



Exploring the Antioxidant Potential of *Fagopyrum esculentum* Moench (Common Buckwheat) Leaf Extract

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: <https://doi.org/10.9734/jabb/2024/v27i111710>

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/127010>

Original Research Article

Received: 17/09/2024

Accepted: 19/11/2024

Published: 25/11/2024

ABSTRACT

This study was conducted in 2022 at the Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India. The objective was to assess the antioxidant potential of the hydromethanolic leaf extract of *Fagopyrum esculentum* Moench (common buckwheat) by evaluating its reducing power and metal ion chelation abilities. Leaves of *Fagopyrum esculentum* were collected from the Pithoragarh district in Uttarakhand and authenticated.

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Cite as: Chand, Priya, Sonu Ambwani, Brij Raj Singh, Tanuj Kumar Ambwani, and Ashutosh Dubey. 2024. "Exploring the Antioxidant Potential of *Fagopyrum Esculentum* Moench (Common Buckwheat) Leaf Extract". *Journal of Advances in Biology & Biotechnology* 27 (11):1243-51. <https://doi.org/10.9734/jabb/2024/v27i111710>.

A 50% hydromethanolic extract (FEE) was prepared from the powdered leaves. The antioxidant potential of FEE was assessed using reducing power and metal ion chelation tests. The hydromethanolic extract yielded 7.17%. FEE exhibited significant antioxidant activity in a concentration-dependent manner, demonstrating both electron-donating ability and metal ion chelation capacity with an IC₅₀ value of 108.846 µg/ml. These results highlight the potential of *F. esculentum* leaf extract as a natural antioxidant for use in the pharmaceutical, cosmetic, and food industries. Further studies are recommended to investigate its effects *In vivo*.

Keywords: *Fagopyrum esculentum*; buckwheat; antioxidant activity; reducing power; metal ion chelation; leaf extract.

1. INTRODUCTION

Throughout human history, plants have played a vital role in various aspects of life, particularly in medicine. Over time, the relationship between humans and plants has evolved, leading to deeper insights into their medicinal properties and therapeutic potential (Kumar et al., 2015). Even today, herbal treatments remain a popular form of traditional medicine, with plant-based systems continuing to play a significant role in healthcare (Ambwani et al., 2018; Pandey and Ambwani, 2022). There is a growing global demand for herbal medicines, dietary supplements, health products, nutraceuticals, pharmaceuticals, and cosmetics (Shakya, 2016). In recent years, interest in natural antimicrobials and antioxidants has surged due to consumer preference for agricultural byproducts free from synthetic additives (Zhao et al., 2018). Functional foods are those that provide health benefits beyond basic nutrition, thanks to natural components with health-promoting and pharmacological properties. These bioactive substances are increasingly added to foods to meet consumer preferences and comply with legal standards. Antioxidants, as functional components, are safe for both the environment and human health while also offering health benefits. They play a vital role as colouring agents, preservatives, antioxidants, and additives in food and medicines, emphasizing their importance in promoting health and enhancing product functionality (Zehiroglu and Ozturk, 2019).

One plant receiving attention in this context is *Fagopyrum esculentum* Moench, commonly known as buckwheat. Native to central and northeastern Asia, buckwheat is a dicotyledonous plant belonging to the Polygonaceae family. However, it is often

classified as a pseudocereal because of its similarities to cereals in terms of processing, chemical composition, use, and grain structure (Begić et al., 2017). It is cultivated across several countries, including China, Japan, Korea, India, Nepal, Bhutan, Canada, and the USA, owing to its adaptability to diverse environmental conditions (Meng, 2015). In India, buckwheat is primarily grown in the North-Western Himalayan region and certain northern states (Dubey et al., 2021). Known by different names across cultures, it is referred to as *ogal* in India, *soba* in Japan, *jawas* in Pakistan, *mitaphapar* in Nepal, and *tianqiaomai* in Mandarin (Ahmed et al., 2014).

Morphologically, buckwheat is a eudicot with significant leaf biomass, and its leaves have long been used for culinary and medicinal purposes (Li et al., 2019). In cooking, the leaves are either consumed as a vegetable dish or dried, ground into powder, and incorporated into gourmet recipes. Buckwheat offers high-quality protein with a favorable amino acid profile, containing more sulfur-containing and essential amino acids than rice or maize (Mota et al., 2016). Additionally, it is a good source of dietary fiber, lipids, minerals, vitamin C, and bioactive compounds, including phenolics, sterols, and anthocyanins (Ahmed et al., 2014; Multari et al., 2016; Alonso-Miravalles et al., 2018; Phull and Gupta, 2023). Several parts of the plant—such as the seeds, leaves, flowers, and sprouts—have been studied extensively for their potential use in functional food development (Prakash and Yadav, 2016; Dražić et al., 2016). Research has particularly focused on the health benefits of these compounds, including their ability to mitigate oxidative stress and regulate blood sugar levels (Huda et al., 2021). In addition to its nutritional value, buckwheat contains a significant amount of flavonoids compared to major cereal crops. Six flavonoids have been identified in buckwheat: rutin, orientin, vitexin,

quercetin, isovitexin, and isoorientin. Among these, rutin, also referred to as vitamin P, is the predominant flavonoid (Sytar *et al.*, 2016; Ganeshpurkar and Saluja 2017). Rutin shields plants from UV radiation due to its potent antioxidant properties (Koja *et al.*, 2018). Although buckwheat leaves and flowers contain high levels of rutin, they also have naphthodianthrone-based alkaloids called fagopyrins, which may exhibit phototoxic properties (Kreft and Jane, 2013). Rutin offers significant benefits for cardiovascular health, particularly by lowering the risk of arteriosclerosis (Rauf *et al.*, 2020).

In addition, compared with other cereals, buckwheat is characterized by a relatively higher nutritional value. Biochemical analyses of buckwheat germplasm have indicated that seeds of these plants are an excellent source of starch, proteins, fats, dietary fiber, vitamins, minerals, and phenolic components (Bobkov, 2016). Pharmacological studies have highlighted the wide range of benefits offered by common buckwheat, including antioxidant, anti-inflammatory, cardiovascular, hypolipidemic, antigenotoxic, antidiabetic, reno-protective, anticancer, antimicrobial, wound-healing, anti-stress, memory-protective, and photoprotective effects. Among these, phenolic compounds produced through secondary metabolism play a key role in countering oxidative stress. These compounds act as antioxidants by donating hydrogen or electrons and forming stable radical intermediates, thereby offering protective effects when included in the diet (Chaves *et al.*, 2020). Research has established a strong link between the antioxidant activity of plant-derived polyphenols and their health-promoting properties. Buckwheat's ability to protect cellular components from oxidative damage may help reduce the risk of degenerative diseases linked to oxidative stress, such as cancer, cardiovascular disease, and osteoporosis (Bystrická *et al.*, 2015). Buckwheat is a gluten-free grain suitable for inclusion in the diets of individuals with celiac disease (Sytar *et al.*, 2016). The study of foods rich in antioxidants and their role in preventing oxidative damage-related diseases has become a crucial area in food and nutrition research. While most studies have focused on the nutritional and therapeutic potential of buckwheat seeds, the medicinal properties of its leaves remain relatively underexplored, despite being rich in bioactive compounds.

In this context, the present study aims to prepare a hydromethanolic extract of *Fagopyrum esculentum* Moench leaves and assess its antioxidant potential. This research seeks to contribute to a deeper understanding of the nutritional and health benefits of buckwheat.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Fagopyrum esculentum Moench leaves were collected from the Pithoragarh district of Uttarakhand. The plant material was authenticated by Dr. D.S. Rawat, Assistant Professor at the Department of Biological Sciences, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India (Voucher specimen No. 1626).

2.2 Preparation of Extract

The method described by Deb *et al.* (2018) was followed to prepare a 50% hydromethanolic extract of *Fagopyrum esculentum* Moench leaves (FEE). The collected leaves underwent thorough cleansing, followed by shade-drying at ambient temperature before being pulverized into a fine powder. A 100g of this powdered leaf material was subjected to extraction using 1 litre of 50% (v/v) methanol solution. The extraction process was conducted in a shaking incubator for a duration of 48 hours under continuous agitation. The resultant mixture was initially filtered through muslin cloth, followed by a secondary filtration using Whatman No. 1 filter paper. The filtrate was then concentrated using a rotary evaporator at 45°C, after which it underwent lyophilization (freeze-drying). The final extract was quantified and stored at -20°C in a deep freezer to preserve its integrity for subsequent analyses.

2.3 Antioxidant Activity of FEE

2.3.1 Reducing power capacity

The reducing power of FEE was evaluated using the method described by Nićiforović *et al.* (2010). The assay protocol involved combining 2.5 ml of varying concentrations of hydromethanolic FEE with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (1%). This mixture was incubated at 50°C for 20 minutes.

Subsequently, 2.5 ml of trichloroacetic acid solution (10%) was introduced, followed by centrifugation at 1000 rpm for 8 minutes. A 5 ml aliquot of the supernatant was then mixed with 1 ml of ferric chloride (0.1%). The absorbance of the resulting solution was measured spectrophotometrically at 700 nm. In this assay, an increase in absorbance correlates with higher reducing activity. Ascorbic acid served as the reference standard for comparative analysis.

2.3.2 Metal Ion chelation assay

The metal ion chelating activity of FEE was assessed using the methodology outlined by Adusei *et al.* (2019). In this assay, EDTA served as the reference standard. The experimental procedure involved combining various concentrations of both the standard and FEE with 200 µL of 0.1 mM ferrous sulphate (FeSO₄) solution. To initiate the reaction, 400 µL of 0.25 mM ferrozine was introduced, resulting in the formation of a Fe²⁺-ferrozine complex. This reaction mixture was then subjected to incubation at room temperature for a duration of

10 minutes. Subsequently, the absorbance of the solution was measured spectrophotometrically at a wavelength of 562 nm.

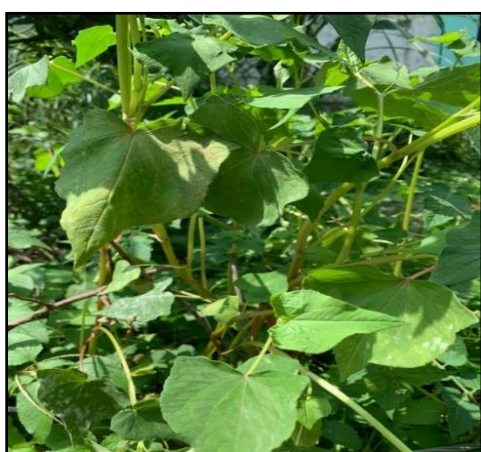
2.3.3 Statistical analysis

Data analysis was conducted using Origin Software, with results presented as mean values accompanied by their standard deviations (±SD). The assessment of statistical differences between experimental and control groups was performed through one-way analysis of variance (ANOVA). Results were deemed statistically significant when probability values fell below 0.05 (p < 0.05).

3. RESULTS

3.1 Percentage Yield of Hydromethanolic Extract of *Fagopyrum esculentum* Moench

The percent yield of authenticated *Fagopyrum esculentum* Moench leaves was obtained as 7.17% (Fig. 1, Table 1).



a) *Fagopyrum esculentum* Moench leaves



b) *Fagopyrum esculentum* Moench leaves dried powder

Fig. 1. Plant material used for *Fagopyrum esculentum* Moench extract preparation

Table 1. Percentage Yield of FEE

S. No.	Plant	Weight of Dried plant leaves (gm)	Weight of hydromethanolic extract obtained (gm)	Percent Yield (%)
1.	<i>Fagopyrum esculentum</i> Moench	100	7.17 gm	7.17

3.2 Determination of Antioxidant Potential of FEE

3.2.1 Reducing power capacity

The capacity of a plant extract to facilitate the reduction of Fe^{3+} to Fe^{2+} serves as a significant indicator of its antioxidant efficacy. In this process, the plant extract interacts with potassium ferricyanide (Fe^{3+}), resulting in the formation of potassium ferrocyanide (Fe^{2+}). This product subsequently reacts with ferric chloride, generating a Prussian blue-coloured ferric-ferrous complex. The intensity of this complex can be quantified spectrophotometrically by measuring its absorbance at 700 nm. A direct proportionality exists between the reducing power capacity and the concentration of FEE. The experimental findings demonstrate that as the concentration of FEE increases, there is a concomitant and significant enhancement in its reducing power capability, as evidenced by the observed increase in absorbance values. This relationship underscores the concentration-dependent nature of FEE's antioxidant activity in this assay (Fig. 2).

3.2.2 Metal ion chelation assay

Antioxidant compounds present in plant extracts interact with ferrous salts and ferrozine, which acts as a chelator for Fe (II), forming a violet-coloured complex. Antioxidants that function through chelation mechanisms decelerate this process, resulting in a reduction of the colour intensity of the ferrozine- Fe^{2+} complex. This phenomenon occurs due to the competition between the extract's chelators and ferrozine for the metal ions. The metal chelating capacity (MCC) of FEE was evaluated. A statistically significant correlation was observed between the concentration of FEE and its metal chelating capacity. This indicates that FEE exhibits substantial metal chelating properties, which may confer a protective effect against oxidative damage induced by metal-catalyzed decomposition reactions. The concentration-dependent increase in FEE's metal chelating capacity suggests its potential as a natural antioxidant agent. These findings are represented in Fig. 2 and in Table 2 of the study.

Table 2. IC₅₀ (Inhibitory Concentration) value of Metal Ion Chelation Assay

S. No.	In vitro Antioxidant Assays	IC ₅₀ (µg/ml)	
		Standard	FEE
1.	Metal Ion Chelation	68.415	108.846

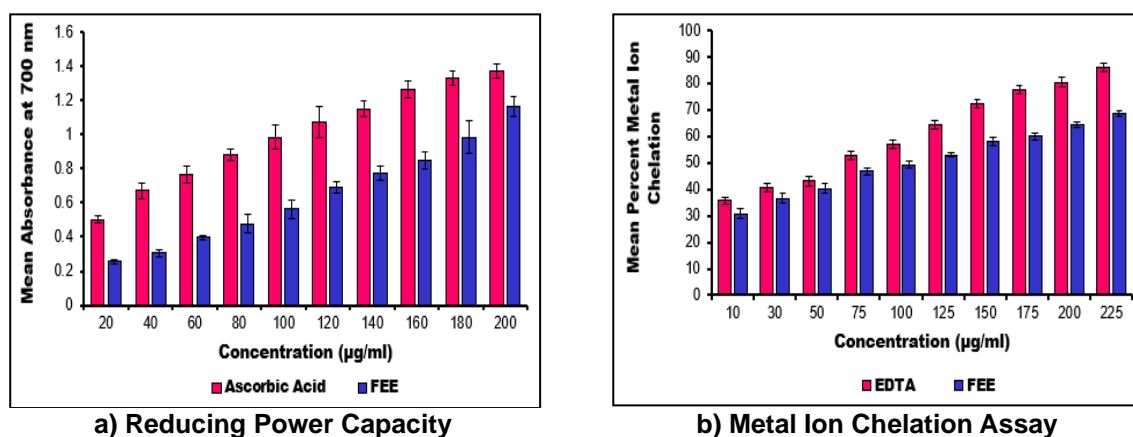


Fig. 2. In vitro antioxidant assays

4. DISCUSSION

The present study aimed to evaluate the antioxidant potential of hydromethanolic extract of *Fagopyrum esculentum* Moench (buckwheat) leaves. The results demonstrate promising antioxidant capabilities of the extract, as evidenced by its reducing power capacity and metal ion chelation ability. The hydromethanolic extract of *F. esculentum* leaves yielded 7.17% (w/w), which is comparable to other plant parts reported in recent literature. Abbasi et al. (2015) reported the extraction yield of *Fagopyrum esculentum* seeds to be 9.8%. However, it's important to note that extraction yields can vary based on factors such as solvent type, extraction method, plant part used, and geographical origin of the plant material.

Recent years have seen a growing interest in the health benefits of buckwheat, particularly its green leaves. Studies have shown that these leaves exhibit a wide range of pharmacological effects, primarily through their antioxidant mechanisms (Prakash and Yadav, 2016). Flavonoids, which are the dominant phenolic constituents in buckwheat, have been identified as the key contributors to its antioxidant activity. This antioxidant effect has been observed not only in the grain and seedlings but also in the green leaves of the buckwheat plant (Kalinova et al., 2006). Antioxidants are essential in both food systems and the human body, as they help mitigate oxidative processes and counteract the damaging effects of reactive oxygen species (ROS). In food systems, nutritional antioxidant molecules play a crucial role in slowing lipid peroxidation and preventing the formation of secondary lipid peroxidation products. This helps preserve the flavor, color, and texture of food products during storage (Gulcin, 2020). Our study builds upon this existing knowledge, focusing specifically on the antioxidant potential of the leaf extract. The results show a significant, concentration-dependent increase in the reducing power of FEE. This trend aligns with findings from other recent studies on buckwheat extracts. For example, Habtemariam (2019) reported similar concentration-dependent reducing power in methanolic extract of common buckwheat leaves. Similarly, Abbasi et al. (2015) also reported a concentration-dependent reducing power capacity of *Fagopyrum esculentum* seeds. The metal ion chelation assay results demonstrate that FEE possesses significant metal-chelating capacity, with an IC_{50} value of 108.846 $\mu\text{g/ml}$. While this is higher than the IC_{50} of the EDTA standard (68.415 $\mu\text{g/ml}$), it still indicates substantial chelating ability. Sedej et al. (2012) reported that different buckwheat grains fraction exhibits a significant chelating activity. The metal-chelating capability observed in various fractions of buckwheat grains, can be attributed to these flavonoids, which offer protection against metal-chelating agents by forming complexes with metal ions. The observed antioxidant potential of FEE can be attributed to the presence of various phytochemicals in buckwheat leaves, particularly flavonoids and phenolic compounds (Chaves et al., 2020). In buckwheat, compounds such as rutin, quercetin, and chlorogenic acid, are known for their strong antioxidant properties. These compounds can donate electrons to reactive free radicals, converting them into more stable products and terminating the radical chain

reaction. The antioxidant and radical scavenging properties of buckwheat herb extract were evaluated against rutin, its primary component. The findings revealed that the extract exhibited notably superior antioxidant activity compared to pure rutin. This suggests that utilizing the buckwheat herb extract may be more advantageous than using pure rutin alone, likely due to the presence of additional minor phenolic compounds in the extract (Al-Snafi, 2017).

5. CONCLUSION

This study demonstrates that the fifty percent hydromethanolic extract of *Fagopyrum esculentum* Moench leaves possesses significant antioxidant potential, as evidenced by its robust reducing power and metal ion chelation abilities. These findings contribute to the growing body of evidence supporting buckwheat's role as a valuable source of natural antioxidants. Future research should focus on fully characterizing the antioxidant profile of the extract and exploring its potential applications across various industries. In the food sector, it could serve as a natural preservative to extend product shelf life by inhibiting lipid peroxidation. The pharmaceutical and cosmetic industries might benefit from incorporating it into formulations targeting oxidative stress-related conditions. Additionally, it could serve as a natural growth promoter or health enhancer in animal feed and agricultural biopesticides. Its environmental applications, such as mitigating oxidative stress in polluted ecosystems, further highlight its versatility, with future research needed to optimize and standardize its use. Crucially, investigating the extract's *in vivo* antioxidant effects through animal model studies is essential to better understand its potential health benefits and pave the way for its use in promoting human health and wellbeing.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ACKNOWLEDGEMENT

We sincerely acknowledge the research facilities provided for this study by the Dean of the College of Basic Sciences and Humanities and

the Director of the Experiment Station at G. B. Pant University of Agriculture and Technology, Pantnagar-263145, Uttarakhand, India.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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