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Assessment of Nutraceuticals Potentials of Protein Isolates from Seed Coat of Four Melon Species

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ABSTRACT: Recently, there has been more attention on the bioactivity of phytochemicals and other metabolites in agro-food by-products for the management of oxidative stress–related conditions. Hence, the objective of this paper is to assess the nutraceuticals potential by investigating antioxidant and α-amylase inhibitory potentials of seed coat protein isolates from four melon species namely: *Citrullus colocynthis*, *Citrullus mucosospermus*, *Cucumeropsis mannii* and *Lagenaria siceraria* using appropriate standard methods. At highest concentration, *Lagenaria siceraria* had the strongest free radical scavenging activity (38% inhibition). The strongest chelating effect was exhibited by *C. mannii* (IC_{50} = 0.59 ± 0.08 mg/ml), which also showed highest ferric reducing potential at all concentrations. At 0.05 mg/ml, *C. colocynthis*, *C. mucosospermus*, *C. mannii*, and *L. siceraria* seed coat protein extracts inhibited alpha-amylase activity by 43.2%, 16.76%, 2.6% and 22.3%, respectively, and inhibition of 60.9%, 28.6%, 38.3% and 29.5% were recorded for the seed protein extracts respectively, at 0.5 mg/ml. In conclusion, the various melon seed coat protein isolates showed appreciable levels of antioxidants and possessed inhibitory activity against α-amylase. Hence, the seed coats of the four melon varieties assessed in this study are promising potential sources of antioxidants for the supplementary treatment of oxidative stress–induced conditions.

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Oxidative stress is a physiological condition, which arises due to an unevenness between the reactive oxygen species generation and the capability of human body to counteract or detoxify their detrimental effects with endogenous antioxidants (Jelinek *et al*., 2021). Temporary instances of oxidative stress can occur in tissues damaged by factors such as infection, trauma, and overexposure to oxygen, toxins, heat, and rigorous physical activity (Gambini and Stromsnes, 2022). Together with genetics, lifestyle, and environmental factors, oxidative stress has been main reference in a wide range of conditions, including aging (Kumar *et al*., 2023), cardiovascular diseases (Franco *et al*., 2022), cancer (Ginckels and Holvoet, 2022), neurodegenerative diseases (Teleanu *et al*., 2022), diabetes (Singh *et al*., 2022), inflammation (Ndrepepa *et al*., 2019), respiratory disorders (Cannavò *et al*., 2021), liver diseases (Karkucinska-Wieckowska *et al*.,

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2022), and eye defects (Ryan *et al*., 2023). Among the types of diabetes in Nigeria, diabetes mellitus (Type 2) is fund in higher percentage (90%) of all reported cases (Galicia-Garcia *et al*., 2020). According to the International Diabetes Federation (IDF), approximately seventy percent of African diabetic patients are undiagnosed (Muhammad, 2020). Diabetes mellitus (DM) is a noncommunicable disease linked with the irregular/abnormal metabolism of carbohydrates leading to postprandial hyperglycemia (Banday *et al*., 2020). This is mostly due to insulin resistance or insulin deficiency. This disease is a major concern around the world because of the high consumption of carbohydrates, chronic course, and disabling complications. Pancreatic β-cells are responsible for generating insulin, which plays a crucial role in facilitating the uptake of glucose into cells to supply energy, and it also participates in various other physiological processes. The consequences of diabetes, including stroke, heart attack, kidney failure, and blindness, are severe (Dal Canto *et al*., 2019**).** Aside, sulfonylureas (SUs) and biguanides which are the most frequently prescribed antidiabetic drugs by primary care physicians (Mehrpour *et al*., 2022), other classes of drugs also exist. Only a few primary care physicians have been reported to prescribe other classes of antidiabetic drugs to their patients (Ugwu *et al*., 2020). The enzymes such as α-amylase and α-glucosidase plays an important role in the breakdown of carbohydrates. Their inhibition is crucial for managing level of glucose in the blood of persons with type 2 diabetes and those on the borderline of diabetes. At present, there is a renewed focus on natural antioxidants found in plants and functional foods that can impact physiological processes, neutralize reactive oxygen species (ROS), and potentially prevent and manage diabetes and obesity. Interestingly, Nigeria is a country with a biodiversity of medicinal plants that is gaining importance in traditional medicine for the management of various oxidative stress-induced diseases, including diabetes. Currently, a significant number of people and health practitioners depend on herbal medicines rather than scientifically proven therapies. Numerous herbal extracts have demonstrated antidiabetic effects and are utilized for diabetes treatment. Such herbal remedies hold promise as sources of innovative compounds for diabetes treatment, offering a more economically feasible approach (Willcox *et al*., 2021). Herbal medicines are potential sources of new molecules for the treatment of diabetes, as they are more cost-effective and have fewer side effects. (Kumar *et al*., 2021). The transformation and conversion of by-products from auxiliary and agricultural waste into the production cycle present a viable solution for the cost-effective utilization of these resources for medical, industrial, agricultural, and other related sectors (Bala *et al*., 2023).

Melons are highly important staple food crops across sub-Saharan Africa and tropical regions (Giller *et al*., 2021). They belong to the Citrullus family, which includes a diverse range of fruits. Melons are commonly consumed, and their seeds find their way into various culinary dishes (Mabaleha *et al*., 2007). Predominant cultivation areas include the Middle East and West Africa, particularly in countries such as Nigeria, Ghana, Togo, and Benin, where they are often interplanted with maize, cassava, and yam to optimize growth (Walters *et al*., 2021). In regions where melon seeds are consumed, they are commonly referred to as "egusi" (Giwa and Akanbi, 2020). Melon seeds have emerged as valuable sources of protein, oil, essential minerals, vitamins, and energy (Rabadán *et al*., 2020). Furthermore, melon seeds are recognized for their abundance of biologically active compounds such as tocopherols, phospholipids, and sterols (Khalid *et al*., 2021). These compounds have traditionally acknowledged properties, including analgesic, anti-inflammatory, antioxidant, and even anticancer effects. Melon seeds are speculated to possess components that can help manage and prevent diabetes (Rolim *et al*., 2018). There are few studies that have been carried out on melon seed coats because seed coats are regarded as agricultural waste that could pose environmental issues, if not properly managed. In an attempt to turn waste to wealth, the present study extracted soluble proteins from the seed coats of four melon species namely: *C. colocynthis*, *C. mucosospermus*, *C. manii*, and *L. siceraria* and investigated their antioxidant and α-amylase inhibitory activities.

MATERIALS AND METHODS

Sample Collection: Four melon species were used for the study, namely, *Lagenaria siceraria*, *Citrullus colocynthis*, *Cucumeropsis mannii*, and *Citrullus mucospermus*. These melon varieties are locally known as "Igba," "Sophi," "Itoo," and "Papa" in the Southwestern region of Nigeria. The melon fruits were purchased from Better-Life Market, Modakeke, Ile-Ife in Osun State, Nigeria. The fruits were subsequently identified and authenticated at The Ife Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

Preparation of Extracts: The seed coats (outer coverings) of the melon seeds were collected by shelling and pulverized into fine powder using an electric blender. The resulting powder underwent a double sieving process to achieve a fine consistency.

The fine powder was homogenized with phosphate-buffered saline (PBS) at a pH of 7.4 for 6 hours. The homogenate was filtered through cheesecloth, and the resulting filtrate was subjected to centrifugation at 10,000 rpm for 30 minutes. The supernatant was subjected to ammonium sulphate precipitation, and the precipitate was obtained by centrifugation and subsequently subjected to dialysis. The dialysate was freeze-dried to yield the final product used for subsequent analyses.

DPPH radical scavenging assay: The stable radical DPPH assay method of Cao *et al*. (2013) was employed to evaluate the radical scavenging capacity of the proteins. Freshly prepared DPPH solutions containing 0.1 mM DPPH in 95% methanol were used daily. In each test tube, 100 µL of protein hydrolysate at various concentrations was mixed with 0.5 mL of the DPPH solution. The mixture was nurtured for half an hour in the darkroom at ambient temperature, after which the optical density was read at 517 nm. The standard radical scavenger used was glutathione, while distilled water was used as the blank. Equation 1 was used in calculating the percentage DPPH radical scavenging (DPPH RS).

$$\text{DPP RS } (\%) = \frac{A_B - A_S}{A_B} \text{ x } 100\% \quad (1)$$

Where DPP RS = DPPH Free radical scavenging (%); AB = absorbance of blank; AS = Absorbance of sample

Ferric Reducing Potential Activity Assay: The reducing power of the protein isolates was tested using a slightly modified method reported by Babu *et al*. (2013). Protein hydrolysates were mixed with phosphate buffer in volumes ranging from 0.2 to 0.5 millilitres and vortexed. Thereafter, potassium ferricyanide (1% (w/v, 0.5 ml) solution was added, and the mixture was left undisturbed for 20 minutes in a regulated water bath set at 50°C. Ten percent TCA (0.5 mL) was added to the mixture. The 0.1% solution of ferric chloride was mixed with equal volume (1.0 mL) of the supernatant and distilled water. The mixture was monitored at 700 nm. Glutathione was used as the positive control.

Metal Chelating Activity Assay: The iron (II) chelating activity of the protein isolates was evaluated using a modified technique according to Dinis *et al.* (1994). One millilitre of ferrous sulphate (2 mM) and various volume of the extracts (0-500 µl) were mixed together. The reaction was initiated by adding 1 ml of ferrozine (0.25 mM), shaking vigorously, and left at 27°C on the laboratory bench for 10 minutes. Ferrozine forms a stable magenta complex when it interacts with divalent iron. In the controls, 1 ml of deionized water was used. The absorbance of the reaction mixture was measured at 517 nm. The chelating activity was calculated using equation 2:

$$\text{CR } (\%) = \frac{A_B - A_M}{A_B} \text{ x } 100\% \quad (2)$$

Where CR = Chelating rate (%); AB = absorbance of the blank; AM = absorbance of the mixture;

Alpha-Amylase Inhibitory Activity Determination: Alpha-amylase inhibitory analysis followed the procedure outlined by Apostolidis *et al*. (2007). In this method, 250 µl of varying concentrations of the extract (dialysate) was transferred into separate test tubes. To these tubes, 0.25 ml of 0.002 M sodium phosphate buffer (pH 6.9) having 500 µg/ml α-amylase was added. The reaction was initiated by introducing 250 µl of a solution consisting of 1% soluble starch dissolved in distilled water. Subsequently, the contents of each test tube were placed in a water bath set at 25°C for 10 minutes. Five hundred microlitres of chromogenic agent, 3,5-dinitrosalicylic acid (DNSA) was added to the reaction to stop it. This is was followed by further incubation in boiling water for 5 minutes. The solution was then placed under running tap to cool them to room temperature. The volume of each test tube was increased with 5 ml of distilled water, before the absorbance was taken at a wavelength of 540 nm.

Statistical analysis: The results are presented as the means ± SDs (standard deviations). All the data were analysed by one-way analysis of variance (ANOVA) using GraphPad Prism software (version 7.0). Mean values were separated using Tukey's test for post hoc analysis at $P < 0.05$. The results are expressed as the mean ± S.E.M.

RESULTS AND DISCUSSION

This study confirmed the presence of soluble proteins within the seed coats of four different seeds of the melon species analysed. The technique employed for extracting the bioactive component likely contributed to a substantial yield of proteins (Jah and Sit, 2022). Numerous plants have been identified to possess antioxidant properties, as evidenced by studies conducted on seed protein isolates from these plants. Similar extracts from different plants have been found to have extensive applications as food additives, preservatives, or dietary supplements (Oriyomi *et al*., 2022). Deledda *et al*. (2021) suggested that increasing the consumption of dietary antioxidants could contribute to maintaining the body's antioxidant status

and normal physiological functions. The recognition of antioxidants as vital phytonutrients is highly recommended due to their protective roles in food and pharmaceutical products against various types of oxidative deterioration (Gulcin, 2020).

DPPH free radical scavenging assay is extensively used to evaluate the antioxidant activity of natural compounds. In the presence of a hydrogen/electron donor (an antioxidant that scavenges free radicals), the absorption intensity decreases, causing the radical solution (the purple chromogen of DPPH radicals) to transition from its original color to a pale-yellow hydrazine. This discolouration occurs in proportion to the number of electrons captured (Locatelli *et al*., 2009). This method serves as a rapid and cost-effective approach for evaluating the antiradical activity of various food products (Rubab *et al.*, 2022). For the DPPH radical scavenging analysis, all protein extracts obtained from melon seed coats showed high DPPH activity, which increased as the concentration increased. Proteins from the seed coats of *L. siceraria* demonstrated the most potent radical scavenging activity **(Fig. 1)**. The results of this study are indicative of the ability of *L. siceraria* melon seed coat proteins to act as the most potent antioxidants by scavenging (neutralizing) ROS or free radicals better than the others. Hence, the protein extract from *L. siceraria* seed coats could be regarded as an antioxidant that is beneficial for human health because it can help protect cells and tissues from oxidative stress and damage (Janciauskiene, 2020) or as a natural antioxidant that can be used in the food and beverage industry to extend the shelf life of products to prevent lipid oxidation (rancidity) and improve the stability of certain ingredients (Petcu *et al*., 2023**)** or as an anti-ageing or skin-protecting agent used in medication and cosmetics (Rusu *et al.*, 2022).

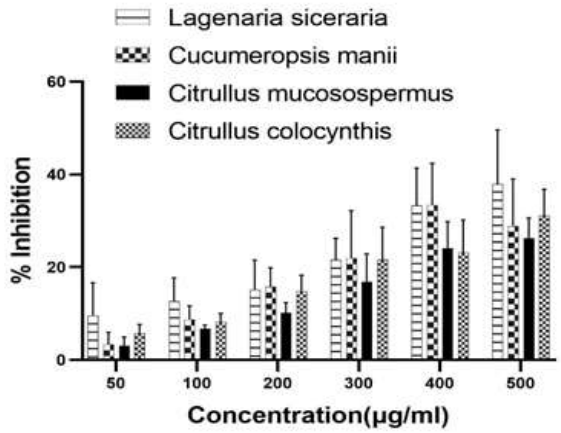

Fig. 1: Comparative DPPH radical scavenging activity of four different species of melon seed coat protein extract. The values are the means ± SEMs (n = 5).

Chelating activity typically refers to the ability of a substance to bind to metal ions, forming stable complexes known as chelates (Adusei *et al*., 2019). Chelating agents are often used in various applications, including in the food industry to prevent metal-induced oxidation and in medicine to remove toxic metal ions from the body. Heavy metals are quickly eliminated within a few hours or days, whereas hazardous metals accumulate over time and are therefore dispersed to many organs (Thakur and Flora, 2023). Excess free ions have been implicated in the stimulation and formation of free radicals in biological systems, and we tested melon seed coat proteins in a metal chelation assay. The melon seed coat proteins of the four species exhibited metal chelating activities in a concentration-dependent manner. However, *C. mucosospermus* and *L. siceraria* demonstrated little to no chelating activity within the concentration range used **(Fig. 2)**. *Citrullus colocynthis* and *Cucumeropsis manii* are excellent chelators with half-maximal inhibitory concentration (IC_{50}) 0.59 ± 0.08 mg/ml. This is possibly due to the presence of amino acid residues such as histidine, cysteine, or glutamic acid in their protein structures that have metal-binding capabilities (Shi *et al*., 2022). Such activity is also typical of antioxidant substances that mitigate oxidative damage by binding to and neutralizing metal ions such as iron and copper, which are involved in oxidative stress reactions responsible for the formation of harmful reactive oxygen species that damage cells and tissues.

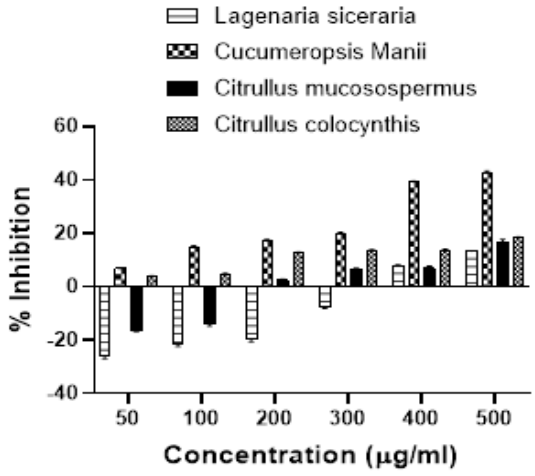

Fig. 2: Comparative metal ion chelating activity of four different species of melon seed coat protein extracts. The values are the means ± SEMs (n = 5).

Reducing power is commonly utilized in assessing the antioxidant activity of plants (Yu *et al*., 2021). The presence of reductants, which exert antioxidant effects by breaking free radicals into smaller chains by donating a hydrogen atom, is often related to the

presence of reducing power. The ferric reducing activity of a substance refers to its ability to donate electrons to ferric ions (Fe^{3+}) and convert them into ferrous ions (Fe^{2+}). This process helps to counteract the damaging effects of oxidative reactions by reducing the levels of reactive oxygen species (Siddeeg *et al*., 2021). The results of the present study showed that melon seed coat proteins contained reductants that decreased the Fe^{3+}/ferricyanide complex to Fe^{2+}/ferrous (Holmes-Hampton *et al.*, 2014). The ferric reducing potential of the seed coat protein extracts of the melon species increased linearly as the concentration of the sample increased (**Fig. 3**), with *C. manii* showing the greatest reducing potential at all concentration levels and *C. colocynthis* showing the least reducing potential which is significantly different from others. Ferric reducing ability is often used as a measure of the antioxidant capacity of samples (Hsieh and Rajashekaraiah, 2021). Substances with high ferric reducing activity are excellent at neutralizing harmful oxidative reactions caused by free radicals and reactive oxygen species (Munteanu and Apetrei, 2021).

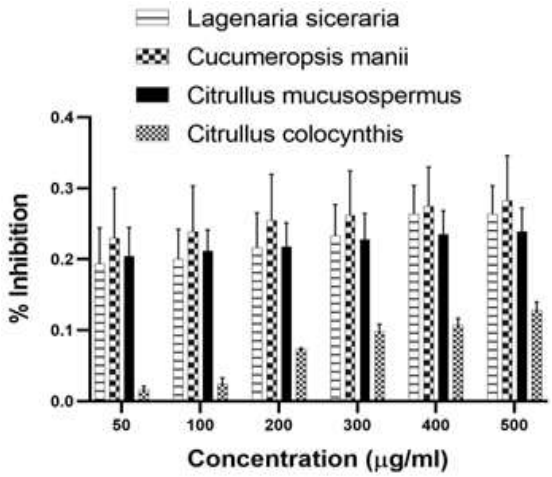

Fig. 3: Ferric reducing activity of four different species of melon seed coat protein extracts. The values are the means ± SEMs (n = 5).

Targeting the inhibition of pancreatic alpha-amylase represents a therapeutic approach to slow the digestion of oligosaccharides into absorbable monosaccharides within the intestinal brush border (Dandeker *et al*., 2021). This approach leads to a decrease in postprandial hyperglycemia (Oyedemi *et al*., 2017). Numerous herbal extracts have been documented for their potential antidiabetic properties and are employed in diabetes treatment (Salehi *et al.,* 2019). The results of the alpha amylase inhibitory studies demonstrated that the four melon seed coat proteins had significant inhibitory potential. The graph of percentage inhibition plotted against different concentrations showed that the enzyme was inhibited in a concentration-dependent manner **(Fig. 4)**. While *C. mannii* showed a significant difference in its percentage inhibition across each concentration, *C. colocynthis*, *C. mucosospermus* and *L. siceraria* showed no significant difference, although their percentage inhibition was concentration dependent. The percentage inhibition obtained for *Citrullus colocynthis* was significantly greater than that for the other species across the different concentrations used. At the lowest concentration (0.05 mg/ml), *C. colocynthis*, *C. mucosospermus*, *C. mannii* and *L. siceraria* inhibited 43.2%, 16.76%, 2.6%, and 22.3% of α-amylase activity, respectively. Also at the highest concentration tested *C. colocynthis*, *C. mucosospermus*, *C. mannii*, and *L. siceraria* inhibited the enzyme activity by 60.9%, 28.6%, 38.3%, and 29.5%, respectively. Proteins that inhibit alpha-amylase have been found to be used either directly or indirectly in the formulation of numerous modern medications in response to biotic stressors (Kasar *et al*., 2022). This study is further supported by Unuofin *et al.* (2018), who reported an IC_{50} value of 0.438 mg/ml for aqueous buffered extracts of *Kedrostis africana*, and Olodude *et al.* (2017), who reported an IC_{50} value of 0.13 ± 0.01 mg/ml for partially purified lectin from *Trilepisium madagascariense ficalho* seeds with significant alpha-amylase inhibitory activity.

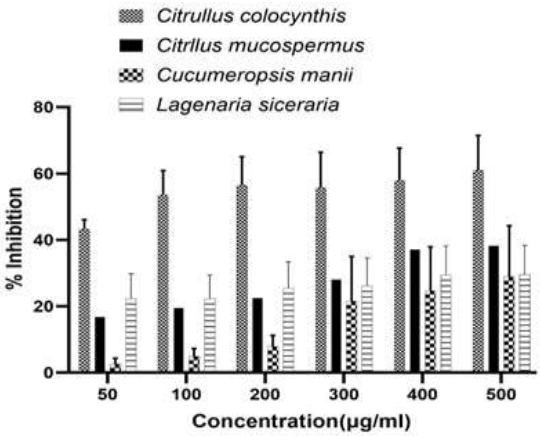

Fig. 4: Comparative α-amylase inhibitory activity of four different species of melon seed coat protein extract. The values are the means ± SEMs (n = 5)

Conclusion: In conclusion, the various melon seed coat proteins showed quantifiable antioxidant and inhibitory effects on alpha-amylase. Hence, the seed coat proteins of melon represent a potential source of complementary therapy against diabetes. Furthermore, a comprehensive and thorough *in-vivo* study is required to investigate the role of these extracts and their bioactive components. This study supports the

use of the melon seed coats used in this research for further studies to determine their potential for diabetes management.

Declaration of Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: Data are available upon request from the corresponding author.

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