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Research Article

# Unlocking the Health Benefits of Browntop Millet: Antioxidant and Anticancer Activity of Phenolic Extracts

**Saddique Choudhury[1], Megha Mallapura Lakshmana[1], Sunil Sihag[1], Jayanthi Mallappa Kumar[2,*], Manu Gangadhar[2]**

[1]Department of Pharmacology, JSS Medical College, JSS Academy of Higher Education and Research, Shivarathreeshwara Nagar, Bannimantap, Mysuru, Karnataka, INDIA.
[2]Department of Pharmacology, Sri Siddhartha Medical College, Tumkur, Karnataka, INDIA.

## ABSTRACT

**Background:** Oxidative stress-an imbalance between Reactive Oxygen Species (ROS) production and the body's defense mechanisms-plays a significant role in aging and chronic diseases, including endocrine system disorders. Antioxidants help mitigate this stress by neutralizing free radicals. Among Indian millets, Browntop Millet (BTM) is particularly rich in phytochemicals, including phenolics and dietary fibers, primarily concentrated in the bran. This study aimed to analyze the distribution of phenolic compounds in BTM and evaluate the antioxidant and anticancer activities of its extracts. **Materials and Methods:** BTM seeds were processed, and free and bound phenolic extracts were prepared from seed, husk, and rice using a rotary evaporator. Phytochemical constituents and total phenolic content were quantified. Antioxidant potential was assessed using DPPH and FRAP assays. *In vitro* anticancer activity was evaluated using the SRB assay on MDA-MB-231 breast cancer cells. **Results:** BTM was found to be rich in phenolic compounds and exhibited strong antioxidant activity. Among the extracts, the bound phenolic-husk extract showed the highest antioxidant activity, with 63.80% DPPH radical scavenging and 361 FRAP units at 1 mg/mL. For anticancer activity, the bound phenolic-rice extract demonstrated the most potent cytotoxic effect, showing 5.046% inhibition of MDA-MB-231 cells at 10 µg/mL. **Conclusion:** The findings underscore BTM's potential as a natural source of antioxidants, as demonstrated by DPPH and FRAP assays. The SRB assay confirmed its antiproliferative activity, highlighting BTM's value in promoting health and potentially preventing disease.

**Keywords:** Antioxidant Activity, Antiproliferative Effect, Bioactive Phytochemicals , Browntop Millet.

**Correspondence:**
**Dr. Jayanthi Mallappa Kumar**
Professor, Department of Pharmacology, JSS Medical College, JSS Academy of Higher Education and Research, Shivarathreeshwara Nagar, Bannimantap, Mysuru-570015, Karnataka, INDIA.
Email: mkjayanthi@jssuni.edu.in

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## INTRODUCTION

Reactive Oxygen Species (ROS) and free radicals play significant roles in the aging process and the progression of various chronic diseases. Oxidative stress has been associated with diseases such as Alzheimer's disease, Parkinson's disease, SMA, cardiovascular diseases and some types of cancers.[1] Oxidative stress, a crucial factor in aging and the development of chronic diseases arises from a discrepancy between the formation of ROS, and the body's ability to remove these reactive substances or mend the ensuing impairment.[2] Furthermore, the previous research result have revealed that oxidative stress may enhance the aging of the endocrine system and relate to the several endocrine diseases.[3] Oxidative stress occurs when there are elevated levels of ROS and the ability of the body to eliminate these intermediates or repair the damaged structures fails. Reactive oxygen species, particularly free radicals, and peroxides, are highly toxic as they can damage DNA, potentially leading to long-term consequences like aging and the development of cancer.[4] UV radiation, ionizing radiations, pollutants, heavy metals and antiblastic drugs and other xenobiotics with ability to generate ROS increases ROS production further leading to oxidative stress and therefore cell and tissue damage.[5] They act as protective chemicals in removing free radicals from the cellular structures of the body with an intention of minimizing oxidative damage.[6] Antioxidants are most actively present and most concentrated in plant foods, especially in the categories of fruits and vegetables.[7] For instance, vitamins A, E, and C, flavonoids, carotenes, and zinc are present in nuts, whole grains, specific meats, poultry, and fish.[8]

Millets, especially, are gaining importance among the growing population due to its nutrient dense quality especially the poor as the antioxidant foods are beyond their reach.[9] In order to make

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the best use of millet seed, husk, and rice in health improvements, it is crucial to comprehend the preferential accumulation of the phenolic compounds.[10] The various sorts of millets cultivated throughout India include Jowar (sorghum), Bajra (Pearl millet), Ragi (Finger millet), Jhangora (barnyard millet), Barri (proso/common millet), Kangni (Italian millet/Foxtail millet), Kodra (Kodo millet), and Browntop millet (*Urochloa ramosa*).[11]

In this context, it is worth to understand that ROS generation is a normal part of oxygen metabolism and it has several physiological functions, one of which is cell signalling.[12] Oxidative damage is more injurious to cellular organelles such as the DNA, proteins, and the lipids, which triggers a series of negative impacts of human health.[13] The consumption of plant products is advocated against these chronic oxidative stress related diseases because of the vast choices and the concentration of antioxidants found in plants. The intake of plant-based antioxidants has many benefits to the general health of a human being and has been well recognized.[14]

Natural compounds are viewed as better substances for use in scientific research due to fewer side effects as compared to the synthetic chemicals. Of the millets, emphasis has been placed on them as the potential future crops due to nutritional value.[15] Research shows that millet grains contain other bioactive compounds apart from proteins and fats and are rich in phenolic compounds that exhibit antioxidant characteristics.[16]

Phenolics and dietary fibre are observed predominantly in the outer coat mainly the bran layers of Browntop millet.[17] Furthermore, there are other potential possibilities for the enhancement of BTM; for instance, including antioxidants (for example, peptides) by germination.[18] Based on these facts, we are concerned with the antioxidant and anticancer possibilities of these compounds in the present study with BTM-whole grain, husk, rice.

Though a lot of work has been done on the nutritional profile and nutritional and cancer-fighting properties of different millets, literature on Browntop Millet (BTM) is limited. Thus, as further information on the antioxidant and anticancer activity of BTM is rather scarce, it is essential to explore its possibilities and applicability. The knowledge of antioxidant properties of BTM may report the role of millet in health and disease prevention in a more effective manner.

## MATERIALS AND METHODS

### Materials

#### *Chemicals*

All solvents and chemicals used were of analytical grade. The DPPH, FRAP, and Folin-Ciocalteu's (FC) reagent were procured from SD Fine Chem Ltd., (Mumbai, India). DNS reagent was procured from SRL (Mumbai, India). Cisplatin injection (1.0 mg/mL) was purchases from Hetero Healthcare Limited (Hyderabad, India). Chemicals such as sodium hydroxide, sodium sulphate, sodium borohydride, sodium carbonate, sodium potassium tartrate, Ethidium Bromide (EtBr), Acridine Orange (AO), tris buffer from Merck Millipore (Darmstadt, Germany). Ethanol, Ethyl acetate extra pure AR, Ethyl ether extra pure, Glacial acetic acid, Hydrochloric acid, Sulfuric acid, Phosphoric acid will be procured from Thermo Fisher Scientific (Waltham, MA, USA).

### Instruments

Dehulling machine, Magnetic stirrers, Digital weighing balance, Mixture grinders, Water bath, Hot air oven, Biosafety cabinet [AC2-4S1. ESCO Lifesciences India Pvt. Ltd., Bangalore, Karnataka, India),, Carbon dioxide (CO) Incubator [Forma 371 Steri Cycle 00: Incubator], Thermo Fisher Scientific, Waltham, MA, USA), Multimode plate reader (EnSpire2300, PerkinElmer Inc, Waltham, MA, USA), UV-Visible Spectrophotometer [Bio Photometer 6131 Eppendorf, Hamburg, Germany], Refrigerated centrifuge (5430R, Eppendorf, Hamburg, Germany), Rotary evaporator (Hel-Vap Core, DBT Builder, India), Freeze dryer [Alpha 2-4 LD plus Martin Christ, Osterode am Harz Germany].

### Plant and extraction

A genuine variety of Browntop Millet (HBR-2) was sourced from the University of Agricultural Sciences, GKVK campus, Bengaluru. The collected seeds were rinsed with regular water to eliminate dirt particles and detritus. The collected grains were dried in the shade. Dehulling of the BTM was performed and separation of seed, husk, and rice was done using an AVM millet dehuller machine.[19] 200 g of dehulled grain was obtained. The dehulled grains were grinded into a fine powder using a suitable grinder. The millet powder was frozen at -80 degrees Celsius in a deep freezer. The free and bound phenolic acids were isolated as described in Subbarao, M.V., Murali Krishna, G. *et al.*[20]

### Extraction of free and bound phenolic compounds

The free phenols were extracted by dissolving the ground powder in 400 mL of 70% ethanol. The mixture was stirred continuously using a magnetic stirrer for 3 hr at room temperature. After extraction, the mixture was filtered, and the filtrate was collected and stored in an amber bottle at -20°C until further analysis.[21] The preparation of pellet for bound phenol was done by adding 100 mL of diethyl ether to the pellet. The mixture was left overnight to facilitate complete evaporation of water. The dried pellet was then stored at -80°C. Both these extracts were preserved at -20°C until use.[22]

### Purification and Isolation of Free Phenolic Extract

The ethanolic extract was concentrated using rotary evaporation to remove ethanol. The residue was partitioned with ethyl acetate in a separating funnel, and the organic phase was collected. Anhydrous sodium sulphate was added to remove residual moisture, followed by filtration. The extract was concentrated

again under reduced pressure, freeze-dried, and lyophilized. The dried residue was carefully scraped from the container walls and stored at -20°C until further analysis.[23]

## Extraction of bound phenolic compounds

The dried pellet was resuspended in water, and a heat-stable α-amylase enzyme was added based on the initial flour weight. The mixture was incubated in a water bath at 50°C to facilitate enzymatic hydrolysis. Complete starch removal was confirmed using the iodine test. Bound phenolics were then extracted using an alkaline hydrolysis method. The resulting supernatant underwent triple condensation and phase separation. The final bound phenolic extract was lyophilized, and the remaining solvent was condensed and removed. The dried extract was stored at -20°C until further analysis.[24]

## Cell culture

Breast cancer cell lines MDA-MB-231 (triple negative), received from the National Centre for Cell Science (NCCS) (Pune, India) were used. 1x104 breast cancer cells (MDA-MB-231) in 100 µL DMEM supplemented with 10% FBS were sown in 96-well plates and cultured at 37°C in a cell culture incubator with 5% CO2 and 90% relative humidity. Phosphate Buffered Saline (DPBS), Glutamax (500 mM), Trypsin-EDTA (0.25%), Sulforhodamine-B (SRB), Dimethyl sulfoxide (DMSO) were the reagents used for the cell culture.

## Methods

### *Determination of Total Phenolic Content (TPC)*

Total phenolic content was measured using the modified Folin-Ciocalteu (F-C) method described by Sánchez-Rangel *et al.*, (2013).[25] Briefly, 70 µL of extract was mixed with 70 µL of F-C reagent (1:1 diluted) and incubated for 5 min at room temperature. Then, 60 µL of 4% sodium carbonate solution was added, and the mixture incubated for 30 min. Absorbance was read at 765 nm using a spectrophotometer. Gallic acid (0-40 µg/mL) was used for the calibration curve. TPC was expressed as mg Gallic Acid Equivalents (GAE)/g of sample, with triplicate measurements for seed, husk, and rice.

## Determination of total flavonoid content

Total flavonoid content was determined using the aluminum chloride colorimetric method.[26] Each 60 µL extract sample was mixed with 60 µL of 2% $AlCl_3$ and incubated for 60 min at room temperature. Absorbance was measured at 420 nm. A standard curve was prepared using quercetin (6.25-200 µg/mL). Results were expressed as mg Quercetin Equivalents (QE)/g of sample. Measurements were done in triplicate.

## Determination of Reducing Sugars (DNS method)

Reducing sugar content was assessed using the DNS method as described by Miller (1959) and Subbarao and Murali Krishna (2001).[27] A 500 µL extract was mixed with 500 µL of DNS reagent and heated in a boiling water bath for 30 min. Absorbance was measured at 540 nm. Glucose (0-800 µg/mL) served as the standard. Measurements were performed in duplicate for each sample type.

## Ferric ion-reducing antioxidant power (FRAP) assay

The FRAP assay was performed following the method of Benzie and Strain (1996).[28] Extracts (10 µL) were added to 190 µL of freshly prepared FRAP reagent and incubated in the dark at room temperature for 30 min. Absorbance was read at 593 nm. A standard curve was constructed using $FeSO_4$ (0-1600 µg/mL). Antioxidant activity was recorded in triplicate.

## DPPH radical scavenging assay

DPPH assay was conducted based on Aksoy *et al.*, (2013).[29] A 20 µL extract was added to 140 µL of 0.1 mM DPPH reagent. The mixture was incubated in the dark at room temperature for 30 min. Absorbance was measured at 536 nm. Vitamin C (0-100 µg/mL) was used to generate the standard curve. All samples were analyzed in triplicate. The percentage inhibition was calculated using the equation:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Blank} - \text{Absorbance of Standard}}{\text{Absorbance of Blank}} \times 100$$

## Antiproliferative assay

The antiproliferative activity of browntop millet extracts on MDA-MB-231 breast cancer cells was evaluated using the SRB assay.[30] Cells were treated with free and bound phenolic extracts (whole grain, husk, and rice) at 10, 100, and 1000 µg/mL for 48 hr. Post-treatment, cells were fixed with cold 50% TCA, incubated at 4°C for 1 hr, washed with distilled water, and air-dried. Fixed cells were stained with 0.4% SRB for 30 min, and excess dye was removed using 1% acetic acid. Bound dye was solubilized with 10 mM Tri's base, and absorbance was measured at 510 nm. Diallyl disulfide (1 mM) served as a positive control. Growth inhibition was calculated relative to DMSO-treated controls. The percentage of cell viability was computed using the following equation:

$$\%\text{Inhibition} = (\text{OD of Control} - \text{OD of Sample}) / (\text{OD of Control}) \times 100$$

# RESULTS

## Polyphenol content of BTM extracts

The polyphenol content of BTM was determined by Folin-Ciocalteau assay.[25] The result showed that traces of polyphenol were found in both free and bound phenol extracts. Comparison of the obtained data indicated that the total phenol content was higher in the Bound Phenolic extracts (BTM-BP) as compared to Free Phenolic extracts (BTM-FP). Particularly, the bound phenolic extract of the whole grain, husk, and rice

was found to be 11.5 mg, 8.4 mg, and 6.1 mg per 100 mg of the sample, respectively, for 1 mg/mL concentration of the extracts. The yield of the free phenolic extract was obtained to be 10.49 mg for whole grain, 8.40 mg for husk, and 6.08 mg for rice extract. The highest concentration of total phenolics was shown to be in the bound phenol extract of whole grain (Figure 1).

### Flavonoid content of BTM extracts

The flavonoid content of BTM extracts was evaluated using Aluminum Chloride (AlCl3) method.[26] The total flavonoid content seemed to be greater in the free phenolic extracts compared with the bound phenolic extracts (Figure 1). Free phenol-Husk showed highest content of flavonoids (Figure 1).

### Reducing substances content of BTM extracts

The reducing substances content of BTM extracts was determined using DNS method.[27] The total reducing sugar content was found to be highest in the free phenol extract of rice (54.31 mg/100 mg) (Figure 2).

### Antioxidant potential of BTM extracts

The bound phenolic extracts displayed a significantly higher potential of the ferric reducing assay than free phenolic extracts as revealed in (Figure 2). Ferric reducing ability of the BTM extracts was analysed using the FRAP assay.[28] The elaborated results proved that both extracts possessed ferric ion-reducing ability (Figure 2).

### Free radical scavenging activity of BTM extracts

The free radical scavenging activity of BTM extracts was analysed using the DPPH assay. The DPPH assay revealed the bound phenolic-husk extract to have the highest scavenging activity among both the extracts with 63.80% inhibition at 1 mg/mL concentration (Figure 3).

### Cancer cell proliferation of BTM extracts

The antiproliferation was tested on MDA-MB-231 breast cancer cell lines.[30] The data established that these phenolic acids effectively mediated the triple negative breast cancer cell line MDA-MB-231. The bound phenol rice extract demonstrated the most potent anticancer activity against MDA-MB-231 cells with an % inhibition value of 5.046% at 10 µg/mL concentration, followed by the free phenol seed extract at 3.454% inhibition and bound phenol husk extract with 3.404% inhibition. Cell viability studies showed that both Free Phenolic Acid and Bound Phenolic Acid (BTM-FP and BTM-BP) have an anticancer property against most of those cancer cell lines (Figures 4-7).

## DISCUSSION

Ethanolic extracts of browntop millet exhibited significant antioxidant and antiproliferative activities, attributed to bioactive phytochemicals like phenolics, flavonoids, and flavones.[31] Similar findings by Madhunapantula *et al.*, and Nirmala *et al.*, on finger millet suggest that phenolic acid structure and processing (e.g., malting) influence antioxidant capacity.[32,33] Compounds with hydroxyl groups and conjugated systems demonstrate stronger

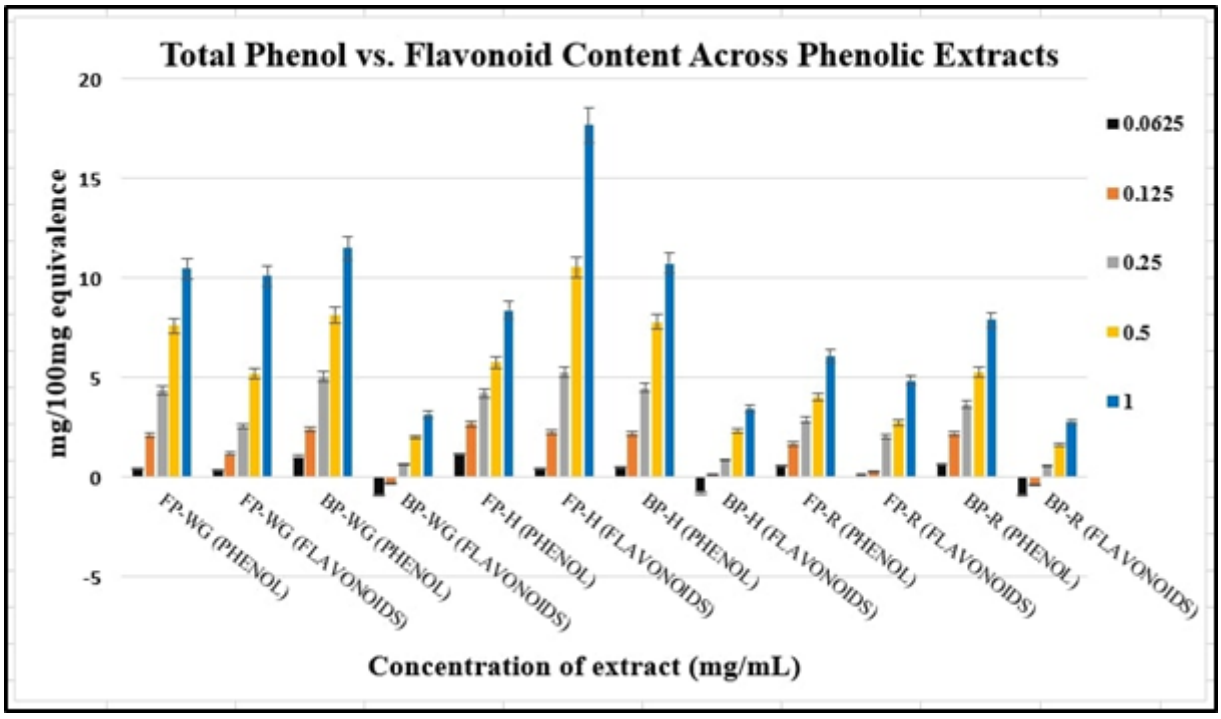


**Figure 1:** Comparison of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) across different phenolic extracts. Data are expressed as mg GAE/g extract for TPC and mg QE/g extract for TFC (FP-W: Free phenol-whole, FP-H: Free phenol-husk, FP-R: Free phenol-rice, BP-W: Bound phenol-whole, BP-H: Bound phenol-husk, BP-R: Bound phenol-rice).

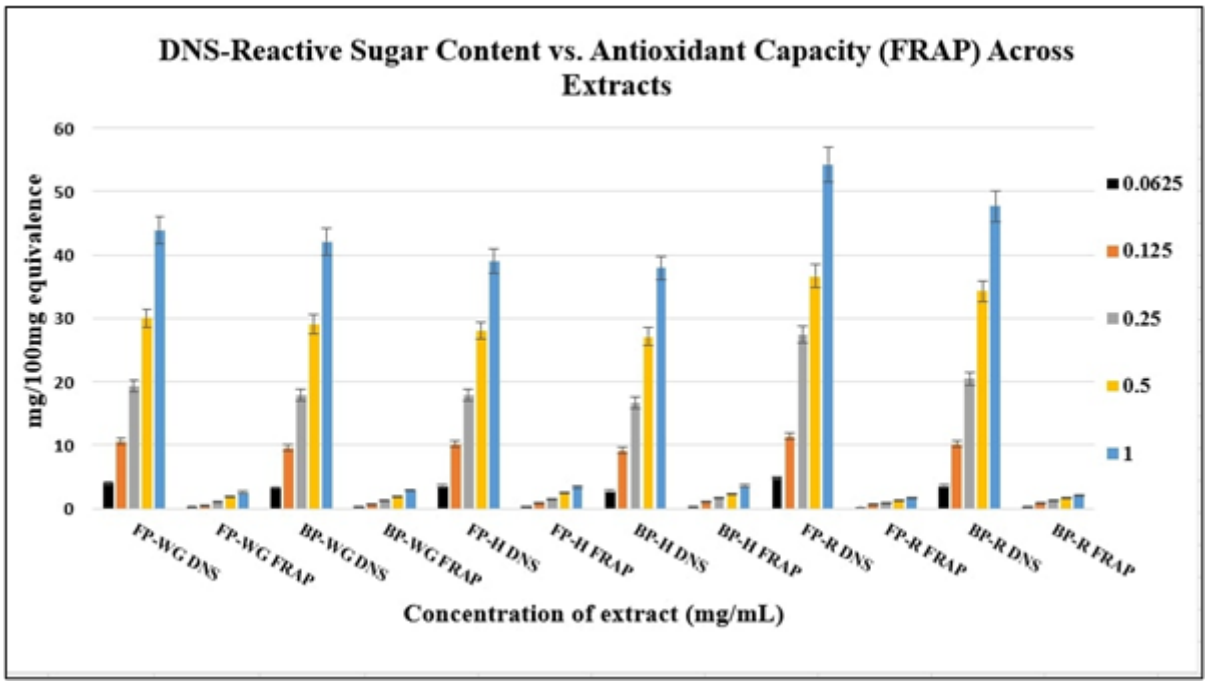


**Figure 2:** DNS method-based quantification of reducing sugars and FRAP assay-based antioxidant potential in phenolic extracts. Reducing substances are expressed in mg glucose equivalents/g extract; FRAP values in µmol $Fe^{2+}$ equivalents/g extract. (FP-W: Free phenol-whole, FP-H: Free phenol-husk, FP-R: Free phenol-rice, BP-W: Bound phenol-whole, BP-H: Bound phenol-husk, BP-R: Bound phenol-rice).

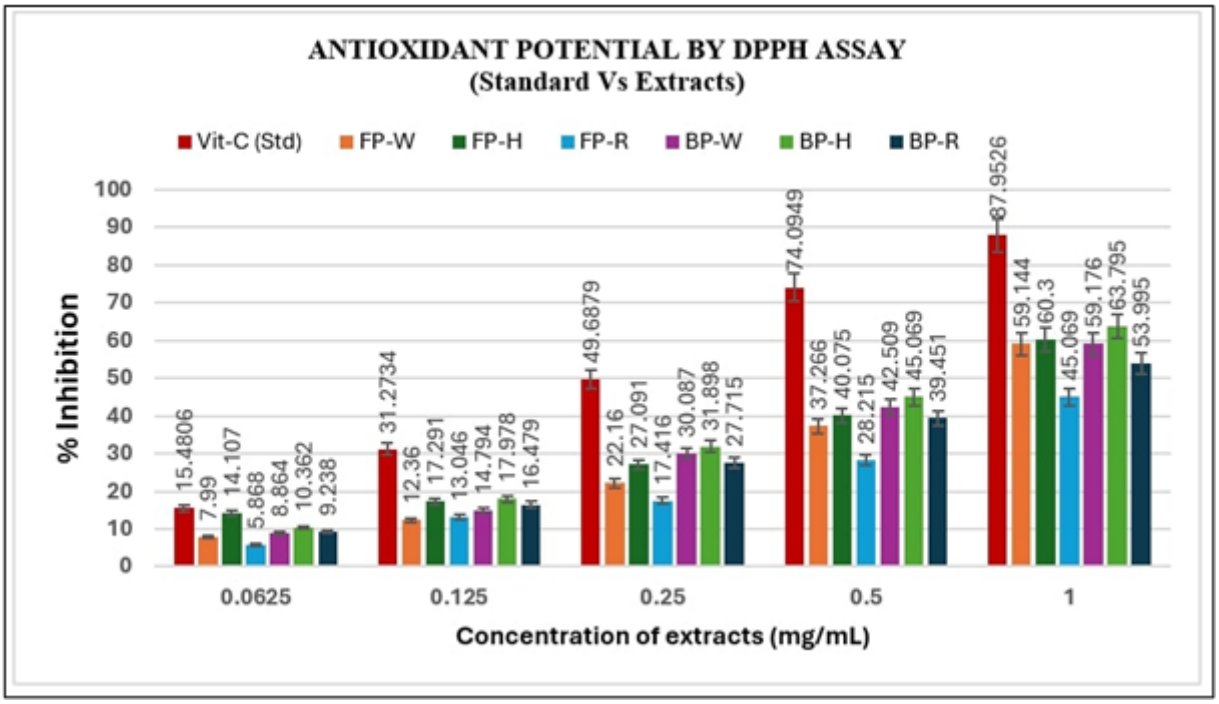


**Figure 3:** Antioxidant activity of millet extracts assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Results are expressed as % inhibition at different concentrations. (FP-W: Free phenol-whole, FP-H: Free phenol-husk, FP-R: Free phenol-rice, BP-W: Bound phenol-whole, BP-H: Bound phenol-husk, BP-R: Bound phenol-rice).

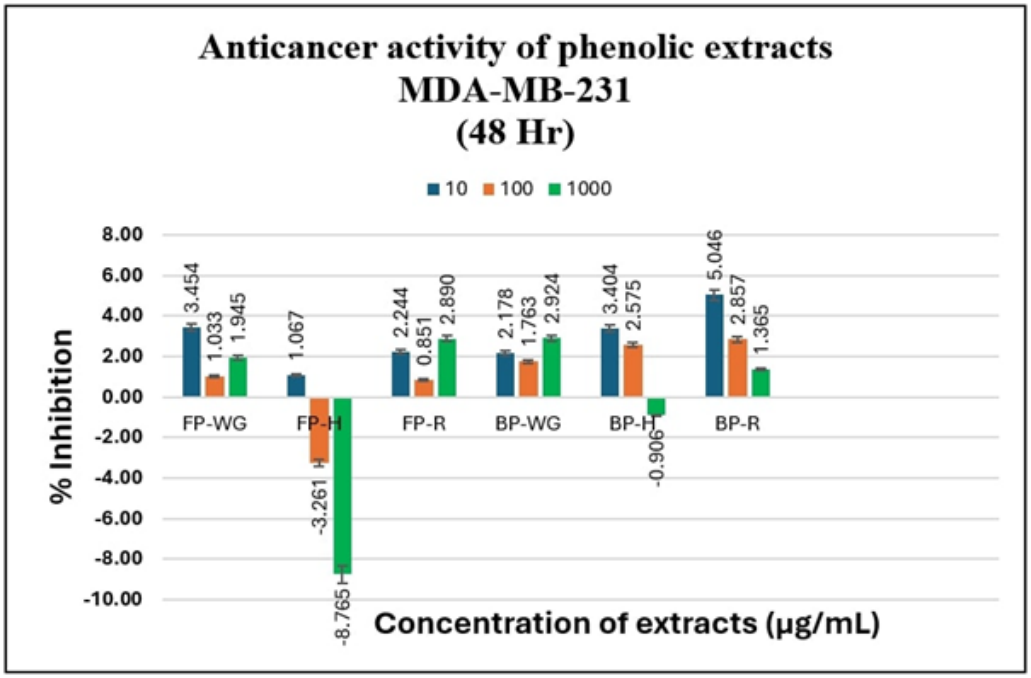


**Figure 4:** Anticancer activity of millet extracts evaluated by Sulforhodamine B (SRB) assay on cancer cell lines. Results are expressed as % inhibition at different concentrations. (FP-W: Free phenol-whole, FP-H: Free phenol-husk, FP-R: Free phenol-rice, BP-W: Bound phenol-whole, BP-H: Bound phenol-husk, BP-R: Bound phenol-rice).

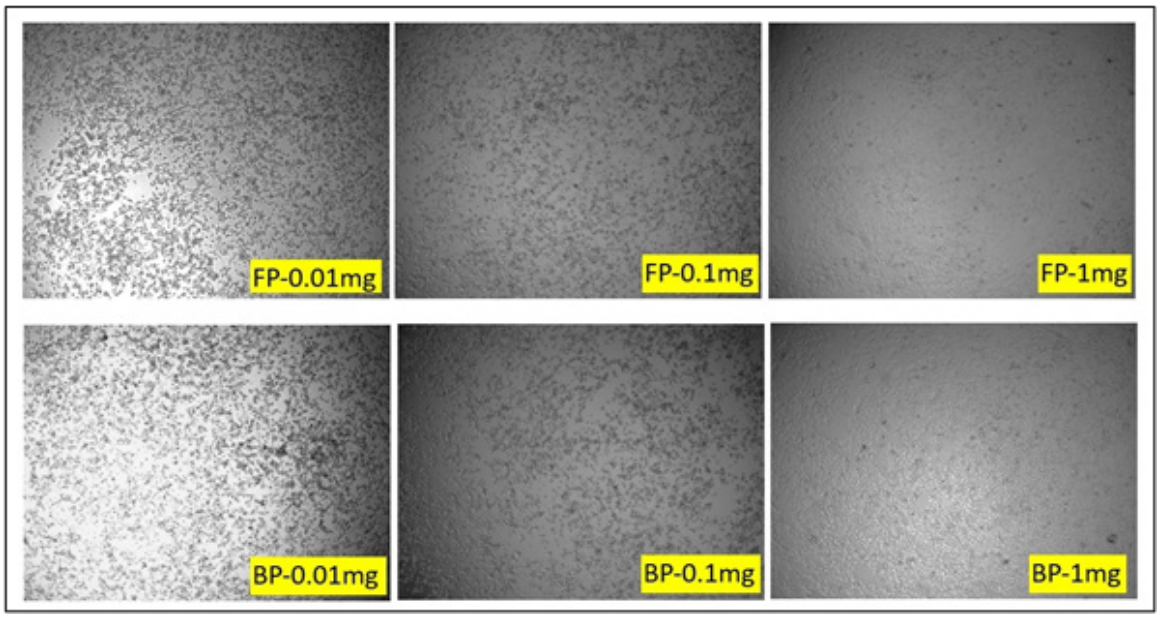


**Figure 5:** Microscopic image showing the anticancer activity of browntop millet rice extract on breast cancer cell lines (MCF-7) assessed by SRB assay. (A) Control (untreated); (B) Vehicle control; (C) Positive control (reference compound). Cell viability is indicated by the intensity of staining. (FP: Free-Phenol, BP: Bound-Phenol).

activity than their methoxylated counterparts.[34] Moreover, benzoic acid derivatives such as gallic and protocatechuic acids were shown to be more potent than cinnamic acids.[35]

Our study aligns with these reports, demonstrating higher antioxidant potential in phenolic-rich extracts, especially in bound phenol fractions rich in ferulic acid and related compounds. These compounds, such as feruloyl arabinoxylans, are known for their antioxidant, anti-inflammatory, and anticancer effects.[36-38] Ferulic acid, a hydroxycinnamic acid prevalent in millet cell walls, scavenges free radicals, chelates metals, and modulates oxidative stress pathways.[39,40] It also shows potential for neuroprotection and cancer therapy by inducing apoptosis and cell cycle arrest in malignant cells.

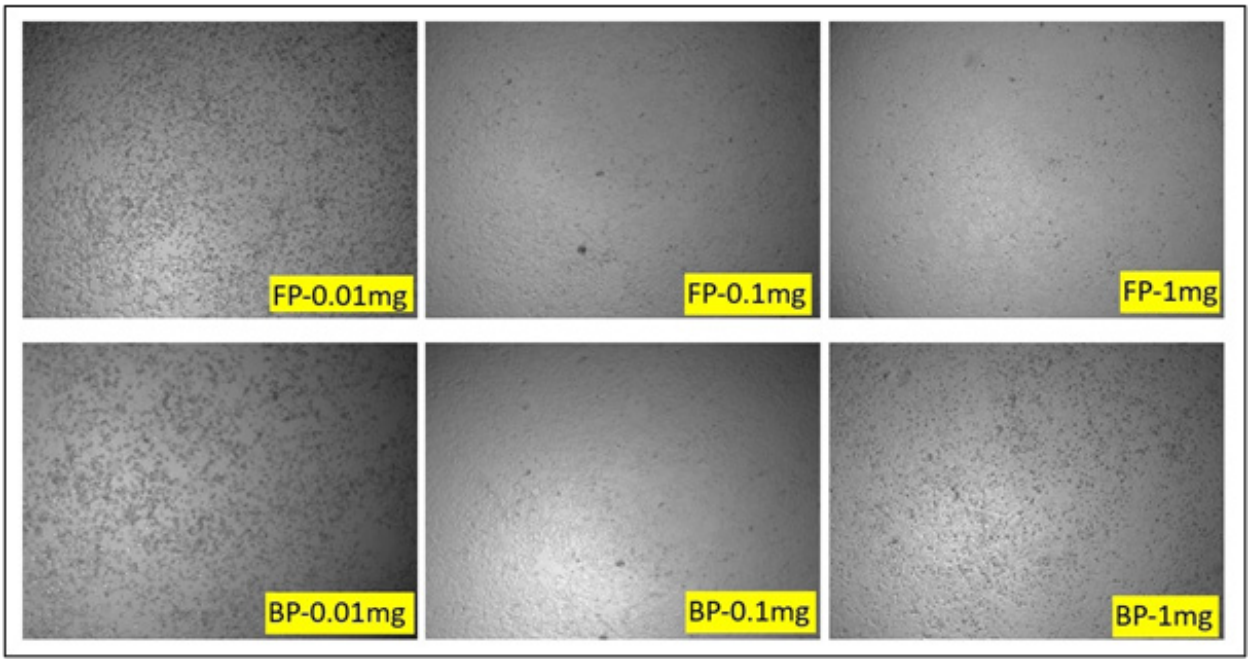


**Figure 6:** Microscopic image showing the anticancer activity of browntop millet whole grain extract on breast cancer cell lines (MCF-7) using SRB assay. (A) Control (untreated); (B) Vehicle control; (C) Positive control (reference compound). Cell viability is depicted based on SRB staining intensity. (FP: Free-Phenol, BP: Bound-Phenol).

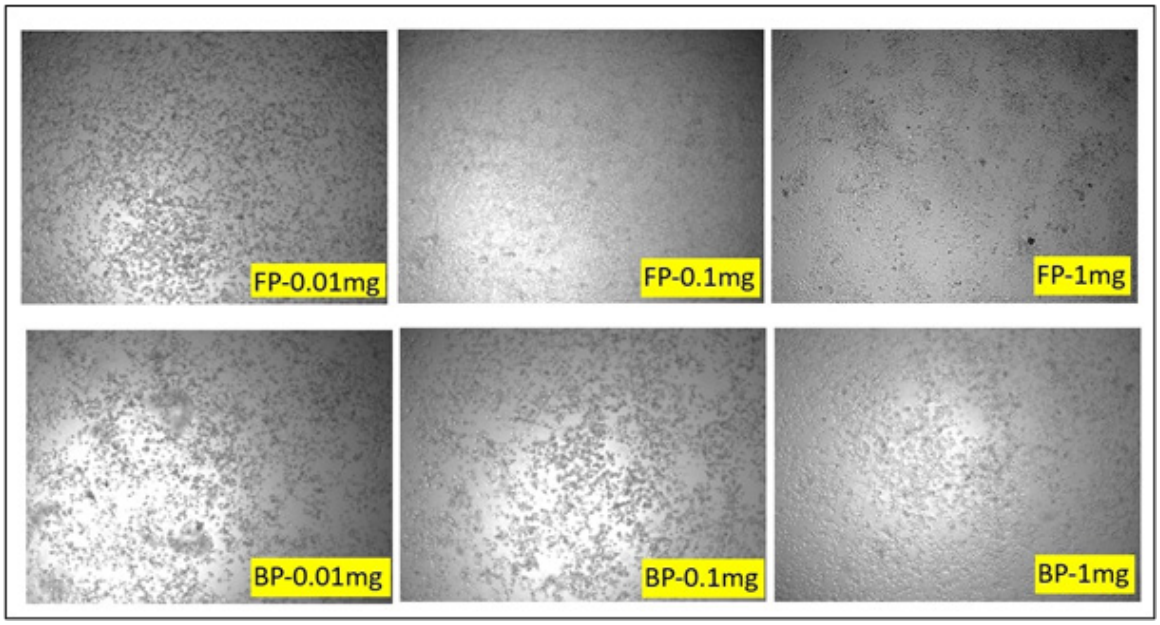


**Figure 7:** Microscopic image showing the anticancer activity of browntop millet husk extract on breast cancer cell lines (MCF-7) assessed by SRB assay. (A) Control (untreated); (B) Vehicle control; (C) Positive control (reference compound). Viability is evaluated through SRB staining intensity. (FP: Free-Phenol, BP: Bound-Phenol).

## Phytochemical Analysis

Phytochemical screening of Browntop Millet (BTM) extracts revealed the presence of alkaloids, glycosides, flavonoids, phenols, saponins, lignans, and others. Total Phenolic Content (TPC), assessed using the Folin-Ciocalteu method, was highest in the bound phenol extract (11.50%), followed by the free phenol (10.49%). Flavonoid content, determined via the $AlCl_3$ method, was notably higher in the free phenol husk extract. The DNS assay revealed considerable levels of reducing sugars in the extracts.

The FRAP and DPPH assays confirmed strong antioxidant potential of the extracts, especially from the husk. The FRAP activity increased dose-dependently, with the highest reducing capacity in bound phenol husk extract. Similarly, DPPH radical scavenging activity peaked at 63.80% at 1 µg/mL concentration. Both assays correlated strongly with TPC and flavonoid content

($R^2$>0.98), emphasizing phenolics' central role in antioxidant activity. Husk extracts consistently showed superior activity, in line with other studies identifying cereal husks as rich in antioxidants.

### Anti-proliferative activity

BTM extracts, especially those rich in ferulic acid, demonstrated significant cytotoxicity against MDA-MB-231 breast cancer cells. Phenolic compounds modulate oxidative stress responses through the Nrf2 pathway. While low concentrations of phenolics activate Nrf2 to protect normal cells by upregulating genes like SOD, GPX, and NQO1, higher doses can inhibit Nrf2, reducing cancer cell proliferation.[41-45] However, as Nrf2 can also support cancer cell survival, cautious application is necessary.

Among the tested extracts, the rice-bound phenolic fraction exhibited the most potent antiproliferative effect against MDA-MB-231 cells, a highly malignant and treatment-resistant line. This suggests a role for specific phenolics in growth inhibition, potentially linked to the unique molecular profiles of the cells. Further investigation into these individual compounds may support their development as adjunct therapies in breast cancer treatment.

## CONCLUSION

The comprehensive phytochemical analysis of the (BTM) grains highlights its rich composition of bioactive compounds, including phenolics, flavonoids, proteins, carbohydrates, and reducing sugars. DPPH, FRAP and SRB assay, demonstrated promising antioxidant and anti-proliferative activity. These findings collectively suggest that BTM grains are a valuable source of various phytochemicals with potent antioxidant properties, making them a promising candidate for functional food applications and further pharmacological studies. The observed dose-dependent increase in antioxidant activities and the strong correlations with total phenolic and flavonoid contents affirm the potential health benefits of incorporating BTM into the diet. Future research should focus on isolating specific bioactive compounds and exploring their mechanisms of action to fully elucidate the therapeutic potential of browntop millet.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**ROS:** Reactive Oxygen Species; **BTM:** Browntop Millet; **DPPH:** 2,2-Diphenyl-1-Picrylhydrazyl; **FRAP:** Ferric Reducing Antioxidant Power; **SRB:** Sulforhodamine-B; **MDA-MB-231:** M.D. Anderson-Metastasis Breast Cancer-231; **HBR-2:** Hagari Base Research Station-2; **GKVK:** Gandhi Krishi Vigyana Kendra, **NCCS:** National Centre for Cell Science; **DMEM:** Dulbecco's Modified Eagle's Media; **DPBS:** Phosphate Buffered Saline; **FBS:** Fetal Bovine Serum; **EDTA:** Ethylenediamine Tetra Acetic Acid; **DMSO:** Dimethyl Sulfoxide; **FCR:** Folin-Ciocalteu Reagent; **DNS:** Dinitrosalicylic Acid; **SOD:** Superoxide Dismutase; **GPX:** Glutathione Peroxidase; **Nrf2:** Nuclear Factor Erythroid 2-Related Factor 2; **NQO1:** NAD(P)H Quinone Dehydrogenase 1; **BTM-FP:** Browntop millet-free phenol, **BTM-BP:** Browntop Millet-Bound Phenol; **FP-WG:** Free phenol-whole grain; **FP-H:** Free phenol-husk; **FP-R:** Free Phenol-Rice; **BP-W:** Bound Phenol-Whole Grain; **BP-H:** Bound Phenol-Husk; **BP-R:** Bound phenol-rice.

## AUTHORS CONTRIBUTIONS

The author's contribution to the paper is as follows: study conception and design: Jayanthi MK, Saddique Choudhury, Megha ML, Sunil Sihag; data collection: Saddique Choudhury, Megha ML, Sunil Sihag; analysis and interpretation of results: Saddique Choudhury, Megha ML, Sunil Sihag, Manu Gangadhar; draft manuscript: Jayanthi MK, Saddique Choudhury. All authors reviewed the results and approved the final version of the manuscript.

## SUMMARY

This study explored the antioxidant and anticancer properties of phenolic extracts from Browntop Millet (BTM), a phytochemical-rich Indian millet. Free and bound phenolic extracts from seed, husk, and rice were analyzed for their phytochemical content and antioxidant activity using DPPH and FRAP assays. The bound phenolic-husk extract exhibited the highest antioxidant activity, while the bound phenolic-rice extract showed the strongest anticancer effect against MDA-MB-231 breast cancer cells. These findings highlight BTM's potential as a natural source of bioactive compounds with significant health benefits, including oxidative stress reduction and anticancer effects.

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