DILLA UNIVERSITY

SCHOOL OF GRADUATE STDIES

DEPARTMENT OF CHEMISTRY

FATTY ACID COMPOSITION, TOTAL PHENOLICS CONTENT, TOTAL FLAVONOIDS AND ANTIOXIDANT ACTIVITY OF NIGER SEED (*Guizotia abyssinica)* COLLECTED FROM SOME AREAS OF ETHIOPIA

M.Sc .THESIS

BY

MEGERSA CHALI MEKURIA

DECEMBER, 2018

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A THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES DEPARTMENT OF CHEMISTRY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN CHEMISTRY (ANALYTICAL)

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ADVISORS' APPROVAL SHEET

I the undersigned certify that I have read and evaluated this thesis entitled "Fatty acid composition, total phenolics content, total flavonoids and antioxidant activity of niger seed (*Guizotia abyssinica)* collected from some areas of Ethiopian" prepared under my guidance by Megersa Chali Mekuria (ID. No PGRAC-009/16). I recommend that it be submitted for defense as fulfilling the thesis requirements.

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EXAMINERS' APPROVAL SHEET

We the undersigned certify that the thesis prepared by Megersa Chali Mekuria entitled **" Fatty acid composition, total phenolic content, total flavonoids and antioxidant activity of niger seed (*Guizotia abyssinica)* collected from some areas of Ethiopian "** and submitted in partial fulfillment of the requirements for the degree of Master 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.

_____	_____	_____
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Name of External Examiner	Signature	Date
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Name of Major Advisor	Signature	Date

AUTHOR'S DECLARATION

I, the undersigned, declare that this thesis, entitled "Fatty Fatty acid composition, total phenolics content, total flavonoids and antioxidant activity of niger seed (*Guizotia Abyssinica)* collected from some areas of Ethiopia " is my original work, that it has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate, that I followed all ethical and technical principles of scholarship in the data collection, data analysis and preparation of this the report, and that all the sources that I have used or quoted have been indicated and acknowledged.

Name: _____

Signature: _____

Date: _____

BIOGRAPHICAL SKETCH OF AUTHOR

Megersa Chali Mekuria was born in West Wolega Zone, Yubdo district Jarso Komto Kebele of Oromia Regional state in 1993 G.C from his father Chali Mekuria and Mother Dibise Xilahun. He attended his elementary school education grade 1−8 in Bikeltu Yawen elementary school. Then he attended Grade 9−10 in Worke Yubdo secondary high school, and grade 11-12 in Guliso preparatory school. After completion of his high school education, he joined Ambo University from 2014−2016 G.C and awarded B.Ed. in Chemistry. Finally he joined Dilla University in 2017 G.C for postgraduate study and currently he is following his postgraduate studies at Dilla University hopeful for M.Sc. degree in Chemistry (Analytical) in 2019 G.C Academic Year.

DEDICATION

I DEDICATED THIS THESIS MANUSCRIPT

TO

MY BELOVED

PARENTS,

BROTHER and SISTERS

ACKNOWLEDGEMENT

First and foremost, I offer my deepest heartfelt thanks to go to almighty God for his invaluable and inestimable favors, especially for the strength and good health that allow me to complete this thesis. I believe that he is the God who opened the door of education to me and who helped and gave me success in all my ways. First of all, I would like to express my deepest appreciation to my supervisor, Doctor Amare Aregahegn for his guidance, encouragement, tremendous support and advice throughout my work.

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LIST OF ACRONYMS AND ABBREVIATIONS

AA	Ascorbic acid
ALA	Alpha-linolenic acid
AOA	Antioxidant Activity
AOAC	Association Official Analytical Chemistry
AOCS	American oil chemists' society
CM	Chloro-methanol
DPPH	2, 2-diphenyl-1-picrylhydrazyl
DW	Dry Weight
EFA	Essential fatty acid
FA	Fatty acid
FAME	Fatty acid Methyl Ester
FFA	Free fatty acid
GA	Gallic acid
GC-MS	Gas Chromatograph-Mass Spectroscopy
HAT	Hydrogen atom transfer
IC	Inhibition concentration
LA	linoleic acid
MSD	Mass spectra detector
MUFA	Monounsaturated fatty acid
ROS	Reactive oxygen species
SF	Saturated fatty acid
PUFA	Polyunsaturated fatty acid
TFC	Total flavonoid contents
TPC	Total phenolic content
UF	Unsaturated fatty acid
UV-VIS	Ultra Violet-Visible

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Abstract

The seed of G. abyssinica is the most important oil crop in Ethiopia, providing 50–60% of the country's indigenous edible oil. The fatty acid types and composition; and the composition and concentration of other phytochemicals including plant secondary metabolites vary with respect to species type, geographical origin, cultivation season and varietal type. However, there are only few data on the fatty acid composition of some varieties of G. abyssinica collected from some selected areas of Ethiopia. The objective of this study was profiling fatty acid composition and to determine total phenolic content, total flavonoids, and evaluate antioxidant activities. Seed samples were obtained from the farmers of selected areas of five zone major producers of G. Abyssinica. The dried seed samples ground to fine powder, sieved with 50mm and subjected to horizontal shaker extraction with chloroform/methanol (2:1, v/v). Then extracted lipid was

derivatized with $HOCH_3/H2SO_4$ in n-hexane. Then Identification of individual FAs was carried out by comparison of their mass spectra with those of the reference mass spectra library NIST-11. The extract was analyzed using GC-MS. The result indicated that a total of 12 FAs have been identified in each G. abyssinica sample in which the fatty acid profile showed a high content of linoleic acid (up to 74.67%) together with palmitic acid (10.66 %), oleic acid (11.02 %), and stearic acid (8.761 %) were major FAs. The FAs concentration of Guizotia abyssinica samples was determined using undecanoic acid internal standard. The total FAs concentration; 240.41, 280.45, 292.24, 312.65 and 347.57mg/g of FAME were determined of sample variety in site of Horo Gudru Wolega, East Wolega, West wolega, East Gojjam and North Gondar respectively. The total FA concentration of Guizotia abyssinica were varied within Guizotia abyssinica location varieties. Ultra sonic extractor was used for extraction of bioactive compounds in two solvent systems (80% methanol extract and 100% methanol) to evaluate antioxidant activities, total phenolic content and total flavonoids contents of G. abyssinica. The result for samples had total phenolic content ranged from (10.89 ± 0.39 - 11.78 ± 0.028 mg GAE/g), total flavonoids contents (5.42 ± 0.03 - 6.67 ± 0.06 mg CE/g) of 80% methanol extract respectively. West wolega scored lower values of TPC and TFC in both solvent systems. 80% methanol extract had better antioxidant activities.

Keywords: *Guizotia abyssinica, total flavonoids, total phenolic, antioxidant activity, fatty acids, GC-MS*

CHAPTER 1: INTRODUCTION

1.1. Background of the study

Oilseed crops are highly valuable basic agricultural trade materials and as the world population expands the fossil resources decline the demand for refined edible oil products and renewable industrial oils continues to grow. Presently the international oilseed market is dominated by soybean (Glycine max), oilseed rape/canola (Brassica napus) and sunflower (Helianthus annuus). However, with increasing production areas the pressure on crop rotations in terms of sustainable production has increased the requirement for greater diversification of crop plants including oilseeds [1].

Interest in newer sources of edible oils has recently grown. So far, a large number of plants have been analyzed and some of these have been cultivated as new oil crops [2]. All, over the world, an ever increasing demand for oil from edible oilseeds is being witnessed. Since the beginning of history, people have made use of oils obtained from oilseeds and used principally as food. India accounts for 9.6% of world's total oilseed production from 25 million ha of land, the largest area under oilseeds in the world [3].

Oilseeds are a mainstay of the rural and national economy in Ethiopia. After coffee, oilseeds are the second largest export earner for the country and more than 3 million smallholders are involved in its production. Niger seed (Guizotia abyssinica), Linseed (Linumusitatissimum L.) and Sesame (Sesamumindicum L.) are major oil seed crops grown in Ethiopia. Niger is an oilseed crop mainly cultivated in different parts of Ethiopia and India. It constitutes about 3% of Indian oilseed production and 50% of the Ethiopian production. The two countries are the first and second Niger seed producers and users in the world, respectively [4]. It is also a minor oil crop in Kenya, Uganda, Sudan, Malawi, and other African countries. In Ethiopia, it is cultivated on waterlogged soils, where most crops and all other oilseeds fail to grow, and contributes a great deal to soil conservation and land rehabilitation [5].

G. abyssinica is used as a human food and also good sources of mineral nutrients for human. In Europe and North America, it is used as a birdseed. It is also used as a human food and today is particularly important to the economy of Ethiopia, where it accounts for 50-60% of its edible oil supply. Its oil is also used in the manufacture of soaps and paints and as a lubricant or lighting fuel [6]. It is used as feed, fertilizer or fuel. In particular, in tropical regions, with their poor quality of basal feed and lack of supplementation with locally available energy and protein sources, niger seed meal is an important supplement for sheep and goats [7, 8].

Figure 1: Niger seed (*Guizotia abyssinica*)

The *G. abyssinica* appears to be nutritionally valuable, as fatty acids known to prevent cardiovascular diseases and to be the precursor of structural components of plasma membranes and of some metabolic regulatory compounds. Additionally, it is accompanied by a significant antioxidant activity, mainly due to sterols and tocopherols [9]. Research shows that phenolic compounds such as flavonoids and phenolic acids exhibit antioxidant properties [10]. Plants rich in antioxidants, including polyphenolic compounds, tocopherols, flavonoids, vitamin C and carotenoids, are attracting to the food industry as replacements for synthetic ones, which use is being restricted due to safety concerns. Phenolic compounds may either occur naturally in the original plant or be formed during processing. The seeds of oil crops, particularly those containing a high percentage of polyunsaturated fatty acids like linoleic and oleic acid are thought to be rich in antioxidants [11].

Oilseed crops are generally grown for the purpose of oil in their seeds and they vary considerably in their oil content, quality, and composition. These factors rely heavily on the plant cultivar or variety), seed quality (maturity, damage caused by harvesting or handling/storage conditions, and upon the environmental conditions in which the crop is grown [12, 13]. Several factors such as; geographical origin, location, species, climate, cultural practices, fertilization, and harvest season have major impact on the fatty acid composition [14, 15]. The fat content of food has traditionally been determined by extraction with organic solvents, followed by extract drying and gravimetric determination. Non-polar organic solvents such as chloroform and *n*-hexane are used for disrupting hydrophobic and ion-dipole interactions (e.g. lipid hydrophobic chain and non-polar amino acids) whereas polar organic solvents with high dielectric constant, such as methanol, for breaking hydrogen bonds (e.g. non-lipid compounds and lipid hydroxyl, carboxyl or amino groups) [16].

Methods employed the composition fatty acid in foods of natural origin are separation of the different isomers containing one or more double bonds may be achieved only using methods mostly based on GC or HPLC. The quantification of FAs in food samples by GC involves transforming the analytes into more volatile and non polar derivatives after extracting the lipids from the food product before analysis [17]. The analysis of fatty acids by GC is complicated by their polarity and inadequate volatility. To permit analysis by GC, the polar carbonyl groups must first be converted to produce more volatile non-polar derivatives. A wide range of alkylation reagents are available for this purpose and fatty acids are frequently converted to their corresponding fatty acid methyl esters (FAMEs). FAME derivatization has been extensively used for fatty acid analysis, especially in connection with mass spectroscopy detection. Thus making the individual FAs available for chromatographic analysis, various methods of derivatization may be employed for the esterification of FAs to FAME [18, 19].

The most widely used techniques, such as diazomethane in ether, acid-catalyzed esterification, and base-catalyzed esterification methods. The acid catalysts such as BF_3, HCl and H_2SO_4, all in methanol were commonly used to methylate fatty acid [20]. In general, the base-catalyzed method for the direct transesterification of lipids has been reported to be more applicable for nutrition analysis because it is easy to use and uses less aggressive reagents than other methods.

However, this method has resulted in poor recoveries of FAMEs because FFAs might remain partially unreacted [21]. The advantage of acid-catalyzed and boron trifluoride methylation, in which $H_2SO_4$and BF_3 are widely used, is that free fatty acids (FFAs) can be catalyzed for esterification [22].

The antioxidant activity of the oils was determined using the DPPH method. Method employed to examine the anti oxidant activity of oil is by measuring the absorbance of the samples, the blank measured at 517 nm and using a UV-Vis spectrophotometer. [23, 24].

Ramadan et al., [3] reported that the phospholipids of different plant seed oil had a strong antioxidant activity because they were involved in synergism with tocopherols, formation of melano-phospholipids, formation of oxygen barrier between oil/air interface and chelation of pro-oxidant metals. They also reported that glycolipids of plant seed oil had a strong antiradical activity due to its reducing sugars. Therefore, the determination of the fatty acids (saturated and unsaturated) composition in Ethiopia *G. abyssinica*, Total phenolic compounds, Total flavonoids and its antioxidant activity is very important in assessing the standard and quality of Ethiopian Niger seed oil as well as any potential implications to health.

1.2. Statement of the problem

The fatty acid types and composition; and the concentration of other phytochemicals were varying with respect to species type, geographical origin, cultivation season and varietal type. Although different varieties of *G. abyssinica* are produced in Ethiopia and exported to the overseas, their chemical constitutes are not documented in depth. There are only few data on the fatty acid composition of some varieties of *G. abyssinica* collected from some selected areas of Ethiopia. However, Niger seeds grown in west and east wolega, Horo Gudru Wolega, East Gojjam and North Gonder areas have not been considered so far. Secondly, in the literature, only two papers have been reported on the level of fatty acids in *G. abyssinica* samples. Of which, one of the paper is too old (Dutta et al., 1994) and the derivatization method they have followed is base catalyzed method which is less efficient as compared to other derivatization techniques. Because fatty acids are might remain partially unreacted and cannot determined as free fatty acid.

While the second paper is a PhD dissertation by Tesfaye Deme, (2017) who used GC-FID for identification of the fatty acids without using standard solution. Although the author followed proper extraction and derivatization protocol, the identification method (GC-FID) he has used seems incorrect. Unlike GC-MS, it is not possible to identify the fatty acids identity from the GC-FID chromatogram alone without analyzing fatty acid standards. In addition, none of the papers described the concentration of the individual fatty acids. Furthermore, the composition of other photochemical (phenolic content, flavonoids and antioxidant activities) of Ethiopian Niger seed have not been reported so far. Thus, it is time to profile the chemical constitutes of niger seeds with respect to varieties and geographical origins so as to promote the seeds with good chemical composition.

1.3. Objectives of the study

1.3.1. General Objective

The general objective of the research was to determine fatty acid composition, total phenolic compounds, total flavonoids and antioxidant activity of Ethiopian Niger seed.

1.3.2. Specific objective

The specific objectives of this study are to:

- Identify the types of fatty acid of Ethiopian *G. abyssinica* by using GC-MS.
- Quantify the composition of individual fatty acid and total fatty acids content of Ethiopian *G. abyssinica*
- Determine total phenolic compound and total flavonoids of Ethiopian *G. abyssinica*.
- Examine the antioxidant activity of Ethiopian *G. abyssinica*.
- Compare the levels of FAs with literature, TPC and TFC of Ethiopian *G. abyssinica* with other seed crops in literature.

1.4. Scope of the study

Ethiopia Niger seed sample were collected from the farmers and stored at room temperature until used. Then, the lipid was extracted using horizontal shaker extraction method, for fatty acid profiling based on iterative percolation of fresh solvent; chloroform and methanol.

The extracted analyte was simultaneous derivatized to FAMEs with low acidity, sodium Sulfuric acid (H_2SO_4) in methanol and identification and quantification of fatty acids. Composition was done using GC-MS with fused- capillary column coated with polyethylene glycol film. Each fatty acid were identified its retention time and quantified by using undecanoic acid as internal standard by the measurement of the integrated peak area.

And also Ultrasonic extraction were used on determination of total phenolic compounds, Total flavonoids and antioxidant activity based on iterative percolation of fresh solvent of 80% aqueous water and 100% methanol. The Antioxidant activity of Ethiopian *G. abyssinica* was evaluated using 1, 1-diphenyl-2-picrylhydrazil using the Patel method. 5-100 µL of different concentrations of the sample and 2 mL of DPPH solution was taken, which was made up to 5 mL with solvent. Thus, the scope of this study includes identifying the types of fatty acid, quantifying the composition of individual and total fatty acids concentration, total phenolic content, total flavonoids and antioxidant activity of Ethiopian Niger seed.

1.5. Significance of the study

This study should be significant in the sense that it will: typical add to the knowledge and understanding of fatty acid composition, antioxidant activities of species under study and significances needed to increase the productivities as well as the income of individuals and groups. And also the typical significances of the research are Oxidation prevention of fat and oil food items, Preservatives/ additives development and Eye breakers for farther study.

CHAPTER 2: LITERATURE REVIEW

2.1. Oil seeds

Edible oils and fats are essential nutrients in the human diet and play a vital role in the supply of essential fatty acids and energy. In addition to their nutritional qualities, the oils and fats contribute consistency and specific binding characteristics to the products that contain them [25]. Oilseeds are leading suppliers of superior quality and specialty vegetable oils to nutritional products, natural food and premium snack food worldwide. Oilseeds, such as soybean, cottonseed, rapeseed (canola), sunflower seed and peanut, are the largest sources of vegetable oils in the World [26].

Ethiopia's oilseed sector, which is rapidly growing to meet both local and foreign demand, plays a vitally important economic role in generating foreign exchange earnings and income for the country. In contrast to grain production, the impact of the drought on oilseed production was minimal. In fact, the production of major oilseeds sesame, Niger seed and soybeans is forecast to increase by 28,000 metric tons to 788,000 metric tons in MY15/16 (Oct-Sep). This increase is attributed to anticipated production gains of sesame and Niger seed, which require relatively less moisture compared to other crops. As for soybeans, production fell slightly because of insufficient moisture. Looking further ahead, production of oilseeds is likely to increase to meet the growing demand for cooking oil and livestock ingredients, most notably soybean meal for poultry production [27, 28].

2.2. Niger seed oils

Niger *G. abyssinica* belongs to the same botanical family as sunflower. It comprises six species which *G. abyssinica* is the only one cultivated [29]. Niger seed is the most important oil crop in Ethiopia and a minor crop in India but it is not involved in the world-wide oilseed trade. Niger seed provides 50 to 60 % of Ethiopia's indigenous edible oil but only3 % of India's total oilseed production. It represents also a minor oilseed crop in some other African countries. Niger has been recognized as one of the 'neglected and underutilized 'crop and thus, so far, it has received little attention from the scientific community [30].

2.3. Fatty acid composition of Niger (*G. abyssinica*)

The fatty acid composition of niger seed was studied by different scholars and reported; as Regarding the fatty acid profile, niger seed oil resembles that of safflower and sunflower with its high content of linoleic acid (C18:2n–6) that was the main unsaturated FA followed by Oleic acid (C18:1n-9). The other predominant saturated fatty acids were palmitic (C16:0) and stearic (C18:0). Geleta et al. [31] studied variation and inheritance of oil content and fatty acid composition in *G. abyssinica*. Duta et al. [32] also reported the Variation in lipid composition of Niger seed (Guizotia abyssinica Cass.) samples collected from different regions in Ethiopia. Dagne and Jonsson [33] found two unsaturated fatty acids [linoleic (54.3–72.8, weight percent of TL) and oleic (5.4–26.8%)], and two saturated fatty acids [palmitic (7.8-10%), and stearic (5.5–8.1%)] which formed about 91–97% of the fatty acids present.

2.4. Importance and classification of fatty acid in food

2.4.1. Importance of fatty acid

Fatty acids represent 30–35% of total energy intake in many industrial countries and the most important dietary sources of fatty acids are vegetable oils, dairy products, meat products, grain and fatty fish or fish oils [34]. With the increasing demands of the consumers towards healthier food, the dietary fats from animal and vegetable origin have been gaining more and more attention as an important source of bioactive compounds. A lower ratio of omega-6/omega-3 fatty acids is more desirable in reducing the risk of many of the chronic diseases of high prevalence in Western societies, as well as in the developing countries, that are being exported to the rest of the world [35]. EFAs form an important constituent of all cell membranes, and confer on membranes properties of fluidity and thus, determine and influence the behavior of membrane-bound enzymes and receptors [36]. Among the main functions of the fatty acids are energy storage, the composition of cellular membranes and being precursor of substances such as prostaglandins, thromboxanes and leukotrienes [37].

2.4.2. Classification of fatty acid

Fatty acids can be grouped in families according to the number of double bonds in the fatty acid. Fatty acids with no double bonds are known as saturated fatty acids. The general formula is: R COOH Where R is CH_3 (CH_2) n; n varies from zero in acetic acid to 24 in cerotic acid and to 86 in mycolic acid. The most commonly occurring saturated fatty acids of the higher plants are palmitic acid (C_{16}) and stearic acid (C_{18}). Those with one double bond are called monounsaturated (MUFA); and those with two or more double bonds are called polyunsaturated (PUFA) fatty acids [38]. Further, the unsaturated ones are classified into omega series, being ω-9, ω-3 and ω-6. While ω-9 are nonessential for humans, certain omega-3 and omega-6 polyunsaturated fatty acids (PUFA) are considered as essential fatty acids, as they are not produced by the human body, and are supplied through the food. Alpha-linolenic (acid omega-3) fatty acid and linoleic acid (LA) omega-6 fatty acid are essential fatty acids to humans [39].

Chemically, a fatty acid is an organic acid that has an acid group at one end of its molecule, and a methyl group at the other end. Fatty acids are typically categorized in the omega groups 3, 6 and 9 according to the location of their first double bond (there's also an omega 7 group, but these are less important to human health). Polyunsaturated fatty acids consist of two different groups, omega-3 and omega-6 fatty acids. The human body needs a balance between omega-3 (n-3) and omega-6 (n-6) acids. The ratio of n 6 to n3 in an average food intake is 25, the ideal being around 5. The composition of omega-3 fatty acids is very important. Alpha linolenic acid, which is the omega-3 fatty acid found mostly in vegetable oils, is converted in the body to the biologically active form and this occurs slowly in healthy people [40].

2.4.3. Extraction of Lipid from food

Various organic solvents or combination of different solvents have been suggested to selectively extract lipids from a complex mixture of organic compound The Folch method , and The Bligh and Dyer method are the methods used to extract all lipids from plants. The Folch method employs the use of chloroform–methanol (2:1 by volume) for extraction of lipids from endogenous cells [41]. Lipid extraction and partitioning are performed simultaneously in the method, wherein proteins are precipitated in the interface of two liquid phases. The Bligh and Dyer method is one of the widely practiced methods for lipid extraction.

It is very similar to the Folch method, but mainly differs in solvent/solvent and solvent/tissue ratios. This procedure is performed by extracting lipids from homogenized cell suspension using 1:2 (v/v) chloroform/methanol [42]. The extraction of lipids may require several steps which include: Pretreatment, including drying, reducing particle size and possibly hydrolysis in order to release the lipid, homogenization of the tissue in the presence of solvents /system solvents, the separation of liquids from the solids, removal of non-lipid contaminants, removal of the solvents and drying, calculations of the amounts of lipids by weight difference [43].

2.4.4. Derivation and determination of Fatty Acid Methyl Esters (FAME) by GC-MS

Derivatization is a process of changing the chemical characteristics of an analyte by reaction with an active agent. Derivatization reactions are meant to transform an analyte for detectability in Gas Chromatography (GC) or other instrumental analytical methods. Derivatization in gas chromatograph analysis can be defined as procedural techniques that primarily modify an analyte's functionality in order to enable chromatographic separations [44].

It is needed to make it possible to analyze a compound(s) of interest using a specific chromatographic technique, like GC and GC-MS. There are several analytical methods that could be used for the identification and quantification of fatty acids in oils. These techniques include gas chromatography–mass spectrometry, gas chromatography, high-performance liquid chromatography–mass spectrometry, high-performance Liquid chromatography, high-performance size exclusion chromatography, nuclear magnetic resonance, Raman spectroscopy, Fourier transform-infrared spectroscopy and near-infrared spectroscopy.

However, GC–MS and GC are the most widely used techniques for the determination of individual fatty acid profiles and contents, respectively, in vegetable and animal oils. Several studies showed that GC–MS, and GC with flame ionization detection (GC–FID) are also applicable for investigating the fatty acid profiles in silkworm pupae oil [45]. The bulk of analytical derivatization reactions used for gas chromatography (GC) fall into three general reaction types: alkylation, acylation and silylation, Esterification and trans-esterification.

Triglycerides of fatty acids cannot be analyzed directly by gas chromatography (GC) they must first be hydrolyzed and derivatized. The ester bonds are hydrolyzed and the free fatty acids that are formed in the process are converted to the corresponding fatty acid methyl esters (FAMEs). FAMEs are moderately a polar and sufficiently volatile to be determined by GC [46, 47].

2.5. Phenolic compounds

Phenolic compounds are secondary products which posses and aromatic ring bearing a hidroxyl substituent and most are of plant origin. Plant phenolics are a chemically heterogonous group: Some are soluble only in organic solvents; some are water-soluble carboxylic acids and glycosides. Another group of phenolics are insoluble polymers (phenolic compound and their importance [48, 49]. Nowadays, phenolic compounds, such as phenolic acids, stilbenes, lignins, and flavonoids, have aroused interest due to their remarkable spectrum of biochemical and pharmacological properties. The compounds are present in various vegetables, fruits and many food supplements. Particular attention has been devoted to the most numerous groups of phenolic compounds, such as flavonoids which have also brought into focus in the current study [50].

2.5.1. Flavonoids

Flavonoid is a general name of a class of more than 6500 molecules based upon a 15-carbon skeleton. The core structure is a 2-phenylbenzopyranone, in which the three-carbon bridge between the phenyl groups is commonly cyclised with oxygen (Figure 2) [51]. Flavonoids are the most common group of polyphenolic compounds are found ubiquitously in plants. Flavonoids are potent antioxidants. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities [52].

The major classes are flavones, isoflavones, flavonols, anthocyanidins, flavanones, flavanols, chalcones and aurones. They are differentiated according to the degree of unsaturation and the degree of oxidation of the three-carbon segment [53].

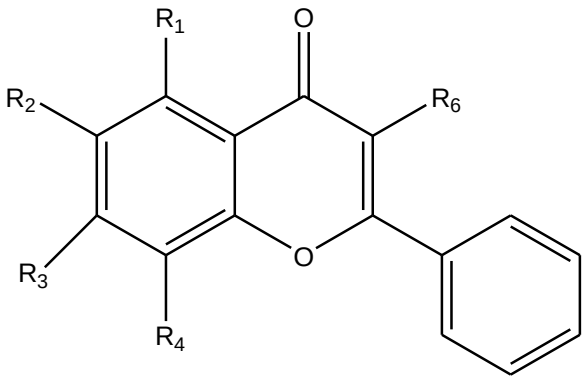


Figure 2: Basic structure of flavonoids

2.5.2. Phenolic compound and flavonoids content determination method

The quantitative analysis of phenolic acids and flavonoids by measurement of ultra-violet absorption is well known [54]. Total flavonoid content was estimated by Aluminium chloride colorimetric method. The principle involved in Aluminium chloride ($AlCl_3$) colorimetric method is that $AlCl_3$ forms acid stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition it also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids.

2.6. Antioxidants and their classification

2.6.1. Antioxidant

Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent [55]. In foods, antioxidants are defined as substances that in small quantities are able to retard or to prevent the oxidation of oxidizable materials such as fats [56]. A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables and tea, etc.

Antioxidant constituents of the plant material are used as radical scavengers, and helps in converting the radicals to less reactive species [57]. Antioxidants slow down the process of degradation so that the energetic action of the environment can lead to higher sustainability and they interact with free radical, making possible their reaction with oxygen.

2.6.2. Classification of antioxidant

Antioxidants are classified into two broad divisions, based upon their solubility in a solvent namely water soluble antioxidants like ascorbic acid, glutathione, lipoic Acid and uric Acid and lipid soluble antioxidants like carotene and ubiquinol. Water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid per oxidation [58].

Antioxidants can be grouped into two classes' synthesis antioxidants and natural antioxidants; the difference between the two categories is that most synthesis antioxidants generate substances that develop cancer or other diseases [59]. Classifying antioxidants can be done depending on their function or on their nature. Depending on their function, there are: Primary antioxidants (antioxidants proper): ascorbic acid and its derivatives, tocopherols, the esters of Gallic acid, erythorbic acid and its sodium salt, BHA, BHT and other substances THBP and TBHQ. Secondary antioxidants (substances with antioxidant action but that have other functions as well). Sulfur dioxide and sulfites as well as lecithin are secondary antioxidants [60]. Primary antioxidant most often acts by donating a hydrogen atom. Secondary or preventive antioxidant can reduce the rate of lipid oxidation by a variety of mechanisms. Secondary antioxidants react with hydro peroxides to yield non-radical, non-reactive products. These may act by binding metal ions able to catalyse oxidative processes by scavenging oxygen, absorbing UV radiation, inhibiting enzymes or decomposing hydroperoxides [61, 62].

Besides the above-mentioned compounds, there are also substances that can strengthen the action of primary antioxidants. Such compounds as lactic acid and lactates, citric acid and citrates, tartric acid and tartrates, etc. have either a synergetic or action of metal complexes that catalysis self-oxidation reactions [63].

Depending on their nature, antioxidants can be natural or synthesis. Tocopherols are natural AOA, as well as a series of compounds with different structures that can be found in plants (flavones). The most important class of synthesis AOA acts by interrupting the lipid self oxidation reaction chain. The phenols substituted belong to this category they react with peroxidic FR and release hydrogen from the OH phenolic group:

$ROO^{\cdot} + Ar - OH \rightarrow ROOH + Ar{-}O^{\cdot}$ ..... (1)

The FR of the antioxidant is stabilized due to the structure of the molecule, the OH group of the antioxidant neighboring, in general, one or several voluminous groups (tert-butyl). Another possibility of stabilizing the radical is through meso-isomerism. These radicals can react with peroxide radicals or between them, forming compounds that no longer have a radical character:

$ROO^{\cdot} + AH \rightarrow ROOH + A^{\cdot}$ and $ROO^{\cdot} + A^{\cdot} \rightarrow ROOA$..... (2)

Upon interaction with the radicals, these substances form radical products that can be stable and persisting.

2.6.3. Extraction and determination method of antioxidant activity

Many herbal plants contains antioxidant compounds which protects cells against degenerative effects of Reactive Oxygen Species (ROS) which is a free radical such as singlet oxygen, superoxide, peroxyl, radicals, hydroxyl radicals. The concept of oxidative stress is that, when a balance between ROS production and antioxidant defenses is lost, 'oxidative stress' result which through a series of events deregulate the cellular function and leads to various diseases such as aging, arthritis, asthma, carcinogenesis, diabetes, rheumatism and various neurons degenerative disease. The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH [65]. DPPH is a stable radical with a deep purple color whose reaction with other radicals, reducing agents, or compounds capable of HAT (hydrogen atom transfer) leads to loss of color at 515 nm. No antioxidant assay is simpler or less expensive to run than the DPPH assay, which accounts for its popularity and extensive use. Needed only are the reagent, some cuvettes, and a UV–vis spectrophotometer, the latter of which are found in even the most rudimentary laboratories. DPPH crystals are dissolved in methanol or ethanol [66].

CHAPTER 3: MATERIALS AND METHOD

3.1. Chemicals and Reagents

All reagents and standards used in the analysis were of analytical grade. Standard grades of fatty acids were used for the laboratory analysis. Other chemicals; methanol(absolute acetone free), chloroform(99.99%), toluene(99.99%), chromatographic grade n-hexane(99.9), acetone(99.5%), sulfuric acid(98%), anhydrous sodium sulfate(99%), and sodium chloride(99.5%), alumnium chloride, sodium nitrite and undecanoic acid as internal standard was used for the laboratory analysis. Catechin, Gallic acid and Ascorbic acid were used as standard, Sodium tungstate ($NaWoO_4.2H_2O$), 85% phosphoric acid and Sodium molybdate ($NaMO_4.2H_2O$) were used for preparation of Folin- Denis reagent. The chemicals and the internal standard were obtained from Sigma Aldrich.

3.2. Apparatus and Instruments

Bench top Shaker (ZHWY-304/334/344), centrifuge (portal centrifuge, Japan), Plastics Bag, GC-MS (Agilent Technologies 7890B-5977A, China), Beaker, Electrical Girder (FW100 High Speed Universal Disintegrator girders), Oven (DAIHAN scientific natural flow type oven), Balance (RADWAG:ps360/c/1), Round Bottle Flask, Spoon, Electricity, Vials, Incubator (constant temperature and humidity incubator), Vacuum Rotary evaporator (Stone Staffordshire, England, ST 1S OSA), Test Tube, Micro Pipit (China), UV-vis model 1601, Shimadzu, Kyoto, Japan, Refrigerator (digital inverter technology, Samsung), aluminum foil, and Crimper (crimper tool 11mm hand crimper, QTY:1) were used for laboratory analysis.

3.3. Description of study are

The samples were collected from five selected areas of Ethiopia namely, West Wolega zone, East Wolega zone, Horo Gudru Wolega Zone in Oromia region, and East Gojjam zone, North Gonder zone in Amhara region from two to three districts. The selection of these areas was based on availability, the consumption and production potential. The Niger seed especially in these areas is as popular as a traditional food used. The map location of the study areas were described blow (Figure 3)

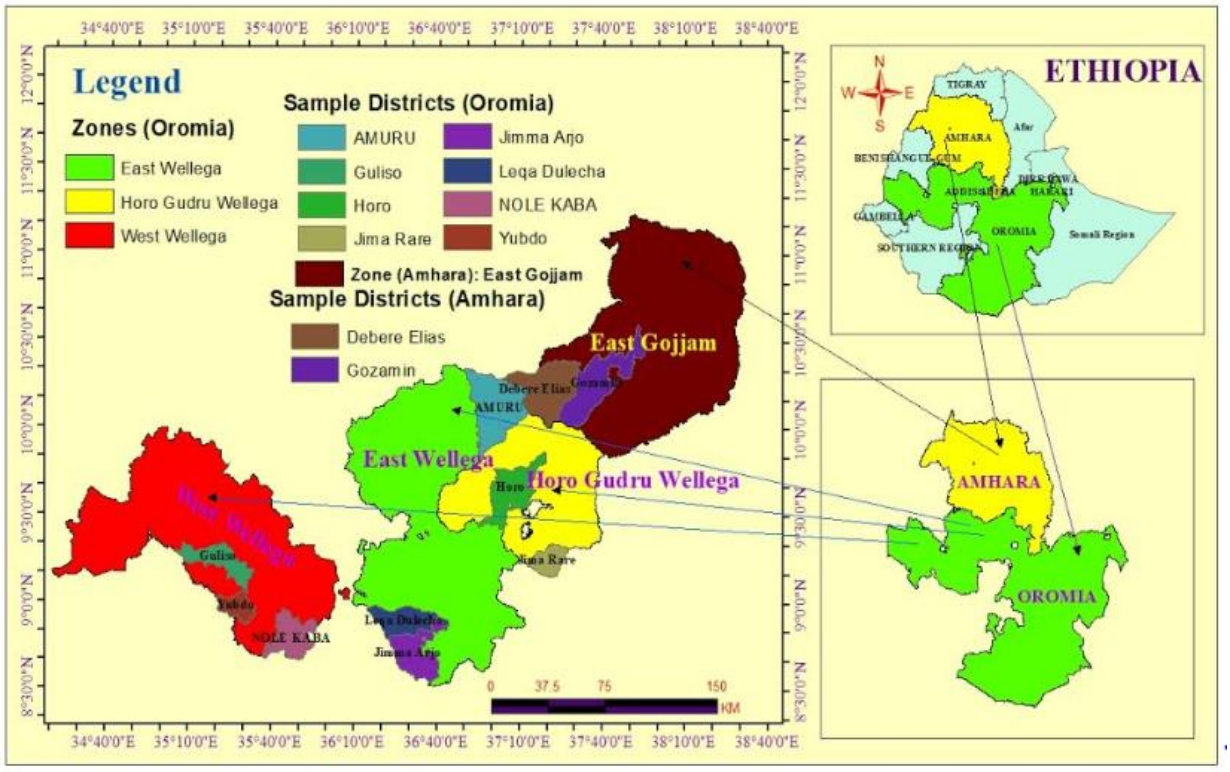


Figure 3: The map location the study areas in selected five area of Ormia and Amhara (source: EMA data using ArcGIS 10.3 software)

3.4. Samples Collection and Sample Preparation

3.4.1. Sample Collection

Seed samples were obtained from the farmers of selected areas of two major producers of G. Abyssinica. The sampling areas were from two region of Ethiopia namely Oromia and Amhara. More briefly; three zones namely, Horo Guduru Welega zone, East Wollega zone, West Wollega zone were selected from Oromia region and two zones namely, North Gondar zone and East Gojjam Zone were selected from Amhara region.

From each five zones two to three districts were selected according to the following; Horo Guduru Welega zone (from Amuru and Jimma Rare districts), East Wollega (from Leka Dulecha and Jimma arjo districts), West Wollega zone (from Yubdo, nolekaba and Guliso distinict) of Oromia region and North Gondar zone (from Lay Armachiho and mirab Armachiho district), East Gojjam Zone (from Guzamn and Debre Elias districts) of Amhara region.

3.4.2. Sample Preparation

The dried seed samples were washed with distilled water to remove dust particles and dried at room temperature. The dried samples were ground to fine powder using electric blender, sieved with 50 mm and stored in an air-tight container before extraction Figure 4.

Figure 4: Sample Preparation process for analysis

3.5. Extraction and derivatisation of fatty acids for GC-MS

3.5.1. Extraction of lipids

Lipids from Niger seeds were extracted according to the method of Folch et al [67] with some modifications. Briefly, a 0.500 g portion of Niger seed powder was extracted with 8 mL chloroform and 4 mL methanol (2:1 ratio by volume) while shaking for 36 h on a platform shaker at 300 rpm. The extract was centrifuged and the filtrate was taken. The lipid phase was separated with the aid of 2 mL of 0.73% aqueous sodium chloride, then the upper phase was removed by using micropipette (siphoning) and the lower phase (chloroform layer) containing the lipids was recovered. The solvent was removed under vacuum rotary evaporator and the residue was reconstituted with 5.0 mL of toluene.

3.5.2. Derivatization of fatty acids

Fatty acids were converted to the corresponding methyl esters prior to GC-MS analysis Christie WW [68] (Figure 5).

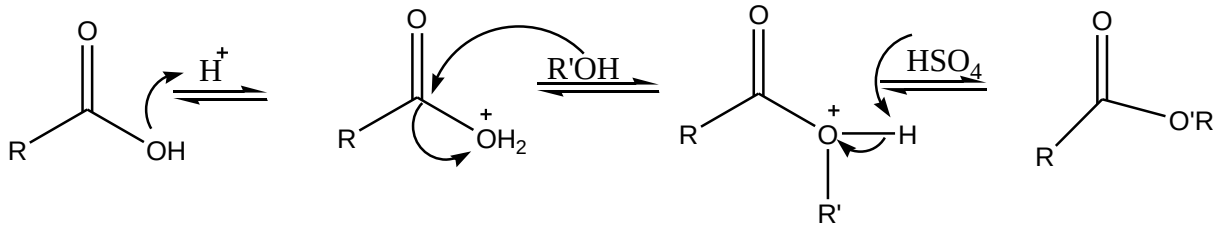


Figure 5: General mechanism of acid-catalyzed esterification of fatty acids

Briefly, a 1.0 mL portion of the lipid extract in toluene was spiked with 175 µL 0.1697 mg/mL of 0.1697 unidecanoic acid and allowed to react with 2.0 mL of 1% methanolic sulfuric acid solution for 12 h, while maintained at 50 °C in an incubator. After that reaction, the mixture was treated with 5 mL of 5% aqueous sodium chloride solution and the required ester was extracted with hexane (2×3 mL) after phase separation, then the upper phase was taken away by using micropipette (siphoning), dried over anhydrous sodium sulfate, filtered by acrodisc syringe membrane filter and transferred in to the vial and analyzed by GC-MS.

3.5.3. GC-MS Analysis

Agilent 7890B gas chromatograph equipped with a 5977A MSD was used. This was coupled to a Mass spectrometer operated in electron ionization mode. A fused-silica capillarity column (30 m, 0.25 mm I.D., 0.25 mm film thickness) with chemically bonded phases DB-5 MS (J&W Scientific) was used for the GC separation. The initial oven temperature was 125 °C, this was held for 2 min then raised at the rate of 30 °C /min to 170 °C, then raised at the rate of 15 ºC/min to 200 °C hold for 2 min, then raised at the rate of 3 °C /min to 230 °C, this was held for 20 min. Carrier gas, He (99.999 %), with a flow rate of 1ml/min; injector temperature, 250 °C , split ratio; 25:1. Mass spectra were recorded at 70 eV and the mass range was from *m*/*z* 40 to 500 amu, ion source temperature 230 °C and quadruple temperature 150 °C and solvent delay time 3.5 min. The constituents were identified by comparison of their mass spectra with those of NIST library data for the GC-MS system.

3.5.4. Quantification of Fatty Acids

The concentrations of all detected fatty acids, with relative percentages higher than 0.1% and which could be quantified accurately Dussert et al ., [69] were determined. Individual fatty acid concentration was determined relative to the internal standard by using the following equation Dussert et al [69].

$$W/W\left(\frac{mg}{g}\right) = \frac{AFA \times MIS}{AIS \times MC} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where AFA is the peak area of the fatty acid, AIS is the peak area of the internal standard, MIS is the mass of the internal standard and MC is the mass of Niger seed used for the analysis. Total fatty acid concentration was determined by sum upping individual fatty acid concentration.

$$TFA = \sum FAi \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where TFA is total fatty acid concentration and FAi is individual fatty acid concentration.

3.6. Preparation of reagents and standard solutions for phenolic compounds analysis

3.6.1. Standard solution preparation

10 mL stock solution of 0.1 mg/mL Gallic acid (GA), 10 mL stock solution of 0.15 mg/mL Catechins and 10 mL stock solution of 0.1 mg/mL Ascorbic acid (AA) were prepared separately by transferring 1mg of GA and AA into 10 mL separate volumetric flask and filled with distilled water to the mark respectively. 10mL Catechins stock solution was prepared by transferring 1.5mg into 10 mL volumetric flack and filled with 100% methanol. These standard stock solutions were used to prepare series of diluted standard solutions that contains different concentrations used for constructing calibration curve for each method.

3.6.2. Preparation of Folin-denis phenol reagent

Folin-denis phenol reagent was prepared according to Seifu et al, [70]. Ten grams of sodium tungstate ($NaWO_4.2H_2O$) and 2.5 g of sodium molybdate ($NaMO_4.2H_2O$) were dissolved in 75 mL of distilled water; then, 5 mL of 85% phosphoric acid and solution was refluxed for 2 hours at 100 ℃. During the reaction process, the hexavalent phosphomolybdic/phosphotungstic complex was formed. 7% of sodium carbonate Na_2CO_3 was prepared by measuring 7g of sodium carbonate in 100mL volumetric flask and filled with distilled water to the mark.

3.7. Preparation of crude *G. Abyssinica* extracts for phenolic compound analysis

The ultrasound assisted extraction procedure, according to Goli et al, [71] was used for sample extraction with slight modification. Briefly, 1 g of powdered Niger seed sample was taken and transferred in to 25 mL separate plastic test tube, 25 mL of 100% methanol and 80% aqueous methanol was added in to each test tube. Then, the sample was extracted in an ultrasonic bath for 60 min at 30 $^{\circ}$C and centrifuged. Then the supernatant were taken after centrifuged and filtration through Whatman number one filter paper in to 25 mL test tube. Finally the test tube was filled up to the mark with respective solvent. The extracting crude extracts were used for the quantification of total phenolic contents, flavonoids, and their antioxidant activity.

3.7.1. Determination of total phenolic content

The total phenolic compounds content was determined by using the method adapted from Waterman and Mole [72] with some modification. Exactly 0.1 mL of the seed extracts (0.667 mg/mL) was mixed with 1.50 mL of distilled water, 0.75 mL of Folin-denis reagent and 3.750 ml of 7% sodium carbonate. The mixture was then incubated in the dark at room temperature for 30 min before the absorbance was measured at 760 nm by a spectrophotometer (UV-vis model 1601, Shimadzu, Kyoto, Japan). The results were expressed in mg Gallic acid per g dry sample.

3.7.2. Determination of total flavonoids content

The total flavonoids content was determined by the aluminium chloride method. The total flavonoids content was determined using the colorimetric method described by Zhishen et al [73] with modifications. The reaction mixture (6.3 mL) comprised of 0.2 mL of sample extract, 3.5 mL distilled water and 0.3 mL of sodium nitrite (5%) was added, then after 5 minute 0.3 mL of aluminium chloride (10%) was added. Finally again after 5 minute 2 mL of 4% sodium hydroxide was added and the mixture were incubated at room temperature for 30 min; absorbance measured at 510 nm. Catechin was used as a positive control. The flavonoids content was expressed in terms of Catechin equivalent (mg/100 g of dry sample).

3.7.3. Determination of antioxidant activity of *Guizotia abyssinica* seed extracts

Antiradical properties of the crude oils under study were compared by using stable free radicals DPPH, according to the method of Ramadan et al [2]. Radical scavenging activity of 100% and 80% aqueous-methanol extract of Guizotia abyssinica seeds against stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) was determined spectrophotometrically. The changes in color (from deep-violet to light-yellow) were measured at 517nm using a UV-visible light spectrophotometer.

Procedure: the hydrogen atoms or electrons donation ability of the extracts were measured from the bleaching of purple colored methanol solution of DPPH. Seven various volume of both 100% and 80% methanol extracts (5, 10, 20, 40, 50 75 and 100 µL) were added to test tube (Table 5 and appendixes). Then 2 mL of 0.004% solution of DPPH radical solution in methanol was added to each test tube with a vortex mixer. Finally the test tubes were filled with 100% methanol and 80% methanol to the mark 5mL. Samples were incubating for 30 min in the dark at

room temperature. The scavenging capacity was read spectrophotometrically by monitoring the decrease in absorbance at 517 nm using a UV-Vis spectrophotometer. Finally, inhibition of free radical DPPH in percent (%) was calculated in following way [2].

$$\% \text{ Inhibition} = 100 \times \frac{(Ao - As)}{Ao} \quad \text{(4)}$$

Where: Ao - the absorbance of the control (blank) sample and, As - the absorbance of the various extract samples. $IC_{50\%}$ value of the extracts, expressed as µg / mL, denote the concentration of crude extract in DPPH methanol solution required to cause 50% inhibition of DPPH radicals. And a graph was plotted for percent DPPH remaining against the concentration of oil in DPPH methanol solution (mg / mL) and IC_{50} value was determined by interpolation of values on the graph.

3.8. Data analysis

In this study, results for each test parameter were interpreted and statistical analysis was done by using SPSS version 20, Origin 6.0 and Excel 2007 soft ware. The samples were analyzed in triplicate. Standard statistical methods were used to determine the mean values and standard deviations. Microsoft Excel spreadsheet was used to record and organize the data. One way analysis of variance (ANOVA) and Tukey was used to compare means among treatments. Significant differences was determined at the $P < 0.05$ levels and results expressed in mean ± SD.

CHAPTER 4: RESULTS AND DISCUSION

4.1. Qualitative Identification of Fatty acids in the samples

Fatty acids analysis of Chloroform -methanol extract of Niger seeds samples were analyzed by GC-MS. The analysis enabled that a total of 12 fatty acids were detected in all of the Niger seeds samples (figure 6 and appendix). The identities of the detected fatty acids were determined by using the NIST-11 spectral library as a reference.

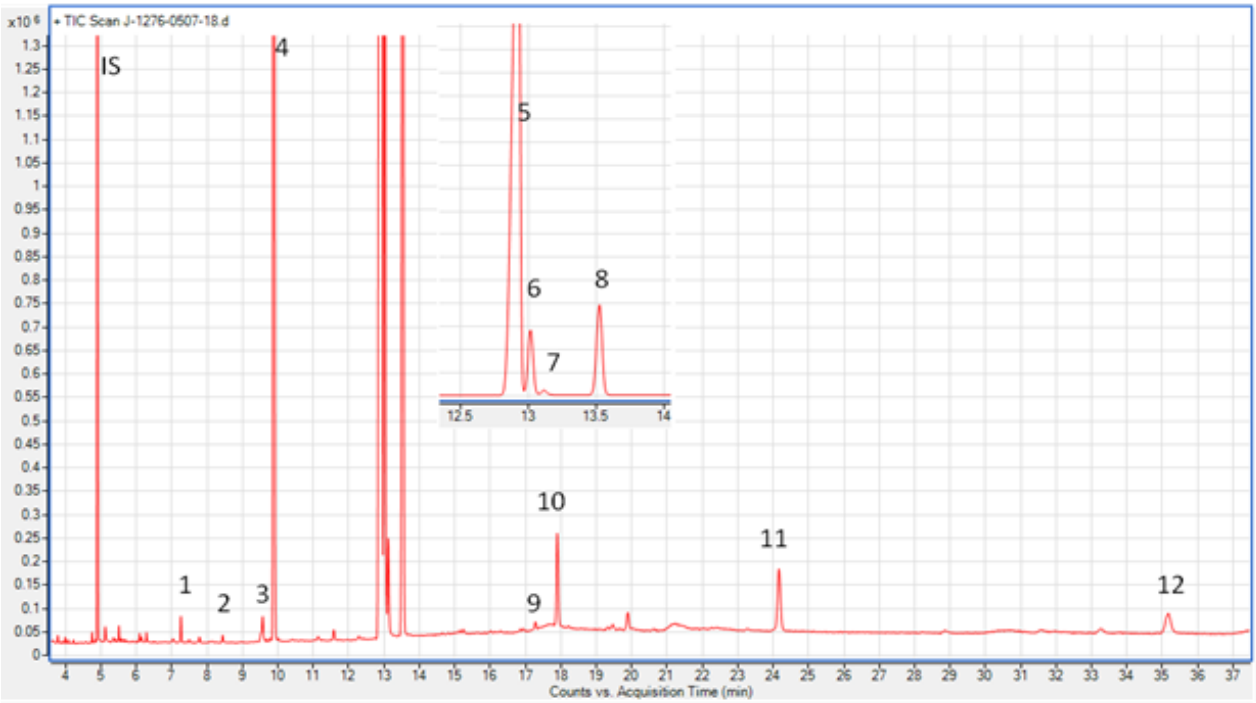


Figure 6: A representative GC – MS Chromatogram of north Gondar sample (where, 1-Myristic acid, 2- Sarcinic acid(12-methyl myristic acid) 3-cis-Hypogeic Acid, 4-Palmitic acid, 5 - Linoleic acid, 6-Vaccenic acid, 7-Oleic acid, 8-Stearic acid, 9-Arachidic acid, 10- Alkynyl Stearic Acid 11-Behenic acid, 12-Lignoceric acid).

The major fatty acids in niger seed are represented by unsaturated FAs such as, Linoleic acid (C18:2); which was the principal unsaturated fatty acid in all niger seed samples ranging from 67.2 to 74.63%., Oleic acid (C18:1n-9) that was the second main unsaturated fatty acid constituting 5.438 – 11.017%, vaccenic acid that was the third unsaturated fatty acid constituting 0.427 – 0.47%, irrespective of variety and location. Palmitic (C16:0) and stearic (C18:0) acids were the major saturated fatty acids in the five Niger seeds sample accounting for 10.31 to 10.66% and 7.59 – 8.76%, respectively (Table 1). This range is within the data report by Dutta et al [32] on Ethiopian Niger seeds varieties where Linoleic acid (C18:2) ranged from 71.4 to 79.2%, Oleic (C18:0) at a range of 6 to 11% each, Palmitic (C16:0) and stearic (C18:0) acids ranged from 9.4 to 16.6% and 6.6 to 8.2% respectively.

These four major fatty acids (linoleic, oleic, palmitic and stearic) constituted about 94% of the total fatty acid in Niger seed oil. The contents of these major fatty acids reported here also concur with published results, of Dagne and Jonsson [33]. Also in addition to these major fatty acids, minor fatty acids in niger seed are; Myristic (C14:0), hypogeic (C16:1n-9), Alkynyl Stearic Acid, arachidic acid (C20:0), vaccenic acid (C18:1n-7), behenic acid(22:0), heptadecanoic acid and lignoceric (C24:0) were identified and constituted about 6.48% of the total fatty acid. This result is in agreement with the work of Geleta et al [31] for the percent composition of the principal fatty acids. The level of Behenic acid (C22:0), Arachidic acid (C20:0), and Lignoceric acid (C24:0) found in the present study is in agreement with the result reported by Ramadan et al and Deme et al [2,5].

The results of the present study concur with those of earlier investigations, which showed detailed fatty acid composition, except for those American varieties, where lower oleic acid content was reported [79]. The extent of other minor fatty acids present in Niger seed of the present study also concurs with those published results, except vaccenic acid and 17-Octadecynoic acid were not report early.

Table 1: The names, retention times (RT) and means of identification of the fatty acids determined in the niger seeds samples.

No	Chemical name	Short hand	% Fatty acids of Niger seeds					RT
			West Wolega	East Wolega	Horogudru Wolega	North Gonder	East Gojjam	
1	Methyl tetradecanoate	C14:0	0.07	0.058	0.049	0.064	0.061	7.257
2	Tetradecanoic acid, 12-methyl-, methyl ester, (S)-	-	0.024	0.018	0.016	0.018	0.015	8.438
3	7-Hexadecenoic acid, methyl ester, (Z)-	C16:1(n-9)	0.146	0.142	0.114	0.155	0.106	9.566
4	Hexadecanoic acid,	C16:0	10.39	10.58	10.32	10.66	10.54	9.883
5	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C 18:2(n-6)	72.887	67.69	74.67	67.30	71.13	12.92
6	11-Octadecenoic acid, methyl ester	C 18:1(n-7)	0.448	0.453	0.429	0.462	0.465	13.02
7	9-Octadecenoic acid (Z)-, methyl ester	C18:1(n-9)	5.769	10.91	5.438	11.02	7.696	13.112
8	Methyl stearate	C18:0	8.761	8.542	7.59	8.703	8.541	13.53
9	17-Octadecynoic acid (17-ODYA)	-	0.0304	-	0.073	-	-	17.28
10	Eicosanoic acid, methyl ester	C20:0	0.532	0.527	0.427	0.064	0.499	17.89
11	Docosanoic acid, methyl ester	C 22:0	0.588	0.682	0.606	0.018	0.577	24.17
12	Tetracosanoic acid, methyl ester	C24:0	0.352	0.399	0.273	0.155	0.311	35.18

The FAs composition of the *G. abyssinica* belongs to the samples study were in general pattern of FAs distribution is similar to within locality samples. However, some differences in the relative proportions of strearic (C18:0), linoleic (C18:2) and Oleic acid are obviously. There was comparatively variation in the FAs composition within individual *G. abyssinica*; within HG/Wolega sample had a lower percentage of stearic acid (7.59%), whereas the high contents of stearic acid (8.76 %) in W/Wolega. Whereas the high contents of oleic acid (11.017 %) in N/Gonder where as HG/Wolega was lowest content (5.43%) compared to those other localities.

However, the higher percentage compositions of *G. abyssinica* were registered for the linoleic acid (C18:2); 67.302, 67.69, 71.128, 72.88 and 74.67% respectively within N/Gonder, E/Wolega, E/Gojjam, W/Wolega and HG/Wolega samples. High linoleic acid content makes the oil of niger seed nutritionally valuable. The minor fatty acids, Myristic (C14:0), cis-hypogeic (C16:1n-9), Alkynyl Stearic Acid, arachidic acid (C20:0), vaccenic acid (C18:1n-7), behenic acid (22:0), heptadecanoic acid and lignoceric (C24:0) were identified in the seed extract and made up 6.24% of the total FAME. On the other hand, Dagne and Jonsson [33] and Deme et al [5] found in trace amounts eurcic (C22:1) and eicosenic (C20:1) acids, which were not detected in the present study. The reason for this variability may be genetic (plant cultivar or variety), seed quality (maturity, damage caused by harvesting or handling/storage conditions), oil processing variables, or accuracy of detection and quantitative techniques.

4.2. Individual Fatty acids and total fatty acid concentration quantification

The concentrations (w/w) of the different FAs were quantified relatively to the internal standard (Undecanoic acid,) by using the equation (3) Dussert et al [69] shown in Table 2. The concentrations of individual fatty acids FAs in *G. abyssinica* samples were determined Table 2. There was a large variation in fatty acid concentration, with in a sampling area. Among the main unsaturated fatty acids (FA) in the niger seed, a linoleic acid (C 18:2(n-6)) was the most abundant ranging from 24.8049 to 233.920 mg/g of sample in HG/Wolega and North Gonder respectively Table 2. Oleic acid concentration had highest 222.425mg/g of east Gojjam and had lowest 16.18mg/g of west wolega sample.

Table 2: The concentration of FAs (µg/g dry weight) determined in *G. abyssinica* samples.

No	Chemical names	Short hands	Concentration of FAs (µg/g) in five sites of *G. abyssinica*				
			West Wolega	East Wolega	Horogudru Wolega	North Gonder	East Gojjam
1	Methyl tetradecanoate	C14:0	197.199	171.680	112.962	222.588	189.655
2	Tetradecanoic acid, 12-methyl-, methyl ester, (S)-	none	67.411	51.082	118.216	61.137	47.129
3	7-Hexadecenoic acid, methyl ester, (Z)-	C16:1(n-9)	408.683	415.010	38.626	538.553	330.557
4	Hexadecanoic acid, methyl ester	C16:0	29142.94	30908.382	275.143	37056.55	32949.46
5	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C 18:2(n-6)	204415.1	197822.677	24804.9	233920.5	138.465
6	11-Octadecenoic acid, methyl ester	C 18:1(n-7)	1256.013	1324.551	13074.97	1603.208	24065.34
7	9-Octadecenoic acid (Z)-, methyl ester	C18:1(n-9)	16181.38	31883.329	179498.6	38293.42	222425.2
8	Methyl stearate	C18:0	24569.92	24964.54529	1029.328	30250.18	1455.608
9	17-Octadecynoic acid	-	85.23863	-	18257.03	-	
10	Eicosanoic acid, methyl ester	C20:0	1492.411	1541.354183	174.577	1833.693	1560.453
11	Docosanoic acid, methyl ester	C 22:0	1651.173	1991.715682	1026.7	2381.423	1804.641
12	Tetracosanoic acid, methyl ester	C24:0	986.5718	1166.875461	1456.862	1409.117	974.259
13	Total FAs	280454	292241.20	240412.4	347570.3	312648.28	280454

The total concentration of FAs in *G. abyssinica* is the sum of each individual fatty acid concentration (saturated and unsaturated fatty acid). Sample from North Gonder had highest total FAs concentration, while sample from HG/ wolega had lowest total fatty acid concentration (Table 2). Niger seed samples from North Gondar had higher total FA contents, 347.57 mg/g of sample followed by sample from East Gojjam, 312.64 mg/g. Total FAs of samples from East Wolega was 292.241mg/g followed by samples from W/Wolega 280.454mg/g, and where as sample from HG/Wolega had the lowest total FA concentration 240.41g/mg. This variability in fatty acid concentration might be due to variety or location (including soil condition, elevation, rainfall and temperature). Although some qualitative data are available on the relative percentage of the individual fatty acids, there was no reported quantitative data on the individual as well as total fatty acid concentration of Niger seeds.

Results of the present study were compared with other literature and common seed crop like, linseed, sesame, Sunflower, flaxseed, safflower grown in different regions of Ethiopia and other counters to in order to evaluate the potential of niger seed nutrients availabilities, phytochemicals contents and fatty acid composition with others (Table 3).

Table 3: Comparison of saturated fatty acid composition with literature and other seed oil crops

Sample	Country	% Fatty acids						Ref.
Niger		C14:0	C16:0	C18:0	C20:0	C22:0	C24:0	
	America	0.3-0.6	8-8.6	5.6-7	0.3-0.5	0.6-2.8	0.5-2	[76]
	German	-	17.5-26.2	5.06-8.77	0.01-0.65	0.02-0.59	0.01-0.9	[2]
	Ethiopia		8.5-9.2	5.6-7	0.1-0.5	0.1-0.6	0.1-0.3	[32]
	Ethiopia		7.56-8.29	6.13-7.13	0.4-0.49	0.55-0.95	0.38-0.48	[5]
Sesame	Ethiopia	-	8.51-9.27	4.87-8.86	0.13-0.61	0.12-0.28	0.08-0.24	[5]
Linseed	Ethiopia		4.92-5.41	3.33-4.05	0.08-0.15	0.11-0.26	0.12-0.45	[5]
Sunflower seed	Brazil	-	5.47-5.90	3.25-3.53	0.04-0.12	0.54-0.70	0.05-0.07	[81]
Safflower seed	Turkey	-	7.9-11	2.3-2.8	-	-	-	[80]
Flax seed		-	7.49	4.59	0.18	0.17	0.25	[82]
Niger seed	Ethiopia	0.05-0.07	10.31-10.6	7.59-8.76	0.06-0.53	0.02-0.68	0.16-0.40	This Study

Table 4: Comparison of unsaturated fatty acid composition with literature and other seed oil crops

Sample	Country	% Fatty acid				Ref.
Niger		C16:1n-9	C18:2n-6	C18:1n-7	C18:1n-9	
	America	-	70.9-71.3	-	0.5-3.3	[76]
	Ethiopia	-	-	-	5.9-11	[32]
	Ethiopia	0.09-0.11	73.32-76.84	-	4.63-6.59	[5]
Sesame	Ethiopia	-	40.16-49.50	-	34.6442.05	[5]
Linseed	Ethiopia	0.04	13.31-16.62		15.9-20.33	[5]
Sunflower	Brazil	0.02	62.33-69.62	-	20.70-26.45	[81]
Safflower seed	Iran	-	62.2-75.6	-	14.5-27.2	[80]
Flaxseed		0.06 ± 0.05	12.25 ± 0.05	0.60	17.97	[82]
Niger seed	Ethiopia	0.11-0.16	67.30-74.67	0.43-0.47	5.44-11.02	This Study

Regarding the fatty acid composition, *G. abyssinica* resembles that of safflower and sunflower with a high content of linoleic acid (18:2), up to 76.84% depending on the origin. From the results of the present and previous studies, it is clear that *G. abyssinica* is a good source of high linoleic acid-containing oil, even higher than sunflower and safflower oils. In addition, it is a good source of bioactive compounds those responsible for antioxidant activity.

4.3. Total phenolic content (TPC) and total flavonoid contents (TFC)

The data produced on the Total phenolic content (TPC) and total flavonoid contents (TFC) of the five niger seed tested in this study are presented in (Table 5), and some differences can be observed among them. The total phenolic content was expressed as milligram of Gallic acid equivalents (mg GAE/g) gram of dry sample. TPC values were obtained from the calibration curve $y = 0.061x - 0.0049$ with $R^2 = 0.9994$, (Figure 5 and Appendix B) where y is the absorbance and x is the concentration of sample solution (mg/mL). Niger seed extracting, contain phenolic compounds to reduce tungusten and molybdium in the Folin-Denis reagent that contain phospho tungestic or molybdic acid as oxidizing agent shows a blue color with maximum absorbance at 730 nm Agbor et al, [78].

The absorbance was proportional to the amount of aromatic phenol group and used for their determination during the oxidation of phenolic in *G. abyssinica* extract. Hence the development of blue colored solution confirmed the presence of phenolic compound in the sample. In general the higher TPC was observed (11.780±0.028 mg GAE/g) for sample from N/Gondar while the lowest was scored in W/wolega sample (10.89±0.39 mg GAE/g) of 80% methanol extract.

In similar way the total phenolic contents of the 100% methanol extract in the five samples (collected from W/wolega, E/wolega, HG/Wolega, N/Gonder, E/Gojjam) were found to be 7.65±0.38, 9.96±0.23, 10.92±0.38, 9.56±0.09 and 10.28±0.52 mg GAE/g, respectively. Samples from E/Gojjam resulted higher (10.92±0.38 mg GAE/g) value, while *G. abyssinica* samples from W/wolega had the lowest concentration (7.65±0.38 mg GAE/g).

Statistically, a significant difference ($p < 0.05$) was noticed for total phenolic content between samples of both 80% methanol and 100% methanol extract from West Wolega and other localities. And also a significant difference ($p < 0.05$) was observed for samples of 100% methanol extract from Horo gudru Wolega and other localities. Methanol (80%) and methanol (100%) extract of Horo gudru Wolega and north Gondar were significant difference of total phenolic content ($p > 0.05$), were observed when one way ANOVA was carried out.

The significant differences of total phenolic compounds content might arise due, climatic conditions, harvesting, temperature and variability of the soil composition of the sampling sites. While no significant difference of total phenolic content ($p > 0.05$) between samples of both solvent system extract from E/wolega, and E/Gojjam.

Table 5: Total phenolics, and total flavonoids contents of G.Absinicca extrac (mean±SD, $n = 3$) expressed per dry mass basis.

Sample site	Total phenolic Contents (mg GAE/g)		Total flavonoid (mgCE/g)	
	80% Methanol extract	100% Methanol extract	80% Methanol extract	100% Methanol extract
W/Wolega	10.89 ± 0.39^{c}	7.65 ± 0.38^{d}	5.64 ± 0.09^{b}	5.00 ± 0.11^{d}
E/Wolega	11.49 ± 0.15^{ab}	9.96 ± 0.23^{bc}	6.44 ± 0.02^{d}	5.87 ± 0.01^{c}
HG/Wolega	11.24 ± 0.08^{b}	10.92 ± 0.38^{a}	6.04 ± 0.22^{c}	6.31 ± 0.22^{b}
N/Gondar	11.78 ± 0.028^{a}	9.56 ± 0.09^{c}	6.67 ± 0.06^{e}	4.98 ± 0.03^{d}
E/Gojjam	11.58 ± 0.04^{ab}	10.28 ± 0.52^{b}	5.42 ± 0.03^{a}	6.76 ± 0.22^{a}

Values in the same column that are followed by a different letter (a–d) are significantly different $p < 0.05$ by Tukey's multiple range tests.

The total flavonoids contents of Niger seed extracts were also determined. The total flavonoids content was expressed in terms of milligrams of catechin equivalent per gram of dry mass of sample (mg GAE/g) and determined from a standard curve of Catechins ($y = 0.054x + 0.003$, $R^2 = 0.999$, Figure 7 Appendix B), and the data are given in table 6. The total flavonoids contents in the studied samples in 80% methanol extract ranged from 5.42±0.03 to 6.67±0.06 from E/Gojjam and E/Wolega respectively.

The 100% methanol extracts were ranged from 4.98±0.11±0.11 to 6.76±0.22 mg GAE/g of dried sample from N/Gonder and E/Gojjam respectively. In case of 100% methanol extract no significant difference ($p < 0.05$) was observed among the studied samples from West Wolega and North Gonder; while the other samples from EW, HGW and EG Showed significant differences between each other when pair wise one-way ANOVA analysis was carried out.

The measured flavonoids contents of both solvent system extract (100% extract and 80% methanol extract of five Niger seed samples were significantly different except between samples from W/Wolega and N/Gondar of 100% methanol extract. These significant differences might be due to differences in the climatic condition of the regions, physicochemical property of the soil and variation of sampling seasons.

Regarding the solvent effect, 80% methanol extract resulted in higher concentration of flavonoids than 100% methanol extract. The TPC of the 80% aqueous methanol extract (10.89±0.39 - 11.780±0.028 mg GAE/g Dry sample) is higher than that of the 100% methanol extract (7.65±0.38- 10.92±0.38mg GAE/g dry sample). The finding of this study was partly in line with the study reported by Matthaus et al [74] who reported that the 70% methanol extract from white mustard had the highest phenolic content, followed by rapeseed and camelinain. And also, the presented study was in agreement with the work of Bekhit et al, [75] TPC, Antioxidant activity and TFC of 80%methanol extract seed cakes shows highest value than 100% methanol extract.

TPC and TFC of present study and other minor seed oil crops in literature were compared to see the nutritional availability of Niger seed (Table 6). Niger seed had shows highest TPC and TFC than other seed except linseeds. So Niger seed had better antioxidant activities.

Table 6: Comparison of TPC and TFC of present study and other minor seed oil crops

Sample	Country	TPC mg/g	TFC	Reference
Safflower seed	Iran	4.72±0.12 - 9.51+0.26	-	[80]
Sesame seed	Indonesia	0.23 - 1.57	0.29 - 4.29	[83]
Sunflower seed	japan	4.39 - 0.20	-	
Soybean seed	Austrail	2.42±0.16 - 3.15+0.01	-	[85]
Corn seed	poland	1.7±0.02 - 5.8+0.1	-	[84]
Linseed	Ethiopia	20.±0.15-22.77±0.05	-	[5]
Sesame seed	Ethioia	10.2±0.03-18.3±0.06	-	[5]
Niger seed	Ethiopia	7.65±0.038-11.78±0.028	4.98±0.03-6.67±0.06	Present study

4.4. Level of Antioxidant activity

4.4.1. DPPH Scavenging Activity assay

The free radical scavenging capacity of the Niger seed extract with 100% methanol and 80% aqueous methanol extract were assayed. Ascorbic acid was used as a positive control to determine percent of inhibition. The scavenging activities (percent of inhibition) of extract with different concentration were determined according to equation (4). The percent inhibition of five niger seed samples of both 100% methanol and 80% aqueous methanol extract considered in the assay were shown in Figure 7 and 8. The percent inhibition was shown to be dependent on the concentration of the extract.

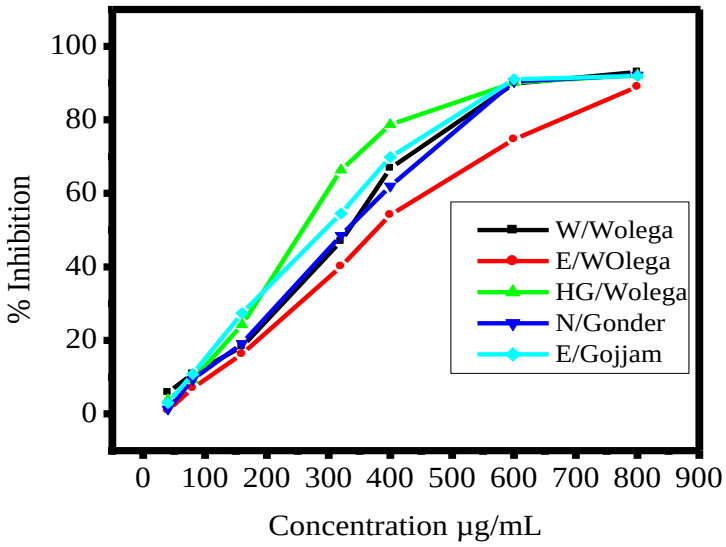


Figure 7: Free radical scavenging of 100%methanolic extract of niger seed.

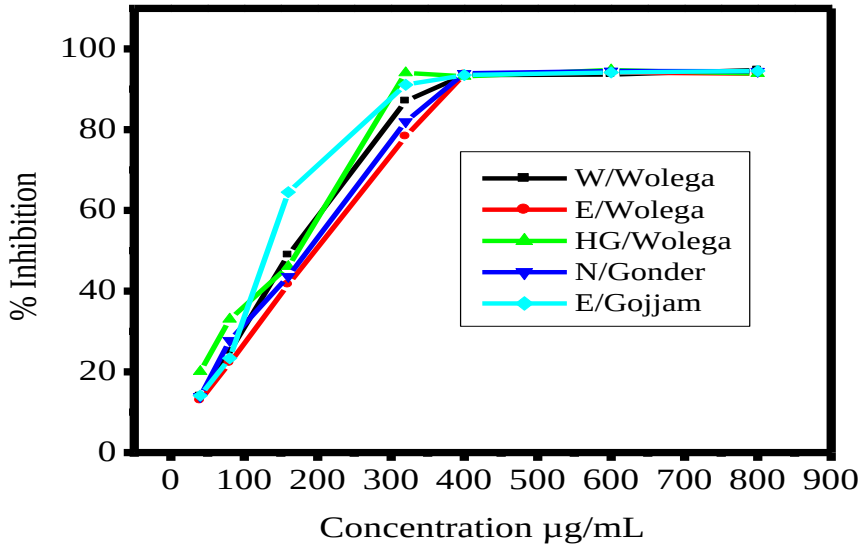


Figure 8: Radical scavenging of 80% methanolic extract of niger seed.

Up on increasing extract concentration the percent inhibition also increased. This has been confirmed from the color change of DPPH from deep violet color to yellow, in which the concentration of extracted sample increasing from 5 to 10µL. The present study showed that the ability of Niger seed of 100% methanol extracts to scavenge the DPPH radical was at the level ranging from 0.91– 92.15 %, and 80% aqueous methanol extract was at the level ranging from 12.912 – 94.526 % (Table 5 and appendix). The E/Wolega sample exhibited the lowest inhibition capacity, while Niger seed from E/Gojjam shows highest percent of inhibition than other sites. Though the varieties in site of *G. abyssinica* for DPPH assay was chosen based on the total flavonoids content, niger seed samples from E/Gojjam with the highest flavonoids content scored the highest percent inhibition.

From the data in Table 7, it can be concluded that extraction with 80% methanol was more effective than with 100% methanol. These results are in agreement with study Terpinc et al [6] who reported 70% methanolic extracts offer better antioxidant activity than 100% methanol extract in terms of reducing capacity, DPPH· radical scavenging. In general, as reported before, the amounts of extractable phenolic substances decreased with decreasing polarity of the solvent.

In the presented study 80% methanol extract showed highest percent inhibition in all *G. abyssinica* extract. This may be due to the presence of Maillard reaction products extracted by the polar solvents could also be contributing to the increased radical scavenging activity. Therefore 80% methanol was the best solvent to extract bioactive molecules like phenolic, flavoniods which have responsible to scavenging free radical in human metabolic system.

4.4.2. The IC_{50} Values of Each Sample

The efficient concentration (EC_{50}) or half maximum inhibitor concentration (IC_{50}) was defined as the concentration of the sample leading to 50% reduction of the initial DPPH concentration, which was obtained from the graph of sample concentration against %inhibition of each Niger seed sample extract, and expressed in µg/mL. The IC_{50} value of 80% aqueous methanol extract was lower when compared with 100% methanol extract. The lowest IC_{50} value the higher is the antioxidant activities of the tested sample and the higher IC_{50} value, the lower is the antioxidant

activities. Hence, Niger seed sample of 80% aqueous methanol and 100% methanol extract, from East Gojjam scored lower IC_{50} value 132.79±3.1 and 294.180±4.08µg/mL respectively. But, samples from East wolega scored higher IC_{50} value 190.503 ±1.461 and 378.297±2.64 for 80% aqueous methanol and 100% methanol extract respectively (Table 7).

Table 7: IC_{50} values of Niger seed extract in 80% of methanol and 100% methanol

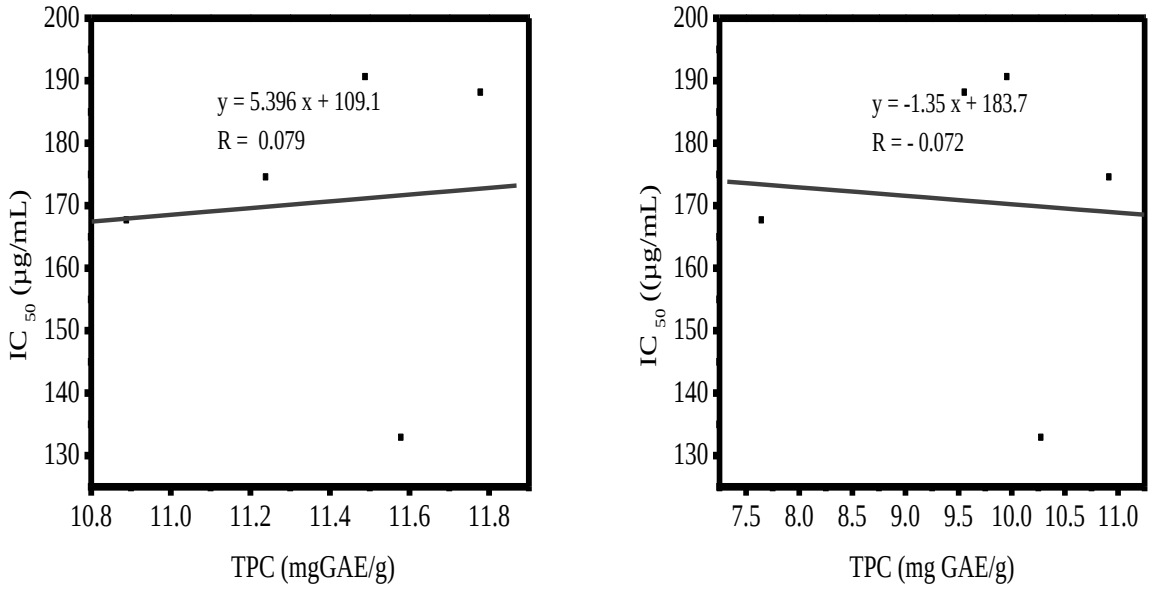
Sample site	DPPH scavenging IC_{50} value (µg/mL) (Mean ± SD, n=3).	
W/Wolega	80% aqueous; methanol	100% methanol
	167.56±3.755[b]	333.177±2.64[c]
E/Wolega	190.503 ±1.461[d]	378.297±2.64[d]
HG/Wolega	174.453 ±3.105[c]	258.717±2.745[a]
N/Gonder	188.017±3.745[d]	330.57±2.64[c]
E/Gojjam	132.79±3.169[a]	294.180±4.08[b]
Ascorbic acid	2.490±0.08	

Note: Values in the same column that are followed by a different letters (a, d) are significantly at $p<0.05$ by Duncan's multiple tests.

E/Wolega- east wolega, w/wolega-west wolega, HG/wolega-Horo Gudru Wolega, N/Gonder-northGonder, E/Gojjam- east Gojjam

The correlation between the TPC, TFC and antioxidant activities

The relationship between antioxidant activities and total phenolic content of five niger seed of 100% methanol extract and 80% aqueous methanol extracts were examined by plotting total phenilc contents versus the IC_{50} values as indicated in Figure 9 below.



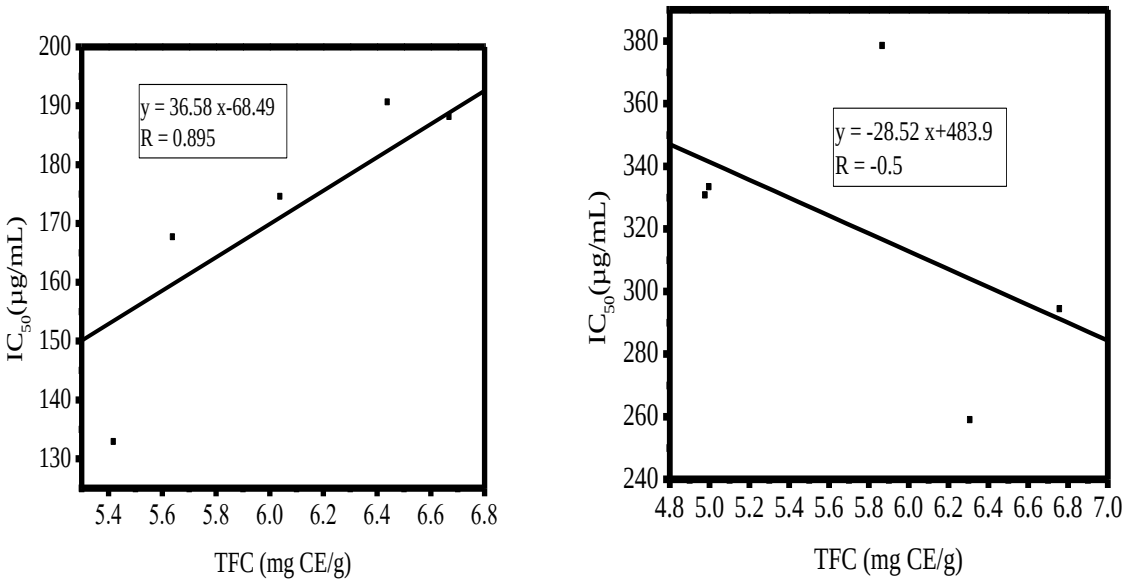
A) B)

Figure 9: Correlation between total phenolic content and antioxidant activities of A) 80% aqueous methanol and B) 100% methanol extract

The antioxidant activity of the 80% methanol and 100% methanol extract had poor correlation with total phenolic content with the correlation coefficient (R) of 0.079 and -0.072 respectively, which indicates that in addition to phenolic compounds, there were other constituents like fatty acids which involved the scavenging effect.

Correlation between total flavonoid contents in G. abyssinica and radical scavenging activity of the five samples, were analyzed by calculating the correlation coefficients (r) between them. The level of total flavonoids contents has been found to positively correlate with the radical scavenging activity of 80% methanol extract and 100% methanol extract shows negative

correlate. The results showed a positive linear correlation between the radical scavenging activity and total flavonoids (R= 0.895) of 80% methanol extract and negative linear correlation (R= -0.5) values of 100% methanol extract extracts Figure 10. However total flavonoids content of 80% methanol extract extracts was strongly correlated (r = 0.894) with radical scavenging activity as compared to the other group of compounds.



A) **B)**

Figure 7: Correlation between total flavonoids content and antioxidant activities of A) 80% aqueous methanol and B) 100% methanol extract

Which indicated that aqueous methanol (80% methanol) is known to be an efficient and widely used solvent system to extract natural antioxidative components, especially the flavonoids among phenolic compounds from plant materials. This is due to the fact that the methanol-water mixture has high polarity and thus greater efficacy towards the extraction of polar phytochemicals such as phenolics and flavonoids Siddiq et al [77]. Polyphenols are the major plant compounds with antioxidant activity, although they are not the only ones (Moure et al., [86]. Some nonphenolic compounds which are known to react with Folin–Ciocalteu reagent but are not effective as free radical scavengers (citric acid, ferrous sulfate, d-glucose) may also interfere with the correlation between TPC and the antioxidant activity of some extracts Magalhaes et al., [87].

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1. CONCLUSIONS

The results of the present investigation indicate that *G. abyssinica* seed oil is a good source of essential fatty acids and natural antioxidant .The study therefore indicates that oil quantity and quality in *G. abyssinica* was comparable to that of other minor oilseed crops like sunflower, safflower and linseed as depicted by oil content and fatty acid profiles. From present study four major fatty acids in *G. abyssinica* are represented; Unsaturated FAs such as, Linoleic acid (C18:2) contain 67.2 to 74.63%., Oleic acid (0.427 – 0.47 %,) and two major saturated fatty acids Palmitic (C16:0) and stearic (C18:0) 10.31 to 10.66 and 7.59 – 8.76%, respectively irrespective of variety of location were determined. It has been reported that the abundance of PUFA's particularly linoleic acid enhances frying flavor intensity of foods due to the presence of volatiles like 2, 4-decadieenal which is the oxidation product of linoleic acid.

And also the individual and total fatty acid concentration of niger seed sample from selected area of Ethiopia was determined. North Gonder samples scored higher total fatty acids than samples from other area, while HG/Wolega resulted in lower total fatty acid concentration 347.57mg/g and 240.41mg/g of FAME respectively. High linoleic acid content makes the oil of *G. abyssinica* nutritionally valuable. Thus, Niger could be nutritionally considered as a new potential source of edible seed oils.

Niger seeds have essential bioactive compounds like polyphenols and flavonoids which impart Niger seeds oil having excellent antioxidant potential. Methanol (100%) and 80% aqueous methanol were used to extract these compounds. The extractability of these bioactive molecules can be increased by the use of polar solvents. 80% aqueous methanol proved to be a good solvent for extraction of *G. abyssinica* rich in bioactive. The inherent bioactive molecules may contribute to the stability and radical quenching ability of *G. abyssinica*. The antioxidant activity of Niger seeds of Methanol (100%) and 80% aqueous methanol extracts were compared with standard Ascorbic acid by varying the concentration of the extracts by free radical scavenging activity, DPPH, using UV-VIS spectrophotometer.

As this study revealed that the antioxidant activity of *G. abyssinica* extracts increased as the concentrations increased, but almost constant after 75 µL of the extracts, as shown on Figure 8 of this manuscript. It could be concluded from the results of the present study that the fatty acid concentration total phenolic content, total flavonoids contents and antioxidant activity of *G. abyssinica* extracts varied considerably depending on the nature of localities and the extracting solvents.

5.2. Recommendation

The use of 80% methanol can be recommended for the extraction of effective antioxidant components from *G. abyssinica*. The extracts derived from *G. abyssinica* were found to be quite effective towards suppressing the primary and secondary oxidation products in the tested sample. This suggests that *G. abyssinica* might be explored as a viable source of potent antioxidants for the protection of vegetable oils from oxidation. For further study; since fatty acid composition, phenolic compounds and flavonoids contents of seeds can varies with area, further research was needed on other areas. Comparative study should be conducted on the extraction and evaluation of antioxidant from niger seed using different organic solvents like Ethanol, water, Hexane, petroleum ether and others with equipment like sohxlet, Supercritical extractor, Microwave assisted extractor and others. Antioxidant activity of seeds can be determined using different techniques rather than, DPPH free radical scavenging activity like ferric reducing ability of plasma (FRAP) assay and Rancimat methods.

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Appendix-A

Chromatogram of five Guizotia abyssinica collected from selected areas of Ethiopia

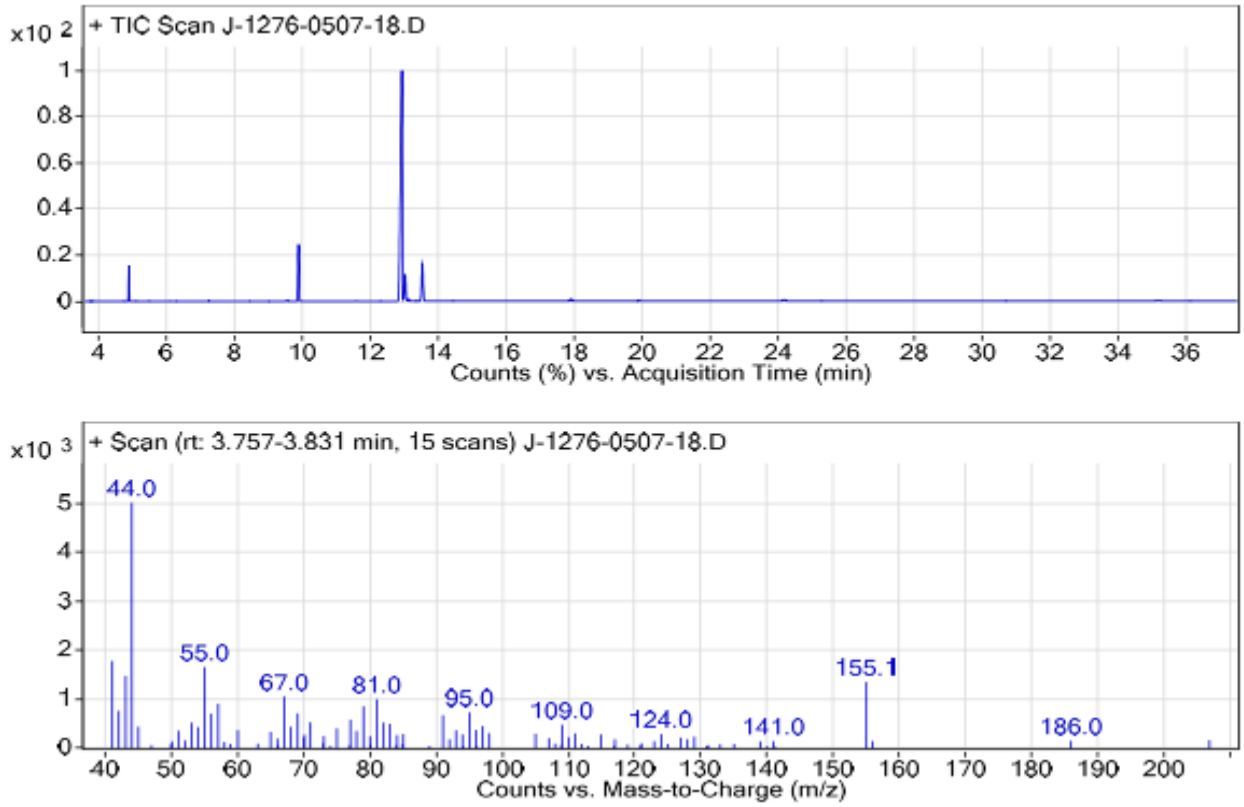

Figure 1**:** GC-MS Chromatogram of west wolega nigerseed sample

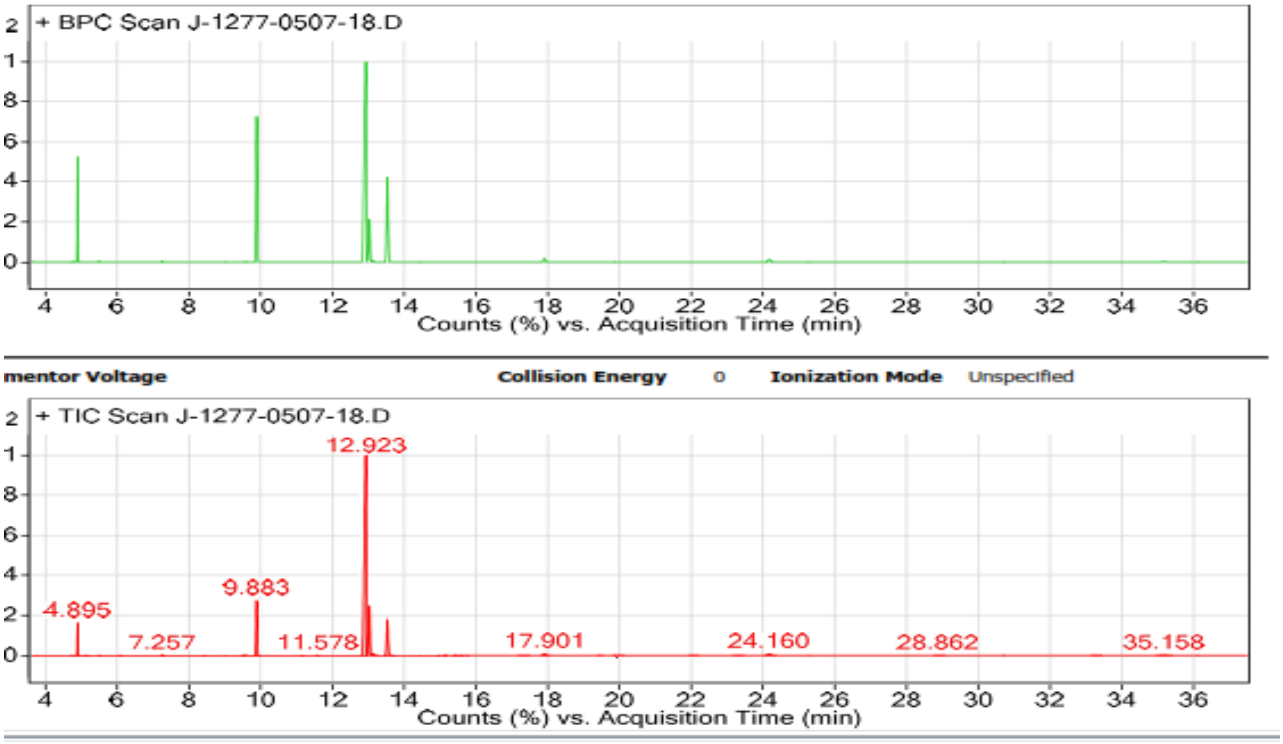

Figure 2: GC-MS Chromatogram of East wolega nigerseed sample

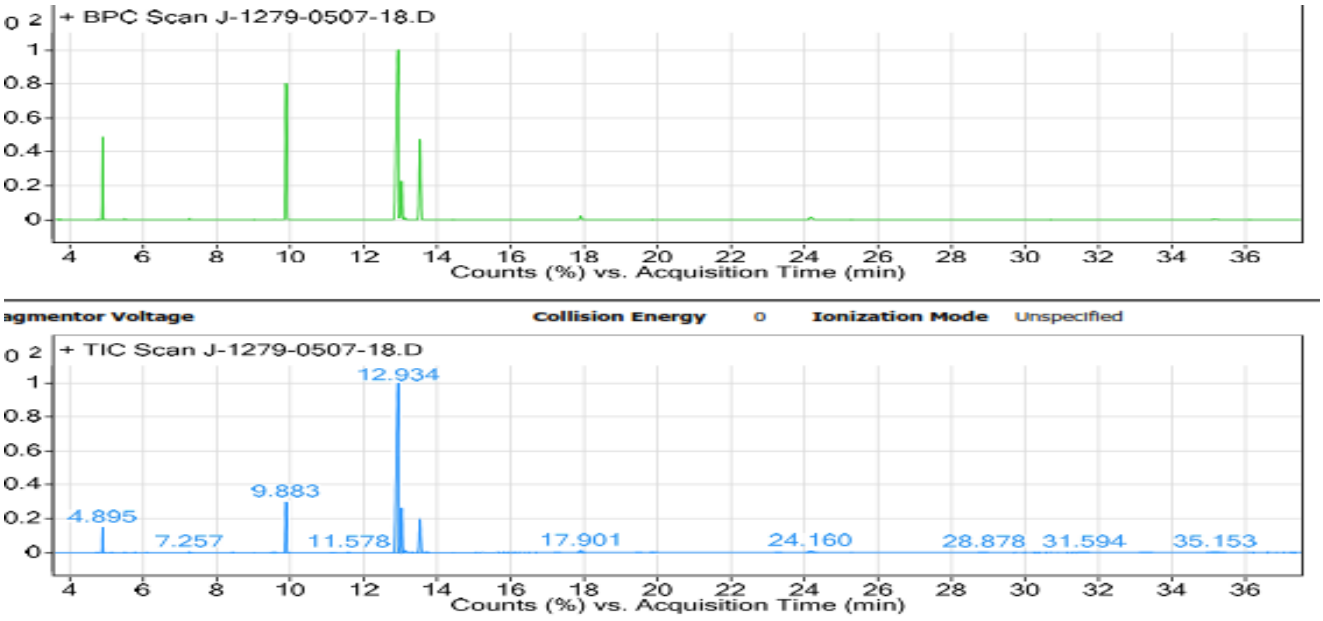

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Figure 3**:** GC-MS Chromatogram of Horo gudru wolega nigerseed sample

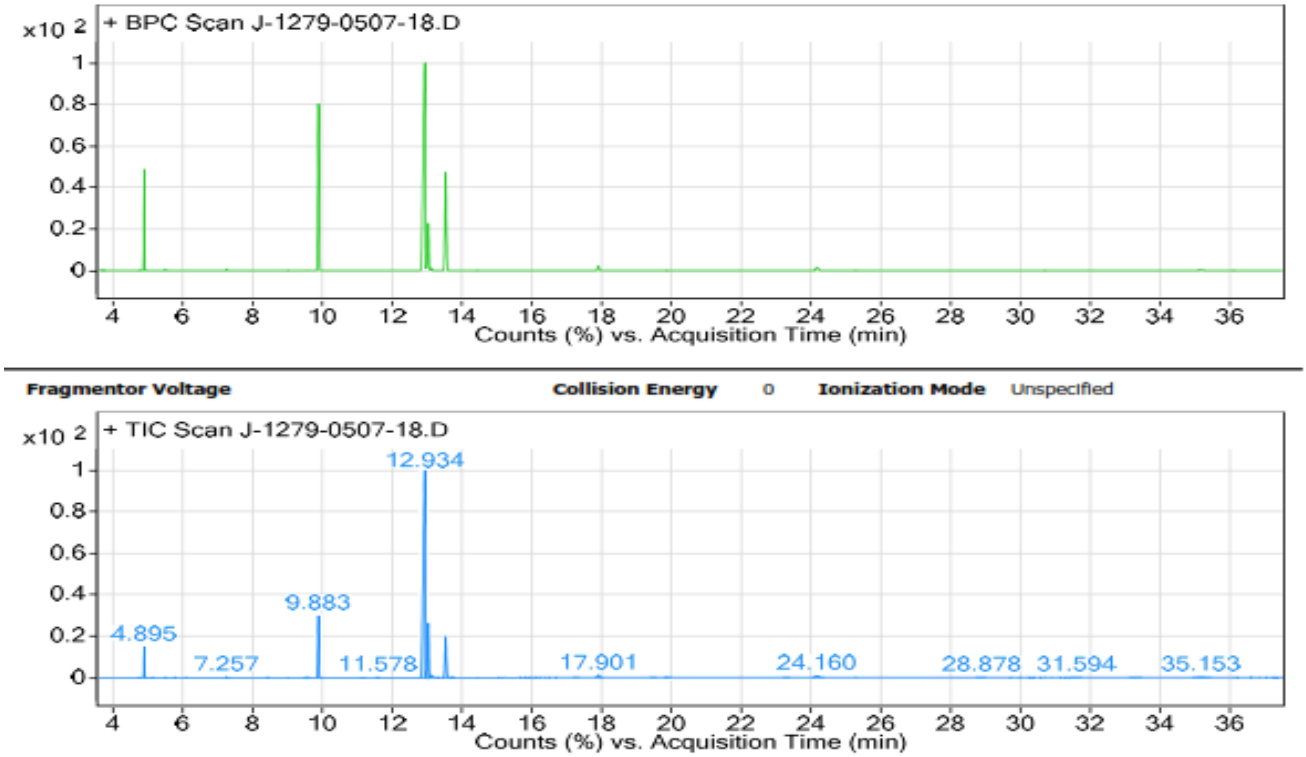

Figure 4: GC-MS Chromatogram of north Gondar nigerseed sample

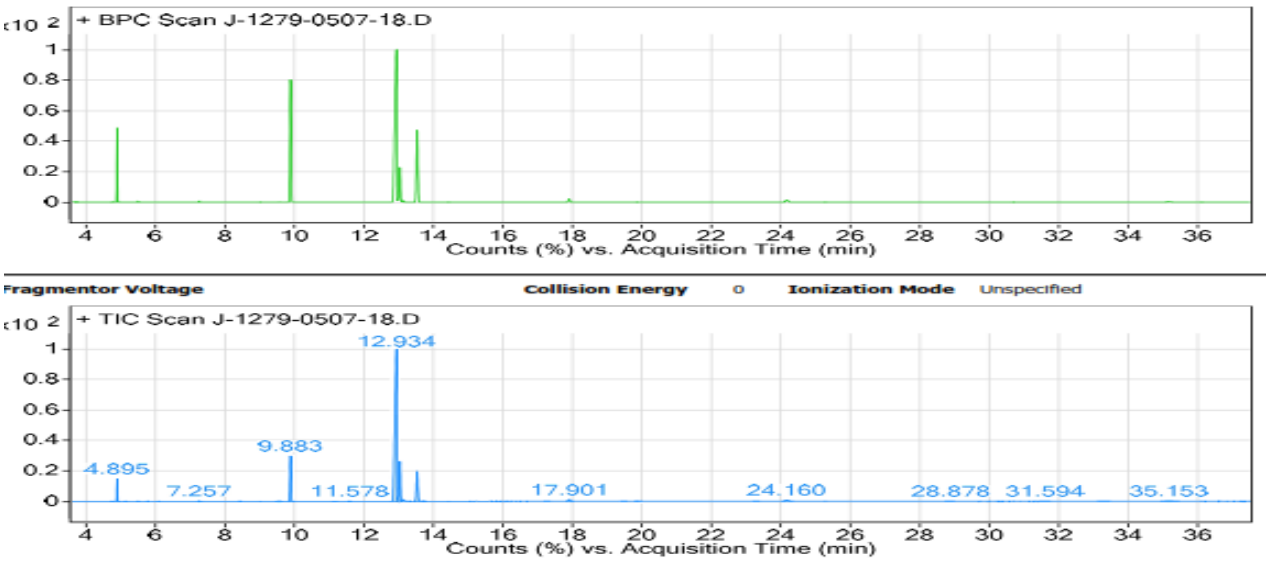

Figure 5: GC-MS Chromatogram of north Goojjam niger seed sample

Appendix-B

Table 1: Calibration curve for flavonoids and total phenolic content determination

Phenolic Calibration data

Tube	Gallic acid 0.1mg/mL (mL)	Gallic acid in µg	H_2O (mL)	Folin-Ciocalteau reagent (mL)	7% Ma_2CO_3 (mL)	Gallic acid in µg/mL	Absorbance at 760 nm
Blank	0.00		1.50	0.75	3.75	-	0.000
T1	0.06	6	1.44	0.75	3.75	1	0.056
T2	0.12	12	1.38	0.75	3.75	2	0.116
T3	0.18	18	1.32	0.75	3.75	3	0.181
T4	0.24	24	1.26	0.75	3.75	4	0.246
T5	0.30	30	1.20	0.75	3.75	5	0.306
T6	0.60	60	0.90	0.75	3.75	10	0.612

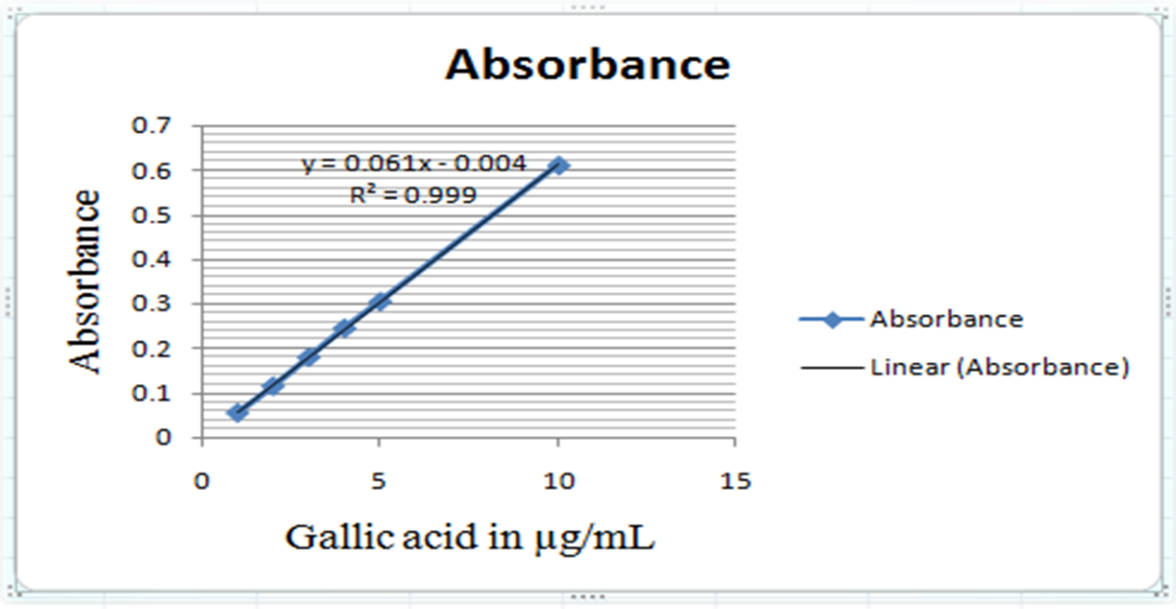

Figure 6: calibration curve of Gallic acid calibration curve

Table 2: Flavonoids Calibration data

Tube	V 1.5mg/mL of (Catechin) µL	V (H_2O) mL	V (7% $NaNO_2$) mL	V (10% $AlCl_3$) mL	V (NaOH) mL	Catechin in µg/mL	Absorbance at 510 nm
Blank	-	3.7	0.3	0.3	2	-	0.00
T1	25	3.675	0.3	0.3	2	0.595	0.023
T1	25	3.675	0.3	0.3	2	0.595	0.035
T1	25	3.675	0.3	0.3	2	0.595	0.028
T2	50	3.65	0.3	0.3	2	1.19	0.063
T2	50	3.65	0.3	0.3	2	1.19	0.07
T2	50	3.65	0.3	0.3	2	1.19	0.062
T3	100	3.6	0.3	0.3	2	2.38	0.120
T3	100	3.6	0.3	0.3	2	2.38	0.123
T3	100	3.6	0.3	0.3	2	2.38	0.124
T4	200	3.5	0.3	0.3	2	4.76	0.252
T4	200	3.5	0.3	0.3	2	4.76	0.256
T4	200	3.5	0.3	0.3	2	4.76	0.251
T5	400	3.3	0.3	0.3	2	9.52	0.506
T5	400	3.3	0.3	0.3	2	9.52	0.515
T5	400	3.3	0.3	0.3	2	9.52	0.510
T6	500	3.2	0.3	0.3	2	11.9	0.632
T6	500	3.2	0.3	0.3	2	11.9	0.641
T6	500	3.2	0.3	0.3	2	11.9	0.638
T7	600	3.1	0.3	0.3	2	14.28	0.791
T7	600	3.1	0.3	0.3	2	14.28	0.789
T7	600	3.1	0.3	0.3	2	14.28	0.782

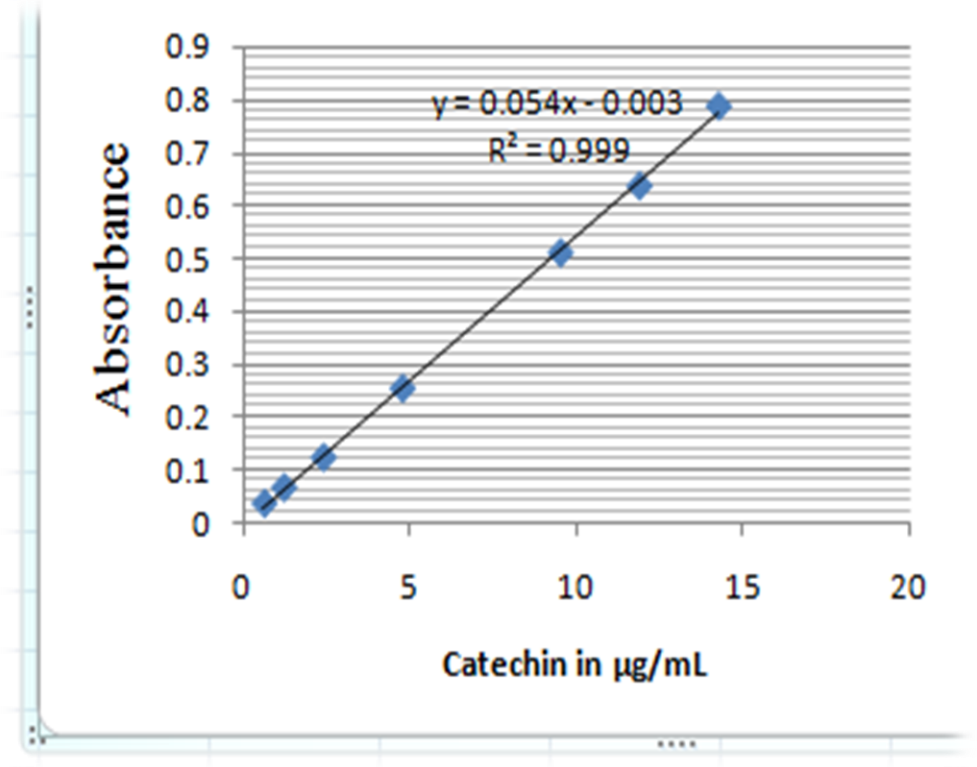

Figure 7: Calibration curve of Catechin standard solution for determination of TFC

Table 3: UV/VIS –Absorbance results of five Niger seed 100% methanol and 80% methanol extract A and B respectively for tolal phenolic determination

A. 100% methanol

Sample type	Measurement triplicate	V(Sample) mL	Sample in µg	Distilled water (mL)	F-C Reagent (mL)	7% Na2CO3 (mL)	Sample in mg/mL	Absorbance at 760nm
Reagent blank		-		1.5	0.75	3.75		0.000
WW	SB	0.1	4000	5.9	-	-	0.667	0.026
	T1	0.1	4000	1.4	0.75	3.75	0.667	0.329
	T2	0.1	4000	1.4	0.75	3.75	0.667	0.351
	T3	0.1	4000	1.4	0.75	3.75	0.667	0.323
EW	SB	0.1	4000	5.9	-	-	0.667	0.002
	T1	0.1	4000	1.4	0.75	3.75	0.667	0.393
	T2	0.1	4000	1.4	0.75	3.75	0.667	0.411
	T3	0.1	4000	1.4	0.75	3.75	0.667	0.406
HGW	SB	0.1	4000	5.9	-	-	0.667	0.004
	T1	0.1	4000	1.4	0.75	3.75	0.667	0.427
	T2	0.1	4000	1.4	0.75	3.75	0.667	0.456
	T3	0.1	4000	1.4	0.75	3.75	0.667	0.450
NG	SB	0.1	4000	5.9	-	-	0.667	0.012
	T1	0.1	4000	1.4	0.75	3.75	0.667	0.395
	T2	0.1	4000	1.4	0.75	3.75	0.667	0.401
	T3	0.1	4000	1.4	0.75	3.75	0.667	0.395
EG	SB	0.1	4000	5.9	-	-	0.667	0.006
	T1	0.1	4000	1.4	0.75	3.75	0.667	0.444
	T2	0.1	4000	1.4	0.75	3.75	0.667	0.413
	T3	0.1	4000	1.4	0.75	3.75	0.667	0.404

B. 80% methanol extract

Sample type	Triplicate	Sample volume (mL)	Sample in µg	Distilled water (mL)	F-C Reagent (mL)	7% Na_2CO_3 (mL)	Absorbance at 760nm	Blank subtract
Reagent blank			-	1.5	0.75	3.75	0.00	
WW	SB	0.1	4000	5.9	-		0.022	
	T1	0.1	4000	1.4	0.75	3.75	0.450	0.428
	T2	0.1	4000	1.4	0.75	3.75	0.454	0.432
	T3	0.1	4000	1.4	0.75	3.75	0.457	0.457
EW	SB	0.1	4000	5.9	-	-	0.020	
	T1	0.1	4000	1.4	0.75	3.75	0.477	0.457
	T2	0.1	4000	1.4	0.75	3.75	0.489	0.469
	T3	0.1	4000	1.4	0.75	3.75	0.484	0.464
SH	SB	0.1	4000	5.9	-	-	0.022	
	T1	0.1	4000	1.4	0.75	3.75	0.472	0.450
	T2	0.1	4000	1.4	0.75	3.75	0.476	0.454
	T3	0.1	4000	1.4	0.75	3.75	0.478	0.456
NG	SB	0.1	4000	5.9	-	-	0.022	
	T1	0.1	4000	1.4	0.75	3.75	0.496	0.474
	T2	0.1	4000	1.4	0.75	3.75	0.498	0.476
	T3	0.1	4000	1.4	0.75	3.75	0.498	0.476
EG	SB	0.1	4000	5.9	-	-	0.023	
	T1	0.1	4000	1.4	0.75	3.75	0.490	0.468
	T2	0.1	4000	1.4	0.75	3.75	0.490	0.468
	T3	0.1	4000	1.4	0.75	3.75	0.487	0.465

Table 4: UV/VIS –Absorbance results of five Niger seed 100% methanol and 80% methanol extract A and B respectively for total flavonoids determination

A. 80 % methanol

Sampling site	Sample volume mL	Trail	Water mL	5 % $NaNO_2$ (mL)	10% $AlCl_3$ (mL)	4% NaOH (mL)	Sample in mg/mL	Absorbance at 510nm	Absorbance blank subtracted
SB	0.2		6.1					0.008	
W/Wolega	0.2		3.5	0.3	0.3	2	1.27	0.396	0.388
	0.2		3.5	0.3	0.3	2	1.27	0.395	0.387
	0.2		3.5	0.3	0.3	2	1.27	0.385	0.377
E/Woleg	0.2		3.5	0.3	0.3	2	1.27	0.439	0.437
			3.5	0.3	0.3	2	1.27	0.441	0.439
			3.5	0.3	0.3	2	1.27	0.441	0.439
HG/Wolega			3.5	0.3	0.3	2	1.27	0.403	0.401
			3.5	0..3	0.3	2	1.27	0.43	0.428
			3.5	0.3	0.3	2	1.27	0.405	0.404
N/Gondar			3.5	0.3	0.3	2	1.27	0.451	0.45
			3.5	0.3	0.3		1.27	0.458	0.457
			3.5	0.3	0.3	2	1.27	0.457	0.456
E/Gojjam			3.5	0.3	0.3	2	1.27	0.37	0.368
			3.5	0.3	0.3	2	1.27	0.37	0.368
			3.5	0.3	0.3	2	1.27	0.373	0.371

B. 100% methanol

Sampling site	Sample volume mL	Trail	Water mL	5% $NaNO_2$ mL	10% $AlCl_3$ mL	4% NaOH mL	Sample in mg/mL	Absorbance at 510nm	Absorbance blank subtructed
SB	0.2		6.1					0.065	
W/Wolega	0.2	T1	3.5	0.3	0.3	2	1.27	0.412	0.347
	0.2	T2	3.5	0.3	0.3	2	1.27	0.403	0.338
	0.2	T3	3.5	0.3	0.3	2	1.27	0.397	0.332
E/Woleg	0.2	T1	3.5	0.3	0.3	2	1.27	0.43	0.399
	0.2	T2	3.5	0.3	0.3	2	1.27	0.43	0.399
	0.2	T3	3.5	0.3	0.3	2	1.27	0.429	0.398
HG/Wolega	0.2	T1	3.5	0.3	0.3	2	1.27	0.466	0.43
	0.2	T3	3.5	0.3	0.3	2	1.27	0.463	0.427
	0.2		3.5	0.3	0.3	2	1.27	0.465	0.429
N/Gondar	0.2	T2	3.5	0.3	0.3	2	1.27	0.351	0.339
	0.2	T3	3.5	0.3	0.3	2	1.27	0.347	0.335
	0.2		3.5	0.3	0.3	2	1.27	0.351	0.339
E/Gojjam	0.2	T2	3.5	0.3	0.3	2	1.27	0.456	0.443
	0.2	T3	3.5	0.3	0.3	2	1.27	0.485	0.472
	0.2		3.5	0.3	0.3	2	1.27	0.477	0.464

Appendix C

Table 5 : Ascorbic Acid Calibration Data for the determination of Antioxidant Activity

Tube (Replicate)	Ascorbic Acid 0.1mg/mL (µL)	Gallic acid in µg	DPPH (mL)	80% aq. Methanol (mL)	Gallic acid in µg/mL	Absorbance at 517nm
Blank	-	-	-	5	-	0.000
Control	-	-	2	3	-	0.614
T1	5	0.5	2	2.995	0.1	0.609
T2	5	0.5	2	2.995	0.1	0.608
T3	5	0.5	2	2.995	0.1	0.608
T1	10	1	2	2.990	0.2	0.599
T2	10	1	2	2.990	0.2	0.597
T3	10	1	2	2.990	0.2	0.600
T1	20	2	2	2.980	0.4	0.562
T2	20	2	2	2.980	0.4	0.560
T3	20	2	2	2.980	0.4	0.559
T1	40	4	2	2.960	0.8	0.513
T2	40	4	2	2.960	0.8	0.511
T3	40	4	2	2.960	0.8	0.510
T1	50	5	2	2.950	1	0.42
T2	50	5	2	2.950	1	0.45
T3	50	5	2	2.950	1	0.41
T1	75	7.5	2	2.925	1.5	0.393
T2	75	7.5	2	2.925	1.5	0.393
T3	75	7.5	2	2.925	1.5	0.391
T1	100	10	2	2.900	2	0.315
T2	100	10	2	2.900	2	0.313
T3	100	10	2	2.900	2	0.316
T1	200	20	2	2.800		0.276
T2	200	20	2	2.800		0.273
T3	200	20	2	2.800		0.281
T1	600	60	2	2.400	12	0.169
T2	600	60	2	2.400	12	0.171
T3	600	60	2	2.400	12	0.167

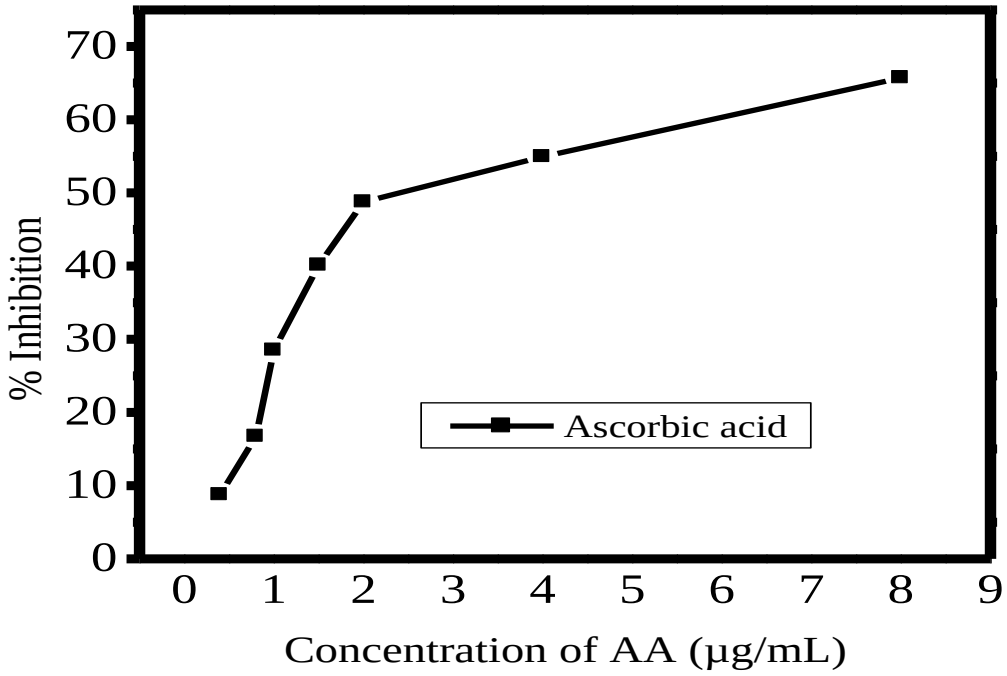

Figure 8: Graph of AA standard solution for determination of AOA

Table 6: Raw data for antioxidant activity evaluation by UV-Spectrophotometer at 517nm 80% methanol and 100% methanol extract A and B respectively

A. 80% methanol extract data

Sample site	Triplicates	V(sample) µl	Sample in µg/mL	V(DPPH) mL	V(80%MeOH) mL	Abs. at 517nm	%IA
Control				2	3	0.475	
West Wollega	T1	5	40	2	2.995	0.41	13.684
West Wollega	T2	5	40	2	2.995	0.41	13.684
West Wollega	T3	5	40	2	2.995	0.406	14.526
West Wollega	T1	10	80	2	2.99	0.362	23.789
West Wollega	T2	10	80	2	2.99	0.361	24
West Wollega	T3	10	80	2	2.99	0.344	27.579
West Wollega	T1	20	160	2	2.98	0.235	50.526
West Wollega	T2	20	160	2	2.98	0.248	47.789
West Wollega	T3	20	160	2	2.98	0.245	48.421
West Wollega	T1	40	320	2	2.96	0.062	86.948
West Wollega	T2	40	320	2	2.96	0.062	86.947
West Wollega	T3	40	320	2	2.96	0.06	87.368
West Wollega	T1	50	400	2	2.95	0.031	93.473
West Wollega	T2	50	400	2	2.95	0.032	93.263
West Wollega	T3	50	400	2	2.95	0.03	93.684
West Wollega	T1	75	600	2	2.925	0.03	93.684
West Wollega	T2	75	600	2	2.925	0.031	93.474
West Wollega	T3	75	600	2	2.925	0.03	93.684
West Wollega	T1	100	800	2	2.9	0.026	94.526
West Wollega	T2	100	800	2	2.9	0.025	94.737
West Wollega	T3	100	800	2	2.9	0.024	94.947

Sample site	Triplicates	V(sample) µl	V(DPPH) mL	V(80%MeOH) mL	Abs. at 517nm	%IA
east Wollega	T1	5	2	2.995	0.417	12.211
east Wollega	T2	5	2	2.995	0.413	13.053
east Wollega	T3	5	2	2.995	0.411	13.474
east Wollega	T1	10	2	2.99	0.376	20.842
east Wollega	T2	10	2	2.99	0.372	21.684
east Wollega	T3	10	2	2.99	0.36	24.211
east Wollega	T1	20	2	2.98	0.28	41.051
east Wollega	T2	20	2	2.98	0.276	41.895
east Wollega	T3	20	2	2.98	0.276	41.895
east Wollega	T1	40	2	2.96	0.099	79.157
east Wollega	T2	40	2	2.96	0.101	78.742
east Wollega	T3	40	2	2.96	0.105	77.895
east Wollega	T1	50	2	2.95	0.028	94.105
east Wollega	T2	50	2	2.95	0.03	93.684
east Wollega	T3	50	2	2.95	0.032	93.263
east Wollega	T1	75	2	2.925	0.024	94.947
east Wollega	T2	75	2	2.925	0.028	94.105
east Wollega	T3	75	2	2.925	0.032	93.263
east Wollega	T1	100	2	2.9	0.029	93.895
east Wollega	T2	100	2	2.9	0.026	94.526
east Wollega	T3	100	2	2.9	0.033	93.053

Sample site	Trails	V(sample)µl	V(DPPH) mL	V(80%MeOH) mL	Abs. at 517nm	%IA
HG Wollega	T1	5	2	2.995	0.382	19.579
HG Wollega	T2	5	2	2.995	0.378	20.421
HG Wollega	T3	5	2	2.995	0.379	20.210
HG Wollega	T1	10	2	2.99	0.32	32.632
HG Wollega	T2	10	2	2.99	0.319	32.842
HG Wollega	T3	10	2	2.99	0.315	33.684
HG Wollega	T1	20	2	2.98	0.257	45.895
HG Wollega	T2	20	2	2.98	0.258	45.684
HG Wollega	T3	20	2	2.98	0.254	46.526
HG Wollega	T1	40	2	2.96	0.03	93.684
HG Wollega	T2	40	2	2.96	0.027	94.316
HG Wollega	T3	40	2	2.96	0.028	94.105
HG Wollega	T1	50	2	2.95	0.031	93.474
HG Wollega	T2	50	2	2.95	0.033	93.053
HG Wollega	T3	50	2	2.95	0.033	93.053
HG Wollega	T1	75	2	2.925	0.026	94.526
HG Wollega	T2	75	2	2.925	0.021	95.579
HG Wollega	T3	75	2	2.925	0.028	94.105
HG Wollega	T1	100	2	2.9	0.025	94.737
HG Wollega	T2	100	2	2.9	0.031	93.474
HG Wollega	T3	100	2	2.9	0.032	93.263

Sample site	Triplicates	V(sample)µl	V(DPPH) mL	V(80%MeOH) mL	Abs. at 517nm	%IA
North Gonder	T1	5	2	2.995	0.411	13.474
North Gonder	T2	5	2	2.995	0.406	14.526
North Gonder	T3	5	2	2.995	0.41	13.684
North Gonder	T1	10	2	2.99	0.330	30.52
North Gonder	T2	10	2	2.99	0.350	26.315
North Gonder	T3	10	2	2.99	0.360	24.210
North Gonder	T1	20	2	2.98	0.268	43.579
North Gonder	T2	20	2	2.98	0.266	44
North Gonder	T3	20	2	2.98	0.269	43.368
North Gonder	T1	40	2	2.96	0.082	82.7368
North Gonder	T2	40	2	2.96	0.077	83.789
North Gonder	T3	40	2	2.96	0.098	79.368
North Gonder	T1	50	2	2.95	0.029	93.895
North Gonder	T2	50	2	2.95	0.025	94.737
North Gonder	T3	50	2	2.95	0.033	93.053
North Gonder	T1	75	2	2.925	0.028	94.105
North Gonder	T2	75	2	2.925	0.022	95.368
North Gonder	T3	75	2	2.925	0.028	94.105
North Gonder	T1	100	2	2.9	0.026	94.526
North Gonder	T2	100	2	2.9	0.021	95.575
North Gonder	T3	100	2	2.9	0.032	

Sample site	Triplicates	V(sample) µl	V(DPPH) mL	V(80%MeOH) mL	Abs. at 517nm	%IA
East Gojjam	T1	5	2	2.995	0.417	12.211
East Gojjam	T2	5	2	2.995	0.414	12.842
East Gojjam	T3	5	2	2.995	0.393	17.263
East Gojjam	T1	10	2	2.99	0.367	22.737
East Gojjam	T2	10	2	2.99	0.362	23.789
East Gojjam	T3	10	2	2.99	0.363	23.579
East Gojjam	T1	20	2	2.98	0.172	63.789
East Gojjam	T2	20	2	2.98	0.166	65.053
East Gojjam	T3	20	2	2.98	0.168	64.632
East Gojjam	T1	40	2	2.96	0.04	91.579
East Gojjam	T2	40	2	2.96	0.036	92.421
East Gojjam	T3	40	2	2.96	0.051	89.263
East Gojjam	T1	50	2	2.95	0.032	93.263
East Gojjam	T2	50	2	2.95	0.026	94.526
East Gojjam	T3	50	2	2.95	0.035	92.632
East Gojjam	T1	75	2	2.925	0.028	94.105
East Gojjam	T2	75	2	2.925	0.026	94.526
East Gojjam	T3	75	2	2.925	0.029	93.895
East Gojjam	T1	100	2	2.9	0.029	93.895
East Gojjam	T2	100	2	2.9	0.024	94.947
East Gojjam	T3	100	2	2.9	0.025	94.737

A. UV/VIS raw data of 100% methanol extract

Sample site	Trail	(V)Samples in μL	DPPH(mL)	100%MeOH(mL)	Absorbance at 517nm	%IA
Control		--	2	3	0.531	
West wolega	T1	5	2	2.995	0.500	5.838
	T2	5	2	2.995	0.503	5.273
	T3	5	2	2.995	0.505	4.896
	T1	10	2	2.990	0.473	10.923
	T2	10	2	2.990	0.475	10.546
	T3	10	2	2.990	0.474	10.734
	T1	20	2	2.980	0.434	18.267
	T2	20	2	2.980	0.435	18.079
	T3	20	2	2.980	0.411	22.599
	T1	40	2	2.960	0.282	46.893
	T2	40	2	2.960	0.282	46.893
	T3	40	2	2.960	0.280	47.269
	T1	50	2	2.950	0.176	66.855
	T2	50	2	2.950	0.172	67.608
	T3	50	2	2.950	0.173	67.420
	T1	75	2	2.925	0.054	89.831
	T2	75	2	2.925	0.049	90.772
	T3	75	2	2.925	0.052	90.207
	T1	100	2	2.900	0.037	93.032
	T2	100	2	2.900	0.032	93.974
	T3	100	2	2.900	0.037	93.032

Sample site	Trail	V(Samples) In (mL)	V(DPPH) In (mL)	V(100%MeOH) In (mL)	Absorbance at 517nm	%IA
Control			2	3	0.531	
East Wolega	T1	5	2	2.995	0.528	
	T2	5	2	2.995	0.526	0.942
	T3	5	2	2.995	0.524	0.318
	T1	10	2	2.990	0.493	7.156
	T2	10	2	2.990	0.495	6.780
	T3	10	2	2.990	0.493	7.156
	T1	20	2	2.980	0.447	15.820
	T2	20	2	2.980	0.443	16.573
	T3	20	2	2.980	0.444	16.384
	T1	40	2	2.960	0.316	40.490
	T2	40	2	2.960	0.317	40.301
	T3	40	2	2.960	0.321	39.548
	T1	50	2	2.950	0.246	53.672
	T2	50	2	2.950	0.240	54.802
	T3	50	2	2.950	0.245	53.861
	T1	75	2	2.925	0.137	74.199
	T2	75	2	2.925	0.132	75.141
	T3	75	2	2.925	0.134	74.765
	T1	100	2	2.900	0.059	88.889
	T2	100	2	2.900	0.060	88.701
	T3	100	2	2.900	0.057	89.265

Sample site	Trail	V (Samples) µL	DPPH(mL)	100%MeOH(mL)	Absorbance at 517nm	%IA
Control	T1		2	3	0.531	
HG/Wolega	T2	5	2	2.995	0.506	4.708
	T3	5	2	2.995	0.503	5.273
	T1	5	2	2.995	0.523	6.247
	T2	10	2	2.990	0.482	9.228
	T3	10	2	2.990	0.479	9.793
	T1	10	2	2.990	0.486	8.475
	T2	20	2	2.980	0.402	24.294
	T3	20	2	2.980	0.404	23.917
	T1	20	2	2.980	0.401	24.482
	T2	40	2	2.960	0.183	65.537
	T3	40	2	2.960	0.176	66.855
	T1	40	2	2.960	0.178	66.478
	T2	50	2	2.950	0.110	79.284
	T3	50	2	2.950	0.109	79.470
	T1	50	2	2.950	0.112	77.401
	T2	75	2	2.925	0.052	90.207
	T3	75	2	2.925	0.053	90.020
	T1	75	2	2.925	0.053	90.020
	T2	100	2	2.900	0.044	91.714
	T3	100	2	2.900	0.044	91.714
	T1	100	2	2.900	0.037	93.032

Sample site	Trail	(V) Samples in µL	DPPH(mL)	100%MeOH(mL)	Absorbance at 517nm	%IA
Control			2	3	0.531	
North Gonder	T1	5	2	2.995	0.527	0.753
	T2	5	2	2.995	0.522	1.695
	T3	5	2	2.995	0.523	1.507
	T1	10	2	2.990	0.468	11.864
	T2	10	2	2.990	0.488	8.098
	T3	10	2	2.990	0.487	8.286
	T1	20	2	2.980	0.431	18.832
	T2	20	2	2.980	0.426	19.774
	T3	20	2	2.980	0.431	18.832
	T1	40	2	2.960	0.275	48.211
	T2	40	2	2.960	0.274	48.399
	T3	40	2	2.960	0.270	49.153
	T1	50	2	2.950	0.207	61.017
	T2	50	2	2.950	0.200	62.335
	T3	50	2	2.950	0.199	62.524
	T1	75	2	2.925	0.051	90.395
	T2	75	2	2.925	0.0522	90.169
	T3	75	2	2.925	0.050	90.584
	T1	100	2	2.900	0.044	91.714
	T2	100	2	2.900	0.038	92.843
	T3	100	2	2.900	0.042	92.090

Sample site	Trail	(V) Samples in µL	DPPH(mL)	100%MeOH(mL)	Absorbance at 517nm	%IA
Control			2	3	531	
East Gojjam	T1	5	2	2.995	0.510	3.955
	T2	5	2	2.995	0.516	2.825
	T3	5	2	2.995	0.520	2.072
	T1	10	2	2.990	0.477	10.169
	T2	10	2	2.990	0.474	10.734
	T3	10	2	2.990	0.470	11.488
	T1	20	2	2.980	0.382	28.060
	T2	20	2	2.980	0.376	29.190
	T3	20	2	2.980	0.398	25.047
	T1	40	2	2.960	0.246	53.672
	T2	40	2	2.960	0.240	54.802
	T3	40	2	2.960	0.238	55.179
	T1	50	2	2.950	0.168	68.362
	T2	50	2	2.950	0.161	69.680
	T3	50	2	2.950	0.151	71.563
	T1	75	2	2.925	0.047	91.149
	T2	75	2	2.925	0.048	90.960
	T3	75	2	2.925	0.048	90.960
	T1	100	2	2.900	0.043	91.902
	T2	100	2	2.900	0.044	91.714
	T3	100	2	2.900	0.042	92.090

Table 7: Raw data of IC50 values extract µg/mL 100%Methanol and 80% aqueous methanol extract

Samplig site	Triplicates	IC50 values 100%Methanol extract µg/mL			IC50 values 80% aqueous methanol extract in µg/mL		
			Average	STD		Average	STD
W/Wolega	S1	333.250	332.33	1.59	157.56	164.6733	6.702181
	S2	333.250			170.87		
	S3	330.50			165.59		
E/Wolega	S1	378.439	377.52	1.58	189.66	189.7433	0.144338
	S2	375.695			189.66		
	S3	378.439			189.91		
HG/Wolega	S1	255.967	257.80	1.59	176.36	175.4433	1.587713
	S2	258.713			176.36		
	S3	258.713			173.61		
N/Gonder	S1	333.251	330.58	2.64	186.91	187.8267	1.587713
	S2	330.506			186.91		
	S3	327.972			189.66		
E/Gojjam	S1	298.621	295.10	3.05	133.70	131.8733	1.58194
	S2	293.342			130.96		
	S3	293.342			130.96		

Appendixes –D

Multiple composition data using SPSS soft ware

Table 8: One way ANOVA test value for TFC content with in five Niger G. abyssinica samples of 100% extract

Descriptive

TFC								
					95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
W/wolega	3	5.0015	.11009	.06356	4.7280	5.2749	4.90	5.12
E/wolega	3	5.8715	.00842	.00486	5.8506	5.8924	5.86	5.88
HG/wolega	3	6.3089	.02227	.01286	6.2536	6.3643	6.28	6.33
N/gonder	3	4.9820	.03367	.01944	4.8984	5.0657	4.94	5.00
E/gojjam	3	6.7610	.21840	.12609	6.2184	7.3035	6.52	6.94
Total	15	5.7850	.73692	.19027	5.3769	6.1931	4.90	6.94

ANOVA

TFC					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.480	4	1.870	151.980	.000
Within Groups	.123	10	.012		
Total	7.603	14			

Multiple Comparisons

Dependent Variable: TFC

	(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	W/wolega	E/wolega	-.79226*	.08824	.000	-1.0827	-.5019
		HG/wolega	-.39370*	.08824	.008	-.6841	-.1033
		N/gonder	-1.02557*	.08824	.000	-1.3160	-.7352
		E/gojjam	.21872	.08824	.172	-.0717	.5091
	E/wolega	W/wolega	.79226*	.08824	.000	.5019	1.0827
		HG/wolega	.39856*	.08824	.008	.1081	.6890
		N/gonder	-.23330	.08824	.135	-.5237	.0571
		E/gojjam	1.01098*	.08824	.000	.7206	1.3014
	HG/wolega	W/wolega	.39370*	.08824	.008	.1033	.6841
		E/wolega	-.39856*	.08824	.008	-.6890	-.1081
		N/gonder	-.63187*	.08824	.000	-.9223	-.3415
		E/gojjam	.61242*	.08824	.000	.3220	.9028
	N/gonder	W/wolega	1.02557*	.08824	.000	.7352	1.3160
		E/wolega	.23330	.08824	.135	-.0571	.5237
		HG/wolega	.63187*	.08824	.000	.3415	.9223
		E/gojjam	1.24429*	.08824	.000	.9539	1.5347
	E/gojjam	W/wolega	-.21872	.08824	.172	-.5091	.0717
		E/wolega	-1.01098*	.08824	.000	-1.3014	-.7206
		HG/wolega	-.61242*	.08824	.000	-.9028	-.3220
		N/gonder	-1.24429*	.08824	.000	-1.5347	-.9539
LSD	W/wolega	E/wolega	-.79226*	.08824	.000	-.9889	-.5956
		HG/wolega	-.39370*	.08824	.001	-.5903	-.1971
		N/gonder	-1.02557*	.08824	.000	-1.2222	-.8290
		E/gojjam	.21872*	.08824	.033	.0221	.4153
	E/wolega	W/wolega	.79226*	.08824	.000	.5956	.9889
		HG/wolega	.39856*	.08824	.001	.2019	.5952

		N/gonder	-.23330*	.08824	.025	-.4299	-.0367
		E/gojjam	1.01098*	.08824	.000	.8144	1.2076
	HG/wolega	W/wolega	.39370*	.08824	.001	.1971	.5903
		E/wolega	-.39856*	.08824	.001	-.5952	-.2019
		N/gonder	-.63187*	.08824	.000	-.8285	-.4353
		E/gojjam	.61242*	.08824	.000	.4158	.8090
	N/gonder	W/wolega	1.02557*	.08824	.000	.8290	1.2222
		E/wolega	.23330*	.08824	.025	.0367	.4299
		HG/wolega	.63187*	.08824	.000	.4353	.8285
		E/gojjam	1.24429*	.08824	.000	1.0477	1.4409
	E/gojjam	W/wolega	-.21872*	.08824	.033	-.4153	-.0221
		E/wolega	-1.01098*	.08824	.000	-1.2076	-.8144
		HG/wolega	-.61242*	.08824	.000	-.8090	-.4158
		N/gonder	-1.24429*	.08824	.000	-1.4409	-1.0477

*. The mean difference is significant at the 0.05 level.

Table 9: Duncan multiple comparisons test for TFC within five Niger G. abyssinica samples of 100% extract.

TFC

	Sample	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	N/gonder	3	4.9820			
	W/wolega	3	5.0015			
	E/wolega	3		5.8715		
	HG/wolega	3			6.3089	
	E/gojjam	3				6.7610
	Sig.		.999	1.000	1.000	1.000
Duncan[a]	N/gonder	3	4.9820			
	W/wolega	3	5.0015			
	E/wolega	3		5.8715		
	HG/wolega	3			6.3089	
	E/gojjam	3				6.7610
	Sig.		.834	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table 10: One way ANOVA test value for TFC content with in five Niger G. abyssinica samples of 80 % extract

Descriptives

TFC

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
W/wolega	3	5.6430	.08870	.05121	5.4227	5.8634	5.54	5.70
E/wolega	3	6.4353	.01684	.00972	6.3935	6.4771	6.42	6.45
HG/wolega	3	6.0367	.21579	.12458	5.5007	6.5728	5.89	6.28
N/gonder	3	6.6686	.05520	.03187	6.5315	6.8057	6.61	6.71

E/gojjam	3	5.4243	.02526	.01458	5.3616	5.4871	5.41	5.45
Total	15	6.0416	.49142	.12688	5.7695	6.3137	5.41	6.71

ANOVA

TFC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.264	4	.816	69.867	.000
Within Groups	.117	10	.012		
Total	3.381	14			

Multiple Comparisons

Dependent Variable:TFC

			Mean Difference			95% Confidence Interval	
	(I) Sample	(J) Sample	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Tukey HSD	W/wolega	E/wolega	-.79226*	.08824	.000	-1.0827	-.5019
		HG/wolega	-.39370*	.08824	.008	-.6841	-.1033
		N/gonder	-1.02557*	.08824	.000	-1.3160	-.7352
		E/gojjam	.21872	.08824	.172	-.0717	.5091
	E/wolega	W/wolega	.79226*	.08824	.000	.5019	1.0827
		HG/wolega	.39856*	.08824	.008	.1081	.6890
		N/gonder	-.23330	.08824	.135	-.5237	.0571
		E/gojjam	1.01098*	.08824	.000	.7206	1.3014
	HG/wolega	W/wolega	.39370*	.08824	.008	.1033	.6841
		E/wolega	-.39856*	.08824	.008	-.6890	-.1081
		N/gonder	-.63187*	.08824	.000	-.9223	-.3415
		E/gojjam	.61242*	.08824	.000	.3220	.9028
	N/gonder	W/wolega	1.02557*	.08824	.000	.7352	1.3160
		E/wolega	.23330	.08824	.135	-.0571	.5237
		HG/wolega	.63187*	.08824	.000	.3415	.9223
		E/gojjam	1.24429*	.08824	.000	.9539	1.5347

	E/gojjam	W/wolega	-.21872	.08824	.172	-.5091	.0717
		E/wolega	-1.01098*	.08824	.000	-1.3014	-.7206
		HG/wolega	-.61242*	.08824	.000	-.9028	-.3220
		N/gonder	-1.24429*	.08824	.000	-1.5347	-.9539
LSD	W/wolega	E/wolega	-.79226*	.08824	.000	-.9889	-.5956
		HG/wolega	-.39370*	.08824	.001	-.5903	-.1971
		N/gonder	-1.02557*	.08824	.000	-1.2222	-.8290
		E/gojjam	.21872*	.08824	.033	.0221	.4153
	E/wolega	W/wolega	.79226*	.08824	.000	.5956	.9889
		HG/wolega	.39856*	.08824	.001	.2019	.5952
		N/gonder	-.23330*	.08824	.025	-.4299	-.0367
		E/gojjam	1.01098*	.08824	.000	.8144	1.2076
	HG/wolega	W/wolega	.39370*	.08824	.001	.1971	.5903
		E/wolega	-.39856*	.08824	.001	-.5952	-.2019
		N/gonder	-.63187*	.08824	.000	-.8285	-.4353
		E/gojjam	.61242*	.08824	.000	.4158	.8090
	N/gonder	W/wolega	1.02557*	.08824	.000	.8290	1.2222
		E/wolega	.23330*	.08824	.025	.0367	.4299
		HG/wolega	.63187*	.08824	.000	.4353	.8285
		E/gojjam	1.24429*	.08824	.000	1.0477	1.4409
	E/gojjam	W/wolega	-.21872*	.08824	.033	-.4153	-.0221
		E/wolega	-1.01098*	.08824	.000	-1.2076	-.8144
		HG/wolega	-.61242*	.08824	.000	-.8090	-.4158
		N/gonder	-1.24429*	.08824	.000	-1.4409	-1.0477

*. The mean difference is significant at the 0.05 level.

Table 11: Duncan multiple comparisons test for TFC within five Niger G. abyssinica samples of 80% extrac

TFC

	Sample	N	Subset for alpha = 0.05				
			1	2	3	4	5
Tukey HSD[a]	E/gojjam	3	5.4243				
	W/wolega	3	5.6430				
	HG/wolega	3		6.0367			
	E/wolega	3			6.4353		
	N/gonder	3			6.6686		
	Sig.		.172	1.000	.135		
Duncan[a]	E/gojjam	3	5.4243				
	W/wolega	3		5.6430			
	HG/wolega	3			6.0367		
	E/wolega	3				6.4353	
	N/gonder	3					6.6686
	Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table 12: One way ANOVA test value for TPC content with in five Niger G. abyssinica samples of 80 % extract

Descriptive

TPC

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
W/wolega	3	10.8880	.38627	.22301	9.9284	11.8476	10.62	11.33
E/wolega	3	11.4861	.14815	.08553	11.1180	11.8541	11.33	11.63
HG/wolega	3	11.2403	.07509	.04335	11.0538	11.4268	11.16	11.31
N/gonder	3	11.7810	.02838	.01639	11.7105	11.8515	11.75	11.80
E/gojjam	3	11.5762	.04257	.02458	11.4704	11.6819	11.53	11.60
Total	15	11.3943	.35578	.09186	11.1973	11.5913	10.62	11.80

ANOVA

TPC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.413	4	.353	9.847	.002
Within Groups	.359	10	.036		
Total	1.772	14			

Multiple Comparisons

Dependent Variable:TPC

	(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	W/wolega	E/wolega	-.59806*	.15466	.020	-1.1071	-.0890
		HG/wolega	-.35228	.15466	.229	-.8613	.1567
		N/gonder	-.89300*	.15466	.001	-1.4020	-.3840

		E/gojjam	-.68818*	.15466	.008	-1.1972	-.1792
	E/wolega	W/wolega	.59806*	.15466	.020	.0890	1.1071
		HG/wolega	.24578	.15466	.535	-.2632	.7548
		N/gonder	-.29493	.15466	.373	-.8039	.2141
		E/gojjam	-.09012	.15466	.975	-.5991	.4189
	HG/wolega	W/wolega	.35228	.15466	.229	-.1567	.8613
		E/wolega	-.24578	.15466	.535	-.7548	.2632
		N/gonder	-.54071*	.15466	.036	-1.0497	-.0317
		E/gojjam	-.33590	.15466	.264	-.8449	.1731
	N/gonder	W/wolega	.89300*	.15466	.001	.3840	1.4020
		E/wolega	.29493	.15466	.373	-.2141	.8039
		HG/wolega	.54071*	.15466	.036	.0317	1.0497
		E/gojjam	.20482	.15466	.684	-.3042	.7138
	E/gojjam	W/wolega	.68818*	.15466	.008	.1792	1.1972
		E/wolega	.09012	.15466	.975	-.4189	.5991
		HG/wolega	.33590	.15466	.264	-.1731	.8449
		N/gonder	-.20482	.15466	.684	-.7138	.3042
LSD	W/wolega	E/wolega	-.59806*	.15466	.003	-.9427	-.2534
		HG/wolega	-.35228*	.15466	.046	-.6969	-.0077
		N/gonder	-.89300*	.15466	.000	-1.2376	-.5484
		E/gojjam	-.68818*	.15466	.001	-1.0328	-.3436
	E/wolega	W/wolega	.59806*	.15466	.003	.2534	.9427
		HG/wolega	.24578	.15466	.143	-.0988	.5904
		N/gonder	-.29493	.15466	.086	-.6395	.0497
		E/gojjam	-.09012	.15466	.573	-.4347	.2545
	HG/wolega	W/wolega	.35228*	.15466	.046	.0077	.6969
		E/wolega	-.24578	.15466	.143	-.5904	.0988
		N/gonder	-.54071*	.15466	.006	-.8853	-.1961
		E/gojjam	-.33590	.15466	.055	-.6805	.0087
	N/gonder	W/wolega	.89300*	.15466	.000	.5484	1.2376
		E/wolega	.29493	.15466	.086	-.0497	.6395

		HG/wolega	.54071*	.15466	.006	.1961	.8853
		E/gojjam	.20482	.15466	.215	-.1398	.5494
	E/gojjam	W/wolega	.68818*	.15466	.001	.3436	1.0328
		E/wolega	.09012	.15466	.573	-.2545	.4347
		HG/wolega	.33590	.15466	.055	-.0087	.6805
		N/gonder	-.20482	.15466	.215	-.5494	.1398

*. The mean difference is significant at the 0.05 level.

Table 13: Duncan multiple comparisons test for TPC within five Niger G. abyssinica samples of 80% extract

TPC

	Sample	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	W/wolega	3	10.8880		
	HG/wolega	3	11.2403	11.2403	
	E/wolega	3		11.4861	11.4861
	E/gojjam	3		11.5762	11.5762
	N/gonder	3			11.7810
	Sig.		.229	.264	.373
Duncan[a]	W/wolega	3	10.8880		
	HG/wolega	3		11.2403	
	E/wolega	3		11.4861	11.4861
	E/gojjam	3		11.5762	11.5762
	N/gonder	3			11.7810
	Sig.		1.000	.064	.098

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table 14: One way ANOVA test value for TPC content with in five Niger G. abyssinica samples of 100 % extract

Descriptives

TFC

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
W/wolega	3	7.6519	.37784	.21815	6.7133	8.5905	7.40	8.09
E/wolega	3	9.9622	.22837	.13185	9.3949	10.5295	9.71	10.15
HG/wolega	3	10.9208	.37624	.21722	9.9861	11.8554	10.49	11.21
N/gonder	3	9.5608	.08514	.04916	9.3493	9.7723	9.51	9.66
E/gojjam	3	10.2817	.51575	.29777	9.0006	11.5629	9.88	10.86
Total	15	9.6755	1.18155	.30508	9.0212	10.3298	7.40	11.21

ANOVA

TFC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	18.326	4	4.581	37.570	.000
Within Groups	1.219	10	.122		
Total	19.545	14			

Multiple Comparisons

Dependent Variable:TFC

	(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	W/wolega	E/wolega	-2.31032*	.28512	.000	-3.2487	-1.3720
		HG/wolega	-3.26886*	.28512	.000	-4.2072	-2.3305
		N/gonder	-1.90888*	.28512	.000	-2.8472	-.9705

		E/gojjam	-2.62983*	.28512	.000	-3.5682	-1.6915
	E/wolega	W/wolega	2.31032*	.28512	.000	1.3720	3.2487
		HG/wolega	-.95854*	.28512	.045	-1.8969	-.0202
		N/gonder	.40144	.28512	.637	-.5369	1.3398
		E/gojjam	-.31951	.28512	.793	-1.2579	.6188
	HG/wolega	W/wolega	3.26886*	.28512	.000	2.3305	4.2072
		E/wolega	.95854*	.28512	.045	.0202	1.8969
		N/gonder	1.35998*	.28512	.005	.4216	2.2983
		E/gojjam	.63902	.28512	.240	-.2993	1.5774
	N/gonder	W/wolega	1.90888*	.28512	.000	.9705	2.8472
		E/wolega	-.40144	.28512	.637	-1.3398	.5369
		HG/wolega	-1.35998*	.28512	.005	-2.2983	-.4216
		E/gojjam	-.72095	.28512	.160	-1.6593	.2174
	E/gojjam	W/wolega	2.62983*	.28512	.000	1.6915	3.5682
		E/wolega	.31951	.28512	.793	-.6188	1.2579
		HG/wolega	-.63902	.28512	.240	-1.5774	.2993
		N/gonder	.72095	.28512	.160	-.2174	1.6593
LSD	W/wolega	E/wolega	-2.31032*	.28512	.000	-2.9456	-1.6750
		HG/wolega	-3.26886*	.28512	.000	-3.9041	-2.6336
		N/gonder	-1.90888*	.28512	.000	-2.5442	-1.2736
		E/gojjam	-2.62983*	.28512	.000	-3.2651	-1.9945
	E/wolega	W/wolega	2.31032*	.28512	.000	1.6750	2.9456
		HG/wolega	-.95854*	.28512	.007	-1.5938	-.3232
		N/gonder	.40144	.28512	.189	-.2339	1.0367
		E/gojjam	-.31951	.28512	.289	-.9548	.3158
	HG/wolega	W/wolega	3.26886*	.28512	.000	2.6336	3.9041
		E/wolega	.95854*	.28512	.007	.3232	1.5938
		N/gonder	1.35998*	.28512	.001	.7247	1.9953
		E/gojjam	.63902*	.28512	.049	.0037	1.2743
	N/gonder	W/wolega	1.90888*	.28512	.000	1.2736	2.5442
		E/wolega	-.40144	.28512	.189	-1.0367	.2339

		HG/wolega	-1.35998*	.28512	.001	-1.9953	-.7247
		E/gojjam	-.72095*	.28512	.030	-1.3562	-.0857
	E/gojjam	W/wolega	2.62983*	.28512	.000	1.9945	3.2651
		E/wolega	.31951	.28512	.289	-.3158	.9548
		HG/wolega	-.63902*	.28512	.049	-1.2743	-.0037
		N/gonder	.72095*	.28512	.030	.0857	1.3562

*. The mean difference is significant at the 0.05 level.

Table 15: Duncan multiple comparisons test for TPC within five Niger G. abyssinica samples of 80% extract

TFC

			Subset for alpha = 0.05			
	Sample	N	1	2	3	4
Tukey HSD[a]	W/wolega	3	7.6519			
	N/gonder	3		9.5608		
	E/wolega	3		9.9622		
	E/gojjam	3		10.2817	10.2817	
	HG/wolega	3			10.9208	
	Sig.		1.000	.160	.240	
Duncan[a]	W/wolega	3	7.6519			
	N/gonder	3		9.5608		
	E/wolega	3		9.9622	9.9622	
	E/gojjam	3			10.2817	
	HG/wolega	3				10.9208
	Sig.		1.000	.189	.289	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.