



ISSN Print: 2664-6064
 ISSN Online: 2664-6072
 IJAN 2023; 5(2): 103-105
www.agriculturejournal.net
 Received: 02-11-2023
 Accepted: 11-12-2023

Tushar Sheshrao Vishe
 Acharya Narendra Deva
 University of Agriculture and
 Technology, Ayodhya,
 Uttar Pradesh, India

Antioxidant and anti-inflammatory properties of tigernut (*Cyperus esculentus*) extract

Tushar Sheshrao Vishe

DOI: <https://doi.org/10.33545/26646064.2023.v5.i2b.165>

Abstract

This study investigates the antioxidant and anti-inflammatory properties of tigernut (*Cyperus esculentus*) extract. Through a series of *in vitro* and *in vivo* experiments, we examine the extract's capacity to scavenge free radicals and its effects on inflammatory markers. The findings suggest that tigernut extract could be a potent natural agent for managing oxidative stress and inflammation, providing a basis for its use in functional foods and therapeutic applications.

Keywords: Tigernut extract, *Cyperus esculentus*, antioxidant, anti-inflammatory, free radicals, inflammation

Introduction

Tigernut (*Cyperus esculentus*) is a tuber crop that has been cultivated for centuries for its edible tubers, which are rich in nutrients and bioactive compounds. Traditionally, tigernuts have been consumed as a food and used in natural remedies across various cultures. In recent years, tigernut has gained significant attention due to its potential health benefits, particularly its antioxidant and anti-inflammatory properties. Oxidative stress and chronic inflammation are recognized as key contributors to the development and progression of numerous chronic diseases, including cardiovascular diseases, diabetes, and cancer. Therefore, the identification and utilization of natural compounds that can mitigate these conditions are of paramount importance.

Tigernuts are known to contain a variety of bioactive compounds, including phenolic acids, flavonoids, and other antioxidants. These compounds are believed to play a crucial role in neutralizing free radicals and reducing oxidative stress. Additionally, they may exert anti-inflammatory effects by modulating inflammatory pathways and reducing the production of pro-inflammatory mediators.

Despite the growing interest in the health benefits of tigernut, there is limited comprehensive data on its antioxidant and anti-inflammatory properties. This study aims to fill this gap by systematically evaluating the antioxidant capacity and anti-inflammatory effects of tigernut extract through a series of *in vitro* and *in vivo* experiments. Understanding these properties can provide valuable insights into the potential therapeutic applications of tigernut extract and support its use as a functional food ingredient.

The antioxidant properties of tigernut extract were assessed using various assays, including the DPPH Radical Scavenging Assay, ABTS Radical Cation Decolorization Assay, and Ferric Reducing Antioxidant Power (FRAP) Assay. These assays provide a comprehensive evaluation of the extract's ability to neutralize free radicals and reduce oxidative damage. The anti-inflammatory properties were evaluated by measuring the inhibition of nitric oxide (NO) production in LPS-stimulated RAW 264.7 macrophages and assessing the reduction of carrageenan-induced paw edema in rats. These models are widely used to study the anti-inflammatory effects of natural compounds and provide relevant data on the extract's potential to modulate inflammatory responses.

By elucidating the antioxidant and anti-inflammatory properties of tigernut extract, this study aims to contribute to the growing body of evidence supporting the health benefits of tigernut and its potential applications in the prevention and management of chronic diseases.

Corresponding Author:
Tushar Sheshrao Vishe
 Acharya Narendra Deva
 University of Agriculture and
 Technology, Ayodhya,
 Uttar Pradesh, India

Objective

To evaluate the antioxidant and anti-inflammatory properties of tigernut (*Cyperus esculentus*) extract through *in vitro* and *in vivo* experiments.

Materials and Methods

Sample Preparation

Tigernuts (*Cyperus esculentus*) were sourced from Koyambedu Market, Chennai, Tamil Nadu, India. The nuts were cleaned, dried, and ground into a fine powder. The powder was extracted using ethanol (70%) through maceration for 48 hours at room temperature. The extract was filtered and concentrated using a rotary evaporator. The concentrated extract was stored at -20 °C until further use.

Antioxidant Activity Assays

The antioxidant activity of the tigernut extract was evaluated using various assays. The DPPH Radical Scavenging Assay measured the ability of the extract to scavenge DPPH free radicals. Different concentrations of the extract were mixed with DPPH solution, and the absorbance was measured at 517 nm after 30 minutes of incubation, with the percentage inhibition calculated. The ABTS Radical Cation Decolorization Assay involved generating ABTS radical cation by reacting ABTS solution with potassium persulfate. The extract was added to the ABTS solution, and the absorbance was measured at 734 nm, expressing the antioxidant activity as the Trolox equivalent antioxidant capacity (TEAC). The Ferric Reducing Antioxidant Power (FRAP) Assay was conducted by preparing the FRAP reagent by mixing acetate buffer, TPTZ solution, and ferric chloride. The extract was added to the FRAP reagent, and the absorbance was measured at 593 nm after 30 minutes, with results expressed as mmol Fe²⁺ equivalents per gram of extract.

Anti-Inflammatory Activity Assays

The anti-inflammatory properties of tigernut extract were assessed using *in vitro* and *in vivo* methods. The Inhibition of Nitric Oxide (NO) Production assay involved stimulating RAW 264.7 macrophages with lipopolysaccharide (LPS) in the presence or absence of the tigernut extract. NO levels in the culture supernatant were measured using the Griess reagent, and the percentage inhibition of NO production was calculated. The Carrageenan-Induced Paw Edema in Rats assay involved dividing male Wistar rats into groups and pre-treating them with different doses of the tigernut extract. Paw edema was induced by injecting carrageenan into the right hind paw, and the paw volume was measured at intervals using a plethysmometer. The anti-inflammatory effect was evaluated by comparing the paw volume changes in treated and control groups.

Statistical Analysis

All experiments were conducted in triplicate, and the results were expressed as mean±standard deviation (SD). Data were analyzed using ANOVA followed by Tukey's post hoc test to determine significant differences between groups ($p<0.05$).

Results and Discussion

Antioxidant Activity

The antioxidant assays revealed that tigernut extract exhibited significant free radical scavenging activity. The results are summarized in Table 1.

Table 1: Antioxidant Activity of Tigernut Extract

Assay	Value (Mean ± SD)	p-Value
DPPH Inhibition (%)	87.3±2.1	< 0.05
TEAC (μmol Trolox/g)	412.5±15.6	< 0.05
FRAP (mmol Fe ²⁺ /g)	5.8±0.3	< 0.05

The DPPH assay showed that the tigernut extract had an inhibition rate of 87.3%, indicating strong radical scavenging activity. The TEAC value was 412.5 μmol Trolox/g, and the FRAP value was 5.8 mmol Fe²⁺/g, both demonstrating the extract's substantial antioxidant capacity. ANOVA analysis confirmed that the differences were statistically significant ($p<0.05$).

Anti-Inflammatory Activity

The anti-inflammatory assays showed that tigernut extract effectively inhibited inflammatory markers. The results are presented in Table 2.

Table 2: Anti-Inflammatory Activity of Tigernut Extract

Assay	Value (Mean ± SD)	p-Value
NO Inhibition (%)	74.6±3.4	< 0.05
Paw Edema Reduction (mm)	1.8±0.2 (high dose)	< 0.05

The NO inhibition assay indicated that the extract reduced NO production by 74.6% in LPS-stimulated macrophages. In the carrageenan-induced paw edema model, the highest dose of tigernut extract significantly reduced paw edema, indicating its anti-inflammatory effect. ANOVA analysis confirmed that these differences were statistically significant ($p<0.05$).

Discussion

The results of this study provide substantial evidence for the strong antioxidant and anti-inflammatory properties of tigernut (*Cyperus esculentus*) extract. The high phenolic and flavonoid content in tigernut extract likely contributes to its potent bioactivity, as these compounds are well-known for their ability to neutralize free radicals and modulate inflammatory responses.

The antioxidant assays, including DPPH, ABTS, and FRAP, consistently demonstrated the significant free radical scavenging activity of tigernut extract. The DPPH assay revealed an inhibition rate of 87.3%, indicating that the extract can effectively donate hydrogen atoms to neutralize DPPH radicals. This high inhibition rate is comparable to that of other potent natural antioxidants, suggesting that tigernut extract is a robust antioxidant source. The ABTS assay further supported these findings, with the tigernut extract showing a TEAC value of 412.5 μmol Trolox/g, indicating its high capacity to scavenge ABTS radicals. Additionally, the FRAP assay demonstrated that the extract had a ferric reducing ability of 5.8 mmol Fe²⁺/g, underscoring its significant reducing power. These results collectively confirm that tigernut extract has a substantial antioxidant capacity, which can help in mitigating oxidative stress and preventing the damage caused by free radicals. The anti-inflammatory properties of tigernut extract were also prominently demonstrated in this study (Alieozaman *et al*, 2023) [3]. The *in vitro* inhibition of nitric oxide (NO) production in LPS-stimulated RAW 264.7 macrophages showed that the extract could reduce NO levels by 74.6%. Nitric oxide is a critical mediator of inflammation, and its overproduction is associated with various inflammatory

diseases. By inhibiting NO production, tigernut extract exhibits a potential mechanism for its anti-inflammatory effects. This finding aligns with previous studies that have reported the anti-inflammatory effects of phenolic and flavonoid compounds through the modulation of nitric oxide synthase (NOS) activity.

In vivo studies using the carrageenan-induced paw edema model further confirmed the anti-inflammatory efficacy of tigernut extract. The extract significantly reduced paw edema in rats, with the highest dose showing a marked reduction of 1.8 mm. Carrageenan-induced paw edema is a widely used model for studying acute inflammation, and the reduction in paw volume indicates the extract's ability to inhibit the inflammatory response. This anti-inflammatory effect could be attributed to the suppression of pro-inflammatory cytokines and mediators, as well as the inhibition of inflammatory cell infiltration, which are commonly influenced by phenolic and flavonoid compounds.

The results of this study are consistent with existing literature on the health benefits of phenolic-rich foods. For instance, previous research has highlighted the role of dietary phenolics and flavonoids in reducing the risk of chronic diseases through their antioxidant and anti-inflammatory actions. The findings from this study provide additional support for the therapeutic potential of tigernut extract, suggesting that it could be incorporated into functional foods or used as a natural supplement to enhance health and prevent disease.

Moreover, the significant antioxidant and anti-inflammatory activities observed in tigernut extract highlight its potential applications in the development of functional foods and nutraceuticals. These products can offer health benefits beyond basic nutrition by providing bioactive compounds that help manage oxidative stress and inflammation. The incorporation of tigernut extract into such products could appeal to health-conscious consumers looking for natural and effective ways to support their well-being.

Future research should focus on the bioavailability and long-term health effects of tigernut extract in human populations. While the current study provides robust evidence of its bioactivity, understanding how these compounds are absorbed, metabolized, and exert their effects in the human body is crucial for developing effective dietary interventions. Additionally, clinical trials are necessary to validate the health benefits observed *in vitro* and in animal models, ensuring that tigernut extract can be safely and effectively used in human health applications.

In conclusion, the study demonstrates that tigernut (*Cyperus esculentus*) extract possesses strong antioxidant and anti-inflammatory properties, making it a promising candidate for functional foods and therapeutic applications. The extract's ability to scavenge free radicals and inhibit inflammatory markers underscores its potential in managing oxidative stress and inflammation-related conditions. These findings support the inclusion of tigernut extract in the development of health-promoting products and encourage further research into its clinical applications and mechanisms of action.

Conclusion

The present study demonstrates that tigernut (*Cyperus esculentus*) extract possesses significant antioxidant and anti-inflammatory properties. Through a series of *in vitro*

and *in vivo* experiments, the extract showed a strong capacity to scavenge free radicals and effectively inhibit inflammatory markers. The high levels of phenolic and flavonoid compounds in the extract likely contribute to these potent bioactivities.

Antioxidant assays, including DPPH, ABTS, and FRAP, confirmed the substantial free radical scavenging ability of tigernut extract. These findings indicate that the extract can play a crucial role in mitigating oxidative stress, which is implicated in the pathogenesis of numerous chronic diseases. The anti-inflammatory properties of the extract were evidenced by the significant inhibition of nitric oxide production in macrophages and the reduction of carrageenan-induced paw edema in rats. These results suggest that tigernut extract can modulate inflammatory responses, potentially benefiting conditions characterized by chronic inflammation.

The study's findings support the potential use of tigernut extract in functional foods and therapeutic applications aimed at enhancing health and preventing disease. The extract's bioactive compounds offer a natural means to combat oxidative stress and inflammation, promoting overall well-being. Future research should focus on the bioavailability, long-term health effects, and clinical applications of tigernut extract in human populations to further validate its efficacy and safety.

In summary, tigernut (*Cyperus esculentus*) extract exhibits strong antioxidant and anti-inflammatory activities, making it a promising candidate for inclusion in health-promoting products. The study provides a foundation for further exploration of tigernut extract's therapeutic potential and its role in functional food development.

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