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Levels of total polyphenol, flavonoid, tannin and antioxidant activity of selected Ethiopian fermented traditional beverages

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Abstract

Indigenous fermented beverages are very popular traditional drinks in Ethiopia. Among these, korefe, tej and tella are very common in northern part of Ethiopia. However, there is no any report on the total polyphenol, flavonoid, tannin and antioxidant activity of these beverages. The purposes of this study were to determine total phenolic, flavonoids, tannins, and their antioxidant activity and to compare these parameters of selected Ethiopian traditional alcoholic beverages (korefe, tej and tella) from different origins of Ethiopia within the same sample type and between different sample types. For these purposes, methanolic extracts (70%) were prepared for semi-solid samples. Total phenolic content was determined by Folin-Ciocalteu's method, total flavonoids content was determined by aluminium chloride ($AlCl_3$) method, total tannin content was determined by indirect method precipitating protein with gelatin, while 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity method was used to determine the total antioxidant activity. The total phenolic content obtained as gallic acid equivalent, GAE mg L^{-1} was: korefe (285–326), tej (369–390) and tella (435–459); the total flavonoids content obtained as catechin equivalent, CE mg L^{-1} was: korefe (190–199), tej (183–190) and tella (211–216); the total tannin content obtained as tannic acid equivalent, TAE mg L^{-1} was: korefe (19.9–25.9), tej (17.9–19.4) and tella (28.8–30.8) and the total antioxidant activity obtained as ascorbic acid equivalent, AAE mgL^{-1} was: korefe (479–498), tej (465–479) and tella (541–561). Statistically at 95% confidence level one way ANOVA displayed that the levels of total polyphenol, flavonoid, tannin and antioxidant activity were significantly different between korefe, tej and tella. For all the determined parameters, tella exhibited a highest value. Pearson correlation showed there were positive correlations between phenolic, flavonoids and tannin with antioxidant activity assayed (r = 0.969, r = 0.931 and r = 0.944, respectively).

Introduction

There are a large number of Indigenous fermented beverages in Ethiopia. These include borde, korefe, shamita, katikala, tej and tella which are very popular traditional drinks. Among these, tella, korefe and tej are very common in northern part of Ethiopia (Alemu *et al*., 1991). Fermented beverages can play an important role, contributing to the livelihoods of rural and perturbing dwellers (Gadaga *et al*., 1999). In Ethiopia, traditional fermentation serves many purposes. It can improve the taste of food, enhance the digestibility of a food, and increase nutritional values. Furthermore, it is used for medical reasons, recreational purposes, in marriages, in religious and non-religious ceremonies, at festivals and social gatherings, at burial ceremonies and as food substitutes.

Polyphenols are common constituents of alcoholic beverages (Šeruga *et al.*, 2011; Abdoul-latif *et al.*, 2012; Lugemwa *et al*., 2013; Sokół-Łętowska *et al*., 2014). Polyphenols are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins (Dai and Mumper, 2010).

In beverages, polyphenols may contribute to the bitterness, astringency, color, flavor, odor and oxidative stability. Polyphenols are the subject of increasing scientific interest because of their possible beneficial effects on human health, abilities of cardio-protective effect, anti-cancer effect, anti-

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diabetic effect, anti-aging effect and neuro-protective effect (Pandey and Rizvi, 2009). Antioxidants may be defined as compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidation chain reactions. They can also protect the human body from free radicals and reactive oxygen species effects (Pandey and Rizvi, 2009). They have also been widely used as food additives to provide protection against oxidative degradation of foods. Food antioxidants such as α-tocopherol, ascorbic acid, carotenoids, amino acids, peptides, proteins, flavonoids and other phenolic compounds might also play a significant role as physiological and dietary antioxidants, thereby augmenting the body's natural resistance to oxidative damage (Gülçin *et al*., 2010).

Ethiopian alcoholic beverages and their types (Alonso-Salces *et al*., 2005; Mahdavi *et al*., 2010), preparation procedure (Peterson, 1979; Alonso-Salces *et al*., 2005; Mahdavi *et al*., 2010; Callemien and Collin, 2010; Abawari, 2013; Sokół-Łętowska *et al*., 2014), alcoholic contents (Mahdavi *et al*., 2010; Callemien and Collin, 2010; Abawari, 2013; Yohannes *et al*., 2013), mineral contents (Woldemariam and Chandravanshi, 2011; Bekele and Chandravanshi, 2012) and physico-chemical properties (Šeruga *et al.*, 2011; Yohannes *et al.*, 2013; Pękal and Pyrzynska, 2014) were studied. However, there is no any report on the total polyphenol, total flavonoid, total tannin and antioxidant activity of traditional Ethiopian alcoholic beverages except one study on samples collected from vending houses at different sub-cities of Addis Ababa, the capital city of Ethiopia and nearby towns (Debebe *et al*., 2016). Therefore, the aim of this work were to establish the profile of phenolic, flavonoid, tannin and antioxidant activities of three traditional alcoholic beverages namely korefe, tej and tella from selected area of northern Ethiopia; to compare profiles of phenolic, flavonoid, tannin and antioxidant activities of these traditional beverages and to evaluate the status of these beverages on the basis of determined parameters with different beverages from literature.

Materials and Methods

Chemicals and reagents

All the reagents used were of analytical grade reagent and the water used was distilled-deionized. Sodium molybdate dihydrate and sodium tungstate (BDH Laboratory Supplies, Poole, England); lithium sulfate, ascorbic acid and gelatin (BDH Chemicals Ltd, Poole, England); phosphoric acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, catechin, aluminium chloride, sodium nitrite, tannic acid, pH standards, sulfuric acid and sodium chloride (Sigma Aldrich, Steinheim, Germany); sodium carbonate, kaolin and sodium sulfate anhydrous (Research-Lab Fine Chem Industries, Mumbai, India); sodium hydroxide and methanol (Scharlau Chemie S.A., Europian Union, Spain); concentrated hydrochloric acid (Fisher Scientific UK Limited, Bishop Meadow Road, UK); and bromine (Guandong Guanghua Chemical Factory Co. Ltd, China) were used as received.

Instruments

Spectrophotometric measurements were performed on a UV-Vis spectrophotometer (Lambda 950, Perkin Elmer, UK) equipped with 1 cm path length quartz cells.

Samples collection

The sample types were selected based on universality in consumption and wide availability in the market and household level than the rest of traditional beverages. Similarly sample areas were selected due to considerations of widely fermentations and consumptions areas of sampled beverages. Though, korefe is mainly familiar in the area of North Gondar, accordingly representative samples were purchased from three different localities namely Chilga, Gondar and Woreta. From each sample area, five representative samples were collected from different vending houses (0.5 L from each) which were selected randomly to prepare a bulk sample by mixing because instead of analyzing separately, analyzing of the mixture reduces the variance and resource consumption and triplicate analysis was performed for each. For tej and tella samples, three sample areas (Debre Birhan, Debre Markos and Gondar city) were selected; from these places five representative samples were purchased for each sample type to have a bulk sample for each type of beverages. All the samples were collected using glass amber bottles and the bulk samples were kept in the fridge at 4^{o}C until analysis. Geographical locations of the sample areas are given in Table 1.

Analyte extraction

Among the samples, korefe was semi-solid. Therefore, it was homogenized with aqueous: methanol solution (30:70%) (Bezuneh and Kebede, 2015). The homogenate was stirred with magnetic stirrer at 900 rpm for 90 min. Then it was put in centrifuge at 3000 rpm for 20 min. Finally the supernatant was taken by decantation and it was stored in the fridge until analysis time at 4^{o}C. The

Table 1. Geographical location of the sample areas

Sample area	Longitude	Latitude	Altitude (m)	Distance from Addis Ababa in km
Chilga	37°4'1"E	12°33'0"N	2,146	784
Debre Birhan	39°31'59"E	9°40'59"N	2,840	130
Debre Markos	37°43'47"E	10°20'1"N	2,446	306
Gondar	37°37'28"E	12°12'39"N	2,133	740
Woreta	37°42'0"E	11°55'1"N	1,810	625

extractions were performed in triplicates.

Total phenolic content

The Folin-Ciocalteu method was used for the determination of total phenolic content (Waterman and Mole, 1994; Blainski *et al*., 2013). Folin-Ciocalteu's phenol reagent was prepared as follow: 10 g sodium tungstate and 2.5 g sodium molybdate were dissolved in 70 mL water. 5 mL 85% phosphoric acid and 10 mL concentrated hydrochloric acid were added to the solution, refluxed for 10 h. Then the following were added 15 g lithium sulfate, 5 mL water and 1 drop bromine, refluxed for 15 min, cooled to room temperature and brought to 100 mL with water. Clear bright yellow solution of Folin-Ciocalteu's phenol reagent was stored tightly capped at room temperature and the reagent was diluted with distilled water as required. A 10% Na_2CO_3 was prepared by dissolving 10 g sodium carbonate in 100 mL distilled water.

To determine the total phenolic content, 0.4 mL of sample/standard gallic acid solutions (700, 550, 400, 250 and 100 mg L^{-1}) was mixed with the 4 mL Folin-Ciocalteu reagent (1:9) diluted with water in 25 mL volumetric flask and after 5 min, 4 mL 10% (w/v) Na_2CO_3 was added. The mixture was made up 25 mL with distilled water, and then incubated for 90 min at room temperature. The absolute absorbance was measured at 760 nm (Abs at 760 nm - Abs at the base line) against an appropriate blank. The concentration of total phenolic compounds was expressed in milligram of gallic acid equivalent (GAE) per liter of sample using the calibration curve equation, y = 0.0012 x + 0.0587 (where y = absorbance and x = concentration, GAE in mg L^{-1}). All the samples were analyzed in triplicates.

Total flavonoid content

Total flavonoid content was determined by spectrophotometric method (Pękal and Pyrzynska, 2014) based on the formation of aluminium-flavonoid complexes using catechin as a standard, 5% sodium nitrite, 10% aluminium chloride, 1 M sodium hydroxide were used as a reagent. Then after appropriate dilution was made with distilled water, the reaction mixture was measured at 415 nm against a blank spectrophotometrically (Lugemwa *et al*., 2013).

Catechin standard solutions were prepared by dissolving catechin in water at a concentration ranging from 50 to 600 mg L^{-1}. Briefly, 1 mL of appropriately diluted aqueous catechin standard solutions or beverage sample was added to 4 mL of distilled water. At time zero, 0.3 mL of 5% (w/v) $NaNO_2$ was added. 0.3 mL of 10% (w/v) $AlCl_3$ was added 5 min later. At 6 min, 2 mL of 1 M NaOH was added and the solution was made up to 20 mL with distilled water and mixed. The spectrum was scanned against blank using spectrophotometer from 850–350 nm. The absolute absorbance (Abs at 415 nm - Abs at the base line) was measured against an appropriate blank. The total flavonoid content was expressed in milligrams of catechin equivalent per liter with calibration equation of y = 0.0008x + 0.049 (y = absorbance and x = concentration, CE in mg L^{-1}). All the samples were analyzed in triplicate.

Total tannin content

Gelatin (0.3% m/v) solution was prepared by soaking 1.5 g of gelatin in 10% sodium chloride solution for 1 h, then warmed to dissolve the gelatin and finally diluted to 0.5 L with 10% (m/v) sodium chloride solution in distilled water after cooling. Acidic sodium chloride solution was prepared by adding 25 mL of concentrated sulfuric acid to 375 mL of 10% sodium chloride solution (Lau *et al*., 1989) and 10% NaCl was prepared by adding of 10 g NaCl in 100 mL distilled water.

The sample blank was obtained as follows: The 0.4 mL of sample solution was pipetted into a 100 mL beaker containing of 5 mL gelatin solution. To the mixture were added 10.0 mL of acidic sodium chloride solution followed by 2.0 g of kaolin added and the mixture was shaken for 15 min. The precipitate was allowed to settle and the mixture was filtered. Then, 10.0 mL of the filtrate, 6.0 mL of distilled water, and 3.0 mL of gelatin solution and 6.0 mL of acidic sodium chloride solution were pipetted

into a 100 mL beaker followed by the addition of 2.0 g of kaolin. After shaking for several minutes, the mixture was filtered and 0.4 mL of the filtrate was treated as described (Lau *et al*., 1989). Tannin was determined from the mean absorbance difference of total polyphenol and non-tannin polyphenol with the calibration equation of tannic acid standard curve. Tannic acid standard, total polyphenol and tannin free polyphenol were determined by Folin-Ciocalteu method as described above.

The procedure for determining the gelatin blank was the same as that for the sample blank except that distilled water was used instead of the sample solution. The difference in absorbance between the sample blank and the gelatin blank gave the net sample blank. The difference in absorbance between the sample and net sample blank was due to tannins in the sample and their concentration was deduced from the calibration graph (Lau *et al*., 1989): y = 0.0017x + 0.0269. All the samples were analyzed in triplicate.

Total antioxidant activity

DPPH radical scavenging method was used for determining the antioxidant activities of the beverages (Gülçin *et al.*, 2010; Mrvcic *et al*., 2012). 160 mg L^{-1} DPPH solutions in methanol was prepared. The solution was vortexes vigorously until all DPPH was dissolved. Subsequently, 2 mL of 160 mg L^{-1} DPPH was mixed with 1 mL of alcoholic beverage samples (1:9) diluted with distilled water/ascorbic acid standards (1:3) diluted (25, 50, 100, 150 and 250 mg L^{-1}), and 3 mL of methanol. The mixture was allowed to react at room temperature in the dark for an hour. The sample blank DPPH solution was prepared with 2 mL of DPPH solution and 4 mL of methanol only. The absorbance values of the compounds changing from violet to yellow color were measured at 517 nm. All the samples were analyzed in triplicates. The antioxidant capacity of compounds was expressed as milligram of ascorbic acid equivalent (AAE) and quantified by using the calibration curve linear equation, y = -0.0032x + 1.3786 (where y = absorbance and x = concentration, AAE in mg L^{-1}). The degree of decolorization of DPPH from purple to yellow indicated the scavenging efficiency of the sample. The percentage inhibition of DPPH free radical scavenging activity was calculated using the following equation (Mrvcic *et al.*, 2012):

$$\% \text{ Inhibition} = [(A_{DPPH} - A_{sample})/A_{DPPH}] \times 100\%$$

Where: A_{DPPH} = Absorbance of DPPH, A_{sample} = Absorbance of sample (sample/ascorbic acid).

Statistical analysis

In this study, a one way ANOVA method was used at 95% confidence level using SPSS software to know the variation of mean of total polyphenol, flavonoid, tannin and antioxidant activities are significantly different between the same and different groups of beverages or not and Pearson correlation coefficient was used to measure the degree of linear relationship and direction of relations between total polyphenol, flavonoid and tannin with total antioxidant of the samples.

Results and Discussion

The results of phyisco-chemical properties, total polyphenols, total flavonoids, total tannins and antioxidant activities of the samples from different areas are summarized in Tables 2-4. From the determined physico-chemical properties of these traditional beverages: korefe has highest ionic strength than the rest of the samples. Whereas the pH value of tej is lower than the other beverages which indicates that it is acidic. But the refractive index and specific gravity of these beverages are comparable. Therefore, the ionic strength and pH value have been taken under consideration, during adjusting of them for gelatin-tannin complexations.

The mean total phenolic content obtained in the beverages are in the order: tella > korefe > tej whereas the total tannin is in the order: tella > korefe > tej. The mean total flavonoids in the beverages is in the order: tella > korefe > tej and the order of antioxidant capacity is: tella > korefe > tej. In most of the investigated assays in comparison with the types of beverages analyzed, tella samples have higher values than the rest. This might be due to the difference in amount of ingredients used such as malt and hop, and their compositions. The total polyphenol levels within the same sample types and between different sample types were significantly different. This might be due to variations of the raw materials and geographical locations (Lo´pez-Laredo *et al.*, 2012; Vagiri, 2014; Gaivelyte *et al*., 2014; Brahmi *et al*., 2014; Mehari *et al*., 2016; Xu *et al*., 2016). Among the korefe samples, Woretas korefe showed lower polyphenol content; this might be due to difference of ingredients phenolic level and topography factors. The total antioxidant activities as AAE, % I result is in the order: tella > korefe > tej. This result indicates that tella is rich in total antioxidant species than the rest sample types.

Different authors have reported the total phenolic contents in mg L^{-1} GAE as: in beer, 270–600 (Berhanu, 2014), 206–374 (Abdoul-latif *et al*., 2012)

Table 2. Some physico-chemical properties of the samples (n = 3, triplicates analysis, p = 0.05)

Sample type	Sample area	Conductivity ($mScm^{-1}$)	pH	Refractive index at 20 °C	Specific gravity
	Chilga	1.06±0.07	4.89±0.05	1.34±0.01	1.02±0.04
Korefe	Gondar	1.09±0.08	4.76±0.01	1.34±0.02	1.04±0.02
	Woreta	1.02±0.05	4.92±0.03	1.34±0.02	1.05±0.01
	Debre Birhan	0.38±0.02	3.98±0.02	1.34±0.01	1.03±0.03
Tej	Debre Markos	0.37±0.06	3.91±0.04	1.34±0.02	1.05±0.06
	Gondar	0.40±0.01	3.87±0.02	1.35±0.07	1.02±0.03
	Debre Birhan	0.76±0.02	4.53±0.05	1.33±0.06	1.02±0.02
Tella	Debre markos	0.66±0.01	4.41±0.03	1.33±0.06	1.02±0.01
	Gondar	0.72±0.03	4.62±0.01	1.33±0.06	1.01±0.05

Table 3. Polyphenol, flavonoids and tannin compounds result (n = 3, triplicates analysis, p = 0.05)

Sample type	Sample area	Total phenolic (mg GAE/L) mean±SD	Total flavonoid (mg CE/L) mean±SD	Total tannin (mg TAE/L) mean±SD
	Chilga	326±1.0	199±1.0	25.9±0.6
Korefe	Gondar	323±0.7	196±1.2	23.2±0.4
	Woreta	285±1.0	190±1.5	19.9±0.9
	Debre Birhan	290±1.0	188±1.5	18.2±0.6
Tej	Debre Markos	269±1.0	183±2.1	17.1±0.5
	Gondar	290±1.0	190±1.0	19.4±0.6
	Debre Birhan	459±0.7	216±1.0	30.8±0.9
Tella	Debre Markos	450±0.8	211±1.0	28.8±0.6
	Gondar	435±0.7	212±1.0	30.6±0.6

and 152–339 (Steinkraus, 1983); in wine, 178–284 (Abdoul-latif *et al*., 2012), 189–3130 (Pandey and Rizvi, 2009) and 1648–4495 (Lugemwa *et al*., 2013) and in dolo, 506 (Hahn *et al*., 1984). The reported total flavonoid in wine ranges from 0.3–680 in mg L^{-1} CE (Koguchi *et al*., 2010), the reported total tannin in TAE mgL^{-1} of sample volume in dolo 34-45 (Abdoul-latif *et al*., 2012), in wine 997-1197 (Abdoul-latif *et al*., 2012), in beer ranges from 73.8–101.5 (Oyaizu, 1986), in alcoholic and non-alcoholic wine 421-1576 and 452-3383, respectively (Nanasombat *et al*., 2015) and the reported total antioxidants in trolox equivalent mgL^{-1} of samples in dolo 14.3-87.3 (Abdoul-latif *et al*., 2012), in wine 82-21362 (Ashenafi, 2006) and in non-alcoholic beverage (wine) 132-933 (Nanasombat *et al*., 2015). In some cases the reported total phenolic, flavonoid, tannin and antioxidant activities in beer and wine are in comparable level with the samples of this study. This might be due to the similarity of composition of the raw materials and brewing process.

ANOVA at 95% (p = 0.05) confidence level shows that between different families of sampled beverages the total polyphenol, flavonoid, tannin and antioxidant mean concentrations are significantly different. Within the same types of beverages from different areas, there were no significant differences except the level of total polyphenol. The variations in the above parameter level are might be due to inconsistency in the preparation process; the difference in topographical location; the samples were collected from five different geographically

Table 4. Total antioxidant activity results (n = 3, triplicates analysis, p = 0.05)

Sample area	Sample type	% of Inhibition mean±SD	Total antioxidant activity (mg AAE/L) mean±SD
Korefe	Chilga	45.6±0.4	508±4.9
	Gondar	44.8±0.5	498±3.1
	Woreta	43.4±0.4	484±3.5
Tej	Debre Birhan	42.9±0.2	479±1.2
	Debre Markos	41.9±0.4	470±2.3
	Gondar	41.6±0.4	465±1.5
Tella	Debre Birhan	50.8±0.1	561±0.7
	Debre Markos	50.3±0.7	556±3.1
	Gondar	48.9±0.3	541±2.1

located areas, which have different soil composition, annual rain fall, cultivation of the raw materials, and the variation of brewing process between groups of samples, the ingredient amount and types. All these lead to the difference in total polyphenol content of the samples. Moreover, the variation is dependent on the type of phenolic compounds present. This is due to the fact that different plants contain different types of phenolic compounds (Lo´pez-Laredo *et al*., 2012; Vagiri, 2014; Gaivelyte *et al*., 2014; Brahmi *et al*., 2014; Mehari *et al*., 2016; Xu *et al*., 2016) and different plant materials are used in the preparation of the three types of fermented beverages studied (Bahiru *et al*., 2001; Yohannes *et al*., 2013; Debebe *et al*., 2016).

Statistical analysis

The analysis of variance (ANOVA) were done with SPSS software revealed that there is a significant difference at 95% (p = 0.05) confidence level between the means of total phenolic, flavonoids, tannin and antioxidant among the alcoholic beverages types and within the samples of each type. This confirmed that the variations might be due to the difference in preparation of the beverages, the amount and kind of ingredients used, and the types and amounts of phenolic compounds present in the raw materials, the variation of environment and soil texture.

Relation between total phenolic groups and antioxidant activities of the beverages

Total polyphenol, total flavonoids and total tannins were found to have correlation coefficients with their antioxidant activity assayed: (r = 0.969 and r = 0.931, r = 0.944, respectively). The results indicate that the presence of positive and very good correlation between these variables. The positive correlation indicates that when the concentrations of total polyphenolic compounds, total flavonoids and total tannins increase, total antioxidant activity also increases. This illustrates that phenolic compounds are important contributors to antioxidant activity of the beverages.

Conclusion

In this study, the level of bioactive compounds: total phenolic, flavonoids, tannins and antioxidant activities of the selected Ethiopian traditional beverages were investigated. The results obtained indicated that at 95% (p = 0.05) confidence level, there were significant difference in the levels of total polyphenol, flavonoids and tannins between different sample types while within the same sample type, the level of total polyphenol was significant different in samples from different location. Similarly total antioxidant activity of these fermented alcoholic beverages was significantly different between different sample types. Among the fermented beverages, tella has showed higher total phenolic and antioxidant capacity than the rest. The results of this study also indicated that the concentration of total tannin is much lower than the concentration of total flavonoid. The investigation indicated that the total phenolic components of alcoholic beverages depend on the raw materials and the brewing processes. According to the data obtained from this study, total polyphenol, flavonoids and antioxidant activity were present remarkably at higher levels while total tannin was very low in the beverages. These research findings conclude that korefe, tej and tella are a promising source of biologically active polyphenol

and flavonoid compounds and contributing effective protection from free radicals, cancer, and aging problems in comparable manner to beer, dolo and few wines which were cited. However, it should be noted that the source of polyphenolic compounds in the fermented beverages is the part of the plant materials used in the preparation of the beverages.

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