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# Effect of Different Solvents Concentrations on Total Phenolics, Flavonoids and Antioxidant Activities of Wheatgrass (*Triticum aestivum*)

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## Abstract:

The purpose of this study was to investigate the effects of different solvent concentrations on the total phenolic, total flavonoid, and antioxidant activities of wheatgrass powder. The spectrophotometric method was used to analyze the total phenolic contents (TPC), total flavonoid contents (TFC), and antioxidant activities in terms of ferric-reducing antioxidant power (FRAP), DPPH radical scavenging activity, reducing capacity, and metal chelating activity. The solvents used were water, absolute ethanol, absolute methanol with aqueous ethanol, and aqueous methanol at different concentrations. The study's findings showed that, among all the solvents at different concentrations, ethanol at a 60% concentration appeared to be the most efficient solvent for extracting phenols and flavonoids from wheatgrass. It was also discovered to be the best extract, demonstrating the highest antioxidant activities. According to study findings, aqueous ethanol and methanol extracts at various concentrations displayed stronger antioxidant activity, phenolic, and flavonoid components than their absolute ones. The extraction of phenols and flavonoids from water extract was shown to be less efficient, and it also has lower antioxidant properties. Moreover, the findings of this study showed that positive and significant correlations were found between the total phenolic and flavonoid compounds and with antioxidant activities of wheatgrass extracts. In summary, it was discovered that ethanol and water mixed in a 60:40 ratio was the best solvent for extracting phenolic and flavonoid components from wheatgrass. It also proved to be the best solvent with the most antioxidant activity.

**Keywords:** wheatgrass, phenolic content, flavonoid content, antioxidant activity.

  

## INTRODUCTION

Wheat is one of the edible grains and cereal-grass crops that is grown most extensively worldwide. Young wheat plantlets (nine to twelve days old) are known as wheatgrass (*Triticum aestivum*). Since ancient times, wheatgrass, a remarkable microgreen, has been used to enhance human health. Wheatgrass contains large amounts of proteins, vitamins, minerals, enzymes, and bioactive compounds such phenolics, flavonoids, alkaloids, glycosides, steroids, carotenoids, tocopherols, saponins, lignins, and tannins (Akcan kardas and Durucasu, 2014; Benincasa *et al.*, 2015; Durairaj *et al.*, 2014; Kulkarni *et al.*, 2006). Since it contains these phytochemicals, it functions as a natural antioxidant agent for scavenging free radicals, lowering the danger of oxidative stress, and associated ailments. Scientific research has demonstrated that wheatgrass has a wide range of medicinal potential, including anti-cancerous activity, anti-inflammatory, anti-arthritic, anti-diabetic, and antibacterial qualities that inhibit the growth of microorganisms (Singhal et al., 2012; Shakya et al., 2014). (Nalini *et al.*, 2011; Sachin *et al.*, 2013; Sundaresan *et al.*, 2015). More study has revealed that it is beneficial for many other degenerative disorders, including thalassemia, distal ulcerative colitis, and hyperlipidemia (Padalia *et al.*, 2010, Marwaha *et al.*, 2004, Afroz *et al.*, 2015). Numerous studies have demonstrated the beneficial effects of frequent wheatgrass consumption on human health.

Several different solvents and processing techniques have been used to extract polyphenols from plant materials (Chavan *et al.*, 2001; Kashif *et al.*, 2011). Water, ethanol, methanol, acetone, chloroform, dimethyl sulfoxide (DMSO), and aqueous combinations of organic solvents are the common solvents used for the extraction. In many scientific experiments, bioactive compounds from fresh and dried wheatgrass powder were extracted using various organic solvents. In one study ethanol and methanol were used and found that methanol was more effective at extracting antioxidant compounds than ethanol(Ove *et al.*, 2021), while, in another study, ethanol extract was found to be the best solvent for the extraction of phenolic and flavonoid compounds than the aqueous extracts of wheatgrass (Kulkarni *et al.*, 2006), whereas in some studies, water solvent was used to extract phenolics and flavonoids from dried wheatgrass powder (Qamar *et al.*, 2018; Durairaj *et al.*, 2014). Other scientific evidence reported that chloroform was found superior to methanol for extraction of the flavonoids from wheatgrass and some studies have demonstrated that aqueous mixtures of organic solvents are the most efficient solvents for extracting phenolics and flavonoids from plant sources (Zendehbad *et al.*, 2014; Venkatesan *et al.*, 2019). These facts indicate that it is unclear which type of solvent is best for extracting phenolics and flavonoids from wheatgrass. The effect of extraction conditions (solvent type and concentration) on the recovery of bioactive components from wheatgrass is poorly supported by scientific data. Thus, to maximize the recovery of phenolic compounds from wheatgrass, our study gives information about the effects of water, absolute (ethanol, methanol), and aqueous mixes of (ethanol, methanol) solvents on phenolic flavonoid and antioxidant activities of wheatgrass.

## MATERIAL AND METHODS

### Collection of chemicals and reagents

All the chemical used in this study includes ethanol and methanol, quercetin, gallic acid, ascorbic acid, DPPH (1,1-diphenyl-2-picrylhydrazyl), folin-ciocalteau reagent, TPTZ (2,4,6-tripyridyl-S-triazine), ferric chloride, sodium carbonate, sodium hydroxide, potassium phosphate, aluminum chloride, ferrous chloride, ferrozine, potassium ferricyanide, sodium hydroxide, trichloroacetic acid, hydrochloric acid and other required chemicals of analytical grade were purchased from HIMEDIA Labs, India, and Merck India.

### Cultivation of wheatgrass

Wheat seeds (Sharbati cultivar) were purchased from the local market of Prayagraj, Uttar Pradesh, India. Wheatgrass was grown indoors until it was needed for the study's experiments. Three inches of a growing medium made up of three parts soil and one part compost were placed inside the clay pot. It was then uniformly coated with seeds that had been soaked overnight and a half-inch of soil was added on top of that. The soil was equally sprinkled with little amounts of water, and three to four hours of indirect sunlight were permitted each day for the growth of grass. The grass is chopped half an inch above the root on the tenth day when it is between six and nine inches tall (Jain and Argal, 2014).

### Preparation of wheatgrass powder

Fresh wheatgrass was cleaned and washed in water before being dried in a cabinet tray dryer for six hours at a temperature of 55 2 °C (Chemida, Mumbai, India). Dehydrated wheatgrass powder was produced after the dry material was ground to powder using a high-speed mixer (Sumeet Domestic Plus, M/s. Sumeet, Nashik, India). For additional analysis, the powder was sealed in metalized polyester polyethylene (MPE) and kept at 4 °C (Durairaj *et al.*, 2014).

### Extraction method

The powdered wheatgrass underwent extraction. The extraction process was carried out by using 100% methanol, ethanol, and water or methanol and ethanol in the range of 80%, 60%, and 20% along with distilled water (vol/vol) as an extraction solvent. Briefly, 20 ml of each solvent was combined with 1 g of sample. After being shaken at 140 revolutions per minute for 24 hours at room temperature, the sample mixtures were filtered using Whatman No. 1 filter papers. Before conducting a chemical analysis, filtered solvents were kept at 4 °C to prevent compound deterioration (Ove *et al.*, 2021).

### Estimation of total phenolic content (TPC)

  

The total phenolic content was determined using the Folin-Ciocalteu technique. 200 µl of sample extract was added to 1 ml of diluted Folin-Ciocalteu reagent (1:10 with distilled water). 800 ml of saturated sodium carbonate (7.5% w/v) was added after 4 minutes. The absorbance at 765 nm was measured after incubation at room temperature for two hours. Results were stated in terms of mg GAE/g of sample and the standard calibration curve used gallic acid (Li *et al.*, 2007).

**Estimation of total flavonoid content (TFC)**

The aluminum chloride ($AlCl_3$) colorimetric method established by Bahorun et al. (1996) was slightly modified to determine the sample's total flavonoid content. Following the procedure, 2 ml of 2% $AlCl_3$ in methanol was combined with 2 ml of the extract and allowed to stand the mixture at room temperature for 5 min. Following that, a reading at 420 nm was taken against a blank sample that contained a 2 ml extract solution and a 2 ml methanol solution. The data were represented in terms of mg QE/g of the sample and the standard calibration curve was made using quercetin.

**Estimation of antioxidant activity**

**Estimation of ferric reducing antioxidant power (FRAP) assay**

With a small modification to Benzie and Strain's (1996) methodology, the FRAP test was used to determine the sample's antioxidant activity. Acetate buffer with a pH of 3.6, 20 mmol/L ferric chlorides, and 10 mmol/L TPTZ composed of 40 mmol/L hydrochloric acids was used to make the FRAP reagent. The three solutions were combined in a ratio of 10:1:1 (vol:vol: vol). 3 ml of the FRAP reagent was added to 100 µl of the sample, and the mixture was thoroughly mixed. After 4 minutes, the absorbance at 593 nm was measured. FRAP values were expressed as mM $Fe^{2+}$ equivalents per g of sample.

**Estimation of DPPH radical scavenging activity**

The method described by Mansouri et al. (2005) was used to estimate the DPPH radical scavenging activity of wheatgrass extracts with a few minor modifications. According to the procedure, 50 ml of DPPH (2.5 mg DPPH in 100 ml of methanol) solution and 50 ml of the extract were combined. The mixture was mixed and let to sit for 30 minutes at room temperature and in the dark. At 517 nm, the absorbance was measured. As a control, DPPH solution without the test chemicals was utilized. This equation was used to determine the free radical scavenging activity percentage.

$$\% \text{ Scavenging Activity} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \text{ X } 100$$

**Estimation of reducing power activity**

  

Reducing power activity was performed according to the method described by Barros et al. (2007). Briefly, 2.5 ml of extracts together with 2.5 ml of 1% potassium ferricyanide and 2.5 ml of 200 mmol/L of Na-phosphate buffer (pH 6.6) were incubated for 20 minutes at 50 °C. After 20 minutes, 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at (1000 r/minute) for 8 minutes. After adding distilled water, the extracted supernatant was used to make up the 10 ml volume. Following that, 1 ml of 0.1% ferric chloride was added to the mixture, and the mixture's reading was measured at 700 nm using the standard ascorbic acid.

**Estimation of metal chelating activity**

The technique used to determine the sample's metal chelating capacity was somewhat modified from that previously described by Danis et al. (1994). Briefly, the reaction mixture contained 1 ml of extracts, 200 µl of 0.6 mM $FeCl_2$ in water, and 1800 µl of methanol. Reaction reagents without the extract or standard were used as a control. As a control, reaction reagents without the extract or standard were utilized. The mixture was shaken and allowed to sit at room temperature for 5 minutes. The mixture was then supplemented with 200 µl of 5 mM ferrozine in methanol, agitated once more, and incubated for an additional 10 minutes at room temperature. At 562 nm, the absorbance was calculated in comparison to a methanol blank. The equation below was used to compute the percentage chelating activity.

$$\% \text{ Chelating Activity} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \text{ X } 100$$

**Statistical analysis**

Statistical analysis was carried out by using one-way ANOVA followed by Tukey's multiple comparison tests with Graph Pad PRISM version 5.01. Values are presented as mean ± standard deviation. All the values with $P < 0.05$ were considered statistically significant. The linear regressions and Pearson correlation coefficient (r) and probability-value (P) were used to show correlation and their significance by using Graph Pad PRISM.

**RESULTS AND DISCUSSION**

**Total phenolic content (TPC)**

Plants are rich in natural antioxidants. Among these, the phenolic compounds can scavenge free radicals, due to the presence of superoxide and hydroxyl radicals through oxidation reactions. Table 1 shows the total phenolic content of different solvents at various concentrations. TPC was observed for methanolic extracts at different concentrations were 18.97±1.00 mg GAE/g (100% methanol), 27.65±0.84 mg GAE/g (80% methanol), 32.16±0.97 mg GAE/g (60% methanol), 12.97±0.99 mg GAE/g (20% methanol) whereas for ethanolic extracts TPC values were found 19.23±1.16 mg GAE/g, 34.42±1.09 mg GAE/g, 38.49±0.95 mg GAE/g, 25.32±0.98 mg GAE/g for 100% ethanol, 80% ethanol, 60% ethanol, 20% ethanol respectively and 17.95±0.99 mg

  

GAE/g for water extract of wheatgrass. The results showed that the maximum TPC (38.48±0.95 mg GAE/g) was found in 60% ethanolic extract and it is significantly different (P<0.001) from the aqueous water extract, while the minimum TPC (17.95±0.99 mg GAE/g) was reported in water extract of wheatgrass, among all the different extracts and concentrations. This is possible since more polar solvents such as aqueous ethanol/ methanol extracted a higher amount of phenolic compounds as compared to absolute ethanol/methanol. Various studies revealed that higher polar solvents were more effective for extracting phenolic compounds from plant sources than less polar solvents (Galanakis *et al.*, 2013). Venkatesan et al. (2019) reported that ethanol at 20% with water could extract the highest TPC from *Pinus densiflora* bark extract, whereas Zhou and Yu (2004) stated that among different solvents tested, 50% acetone extracts contained the greatest level of total phenolics from wheat. This is in concurrence with our results, in the present study, we employed extraction of wheatgrass to get maximum TPC using an aqueous mixture of ethanol, methanol absolute ethanol, methanol, and water extracts and observed that 60% ethanol: water solvent mixture was found most excellent for maximum extraction of phenolic compounds.

**Total flavonoid content (TFC)**

Flavonoids are naturally occurring compounds of the polyphenolic groups, and are especially found in plants. Flavonoids always are obligated an excellent impact on human health; flavonoid derivatives have revealed a wide range of antioxidant, antiviral, anti-inflammatory, antibacterial, allergic, and anticancer activities. Table 1 presents the total flavonoid content of wheatgrass extracts. The range of TFC in extracts is from 10.56±1.15 mg QE/g to 30.64±0.85 mg QE/g. 60% aqueous ethanolic extract (30.64±0.85 mg QE/g) showed the highest total flavonoid content while 80% ethanolic extract (27.46 ± 1.49) was found as second and 60% methanolic extract (25.26 ± 1.03) found third-best solvents for TFC extraction and these values are significantly different (P<0.001) from the aqueous water extract. The results of the study indicated that the highest amount of total flavonoid content of wheatgrass extracts was observed in aqueous mixtures of solvent followed by absolute one this is because aqueous methanol and aqueous methanol extracts of wheatgrass contained more polyphenols as compared to their absolute extract. The findings of our study are in good agreement with Afshar et al. (2019) who found in their study that there is a positive correlation between phenolic content and flavonoid content, and antioxidant activity. Accordingly, the lower phenolic content led to a decrease in the flavonoid content of wheatgrass extracts, therefore, the result suggested phenolics and flavonoids compounds are latent sources of natural antioxidants and maybe the foremost contributors to the antioxidative potential and protective properties of the *in vivo* and *in vitro* oxidative damages.

Table 1 Effect of different solvents concentrations on total phenolic and flavonoid content of wheatgrass extracts

  

| S. no. | Extracts name | Extraction solvents | TPC (mgGAE/g) | TFC (mgQE/g) |
|---|---|---|---|---|
| 1 | M (100% Methanol) | Methanol | $18.97 \pm 1.00^{ns}$ | $11.35 \pm 1.15^{ns}$ |
| 2 | M80 (80% Methanol) | Methanol and water (80:20 vol/vol) | $27.65\pm 0.84^{***}$ | $21.65 \pm 0.86^{***}$ |
| 3 | M60 (60% Methanol) | Methanol and water (60:40 vol/vol) | $32.16\pm 0.97^{***}$ | $25.26 \pm 1.03^{***}$ |
| 4 | M20 (20% Methanol) | Methanol and water (20:80 vol/vol) | $22.97\pm 0.99^{***}$ | $16.54 \pm 1.21^{***}$ |
| 5 | E (100% Ethanol) | Ethanol | $19.23 \pm 1.16^{ns}$ | $13.74 \pm 0.96^{***}$ |
| 6 | E80 (80% Ethanol) | Ethanol and water (80:20 vol/vol) | $34.42\pm 1.09^{***}$ | $27.46 \pm 1.49^{***}$ |
| 7 | E60 (60% Ethanol) | Ethanol and water (60:40 vol/vol) | $38.49 \pm 0.95^{***}$ | $30.64 \pm 0.85^{***}$ |
| 8 | E20 (20% Ethanol) | Ethanol and water (20:80 vol/vol) | $25.32 \pm 0.98^{***}$ | $18.94 \pm 1.00^{***}$ |
| 9 | W (100% Water) | water | 17.95 ± 0.99 | 10.56 ± 1.15 |

Values are expressed as gallic acid equivalent (mgGAE/g) extracts and quercetin equivalent (mgQE/g) extracts. Values are mean ± SD from three independent experiments. $^{***}$ ($P<0.001$), $^{ns}$ (non-significant), compared to water extract (A100).

**Antioxidant activity of wheatgrass extracts**

**Antioxidant activity (AOA) by FRAP assay**

The antioxidant capacity was determined by measuring the reducing power of extracts to ferric into ferrous ions and it reflects the antioxidant capability of the test extract, and the reducing power of extracts was confirmed by the changing in color from yellow to shades of greens and blues (Durairaj *et al.*, 2014).

  

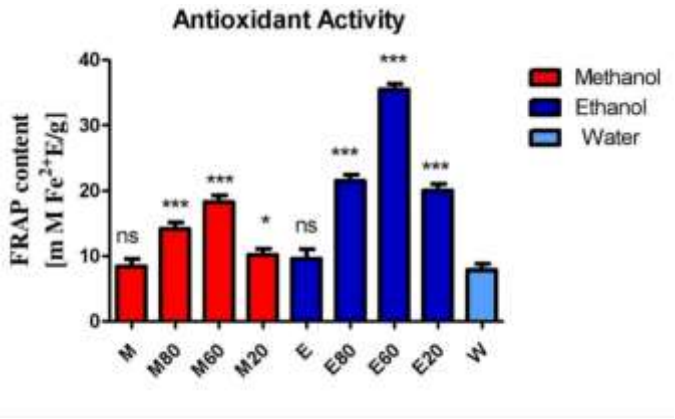


Figure 1 Antioxidant activity of wheatgrass extracts as measured using the FRAP assay

Figure 1 presents the FRAP value of different solvents at different concentrations. The FRAP value of extracts decreased in the following order, 60% ethanolic extract > 80% ethanolic extract > 20% ethanolic extract > 60% methanolic extract > 80% methanolic extract >20 % methanolic extract >100 % ethanolic extract >100 % methanolic extract > water extract. FRAP values were observed for methanolic extract at different concentrations ranging from 8.41±1.15 mM $Fe^{2+}$E/g to 18.25±1.05 mM $Fe^{2+}$E/g while, for ethanolic extract at different concentrations FRAP values were estimated ranging from 9.59 ±1.41 mM $Fe^{2+}$ E/g to 35.45 ±0.86 mM $Fe^{2+}$ E/g and 7.84±0.98 mM $Fe^{2+}$E/g for aqueous water extract of wheatgrass. The results showed that the maximum FRAP value (35.45±0.86 mM $Fe^{2+}$E/g) was found in 60% ethanolic extract of wheatgrass and the value is significantly different ($P<0.001$) from the water extract, while the minimum FRAP value (7.84 ±0.98 mM $Fe^{2+}$E/g) was reported in the water extract.

**Radical scavenging activity (RSA) using DPPH radical**

The DPPH radical scavenging method was used to determine the antioxidant activity of the extracts in this study. This method is based on the reducing ability of antioxidants toward DPPH and is widely used to evaluate the antioxidant activity of phenolic compounds of extract.

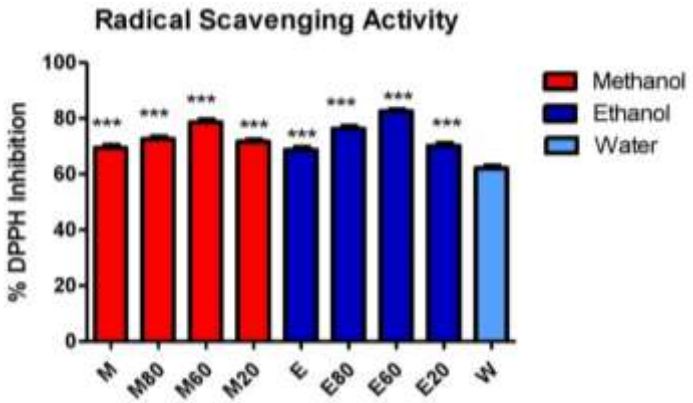


Figure 2 Scavenging effects of wheatgrass extracts on DPPH radical

  

Figure 2 presents the effects of different solvent extracts on DPPH radical scavenging activities, which are determined based on the hydrogen-donating ability of extracts. The recorded mean value of DPPH radical scavenging activity for methanolic extracts at different concentrations were 69.41± 1.04%, 72.53±1.02%, 78.56±1.13%, 71.62±0.75% respectively, while 68.65±1.18%, 76.20± 1.02%, 82.53±0.77%, 70.15±1.09% respectively, for ethanolic extracts, and 62.12±1.03%, for water extract of wheatgrass. 60% ethanolic extract (82.53±0.77%) was the most potent for DPPH radical scavenging activity, while water extract (62.12±1.03%) depicted the lowest DPPH radical scavenging activity. The findings of the study revealed that the extracts obtained using high-polarity solvents were considerably more effective radical scavengers than those using fewer polarity solvents, indicating that phytochemicals of different polarities could be present in wheatgrass. A change in solvent polarity alters its ability to dissolve a selected group of bioactive compounds and influences the antioxidant activity estimation (Zhou and Yu, 2004). The DPPH radical scavenging activity of extracts decreased in the following order, 60% ethanolic extract > 60% methanolic extract > 80% ethanolic extract > 80% methanolic extract > 20% methanolic extract >20 % ethanolic extract >100 % methanolic extract >100 % ethanolic extract > water extract. In our study, this trend was similar to that observed for the content of phenolics and flavonoids, indicating that the phenolics and flavonoids act as an antioxidant as well as pro-oxidants and are highly efficient scavengers and neutralizers of high oxidizing molecules (Arora *et al.*, 2000). These bioactive compounds are strongly correlated with antioxidant activity. The results of the study are in agreement with Turkmen et al. (2006) who observed that the content of polyphenols in the extracts of black and mate tea correlates with their antioxidant activity, confirming that polyphenols act as an antioxidant likely to contribute to the radical scavenging activity of the plant extracts. Similar results have also been reported for different plants by various studies (Katalinić *et al.*, 2004; Maksimović *et al.*, 2005; Yu *et al.*, 2005).

**Reducing power activity**

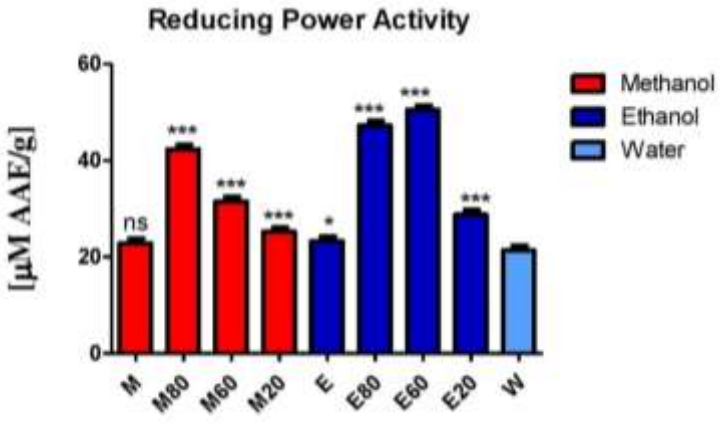


Figure 3 Reducing power activity of wheatgrass extracts

  

The reducing property of the cell reinforcement depends on the ability of binding transition metal ion catalysts, giving a hydrogen atom to the free radical, and along these lines balancing out them and breaking the free radical chain and radical scavenging potential have been claimed to explain the antioxidant activities (Jamuna *et al.*, 2011). Figure 3 represents the reducing properties of varying concentrations of different solvent extracts of wheatgrass. The reducing capacity was found in the following order, 60% ethanolic extract > 80% ethanolic extract > 80% methanolic extract > 60% methanolic extract > 20% ethanolic extract >20 % methanolic extract >100 % ethanolic extract >100 % methanolic extract > water extract. The results showed that the maximum reducing capacity (50.46±0.94 umolAAE/g) was found in 60% ethanolic extract and the value is significantly different (*P<0.001*) from the water extract other, while water extract showed the least reducing capacity (21.36±1.08 umolAAE/g). These results indicated that aqueous ethanol was a better solvent for the extraction of reducing Fe ion compounds from wheatgrass and it was found in our study that the aqueous ethanolic and methanolic extracts may contain higher levels of antioxidant compounds with high reducing capacity than their absolute ones. Kobus-Cisowska et al., (2020) found that extracts with high reducing power contained the highest amounts of total phenolic and flavonoid compounds. The concentration of bioactive compounds is mostly correlated to reducing power. Various studies have shown that bioactive compounds act as an antioxidant by various mechanisms through the sequencing of the free radicals, by reduction and oxidation process by donating electrons reflecting the reducing power. It has been reported that reducing power is generally associated with the presence of reductions, which have been shown to exert antioxidant action by breaking the free radical chain via the donation of a hydrogen atom (Zhang *et al.*, 2018). A similar trend was found in this study therefore, it may be concluded that extracts have a high level of bioactive compounds their reducing capacity will also be high or extracts have high levels of reduction capacity which are considered to be an abundant amount of polyphenols.

**Metal chelating activity**

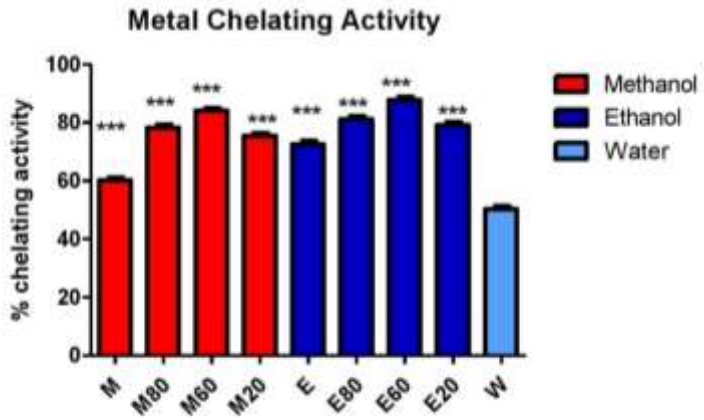


Figure 4 Metal chelating activity of wheatgrass extracts against $Fe^{2+}$

  

Metal ions can start lipid peroxidation; initiate a chain reaction and cause malignant growth and joint torment (Halliwell *et al.,* 1995). Several studies revealed that wheatgrass showed a greater ability to chelate ferrous ions. This may be contributed to a large amount of chlorophyll which is one of the most efficient natural chelating molecules. Figure 4 represents the metal chelating activity of extracts. Metal chelating activity was expressed as % inhibition of metal ions. Observed values of metal chelating activity for methanolic extracts at different concentrations were 60.21 ± 0.98%, 78.25± 1.09%, 84.11± 0.97%, 75.54± 0.93% respectively, while for ethanolic extracts at different concentrations values were 72.65±1.12%, 81.16± 1.11%, 87.79± 1.05%, 79.12± 1.10% respectively, and 50.21±1.07%, for aqueous extract of wheatgrass. Results of the study demonstrated that 60% ethanolic extract was determined highest (87.79± 1.05%) ion chelating activity and significantly different ($P<0.001$) from aqueous water extract, while water extract was found minimum (50.21±1.07) metal chelating activity. The decreasing order of metal chelating activity was found as 60% ethanolic extract > 60% methanolic extract > 80% ethanolic extract > 20% ethanolic extract > 80% methanolic extract >20 % methanolic extract >100 % ethanolic extract >100 % methanolic extract > water extract.

**Correlation analysis between total phenolic content, flavonoid content, and antioxidant activities**

Pearson's correlation coefficient was applied to estimate the relationship between the total phenolic, flavonoid content, and antioxidant activities shown in Table 2. The total phenolic content depicted a significant positive correlation with antioxidant activities including FRAP (r=0.90, $p<0.001$), reducing capacity (r=0.90, $p<0.001$), and metal chelating activity (r=0.83, $p<0.01$). The flavonoid content exhibited a highly significant positive correlation with antioxidant activities using FRAP assay(r=0.89, $p<0.01$), reducing capacity (r=0.91, $p<0.001$) and metal chelating assays (r=0.87, $p<0.01$), confirming that the phenolic and flavonoid compounds contributed significantly to the antioxidant activities of the wheatgrass extracts. Results revealed that total flavonoid content was strongly correlated with antioxidant activities of wheatgrass extracts in terms of reducing capacity and metal chelating assays than total phenolic content, whereas total phenolic content was strongly correlated with the FRAP assay. Various scientific studies have reported that different types of bioactive compounds such as polyphenols and flavonoids are present in plants and plants products, contributing its antioxidant activities and properties, which was confirmed by this study, results of the study revealed that total phenolic and flavonoids content was positive significantly correlated with the antioxidant activities of the wheatgrass extracts, which were assessed by the different methods. The results of the current study are in good agreement with those of other studies that revealed a relationship between antioxidant activity and total phenol and flavonoid content. In other research, DPHH, FRAP, BTS, reducing capacity, and metal chelating assays have shown linear relationships between phenolic components and antioxidant capacity in a variety of plants and herbs (Rezaei and Ghasemi Pirbalouti, 2019; Gan *et al.,* 2017; Osman *et al.,* 2021).

  

Table 2 The correlation coefficient among total phenolics, flavonoids, and various antioxidant activities

| | FRAP assay | Reducing capacity assay | Metal chelating assay |
|---|---|---|---|
| TPC | 0.90*** | 0.90*** | 0.83** |
| TFC | 0.89** | 0.91*** | 0.87** |

Correlations are significant at the **0.01 and ***0.001 levels

Linear regression analysis was used to further assess the contributions of phenolic and flavonoid components to the antioxidant activity (DPPH radical scavenging antioxidant activity) of wheatgrass extracts. Figure 5 demonstrates a linear, positive correlation ($R^2$=0.97, P0.001) between total phenolic and total flavonoids, demonstrating that flavonoids are mostly made up of phenolic units. The phenolic and flavonoid compounds, which are the main bioactive compounds, significantly contributed to the antioxidant capacity of the wheatgrass extracts, as shown by linear and positive responses between total phenolic and total flavonoids with DPPH assay ($R^2$=0.86, P0.001) ($R^2$=0.88, P0.001), respectively.

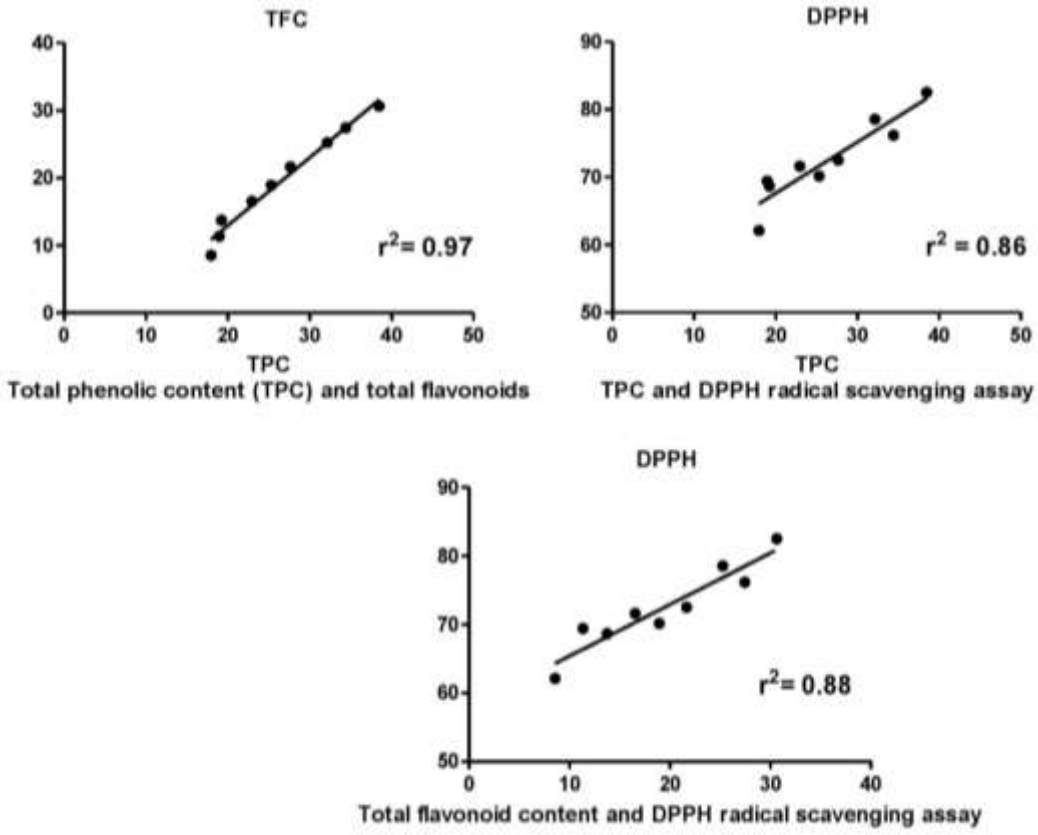


Figure 5 Linear regression between total phenolic, total flavonoids, and antioxidant activity by DPPH assay

  

## CONCLUSION

60% aqueous ethanolic extract was shown to be the most effective solvent for the extraction of phenolic and flavonoid components, whilst water extract exhibited the least effectiveness. Antioxidant assays in terms of DPPH, FRAP, metal chelating activity, and reducing capacity indicated that the wheatgrass extracts obtained from 60% ethanol have the highest antioxidant activity and are significantly different ($P<0.001$) from water extract, while water, absolute ethanol, and methanol extracts showed less antioxidant activities compare to their aqueous ones. Therefore, our results clearly showed that the extraction of phenolic and flavonoid compounds and their antioxidant activities are significantly affected by solvent combinations. Moreover, the results of the study indicated that the antioxidant activities of wheatgrass extracts positively correlate with total phenolic and flavonoid compounds. Hence, it could be used as a natural antioxidant agent in various supplements and nutraceuticals.

### Conflict of interest

The authors have no conflicts of interest.

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