometer (Milton Roy Spectronic 601, USA) at 760 nm. The concentration of total phenol was expressed as gallic acid equivalent (GAE) (mg/g of dry mass) which is a commonly used reference value.

2.5 Total Flavonoid Content Estimation

Total soluble flavonoid of the extract was determined using aluminum chloride colorimetric method using quercetin as standard [29]. One milliliter of sample solution (100μg/ ml) was mixed with 3 ml of methanol, 0.2 ml of 10% Aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. The resulting mixture was incubated at room temperature for 30 minutes and the absorbance of the reaction mixture was measured on a UV-visible spectrophotometer (Milton Roy Spectronic 601, USA) at 415 nm. The calibration curve was prepared by preparing quercetin solutions at various concentrations in methanol.

2.6 Total Antioxidant Capacity Determination

The total antioxidant capacity of the extracts was determined using the method of [30]. A sample of the extract (0.3 ml) was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95^0 C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm. The total antioxidant capacity was expressed as equivalent of ascorbic acid.

2.7 Statistical Analysis

Statistical significance was established using student t test paired comparison and data were reported as mean ± of standard error (SEM) of three determinations using Microsoft Excel 2010. The p values of $p < 0.05$ were considered statistically significant for differences in mean between the standard Ascorbic acid and the seed extract. The IC_{50} values were estimated from the % inhibition versus concentration plot using linear regression analysis.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Constituents

The phytochemical screening of the seed extracts revealed the presence of different secondary metabolites like alkaloids, flavonoids, steroids, phenols, cardiac glycosides and terpenoids as presented in Table 1. The other phyto-constituents such as saponin, tannins and phlobatannins were totally absent.

Table 1. Qualitative and quantitative phytochemical screenings of aqueous extract of golden melon *(Cucumis melo* L*)* seed

Compound	Qualitative	Quantitative (mg/100 g)
Flavonoid	+	20.67±0.19
Alkaloid	+	18.85±0.12
Phenol	+	29.39±0.11
Saponin	-	ND
Cardiac glycoside	+	11.34±0.18
Phlobatanin	-	ND
Tannin	-	ND
Steroid	+	22.63±0.22
Terpenoid	+	19.49±0.14

Values are means ± SDs of triplicate determinations
ND- Not Detected

This present study showed that the predominant phytochemical of the seed extract is phenol, which was followed by steroid, then flavonoids, terpenoids and alkaloids while cardiac glycoside was the least compound.

Presence of flavonoids, tannins, and alkaloids has been observed to exhibit various biological properties related to antioxidant mechanisms [31]. They are effective hydrogen donors that inhibit the lipid oxidation and chelate metal ions, making them good anti-oxidants [32]. The cardiac glycosides are basically steroids with an inherent ability to afford a very specific and powerful action mainly on the cardiac muscle when administered through injection into man or animal. Cardiac glycosides have been reported to be used to treat congestive heart failure and cardiac arrhythmia [33]. Their mode of action starts by inhibiting Na+/K+ pump which then increases the level of calcium ion, so more Ca^{+2} would be available for the contraction of heart muscles which recover cardiac output and reduce the distension of heart [34,35].

Alkaloids are the largest group of secondary chemical constituents made largely of ammonia compounds comprising basically of nitrogen bases synthesized from amino acid building blocks with various radicals replacing one or more of the hydrogen atoms in the peptide ring, most containing oxygen.

Steroids (anabolic steroids) have been observed to promote nitrogen retention in osteoporosis and in animals with wasting illness [36,37]. Similarly, steroids derived from plants are known to have cardiotonic effect and also possess antibacterial and insecticidal properties [38]. They are very often used in medicines due to their well-known biological activities

The total phenolic content expressed as mg per 100 g of the seed extract was found to be 29.39 mg/100 g while the total flavonoid content was 20.67 mg/100 g. The phenolic content can be used as an important indicator of antioxidant capacity and can be used as a primary screening for any product when intended for use as a natural source of antioxidants in functional foods.

Plants contain a wide variety of free radicals scavenging molecules including phenols, flavonoids, vitamins, terpenoids that are rich in antioxidant activity [39,40]. Antioxidants protect cells against the damaging effects of reactive oxygen species otherwise called, free radicals such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynite which result in oxidative stress leading to cellular damage [41]. Natural antioxidants play a key role in health maintenance and prevention of the chronic and degenerative diseases, such as atherosclerosis, cardiac and cerebral ischemia, carcinogenesis, neurodegenerative disorders, diabetic pregnancy, rheumatic disorder, DNA damage and ageing [42]. Plant polyphenols are the significant group of compounds acting as free radical scavenging or primary antioxidants; therefore, it is justifiable to determine phenolic content in plant extract.

Phenolics essentially represent a host of natural antioxidants and are used as nutraceuticals. They are found in apples, green tea, and red wine for their enormous ability to combat cancer and are also thought to prevent heart ailments to an appreciable degree and sometimes are anti-inflammatory agents. The most common group of plant phenolics is the flavonoids, the structures of which are based on that of flavone, consisting of two benzene rings linked through a three-carbon γ-pyrone ring. Common classes of flavonoids include flavones, flavonols, isoflavones, anthocyanins, catechins (flavanols) and flavanones. Flavonoids are a group of phenolic compounds with antioxidant activity that have been identified in fruits, vegetables, and other plant foods and have been linked to reducing the risk of major chronic diseases.

The health-related properties of phenolic compounds, particularly flavonoids, are believed to be based on their antioxidant activity as hydrogen donating free radical scavengers [43,44].

3.2 Free Radical Scavenging Activity

The antioxidant potential of the *Cucumis melo* seed extracts was examined by determining1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, *in vitro* lipid peroxidation, and nitric oxide (NO) scavenging assay. The free radical scavenging capacity was observed at 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml of methanol extracts. The results showed that the seed extracts of test fruit had radical scavenging activity. A number of assays were conducted to analyze antioxidant activities of the extract since antioxidant activities of different types of substances involve different mechanisms.

Scavenging ability of the methanolic extract was observed to increase in proportion to concentration for the sample and at the highest concentration of100 µg/ml.

The DPPH free radical scavenging activity of *Cucumis melo* extract at concentrations from 20 to 100µg/ml differed significantly ($p < 0.05$) compared to the reference standard which is ascorbic acid. It ranged from 37.86 % ± 0.64 to 75.20% ± 0.72 while the reference standard (Ascorbic acid) ranged from 44.72% ± 0.23 to 83.24% ± 0.31 as was depicted in Fig 1. *Cucumis melo* seed showed maximum inhibition (75.20%) of DPPH free radical at 100 µg/ml while the IC_{50} values for the extract and the reference standard (Ascorbic acid) were found to be 53.28 ± 0.56 µg/ml and 46.77 ± 0.34 µg/ml respectively. IC_{50} value is defined as the concentration of the sample required to scavenge DPPH radical by 50%, and was obtained from a calibration curve for the extract. The higher the antioxidant activity, the lower is the value of IC_{50} value.

It was clear that the free radical scavenging activity of the extract was in a concentration dependent manner. This high scavenging ability in the seed extracts may be attributed to the presence of phenolic and flavonoids compounds in the extract, which in-vitro studies have shown that flavonoids compounds may have positive effects against inhibition oxidation of biomolecules by its antioxidative potential, anti-inflammatory, anti-allergic, and antifungal effect [45,46,47]. This study is in agreement with the result obtained by [48] who worked on the free radical scavenging activity of *Bergenia stracheyi* extracts.

Free radicals are known to be scavenged by synthetic antioxidants, but due to their adverse side effects leading to carcinogenicity, search for effective and natural antioxidants has become crucial [49]. Natural antioxidants are believed to be safer and bioactive [50]. DPPH assay was carried out to measure the primary antioxidant activity of the sample in the present study. The ability of a substance to remove or scavenge free radicals is classified as a primary antioxidant [51]. DPPH assay reaction depends on the ability of the samples to scavenge free radicals which is visually noticeable as the colour change from purple to yellow due to hydrogen donating ability [52]. The more rapid the absorbance decreases, the more potent the primary antioxidant activity [53].

Fig. 2 presents lipid peroxidation inhibition ability of *Cucumis melo* seed extract at different concentrations of 20 to100 µg/ml. It ranged from 47.18±0.13 - 87.18%±0.16 while the standard (ascorbic acid) ranged from 45.55%±0.12-94.96%±0.16. The IC_{50} was found to be 50.34± 0.36 µg/ml for the extract, and ascorbic acid which is the standard reference antioxidant as 45.75±0.42 µg/ml. Lipid peroxidation scavenging activity of *Cucumis melo* seed showed maximum inhibition (94.96%) at 100 µg/ml in a concentration dependent manner and differed significantly ($p< 0.05$) from the standard antioxidant. Extensive research studies have shown that lipid peroxidation is probably the most investigated free radical induced process [54,55,56,57]. The production of malondialdehyde (MDA) becomes elevated after oxidative stress has initiated lipid peroxidation [58]. MDA is expressed when the oxidative insult exceeds the threshold value [59]. Antioxidants are important inhibitors of lipid per-oxidation not only as a defense mechanism of living cells against oxidative damage but also for food preservation [48].

Reducing power of the methanolic of *Cucumis melo* seed extract was determined and the results are shown in Fig. 3. Significant reduction ($p< 0.05$) was observed in the reducing power of the seed extract when compared with the standard reference ascorbic acid. In the same manner, the methanolic extract displayed the highest reducing power at 100 µg/ml in a concentration dependent manner. In this assay, the presence of reducers (i.e., antioxidants) causes the reduction of the $Fe3+$/ferricyanide complex to the ferrous form. Therefore, measuring the formation of Perl's Prussian blue at 700 nm can monitor the $Fe2+$ concentration [41]. The mechanisms of antioxidant properties of plant extracts are assumed to be based on their ability to trap positively charged electrophilic species, to scavenge oxygen radicals, to have reducing power, and to chelate metals to form inactive complexes [60] and these properties are majorly dependent on phenolic compounds found in the extracts. Some studies have however suggested that a proper and reasonable heat treatment application to food materials such as citrus peel could be used to enhance its antioxidant properties. In addition, polyphenols, including flavonoids that are soluble in less polar solvents, may be able to chelate metal ions such as iron and copper because of the large number of hydroxyl groups (OH) of their chemical structure, which are responsible for conferring the chelating ability [61].

In order to evaluate the antioxidant potency through NO scavenging by the test samples, the change of optical density of NO was monitored. Results obtained from this study showed that the Nitric Oxide scavenging ability of the extract ranged from 33.19- 80.50%±0.63 while the standard antioxidant ranged from 44.97% ±0.74 - 85.94% ± 0.54. There was a significant difference ($p<0.05$) between the seed extract and the standard antioxidant at the different concentrations studied. The IC_{50} value was estimated as 58.36 ± 0.34 µg/ml for the extract and 51.66±0.22 µg/ml for ascorbic acid which was the standard reference antioxidant.

Nitric oxide scavenging activity of *Cucumis melo* seed showed maximum inhibition (80.50%) at 100µg/ml in a concentration dependent manner as well (Fig. 4). These demonstrated the ability of the seed in arresting the chain of reactions initiated by excess generation of reactive nitric oxide species (NO.) that are deleterious to the human health [62]. Nitric oxide is classified as a free radical because of its unpaired electron and displays important reactivity with certain types of proteins and other free radicals. This result was in agreement with study conducted by [22].

In all the assays, *Cucumis melo* extract showed maximum percentage of antioxidant potentials at 100µg/ml with the IC_{50} values well comparable to that of the ascorbic acid.

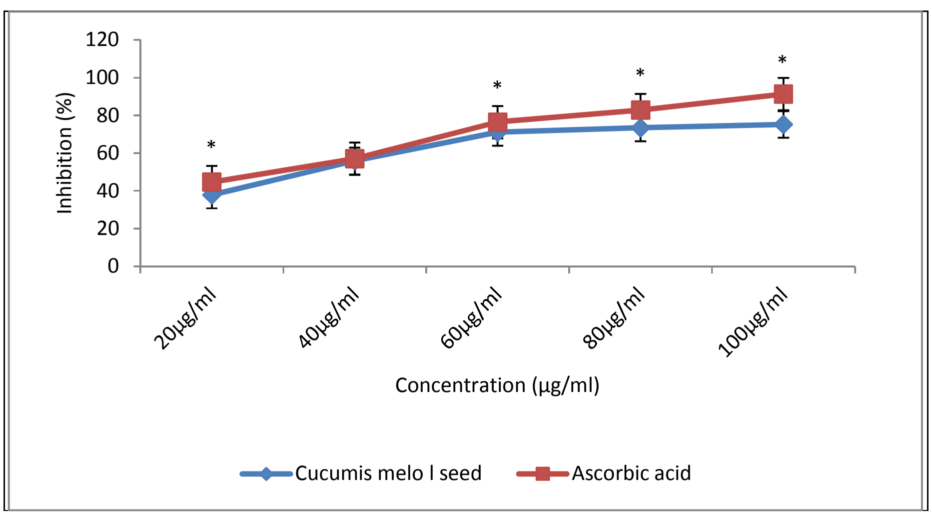

Fig. 1. DPPH radical scavenging activity of *Cucumis melo* L seed extract at different concentrations

Ascorbic acid was used as positive control. Bars represent standard error (SEM) of three replications.
**Statistically significant at $p < 0.05$*

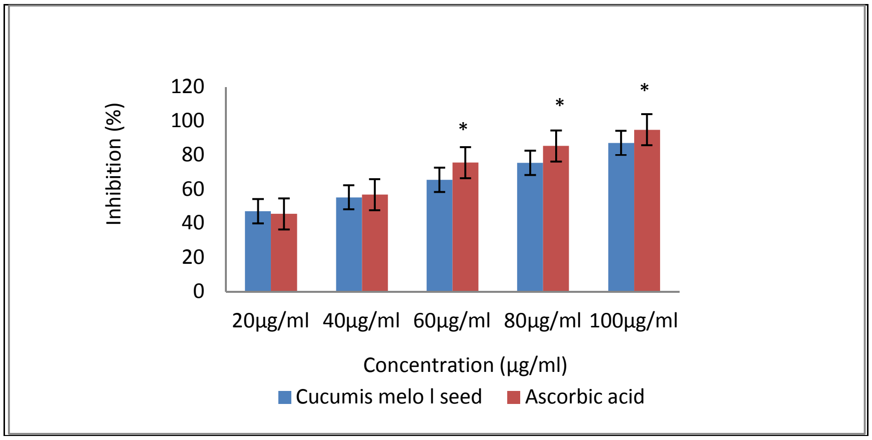

Fig. 2. Lipid peroxidation inhibition by *Cucumis melo* L seed extract at different concentrations
*Bars represent standard error (SEM) of three replications; *Statistically significant at p < 0.05; Ascorbic acid was used as positive control.*

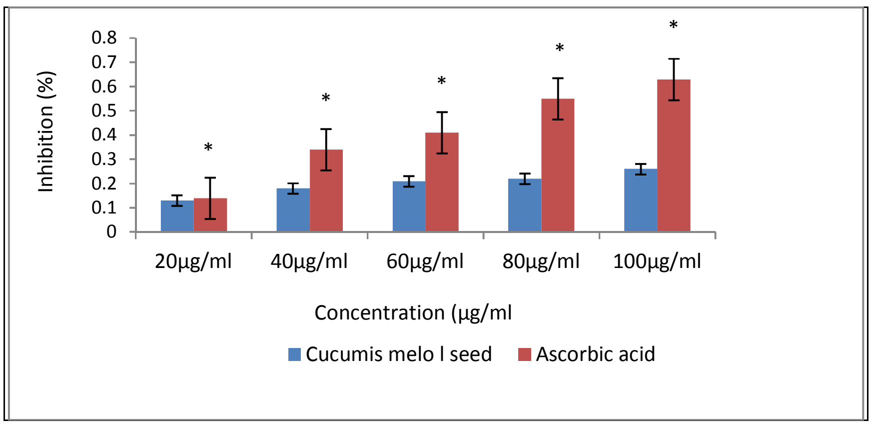

Fig. 3. Reducing power of different concentration of *Cucumis melo* L seed extract which was estimated by potassium ferricyanide method
*Bars represent standard error (SEM) of three replications; *Statistically significant at p < 0.05; Ascorbic acid was used as positive control*

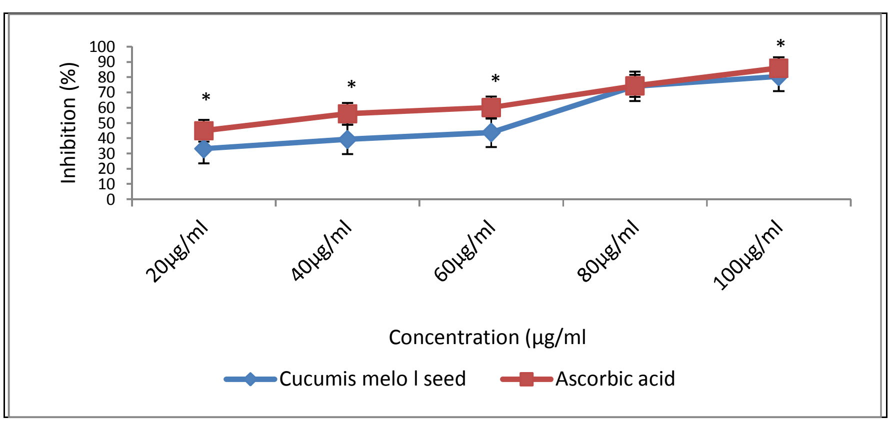

Fig. 4. Nitric oxide scavenging activity of *Cucumis melo L* seed extract at different concentrations
*Bars represent standard error (SEM) of three replications; *Statistically significant at p < 0.05; Ascorbic acid was used as positive control*

4. CONCLUSION

The results of phytochemical screening of methanolic extracts of *Cucumis melo* L seed reveals the presence of alkaloids, flavonoids, phenolics, steroids, cardiac glycosides and terpenoids. The free radicals scavenging activity results show that *Cucumis melo* L seed has compounds of radical scavenging potential. Antioxidant activity of plant material is one of the key indicators of the health beneficial effects in prevention and reduction of risk of diseases which is becoming increasingly rampant even in developing countries. The phenol and flavonoid contents and radical scavenging properties of the seed are associated with its antioxidant properties. Hence, this study had revealed that golden melon seed flour possess appreciable amount of phenols and flavonoids and high antioxidant properties which could be explored and incorporated in functional food applications particularly in baked products which serve as potential delivery systems for food fortification, enhancement and functionality.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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