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TOTAL CONTENTS OF PHENOLICS, FLAVONOIDS, TANNINS AND ANTIOXIDANT CAPACITY OF SELECTED TRADITIONAL ETHIOPIAN ALCOHOLIC BEVERAGES

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ABSTRACT. The aim of this study was to determine the total contents of phenolics, tannins and flavonoids and antioxidant capacity and their relationships in traditional Ethiopian alcoholic beverages. They have been determined utilizing Folin–Ciocalteu assay, aluminum chloride precipitating agent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, respectively. The most widely consumed beverages and which have many varieties were selected for this study. These are gesho fermented and non-gesho beverages *tella*, *tej*, *borde*, *keribo*, *birz*, *korefe* and *areke*. The total phenolic content obtained in gallic acid equivalent (GAE) μg mL^{-1} was: *areke* (0.2–0.62), *tella* (10.1–19.1), *tej* (5.8–9.5), *keribo* (10.4–14.9), *birz* (10.5–12.2), *korefe* (9.2–10.7) and *borde* (8.4–10.6). The majority of phenolic compounds in the alcoholic beverages are non-tannic and non-flavonoid compounds. The antioxidant capacity obtained in ascorbic acid equivalent (AAE) μg mL^{-1} was: *areke* (-0.28–284), *tella* (31.6–201), *tej* (1.73–73.7), *keribo* (39.21–90.11), *birz* (41.95–63.08), *korefe* (58.25–96.45) and *borde* (180–217). The variation in the antioxidant activity among the beverages is due to the types and amount of ingredients used, disparity in the preparation process and the types of phenolic compounds found. The relationship between total phenolics and antioxidant activities was investigated using Pearson correlation at 95% confidence level. The results obtained indicate that the non-gesho fermented beverages such as *keribo* (-0.714), *birz* (-0.686) and *borde* (-0.212) have negative antioxidant correlation with the total phenolic, whereas, fermented beverages with gesho such as *tella* (0.539), *tej* (0.385) and *korefe* (0.557) have positive correlations. *Areke* has an overall positive correlation (0.609), but, the cereal *areke* which does not have medicinal plants has negative correlation.

KEY WORDS: Phenolic content, Antioxidant capacity, Traditional alcoholic beverages, Ethiopian beverages

INTRODUCTION

Traditional alcoholic beverages, *tella*, *tej*, *keribo*, *birz*, *borde*, *korefe* and *areke* are indigenous to Ethiopia [1]. *Areke* is distilled whereas the rest are fermented alcoholic beverages [2–4]. *Tella* and *korefe* (a malt beverage like beer) are made from a mixture of *enkuro* (a dark brown toasted flour of barely, maize or sorghum), germinated wheat grain (*bikil*), *gesho* (*Rhamnus prenoids*) and water [4]. *Tej* and *birz* (honey beer) are prepared from honey and water. But, *tej* has leaves or stem of *gesho* (*Rhamnus prenoides*) as an additional ingredient [2, 4]. *Borde* and *keribo* are made from a mixture of roasted grain (barely, wheat, maize or sorghum) and their malts [4, 5]. *Areke* is a colorless, clear, distilled traditional alcoholic beverage which is prepared in almost the same way as *tella* [3]. The beverages were selected by two major points. One they are the most widely consumed beverages. Second, the beverages especially the distilled, *areke* has many varieties.

Alcoholic beverages beside their main components, ethanol and water, contain a large number of minor compounds such as phenolics [6–8]. The characteristics of alcoholic beverages are determined by their chemical composition [9]. In this sense, phenolic compounds have impacts in alcoholic beverages quality [9].

Phenolic compounds, the secondary metabolites, are widely distributed in a variety of beverages [8]. Natural phenolic compounds can range from simple molecules, such as phenolic

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acids, to highly polymerised compounds, such as tannins [10]. Phenolic compounds, i.e. anthocyanins, resveratrol, gallic acid, catechin, myricetin, quercetin, etc. are abundant in red wines [11]. They occur primarily in conjugated form, with one or more sugar residues linked to hydroxyl groups, although direct linkages of the sugar unit to an aromatic carbon atom also exist [10]. The presence of phenolic compounds in plant foods is largely influenced by genetic factors and environmental conditions. Other factors such as germination, degree of ripeness, variety, processing, and storage also influence the content of plant phenolics [10, 12].

Phenolic compounds are important components of beverages, to which they contribute flavor, color, and sensory properties such as bitterness and astringency [9, 11, 13–15], aroma and haze formation during storage [9]. Moreover, phenolic compounds are known to play an important role in alcoholic beverages making processes, since they participate in clarification, and present an inhibiting effect on spoilage micro-organisms, as well as, on clarification enzymes [9, 11].

In contrast to their ability of reducing food digestibility caused by the potential phenolics to bind and precipitate macromolecules; interest in food phenolics has increased, because of their antioxidant and free radical scavenging abilities [10], metal chelators and enzyme modulators [11]. Many phenolics can exhibit antioxidant activity as their extensive, conjugated π-electron systems allow ready donation of electrons, or hydrogen atoms, from the hydroxyl moieties to free radicals. However, the antioxidant efficacy, in terms of reaction stoichiometry and reaction kinetics may vary considerably [10]. This is dependent on structural features, such as the number and positions of the hydroxyl moieties on the ring systems, and the extent by which the unpaired electron in the oxidized phenolic intermediate can delocalise throughout the molecule. Thus, most phenolics, especially flavonoids are very effective scavengers of hydroxyl and peroxyl radicals. Phenolics are chelators of metals and inhibit the Fenton and Haber-Weiss reactions [10, 11], which are important sources of active oxygen radicals. In addition, flavonoids retain their free radical scavenging capacity after forming complexes with metal ions [10]. On account of their antioxidant effects, phenolic compounds inhibit the development of cancerous tumours, reduce a risk for cardiovascular disease, and have showed anti-bacterial, anti-inflammatory, anti-spasmodic and anti-diarrhoeic properties [16].

Total phenolic compounds in liqueurs made from red fruits [17], wine [18–21], worts and beer [13, 22, 23], traditional fermented sorghum beers "dolo" [16], alcoholic beverages made from purple rice [24], ciders [25] and spirit drinks [26]; anthocyanins in liqueurs made from red fruits [17], dolo [16] and wine [21]; total flavonoids in wines [21]; and antioxidants capacity in wine [12, 18, 20, 21], beer [23], liqueurs made from red fruit [17], alcoholic beverages made from purple rice [24] were determined.

Ethiopian alcoholic beverages, their types [4, 27], preparation [1, 4, 5, 27, 28], alcoholic contents [4, 27, 28], and physico-chemical properties [27, 28–30] were studied. These are summarized in Table 1 for the studied beverages (Table 1). Nevertheless, there is no any report on the total phenolics, total flavonoids, total tannins and antioxidant capacity of Ethiopian alcoholic beverages. Moreover, there is no any report on the total tannin contents of alcoholic beverages in general.

Phenolic compounds have tremendous importance. Traditionally, the Ethiopian alcoholic beverages *areke* and *tej* have been used as medicine. However, data on their phenolics and antioxidant effects are scarce. Therefore, the objectives of present work were to: (i) determine the total phenolic, total flavonoid and total tannin contents, (ii) study the antioxidant characteristics, (iii) establish the relationship between the characteristics mentioned and (iv) develop a procedure for total tannin determination in some selected traditional alcoholic beverages. To the best of our knowledge, this study represents the first of its kind on total phenolics and related assays and antioxidant effects of Ethiopian traditional alcoholic beverages.

Table 1. Physico-chemical properties, raw materials and production process of some Ethiopian traditional beverages.

S. No.	Samples	Physico-chemical properties	Raw materials	Production process	Reference
1	*Tella*, *Korefe*	Dark brown in color pH 4–5	*Kita* (a thin, 5–10 mm thick, pancake-like bread), *enkuro* (a dark brown toasted flour), *bikil* (germinated grain), powdered *gesho* (*Rhamnus prenoids*)	A four phase fermentation for 10–12 days	4, 28
2	*Tej*	Yellow, sweet, effervescent and cloudy pH 3.07–4.90	Honey or mixture of sugar with honey and leaves of *gesho* (*Rhamnus prenoides*)	Mixing boiled must (with *gesho* (*Rhamnus prenoides*) and unboiled must and then allow to ferment for 5 days in warm or for 15–20 days in colder weather	2, 4
3	*Birz*	Yellow, sweet, effervescent and cloudy	Honey or mixture of sugar with honey	Has a short fermentation period, usually overnight	4
4	*Borde*	Opaque, effervescent, whitish-grey to brown coloured with a thick consistency and a sweet-sour taste	Unmalted maize (*Zea mays*), barley (*Hordeum vuldare*), wheat (*Triticum sativum*), finger millet (*Eleusine coracana*), sorghum (*Sorghum bicolor*) and/or *tef* (*Eragrostis tef*) and their malt; additional ingredients, garlic, fresh chili (*Capsicum minimum*), ginger and salt	A four phase fermentation for less than 4 days	1, 4, 27
5	*Keribo*	Dark brown coloured pH 3.20–5.17	Unmalted roasted barley (*Hordeum vuldare*), sugar and yeast	Has a short fermentation period, usually overnight	5
6	*Areke*	Clear and colorless (sometimes colorful) pH 4.30-4.51	*Kita* (a thin, 5–10 mm thick, pancake-like bread), *bikil* (germinated grain), powdered *gesho* (*Rhamnus prenoids*); additional ingredients, *koso* (*Hagenia abyssinica*), *gibto* (*Lupinus albu* or white lupin), mar (honey), and tenaadam (*Ruta chalepensis*)	Fermentation in the same way as *tella* and then distillation	4, 28

EXPERIMENTAL

Chemicals

Folin-Ciocalteu's reagent (BDH Chemicals Ltd, Poole, England), 2,2 diphenyl-1-picrylhydrazyl, DPPH (Sigma Aldrich, Steinheim, Germany), sodium tungstate (BDH Laboratory Supplies, Poole, England), sodium molybdate dehydrate (BDH Laboratory Supplies, Poole, England), lithium sulfate (BDH Chemicals Ltd, Poole, England), bromine water (Guandong Guanghua Chemical Factory Co. Ltd, China), lithium sulfate anhydrous (Research–Lab Fine Chem

Industries, Mumbai, India), sodium carbonate (Reaserch–Lab Fine Chem Industries, Mumbai, India), gallic acid (Sigma Aldrich, Steinheim, Germany), ascorbic acid (BDH Chemicals Ltd, Poole, England), catechin (Sigma-Aldrich, Steinheim, Germany), aluminium chloride (Sigma-Aldrich, Steinheim, Germany), sodium nitrite (Sigma-Aldrich, Steinheim, Germany), sodium hydroxide (Scharlau Chemie S.A., Eurprean Union), egg albumin (BDH Chemical Ltd, Poole, England), tannic acid (Sigma Aldrich, Steinheim, Germany), acetate buffer (prepared using acetic acid and sodium acetate, BDH Laboratory Supplies, Poole, England) and sodium chloride (Sigma Aldrich, Steinheim, Germany).

Instruments

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

Beverages sampling

For this study, seven most popular Ethiopian alcoholic beverages, *tej* (honey wine), *tella* (a malt beverage like beer), *areke* (distilled beverage), *keribo*, *birz*, *borde*, and *korefe* were selected. A total of 49 fermented beverages samples: 15 *tej*, 15 *tella,* 3 *borde*, 4 *birz*, 6 *korefe* and 6 *keribo* were collected randomly from vending houses at different sub-cities of Addis Ababa, the capital city of Ethiopia and from five nearby towns (Sebeta, Dukem, Sululta, Sendafa, and Burayu) of Oromia Regional State and 15 *Areke* (distilled beverage) from Addis Ababa and other potential producing areas such as Arsi Negele (Ormiya Regional State), Butagira (SNNPR Regional State) and Debere Birhan (Amhara Regional State). The collected samples of *areke* include: Yekoso (*Hagenia abyssinica*), Cereal (only cereal based), and Yegibto (*Lupinus albu* or white lupin), Yemar (honey), Yetenaadam (*Ruta chalepensis*). 150 mL of fermented beverages of each type were taken from the three places of a particular site and then 450 mL bulk samples were prepared. Likewise, 250 mL of the beverages were taken and then, 750 mL bulk samples were prepared for distilled beverages. All the *areke* samples except cereal based have additional ingredient as their names indicate. All the samples were collected using glass amber bottles and kept at 4 ºC until the analysis time. For liquid samples such as *tella*, *tej*, *birz*, *keribo* and *areke* no sample pretreatment was made except filtration, whereas, for semi-liquid samples such as *borde* and *korefe* extraction and filtration were done.

Phenolic compounds extraction

For the extraction of phenolic compounds, several solvents have been employed. The solvents used were acetone, chloroform, ethanol, methanol, ethyl acetate, 80% acetone, 80% ethanol and 80% methanol. The preliminary assays showed that the best extraction was achieved with ethyl acetate. The extraction time (30–150 min) and the volume of extraction solvent (ethyl acetate) (5–25 mL) and centrifugation time (5, 10 and 15 min) were recorded. The optimal results were achieved using 10 mL sample, 10 mL ethyl acetate, 120 min reaction time and 10 min centrifugation.

Total phenolic content

The total phenolic content in the samples/extracts was determined by the Folin–Ciocalteu colorimetric method using gallic acid as the standard [31]. The method is based on the reduction of phosphotungstate–phophomolybdate complex by phenolics to blue reaction products [22]. The modified method in brief, 0.1 mL of sample/standard solution (500, 250, 125, 62.5, 31.25 and 15.63 μg mL^{-1}) in 5% (v/v) methanol was mixed with 1 mL Folin–Ciocalteu reagent (1:9

diluted with water) and after 5 min 1 mL 10% (w/v) Na_2CO_3 was added. The mixture was diluted with 8.4 mL de-ionized water. The spectrum was scanned against blank using spectrophotometer from 1396–400 nm after 90 min of incubation at room temperature. The concentration of total phenolic compounds was expressed in microgram of gallic acid equivalent (GAE) per milliliter using the calibration curve equation, y = 0.36397x + 0.0287 (where y = absorbance and x = concentration, GAE in μg mL^{-1}). The absolute absorbance was taken around 760 nm (Abs at $_{max}$ – Abs at $_{base}$) against an appropriate blank. All samples were analyzed in triplicates.

Antioxidant assays

The evaluation of antioxidant capacity may vary according to the methods used [16]. The antioxidant capacity can be estimated with 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, trolox equivalent antioxidant capacity (TEAC) assay using 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid radical) [16] and ferric reducing/antioxidant power (FRAP) assay [32]. In this study DPPH assay was used. This test is based on the capacity of stable free radical 2,2-diphenyl-1-picrylhydrazyl to react with hydrogen (H) donors, including phenols. It is used for the quantification of antioxidants in the complex of biological systems, beverages [16].

For this routine assay, 160 μg mL^{-1} DPPH solution in methanol was prepared. The solution was vortexed vigorously until all DPPH was dissolved. Afterward, 2 mL of 160 μg mL^{-1} DPPH was mixed with 1 mL of ascorbic acid standards (25, 12.5, 6.25, 3.13 and 1.56 μg mL^{-1}) per alcoholic beverage samples, and 3 mL of methanol. The mixture was allowed to react at room temperature in the dark for one hour. The positive control assays were 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. Quantification of antioxidant capacity was made by calibration curves obtained from methanolic solutions of ascorbic acid; the antioxidant capacity of compounds was expressed as microgram of ascorbic acid equivalent (AAE) using the calibration curve equation, y = 18.23x + 17.66 (where y = absorbance and x = concentration, AAE in μg mL^{-1}). All samples were analyzed in triplicates.

Total flavonoid content

The total flavonoid concentration was measured using a colorimetric assay [32]. Catechin standard solutions were prepared by dissolving catechin in water at a concentration ranging from 10 to 50 μg mL^{-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 mol L^{-1} NaOH was added and the solution was made up to 10 mL with distilled water and mixed. The spectrum was scanned against blank using spectrophotometer from 850–290 nm. The absolute absorbance (Abs at $_{500}$ – Abs at $_{base}$) was against an appropriate blank. The total flavonoid content was expressed in micrograms of catechin equivalent per millilitre with calibration equation of y = 0.02228x + 0.06345 (y = absorbance and x = concentration, CE in μg mL^{-1}). All samples were analyzed in triplicate.

Total tannin content

The total tannin determination in alcoholic beverages was developed using egg albumin solution and Folin-Ciocalteu reagent as a precipitating reagent and color forming moiety, respectively. In this case the indirect approach was followed. That is, total polyphenolic compounds were determined using Folin-Ciocalteu method. For the determination of total tannin, first the same sample/extract was treated with 2 mL egg albumin (25 mg L^{-1}) solution under optimized

conditions (1:5 concentration ratio, 3.5 pH, 1 hour reaction time and 10 min centrifugation). 0.5 mL, 0.1 mL and 0.02 mL were taken for *tella*, *borde*, *korefe* and *keribo*; *tej* and *birz;* and *areke* samples, respectively. Then, the tannin–protein precipitate was separated via centrifugation and the supernatant (the non-tannin solution) was treated with Folin-Ciocalteu method as stated above. The difference in absorbance value before and after tannin removal was regarded as absorbance value of total tannins. All samples were analyzed in triplicate.

Statistical analysis

In this study, a one way ANOVA and Pearson correlation coefficient were used at 95% confidence level using SPSS software to know the variation between samples analyzed was significant or not.

RESULTS AND DISCUSSION

Quantities of total phenol and related assays and antioxidant capacity

Distilled beverage, areke

Levels of phenolic content are expressed in terms of gallic acid equivalent (GAE). The total phenolic content was determined by the method of Folin-Ciocalteu from calibration curve equation.

Table 2. Total phenolics, total flavonoids, total tannins and antioxidant capacity of *areke* samples.

Types of samples	Total phenolics [μg mL^{-1} GAE]	Total flavonoids [μg mL^{-1} CE]	Total tannins [μg mL^{-1} GAE]	Antioxidant capacity [μg mL^{-1} AAE]
Arsi Negele Cereal	0.25 ± 0.0008	0.08 ± 0.004	0.01 ± 0.006	0.19 ± 0.10
Arsi Negele Yekosso	0.26 ± 0.0014	0.08 ± 0.007	0.01 ± 0.033	271 ± 2
Butagera Cereal	0.22 ± 0.0045	0.07 ± 0.004	0.05 ± 0.009	-4.80 ± 0.06
Butagera Yekosso	0.20 ± 0.0003	0.07 ± 0.003	0.02 ± 0.001	-1.49 ± 0.05
Addis Ababa Cereal	0.22 ± 0.0002	0.07 ± 0.001	0.07 ± 0.003	-2.87 ± 0.03
Addis Ababa Yekosso	0.26 ± 0.0007	0.08 ± 0.008	0.08 ± 0.011	-1.61 ± 0.04
Addis Ababa Yegibto	0.27 ± 0.0015	0.07 ± 0.011	0.04 ± 0.003	0.62 ± 0.02
Debre Birhan Cereal	0.22 ± 0.0009	0.07 ± 0.006	0.04 ± 0.003	-0.86 ± 0.06
Debre Birhan Yekosso (white)	0.27 ± 0.0017	0.06 ± 0.005	0.00 ± 0.002	-3.39 ± 0.04
Debre BirhanYekosso (brown)	0.40 ± 0.0000	0.11 ± 0.197	0.05 ± 0.001	256 ± 4
Debre Birhan Yemar	0.62 ± 0.0021	0.07 ± 0.001	0.03 ± 0.001	284 ± 3
Debre Tsige Cereal	0.21 ± 0.0001	0.07 ± 0.003	0.08 ± 0.005	10.6 ± 0.01
Debre BirhanYenechshinkurt	0.24 ± 0.0019	0.12 ± 0.022	0.02 ± 0.001	249 ± 0.1
Dembecha Yegibto	0.32 ± 0.0008	0.07 ± 0.001	0.04 ± 0.002	217 ± 2
Dembecha Dagim	0.27 ± 0.0032	0.07 ± 0.015	0.06 ± 0.002	220 ± 4

Total phenolics, total flavonoids, total tannins and antioxidant capacity of *areke* samples ranged from 0.20–0.62 μg mL^{-1} GAE, 0.06–0.12 μg mL^{-1} CE, 0.02–0.08 μg mL^{-1} GAE and -0.86–284 μg mL^{-1} AAE with average values 0.28 μg mL^{-1} GAE, 0.077 μg mL^{-1} CE, 0.04 μg mL^{-1} GAE and 99.5 μg mL^{-1} AAE, respectively. Among the investigated types of *areke* samples, Yemar *areke* has the highest amounts of total phenolic and followed by Debre Birhan Yekosso (brown) *areke*. In most cases the *areke* types that have additional ingredients are higher in total phenolics and antioxidant capacity than the normal (cereal). However, there are some anomalies such as Addis Ababa Yekosso, Butagira Yekosso and Debre Birhan Yekosso (white) *areke* which have negative antioxidant capacity. This is most probably the expected one unless these

ingredients are combined after the distillation of *areke* is over using the distillate as extracting solvent (Table 2).

The result obtained in this study is comparable with the report on total phenolic contents of spirits, 0–22 μg mL^{-1} GAE [26]. Both spirits and *areke* have low content of total phenolics. The reasons are the depletion of phenolic compounds present in the raw materials with the high temperatures used in the distillation process while spirits were produced. Furthermore, the volatility of phenolic compounds is lower than that of ethanol and specific aroma compounds, that is why during the distillation process phenolic compounds do not accumulate in spirit drinks [26].

The antioxidant results of *areke* samples are in agreement with the low antioxidant activities of pure distillates determined by DPPH and FRAP assays. Rum, vodka, gin and other distillates showed no antioxidant behavior [33] or have negative values which is consistent with the low concentrations of phenolic compounds [34]. Values obtained by DPPH method were negative due the ethanol prooxidant activity and enhanced free radical formation [26].

Fermented beverages

The fermented beverages, *non-gesho* and with *gesho* phenolics and antioxidant assays are given in Table 3–6.

Table 3. Total phenolics, total flavonoids, total tannins and antioxidant capacity of *non-gesho* fermented samples.

Types of samples	Total phenolics [μg mL^{-1} GAE]	Total flavonoids [μg mL^{-1} CE]	Total tannins [μg mL^{-1} GAE]	Antioxidant capacity [μg mL^{-1} AAE]
Keribo				
Sample 1	14.9 ± 0.04	2.62 ± 0.01	0.68 ± 0.08	39.2 ± 1.32
Sample 2	12.4 ± 0.02	2.77 ± 0.01	4.01 ± 0.13	39.2 ± 1.32
Sample 3	11.8 ± 0.03	2.65 ± 0.01	4.98 ± 0.03	39.2 ± 1.32
Sample 4	10.4 ± 0.01	2.59 ± 0.01	3.66 ± 0.03	90.1 ± 5.24
Sample 5	12.1 ± 0.03	2.61 ± 0.05	4.60 ± 0.02	62.9 ± 1.89
Sample 6	10.9 ± 0.02	2.61 ± 0.05	4.36 ± 0.01	62.8 ± 5.42
Birz				
Sample 1	12.2 ± 0.14	2.56 ± 0.01	0.25 ± 0.02	42.0 ± 1.01
Sample 2	10.5 ± 0.02	2.57 ± 0.01	0.41 ± 0.02	52.5 ± 1.70
Sample 3	11.0 ± 0.08	2.56 ± 0.01	0.31 ± 0.08	63.1 ± 1.12
Sample 4	11.1 ± 0.02	2.56 ± 0.01	0.59 ± 0.05	50.7 ± 0.32
Borde				
Sample 1	8.56 ± 0.06	0.90 ± 0.09	0.11 ± 0.03	217 ± 13
Sample 2	9.03 ± 0.01	0.75 ± 0.01	0.41 ± 0.10	180 ± 5
Sample 3	10.6 ± 2.10	0.81 ± 0.02	3.67 ± 2.07	199 ± 9

In this study, the total phenolic content obtained in μg mL^{-1} GAE is: *tella* (10.1–19.1), *tej* (5.8–9.5), *keribo* (10.4–14.9), *birz* (10.5–12.2), *korefe* (9.2–10.7) and *borde* (8.4–10.6). Based on the mean total phenolics content obtained the beverages are in the order: *tella* > *keribo* > *birz* > *korefe* > *borde* > *tej*. According to the result obtained *tella* is the leading in the total phenolics content; this might be due to its more toasted grain flour (*enkuro*), germinated grain (*bikil*) and *gesho* (*Rhamnus prenoids*) in the composition [4]. Whereas *tej* is the least, this may be mainly by its preparation and less *gesho* (*Rhamnus prenoids*) content and nowadays by the using of sugars in place of honey during preparation [4]. The reported total phenolic contents in μg mL^{-1} GAE are: in beer, 270–600 [10], 206–374 [16] and 152–339 [23]; in wine, 178–284 [16], 189–3130 [21], 103–2261 [12] and 1648–4495 [35] and in dolo, 506 [16]. In all cases the reported

total phenolics in beer and wine are higher than the total phenolic compounds in the studied traditional alcoholic beverages. This is mostly due to differences in the composition of the raw materials, storage time and brewing process [36–38].

Table 4. Total phenolics, total flavonoids, total tannins and antioxidant capacity of *tella* samples.

Types of samples	Total phenolics [μg mL^{-1} GAE]	Total flavonoids [μg mL^{-1} CE]	Total tannins [μg mL^{-1} GAE]	Antioxidant capacity [μg mL^{-1} AAE]
Akaki Kaliti	13.3 ± 0.8	7.49 ± 1.45	0.87 ± 0.05	201 ± 3
Kirkos	13.0 ± 1.7	8.09 ± 2.15	1.69 ± 0.04	109 ± 2
Lafto	15.6 ± 0.4	5.89 ± 0.96	1.52 ± 0.48	136 ± 1
Bole	12.2 ± 1.3	4.62 ± 0.30	0.18 ± 0.11	87.5 ± 1.8
Sendafa	11.3 ± 1.3	4.75 ± 0.03	0.42 ± 0.35	133 ± 4
Sululta	18.3 ± 0.6	5.41 ± 0.06	1.21 ± 0.53	110 ± 3
Arada	11.2 ± 1.1	5.28 ± 0.01	2.29 ± 0.07	97.8 ± 1.2
Kolfe	15.3 ± 0.9	4.97 ± 0.01	2.10 ± 1.78	186 ± 4
Burayu	19.1 ± 0.9	4.77 ± 0.03	2.21 ± 0.06	195 ± 1
Gulele	14.3 ± 1.6	6.10 ± 0.04	8.84 ± 1.89	151 ± 4
Addis Ketema	12.5 ± 0.8	4.78 ± 0.05	0.28 ± 0.24	79.2 ± 0.9
Sebeta	11.0 ± 1.1	4.89 ± 0.01	0.29 ± 0.10	74.9 ± 2.6
Yeka	16.5 ± 0.9	5.37 ± 0.03	3.79 ± 2.86	82.7 ± 2.3
Lideta	12.2 ± 1.6	4.40 ± 0.01	2.25 ± 1.41	75.7 ± 3.2
Dukem	10.1 ± 1.6	5.05 ± 0.01	1.71 ± 0.09	31.6 ± 2.1

Table 5. Total phenolics, total flavonoids, total tannins and antioxidant capacity of *tej* samples.

Types of samples	Total phenolics [μg mL^{-1} GAE]	Total flavonoids [μg mL^{-1} CE]	Total tannins [μg mL^{-1} GAE]	Antioxidant capacity [μg mL^{-1} AAE]
Akaki Kaliti	8.48 ± 0.03	3.63 ± 0.01	2.36 ± 0.03	26.5 ± 0.1
Kirkos	7.59 ± 0.04	4.14 ± 0.01	0.93 ± 0.41	33.3 ± 0.02
Lafto	7.42 ± 0.29	4.08 ± 0.01	0.38 ± 0.16	25.5 ± 0.3
Bole	7.39 ± 0.11	4.11 ± 0.01	0.15 ± 0.09	27.7 ± 0.5
Sendafa	6.06 ± 0.20	2.86 ± 0.01	0.36 ± 0.12	3.49 ± 0.04
Sululta	6.95 ± 0.08	3.42 ± 0.01	0.65 ± 0.17	12.9 ± 0.1
Arada	9.47 ± 0.06	3.97 ± 0.01	2.26 ± 0.05	37.7 ± 0.5
Kolfe	6.44 ± 0.36	3.55 ± 0.01	1.08 ± 0.19	3.27 ± 0.36
Burayu	7.33 ± 0.52	3.26 ± 0.01	1.79 ± 0.27	7.79 ± 0.04
Gulele	6.21 ± 0.07	3.08 ± 0.01	0.27 ± 0.07	32.9 ± 0.2
Addis Ketema	6.84 ± 0.22	3.09 ± 0.01	1.14 ± 0.09	28.5 ± 0.1
Sebeta	6.07 ± 0.67	3.02 ± 0.01	0.51 ± 0.60	23.3 ± 0.3
Yeka	6.87 ± 0.06	3.97 ± 0.01	0.94 ± 0.08	7.51 ± 0.07
Lideta	8.69 ± 0.17	3.77 ± 0.01	1.77 ± 0.30	3.77 ± 0.33
Dukem	5.77 ± 0.08	3.16 ± 0.01	0.27 ± 0.18	1.73 ± 0.08

Table 6. Total phenolics, total flavonoids, total tannins and antioxidant capacity of *korefe* samples.

Types of samples	Total phenolics [μg mL^{-1} GAE]	Total flavonoids [μg mL^{-1} CE]	Total tannins [μg mL^{-1} GAE]	Antioxidant capacity [μg mL^{-1} AAE]
Sample 1	10.7 ± 0.01	3.01 ± 0.04	0.25 ± 0.02	73.9 ± 0.8
Sample 2	9.97 ± 0.02	2.61 ± 0.02	1.62 ± 0.06	96.5 ± 0.4
Sample 3	9.20 ± 0.03	2.79 ± 0.11	0.66 ± 0.01	58.4 ± 0.2
Sample 4	9.25 ± 0.05	2.80 ± 0.05	1.03 ± 0.04	58.3 ± 2.5
Sample 5	9.85 ± 0.02	2.91 ± 0.02	0.55 ± 0.02	69.2 ± 0.5
Sample 6	10.2 ± 0.02	2.78 ± 0.02	0.21 ± 0.04	75.1 ± 0.2

The total flavonoids content obtained in μg mL^{-1} CE is: *tella* (4.4–8.1), *tej* (2.9–4.1), *keribo* (2.6–2.8), *birz* (2.6–2.7), *korefe* (2.6–3.0) and *borde* (0.8–0.9). For mean total flavonoids the beverages are in the order: *tella* > *tej* > *korefe* > *keribo* > *birz* > *borde*. The reported total flavonoids in wine range from 0.3–680 in μg mL^{-1} CE [21]. Though, the obtained total flavonoids in the traditional alcoholic beverages is in the range reported by Li *et al.* [21], the value in wine is too high. *Tella* is the leading in the total flavonoids content than the rest. This might be due to its more *gesho* (*Rhamnus prenoids*) in the composition that is most probably a major source for total flavonoids.

Likewise in this investigation, the orders of the beverages based on the total tannins and antioxidant capacity are: *keribo* > *tella* > *borde* > *tej* > *korefe* > *birz* and *borde* > *tella* > *korefe* > *keribo* > *birz* > *tej*, respectively. *Keribo* is the leading in total tannins. This is most probably by its less fermentation time and preparation process [5]. In antioxidant capacity case, *borde* is first, this is most probably by the spices added when it is ready for serve, prepared from a wide variety of cereal crops and low alcoholic beverage [1].

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 amount of ingredients such as malt and hop, and their compositions [39]. Hop is known to be a major source of better antioxidant such as *p*-coumaric, caffeic, and ferulic acids [39].

The total phenolics and related assays of fermented beverages such as *tella*, *keribo*, *birz*, *korefe*, *borde* and *tej* results might be an over estimation. This might be due to the interference of the total phenolics, flavonoids, and antioxidant activity assays by substances which are present in the beverages such as sulfur dioxide, ascorbic acid, ethanol, and reducing sugars. The high concentrations of sugars (mainly glucose and fructose) and Maillard reaction products present could also interfere with these assays [32].

In all the beverages the total phenolic compounds reported in comparison with the total flavonoids and total tannins is too much and even incomparable. This has an agreement with; the majority of phenolic compounds in beer are non-tannic and non-flavonoid compounds (98% of total phenolic compounds) such as phenolic acids [37]. The variation among beverages and samples of the same beverages were obtained at 95% confidence level. This is due to the variation in the types and amount of ingredients used, and disparity in the preparation process [12, 40]. Moreover, the variation is dependent on the type of phenolic compounds present. For instance, flavonoids commonly constitute > 85% of the phenolics content (≥ 1 g L^{-1}) in red wines. In white wines, flavonoids typically comprise < 20% of the total phenolics content (≤ 50 mg L^{-1}) [12].

Relation between total phenolics and antioxidant activities of the beverages

The relationship between total phenolics and antioxidant activities was investigated using Pearson correlation at 95% confidence level. The results obtained indicate that the *non-gesho* fermented beverages such as *keribo* (-0.714), *birz* (-0.686) and *borde* (-0.212) have negative antioxidant correlation with the total phenolic. In contrast *areke* (0.609), *tella* (0.539), *tej* (0.385) and *korefe* (0.557) have positive correlations. In *areke* case the normal *areke* has negative correlation as reported; however, the overall correlation is positive. This is due to the phenolic compounds found in the added medicinal plants. In general, this demonstrated that the antioxidant activity of the beverages is dependent not only on the total phenolics content but also on the relative amounts of individual phenolics [14, 32]. Furthermore, the radical scavenging capacity of a group of phenolic compounds is dependent on their structure. For instance, the radical savaging capacity of the phenolic acids decreased in the order caffeic acid > protocatechuic acid > ferulic acid > vanillic acid > *p*-coumaric acid. From this sequence it can be concluded that cinnamic acid derivatives are better antioxidants than their benzoic acid

counterparts. This can be explained in terms of the CH=CHCOOH group, which participates in stabilizing the radicals of cinnamic acid derivatives by resonance [14].

Statistical analysis

The analysis of variance (ANOVA) revealed that there is a significant difference at 95% confidence level between the mean values of total phenolic, total favonoids, total tannin and antioxidant among the alcoholic beverages types and within the samples of each type. In addition, the relationship between total phenolic and antioxidant activities was investigated using Pearson correlation at 95% confidence level. The results obtained indicated that the *non-gesho* fermented beverages *keribo* (-0.714), *birz* (-0.686) and *borde* (-0.212) have negative antioxidant correlation with the total phenolic while *areke* (0.609), *tella* (0.539), *tej* (0.385) and *korefe* (0.557) have positive correlations. The variation in mean values of investigated parameters and the nature of correlation they have are due to the differences in preparation of the beverages, the amount and kind of ingredients used, and the types of phenolic compounds present in the raw materials and so on.

CONCLUSION

In this study the total phenolics, total flavonoids, total tannins and antioxidant activities of the beverages were investigated. In all beverages the total phenolic compounds were higher than the total flavonoids and total tannins. This showed that the majority of phenolic compounds in the beverages are non-tannic and non-flavonoids. The results obtained indicated that the distilled alcoholic beverage, *areke* has less antioxidant capacity and total phenolic content relative to fermented beverages. *Gesho*-containing fermented beverages have more total phenolic assays than the *non-gesho* fermented beverages. Among the fermented beverages, *tella* showed higher total phenolic level and antioxidant capacity than the rest. The investigation indicated that the total phenolic components of alcoholic beverages depend on the raw materials used and the brewing processes.

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