

Phytochemical Profiling and Antioxidant Potential of Dried and Germinated *Sorghum bicolor* Seeds

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ABSTRACT

The present study is to evaluate compositions of phytochemicals, antioxidant potential, GC-MS profile of dried seeds and germinated sprouts of *Sorghum bicolor*, commonly known as ragi. Coarse powder from dried seeds and germinated seeds (24, 48, and 72 hours) extracted using 70% of ethanol. These extracts subjected to qualitative phytochemical screening and seven antioxidant assays. Analysis of phytochemicals revealed the presence for bioactive molecules, that includes steroids, glycosides, flavonoids, tannins, saponins, volatile oils, alkaloids, across all germination stages and dried seeds. Quantitative estimation indicated higher concentrations of total flavonoids and tannins in dried seeds compared to germinated samples. Antioxidant evaluation through seven assays demonstrated that the dried seed extract exhibited the highest radical-scavenging potential. It showed the most effective DPPH quenching activity (IC_{50} : 246.65 μ g/mL), followed by nitric oxide scavenging (IC_{50} : 260.9 μ g/mL), lipid peroxidation inhibition (IC_{50} : 261.5 μ g/mL), ABTS scavenging of (IC_{50} : 272.2 μ g/mL), reducing power (IC_{50} : 273.9 μ g/mL), hydroxyl radical scavenging (IC_{50} : 275.5 μ g/mL), and metal chelation (IC_{50} : 484.7 μ g/mL). Among all samples, the dried seed extract consistently exhibited superior antioxidant activity compared to germinated seed extracts. These findings suggest that *Sorghum bicolor* dried seeds possess significant phytochemical and antioxidant potential, making them a promising source for nutraceutical and therapeutic applications.

Keywords: Phytochemicals, *Sorghum bicolor*, glycosides, flavonoids, tannins, saponins, volatile oils, and alkaloids

Introduction

Sorghum bicolor, commonly known as ragi/finger millet, is nutritious cereal widely cultivated in semi-arid regions of Asia and Africa. Known for its resilience to harsh environmental conditions, ragi gained attention not only for rich nutritional profile but for its potential healthy benefits and an excellent source of proteins, dietary fiber, vitamins, and essential minerals such as calcium, iron, and phosphorus [1-2], *Sorghum bicolor* is recognized for its abundance of bioactive molecules that comprises polyphenols, flavonoids, tannins, and antioxidants, which contribute to its therapeutic properties. Germination is a well-known process that enhances the bioavailability of nutrients and bioactive compounds in cereal grains [3]. During germination, biochemical changes lead to the breakdown of complex macromolecules, resulting in increased concentrations of free amino acids, vitamins, and antioxidants. This process also stimulates the synthesis of secondary metabolites are flavonoids and phenolic compounds, that plays significant role in combating oxidative stress [4]. Thus, evaluating the phytochemical composition and

antioxidant potential of ragi at different germination stages can provide valuable insights into its nutritional and functional properties.

The present work is to analyze the phytochemical profile and its antioxidant potential of dried seeds and germinated seeds of *Sorghum bicolor* at 24, 48, and 72 hours. The seeds were extracted using 70% ethanol and subjected to qualitative phytochemical screening, quantitative estimation of flavonoids and tannins, and seven different antioxidant assays [5], the study employed GC-MS analysis to identify bioactive molecules present in extracts. The findings of this study are expected to highlight the impact of germination on the phytochemical composition and antioxidant efficacy of ragi, providing a scientific basis for its use as a functional food and nutraceutical ingredient.

Plant-derived antioxidants are gaining attention for their role in combating oxidative stress, which contributes to diseases like cancer, cardiovascular disorders, and neurodegeneration. Compounds such as polyphenols and flavonoids help neutralize free radicals, reducing cellular damage and promoting health.

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Cereals like *Sorghum bicolor*, rich in polyphenols and other phytochemicals, have emerged as promising candidates for dietary antioxidants. The germination process further enhances the antioxidant potential by activating metabolic pathways that increase the synthesis of bioactive compounds [6]. Therefore, understanding how germination affects the phytochemical composition and antioxidant properties of ragi is crucial for promoting its consumption as a functional food. The utilization of ragi in health foods and nutraceutical formulations requires a comprehensive understanding of its phytochemical profile and antioxidant potential across different stages of germination [7]. While previous studies have highlighted the nutritional benefits of finger millet, limited research has focused on the comparative evaluation of its bioactive compounds and antioxidant activity during germination. This study aims to provide detailed insights of phytochemical variations, antioxidant potential, and bioactive compound profiles of dried and germinated ragi seeds. These findings contribute to the growing body of evidence that supporting the inclusion of the ragi in health-promoting diets and its potential applications in functional food development.

Materials and Methods

Collection of Seeds

Dry seeds of *Sorghum bicolor* (commonly known as jowar) varieties Jowar CSV-36, Jowar CSV-31, Jowar CSV-39, Jowar CSV-27, and Jowar CSV-15 were procured from the Agriculture Research Institute, Professor Jayashankar Telangana State Agricultural University, Hyderabad, TS-India.

Sample Preparation

The collected jowar seeds were manually cleaned to remove dust and impurities. A total of 100 g of dried seeds were finely ground using a mechanical grinder and stored in an airtight container for phytochemical and antioxidant analysis [8]. For the germination study, 1.5 kg of dried seeds were divided into three equal 500 g batches and soaked in distilled water (1:3 w/v) for 24 hours. The soaked seeds were spread on a moist cotton cloth and kept hydrated with frequent water spraying. Germination was conducted for 24, 48, and 72 hours for the respective batches. After each period, seeds were dried in a hot air oven at 45°C until moisture was fully removed, then ground into a fine powder and stored for further analysis.

Extraction of Plant Material

The extraction of the jowar seed samples (*Sorghum bicolor* varieties JCSV-36, JCSV-31, JCSV-39, JCSV-27, and JCSV-15) was carried out following the method described by [9], with slight modifications. For each sample, 200 g of finely ground seed powder was weighed and subjected to extraction using 70% ethanol as a solvent. The extraction process was performed using a Soxhlet apparatus (Buchi, Flawil, Switzerland) for 6–8 hours to ensure the complete extraction of the bioactive compounds. After extraction, the solvent evaporated under pressure with the help of rotary evaporator at 40°C and to obtain the crude extracts. The crude extract yield was recorded for each sample, and the residues were air-dried and stored in desiccators for 24–48 hours to remove residual moisture. They were then sealed in airtight containers and stored at -20°C until further analysis. The extracts were labeled as follows:

1. **R1:** Ethanolic extract of dried seeds
2. **R2:** Ethanolic extract of 24-hour germinated seeds
3. **R3:** Ethanolic extract of 48-hour germinated seeds
4. **R4:** Ethanolic extract of 72-hour germinated seeds

Qualitative Phytochemical Analysis

The ethanolic extracts (R1, R2, R3, and R4) were qualitatively analyzed for bioactive compounds, including alkaloids, flavonoids, tannins, saponins, steroids, glycosides, and volatile oils. The analysis was conducted according to the standard procedures described by [10].

For each test, 1 mL of the respective extract was dissolved in 10 mL of distilled water or appropriate solvents and subjected to the following phytochemical tests:

1. **Alkaloids:** Dragendorff's and Wagner's tests
2. **Flavonoids:** Shinoda test
3. **Tannins:** Ferric chloride test
4. **Saponins:** Foam test
5. **Steroids:** Salkowski test
6. **Glycosides:** Keller-Kiliani test
7. **Volatile oils:** Spot test

The results were noted based on the intensity of the color change or precipitate formation, indicating the presence or absence of specific phytochemicals across different germination stages and the dry seed extracts.

Quantitative Phytochemical Analysis

Total Phenolic Content

The total phenolic content (TPC) of the ethanolic extracts (R1, R2, R3, and R4) was assessed using a modified Folin-Ciocalteu method. A 0.5 mL aliquot of each extract (1 mg/mL) was combined with 2.5 mL of diluted Folin-Ciocalteu reagent and allowed to react for 5 minutes. Following this, 2 mL of a 7.5% sodium carbonate solution was added, and the mixture was incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer. TPC was quantified based on a gallic acid calibration curve and expressed as mg gallic acid equivalents (GAE) per gram of extract.

Total Flavonoid Content

The total flavonoid content (TFC) of the ethanolic extracts (R1, R2, R3, and R4) was determined using the aluminum chloride colorimetric method, as outlined by Basniwal et al. (2009) with slight modifications. A 0.5 mL aliquot of each extract (1 mg/mL) was combined with 2 mL of distilled water and 0.15 mL of 5% sodium nitrite solution. After 5 minutes, 0.15 mL of 10% aluminum chloride was added, followed by 1 mL of 1 M sodium hydroxide after 6 minutes. The total volume was adjusted to 5 mL with distilled water, and absorbance was measured at 510 nm using a UV-Vis spectrophotometer. The flavonoid content was quantified using a rutin standard curve and expressed as mg rutin equivalents (RE) per gram of extract.

Total Tannin Content

The total tannin content (TTC) of ethanolic extracts was assessed following the method of [11] with slight modifications. A 0.5 mL aliquot of each extract was combined with 3 mL of 4% vanillin in methanol and 1.5 mL of concentrated hydrochloric acid. The reaction mixture was incubated at room temperature for 15 minutes, and absorbance was recorded at 500 nm using a UV-Vis spectrophotometer. The tannin content was determined using a tannic acid standard curve and expressed as micrograms of tannic acid equivalents per milligram of dry weight ($\mu\text{g TAE/mg}$).

Results and Discussion

Qualitative Phytochemical Analysis

The ethanolic extracts of dried sorghum seeds (R1) and germinated seeds at different stages (R2, R3, and R4) revealed presence of the significant bioactive compounds present. The phytoconstituents identified are alkaloids, flavonoids, tannins, glycosides, steroids, saponins, phenols, and volatile oils. The presence or absence of these compounds across the different germination stages is summarized in Table 1.

Alkaloids were detected in all extracts, with a higher intensity observed in the dried seeds (R1) compared to germinated seeds. This suggests that alkaloid concentration may decline during germination, possibly due to metabolic conversion into simpler compounds. Flavonoids and tannins, known for their antioxidant properties, were present across all samples, though their concentration was comparatively higher in dried seeds. The reduction in flavonoid and tannin content during germination could be attributed to their utilization as protective agents during seedling growth.

Saponins and steroids were consistently present across all extracts, indicating that germination did not significantly affect their synthesis. Glycosides, however, were more prominent in 24-hour germinated seeds (R2), suggesting an increase in metabolic activity during the early stages of germination. Volatile oils, known for their antimicrobial properties, were detected in all samples, though with varying intensities [11]. The presence of phenolic compounds across all extracts highlights the potential antioxidant capacity of sorghum seeds, irrespective of germination, the higher concentration in dried seeds suggests that phenolics may be partially degraded or utilized during germination. The qualitative analysis thus demonstrates that while germination alters the composition of some phytochemicals, the bioactive potential of sorghum remains significant across different stages. The quantitative analysis will provide deeper insights into these variations and their potential health benefits.

Table 1: Sorghum bicolor (Jowar) Seedling Phytochemical Analysis

S. No.	Constituents	CSV-15	CSV-27	CSV-31	CSV-36	CSV-39
1	Steroids	+	+	+	+	+
2	Glycosides	+	+	+	+	+
3	Volatile Oils	+	+	+	+	+
4	Saponins	+	+	+	+	+
5	Tannins	+	+	+	+	+
6	Flavonoids	+	+	+	+	+
7	Alkaloids	--	--	--	--	--

Note: "+" indicates the presence of the constituent, while "--" indicates its absence.

GC-MS Analysis

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis was employed to identify the bioactive compounds present in the ethanolic extracts of different *Sorghum bicolor* (jowar) genotypes. The analysis provided detailed spectra for each sample, highlighting retention times, peak areas, and compound identities based on NIST library comparisons. The major compounds detected, along with their molecular weight, molecular formula, and nature, are presented in the respective Fig 28 (Marked Fig 28 from my PhD thesis). Make it Fig 1

Jowar (J CSV-36 Genotype)

The GC-MS spectrum of the chloroform extract from the J CSV-36 genotype revealed the presence of multiple bioactive compounds. The important compounds identified included Dextroamphetamine, Tetraborane, Carbonochloridic acid, 2-Heptanamine, Oxirane, Tartonic acid, Uronic acid, Benzene acetic acid, Benzoic acid, Sarpagan-17-ol, Aldicarb, Acetic acid, Argon, and Mesitol. These compounds exhibited varying retention times and peak areas, suggesting differences in their concentration. Notably, the presence of alkaloids, phenolic acids, and terpenoids highlights the potential therapeutic applications of this genotype.

Jowar (J CSV-31 Genotype)

GC-MS analysis of the J CSV-31 genotype extract demonstrated the presence of a diverse range of secondary metabolites, including carbonic acids, amino acids, alkaloids, sesquiterpenes, phenylpropenes, and phenylamines. Different alkaloids were detected at varying retention times, corresponding to different peak areas. The major compounds identified were Linalool, Hexadecanoic acid, Octadecanoic acid, 2,4-Di-tert-butylphenol, and Phytol.

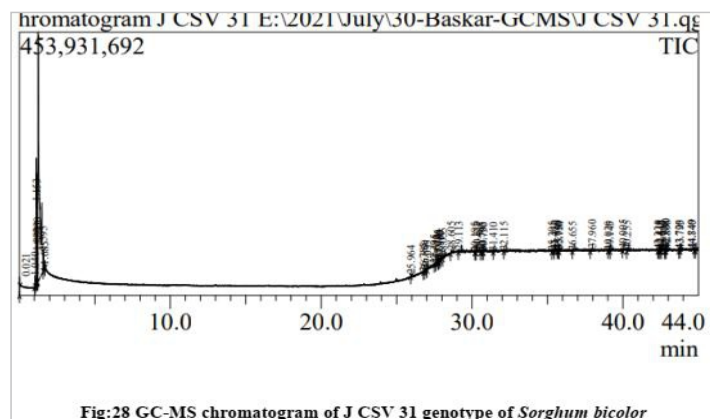


Table 2: GC-MS compounds identified in *Sorghum bicolor* J CSV-31 genotype

S. No	Name (J CSV-31)	Retention Time (min)	Area (%)	Molecular Weight (g/mol)	Molecular Formula	Compound Class
1	Dextromphetamine	0.021	0.78	135	Dextromphetanmine - C ₉ H ₁₃ N	Phenyl Amine
2	Trichloroethylene	1.50	19.27	131	Trichloroethylene - C ₂ HCl ₃	Hydrocarbon
3	Oxirane	1.52	12.97	60	Oxirane - C ₃ H ₆ O	Ether
4	Imidazole-5-carboxylic acid, 2-amino-	1.67	0.76	127	Imidazole-5-carboxylic acid, 2-amino - C ₄ H ₅ N ₃ O ₂	Carboxylic Acid
5	Tartaric acid, 4-(dimethylthioethylsilyl)perhydro-1, dimethyl ester	28.88	13.10	318	Tartronic acid, 4-(dimethylethylsilyl)perphenyl-dimethyl ester - C ₁₅ H ₂₂ O ₅ Si	Carboxylic Acid
6	6-Bromoquinoxaline	29.67	0.13	208	6-Bromoquinoxaline - C ₈ H ₅ BrN ₂	Quinoxalines
7	Morphinan, 6,7,8,14-tetrahydro-3-methoxy-17-methyl-	34.11	11.06	285	Morphinan, 6,7,8,14-tetrahydro-3-methoxy-17-methyl- - C ₁₈ H ₂₁ NO	Alkaloid
8	Spargan-17-ol	34.11	0.45	426	Sarpagan-17-ol, 3-methoxy - C ₂₄ H ₂₈ N ₂ O ₃	Alkaloid
9	Morphinan, 6,7,8,14-tetrahydro-3-methoxy-17-methyl-	33.54	10.36	267	Morphinan, 6,7,8,14-tetrahydro-17-methyl- - C ₁₈ H ₂₁ NO	Alkaloid
10	1,2-Cinnoline dicarboxylic acid	33.94	0.18	330	1,2-Cimino thioendicarboxylic acid - C ₁₇ H ₃ NO ₂ S ₅ Si (Correction: It should be C ₁₇ H ₁₃ NO ₂ S ₅ Si)	Carboxylic Acid
11	Cinnamic acid, 3,4-dimethoxy-	34.93	0.88	208	Cinnamic acid, 3,4-dimethoxy - C ₁₄ H ₁₂ O ₄ Si (Correction: It should be C ₁₀ H ₁₂ O ₄)	Unsaturated Carboxylic Acid
12	Ulene	38.09	9.93	272	Ulene - C ₁₆ H ₂₁ N ₂ (Correction: It should be C ₁₆ H ₂₂ N ₂)	Alkaloid
13	Thebenine	44.90	0.16	289	Thebenine - C ₁₈ H ₁₉ NO ₃	Alkaloid

Total Phenolic Content

The total phenolic content (TPC) in the ethanolic extracts of *Sorghum bicolor* seeds varied across different germination stages. The dried seeds exhibited the highest phenolic content, measuring 8.45 mg GAEq/g (Table 2). In contrast, the 24-hour germinated seed extract contained 6.13 mg GAEq/g, followed by 5.84 mg GAEq/g in the 48-hour germinated seeds. Interestingly, the TPC slightly increased to 5.90 mg GAEq/g after 72 hours of germination.

These results indicate that germination reduces phenolic content initially, but prolonged germination (72 hours) leads to a slight resurgence. Overall, the dried seeds demonstrated the highest concentration of phenolic compounds, highlighting their potential as a rich source of natural antioxidants. The trend observed was dried seeds > 24-hour germination > 48-hour germination < 72-hour germination, suggesting a dynamic change in phenolic metabolism during sprouting.

Total Flavonoid Content

TFC in the ethanolic extract of *Sorghum bicolor* seeds varied significantly across different germination stages. The dried seeds exhibited the highest flavonoid content, measuring 5.89 mg RE/g (Table 2). However, germination led to a progressive decline in TFC, with the 24-hour germinated seed extract showing 3.38 mg RE/g, followed by 2.19 mg RE/g after 48 hours and 2.01 mg RE/g after 72 hours of germination. These findings suggest that the flavonoid content in ragi decreases during malting and germination, likely due to enzymatic degradation or metabolic utilization of flavonoid compounds during the sprouting process. Thus, dried seeds appear to be the richest source of flavonoids, emphasizing their potential for antioxidant applications.

Total Tannin Content

TTC in the ethanolic extract of *Sorghum bicolor* seeds exhibited significant variations across different stages of germination.

The dried seeds showed the highest tannin concentration, measuring 5.12 mg TAE/g (Table 2). Upon germination, the tannin content initially decreased to 3.78 mg TAE/g after 24 hours and further declined to 2.19 mg TAE/g at 48 hours. However, an increase was observed after 72 hours of germination, with the tannin content rising to 3.07 mg TAE/g. This trend suggests that tannins in ragi are abundant in dried seeds and undergo degradation during the early germination stages. The subsequent increase at 72 hours may be attributed to metabolic changes and the release of bound tannins during prolonged germination.

Detection of Volatile Compounds from Ragi Varieties using GC-MS Analysis

The volatile compounds in five ragi (*Eleusine coracana*) varieties—GPV 67, ICMR-301, R-VL352, R-CF, and R-VR847—were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The analysis covered different plant parts, including the leaf, neck, and finger, and was conducted at the GC-MS Central Analysis Facility, University College of Technology, Osmania University, Hyderabad. A Thermo Scientific Trace GC Ultra DSQ II system with a 30 mm × 0.25 mm × 0.25 μm column was used. Helium served as the carrier gas at a flow rate of 1 mL/min, and 1 μL of each sample was injected in split-less mode. The column temperature was programmed from 50°C to 200°C at a rate of 10°C per minute, with a 2-minute hold. The system maintained a surge pressure of 3 kPa, while the source and MS transfer line temperatures were set at 200°C and 250°C, respectively. Electron impact ionization (70 eV) and mass scanning between 45 and 450 m/z enabled compound identification.

The GC-MS analysis identified various bioactive compounds across the five ragi varieties. These included alkaloids (hexane and ethyl acetate fractions), phenols, tannins, steroids, terpenoids, carbohydrates, cardiac glycosides, and balsams,

with ethanol extract showing a high presence of phenols and tannins [12]. These secondary metabolites contribute to the medicinal and nutritional value of plants. Alkaloids aid in plant defense and have pharmacological significance, often serving as the basis for various medications. Steroidal compounds, structurally similar to sex hormones, hold pharmaceutical importance, while terpenoids are widely studied for their antibacterial and therapeutic potential [14-15]. The ethanolic extract's high phenolic content is significant due to its potent antioxidant and free radical scavenging properties, as well as its antimicrobial effects [17]. Tannins, despite potentially reducing in vitro protein digestibility, contribute to plant defense mechanisms. Additionally, cardiac and anthraquinone glycosides are known for their antibacterial and antifungal activities, further enhancing the extract's potential therapeutic value [18-19].

The antioxidant activity

The antioxidant activity was found to be highest in ethanolic extract of dried ragi seeds, consistent with previous studies. [20] reported a significant decrease in phytic acid content upon germination, while [21] observed a 54% reduction in tannin content during malting. In the present investigation, antioxidant activity decreased during germination, but a slight increase was observed after 72 hours of germination. This suggests that while malting initially reduces antioxidant capacities, prolonged germination can lead to a resurgence of antioxidant potential. The GC-MS analysis confirmed that ragi seeds are rich in bioactive compounds with significant pharmacological and nutritional benefits. The study highlights the impact of germination on the phytochemical composition of ragi, emphasizing the need for further research to isolate and characterize the specific compounds responsible for changes in antioxidant capacities at different growth stages.

Conclusion

The study highlights the significant phytochemical composition and antioxidant properties of ragi (*Eleusine coracana*) seeds, with notable variations observed during different stages of germination. The dried ragi seeds exhibited the highest levels of total phenolic, flavonoid, and tannin content, which gradually decreased during germination, a slight increase in these compounds was noted after 72 hours of germination, suggesting a dynamic change in phytochemical composition throughout the malting process. The GC-MS analysis revealed the presence of several bioactive compounds, including alkaloids, phenols, tannins, steroids, terpenoids, carbohydrates, and cardiac glycosides, which contribute to the nutritional and medicinal value of ragi. The findings also confirm that while germination reduces certain antinutritional factors, it simultaneously enhances the digestibility and bioavailability of nutrients. The increase in antioxidant activity after prolonged germination suggests the potential for optimizing germination conditions to maximize the nutritional benefits of ragi.

Declaration of Competing Interest

The authors declare no known financial or personal conflicts of interest that could have influenced the work presented in this paper.

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