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Effects of Roasting on Antioxidant Activities of Ethanol Extracts of Jack Beans (*Canavalia ensiformis)* Seeds

Ogundele, Joan Olayinka[1*] Jegede, Titilayo Faith [1] Akinyelu, Jude[2] Oseni, Margaret Oladunni [1] Adesemuyi, Florence Mebinuola [1] Abiodun, Oluyide Michael[1] Momod, Daniel Uwaremhevho [1] and Oyebanji, Adedayo Olamide [3]

[1]Department of Chemistry, Faculty of Science, Federal University Oye Ekiti, Ekiti State, Nigeria.
[2]Department of Biochemistry, Faculty of Science, Federal University Oye Ekiti, Ekiti State, Nigeria.
[3]Department of Chemical Sciences, Faculty of Science, Joseph Ayo-Babalola University, Ikeji-Arakeji, Osun State, Nigeria

(*)Corresponding Author's: joan.ogundele.joko@gmail.com+2348034752350

Abstract

Underutilized legumes are rich sources of essential nutrients and bioactive compounds, yet their potential remains largely untapped. Processing techniques such as roasting and fermentation can enhance their nutritional quality and functional properties. This study aims to evaluate the effect of roasting on the antioxidant properties of the ethanolic extract derived from the seeds of *Canavalia ensiformis* (Jack bean, JB). Total phenolic content (TPC) and total antioxidant capacity (TAC) were assessed using the Folin–Ciocalteu and phosphomolybdenum methods, respectively, while antioxidant activity was evaluated via the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Results showed that roasting led to a reduction in TPC (roasted JB: 37.35 ± 3.75 mgGAE/g; raw JB: 42.20 ± 4.65 mgGAE/g) and TAC (roasted JB: 48.00 ± 4.50 mgAAE/g; raw JB: 53.15 ± 4.30 mgAAE/g), likely due to thermal degradation of heat-sensitive phenolic compounds. Both extracts exhibited concentration-dependent DPPH scavenging activity, though their potency was lower than sodium ascorbate (standard). At the highest studied concentration of 100 µg/mL, scavenging activity followed the order: sodium ascorbate (75%) > RAJB (60%) > ROJB (58%). Although roasting led to a slight reduction in antioxidant activity, a substantial level of antioxidant potential was preserved, indicating that roasted JB seeds can still serve as a valuable dietary source of natural antioxidants with potential applications in functional foods and nutraceuticals for managing oxidative stress-related disorders.

Keywords:, Underutilized, Legumes, Roasting, Antioxidant.

Introduction

Antioxidants are employed as dietary supplements to neutralize the adverse effects of oxidative stress. Based on their mechanism of activity, antioxidants can be classified as chain breaker (or free radical inhibitor), peroxide decomposer, metal inactivator, or oxygen scavenger. The relationship between the intake of food rich in antioxidant components and

the alleviation of illness caused by oxidative damage has become an important topic in food and medicinal research. Natural antioxidants can be obtained from vegetables, fruits, spices, herbs, nuts, oilseeds, cereals, legumes, animal and microbial sources [1].

In recent years, antioxidants derived from natural sources, mainly plants have been intensively used to prevent oxidative damage because of their advantages over synthetic ones [2]. They are easily obtained, economical and have slight or negligible side effects [2]. Bioactive antioxidant agents present in herbs and spices may offer resistance against oxidative stress by scavenging free radicals and therefore inhibiting or preventing the deleterious consequences of oxidative stress [3].

Underutilized legumes can serve as valuable sources of essential nutrients, bioactive compounds, and dietary antioxidants, contributing to improved nutrition and health. Jack beans (JB) (*Canavalia ensiformis*) rank among the underutilized legumes that could improve overall health status, particularly in developing countries. A study reported that various collections of JB seeds contained a minimum of 29% protein, 31% starch, and 15% fiber [4]. In Nigeria, JB is currently grown as ornamental plant, or they are planted near residential houses and allowed to trail on walls and trees, as it is believed to repel snakes [5]. JB contain anti-nutrients such as protease inhibitors, lectins, phytic acid, and tannins [6]. These compounds can interfere with nutrient absorption, inhibit digestive enzymes, and reduce the bioavailability of essential minerals [7]. The use of processing methods such as soaking, boiling, fermentation, and microwaving can significantly reduce antinutrients in JB seeds, thus enhancing their digestibility and nutrient absorption [6]. In particular, heat treatment can deactivate or reduce the levels of anti-nutritional factors such as trypsin inhibitors, lectins, and tannins, which may interfere with nutrient digestion and utilization [8].

Although heating is effective in reducing the anti-nutritional factors of certain underutilized legumes, it may also affect their overall nutritional value. Therefore, it is essential to establish an optimal processing method that minimizes anti-nutritional compounds while preserving or enhancing their nutritive benefits. Despite its abundant nutritional and bioactive compounds, JB remains an underutilized legume, largely due to a lack of awareness regarding its health benefits and inadequate research on its processing methods. Therefore, this study aimed to evaluate the effect of roasting on the antioxidant capacity of *JB* seeds by assessing changes in total phenolic content (TPC), total antioxidant capacity (TAC), and DPPH radical scavenging activity. The findings will provide valuable insights into the potential of roasted JB seeds as a source of dietary antioxidants and contribute to strategies for improving the nutritional value of underutilized legumes.

Materials and Methods

Methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH, $C_{18}H_{12}N_5O_6$), sodium carbonate, gallic acid. All the reagents used were of analar grade.

Collection and Sample preparation

Briefly, JB seeds were freshly harvested from a farm in Isua Ibodi, Atakumosa-East Local Government Area, Osun State, Nigeria (Figures 1a and b). Exactly 500 g of JB seeds were collected and evenly divided into two portions of 250 g each. One portion was roasted in an aluminum pot over low heat on a charcoal burner until it attained a golden-brown color (Figure 1c, while the other portion was retained as a raw sample. Both dried samples were separately ground into powder using a grinder, packed in plastic containers, and labeled as roasted JB (ROJB) (Figure 1d) and raw JB (RAJB) (Figure 1e). The samples were then stored in a refrigerator for further analyses.

Figure 1. (a) JB pod; (b) JB seed; (c) roasted JB seed; (d) roasted JB flour; and (e) raw JB flour

Preparation of Extract Solutions

Exactly 10 g of RAJB and ROJB were individually weighed and immersed in 50 mL of ethanol. The mixtures were then left to stand for 48 hours before being filtered through muslin cloth. The resulting filtrates were separately concentrated using a rotary evaporator under reduced pressure at 40 °C. The concentrated extracts were subsequently dried and stored in a refrigerator for future use. A stock solution of 1 mg/mL was prepared for both ROJB

and RAJB, from which various concentrations (20–100 µg/mL) were formulated.

Determination of total phenol content (TPC)

The TPC of the ROJB and RAJB was determined by the Folin–Ciocalteu method [9]. Briefly, 300 µL of each extract samples (50 µg/mL and 100 µg/mL) were made up to 1 mL with distilled water, mixed with 300 µL Folin–Ciocalteu reagent (diluted with water 1:1 v/v) for 5 minutes. Subsequently, 1 mL of 8% sodium carbonate was added. The mixtures were allowed to stand for 45 minutes in a dark cupboard, and the absorbance (Abs) was read at 765 nm (SP- UV1100, China). The TPC was expressed as mg of gallic acid equivalent per gram dry weight of extract (mgGAE/g).

Determination of total antioxidant capacity (TAC)

The TAC the extracts was determined spectrophotometrically using the phosphomolybdenum method [10]. Briefly, 1 mL of each extract samples (50 µg/mL and 100 µg/mL) was combined with 3 mL of reagent solution containing 0.6 M H_2SO_4, 28 mM sodium phosphate, and 4 mM ammonium molybdate. A blank solution was prepared using 4 mL of the reagent solution without the extract. The mixtures were incubated at 95°C for 150 minutes, then allowed to cool to room temperature. Absorbance (Abs) was measured at 695 nm (SP- UV1100, China), and TAC was expressed as mg of ascorbic acid equivalent per gram dry weight of extract (mgAAE/g)

Determination of the Antioxidant Activities of the samples

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed according to a reported method [11]. Briefly, 2 ml of DPPH (0.1 mM in methanol) was added to 2 mL of various concentrations of ROJB and RAJB (20 -100 µg/mL). An equal amount of DPPH and methanol served as control. The reaction mixture was kept in the dark at 30 °C for 20 minutes, and the absorbance (Abs) was read at 517 nm (SP- UV1100, China). Sodium ascorbate was used as standard. The percentage of DPPH radical scavenging activity was determined by the equation below:

% scavenging activity = [(Abs of control –Abs of sample)/(Abs of control)] x 100

Statistical Analysis

All the data are expressed as the mean ± SD of three individual experiments. Significant differences between treatment groups and control were determined using a one-way analysis of variance. Statistical analysis was performed using GraphPad statistical software version 6.0. $p < 0.05$ was considered as significant.

Results and Discussion

Phenolic compounds are vital bioactive constituents of plants, known for their redox properties that contribute significantly to antioxidant activity. Their ability to scavenge free radicals is primarily attributed to the presence of hydroxyl (OH) groups, which donate hydrogen atoms to neutralize reactive oxygen species (ROS) and prevent oxidative damage [12]. These properties make phenolics essential in protecting biological systems from oxidative stress-related disorders [12]. As presented in Table 1, the TPC of ROJB was measured at 37.35 ± 3.75 mgGAE/g, which was lower than that of RAJB, recorded at 42.20 ± 4.65 mgGAE/g. This reduction in TPC following roasting suggests that heat treatment may have caused the degradation or transformation of certain phenolic compounds. The decrease in phenolic content upon roasting could be attributed to various factors, including thermal degradation, polymerization, or interactions with other components within the seed matrix. High temperatures may break down heat-sensitive phenolics, leading to their volatilization or conversion into non-extractable bound forms [13, 14], thereby reducing their overall availability in ROJB extract. Additionally, roasting may facilitate the oxidation of phenolic compounds [13], further contributing to the observed decline in TPC. Our findings in this study is in contrast with those of a previous study, which reported a TPC of 0.35±0.01 mgGAE/g in fermented juice from JB seeds. This value is lower than the TPC observed in our study, highlighting the significant influence of processing methods on the phenolic composition of JB seeds. These variations suggest that different processing techniques may either enhance or degrade phenolic compounds, ultimately affecting the antioxidant potential of the seeds.

Table 1: Total phenolic content

SAMPLE	100 µg/mL	50 µg/mL	Mean TPC (mgGAE/g)
ROJB	43.30 ± 3.20	31.40 ± 4.30	37.35 ± 3.75
RAJB	49.10 ± 4.10	35.30 ± 5.20	42.20 ± 4.65

The Total Antioxidant Capacity (TAC) assay, determined using the phosphomolybdenum method, is based on the reduction of molybdenum (VI) [Mo(VI)] to molybdenum (V) [Mo(V)] by antioxidant compounds present in the sample. This reduction leads to the formation of a green phosphate/Mo(V) complex under acidic conditions, which can be quantitatively measured [10]. The assay is particularly effective in detecting various antioxidant compounds, including

phenolics, ascorbic acid, n-tocopherol, and carotenoids [10]. As presented in Table 2, ROJB exhibited a TAC value of 48.00 ± 4.50 mgAAE/g, which was lower than the RAJB, which had a TAC value of 53.15 ± 4.30 mgAAE/g. This difference suggests that roasting leads to a reduction in the total antioxidant capacity of the sample. The decrease in TAC may be attributed to the thermal degradation of heat-sensitive antioxidants, such as ascorbic acid and certain phenolic compounds, which are susceptible to oxidation and decomposition at elevated temperatures [13].

Table 2: Total antioxidant capacity (TAC)

SAMPLE	100 µg/mL	50 µg/mL	Mean TAC (mgAAE/g)
ROJB	53.40 ± 4.70	42.60 ± 4.30	48.00 ± 4.50
RAJB	59.70 ± 3.40	46.60 ± 5.20	53.15 ± 4.30

The DPPH assay is widely recognized as the gold standard for assessing free radical scavenging activity *in vitro*. This method relies on the ability of antioxidants to reduce DPPH free radicals by donating hydrogen atoms or transferring electrons, thereby stabilizing the radicals and leading to a measurable decrease in absorbance [11].

As illustrated in Figure 2, both ROJB and RAJB extracts exhibited a dose-dependent increase in DPPH radical scavenging activity. However, their scavenging potential was lower in comparison to the standard antioxidant, sodium ascorbate. At the highest tested concentration (100 µg/mL), the DPPH radical scavenging activity followed the trend: sodium ascorbate (75.6%) > RAJB (60%) > ROJB (58%). The slight reduction in the scavenging activity of JB seeds after roasting may be attributed to the decline in total phenolic content, as previously demonstrated in this study.

Heat exposure during roasting can degrade phenolic compounds, which are key contributors to antioxidant activity, thereby diminishing the radical-scavenging potential of the extract. Despite the superior performance of sodium ascorbate, both ROJB and RAJB displayed significant free radical scavenging potential, suggesting their notable antioxidant properties. Notably, while roasting resulted in a slight decrease in the DPPH radical scavenging potential of JB seeds, a significant level of antioxidant activity was still retained. A previous study reported the concentration-dependent DPPH radical scavenging activity of JB [15]. However,

while fermentation reduced DPPH radical scavenging activity in that study, it enhanced nitric oxide radical scavenging activity. Interestingly, our study indicates that although heat treatment can lead to some degradation of bioactive compounds, it does not eliminate the inherent antioxidant capacity of the seeds. Moreover, roasting which is form of heating is known to influence the nutritional composition of seeds, often leading to changes in the levels of phenolic compounds, flavonoids, and other antioxidants [13]. While some heat-sensitive antioxidants may degrade during roasting, other compounds could undergo structural modifications that enhance their bioavailability or generate new antioxidant-active compounds through Maillard reaction products [16]. This considerable retention of antioxidant potential implies that roasted JB seeds could still serve as a valuable dietary source of natural antioxidants, supporting their potential application in functional foods and nutraceuticals aimed at combating oxidative stress-related disorders.

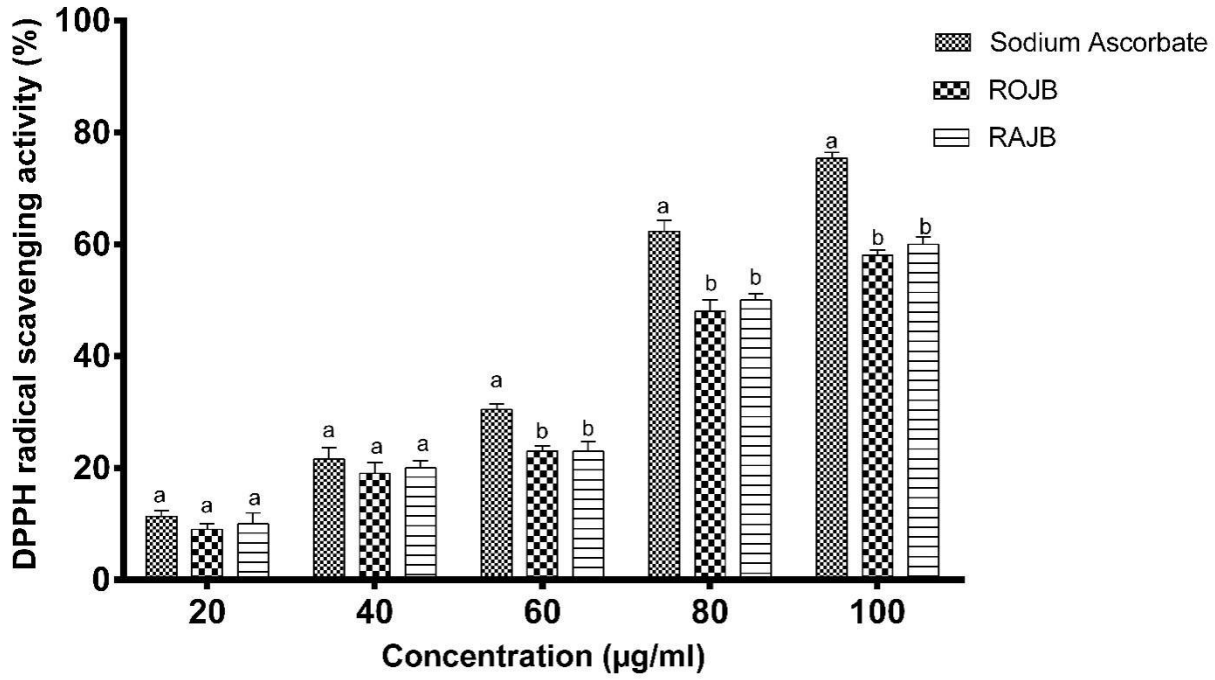

Figure 2: Antioxidant activity of JB extracts and sodium ascorbate based on DPPH radical scavenging. Data are represented as mean ± SD. For every grouped concentration, bars containing different alphabets are significantly different from each other at $p < 0.05$.

Conclusion

Roasting led to a reduction in phenolic content and antioxidant potential of *JB* seeds, likely due to thermal degradation and oxidation of heat-sensitive antioxidants. Despite this decline, substantial antioxidant activity remained, indicating that roasting does not entirely deplete the seeds of their bioactive compounds. Both raw and roasted *JB* seeds exhibited significant antioxidant potential, suggesting their continued relevance as natural sources of antioxidants. Optimizing roasting conditions could help preserve their functional benefits.

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