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Phytochemical Analysis And Evaluation Of Antioxidant Activity In *Fagopyrum Esculentum*

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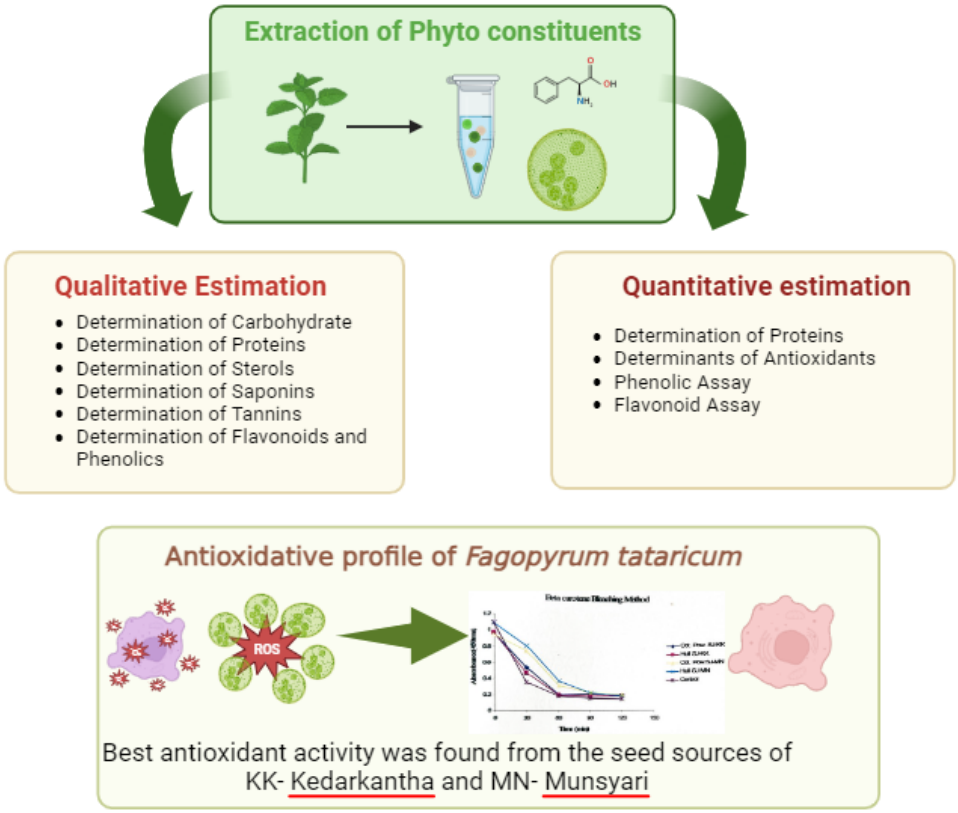
Abstract

Herbal nutraceutical products have become increasingly popular in recent years, particularly in the dermatology and cosmetics fields, because of their potential to prevent skin photodamage and their photoprotective qualities against UV radiation. Standardized herbal extracts are necessary for modern phytopharmaceutics and phytocosmetics, and buckwheat herb, which is high in flavonoids, has shown promise as an antioxidant source. The objective of the research is to ascertain the existence of various nutraceutical components in buckwheat, such as proteins, carbohydrates, sterols, alkaloids, saponins, and tannins, both quantitatively and qualitatively. The study assesses possible interactions between these components, food, and prescribed medications as the body of research on their health benefits grows. Through a variety of phytochemical tests, the results show the high antioxidant and nutraceutical qualities of Fagopyrum esculentum, with methanolic extracts showing greater activity than water extracts. Notably, the study highlights the high antioxidant activity, alkaloids, flavonoids, tannins, and phenolic compounds of F. esculentum as potential major food supplement. This study offers a biochemical justification for its application in ethnopharmacology and as a nutraceutical to improve health and prevent a variety of ailments.

Keywords: Neutraceutical, Antioxidant activity, ethnopathology



1. Introduction

Known for their medicinal qualities and less adverse effects than conventional pharmaceutical agents, nutraceuticals are bioactive chemical compounds obtained from natural sources, mostly plants. These drugs are necessary for promoting medical objectives, preventing disease, and preserving health. The term "nutraceutical" was coined in 1994 by Stephen DeFelice. It blends the terms "pharmaceutical" with "nutrition." Based on their source and therapeutic qualities, these compounds can be grouped into categories that include probiotics, vitamins, antioxidants, plant extracts, and more. Nutraceuticals have been extensively researched and used to treat a wide range of health conditions, including those pertaining to the bones and joints, coronary heart disease, cancer risk, cognitive function, diabetes control, and many more.

One particular nutraceutical of interest is buckwheat (*Fagopyrum esculentum*), a non-cereal, pseudo-cereal plant with remarkable nutritional properties (Habtemariam, 2019; Luo *et al.*, 2020). It provides elements such vitamins, minerals, antioxidants, dietary fiber, and protein that are essential to human health. Buckwheat is a crop that can be grown in adverse weather conditions, making it excellent for subsistence farming. It is particularly prevalent in the high-altitude areas of the Indian Himalayas. The many components of buckwheat, including rutin, highlight its nutraceutical potential and may provide health benefits such cancer prevention and diabetes management (Zhang *et al.*, 2012). Because of its phytochemicals, which have been shown to slow down aging and reduce the risk of different diseases, buckwheat is an essential component of a healthy diet.

In the context of nutraceuticals, phytochemicals are naturally occurring, bioactive compounds found in plants that serve as a plant's defense against disease. These compounds, which are often found in fruits, vegetables, medicinal plants, flowers, leaves, and roots, shield the body against damage along with nutrients and dietary fiber (Khalaf *et al*., 2008). Based on their role in plant metabolism, phytochemicals are divided into primary and secondary constituents. Primary constituents include things like sugars, proteins, amino acids, and chlorophyll; secondary constituents include things like tannins, alkaloids, terpenoids, flavonoids, and phenolic compounds. The study aims to validate the plant's traditional therapeutic use by investigating the phytochemical content and potential health advantages of Fagopyrum esculentum, also known as buckwheat. This study encompasses the collection of plant material, preparation of plant extracts, phytochemical analysis of methanolic extracts, and the assessment of antioxidant activity in *F. esculentum*.

2. Materials & Methods

2.1. Materials

2.2. The plant materials were collected from different phyto geographical regions of Uttarakhand. The plant sources used for the study are as follows: MKD, DC, MN, AL, KK, MIX
All the consumables including reagents and chemicals were purchased from Hi-Media.

2.3. Methods

2.3.1. Processing of Plant Material

A range of plant seed sources for *Fagopyrum esculentum* were analyzed for their phytochemical composition (carbohydrates, proteins, alkaloids, saponins, flavonoids, phenols, steroids, and tannins) and quantitative antioxidant assessment. We sourced our seeds from six different locations in Uttarakhand. The seeds were cleaned, let to air dry, and then sealed plastic bags were stored in a cool location for later use.

2.3.2. Isolation/ Extraction

The seeds were dried at 25°–30° ± 2°C for an hour. To create a fine flour and hull, samples that had been dry-heated were ground into a fine powder with a 0.5 mm particle size and sieved. For two days, these samples were shaken at 90 rpm to extract the contents in 80 percent methanol and water, respectively. Filtered extracts were stored at -20°C until additional analysis was performed.

2.3.3. Qualitative Analysis of Phyto-constituents

Qualitative phytochemical tests were performed on all solvent extracts of *Fagopyrum esculentum* seeds (powder/hull) to identify different plant nutraceutical constituents such as proteins, carbohydrates, alkaloids, sterols, saponins, tannins, phenolic and flavonoids, etc. (Brower, 1998; Shahidi *et al*., 2005). The various tests performed for the qualitative analysis include:

a) Determination of Carbohydrate
b) Determination of Proteins
c) Determination of Sterols
d) Determination of Saponins
e) Determination of Tannins
f) Determination of Flavonoids and Phenolics

2.3.4. Quantitative Analysis of Phytochemicals

2.3.4.1. Aqueous (water) and methanolic seed powder and hull extracts were quantified for a range of phytochemicals or nutrients (flavonoids, phenolic compounds, and proteins) (thin layer chromatography, Lowry method, BSA as standard). The total antioxidant activity was measured using the DPPH method (DPPH + BSA) and the β-Carotene Bleaching method (BSA).

2.3.4.2. Determination of Proteins

Reagent C was obtained by adding Reagent B (0.5% v/v $CuSO_4.5H_2O$ + 1% v/v Na-K-tartarate) in a 50:1 ratio to Reagent A (2.0 g Na_2CO_3 + 0.1N NaOH and make up the volume to 100 ml). Five milliliters of test solutions were taken, and Reagent C and an equal volume of distilled water were added. The mixture was incubated for 30 minutes at room temperature. Addition of 0.5 milliliter of Folin's Reagent initiated the reaction. Next, the protein content was calculated using spectrophotometry at 660 nm. BSA was taken as a standard (Hayes, 2020).

2.3.4.3. Determinants of Antioxidants

The antioxidant activity was determined using β-carotene bleaching and DPPH method.

a) DPPH Method

Using DPPH assay, the total potential antioxidant activity of the prepared aqueous and methanolic seed extracts was evaluated by means of their ability to scavenge 1,1-diphenyl1-2-picrylhydrazyl (DPPH) free radicals(Nooman A et al., 2008). For each concentration (0 µl - 28 µl), an aliquot of extract (1 ml) was combined with methanol DPPH solution (780 µg/ml). The tubes were then left in the dark at room temperature, and the absorbance was measured every 30 minutes at 520 nm. For every time interval, an A_{520} vs. concentration plot was created. For ultimate antiradical activity, the sample concentration that had an initial absorbance that was most similar to the blank (DPPH + solvent) was selected (Oomah, and Martinez, 2005).

ARA = 100 x 1- (Absorbance of sample/ Absorbance of control)

b) β-carotene Method

A circular-bottomed flask containing β-carotene (0.2 mg in 1 ml chloroform), 0.2 ml linoleic acid, and 0.2 ml tween-20 was submerged in water to remove the chloroform. To create a clear β-carotene solution, add 20 milliliters of distilled water and vortex. After that, 80 µl of buckwheat powder and hull extract or methanol/water were added as a control before the β-carotene mixture was added. A spectrophotometer was used to measure the absorbance at 30-minute intervals at 450nm (Schierle *et al*., 2004). Degrading rate (DR) was computed using the following formula, which was based on Al-saikhan, Howard, and Miller (1995), in accordance with first order kinetics.

In (a/b). 1/t =DR sample or DR standard

Where the:
In : Natural log.
a : initial absorbance (450 nm) at time 0,
b: the absorbance (450 nm) at 30, 60, 90, 120 or 150 minutes
t: the initial absorbance (470 nm) at time 0.
Antioxidant activity (AA) was expressed in percent of inhibition relative to the control, using the following formula:

AA = [{DR (control) - DR (sample or standard)} x 100] / DR (control)

2.3.5. Phenolic Assay

Using the Folin-Ciocalteaeu technique, total phenolics were calculated.Aqueous and methnol extracts, or blank, were taken in 20 µl triplicate aliquots, and their volume was increased to 1.60 ml with distilled water. Each tube holding aliquots received 100 µl of FC-reagent. After correctly vortexing, the tubes were left at room temperature for one to three minutes. To obtain a blue color, the reaction was initiated by adding 20% aqueous sodium carbonate to each aliquot and incubating them for two hours. The control tube contained no test sample. In comparison to the gallic acid standard, a mixture with a blue color was measured at 765 nm (Zaidul and Tatsuro, 2008).

2.3.6. Flavonoid Assay

Flavonoid content was determined using 100 aliquots of seed extract and 100 aliquots of methanol extract. The samples were then placed into test tubes in triplicate, and the volume was increased to 175, using $NaNO_3$ solution. 150 of the prepared $AlCl_3$ (5%) solution was added to each aliquot, and the reaction was initiated by adding 0.5 ml of 1M. The absorbance at 510 nm was measured against a blank after aliquots were filled with one drop of distilled water and left to stand for five to ten minutes(B. Dave Oomah, Giuseppe Mazza, 1996). The amount flavonoids was calculated as equivalent from the calibration curve rutin standard solutions.

3. Result & Discussion

The antioxidant potential and phytochemical analysis of buckwheat (*Fagopyrum esculentum*) varieties collected from various provenances of Uttarakhand (India) were assessed. Extracts of methanol and water were made to test for antioxidant and phytochemical properties. The altitudinal variation with respect to the anti-oxidant activity and other nutritional parameter have been discussed (Oomah *et al*., 2005; Jing *et al*., 2016; Musilová *et al*., 2013). Using a fine sieve, the ground seeds were separated into the hull and powdered samples. It has been done to estimate phytochemicals both quantitatively and qualitatively. These extracts underwent additional testing to determine their antioxidant potential using the β-carotene bleaching method and DPPH radical scavenging activity. Results of Qualitative estimation have been summarized in Table 2.

Protein, polyphenols, flavonoids, and other phytochemicals quantitative estimation with the exception of phenolics, which were more prevalent in the hull samples, the powder sample showed the highest activity out of the six samples. The findings indicated that there was a high concentration of flavonoids, alkaloids, and tannins in the samples MN and KK. The variety is beneficial because of the significant amount of flavonoids in the two samples; flavonoids are linked to antioxidant, antipyretic, and analgesic activity (Chopra *et al*., 1986;

Kim *et al*., 2008). Other phytoconstitutes, such as tannins and alkaloids, suggest using plant seeds as a source of antihypertensive properties.

3.1 QUALITATIVE ANALYSIS:

Qualitative analysis was carried out with Methanolic and aqueous extract of powder and hull of seed sample of different locations they showed the presence of phytochemical constitute except saponins, in both the extracts.

As shown in Table 1 and 2, tannins flavonoids, phenols, alkaloids and protein are present intensely in methanolic extracts of all the seed sample in contrast to the aqueous extract. The phytochemical contents of MN and KK (powder & hull) were revealed by the indicator color's intensity in the methanolic extract. These two samples were thus chosen for additional examination. The results of the carbohydrate test indicated that all of the sample's methanolic and aqueous extracts contained simple sugars and no reducing sugar.

Table 1 Quantitative Phytochemical analysis of different Methanol sample extracts of *Fagopyrum esculentum* MKD- 1; DC- 2; MN- 3; AL- 4; KK- 5; MIX- 6

Phytochemical Analysis	Name of the test	Water Extract of plant sample											
		Powder Sample						Hull sample					
		1	2	3	4	5	6	1	2	3	4	5	6
carbohydrate	Anthron Test	++	++	++	++	++	++	++	++	++	++	++	++
	Fehling's Test	-	-	-	-	-	-	-	-	-	-	-	-
	Benedict Test	-	-	-	-	-	-	-	-	-	-	-	-
Phenolic/ Flavanoids	$FeCl_3$ (HCL)	-	-	-	-	-	-	++	+	++	+	++	+
	Vanillin Test	++	+	+++	++	+++	++	+	+	+	+	+	+
Alkaloids	Wagener's Test	++	++	+++	+	+++	+	+	+	++	+	++	+
	Heger's Test	+	+	++	++	+++	+	++	+	++	+	+++	+
Tannin	Tannin Test	+	++	+++	++	+++	+	+	++	+++	++	+++	++
Saponin	Saponin Test	-	-	-	-	-	-	-	-	-	-	-	-
Sterol	Salkowaski Test	++	++	++	++	++	++	++	++	++	++	++	++
	Hensen Test	++	++	++	++	++	++	++	++	++	++	++	++
Protein	Ninhydrin Test	++	++	++	++	++	++	+	+	+	+	+	+

Table 2 Qualitative phytochemical analysis of different water sample extracts of *Fagopyrum esculentum* MKD- 1; DC- 2; MN- 3; AL- 4; KK- 5; MIX- 6

Phytochemical Analysis	Name of the test	Water Extract of plant sample											
		Powder Sample						Hull sample					
		1	2	3	4	5	6	1	2	3	4	5	6
carbohydrate	Anthron Test	++	++	++	++	++	++	++	++	++	++	++	++
	Fehling's Test	-	-	-	-	-	-	-	-	-	-	-	-
	Benedict Test	-	-	-	-	-	-	-	-	-	-	-	-
Phenolic/ Flavanoids	$FeCl_3$ (HCL)	+	++	+++	++	+++	+++	+	+	+++	++	++	++
	Vanillin Test	++	++	+++	++	+++	+	-	-	-	-	-	-
Alkaloids	Wagener's Test	++	+	++	+	++	+	+	+	++	+	+++	+
	Heger's Test	+	++	+++	++	+++	++	+	++	+++	++	+++	+
Tannin	Tannin Test	++	+	+++	++	+++	++	+	+	++	++	+++	+
Saponin	Saponin Test	-	-	-	-	-	-	-	-	-	-	-	-
Sterol	Salkowaski Test	+	+	+	+	+	+	+	+	+	+	+	+
	Hensen Test	+	+	+	+	+	+	+	+	+	+	+	+
Protein	Ninhydrin Test	+++	+++	+++	+++	+++	+++	+	+	++	++	++	++

3.2 QUANTITATIVE ANALYSIS:

The hull and powder methanolic extracts from seed samples were quantitatively estimated for the following parameters:

3.2.1 Protein Quantitative Estimation

Table 3 presents the results of the folin's lowry method for determining the protein content of the powder and hull of all the seed samples. MN and KK demonstrated extremely high protein contents of 234.907 mg/g of dry weight and 235.547 mg/g of dry weight, respectively. Further research was conducted on these two seed samples.

Table 3 Quantitative Estimation of Protein in Powder and Hull

S. No.	Name of the Sample	Protein Concentration (mg/g of dry weight)	
		Powder	Hull
1	MKD	233.466 ± 1.24	114.708 ± 0.98
2	DC	234.907 ± 1.27	115.989 ± 0.99
3	MN	234. 907 ± 1.22	66.213 ± 0.93
4	AL	233.466 ± 1.23	72.455 ± 0.95
5	KK	235.547 ± 1.23	74.855 ± 0.92
6	MIX	233.946 ± 1.25	58.210 ±0.93

3.3 QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF METHANOLIC EXTRACT:

The total polyphenolic content was calculated using the standard gallic acid. The highest polyphenolic content (23 mg/g of dry sample) was found in the methanolic extract of the MN powder. Due to its high polyphenol content, buckwheat may be used as a stimulating agent and to cleanse the blood of toxins and other harmful substances (Kenner & Requena, 1996). Using rutin as the standard, the methanolic extract of MN and KK had a higher total flavonoid content of about 4.30 mg/g.

DPPH and the β-carotene bleaching method were used to measure the antiradical activity: The amount of polyphenolic content was directly correlated with the DPPH radical scavenging activity. This pattern indicates that the antiradical activity of the methenolic extract of the hull of KK is due to polyphenolic constituents, as evidenced by the lower activity (EC50 of 13.91 mg) and higher activity (25.85 mg) in MN.

The highest percent inhibition of β-carotene bleaching was obtained from the methanolic extract of MN cotyledon powder (21.26%). The hull extract had the lowest percent inhibition (4.10%), but in KK, the percentage inhibition of both the hull and powder methanolic extracts was nearly identical, as seen in the figure.

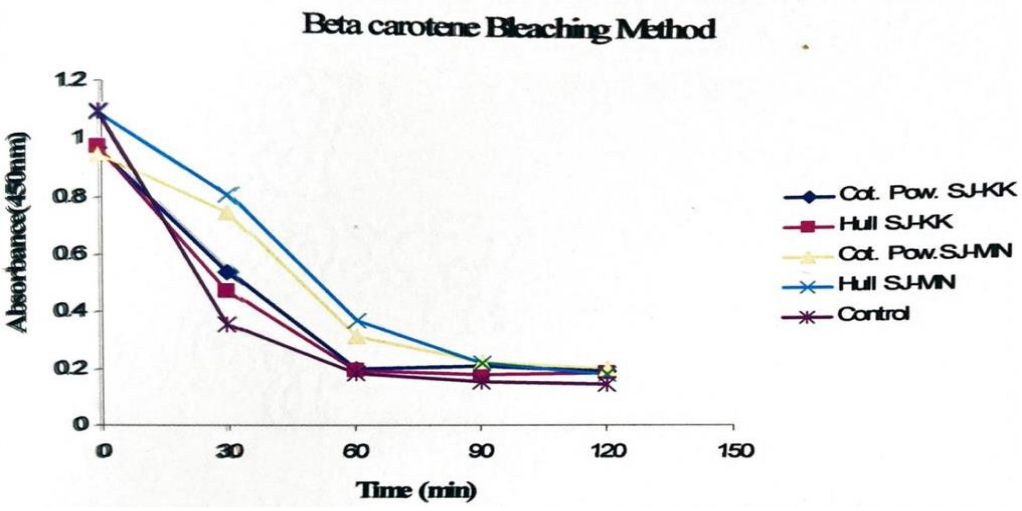


Figure 1 β-carotene Bleaching Method

Table 4 Quantitative Phytochemical Analysis of Methanolic extract of Fagopyrum esculentum

S. No.	Sample Number	Total Polyphenolic content (GAE mg/g of dry weight)	
		Hull	Cotyledon Powder
1	MN	17.05 mg	25.83 mg
2	KK	13.91 mg	24.15 mg
		Total Flavonoid Content (GAE mg/g of dry weight)	
		Hull	Cotyledon Powder
1	MN	1.61	2.67
2	KK	0.65	4.30
		Total Polyphenolic content (GAE mg/g of dry weight)	
		Hull	Cotyledon Powder
1	MN	14.6	23.8
2	KK	7.10	12.5
		% Inhibition of Beta carotene Bleaching	
		Hull	Cotyledon Powder
1	MN	4.10	21.26
2	KK	16.84	18.31

The research concludes that *Fagopyrum esculentum* (buckwheat) has significant antioxidant and nutraceutical potential, especially in its methanolic extracts (MN and KK). As a result, it is a promising candidate for use as a dietary supplement and in the prevention of various disorders. High levels of antioxidant activity and important bioactive compounds highlight its potential as a source of components that could improve health.

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