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Determination of Total Phenolic Compounds, Total Flavonoids and Antioxidant Activity of Barley Grain (Hordeum Vulgare L.) Commercially Available In Derahamusit, Burie and Jabitehena Districts of Amhara Region, Ethiopia

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BAHIR DAR UNIVERSITY

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M.SC THESIS

DETERMINATION OF TOTAL PHENOLIC COMPOUNDS, TOTAL FLAVONOIDS AND ANTIOXIDANT ACTIVITY OF BARLEY GRAIN (*HORDEUM VULGARE L.*) COMMERCIALLY AVAILABLE IN DERAHAMUSIT, BURIE AND JABITEHENA DISTRICTS OF AMHARA REGION, ETHIOPIA

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AUGUST, 2020
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A THESIS SUBMITED TO COLLEGE OF SCIENCE POST GRADUATE PROGRAM IN PARTIAL FULFILMENT TO THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ANALYTICAL CHEMISTRY

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ADVISOR'S APPROVAL SHEET

This is to certify that thesis entitled **"DETERMINATION OF TOTAL PHENOLIC COMPOUNDS, TOTAL FLAVONOIDS AND ANTIOXIDANT ACTIVITY OF BARLEY GRAIN (*HORDEUM VULGARE L.*) COMMERCIALLY AVAILABLE IN DERAHAMUSIT, BURIE AND JABITEHENA DISTRICTS OF AMHARA REGION, ETHIOPIA''** submitted in partial fulfillment of the requirements for the degree of Masters of Science in Analytical Chemistry to the Graduate Program in Department of Chemistry "college of science" Bahir Dar University and has been carried out by Banchayehu Baylie/BDU1100918PR/**,** under my supervision. Therefore, I recommend that the student has fulfilled the requirements and hence hereby can submit the thesis to the department.

Minaleshewa Atlabachew _____ _____

Name of Advisor Signature Date

EXAMINERS' APPROVAL SHEET

We, the undersigned, members of the Board Examiners the final open defense by Banchayehu Baylie Siferh have read and evaluated she's thesis entitled**" DETERMINATION OF TOTAL PHENOLIC COMPOUNDS, TOTAL FLAVONOIDS AND ANTIOXIDANT ACTIVITY OF BARLEY GRAIN (*HORDEUM VULGARE L.*) COMMERCIALLY AVAILABLE IN DERAHAMUSIT, BURIE AND JABITEHENA DISTRICTS OF AMHARA REGION, ETHIOPIA",** and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree of Masters of Science in Chemistry (Analytical) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Board of Examiners

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DECLARATION

I, the undersigned, declare that this thesis, entitled **"DETERMINATION OF TOTAL PHENOLIC COMPOUNDS, TOTAL FLAVONOIDS AND ANTIOXIDANT ACTIVITY OF BARLEY GRAIN SAMPLE (*HORDEUM VULGARE L.*) COMMERCIALLY AVAILABLE IN DERAHAMUSIT, BURIE AND JABITEHENA DISTRICTS OF AMHARA REGION, ETHIOPIA"** is my original work under the supervision of Dr. Minaleshewa Atlabachew, except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at BDU and other institution.

Acknowledgements

This thesis could not have been accomplished without assistance of a number of persons and I would like to take these few lines to thank them warmly for their help.

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Abbreviation and Acronyms

AA…….Antioxidant activity

AAE……..Ascorbic acid equivalent

BHA……...Butylated hydroxyanisole

BHT……Butylated hydroxytoluene

CE…….. Catechin equivalent

CSA…….Central statistical agency

DPPH……..2, 2-diphenyl-1-picrylhydrazyl

FCR ……. . Folin-Ciocalteu's Reagent

GAE ……..Gallic acid equivalent

HAT……Hydrogen atom transfer

ROS…….Reactive oxygen species

rpm…….....Revolution per minute

TPC…….Total phenolic content

TAA……. Total Antioxidant activity

UV-Visible……Ultraviolet visible

V/V……Volume per volume

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Abstract

Barley is among the most ancient cereal crops, evolving over eons. The purposes of this study was to determine the total phenolic content, total flavonoids and total antioxidant capacity of barley grain collected from selected areas of Amhara region; and to compare it with other country barley and other cereal grains. A70% acetone in water was used to extract the phytochemicals. Total phenolic content was determined by Folin-Ciocalteu's method, total flavonoids was determined by aluminium chloride methods while DPPH radical scavenging activity method was used to determine the total antioxidant activity and results were expressed as gallic acid equivalent), catechin equivalent and ascorbic acid equivalent respectively. Results of the determination revealed that total phenolic content, total flavonoids and total antioxidant capacity was ranging from 4.85±0.4 to 5.65±0.7 mg GAE/g, 3.45±0.4 to 4.01±0.7mg CE/g, and 2.98±0.8 to 3.69±0.4 mg AAE/g of sample respectively. Among the studied samples, sample from Derahamusite district exhibited a highest scavenging effect and the reducing capability on DPPH solution compared to samples from other district. Pearson correlation showed there were positive correlations between total phenolic content and total flavonoids content with antioxidant capacity. At 95% confidence level one way ANOVA displayed that the levels of total polyphenol, total flavonoid and antioxidant activity were not significantly varied between sampling sites. Furthermore, the magnitude of these bioactive chemicals are comparable amount with the other barley grain and other cereals reported in other literature.

***KEY WORDS**: Polyphenol level, Flavonoid level, Antioxidant capacity, Barley grain, Catechin, Folin-Ciocalteu*

1. Introduction

1.1 Background of the Study

Barley is among the most ancient cereal crops, evolving over eons. Cultivated barley (*Hordeum vulgare L.)* is derived from its wild relative (*Hordeum spontaneum C. Koch*) and domesticated about 10 thousand years ago in the Fertile Crescent of the Middle East (Newman and Newman, 2008). The progenitor of cultivated barley is a brittle two-row spike and a hulled grain. The first cultivated barley is 2-rowed types. In high production irrigated and rain fed areas, 6-rowed types quickly became the predominant barleys (Kleinhofs and Graner, 2001). The weed forms of barley are *Hordeum spontaneum K. Koch, Hordeum agrocrithon Aberg, Hordeum intermedium, Hordeum bulbosum*, etc. *Hordeum spontaneum C. Koch* represents a genetically diverse source of diverse phenotypes for barley improvement (Henry and Brown, 1987).

Presently, production wise, barley occupies fourth position among the cereal crops in the world. Although barley has been used extensively as a food in the past, It is now been relegated to animal feed (about 60%), malt (about 30%), or seed (about 7%), with only a small amount (about 3%) for human food in most countries (Baik and Ullrich, 2008). Predominance of maize, wheat, and rice as main food grains has presently demoted barley to the status of "poor man's bread" Besides the usual nutritional benefits of cereal grain, most importantly, barley cell wall is good amount of biologically active compounds, including soluble dietary fiber (Newman and Newman, 2008), ß-glucans, chemically (1-3,1-4)-ß-D-glucans, microelements, sterols, phenolic compounds, peptides, vitamins, minerals, proteins, phytonutrients include vitamin E, Zn, Fe, Se, Co, Mn, S, folates, carotenoids, phytic acid, lignins and lignans distributed throughout the entire kernel (Sullivan et al., 2013). Barley is easily digestible due to low gluten contents (Šimić *et al.*, 2017).

Phenolic compounds are secondary metabolites created by plants during normal development and under stress conditions (Sathishkumar et al., 2010). They can be found in a free, esterified or in an insoluble bound form and they are quantitatively distributed between different tissues of the grains (Lahouar *et al*., 2014). A large and heterogeneous group of polyphenols including phenylpropanoids, condensed tannins, lignin, flavonoids and hydroxycinnamic acids (Bragazza and Freeman, 2007).

The major portion of phenolic compounds is located in outer parts of grains, where they are involved in defense against ultraviolet radiation, pathogen invasion and in modification of mechanical properties (Manach et al., 2004). Phenolic compounds have strong anti- oxidant properties in vitro, associated with their ability to scavenge free radicals and chelate transition metal ions (Andreasen et al., 2001).

The presence of bioactive compounds and non-digestible carbohydrate in outer layer of the grains and in the germ fraction always make them be the first choice when human looking for a healthy and balance diet. The presence of bioactive compound significant antioxidant effects. The antioxidants in grain products could act independently or synergistically with fiber for prevention of chronic diseases. Thus, grains are a very convenient way to fulfill the requirement of daily of antioxidant intake for us (Miller et al., 2000) and non-digestible carbohydrate gives beneficial effects in human physiology and important for normal digestive function. It is the major fiber fraction in most cereal products and made of insoluble hemicelluloses, cellulose, resistant starch and lignin. On the other hand, the soluble dietary fiber also helps to lower cholesterol and glycemic index (Shimizu *et al*., 2002).

Barley contains a relatively high concentration of β- glucans compared with other grains. Barley is an abundant source of phenolic compounds and can be considered an excellent dietary matrix of natural antioxidants for disease prevention and health promotion. Additionally, barley phenolic are very important due to their influence in several stages of the brewing process and the overall beer stability (e.g., formation of haze, color, taste, filtration, foam stability and redox state) (Carvalho et al., 2015). It is known that over 60% of the total poly phenolic content found in beer comes from barley (Quifer-Rada *et al.*, 2015).

1.2 Statement of the Problem

Like many other foods we eat and drink, the composition of barley grain there are many compounds in barley grain that are often thought to have implications upon human health. So we have to analyze some of this phytochemicals like phenolic compounds, total flavonoids and antioxidant activity. This is because, phenolic compounds and flavonoids are essential to prevent chronic diseases. There are some reports on the amount of phenolic compounds, total flavonoids and antioxidant activity in the worldwide barley grain collected from different countries (Miller et al., 2000). These data reveals that the phenolic compounds, total flavonoids

and antioxidant activity of barley grain varies significantly with region of origin. Although barley grain cultivation is expanding in Ethiopia at different areas, the total phenolic compound, total flavonoids and antioxidant activity of these barley grain types have not been investigated so far. Hence, it is a pressing demand to quantify the total phenolic compound, total flavonoids and antioxidant activity of the barley grain in Ethiopia. In general, the main purpose of this study to establish the chemical profile of food barley in the selected area of Ethiopia.

1.3 Objective Of The Study

1.3.1 General Objective

The main objective of this study was to determine the levels of total phenolic content, total flavonoids and antioxidant activity in barley grain calorimetrically.

1.3.2 Specific Objective

- To determine the levels of total polyphenol in barley grain from selected area of Amhara region.
- To determine total antioxidant capacity in barley grain from selected area of Amhara region.
- To determine the levels of total flavonoids in barley grain from selected area of Amhara region.
- To investigate the variations of total polyphenol, total flavonoids and antioxidant capacity between the different sampling areas.
- To compare the levels of mentioned bioactive chemicals of these barley grain with other barley grain and other cereals from different areas.

1.4. Significance of the Study

This study reveals that ability of barley to fight against oxidation stress leads to have properties that improve to decrease the occurrence of a number of age-related diseases such as coronary heart disease, diabetes and certain types of cancer, increases running endurance, decrease body fat composition while increasing one's energy expenditure and reduce mortality caused by degenerative diseases (Hue, 2013).

These findings point to various polyphenols, total flavonoids and total antioxidant in many barley grains as a causative factor for these beneficial results. However, there is no report in Ethiopian to support this assumption. Therefore, this study was designed to determine total polyphenols, total flavonoids and total antioxidant levels in selected Ethiopian barley grain. The findings will give credence to the hypothesis that described barley has beneficial qualities. Consumers will then be able to production of barley with successful efforts in obtaining the greatest concentrations of health beneficial. So, this study will provide baseline information and make recommendation for the future on these barley grain.

2. Literature Review

2.1 World Production and Distribution of Barley

Barley is a widely grown cereal crop in the world mostly used as feed for animals and humans, raw material in the production of malt for the brewing industry, and in the bakery industry (Gebremariam et al., 2014). The exact origin of barley is not exactly known, presumed to be originated either in Egypt, Ethiopia, the Near East. There is, however, compelling evidence of the possibilities of multi centers of origin of barley, initiating in the Iberian Peninsula, extending across North Africa. But it can be surely said that it is one of the earliest cultivated grains. Barley is grown in the Middle East prior to 10,000 BC, but barley's cultivation in China and India probably occurred later. Barley is grown on the Korean Peninsula by 1500-850 BC along with millet, wheat, and legumes. Six-rowed barley did not come about until after 6000 BC. In ancient Egypt (3200 BC to 30 BC) barley bread and beer (made from malt) constituted a complete diet (Vasan et al., 2014).

The major producers of barley in the world during the year 2011 E.C are Russian Federation, Ukraine, France, Germany, Spain, Australia and Canada. Russian Federation produces the greatest quantities of barley with an estimated production of approximately 16.94 million tons followed by Ukraine with a production of about 9.1 million tons in 2011 E.C. Of all countries that produce barley, the least producer of barley is Bangladesh, which produced only 484 tons of barley in 2011. Ethiopia is the second in the Africa in terms of barley production and produced only 1.7million tons of barley during the year 2011 E.C (Holland *et al*., 2001).

The world and African barley production is listed in Figure1 and Figure 2

Figure 1: world barley production in 2011 E.C

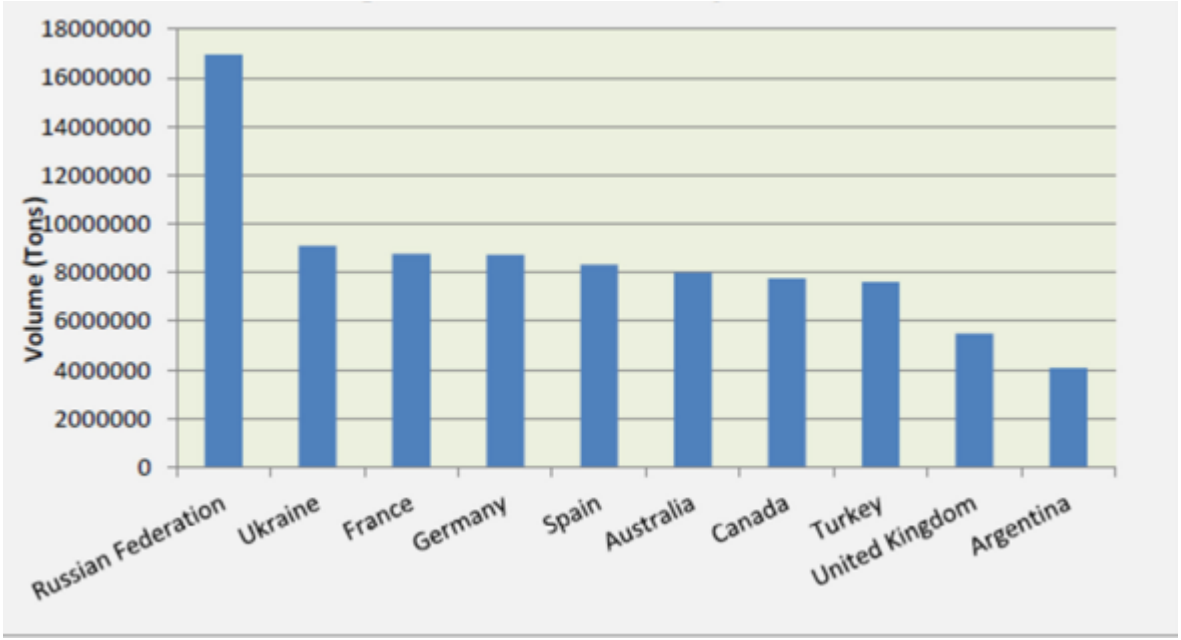
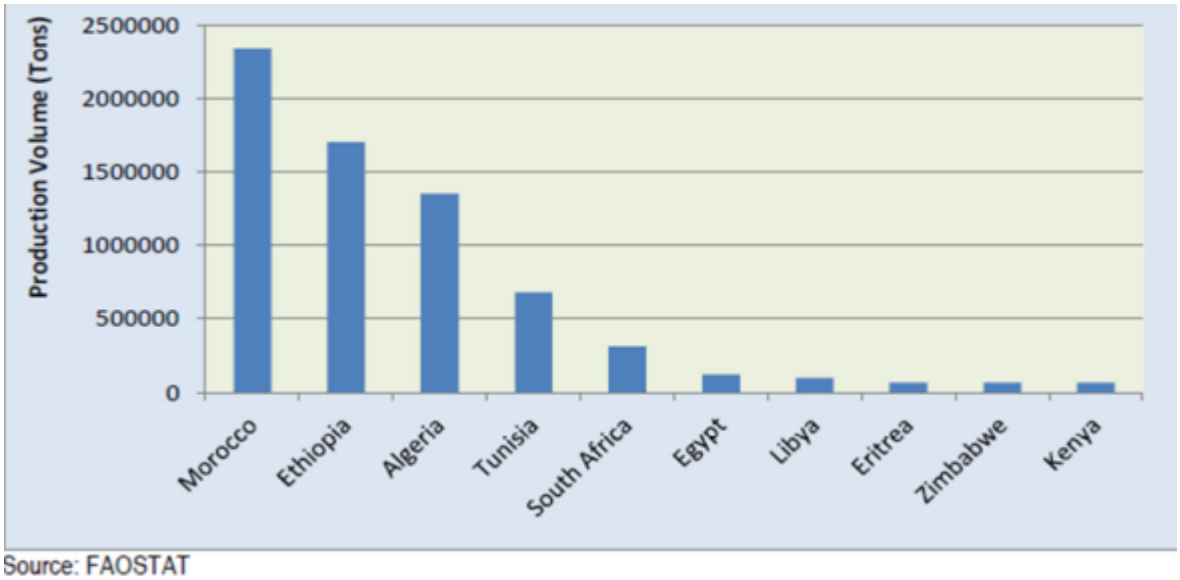


Figure 2: Africa barley production in 2011 E.C



Source: FAOSTAT

2.2 Barley production in Ethiopia

Barley (*Hordeum vulgare*) is cultivated by small holders in every region of Ethiopia, since it is able to grow at all elevations, but it performs best at the higher elevations in the northern and central regions of the country. Barley is a major staple food crop in the highlands of Ethiopia and the fifth most important cereal crop in the country (Kaso and Guben, 2015). Ethiopia is ranked twenty-first in the world in barley production with a share of 1.2% of the world's total production. There are two varieties of barley in Ethiopia: food barley for human consumption and malt barley. The crop is used for preparing various types of traditional foods such as *Kita, Kolo, Beso, Enjera, , cuko (shakeka)* and many others (Tefera, 2014).

Agriculture in Ethiopia contributes up to 46% of the national gross domestic product and 80% of export earnings (Abraham *et al.*, 2014). It occupies 1.02 million ha of land in terms of production area. In Ethiopia, barley grain is produced mainly for human consumption and it is one of the most important staple food crops (Birhanu *et al*., 2005).

The major barley production in Ethiopia and Amhara Region is listed in Table 1 and Table 2 respectively.

Table 1: In 2017/2018 (2010 E.C) major barley production in Ethiopia

Regio*n*	Production In Quintals
Tigray	1,694,179.80
Afar Region	**
Amhara Region	6,394,523.75
Oromia Region	10,884,876.60
Somali Region	**
Benishangul-Gumuz Region	10,641.21
S.N.N.P. Region	1,545,047.18
Gambela Region	**
Harari	**

Source: CSA agricultural sample survey 2017/2018 (2010E.C)

** means very minimum amount

Table 2: In 2015 - 2016 (2008 E.C) major barley production in Amhara Region

Zone	Production In Quintals
North Gondar	1,010,157.56
South Gondar	470,898.65
North Wollo	477,423.79
South Wollo	522,945.00
North Shewa	482,344.49
East Gojam	879,655.28
West Gojam	1,570,324.77
Waghimra	151,515.76
Agwawi	189,016.72

Source: CSA agricultural sample survey 2015 - 2016 (2008E.C)

2.3 Variety of Barley in Ethiopia

There are two varieties of barley in Ethiopia: food barley for human consumption (Six-row barley) and malt barley. Six-row barley is the most cultivated barley in Ethiopia and it produce 25-60 grains and it has high content of proteins. Based upon the fertility of the florets on the spike in six-rowed barleys all of the florets are fertile leading to six vertical rows of seeds on the spike (Meints et al., 2016). Malt barley is made by soaking and drying barley kernels. The kernels are then allowed to germinate or sprout in a controlled environment. Malting barley is used to produce malt for the brewing and food industries. (Vasan *et al*., 2014).

Consequently, in 2012/2013 cropping season, there are different improved food barley varieties which includes *Biftu, temeshi gebs, Dafo, Abdane, white barley, Dimtu, HB-1307(Tebel gebs), Ardu-1260B, Shege, Dinsho, Senef kolo, HB-42 Diribe and cross-4198* are evaluated against local check. Six-rowed food type barley, *HB-1307(Tebel gebs),* is developed by Holetta Agricultural Research Center from a cross between *a landrace line and exotic germplasm (Awra gebs-1 x IBON93/91)* and released in 2006 for mid and high altitude areas. This variety has been released for mid and high altitude barley growing areas due to its superiority in grain

yield performance, stability, and wide adaptation. It has good physical grain quality (Figure 3), resistance to leaf rust and scald, moderate resistance to net and spot blotch, lodging tolerance, and good biomass yield (Bekele *et al*., 2005). Some agronomic characteristic and agro-ecological requirements of *HB-1307(Tebel gebs)* food barley is presented in Table 3 and the variety is shown in Figure 3.

Table 3: Some agronomic characteristics of HB-1307 and ecological requirement

Parameters	Descriptions
Year of release	2006 G.C
Maturity time	137 days
Rain fall requirement	700-800mm
Altitude range	2000-3000m above sea level

Figure 3: *HB-1307* food barley improved variety seed/grain.

Source: Agricultural production in Ethiopia

2.4. Nutritional Composition and Health Benefits of Barley

The health benefits and medical applications of barley foods are referred to in ancient Arabic, Chinese, Egyptian, Ethiopian and Greek literature and have been reported by more recent civilizations from Asia to Europe (Newman and Newman, 2008). Whole barley grain consists of about 65 – 68 % starch, 10 – 17 % protein, 2 – 3 % free lipids, 4 - 9 % β-glucans, total flavonoid 0.53 %, total polyphenol 1.06 %, fat 4.57 % and 1.5 - 2.5 % minerals (Abdel-Aal and Choo, 2014).

Generally, the content of nutritious and functional ingredients is very different depending on the growth stage of barley grains or processing technology or various cultivars (Koga *et al.*, 2013). Barley contains protein similarly to other cereals but like oats contains higher levels of the soluble fiber β-glucan compared with other cereal grains such as wheat and rye. The β-glucans found in barley can lower blood cholesterol levels and thereby reduce the risk of coronary heart disease (Ames and Rhymer, 2008).

The β-glucans also lower blood sugar which is important in the prevention and management of type 2 diabetes (Tosh and Miller, 2019). Barley have a soluble fiber in the form of β-glucans and also an excellent source of insoluble fiber which is important in maintaining digestive health and protecting against colon cancer (Aune *et al*., 2011).

Barley also has high levels of tocotrienols, phenolic compounds and lignans which are responsible to reduce the risk of coronary heart disease, diabetes, and certain cancers. Barley is a good source of many essential vitamins and minerals, including thiamin, niacin, folate, riboflavin, iron, phosphorus, magnesium, zinc, and selenium, all of which are important in maintaining good health and weight control. Barley contains levels of fat that are similar to other cereal grains with the exception of oats which has higher levels of fat than any other cereal grains (McGuire, 2011).

2.5. Total phenolic Compound

Phenolic compounds are secondary metabolites that are created by plants during normal development and under stress conditions such as wounding, infection and ultraviolet radiation (Sathishkumar et al., 2010). Polyphenols are naturally occurring organic compounds found largely in the fruits, vegetables, cereals and beverages. More than 8000 phenolic structures are currently known and among them over 4000 flavonoids have been identified (Berhanu, 2014). Flavonoids are a type of polyphenols, which made up approximate two-thirds of the dietary phenols. Apart from grains they also exist in vegetables, nuts, spices and beverages such as wine, beer and tea. Flavonoids have high potential in antioxidant and anticancer activities (Hue, 2013).

Although polyphenols are chemically characterized as compounds with phenolic structural features, this group of natural products is highly diverse and contains several sub-groups of phenolic compounds. Fruits, vegetables, whole grains and other types of foods and beverages

such as tea, chocolate and wine are rich sources of polyphenols. The diversity and wide distribution of polyphenols in plants have led to different ways of categorizing these naturally occurring compounds. Polyphenols have been classified by their source of origin, biological function and chemical structure. Also, the majority of polyphenols in plants exist as glycosides with different sugar units and acylated sugars at different positions of the polyphenol skeletons (Dai and Mumper, 2010).

Polyphenols are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or violence by pathogens. In food, 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 (Dai and Mumper, 2010). Examples of polyphenol compounds are shown in Figure 4.

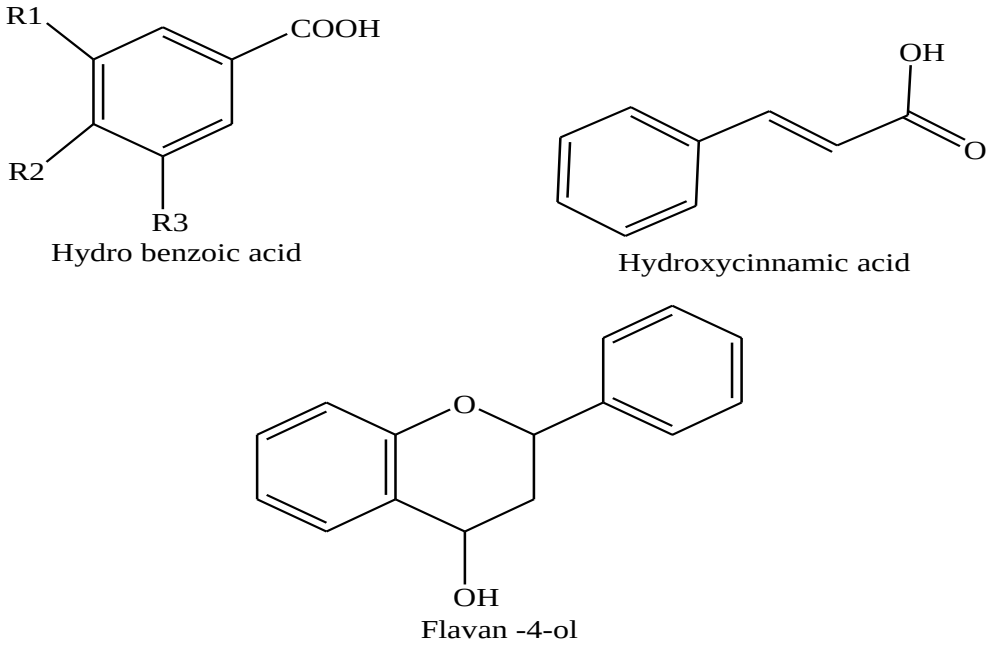


Figure 4:Examples of polyphenolic compounds which is hydroxybenzoic acid, hydroxycinnamic and Flavan-4-ol (Dai and Mumper, 2010).

2.6 Flavonoids

Flavonoids comprise the most studied group of polyphenols. Flavonoids have the C6–C3–C6 general structural backbone in which the two C6 units (ring A and ring B) are of phenolic

nature (Figure 5). Due to the hydroxylation pattern and variations in the chromane ring (ring C), flavonoids can be further divided into different sub-groups such as flavan-3-ols, flavones, flavanones and flavonols. While the vast majority of the flavonoids have their ring B attached to the C2 position of ring C, some flavonoids such as isoflavones and neoflavonoids, whose ring B is connected at the C3 and C4 position of ring C, respectively, are also found in plants. These basic structures of flavonoids are aglycones; however, in plants, most of these compounds exist as glycosides. Biological activities of these compounds, including antioxidant activity, depend on both the structural difference and the glycosylation patterns. Quercetin, myricetin, Catechins, etc. are some most common flavonoids (Abegaz et al., 2002).

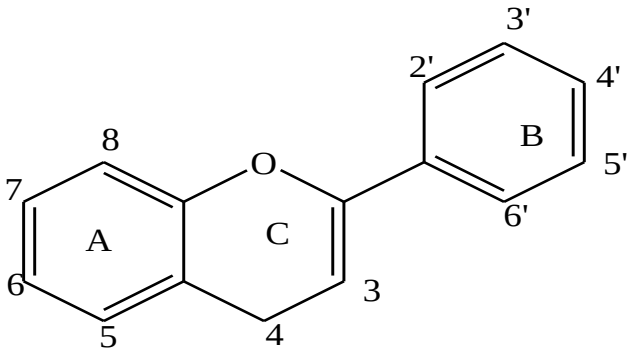


Figure 5: Basic flavonoid structure(Abegaz *et al*., 2002)

2.7 Antioxidant Activity

Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Antioxidants can also protect the human body from free radicals and ROS effects. They retard the progress of many chronic diseases as well as lipid peroxidation. Also, antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods. Food antioxidants such as ascorbic acid, amino acids, proteins, flavonoids and other phenolic compounds might also play a significant role as physiological and dietary antioxidants, there by supplementing the body's natural resistance to oxidative damage (Gülçin *et al*., 2010).

There are mainly two types of antioxidant, which are natural antioxidant and synthetic antioxidant. Some natural antioxidants are vitamin C, vitamin E, β- carotene, nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, amines) and phenolic compounds. Phenolic compounds are common antioxidants, which can be found in fruits, vegetables and

grains. Synthetic antioxidants are phenolic compounds that have different degrees of alkyl substitution. Instances of synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate, and tertbutylhydroquinine (Velioglu et al., 1998). Figure 6 illustrates how antioxidant scavenges the DPPH radical and reduces its amount.

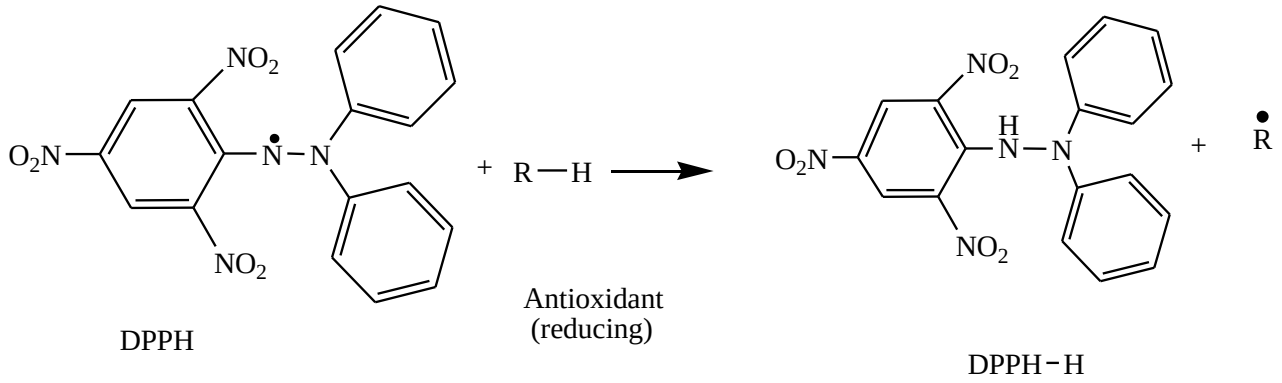


Figure 6: simple illustration on how antioxidants scavenge DPPH radical (Cian et al., 2014)

2.8 Sample preparation

Sample preparation is an important step for any solvent-based extraction procedure. Although many samples can be effectively extracted without any pretreatment, other samples require some manipulation for efficient extraction. (Hyötyläinen, 2009).

2.8.1 Extraction Methods

Extraction methods are selected mainly depending on the nature of the compounds, the importance of the facilities, and the end use of the manufactured substance. Several pretreatment steps have been also applied in different studies including drying, maceration, homogenization, grinding, and milling. These processes increase the interaction between the solvent and the analyte (the polyphenols) during the step of extraction. Drying generally reduces the mass of the raw material required to be used for extraction. Pretreatment methods such as maceration and grinding lead to breakdown of cellular structure to increase the surface area of the biomaterial exposed for extraction and increase the amount of polyphenols extracted (Verma *et al*., 2012). The most common method of extraction is solvent extraction. There are several other advanced methods of extraction for polyphenols which have been found to be more efficient methods than standard solvent extraction. These advanced methods of extraction are also based on solvent extraction that is they use the most suitable solvents with advanced processes to drive the extraction. With concerns regarding energy conservation, lower solvent

consumption, and prevention of pollution, methods such as microwave extraction, ultrasonic extraction, accelerated solvent extraction, supercritical fluid extraction and others (Stalikas, 2007).

2.8.2 Extraction of Phenolic Compounds

Scientists have studied and analyzed the impact of different types of solvents, such as methanol, hexane, ethanol, acetone and ethyl alcohol, for the purpose of antioxidant extraction from various plants parts, such as leaves and seeds (cereals). In order to extract different phenolic compounds from plants with a high degree of accuracy, various solvents of different polarities must be used (Wong and Kitts, 2006). Moreover, scientists have discovered that highly polar solvents, such as methanol, acetone and N, N dimethylformamide (DMF) are highly effective at extracting antioxidant activity's different plant and seed (cereal) extract (Koffi et al., 2010).

Multiple solvents have been commonly used to extract phytochemicals, and scientists usually employed a dried powder of plants to extract bioactive compounds and eliminate the interference of water at the same time. Solvents used for the extraction of biomolecules from plants are chosen based on the polarity of the solute of interest. A solvent of similar polarity to the solute will properly dissolve the solute. Multiple solvents can be used sequentially in order to limit the amount of analogous compounds in the desired yield. The polarity, from least polar to most polar, of a few common solvents is as follows:

Hexane < Chloroform < Ethylacetate < Acetone < Methanol < Water. For this work Acetone is the most preferable solvent. The reason to choose aqueous acetone is antioxidant compounds existed in the cereals were complex and their activity and mechanism were greatly affected by composition and conditions of the test systems. Acetone managed to extract the antioxidants in the cereal efficiently since the recommended solvent suggested by (Vaher *et al*., 2010).

2.9. Methods of Analysis of phenolic Compounds and Antioxidant Activity

2.9.1. Method Analysis of Total Polyphenols

The Folin-Ciocalteu method is a widely used method for the determination of total phenolic content. The Folin-Ciocalteu is chosen for this purpose due to its wide applicability for biological materials and its simplicity to use in the lab (Waterman, 1994). The method also provides reasonably good and reliable estimates of concentration of total reducing phenolic groups. The method is based on the reducing power of the phenolic hydroxyl groups (Hahn et al., 1984) which react with Folin- Ciocalteu phenol reagent (an oxidizing agent comprised of hetero polyphosphotungstate- molybdate) under basic conditions to form chromogens that can be detected spectrophotometrically at 760 nm. The reaction forms a blue chromophore constituted by a phosphotungstic phosphomolybdenum complex, Folin and Ciocalteu included lithium salts in the reagent, which prevented the turbidity due to excess Folin-ciocalteu reagent (Blainski *et al.*, 2013)

Sodium carbonate, which yields an appreciable concentration of the phenolate ions, the phenolates reduces the yellow Folin-Ciocalteu reagent; the reaction changing it into a blue pigment. The absorbance of extracts is measured by using single beam spectrophotometer and expressed as mg of GAE per gram of grain. TPC values ranged from 169 to 10548 mg/100 g of dry product (Cicco and Lattanzio, 2011).

2.9.2 Method Analysis of Antioxidant Activity

Various methods are created to quantify antioxidant capacity of food constituents. Radical scavenging activities are very important due to the deleterious role of free radicals in foods and in biological systems. Diverse methods are currently used to assess the antioxidant activity of plant phenolic compounds. But DPPH radical scavenging methods are common spectrophotometric procedures for determining the antioxidant capacities of components because of DPPH radical is the stable chromogen compounds to be measured with UV-Vis spectroscopy (Li *et al*., 2009), the violet DPPH radical is easy to use, have a high sensitivity, and allow for rapid analysis of the antioxidant activity of a large number of samples, inexpensive and provides firsthand information on the overall antioxidant capacity of the test system (Gülçin *et al*., 2010).

In the DPPH assay, the antioxidants able to reduce the stable radical DPPH to the yellow colored diphenyl-picrylhydrazine. The method is based on the reduction of alcoholic DPP· (Radical) solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH–H by the reaction. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Brighente *et al*., 2007). With this method it is possible to determine the antiradical power of an antioxidant by measuring of a decrease in the absorbance of DPPH-at 517 nm. The resulting color change from purple to yellow, the absorbance decreased when the DPPH- is scavenged by an antioxidant through donation of hydrogen to form a stable DPPH-H molecule in the radical form, this molecule has an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule (Gülçin *et al*., 2010).

Spectrophotometry is used and the level of the mixture of antioxidants can be determined with ascorbic acid or gallic acid equivalent. The DPPH radical scavenging activity in terms of percentage is calculated according to the following equation (Mitra and Uddin, 2014).

$$\text{Percent inhibition} = \frac{\text{ADPPH} - \text{A sample}}{\text{ADPPH}} \text{ X } 100\%$$ Where: ADPPH = Absorbance of DPPH, A sample = Absorbance of sample (sample/ascorbic acid).

2.9.3 Method Analysis of Total Flavonoids

The flavonoid content is usually determined according to mostly applied spectrophotometric methods based on the formation of aluminum-flavonoid complexes; complexion reaction is carried out in the presence of $NaNO_2$ in alkaline medium (Pękal and Pyrzynska, 2014).

Total flavonoid content is determined by aluminum chloride method, using catechin as a standard, 5% sodium nitrite, 10% aluminum chloride, 1M sodium hydroxide is used as a reagent. Here the method is based on the nitration of any aromatic ring bearing a catechol group with its three or four positions un-substituted or not satirically blocked. After addition of $AlCl_3$, a yellow solution of complex is formed, which then turned immediately to red after addition of NaOH, 5% $NaNO_2$ is added because the complexation of $AlCl_3$ with flavonoid, in the presence of $NaNO_2$ in alkaline medium is very selective. 1M sodium hydroxide is used for neutralization of the mixture (Pękal and Pyrzynska, 2014).

3. Materials and Methods

3.1 Description of Study Area

The study was conduct in Amahara region which is Dera, Burie, and Jabitehenan districts. Dera located in north western Ethiopia, in Amhara national regional State its distance is 40 km from Bahrdar. Jabitehenan, is located in north western Ethiopia, in Amhara national regional state, at a distance of 172 km from Bahrdar. Burie is located in north western Ethiopia, in Amhara national regional State, at a distance of 152 Km from Bahrdar.

3.2. Sample Collection

One variety of barley grain (HB-1307) samples was collected from three districts of Amhara region. The districts are: Dera, Burie and Jabitehenan districts. For each district three samples from different sub-districts were considered. A total of nine barley grain samples was collected from the market of each sub-districts. The samples were collected in November 2019. The districts of the sample is listed in Table 4.

Table 4: District of study area with varieties of sample (taken by author)

Zone	District	Sub-district	Sample
North Gondar	Dera hamusit	Aribgebeya	*Tebel gebs* *(HB-1307)*
		Gelawudiwes	
		Sene Mariam	
West Gojam	Burie	Fereda	
		Wundiy	
		Bure zuria	
	Jabitehena	Mendereslasie	
		Abragit	
		Kuarit	

3.3. Instruments and Materials

Refrigerator, measuring cylinder (Karter Scientific 213I10), Mill (ADITEK), Analytical balance (Model ESJ200-A), Centrifuge (Model-1), polyethylene plastic bags, Micro Pipette (China), Water bath shaker (DAIHAN Scientific), 0.5 mm sieve, quantitative filter paper and UV Spectrophotometer (Agilent technology, Cary 60UV-Vis) were used for laboratory analysis.

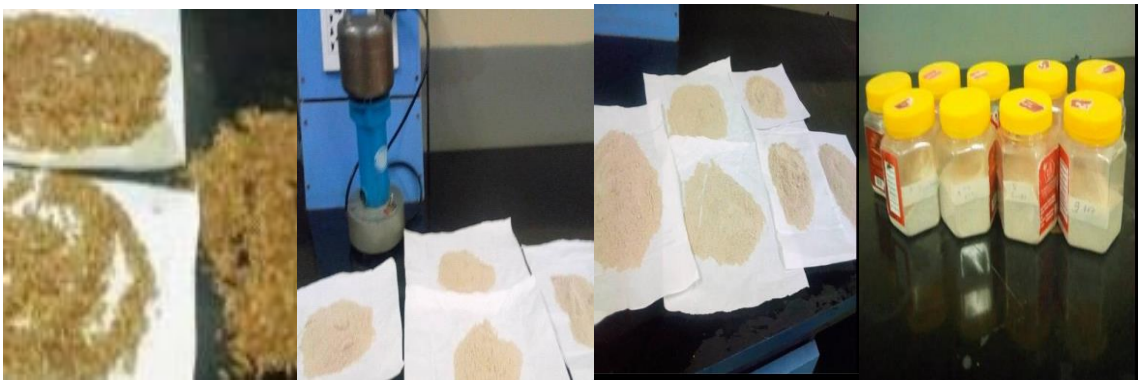
3.4. Chemicals and Reagents

All reagents and standards that used in the analysis were at analytical grade. Gallic acid (CARLOERBA, France), Folin- Ciocalteu's Reagent (Maharashtra, India), Sodium Carbonate (DELHI-110092, INDIA), 2, 2–diphenyl-1-picrylhydrazyl (DPPH) (sigma Aldrich) Acetone, Ethanol, sodium nitrite (Blulux), Aluminum chloride, sodium hydroxide, Ascorbic acid (India), chatichin (Blulux), Distilled and deionized water.

3.5 Methods of Analysis

3.5.1 Sample preparation

Some impurities like soil, sand and other external wastes were sorted out manually from the grain of barley. The pure barley grain samples were ground by electric miller (grinder) and sieved using 0.5 mm sieve. Finally, the powder samples were kept in plastic container until the next step (extraction) could takes place (Figure 7).



A) Cleaned sample B) Grinding C) Sieved samples D) packed sample

Figure 7: barley grain powder (Tebel gebs) sample preparation

3.5.2 Analyte Extraction

For extraction of phenolic compounds from solid samples, 70% acetone is frequently reported as an extraction solvent (Vaher *et al*., 2010). Hence in this study, about 1.5 g of sample was extracted with 50 mL of aqueous acetone (70%) under water bath shaker for 30 min. After extraction, the mixture was centrifuged, decanted and filtered. The filtrate was stored at 4^{o}C until analysis. The preparation is shown in figure 8.

A) Water bath shaker B) Centrifuge apparatus C) Filterd sample

Figure 8: Barley grain extraction preparation

3.5.3 Preparation of Standard Solution

0.06 g of Gallic acid was dissolved in 100 mL of distilled water to get 0.06 % solution of Gallic acid (0.6 mg/mL) termed as standard 1 solution. Similarly 0.013 g of Cathechin was dissolved in 13 mL of distilled water separately to get 0.013% solution of cathechin (1mg/mL) termed as standard 2 solution. 0.02 g of ascorbic acid dissolved in 100 mL of deionized water to get 0.02 % solution ascorbic acid (0. 2 mg/mL) termed as standard 3 solution.

3.5.4 Preparation of DPPH Solution

Stock solution of 0.4 mM DPPH was prepared by dissolving 7 mg of DPPH in 100 mL ethanol and kept in the refrigerator until use.

3.6 Quantitative Determination of Bioactive Compounds

3.6.1 Determination of Total Phenolic Content in Barley Grain Samples

Total phenolic level was determined by Folin-Ciocalteu's method reported by (Cicco and Lattanzio, 2011) and (Atlabachew *et al*., 2014) with some modifications. About 100 µl of barley grain sample extract/standard gallic acid (1.2, 6, 12, 18, 24 µg/ml) was positioned into volumetric flask and 1ml Folin-Ciocalteu's reagent was mixed and shaken with hand. After 5 min, 2 ml of 7 % sodium carbonate was added to the mixture and volume was adjusted with distilled water. After incubation for 1 hr. the spectrum was scanned against blank using spectrophotometer from 200–800 nm. The absorbance measurement was performed in duplicate and the average absorption was taken at 725nm for each sample in duplicate.

The calibration curve was plotted using standard Gallic acid. Quantitative analysis was done based on the equation of regression line: y = 0.0662x - 0.0815 (Where y = absorbance (Abs. at max - Abs. at base) and x = concentration in µg/ml. The data for total polyphenol contents of barley grain was expressed as mg GAE/g. $T = C\frac{V}{M}$ Where T is the total phenolic content in mg GAE/g of the extracts, C(x) is the concentration of Gallic acid established from the calibration curve in µg/ml, V is the volume of the extract solution in mL and M is the weight of the ex-tract in g.

3.5.4 Determination of Antioxidant Activity in Barley Grain Sample

Antioxidant activity of barley in the extracted samples were determined according to the procedure reported by (Cicco and Lattanzio, 2011) and (Atlabachew *et al*., 2014) with some modification. 60µl sample/ standard ascorbic acid solution (0.5, 0.8, 0.9, 1.2, 1.3 µg/mL) diluted with ethanol was mixed in test tubes with 2 ml of DPPH solution (7 mg/100 mL) and made up to final volume of 6.015 ml with ethanol. 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 4.015 mL of ethanol only. Ethanol was used as a blank. The absorbance was measured by scanning from 400 - 600nm. The experiment was performed in duplicate and the average absorbance was recorded at 517 nm. Quantification of antioxidant capacity was made by calibration curves obtained from ethanolic solutions of ascorbic acid and the antioxidant capacity was expressed as milligram of ascorbic acid equivalent (AAE) using the equation of: y = 84.941X – 40.566(Where y = % inhibition and x = concentration in µg/ml).

$$\text{Percent inhibition} = \frac{\text{ADPPH - A sample}}{\text{ADPPH}}$$ X 100% Where: ADPPH = Absorbance of DPPH, A sample = Absorbance of sample

3.5.6 Determination of Total Flavonoid in Barley Grain Sample

Total flavonoids was measured by colorimetric assay according to the procedure reported by (Dewanto *et al*., 2002) and (Atlabachew *et al*., 2014) with some modifications. 1.5 mL of sample/standard catechin solution (2.4, 4.7, 9.4, 33.9, and 50.9 µg/ml) was mixed with 1.5 mL of distilled water and 0.15 mL of 5% sodium nitrite solution ($NaNO_2$) was added After 5 min, 0.15 mL of 10% aluminum chloride was added. After 6 min, 2 mL of 4% sodium hydroxide (NaOH) was added and mixed well. The samples were performed in duplicates. It was allowed to incubate for 10 min. Orange yellowish color was developed. The absorbance was measured at 510 nm spectrophotometerically using UV-Visible instrument against the blank sample that contain distilled water instead of sample and all reagents. The spectrum was scanned from 300 – 600 nm. The absorbance measurement was performed in duplicate and the average absorption was taken. Quantitative determination was done based on the equation of regression line: Y = 0.00512X + 0.08077 (Where y = absorbance and x = concentration in µg/ml). The total flavonoids content of the samples were expressed as mg of catechin equivalent (mg CE/g) of

barley grain. $T = C\frac{V}{M}$ Where T is the total flavonoids content in mg CE/g of the extracts, C is the concentration of catechin established from the calibration curve in µg/mL, V is the volume of the extract solution in ml and M is the weight of the ex-tract in g.

3.7 Statistical Analysis

Analysis of variance (ANOVA) is a statistical method used to test differences between two or more means. In this study, a one way ANOVA analysis was used at 95% confidence level using version 20 SPSS software to know the variation of mean of total polyphenol, flavonoid and antioxidant activity are significantly different or not within and between groups. Pearson correlation coefficient was also used to measure the degree of linear relationship and direction of relations between total polyphenol, flavonoid with total antioxidant activity of the samples by using XL software.

4. Results and Discussion

4.1 Determination of Total Phenolic Content in Barley Grain Sample

The calibration curve was constructed with standard gallic acid in the concentration range of 1.2-24 µg/ml. The levels of total polyphenols in the samples were determined with the regression line equation the data is shown in Table 5 and Figure 9. Results of the analysis are shown in Table 6 and Figure 10. The data of the sample are shown in the appendix Table 17.

Table 5: Data for calibration curve construction using standard gallic acid for the determination of total polyphenols in the sample (n = 2 duplicate analysis).

Tube	Volume of GA(0.06 mg/mL) in mL	Gallic acid in µg	Volume H_2O (mL)	V Folin-Ciocalteu reagent (mL)	Volume 7% Na_2CO_3 (mL)	Gallic acid in (µg/mL)	Absorbance at 725 nm
Blank	-	-	2	1	2	-	0.0000
T1	0.01	6	1.990	1	2	1.2	0.0171
T2	0.05	30	1.950	1	2	6	0.3115
T3	0.10	36	1.900	1	2	12	0.6908
T4	0.15	90	1.850	1	2	18	1.0949
T5	0.20	120	1.800	1	2	24	1.5304

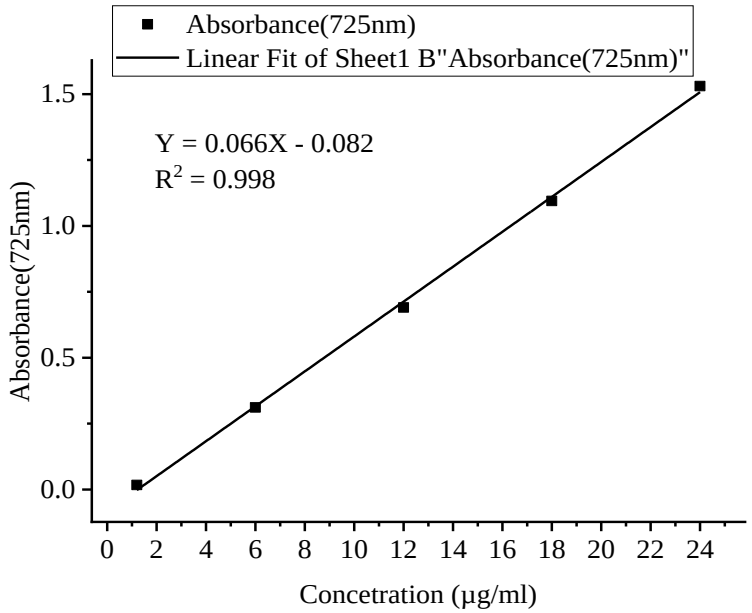


Figure 9: Calibration curve of gallic acid for the determination of total polyphenol

Table 6: TPC determination results of the samples expressed as mg GAE/g (n = 2)

Sampling district	Sampling sub-district	Concentration in µg/ml Mean ± SD	TPC(mg GAE/g) Mean ± SD	Average Mean ± SD
Jabitehena	Mendereslasie	2.863 ± 0.021	4.796±0.03	5.61 ± 0.8[b]
	Abragit	3.362 ± 0.171	5.602 ± 0.2	
	Kuarit	3.867 ± 0.075	6.445 ± 0.1	
Burie	Fereda	2.855 ± 0.395	4.759 ± 0.4	4.85 ± 0. 4[b]
	Wundiy	3.195 ± 0.277	5.325 ± 0.3	
	Bure zuria	2.681 ± 0.064	4.468 ± 0.1	
Dera hamusit	Aribgebeya	3.346 ± 0.064	5.576 ± 0.1	5.64 ± 0.7[b]
	Gelawudiwes	2.961 ± 0.288	4.934 ± 0.3	
	Sene Mariam	3.844 ± 0.342	6.407 ± 0.4	

Results are expressed as mean ± SD for P- value was calculated using one-way ANOVA Tukey HSD[a] among the barley grain

Mean in the same column between the same letters are not significant

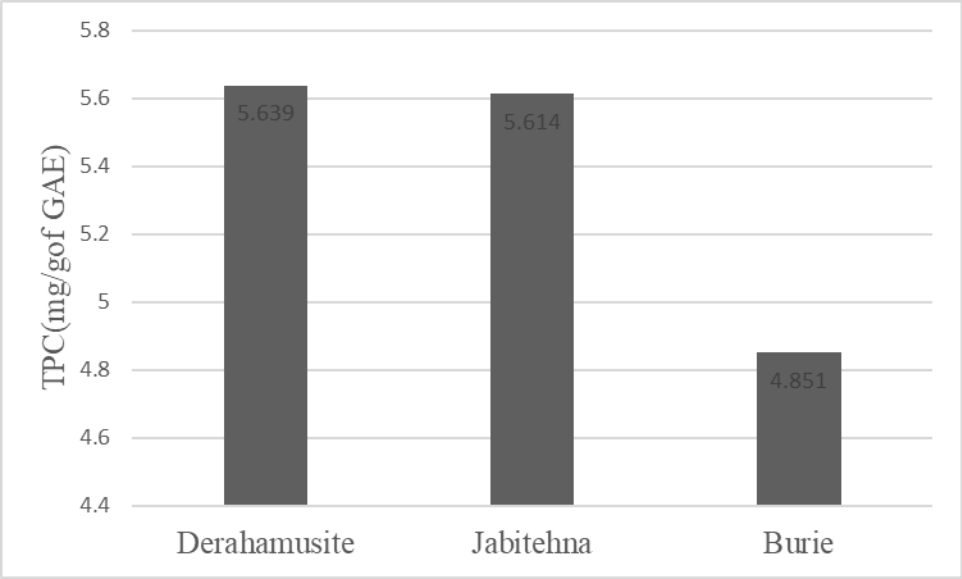


Figure 10: Average TPC (mg GAE/g) in the sampling district

In the current study the selected district barley grain exhibits their total phenolic compounds. In general barley contained a good level of phenolic compounds and insignificant difference was observed among the selected district barley grain (Table 6 and figure 10). Analysis of ANOVA at 95% confidence level showed that the mean of total Polyphenols within the samples were insignificant. The Absence of significant difference might be due to similarity in factors like soil composition, annual rain fall, cultivation of the raw materials, and barley growth process between groups of samples. But a little bit difference was observed with each sampling district. The presence of this difference might be due to preharvest factors (environmental fluctuations, location and irrigation conditions), storage conditions, high temperature and low rain full (Gamel and Abdel-Aal, 2012). Contrarily could temperature destroy or block the metabolic pathway of certain phenolic compounds (Christie et al. 1994).On the level of average TPC in the three districts are in the order Derahamusit > Jabitehena > Burie the data is shown in Table 6 and Figure 10. Barley grain obtained from Derahamusite district cultivars were found to exhibited higher amount of total phenolic compounds as compared to other two district cultivars with the value of 5.64 ± 0.7 mg GAE/g of sample and the lowest concentration of TPC was found in sample from Burie district with the value of 4.85 ± 0.4 mg GAE/g of sample.

4.1.1 Comparison of Total Phenolic Content of Barley Grain with Other literatures

This study revealed that the barley grain contained total phenolic contents and the results were compared with other literatures (Table 7). The results shown that the total phenolic content of

barley grain samples of this study agree with Chinese barley grain done by Hue (2013). On the other hand the reason of agree is TPC values of cereals ranged from 169 to 10548 mg/100 g of dry product (Cicco and Lattanzio, 2011).

Table 7: Comparisons results of the total phenolic content with other related literature data.

Country	TPC(mg GAE/g)	Solvent	Ref.
Ethiopia	4.8– 5.6	70 %Acetone	In this study
Saudi Arabia	2.31	65 % Ethanol	(Ghafoor, 2015)
Francis	3.41	50 % Acetone	(López-Perea et al., 2019)
Eastern Canada	3.07- 4.48	Methanol	(Šimić et al., 2017)
Chinese	5 - 8	70% Acetone	(Hue, 2013)
Tunisian	1.95	70 % Ethanol	(Abidi et al., 2015)

In this work, the total phenolic content in barley grain was 4.8 - 5.6 mg GAE/g) (Table 7), were agree with the total phenolic content of barley grain, 5 - 8 mg GAE/g by Hue (2013). In another study lower concentration of total phenolic content was reported (1.95 mg GAE/g) by Abidi *et al*., 2015 and others. These differences might be due to several factors such as climatic conditions of the districts, cropping year, soil chemistry of the regions and different agronomic practices.

4.1.2 Comparison of Total Phenolic Content of Barley Grain with Other Cereals with literatures

This study revealed that the barley grain contained total phenolic contents and the results were compared with others cereals reported by different literatures (Table 8).

Table 8: Comparison results of the total phenolic content with other cereals

Varity	Country	TPC(mg GAE/g)	Solvent	Ref.
Barley	Ethiopian	4.85 - 5.64	Acetone	in this study
Millet	Portugal	0.44 – 3.73	Acetone	(Luthria, 2008)
Rice	Portugal	0.24 – 3.87	Acetone	(Abdel-Aal et al., 2014)
Sorghum Brans	Canada	36 – 45	aqueous acetone	(Anese et al., 1999)
Corn	Mexico	2.1 – 3.4	Ethanol	(Lopez-Martinez et al., 2009)
Oat	Chinese	3.9 - 4.3	70% acetone	(Hue, 2013)
Wheat	Portugal	0.21– 5.95	70% acetone	(Hue, 2013)

In this work, the total phenolic content in barley grain was 4.8 -5.6 mg GAE/g) (Table 8), were agree with the total phenolic content of wheat, 0.21 – 5.95 mg GAE/g Hue (2013). In another study lower concentration of total phenolic content was reported in the cereal of Millet, rice, corn and oat (3.4, 2.3 and 4.3 mg GAE/g). In another study there is high amount of phenolic compounds observed in sorghum brans (36 – 46 mg GAE/g) These differences might be due to several factors such as cropping year, genotype and environment have also been found to significantly influence total phenolic compounds, antioxidant capacity and phenolic acid (Mpofu et al., 2006).

4.2 Determination of Total Flavonoids in Barley Grain Sample

The calibration curve was constructed with standard catechin in the concentration range of 2.34 - 50.94 µg/ml. The calibration curve of standard catechin the data is shown in Table 9 and Figure 11. The levels of total flavonoids were determined with the regression line equations. The sample data is shown in the appendix Table 18. The results of total flavonoids of the samples from different areas have been summarized in Table 10 and the average TFC in the sampling districts is shown in Figure 12.

Table 9: Data for the calibration curve construction with standard catechin for total flavonoid (n = 2)

Tube name	Catechin in µg/mL	V/Catechin in mL	V/$NaNO_2$in mL	V/H_2O in mL	V/10% $AlCl_3$in mL	V/1M NaOH in mL	Absorbance at 510 nm
Blank	-	-	0.15	3.000	0.15	2	-
T1	2.359	0.0125	0.15	2.985	0.15	2	0.088
T2	4.717	0.025	0.15	2.975	0.15	2	0.113
T3	9.434	0.05	0.15	2.95	0.15	2	0.127
T4	33.962	0.09	0.15	2.910	0.15	2	0.252
T5	50.943	0.27	0.15	2.730	0.15	2	0.343

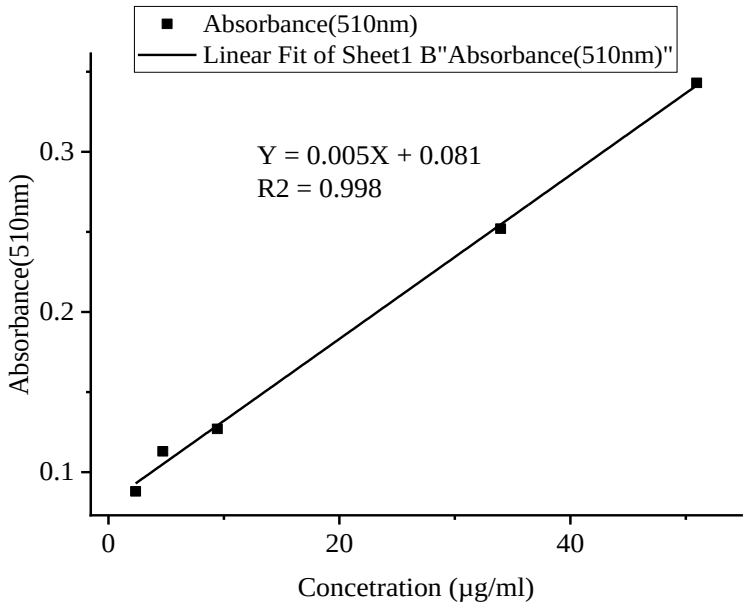


Figure 11: Calibration curve of standard catechin for the determination of total flavonoid

Table 10: Total flavonoid concentration in mg CE/g of samples (n = 2)

Grain sample district	Sampling sub - district	TFC (mg/g) Mean ± SD	Average TFC (mg/g) in the district Mean ± SD
Dera hamusit	Aribgebeya	3.604 ± 0.2	3.72 ± 1.1[b]
	Gelawudiwes	4.835 ± 0.2	
	Sene Mariam	2.731 ± 0.1	
Burie	Fereda	3.397 ± 0.1	4.01 ± 0. 7[b]
	Wundiy	3.812 ± 0.1	
	Bure zuria	4.812 ± 0.1	
Jabitehena	Mendereslasie	3.191 ± 0.1	3.45 ± 0. 4[b]
	Abragit	3.857 ± 0.1	
	Kuarit	3.294 ± 0.1	

Results are expressed as mean ± SD for P- value was calculated using one-way ANOVA Tukey HSD[a] among the barley grain

Mean in the same column between the same letters are not significant

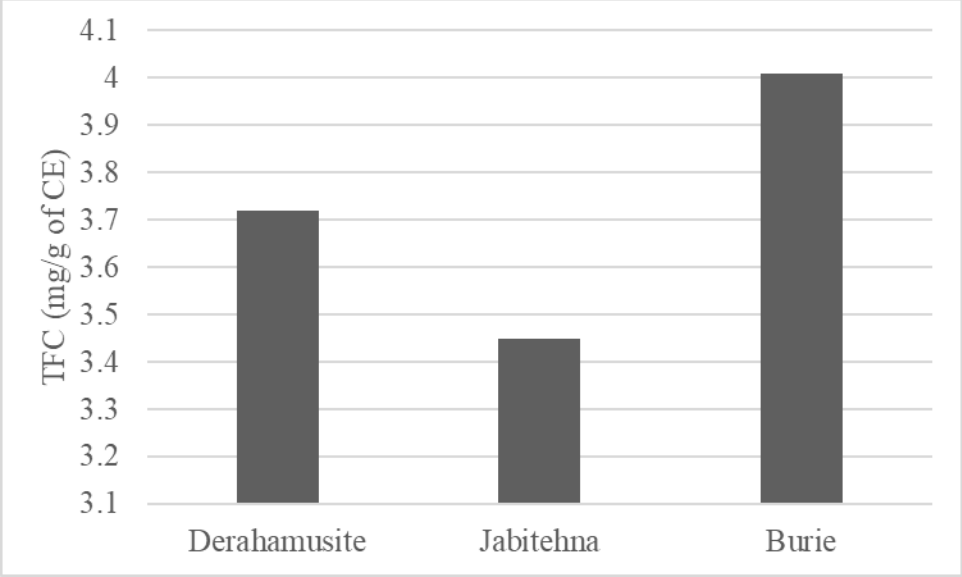


Figure 112: Average TFC (mgCE/g) in the sampling districts

Barley consists of a subclass of flavonoids, flavan-3-ols which was absent in other cereals such as oats and wheat (Zilic et al., 2011).

In the current study the selected district barley grain exhibits there total flavonoid compounds.

In general barley contained a good level of flavonoid compounds and insignificant difference was observed among the selected district barley grain (Table 10 and figure 12). Analysis of ANOVA at 95% confidence level showed that the mean of total flavonoids within the samples were insignificant. The Absence of significant difference might be due to similarity in factors like soil composition, annual rain fall, cultivation of the raw materials, and barley growth process between groups of samples. But a little bit difference was observed with each district sample. The presence of this difference might be due to cropping year, storage condition, preharvest factor, high temperature and low rain full (Botelho *et al*., 2017). Contrarily could temperature can destroy or block the metabolic pathway of certain phenolic compounds (Christie *et al*. 1994).

On the level of average TFC in the selected district are in the order of Burie > Derahamusit > Jabitehena the data is shown in Table 10 and Figure 12.

The highest concentration of total flavonoid was found in sample collected from Burie with the value of 4.01 ± 0.7 mg CE/g while the lowest concentration was found in sample from Jabitehenan district with a value of 3.45 ± 0.4 mg CE/g of sample.

4.2.1 Comparison of Total flavonoid Content of Barley Grain with Other literatures

This study revealed that the barley grain contained total flavonoid contents and the results were compared with others reported data (Table 11).

Table 11: Comparisons results of the total flavonoid content with other related literature.

Country	TFC (mg CE/g)	Solvent	Ref.
Ethiopia	3.5 – 4	70%Acetone	In this study
Tunisian	1.24	70 % Ethanol	(Abidi *et al*., 2015)
Francis	0.313	50 % Acetone	(López-Perea *et al*., 2019)

The total Flavonoid content of barley grain (1.24 mg CE/g and 0.313 mg CE/g) were reported from Tunisian and Francis (Abidi *et al*. 2015 and Lopez-perea et al.,2019) respectively. Those values are lower than present studies (3.5 – 4 mg CE /g barley grain) reported from Ethiopia (in this study). The reason of the difference are discussed in the comparison of the total phenolic compounds.

4.3 Determination of Antioxidant Activity in Barley Grain Samples

The calibration curve was constructed with ascorbic acid standard in the concentration range of 0.5 to 1.3 µg/ml (Table 12) and the calibration curve of standard ascorbic acid is shown in Figure 13. The levels of total antioxidant were determined with the regression line equations. The sample data is shown in the appendix Table 19. The results of total antioxidant activity of the samples from different districts are summarized in Table 13 and figure 14.

Table 12: Data for construction calibration curve using standard ascorbic acid solutions (n= 2).

Tube name	Ascorbic Acid (µg /mL	Volume of ascorbic Acid in mL	DPPH (ml)	99.99%aq. Ethanol (mL)	Absorbance at 517 nm	% Inhibition
Blank	-	-	-	6.015	-	-
Control	-	-	2	4.015	0.5551	-
T1	0.499	0.015	2	4	0.4846	12.5
T2	0.831	0.025	2	3.990	0.4170	24.88
T3	0.998	0.030	2	3.985	0.3823	31.13
T4	1.164	0.035	2	3.980	0.2968	46.53
T5	1.330	0.040	2	3.975	0.0455	91.8

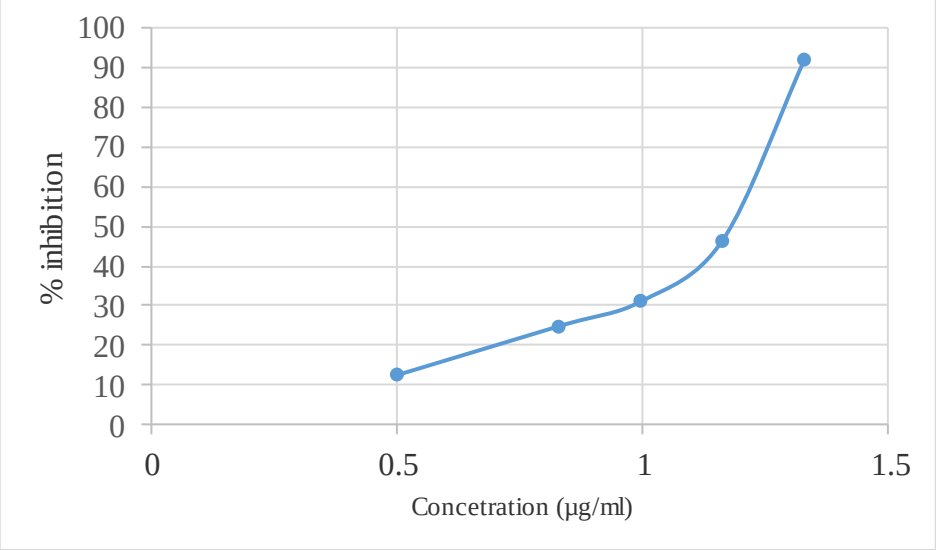


Figure 12: Calibration curve of AA to determine total antioxidant activity of the samples.

% inhibition verses concentration of ascorbic acid equation is Y = 84.941X – 40.566

$R^2 = 0.7801$

Table 13: Total antioxidant activity in mg AAE/g of sample (Mean ± standard deviation, n = 2)

Grain sample district	Sampling sub - district	TAA (mg AAE/g)	Average TAA (mg AAE/g) in the district	Average % inhibition in the district
Dera hamusit	Aribgebeya	3.939	3.69 ± 0.42^{d}	53.57 ± 10^{e}
	Gelawudiwes	3.205		
	Sene Mariam	3.939		
Jabitehena	Mendereslasie	3.958	3.28 ± 0.59^{d}	43.07 ± 15^{e}
	Abragit	2.824		
	Kuarit	3.064		
Burie	Fereda	3.911	2.96 ± 0.83^{d}	34.80 ± 21^{e}
	Wundiy	2.475		
	Bure zuria	2.487		

Results are expressed as mean ± SD for P- value was calculated using one-way ANOVA Tukey HSD[a] among the barley grain

Mean in the same column between the same letters are not significant

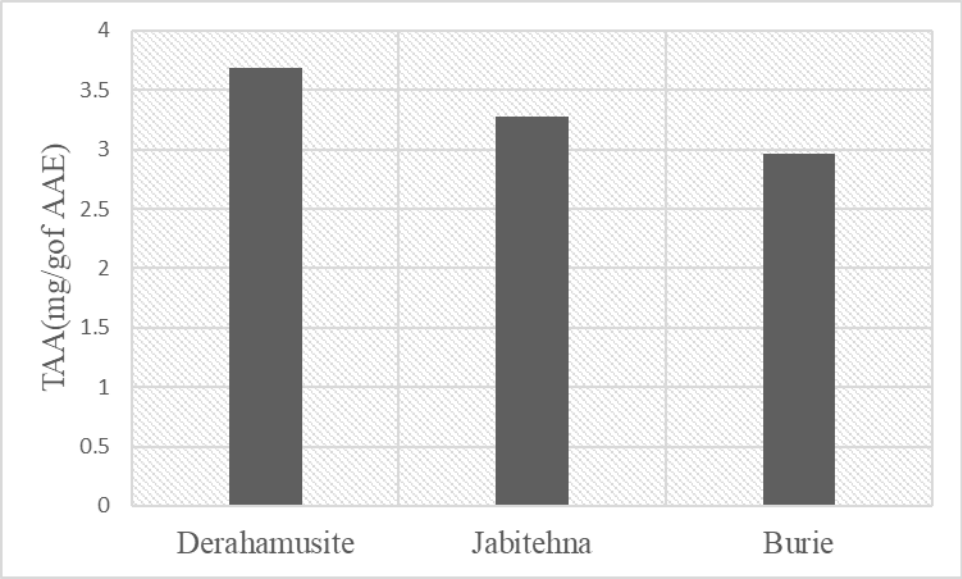


Figure 13: Average TAA (mg AAE/g of samples from the three districts

Antioxidant activity of barley grains contributes to mechanisms that against chronic diseases (Miller *et al*., 2000). The AA of food barley grains is contributed majorly by total phenolic contents. The presences of polyhydroxyl groups in flavonoids make them become good radical scavengers for anti-inflammation and other chronic diseases (Žilić *et al*., 2011). The antioxidant activity on DPPH by barley grain samples was presented in (mg AAE/g) in Table 13. The higher the TAA, the more DPPH radicals are able to be scavenged by the antioxidants. Hence, this antioxidant is excellent in prevent cell damage by reduce the oxidation damage caused by radicals in human body.

ANOVA at 95% confidence level showed that the mean of TAA within the samples were insignificant. The Absence of significant difference might be due to the similarity in the types and amount of ingredients used, and inconsistency in the growth process. Moreover, the similarity is dependent on the type of phenolic compounds present. But a little bet difference observed with each district sample. The presence of this difference might be due to high temperature and low rain full (Gamel and Abdel-Aal, 2012).

On the level of average TAA in the selected district are in the order of Derahamusit > Jabitehena > Burie > the data is shown in Table 13 and Figure 14.

Among the barley samples, Derahamusit district barley sample seems to be able to quench the highest amount of DPPH free radicals since it showed the highest TAA, 3.69 ± 0.4 mg AAE/g (53.57 % inhibition power) . Burie district barley sample seems to be able to quench the lowest

amount of DPPH free radicals since it showed the lowest TAA, 2.96 ± 0.8 mg AAE/g (34.8 % inhibition power) .

4.3.1 Comparison of TAA Content of Barley Grain with Other literatures

This study revealed that the barley grain contained TAA and the results were compared with others reported data (Table 14). The results shown that the TAA of barley grain samples of this study agree with Saudi Arabia barley grain done by (Ghafoor, 2015).

Table 14: Comparison results of the TAA with other literature.

Country	% inhibition	Solvent	Ref.
Ethiopia	34.80 – 53.57	70 %Acetone	In this study
Saudi Arabia	30 – 65	65 % Ethanol	(Ghafoor, 2015)
Francis	28- 36	50 % Acetone	(López-Perea *et al*., 2019)
Eastern Canada	62.1 – 75.6	HCl/methanol, 1 : 100, v/v)	(Šimić *et al*., 2017)
Chinese	72.79 - 80.44	70% Acetone	(Hue, 2013)

In this work, the TAA in barley grain was 34.8 -53.6% (Table 14), were agree with the TAA, 30 – 65% by Ghafoor, 2015. In another study lower TAA was reported 28 – 36% by Lopez-Perea *e t al*., 2019. These differences might be due to several factors such as the amount of phenolic compounds, climatic conditions of the districts, soil chemistry of the regions and different agronomic practices (Mapofu *e t al.*, 2006).

4.3.2 Comparison of TAA of Barley Grain with Other Cereals in the literatures

This study revealed that the barley grain contained TAA and the results were compared with others reported data (Table 15). The results shown that the TAA of barley grain samples of this study agree with wheat grain done by Hue, 2013.

Table 15: Comparisons results of the TAA with other cereals

Varity	Country	TAA	Solvent	Ref.
Barley	Ethiopian	34.80 – 53.57	70% Acetone	In this study
Oat	Chinese	80.92 - 82.2	70% Acetone	(Hue, 2013)
Wheat	Chinese	21.22 - 56.4	70% Acetone	(Hue, 2013)

In this work, the % inhibition barley grain was 34.80 - 53.57% (Table 15), were agree with the % inhibition of wheat, 51.2 – 56.4% done by Hue, 2013. In another study higher % inhibition was reported in the cereal of oat by Hue (2013). This was due to the fact that oats contained avenanthramides, which was found in oats only. These compounds were cinnamoyl conjugates that able to give rise to high AA (Adom and Liu, 2002). Those differences might be due to several factors such as genotype, cropping year, types of sample and environment have also been found to significantly influence total phenolic compounds, antioxidant capacity and phenolic acid (Mapofu e t al.2006).

4.4 Correlation Between Antioxidant activity with Total Flavonoid content and Total phenolic contents

The relationship between total phenolic contents and total flavonoids with antioxidant activities were investigated using Pearson correlation. The correlations of the samples are compared in Table 16. Antioxidant activity of phenolic compounds is mostly associated with their redox properties which allow them to act as ant oxidative agents (Siddhuraju, 2007).

Table 16: Pearson correlation between determined parameters of the samples.

	TPC	AOA	TFC
TPC	1		
AOA	0. 381	1	
TFC	0.889	0.423	1

Total polyphenol and total flavonoids were found to have correlation coefficients with their antioxidant activity assayed :(r = 0. 381 and r = 0.423, respectively). The results indicate that the presence of positive which indicates good correlation between these variables. The positive correlation indicates that when the concentrations of total polyphenolic compounds and total flavonoids increase, total antioxidant activity also increases. This illustrates that phenolic compounds are important contributors to antioxidant activity of the barley sample.

5. Conclusion and Recommendations

5.1 Conclusion

In this study, the level of bioactive compounds: total phenolic, total flavonoids, and antioxidant activities of barley grain were investigated. The results obtained indicated that at 95% (P = 0.05) confidence level, there were insignificant on the level of total polyphenol, total flavonoids within the same sample type, Similarly total antioxidant activity of these barley grain was insignificantly between the same sample types. Among the three districts barley, Derahamusite food barley has showed higher total phenolic and antioxidant capacity than the other two districts. The investigation indicated that the total phenolic components of barley grain depend on the climatic condition and the growth processes.

According to the data obtained from this study, the content of total polyphenol, total flavonoids and total antioxidant activity also present remarkably. These research findings conclude that barley grain (HB-1307) (Tebel gebs) Ethiopian growth product are as a promising source of biologically active polyphenol and flavonoid compounds and contributing effective protection from free radicals, cancer, and aging problems in comparable manner of corn, oats and rice.

5.2 Recommendations

1. Even if, all phenolic compounds have similar functions like antioxidant, anticancer, antiaging, etc.; they differ on absorption site of body and rate of mentioned functions based on structures and size. So these barley grain need further individualizations studies of phenolic compounds.
2. Apart from the one kinds of grains examined in this study other kinds of barley grains such as malt, Biftu, temeshi gebs, Dafo, Abdane, white barley, Dimtu, Ardu-1260B, Shege, Dinsho, Senef kolo, HB-42 Diribe and cross-4198 should be the bioactive compounds assessed to further.
3. Different solvents such as methanol, ethanol, water and petroleum-ether are other choices to extract the antioxidants and phenolic compounds in grain samples. The results will vary due to different solubility of these compounds in different solvent. TOSC assay, ß-carotene bleaching method and ABTS assay are other efficient method to quantify antioxidant capacity. The results can be compared to get a more consistent result regarding barley grains. Since grains are full of soluble and insoluble fiber, further studies regarding these compounds should be proposed to compare their amount in the grains.

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Appendix 1

Determination of total phenolic content, total Flavonoid content and antioxidant activity of food barley grain samples in duplicate measurement.

Table 17: 70% acetone extract for determination of total phenolic content.

Barley grain Sample site	Trail	V(Sample) in ml	Sample in µg	V(H_2O) in ml	V(7%Na_2CO_3)in ml	Folin ciocalutu's in ml	Absorbance at 725 nm
Mendereslasie	T1	0.1	600	1.9	2	1	0.109
	T2	0.1	600	1.9	2	1	0.107
Abragit	T1	0.1	600	1.9	2	1	0.133
	T2	0.1	600	1.9	2	1	0.149
Kuarit	T1	0.1	600	1.9	2	1	0.178
	T2	0.1	600	1.9	2	1	0.171
Fereda	T1	0.1	600	1.9	2	1	0.089
	T2	0.1	600	1.9	2	1	0.126
Wundiy	T1	0.1	600	1.9	2	1	0.117
	T2	0.1	600	1.9	2	1	0.143
Bure zuria	T1	0.1	600	1.9	2	1	0.093
	T2	0.1	600	1.9	2	1	0.0.99
Aribgebeya	T1	0.1	600	1.9	2	1	0.137
	T2	0.1	600	1.9	2	1	0.143
Gelawudiwes	T1	0.1	600	1.9	2	1	0.128
	T2	0.1	600	1.9	2	1	0.101
Sene Mariam	T1	0.1	600	1.9	2	1	0.189
	T2	0.1	600	1.9	2	1	0.157

Table 18: 70% acetone extract for determination of total Flavonoid content.

Barley grain Sample site	Trail	V(Sample) in ml	Sample in µg	V(H_2O) in ml	V(5% $NaNO_2$)in ml	V(10 % $AlCl_3$) in ml	V(NaOH) in Ml	Absorbance at 510 nm
Aribgebeya	T1	1.5	8491	1.5	0.15	0.15	2	0.229
	T2	1.5	8491	1.5	0.15	0.15	2	0.246
Gelawudiwes	T1	1.5	8491	1.5	0.15	0.15	2	0.299
	T2	1.5	8491	1.5	0.15	0.15	2	0.283
Sene Mariam	T1	1.5	8491	1.5	0.15	0.15	2	0.200
	T2	1.5	8491	1.5	0.15	0.15	2	0.197
Fereda	T1	1.5	8491	1.5	0.15	0.15	2	0.255
	T2	1.5	8491	1.5	0.15	0.15	2	0.231
Wundiy	T1	1.5	8491	1.5	0.15	0.15	2	0.252
	T2	1.5	8491	1.5	0.15	0.15	2	0.241
Bure zuria	T1	1.5	8491	1.5	0.15	0.15	2	0.291
	T2	1.5	8491	1.5	0.15	0.15	2	0.289
Mendereslasie	T1	1.5	8491	1.5	0.15	0.15	2	0.215
	T2	1.5	8491	1.5	0.15	0.15	2	0.224
Abragit	T1	1.5	8491	1.5	0.15	0.15	2	0.249
	T2	1.5	8491	1.5	0.15	0.15	2	0.248
Kuarit	T1	1.5	8491	1.5	0.15	0.15	2	0.222
	T2	1.5	8491	1.5	0.15	0.15	2	0.226

Table 19: 70% acetone extract for determination of antioxidant activity.

Barley grain Sample site	Trail	V(Sample) in µl	Sample in µg	V(DPPH) in ml	V(ethanol)(100 %) in ml	Absorbance at 517 nm	Inhibition (%)
R-blank	-	-	-	-	6.015	0.00	-
Control	-	-	-	2	4.015	0.555	-
Aribgebeya	T1	60	300	2	3.955	0.217	60.9
	T2	60	300	2	3.955	0.229	58.7
Gelawudiwes	T1	60	300	2	3.955	0.333	40.7
	T2	60	300	2	3.955	0.323	41.5
Sene Mariam	T1	60	300	2	3.955	0.234	57.8
	T2	60	300	2	3.955	0.212	61.8
Mendereslasie	T1	60	300	2	3.955	0.224	59.6
	T2	60	300	2	3.955	0.217	60.9
Abragit	T1	60	300	2	3.955	0.389	31.1
	T2	60	300	2	3.955	0.379	31.7
Kuarit	T1	60	300	2	3.955	0.344	38.1
	T2	60	300	2	3.955	0. 351	36.8
Fereda	T1	60	300	2	3.955	0.217	60.9
	T2	60	300	2	3.955	0.237	57.3
Wundiy	T1	60	300	2	3.955	0.431	22.3
	T2	60	300	2	3.955	0.429	22.7
Bure zuria	T1	60	300	2	3.955	0.424	23.6
	T2	60	300	2	3.955	0.433	21.9

Appendix 2

Table 20: Tukey HSD[a] multiple comparisons test for TPC of 70% acetone extract in barley (Seed) sample in the district.

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Jabitehna	Burie	.763667	.393880	.162	-.25942	1.78676
	Jabitehna	-.025333	.393880	.998	-1.04842	.99776
	Derahamusite	-.763667	.393880	.162	-1.78676	.25942
Burie	Jabitehna	-.789000	.393880	.146	-1.81209	.23409
	Derahamusite	.025333	.393880	.998	-.99776	1.04842
Derahamusite	Burie	.789000	.393880	.146	-.23409	1.81209

Table 21:Tukey HSD[a] multiple comparisons test for TFC of 70% acetone extract in barley (Seed) sample in the district.

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Derahamusite	Burie	-.283500	.403266	.765	-1.33097	.76397
	Jabitehna	.276333	.403266	.776	-.77114	1.32380
	Derahamusite	.283500	.403266	.765	-.76397	1.33097
Burie	Jabitehna	.559833	.403266	.372	-.48764	1.60730
	Derahamusite	-.276333	.403266	.776	-1.32380	.77114
Jabitehna	Burie	-.559833	.403266	.372	-1.60730	.48764

Table 22: Tukey HSD[a] multiple comparisons test for TAA of 70% acetone extract in barley (Seed) sample in the district.

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Derahamusit	Jabitehna	.412333	.520339	.721	-1.18421	2.00888
	Burie	.736667	.520339	.392	-.85988	2.33321
Jabitehna	Derahamusit	-.412333	.520339	.721	-2.00888	1.18421
	Burie	.324333	.520339	.813	-1.27221	1.92088
Burie	Derahamusit	-.736667	.520339	.392	-2.33321	.85988
	Jabitehna	-.324333	.520339	.813	-1.92088	1.27221