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TOTAL PHENOLICS AND ANTIOXIDANT CAPACITY OF THE WHITE AND BROWN TEFF [*ERAGROSTIC TEF* (ZUCCAGNI) TROTTER] VARIETIES CULTIVATED IN DIFFERENT PARTS OF ETHIOPIA

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ABSTRACT. This study was aimed to determine the total phenolics contents and antioxidant capacity of white and brown teff grain samples. The free total polyphenolic content (TPPC), bound TPPC and total flavonoids content ranged from 62.1–129.9, 84.6–189.6 mg GAE/100 g and 84.4–195.1 mg QE/100 g, respectively, in white teff samples, and, 118.6–196.7, 141.1–195.1 mg GAE/100 g and 97.8–202.5 mg QE/100 g, respectively, in brown teff samples. Besides, the free 2,2-diphenyl-1-picrylhydrazyl (DPPH) and bound DPPH ranged from 74.8–98.3, 77.1–99.9 mg AAE/100 g, respectively, for white teff samples, and, 68.7–93.1 and 71.2–99.4 mg AAE/100 g, respectively, for brown teff samples. This study revealed that total phenolics content was higher in brown teff samples than white teff samples and is in agreement with other reports. However, their DPPH scavenging activities were nearly equal, indicating that both varieties of teff are relevant for human nutrition and health. Furthermore, the dendrogram has shown sharp separation of the samples based on their origin and variety related to the total phenolics parameters. Hence, findings of this study can help consumers appreciate the nutritional value of white and brown teff grains; provide them guidance for teff purchase and production.

KEY WORDS: Teff, Phenolics, Gallic acid, Ascorbic acid, Anti-oxidant, DPPH

INTRODUCTION

Cereals are extremely versatile foodstuffs and are processed into a very wide range of traditional food and beverage products [1]. Teff [*Eragrostis tef* (Zuccagni) Trotter] is an ancient tropical cereal that has been originated in the northern Ethiopian highlands [2]. It is a member of the Poaceae family that can be considered as a low-risk crop since it can be cultivated and thrived in a wide range of ecological conditions including under tough environmental conditions where many other cereals fail [3]. Like sorghum and maize, teff is a C4 plant which utilizes CO_2 very tefficiently during photosynthesis [4]. Teff tolerates anoxic situations better than maize, wheat and sorghum and is resistant to many pests and diseases during storage [5, 6]. It has the potential of growing in every part of the world [6]. It is a predominant source of nutrients like minerals, amino acids, dietary fibers, proteins, dietary polyphenols, starch, carbohydrates and vitamins [7], volatiles like aldehydes, ketones and alcohols [8], and fatty acids [9].

Teff is the major food crop native to Ethiopia [4], in which domestication took place between 4000 to 1000 BC [10]. Recently, there has been increasing interest and global demand of teff throughout the world due to its perceived better nutritional quality which plays an important role in the food security compared to other cereal grains [8, 11, 12]. Its cultivation has been successfully adapted to other parts of the world like Australia, Cameroon, Canada, China, India, Netherlands, South Africa, the UK, Uganda and the USA [13].

Teff grain is one of the smallest in cereals comprised of pericarp, endosperm and germ layers, and its flour is a rich source of bioactive compounds like phenolics [8, 14]. Based on their color, there are two main types of teff grains: white teff and brown/red teff, both belonging to the group of millets [15]. Epidemiological studies and their meta-analyses have consistently reported that

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high consumption of whole grain is very crucial to lower risk of chronic diseases and mortality, obesity, type 2 diabetes, cardiovascular disease, certain cancers [16-18], and plays a role in body weight management and digestive health [19]. Among the whole grain components, the phenolic compounds have been revealed great health benefits by preventing chronic diseases, determine the sensory properties of the whole grain-based products and being the major predictors of biological functions [19, 20]. The key role and wide range of biological and pharmacological properties of phenolics in cereals arise due to their strong anti-oxidant, anti-inflammatory, anti-carcinogenic and anti-microbial potential properties [21, 22]; anti-allergic, anti-viral, anti-thrombotic, hepatoprotective, food additive, signaling molecules [22] and boosting the body's immune system [23].

Polyphenols and flavonoid rich natural diets including teff exhibit comparatively high antioxidant activity which fostered high interest in nutrition and food science [24]. This is due to their conjugated-electron system enabling them to donate electrons or hydrogen atoms from the hydroxyl moieties to free radicals, which reveal free radical inhibition, peroxide decomposition, metal inactivation or oxygen scavenging in biological systems and prevent oxidative disease burden [25]. However, in terms of reaction stoichiometry and reaction kinetics, antioxidant efficacy might vary significantly. This is determined by structural characteristics such as the number and positions of the hydroxyl moieties on the ring systems, the degree to which the unpaired electron in the oxidized phenolic intermediate can delocalize across the molecule [26]. The natural phenolic and flavonoid compounds are the most abundant plant secondary metabolites that hold an aromatic ring bearing at least one hydroxyl group [27, 28]. This principally protect against UV radiation, competitive warfare [22], degenerative diseases (heart diseases and cancer) in which reactive oxygen species are involved [1]; acting as phytoalexins, attractants for pollinators, and contributors to plant pigmentation [29]. They are also responsible for the organoleptic properties of cereal products [22].

Due to the exclusive chemical compositions and gluten-free property, teff is getting acceptance as medicinal ingredient to aid people with celiac disease and has revealed in vitro anti-oxidant activities, can improve the hemoglobin level in human body because it has high levels of iron, help to prevent malaria and incidence of anemia, it has a lower glycemic index and can avoid diabetes [8, 30]. Because of this reason, the natural functional bioactive compounds like phenolics present in cereals have received an increasing interest from researchers owing to their safety, nutritional efficacy and potential pharmacodynamics [31].

The majority of teff research to date has been in the genomics sector, with a focus on improving teff breeding, particularly in terms of grain production and grain color quality [32]. Nevertheless, there has been a surge in interest in teff phenolics in the last decade, starting with antioxidant potential and moving ahead to analyze phenolic profiles [15, 33, 34]. As compared to the common cereals like rice, wheat, maize and the positive health outcomes of the teff consumption, however, the information available about its phenolic content and the related antioxidant potential is very few, scattered and not comprehensively addressed. Hence, the objectives of the study were: (1) to optimize the suitable extraction procedures of the teff grain samples, (2) to determine the total polyphenolics contents in the white and brown teff grain samples using Folin-Ciocalteu assay, (3) to determine the total flavonoids contents in the white and brown teff grain samples using aluminum chloride assay, and (4) to investigate the antioxidant capacity of phenolic compounds in the white and brown teff grain samples cultivated in different parts of Ethiopia using DPPH assay.

EXPERIMENTAL

Apparatus and instrument

Electronic balance (Model: ARA520, China), grinder (High speed multifunctional grinder, Shanghai, China), water deionizer system (Model: Molatom510d, Molewater System Co., Ltd),

ultrasonic cleaner (Model: K240HTD, China), centrifuge (Model: 80-2, China), pH meter (Eutech Instruments, pH 700) were used throughout the experiment. UV–VIS–NIR spectrometer (Model: Lambda 950, Perkin Elmer, UK) was used for measuring the absorbance of the prepared standards and samples.

Chemicals

Folin-Ciocalteu's reagent (BDH Chemicals Ltd, Poole, England), DPPH (Sigma Aldrich, Steinheim, Germany), gallic acid (Carlo Erba reagents, France), ascorbic acid (BDH Chemicals Ltd, Poole, England), quercetin (Sigma Aldrich, Steinheim, Germany), aluminium chloride hexahydrate (97%, *AvonChem* Ltd, Banbury, Oxon, UK), sodium hydroxide (Scharlau Chemie S.A., European Union), potassium acetate (BDH Laboratory Supplies, Poole, England), methanol (≥99.9%, Carlo Erba, Italy), sodium carbonate (Research Lab Fine Chem Industries, Mumbi, India) were used as received. Deionized water was used throughout the experiment.

Sample collection and preparation

There are nineteen significant teff production zones in Ethiopia, including the Amhara Region's North Shewa, South Wollo, and East Gojam, the Oromia Region's East Shewa, Arsi, and West Arsi, and the Southern Nations Nationalities and Peoples (SNNP) Region's Haddiya zone, among others [35]. In the present study, 18 white teff grain samples and 18 brown teff grain samples, totalling 36 teff grain samples from these three regions and 18 districts, were collected as indicated in Table 1.

The study area is shown in Figure 1. For statistical analysis, sampling regions for Amhara, Oromia, and SNNP Regions were represented by group 1, group 2, and group 3, respectively. All samples containing 500 g of each teff grain were collected from local markets of the respective districts during December 18, 2020 to January 18, 2021 and stored in paper bags at room temperature (22 ± 2 ºC). Immature seeds, straw, and soil were sieved out of the teff grains. Then, using an electronic grinder, material was ground to a mesh size of 300 μm for extraction.

Extraction of free and bound phenolics fractions

Free phenolics were extracted using the method reported by Sumczynski *et al.* [36] with minor modifications. Briefly, teff flour (1.0 g) was added to 10 mL of extraction solvent (methanol and deionized water in the ratio of 80:20), and soaked for 60 min. The phenolics were extracted from the wetted sample in an ultrasonic bath for 30, 40 and 60 min at 40 ºC for the optimization purpose and the mixture was centrifuged at 4000 rpm for 20 min. This procedure was repeated once more and the supernatants from the two extracts were combined and filtered through a nylon membrane syringe filter having a 0.45 μm pore size filter. Then, the pH of the supernatant was adjusted at 4.5–5.0 by using 0.6 M HCl. This extract solution was analyzed as a free phenolics fraction.

After the free phenolics extraction, obtained residues were rewashed twice with 10 mL of deionized water to extract bound phenolic compounds. Water was then removed and samples were blended with 10 mL of 0.4 M NaOH for 30, 40 and 60 min to liberate ester or ether linked phenolics in an ultrasonic bath. The mixture was centrifuged at 4000 rpm for 20 min. This procedure was repeated once more. The supernatants from the two extracts were combined and adjusted to pH 4.5–5.0 by using 0.6 M HCl and was used as a bound phenolics fraction [37].

The extraction of total phenolics was studied at 30, 40 and 60 min. It was found that 60 min was the optimized time for the extracts of free and bound polyphenolics in the brown teff sample and bound polyphenolics in the white teff sample whereas 40 min was the optimized time for the free polyphenolics in the white teff samples based on the content determined.

Determination of total polyphenolics content by Folin–Ciocalteu assay

The Folin–Ciocalteu assay was performed to estimate the total polyphenolic content in the extracts following the procedure of Ibrahim *et al.* [38] with some modifications. Briefly, 0.5 mL of the extract was mixed with 1 mL of Folin–Ciocalteu reagent and 5 mL deionized water. After 5 min, 5 mL of 8% Na_2CO_3 solution was added and incubated in dark for 2 h at room temperature before the absorbance was taken at 760 nm. Similarly, a calibration curve was constructed using gallic acid standard over a concentration range of 1, 5, 10, 20, 30, 40, 60, 80, 100, and 120 mg/L and the result was reported as milligrams of gallic acid equivalent (mg GAE/100 g of flour on dry basis) of the samples. All the determinations were carried out in triplicate.

Table 1. Sampling areas and sample ID of the white and brown teff grains (*Eragrostis tef* (Zuccagni) Trotter)) varieties cultivated in Ethiopia.

No.	Geographical region	Administrative zone	District	Variety of teff	Sample ID
1	Group 1	North Shewa	Minjar Shenkora	White	AW1
				Brown	AB19
			Tar Mahber	White	AW2
				Brown	AB20
			Antsokiyana Gemza	White	AW3
				Brown	AB21
			Ankober	White	AW4
				Brown	AB22
		South Wollo	Dessie Zuria	White	AW5
				Brown	AB23
			Were Ilu	White	AW6
				Brown	AB24
			Legahida	White	AW7
				Brown	AB25
		East Gojam	Goncha Siso Enese	White	AW8
				Brown	AB26
2	Group 2	East Shewa	Boset	White	OW9
				Brown	OB27
			Adama Zuria	White	OW10
				Brown	OB28
		Arsi	Jeju	White	OW11
				Brown	OB29
		West Arsi	Negelle Arsi	White	OW12
				Brown	OB30
		East Shewa	Ada'a	White	OW13
				Brown	OB31
			Bishoftu	White	OW14
				Brown	OB32
			Gimbichu	White	OW15
				Brown	OB33
			Dugda	White	OW16
				Brown	OB34
3	Group 3	Haddiya	Soro	White	SW17
				Brown	SB35
			Gomibora	White	SW18
				Brown	SB36

Note: Amhara region = Group 1, Oromia region = Group 2, SNNP region = Group 3, ID = Identification.

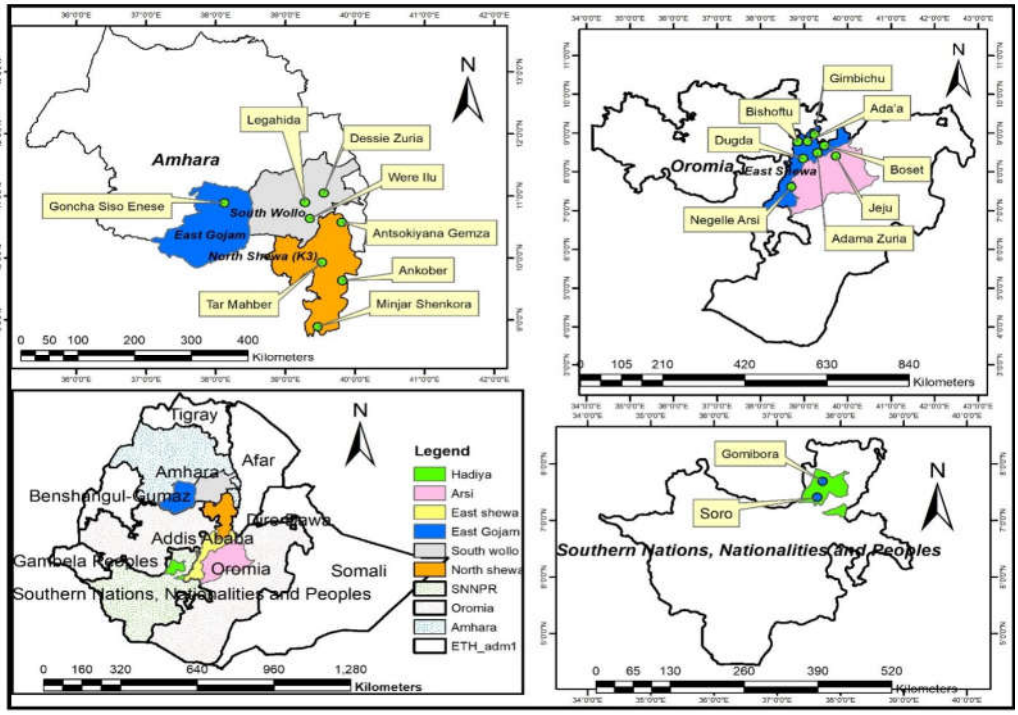

Figure 1. Map of the regions, administrative zones and districts showing the sampling area of teff.

Determination of total flavonoid content as quercetin equivalent

The total free flavonoid contents of the white and brown teff extracts were successfully determined as per the procedure reported by Abozed *et al.* [39] with some modifications. Briefly, 1 mL of the extract solution or quercetin (1–120 mg/L) was mixed with 0.2 mL of 10% (w/v) $AlCl_3 \cdot 6H_2O$ solution in methanol followed by the addition of 0.2 mL (1 M) potassium acetate and 6 mL of deionized water. The mixture was incubated for 60 min at room temperature. Then, measurement of the absorbance was conducted at 430 nm against the blank. The outcome data were expressed as milligrams of quercetin equivalents per 100 g (mg QE/100 g of flour on dry basis) of the samples extract by using the calibration curve equation and each sample was analyzed in triplicate.

Determination of antioxidant activities of the white and brown teff samples using DPPH assay

The antioxidant activity of the white and brown teff sample extracts were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity method reported by Haile *et al.* [40] with some modifications. A mass of 0.04 g of DPPH was dissolved with little amount of methanol in a 200 mL volumetric flask. After the DPPH was fully dissolved, the flask was filled up to the mark using methanol to get a concentration of 200 mg/L solution. The control was measured using 4 mL of methanol and 2 mL of DPPH solution. Besides, a stock solution of ascorbic acid (400 mg/L) was prepared by dissolving 0.04 g of ascorbic acid in 100 mL of volumetric flask using methanol. A calibration curve was established by preparing different

concentrations, 50.0, 25.0, 12.5, 6.25 and 3.125 mg/L, from the stock solution. From each standard solution of ascorbic acid a volume of 1 mL was transferred in to five different 25 mL volumetric flasks and to each flask 4 mL of methanol and 2 mL of DPPH solution was added and then the solution was incubated in the dark at room temperature for 40 min. Finally, the absorbance was measured at 517 nm. For the samples, a 1 mL portion of the extract was mixed with 4 mL of methanol and 2 mL of DPPH solution. The mixture was kept in the dark at room temperature for 40 min. It was noted that the bound phenolics extract has been centrifuged for 5 min at 3000 rpm and the absorbance of the supernatant was measured at 517 nm. The results were expressed as milligrams of ascorbic acid equivalent/100 g (mg AAE/100 g of flour on dry basis) by means of calibration curve. Each sample was analyzed in triplicate.

Statistical analysis

Results were presented as mean ± SD on dry basis from triplicate measurements. Statistical data analysis was performed using Minitab 17 software. One-way analysis of variance (ANOVA) was performed to test the presence of significant differences ($\alpha = 0.05$), in the mean concentration of total polyphenolics, total flavonoids and total antioxidant activities among the sampling regions (groups) and between the two varieties of teff. Tukey's Honestly Significant Difference Post Hoc test was used to check if there were significant differences among the three regions. Hierarchical cluster analysis (HCA) was performed using Euclidean distances as a measure of similarity to show the natural groupings of samples. Pearson correlation has been applied to observe the relationship between the total polyphenolics, total flavonoids and total antioxidant activities in both varieties of teff.

RESULTS AND DISCUSSION

The extracts of free and bound phenolics for both the white and brown teff samples has been adjusted to pH 4.5–5.0 after centrifugation. Optimization of reaction time at 30 min, 40 min and 60 min has been conducted for the determination of total free and bound polyphenolics in the white and brown teff samples. The free total polyphenolics content (TPPC) determined in the white teff sample was at 30 min (75.77±0.22 mg GAE/100 g), 40 min (80.99±0.59 mg GAE/100 g) and 60 min (75.83±0.83 mg GAE/100 g). While the bound TPPC was at 30 min (77.37±0.93 mg GAE/100 g), 40 min (123.7±0.1 mg GAE/100 g) and 60 min (136.2±0.3 mg GAE/100 g), respectively. Besides, the free TPPC in the brown teff sample was at 30 min (151.8±0.6 mg GAE/100 g), 40 min (152.7±0.3 mg GAE/100 g) and 60 min (154.1±0.5 mg GAE/100 g), while the bound TPPC was at 30 min (85.4±0.8 mg GAE/100 g), 40 min (179.4±0.3 mg GAE/100 g) and 60 min (199.0±0.4 mg GAE/100 g). 60 min optimized time has provided higher free and bound polyphenolics content for the brown teff sample and higher bound polyphenolics content for the white teff sample whereas 40 min optimized time has provided higher free polyphenolics content for the white teff sample. Hence, 60 min was used as an optimum extraction time for all the free and bound polyphenolics in the brown teff sample and higher bound polyphenolics in the white teff sample while 40 min was used for the extraction of free polyphenolics in the white teff samples.

Determination of total polyphenolics content by Folin–Ciocalteu assay

The total free and bound polyphenolics content (TPPC) of the white and brown teff grain samples measured by Folin–Ciocalteu method are shown in Table 2. The calibration curve equation (Figure 2) established for the gallic acid standard was $y = 0.00787x + 0.10065$. The coefficient of determination ($R^2 = 0.9999$) indicated a strong relationship between the concentration ratio and response in the linear range of 1–120 mg/L for gallic acid.

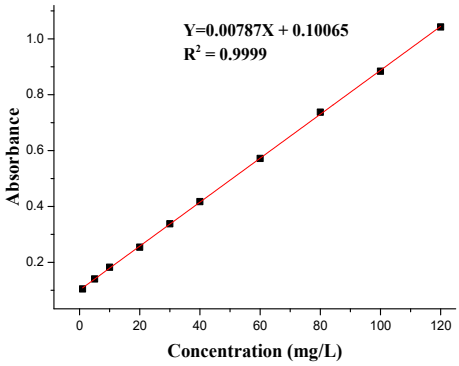

Figure 2. Calibration curve of gallic acid standard.

The total polyphenolic content (TPPC) was reported as milligrams of gallic acid equivalent per 100 g of flour on dry basis (mg GAE/100 g). The TPPC of the free extract in the white and brown teff grain samples ranged between 62.1–129.9 mg GAE/100 g and 118.6–196.7 mg GAE/100 g, respectively, which was higher than the content in the white maize grain sample (35 mg GAE/100 g) [27], white teff grain sample (37.1 mg GAE/100 g) and brown teff grain sample (71.4 mg GAE/100 g) [33]. The TPPC of the bound extract in the white and brown teff grain samples was in the range of 84.6–189.6 mg GAE/100 g and 141.1–195.1 mg GAE/100 g, consequently, which was lower than the report made by Shumoy and Raes [33] and Salinas-Moreno *et al.* [27] but higher than the report data of Kotaskova *et al.* [15]. The lowest TPPC in the free extract (62.1 mg GAE/100 g) and bound extract (84.6 mg GAE/100 g) were determined in the white teff grain samples from group 1 districts of Antsokiyana Gemza, Minjar Shenkora, respectively, of the North Shewa zone. The highest TPPC in the free extract (196.7 mg GAE/100 g) and bound extract (195.1 mg GAE/100 g) were found in the brown teff grain samples from group 2 district of Dugda, East Shewa zone and group 1 district of Legahida, South Wollo zone, respectively.

In the present study, the determined total TPPC varied between 155.8–293.7 mg GAE/100 g and 285.0–367.7 mg GAE/100 g in the white and brown teff grain samples of the three regions (groups), which is comparable with the total TPPC of finger millet (199.1–284.1 mg GAE/100 g), pearl millet (268.4 mg GAE/100 g), barley (211.3 mg GAE/100 g), red rice (224.9 mg GAE/100 g), oat (180.3 mg GAE/100 g), sorghum (81.4–189.9 mg GAE/100 g) reported by Kumar and Kaur [41] and hulless barley grain (139–178 mg GAE/100 g) [42]; but higher than the total TPPC of wheat (37 mg GAE/100 g), corn (65.8 mg GAE/100 g), teff (123.6 mg GAE/100 g) [43] and foxtail millet (23.0–45.7 mg GAE/100 g) [44]. Besides, the total TPPC in aromatic rice (268.7–474 mg GAE/100 g) assessed by Asaduzzaman *et al.* [45] is slightly higher than the present study. In the present study, the lowest total TPPC (155.8 mg GAE/100 g) and the highest total TPPC (367.7 mg GAE/100 g) were found in the white and brown teff grain samples, respectively, from group 1 districts of Minjar Shenkora, North Shewa zone and Were Ilu, South Wollo zone, respectively. This implied that the brown teff grain samples from group 1 (from Were Ilu, South Wollo zone) contained a higher total TPPC than the other teff grain samples from group 1, 2 and 3. Though making direct comparisons of values in literature is difficult owing to the fact that many researchers use different standards for analysis and solvents to prepare extracts, they offer a useful way of characterizing the grain material regarding the content of phenolics. Punia *et al.* [46] have also reported the total TPPC in the white sorghum pericarp (191.2 mg GAE/100 g) and in brown sorghum pericarp (173.7 mg GAE/100 g) which is in agreement to the present result.

Table 2. Total polyphenolics content in the white and brown teff samples.

Sample region	Variety of teff	Sample ID	Free TPPC (mg GAE/100 g)	Bound TPPC (mg GAE/100 g)	Total TPPC (mg GAE/100 g)
Group 1	White teff	AW-1	71.20±0.94	84.63±0.12	155.8±1.1
		AW-2	81.00±0.59	141.6±1.5	222.6±2.1
		AW-3	62.10±0.23	160.8±1.5	222.8±1.8
		AW-4	72.80±1.33	158.0±0.4	230.8±1.7
		AW-5	94.80±0.08	128.3±1.4	223.16±1.5
		AW-6	81.90±0.68	160.9±1.4	242.8±2.1
		AW-7	80.00±0.24	167.7±1.4	247.7±1.6
		AW-8	64.90±0.81	113.0±0.6	177.8±1.4
	Brown teff	AB-19	187.4±0.4	142.6±0.3	330.0±0.6
		AB-20	169.6±0.6	171.18±0.7	340.74±1.4
		AB-21	118.6±0.2	174.8±2.4	293.4±2.6
		AB-22	189.9±0.6	163.5±0.3	353.4±0.8
		AB-23	161.9±0.1	141.5±0.4	303.3±0.5
		AB-24	178.4±0.2	189.3±0.8	367.7±1.0
		AB-25	161.0±0.1	195.1±0.4	356.1±0.5
		AB-26	154.0±0.6	156.3±0.5	310.2±1.1
Group 2	White teff	OW-9	104.2±0.3	189.6±0.4	293.7±0.7
		OW-10	84.50±0.76	145.8±0.1	230.3±0.9
		OW-11	122.0±2.3	143.8±1.3	265.8±3.6
		OW-12	102.1±0.3	123.6±0.3	225.7±0.6
		OW-13	112.4±0.4	148.3±0.1	260.7±0.5
		OW-14	127.7±0.1	160.8±1.0	288.4±1.2
		OW-15	105.3±1.0	175.6±0.2	280.9±1.1
		OW-16	129.9±0.6	159.3±0.2	289.2±0.8
	Brown teff	OB-27	156.1±0.5	151.3±0.3	307.4±0.8
		OB-28	160.4±0.3	172.0±1.0	332.4±1.3
		OB-29	164.9±0.1	186.5±0.4	351.3±0.5
		OB-30	172.7±0.4	145.7±0.3	318.4±0.7
		OB-31	157.2±0.1	180.4±1.3	337.7±1.3
		OB-32	159.0±0.6	188.4±0.6	347.3±1.2
		OB-33	145.3±0.1	171.1±0.2	316.4±0.3
		OB-34	196.7±0.7	162.7±1.8	359.4±2.5
Group 3	White teff	SW-17	121.4±0.4	110.8±0.1	232.2±0.5
		SW-18	116.9±0.2	127.0±1.0	243.9±1.2
	Brown teff	SB-35	143.9±0.3	141.1±1.0	285.0±1.3
		SB-36	137.5±0.1	157.9±0.2	295.4±0.3

Note: Group 1 = Amhara; Group 2 = Oromia; Group 3 = SNNPR; TPPC = total polyphenolics content; mg GAE = milligrams gallic acid equivalent; ID = identification number.

Statistical analysis using one-way ANOVA ($\alpha = 0.05$) was performed to test the presence of significant differences among the mean concentration of the free TPPC, bound TPPC and total TPPC both in the white and brown teff grain samples of the three sampling regions (groups) (Table 3) and between the two teff varieties. The results of one-way ANOVA ($\alpha = 0.05$) indicated that there was statistically significant difference in the total polyphenolics content between the white and brown teff samples. The test has confirmed that the mean value of the total polyphenolics in the brown teff samples was found 1.37 fold higher than that of the white teff samples. This result was consistent with the report of Zhu [4] and Shumoy and Raes [33] stating that brown teff has a higher total phenolics content than the white teff.

To the detail interpretation of the one-way ANOVA ($\alpha = 0.05$) result, the brown teff grain samples from group 1 had shown higher free TPPC than the white teff grain samples of group 1,

2 and 3 and that of brown teff grain samples of group 2 and 3. Likewise, one-way ANOVA (α = 0.05) revealed that brown teff grain sample from group 2 exhibited higher bound TPPC and total TPPC than the white teff grain samples of the other three groups as well as brown teff grain samples of group 1 and 3.

The Tukey's test has confirmed that the free TPPC determined in the brown teff samples from group 1 and 2 was statistically significantly different (α = 0.05) with that of the white teff samples from the three groups. However, Tukey's test has indicated that there was no significant differences (α = 0.05) in the free TPPC of the brown teff samples from group 3 with the brown teff samples of group 1 and 2 as well as with the white teff samples of group 2 and 3. Besides, no significant differences (α = 0.05) of the free TPPC in the brown teff grain samples has been observed among the three sampling groups. But there was significant differences (α = 0.05) in the free TPPC of the white teff samples in group 1 with that of group 2 and 3. While no significant differences (α = 0.05) of the free TPPC in the white teff grain samples from group 2 and 3 has been observed. Tukey's test has also shown that there was no significant differences (α = 0.05) in the bound TPPC of the two varieties of teff samples among the three regions (groups). In addition, the test has assured that the total TPPC determined in the brown teff grain samples from group 1 and 2 was statistically significantly different (α = 0.05) with that of the white teff grain samples from group 1, 2 and 3. Nevertheless, Tukey's test has indicated that there was no significant differences (α = 0.05) in the total TPPC of the brown teff grain samples among the three groups. Besides, there was no significant differences (α = 0.05) in the total TPPC of the brown teff grain samples from group 3 with the white teff samples of group 2 and 3. The test has also shown that there was no significant differences (α = 0.05) in the total TPPC of the white teff samples from group 2 and 3. However, the total TPPC in the white teff samples from group 1 was statistically significantly different (α = 0.05) with that of the white teff samples from group 2.

Determination of total flavonoid content by aluminium chloride hexahydrate

The total flavonoids content (TFC) of the dry basis white and brown teff flour samples measured by aluminium chloride hexahydrate ($AlCl_3 \cdot 6H_2O$) colorimetric method are shown in Table 3. The calibration curve equation (Figure 3) established for quercetin standard was y = 0.01507x – 0.01152). The coefficient of determination (R^2 = 0.9997) indicated very strong relationship between the concentration ratio and response in the linear range of 1–120 mg/L.

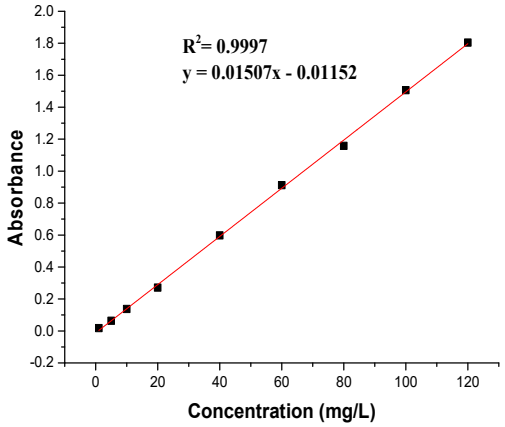

Figure 3. Calibration curve of quercetin standard.

Flavonoids have a π–π* transition which might be expected to appear at around λ_{max} = 280 nm. The π–π* transition is, generally, less dependent on solvent polarity while the charge transfer transition is solvent polarity dependent. Furthermore, the charge transfer transition is dependent on the extent of conjugation on the flavonoid compounds. The charge transfer transition band's λ_{max} (Figure 4) investigated for the total flavonoids in the brown teff sample was appeared at around 405 nm which was shifted by 25 nm to shorter wavelength as compared to the λ_{max} of the quercetin standard (430 nm). This indicated that the average total flavonoids in the brown teff sample constitute relatively less conjugated molecules as compared to the standard quercetin. The situation in the white teff sample is even more dramatic that the shift in λ_{max} was about 50 nm of the standard (quercetin). This implied that the average constituents of the total flavonoids are less conjugated as compared to both the brown teff sample and the standard. This fact could be used to differentiate the two varieties of the teff samples, though further investigation might be required.

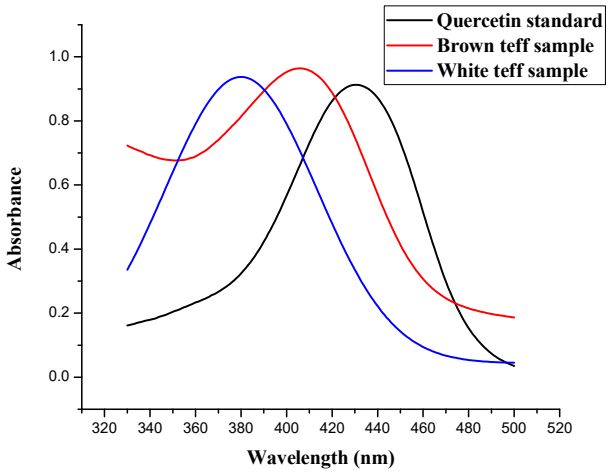

Figure 4. UV-VIS absorption spectra of the free total flavonoids in the white teff sample, brown teff sample and quercetin standard.

The total flavonoids content (TFC) was reported as milligrams of quercetin equivalent per 100 g of flour on dry basis (mg QE/100 g) (Table 3). The TFC of the free extract in the white and brown teff grain samples ranged between 84.4–195.1 mg QE/100 g and 97.8–202.5 mg QE/100 g, respectively, which is higher than the total TFC in the finger millet (22.82 mg CE/100 g), pearl millet (9.6 mg CE/100 g), sorghum (7.41 mg CE/100 g), barley (2.62 mg CE/100 g), oat (16.86 mg CE/100 g), red rice (19.1 mg CE/100 g) reported by Kumar and Kaur [41], red sorghum (42.8 mg RE/100 g), brown sorghum (36.7 mg RE/100 g) [46], but lower than aromatic rice (680–1280 mg RE/100 g) [45].

In the present study, the lowest TFC in the free extract of the white (84.4 mg QE/100 g) and brown (97.8 mg QE/100 g) teff grain samples was from group 3 (from Gomibora district, Haddiya zone) and group 1 (from Tarmaber district, North Shewa zone), respectively, while the highest TFC of the white and brown teff grain samples was from group 2 of Negelle Arsi district, West Arsi zone. Comparing the two varieties of teff samples, the brown teff grain sample from group 2 has revealed the highest total flavonoid content (202.5 mg QE/100 g). It is to be noted that an attempt was made to investigate and determine the bound total flavonoids content in the white and brown teff grain samples. However, bound total flavonoids were not detected by the method in all the samples. This might be due to the fact that the amount of bound flavonoids in the teff samples could be very small which may not be detected by the method.

Table 3. Total flavonoid contents of the white and brown teff samples.

Region	Variety of teff	Sample ID	TFC (mg QE/100 g)
Group 1	White teff	AW-1	99.13±2.7
		AW-2	103.4±3.8
		AW-3	111.6±5.1
		AW-4	112.3±5.5
		AW-5	125.5±4.4
		AW-6	129.2±2.6
		AW-7	131.6±3.2
		AW-8	116.8±4.9
	Brown teff	AB-19	163.7±1.7
		AB-20	97.78±2.4
		AB-21	107.8±3.3
		AB-22	135.6±5.4
		AB-23	105.0±1.5
		AB-24	157.5±4.0
		AB-25	155.1±5.8
		AB-26	176.4±6.2
Group 2	White teff	OW-9	130.3±6.9
		OW-10	158.2±5.2
		OW-11	131.5±3.1
		OW-12	195.1±3.7
		OW-13	107.7±3.8
		OW-14	110.5±5.5
		OW-15	111.0±6.3
		OW-16	140.6±5.3
	Brown teff	OB-27	122.3±3.2
		OB-28	143.3±3.6
		OB-29	168.8±6.6
		OB-30	202.5±4.5
		OB-31	117.8±1.6
		OB-32	168.5±7.3
		OB-33	140.5±6.0
		OB-34	201.3±5.1
Group 3	White teff	SW-17	95.27±4.0
		SW-18	84.43±3.9
	Brown teff	SB-35	106.3±6.6
		SB-36	105.8±1.6

Note: Group 1 = Amhara; Group 2 = Oromia; Group 3 = SNNPR, ID = identification, TFC = total flavonoid contents, mg QE = milligrams quercetin equivalent.

One-way ANOVA ($\alpha = 0.05$) indicated the presence of statistically significance difference of the mean TFC between the white and brown teff samples and the mean value of the TFC in the brown teff samples was found 1.18 times higher than that of the white teff samples. Tukey's test has confirmed that there was a significant difference ($\alpha = 0.05$) between the brown teff samples from group 2 with that of white teff samples from group 1 and 3. However, there was no significant differences ($\alpha = 0.05$) in the mean TFC in the white and brown teff samples among the three sampling groups.

The hierarchical cluster analysis (HCA) performed on the total phenolics contents (free total polyphenols, bound total polyphenols and total flavonoids) in the 36 teff samples was based on the Euclidean distances as a measure of similarity. As can be seen from the dendrogram in Figure

5, the first cluster (cluster 1) and second cluster (cluster 2) enclosed by the black and violate colors rectangles were homogenously composed of the white teff samples collected from the SNNP region and Amhara region, respectively. The third cluster (cluster 3) encircled by the blue color rectangle was almost composed of the white teff samples collected from the Oromia region. While the fourth cluster (cluster 4) and fifth cluster (cluster 5) encircled by the red and green colors rectangles were comprised of mostly brown teff samples from Amhara and Oromia regions, respectively. Furthermore, the varieties of teff (white and brown) were clearly separated in the dendrogram, indicated that cluster 1, 2 and 3 were composed of almost white teff while cluster 4 and 5 homogenously comprised of brown teff samples. Therefore, it is possible to conclude that the dendrogram has evidenced a quite sharp separation of the samples based on their origin (Amhara, Oromia and SNNPR) and variety (white and brown teff) with respect to total phenolics contents.

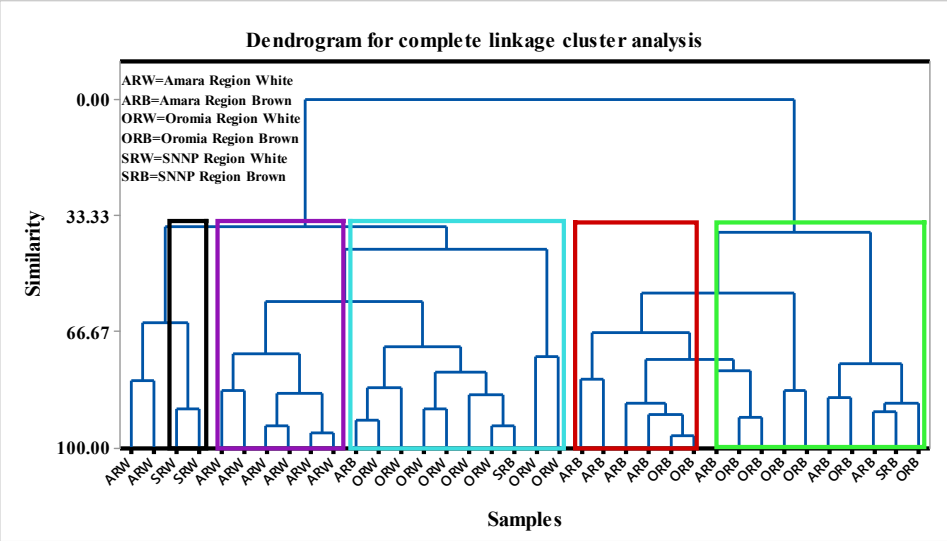

Figure 5. Dendrogram of the hierarchical cluster analysis (HCA) performed on the total phenolics parameters determined in all the 36 teff samples (both in the white teff (W) and brown teff (B) samples).

Determination of antioxidant activities of the white and brown teff *samples using DPPH assay*

In the present study, antioxidant activities of the free and bound phenolic fractions of the white and brown teff grain samples were evaluated by DPPH assay (Table 4). The calibration curve (y = -0.02581x + 1.86228) of ascorbic acid standard DPPH scavenging activity established is depicted in Figure 6.

The DPPH radical scavenging activities of the free fractions in the dry basis of white and brown teff grain samples ranged from 74.8–98.3 mg AAE/100 g and 68.7–96.7 mg AAE/100 g, respectively, which showed no significant differences ($\alpha = 0.05$) between the two teff varieties. The white teff grain samples from group 2 and 1 had revealed the lowest value (74.8 mg AAE/100 g) (from Jeju district, Arsi zone) and the highest value (98.3 mg AAE/100 g) (from Antsokiyana Gemza district, North Shewa zone) DPPH free radical scavenging activities, sequentially. Likewise, the brown teff grain samples from group 2 and 1 had revealed the lowest

(68.7 mg AAE/100 g) (from Dugda district, East Shewa zone) and the highest (96.7 mg AAE/100 g) (from Legahida district, South Wollo zone) DPPH scavenging activities, respectively. Furthermore, the DPPH radical scavenging activities of the bound fractions of the white and brown teff grain samples ranged from 77.1–99.9 mg AAE/100 g and 68.7–96.7 mg AAE/100 g, respectively, while the lowest and the highest value DPPH radical scavenging activities for both varieties of teff grains were found in the sampling areas of group 1 and 2, respectively. One-way ANOVA indicated that there was no significant differences ($\alpha = 0.05$) in the mean bound DPPH content between the white and brown teff varieties.

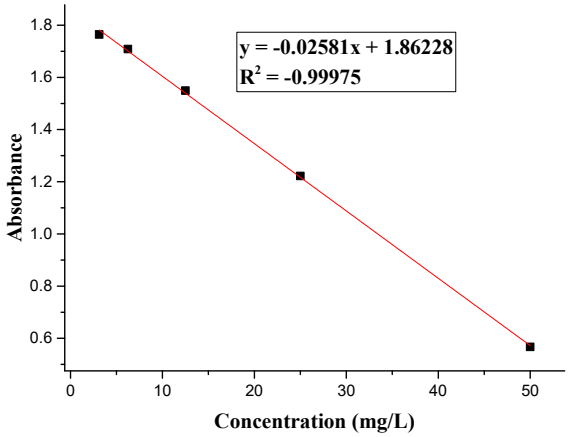

Figure 6. The calibration curve of ascorbic acid standard DPPH scavenging activity.

The total DPPH radical scavenging activities of the white and brown teff grain samples ranged from 155.2–193.0 mg AAE/100 g and 153.8–187.0 mg AAE/100 g, respectively. The lowest (155.2 mg AAE/100 g) and the highest (193.0 mg AAE/100 g) total values of the DPPH radical scavenging activities in the white teff grain samples were determined from group 3 and group 1, respectively. While the brown teff grain samples has resulted the lowest (153.8 mg AAE/100 g) and the highest (187.0 mg AAE/100 g) total DPPH radical scavenging activities from the sampling areas of group 1 and 3, respectively, which revealed no significant differences ($\alpha = 0.05$) between the two teff varieties. Results of the present study are consistent with the previous study which exhibited the antioxidant capacities contributed by the free (33.0–180.0 mg TE/100 g) and bound fractions (55.0–142.0 mg TE/100 g) in red sorghums [47] but higher than the white sorghum pericarp (8.0 mg TE/100 g) and black sorghum pericarp (14.6 mg TE/100 g) [46]. The white teff grain has higher consumer preference than the brown teff grain [48, 49]. It is the costliest type of teff which is normally held for the richest and most prestigious families in Ethiopia [49]. From the nutritional point of view, however, the present study revealed that the white teff and brown teff grain have no difference in antioxidant capacity. Furthermore, Yisak *et al.* [9] have reported that the white and brown teff grain oil is rich in fat and essential fatty acids as compared to the other cereal grains. The average ratio of ω-6/ω-3 found in white (2.48) and brown teff (2.42) flour was comparable with the suggested 1–2:1 ratio for the normal human growth and development. The present findings of the study, therefore, indicated that both varieties of teff are rich in antioxidant capacity which is very important for health.

Table 4. DPPH radical scavenging activities of the phenolics fractions of the white and brown teff varieties.

Sample region	Variety of teff	Sample ID	Free DPPH (mg AAE/100 g)	Bound DPPH (mg AAE/100 g)	Total DPPH (mg AAE/100 g)
Group 1	White teff	AW-1	85.91±0.98	84.70±1.81	170.6±2.8
		AW-2	97.88±1.38	80.36±1.90	178.2±3.3
		AW-3	98.25±0.53	94.77±3.07	193.0±3.6
		AW-4	84.02±0.74	94.46±3.28	178.5±4.0
		AW-5	95.00±0.70	77.10±1.23	172.1±1.9
		AW-6	86.92±1.35	89.82±4.11	176.7±5.5
		AW-7	82.38±1.95	88.13±1.80	170.5±3.8
		AW-8	83.63±0.79	97.04±1.25	180.7±2.0
	Brown teff	AB-19	86.56±0.67	80.85±4.51	167.4±5.2
		AB-20	91.05±0.54	84.25±2.95	175.3±3.5
		AB-21	93.49±1.67	81.32±3.05	174.8±4.7
		AB-22	82.59±0.85	71.24±2.62	153.8±3.5
		AB-23	93.17±0.22	80.21±0.78	173.4±1.0
		AB-24	87.46±1.42	80.28±1.15	167.7±2.6
		AB-25	96.68±0.78	85.21±2.02	181.9±2.8
		AB-26	79.39±0.61	94.64±2.01	174.0±2.6
Group 2	White teff	OW-9	90.98±0.55	77.53±1.77	168.5±2.3
		OW-10	83.63±0.99	83.42±2.05	167.1±3.0
		OW-11	74.82±0.38	96.15±3.48	171.0±3.9
		OW-12	76.44±0.69	81.12±1.82	157.6±2.5
		OW-13	79.45±1.53	79.68±2.14	159.1±3.7
		OW-14	85.88±1.50	99.00±0.83	184.9±2.3
		OW-15	91.48±1.81	91.88±2.64	183.4±4.5
		OW-16	83.20±0.66	99.89±1.62	183.1±2.3
	Brown teff	OB-27	84.70±0.77	93.14±2.12	177.8±2.9
		OB-28	81.46±0.84	91.41±2.00	172.9±2.8
		OB-29	81.51±0.47	80.47±2.20	162.0±2.7
		OB-30	83.46±0.50	96.60±1.20	180.1±1.7
		OB-31	93.14±0.42	85.38±0.83	178.5±1.3
		OB-32	85.00±0.30	99.40±1.27	184.4±1.6
		OB-33	86.07±0.63	87.78±1.07	173.9±1.7
		OB-34	68.73±2.26	96.41±1.62	165.1±3.9
Group 3	White teff	SW-17	93.36±0.87	81.63±1.40	175.0±2.3
		SW-18	75.75±2.49	79.48±0.99	155.2±3.5
	Brown teff	SB-35	89.15±0.66	97.84±1.22	187.0±1.9
		SB-36	82.49±1.23	90.75±1.86	173.2±3.1

Note: Group 1 = Amhara; Group 2 = Oromia; Group 3 = SNNPR, ID = identification, DPPH = 2,2-diphenyl-1-picrylhydrazyl, mg AAE = milligrams ascorbic acid equivalent.

Antioxidant activities and their correlation with total phenolics

The relationship between the total phenolics and antioxidant activities has been investigated using Pearson correlation at 95% confidence level. The results found in the white teff grain samples exhibited a positive correlation between the free TPPC and free DPPH (r = 0.278, p = 0.264), bound TPPC and bound DPPH (r = 0.360, p = 0.221), total TPPC and total DPPH (r = 0.489, p = 0.039) but negative correlation between TFC and total DPPH (r = -0.230, p = 0.359). Likewise, the results obtained in the brown teff grain samples revealed that the free TPPC has positive but weak correlation with the free DPPH (r = 0.382, p = 0.118) radical scavenging activity. However, the bound TPPC and bound DPPH (r = -0.530, p = 0.024), total TPPC and total DPPH (r = -0.264, p = 0.290), the TFC and total DPPH (r = -0.194, p = 0.441) have negative antioxidant correlation.

This indicated that the total phenolics is not the dominant contributing factor for the radical scavenging activity resulting very weak functional relationship in the present study. In addition, it should be noted that the antioxidant activity of the white and brown teff samples is dependent not only on the total phenolics content but also on the relative amounts of individual phenolics. The radical scavenging capacity of the phenolics is dependent on their structure and composition [47].

CONCLUSION

The present study has reported the optimum extraction procedure, total content of phenolics (polyphenols and flavonoids) and the antioxidant capacity of the white and brown teff varieties. 60 min was the optimum extraction time for the determination of all the free and bound polyphenolics in the brown teff sample and bound polyphenolics in the white teff sample, while 40 min was optimum for the extraction of free polyphenolics in the white teff samples. The mean free TPPC, bound TPPC, total TPPC and TFC determined in the brown teff samples were statistically significantly different ($\alpha = 0.05$) and even higher than the white teff samples. These differences might be due to the variations in their cultivars, varieties, types of soil, and their geographical locations. The lowest and the highest total TPPC was determined in the white and brown teff samples, respectively, from the sampling area of group 1. In addition, the lowest and the highest TFC were quantified in the white and brown teff samples from group 3 and 2, sequentially. This study indicated that the brown teff variety is rich in polyphenolics and flavonoids content than the white teff samples. The dendrogram has revealed clear separation of the samples based on their origin and variety with respect to total phenolics parameters. The DPPH radical scavenging activities of the free and bound fractions in the white and brown teff grain samples has exhibited no significant differences ($\alpha = 0.05$) between the two teff varieties. Besides, the TPPC, TFC and total DPPH radical activity were not strongly correlated. This might be due to the fact that the antioxidant activity of the white and brown teff samples is dependent not only on the total phenolics content but also on the relative amounts of individual phenolics. Furthermore, the radical scavenging capacity of the phenolics is dependent on their structure and composition.

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