Biotech Studies 31(2), 87-94
http://doi.org/10.38042/biotechstudies.1222785

BIOTECH STUDIES

RESEARCH PAPER

Juglans regia kernel meal; A prospective nutraceutical feed supplement

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How to cite

Oloruntola, O. D. (2022). *Juglans regia* kernel meal; A prospective nutraceutical feed supplement. *Biotech Studies, 31*(2), 87-94. http://doi.org/10.38042/biotechstudies.1222785

Article History
Received 27 October 2022
Accepted 20 December 2022
First Online 22 December 2022

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Keywords
Botanicals
Feed supplements
Nutraceuticals
Phytobiotics
Walnuts

Abstract

This study aims to characterize the proximate composition, antioxidant activity, phytochemical profile, anti-diabetic, and anti-inflammatory properties of *Juglans regia* kernel meal (JKM). The examination of the proximate composition reveals that JKM contains moisture (7.74%), ash (4.46%), crude fat (31.26%), crude fiber (8.41%), crude protein (8.99%) and nitrogen-free extract (39.14%). The analysis of JKM for antioxidant properties shows lipid peroxidation inhibition (63.78%), ferric ion reducing antioxidant power (103.44 mg/g), 2,2-diphenyl-1-picrylhydrazyl hydrate (57.91%), and vitamin C (152.87 mg/g). The phytochemical compositional analysis shows that JKM has alkaloids (12.08%), saponins (43.49 mg/g), steroids (4.84 mg/g), flavonoids (14.74 mg/g), tannins (1.69 mg/g) and phenol (35.93 mg/g). The JKM also demonstrated alpha-amylase inhibition and alpha-glucosidase inhibition activities of 61.06% and 67.76%, respectively; while 62.71% and 79.17% were reported for the albumin denaturation inhibition and antiproteinase activity of JKM, respectively. JKM dietary supplementation may enhance the animals' welfare. It is advised to employ it in an animal model, though.

Introduction

The term "nutraceutical," which combines the words "nutrition" and "pharmaceutical," refers to either a food or a component thereof that has the ability to both prevent and/or treat diseases (Alagawany et al., 2021). Numerous bioactive substances included in nutraceuticals frequently have a variety of pharmacological actions, including anti-inflammatory, antibacterial, adaptogenic, free radical scavenging and antioxidative, immunomodulatory, and sedative properties (Gupta 2016). Due to the current limitation on the use of antibiotics in feed supplements for animal production and the growing need to improve production performance in both animals and humans, nutraceuticals have recently acquired relevance (Alagawany et al., 2021).

A significant portion of the nutraceuticals category consists of botanicals, which are whole, fragmented, or cut plants, algae, fungi, lichens, and botanical preparations made from these materials through extraction, distillation, expression, fractionation, purification, concentration, and fermentation (Gulati et al., 2014).

Juglans regia commonly known as walnuts is from the Juglandaceae family and most widespread tree nut in the world (Aryapak & Ziarati, 2014). When the plant reaches reproductive maturity, it becomes a woody perennial climber or climbing shrub that can grow to be between 6 and 18 meters tall. When young, the stem is

green and glabrous, but as it ages, it can girth up to 16 cm (Nwachoko & Jack 2015). Nearly all parts of walnuts are employed in ethnomedicine, including the leaves, seeds, stem barks, and roots. Specifically, the stem bark, seed kernel, and leaf are used as a mild laxative (Janick & Paul, 2008), aphrodisiac and tonic (Aiyeloja & Bello, 2006), fertility enhancer in women (Nwauzoma & Dappa, 2013), respectively.

The chemical and bioactive makeup of botanicals may affect how therapeutically effective they are (Adeyeye et al., 2020; Oloruntola & Ayodele, 2022). Comparatively to other botanicals such as sunflower, goat weed (Adeyeye et al., 2020), ginger (Anwar et al., 2020), *Corchorus capsularis* and *Corchorus olitorius* (Biswas et al., 2020), *Moringa oleifera* (Iqbal & Bhanger, 2006), *Anacardium occidentale* (Oloruntola, 2021), nettle (Otles & Yalcin, 2012), *Niebuhria apetala* (Rajesh et al., 2019), etc.; *Juglans regia* kernel chemical composition has received less research. Given that feed additives are a variety of groups of chemicals, substances, or organisms that support growth as well as alter physiological processes like stress resistance, reproduction, and immunological function. Additionally, using plant components as phytogenic additions is growing in popularity lately. Consequently, regular testing and characterisation of plant parts for phytochemical composition, antioxidant properties, antibiotic properties, and anti-cholesterol properties, among others are also necessary to support the use of plant parts as nutraceutical feed additives and supplements (Oloruntola, 2021; Oloruntola & Ayodele, 2022). Therefore, the objective of this study is to investigate the proximate, and phytochemical composition, antioxidant, alpha-amylase inhibitory, alpha-glucosidase inhibitory, albumin denaturation inhibitory and antiproteinase activities of *Juglans regia* kernel meal.

Materials and Methods

Processing of *Juglans regia* kernel

Juglans regia fruits were obtained from villages in Akungba Akoko, Nigeria. Raw kernels were extracted from the *Juglans regia* fruits, finely chopped, sparingly scattered, allowed to air dry in the shade for 14 days, milled and referred to as *Juglans regia* kernel meal (JKM). The JKM was thereafter stored for laboratory analysis. Four hundred grams (400 g) of JKM was added to 2000 ml of 70% ethanol, shaken for 6 hours, allowed to stand still for an additional 48 hours, and then filtered through Whatman No 1 filter paper. A rotary evaporator was used to vacuum condense the JKM ethanolic extract at 35–40°C.

Chemical analysis

The analytical reagent grade reagents used for chemical analysis were purchased from Sigma-Aldrich.

Proximate and vitamin C composition determination

The AOAC (2010) method was used to examine JKM for ash, crude fiber, crude fat, crude protein, and nitrogen-free extract. Vitamin C (Benderitter et al., 1998) was determined in JKM and the procedures were earlier reported by Oloruntola (2021).

Lipid peroxidation inhibition

The Bajpai et al., (2015) method was used to evaluate the JKM extracts' ability to inhibit lipid peroxidation. In both the absence and addition of JKM extract (50-250 g/mL) or reference compound, the reaction mixture of 1 mM $FeCl_3$, 50 µL of bovine brain phospholipids (5 mg/L), and 1 mM ascorbic acid in 20 mM phosphate buffer was incubated at 37°C for 60 minutes. Malondialdehyde (MDA), which was measured by the 2-thiobarbituric acid (TBA) reaction, was created as a byproduct of the process as hydroxyl radicals, which led to lipid peroxidation. The percentage of inhibitory activity was calculated.

$$\text{Percent inhibition } (\%) = \frac{(A\ control - A\ test)}{(A\ control)} x100$$

A control: Absorption of the controlling reaction

A test: Test reaction absorbency.

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH)

The DPPH radical degradation activity method was used to assess the JKM's DPPH antioxidant activity (Otles & Yalcin, 2012). Pure methanol ($6x10^{-5}$ M), sample extract or standard solution (100 liters), and methanolic DPPH solution (2 liters) were used to form the DPPH radical. The DPPH radical was kept in the dark for 20 minutes. The sample absorbance was calculated at 515 nm. As a control, a blank solution made entirely of methanol was employed. 100 µL of pure water were substituted for 100 µL of extract in the control solution. To examine the antioxidant capacities of JKM extracts, a calibration curve with various gallic acid solution concentrations (10-100 ppm) was created.

Ferric-reducing antioxidant power (FRAP)

Using the Benzie and Strain (1996) method, the spectrophotometric antioxidant capacity of the JKM was calculated. To make the FRAP reagent, 300 mM acetate buffer was combined with 10 mL TPTZ in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ at 37°C in the ratio of 10:1:1. Using a 1–5 mL variable micropipette, 3.995 mL freshly made functioning FRAP reagent was pipetted and fully blended with 5 µL of the properly diluted JKM. When the ferric tripyridyl triazine (Fe^{3+} TPTZ) complex was converted to a ferrous (Fe^{2+}) form, a strong blue color complex was created. The absorbance at 593 nm was measured in comparison to a reagent blank (3.995 mL FRAP reagent and 5 µl distilled water) after 30 minutes of 37°C incubation. Plotting the absorbance at 593 nm vs various $FeSO_4$ concentrations produced the calibration curve. The levels of $FeSO_4$ were then compared to the levels of the common antioxidant Trolox. The FRAP values, which are reported as mg of

Trolox equivalent per gram of material, were calculated by comparing the absorbance change in the test mixture with those obtained from increasing concentrations of Fe^{3+}.

Phytochemical analysis

The detailed outlines for determining the phytochemicals (alkaloids, saponins, steroids, flavonoids, tannins, and phenol) have been reported by Oloruntola & Ayodele (2022). The JKM was analyzed for alkaloids using the gravimetric method (Adeniyi et al, 2009), total saponins with vanillin and concentrated sulfuric acid colorimetric technique (He et al., 2014); steroids (Madhu et al., 2016); total flavonoids (Surana et al., 2016); total tannins (Biswas et al., 2020) and total phenol (Otles & Yalcin, 2012).

Alpha-amylase inhibitory activity

The α-amylase inhibition study was carried out using the 3,5-Dinitrosalicylic acid (DNSA) method (Wickramaratne et al., 2016). After being treated with at least 10% Dimethylsulfoxide, the JKM extract was diluted in buffer ((Na_2HPO_4/NaH_2PO_4 (0.02 M, at pH 6.9), (NaCl (0.006 M)) to provide concentrations between 10 and 1000 g/mL. 200 mL of extract and 2 mL of α-amylase solution were mixed and incubated at 30°C for 10 minutes. After that, each tube received 200 µL of the starch solution (1 percent in water (w/v)) and was incubated for 3 minutes. The process or reaction was halted by adding 200 µL DNSA reagent (12 g sodium potassium tartrate tetrahydrate in 8.0 mL 2 M NaOH and 20 mL 96 mM 3,5-Dinitrosalicylic acid solution) boiled for ten minutes in a water bath between 85 and 90°C. After being brought to room temperature, the mixture was diluted with 5 mL of distilled water and then subjected to a UV-Visible spectrophotometer analysis at 540 nm. The JKM extract was swapped out for 200 L of buffer, yielding a blank with 100% enzyme activity. A blank reaction was produced using the JKM extract at each concentration in the absence of the enzyme solution. As a positive control sample, acarbose (100–200 µg/mL) was employed, and the reaction was conducted in the same manner as the JKM extract reaction. Using the equation below, the inhibitory activity of α-amylase was calculated and reported as a percentage of inhibition. By graphing the percentage of α-amylase inhibition versus the extract concentration, the IC_{50} values were determined.

$$\text{Percentage } \alpha - \text{amylase inhibition} = 100\ X \frac{Abs\ 100\%\ Control - Abs\ Sample}{Abs 100\%\ Control}$$

Alpha-glucosidase inhibitory activity

An assay for assessing α-glucosidase inhibitory activity was described by Dej-adisai and Pitakbut (2015). The glucosidase enzyme converts the substrate, p-nitrophenol-D-glucopyranoside (pNPG), into the yellow product, p-nitrophenol (pNP), which was used to analyze the α-glucosidase reaction. 50 µL of a 10 mM phosphate buffer solution (pH 7) containing 0.2 mg/mL sodium azide and 2 mg/mL bovine serum albumin were added to a well plate. One unit/mL of Saccharomyces cerevisiae α-glucosidase (Type I, lyophilized powder, Sigma, EC 3.2.1.20) and 50 µL of an 8 mg/mL sample solution were added to the phosphate buffer solution. The solvent control was a 5% DMSO solution, and the positive control was 8 mg/mL of acarbose in each well. The mixes were incubated at 37°C for 2 minutes. 50 µL of 4 mM pNPG were then put into the well. The mixture has to incubate for a further five minutes in the same circumstances. For 5 minutes, the pNP was carried out and timed using a microplate reader at 405 nm every 30 seconds. The mixture has to incubate for a further five minutes in the same circumstances. For 5 minutes, the pNP was carried out and timed using a microplate reader at 405 nm every 30 seconds. The following linear relationship equation between absorbance and time was used to calculate the velocity (V).

$$\text{Velocity} = \frac{\Delta Absorbance\ at\ 405\ nm}{\Delta\ Time}$$

Each sample's initial reaction's highest velocity was gathered, and the equation below was used to calculate the percentage of inhibition.

$$\%\ \text{Inhibition} = \frac{\text{V control} - \text{V sample}}{V\ control} X\ 100$$

Anti-inflammatory activities

Albumin denaturation inhibition

The assay was performed as specified by Osman et al., (2016). Ibuprofen and diclofenac, two positive standards, were prepared at a concentration of 0.1 percent each (1.0 mg/mL), along with the JKM extract. The reaction vessel for each mixture contained 200 µL of egg albumin, 1400 µL of phosphate-buffered saline, and 1000 µL of JKM extract. Instead of the JKM extract, distilled water was used as a negative control. After 15 minutes of incubation at 37°C, the mixtures were then heated for 5 minutes at 70°C. After cooling, their absorbances at 660 nm were assessed. The percentage of protein denaturation inhibition was calculated using the following formula:

% Denaturation inhibition=(1-(Absorbance reading of the test sample)/(Absorbance reading without test sample (-ve control)))*100%

Antiproteinase activity

Rajesh et al., (2019) guidelines for the antiproteinase activity experiment were followed. The reaction mixture (2 mL) contained 1 mL of the test sample at various concentrations (100-500 g/mL), 0.06 mg of trypsin, and 20 mM Tris-HCl buffer (pH 7.4). The mixture was maintained warm at 37°C for 5 minutes. The mixture was then given 1 mL of casein which was 0.8 percent (w/v). The combination was maintained at a high temperature for another 20 minutes. 2 mL of 70% perchloric acid was added to the mixture to halt the

reaction. After that, the murky suspension was centrifuged. Then, using a buffer as a blank, the absorbance of the supernatant was measured at 210 nm. The investigation was carried out three times. Proteinase inhibitory activity was calculated using the formula shown below:

% inhibition= (Abs control-Abs sample)*100/Abs control

Statistical analysis

Three times each of the assays were run, and the average mean of the results was given. Descriptive statistics were adopted to analyze the data. Excel was used to create bar graphs for a better understanding of the average mean.

Results and Discussion

It is crucial to quantify the approximate amounts of a common feed/food ingredient or supplement to show its nutritional profile and choose the appropriate amount to add to a compounded feed/food (Oloruntola, 2021). Figure 1 depicts the proximate composition of *Juglans regia* kernel meal. *Juglans regia* kernel meal (JKM) proximate profile are nitrogen-free extract (39.14%), crude fat (31.26%), crude protein (8.99%), crude fiber (8.41%), moisture (7.74%) and ash (4.46%) in decreasing level of concentration. The values for various aspects of proximate composition in this study differ from the values previously reported for carbohydrates (24%), crude fat (52-77.5%), crude protein (11-25%), and ash (1.3-2.5%) (Ozkan & Koyuncu, 2005; Martinez & Maestri, 2008; Savage, 2001). This variance may result from various factors, such as processing techniques (Osum et al., 2013), geographic location (Khattak & Rahman, 2015), or developmental stage (Kiskini et al., 2016).

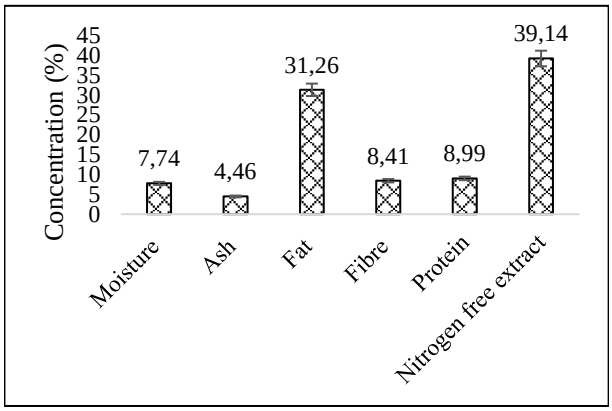

Figure 1. Proximate compositon of *Juglans regia* kernel meal.

Figure 2 depicts the antioxidant activity of JKM. There is evidence that plants are abundant sources of natural antioxidants such as vitamin C, flavonoids, carotenoids, tocopherols, and other phenolic compounds (Iqbal & Bhanger, 2006).

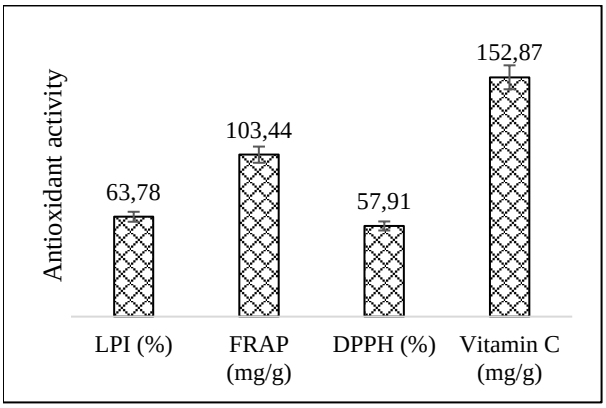

Figure 2. Vitamin C concentration and antioxidant activities of *Juglans regia* kernel meal.

LPI: Lipid peroxidation inhibition; FRAP: Ferric ion reducing antioxidant power; DPPH: 2,2-diphenyl-1-picrylhydrazyl hydrate.

Atherosclerosis, rheumatoid arthritis, atherosclerotic disease, and neurological illnesses have all been linked to lipid peroxidation. Therefore, the function of antioxidants in preventing the production of active oxidants, quenching and removing active oxidants, scavenging, repairing damage, excreting toxic oxidants, and promoting adaptive responses has garnered considerable attention (Niki et al., 2005). This study's discovery of JKM's significant lipid peroxidation inhibition properties of 63.78% (Figure 2) reveals a crucial nutraceutical property of health significance. This corroborated an earlier discovery that some foods and feeds include different antioxidants that scavenge free radicals (Niki & Noguchi, 2000). Additionally, the study's detection of JKM's FRAP (103.44 mg/g), DPPH (57.91%), and vitamin C (152.87mg/g) shows that the phytogens have bioactive compounds that exhibit antioxidant activities. The DPPH and FRAP assays evaluate the primary (an activity that scavenges free radicals) and secondary (mitigation of radical production and defence against oxidative damage) antioxidant abilities of phytogens, respectively (Lim et al., 2007; Yin et al., 2016). The DPPH value observed in this study is less than the 75.02–85.96% reported by Kabiri et al (2019). The JKM's DPPH concentration in this study was greater than 42.24% reported for *Anacardium occidentale* L. leaf powder (Oloruntola, 2021), but lower than 67.32%, 87.15% and 69.17% reported for *Ficus carica, F. exasperata* and *F. thonningii* leaf meals, respectively (Osowe et al., 2021). Furthermore, the JKM's vitamin C concentration in this study was greater than 7.74 mg/g reported for *Anacardium occidentale* L. leaf powder (Oloruntola, 2021) and 28.07 mg/g, 7.15 mg/g and 5.91 mg/g reported for *Ficus carica, F. exasperata* and *F. thonningii* leaf meals, respectively (Osowe et al., 2021).

The antioxidant capacity of JKM is demonstrated by its phytochemical makeup, which includes alkaloids (120.80 mg/g), saponins (43.49 mg/g), steroids (4.84 mg/g), flavonoids (14.72 mg/g), tannins (1.69 mg/g), and phenol (35.93 mg/g) (Figure 3). According to reports, phytochemicals (such as carotenoids, flavonoids, and alkyl sulphide) have antioxidant action

and lower the risk of a variety of diseases (Agbafor & Nwachukwu, 2011). Total phenolic content and overall antioxidant activity in phytogens are directly correlated (Sun et al., 2002). The essential enzyme for the cellular generation of reactive oxygen species, NADPH-oxidase, has also been shown to be inhibited by alkaloids. This inhibition can occur by preventing the synthesis, activation, or translocation of NADPH-oxidase subunits (Macakova et al., 2019). Xu and Yu (2021) also reported that saponins exhibit a wide range of pharmacological actions, such as antioxidant, anti-inflammatory, antiviral, anticancer, antifungal, antibacterial, and immunomodulatory properties. Tannins have been reported to be active in the modulation of immunological response, increasing blood flow, lowering blood pressure and serum cholesterol concentration, and producing antimicrobial, anti-allergic, anti-cancerous and anti-inflammatory properties, even though they have depressing effects on feed intake, digestibility and metabolism in experimental animals (Sharma et al., 2019; Oloruntola, 2021).

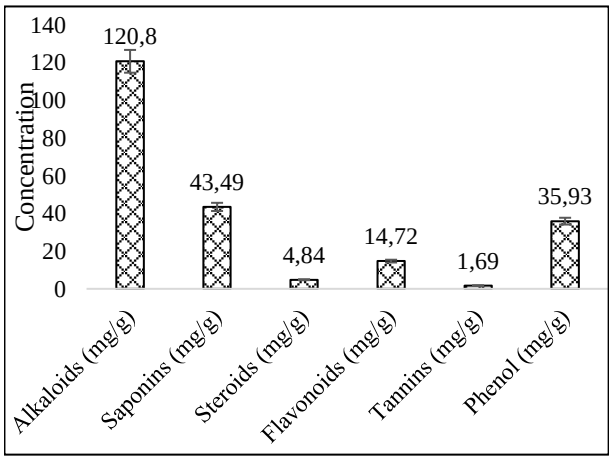

Figure 3. Phytochemical composition of *Juglans regia* kernel meal.

In this study, the JKM had an alkaloid content that was higher than the amounts reported for leaf meals from *Ficus carica, F. exasperata*, and *F. thonningii*, which were 103 mg/g, 81 mg/g, and 59.50 mg/g, respectively (Osowe et al., 2021); While JKM's saponin concentration was less than that of *F. carica, F. exasperata*, and *F. thonningii* leaf meals (60.17 mg/g, 80.72 mg/g, and 51.42 mg/g, respectively) (Osowe et al., 2021). The flavonoid concentration of JKM in this study is at variance with 0.80-1.10 g/100g reported by Mo et al., (2022) and 12.59-62.11 mg/100g by Kabiri et al., (2019). Additionally, this study's JKM phenol content differed from the 1017–3739 mg/100g reported by Kabiri et al (2019). This variation might be brought on by processing methods (Osum et al., 2013), place (Khattak & Rahman, 2015), or developmental stage (Kiskini et al., 2016).

The chemical compounds known as alpha-glucosidase inhibitors prevent the enzymes glucoamylase, sucrase, maltase, and isomaltase from converting complex, non-absorbable carbohydrates into simple, absorbable carbohydrates. This delays the absorption of carbohydrates and lowers the rise in postprandial blood glucose levels (Kumar et al., 2011; Derosa & Maffioli, 2012); while the alpha-amylase inhibitors' function as carbohydrate blockers, they restrict the gastrointestinal tract's ability to digest and absorb carbohydrates. They can be used to stop disorders including obesity, diabetes, hyperglycemia, and hyperlipemia (Gong et al., 2020). The nutraceutical properties of JKM, when used as food/feed supplements or ingredients, are revealed by the alpha-amylase inhibition (61.06%) and alpha-glucosidase inhibition (67.76%) capacities of JKM, as reported in this study (Figure 4). Previous research showed that phytogens had alpha-amylase and alpha-glucosidase inhibitory activities (Boulfia et al., 2021; Oloruntola & Ayodele, 2022). The phytochemical makeup of JKM may be responsible for its alpha-amylase and alpha-glucosidase inhibitory capabilities; for example, peptides derived from cereals, phenolic compounds, non-starch polysaccharides, and lipids were confirmed to inhibit alpha-glucosidase and alpha-amylase activities (Gong et al., 2020).

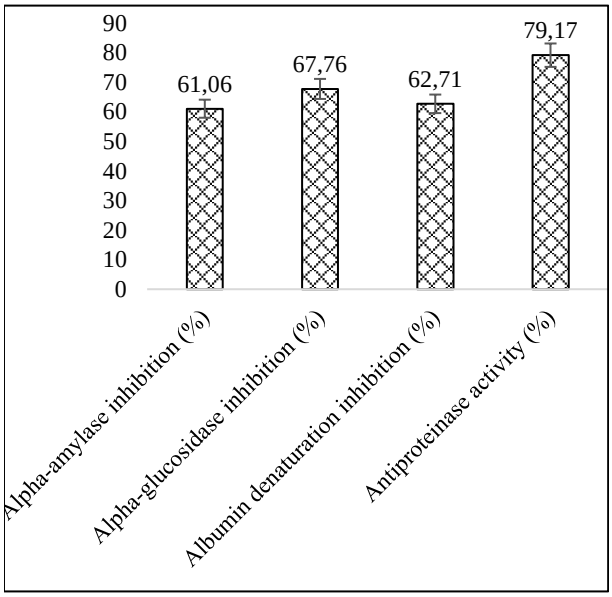

Figure 4. The alpha-amylase inhibition, alpha-glucosidase inhibition, albumin denaturation inhibition and antiproteinase activity of *Juglans regia* kernel meal.

The complex process of inflammation, which commonly entails pain, includes things like increased vascular permeability, increased protein denaturation, and membrane modification (Ruiz-Ruiz et al., 2017). Anti-proteinase activity is also thought to be involved in controlling inflammatory disorders because of its capacity to protect against proteinase-induced tissue damage. Protein denaturation may be a significant contributor to inflammation because it may result in the production of auto-antigens in certain inflammatory conditions (Anwar et al., 2020). Inhibition of albumin denaturation (62.71%) and antiproteinase activity

(79.17%) by JKM in this study demonstrate the potential for JKM to have anti-inflammatory effects.

Conclusions

These findings, therefore, showed that JKM has anti-inflammatory, antioxidant, and anti-diabetic properties. In feeding studies using an animal model, the JKM is suggested as a dietary supplement to validate its usefulness as a nutraceutical feed supplement.

Conflict of Interest

The author declares that he has no known competition for financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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