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Comparative Analysis of Antioxidant, Nutritional, Phytochemical and Enzyme Inhibition Properties of *Justicia carnea* and *Alchornea cordifolia* Leaf Meals

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Abstract

In recent years, there has been an increasing interest in plant-derived compounds for their potential health benefits and therapeutic applications. In this study, two botanical species, *Alchornea cordifolia* and *Justicia carnea* leaf meals were examined for their antioxidant characteristics, phytochemical and proximate composition, anti-proteinase properties and lipase, albumin, alpha-amylase and alpha-glucosidase inhibitory properties. *A. cordifolia* exhibited significantly higher levels of vitamin C, ferric ion-reducing antioxidant power, 2, 2-diphenyl-1-picrylhydrazyl hydrate scavenging activity and flavonoid content compared to *J. carnea*, indicating superior antioxidant potential. Conversely, *J. carnea* showed higher saponin and cardiac glycoside content. Notably, *A. cordifolia* demonstrated stronger inhibition of lipase albumin and anti-proteinase activities, as well as higher inhibition of alpha-amylase and alpha-glucosidase enzymes compared to *J. carnea*. Proximate composition analysis revealed differences in moisture, nitrogen-free extract, crude fat, crude fiber and crude protein contents between the two leaf meals. In conclusion, *A. cordifolia* emerges as a promising source of antioxidants and enzyme inhibitors, highlighting its potential as a valuable nutraceutical resource. Its superior properties to *J. carnea* suggest its potential application as a functional food ingredient for promoting health and preventing metabolic disorders. This study provides valuable insights into the biochemical and nutritional composition of these leaf meals, contributing to the understanding of their potential health benefits.

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Introduction

Recently, there has been an increasing interest in exploring natural sources, particularly plant-derived compounds, for their potential health benefits and therapeutic applications [1, 2]. *Justicia carnea* and *Alchornea cordifolia*, two botanical species commonly found in various regions, have attracted attention for their traditional uses in folk medicine and their purported hepato-protective, antioxidant and blood-boosting properties [3, 4]. As global interest in functional foods and nutraceuticals continues to grow [5], there is a pressing need to scientifically evaluate the biochemical, nutritional, and phytochemical compositions of these botanicals to elucidate their potential health-promoting properties. *Justicia carnea*, commonly known as "Brazilian plume flower" or "Jacobinia," is a flowering plant indigenous to South America and widely distributed in tropical and subtropical regions. Traditionally, various parts of the plant, including its leaves, have been used in folk medicine for their purported medicinal properties, such as their role in improving blood circulation and treating anaemia [6]. Phytochemical studies have revealed the presence of bioactive compounds such as flavonoids, alkaloids, and phenolic compounds in *J. carnea*, which contribute to its antioxidant and pharmacological activities [4,7].

A. cordifolia, also known as "Christmas bush" or "African holly," is a shrub or small tree native to tropical Africa. In traditional African medicine, various parts of *A. cordifolia*, including its leaves, bark, and roots, have been used for their medicinal properties, including their purported ability to enhance blood circulation and treat various ailments [3, 8]. Phytochemical analyses have identified a diverse array of bioactive compounds in *A. cordifolia*, including flavonoids, tannins, alkaloids, and terpenoids, which contribute to its pharmacological properties [9]. Despite their traditional uses and anecdotal evidence of health benefits, there is a dearth of scientific studies comprehensively comparing the biochemical, nutritional and phytochemical profiles of *J. carnea* and *A. cordifolia* leaf meals. Additionally, the potential enzyme inhibition properties of these botanicals, particularly their effects on key enzymes involved in metabolic disorders such as diabetes and obesity, remain largely unexplored.

This comparative analysis aims to address these knowledge gaps by systematically evaluating and comparing the biochemical, nutritional and phytochemical profiles of *J. carnea* and *A. cordifolia* leaf meals. Furthermore, enzyme inhibition assays were conducted to assess their potential therapeutic applications in the management of metabolic disorders. By elucidating the bioactive components and enzyme inhibition properties of these botanicals, this study seeks to provide valuable insights into their potential as functional food ingredients and nutraceuticals for promoting animal and human health and well-being.

Materials and Methods

Collection of *Alchornea cordifolia and Justicia carnea* leaves and processing

Fresh *A. cordifolia* and *J. carnea* leaves were collected separately from their mother plants within the premises of Adekunle Ajasin University, Akungba Akoko (AAUA), Nigeria. The leaves were authenticated by a Crop Scientist from the Department of Agronomy, AAUA, Nigeria. The leaves were washed in clean running water, drained, sliced with a clean stainless knife and spread lightly on polythene under shade to air-dry for 12 days. Thereafter, the dried *A. cordifolia* and *J. carnea* leaves were powdered with a blender to form *A. cordifolia* leaf meal (ALM) and *J. carnea* leaf meal (JLM), respectively. The ALM and JLM were stored in plastic rubber in the freezer for analysis. Each analysis was conducted in triplicate.

Antioxidant properties analysis

The Roe and Kuether method was used for the determination of vitamin C [10]. Thiobarbituric acid reactive substances assay was used for the determination of lipid peroxidation inhibition [11]. Ferrozine-based iron chelating assay was used for the determination of ferric ion reducing antioxidant power [12] and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical degradation activity [13] was determined as described by Oloruntola [14] and Oloruntola et al. [15].

Proximate and phytochemical analysis

Chemicals and reagents for chemical analysis were all bought from Sigma-Aldrich. In this experiment, only analytical reagent-grade chemicals were employed. The concentrations of saponins [16], cardiac glycosides [17], tannins [18], alkaloids [19], flavonoids [20] and phenol [13] in ALM and JLM were determined using the procedures outlined by Oloruntola [14]. Steriods [21] concentrations were

determined as outlined by Oloruntola et al. [22] and Tofighi et al. [23]. Later, ALM and JLM were analyzed for moisture, crude protein, ash, crude fat, crude fiber, and nitrogen-free extract using the AOAC methods [24].

Lipase and albumin inhibition properties and anti-proteinase property

Lipase inhibitory property was determined using the fluorometric lipase assay as outlined by Nakai et al. [25]. The protein denaturation inhibition method was used for determining the albumin inhibition and the caseinolytic method was used for determining the anti-proteinase properties as outlined by Osman et al. [26] and Rajesh et al. [27], respectively.

Alpha-amylase and alpha-glucosidase inhibition properties

The α-amylase activity assay for the determination of alpha-amylase [28] and the α-glucosidase inhibition assay for the determination of alpha-glucosidase [29] inhibition properties were determined as outlined by Oloruntola and Ayodele [30].

Statistical analysis

The results were obtained by averaging triplicate measurements for each data point. Statistical analysis was conducted using SPSS (Version 20.0. Chicago, SPSS Inc.). Significant differences in mean values were assessed using a one-way analysis of variance.

Results and Discussion

The antioxidant properties of *A. cordifolia* and *J. carnea* leaves were investigated and are summarized in Table 1. The vitamin C content in *A. cordifolia* leaf was significantly (P<0.05) higher compared to *J. carnea* leaf. *A. cordifolia* leaf exhibited a higher percentage of lipid oxidation compared to *J. carnea* leaf with a significant (P<0.05) difference. The ferric ion-reducing antioxidant power (FRAP) of *A. cordifolia* leaf was significantly (P<0.05) greater than that of *J. carnea* leaf. *A. cordifolia* leaf demonstrated higher DPPH scavenging activity compared to *J. carnea* leaf with a significant (P<0.05) difference. The results obtained from the study comparing the antioxidant properties of *A. cordifolia* and *J. carnea* leaves provide valuable insights into their potential scientific benefits and implications. Vitamin C is a potent antioxidant that plays a crucial role in scavenging free radicals, boosting the immune system, and promoting collagen synthesis for healthy skin and tissue repair [31]. *A. cordifolia*'s higher vitamin C content indicates its potential as a more effective natural source of this essential nutrient, offering enhanced antioxidant and immune-boosting benefits compared to *J. carnea* leaf. The high vitamin C content in *A. cordifolia* supports its use as a nutraceutical feed supplement for livestock, offering antioxidant protection, collagen synthesis and immune support, potentially improving overall health and productivity [9].

Lipid oxidation inhibition is essential for maintaining the quality and nutritional value of feed ingredients, as lipid oxidation leads to rancidity and loss of nutrients [32]. In this comparative study, *A. cordifolia* exhibited higher inhibition of lipid oxidation compared to *J. carnea* leaf, indicating its stronger antioxidant potential in preserving lipid integrity. *A. cordifolia*'s higher inhibition of lipid oxidation suggests its superior ability to protect feed ingredients from oxidative degradation, ensuring better nutrient retention and feed stability [32]. In addition, the superior lipid oxidation inhibition of *A. cordifolia* supports its use as a natural antioxidant phytogenic feed supplement, helping to maintain feed quality and nutrient integrity in livestock diets. By implication, *A. cordifolia* leaves, when compared to *J. carnea* have superior potential in preventing oxidative damage to lipids, which is a crucial factor in various diseases, including cardiovascular diseases and neurodegenerative disorders [33]. FRAP assay measures the ability of antioxidants to reduce ferric ions, reflecting their capacity to neutralize free radicals and protect against oxidative damage [34]. In this study, *A. cordifolia* exhibited higher FRAP values compared to *J. carnea* leaf, indicating its stronger reducing power against oxidative stress. Furthermore, the higher FRAP values of *A. cordifolia* support its potential as an effective natural antioxidant phytogenic feed supplement, offering enhanced protection against oxidative stress-related disorders in animals [34, 35]. DPPH assay is a widely used method for evaluating the free radical scavenging capacity of antioxidants, reflecting their potential health-promoting effects [36]. *A. cordifolia* exhibited higher DPPH scavenging activity compared to *J. carnea* leaf, indicating its superior ability to neutralize free radicals. *A. cordifolia*'s higher DPPH scavenging activity suggests its greater antioxidant efficacy, making it a valuable natural source of antioxidants for promoting animal health and well-being [37].

J. carnea leaves had a substantially (P<0.05) greater saponin content than *A. cordifolia* leaves (Table 2). *J. carnea* leaf had a considerably (P<0.05)

Table 1 The antioxidant properties of *Alchornea cordifolia* and *Justicia carnea* leaf meal.

Parameters	*Alchornea cordifolia* leaf	*Justicia carnea* leaf	SEM	*P* Value
Vitamin C (mg/g)	0.87[a]	0.52[b]	0.08	0.01
Lipid oxidation (%)	26.51[a]	15.28[b]	2.64	0.04
Ferric ion reducing antioxidant power (mg/g)	66.69[a]	57.68[b]	2.01	0.01
2,2-diphenyl-1-picrylhydrazyl hydrate (mg/g)	55.24[a]	27.22[b]	6.27	0.01

Means with different superscripts within a column are significantly different.
SEM: Standard error of the means.

Table 2 The phytochemical composition of *Alchornea cordifolia* and *Justicia carnea* leaf meal.

Phytochemicals	*Alchornea cordifolia* leaf	*Justicia carnea* leaf	SEM	P Value
Saponin (mg/g)	30.59[b]	57.33[a]	5.98	0.01
Cardiac glycosides (mg/g)	36.61[b]	38.31[a]	0.44	0.03
Tanins (mg/g)	1.30[a]	0.21[b]	0.24	0.01
Alkaloid (%)	10.54[a]	3.59[b]	1.55	0.01
Steroids (mg/g)	0.98[b]	1.24[a]	0.06	0.01
Flavonoid (mg/g)	40.71[b]	10.03[a]	6.87	0.01
Phenol (mg/g)	189.58[a]	84.48[b]	23.53	0.01

Means with different superscripts within a column are significantly different.
SEM: Standard error of the means.

greater number of cardiac glycosides than *A. cordifolia* leaf. When comparing the tannin content of *A. cordifolia* leaf to *J. carnea* leaf, a significant (P<0.05) difference was observed. The alkaloid content in *A. cordifolia* leaf was significantly (P<0.05) higher compared to *J. carnea* leaf. *J. carnea* leaves have a concentration of steroids, which was substantially (P<0.05) more than that of the *A. cordifolia* leaves. *A. cordifolia* leaf flavonoid concentration was significantly (P<0.05) greater than *J. carnea* leaf flavonoid content. *J. carnea* leaf and *A. cordifolia* leaf had substantially (P<0.05) different phenol contents. Saponins, bioactive substances are known for their extensive pharmacological characteristics, exhibit natural surfactant activity and have antibacterial, anti-inflammatory, and immunomodulatory effects; they also regulate the immune system, reduce cholesterol, and protect against gastrointestinal diseases [38]. Compared to *A. cordifolia* leaf, *J. carnea* leaf boasts a higher saponin concentration, potentially indicating its greater efficacy as a natural surfactant and immune-modulating agent. Moreover, *J. carnea* leaf could be valuable in animal diets, as saponins can influence feed palatability and nutrient absorption, particularly gut health and immunological function. Natural substances called cardiac glycosides have beneficial inotropic effects, which makes them useful in the treatment of heart diseases including congestive heart failure [39]. By raising myocardial contractility and lowering heart rate, cardiac glycosides can enhance cardiac function by improving circulation and tissue oxygen delivery [40]. Compared to *A. cordifolia* leaf, *J. carnea* leaf has a higher number of cardiac glycosides, which may indicate that it has more potent cardiovascular effects. The cardiac glycosides found in *J. carnea* leaf may help livestock's cardiovascular health by enhancing general heart health and performance, especially in stressed or heart-related animals.

Tannins, polyphenolic substances renowned for antibacterial and antioxidant properties, exhibit an affinity for minerals, proteins, and carbohydrates, potentially affecting nutrient absorption. Their health benefits encompass defense against free radicals, inflammation reduction and modulation of gut microbiota [41]. Compared to *J. carnea* leaf, *A. cordifolia* leaf has a higher tannin concentration, which suggests that it may have more significant antioxidant and antibacterial properties. Because of their antibacterial qualities, the tannins in *A. cordifolia* leaves can help animals' immune systems and digestive health while also enhancing feed stability and shelf life [42]. Nitrogenous alkaloids exhibit diverse pharmacological properties, serving as potent analgesics, anti-inflammatory and antiparasitic agents. Their potential health benefits extend to inflammation reduction, parasite control and pain alleviation [43]. *A. cordifolia* leaf, boasting a higher alkaloid concentration than *J. carnea* leaf, potentially offers more robust therapeutic benefits. These alkaloids provide natural pain relief, anti-inflammatory support, and parasite control in animals, enhancing overall health and welfare [43]. Steroids, recognized for their physiological effects, encompass immunomodulatory, anti-inflammatory, and metabolic regulatory properties. Their potential health benefits span immune system modulation,

metabolic regulation, and inflammation mitigation [44]. *J. carnea* leaf, with a higher steroid concentration compared to *A. cordifolia* leaf, may offer enhanced anti-inflammatory and immunomodulatory advantages. These steroids in *J. carnea* leaf hold promise for reducing inflammation and bolstering immunity in animals, particularly during periods of stress or medical challenges [22]. Flavonoids, potent polyphenolic compounds, possess remarkable antioxidant, anti-inflammatory, and anticancer properties, offering significant health benefits such as antioxidant protection, inflammation reduction, and cancer prevention [45]. The higher flavonoid content in *A. cordifolia* leaf suggests its potential for more robust antioxidant and anti-inflammatory effects compared to *Justicia carnea* leaf. These flavonoids in *A. cordifolia* leaf can deliver potent antioxidant support, effectively reducing oxidative stress and inflammation in livestock, and thereby promoting overall health and performance [9]. Phenolic chemicals are antioxidants that have a variety of positive health effects, such as anti-inflammatory, anti-cancer, and cardiovascular protection. Potential health advantages of phenolic substances include reduced inflammation, illness prevention, and antioxidant defense [46]. Compared to *J. carnea* leaf, *A. cordifolia* leaf has a higher phenol concentration, which suggests that it may have more potent antioxidant and health-promoting benefits. *A. cordifolia* leaf phenolic compounds can offer strong antioxidant protection, warding off illnesses linked to oxidative stress and enhancing livestock longevity and general health [47].

A. cordifolia leaves had significantly stronger (P<0.05) lipase inhibitory, albumin inhibitory and anti-proteinase properties than *J. carnea* leaves (Fig. 1). The lipase inhibitory activity of *A. cordifolia* leaves was found to be substantially higher than that of *J. carnea* leaves. Inhibiting lipase may aid in controlling lipid metabolism, hence limiting the absorption of too much fat and aiding in animal weight control. This characteristic is especially helpful for animals that are prone to metabolic problems or obesity [48]. Because of their greater lipase inhibitory activity, *A. cordifolia* leaves can be used as natural supplements to help livestock maintain a healthy weight and metabolic state. In addition, compared to *J. carnea* leaves, *A. cordifolia* leaves demonstrated a significantly stronger albumin inhibitory activity. Because albumin inhibition slows down the pace at which carbohydrates are absorbed and digested, it is crucial for managing blood glucose levels. This characteristic may be especially helpful

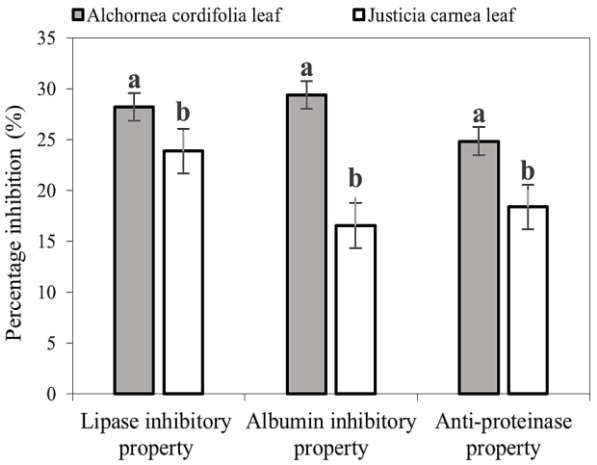

Fig. 1 Lipase, albumin and proteinase inhibitory properties of *Alchornea cordifolia* and *Justicia carnea* leaves. Bars with different letters are significantly different at P<0.05.

for animals suffering from diabetes or insulin resistance [49]. Because *A. cordifolia* leaves have a greater albumin inhibitory effect, they can improve metabolic health in livestock by regulating blood glucose levels and lowering the risk of problems from diabetes. Compared to *J. carnea* leaves, *A. cordifolia* leaves showed noticeably stronger anti-proteinase characteristics. Protease enzymes are involved in the degradation of proteins and the injury of tissues; anti-proteinase activity aids in their inhibition. This characteristic is crucial for lowering inflammation and encouraging tissue regeneration [50]. With higher anti-proteinase qualities, *A. cordifolia* leaves can help stimulate tissue repair and reduce inflammation in animals, especially after trauma or injury.

A. cordifolia leaves exhibited significantly (P<0.05) stronger alpha-amylase and alpha-glucosidase inhibition than *J. carnea* leaves (Fig. 2). The observed higher inhibition of alpha-amylase and alpha-glucosidase by the *A. cordifolia* leaf compared to the *J. carnea* leaf holds significant scientific implications, particularly in the context of their nutraceutical potential and natural antioxidant properties. Alpha-amylase and alpha-glucosidase are key enzymes involved in the breakdown of complex carbohydrates into simpler sugars during digestion. Inhibition of these enzymes can lead to a slower rate of glucose absorption, which is beneficial in managing blood sugar levels, especially in individuals with diabetes or those at risk of developing the condition [51]. The higher inhibition values exhibited by the *Alchornea cordifolia* leaf suggest a greater potential to modulate carbohydrate metabolism compared to the *Justicia carnea* leaf. This suggests that *A. cordifolia* may offer more

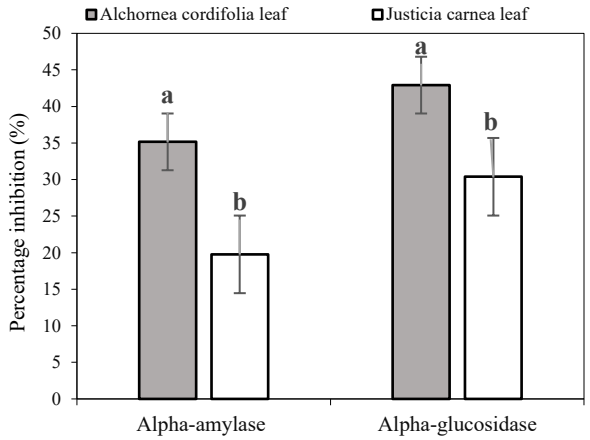

Fig. 2 Alpha-amylase and alpha-glucosidase inhibition of *Alchornea cordifolia* and *Justicia carnea* leaves. Bars with different letters are significantly different at $P<0.05$.

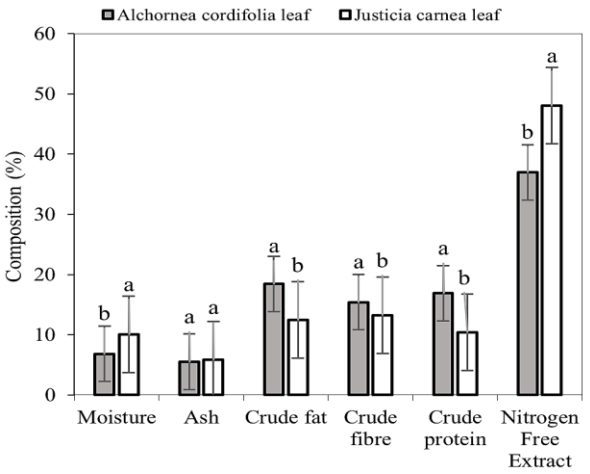

Fig. 3 Proximate composition of *Alchornea cordifolia* and *Justicia carnea* leaves. Bars with different letters are significantly different at $P<0.05$.

substantial benefits in controlling postprandial glucose spikes and managing conditions related to glucose metabolism disorders [52]. Furthermore, the nutraceutical benefits of these leaves extend beyond their ability to modulate carbohydrate digestion. Both leaves likely contain a range of bioactive compounds such as polyphenols, flavonoids and other antioxidants, which contribute to their overall health-promoting properties. These compounds are known for their ability to scavenge free radicals, reduce oxidative stress, and protect against chronic diseases such as cardiovascular disease, cancer, and neurodegenerative disorders [52]. The higher inhibition values observed in *A. cordifolia* suggest that it may possess a more potent antioxidant and health-promoting profile compared to *Justicia carnea*. This highlights its potential as a natural antioxidant phytogenic feed supplement, which could be utilized in animal nutrition to promote health and improve overall well-being.

Fig. 3 illustrates the proximate composition of *A. cordifolia* and *J. carnea* leaves. *Justicia carnea* leaves have a significantly ($P<0.05$) higher moisture content compared to *A. cordifolia* leaves. This is of importance because higher moisture content can influence the shelf life and susceptibility to microbial growth, which is important for the storage and processing of food supplements or ingredients [53]. In this study, the nitrogen-free extract (*i.e.*, carbohydrates such as sugars and starches) being significantly ($P<0.05$) higher in *J. carnea* leaves, when compared to *A. cordifolia* indicates that *J. carnea* could be a better energy source, making it beneficial for providing quick energy in diets [54]. *A. cordifolia* leaves have a significantly ($P<0.05$) higher crude fat content. Since fats are essential for providing energy, aiding in the absorption of fat-soluble vitamins, and contributing to cell structure and function [55]; *A. cordifolia* leaves could be advantageous in diets requiring higher energy and essential fatty acids. Dietary fiber is crucial for maintaining digestive health, preventing constipation, and regulating blood sugar levels [56]. According to this study, *A. cordifolia* leaves had a greater crude fiber content ($P<0.05$) than *J. carnea* leaves. *A. cordifolia* may therefore be more advantageous for enhancing digestive health and treating metabolic problems. *A. cordifolia* leaves have a significantly ($P<0.05$) higher crude protein content. Proteins are vital for the growth, repair, and maintenance of body tissues, as well as for enzymatic and hormonal functions [57]. *A. cordifolia* may therefore be a more beneficial source of protein, particularly for diets low in protein. There is no significant ($P>0.05$) difference in the ash content of *A. cordifolia* and *J. carnea* leaves. Ash content is indicative of the total mineral content in the leaves [22], suggesting that both leaves provide a comparable mineral contribution to the diet.

Conclusions

In conclusion, *A. cordifolia* demonstrates superior antioxidant activity, with higher levels of Vitamin C, FRAP, DPPH scavenging, and flavonoid content compared to *J. carnea*. Additionally, it exhibits stronger inhibition of key enzymes involved in metabolism. These findings underscore its potential as a valuable nutraceutical resource. *A. cordifolia* leaves offer superior antioxidant activity and enzyme inhibition compared to *J. carnea* leaves, suggesting potential as functional food ingredients.

Conflict of interest

The authors claim no conflicts of interest.

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