



## EVALUATION OF ANTIOXIDANT, TOXICITY, AND ANTIDIABETIC ACTIVITIES OF YOUNG SPROUTS OF *Hordeum vulgare*, *Triticum aestivum*, AND *Zea mays*

Surya Kant Kalauni\*<sup>1</sup>, Gita Bhattarai<sup>2</sup>, Lekha Nath Khanal\*<sup>2</sup>

<sup>1</sup>Central Department of Chemistry, Institute of Science and Technology, Tribhuvan University, Kathmandu, Nepal

<sup>2</sup>Department of Chemistry, Prithvi Narayan Campus, Tribhuvan University, Pokhara, Nepal

\*Correspondence: [skkalauni@gmail.com](mailto:skkalauni@gmail.com) and [lnkhanal@pncampus.edu.np](mailto:lnkhanal@pncampus.edu.np)

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### ABSTRACT

The germination of dormant seeds triggers various metabolic reactions, resulting in the production of essential phytochemicals with diverse biological activities. This contributes to the inclination to consume sprout juices among individuals seeking to enhance their immune system, manage oxidative stress, and prevent complaints associated with metabolic disorders. In this study, we evaluated the antioxidant, toxicity, and antidiabetic activity of young sprout extracts of *Hordeum vulgare*, *Triticum aestivum*, and *Zea mays* by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, brine shrimp lethality assay, and  $\alpha$ -amylase inhibition methods, respectively. The ethanolic extracts showed the presence of alkaloids, flavonoids, tannins, and polyphenols. The extracts showed moderate antioxidant activity, with *Z. mays* having the highest capacity, followed by *T. aestivum* and *H. vulgare*. Their half-maximal concentration (IC<sub>50</sub>) values were  $54.24 \pm 3.35$ ,  $95.94 \pm 3.29$ , and  $129.26 \pm 5.97$   $\mu\text{g/mL}$ , respectively. The same trend of toxicity against brine shrimp nauplii was obtained with half-maximal lethal concentration (LC<sub>50</sub>) values of 326.41, 473.61, and 6768.75 mg/mL respectively. The antioxidant activity across various extracts displayed a positive correlation with the total phenolic and total flavonoid contents. The extracts demonstrated moderate activity in the  $\alpha$ -amylase inhibition assay conducted through the starch-iodine method. The outcomes of this study underscore the presence of significant phytochemicals in the young sprouts of commonly consumed cereals, suggesting their potential use as immune boosters and in treating diseases associated with free radicals.

**Keywords:** Antidiabetic activity, antioxidant activity, *Hordeum vulgare*, *Triticum aestivum*, young sprout, *Zea mays*

### INTRODUCTION

Although oxygen and nitrogen are essential elements for life on earth, certain conditions can lead to the formation of extremely reactive structures of these elements. In such cases, these reactive species can act as natural killer cells, inducing necrosis and cell death (Asmat *et al.*, 2016). The reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in living cells by various cellular reactions. Our body has a natural self-regulating system to control the concentration of these species. When the populace of free radicals surpasses the critical limit, a state of oxidative stress is set up in the body. Oxidative stress is a major cause of the genesis of several irreversible consequences of diabetes like the expansion of the extracellular matrix, cellular hypertrophy, hyperplasia, and vascular complaints (Naris, 2022). Antioxidants are a useful group of chemical and biological agents that counterbalance the detrimental actions of free radicals. They moderate or face oxidative stress by various pathways such as catalytic activities to neutralize ROS, inactivation of metal ions that generate ROS by Haber-Weiss reaction, endowing suicidal and chain-breaking reactions, etc. (Kanwugu *et al.*, 2021).

Diabetes mellitus (DM) is characterized by a long-lasting metabolic disorder that leads to chronic hyperglycemia. It arises due to defects in the secretion of insulin, its metabolic action, or both (Usai *et al.*,

2022). Insulin, a peptide hormone that is secreted by the pancreatic  $\beta$ -cells is crucial for maintaining blood glucose metabolism and controls the development of type 2 diabetes. Several factors like high glucose, inflammatory cytokines, free fatty acids, and islets of amyloid polypeptides, etc. contribute to the impairment of pancreatic  $\beta$ -cells in terms of their mass, and action (Khin *et al.*, 2023). In recent times, diabetes mellitus (DM) has emerged as a significant health problem, characterized by elevated blood glucose levels due to insulin metabolism. This condition gives rise to various complications such as cardiovascular issues, renal problems, blindness, and heart-related ailments. As a result, diabetes has become an alarming burden of illness and death worldwide (Yedjou *et al.*, 2023). Rapid urbanization, technological advancement, and globalization of the food market have resulted in many disapproving consequences in the lifestyle of modern people. A polluted environment, increasing consumption of processed fast foods, lack of physical exercise, obesity, drug abuse and smoking, poor sleeping, stress, etc. have augmented a myriad of adverse impacts on human health (Gherasim *et al.*, 2023).

Phenolic and flavonoid compounds represent an extensive category of plant secondary metabolites found abundantly in various parts such as bark, roots, leaves, flowers, and shoots. Given their notable

biological activities, they are recognized as essential components of both human and animal diets (Huyut *et al.*, 2017). Phenolic acids, tocopherols, and flavonoids are the most widespread classes of plant phenolic compounds known for their remarkable antioxidant properties. These compounds possess various beneficial biological activities, including anti-allergic, anti-diabetic, anti-inflammatory, antimicrobial, vasodilatory effects, etc. So, they have excessive potential to be used in the management of numerous chronic conditions such as cancer, cataracts, Parkinson's disease, Alzheimer's disease, cardiac and hepatic issues, as well as neurodegenerative disorders (Mutha *et al.*, 2021; Shahidi & Ambigaipalan, 2015).

*T. aestivum* L. is a common crop belonging to the Poaceae family and is commonly known as wheatgrass. It contains high chlorophyll contents with about 70% of its chemical components (Mohan *et al.*, 2013). The juice of the young sprout of the plant is consumed to enhance the immune system and overall bodily strength. It is believed to improve hemoglobin deficiency, offering substantial nourishment and showcasing diverse biological effects. This juice is suggested for potential applications in treating several disease conditions such as cancer, diabetes, ulcers, anemia, kidney stones, asthma, and digestive disorders, including diabetes (Choudhary *et al.*, 2021). *Hordeum vulgare* (Nepali name: Uwa, Jau) is one of the historical crops grown in the marginal agricultural fields as the fourth most important cereal after wheat, rice, and maize (Yadav *et al.*, 2018). The sprout of the plant is reported to contain 52.6% of polysaccharides, 34.1%, protein, 4.9% fat, and several vitamins, polyphenols, and minerals (Byun *et al.*, 2015). Consumption of the leaf juice is supposed to improve cholesterol metabolism. The plant is native to China, where it has been traditionally used for treating a range of skin, blood, liver, and gastrointestinal disorders (Singh *et al.*, 2023). *Z. mays* (maize) is one of the significant cereal crops that is grown in different parts of the world. In terms of human nutrition, maize contributes a minimum of 30% of dietary calories to over 4.5 billion individuals, especially in developing nations, establishing it as a fundamental staple food. Maize contains a substantial quantity of starch and various phenolic compounds, including phenolic acids,  $\beta$ -carotene, lutein, zeaxanthin, and others, which contribute to their antioxidant properties (D'Amato *et al.*, 2019).

Sprouting is one of the techniques adopted by the food industries for the germination of cereals, veggies, and oilseeds to improve their nutritive content (Zhang *et al.*, 2021). Sprouting leads to the decomposition of several micronutrients to increase the proportion of polyphenols, vitamins C, amino acids, simple sugars, etc., and reduce the concentrations of anti-nutritional components. In the last decades, the consumption of sprouts has been popular due to their functional features and digestive attributes directly related to the health benefits (Liu *et al.*, 2019; Mir *et al.*, 2021).

This study aims to evaluate the toxicity, antioxidant, and antidiabetic potency of the young sprouts of *H. vulgare*, *T. aestivum*, and *Z. mays* which are common among Nepalese consumers in recent days. The toxicity was assessed by the Brine shrimp lethality assay (BSLA) method. The antioxidant and antidiabetic activities were evaluated by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and  $\alpha$ -amylase inhibition capacity by the iodine-starch technique respectively.

## MATERIALS AND METHODS

### Growing of mature sprouts and sample preparation

The seeds of corn, wheat, and barley of good quality were purchased from a local market in Kirtipur, Kathmandu, Nepal. The young sprouts of the plants were grown according to Randhir *et al.* (2008) with slight modification. Briefly, the grains were washed with distilled water and drenched at room temperature for 24 hours to remove residual water. The wetted seeds were cultivated individually in darkness, positioned between moist cotton fabrics. Additional damp cotton layers were placed over them two times a day to preserve humidity. To optimize growth, the grasses were exposed to only three hours of daylight in a room, avoiding direct sunlight. The 8-day-old grass was chopped, cleaned with tap water, and then chopped into little pieces before being air-dried in the shade for 15 days, dried, and ground into powder.

### Preparation of the extracts

The extracts of the dry samples of the sprouts were prepared by using the Soxhlet apparatus—using 99 % ethanol. The extract was concentrated through a rotary evaporator and dried over a water bath at around 35°C. The semisolid crude extracts were separately stored in the airtight glass bottles at 4°C for further study.

### Phytochemical analysis

The phytochemical screening of the sprout extracts was carried out by adopting the standard protocol. The different phytochemicals present in the extract were identified by various color reactions with different reagents (Khanal *et al.*, 2022; Tiwari *et al.*, 2020). The tests were performed for the presence of alkaloids, polyphenols, tannins, saponins, flavonoids, glycosides, quinones, terpenoids, and reducing sugars.

### Estimation of total phenolic content

The quantitative estimation of total phenolic content (TPC) in the ethanolic extracts of *T. aestivum*, *H. vulgare*, and *Z. mays* was conducted by using the Folin-Ciocalteu method with slight adjustment (Ainsworth & Gillespie, 2007; Singh *et al.*, 2016). Briefly, 20  $\mu$ L of gallic acid solutions of each concentration and the test solutions (5 mg/mL) were poured into each well of a 96-well plate. Then, 100  $\mu$ L of 10% Folin-Ciocalteu reagent (FCR) and 80  $\mu$ L of 7% Na<sub>2</sub>CO<sub>3</sub> were added to each well to make the volume of 200  $\mu$ L. The blue-colored mixture was incubated at room temperature for 25 minutes. Using a spectrophotometer (Synergy

LX, BioTek, Instruments, Inc., USA), the absorbance of the solution was measured at 765 nm against a blank containing all reagents except gallic acid. All of the experiments were done in triplicate. The total phenolic content in the extract was calculated from the standard calibration curve and expressed as mg GAE/g of the dry weight of the extract.

#### Estimation of total flavonoid content

The total flavonoid content (TFC) of the crude extracts of *H. vulgare*, *T. aestivum*, and *Z. mays* were determined by an Aluminum chloride colorimetric assay with little change (Bhandari *et al.*, 2021; Maharjan *et al.*, 2021). Briefly, 130  $\mu$ L of quercetin of each concentration (10–80  $\mu$ g/mL) and 20  $\mu$ L of the plant extract (5 mg/mL) with 110  $\mu$ L of distilled water were transferred into the wells of a 96-well microplate. Then 60  $\mu$ L ethanol, 5  $\mu$ L 10% AlCl<sub>3</sub>, and finally, 5  $\mu$ L of 1M potassium acetate was added to the mixture. The mixture was shaken and incubated in the dark for 25 minutes at room temperature. Finally, the absorbance of the pink color mixture was determined at 415 nm wavelength versus blank containing all the reagents except quercetin using a spectrophotometer (Synergy LX, BioTek, Instruments, Inc., USA). The absorbance of the quercetin was used to construct a calibration curve and the total flavonoid content was calculated by using the linear regression equation and expressed as mg QE/g.

#### Brine shrimp lethality test

The toxicity of the crude alcoholic extracts of the sprouts of *T. aestivum*, *H. vulgare*, and *Z. mays* was evaluated by the Brine shrimp lethality assay (BSLA) method with little modification (Meyer *et al.*, 1982;

Adhikari *et al.*, 2023). About 5 mg of brine shrimp (*Artemia salina*) eggs were allowed to hatch into artificial seawater in a beaker at 28–30°C for about 48 hours. Aliquots of 10 freshly hatched nauplii were introduced into 5.0 mL of the crude extract solutions of 10, 100, and 1000 mg/mL and the solvent as a control in separate test tubes. The test samples were incubated under controlled conditions for 24 hours. The next day, the number of survivors was counted in each of the treatments and the percentage mortality was calculated. The dose-response results were converted into a straight line by Probit transformation and the concentration causing 50% death of larvae (LC<sub>50</sub>) was calculated from the best-fit line attained from linear regression analysis.

#### Measurement of DPPH free radical scavenging activity

The antioxidant capacity of the extracts was evaluated by the 2, 2-diphenyl-2-picrylhydrazyl free radical scavenging method using ascorbic acid as standard (Brand-Williams *et al.*, 1995; Pandey *et al.*, 2017). Each of the extracts was dissolved in 50% dimethyl sulphoxide (DMSO) to prepare the test solutions of 250, 125, 62.5, 31.25, 15.62, and 7.8  $\mu$ g/mL. Aliquots of 100  $\mu$ L of ascorbic acid or the test solutions of each concentration were mixed with 100  $\mu$ L of 0.1mM DPPH solution in triplicate and kept in the dark for 30 minutes. Their absorbance values were measured at 517 nm using a spectrophotometer (SynergyLX, BioTek, Instruments, Inc., USA) against control. The percentage scavenging capacity was calculated by using the formula:

$$\% \text{ Scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The half-maximal concentration (IC<sub>50</sub>) was calculated by using the Graph Pad Prism9 software.

#### $\alpha$ -Amylase inhibition assay

The iodine-starch method was used with certain modifications to determine the effect of crude extract on  $\alpha$ -amylase inhibition (Sudha *et al.*, 2011; Tayab *et al.*, 2021). Acarbose and the extracts were dissolved in 50% DMSO to the concentrations of 100, 80, 40, 20, and 10  $\mu$ g/mL, were prepared by dilution from a stock solution of 1 mg/mL. Aliquots of 20  $\mu$ L of plant extract or acarbose were incubated at 40°C for about 5 minutes with 40  $\mu$ L of starch solution. The 20  $\mu$ L of the 3U/mL  $\alpha$ -amylase dissolved in phosphate buffer was also incubated for 15 minutes at 37°C. Aliquots of 20

$\mu$ L of enzyme, 20  $\mu$ L (3U/mL) of plant extract or acarbose (standard), and 10  $\mu$ L sodium phosphate buffer (pH 6.9 containing 6 mM NaCl), were mixed and incubated at 37°C for 10 minutes. After pre-incubation, 30  $\mu$ L of starch (0.5%) was added and again incubated at 37°C for 15 minutes, after that, 20  $\mu$ L of HCl (0.1M) followed by 100  $\mu$ L iodine (2.5M) was added and absorbance was measured at 620 nm using a spectrophotometer (Synergy LX, Bio Tek, Instruments, Inc., USA) against the control that contains buffer instead of sample. The % inhibition was calculated as:

$$\text{Percentage inhibition} = \frac{(\text{Absorbance of starch} - \text{absorbance of sample})}{(\text{Absorbance of starch} - \text{absorbance of Starch} + \text{enzyme})} \times 100$$

#### Statistical analysis

The Gen 5 microplate and analysis software of the microplate reader along with MS Excel was used to analyze the data. The concentration causing 50%

inhibition (IC<sub>50</sub>) and concentration causing 50% death (LC<sub>50</sub>) were calculated by using the linear regression equation  $y = mx + C$ . All the tests were carried out in triplicates and the results were expressed as mean  $\pm$

standard deviation (SD).

## RESULTS AND DISCUSSION

### The percentage yield of the extract

The percentage of yields of ethanolic extracts of *H. vulgare*, *T. aestivum*, and *Z. mays* are shown in Table 1. The highest quantity (28.80%) of the extract was obtained from *T. aestivum* followed by *Z. mays* (22.29%) and the lowest quantity of the extract was obtained for *H. vulgare* (12.27%). The polarity of the extracting solvent, extraction method, and the type of compounds to be extracted greatly affect the amount of compound extracted (Abbas *et al.*, 2021). This research aimed to extract polar phenolic and flavonoid compounds with strong antioxidant properties. We chose ethanol as the extracting solvent for this purpose. Although methanol could also be used, its toxic nature in comparison to ethanol led us to prefer the latter.

**Table 1. Percentage yield of ethanolic extract**

Name of the plants	Yield (%)
<i>T. aestivum</i>	28.80
<i>H. vulgare</i>	12.27
<i>Z. mays</i>	22.29

### Phytochemical screening

Phytochemicals are the bioactive compounds in plants exhibiting significant therapeutic properties like antioxidant, antimicrobial, antidiabetic, and anti-inflammatory activities. The results obtained in the phytochemical screening of each of the ethanolic extracts of *H. vulgare*, *T. aestivum*, and *Z. mays* are shown in Table 2. All of the extracts were found to contain most of the phytochemicals tested in the study. Similar results were reported by Tessema and Tura (2018) in *T. aestivum*, and *H. vulgare* and Solihah *et al.* (2012) in *Z. mays*. The result obtained here resemble the previous studies on *T. aestivum* and *H. vulgare*.

**Table 2. Results of phytochemical screening**

S.N	Phytochemicals	<i>T. aestivum</i>	<i>H. vulgare</i>	<i>Z. mays</i>
1.	Alkaloids	+	+	+
2.	Flavonoids	+	+	+
3.	Terpenoids	+	+	+
4.	Tannin	+	+	+
5.	Glycosides	+	+	+
6.	Quinones	+	+	+
7.	Reducing sugar	+	+	-
8.	Polyphenols	+	+	+
9.	Saponins	-	-	-

Existing literature confirms the existence of carbohydrates, proteins, alkaloids, tannins, and phenols in both methanolic and aqueous extracts of *H. vulgare*. This lines up with our findings, supporting the presence of alkaloids, tannins, quinones, and polyphenols. This study also shows the presence of indole, anthraquinones, and anthrones in wheatgrass and *H. vulgare* grass (Lirazan *et al.*, 2018).

### Total phenolic and flavonoid content

Phenolics and flavonoids are the chief compounds in plants that are responsible for different biological activities like antioxidant, antimicrobial, antidiabetic, anticancer, anti-inflammatory, etc. (Kumar & Pandey, 2013; Mutha *et al.*, 2021). The quantitative assessment of phenolic and flavonoid content in the extracts of *Z. mays*, *T. aestivum*, and *H. vulgare* sprouts was carried out and the findings are listed in Table 3. The results reveal that *Z. mays* exhibited the highest total phenolic content  $42.44 \pm 4.11$ , followed by *T. aestivum* with  $32.21 \pm 0.90$ , while the extract of *H. vulgare* showed the lowest value of  $26.61 \pm 1.96$  mg GAE/g. Likewise, the sprout

extract of *Z. mays* exhibited the highest flavonoid content of  $30.43 \pm 1.97$ , followed by *T. aestivum* and *H. vulgare* with  $14.71 \pm 1.23$  and  $12.55 \pm 0.90$  mg QE/g, respectively. Niroula *et al.* (2019) reported a higher quantity of phenolics in the 7-10-day-old sprouts of the seed obtained from the Food Research Division, Nepal in comparison to their seed. The sprouts of *T. aestivum*, *H. vulgare*, and *Z. mays* had TPC values of  $495.66 \pm 20.49$ ,  $479.02 \pm 11.7$ , and  $404.05 \pm 24.16$  mg GAE/100g respectively. In this case, we randomly chose the seeds from the local market of Kathmandu and the obtained results align with our study outcomes, despite not directly comparing the values with those of the corresponding seeds. When the dormant seeds get water several quiescent enzymatic reactions of ester-linked phenolic moieties are activated to form phenolic compounds leading to the increase of total phenolic content during growth. The higher proportion of these active compounds in the germinating seeds increases the nutrient content of the sprouts of cereals (Schendel, 2019).

**Table 3. List of total phenolic and flavonoid content and antioxidant capacity of different extracts**

S. No.	Extracts	TPC(mg GAE/g)	TFC(mg QE/g)	DPPH scavenging capacity (IC <sub>50</sub> in $\mu\text{g}/\text{mL}$ )
1	<i>Z. mays</i>	42.44 $\pm$ 4.11	30.43 $\pm$ 1.97	54.24 $\pm$ 3.35
2	<i>T. aestivum</i>	32.21 $\pm$ 0.90	14.71 $\pm$ 1.23	95.94 $\pm$ 3.29
3	<i>H. vulgare</i>	26.61 $\pm$ 1.96	12.55 $\pm$ 0.90	129.26 $\pm$ 5.97
4	Ascorbic acid	--	--	33.92 $\pm$ 0.74

### Brine shrimp lethality test

The toxicity of the crude alcoholic extracts of the sprouts of *T. aestivum*, *H. vulgare*, and *Z. mays* was assessed by the BSLA method, and the results are shown in Table 4. The toxicity powers varied with concentrations among the extracts, with *Z. mays* showing the highest toxicity at 326.41 mg/mL, followed by *T. aestivum* at 473.61 mg/mL. The lowest toxicity was observed in the extract of *H. vulgare*, with a concentration of 6768.75 mg/mL. In this bioassay, shrimp larvae consume their yolk sac without external food for a duration of up to 48 hours, and any mortalities were attributed to the test samples and not to starvation (Carballo *et al.*, 2002). The active compounds in the plant extract cause cytotoxicity to the newborn larvae of brine shrimp. The standard BSLA specifies that any sample causing LC<sub>50</sub> value < 1000  $\mu\text{g}/\text{mL}$  is considered as potent bioactive (Kabubii *et al.*, 2015). Based on this notion, our samples cannot be considered to exhibit strong toxicity. Analogous to our observation, the alcoholic and aqueous extracts of *Allium fistulosum* had LC<sub>50</sub> values of 13.433mg/mL and 1846.550 mg/mL. The *Brassica oleracea* extracts prepared by using alcohol and distilled water also had

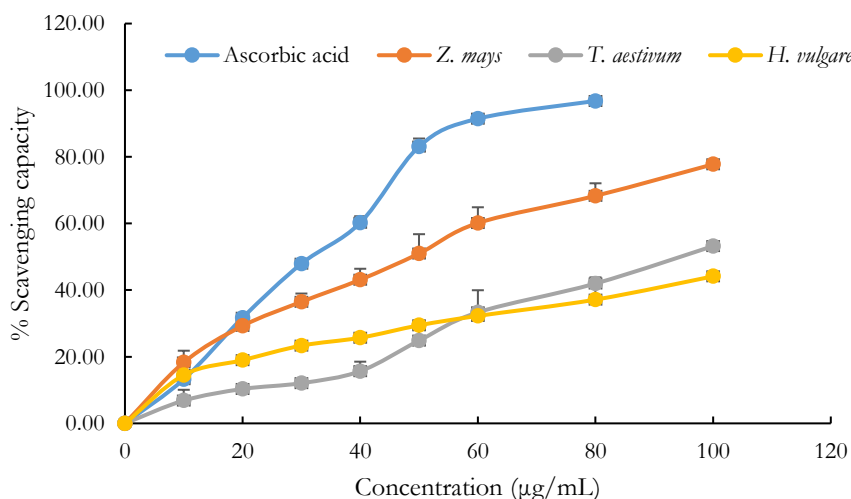
higher toxicity with LC<sub>50</sub> values of 10.81 and 64.83 mg/mL respectively. The abundance of bioactive compounds such as certain alkaloids, flavonoids, and tannins is accountable for the cytotoxicity of the plant extract (Waghulde *et al.*, 2019).

**Table 4. Results of BSLA test**

Sample	LC <sub>50</sub> (mg/mL)
<i>T. aestivum</i>	473.61
<i>H. vulgare</i>	6768.75
<i>Z. mays</i>	326.41

### Antioxidant activity

The antioxidant compound transfers electrons or hydrogen to DPPH and change the violet color of the free radical into yellow. The intensity of color change that is proportional to concentration is measured by the absorbance precisely using a spectrophotometer. So, it is one of the simple, reliable, and popular assays to measure antioxidant capacities by scholars (Kim *et al.*, 2022). The increased concentration of phenolic compounds in the sprouts of oats, barley, wheat, and rye were reported to enhance antioxidant potential (Nemzer *et al.*, 2018).

**Figure 1. Dose-dependent variation of scavenging capacity**

Here we determined the antioxidant potential of ethanolic extracts of *H. vulgare*, *T. aestivum*, and *Z. mays* by DPPH method taking ascorbic acid as a standard. The dose-dependent variation of the radical scavenging capacity of the extracts is plotted with ascorbic acid (Figure 1). The curve of *Z. mays* extracts is closer to that of ascorbic acid and the others also exhibit positive correlations with concentration. The results of the study showed the highest antioxidant

capacity of *Z. mays* followed by *T. aestivum*, and *H. vulgare* with half-maximal concentrations of 54.24  $\pm$  3.35, 94.94  $\pm$  3.29, and 129.26  $\pm$  5.97  $\mu\text{g}/\text{mL}$  respectively (Table 3). According to the half-maximal concentration (IC<sub>50</sub>) values, the position of antioxidant capacities of the extracts was found as *Z. mays* > *T. aestivum* > *H. vulgare*, along with ascorbic acid having the highest capacity (Figure 2).

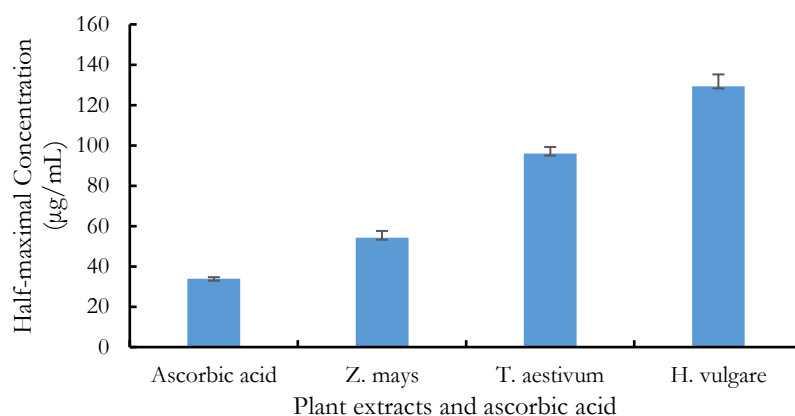


Figure 2. Half-maximal concentrations (IC<sub>50</sub>) of the extracts and ascorbic acid

The relationship between the antioxidant capacity of three young sprout extracts with TPC and TFC was analyzed by a curve in Figure 3. The correlation of antioxidant capacity with TPC was strong with ( $R^2 =$

0.9895) and that of TFC was found to be less ( $R^2 = 0.8835$ ). This observation indicates the higher contribution of phenolic compounds in the extract.

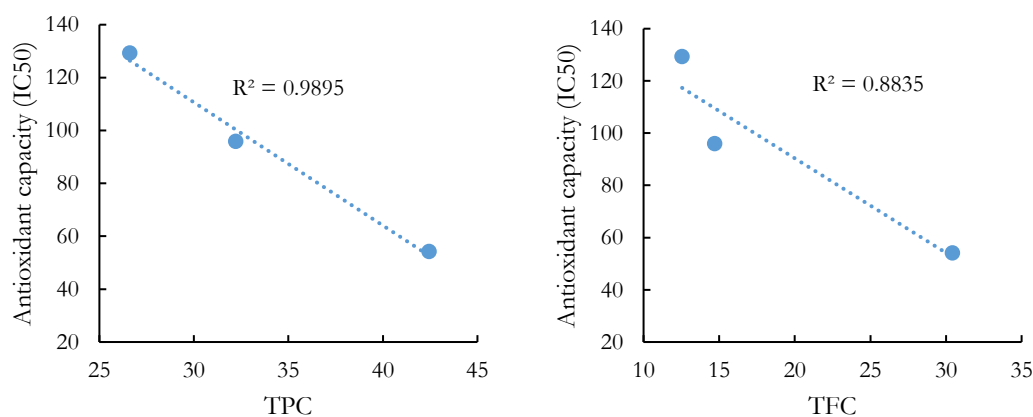


Figure 3. Correlation of TPC and TFC with antioxidant activity

In a similar study carried out by Niroula *et al.* (2019), the antioxidant capacity in terms of IC<sub>50</sub> values was evaluated. The (IC<sub>50</sub>) value of the extracts of the 10-day sprouts of wheat grass, and 13-day sprout of barley grass were  $88.82 \pm 8.93$  and  $103.10 \pm 11.37$  mg/mL respectively were quite higher than that of the standard ascorbic acid. The extracts of *T. aestivum* were found to exhibit significant antioxidant and DPPH radical scavenging capacity of 1.2 mM and 1.8 mM ascorbic acid equivalents respectively (Lee *et al.*, 2009). The antioxidant activity of the sprout is greatly enhanced during the germination of seeds due to the rejuvenation of various metabolic processes several food industries have incorporated the germinating seeds in their products (Nemzer *et al.*, 2018).

#### α-Amylase inhibition test

The antidiabetic activity of the different sprouts was

evaluated by α-amylase inhibition capacity taking acarbose as standard. The dose-dependent variation of the inhibitory capacity of the extracts were compared with that of acarbose in Figures 4 and 5. The extracts of all the cereals in this study show relatively weaker scavenging power than that of the standard acarbose.

The half-maximal concentration (IC<sub>50</sub>) of the extracts inhibiting α-amylase enzyme was calculated and presented in Figure 4. In this study, we observed the highest activity by the extract of *T. aestivum* with the lowest IC<sub>50</sub> value of  $7.19 \pm 0.28$  followed by *H. vulgare* and *Z. mays* with IC<sub>50</sub> values of  $7.82 \pm 0.60$  and  $14.25 \pm 0.07$  mg/mL respectively. Acarbose was taken as a standard and exhibited the strongest inhibiting power with the lowest half-maximal concentration of  $0.43 \pm 0.03$  mg/mL which is depicted in the bar diagram Figure 6.

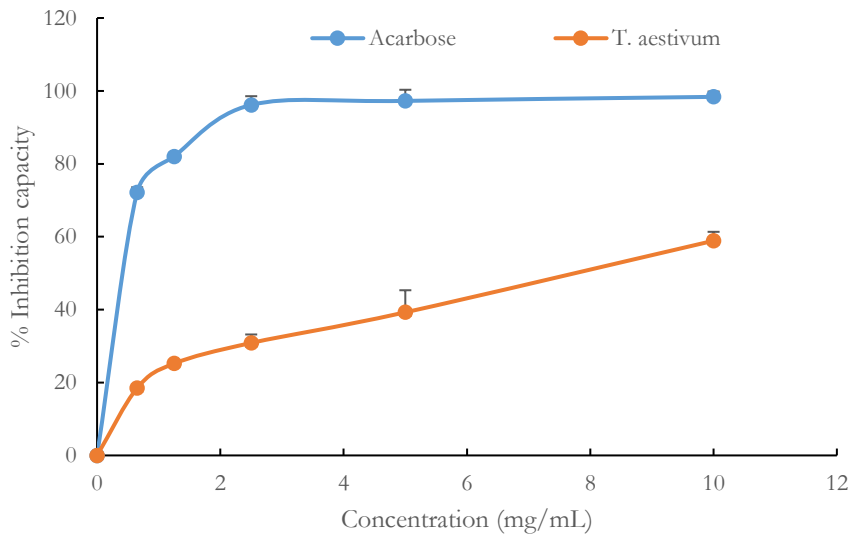


Figure 4. Dose-dependent variation of percentage scavenging capacity

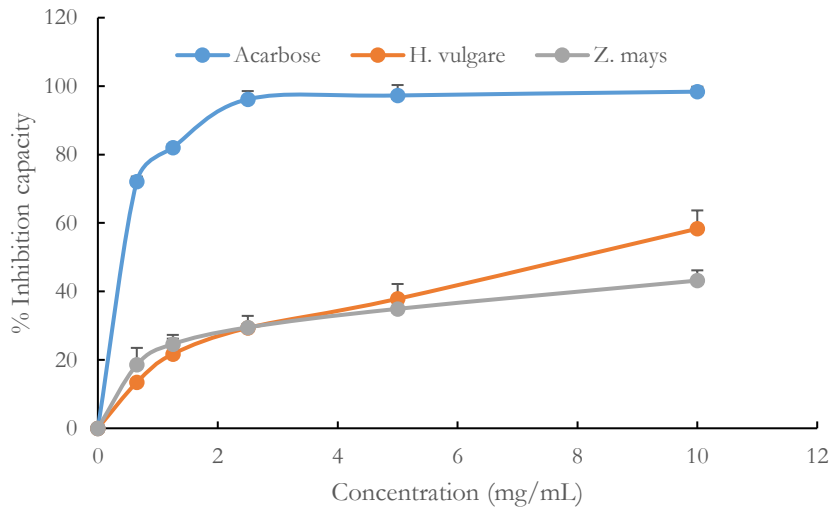


Figure 5. Dose-dependent variation of percentage scavenging capacity

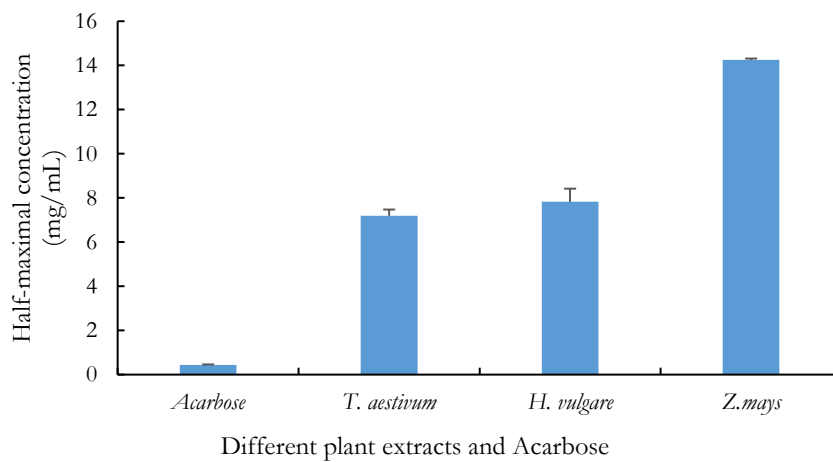


Figure 6. Half-maximal concentrations of the extracts and acarbose

The extract of dark-germinated sprouts of *H. vulgare* was found to exhibit good *in vitro*  $\alpha$ -amylase and  $\alpha$ -glucosidase enzyme inhibitory activities due to the higher concentration of phenolic compounds (Ramakrishna *et al.*, 2017). The water and ethyl acetate fractions of *T. aestivum* extract revealed superior  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition activities compared to other fractions. Through preparative HPLC of these fractions, two potent compounds: ferulic acid and  $\gamma$ -aminobutyric acid were isolated. Their structures were identified using FTIR, 1H-NMR, and 13C-NMR data (Jeong *et al.*, 2012).

## CONCLUSIONS

The ethyl alcohol extracts of *H. vulgare*, *T. aestivum*, and *Z. mays*, prepared by the maceration method, were identified to contain significant secondary metabolites on preliminary phytochemical screenings. The observed antioxidant capacity and toxicity towards brine shrimp nauplii followed the order *Z. mays* > *T. aestivum* > *H. vulgare*. The antioxidant activities were found to have a good correlation when plotted with IC<sub>50</sub> values. The extracts exhibited a moderate  $\alpha$ -amylase inhibitory activity. The findings of this study emphasize the antioxidant, toxicity, and antidiabetic capabilities of sprouts from commonly used cereals. Additionally, it underscores that incorporating young sprouts into diets could enhance nutritional supplements and open avenues for creating novel pharmaceuticals derived from natural resources.

## AUTHOR CONTRIBUTIONS

SKK: conceptualized the research project; GK and LNK: prepared the manuscript and analyzed the data.

## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

## DATA AVAILABILITY

The data of this study can be obtained from the corresponding author upon reasonable request.

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