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Comparative analysis and determination of antioxidant profile of sesame seed, date seed and chestnut

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ABSTRACT

This study comparatively analyzed the antioxidant profiles and phytochemical composition of Sesame seed (Sesamum indicum), Date seed (Phoenix dactylifera), and Chestnut (Castanea sativa) using various antioxidant assays and analytical techniques which included 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and the total phenolic content. The results showed significant variations in the antioxidant capacities of the three samples. The antioxidant profile of each sample was further characterized by identifying and quantifying the major antioxidant compounds present, including phenolic acids, flavonoids, carotenoid, anthocyanin and ascorbic acid. Results indicated that Chestnut seed exhibited the highest antioxidant activity for DPPH with (51.3%) followed by Sesame seed (42.8%) and Date seed (38.6%). The ascorbic acid content in Chestnut seed was very high (310.68mg/100g) but extremely low in sesame seed (0.03mg/100g).Carotenoid content in Chestnut was very high (28.74µg) but low in date seed (1.96µg). Phytochemical analysis revealed that Date seed had the highest total phenolic content (1350. 64 mg GAE/100g) and flavonoid concentration (742.18 mg /100g). This suggests that dietary intake of Chestnut, Date and Sesame seeds can go a long way to protecting against diverse disease and their seeds could be valuable in functional foods, nutraceuticals, and pharmaceutical applications.

Keywords: *Antioxidants, phytochemicals, nutraceutical, assays, functional foods*

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INTRODUCTION

The increasing prevalence of oxidative stress-related conditions such as cardiovascular diseases, cancer, diabetes, and neurodegenerative disorders has drawn significant attention to antioxidants as natural defense mechanisms (Halliwell, 2024). Antioxidants are molecules that inhibit the oxidation of other molecules, protecting cells from the damaging effects of free radicals and reactive oxygen species (ROS). Oxidation is a natural chemical process in the body that generates ROS as byproducts of metabolic activities. While these reactive species are essential for normal physiological functions, excessive production leads to oxidative stress, which can harm proteins, lipids, and DNA (Forman and Zhang, 2021). Antioxidants, which include vitamins, phenolic compounds, flavonoids, and other bioactive constituents, have the capacity to neutralize reactive oxygen species (ROS) and free radicals, thereby protecting cells from damage (Olaokun et al., 2020). While synthetic antioxidants are commonly used in food and pharmaceutical industries, concerns about their potential side effects have spurred interest in natural antioxidant sources (Shahidi and Naczk, 2014). Plants have emerged as valuable reservoirs of natural antioxidants due to their diversity in bioactive compounds. Seeds, fruits, and nuts, in particular, are rich in phytochemicals that exhibit antioxidant properties (Adebayo et. al., 2020). Antioxidants are broadly classified into two categories: endogenous antioxidants and exogenous antioxidants. Endogenous antioxidants are produced within the body and include enzymatic antioxidants like superoxide dismutase (SOD), catalase, and glutathione peroxidase, as well as non-enzymatic antioxidants such as glutathione and uric acid. Exogenous antioxidants are obtained from external sources, primarily diet, and include vitamins (e.g., vitamin C and E), minerals (e.g., selenium and zinc), and plant-derived compounds like flavonoids, polyphenols, and carotenoids (Pisoschi et al., 2016).

The key functions of antioxidants include:

- Neutralizing ROS: Antioxidants donate electrons to ROS, neutralizing their reactivity and preventing cellular damage.
- Preventing lipid peroxidation: They protect cell membranes by inhibiting the oxidation of lipids, a process that can lead to cell dysfunction.
- DNA protection: Antioxidants reduce the risk of mutations and DNA damage caused by oxidative stress, thereby lowering the risk of diseases such as cancer.

The benefits of antioxidants are well-documented in the prevention and management of chronic diseases. For instance, they play a significant role in reducing inflammation, supporting immune function, and improving cardiovascular and neurological health. Dietary sources of antioxidants, such as fruits, vegetables, seeds, and nuts, are considered the best options due to their bioavailability and minimal side effects compared to synthetic antioxidants (Shahidi and Ambigaipalan, 2015).

In recent years, the exploration of plant-based antioxidants has gained prominence due to their high efficacy and diverse bioactive compounds. Natural antioxidants, including those found in Sesame seed, Date, and Chestnut, have become increasingly popular for their role in promoting health and combating oxidative stress. Among the natural sources of antioxidants, Bene seed (Sesame seed), Date (Phoenix dactylifera), and Chestnut (Castanea species) stand out due to their exceptional nutritional profiles and medicinal benefits. These plant-derived foods are rich in bioactive compounds, including vitamins, minerals, phenolics, and flavonoids, which contribute to their antioxidative, anti-inflammatory, and overall health-promoting properties.

Sesame seed is a highly nutritious oilseed known for its rich composition of antioxidants, particularly sesamin, sesamol, and sesamolin, which are potent lignans with antioxidant properties. These compounds protect against lipid peroxidation, reduce oxidative stress, and exhibit anti-inflammatory effects (Nwanna and Agboola, 2018; Dravie et al., 2020). Sesame seed is also an excellent source of vitamin E, a fat-soluble antioxidant that prevents cell membrane damage. Additionally, the seed is known for its cardiovascular benefits, such as lowering cholesterol levels and improving heart health, due to its high content of unsaturated fatty acids (Oboh et al., 2019).

Date fruits are celebrated for their remarkable antioxidant and anti-inflammatory properties. They are rich in phenolic acids, flavonoids, and carotenoids, all of which contribute to their ability to neutralize reactive oxygen species (ROS) and reduce oxidative stress (Al-Farsi et al., 2015). Dates are also a good source of dietary fiber, which promotes digestive health, and natural sugars, which provide sustained energy. Moreover, studies have shown that date fruits possess antimicrobial and neuroprotective properties, making them a valuable functional food (Baliga et al., 2011).

Chestnuts are unique among nuts for their low fat content and high carbohydrate content, but they are equally potent in antioxidants. Chestnuts are particularly rich in vitamin C, which is unusual for nuts, as well as phenolic compounds like gallic acid and ellagic acid. These compounds contribute to their antioxidant capacity and help combat oxidative stress-related conditions such as cardiovascular diseases and certain cancers (Borges et al., 2017). Chestnuts are also a good source of dietary fiber, essential minerals like potassium and magnesium, and exhibit anti-inflammatory properties, supporting metabolic and cardiovascular health.

Incorporating antioxidants into diets or consumables is an effective strategy to counter oxidative stress. Antioxidants neutralize ROS, prevent cellular damage, and promote overall health. These compounds are abundant in natural foods, especially plant-based sources like fruits, seeds, and nuts. The benefits of antioxidants include reducing inflammation, as many antioxidants such as flavonoids and phenolic compounds have anti-inflammatory properties, which help mitigate chronic diseases (Halliwell, 2024). Antioxidants also boost immune function, as seen with vitamin C, which enhances the body's defense mechanisms (Alum et al. 2023). They improve cardiovascular health by reducing LDL oxidation, a key factor in the development of atherosclerosis (Forman and Zhang, 2021). Additionally, antioxidants enhance skin health by protecting against damage caused by ultraviolet (UV) radiation, reducing the risk of premature aging and skin cancer (Michalak, 2022). This study focuses on the antioxidant profiles of Sesame seed, Date, and Chestnut, which are known to be rich in bioactive compounds. A comparative analysis of these plant products will provide valuable insights into their potential as natural antioxidants in promoting health and preventing oxidative stress-related conditions.

MATERIALS AND METHODS

Materials/Chemicals/Reagents

Chemicals and Reagents: Methanol, Folin-Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), Aluminum chloride, Sodium carbonate, Hydrochloric acid, Standard antioxidants (e.g. Gallic acid, Ascorbic acid), Sulphuric acid, Ammonium molybdate

Equipment: Spectrophotometer, pH meter, evaporator, centrifuge, cuvettes

Sample size

The samples used were: Three samples of sesame seeds, three samples of date seeds and three samples of chestnuts.

Sample collection and preparation

Selection of Samples: Dried samples of Sesame seed, Date seed and chestnut were obtained from local markets in Port Harcourt. They were washed, sun-dried for 4 days and milled to a fine powder. The powdered samples were then kept in a sterile sample bag at - 4ºc until further use. All laboratory analyses were conducted at Austino-Research and Analysis Laboratory Nigeria Limited, Alakahia, Uniport , Port Harcourt.

Extraction of chestnut, date seed and sesame seeds

The seeds were extracted in 70% methanol as followed; To 10g of each powdered seed, 50ml of 70%v/(v) of methanol was added and allowed to stand in a conical flask for 48 hours with occasional shaking after which the solvent was filtered to remove solid particles. The filtrate was then evaporated at 50ºc to dryness in an oven and the extract stored at -4ºC.

Determination of carotenoid content

To determine the total amount of carotenoids, approximately 15 g of the samples, plus 3 g of celite 454 (Tedia, Ohio, USA) were weighed in a mortar on a digital balance (Model MA0434/05). For the carotenoid extraction, successive additions of 25 mL of acetone were made to obtain a paste, which was transferred into a sintered funnel (5 µm) coupled to a 250 mL Buchner flask and filtered under vacuum. This procedure was repeated three times until the sample became colorless. The extract obtained was transferred to a 500 mL separatory funnel containing 40 mL of petroleum ether. The acetone was removed through the slow addition of ultrapure water (Milli-Q - Millipore) to prevent emulsion formation. The aqueous phase was discarded. This procedure was repeated four times until no residual solvent remained. Then, the extract was transferred through a funnel to a 50 mL volumetric flask containing 15 g of anhydrous sodium sulfate. The volume was made up by petroleum ether, and the samples were read at 450 nm. The total carotenoid content was calculated using the following formula:

$$Carotenoids\ Content\ (\mu g/g) = \frac{A\ x\ Vx\ 10000}{A1\%\ x\ P(g)}$$

Where A = Absorbance; V = Total extract volume; P = sample weight; $A_{1cm}^{1\%}$ = 2592 (β-carotene Extinction Coefficient in petroleum ether)

Determination of total anthocyanin content

According to Metoui et al. (2019) procedure, the total anthocyanin content was determined by pH differential method using two buffer systems: Potassium chloride buffer (pH 1.0, 0.025m) and sodium acetate buffer (pH 4.5, 0.4m). Methanolic extract was mixed with 3.6ml of corresponding buffers and read against water as a blank at 520 and 700nm. Absorbance was calculated using this formula:

A= (A520-A700) pH1.0 - (A520-A700) pH4.5

With a molar extinction coefficient of 2960. Results were expressed as mg of Cyanidin-3-
glucoside equivalent per 100g dry weight.

Ascorbic acid determination

About 5g of each powdered samples (chestnut, Date seed and sesame seeds) were dissolved in distilled water. The solution was stirred continuously using magnetic stirrer over a hot plate for 30 minutes at room temperature to facilitate solubility of ascorbic acid. The mixture was filtered through Whatman filter paper number 41. The filtrate was transferred into clean dried centrifuge tubes and centrifuged at 4000 rpm for 10 mins at room temperature. The supernatant was collected into vials while the residue was discarded. Finally, the extract was directly used for qualitative and quantitative analysis. Exactly 0.25ml of 3% bromine water was added into 5ml of centrifuged sample solution to oxidize the ascorbic acid to dehydro ascorbic acid and after that, 0.15ml of 10% thiourea was to remove the excess of bromine. 2ml of 2, 4-DNPH solution was added to form osazone. All standards, samples and blank solution were kept at 37ºc temperature for 3 hours in a thermostatic bath. After that, all were cooled in ice bath for 30 minutes and treated with 5ml chilled 85% H_2SO_4 with constant stirring. As a result, a colored solution absorbance was taken at 540nm.
The sample procedure was carried out for standard and blank. The ascorbic acid content is calculated in mg%.

Determination of 2, 2-diphenyl -1-Picrylhydrazyl (DPPH) radical scavenging activity

The DPPH scavenging activity was determined as described by Olaokun et al. (2020). A 200µl aliquot of each extract (2mg/ml) was added to 800µl of 0.1mm DPPH for 20 min at room temperature. The absorbance was determined at 517nm against the corresponding blank solution using a VERSA max microplate reader. The experiment was repeated thrice (triplicates) and the mean values recorded. The radical scavenging activity was calculated and expressed as a percentage of the control.

Estimation of total phenolic content

The total phenolic content was measured using the method by Metoui et.al. (2019). Exactly 0.5g of each extract was dissolved in 5.0 ml methanol and added to 0.5ml Folin-ciocalteau reagent for 5 minutes. Sodium carbonate (1ml of IN) was added and incubated for 1 hour at room temperature. The absorbance was measured 765nm using a spectrometer. The calibration curve was prepared using 100, 200, 400, 600, 800 and 1000µg/ml solution of galllic acid in methanol. Total phenolic contents were presented as milligrams as gallic acid (standard phenolic compound) per gram dry weight of the sample (using the standard curve).

Estimation of total flavonoid content

The total flavonoid content was determined by the aluminum chloride colorimetric assay with modification as described by Dravie et.al. (2020) Aliquots of 60µl of each extract were added to 10ml test tube containing 2ml of Ethanol. About 150ml of 5% sodium nitrite solution (5% $NaNO_2$) was added to each mixture and rested for 5 minutes before the addition of 150µl 10% of aluminum chloride (10% $A1C1_3$). The resulting mixture was then allowed to stay for another 5 minutes before adding 1ml of 1m sodium hydroxide (1.0M NaOH) and vortexed for 10 seconds. The absorbance of the aliquot was measured against prepared reagent blank at 510nm using a UV spectrophotometer and the total flavonoid content expressed as catechin equivalent (CE) mg/g on dry mass.

Estimation of ferric reducing antioxidant potential (FRAP)

The ferric reducing antioxidant potential (FRAP) assay was carried out according to the method of Luqman et al. (2009). The working FRAP reagent was prepared by mixing of 300mm acetate buffer, pH 3.6 10mM TPTZ (2, 4, 6-Tripyridyl-5-triazine) in 40mM HCI and with 20mM ferric chloride ($FeCl_3$. 6H20) in the ratio of 10:1:1 100µl of each sample (2mg/ml) was added to 3ml of prepared FRAP reagent. The reaction mixture was incubated in a water bath for 30 min at 37ºc. Then, the absorbance of the sample was measured at 593nm. The difference between the absorbance of the sample and the absorbance of blank was calculated at the FRAP value. FRAP value was compared to that of ascorbic acid. All measurements were calculated from the value obtained from triplicate assays.

Statistical analysis

Values expressed are mean of three replicate determinations and are presented in statistical table.

RESULTS AND DISCUSSION

Phytochemical and antioxidant activity of chestnut, date and sesame seeds

The samples (seeds) were extracted in methanol and the phytochemical and antioxidant activities are presented below (Table 1). The total phenolic, total flavonoids, anthocyanin, carotenoids and vitamin C contents were also investigated. The result presented in (Table 1) indicated that Chestnut had the highest antioxidant activity by scavenging 51.3% 2,2-diphenyl -1-Picrylhydrazyl (DPPH) and had a ferric reducing antioxidant potential (FRAP) value of 148.63 mmol TE/100g. This was followed sesame seed which scavenged 42.8% DPPH, with a FRAP value of 80.26 mmol TE/100g Date seed showed the least antioxidant effect by scavenging 38.6% DPPH, with a FRAP value of 19.32 mmol TE/100g. The total phenolic content was very high in Date seed (1350.64mg GAE/100g), followed by sesame seed (274.31mg GAE/100g) and the least was recorded in Chestnut seed (75.96 mg GAE/100g). The flavonoid content was very high in Date seed (742.18mg/100g), followed by Chestnut seed (182.04mg/100g) and the least value of 37.59mg/100g was recorded in sesame seed. Similarly, anthocyanin was very high in Date seed, followed by chestnut and the least in sesame seeds. The carotenoid content was notably high in chestnut seed (28.74µg/g) when compared with sesame seed (8.94µg/g) and Date seed (1.96µg/g). The ascorbic acid content in Chestnut seed was very high (310.68mg/100g) but extremely low in sesame seed (0.03mg/100g). Chestnut seeds possessed significant antioxidant followed by sesame seed significant amount of phenolics, flavonoids and anthocyanin was observed in Date seeds. Equally, Chestnut seeds contained rich amount of Ascorbic acid, carotenoids when compared with Date and sesame seeds.

The results of the comparative analysis revealed significant differences in the antioxidant profiles of the three seed types. Date seed exhibited the highest total phenolic content (TPC) and flavonoid content, followed by sesame seed and chestnut. The antioxidant activity, measured using the DPPH and FRAP assays, was high in chestnut. The higher total phenolic content and flavonoid content in date seed may be attributed to its higher content of polyphenolic compounds, such as flavonoids and phenolic acids. Typically, dates contain 70–80% sugar by dry weight, depending on the variety and ripeness (Al-Farsi et al., 2015. The comparison of the antioxidant activities of Sesame seed, Date, and Chestnut revealed that Chestnut seed exhibited the highest antioxidant activity across various assays, including DPPH (51.3%) scavenging ability and FRAP which was carried out on

Table 1: Result of samples and their parameters.

Sample	Chestnut seed	Date Seed	Seseme seed
Flavonoid (mg/100g)	182.04	742.18	37.59
Carotenoid (µg/g)	28.74	1.96	8.94
Anthocyanins (mg/100g)	118.53	157.14	0.26
Ascorbic acid (mg/100g)	310.68	28.17	0.03

Sample	Chestnut seed	Date Seed	Sesame seed
DPPH (%)	51.3	38.6	42.8
Total Phenolics (mg GAE/100g)	75.96	1350.64	274.31
FRAP (mmol TE/100g)	148.63	19.32	80.26

Chestnut seed by (148.63mmol TE100g) followed by Sesame seed (80.26mmol TE/100g) and Date seed (19.32mmol TE/100g). Phytochemicals such as flavonoids, phenolic acids, carotenoids contribute significantly to the antioxidant activity of plant-based foods. The study found that Date seed had the highest Anthocyanin content of (157.14mg/100g) when compared to Chestnut seed (118.53mg/100g) and Sesame seed(0.26mg/100g) , indicating its strong potential as a natural antioxidant source. Chestnut exhibited a more balanced polyphenolic profile, consistent with previous studies by Barreira et al. (2018), which highlighted its role in reducing oxidative stress-related disorders. The study revealed that sesame seed was richer in DPPH (42.8%) and FRAP (80.26mmol TE/100g) as compared to Date which had DPPH value of (38.6%) and FRAP value of (19.32mmol TE/100g). The total phenolic in sesame seed (274.37 mg GAE /100g) was much higher than in Chestnut (75.96mg GAE/100g) as present in (Table 1). Sesame seeds are a rich source of essential nutrients, including vitamin E, a fat-soluble antioxidant that protects cell membranes from oxidative stress and supports immune function (Nwanna and Agboola, 2018; Oboh et al. 2019). The antioxidant properties of Sesame seed, Date, and Chestnut indicate their potential applications in functional foods, pharmaceuticals, and nutraceuticals. Flavonoids possess a number of medicinal benefits, including anticancer, antioxidant, anti-inflammatory, and antiviral properties They also have neuro-protective and cardio -protective effects (Zhang and Mine, 2015) Flavonoids are phytochemical compounds present in many plants, fruits, vegetables, and leaves, with potential applications in medicinal chemistry. Flavonoids provide a wide range of health benefits, from fighting cancer and lowering the risk for heart diseases to preserving brain function. The key reason flavonoids are good for us is that they have anti-inflammatory effects and are antioxidants (Adebayo et al. 2020)

Prevalently, carotenoid consumption and absorption help to fight oxidative stress and reduce the risk of several chronic diseases, including cardiovascular and neurological disorders, type 2 diabetes, and different types of cancer (Gulcin, 2020; Michalak, 2022). Carotenoids are beneficial antioxidants that can protect one from disease and enhance your immune system. Pro-vitamin A carotenoids can be converted into vitamin A, which is essential for growth, immune system function, and eye health. Carotenoids are antioxidants, lowering inflammation in the body. Though it's still being researched, carotenoid anti-inflammatory properties have been associated with improving cardiovascular health. Reducing inflammation helps to protect against heart diseases and prevents arterial walls from being blocked (Gulcin, 2025)

Anthocyanins are a group of antioxidants found in red, purple, and blue fruits and vegetables. They belong to the flavonoid family (Borges et al., 2017). Anthocyanin rich foods are an important part of a healthy diet. Overall, evidence suggests that anthocyanins provide a range of health benefits: lowers blood pressure, reduces risk of heart disease, prevents neurological diseases, and slows down cancer growth (Al-Farsi et al., 2015). The human body absorbs anthocyanins along the gastrointestinal tract, especially in the large intestine, where they interact with the gut bugs that live there. By doing so, they help support gut health and are themselves transformed into compounds that may have many health benefits, including helping to prevent age related bone loss and cancer (Belwal et al., 2017).

The Ascorbic acid antioxidant capacity protects the cellular components from free radical damage in the aqueous phases of cells and the circulatory system (Alagendran et.al. 2019; Gulcin, 2025)). Vitamin C (ascorbic acid) is a nutrient your body needs to form blood vessels, cartilage, muscle and collagen in bones. Vitamin C is also vital to your body's healing process. Free radicals might play a role in heart disease, cancer and other diseases. Vitamin C also helps the body to absorb and store iron. Vitamin C has so many benefits which includes; boosting of the immune system, supports the production of white blood cells, which fight infections and diseases. It is also responsible for the enhancement of antibody production; helps produce antibodies, which neutralizes the pathogens and foreign substances. It protects against oxidative stress, reduces inflammation, enhances wound healing, lowers blood pressure, and improves lipid profiles. It may also help improve cognitive function and reduce the risk of age related cognitive decline. Vitamin C enhances iron absorption, reducing the risk of iron deficiency anemia (Alum et al, 2023).

Conclusion

The comparative analysis of Sesame seed, Date, and Chestnut provided valuable insights into their antioxidant properties, phytochemical composition, and potential health benefits. Each seed exhibited unique polyphenolic and flavonoid compositions, contributing to varying antioxidant capacities. Sesame seeds demonstrated notable antioxidant activity, likely attributed to their high sesamin and sesamolin content. Date seeds showed significant levels of phenolic acids and flavonoids, while chestnut displayed a robust antioxidant profile with high total phenolic content.. These findings suggest that these seeds can be effectively utilized in developing natural antioxidant-based therapeutics and functional foods, further reinforcing their significance in nutritional and pharmaceutical applications.

Recommendations

Based on the findings, several recommendations can be made to maximize the benefits of Sesame seed, Date, and Chestnut as natural antioxidants:

- Sesame seed, with its antioxidant activity and phenolic content, should be further explored for its potential in functional food and nutraceutical development. It could be incorporated into dietary supplements or used as a natural antioxidant in food preservation to enhance shelf life and nutritional value.
- Since Date exhibits strong antimicrobial properties, it should be considered for applications in natural food preservatives and pharmaceutical formulations. Further research can focus on its role in inhibiting microbial growth in food products and medicinal preparations.
- Chestnut, with its balanced polyphenolic profile and ability to mitigate oxidative stress-related disorders, could be promoted as a dietary supplement for individuals at risk of neurodegenerative diseases and chronic inflammatory conditions. Its potential in the development of anti-aging and neuro-protective therapies should be explored.
- Given the strong correlation between phenolic content and antioxidant activity, future research should focus on optimizing extraction methods to maximize the yield of bioactive compounds from these plant sources. Advanced techniques such as enzymatic extraction or nano-encapsulation could enhance the bioavailability and stability of these antioxidants.
- Public health campaigns and nutritional awareness programs should emphasize the importance of incorporating these plant-based antioxidants into daily diets. Encouraging the consumption of Sesame seed, Date, and Chestnut could contribute to better health outcomes and disease prevention. Further studies are recommended to explore their therapeutic potential and mechanisms of action in human health.
- Collaboration between food scientists, pharmaceutical industries, and policymakers is necessary to develop standardized formulations and regulatory guidelines for integrating these natural antioxidants into commercial health products. Further clinical studies are required to validate their efficacy and safety for widespread use.

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