NTNU

Norwegian University of
Science and Technology

Essential oil and biodiversity study of Securidaca longipedunculata

Teklebrihan Lijalem Bahta

MSc in Biology
Submission date: May 2017
Supervisor: Richard Strimbeck, IBI
Co-supervisor: Jens Rohloff, IBI

Norwegian University of Science and Technology
Department of Biology

Abstract

Securidaca longipedunculata Fresen, also named as Violet tree, is semi-deciduous shrub or small tree that belonging to the family Polygalaceae and found in rainfall and equatorial humid areas of Africa. It is an indigenous medicinal plant in Africa and has important place in the traditional medicine as well as modern medicine. This plant is rich in essential oils and phytochemicals, so studying the essential oils is important to use this plant for medicinal purposes. Thus, the objective of this study is to characterize and show chemical difference between root and leaf from three region collections and to identify chemical compounds for potential medicinal use. The methods were GC/MS for the extraction of phytochemicals and phenolics, SPME for the essential oil extraction. 270 compounds were extracted from the GC/MS metabolite profiling and from those 44 were phenols and flavonoids. 125 volatiles compounds were extracted using the SPME. Leaf extracts were higher in concentration of phenolics and oils. All the extracted compound was detected in the three region samples. Tigray region samples were relatively higher in concentration of phenolics and volatiles.

Acknowledgments

My deepest gratitude goes to my advisor, Asso. Prof. Richard Strimbeck and Jens Rohloff for their continuous, unreserved, constructive guidance and support as well as providing the necessary resources unlimitedly. I am very fortunate to have worked under their guidance and am proud to have studied in their laboratory.

Table of Contents

1. Introduction

Plants that have therapeutic properties or use for beneficial pharmacological effects on the human body are called medicinal plants. These plants have been used for many years by humans of different groups in traditional ways. The application of medicinal plants is almost as old as the history of mankind. More than 80% of the world's population depends on traditional medicine to meet daily health needs (WHO, 2001). Traditionally Chinese and Indians start to use for the treatment of healing pains and diseases, and now most of the world uses herbs for healing from different diseases (Hosseinzadeh, Et al., 2015). Traditional use of herbs is still widely practiced in Africa by different ethnic groups.

Plants produce essential oils and chemicals which makes them important for healing process. *Securidaca longipedunculata* (Polygalaceae) is a semi-deciduous savanna shrub or small tree up to 10 meters high with many branches with an open and sprawling crown (Iwu, 1986). The leaves are alternate lateral branchlets, with fine hairs while young but losing because of maturity, small flowers, pink or purple, pleasantly perfumed, produced in excess in terminal axillary sprays and is monecious (Van Wyk et al., 2005). It has round, straw-colored fruit (Coates-Palgrave, 2005).
The common English names are violet tree, fiber tree and Rhodesian violet and the common name in Ethiopia is 'Ets a manahi' in Amharic and 'shotora' in Tigrigna (Keshebo etal., 2014).
S. longipedunculata is native to most parts of Africa in a wide range of climates from subtropical to arid areas (Orwa etal., 2009). It is distributed in all sub-Saharan African countries (Tshisikhawe et al., 2012). It is found in Ethiopia in different regions with a lot of variation in distribution and use for traditional medicine in different localities.

1.1 The study area, Ethiopia

Ethiopia is located at the Horn of Africa between 3° to 18° N latitude and 33° to 48° E longitude and extends over 1,127,127 km² including a water surface area of 7,444 km². It has dry and rainy climatic conditions with warm and moist winds from the influence of Indian and Atlantic oceans. Ethiopia has six different agro-ecological zones (Alemayehu, 2006) (: Bereha (desert), Kolla (hot zone), Weyina-Dega (warm zone), Dega (cold zone), Wurch (very cold zone) and Kur (extremely cold with ice). These can also be classified in lowland, midland and highland zone with different characteristic tree and crop species. *S. longipedunculata* grows in the Bereha, Kolla and Weyina Dega zones. The climatic conditions in which this plant grows are similar and with sunlight throughout the year. This plant grows abundantly in the low lands and warm areas of Tigray, Amhara, Oromia, Benshangul Gumz, Afar, Gambela and the southern nation and nationalities regions. Based on the abundance of this plant we chose Tigray, Amharic and Oromia regions for this study.

Tigray region is in the northern part of the country bordering with Eritrea in the North, Sudan in the West, in South and Southwest with Amhara and with Afar region in East (VAM- Ethiopia, 2009). The climate is characterized by large spatial variations with precipitation ranging from about 1000-1260 mm in some areas (Southwest) to less than 300 mm per year in the Northeast lowlands (Gebrehiwot and Anne van der Veen, 2013). Most parts are with low rain fall amount and short rainy periods. This region varies from temperate rainy to arid with the annual average temperature variation from 13.4 to 28°C (Gebrehiwot and Anne van der Veen, 2013). *S. longipedunculata* is most abundant in Western Tigray. In this study, samples were collected in May Tsebri, which is found in the western part of the region, with the maximum rainfall of 200 mm per month for summer season only and low to no rain fall in winter and spring and an annual

mean temperature of 12.23 °C (http://ru.worldweatheronline.com/). This village is grouped under kola (hot zone).

Amhara region is in the North West bordering with Sudan in west, Tigray in north, Oromia in the south and Afar in the West and most of this plant found in North west part (VAM- Ethiopia, 2009). The climate of the Amhara region, based on altitude, includes: Kola (hot zone) covers 31% of the region, Weyina Dega (warm zone) - covering 44% and Dega (cold zone) and it covers 25% of the region and the mean annual temperature of the region is between 15 °C and 21 °C. Samples from this region were collected from Metema, which is found Northwest part bordering Sudan, in the kola zone. The climate is classified as tropical and the summers have a good rainfall, while the winters have very little and the average temperature 28.1 °C and the average annual rainfall is 896 mm.

Oromia region shares boundaries with all regions of Ethiopia except Tigray, including the Amhara and Afar regions to North and Gambela and Southern regions to the west, and with Somalia to the east, Kenya in the south and Sudan to the southwest (VAM- Ethiopia, 2009). It includes the dry climate or the hot arid (kola zone), semi-arid, dry sub-humid climates, tropical rainy climate or the tropical humid and tropical sub-humid climates and temperate rainy climate or the warm temperate humid, the warm temperate per humid and the cool highland climates and the rainfall varies due to high variation in different parts and ranges from below 500mm to 2400 mm per year. Samples were collected in Gebre Guracha, which is located near Abay desert (Bereha), with around 200 mm per month for the summer months and lower for winter and spring season, and the annual temperature is 9.25 °C(http://www.worldweatheronline.com).

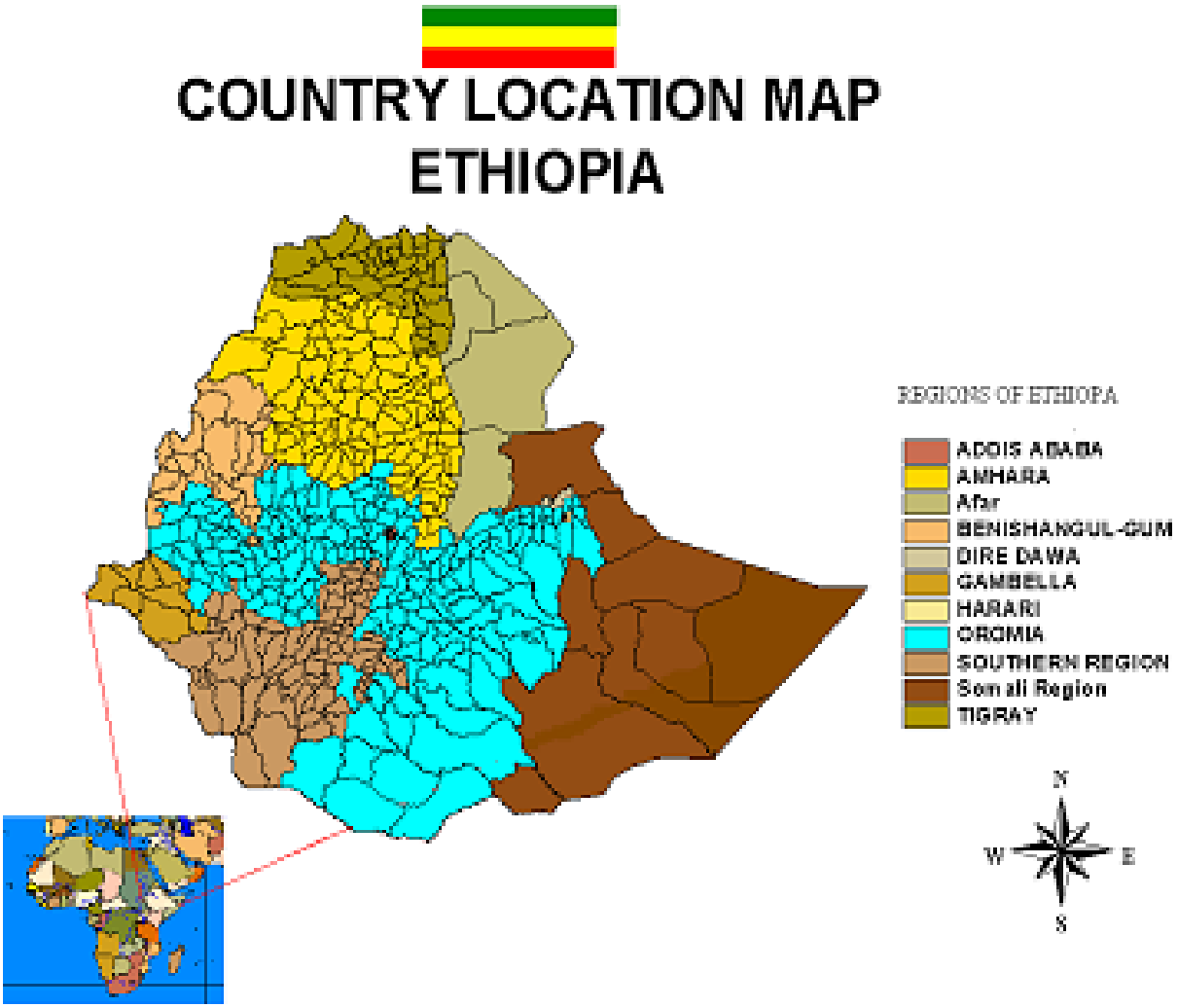


Figure 1: Location map for regions of Ethiopia (MoA, 2000)

1.2 Traditional uses

S. longipedunculata is an indigenous medicinal plant in Africa and has an important place in the traditional medicine as well as modern medicine. It is used as treatment for a variety of diseases including malaria, tuberculosis and sexually transmitted diseases, stomach problems, snake bite fever, headache coughs arrow poisons and for insect pest control in stored grain (Afful, 2012). In different countries, it is used for treatment of diseases. For instance, In Zimbabwe the roots are

used for venereal diseases, syphilis, pains, fever, epilepsy, pneumonia, tuberculosis; (Mustapha, 2013a). In Nigeria, the roots are used for abortion, constipation, coughs, fever, pneumonia, sexual boost, toothache (Mustapha, 2013b) and the bark is used to treat infections related to nervous system, typhoid and frequent stomach ache (Borokini et al., 2013), and the leaves used in dislocated jaw, headaches, skin cancer (Mustapha,2013a; Mustapha,2013b). In South Africa, the root uses for Flu, blood purifier, aphrodisiac, psychoactive purposes (Moeng, 2010).

In Ethiopia, this plant is traditionally used for different purposes. *S. longipedunculata* is used in the form of root powder in traditional medicine and sold in markets the root is used by traditional healers to heal different diseases by different ethnic groups. Among the ethnic groups Kunamas in north Tigray use it for treating human and livestock ailments (Gidey et al., 2015). The Kunamas are well known by their traditional knowledge and preparation. There are other groups that knew about the use this plant for healing different diseases and animal bites (Gidey et al., 2015).

1.3 Use in modern medicine

S. longipedunculata has been studied for preparation of medicine for many diseases and antimicrobials. Some studies showed about the use of *S. longipedunculata* for treatment of human diseases. It is an important plant with the potential benefits in the treatment of communicable and transferrable diseases like malaria, tuberculosis, and caused by public acquired microorganisms (Mongalo et al., 2015).

Leaf extracts have anticonvulsant properties for healing of the neurological disorder epilepsy (Odebiyi and Sofowora, 1978). There are studies on the use of this plant for antimicrobial activities. *S.* l*ongipedunculata* root extracts and found that the extracts inhibit gram positive and

gram negative and multidrug resistant bacteria and fungi (Ngonda 2012). The high antimicrobial activity of *S. longipedunculata* roots extracts were also testified by (Ajali and Chukwurah, 2004).

S. longipedunculata is used for treatment of rheumatic fever and for pain reliever and stiffness in muscular sprains and skin diseases and the powdered roots or wood scrapings are used to treat headache by rubbing them on the forehead, while infusions from the roots are used to wash tropical ulcers (Kokwaro, 1976). There are many other studies on medicinal use of chemical components which extracted from this plants, but it is enough for this study.

Table1: Some publications on medicinal uses of *S. longipedunculata*

Part	**Uses**	**Reference**
Root	Anti-microbial activities	(Ngonda et al., 2012)
Whole plant	For the treatment of malaria	(Wanzala et al.,2012; Nguta et al., 2010a; Nguta et al., 2010b)
Stem and root bark	For the treat infections related to nervous and circulatory system	(Borokini et al., 2013)
Stem bark	Dysentery, malaria, typhoid and frequent stomach ache	(Mustapha, 2013a)
Roots	For sexual boost, abortion, tuberculosis, cough, fever and constipation	(Mustapha, 2013a; Ogunmefun and Gbile, 2012)
Leaves	For headache and as contraceptive purpose	(Mustapha, 2013b)
Root extracts	For anti-parasitic activities	(Fernandes et al., 2008)
Leaves and root extracts	For anti-plasmodial activity	(Bah et al., 2007)
Root barks extracts	Anti-inflammatory	(Okoli et al., 2005)

Root extracts	For enzyme inhibition	(Bangou et al., 2011)
Root	For epilepsy	(Kadir et al., 2013)
Root and leaves	Infertility, placenta expulsion, stomach ache and toothache	(Augustino et al., 2011)
Seeds	for treating headache, fever and rheumatism	(Orwa *et al.*, 2009)

Plant essential oils also called secondary metabolites are chemicals secreted by plants for the benefit of the plant itself and found as complex mixtures. These compounds are important in the interaction of the plant with the environment such as defense compounds for deterrence, toxic and anti-feeding for herbivores and microbes. It also used as inhibition of germination and growth of parasitic plants.

1.4 Classification of essential oils in plants

Phenols: are chemical compounds which have a phenyl ring bearing one or more hydroxyl substituents. These compounds are one of largest group of plants secondary constituents synthesized by fruits, vegetables and other plants that have certain health benefits. These are involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contributing to plants' colors. These are also synthesized by microorganisms and industrially.

These compounds serve as signaling molecules in plant microbe interactions (Mandal, 2010).

Phenolics are classified as phenolic acid, flavonoids, tannins and lignans. The phenolic acid has two classes of derivatives of benzoic acid such as gallic acid, and the derivatives of cinnamic acid

such as caffeic and ferulic acid. Caffeic acid is the most abundant phenolic acid in many fruits and vegetables, which is the major phenolic compound in coffee.

Phenolics acids have many biological activities, for example, anti-ulcer, anti- inflammatory, anti-oxidant (Silva et al, 2007), cytotoxic and antitumor, anti-spasmodic, and anti-depressant activities (Ghasemzadeh et al, 2010). These compounds have diverse functions and are vastly significant in plant-microbe interactions or symbiosis and act as signaling molecules in the initiation of legume rhizobia symbioses, establishment of arbuscular mycorrhizal symbioses and these also can use as agents in plant defense activities (Mandal, 2010). Generally, many studies have shown that plant defense against soil borne pathogens, nematodes, plant feeding insects is based on the release of phenolic compounds in soil system (Mandal, 2010).

Flavonoids are the most abundant polyphenol in our diet, and divided into six groups, flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins. Dietary flavonoids are mostly found in berry fruits, soybean fruits and beverages such as tea coffee, tea and fruit drinks (Dai and Mumper, 2010). These compounds have been used as medical treatments since ancient times, and their use has persisted up to now and use anti-microbial, cytotoxicity, anti-allergic, anti-inflammatory as well as antitumor activities and act as dominant antioxidants which can protect the human body from free radicals and reactive oxygen species (Saxena et al, 2013). These compounds also play important roles in protecting biological systems against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, lipids and DNA (Atmani et al, 2009).

Tannins are a group of polyphenols in our diets and usually subdivided into two groups: hydrolysable tannins and condensed tannins. The hydrolysable tannins containing a central core of glucose and due to the formation of oxidative linkage it has great variety of structure (Dai and

Mumper, 2010). The condensed tannins are oligomers or polymers of flavan. They are decomposed through acid-catalyzed oxidation reaction when heating in acidic alcohols. These compounds are commonly found in fruits such as grapes, persimmon, blueberry, tea, chocolate, legume forages, legume trees and in grasses (Giner-Chavez, 1996). They have several health benefits been recognized for the intake of these compounds and some epidemiological relations with the decreased incidence of chronic diseases have been recognized (Serrano et al, 2009).

Uses of phenols for the plant: UV protection, pigmentation, stimulation of nitrogen fixation and for disease resistance (Koes et al., 1994).

Alkaloids: are naturally occurring compounds having basic nitrogen atoms. These are produced by plants and other organisms, including bacteria, fungi and animals (Goyal, 2013). The most known natural occurring plant alkaloids are, cocaine, caffeine, heroine, atropine, nicotine and morphin. Plants which are rich with alkaloids are cannabis, cocoa, coffee, Datura stramonium, potatoes, tomatoes, Datura, Khat, Opium, tea and tobacco Atropa belladonna, Datura stramonium, Mandragora officinarum, eggplant, bittersweet nightshade and other plants of the family Solanaceae. Alkaloids are classified in pyridine, pyrrolidine, tropane, indolizidine, quinoline, isoquinoline, phenanthrene, phenethylamine, indole group, purine groups.

Most of these compounds are applicable in medicine the treatment of different diseases such as quinines use for anti-malaria, anti-hypertensive effects (many indole groups), anti-arrhythmic effect (quinidine, spareien), and anti-cancer actions (indoles, vincristine, vinblastine) (Saxena et al, 2013). These are few example showing the great economic use of alkaloids of plant constituents. Some alkaloids have stimulant property as caffeine and nicotine, morphine are used as the analgesic and quinine as the antimalarial drug (Saxena et al, 2013).

Alkaloids have many ecological roles. Allelopathy strengthens the competitiveness of invasive plant species by inhibiting the growth of neighboring plants, this helps for crops warding off weeding (Goyal, 2013) and barley releases alkaloids exude from living roots, inhibiting weed growth.

Alkaloids have also ecological roles on herbivores, insects and microorganisms. Some alkaloids deter the vertebrate herbivores, shows a bitter or pungent taste (Wink ,2008). Ragwort species are the major example for their success throughout the world by using alkaloids to defend them against herbivores.

Terpenes: are aromatic compounds which are found in many of plant species and encompassing many flavours, fragrances and antibiotics. These are the most numerous and divers group (Zwenger.S and Basu C., 2008). In woody plants terpenes are abundantly found like pine tree species (Zwenger R. S and Basu C, 2008). These are classified as sterols, triterpenes, sesquiterpenes, diterpenes and carotenoids (Humphrey J.A and Beale H., 2007). The diterpenes are important for the plant as growth hormones or defence compounds and for humans serve as antibacterial or antifungal activity (Humphrey J. A and Beale H., 2007). The triterpene

Carotenoids are tetraterpenes which make as accessory for pigments in photosynthesis, use for attraction of insects, animals and birds to their flowers and fruits (Humphrey J.A and Beale H., 2007) and use as food additives. In human medicine carotenoids serve as vitamin deficiency medication.

The functions of terpenes for the plants are attract specific insects for pollination or expel some animals which uses the plat as food, the bitter- tasting use to protect some plants from being eaten by animals and play vital role as signal compounds and growth regulators of plants (Breitmaier, 2006).

Glycosides: are natural organic compounds found in a lot of plants and some animals, and these compounds upon hydrolysis gives one or more sugars (glycone) β form and non-sugar (aglycone). Glycosides are termed as a sugar molecules bonded to another functional group through glyosidic bond. Glycosides are defined as the condensation products of sugars with a host of different varieties of organic hydroxy compounds, in such a way that the hemiacetal unit of the carbohydrate must participate in the condensation (Doughari, 2012). Plants by nature store the inactive forms of these chemical compounds and activated by enzymes through the process of hydrolysis which broken off the sugar part.

The best example of glycosides in plants which have large effect on human life is *Manihot esculenta*. The acute *Manihot esculenta* poisoning has been reported in Nigeria since 1989 and many patients died after eating a meal of *this plant* product directly by vomiting and complaining of abdominal pain, with acute renal failure and died of cardio-pulmonary arrest (Vetter, 2000). Glycosides also have ecological role in plants is the participation in defense mechanisms against different phytopatogens (Vetter, 2000). Glycosides have also use as sources of energy or sugar, regulation for growth and for pollinations process due to some have beautiful colours.

Saponins are glycosides with a distinctive foaming properties. They are found in many plants, but get their name from the Saponaria officinalis, the root of which was used historically as a soap. They consist of a polycyclic aglycone attached via to a sugar side chain.

Saponins are found in most vegetables, beans and herbs and the best-known sources of saponins are peas, soybeans, and some herbs with names indicating foaming properties such as soapwort, saoproot, soapbark, soapberry and higher plants.

These compounds traditionally used as a natural detergent and have several industrial and commercial use such as, as sources of raw materials for steroid hormones production, as food

additives, as ingredients in photographic emulsions, fire extinguishers and other industrial applications (Tamura et al., 2012).

Saponins have many medication applications such as haemolytic activity, molluscicidal, Anti-inflammatory, anti-fungal, anti-yeast and antimicrobial anti-parasitic, Cyto toxicity, anti-tumor, antiviral activity and other biological activities (Sparg et al., 2004). The steroid saponins of most plants use for the treatment of different tumor cells in humans such as the cycloartane saponins displayed anti-cancer effect and act as chemotherapeutic agent in the treatment of tumors (Man et al., 2010).

1.5 Extraction

Extraction is the isolation of medicinally active plant portion using different selective solvents and the aim is to separate soluble plant chemicals. There are two types of extraction methods in general category, the traditional and modern methods. Traditional extractions are most widely used on commercial scale. These are hydrodistilations like water distillation, water and steam distillation and direct steam distillation and maceration. In recent days there are modern ways of extracting metabolites from plants like Headspace trapping, Static headspace, Vacuum headspace, dynamic headspace, solid phase micro-extraction (SPME), supercritical fluid extraction (SFE), Phytosol (phytol) extraction, protoplast technique, simultaneous distillation extraction (SDE), microwave distillation controlled instantaneous decomposition (CID) , thermo micro distillation, micro distillation, molecular spinning band distillation and membrane extraction.

1.6 Solid-Phase Micro extraction (SPME) and Gas chromatography/mass spectrometry (GC/MS)

Sample preparation is an essential step in analysis, greatly influencing the reliability and accuracy of the results. The isolation of essential oils and other plant volatiles by using solid-phase micro extraction (SPME) is a very simple and efficient solventless sample preparation method, invented by Pawliszyn in 1989 in an attempt to address restrictions inherent in solid-phase extractions and liquid–liquid extraction techniques (LLE) (Vas and Vekey, 2003). SPME can be used for integrated sampling, extraction, concentration since analytes of the sample are straightly extracted and concentrated to the extraction fibre. The method generally saves preparation time, disposal costs and can improve detection limits (Vas and Vekey, 2004).

SPME method is commonly used in combination with gas chromatography /mass spectrometry (GC/MS) and effectively useful to a wide variety of compounds, particularly for the extraction of volatile and semi-volatile organic compounds from different samples (Vas and Vekey, 2004). There are many applications of SPME, sampling gases or sampling solutions are the major once.

On the other hand, sample preparation procedures using solvent liquid–liquid extraction are time consuming, labor-intensive and multi-stage operations, with each step with errors. So, using appropriate sampling is the only option to reduce the time spent on analysis. Using solid-phase extraction reduces many limitations of classical liquid–liquid extraction methods needs less solvent but it is a time-consuming multi-step process and often requires a concentration step, which may result in a loss of volatile components and longtime usage. When we compare the different sampling techniques, SPME coupled with GC/MS is a very efficient method to extract and analyze volatile and semi-volatile organic compounds. The application of SPME can cover the analysis of

solvents and inhalation narcotics, amphetamines, cocaine metabolites, cannabinoids, methadone and other opioids, fatty acids and various other therapeutic drugs, pesticides, chemical warfare agents, cyanide, sulfide and metal ions.

Due to the above advantages in simplifying sample preparation, increasing reliability, selectivity, sensitivity and reducing the cost and time of analysis, using SPME was considered the most cost effective and error-reducing method and was therefore chosen for sample extraction.

There are many compounds extracted from root and leaf of *S. longipedunculata* as reported from different studies with major focus on root parts of this plant.

The first objective of this study is to characterize different compound composition between the three regions. The second goal is to determine the chemical difference between tissues from all regions. The third objective is to identify chemical compounds for potential medicinal use.

2. Materials and methods

2.1 Plant material collection

Plant material was collected in three regions of Ethiopia, which were selected based on the availability of this plant: Tigray, Amhara and Oromia regions. In all regions, the plant grows under similar climatic conditions. Samples were collected in selected areas of each region, from Tigray region in Maytsebri district, from Amhara region in Metema area, and from Oromia region samples were collected in Abay Bereha district.

Collection and chemical composition of plant material depends on season and timepoint, type of plant tissue and metabolites to be extracted. The study focused on the root and leaves, where the largest amounts of potential bioactive metabolites is produced. These parts needed to be collected during the humid season, since the roots would be growing sufficiently and unstressed, leaves receive enough nutrients and water to produce biomass and metabolites. The harvesting time was in the morning around 7:00 to 9:00 Am, as recommended by the traditional healer.

Root sample collection was performed by removing parts of the root bark without removing the whole root from the natural stand. This is important to preserve the plant from damage and potential death. The traditional healers take the whole root by killing the plant, which increases the extinction of the plant. Leaf material was collected by sampling leaves of different age from the stand.

For each sample 30 g from roots of different *S. longipedunculata* plants and 25 g of leaf samples from different parts of branches were collected without damaging the plant stand. 10 samples from root and leaf were collected from each district, for a total of 30 root and 30 leaf samples. For root

samples, the bark was peeled by hand by using gloves and retained for drying and analysis. Green leaves were cut at the petiole and taken from all parts of the branches.

2.2 Sample drying

Drying is the most common method for post-harvest preservation of medicinal plants in order to preserve the phytochemical efficiency of the plant materials. It is an important step to keep the samples with the required chemical compositions. Different methods are used for the drying of medicinal plants, the most common methods are sunlight, shade, mechanical ovens and by freeze-drying. Sun drying uses sun light with air in outdoor and some shade. Samples are spread on a tray covered with cotton sheets to keep away from external pests and dirt and are exposed to sunlight until the samples are dried (2 days). Secondly, room drying where samples are spread on a tray and covered with cotton sheets and left to dry in a room with appropriate air flow until all samples are dried (4 days). Thirdly, using oven drying samples are spread on shelves in an oven and left overnight at 40-60ºC and fourthly, freeze drying also called lyophilization, using specially designed freezers where samples in aluminum foil are left for three days at -45ºC (Bernard et al., 2014). In some cases, integrated methods are used like sun light and shade with ventilation (Keshebo et al., 2014). Generally, lower temperatures are mostly recommended compared to higher temperatures in order to preserve phytochemicals

Based on the above described studies, the best way to drying of medicinal plants was in the room under sunlight exposure (not directly under sunlight) with good air ventilation. drying plant parts in the shed with air under low light condition. So, for this study we used this method from among all methods by considering the susceptibility of the plant and the medicinal chemicals we needed to extract.

All samples were labeled and subjected directly to drying. Fresh leaves and roots of 30 grams were used for all treatments with five replicates. For the leaf samples the drying process required shorter time than the root, in total 8 days compared to roots with 10 to 12 days. All samples were dried in Addis Ababa University biotechnology tissue culture laboratory. Drying process was performed by putting all the samples on separate position on the table near to the windows to get sun light and air. Changing positions every day and retrace each sample and check all samples to dry uniformly. In total, the drying process took one month for all samples. The drying condition in the laboratory were around room temperature (25 ºC)

2.3 Sample storage

Storage is the most important step to keep the sample from damage. Storage conditions affect the chemical quality of the sample (Keshebo et al., 2014). Different storage methods are used for medicinal plants like paper bags, cloth bags, glass jars, boxes and other containers. The paper bags are used for the root, leaf and seed samples and this is best for all samples to store for long term. The cloth bags are good for seed samples because it has pores to aerate the samples, but not suitable for leaf and root samples which leads to loss of moisture and oils during storage. Glass jars are used when plants are needed for essential oils or other volatile substances, e.g. jars with a tight-fitting lid. Boxes and containers are generally used for all sample types but with some limitations regarding to transportation and storage space.

For this study, we used paper bags due to feasible sample collection and ease of transportation. Two ways of samples storage were used, one storage before grinding the samples which starts from collections of natural stands of trees and after grinding specific amount from each sample.

All dried samples were stored in paper bags in shed at room temperature. After grinding all samples were stored in test tubes and stored in the dark at room temperature.

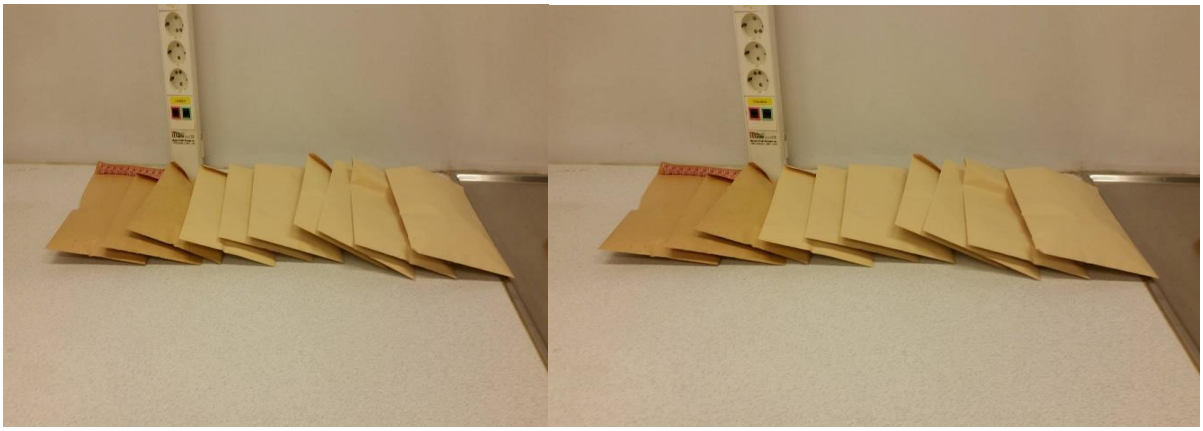


Figure 2: Dried samples stored in paper bags

2.4 Sample transportation

Transportation is an important step to safeguard samples from the site of collection to the laboratory. Transportation from sampling areas to Addis Ababa university by using paper bags with each sample labeled immediately after collection to prevent the loss of moisture. The bags were only partially closed to aerate the samples. Transportation from the sampling areas to the sample drying place (Addis Ababa University) was performed by collection and storage in paper bags, which were later shipped to NTNU for analysis.

2.5 Sample grinding

Grinding is the most important part of sample preparation. Appropriate grinding is very critical step for the extraction of different chemicals from plant samples easily. Grinding produces smaller particles; powdered samples have a very small particle size with relatively large surface for better

contact with the solvents for an efficient extraction. A suitable particle size is <0.5 mm, which is ideal for the process of extraction (Azwanida, 2015).

Different types of grinders are used for medicinal plant samples like mortar and pestle, blenders and mills. Electric blenders or pestles are good for crushing plant materials. For this study, we used a Wiley hammer mill for all root and leaf samples. We used 1 gram for root samples and 500 mg for leaf samples. A steel mesh with 2 mm holes was utilized for all samples. The mill was cleaned after each run, and the ground samples were transferred to labelled test tubes.

The Wiley mill has weaknesses when grinding plant material of very small size, deliquescent matrix or fibrous texture. Therefore, root samples were prepared by peeling the internal part and removing the stem part, while the leaf samples where easy to grind. Regardless of the weaknesses of the Wiley mill, it was the best option for sample processing and used for this study. After the grinding process, samples collected in glass vials with screw cork and septum, were immediately transferred to dark boxes and stored until the actual extraction work.

2.6 Extraction Methods

Extraction is the isolation of medicinally active plant compounds by using different selective solvents or devices with the aim of extraction for separation of soluble plant chemicals. There are two types of extraction methods for plant volatiles in general, these are traditional and modern methods. Traditional methods of extraction are most widely used on commercial scale. These are hydrodistilations like water distillation, water and steam distillation and direct steam distillation and maceration. In recent years, more advanced methods have been established like Headspace trapping, Static headspace, Vacuum headspace, dynamic headspace, solid phase micro extraction (SPME), supercritical fluid extraction (SFE), Phytosol (phytol) extraction, protoplast technique,

simultaneous distillation extraction (SDE), microwave distillation controlled instantaneous decomposition (CID), thermo micro distillation, micro distillation, molecular spinning band distillation and membrane extraction. Based on available technology and established lab routines, we chose the SPME method for this study.

2.7 Solid Phase Micro extraction

SPME is a powerful sample preparation tool prior to mass spectrometric analysis and a very simple, efficient and solventless method. Due to its advantage for the extraction of essential oils from medicinal plants and accessibility in the university lab we chose this method for our study. After all the samples were ground, samples were subjected to SPME extraction using a manual fiber sample holder and a 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber (Supelco Inc., Bellefonte, PA). Prior to extraction, the SPME fiber was rinsed in the injection port of the gas chromatograph at 220°C. Samples were heated in a water bath, a 250-ml beaker glass with water temperature adjusted to 60°C. under thermometer control. An extraction time of 30 min was used for all samples. Immediately after extraction, the SPME fiber was retracted and the sample was introduced into the injection port of the gas chromatograph.

2.8 Extraction of polar compounds and derivatization

Powdered leaf (500 mg) and root samples (1000 mg) were transferred to 15 ml sample tubes. Then, 5000 µl of ethanol: water (80:20) containing the internal standard ribitol (150 µg/ml) were added to the tubes and vortexed. Samples were then treated for 60 min at 50°C in an ultrasonic bath. After extraction and cooling down to room temperature, samples were centrifuged at 3100 rpm at 4°C. 1000 µl aliquots (leaf) or 500 µl aliquots (root) from the clear supernatant were transferred into 1.5 ml round-bottomed Eppendorff-tubes and dried in a SpeedVac overnight without heating.

Residues were redissolved in 80 µl of 20 mg/ml methoxyamine hydrochloride in pyridine and treated at 30°C for 90 min. Then, samples were derivatized with 80 µl of MSTFA (N-Methyl-N-(trimethylsilyl) trifluoroacetamide) at 37°C (incubator) for 30 min. Finally, samples are being transferred to 1.5 ml auto sampler vials with glass inserts, and stored at -20°C prior to gas chromatography/mass spectrometry (GC/MS).

2.9 Gas Chromatography/ Mass Spectrometry (GC/MS)

Gas chromatography is one of the key methods used for screening, identification and quantification of many chemical compounds from different sources. The separation power is high and used in combination with different detector types. So, for this study we use gas chromatography-mass spectrometry (GC/MS) for identifying the VOCs.

Analysis of essential oil volatiles: An Agilent 6890/5975 GC/MS (Agilent Technologies Inc., Palo Alto, CA) was used for all analyses. Absorbed analytes were desorbed in the GC injection port for 3 min using split less conditions for 1 min followed by a split ratio of 25:1. GC separations were carried out using a HP-5MS capillary column (30 m × 0.25 mm and film thickness 0.25 mm). Injection temperature was 220°C, and the interface was set to 220°C. The carrier gas was He at a constant flow rate of 1 ml/min. GC temperature was ramped from 40° to 220°C at a rate of 10°C/min, and finally held at 220°C for 5 min (analysis time: 23 min). The MS source was adjusted to 230°C, and a mass range of *m/z* 35–300 was recorded. Mass spectra were acquired in electron impact ionization mode at 70 eV.

Chromatogram visualization and peak identification was carried out using the GC/MS software packages Agilent ChemStation software (Agilent Technologies, Waldbronn, Germany) and AMDIS software (version 2.71; National Institute of Standards and Technology, Boulder, CO,

USA). The following mass spectral libraries, in combination with retention indices were used for tentative compound identification: NIST05 spectral library (National Institute of Standards and Technology, Gaithersburgh, MD) and a customized *in-house* terpene MS library. GC/MS data integration, normalization (total signal) and alignment was carried out using the Metalign software (PRI-Rikilt, Wageningen, The Netherlands). Based on distinct quantifier ions, detected volatile analytes were assessed semi-quantitatively based on MS detector response (arbitrary units).

GC/MS based metabolite profiling of polar compounds: The same instrument type and capillary column were used for derivatized samples. Sample volumes of 2 µl were injected with a split ratio of 25:1. Injection temperature was set at 230°C, and the interface was set to 250°C. The carrier gas used was He at a constant flow rate of 1 ml/min. The GC temperature programme was held isothermically at 70°C for 5 min, ramped from 70 to 310°C at a rate of 5°C/min and finally held at 310°C for 7 min (analysis time: 60 min). The MS source was adjusted to 230°C, and a mass range of *m/z* 70–700 was recorded. All mass spectra were acquired in electron impact ionization (EI) mode (70 eV). Chromatogram visualization and peak area integration was carried out using Agilent ChemStation software (Agilent Technologies, Waldbronn, Germany) and the data alignment software MetAlign (Wageningen UR, The Netherlands). Detected metabolites (selected MS intensities) were quantitatively determined based on the internal standard ribitol and concentrations finally expressed as µg/g dry weight. For mass spectra evaluation and metabolite peak identification, AMDIS software (National Institute of Standards and Technology, Boulder, CO, USA) was used in combination with the Golm Metabolome Database GMD (Max-Planck Institute for Molecular Plant Physiology, Golm, Germany), and the NIST05 spectral library.

2.10 Statistical tests

The average mean values for each metabolites and volatiles were done by Excel and the statistical significance (p-value, which were regarded as significance at $p<0.05$) were done by Kruskal-Wallis statistical test by using Minitab software (version16.0) and the figures for each compound were done by One way analysis of variance (ANOVA).

For the regional variation test we use the principal component analysis and the discriminant analysis, were performed using Biostatflow (version 2.7.7, 2015).

3. Results

3.1 Metabolite profiling of polar compounds

Based on metabolite profiling, 270 different compounds were isolated from the GC-MS extraction including identified metabolites (130 compounds), those identified by structure (96), and unknowns (44). Phenols and flavonoids (44 compounds), non-N-containing acids (46), amino acids (23), and lipids (20) were the most abundant metabolites in the plant sample extracts. Disaccharides were most abundant and phenols and flavonoids were found in significant amounts. In all, 36 phenols, 9 flavonoids, 7 sterols, 2 terpenes and 2 carotenoids were identified (Table 1 and 2). In this study, especial focus was given to the phenols, flavonoids, sterols and terpenes, which have known or potential medicinal applications.

3.2 Tissue and regional variations

There were variations in concentration of compounds between the two tissues of leaf and root and between the three regions (Table 2 and 3). From the two tissues, the root samples were abundant in certain phytochemical compounds, while the concentrations were generally higher in leaf samples (Table 2 and 3). From all the extracted compounds in roots 29 were found in reasonable amounts including phenols, flavonoids and sterols (Table 2 and 3) in the range between 50 to 500 µg/g dry weight. There were also other phenols and flavonoids extracted and screened but at very low concentrations, and therefore considered not that important for this study.

Simple phenols, phenolic acids and flavonoids were extracted very well in relatively large concentration in both tissues of root and leaves, and found in samples of the three regions, whereas sterols, carotenoids and terpenes were less abundant in roots and leaves, but also found in all regions (Table 2 and 3).

Generally, the majority of the metabolites high in concentration were found in Tigray region and lower in Amhara regions for root samples (Table 2). Some of the compounds without significant difference between regions had high concentration in all region samples, like (E)-sinapic acid, quercetin and stigmasterol. The highest concentration was found for a structurally-identified Alkaloid1, which found in Oromia region roots (Table 2).

Many compounds used in human medicine were found in relatively high concentration in the root samples including salicylic acid, gentisic acid, (E)-sinapic acid, protocatechuic acid and stigmasterol (Table 2).

Concentrations of 18 compounds differed significantly between regions (Table 1). Here we focus only on the important phenols, flavonoids and sterols.

The following were the most significant compounds: salicylic acid, gentisic acid, α-tocopherol hydroquinone, hydroquinone, protocatechuic acid and caffeoyl quinic acid derivative (Figures 3-5). Some compounds were found in highest concentration in roots collected in Tigray (gentisic acid, Figure 3; a-tocopherol hydroquinone and hydroquinone, Figure 4 and caffeoyl quinic acid derivative1, Figure 5).

Generally, sample tissue type had a strong effect on concentrations of compounds. Roots and leaves had high concentrations for most compounds. but when we saw the variations between regions, root samples were highly differed (Table 1 and 2).

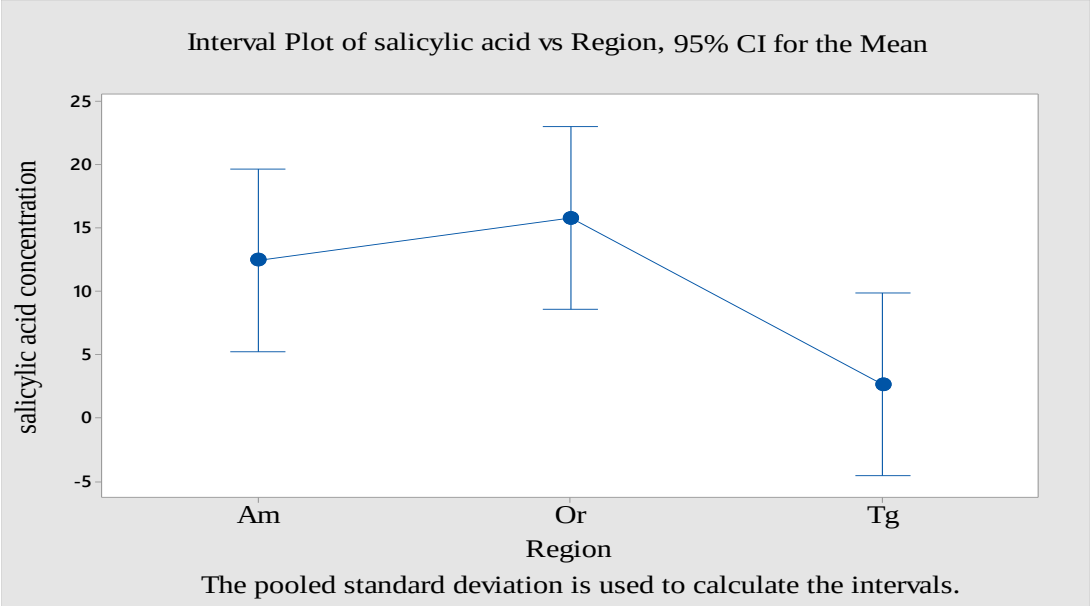
The distribution of root samples from the three regions are depicted in Figure 9, using Principal Component Analysis (PCA). Based on the metabolite composition of root extracts, sample clusters from the three regions were overlapping (Figure 9). Amhara and Tigray region extracts were somewhat similar, while the Oromia region samples differed more from the other two regions.

Table 2. Metabolite concentrations of 29 phytochemicals in root extracts of *S. longipedunculata* from the three regions of Ethiopia. Average values for the three regions were calculated (n=5). and expressed in µg/g dry weight. The metabolites were arranged in order of higher to lower average values within the same category. Phen stands for phenol, flav for flavonoids, ster for sterols, car for carotenoids, alkld for alkaloid, met for metabolite, str for structurally identified. Sig. stands for statistical significance (*p*-value), which was calculated as for less than or equal to 0.001, ***, for less than or equal to 0.01, ** and for less than or equal to 0.05, *.

Compound	Class	Category	Average Amhara Root	Average Oromia Root	Average Tigray Root	p-value	Sig.
salicylic acid	Phen	met	12.46	15.88	2.67	0.009	**
4-hydroxy-2-methylbenzoic acid	Phen	met	6.71	4.98	12.58	0.264	
gentisic acid	Phen	str	1.34	3.39	6.95	0.005	**
(E)-sinapic acid	Phen	met	3.86	1.79	5.54	0.137	
phenolic compound2	Phen	str	4.32	3.07	0.94	0.007	**
phenolic compound1	Phen	str	2.03	1.52	1.24	0.432	
(E)-ferulic acid	Phen	met	1.62	1.02	1.57	0.181	
phenyl pyruvic acid	Phen	met	1.99	0.96	0.88	0.228	
benzoic acid	Phen	met	0.88	0.80	0.63	0.23	
4-hydroxybenzoic acid	Phen	met	0.02	0.04	1.98	0.005	**
hydroquinone	Phen	met	0.34	0.20	1.36	0.008	**
m-coumaric acid	Phen	met	0.61	0.44	0.79	0.543	
α-tocopherol	Phen	met	0.02	0.21	0.37	0.432	
α-tocopherol hydroquinone	Phen	met	0.02	0.21	0.37	0.008	**
caffeoyl quinic acid derivative1	Phen	str	0.02	0.10	0.18	0.008	**
phyllodihydroquinone	Phen	met	0.02	0.04	0.03	0.005	**
protocatechuic acid	Phen	met	0.02	0.04	0.02	0.009	**
flavonoid5	Flav	str	19.66	2.23	0.02	0.002	**
quercetin	Flav	met	9.03	3.91	8.44	0.403	
kaempferol	Flav	met	2.72	0.35	0.03	0.039	*
flavonoid3	Flav	str	0.59	1.38	0.61	0.013	*
flavonoid4	Flav	str	0.79	0.15	0.02	0.002	**
(E)-phytol	Ter	met	0.02	0.03	0.03	0.009	**
stigmasterol	Ster	met	2.45	0.89	1.58	0.174	

sterol2	Ster	str	0.23	0.42	0.97	0.046	*
β-sitosterol derivative1	Ster	met	0.08	0.12	0.39	0.056	
sterol 4	Ster	met	0.02	0.04	0.20	0.005	**
alkaloid1	Alkld	str	2.15	39.16	0.02	0.034	*
carotenoid2	Car	str	0.39	0.47	1.38	0.03	*

a.



b.

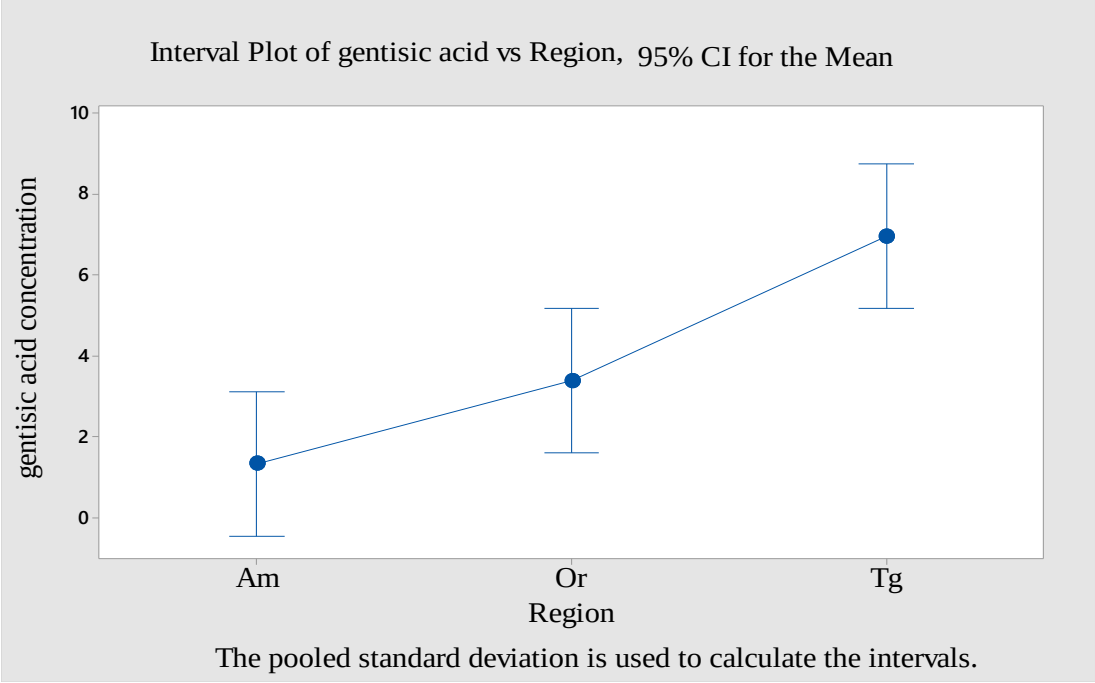
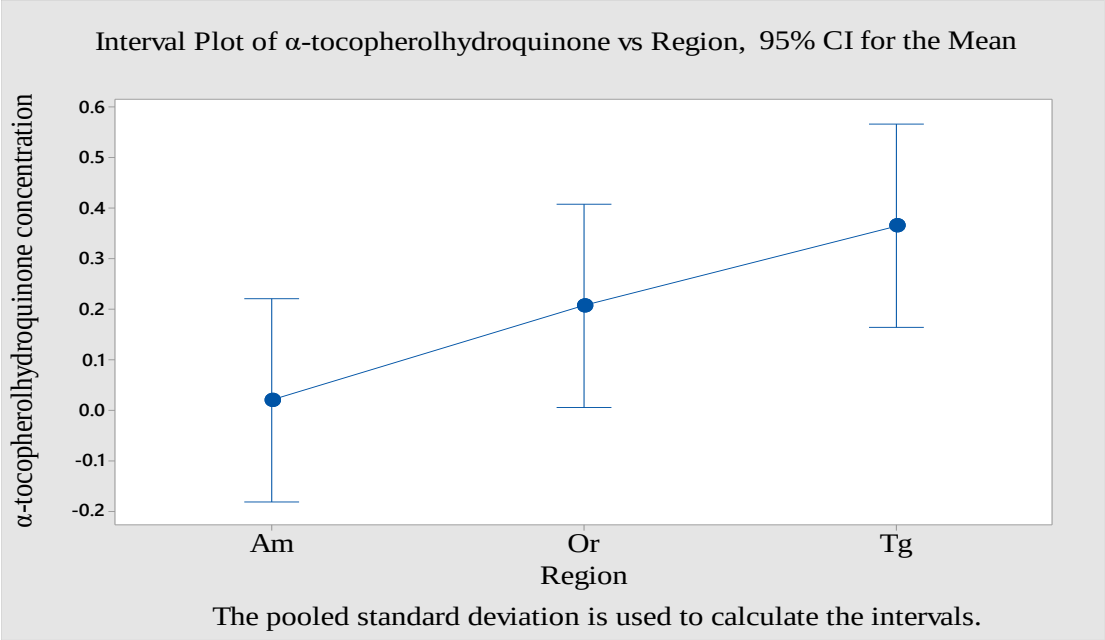


Figure 3: Concentrations of salicylic acid (a) and gentisic acid (b) extracted from *S. longipedunculata* roots in three region samples (n=5). The vertical axis shows the concentration (µg/g dry weight) and horizontal axis the three region samples. Am stands for Amhara samples, Or for Oromia and Tg for Tigray region samples.

a.



b.

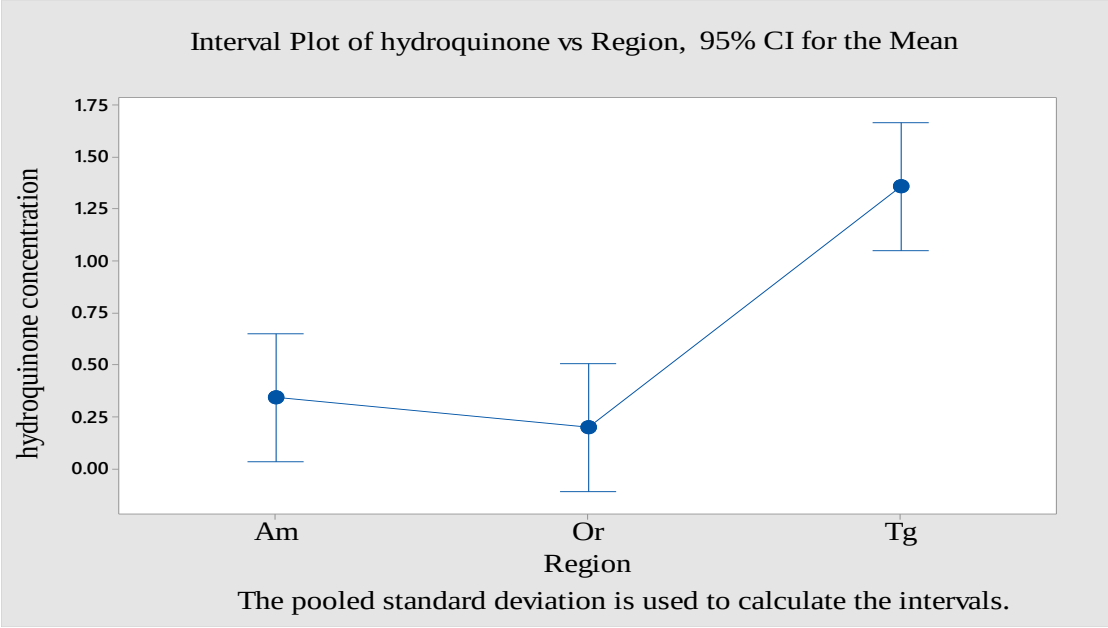
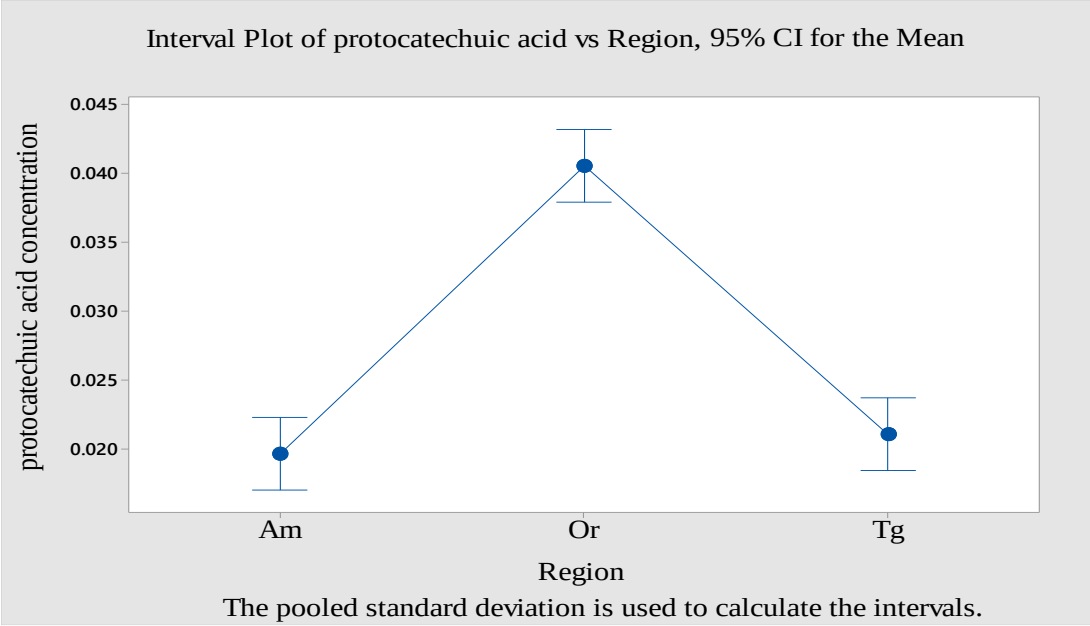


Figure 4: Concentrations of α-tocopherol hydroquinone (a) and hydroquinone (b) extracted from *S. longipedunculata* roots in three region samples (n=5). The vertical axis shows the concentration (µg/g dry weight) and horizontal axis the three region samples. Am stands for Amhara samples, Or for Oromia and Tg for Tigray region samples.

a.



b.

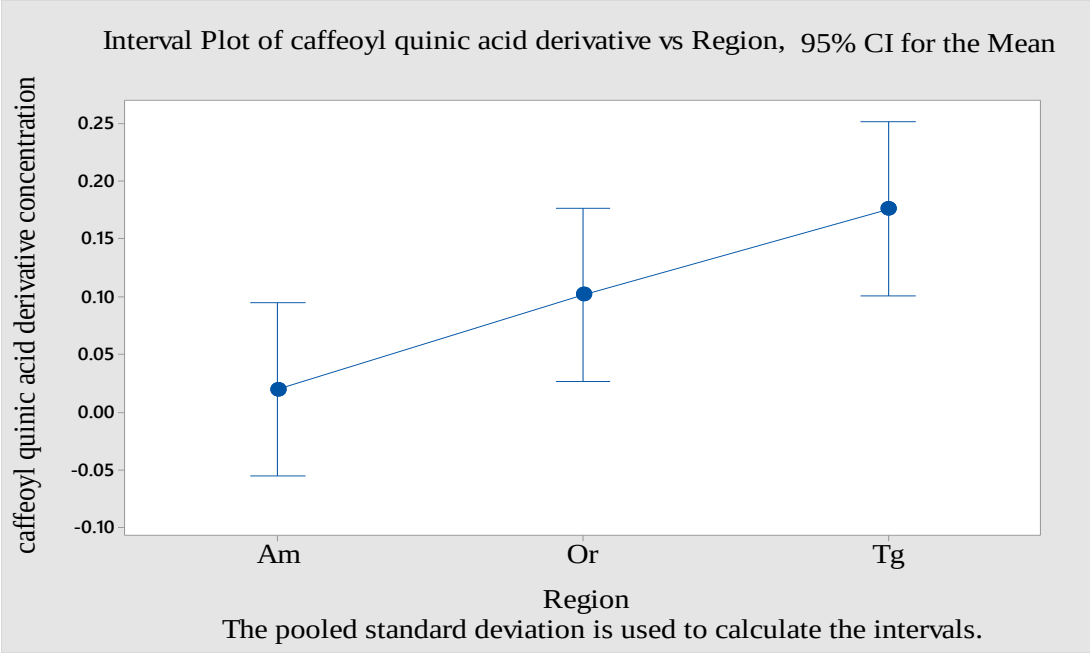


Figure 5: Concentrations of protocatechuic acid (a) and caffeoyl quinic acid derivative1(b) extracted from *S. longipedunculata* roots in three region samples (n=5). The vertical axis shows the concentration (µg/g dry weight) and horizontal axis the three region samples. Am stands for Amhara samples, Or for Oromia and Tg for Tigray region samples.

The leaf samples showed lower regional variability but concentrations were higher as compared to the root sample extracts. Highest metabolite concentrations in leaves were found for α-tocopherol hydroquinone followed by salicylic acid (Table 3).

Medicinally most important compounds which showed relatively high concentration in the three regions leaf samples were α-tocopherol hydroquinone, salicylic acid, α-tocopherol, gentisic acid, quercetin and kaempferol (Figure 6-8). Concentrations of the first two phenolic compounds were differed significantly between regions (Table 3) but these were the structurally-identified phenolic compounds 1 and 2, but since they were not identified, no focus was put on these metabolites.

The most significant compounds from the leaf extracts were α-tocopherol hydroquinone, salicylic acid, α-tocopherol, gentisic acid, quercetin and kaempferol (Figures 6-8).

Some compounds were found in highest concentration in leaves collected in Oromia (α-tocopherol hydroquinone and salicylic acid, Figure 6, kaempferol, Figure 8).

Using the PCA method, the distribution of samples based on the metabolite profiles of leaf extracts from the three regions (Figure 10) indicated different but partly overlapping sample clusters. Amhara and Oromia region extract were to some extent similar, while the Tigray region samples differed more from the other two regions.

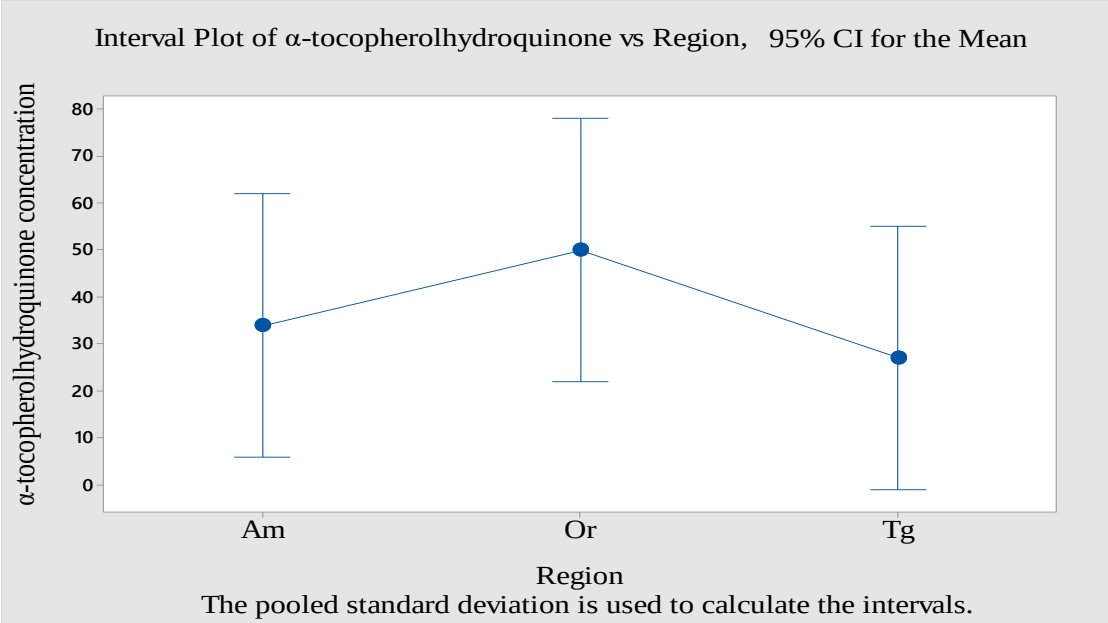
Table 3: Metabolite concentrations of 29 phytochemicals in leaf extracts *S. longipedunculata* from the three regions of Ethiopia. Average values for the three regions were calculated (n=5) and expressed in µg/g dry weight. The metabolites were arranged in order of higher to lower average values within the same category.

Phen stands for phenol, flav for flavonoids, ster for sterols, car for carotenoids, alkld for alkaloid, met for metabolite, str for structurally identified. Sig. stands for statistical significance (*p*-value),

which was calculated as for less than or equal to 0.001, ***, for less than or equal to 0.01, ** and for less than or equal to 0.05, *.

Compound	Class	Category	Average Amhara Leaf	Average Oromia Leaf	Average Tigray Leaf	p-value	Sig.
α-tocopherol	Phen	met	33.884	49.93	27.09	0.533	
hydroquinone							
salicylic acid	Phen	met	16.35	44.18	10.75	0.403	
4-hydroxy-2-methyl benzoic acid	Phen	met	0.41	0.25	10.10	0.827	
α-tocopherol	Phen	met	3.86	2.29	3.43	0.326	
gentisic acid	Phen	str	1.67	3.42	3.96	0.961	
caffeoyl quinic acid derivative1	Phen	str	2.11	2.34	1.68	0.852	
protocatechuic acid	Phen	met	1.65	0.86	3.30	0.472	
phyllodihydroquinone	Phen	met	2.20	2.02	1.58	0.691	
phenyl pyruvic acid	Phen	met	1.82	0.44	3.36	0.533	
benzoic acid	Phen	met	1.88	1.42	1.84	0.733	
(E)-ferulic acid	Phen	met	0.78	0.91	1.98	0.779	
m-coumaric acid	Phen	met	0.91	0.64	1.75	0.368	
(E)-sinapic acid	Phen	met	0.27	1.35	1.15	0.164	
phenolic compound1	Phen	str	0.41	1.73	0.03	0.007	**
4-hydroxybenzoic acid	Phen	met	0.55	0.70	0.66	0.677	
phenolic compound2	Phen	str	0.54	1.04	0.12	0.024	*
hydroquinone	Phen	met	0.27	0.23	0.21	0.472	
quercetin	Flav	met	75.24	50.34	59.53	0.403	
flavonoid5	Flav	str	33.42	31.99	76.72	1	
flavonoid3	Flav	str	7.83	10.76	10.24	0.651	
flavonoid4	Flav	str	3.16	4.42	4.38	0.811	
kaempferol	Flav	met	3.64	4.45	3.80	0.811	
(E)-phytol	Ter	met	5.53	4.70	3.55	0.482	
β-sitosterol derivative1	Ster	met	9.36	11.10	7.88	0.779	
sterol2	Ster	str	2.97	2.17	10.26	0.595	
stigmasterol	Ster	met	4.08	3.05	3.25	0.533	
sterol 4	Ster	met	3.76	2.66	2.94	0.691	
alkaloid1	Alkld	str	3.81	1.48	1.87	0.445	
carotenoid2	Car	str	0.48	0.34	4.23	0.472	

a.



b.

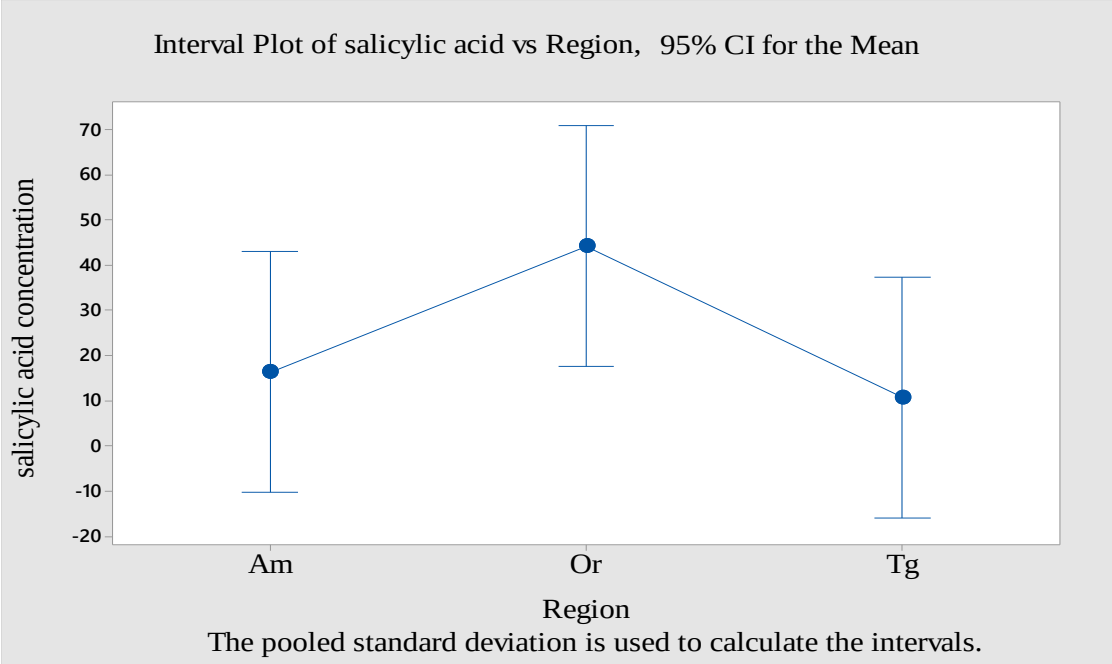
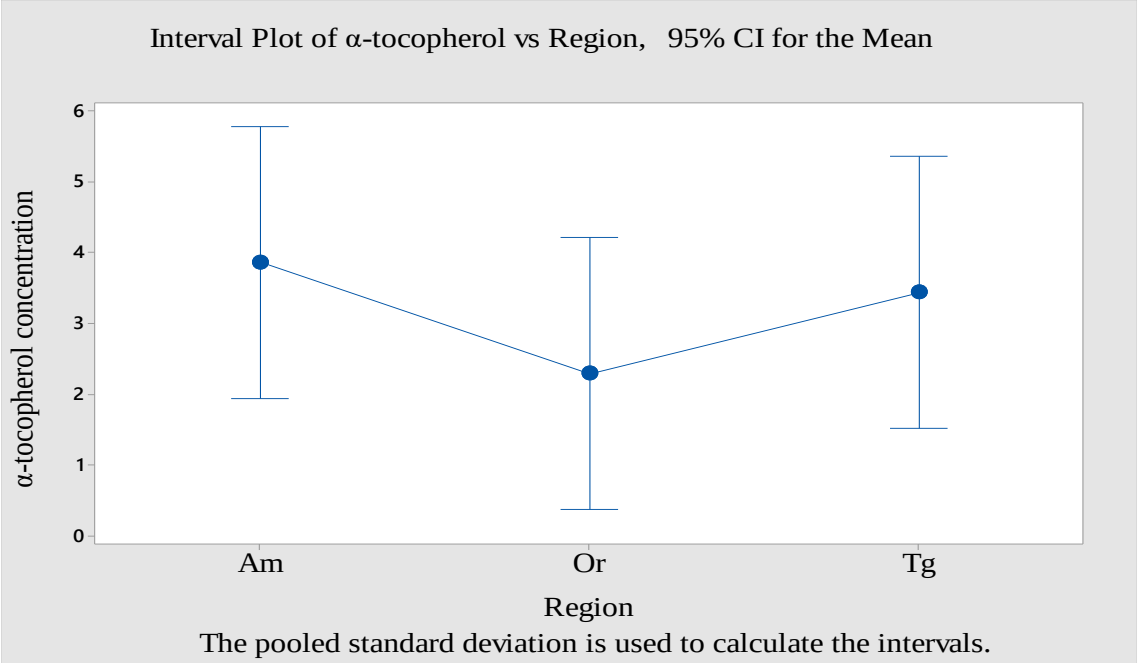


Figure 6: Concentrations of α-tocopherol hydroquinone (a) and salicylic acid (b) extracted from *S. longipedunculata* leaves in three region samples (n=5). The vertical axis shows the concentration (µg/g dry weight) and horizontal axis the three region samples. Am stands for Amhara samples, Or for Oromia and Tg for Tigray region samples.

a.



b.

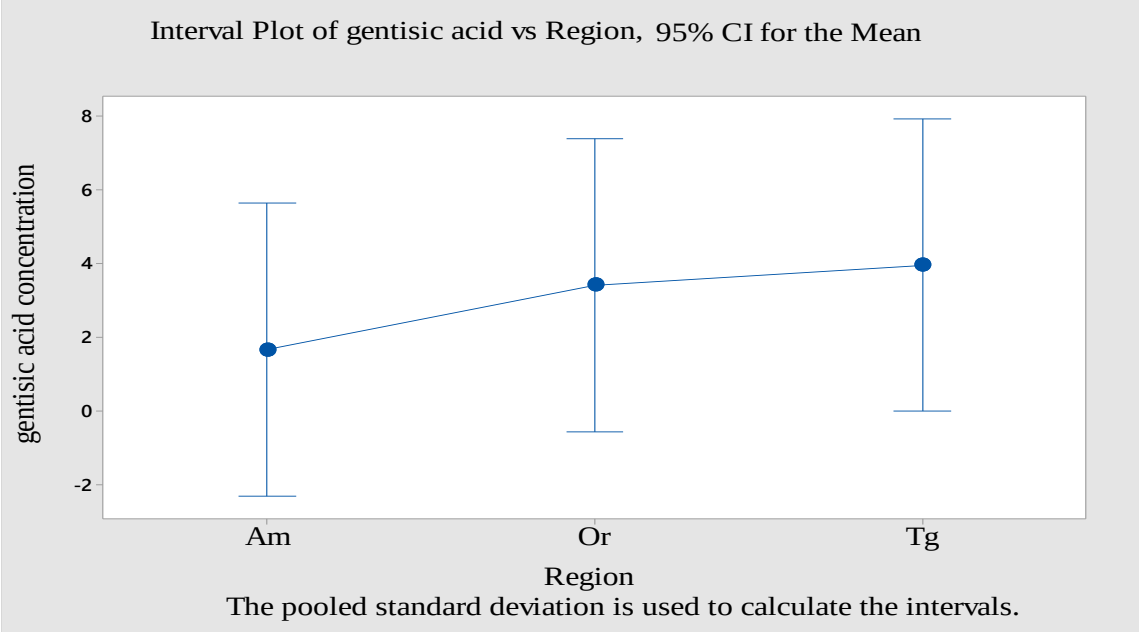
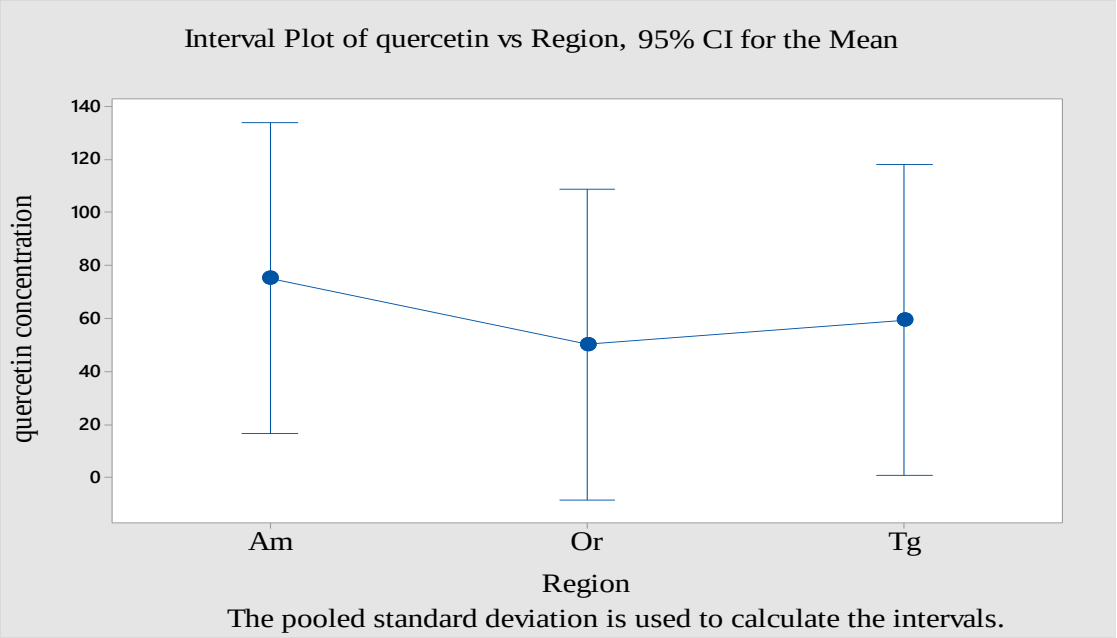


Figure 7: Concentrations of α-tocopherol (a) and gentisic acid (b) extracted from *S. longipedunculata* leaves in three region samples (n=5). The vertical axis shows the concentration (µg/g dry weight) and horizontal axis the three region samples. Am stands for Amhara samples, Or for Oromia and Tg for Tigray region samples.

a.



b.

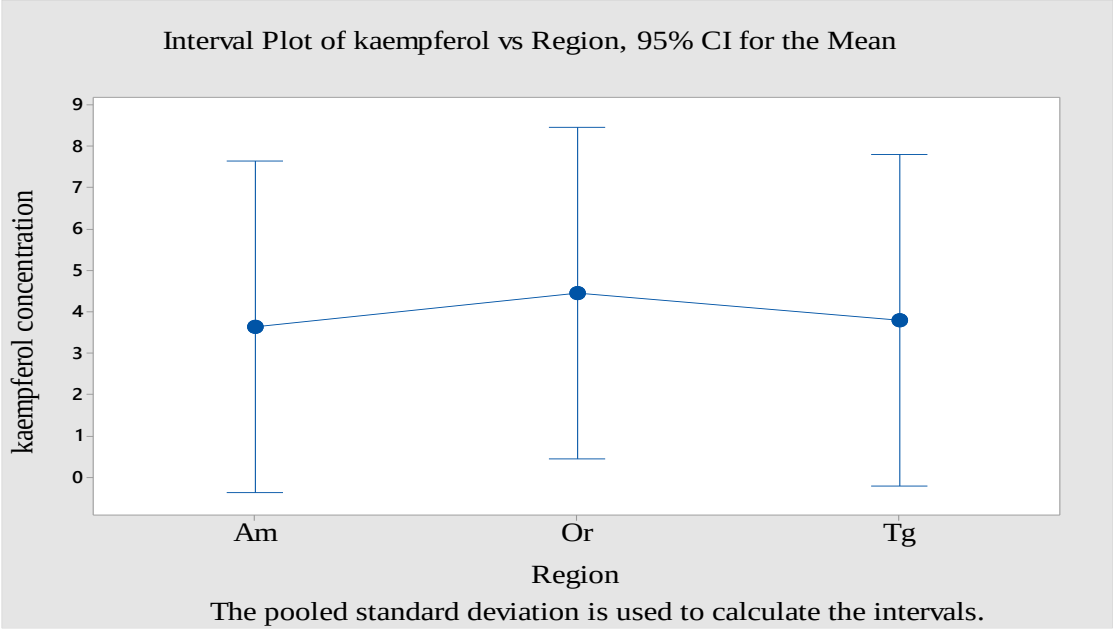


Figure 8: Concentrations of quercetin (a) and kaempferol (b) extracted from *S. longipedunculata* leaves in three region samples (n=5). The vertical axis shows the concentration (µg/g dry weight) and horizontal axis the three region samples. Am stands for Amhara samples, Or for Oromia and Tg for Tigray region samples.

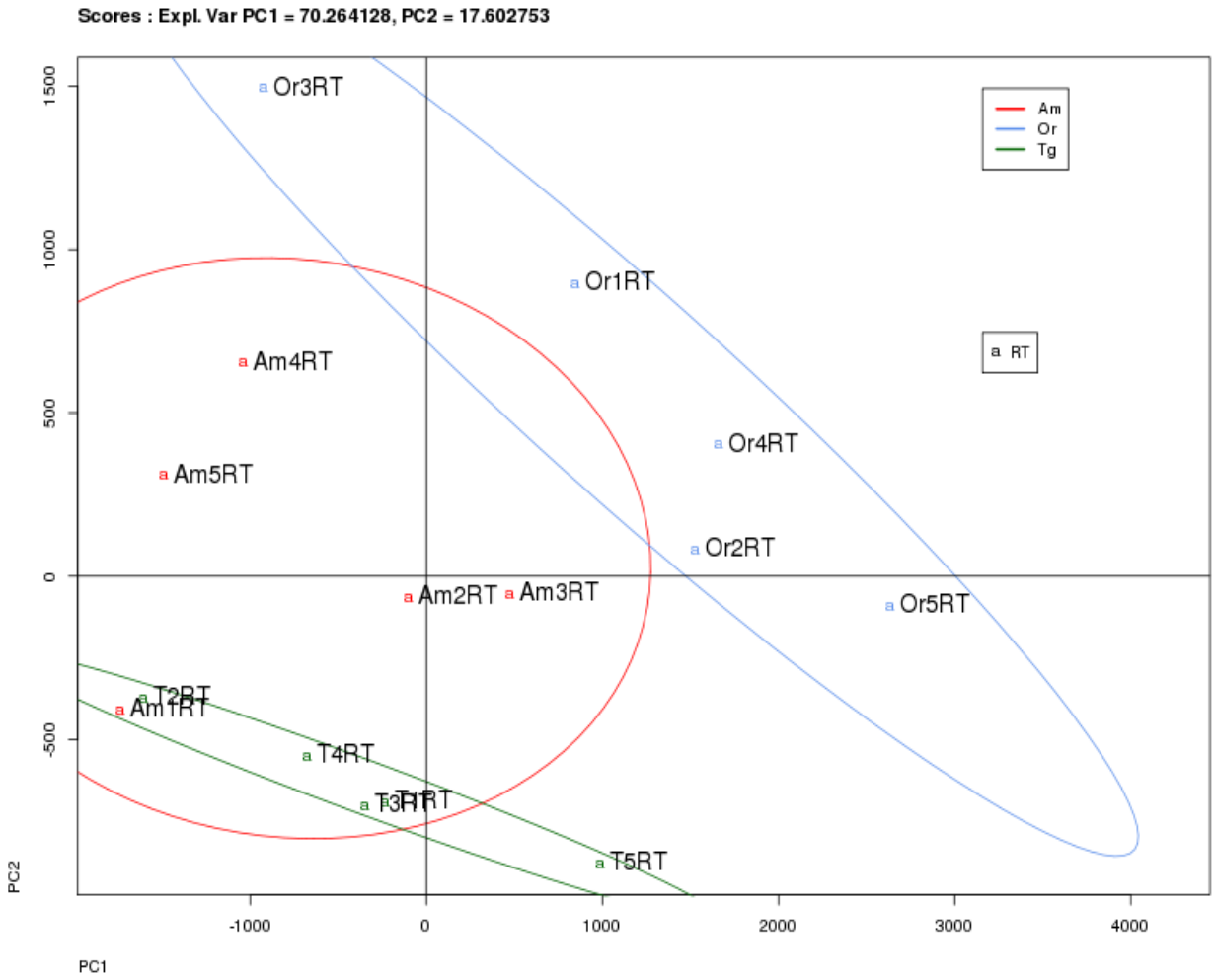


Figure 9: The distribution of samples based on metabolite profiles of 110 compounds extracted from roots in the three regions. The circles indicate the distributional separation between regions. Principal component 1 (PC1) explains the distribution of samples horizontally, which covers 70.3% of variation in the data set. PC2 indicates the distribution of samples vertically, covering 17.6% of variation.

PC1 stands for principal component one, PC2 for principal component two, Am for Amhara region samples, Or for Oromia region samples and Tg for Tigray region samples, AmRT for Amhara roots, OrRT for Oromia roots and OrRT for Oromia root samples.

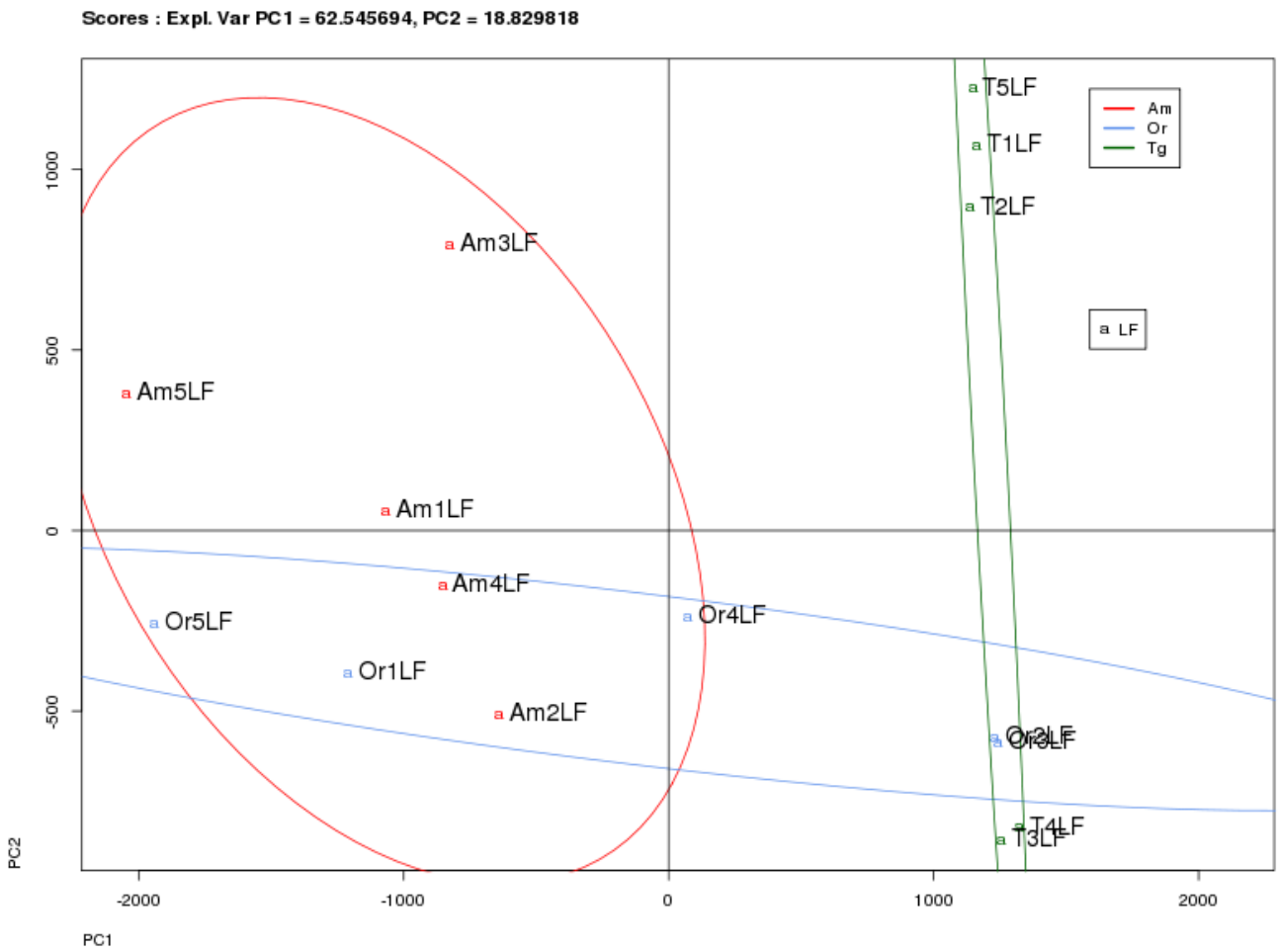


Figure 10: The distribution of samples based on metabolite profiles of 110 extracted compounds from leaves in the three regions. The circles indicate the distributional separation between regions. PC1 explains the distribution of samples horizontally, which covers 62.6% of variation in the data set. PC2 affects the distribution of samples vertically, covering 18.8% of variation.

PC1 stands for principal component one, PC2 for principal component two, Am for Amhara region samples, Or for Oromia region samples and Tg for Tigray region samples, AmLF = Amhara region leaf, OrLF = Oromia region leaf, TLF = Tigray region leaf extracts.

3.3 Essential oil volatiles

Essential oil volatiles were extracted by SPME and 125 compounds were detected. 96 volatile compounds could be tentatively identified, 22 structurally identified, and 7 remained unknown. In total, 53 volatiles showed significant differences between regions from the root samples (Table 3), while 47 volatiles showed significant differences between regions for the leaf samples (Table 4). This plant was rich in many volatile compounds found in both tissues and all region samples. Some volatiles were higher in concentration but without showing significant difference between regions, like methyl salicylate and methyl 4-methoxysalicylate in the root samples (Table 3) and methyl 4-methoxysalicylate in the leaf sample extracts (Table 4).

3.4 Tissue and regional variations

There were variations in concentration of volatile compounds between the two tissues of leaf and root and between the three regions. From all the extracted volatile compounds showing significant difference, including the two major non-significant salicylates, 18 phenols were found in the root samples and 16 phenols in leaf samples (Table 3 and 4). Reasonable amounts of mono terpenes were found in both the roots and leaf samples. Moreover, aldehydes, alkanes, ketones, esters, acids and alcohols were also found in significant amount in samples of all regions. Most of the volatiles detected in both the leaves and roots were phenols, followed by monoterpene compounds as shown in Table 3 and 4.

Some of the volatiles were higher in concentration in Tigray root samples (methyl salicylate and benzyl alcohol, Figure 11, phenyl ethyl alcohol and geranyl acetone, Figure 12, and benzaldehyde and acetophenone, Figure 13).

The following volatiles were detected at higher levels in Tigray leaves: dihydro actinidiolide, Figure 14, phenyl ethyl alcohol and geranyl acetone, Figure 15, and cumin aldehyde, Figure 16. Variation of volatiles between the three region samples was observed indicating differences in average values of concentration. The concentration of volatiles was high in Tigray region sample extracts for majorities of the compounds. For large number of volatiles, the regional variability indicated significant differences.

The most abundant volatile compound in the roots and leaf extracts was methyl salicylate, followed by methyl 4-methoxysalicylate (Table 4 and 5). Levels of methyl salicylate were not significantly different between regions in root samples but were significant different in leaf samples. Methyl 4-methoxysalicylate did not show differences between regions (Table 4 and 5).

Based on PCA analysis of 125 volatiles in the three regions, root samples showed different but overlapping clusters (Figure 17). Volatile profiles of leaf samples from the three regions were to some extent similar, showing more clearly separated sample groups but also overlapping clusters (Figure 18). Amhara and Oromia region extract were somewhat similar, while the Tigray region samples differed more from the other two regions.

Table 4: Average levels of 49 volatile compounds (detector response, arbitrary units) in root samples of *S. longipedunculata* from the three regions of Ethiopia (n=5). The volatiles were arranged in the order from higher to lower average values within the same category.

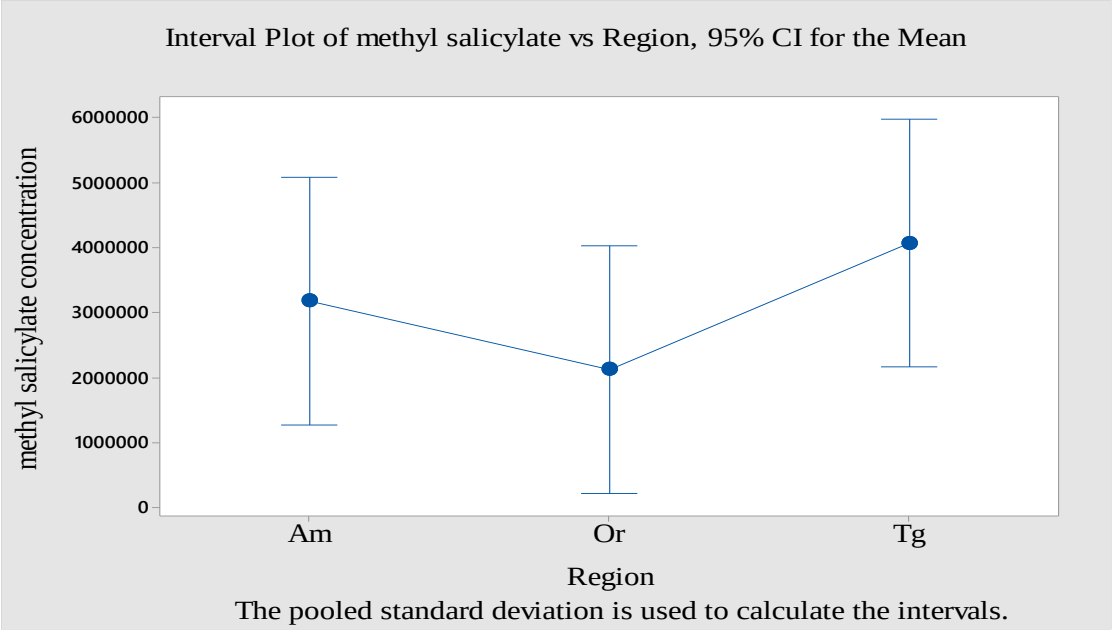
Phen stands for phenol, mt for monoterpenes, st for sesquiterpene, ald for aldehyde, alk for alkane, ket for ketone, est for ester, ac for acid, alc for alcohol, na for not annotated, met for metabolite, str for structurally identified, ns for not significant, Sig. stands for statistical significance (p-value),

which was calculated as for less than or equal to 0.001, ***, for less than or equal to 0.01, ** and for less than or equal to 0.05, *.

Compound	Class	Category	Average Amhara Root	Average Oromia Root	Average Tigray Root	P-value	Sig.
methyl salicylate	phen	met	3179327	2131657	4067866	0.249	Ns
methyl 4-methoxysalicylate	phen	met	453004	903570	377269	0.054	Ns
benzyl alcohol	phen	met	1300	4449	41278	0.018	*
3-(benzyloxy)-6-(hydroxymethyl)-2,5-dimethylphenol	phen	met	3967	4036	22775	0.009	**
phenyl ethyl alcohol	phen	met	1492	2434	24162	0.013	*
benzaldehyde	phen	met	1248	1257	21168	0.008	**
phenanthrene derivative3	phen	str	1584	1604	12840	0.009	**
1,2-diacetoxy-4-allylbenzene	phen	met	2131	1383	11601	0.008	**
phenanthrene derivative2	phen	str	1474	1598	11769	0.009	**
cumin aldehyde	phen	met	1892	1663	11110	0.019	*
dihydro actinidiolide	phen	met	1973	1925	9188	0.019	*
1-hydroxycumene	phen	met	230	566	11432	0.009	**
phenanthrene derivative1	phen	str	1217	1280	9689	0.007	**
acetophenone	phen	met	190	748	10903	0.008	**
4-methoxybenzaldehyde	phen	Met	1377	1360	8135	0.026	*
naphthalene	phen	met	504	210	8760	0.009	**
benzyl acetate	phen	met	434	210	5836	0.019	*
benzoic acid derivative1	phen	met	842	621	150	0.042	*
geranyl acetone	mt	met	18370	15430	73582	0.042	*
dihydro myrcenol	mt	met	2202	9098	76458	0.009	**
3,5-octadien-2-one	mt	met	723	2981	33544	0.009	**
β-ionone	mt	met	4769	2566	19415	0.017	*
linalool	mt	met	4032	575	13291	0.03	*
β-methyl ionone	mt	met	190	495	3909	0.023	*
α-terpineol	mt	met	482	170	3760	0.047	*
hexahydrofarnesyl acetone	St	met	1280	4651	16454	0.037	*
nonanal	ald	met	2066	6477	236548	0.009	**
decanal	ald	met	6814	1448	171800	0.008	**
octanal	ald	met	492	1757	99243	0.008	**
hexanal	ald	met	685	2173	59911	0.008	**
(E, E)-2,4-heptadienal	ald	met	210	1596	15512	0.009	**
(E)-2-nonenal	ald	met	150	210	8481	0.005	**
undecanal	ald	met	170	797	7065	0.015	*
hexadecane	alk	met	1996	1198	12243	0.008	**

tetradecane	alk	met	1094	1015	10737	0.009	**
alkane5	alk	str	948	687	5896	0.009	**
6-methyl-5-hepten-2-one	ket	met	3356	2283	49738	0.009	**
(E)-6-methyl-3,5-heptadien-2-one	ket	met	1489	3976	48204	0.013	*
1-acetylcyclohexene	ket	met	210	1506	10210	0.009	**
2-hydroxy-5-ethyl-5-methylcyclopent-2-en-1-one	ket	met	210	671	4734	0.03	*
3-methylbutyl butanoate	est	met	801	17541	203	0.03	*
nonanoic acid	Ac	met	1031	1144	20955	0.009	**
hexanoic acid	Ac	met	210	1155	18614	0.009	**
alcohol3	alc	str	5569	2283	17980	0.039	*
2-ethyl hexanol	alc	met	1003	249	10362	0.008	**
1-hexanol	alc	met	1084	2519	170	0.048	*
NA4	Na	na	2191	1351	42219	0.008	**
NA1	Na	na	210	927	19392	0.009	**
NA3	Na	na	403	210	2854	0.03	*

a.



b.

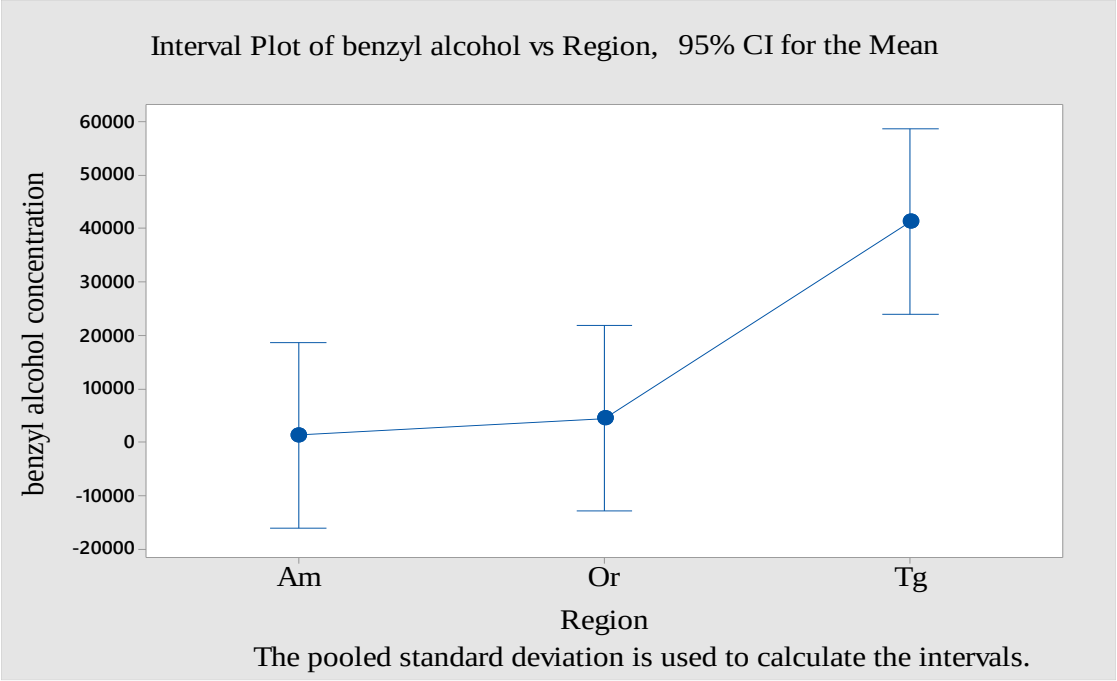
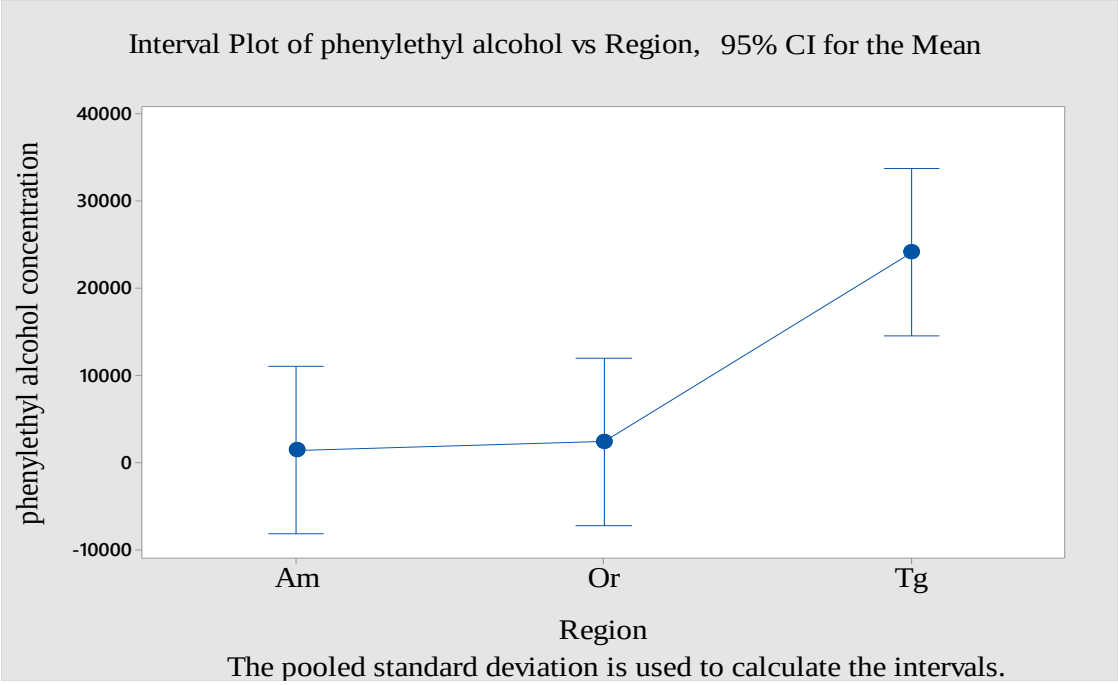


Figure 11: Concentrations of methyl salicylate (a) and benzyl alcohol (b) extracted from *S. longipedunculata* roots in three region samples (n=5). The vertical axis shows the concentration (detector response, arbitrary units) and horizontal axis the three region samples. Am stands for Amhara samples, Or for Oromia and Tg for Tigray region samples.

a.



b.

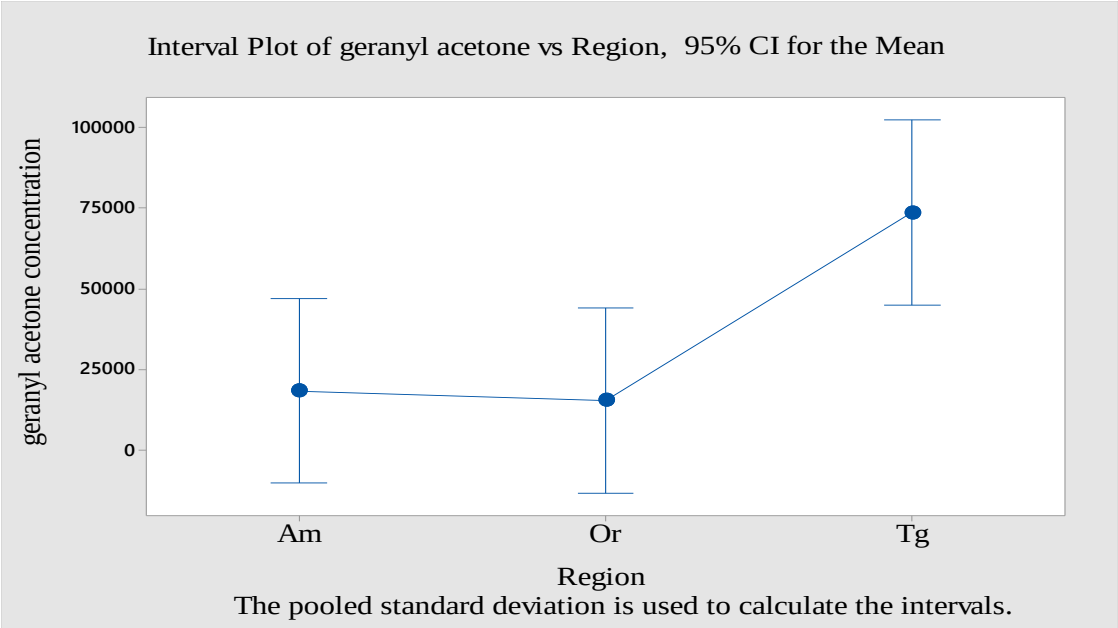
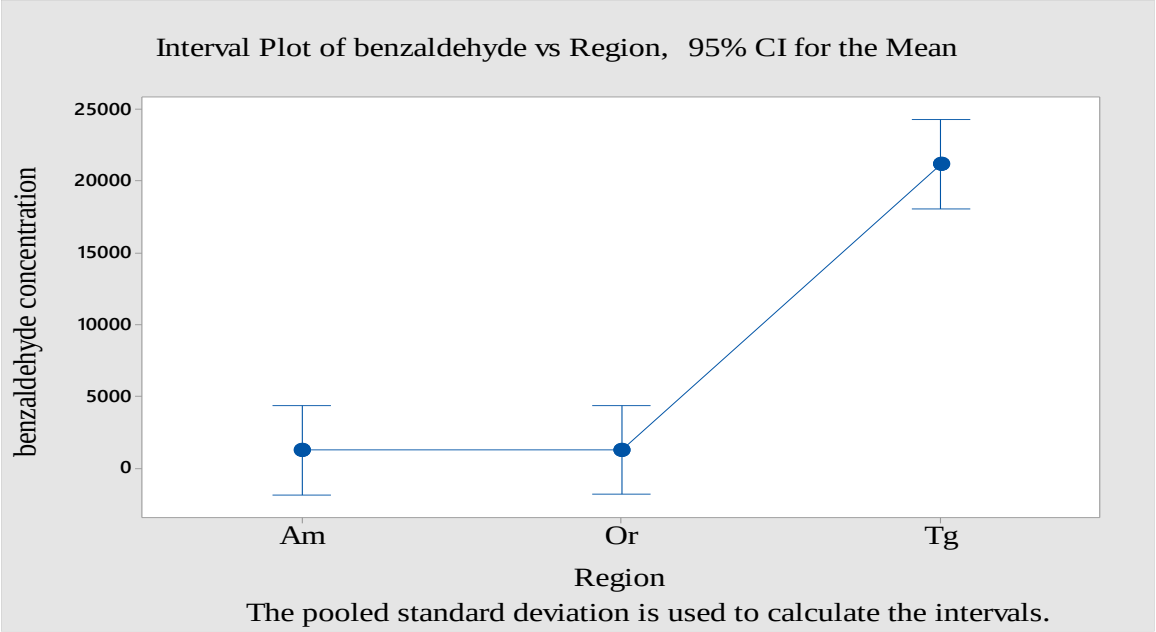


Figure 12: Concentrations of phenyl ethyl alcohol (a) and geranyl acetone (a) extracted from *S. longipedunculata* roots in three region samples (n=5). The vertical axis shows the concentration (detector response, arbitrary units) and horizontal axis the three region samples. Am stands for Amhara samples, Or for Oromia and Tg for Tigray region samples.

a.



b.

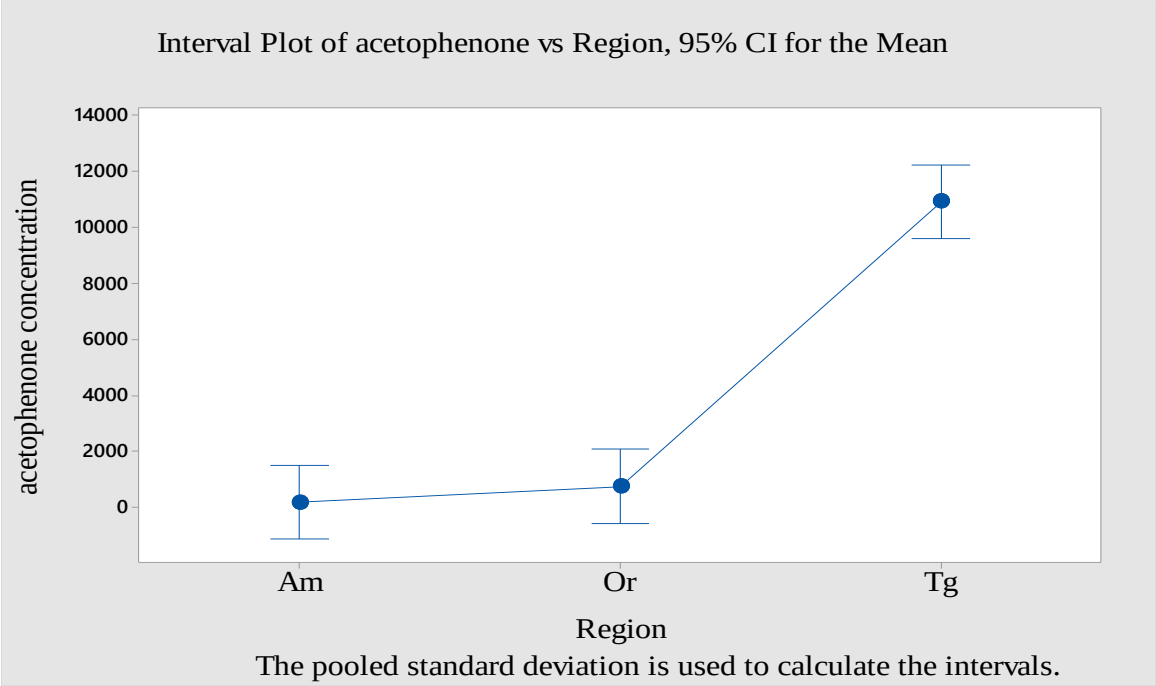


Figure 13: Concentrations of benzaldehyde (a) and acetophenone (b) extracted from *S. longipedunculata* roots in three region samples (n=5). The vertical axis shows the concentration (detector response, arbitrary units) and horizontal axis the three region samples. Am stands for Amhara samples, Or for Oromia and Tg for Tigray region samples.

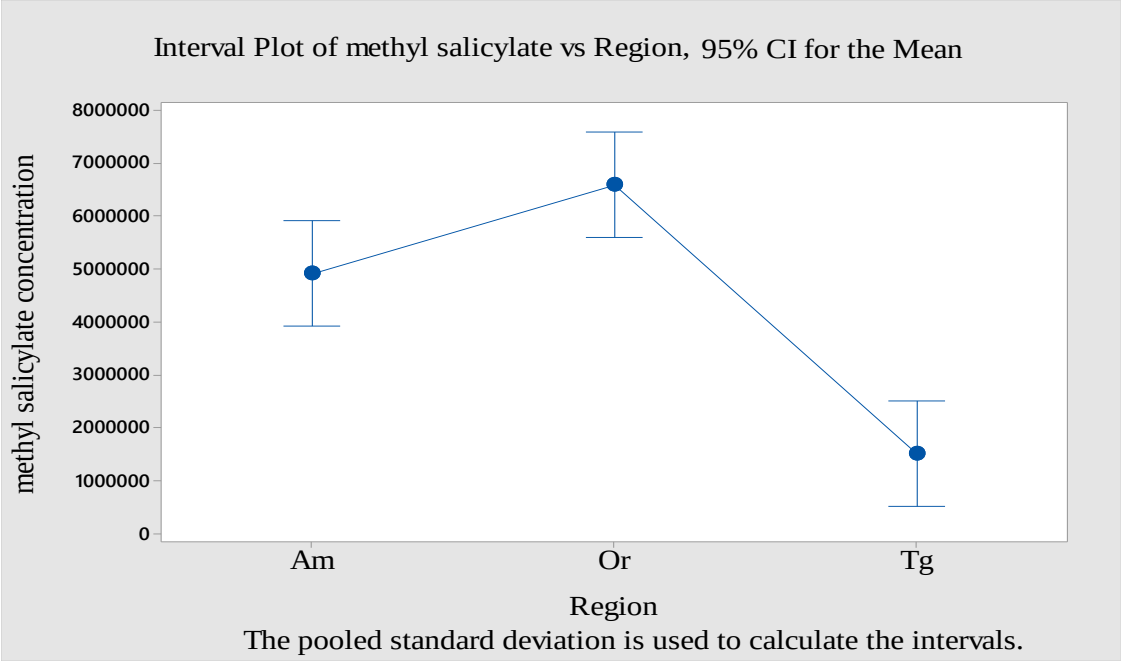
Table 5: Average levels of 54 volatile compounds (detector response, arbitrary units) in leaf samples of *S. longipedunculata* from the three regions of Ethiopia (n=5). The volatiles were arranged in the order from higher to lower average values within the same category.

Phen stands for phenol, mt for monoterpenes, tt for triterpene, st for sesquiterpene, ald for aldehyde, alk for alkane, ket for ketone, est for ester, ac for acid, alc for alcohol, fur for furanone, na for not annotated, met for metabolite, str for structurally identified, ns for not significant, Sig. stands for statistical significance (p=value), which was calculated as for less than or equal to 0.001, ***, for less than or equal to 0.01, ** and for less than or equal to 0.05, *.

Compound	Class	category	Average Amhara Leaf	Average Oromia Leaf	Average Tigray Leaf	P-value	Sig.
methyl salicylate	phen	met	4919777	6593653	1507936	0.004	**
methyl 4-methoxysalicylate	phen	met	135296	228029	461598	0.181	ns
dihydro actinidiolide	phen	met	26481	23045	87465	0.032	*
phenyl ethyl alcohol	phen	met	45104	20108	61475	0.022	*
aromatic compound1	phen	str	12857	49441	40747	0.046	*
phenanthrene derivative3	phen	str	12849	7472	37562	0.006	**
phenanthrene derivative2	phen	str	12771	7923	31270	0.008	**
6-ethyltetralin	phen	met	1178	170	42926	0.007	**
tetralin derivative1	phen	str	1086	170	42186	0.007	**
phenanthrene derivative1	phen	str	10097	5415	25930	0.011	*
p-methyl anisole	phen	met	170	2045	37332	0.006	**
cumin aldehyde	phen	met	12933	6830	16796	0.014	*
naphthalene	phen	met	12291	3733	18240	0.012	*
hexyl salicylate	phen	met	16374	3936	13000	0.026	*
benzyl acetate	phen	met	17854	1519	10679	0.033	*
3-methoxyphenol	phen	met	210	4864	24485	0.022	*
geranyl acetone	Mt	met	84442	109704	422510	0.008	**
dihydro myrcenol	Mt	met	23041	27870	122947	0.013	*
β-ionone	Mt	met	61513	24867	73135	0.023	*
linalyl acetate	Mt	met	44023	6921	26223	0.035	*
linalool	Mt	met	40124	8023	19987	0.032	*
limonene	Mt	met	29167	7110	28850	0.032	*
3,5-octadien-2-one	Mt	met	4445	5530	28503	0.009	**
β-methyl ionone	Mt	met	8532	4224	18694	0.008	**

terpene1	Mt	str	617	716	6452	0.008	**
triterpene	Tt	str	170	514	5771	0.017	*
hexahydrofarnesyl acetone	St	met	57087	114737	523697	0.009	**
β-cedrene epoxide	St	met	24243	50657	53360	0.048	*
hexahydrofarnesol	St	met	5804	2354	17278	0.006	**
sesquiterpene oxygenated1	St	str	11763	1204	5302	0.008	**
decanal	Ald	met	39845	84720	124438	0.038	*
nonanal	Ald	met	29501	31442	148124	0.009	**
(E, E)-2,4-heptadienal	Ald	met	14531	10374	52299	0.008	**
hexanal	Ald	met	6158	5343	32633	0.012	*
(E)-2-hexenal	Ald	met	3846	3058	20783	0.024	*
tetradecane	Alk	met	16886	10256	30615	0.021	*
hexadecane	Alk	met	16049	6557	29555	0.008	**
alkane1	Alk	str	9160	5555	29518	0.011	*
alkane4	Alk	str	2585	6521	28774	0.017	*
(E)-6-methyl-3,5-heptadien-2-one	Ket	met	37001	69222	460368	0.008	**
6-methyl-5-hepten-2-one	Ket	met	74664	68673	293008	0.009	**
1-acetylcyclohexene	Ket	met	2232	16188	80752	0.006	**
cyclohexenone derivative	Ket	str	42924	23337	830	0.015	*
2-hydroxy-5-ethyl-5-methylcyclopent-2-en-1-one	Ket	met	2144	6377	43466	0.008	**
1-methylbutyl butanoate	Est	met	31059	1810	1335	0.042	*
2-pentyl acetate	Est	met	20682	542	230	0.041	*
nonanoic acid	Ac	met	22687	16049	46806	0.009	**
hexanoic acid	Ac	met	16935	11406	25486	0.022	*
decanoic acid	Ac	met	6595	6734	17021	0.009	**
2,6-dimethylcyclohexanol	Alc	met	25498	5001	18451	0.013	*
furan derivative1	Fur	str	7802	4425	23628	0.01	**
NA5	Na	na	19763	12977	37191	0.022	*
NA1	Na	na	6485	9237	42644	0.009	**

a.



b.

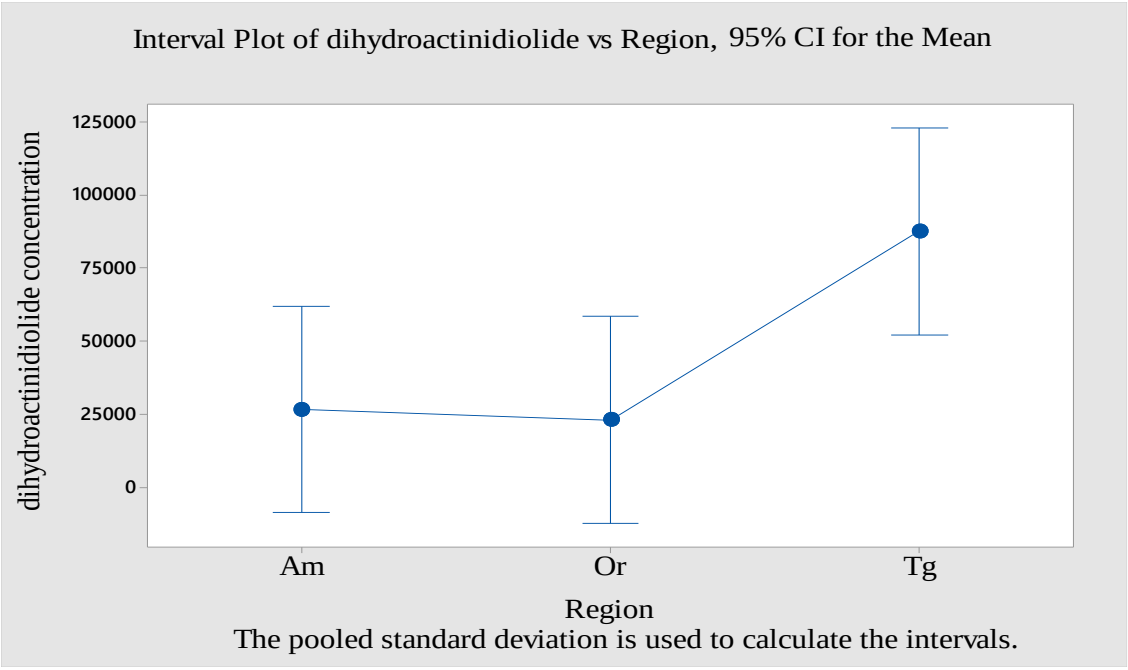
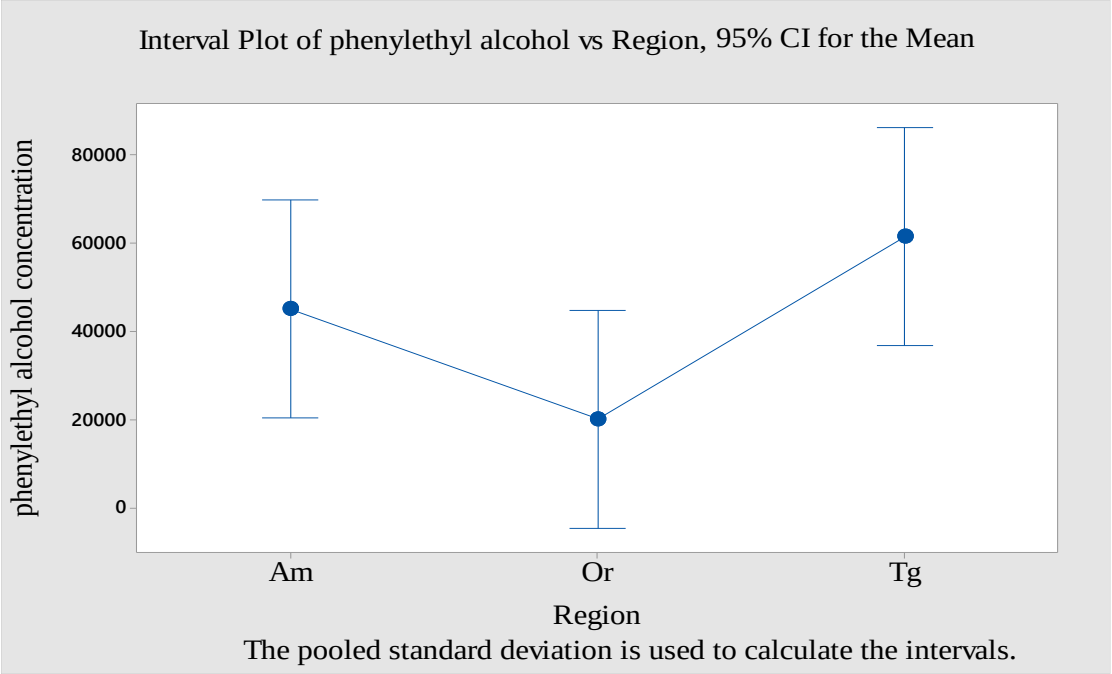


Figure 14: Concentrations of methyl salicylate (a) and dihydro actinidiolide (b) extracted from *S. longipedunculata* leaves in three region samples (n=5). The vertical axis shows the concentration (detector response, arbitrary units) and horizontal axis the three region samples. Am stands for Amhara samples, Or for Oromia and Tg for Tigray region samples.

a.



b.

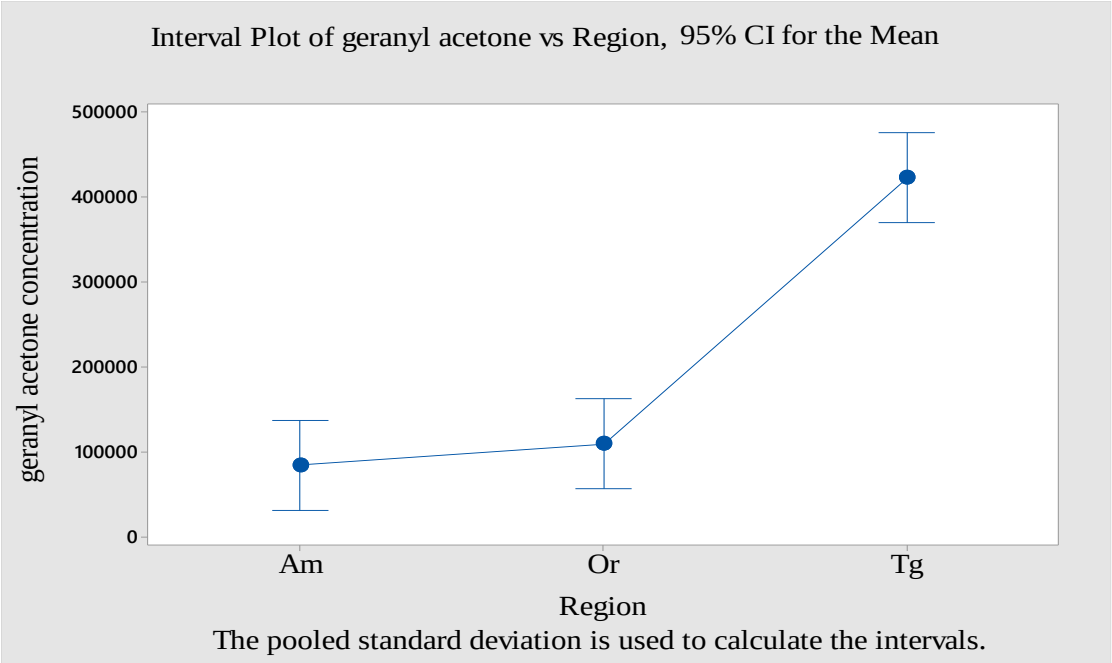
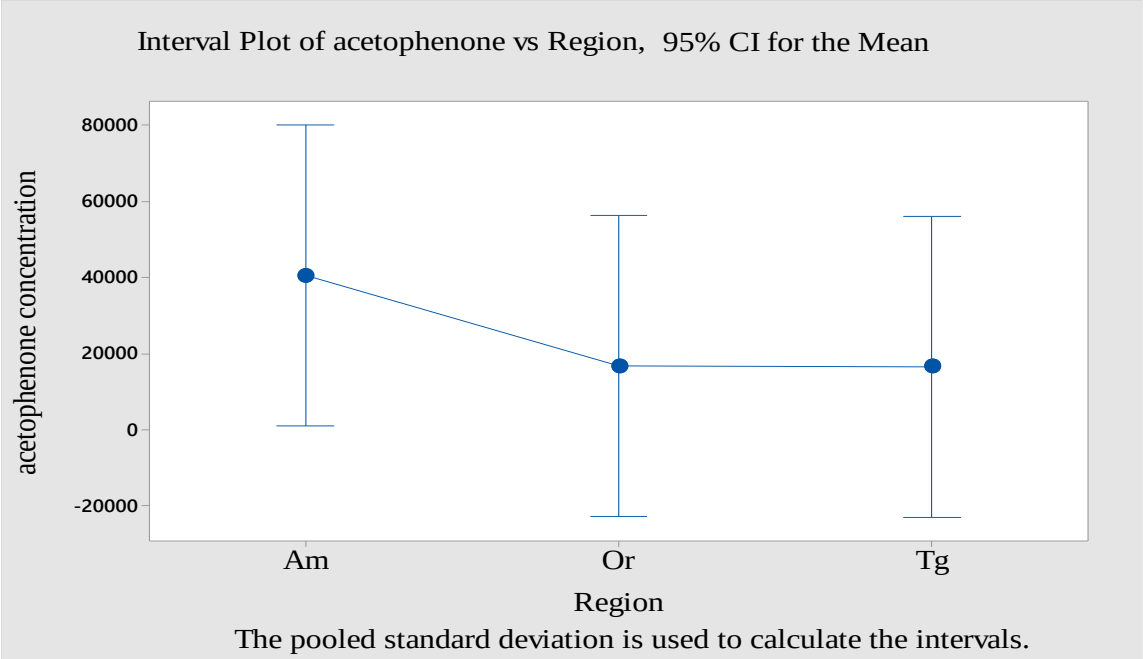


Figure 15: Concentrations of phenyl ethyl alcohol (a) and geranyl acetone (b) extracted from *S. longipedunculata* leaves in three region samples (n=5). The vertical axis shows the concentration (detector response, arbitrary units) and horizontal axis the three region samples. Am stands for Amhara samples, Or for Oromia and Tg for Tigray region samples.

a.



b.

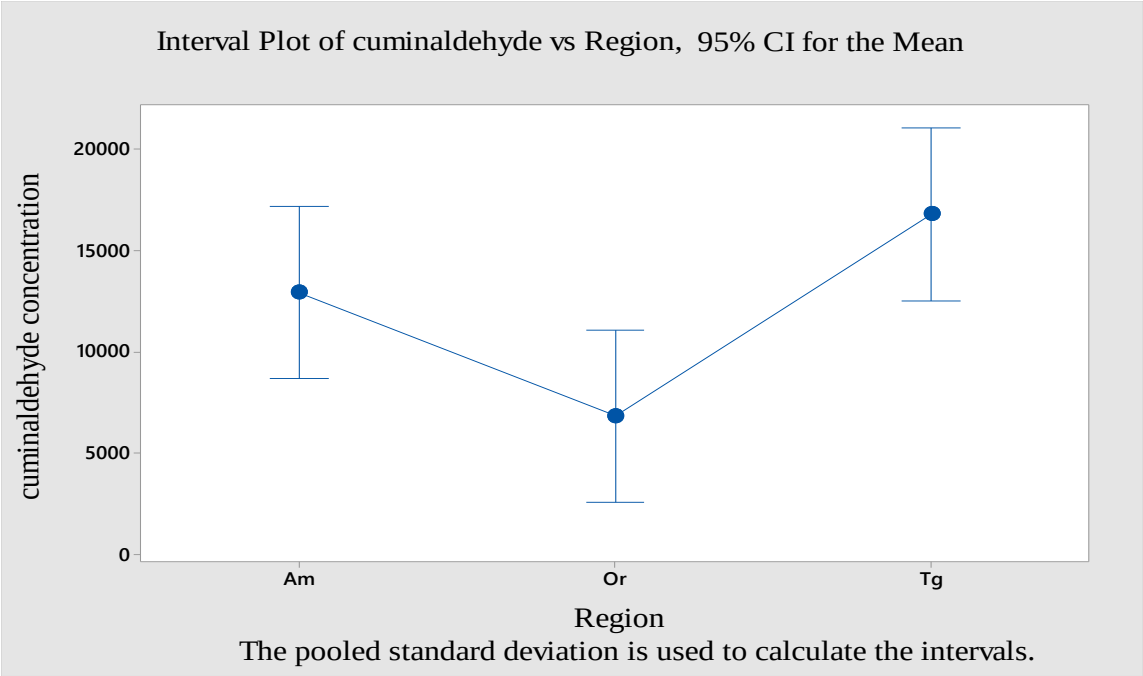


Figure 16: Concentrations of acetophenone (a) and cumin aldehyde (b) extracted from *S. longipedunculata* leaves in three region samples (n=5). The vertical axis shows the concentration (detector response, arbitrary units) and horizontal axis the three region samples. Am stands for Amhara samples, Or for Oromia and Tg for Tigray region samples.

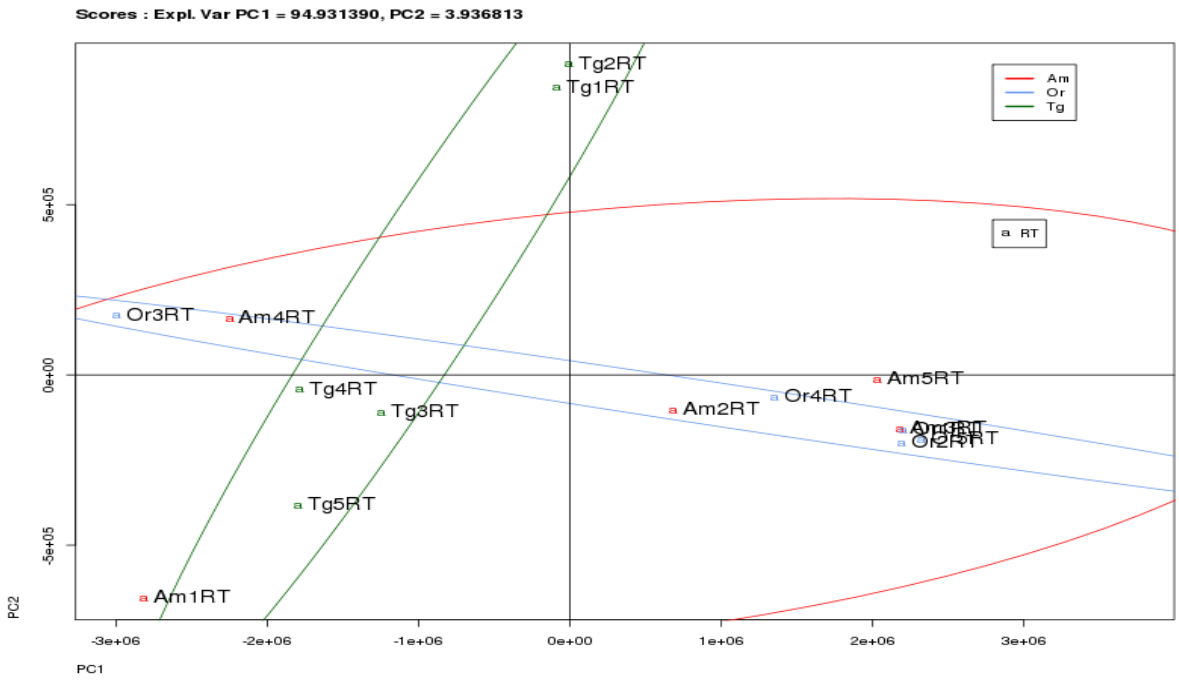


Figure 17: The distribution of samples based on volatile profiles of 125 extracted compounds from *S. longipedunculata* roots in the three regions using SPME. The circles indicate the distributional separation between regions. The PC1 explains the distribution of samples horizontally, which covers 94.9 % of variation in the data set. PC2 affects the distribution of samples vertically, covering 3.9 % of variation.

PC1 stands for principal component one, PC2 for principal component two, Am for Amhara region samples, Or for Oromia region samples and Tg for Tigray region samples, AmRT for Amhara roots, OrRT for Oromia roots, TgRT for Oromia root samples and five samples from each region (AmRT 1-5, OrRT 1-5 and TgRT 1-5).

Root samples from the three regions showed different but overlapping clusters (Figure 15). All the three regions extract were to some extent similar. Most of the variation was explained by PC1 (94.9 %) and to less extent by PC2 (3.9 %).

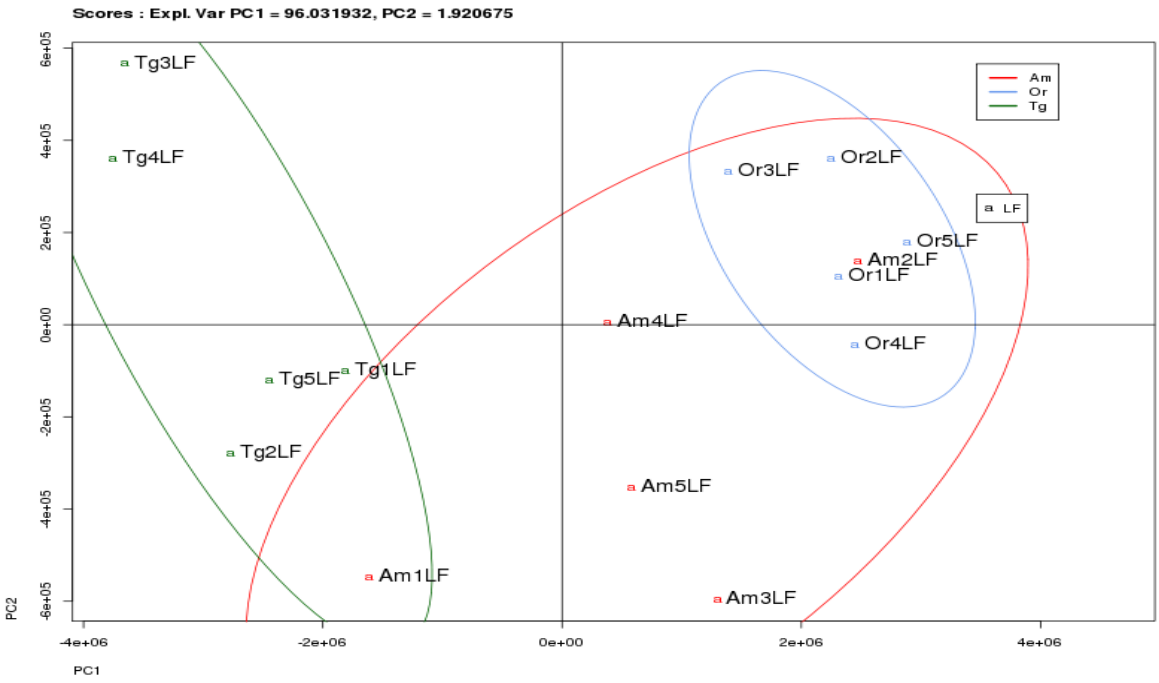


Figure 18: The distribution of samples based on volatile profiles of 125 extracted compounds from *S. longipedunculata* leaves in the three regions using SPME. The circles indicate the separation between the regions. The PC1 explains the distribution of compounds horizontally, which covers 96.0 % of variation in the data set. PC2 explains the distribution of compounds vertically, covering 1.9 % of variation.

PC1 stands for principal component one, PC2 for principal component two, Am for Amhara region samples, Or for Oromia region samples and Tg for Tigray region samples, AmLF = Amhara region leaf, OrLF = Oromia region leaf, TLF = Tigray region leaf samples and five samples from each region (AmLF 1-5, OrLF 1-5 and TgLF 1-5).

Leaf samples from the three regions showed clear cluster groups partly overlapping (Figure 16). Amhara and Oromia region samples were somewhat similar, while the Tigray region samples differed more from the other two regions. Most of the variation was explained by PC1 (96.0 %) and to less extent by PC2 (1.9 %).

4. Discussion

4.1 Metabolite profiling of polar compounds

The results showed that many phytochemicals (phenolic compound, phenols and flavonoids, 44) were extracted and identified, using a GC/MS metabolite profiling technique. From this plant, there were some phytochemicals identified from previous works like phenolic acids of sinapic acid, 4,5-dicaffeoyl-D-quinic acid, caffeic acid and 3,4,5-tricaffeoyl-D-quinic acid from quercetin, p-coumaric acid, cinnamic acid, caffeic acid and chlorogenic acid from Muanda et al. (2010). From Okoli et al (2006) studied anti-inflammatory activity of extracts of root bark of *Securidaca longipedunculata*, found extracts had flavonoids, saponins, terpenoids and sterols. There were many other phytochemicals were extracted from this plant but by using the method of GC/MS metabolite profiling technique but there were no available works which shows the extraction of so many compounds by using the GC/MS method in this plant.

From the results the extracted compounds were rich in disaccharides, phenols, flavonoids and amino acids. Since the study was aimed at the biodiversity of potentially bioactive compounds, my focus was on the phenols, flavonoids and other phytochemicals. For this plant the previous works were limited on the chemical composition studies but in some studies the type of metabolites extracted were very small number, Ekwy et al (2015) who studied the phytochemical and GC/MS analyses of the bioactive components of *Securidaca longepedunculata* roots for anti-breast cancer activity, found the ethanol roots extracts show the presence of tannins, alkaloids, saponins, saponins glycosides, cardiac glycosides, terpenes, and steroids.

Muanda et al. (2010) studied polyphenolic compounds, in vitro antioxidant and anti-inflammation properties of *Securidaca longepedunculata* root barks, and measured gallic acid, chlorogenic acid,

caffeic acid, epicatechin, p-coumaric acid, rutin, quercetin glucosyl, quercetin dihydrat, cinnamic acid, quercetin and apigenin by RP-HPLC and UV spectrophotometry.

Mongalo et al. (2015) investigated the ethnomedicinal uses and chemical composition of this plant, the root extracts yielded flavonoids, saponins, terpenoids and some steroids. This shows that our results are better in number of compounds and types for this plant.

4.2 Tissue and regional Variations

The results showed variations in concentration of metabolites (phenolics) between the two tissues of leaves and roots, the leaf extracts were somewhat higher.

In this study, the higher concentration of phenolics extracted from the leaf samples were the new very important result for this plant. The most studied part was the root bark and its phytochemicals were used for medicinal purposes in most studies. Using the root was dangerous to the plant survival as the traditional healers use this part by killing the whole plant. So, based on the identified phenolics, the leaf part had higher concentrations, which was important to solve the problem of losing the whole plant for the root purposes and instead we suggest to use the leaf part without damaging the whole plant part.

There were some studies on the leaves of this plant which had reasonable concentrations for some phenolics. Olaleye et al. (1998) studied the toxicity and antimicrobial effect of this plant, high concentrations of glycosides, saponins, tannins and alkaloids were found in the leaf extracts, and the root extracts contained saponins, tannins, and alkaloids in significant amounts. And from Debella et al., (2000) who studied the leaf composition of this plant, found quercetin indicating that use of the leaf might be a good source of phytochemicals. Our results showed that most important bioactive metabolites used for medicinal purpose were found in both tissues in almost

similar amounts emphasizing that focus of medicinal investigations might be on the leaf tissue of *S. longipedunculata*.

Variation of phenolics concentrations showed significant differences in the three region extracts. From the results, we focused on the most of the important phenolics, which showed differences between the regions. The three regions had variations in climate and soil type and composition, which may lead to the variations. Sivaci and Duman (2014) studied the evaluation of seasonal antioxidant activity and total phenolic compounds in stems and leaves of *Prunus amygdalus* L. varieties, showed the antioxidant activity and phenolic amount (concentration) were affected by the regional and precipitation variations. Precipitation is known to influence plant growth and development and production of secondary metabolites.

The climatic conditions of the three regions make big differences for the concentration difference between regions for the same phenolic compound. From Liu et al. (2015) studied the influence of ecological factors on the production of active substances, biosynthesis and accumulation, showed the active ingredients of medicinal plants were affected by different ecological factors. Soil factors had differences in the three regions of the country, Ethiopia. The altitude and latitudinal difference also influenced the plant metabolite concentration. The stage (young or old) of the plant part in which the samples were taken also had influence on the concentration.

From the leaf extracts, quercetin, α-tocopherol hydroquinone and salicylic acid were the most abundant phenolics. Those metabolites have a lot applications in modern medicine, and are discussed below in the medicinal uses of metabolites.

Root samples shows high variability in concentration in the three regions. Among 29 compounds, 18 were shown to be significantly different between regions. This means 60 % of the tested

metabolites shows significant difference between regions, and this shows that active metabolite production in this plant is sensitive to the regional climatic conditions. Among the phenolics, the highest concentration in the root extracts were salicylic acid, 4-hydroxy-2-methylbenzoic acid and gentisic acid. The medicinal uses of these phenolics are also discussed later.

In general, the majority of samples from Tigray region showed higher concentrations for large numbers of metabolites. Tigray region is relatively drier and lower rainfall for most parts. The variation of metabolites was probably influenced by the soil factors and climatic conditions of the sampling site. The Tigray region was characterised by red soils, which had high water capacity and thus, providing good growth conditions regarding essential nutrients to produce larger concentrations of compounds. As I observe the region, the sampling site (Maytsebri) showed relatively good soil fertility from the other regions sampling areas, but this can't be supported by literature sources due to lack of research on the region. If one intends to use this plant for medicinal purposes and for the extraction of medicinally important compounds, the Tigray region might be a preferred site for plant sampling.

The discriminant analyses of the GC/MS results were done for only selected 112 metabolites to show the distribution in the three regions. From the root samples used to show the regional distributions, Tigray and Amhara region samples had similarity but smaller similarities with the Oromia region samples. The reason for this similarity for the two region extracts might be due to the regions bordered on each other and the sampling areas from those regions were nearby. So, this might be one reason for the similarities of distribution of samples. The sampling sites of the two regions had similarities in conditions and the overall climatic conditions were somewhat similar for the three regions. There is no literature which shows the climatic variations between the two regions.

From the PCA figure 8, the distribution of leaf extracts from the three regions, indicated different but partly overlapping sample clusters. Amhara and Oromia region extract were to some extent similar, while the Tigray region samples differed more from the other two regions. There was no clear reason why the Tigray region extracts differed from the others, but I can suggest these come from the sampling, transporting and drying problems.

3.3 Essential oil volatiles

From the results 96 volatile compounds were extracted by SPME. For *Securidaca longipedunculata* there are limited works on volatile extraction using SPME. Alitonou et al. (2012) worked on this plant and used the same method of GC/MS but extracted only seven compounds, which was very low number when compared to our results. This shows that, the large number of volatiles comes from our effective sampling and extraction methods to this plant.

There are many volatile compounds extracted from this plant by using other methods, from Mongalo et al. (2015) studied the ethnomedicinal uses, photochemistry, pharmacological properties and toxicology, found the root bark contains xanthones, some benzyl benzoates and triterpene saponins. Furthermore, the roots contain flavonoids 1,7-dimethoxy-2-hydroxyxanthone and 1,4-dihydroxy-7- methoxy-xanthone, saponine aglycones presenegin, elymoclavine, sinapic acid, 3,4,5-O-caffeoyl-quinic acid, securinine, methyl salicylate. (http://www.mmhmms.com/downloads/mp16securidacalongipedunculatapdf.pdf). The major volatile for this plant is the methyl salicylate and from our result this is similar with other studies.

4.4 Tissue and regional variations

The result shows there were variations in concentration of volatiles between the root and leaf extracts. Among the volatiles, 18 phenols were found in the root extracts and 16 phenols in leaf

samples. These shows both the root and leaf were abundant in volatile compounds. The root extracted volatiles were the most researched ones, but the leaf volatiles were not well studied previously from this plant. Alitonou et al (2012) studied the volatile constituents and biological activities of essential oil, found the root bark extracts contain methyl salicylate, benzyl salicylate, p-α-dimethyl styrene, eugenol, γ-terpinene, p-cymene and germacrene D, and from these extracted volatiles, methyl salicylate covers 97 %. From our results the methyl salicylate was extracted with highest concentrations in both the root and leaf tissues, which was related to the previous studies on this plants.

Previous studies of leaf extracts have not used the SPME method. Adebayo et al. (2007) analysed leaf oil of Nigerian *Securidaca longipedunculata* using GC/MS method and found about 14 volatiles were extracted including methyl salicylate, alpha- pinene, beta-pinene, 1, 8-cineole, alpha-cadinol and other compounds. Methyl salicylate was the major one and comprised 89.6 %. When we compare our leaf volatiles with this study, our results from leaf contain a lot of volatile compounds which were 125 and the most important medicinal volatiles were found.

Concentrations of the volatiles methyl salicylate, methyl 4-methoxysalicylate, geranyl acetone and benzyl alcohol were among the highest in the leaf extracts.

When we see the regional variabilities of volatile concentrations, Tigray region sample extracts were high for the majority of the compounds. For large number of volatiles, the regional variability indicated significant differences. There are limited literatures describing the regional effects on the extraction of volatiles. So, as discussed above from the volatiles, the reason for the higher concentrations in Tigray region samples might be from natural climatic conditions, the soil and might be from the sample handling procedures. From my point of view, the volatiles are highly sensitive and needs high care and proper handling of samples from collection to extraction of oils,

and as I collect from highly hot areas there may be some loss of volatiles and the drying and transportations also contribute some part to the loss.

When we see the most important volatiles and their variability in concentrations through the three regions, for example, methyl salicylate, it was higher in Amhara root extracts but doesn't show significant differences between the regions and higher in Oromia leaf extracts and had significant difference between the regions. So, for methyl salicylate the regional factors were influenced more for the leaf tissues than the roots. Methyl 4-methoxysalicylate was the second highest compound in concentration for both tissues but doesn't show significance difference between regions for both tissues.

There are some studies showing that extraction of volatiles is influenced by the regional differences of the sample collected or growth. Nezhadali et al. (2014) studied the chemical variation of leaf essential oil at different stages, found the essential oils were differed significantly in extracts of Thymus species from different countries and regions.

From the PCA analysis of 125 volatiles for the three regions extracts, root samples showed different but overlapping clusters. The Amhara and Oromia samples somewhat show similarities but samples from Tigray were different. The overlapping cluster shows the sample cluster of one region samples had similarities in distribution with another samples and most volatiles were not showed significance difference between the three regions.

The volatile profiles of leaf samples from the three regions were to some extent similar, showing more clearly separated sample groups but also overlapping clusters. The clear separation of samples for the three regions shows the difference between regions and the clusters shows the similarities. Amhara and Oromia region extracts were somewhat similar, while the Tigray region

samples differed more from the other two regions. Generally, the distribution for the leaf samples were better than the root samples.

4.5 Some important phenolics and medicinal uses

Salicylic acid: this compound was found in high concentrations in both tissues and shows significant differences between the three regions in root extracts. This was the major phenolic compound in all samples extracts. Salicylic acid also used to treat acne and as skin care medications. Arif (2015) studies the salicylic acid as a peeling agent, found that the ability of breakup of clogged pores without the disrupting internal cellular parts, makes salicylic acid useful as peeling agent for patients with acne. From Abdel- Motaleb et al. (2017), found the use of salicylic acid for the treatment of skin disorders and used to rejuvenate the skin. Salicylic acid is also used as an ingredient in shampoo to control dandruff (Nowicki, 2006).

Jnawali and Ryoo (2013), in a study on the first and second line drugs and drug resistance, found that amino salicylic acid can be used to treat tuberculosis.

Danielson and Walter (2005) studied the use of salicylic acid and hydrogel in limiting scar tissue formation, and found that patients were healing from scars in shorter time by topical application without side effects.

Salicylic acid was used to prepare aspirin (the derivative of salicylic acid). Mahdi (2010) found salicylic acid as drug.

Benzoic acid: found in the root and leaf samples in all the three region sample extracts. This is very important medicinal phenol after salicylic acid. It is used for the treatment of inflammation, fungal skin diseases and foot skin infections.

Benzoic acid used in fungal skin disease and foot skin infections. World health reports both benzoic acid and methylrosanilinium chloride solution were effective in treatment of fungi and foot worms.

α-tocopherol hydroquinone is the oxidation product of α-tocopherol and found in both tissue sample extracts and which shows significant difference between the three regions. In leaf extracts it was the major phenol with the highest concentration, whereas in the root extracts the concentration was low. This metabolite has importance for human medication.

Different literatures show it used as antioxidant activities in biological systems and serve as vitamin E in cosmetics sectors. Neuzil et al. (1997) found that the α-tocopherol hydroquinone showed strong antioxidant activity for the low-density lipoproteins, these lipoproteins were the not good cholesterol for human's blood circulation system.

Thiele and Ekanayake-Mudiyanselage (2007) found the use of α-tocopherol hydroquinone as vitamin E in human skin in cosmetics and this compound is highly used in the cosmetics industry.

Quercetin: found in both root and leaf samples, with higher concentrations in leaves and moderate amounts in root extracts. Medicinally quercetin was the most widely used one among flavonols, and is used to reduce inflammation, reduce allergies, for treatment of cancer, and other uses.

Lakhanpal and Rai (2007) found that quercetin had many medicinal applications like to improve the cardiovascular system health, reduce the risk of cancer, serve as antioxidant, help for gene expression as modulator, as therapeutic uses for allergies, asthma and hay fever, as anti-bacterial activity, reducing arthritis, to reduce diabetic complications and prevention of osteoporosis by improving the bone mineral density.

Maalik et al. (2014) studied pharmacological applications of quercetin and its derivatives, and investigated quercetin for antiviral activity, anti-inflammatory activity, neuroprotective effect and hepatoprotective activity to protect liver from damage.

4.6 Important volatiles and medicinal uses

The following volatile compounds were chosen based on its medicinal importance, abundance in both tissues and regional availability.

Methyl salicylate: this compound was found in large amount in both tissues of root and leaf extracts and in all regions. This compound is used in treatment of different illnesses such as liniments to treat muscle pains and give relieve acute or chronic pain. As this compound was found in large amount it can be important to make medication for the above human pains.

Paudel et al. (2013) compared of leaf essential oil compositions and biological activities in *Juglans regia* and *J. nigra*, two trees important in traditional medicine and found that those plants were rich with the methyl salicylate and eugenol, leadin to the traditional use of the plant in treating toothache, rheumatism and fungal infections.

Higashi et al. (2010) studied the efficacy and safety profile of a topical methyl salicylate and menthol patch in adult patients with mild to moderate muscle strain, and found that the combined use of methyl salicylate and menthol gives relief of pain associated with mild to moderate muscle strain in these adult patients compared with patients receiving a placebo patch.

Benzyl alcohol: found in both tissues but significantly in root extracts and found in the three region sample extracts. From different literatures, it has medicinal importance as a bacteriostatic preservative and for the treatment of headlice infestation in children.

Benzyl alcohol is used to reduce pain associated with lignocaine injection and cosmetic formulations as a fragrance component, preservative, solvent, and viscosity-decreasing agent. Benzyl alcohol is used for the treatment of headlice infestations in children, where it kills head lice by asphyxiation (Verma and Namdeo 2015).

Phenyl ethyl alcohol: found in significant amount in both root and leaf extracts and all the three region samples. It has many uses but in this study the focus was for medicinal importance, different literatures show that it used as anti-microbial activity. Malhadas et al. (2017) attributed the antimicrobial potential of the olive tree leaves to 3-methyl-1-butanol and phenyl ethyl alcohol.

Acetophenone: this phenolic compound was found in root and leaf sample extracts but with significant differences in the three regions in the root samples only. The amounts were high in the root samples. Acetophenone is used in human medicine for making of different pharmaceuticals like dextropropoxyphene and aspaminol. Balhara (2014), who studied the dextropropoxyphene ban in India, investigated the use of acetophenone in production of the medication, dextropropoxyphene. This compound lacks available literatures to discuss more on the medicinal uses. Thioureido derivatives of acetophenone semi carbazone are effective as anti-seizure drugs (Pandeya et al. 1998).

Limonene was found in both tissues of root and leaf samples but significantly in leaf samples, found in all region sample extracts and is used to treat gastroesophageal reflux and heartburn. Sun (2007) studied safety and clinical applications of D-Limonene, including preventive activity against cancer and to dissolve gallstones with cholesterols.

Conclusion

From the results, the extractions are effective to get maximum number of volatile compounds. Regional difference has significant influence on the concentration of extracted compounds. The leaves show larger concentrations in all compounds so using for extraction of medicinal compounds is better option than the roots. Characterizing compound compositions of the three regions is effective. The chemical difference between tissues are low or not much. Some of the extracted chemical compounds has potential for medicinal uses. Some of the most medicinally important metabolites and volatiles are abundant from this plant.

References

Abdel-Motaleb, AA et al. J Cosmet Dermatol.(2017). Dermal morphological Changes following salicylic acid peeling and microdermabrasion.

Adebayo, M. A., A. Karioti, H. Skaltsa and I. A. Ogunwande (2007). "Essential oils of Nigeria II: Analysis of the leaf oil of Securidaca longependuculata fers." *Journal of Essential Oil Research* **19**(5): 452-454.

Nowicki, R. (2006). Modern management of dandruff, Pol Merkur Lekarski, 20(115):121-4. Review. Polish.

Afful, E., Owusu, E.O., Obeng-Ofori, D., 2012. Bioactivity of Securidaca longepedunculata Fres. against Callosobruchus maculatus Fab. (Coleoptera: Bruchidae) and Sitophilus zeamais Motsch (Coleoptera: Curculionidae). International Journal of Agricultural Science Research 1, 046–054.

Ajali, U. and B. K. C. Chukwurah (2004). "Antimicrobial activity of Securidaca longipedunculata." Phytomedicine **11**(7-8): 701-703.

Alain G.A., Alain Yaya A.K., Sossou D.J., Yehouenou B., Avlessi B., Adeoti S., Menut C , C.K. Sohounhloue D.C.K. (2012). Volatile constituents and biological activities of essential oil from securidaca longepedunculata fers. growing in benin. Scientific Study & Research. 2012, 13 (1), pp. 033 – 042.

Alemayehu, M. (2006) Country Pasture/Forage Resource Profiles. MoA, Addis Ababa, Ethiopia.

Arif, T (2015) Salicylic acid as a peeling agent: a comprehensive review. Clin Cosmet Investig Dermatol. 2015; 8: 455–461.

Atmani, D., N. Chaher, M. Berboucha, K. Ayouni, H. Lounis, H. Boudaoud, N. Debbache and D. Atmani (2009). "Antioxidant capacity and phenol content of selected Algerian medicinal plants." Food Chemistry **112**(2): 303-309.

Augustino, S., Hall, J.B., Makonda, F.B.S., Ishengoma, R.C., 2011. Medicinal resources of the Miombo woodlands of Urumva. Tanzania: Plants and Its Use, Journal of Medicinal Plants Research 5, 6352–6372.

Azwanida, NN (2015). A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Azwanida, Med Aromat Plants 2015, 4:3. Retrived on January.

Bah, S., Jäger, A.K., Adsersen, A., Diallo, D., Paulsen, B.S. (2007). Antiplasmodial and GABA A-benzodiazepine receptor binding activities of five plants used in traditional medicine in Mali, West Africa. Journal of Ethnopharmacology 110, 451–457.

Balhara, Y. P. S. (2014). Dextropropoxyphene ban in India: Is there a case for reconsideration? *Journal of Pharmacology & Pharmacotherapeutics*, *5*(1), 8–11. Retrived on 4th of October, 2017, From: http://doi.org/10.4103/0976-500X.124406.

Bangou, M.J., Kiendrebeogo, M., Meda, N.T.R., Coulibaly, A.Y., Compaoré, M., Zeba, B., Millogo-Rasolodimby, J., Nacoulma, O.G., 2011. Evaluation of enzymes inhibition activities of medicinal plant from Burkina Faso. Pakistan Journal of Biological Science 14, 99–105.

Bernard, D., Asare Isaac Kwabena A.I., Osei O.D., G. Achel Daniel G.A., Elom S. A., and Sandra A. (2014). The Effect of Different Drying Methods on the Phytochemicals and Radical Scavenging Activity of Ceylon Cinnamon (Cinnamomum zeylanicum) Plant Parts. *European Journal of Medicinal Plants* 4(11): 1324-1335, 2014.

Borokini, T.I., Clement, M., Dickson, N.J., Edagbo, D.E., 2013. Ethnobiological survey of traditional medicine practice for circulatory and nervous system related diseases in Oyo State, Nigeria. Topclass Journal of Herbal Medicine 2, 111–120.

Breitmaier, E. (2006). Terpenes: Importance, General Structure, and Biosynthesis. Book published by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim ISBN: 3-527-31786-4. Retrived on 4th of Decempber, 2016. From: https://application.wiley-vch.de/books/sample/3527317864c01.pdf.

Coates-Palgrave, M., 2005. Keith Coates Palgrave Trees of Southern Africa, Third Edition, Struik Publishers, Cape Town, pp. 458-459.

Ekwy, O.C, samuel O.E., Brendan E.O., (2015) phytochemical and gc-ms analyses of the bioactive components of securidaca longepedunculata (fresen) roots for anti-breast cancer activity. *World journal of pharmaceutical research*. *volume 4, issue* 12, 1503-1518.

Dai J, Mumper R (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules, 15: 7313-7352.

Debella, A., O. Kunert, M. G. Schmid, G. Michl, F. Bucar, D. Abebe and E. Haslinger (2000). "A diterpene, a flavonol glycoside, and a phytosterol glycoside from Securidaca longipedunculata and Entada abyssinica." Monatshefte Fur Chemie **131**(4): 401-408.

Doughari, J. H., P. A. Ndakidemi, Human I. S. and Benade S. (2012). "Antioxidant, antimicrobial and antiverotoxic potentials of extracts of Curtisia dentata." *Journal of Ethno pharmacology* **141**(3): 1041-1050.

Fernandes, L., Hoosen, A.A., van Rensburg, C.E.J., Steenkamp, V., 2008. In vitro activity of medicinal plants of the Venda region, South Africa, against Trichomonas vaginalis. South African Journal of Epidemiology and Infections 23, 26–28.

Gebrehiwot, T and Anne van der Veen (2013) Assessing the evidence of climate variability in the northern part of Ethiopia. Journal of Development and Agricultural Economics Vol. 5(3), pp. 104-119.

Ghasemzadeh, A., H. Z. E. Jaafar and A. Rahmat (2010). "Antioxidant Activities, Total Phenolics and Flavonoids Content in Two Varieties of Malaysia Young Ginger (Zingiber officinale Roscoe)." Molecules **15**(6): 4324-4333.

Giner-Chavez B.I., 1996. Condensed tannins in tropical forages. Doctoral Thesis. Cornell University. Ithaca, NY, USA.

Goyal M., Ghosh, M., Nagori B.P.,, and Sasmal D.,(2013). Analgesic and anti-inflammatory studies of cyclopeptide alkaloid fraction of leaves of *Ziziyphus nummularia. Saudi J Biol Sci.* 2013 Oct; 20(4): 365–371.

Humphrey A.J., Beale M.H. Terpenes. (2007). In: Crozier A., Clifford M.N., Ashihara H., editors. Plant Secondary Metabolites: Occurrence, Structure and Role in the Human Diet. Blackwell Publishing, Ltd.; Oxford, UK: 2007. pp. 47–101.

Iwu, M. M. (1986). African ethnomedicine. CECTA Nig. Ltd., Enugu, 34-37.

Jnawali H.N.and Ryoo S. (2013). First– and Second–Line Drugs and Drug Resistance. Retrived from February1, 2017. From; http://dx.doi.org/10.5772/54960.

J*ohn R. Danielson and Robert J. Walter (2005)* Case Studies: Use of Salicylic Acid (Avosil) and Hydrogel (Avogel) in Limiting Scar Formation.

Neuzil, J., P. K. Witting and R. Stocker (1997). "alpha-tocopheryl hydroquinone is an efficient multifunctional inhibitor of radical-initiated oxidation of low density lipoprotein lipids."

Proceedings of the National Academy of Sciences of the United States of America **94**(15): 7885-7890.

Kadiri, A.B., Agboola, O.M., Fashina, F.O., 2013. Ethnobotanical survey and phytoanatomical studies of some common plants used for the treatment of epilepsy in some rural areas of South West Nigeria. Journal of Pharmacy and Phytochemistry 2, 175–182.

Keshebo, D.L., Choundhury, M.K., Dekebo, A.H., 2014. Investigation on toxicity, hypoglycemic effect of the root bark of Securidaca longipedunculata Fresen (Polygalaceae) and determination of heavy metals. Annals of Biological Research. 5, 15-19.

Koes, R.E., Quattrocchio, F. and Mol, J.N.M. (1994) The flavonoid biosynthetic pathway in plants: function and evolution. BioEssays, 16, 123–132.

Kokwaro J O. (1976) Medicinal plants in East Africa. Nairobi: East African Literature Bureau. Lakhanpal and Rai (2007). "Quercetin: A Versatile Flavonoid." Internet Journal of Medical Update **2 (**2**)**: 22-37.

Light S. G., M. E. and Staden J. (2004). "Biological activities and distribution of plant saponins."

Journal of Ethnopharmacology **94**(2-3): 219-243.

Liu W, Liu J, Yin D, Zhao X (2015) Influence of Ecological Factors on the Production of Active Substances in the Anti-Cancer Plant *Sinopodophyllum hexandrum*(Royle) T.S. Ying. PLoS ONE 10(4): e0122981. Retrived on 3rd of October, 2017, from: https://doi.org/10.1371/journal.pone.0122981.

Mahdi, J.G (2010) Medicinal potential of willow: A chemical perspective of aspirin discovery. Journal of Saudi Chemical Society (2010) 14, 317–322.

Maalik, A., F. A. Khan, A. Mumtaz, A. Mehmood, S. Azhar, M. Atif, S. Karim, Y. Altaf and I. Tariq (2014). "Pharmacological Applications of Quercetin and its Derivatives: A Short Review." *Tropical Journal of Pharmaceutical Research* **13**(9): 1561-1566.

Malhadas, C., R. Malheiro, J. A. Pereira, P. G. de Pinho and P. Baptista (2017). "Antimicrobial activity of endophytic fungi from olive tree leaves." *World Journal of Microbiology & Biotechnolo*gy **33**(3).

Man S., Gao W.,, Zhang Y., Liu C.(2010). Chemical study and medical application of saponins as anti-cancer agents. Fitoterapia 81 (2010) 703–714.

Mandal SM, Chakraborty D, Dey S.(2010) Phenolic acids act as signaling molecules in plant-microbe symbioses. Plant Signal Behav. 2010 Apr;5(4):359-68.

Meaza, Gidey, Tadesse Beyene2, Maria Adele Signorini3, Piero Bruschi4 and Gidey Yirga2(2015) Traditional medicinal plants used by Kunama ethnic group in Northern Ethiopia. Journal of Medicinal Plants Research Vol. 9(15), pp. 494-509.

Ministry of Agriculture (MoA) (2000) Agroecological Zonations of Ethiopia. Addis Ababa, Ethiopia.

Moeng, T.E., 2010. An investigation into the trade of medicinal plants by muthi shops and street vendors in the Limpopo Province, South Africa Master of Science dissertation. University of Limpopo, South Africa.

Mongalo, N. I., L. J. Mcgaw, J. F. Finnie and J. Van Staden (2015). "Securidaca longipedunculata Fresen (Polygalaceae): A review of its ethnomedicinal uses, phytochemistry, pharmacological properties and toxicology." Journal of Ethnopharmacology **165**: 215-226.

Muanda, F. N., A. Dicko and R. Soulimani. (2010). "Assessment of polyphenolic compounds, in vitro antioxidant and anti-inflammation properties of Securidaca longepedunculata root barks." Comptes Rendus Biologies **333**(9): 663-669.

Mustapha, A.A., 2013a. Ethno-medico-botanical uses of Securidaca longepedunculata (Fresen) (Family Polygalaceae) from Keffi local government, Nasarawa State, Nigeria. Journal of Natural Remedies 13, 133–137.

Mustapha, A.A., 2013b. Ethno-medicinal field study of anti-fertility medicinal plants used by the local people in Keffi local government, Nasarawa State, Nigeria. International Journal of Medicinal Plants Research 2, 215–218.

Neuzil, J., P. K. Witting and R. Stocker (1997). "alpha-tocopheryl hydroquinone is an efficient multifunctional inhibitor of radical-initiated oxidation of low density lipoprotein lipids."

Proceedings of the National Academy of Sciences of the United States of America **94**(15): 7885-7890.

Nezhadali A., Nabavi M., Rajabian M., Akbarpour M., Pourali P., Amini F. (2012). Chemical variation of leaf essential oil at different stages of plant growth and in vitro antibacterial activity of Thymus vulgaris Lamiaceae, from Iran, Beni-Suef University *Journal of Basic and Applied Sciences*, Volume 3, Issue 2, June 2014, Pages 87-92, ISSN 2314-8535, https://doi.org/10.1016/j.bjbas.2014.05.001.

Ngonda, F., Magombo, Z., Mpeketula, P., Mwatseteza, J., 2012. Evaluation of Malawian Vernonia glabra (Steetz) Vatke leaf and Securidaca longepedunculata (Fresen) root extracts for antimicrobial activities. Journal of Applied Pharmaceutical Science 2, 026–033.

Nguta, J.M., Mbaria, J.M., Gakuya, D.W., Gathumbi, P.K., Kiama, S.G., (2010a). Antimalarial herbal remedies of Msambweni, Kenya. Journal of Ethnopharmacology 128, 424–432.

Nguta, J.M., Mbaria, J.M., Gakuya, D.W., Gathumbi, P.K., Kiama, S.G., (2010b). Traditional antimalarial phytotherapy remedies used by the South Coast community, Kenya. Journal of Ethnopharmacology 131, 256–267.

Odebiyi, O. O. and E. A. Sofowora (1978). "Phytochemical Screening of Nigerian Medicinal-Plants .2." Lloydia-the Journal of Natural Products **41**(3): 234-246.

Ogunmefun, O.T., Gbile, Z.O., 2012. An ethnobotanical study of anti-rheumatic plants in South Western States of Nigeria. Asian Journal of Science and Technology 4, 63–66.

Okoli, C.O., Akah, P.A., Ezugworie, U., 2005. Anti-inflammatory activity of extracts of root bark of Securidaca longipedunculata Fres (Polyalaceae). African Journal of Traditional Complementary and Alternative Medicine 2, 54–63.

Okoli R. I., Turay, A. A., Mensah J.K., and Aigbe A.O. (2006). Phytochemical and Antimicrobial Properties of Four Herbs from Edo State, Nigeria. Report and Opinion,2009;1(5).

Olaleye, S. B., Akinniyi, J. A., Salami, H. A., Mbajiorgu, F. E., & Ameh, J. A. (1998). Preliminary studies on the toxicity and antimicrobial effects of extract of Securidaca longipedunculata. *Afr. J. Biomed. Res*, *1*(1), 39-43.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., Simons, A., 2009. Agroforestree Database: a tree reference and selection guide, version 4, 0 (accessed 26.01.15).

Pandeya, S. N., V. Mishra, Singh P. N. and. Rupainwar D. C. (1998). "Anticonvulsant activity of thioureido derivatives of acetophenone semicarbazone." Pharmacological Research **37**(1): 17-22.

Hosseinzadeh, S., Azizollah Jafarikukhdan, Ahmadreza Hosseini, Raham Armand (2015). The Application of Medicinal Plants in Traditional and Modern Medicine: A Review of Thymus vulgaris. International Journal of Clinical Medicine 6(9): 635-642.

Saxena M, Saxena J, Nema R, Singh D and Gupta A (2013). Phytochemistry of medicinal plants J. Pharm. Phytochem., 1(6): 168 - 82.

Serrano M, Díaz-Mula HM, Zapata PJ, Castillo S, Guillén F, Martínez-Romero D, Valverde JM, Valero D(2009) Maturity stage at harvest determines the fruit quality and antioxidant potential after storage of sweet cherry cultivars. J Agric Food Chem. 2009 Apr 22;57(8):3240-6.

Silva EM, Rogez H, Larondelle Y. Optimization of extraction of phenolics from *Inga edulis* leaves using response surface methodology. Sep Purif Technol. 2007;55(3):381–387.

Sivaci, A. and S. Duman (2014). "Evaluation of seasonal antioxidant activity and total phenolic compounds in stems and leaves of some almond (Prunus amygdalus L.) varieties." Biological Research **47**.

Sun, J (2007). D-Limonene: Safety and Clinical Applications Alternative Medicine Review Volume 12: 259-264.

Tamura Y., Miyakoshi M., and Yamamoto M., *(2012).* Application of Saponin-Containing Plants in Foods and Cosmetics. Complementary and Alternative Medicine. *book edited by Hiroshi Sakagami, ISBN 978-953-51-0903-7.*

Thiele, J. J. and Ekanayake-Mudiyanselage S. (2007). "Vitamin E in human skin: Organ-specific physiology and considerations for its use in dermatology." Molecular Aspects of Medicine **28**(5-6): 646-667.

Tshisikhawe, M. P., Olivia Baloyi and Maanda Hadzhi Ligavha-Mbeleng (20129) Ecología poblacional de Securidaca longepedunculata Fresen. en la Reserva Natural Nylsvley, Provincia Limpopo, Sudáfrica. ResearchGate. 81: 51-58.

Van Wyk, B.E. de Wet, H. Van Heerden c,(2005) An ethnobotanical survey of medicinal plants in the southeastern Karoo, South Africa. South African Journal of Botany 74 (2008) 696–704.

Vas G, Vekey K. (2004). Solid-phase micro extraction: a powerful sample preparation tool prior to mass spectrometric analysis. *Journal of Mass Spectrometry* 39:233–254.

Vas, G. and Vekey, K. (2003) Solid-phase micro extraction: a powerful sample preparation tool prior to mass spectrometric analysis. *J. Mass Spectrom* 2004; 39: 233–254.

Verma, P. and C. Namdeo (2015). "Treatment of Pediculosis Capitis." Indian Journal of Dermatology **60**(3): 238-247.

Vetter, J (2000) Plant cyanogenic glycosides**.** Toxicon. ;38(1):11-36.

Vulnerability Assessment and Mapping Ethiopia (2009) Food Security and Vulnerability in Selected Towns of Tigray Region. Founds online: http://vam.wfp.org/CountryPage_assessments.aspx?iso3=eth.

Wanzala, W., Takken, W., Mukabana, W.R., Pala, A.O., Hassanali, A., 2012. Ethnoknowledge of Busuku community on livestock tick prevention and control in Bungoma district, Western Kenya. Journal of Ethnopharmacology 140, 298–324.

WHO (World health organization) (2001). Introduction to traditional medicine.

Wink, M. (2008). Ecological roles of alkaloids, in modern alkaloids. Structure, Isolation, Synthesis and biology (eds E. Fattorusso, O.Taglialatela-Scafati.),Willey-Vch,Weinheim,pp.3-24.

Zwenger.S and Basu C., (2008) Plant terpenoids: Applications and future potentials.

Appendex

Table 1: Metabolite profiling of polar compounds

Compound	Class	Category	Average Amhara Root	Average Oromia Root	Average Tigray Root	P value	Sig
glutamic acid	aa	met	781,09	2324,46	1242,62	0,121	
4-aminobutyric acid	aa	met	199,65	85,02	9,68	0,011	*
aspartic acid	aa	met	692,11	1352,69	0,03	0,000	***
alanine	aa	met	318,20	1283,32	476,15	0,002	**
proline	aa	met	256,64	845,02	649,38	0,024	*
valine	aa	met	133,11	25,78	21,75	0,014	*
glycine	aa	met	61,31	27,82	3,08	0,013	*
leucine	aa	met	26,20	17,99	0,02	0,112	
isoleucine	aa	met	8,88	11,81	72,93	0,003	**
sugar acid derivative5	ac	str	339,83	234,18	30,18	0,100	
gluconic acid	ac	met	85,29	75,48	36,02	0,115	
malic acid	ac	met	78,78	234,74	80,69	0,015	*
arabinonic acid	ac	met	129,07	162,00	35,80	0,120	
sugar acid derivative4	ac	str	6,25	31,83	74,51	0,000	***
succinic acid derivative2	ac	str	6,93	5,53	25,10	0,005	**
sugar acid derivative6	ac	str	11,83	18,46	25,18	0,003	**
acid4	ac	str	9,03	3,91	8,44	0,350	
lactic acid	ac	met	28,85	78,15	20,99	0,000	***
galactonic acid	ac	met	6,35	4,38	21,52	0,009	**
citric acid	ac	met	19,66	2,23	0,02	0,009	**
fumaric acid	ac	met	7,66	10,24	22,86	0,036	*
succinic acid	ac	met	0,41	2,45	74,19	0,005	**
3-hydroxypropanoic acid	ac	met	7,62	2,00	1,05	0,026	*
glyceric acid	ac	met	9,51	18,06	17,93	0,001	***
ribonic acid	ac	met	0,02	0,21	0,36	0,063	
sorbitol	alc	met	27,16	47,96	9,49	0,001	***
erythritol	alc	met	12,46	15,81	2,67	0,040	*
glycerol	alc	met	19,34	53,46	13,98	0,001	***
myo-inositol	alc	met	13,18	12,78	2,84	0,176	
scyllo-inositol	alc	met	5,23	12,66	25,72	0,039	*
A170001 (pentitol)	alc	str	3,33	1,73	3,95	0,049	*
ethanolamine	am	met	31,68	19,95	5,11	0,099	
N-carboxymethylamine	ph	met	15,62	13,60	25,50	0,219	

putrescine	am	met	4,92	4,14	20,69	0,006	**
carotenoid2	car	str	16,87	6,38	0,02	0,052	
sucrose	disac	met	11,79	37,28	5,01	0,003	**
maltose	disac	met	18,31	11,97	1,08	0,025	*
disaccharide6	disac	str	13,58	14,53	6,40	0,119	
disaccharide13	disac	str	10,91	25,84	2,71	0,023	*
disaccharide10	disac	str	25,61	13,96	8,05	0,100	
disaccharide1	disac	str	0,98	0,62	0,80	0,880	
disaccharide14	disac	str	12,72	31,56	7,47	0,214	
disaccharide7	disac	str	6,14	9,17	4,35	0,151	
disaccharide9	disac	str	2,15	6,38	4,72	0,160	
disaccharide3	disac	str	29,85	11,69	0,19	0,001	***
hexadecanoic acid (C16:0)	fa	met	23,13	4,29	0,55	0,088	
octadecanoic acid (C18:0)	fa	met	1,77	1,47	4,88	0,142	
octadecadienoic acid, 9,12-(Z,Z)- (18:2)	fa	met	6,70	4,98	12,58	0,212	
octadecenoic acid, 9-(Z)- (C18:1)	fa	met	0,97	0,49	4,30	0,002	**
fatty acid derivative1	fa	str	1,59	0,99	1,64	0,212	
octadecatrienoic acid, 9,12,15-(Z,Z,Z)- (C18:3)	fa	met	0,59	1,38	0,61	0,002	**
tetracosanoic acid	fa	met	3,09	1,24	3,93	0,046	*
heptanoic acid	fa	met	0,08	0,12	0,39	0,031	*
tetradecanoic acid (C14:0)	fa	met	0,38	0,17	0,91	0,026	*
docosanoic acid	fa	met	5,57	10,24	5,60	0,057	
quercetin	flav	met	5,03	3,64	1,39	0,166	
flavonoid5	flav	str	0,63	5,57	6,60	0,040	*
flavonoid3	flav	str	4,62	3,39	5,66	0,382	
flavonoid1	flav	str	2,34	1,65	14,42	0,010	**
flavonoid2	flav	str	1,70	7,85	2,48	0,002	**
flavonoid4	flav	str	1,34	1,38	10,33	0,006	**
1-deoxyglucose	hex	met	1,64	8,05	2,49	0,001	***
glucose1	hex	met	9,11	6,66	5,00	0,559	
fructose3	hex	met	5,18	5,77	9,02	0,464	
sugar5	hex	str	3,33	4,19	2,01	0,164	
fructose2	hex	met	1,34	3,39	6,95	0,001	***
glucose2	hex	met	3,35	2,42	3,46	0,663	
glucose3	hex	met	7,21	7,84	0,65	0,006	**
sugar8	hex	str	3,01	1,26	2,92	0,146	
galactose1	hex	met	0,85	0,75	1,51	0,234	

galactose2	hex	met	0,23	0,42	0,97	0,028	*
1-monopalmitin	mgl	met	2,45	0,89	1,58	0,175	
1-monostearin	mgl	met	0,41	0,69	3,66	0,000	***
A203003	na	na	1,47	4,17	1,05	0,089	
NA31	na	na	2,11	0,80	3,08	0,029	*
A136002	na	na	3,86	1,79	5,54	0,098	
NA7	am	met	2,82	0,43	5,59	0,042	*
NA15	na	na	0,79	0,15	0,02	0,001	***
NA39	na	na	0,96	1,49	1,85	0,104	
ribose	pen	met	1,53	0,56	0,77	0,060	
pentose2	pen	str	2,07	1,77	1,49	0,525	
phosphoric acid	ph	met	2,00	2,15	1,07	0,220	
ethyl phosphoric acid	ph	met	0,46	0,25	0,43	0,647	
glycerol-3-phosphate	ph	met	4,32	3,07	0,93	0,001	***
?-tocopherolhydroqui-none	#N/A	#N/A	1,99	0,96	0,88	0,120	
salicylic acid	phen	met	0,79	0,99	0,89	0,865	
4-hydroxy-2-methylbenzoic acid	phen	met	0,34	2,38	2,48	0,007	**
?-sitosterol derivative1	#N/A	#N/A	1,62	1,02	1,57	0,367	
gentisic acid	phen	met	0,63	2,04	1,29	0,138	
(E)-sinapic acid	phen	met	0,88	0,80	0,63	0,328	
?-tocopherol	#N/A	#N/A	0,72	0,41	1,18	0,044	*
phenolic compound2	phen	str	0,39	0,47	1,38	0,023	*
phenylpyruvic acid	phen	met	1,05	1,12	1,03	0,972	
(E)-ferulic acid	phen	met	2,03	1,52	1,24	0,472	
benzoic acid	phen	met	0,21	0,49	0,55	0,287	
phenolic compound1	phen	str	0,66	0,80	0,90	0,645	
m-coumaric acid	aa	met	0,61	1,40	0,59	0,058	
uridine	pym	met	0,37	0,23	0,37	0,623	
sterol2	ster	str	0,60	0,44	0,79	0,583	
stigmasterol	ster	met	0,32	0,19	0,81	0,096	

Table 2. Metabolite profiling of polar compounds

Compound	Class	Category	Average Amhara Leaf	Average Oromia Leaf	Average Tigray Leaf	P value	Sig
glutamic acid	aa	met	15,36	14,09	247,08	0,358	
4-aminobutyric acid	aa	met	2082,65	1287,58	43,42	0,008	**
aspartic acid	aa	met	217,45	106,84	504,02	0,311	
alanine	aa	met	0,04	239,19	96,30	0,283	
proline	aa	met	0,58	2,61	0,32	0,001	***
valine	aa	met	685,19	0,45	550,54	0,110	
glycine	aa	met	833,59	403,70	6,05	0,002	**
leucine	aa	met	193,09	201,33	776,16	0,088	
isoleucine	aa	met	481,90	210,91	302,22	0,486	
sugar acid derivative5	ac	str	119,08	115,08	25,14	0,119	
gluconic acid	ac	met	187,62	174,73	73,13	0,207	
malic acid	ac	met	0,14	1,20	1,94	0,551	
arabinonic acid	ac	met	10,18	9,34	8,89	0,920	
sugar acid derivative4	ac	str	5,52	120,64	108,44	0,309	
succinic acid derivative2	ac	str	67,26	47,21	61,71	0,714	
sugar acid derivative6	ac	str	13,16	88,95	53,46	0,372	
acid4	ac	str	75,24	50,34	59,53	0,807	
lactic acid	ac	met	1,78	56,71	19,67	0,128	
galactonic acid	ac	met	16,75	86,51	41,26	0,288	
citric acid	ac	met	33,42	31,99	76,72	0,469	
fumaric acid	ac	met	20,76	93,95	1,98	0,046	*
succinic acid	ac	met	5,46	2,56	42,27	0,278	
3-hydroxypropanoic acid	ac	met	21,59	25,54	61,10	0,209	
glyceric acid	ac	met	8,73	37,42	25,02	0,464	
ribonic acid	ac	met	33,88	49,93	27,08	0,459	
sorbitol	alc	met	6,91	10,98	1,67	0,053	
erythritol	alc	met	16,35	44,18	10,74	0,160	
glycerol	alc	met	1,86	4,50	3,98	0,372	
myo-inositol	alc	met	24,68	24,12	12,27	0,444	
scyllo-inositol	alc	met	3,18	35,63	6,23	0,186	
A170001 (pentitol)	alc	str	18,66	13,83	47,12	0,113	
ethanolamine	am	met	10,56	9,26	3,91	0,166	
N-carboxymethylamine	ph	met	10,48	9,07	4,09	0,241	
putrescine	am	met	14,93	19,68	12,84	0,804	
carotenoid2	car	str	35,91	11,96	0,93	0,191	
sucrose	disac	met	10,04	6,10	0,99	0,247	

maltose	disac	met	14,16	18,80	3,32	0,067	
disaccharide6	disac	str	1,68	0,67	28,26	0,051	
disaccharide13	disac	str	5,06	3,95	6,32	0,859	
disaccharide10	disac	str	4,45	2,33	0,03	0,148	
disaccharide1	disac	str	22,59	7,55	21,52	0,184	
disaccharide14	disac	str	0,44	0,51	0,46	0,974	
disaccharide7	disac	str	15,77	15,64	2,07	0,001	***
disaccharide9	disac	str	13,45	5,82	17,32	0,149	
disaccharide3	disac	str	0,87	0,41	0,03	0,000	***
hexadecanoic acid (C16:0)	fa	met	2,27	2,08	5,63	0,159	
octadecanoic acid (C18:0)	fa	met	3,65	3,40	20,01	0,049	*
octadecadienoic acid, 9,12-(Z,Z)- (18:2)	fa	met	0,41	0,25	10,10	0,022	*
octadecenoic acid, 9-(Z)- (C18:1)	fa	met	3,45	19,52	6,07	0,215	
fatty acid derivative1	fa	str	15,52	10,21	3,23	0,062	
octadecatrienoic acid, 9,12,15-(Z,Z,Z)- (C18:3)	fa	met	7,83	10,76	10,24	0,618	
tetracosanoic acid	fa	met	5,06	8,40	9,57	0,503	
heptanoic acid	fa	met	9,35	11,10	7,88	0,724	
tetradecanoic acid (C14:0)	fa	met	12,52	8,39	4,72	0,095	
docosanoic acid	fa	met	2,74	0,82	1,98	0,410	
quercetin	flav	met	8,00	7,39	1,11	0,026	*
flavonoid5	flav	str	2,25	4,67	6,59	0,052	
flavonoid3	flav	str	4,57	6,80	1,26	0,009	**
flavonoid1	flav	str	1,32	0,22	4,32	0,057	
flavonoid2	flav	str	1,69	7,14	1,47	0,017	*
flavonoid4	flav	str	1,36	6,51	1,20	0,018	*
1-deoxyglucose	hex	met	1,57	6,51	1,36	0,014	*
glucose1	hex	met	0,18	0,28	0,38	0,664	
fructose3	hex	met	0,44	0,50	0,65	0,813	
sugar5	hex	str	4,70	6,00	1,25	0,000	***
fructose2	hex	met	1,67	3,42	3,96	0,659	
glucose2	hex	met	3,89	5,70	1,42	0,002	**
glucose3	hex	met	1,73	0,91	0,71	0,389	
sugar8	hex	str	2,87	2,42	6,51	0,002	**
galactose1	hex	met	6,02	5,06	3,23	0,476	
galactose2	hex	met	2,96	2,17	10,26	0,120	
1-monopalmitin	mgl	met	4,08	3,05	3,25	0,748	
1-monostearin	mgl	met	2,92	4,32	3,21	0,653	
A203003	na	na	1,74	5,36	0,74	0,049	*

NA31	na	na	2,77	2,88	2,63	0,987	
A136002	na	na	0,27	1,35	1,15	0,292	
NA7	am	met	1,26	0,55	3,18	0,103	
NA15	na	na	3,16	4,42	4,38	0,743	
NA39	na	na	2,06	3,82	2,24	0,363	
ribose	pen	met	2,94	1,97	4,57	0,344	
pentose2	pen	str	1,67	3,29	2,03	0,221	
phosphoric acid	ph	met	2,66	2,69	1,31	0,080	
ethyl phosphoric acid	ph	met	3,86	2,29	3,44	0,453	
glycerol-3-phosphate	ph	met	0,54	1,04	0,12	0,140	
?-tocopherolhydroqui-none	N/A	N/A	1,82	0,44	3,36	0,358	
salicylic acid	phen	met	3,20	1,57	1,63	0,550	
4-hydroxy-2-methylben-zoic acid	phen	met	0,85	0,44	1,41	0,339	
?-sitosterol derivative1	N/A	N/A	0,78	0,91	1,98	0,191	
gentisic acid	phen	met	0,72	2,69	0,23	0,044	*
(E)-sinapic acid	phen	met	1,88	1,42	1,84	0,811	
?-tocopherol	N/A	N/A	0,79	0,92	3,29	0,000	***
phenolic compound2	phen	str	0,48	0,34	4,23	0,030	*
phenylpyruvic acid	phen	met	1,83	1,35	0,82	0,484	
(E)-ferulic acid	phen	met	0,41	1,73	0,03	0,215	
benzoic acid	phen	met	0,81	0,51	4,24	0,060	
phenolic compound1	phen	str	2,03	1,98	0,31	0,017	*
m-coumaric acid	aa	met	0,73	2,04	0,40	0,100	
uridine	pym	met	1,44	1,22	1,83	0,730	
sterol2	ster	str	0,91	0,64	1,75	0,140	
stigmasterol	ster	met	0,98	0,84	1,83	0,308	

Table 3: Oils from root using SPME

RI	Compound	Average Amhara Root	Average Oromia Root	Average Tigray Root	P.valus	Sig.
602	acetic acid	170	190	190	0,783	
807	hexanal	685	2173	59911	0,001	***
843	2-pentyl acetate	449	331	3706	0,256	
854	(E)-2-hexenal	170	190	210	0,493	
870	1-hexanol	1084	170	2519	0,139	
903	NA1	210	927	19392	0,006	**
931	1-acetylcyclohexene	210	1506	10210	0	***
945	NA2	250	210	1890	0,167	
958	dihydro-3-methyl-2(3H)-furanone	386	599	7386	0,021	*
960	NA3	403	210	2854	0,007	**
971	benzaldehyde	1248	1257	21168	0	***
986	6-methyl-5-hepten-2-one	3356	2283	49738	0	***
996	NA4	2191	1351	42219	0	***
1004	hexanoic acid	210	1155	18614	0	***
1005	p-methylanisole	230	210	3150	0,004	**
1007	octanal	492	1757	99243	0,023	*
1013	(E,E)-2,4-heptadienal	210	1596	15512	0	***
1016	1-methylbutyl butanoate	1652	509	13874	0,096	
1030	2-ethyl hexanol	1003	249	10362	0	***
1033	limonene	1043	515	9157	0,09	
1037	benzyl alcohol	1300	4449	41278	0,007	**
1045	phenylacetaldehyde	190	1182	7239	0,062	
1056	3-methylbutyl butanoate	801	203	17541	0,147	
1065	acetophenone	190	748	10903	0	***
1076	dihydromyrcenol	2202	9098	76458	0,001	***
1090	1-hydroxycumene	230	566	11432	0,001	***
1098	3,5-octadien-2-one	723	2981	33544	0,001	***
1101	linalool	4032	575	13291	0,034	*
1108	nonanal	2066	6477	236548	0	***
1109	(E)-6-methyl-3,5-heptadien-2-one	1489	3976	48204	0	***
1113	2-hydroxy-5-ethyl-5-methyl-cyclopent-2-en-1-one	210	671	4734	0,003	**
1114	2,6-dimethylcyclohexanol	1181	727	6175	0,017	*
1117	furan derivative1	190	210	2002	0,115	

1122	phenylethyl alcohol	1492	2434	24162	0,005	**
1155	β-citronellal	230	190	658	0,412	
1164	(E)-2-nonenal	150	210	8481	0,001	***
1172	benzyl acetate	434	210	5836	0,005	**
1174	1,3-dimethoxybenzene	6783	343	74792	0,027	*
1178	menthol	247	230	2089	0,039	*
1182	octanoic acid	210	1239	11616	0,043	*
1192	α-terpineol	482	170	3760	0,007	**
1192	naphthalene	504	210	8760	0	***
1198	methyl salicylate	3179327	2131657	4067866	0,327	
1201	2,2-dimethoxyethyl benzene	9683	3583	150	0,175	
1206	decanal	6814	1448	171800	0	***
1213	alcohol1	13757	19574	16451	0,923	
1218	alcohol2	21849	3846	1110	0,085	
1224	citronellol	6073	5108	3818	0,662	
1229	3-methoxyphenol	3122	843	4361	0,58	
1231	NA5	11505	10508	15875	0,836	
1235	cuminaldehyde	1892	1663	11110	0,01	**
1246	D-carvone	3978	409	5898	0,206	
1255	linalyl acetate	4992	887	5157	0,274	
1260	2-phenylethyl acetate	664	230	1707	0,287	
1263	4-methoxybenzaldehyde	1377	1360	8135	0,011	*
1278	geranial	992	1220	4902	0,271	
1279	3-methoxybenzyl alcohol	1087	2258	8652	0,132	
1282	nonanoic acid	1031	1144	20955	0,003	**
1284	p-menth-4(8)-en-9-ol	1446	295	230	0,433	
1286	ethyl salicylate	3193	4556	150	0,226	
1289	(E)-anethole	4309	230	1567	0,452	
1296	thymol	621	402	2675	0,07	
1297	α,α-dimethylphenethyl acetate	230	230	5106	0,106	
1304	syringol	53786	13448	68700	0,23	
1305	undecanal	170	797	7065	0,001	***
1310	2,7-dimethyltetralin	230	230	2016	0,408	
1317	methylsyringol	9662	708	5427	0,381	
1334	piperonal	6148	5106	190	0,34	
1340	tetralin derivative1	210	230	8196	0,106	
1345	6-ethyltetralin	210	230	7488	0,09	
1346	methyl 2-methoxybenzoate	28279	4231	13951	0,371	
1352	methyl 3-methoxybenzoate	14625	27682	150	0,387	

1354	α-terpinylacetate	958	371	3253	0,273	
1360	eugenol	1132	1690	23200	0,247	
1362	NA6	321	533	6510	0,013	*
1367	alkane1	365	561	5779	0,111	
1372	decanoic acid	212	205	2998	0,086	
1373	alkane2	615	810	3053	0,287	
1382	terpene1	170	230	190	0,178	
1393	benzoic acid derivative1	842	621	150	0,014	*
1397	alcohol3	5569	2283	17980	0,03	*
1400	tetradecane	1094	1015	10737	0,003	**
1407	alkane3	763	210	541	0,448	
1420	thujopsene	1181	8289	58385	0,106	
1422	(Z)-α-bergamotene	862	692	28130	0,121	
1423	indan-1,3-diol monoacetate	760	517	2288	0,128	
1426	sesquiterpene1	230	230	50357	0,382	
1436	methyl 4-methoxysalicylate	453004	377269	903570	0,153	
1438	(E)-α-bergamotene	2430	411	57329	0,128	
1444	NA7	1815	230	705	0,27	
1450	alkane4	1850	1723	2629	0,744	
1454	geranyl acetone	18370	15430	73582	0,014	*
1468	β-chamigrene	336	1575	7625	0,232	
1478	β-chamigrene	2844	652	6805	0,071	
1481	cyclohexenone derivative	5118	5766	230	0,189	
1483	methyl 2,3-dimethoxybenzo-ate	335	559	250	0,055	
1486	β-ionone	4769	2566	19415	0,002	**
1509	(E,E)-α-farnesene	1416	1391	833	0,84	
1516	indan-1,3-diol monopropio-nate	519	230	1536	0,092	
1517	sesquiterpene oxygenated1	841	190	1514	0,316	
1520	2-ethoxynaphthalene	1131	230	476	0,495	
1525	sesquiterpene oxygenated2	1027	567	3937	0,365	
1536	methyl 2,6-dimethoxybenzo-ate	1930	450	26891	0,122	
1542	dihydroactinidiolide	1973	1925	9188	0,002	**
1554	elemicine	913	190	1671	0,249	
1555	amyl salicylate	1445	407	1461	0,578	
1556	β-methylionone	190	495	3909	0,007	**
1556	isoamyl salicylate	1240	230	664	0,575	
1563	hexahydrofarnesol	294	373	638	0,666	
1600	hexadecane	1996	1198	12243	0	***

1610	β-cedrene epoxide	2742	5564	62561	0,291	
1648	methyl dihydrojasmonate	604	680	1258	0,594	
1648	1,2-diacetoxy-4-allylbenzene	2131	1383	11601	0	***
1652	hexyl salicylate	718	190	2937	0,045	*
1685	phenanthrene derivative1	1217	1280	9689	0	***
1691	phenanthrene derivative2	1474	1598	11769	0	***
1694	alkane5	948	687	5896	0	***
1743	phenanthrene derivative3	1584	1604	12840	0	***
1747	α-hexylcinnamaldehyde	950	440	190	0,306	
1843	hexahydrofarnesyl acetone	1280	4651	16454	0,018	*
1869	benzyl salicylate	3914	4551	55819	0,059	
1955	alcohol4	462	230	250	0,444	
1967	triterpene	369	210	3382	0,001	***
2021	aromatic compound1	5053	6914	73026	0,367	
2345	3-(benzyloxy)-6-(hydroxymethyl)-2,5-dimethylphenol	3967	4036	22775	0,002	**

Table 4: Oil from leaf using

RI	Compound	Average Amhara Leaf	Average Oromia Leaf	Average Tigray Leaf	P.value	Sig.
602	acetic acid	230	5323	24834	0,783	
807	hexanal	6158	5343	32633	0	***
843	2-pentyl acetate	20682	542	230	0,148	
854	(E)-2-hexenal	3846	3058	20783	0	***
870	1-hexanol	7691	866	210	0,001	***
903	NA1	6485	9237	42644	0	***
931	1-acetylcyclohexene	2232	16188	80752	0,001	***
945	NA2	646	826	4548	0,005	**
958	dihydro-3-methyl-2(3H)-furanone	8687	2390	11055	0,248	
960	NA3	3924	4060	579	0,406	
971	benzaldehyde	27361	9638	28272	0,124	
986	6-methyl-5-hepten-2-one	74664	68673	293008	0	***
996	NA4	14130	5335	9512	0,354	
1004	hexanoic acid	16935	11406	25486	0,011	*
1005	p-methylanisole	170	2045	37332	0	***

1007	octanal	3621	5611	17779	0,031	*
1013	(E,E)-2,4-heptadienal	14531	10374	52299	0,001	***
1016	1-methylbutyl butanoate	31059	1810	1335	0,237	
1030	2-ethyl hexanol	19357	5461	15765	0,068	
1033	limonene	29167	7110	28850	0,05	*
1037	benzyl alcohol	32139	11354	23698	0,422	
1045	phenylacetaldehyde	19231	23085	80550	0,192	
1056	3-methylbutyl butanoate	11795	2671	17448	0,043	*
1065	acetophenone	40475	16698	16592	0,577	
1076	dihydromyrcenol	23041	27870	122947	0	***
1090	1-hydroxycumene	7447	2508	7088	0,633	
1098	3,5-octadien-2-one	4445	5530	28503	0	***
1101	linalool	40124	8023	19987	0,235	
1108	nonanal	29501	31442	148124	0	***
1109	(E)-6-methyl-3,5-heptadien-2-one	37001	69222	460368	0	***
1113	2-hydroxy-5-ethyl-5-methylcyclopent-2-en-1-one	2144	6377	43466	0	***
1114	2,6-dimethylcyclohexanol	25498	5001	18451	0,023	*
1117	furan derivative1	7802	4425	23628	0,001	***
1122	phenylethyl alcohol	45104	20108	61475	0,068	
1155	β-citronellal	6697	3001	28112	0,023	*
1164	(E)-2-nonenal	1600	1141	6926	0,029	*
1172	benzyl acetate	17854	1519	10679	0,515	
1174	1,3-dimethoxybenzene	4299	170	15643	0,054	
1178	menthol	3863	170	170	0,125	
1182	octanoic acid	14813	9945	20344	0,086	
1192	α-terpineol	8237	586	6929	0,079	
1192	naphthalene	12291	3733	18240	0,001	***
1198	methyl salicylate	4919777	6593653	1507936	0	***
1201	2,2-dimethoxyethyl benzene	9305	716	250	0,175	
1206	decanal	39845	84720	124438	0	***
1213	alcohol1	32976	22167	36933	0,923	
1218	alcohol2	6311	418	210	0,085	
1224	citronellol	22655	3486	4736	0,662	
1229	3-methoxyphenol	210	4864	24485	0,58	
1231	NA5	19763	12977	37191	0,836	
1235	cuminaldehyde	12933	6830	16796	0,01	**
1246	D-carvone	57568	4895	6104	0,206	
1255	linalyl acetate	44023	6921	26223	0,274	
1260	2-phenylethyl acetate	7818	170	5735	0,287	

1263	4-methoxybenzaldehyde	52810	7750	18900	0,011	*
1278	geranial	6147	4792	2170	0,271	
1279	3-methoxybenzyl alcohol	190	3245	9747	0,132	
1282	nonanoic acid	22687	16049	46806	0,003	**
1284	p-menth-4(8)-en-9-ol	15696	2628	4886	0,433	
1286	ethyl salicylate	190	210	250	0,226	
1289	(E)-anethole	78756	5565	4095	0,452	
1296	thymol	6720	2053	8140	0,07	
1297	α,α-dimethylphenethyl acetate	170	170	7563	0,106	
1304	syringol	30027	45282	36800	0,23	
1305	undecanal	230	2085	3336	0,001	***
1310	2,7-dimethyltetralin	170	170	17753	0,408	
1317	methylsyringol	4177	2876	7990	0,381	
1334	piperonal	6574	540	3347	0,34	
1340	tetralin derivative1	1086	170	42186	0,106	
1345	6-ethyltetralin	1178	170	42926	0,09	
1346	methyl 2-methoxybenzoate	9370	11227	8338	0,371	
1352	methyl 3-methoxybenzoate	190	230	250	0,387	
1354	α-terpinylacetate	5702	696	6272	0,273	
1360	eugenol	3610	5393	10788	0,247	
1362	NA6	3066	3956	2651	0,013	*
1367	alkane1	9160	5555	29518	0,111	
1372	decanoic acid	6595	6734	17021	0,086	
1373	alkane2	3950	5072	25033	0,287	
1382	terpene1	617	716	6452	0,178	
1393	benzoic acid derivative1	365	230	250	0,014	*
1397	alcohol3	47905	31521	113300	0,03	*
1400	tetradecane	16886	10256	30615	0,003	**
1407	alkane3	2296	1950	7965	0,448	
1420	thujopsene	1705	1793	2494	0,106	
1422	(Z)-α-bergamotene	901	585	3087	0,121	
1423	indan-1,3-diol monoacetate	7959	170	1782	0,128	
1426	sesquiterpene1	170	170	11286	0,382	
1436	methyl 4-methoxysalicylate	135296	228029	461598	0,153	
1438	(E)-α-bergamotene	6979	610	8568	0,128	
1444	NA7	7297	1046	230	0,27	
1450	alkane4	2585	6521	28774	0,744	
1454	geranyl acetone	84442	109704	422510	0,014	*
1468	β-chamigrene	501	170	210	0,232	
1478	β-chamigrene	29104	4426	12024	0,071	

1481	cyclohexenone derivative	42924	23337	830	0,189	
1483	methyl 2,3-dimethoxybenzoate	170	170	150	0,055	
1486	β-ionone	61513	24867	73135	0,002	**
1509	(E,E)-α-farnesene	335162	119882	9649	0,84	
1516	indan-1,3-diol monopropionate	8224	170	2886	0,092	
1517	sesquiterpene oxygenated1	11763	1204	5302	0,316	
1520	2-ethoxynaphthalene	15809	1439	466	0,495	
1525	sesquiterpene oxygenated2	19423	3397	1714	0,365	
1536	methyl 2,6-dimethoxybenzoate	210	170	56593	0,122	
1542	dihydroactinidiolide	26481	23045	87465	0,002	**
1554	elemicine	24886	5260	7674	0,249	
1555	amyl salicylate	18931	2290	5629	0,578	
1556	β-methylionone	8532	4224	18694	0,007	**
1556	isoamyl salicylate	29989	2128	2830	0,575	
1563	hexahydrofarnesol	5804	2354	17278	0,666	
1600	hexadecane	16049	6557	29555	0	***
1610	β-cedrene epoxide	24243	50657	53360	0,291	
1648	methyl dihydrojasmonate	8266	4270	4878	0,594	
1648	1,2-diacetoxy-4-allylbenzene	24884	11291	28150	0	***
1652	hexyl salicylate	16374	3936	13000	0,045	*
1685	phenanthrene derivative1	10097	5415	25930	0	***
1691	phenanthrene derivative2	12771	7923	31270	0	***
1694	alkane5	6173	2482	8645	0	***
1743	phenanthrene derivative3	12849	7472	37562	0	***
1747	α-hexylcinnamaldehyde	5184	567	2911	0,306	
1843	hexahydrofarnesyl acetone	57087	114737	523697	0,018	*
1869	benzyl salicylate	1202	4836	20414	0,059	
1955	alcohol4	15075	1842	150	0,444	
1967	triterpene	170	514	5771	0,001	***
2021	aromatic compound1	12857	49441	40747	0,367	
2345	3-(benzyloxy)-6-(hydroxyme-thyl)-2,5-dimethylphenol	1092	1822	13151	0,002	**