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GC-MS and ICP-OES Analysis of Secondary Metabolites from Selected Wheat Varieties in India

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Abstract

Triticum aestivum L. serves as a major global commodity with annual trade volumes reaching around 150 million tons. Wheat varieties including Sharbati, Black Wheat, Chawal Keti, and Sonamoti are extensively cultivated across many regions in India. Besides its nutritional value, wheat has medicinal importance especially for diabetic, cancer, and cardiac patients. These medicinal values are because of the presence of phytochemicals, which are natural compounds that offer health benefits, including antioxidant properties. This study focuses on the qualitative and quantitative assessment of phytochemicals from selected wheat varieties using standard protocols and analyzed with their ICP-OES and GC-MS reports. Qualitative and quantitative analysis emphasized significant levels of proteins, carbohydrates, flavonoids, ascorbic acid, and fattyacids. Among all Wheat varieties tested, Black Wheat and Sharbati aqueous extract were found to be rich in secondary metabolites. The ICPOES analysis revealed that Black wheat possesses Iron and Magnesium in rich amounts where as GCMS analysis showed the presence of 1,2 -dimethoxy propane, 2,2 - dimethoxy propane, Ethanol, Carbonochloridic acid, ethyl ester and Carbondioxide in higher concentrations. Further exploration of these could enhance our understanding of their potential health benefits of wheat.

Keywords: *Triticum aestivum*, Phytochemicals, Nutritional and Medicinal Properties.

Introduction

In 2013, the Food and Agriculture Organization (FAO) projected that global cereal production would approach 2.5 billion tons, with wheat contributing approximately 700 million tons of this total (FAO, 2014). Key grains include wheat, rice, corn, oats, rye, barley, sorghum, triticale, millet, amaranth, and teff. While rice dominates grain consumption in Asia, wheat is the primary grain in Europe and North America. Consuming grain-based foods is linked to a reduced risk of obesity (Koh-Banerjee *et al.*, 2004). Whole grains and wheat bran are recognized for their high fiber content, which is beneficial in reducing the risk of colon cancer (Kritchevsky, 2001). Wheat bran aids in digestion and may help in the prevention of colorectal cancer (Ferguson & Harris, 1999).

Grains and their processed forms are essential sources of energy globally. They supply a large proportion of carbohydrates, some proteins, oils, dietary fiber, and various micronutrients in many diets. Recent advocacy from universities, government bodies, non-profit organizations, industry, and trade groups supports the increased consumption of whole grain foods due to their health benefits (Chanson-Rolle *et* *al*., 2014). The U.S. Food and Drug Administration (FDA) defines whole grain foods as those where the main parts of the grain, the endosperm, germ and bran, are retained in the same proportions as found in the whole grain. The germ includes the plant embryo, the endosperm contains starch and storage proteins, and the bran, the outer layer, protects the grain from environmental factors like weather, insects, and microorganisms(FDA, 2006).

Wheat production exceeds that of rice, maize, and potatoes, with the majority of production concentrated in a few countries. For the 2023/2024 period, global wheat production is projected to be around 789.5 million tons, with the leading producers being the United States, Australia, Canada, France, and Russia. In India's wheat productivity is currently lower compared to neighboring countries like Bangladesh and Myanmar (Geeks for Geeks, 2023).

Research indicates that consuming whole grains can significantly lower the risk of chronic conditions such as type 2 diabetes, cardiovascular diseases, and colorectal cancer (Koh-Banerjee *et al*., 2008; Landberg *et al*., 2014). Initially, these benefits were thought to stem mainly from fiber content, but more recent studies suggest that the benefits are due to a combination of fiber, vitamins, phenolics, carotenoids, alkylresorcinols and other phytochemicals (Piironen *et a*l., 2009).

Global production for wheat was around 699.4 million metric tons for 2011-2012 (FAO, 2012). The International Grains Council estimates that global production will reach 770 million tons, down from 781 million tons in the previous year, with 195 million tons available for trade. Spelt is increasingly viewed as a "healthier, more natural" grain compared to modern varieties. In diverse environmental conditions and food habits of people in India supports the cultivation of three types of wheat (bread, durum and dicoccum). Wheat crop in India is grown under six diverse agro-dimatic zones, where in Indo-gangetic plains(IGPs), comprising the two zones namely; North Western Plains Zones(NWPZ) and the North eastern Plains zone (NEPZ) form the major wheat tract followed by the Central zone (CZ) and the Peninsular zone(PZ) (Randhir singh poswal, 2012).

The wheat plant has long slender leaves and stems that are hollow in most varieties. The plant is made up of a root and shoot system. Two types of roots are found, the seminal roots and the nodal roots (adventitious or crown roots), which arise from the lower nodes of the shoot. The shoot is made up of a series of repeating units or phytomers, each potentially having a node, a leaf, an elongated internode and a bud in the axil of the leaf(Kirby, Ear 1974). The inflorescences are composed of varying numbers of minute flowers, ranging from 20 to 100. The flowers are borne in groups of two to six in structures known as spikelets, which later serve to house the subsequent two or three grains produced by the flowers. Though grown under a wide range of climates and soils, wheat is best adapted to temperate regions with rainfall between 30 and 90 cm (12 and 36 inches). Winter and spring wheat are the two major types of the crop, with the severity of the winter determining whether a winter or spring type is cultivated. Winter wheat is always sown in the fall; spring wheat is generally sown in the spring but can be sown in the fall where winters are mild(encyclopedia britannica,2024).

Wheat contributes significantly to the daily caloric intake, providing around 9% of total calories). It contains beneficial phytochemicals and natural compounds with antioxidant properties that help protect the body from disease by mitigating cellular damage. Due to concerns about synthetic antioxidants, there is growing interest in natural antioxidants (Ankanna & Savithramma, 2011). Gas chromatography-mass spectroscopy(GC-MS)is a combined analytical technique used to determine and identify compounds present in a plant sample(Uma and Balasubramaniam,2012). It plays an essential role in the phytochemical

analysis and chemotaxonomic studies of medicinal plants containing biologically active compounds(Hethelyi *et al.*, 1987).
Wheat is a substantial source of carbohydrates and also provides protein, fat, ash, fiber, and various vitamins (Kumar *et al*., 2011). Essential minerals found in wheat include macro minerals (e.g., calcium, magnesium, potassium) and micro minerals or trace elements (e.g., copper, zinc, iron, boron, selenium), which are crucial for human health (Martinez-Ballesta *et al*., 2009). Nutritional quality of wheat is much important as itis basic staple food for the masses. Nutritional attributes play a vital role toward thehealth status of the consuming population, which ultimately affect the economy ofthe nation(Muhammadjavid, *et al* 2022). Inductively Coupled Plasma Optical Emission Spectroscopy(ICP-OES) is an analytical technique used to determine nutritional elements are in a sample(Bulka et all., 2016).
Despite this, there is a lack of scientific data on the nutritional and medicinal properties of popular wheat varieties in India, Therefore, this study aims to evaluate these wheat varieties for various phytochemicals, both qualitatively and quantitatively.

MATERIALS AND METHODS

Extraction of Phytochemicals from Wheat

Raw Material Collection: This study utilized four wheat varieties Sharbati, Black Wheat, Chawal Keti, and Sonamoti—sourced from various regions across India. The samples were cleaned to remove any impurities, including sand particles. After cleaning, the wheat was ground into a fine powder through a 0.5 mm mesh. This powder was then used for subsequent phytochemical analysis following established protocols and nutritional properties were estimated by ICP-OES and GC-MS method Fig 1.
Extraction Procedure: To investigate the bioactive components and their potential health benefits, multiple solvents and extraction methods were used (Herrero *et al*., 2006; Luthria, 2012). Extracts were prepared using both polar solvents (water and methanol) and non-polar solvents (benzene and hexane) (Peeriga & Banoth, 2016; De Silva *et al*., 2017).
Phytochemical Screening: Various tests were performed on the extracts to detect the presence of different metabolites, including carbohydrates, lipids, proteins, reducing sugars, ascorbic acid, alkaloids, phenols, flavonoids, saponins, tanins, coumarins and terpenoids, following the methods outlined by Harborne (1973), Savithramma et al. (2011), Shaik and Patil (2020), and Sharma et al. (2020).
Statistical Analysis: All the experiments were performed three times and the data were reported as the mean ± SD of three measurements. Statistical analysis was performed using KyPlot version 2.0 beta 15 (32 bit).

Results and Discussion

The study revealed that the extracts from all four wheat varieties contained a significant number of phytochemicals. Among the solvents used, aqueous extracts generally exhibited the highest number of identified compounds, followed by methanol, Benzene and hexane extracts contained fewer compounds Table-1.
Aqueous extracts of all wheat varieties contained primary metabolites like carbohydrates, proteins, ascorbic acid, reducing sugars, and fats, as well as secondary metabolites such as alkaloids, flavonoids, glycosides, terpenoids and steroids (notably in Black Wheat), phenols (also in Black Wheat), tannins, and coumarins.

Methanolic extracts showed a higher number of primary and secondary metabolites proteins, aminoacids, carbohydrates, ascorbic acid, alkaloids, flavonoids, glycosides and tannins were in all wheat varieties.

Benzene extracts contained a moderate number of primary secondary metabolites proteins present in Black wheat, Sonamoti and Chawal keti and alkaloids, flavonoids, glycosides, saponins, terpenoids and steroids in Black wheat respectively.

Hexane extracts showed the minimum number of compounds present compared to the other extrats, secondary metabolites alkaloids and tannins in all varieties. Among all tested solvents, aqueous and methanolic extracts possess a greater variety of phytochemicals compared to benzene and hexane extracts. These results align with previous studies on wheat and wheat grass extracts in methanol solvents(Suriyavathana *et a*l., 2016).

Among all tested varieties Black wheat and Sharbati having more number of secondary metabolites followed by Sonamoti and Chawal keti. Primary metabolites like fats, proteins, carbohydrates were found to bo present in more number of solvents. Secondary metabolites alkaloids, glycosides, saponins and flavonoids were found to be present in more number of solvents where as hexane extracts showed tannins from Black wheat, Sonamoti and Chawal keti where as Black wheat and Chawal keti varieties having flavonoids. Flavonoids are recognized for their antimicrobial, antiulcer, antiarthritic, and anticancer effects, while tannins offer analgesic and anti-inflammatory benefits and assist in wound healing and treating inflamed mucous membranes (Zhou *et al*., 2024). Hence Black wheat proved high contents of proteins, ascorbic acid and lipids than the other. The protein and vitamins present in wheat are essential for muscle tissue development and maintenance(Muhammad Javid Iqbal *et al*, 2022) Table 2, Graph 1.

Black wheat found to be rich in Iron and Magnesium than other varities. Sonamoti having sodium, phosphurus, sulphur, manganese and zinc. Sharbati having high calcium and copper were recorded in than other varieties. The GC-MS analysis revealed that compounds were identified in aqueous extract of Wheat. Among these 81.36% area 1, 2 - dimethoxy propane, 2,2- dimethoxy propane, Ethanol, Carbonochloridic acid, ethyl ester and Carbondioxide showed higher compounds. ICP-OES analysis revealed that Black Wheat posseses rich in Iron and Magnesium Table 3, 4.

Conclusion

The study concluded that the Black Wheat and Sharbati varieties are rich in bioactive compounds that may enhance immune function and support various metabolic processes, potentially countering oxidative stress caused by free radicals. The results helps for the researches for further studies.

Table 1: Metabolomics present in different solvents of wheat grains

Type of plant	Sharbati wheat				Black wheat				Chawal keti				Sonamoti			
	A	M	B	H	A	M	B	H	A	M	B	H	A	M	B	H
Carbohydrate	+	+	−	−	+	+	+	+	+	+	−	−	+	+	−	−
Proteins	+	+	−	−	+	+	+	−	+	+	+	−	+	+	+	−
Reducing sugars	+	−	−	−	+	+	−	−	+	+	−	−	+	−	−	−
Fats	+	−	+	+	+	−	+	+	+	+	+	+	+	−	+	−
Aminoacids	−	+	−	−	−	+	−	−	−	+	−	−	−	+	−	−

Alkaloids	+	+	+	+	−	+	+	−	−	+	+	+	+	+	−	−
Flavonoids	−	+	+	−	+	+	+	+	+	+	−	−	+	−	−	−
Tannins	−	−	+	−	−	+	+	+	−	+	−	+	−	+	+	+
Glycosides	−	+	+	−	+	+	+	+	+	+	−	−	+	+	+	−
Saponins	+	−	+	−	+	−	+	−	+	+	−	+	−	−	+	+
coumarins	+	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−
terpenoids	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−
steroids	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−
Ascorbic acid	+	+	−	−	+	+	−	−	+	+	−	−	+	+	−	−
phenols	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−

A=Aqueous, M=Methanol, B= Benzene, H= Hexane

Table 2: Quantitative estimation of secondary metabolites from selected Wheat varieties(mg/g)

Compounds	Black wheat	Sharbati	Chawal keti	Sonamoti
Flavonoids	0.48±0.02	0.44±0.02	0.48±0.02	0.46±0.02
Proteins	1.86±0.25	1.53±0.20	1.55±0.20	1.57±0.20
Carbohydrates	10.22±0.52	12.88±0.62	9.33±0.45	7.33±0.35
Lipids	0.12±0.03	0.08±0.02	0.03±0.01	0.10±0.01
Ascorbic acid	6.63±0.50	3.25±0.31	0.43±0.04	6.5±0.12

Graph 1: Quantitative analysis of metabolites of different Wheat varieties

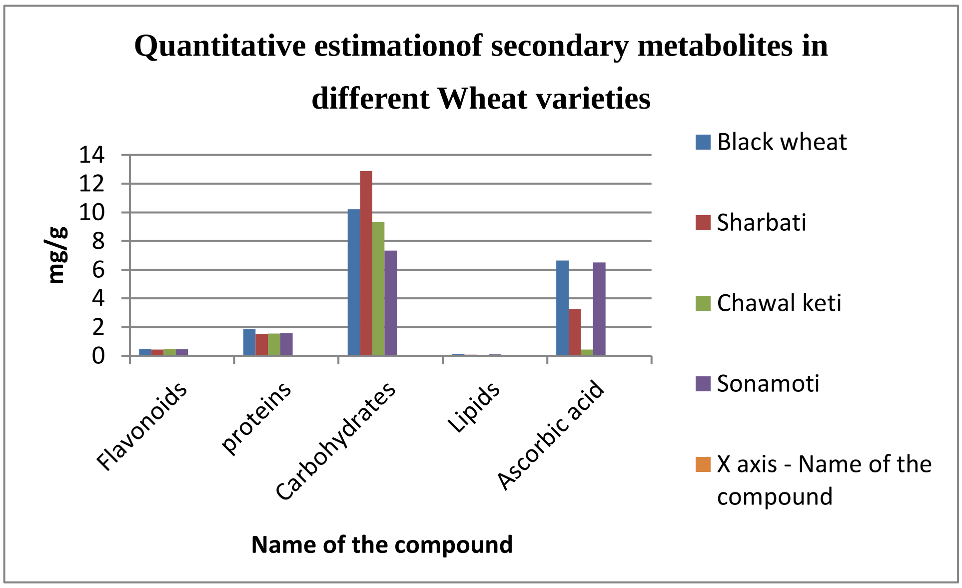

Fig: 1 Grains of different Wheat varieties

Table 3 GC-MS Identification of Bioactive compounds in Black Wheat

SN	Name of the compound	RT	Compound Nature	PA%	MF	MW g/mol	Biological Activity
1	1,2-dimethoxy propane	1.17	Volatile	81.36	$C_5 H_{12}O_2$	104	Hypnotic, Analgesic, Anticonvulsant
2	2,2- dimethoxy propane	1.60	Volatile	17.60	$C_5 H_{12}O_2$	104	Anesthetic, Sedative,Hypnotic
3	Ethanol	1.35	Volatile	0.15	C_2H_6O	46	Antimicribial, Analgesic, psychoactive Energizer
4	Carbanochloridic acid	1.09		0.24	$C_3H_5ClO_2$	108	Antiseptic, Disinfectant
5	Ethyl ester	1.09	Aromatic	0.24	H_5COOR	88.12	Analgesic, Antioxidant
6	Carbondioxide	1.05	Biological molecule	0.64	CO_2	44	Alkalizer, vasodilation, Neuroprotective, Cardiovascular

RT: Retention time MF: Molecular formula MW: Molecular weight

Table 4 ICP-OES Identification of Nutrient component in Black Wheat mg/kg

Plant name/Nutrient name	Sodium Na	Potassium K	Phosphurus P	Sulfur S	Calcium Ca	Magnesium Mg	Copper Cu	Mangenese Mn	Iron Fe	Zinc Zn
Black Wheat	1.27	1.20	0.13	0.03	0.16	0.04	17.5	41.4	275.8	22.9

References

1. FAO, 2014; world cereal Production in 2014 to surpass the record in 2013 Available form
i. http://www.fao.org/world food situation/csdb/en-(2014).
2. Koh-Banerjee Pauline, Franz Mary, Sampson Laura, Liu Simin, Jacobs David R Jr, Spiegelman Donna, Willett Walter, Rimm Eric(2004). Changes in Whole-grain bran and cereal fiber consumption

in relation to 8-y weight grain among men. *The American Journal of Clinical Nutrition 80*,(5):1237-1245.

3. Kritchevsky D(2001), Effect of malted Sorghum on quality Characteristics of Wheat-sorghum-Soyabean flour for potential use in confectioneries. *Food and Nutrition Sciences*, 7, 13
4. Ferguson L R, Harris P(1999). Protection against cancer by Wheat bran: role of dietary fibre and Phytochemicals DOI: 10.1097/00008469-199902000-00003 · Source: *PubMed*
5. Chanson-Rolle A, Lappi J, Meynier A, Pountance K, Vinoy S, Braesco V(2014). Health benefits of Whole grain: A systematic review of the evidence to propose a daily intake recommendation. *The FASEB Journal* 28, 11-17.
6. FDA, 2006; Food and drug adminsration Draft guidance: Whole grain label statements Available form http://www.fda.gov/OHRMS/DOCKETS/98FR/06d-0066-gd100001.pdf.
7. Geeks for Geeks, 2023, https:www.geeksforgeeks.org>tog-wheat producing states in India.
8. Rikard Landberg, Matti Marklund, Afaf Kamal-Eldin, Per Aman(2014) An update on alkylresorcinols-Occurrence, bioavailability, bioactivity and utility as biomarkers, *Journal of Functional foods*, 7,77-89.
9. Vieno Piironen, Lampi A.M, Ekholm P, Salmenkallio-Marttila M(2009), Micronutrients and Phytochemicals in Wheat Grain. *Research gate* DOI:10.1016/B978-1-891127-55-7.50014-8.
10. **Abu Saeid** M, Hoque S, Kumar U, Das M(2015),Comparative studies on nutritional quality of commercial wheat flour in Bangladesh, *Bangladesh Journal of Scientific and Industrial Research* 50(3):181 DOI:10.3329/bjsir.v50i3.25581.
11. FAO, 2012 *Food and Agricultural Organisation* (https://www.fao.org).
12. Randhir singh poswal(2012) Exploring the performance of wheat production in India, *Agriculture Science*,4(2):37-44
13. Kirby, E.J.M. 1974. Ear development in spring wheat. *J. Agric. Sci.*, 82: 437-447.
14. Encyclopediabritanica, 2024 www.britannica.com.,wheat growing season.
15. Ankanna S, Savithramma N (2011), Quantitative analysis of some secondary metabolites of *Shorea tunbuggaia ROXB*. An endemic, endangered & globally threatened medicinal tree species, *Journal of Pharmacy research* 4 (10) 3582-3584.
16. Uma G, Balasubramaniam V(2012) GC-MS analysis os *Nothapodytes nimmoniana*, Mabberly leaves. J Chem Pharma 4(9);4417-4419.
17. Hethelyi E, Tetenyi P, Dabi E, Danos B(1987) The role of mass specroscopy in medicinal plant research. Biomed Environ Mass Spectrom 14(11):672-632.
18. Martinez-Ballesta, Dominguez-Perles, D.A.Moreno D A, Muries B, Alcaraz-L opez, Bast´ ias3, Garc Viguera, Carvajal M, Minerals in plant food: effectof agricultural practices and role in human health. DOI:10.1051/agro/2009022.
19. Muhammad Javid Iqbal M, Naureen Shams, Kalsoom Fatima(2022), Nutritional quality of Wheat. *Nutrition and Dietetics*, DOI: 10 5772/intechopen. 104659.
20. Bulka E et al.(2016) Quantitative aspects of inductively coupled plasma mass spectrometry: Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences.
21. Peeriga & Banoth, 2016;Peeriga & Banoth, 2016 Pharmacognostical investigation and preliminary Phytochemical screening of leaves of Pumburus missioniss. *Journal of comprehensive Pharmacy* 3(3); 85-90.
22. De Silva, G. O., Abeysundara, A. T., & Aponso, M. M. W. (2017). Extraction methods, qualitative

and quantitative techniques for screening of phytochemicals from plants. *American Journal of Essential Oils and Natural Products, 5*(2), 29-32.

23. Harborne (1973)Harbone, J. B. (1973). *Phytochemical methods: A Guide to Modern techniques of Plant analysis.* London, UK: Chapman and Hill. https:// doi.org/10.1007/978-94-009-5570-7.
24. Shaikh, J. R., & Patil, M. K. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies, 8*(2), 603-608. https://doi.org/10.22271/ chemi.2020.v8.i2i.8834
25. Sharma, T., Pandey, B., Shrestha, B. K., Koju, G. M., Thusa, R., & Karki, N. (2020). Phytochemical Screening of Medicinal Plants and Study of the Effect of Phytoconstituents in Seed Germination. *Tribhuvan University Journal, 35*(2), 1-11. https://doi.org/10.3126/tuj.v35i2.36183
26. Suriyavathana M, Roopavathi I, Vinu vijayan(2016), Phytochemical Characterization of Triticum aestivum(wheat grass), *Journal of pharmacognosy and phytochemistry,* 5(1): 283-286.
27. Zhou Y., Zheng J, Li Ya, Dong-Ping Xu, Sha Li, Yu-Ming Chen, Hua-Bin Li, (2016). Natural Polyphenols for Prevention and Treatment of Cancer. *Nutrients, 22;8*(8), 515. https://doi.org/10.3390/nu8080515