Original Research Article

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

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. A 50% hydromethanolic extract (FEE) was prepared from the powdered leaves. The antioxidant potential of FEE was assessed using *in vitro* assays, including 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. 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).

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, *mitephapar* 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). 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).

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). 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 ($FeSO_4$) solution. To initiate the reaction, 400 µL of 0.25 mM ferrozine was introduced, resulting in the formation of a Fe^{2+}-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).

Table 1: Percentage Yield of FEE

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

a) ***Fagopyrum esculentum*** **Moench leaves** **b)** ***Fagopyrum esculentum*** **Moench leaves dried powder**

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

3.2 Determination of Antioxidant Potential of FEE

3.2.1 Reducing Power Capacity

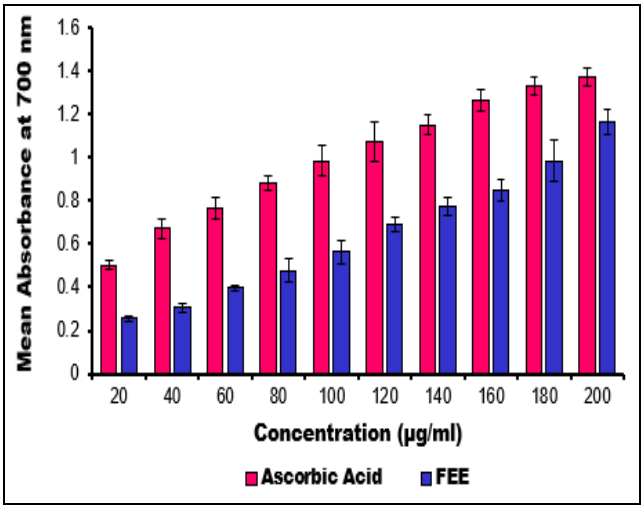
The capacity of a plant extract to facilitate the reduction of Fe^{3+} to Fe^{2+} serves as a significant indicator of its antioxidant efficacy. In this process, the plant extract interacts with potassium ferricyanide (Fe^{3+}), resulting in the formation of potassium ferrocyanide (Fe^{2+}). This product 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

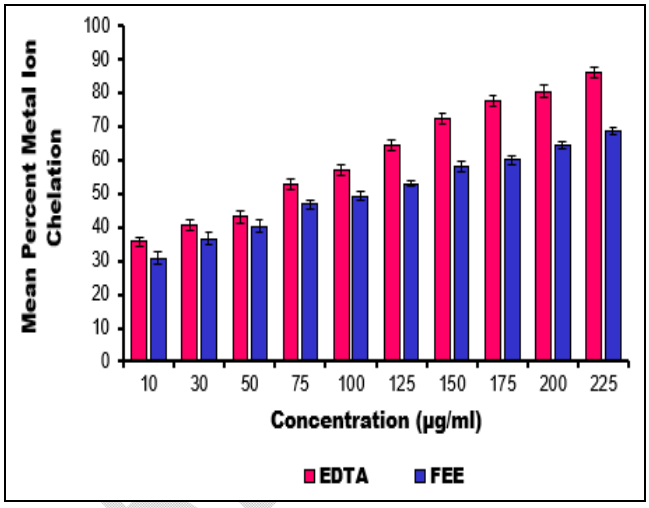
Antioxidant compounds present in plant extracts interact with ferrous salts and ferrozine, which acts as a chelator for Fe (II), forming a violet-coloured complex. Antioxidants that function through chelation mechanisms decelerate this process, resulting in a reduction of the colour intensity of the 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: IC_{50} (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



a) Reducing Power Capacity



b) Metal Ion Chelation Assay

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). 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 µg/ml. While this is higher than the IC_{50} of the EDTA standard (68.415 µg/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. 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.

REFERENCES

1. Kumar, N., Wani, Z. A., & Dhyani, S. (2015). Ethnobotanical study of the plants used by the local people of Gulmarg and its allied areas, Jammu & Kashmir, India. International Journal of Current Research in Bioscience and Plant Biology, 2(9), 16-23.
2. Ambwani, S., Tandon, R., Ambwani, T. K., Malik, Y. S. (2018). Current knowledge on nanodelivery systems and their beneficial applications in enhancing the efficacy of herbal drugs. Journal of Experimental Biology and Agriculture Science, 6(1), 87–107. doi:10.18006/2018.6(1).87.107
3. Pandey, Y., & Ambwani, S. (2022) Nano metal based herbal theranostics for cancer management: coalescing nature's boon with nanotechnological advancement. Current Pharmaceutical Biotechnology, 23(1), 30-46. DOI: 10.2174/1389201022666210122141724
4. Shakya, A. K. (2016). Medicinal plants: Future source of new drugs. International Journal of Herbal Medicine, 4(4), 59-64.
5. Zhao, J., Jiang, L., Tang, X., Peng, L., Li, X., Zhao, G., & Zhong, L. (2018). Chemical composition, antimicrobial and antioxidant activities of the flower volatile oils of Fagopyrum esculentum, Fagopyrum tataricum and Fagopyrum cymosum. Molecules, 23(1), 182. https://doi.org/10.3390/molecules23010182.
6. Begić, M., Oručević Žuljević, S., Akagić, A., & Spaho, N. (2017). Relationship between the Physical and Chemical Quality Parameters of Common Buckwheat (Fagopyrum esculentum Moench) and Tatary Buckwheat (Fagopyrum esculentum Moench). International Journal of Research in Agricultural Sciences, 4(3), 115-159.
7. Meng, Y., Comparison of Immunomodulatory Effects Among Three Tissue Extracts of Tartary Buckwheat in Mice.
8. Dubey, S., Chaurasia, A. K., Shukla, P. K., Kumari, A., & Patil, P. D. (2021). Evaluation of buckwheat (Fagopyrum esculentum L.) genotype on the basis of quality parameters. The Pharma Innovation Journal, 10(10), 1114-1116.
9. Ahmed, A., Khalid, N., Ahmad, A., Abbasi, N. A., Latif, M. S. Z., & Randhawa, M. A. (2014). Phytochemicals and biofunctional properties of buckwheat: a review. The Journal of Agricultural Science, 152(3), 349-369. https://doi.org/10.1017/S0021859613000166.
10. Li, H. (2019). Buckwheat. Bioactive factors and processing technology for cereal foods, 137-149.
11. Mota, C., Santos, M., Mauro, R., Samman, N., Matos, A. S., Torres, D., & Castanheira, I. (2016). Protein content and amino acids profile of pseudocereals. Food Chemistry, 193, 55-61. https://doi.org/10.1016/j.foodchem.2014.11.043.
12. Multari, S., Neacsu, M., Scobbie, L., Cantlay, L., Duncan, G., Vaughan, N., et al. (2016). Nutritional and phytochemical content of high-protein crops. Journal of Agricultural and Food Chemistry, 64(41), 7800-7811. https://doi.org/10.1021/acs.jafc.6b00926.
13. Alonso-Miravalles, L., & O'Mahony, J. A. (2018). Composition, protein profile and rheological properties of pseudocereal-based protein-rich ingredients. Foods, 7(5), 73. https://doi.org/10.3390/foods7050073.
14. Phull, T., & Gupta, M. (2023). Extraction process optimization for antioxidants, nutritional, physicochemical and functional properties in Fagopyrum esculentum Moench leaves of Himalayan region. Food and Humanity, 1, 696-709.
15. Prakash, S., & Yadav, K. (2016). Pharmacobiology and Medical Sciences. International Journal of Current Trends in Pharmacobiology and Medical Sciences, 1(3), 1-15.
16. Dražić, S., Glamočlija, D., Ristić, M., Dolijanović, Ž., Drazić, M., Pavlović, S., ... & Jaramaz, D. (2016). Effect of environment of the rutin content in leaves of Fagopyrum esculentum Moench. Plant Soil and Environment, 62(6), 261-265.
17. Huda, M. N., Lu, S., Jahan, T., Ding, M., Jha, R., Zhang, K., et al. (2021). Treasure from garden: Bioactive compounds of buckwheat. Food Chemistry, 335, 127653. https://doi.org/10.1016/j.foodchem.2020.127653.
18. Kreft, S., Janeš, D., & Kreft, I. (2013). The content of fagopyrin and polyphenols in common and tartary buckwheat sprouts. Acta Pharmaceutica, 63(4), 553-560. https://doi.org/10.2478/acph-2013-0031.

19. Chaves, N., Santiago, A., & Alías, J. C. (2020). Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity and hierarchization based on the method used. Antioxidants, 9(1), 76. https://doi.org/10.3390/antiox9010076.
20. Bystrická, J., Vollmannová, A., Kupecsek, A., Musilová, J., Poláková, Z., Čičová, I., & Bojňanská, T. (2011). Bioactive compounds in different plant parts of various buckwheat (Fagopyrum esculentum Moench.) cultivars. Cereal Research Communications, 39, 436-444. https://doi.org/10.1556/CRC.39.2011.3.13.
21. Deb, K., Kaur, A., Ambwani, S., & Ambwani, T. K. (2018). Preliminary phytochemical analyses of hydromethanolic leaf extract of Melia azedarach L. Journal of Medicinal Plants Studies, 6(3), 4-8.
22. Nićiforović, N., Mihailović, V., Mašković, P., Solujić, S., Stojković, A., & Muratspahić, D. P. (2010). Antioxidant activity of selected plant species; potential new sources of natural antioxidants. Food and Chemical Toxicology, 48(11), 3125-3130. https://doi.org/10.1016/j.fct.2010.08.007.
23. Adusei, S., Otchere, J. K., Oteng, P., Mensah, R. Q., & Tei-Mensah, E. (2019). Phytochemical analysis, antioxidant and metal chelating capacity of Tetrapleura tetraptera. Heliyon, 5(11), e02779. https://doi.org/10.1016/j.heliyon.2019.e02762 .
24. Abbasi, R., Janjua, S., Rehman, A., William, K., & Khan, S. W. (2015). Some preliminary studies on phytochemichals and antioxidant potential of Fagopyrum esculentum cultivated in Chitral, Pakistan. The Journal of Animal & Plant Sciences, 25(3), 576-579.
25. Kalinova, J., Triska, J., & Vrchotova, N. (2006). Distribution of vitamin E, squalene, epicatechin, and rutin in common buckwheat plants (Fagopyrum esculentum Moench). Journal of Agricultural and Food Chemistry, 54(15), 5330-5335. https://doi.org/10.1021/jf060521r.
26. Habtemariam, S. (2019). Antioxidant and rutin content analysis of leaves of the common buckwheat (Fagopyrum esculentum Moench) grown in the United Kingdom: a case study. Antioxidants, 8(6), 160. https://doi.org/10.3390/antiox8060160.
27. Sedej, I., Sakač, M., Mandić, A., Mišan, A., Tumbas, V., & Čanadanović-Brunet, J. (2012). Buckwheat (Fagopyrum esculentum Moench) grain and fractions: antioxidant compounds and activities. Journal of Food Science, 77(9), C954-C959. https://doi.org/10.1111/j.1750-3841.2012.02867.x.
28. Al-Snafi, A. E. (2017). A review on Fagopyrum esculentum: A potential medicinal plant. IOSR Journal of Pharmacy, 7(3), 21-32.