ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES
CENTRE FOR FOOD SCIENCE AND NUTRITION

NUTRITIONAL COMPOSITION, ANTIOXIDANT PROPORTY AND TOXICITY EVALUATION OF THE ROOT AND GEL OF *Aloe percrassa* Tod.

BY
HABTAMU ADDNEW

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Board of Examiners

Name	Signature	Date
1. Dr. Paulos Getachew (PhD)		09/02/2017
2. Mr. Kelbesa urga		9/02/017
3. Dr. Yihunie Ayele (PhD)		10/02/017
4. Prof. Yalemtsehay Mekonnen		

DECLARATION OF ORIGINALITY

I, the undersigned, hereby declare that this thesis is my original work. The work has not been presented in any university or institution for similar degree or other purposes and all source of materials used for the thesis have been correctly acknowledged.

Name of candidate: Habtamu Addnew

Signature: _____

Date of Submission 10/02/17

This MSc thesis has been submitted for the evaluation with my approval as a university advisor.

Name of the advisor	Signature	Date
1. Dr. Yihunie Ayele	_____	10/02/017
2. Prof. Yalemtsehay Mekonnen	_____	_____

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LIST OF ABBREVIATIONS

ALTL	Alanine aminotransferase
AOAC	Association of Official Analytical Chemists
ASTL	Aspartate aminotransferase
CSA	Central Statistics Agency
DPPH	2, 2 diphenyl-1-picrylhydrazyl
EARs	Estimated Average Requirements
EDHS	Ethiopia Demographic and Health Surveys
HCT	Hematocrit
HGB	Hemoglobin
IC_{50}	The 50% inhibitory concentration
LD_{50}	Lethal Dose for 50%
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
OECD	Organization for Economic Cooperation and Development

ABSTRACT

Different Aloe species can be utilized in various forms. However, A. percrassa has not been utilized to its fullest due to lack of information. Thus, in this study the proximate, anti-nutritional, antioxidant composition and toxicity of methanol extract of the gel and root of A. percrassa were determined. The proximate composition analysis involved the moisture, crude protein, crude fibre, crude fat, ash and carbohydrate contents while the anti-nutritional assessment involved tannins and phytate. The antioxidant property was determined using DPPH free radical scavenging method. The acute and sub-acute toxicities were evaluated on mice by oral administration of extracts for 24 hr and 28 days, respectively. Histopathological changes on the liver and kidney of mice were examined on haematoxylin-eosin stained tissue sections. The moisture content covered the uppermost portion of the plant part, that is, 97 and 82.27% of the gel and root, respectively. The gel of A. percrassa was found to contain carbohydrate (2.27%) and ash (0.45%) content while the root has carbohydrate (8.41%), fiber (7.59%) and ash (0.88%). The protein and fat content were found to be relatively low, 0.13 and 0.08% in the gel and 0.59 and 0.26% in the root, respectively. The level of minerals such as calcium, potassium, sodium and iron in the root extract were 871.03, 387.86, 378.03 and 9.39 mg/100 g powder, respectively. The gel is also rich in K, Ca, Na, P, Zn and Fe with 4765.23, 782.76, 280.15, 3.55 and 3.02 mg/100 g powder, respectively. The antioxidant results also indicated that both extracts displayed concentration dependent inhibitory activities, though not high, with IC_{50} values of 108.76 for the root and 316.92 µg/L for the gel exceeding the standard ascorbic acid with IC_{50} value of 41.82 µg/L. In the gel, tannin and phytate contents were 5.61 mg/100 g and 127.90 mg/100 g, respectively whereas the root part contains lower tannin content (49.26 mg/100 g) but higher phytate content of 1497.20 mg/100 g on dry weight bases. Concerning toxicity studies, the acute toxicity study both root and gel extracts at a dose rate of 2, 4 of 6 g/kg didn't cause mortality or induce physical signs of toxicity on mice during the 24 hr observation period. The LD_{50} of extracts of the root and gel of A. percrassa is estimated to be greater than 6 g/kg. The sub-acute toxicity study also showed neither death of the mice nor significant difference in haematological and blood chemistry results showed compared to the control group. However, histopathological examination slight focal defects were observed in the sectioned liver and kidney of four test mice. In conclusion, the findings of the present study suggest that there is an indication that A. percrassa contains important proximate and mineral compounds that may be linked to its beneficial effects on diet and health. It would be worthwhile embarking on further scientific experimentation and investigation on this valuable nutritional plant and to promote its large-scale utilization so that it can be used as a good source of carbohydrates, minerals and fibres.

Key words: *Aloe percrassa, Antioxidant, Anti-nutrient and Toxicity*

1. INTRODUCTION

1.1 Background

Ethiopia is one of populous countries in Africa. The population has increased steadily from time to time, from 42.6 million in 1984 to 53.5 million in 1994 and 73.8 million in 2007 (CSA and ICF, 2011). Also based on the 2007 Population and Housing Census and the 2012 Inter-Censual Population Survey the medium variant projection projected the country's total population to be 85.8 million for 2013, 87.9 million for 2014 and 90 million for 2015. The population growth is contributing to the challenge of providing nutritious and affordable food. The increasing population growth could place extreme pressure on the agricultural land causing land degradation or erosion (Waithaka *et al.*, 2013). The balance between food provision and population growth can be addressed through using alternative food sources.

Utilization of underutilized plant species remains the major option in increasing food supply and diversity. Ethiopia is endowed with many bio-resources of huge potential and high prospects. Especially, plant species which can be used as the main sources of food are abundant in different part of the country. However, these potential bio-resources have been marginalized by mainstream research institutes and universities (Lulekal *et al.*, 2011).

Among plant species, *Aloe* contains numerous vitamins, minerals, enzymes, amino acids, natural sugars and other bioactive compounds with soothing the skin, laxative and antimicrobial activities (Mariappan and Shanthi, 2012), anti-inflammatory, antioxidant, aphrodisiac, anthelminthic, antifungal, antiseptic and cosmetic values for health care (Sahu *et al.*, 213). This plant has potential to cure sunburns, burns and minor cuts, and even skin cancer. The external use in cosmetic primarily acts as skin healer and prevents injury of epithelial tissues, cures acne and gives a youthful glow to skin, also acts as extremely powerful laxative (Rajeswari, 2012; Pankaj *et al.*, 2013). One of the extensively studied species, *Aloe vera*, is applied as a postharvest treatment to inhibit microbial spoilage and reduce decay incidence during postharvest storage of grapes, because of its bioactive molecules, such as *Aleonin* and *Aloe-emodin*. These chemicals have shown antifungal activity against *Aspergillus*, *Cladosporium* and *Fusarium* (Chauhan *et al.*, 2014). In addition some beverages can be prepared from the gel extracts after removing the toxic chemical *Aloin*. *Aloin* is found in leaf part of the plant, especially in exudates. To avoid health hazard,

removing of the exudates before preparation of the drink is very important (Lachenmeier *et al.*, 2005).

Aloe percrassa is also one of the potential candidate species for food, medicine and cosmetics applications. The plant is unique in that it grows in the marginalized land especially in rocky mountains. *A. percrassa* is traditionally used to treat malaria, wound, eye inflammation, pain, treatment, dermatological and gastro intestinal problems. However, very little scientific evidences support these claims. Nonetheless, few studies showed that this species of *Aloe* has analgesic, anti-inflammatory activities and this could partly contribute to their traditional use against infectious and inflammatory related disorders (Sibhat and Periasamy, 2015).

The root of *A. percrassa* is usually used as animal feed at times of animal food shortage in Southern Tigray. Besides, some shepherds used to consume it and express it is sweet. These are evidences for the existence of a tremendous opportunity to develop food from the gel and root parts of *A. percrassa*. To secure adequate food that is healthy, safe and high nutritional quality, the root and gel extract of this plant is the right candidate to serve as functional ingredient in the development of new food product. However, lacking scientific information on the nutritive values and health benefits is a major constraint in its utilization.

Diet in Ethiopia is characterized by a heavy reliance on cereals and grains as the primary source of energy. This contributes for the absence of food diversity in the country. Unlike *A. percrassa* other species of aloe are well studied and have scientific data on chemical composition and biochemical activities (Ahmed and Hussain, 2013), antioxidant property (Vidic *et al.*, 2014), safety (Mwale and Masika, 2012) and nutritional benefits. The absence of data may affect the likely contribution of the plant in the diet of Ethiopians. Therefore there is a need to investigate the potential uses of *A. percrassa* in the development of new foods, though its medicinal use was shown in very few studies (Gebrelibanos *et al.*, 2014)

1.2 Statement of the Problem

Several species of *Aloe* have been widely studied and some of them are used as a food, cosmetics and medicine but due to absence of studies and lack of awareness on its food composition and edibility, the use of *A. percrassa* is limited to animal feed only. Thus, assessing its potential as a food for human and maximizing its production will help in fighting food insecurity.

2. OBJECTIVE OF THE STUDY

2.1 General objective

The main objective of this study is to evaluate the nutritional value, antioxidant capacity and toxicity level of *Aloe percrassa* Tod.

2.2 Specific objectives

The specific objectives of this study are to:

- analyse the nutritive values of the root and gel of *A. percrassa.*
- determine the antioxidant potential of the root and gel of *A. percrassa.*
- assess the anti-nutritive effect of the root and gel of *A. percrassa.*
- investigate the toxicity of root and gel extract of *A. percrassa.*

3. LITERATURE REVIEW

3.1 Contributions of Edible Wild Plants in Food Security

Aloe species are underutilized plant species in different part of Ethiopia. According to the World Food Summit organised in Rome (1996), the consensus definition of food security was coined as "food security exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets their dietary needs and food preferences for an active and healthy life". The Food and Agriculture Organization of the United Nations (FAO) State of Food Insecurity for 2010 assesses that nearly one billion people are estimated to be undernourished, representing almost 16% of the population of developing countries. This problem needs some solution.

Edible wild plants refer to species that are neither cultivated nor domesticated, but are available from their wild natural habitat and used as sources of food (Beluhan and Ranogajec, 2010). Edible wild plants had high importance to local populations and the availability of edible wild plants plays an important role in rural livelihoods through ensuring food security, dietary diversity and sustained income. So far, 413 wild edible plants of different species are identified in Ethiopia, 129 species of shrubs, representing the dominant growth form, followed by 127 trees, 121 herbs, and 36 climbers. Fruits were the most commonly reported edible parts of about 210 of wild plants reported for food, followed by leaves 97 and seeds 43. Moreover, other parts or products such as gum, nectar, bark, inflorescence, tubers or a combination of two or more of these parts or products were also reported for edibility in different communities (Lulekal *et al.*, 2012).

Edible wild plants are not only relevant for certain groups but for everyone in the community. They contribute significantly to the food supply, particularly, in low income households. In some rural area the households harvest edible wild plants for domestic use and for sale as a primary source of income with the wealthy earning more than the poor (Neudeck *et al.*, 2012). Edible wild plants are not used during normal times when cultivated food is abundant (Nagwa and Hassan, 2014), but during seasonal food shortages when household stocks were empty and the new crop were still in the field (commonly from July to September), collecting, selling and consuming wild edible plants largely practiced in different parts of the country including Michew, the place where *A. percrassa* was collected (Gebrezegiabher and Tsegay, 2015). Many wild fruits that ripen at this time, therefore important back-ups of household

food and animal feed are there. Generally, the year-round availability of edible wild plants of different species within and across the study areas provides supplementary food and nutrition and presents an opportunity for trade if properly supported by extension services (Feyssa *et al.*, 2011).

Despite their high value, populations of some edible wild plants have been reported to be declining due to deforestation, expansion of cultivated areas, and over-harvesting being major threats. Sustainable utilization and awareness are needed to respond to these threats. The values of edible wild plants need to be recognized so that they can receive the conservation status they deserve and be developed both *in situ* and *ex situ*, e.g., through domestication (Neudeck *et al.*, 2012).

3.2 General Descriptions of *Aloe*

3.2.1 Family "Liliacea"

Family *Liliacea* is a widely distributed family of about 250 genera and 3,700 species; mostly perennial herbs with a rhizome or bulb. The flowers usually have six stamen and a superior (rarely semi-inferior) ovary, with numerous anatropous ovules attached to axile placentas. The fruit is loculicidal or septicidal capsule, or aberry (Bhuvana and Hema, 2014). But according to the most recent and widely accepted taxonomic information, the genus *Aloe* belongs to family *Aloaceae* (Alessandro and Stefano, 2005).

3.2.2 Genus "Aloe"

Aloe, the semi-tropical plant, has a long and illustrious history dating from biblical times. It has been mentioned throughout recorded history and given a high ranking as an all-purpose herbal plant. *Aloe* has thick, tapered and spiny leaves that grow from a short stalk near ground level. It is not a cactus, but a member of the tree Lily family and is related to other members the family such as the onion, garlic and turnip families. *Aloe's* relationship to the lily family is evident from the tubular yellow flowers produced annually in the spring that resemble those of the Easter lily (Vogler and Ernst, 1999).

Aloe has rosette of large, thick, succulent leaves, which are sometimes spotted. The rosettes are situated on the ground or on trunks up to 2 m. In rare cases, the thick leaves are spaced along a stem rather than crowded in a rosette. In most species the leaves are D-shaped in cross

section, but some have leaves more or less V-shaped in cross section. The leaf margin is almost always armed with sharp teeth. The inflorescence is usually branched (occasionally simple), the lower branches sometimes branching again. Each flower is supported by a bract, the shape and size of which are important for the identification of the species. Flower colouration is most often red, orange or yellow, rarely white. The tepals are fused to form a tube (but free to the base in *Aloe steudneri*). The upper parts of the tepals are more or less reflexed. The 3+3 stamens are free, inserted at the base of the ovary, exerted in the flowering stage. The capsule wall is papery or slightly woody when mature. The seeds are irregularly 3-sided to flattened, narrowly to broadly winged (Demissew and Nordal, 2010).

Despite the fact that many species of *Aloe* grown around the world, only two species are grown today commercially, with *Aloe barbadensis* Miller (*Aloe vera*) and *A. aborescens* being the most popular. *A. vera* is as old as civilization and throughout history it has been used as a popular folk medicine. It is present in the arid regions of the world, and is believed to be effective in treating stomach ailments, gastrointestinal problems, skin diseases, constipation, for radiation injury, for its anti-inflammatory effect, for wound healing and burns, as an anti-ulcer and diabetes. Currently, the plant is widely used in skin care, cosmetics and as nutraceuticals (Noor *et al.*, 2008).

The *Aloe* leaf can be divided into two major parts, namely the outer green rind, including the vascular bundles, and the inner colourless parenchyma containing the *Aloe* gel. Description of the inner central part of the aloe leaf may sometimes be confusing, due to the different terms that are used interchangeably such as inner pulp, mucilage tissue, mucilaginous gel, mucilaginous jelly, inner gel and leaf parenchyma tissue. Technically, the term pulp or parenchyma tissue refers to the intact fleshy inner part of the leaf including the cell walls and organelles, while gel or mucilage refers to the viscous clear liquid within the parenchyma cells (Casper *et al.*, 2012). Chemical analysis has revealed that this clear gel contains amino acids, minerals, vitamins, enzymes, proteins, polysaccharides and biological stimulators.

Public interest in *Aloe* has grown quickly, and now there is a considerable amount of research into the various components of *Aloe* to find out more about their properties and to characterize these components so that more specific research can provide clues to the "magic" that is attributed to *Aloe* plant (Arunkumar and Muthuselvam, 2009).

3.2.3 Species "percrassa"

About 46 species of *Aloe* are known in Ethiopia and Eritrea; of which 31 species are endemic. *Aloe percrassa* also known as *Aloe abyssinica* var. *percrassa baker* is one of the species native to Ethiopia (Demissew and Nordal, 2010). In some rural locations, around Adigrat (a northern Ethiopia), *A. percrassa* is known as *"Ere-Meeraat"; "Ere"* means *Aloe*, and "*Meraat*" means bride. The name is believed to have been derived from the fact that gel of the plant was widely used as traditional shampoo for brides (Abrha *et al.*, 2014). Around Maichew (Southern Tigray), *A. percrassa* is also known as "*Abyi-Ere*", means that *large-Aloe*, since it is larger than the other *Aloe* species around. The species was described in 1875 based on a plant grown in St. prtersburg (Leningrad) from seeds sent by Schimper from Tigray floristic region in Ethiopia (Demissew and Nordal, 2010).

3.2.4 Botanical Description of Aloe percrassa

Aloe percrassa Tod is a succulent herb, suckering from base to form small groups, commonly stemless but sometimes developing erect or decumbent stem up to 80 cm long, 10-15cm thick (Figure 1). It' leaves are crowded, 40-55x13-15cm or longer, glaucous-green or grey-green, often suffused red, old leaves brown when drying (Figure 1 B). It's marginal spine 6-16 per 10cm 2-3-3mmlong, with pale pink to brown tips. It's florescence is 60-80cm high with 5-12 racemes. Its racemes are cylindrical to conical, 6.5-25cm long, with 2-5 flowers per cm (Figure 1 A). It's bracts are ovate acuminate, 8-10-16-20x2.5-3-6mm, pedicels 11-17-20mm long, It's perianth are cylindrical,17-23 mm long, 4-6mm wide pressed; outer lobes free for 5-7 mm and flowers in September and October and occasionally in March and April (Figure 1) (Demissew and Nordal, 2010).

Figure 1. *Aloe percrassa* (plant), (A) Flower (B) Leaf
(Photo taken on January 10, 2016 from Belago area, Maichew)

3.2.5 Geographical distribution

The genus *Aloe*, which includes about 400 species, has wide distribution in Africa especially south of Sahara, including Madagascar and Mascarenes. Few species reach the Arabian Peninsula and Socotra. The genus was introduced to the Mediterranean region and the West Indies a long time ago. *A. percrassa* grows in sparsely vegetated rocky slopes and outcrops between 2100 and 2700m in Ethiopia (Tigray, Gonder) and Eritrea (Demissew and Nordal, 2010).

3.3 Phytochemical Composition

Phytochemicals are non-nutritive plant chemicals, naturally occurring biologically active compounds in plants (Rajeshwari *et al.*, 2014). *Aloe* plants are store houses of different secondary metabolites belonging to different classes of compounds. They elaborate many types of compounds, such as *alkaloids*, *anthraquinones*, *pre-anthraquinones*, *anthrones*, *bianthraquinoids*, *chromones*, *flavonoids*, *coumarins* and *pyrones* (Dagne *et al.*, 2000).

Anthraquinones are main active constituent of *Aloe* plants latex, including the *hydroxyanthracene* derivatives *aloins* A and B, *barbaloin*, *isobarbaloin*, and *emodin*, 7-*hydroxyaloins* A and B, *aloeemodin*, *resins*, *aloesin* and its *aglycone aloesone*, *chromone* derivatives and *chrysophanol* (Sikarwar Mukesh *et al.*, 2010).

Aloe plant products are only suitable for human consumption if they are free from *aloin*, a native *Aloe* constituent that acts as a laxative and is supposed to be a DNA-damaging and carcinogenic agent. Entering of *aloin* can be prevented during production process if the outer layers of the Aloe plant, contains the highest quantities of *aloin*, are carefully discarded before extracting the juice (Lanchenmeier *et al.*, 2005). *A. vera* products may be considered *aloin*-free if it is below the maximum limit of 0.1 mg/l (Logaranjan *et al.*, 2013). Treated with activated carbon is important to remove *aloin* from the extract, in case of possible contamination of the latex with the gel. There are basic methods of extraction of aloe leaf; such as Traditional hand filleted Aloe processing, whole leaf Aloe processing and total process Aloe processing, with different unit operation (Ramachandra and Rao, 2008). In general, *Aloe* plant is rich in phytochemicals such as saponins, Phenols, Alkaloids and Flavonoids that are important ingredients in pharmaceutical and cosmetics industries (Adesuyi, 2012).

3.4 Nutritional Composition

The nutritional constituent of *A. percrassa* is not well studied, but some *Aloe* species are studied and have important nutritional value. Two of the species: *A. vera* and *A. ferox*, are the most wildly used species of *Aloe* plant as source of nutrients in the form of juice, beverage and food additives (Lachenmeier *et al.*, 2005). Both *A. vera* and *A. ferox* has different nutritional constituents like carbohydrate, important amino acids, minerals and vitamins (Haque, 2010). *A. vera* gel contains a large range of vitamins such as vitamin A, B-Group vitamins including vitamin B12, vitamin C, vitamin E and folic acid and also it contains important amino acids including 19 of the 20 amino acids needed by the human body and seven out of eight essential ones (Table 1). *A. vera* also includes quite an impressive range of fatty acids. Aloe contains three plant sterols, which are important fatty acids, cholesterol (which lowers fats in the blood), campesterol, and B-sitosterol. Other fatty acids include linoleic, linolenic, myristic, caprylic, oleic, palmitic, and stearic are also found in Aloe plant (Rajeswari, 2012).

3.4.1 Carbohydrates

Aloe vera gel is a popular dietary supplement ingredient. Sugars are derived from the mucilage layer of the plant under the rind, surrounding the inner parenchyma or gel. They form 25 percent of the solid fraction and comprise both monosaccharide and polysaccharides. The most important are the long chain polysaccharides, comprising glucose and mannose, known as the gluco-mannans. The polysaccharides are absorbed complete and appear in the blood stream unchanged hence they act as Immunomodulatory (Duncan *et al.*, 2008). Commonly *Aloe* has two sugary elements, monosaccharide's (glucose and mannose), are simple molecules, and polysaccharides (acemannan and cellulose) are complex molecules (Bassetti and Sala, 2005). In addition, *A. barbadensis* is also rich in carbohydrate (73.07% of dry base matter) and can be used as a good source of carbohydrate (Adesuyi, 2012).

3.4.2 Minerals

In *A. Vera*, about 20 minerals are found including calcium, magnesium, zinc, chromium, selenium, sodium, iron, phosphorus, potassium, copper, and manganese (Nwaoguikpe *et al.*, 2010). According to Pawar and Kamble (2015), *A. Vera* is free from some heavy and toxic metals like mercury, silver, cadmium, lithium, beryllium and bismuth.

Table 1. Food constituents of *Aloe* (Bhuvana *et al.*, 2014)

Saccharides	**Vitamins**	**Essential-amino acids**	**Enzymes**	**Minerals**
Cellulose	B1, B2, B6, B12	Lysine	Cyclooxygenase	Calcium
Glucose	Ascorbic Acid	Threonine	Oxidase	Sodium
Mannose	Choline	Valine	Amylase	Chlorine
L-rhamnose	α-tocopherol	Leucine	Lipase	Potassium
Aldopentose	β-carotene	Isoleucine	Alkaline Phosphatase	Copper
	Folic acid	Phenylalanine	Carboxypeptidase	Magnesium
		Methionine	Catalase	Iron

3.4.3 Vitamins

The number of people who take vitamin supplements is increasing due to a greater awareness of its benefit. In the USA it is estimated currently that between 51% and 61% of the

population consume vitamin supplements. The elderly population is greatly increasing in developed nations. This group is especially vulnerable to vitamin deficiency, due to age-related decreases in absorption, reduced food intake, and increased drug uses (Vinson *et al.*, 2005). *A. Vera* is a useful source of vitamins and it contains a large range of vitamins, taking of *Aloe* juice contains many vitamins, including the important antioxidant vitamins A, C and E, vitamins B1 (thiamine), vitamin B2 (riboflavin), vitamin B12, niacin, Choline and folic acid (Rajeswari *et al.*, 2012).

3.4.4 Amino acids

Aloe gel contains important amino acids including 19 of the 20 amino acids needed by the human body and seven of the nine (Table 1) essential ones that just cannot be synthesized by human body (Nwaoguikpe *et al.*, 2010; Rajeswari *et al.*, 2012). Using HPLC the essential amino acids such as threonine (7.4 ppm), valine (12.5 ppm), methionine (11.6 ppm), isoleucine (14 ppm), leucine (17.7 ppm), phenylalanine (13.8 ppm), and lysine (36.5 ppm) are studied from *A. vera* gel extract (Sugiastuti, 2012).

3.5 Commercial Use of *Aloe*

Commercially, thousands of *Aloe* based products are available in the world market, some of them can be found in the form of pills, sprays, ointments, lotions, liquids, drinks, jellies, and creams (Nandal and Bhardwaj, 2012). Global turnover of raw *Aloe* leaves attains 70-80 million USD, for the next five years which is expected to grow at a rate of 35%. USA supplies the major bulk of *Aloe* in the world market having a share of 60-65%, whereas Latin American countries with 20-25% and Australia, China and India have a market share of only 10% (Das and Chattopadhy, 2004).

The unique properties of this plant are deep penetration antiseptic, stimulates cell growth and new tissues, clearing effects on nervous system, dietary fibre, normalization of metabolic path way. Due to this property Aloe plant is extensively preferred as a natural product with medicinal and therapeutic properties; dietary supplements and cosmetics. Also it has been used as a novel functional foods production of gel containing healthy drinks/beverages. (Banjare *et al.*, 2014). Many of the health benefits of this plant have been associated with polysaccharides contained in the gel of the leaves (Singh, 2010; Banjare *et al.*, 2014).

3.5.1 Medicinal use

Studies showed that different *Aloe* species have different medicinal effects. For example, *A. vera* has antimicrobial activities (Shireen *et al.*, 2015), antioxidant activity, anti-diabetic effects, immunomodulatory effects, lipid lowering effects, hepatoprotective activities, analgesic and anti-inflammatory effects (Mwale and Masika, 2010; Banjare *et al.*, 2014), wound healing effects, anti-cancer effects (Harrison, 2009), relieving intestinal problems, increasing high density lipoprotein (HDL), reducing low density lipoprotein (LDL), fighting acquired immune deficiency syndrome (AIDS), minimizing allergies and improving immune system (Ahlawat and Khatkar, 2011). Dermatological health benefits of *A. Vera* include its application in wound healing, treating burns, protection against skin damage from x-rays and also used for skin hydration (Ahlawat and Khatkar, 2011).

The latex of *A. Vera* is officially approved as a laxative in the U.S., England, and Germany. The German commission recommends the use of *Aloe* for occasional constipation and for conditions that require a soft stool, such as anal fissures, haemorrhoids, and after rectal or anal surgery. Externally, the latex is used like the gel and as a soothing agent in treating burns and mild cuts. (Sikarwar Mukesh *et al.*, 2010).

Aloe powder has an important application in pharmaceutical product preparation, use as excipients, in sustained release pharmaceutical dosage forms; enhance the intestinal absorption and bioavailability of co-administered compounds (Hamman, 2008; Singh *et al.*, 2010).

There is paucity of information on the medicinal effect and food value of *A. percrassa* worldwide. But the existing few studies showed the medicinal effect of the leave extracts of this plant. To mention some of them, the analgesic activity of the leaf latex of *A. percrassa* demonstrated by Geremedhin and Periasamy (2015) justified their traditional use for treatment of pain. Gebrelibanos *et al.*, (2014) also proved that their traditional uses against infectious and inflammatory related disorders and Geremedhin (2014) justified the traditional use for treatment of malaria.

3.5.2 Cosmetics

The use of *Aloe* plant in the field of cosmetic industry is increasing and wide variety of products contain *Aloe* in one form or in another for delivering specific activity (Basmataker *et*

al., 2011). It is commercially used as soaps, shampoos, creams, lotions and other beauty purposes products. Because of its high penetrative ability *Aloe* is used as a base to carry other active ingredients deep in to the skin to nourish the dermis (Joseph and Justin, 2010). Due to high water content, it hydrates or moisturizes the skin, when applied topically. It also removes dead skin cells and rejuvenates the skin. *Aloe* increases the elasticity of the skin through *collagen* and *elastin* production (Das and Chattopadhy, 2004). It helps supply oxygen to the skin cells, increasing the strength and synthesis of skin tissue and induces improved blood flow to the skin through capillary dilation (Pounikar, 2012). It also reduces the formation of melanin and any tendency to hyper-pigmentation. People with oily skin can prevent formation of pimples and acne by using this gel. Scarring and scratch marks are some of the signs of aging which can be prevented by the antioxidants present in this plant. It also used as natural treatment for dry hair and dandruff (Rajeswari, 2012).

3.5.3 Food and feed source

Aloe vera gel has wide application in food industry such as manufacturing of soft drinks, yoghurts and jams (Jayabalan and Karthikeyan, 2013), instant tea granules, candies, alcoholic beverages, ice cream, ready to use drink, health drink, soft drink, laxative drink, *A. vera* lemon juice, sherbet, *Aloe* sports drink with electrolyte, diet drink with soluble fiber, hangover drink with B vitamin, healthy vegetable juice mix, tropical fruit juice with aloe vera, aloe vera yoghurts, aloe vera mix for whiskey and white bread, cucumber juice with *A. vera* (Hamman, 2008; Ahlawat and Khatkar, 2011). FDA (Food and Drug Administration) approved the gel of *A. vera* whole leaf extract (which combines both the gel and latex) and *A. vera* decolorized whole leaf extract (from which most of the latex components have been removed) are popular as dietary supplements for various systemic ailments (Steenkamp and Stewart, 2007; Mukesh *et al.*, 2010). *A. vera* gel used in beverages industry as bitter flavouring agent. In the USA, it is becoming a common phenomenon for people to add the powdered extracts of *Aloe* to their food or take one of the many flavoured *A. vera* drinks (Eshun and He, 2004).

3.6 Use of *A. Percrassa*

3.6.1 A. percrassa as animal feed

Livestock in Tigray region mainly get their fed from crop residues after the crops are harvested, during the time when crops are not yet harvested and most of the grazing lands are covered by crops scarcity of feed is very common. The problem of feed is more aggravated in arid and semi-arid areas of the country, with erratic and unreliable rainfall, where moderate and severe droughts are common (Birhane, 1997; Haile *et al.*, 2002). The farmers find different solutions for their animal feeds. Root of *A. percrassa* is one of the plants that are used by the farmers in Maichew, Southern Tigray, during that period. Preparing this plant ready to feed to the animals starts from gathering the root from the field, removing the outer part of the root and then chopped in to small pieces and finally makes it ready for animal feed. Since this plant is found mainly in Rocky mountain of Michew and areas where the feed scarcity is common, use of *A. percrassa* root for animal feed is common.

3.6.2 *A. percrassa* as soil and water conservation

Land degradation is a serious global problem. Tigray contains the largest degraded land in Ethiopia's highlands (Fitsum *et al.,* 1999). The landform is generally rugged and broken; the soils are shallow, stony and rocky (SAERT, 1994). They are characterized as marginal land. Steep slopes favour runoff. For the last few years' the government and non-governmental organizations have implemented various land rehabilitation programs to address these problems through the introduction of conservation methods such as stone terraces, soil bunds, area enclosure and A-forestation. Among this the predominant one is area closures, through tree planting and physical conservation measures such as terracing (Mebrat, 2015). This method has indispensible role for rehabilitation of degraded lands, animal conservation, soil development and conservation (Tiki *et al.*, 2015). Some steep and mountainous areas in southern Tigray have been covered with natural Aloe plant thus preventing the area from severe run-off as well as erosion. A-forestation using endogenous plants is very important in water and soil conservation. *A. percrassa* is one of the plants that grow in the rocky mountains of Maichew, Southern Tigray, and traditionally used in terracing.

3.6.3 A. percrassa as source of honey

According to Crane (1990), from 250,000 plants in the world, about 40,000 plant species are important for honey bee as a food source. Due to its wide climatic variability, Ethiopia is endowed with diverse and unique flowering plants of 6,000 to 7,000 species thus making it highly suitable for large number of colonies and long practice in beekeeping (Gezhagn, 2007; Gidey and Mekonen, 2010). Tigray region has also various agro ecological zones that are suitable for the growth of different bee flora and development of apiculture. It is estimated that the region has about 202,000 bee colonies across the different ecological zones (Ayalew, 2004) contributing about 5% of the Ethiopian honey and bee wax production (Melaku *et al.*, 2008). Based on the studies of Equar *et al.*, (2015) and Gebremeskel *et al.*, (2015) one species of Aloe, *A. berhana,* is one of those common honey bee flora plant spices in Tigray. Scientifically use of *A. percrassa* plant as resource of bee flora is not yet studied, but from indigenous knowledge of beekeepers, *A. percrassa* is one of sources of red and yellow colour honeys in mountainous area of Maichew, Southern Tigray.

4. MATERIALS AND METHODS

4.1. Collection of Plant Material

Aloe percrassa plant was collected from Bolago area in Maichew, Southern Tigray, Ethiopia (Figure 2). Maichew is located at 665 km north of Addis Ababa along the highway which runs to Mekelle. It is located at 12°47′N longitude and 39°32′E latitude. Its elevation is 2,479 meters above sea level whereas Bolago is 3,500 m.a.s.l. (http://en.wikpedia.org/wik/ Maychew; Meire *et al.*, 2012). The samples were identified and authenticated by Prof. Sebsibe Demissew at National Herbarium of Ethiopia, Department of Biology, College of Natural and Computational Sciences, Addis Ababa University.

Figure 2. Map of Tigray Regional State
(Source: www.tigraionline.com/tigraistat.html)

4.2. Preparation of Samples

The whole leaves were cleaned by washing them individually with tap water (Appendix 1 A), after removing the dirty matter by washing, weighed individual leaf using Fx-320 Electronic balance, (made by A and B company Limited, Japan). The cortex was carefully separated from the parenchyma using plastic knife manually (Appendix 1 B) (Ramachandra and Rao, 2008 and Muñoz *et al.*, 2015). The extracted gel was weighed to calculate the gel yield. The gel was collected in a plastic container for further lyophilizating using Chris alpha 2-4LD plus lyophilizer (Martin Christ Gefriertrocknungsanlagen GmbH, Germany) under vacuum (Appendix 2). The lyophilized material (Appendix 3 A) was reduced to powder using mortar and pestle (Appendix 3 B) and stored at room temperature in closed glass flasks to prevent gel humidification until analysis were conducted

The root of *A. percrassa* was thoroughly washed with tap water and removed the dirty matters with its outer part using knife (Appendix 4 A). Then chopped into small pieces (Appendix 4 B) and dried in an oven at 40°C and the dry material was powdered using grinder for proximate and antioxidant studies (Appendix 5).

Powders of both the gel and root were re-extracted by 80% methanol using maceration technique, and allowed to stand at room temperature for 72 hrs with frequent agitation using shaking water both, until the soluble matter were dissolved. The mixtures were filtered using Whatman filter paper (0.45 µ filter units). Finally the extracts were concentrated using a rotary evaporator at 40°C under reduced pressure. Toxicity studies were conducted (Handa, 2008; Arunkumar and Muthuselvam, 2009; Doughari, 2012).

4.3 Proximate Chemical Analysis

4.3.1 Determination of the moisture content

Moisture content of the samples determined according to (AOAC, 2000) using the official method 925.09. A clean dried and covered flat aluminium dish was weighed and about 5g of the sample was transferred to the dish. The dish was placed in the oven (Memmert 854 Schwabach, Germany) at 105°C for 5hrs and cooled in desiccators and re-weighed. Then, the moisture content was calculated by the formula:

$$\text{Moisture content (\%)} = [((\text{weight of fresh sample} - \text{weight of dry sample})/(\text{weight of fresh sample}))] \times 100$$

4.3.2 Determination of crude protein

Protein content was determined according to (AOAC, 2005) using the official method 979.09. A digestion flask containing about 1 g of sample, to which 6 mL of acid mixture (conc. Sulphuric acid and conc. Orthophosphuric acid) and about 3 g of catalyst mixture (K_2SO_4 and Selenium) was added and heated to about 370°C. Digestion was continued until clear solution was obtained. Distillation was take place by adding 25 mL of 40% NaOH and using 285 mL of boric acid with 10 drops of indicator solution. Finally, the distillate was titrated with standardized 0.1N sulphuric acid to a reddish colour. Then, crude protein content was estimated using the formula:

$$\text{Total nitrogen} = ((V_2 - V_1) \times N \times 14.007 \times 100)/W)$$

Where,

V_1 = Volume in of standard sulfuric acid solution used in the titration for the blank determination.

V_2 = Volume in mL of standard sulfuric acid solution used in the titration for the test material.

N = Normality of standard sulfuric acid.

W = Weight in grams of the test material.

N.B. Crude protein content percent per weight = total nitrogen × 6.25.

4.3.3 Determination of crude fat

Clean and dried thimbles containing about 2 g of dried samples were covered with fat free cotton at the bottom and top and placed in the extraction chamber. Extraction was taken place for at least 4hr according to (AOAC, 2000) official method 450.1. The crude fat content was determined by the formula:

$$\text{Weight of fat } (W_f) = W_a - W_b$$

Where,

W_a = Weight of extraction flask after extraction.

W_b = Weight of extraction flask before extraction.

Crude fat content [g/100] = (W_f [100 - moisture, %]/W_d)

W_d = Dried sample obtained after determination of moisture.

4.3.4 Determination of crude fiber

Analysis of crude fiber of the samples was conducted using the method of (AOAC, 2000) official method 962.09. Briefly, about 1.5 g weighed samples was transferred into a 600 mL beaker and 200 mL 1.25% sulfuric acid was added and boiled for 30 min. Recording was taken by placing a watch glass over the mouth of the beaker for 30 min heating by gently keeping the level constant with distilled water, 20 mL of 28% KOH was added and boiled gently for further 30 min. Subsequently, washing was conducted with 1% sulfuric acid, NaOH and aceton. Then, it was filtered and dried in the electric oven at 130°C for 2hrs. Furthermore, it was cooled at room temperature for 30 min in a desiccator and weighed, and then transferred to a crucible muffle furnace for 30 min for ashing at 550°C. Finally, it was cooled again in desiccators and re-weighed. The crude fiber content was calculated by using the formula

$$\text{Crude fiber content } [(g/100)] = [(((w_1-w_2) *(100 - m))/w_3)]$$

Where,

w_1 = Crucible weight after drying

w_2 = Crucible weight after ashing

w_3 = Dry weight

m = % moisture of the sample

4.3.5 Determination of total ash

A dry porcelain dish containing about 2.5g sample was placed in a muffle furnace at 550°C for 5hrs and allowed to cool in desiccator and weighted, the ash content was determined by (AOAC, 2000) using the official method 923.03 and applying a simple formula:

$$\text{Total ash } [\%] = [((w_2-w)/ (w_1-w))] \times 100$$

Where,

w = Weight in grams of empty dish

w_1 = Weight in grams of the dish plus the dried test material

w_2 = Weight in grams of the dish plus ash

4.3.6 Determination of total carbohydrates

Total carbohydrate contents of the samples were determined by subtraction of the above measured parameters from 100% as per Otitoju (2009).

Total carbohydrates [%] = 100 - [%Moisture + % Protein+ % Fat + % Ash +% fiber]

4.3.7 Mineral analysis

Calcium, phosphors, iron and zinc were determined by Atomic Absorption Spectrophotometer, while sodium and potassium were determined using Flame Photometry (Osborne and Voogt, 1978). The ash obtained after dry ashing at 525°C was treated with 7 mL of 6N HCl to wet it completely and 15 mL of 3N HCl was added and the dish was heated on the hot plate until the solution just boils. Then, it was cooled and filtered again 10 mL of 3N HCl was added to the dish and heated until the solution just boils. Finally, it was cooled and filtered into the graduated flask. Calibration curves were prepared by plotting the absorption or emission values against the metal concentration in mg/100g. Readings were taken from the graph which depicted the metal concentrations that correspond to the absorption or emission values of the samples and the blank.

The metal contents were calculated by using the formula:

$$\text{Metal content [(mg/100g)]} = [(((A-B)*V)/10W)]$$

Where, W = Weight of sample in (g)

V = Volume of extract (mL)

A = Concentration of sample solution (µg/mL)

B = Concentration of blank solution (µg/mL)

4.4 Antioxidant Activity

The antioxidant activity of the samples was evaluated spectrophotometrically by using DPPH method (Williams *et al.*, 1995). Different concentrations of the extracts 20, 40, 80, 120, 140, 160 and 200µg/L were prepared. From this solution 1 mL was taken and added to 2 mL of a freshly prepared DPPH solution (0.01mM). Each particular sample was mixed thoroughly and kept in the dark for 30 min, at room temperature, then after, each mixture was tested for the DPPH radical scavenging activity by reading the absorbance at 517nm on a UV-VIS

spectrophotometer. A blank was prepared by mixing 1 mL of methanol with 2 mL of the DPPH solution (0.01mM) and reading was taken at same wavelength. In addition, blank samples were prepared with 1 mL of each extract and 2 mL of methanol. All experiments were carried out in triplicate. The antioxidant activity was calculated by the following formula:

$$\text{Antioxidant activity (\%)} = [(AC - AE) / AC] \times 100$$

Where,

AC = is the absorbance of a DPPH solution without extract

AE = is the absorbance of the tested extract, which is equal to the absorbance of the plant extract plus the DPPH (0.01mM) minus the blank extract absorbance (Ribeiro *et al.*, 2005).

As standard, ascorbic acid at different concentration 20-240 µg/L was used. The IC_{50} value, defined as the concentration of the sample leading to 50% reduction of the initial DPPH concentration, was calculated from the linear regression of plots of concentration of the test extracts.

4.5 Anti-nutritional composition of *A. percrassa*

4.5.1 Determination of Tannins

One gram of sample in a screw cap test tube was weighed, 10 mL 1%HCl in methanol was added to the tube containing the sample, the tube was placed on mechanical shaker for 24 hrs at room temperature, and centrifuged at 1000g for 5 min. One millilitre supernatant was taken and mixed with 5 mL of vanillin-HCl reagent in another test tube. Waited for 20 min to complete the reaction. The absorbance was read at 500nm.

Standard solution was prepared as follows; 40 mg D-Catechin was weighed and dissolved in 100 mL of 1%HCl in methanol (stock) and 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of stock solution was taken using a test tube. The solution was adjusted to 1 mL with 1% HCl in methanol, 5 vanillin-HCl reagent was added to each tube, and waited for 20 min to complete the reaction. Finally the absorbance was read at 500 nm. Using SPSS Plot the calibration curve (absorbance Vs concentration) and slope and the intercept were found out (Burns, 1971; Maxson and Rooney, 1972).

$$\text{Tannin in mg/g} = \frac{(A_s - A_b)\text{-Intercept}}{\text{Slope} \times d \times w}$$

Where,

A_s= Sample absorbance

A_b= Blank absorbance

d = Density of solution

W = Weight of sample in gram

4.5.2 Determination of phytate

About 0.5 g of dried samples were extracted with 10 mL of 0.2N HCl for 1 hr at an ambient temperature and centrifuged in 3000 rpm for 30 min; the clear supernatant was used for the Phytate estimation. 2 mL of wade reagent was added to 3 mL of the supernatant sample solution then homogenized and centrifuged the solution in 3000 rpm for 10 min. The absorbance at 500 nm was measured using UV-Vis spectrophotometer. The Phytate concentration was calculated from the difference between the absorbance of the blank (3 mL of 0.2N HCl + 2 mL of Wade reagent) and that of assayed sample. The amount of phytic acid was calculated using phytic acid standard curve and result was expressed as phytic acid in µg/g fresh weight (Latta and Eskin, 1980; Vaintraub and Lapteva 1988).

4.6 Toxicity Studies

4.6.1 Acute toxicity

The crude methanol extracts of the gel and root of *A. percrassa* were evaluated for their toxicity in female non-infected Swiss albino mice 6-8 week age and with weight of 25-35 g. For each extract test, 20 mice were used by randomly allocating them into four groups each with five mice. Prior to oral administration of a single dose of each extract, the mice were deprived from food for 3 hrs (OEDC 423, 2001).

A single dose of the extract 2g/kg, 4g/kg and 6g/kg were given. Feed was provided immediately after dosing and signs of intoxication was observed for 1 hr and continued for 4 hrs, and thereafter follow up continued over a period of 24 hrs for physical and behavioural changes such as hair erection, reduction in motor activity, reduction in feeding activity, rigidity, sleep, mortality, and other visible signs of acute toxicity. Intoxication follow up was extended up to 14 days (OEDC 423, 2001). For each extract dose levels, body weight

parameter was considered at day zero (before the extract was given) and day fourteen. Finally, the LD_{50} were calculated (Akhila *et al.*, 2007).

4.6.2 Sub-acute toxicity

Female Swiss albino mice were divided into seven groups of five mice each for the sub-acute toxicity studies of the root and the gel. Oral administration of various concentrations of methanol extracts of the gel and root of *A. percrassa* was made on daily basis for consecutive 28 days (Appendix 6). Group 1 received distilled water and serve as the control while groups 2, 3 and 4 were received gel extract (2, 4 and 6 g/kg body weight) and group 5, 6 and 7 methanol extract of root (2, 4 and 6g/kg body weight). The weights of the mice were taken on weekly bases. The mice were fasted for four hrs on the 28th day, weights were taken and blood samples were collected from jugular vine for further analysis of haematology and blood chemistry. Finally, they were euthanized and the liver and kidney were collected for histopathological analysis (OECD 407, 2008).

4.6.2.1 Haematological analysis

At the end of the 28th days of treatment, blood was collected using vacutainer coated with ethylene-diamine-tetra-acetic acid (EDTA) to prevent adhesion proteins (coagulation factors) in cell-cell and cell-matrix interactions for haematological studies. Haematological analysis was performed using an automatic haematological analyzer (XT-1800i, Sysmex, Japan) at Ethiopian Public Health Institute, Addis Ababa, Ethiopia. The blood parameters measured were: white blood cell (WBC), red blood cell (RBC), platelet, mean cell volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) (Hoff and Rlagt, 2000).

4.6.2.2 Blood chemistry analysis

The parameters analysed from the blood samples collected at the end of the sub-acute toxicity studies were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) , urea, and creatinine. The analysis was performed using (COBAS 6000, C 501, Roche, USA).

4.6.2.3 Histopathological analysis

Tissue samples (liver and kidney) were taken immediately after sacrifice and transferred by a blunt forceps to a test tube containing 10% buffered neutral formalin that completely immerses the tissues for the purpose of fixation after blood collection (Lamberg and Rohstein, 1978).The fixed tissues were washed in a running tap water for 40 min to remove the fixative agent. Following washing, tissues were dehydrated in a series of an increasing graded ethanol (i.e. in 70%, 80%, 95%, 96%, 96%, 100% I and 100% II) for one hr each based on the guideline of histological techniques. The tissues were immersed with xylene two times for one hr each to remove ethanol from the tissue and replace it with fluid miscible with paraffin (Singh, 2006). Tissues were infiltrated by two changes of paraffin wax which had a melting point of 56°C-60°C for one hr and thirty min in each change (Mohan, 2007). The tissues were embedded in paraffin wax with the help of Electro-thermal Wax Dispenser to form tissue blocks in squared metallic plates block moulds. The blocks were then labelled, and placed in a refrigerator until sectioned (Mohan, 2007). Then, the tissue blocks were sectioned manually at a thickness of 5µm using a rotary microtome. The ribbon of the sections was carefully picked from the knife using forceps to float in a water-bath of 40°C (slightly below the melting point of wax) to remove folds in the sections. The unfolded sections were picked by clean microscopic glass slides and placed in an oven maintained at a temperature of 56°C for 20-30 min for a proper drying and better adhesion.

The paraffin wax was removed from the tissue sections. The sections were then immersed in Xylene and then series of descending alcohol concentration to remove xylene after which distilled water was used to hydrate the tissue. The hydrated sections were immersed in Mayer's hematoxylin staining solutions that was prepared as per Clopton (2006) for 3-5 min and then immersed in water for 5 min to make the cell nucleus becoming blue, and eosin counterstained for 1-2 min to make colourful background the whole cell structure except nucleus. Following the staining, rinsed in tap water (Clopton, 2006; Mohan, 2007). Finally, the tissue sections were dehydrated in 80%, 90%, 95% ,96% 100% I and 100% II alcohol, cleared in xylene, and mounted by adding a drop of Dibutyl phthalate in xylene (DPX) mounting medium on the section to cover the microscopic glass with cover glass and to increase the refractive index of the tissue under light microscope (Singh, 2006).

4.7 Ethical Consideration

The Mice were treated in accordance to the national Guidelines for Handling Laboratory Animal (Guide for the care and use of laboratory animals, 2010). The mice used in this research were fed on standard diet and water *ad libitum*. Besides, they were on crude extracts of the root and gel (from leaf) of the plant *A. percrassa*. Then, blood was collected gently using tubes for PCV determination, haematology and blood chemistry analysis based on the guideline. Finally, they were euthanized using chloroform for organ collection accordingly.
The ethical clearance was approved from the College of Natural and computational Science Institutional Review Board (CNS-IRB) Committee, in its meeting held on 27/10/2016 and minutes number IRB/023/2016.

4.8 Data Analysis

The data generated in this research were collected using pre-prepared formats, entered, edited and stored into a Microsoft Excel-spread sheet. The data analysis results were reported as mean ± standard error of the mean (SEM) for body weights, haematology, and blood chemistry data. The mean differences between body weight values of the tested and control mice were analysed using Student-*t*-test. Changes in haematology and blood chemistry between the test and control groups were assessed using one-way analysis of variance (ANOVA) using SPSS software package version 20 and differences between groups were considered to be significant if $P < 0.05$.

5. RESULTS AND DISCUSSION

5.1 Gel Production Yield

The average weight of fresh single leaf of *A. percrassa* was weighed 878 g, form this 573 g was covered by the gel. Gel production yield was calculated considering the average weight of the leaves to be 100%. The gel represented 65.3% of the total leaf, while the remaining 34.73% accounted to the cortex and other parts of the leaf. The gel yield obtained in this study is comparable to yields obtained from other species of commercially used *Aloe* plants like *A. vera* (64.03.3%) and others (61.3%) (Muñoz, *et al.*, 2015).

5.2 Nutritional Content

The highest constituent of the gel and root of *A. percrassa* is water which accounts for 97.00 ± 0.15% and 82.27 ± 0.02%, respectively (Table 2). After removing the water from both samples, carbohydrate represented the highest content, that is, 75.7% in gel and 47.4% in root (Table 3). The gel of *A. percrassa* comprised higher carbohydrate concentration than other species of *Aloe*, such as *A. vera* gel powder ranging from 56.27 to 73.08% on dry weight basis (Adesuy *et al.*, 2012; Haque *et al.*, 2014) and with plants high moisture content like pumpkin pulp (66.67%) (Adebayo *et al.*, 2013), but less than that of watermelon (*Citrullus lanatus*) (89.77%) (Fila *et al.*, 2013). The carbohydrate content of the root of *A. percrassa* as shown in Table 3 is high, but lower compared to tuber plants, like *Enset ventricoum* with 69.3 – 78.92% carbohydrate content (Tsehaye and Kebebew, 2006; Daba and Shigeta, 2016).

As indicated in Table 2, ash content of the gel was the third highest parameter recorded in the present study (0.45%). The total ash content of *A. percrassa* gel is higher than that of *A. vera* (0.25%). But other constituents as shown in Table 2 are almost similar to *A. vera* gel powder i.e. total protein 0.11%, crude fiber 0.1% and total fat 0.09% (Chandegara and Varshney, 2013).

Crude fiber was the third largest constituent in the root part of *A. percrassa*. The crude fiber concentration of the root (42.88 g/100 g) was higher than that of *Enset* (*E. ventricoum*) (18.45 g/100 g) powder. In addition, ash (4.96 mg/100 g) and fat (1.47 mg/100 g) contents were slightly higher than that of *E. ventricoum* 3.3 g/100 g and 0.64 g/100 g ash and fat, respectively. But, protein content of *A. percrassa* root (3.3 g/100 g) was lower than *E.*

ventricoum (8.8 g/100 g) (Daba and Shigeta, 2016). Generally, *A. percrassa* root was high in fiber composition and might be candidate food for fiber source but not for protein.

Table 2. Proximate analysis of the gel and root of *A. percrassa*

	Percentage of Nutrient composition					
Plant parts	Crude protein (%)	Crude Fiber (%)	Moisture (%)	Crude Fat (%)	Total ash (%)	Carbohydrate (%)
Root	0.59±0.05	7.59±0.14	82.27±0.02	0.26±0.13	0.88±0.44	8.41±0.4
Gel	0.13±0.12	0.07±0.11	97±0.15	0.08±0.044	0.45±0.73	2.27±0.34

Table 3. Proximate analysis of the gel and root of *A. percrassa* on dry weight basis

	Percentage of Nutrient composition on dry weight basis				
Plant parts	Crude protein (%)	Crude Fiber (%)	Crude Fat (%)	Total ash (%)	Carbohydrate (%)
Root	3.33±0.05	42.8±0.14	1.47±0.13	4.96±0.44	47.4±0.4
Gel	4.3±0.12	2.3±0.11	2.7±0.04	15.0±0.73	75.7±0.34

5.3 Mineral Content

Minerals are important elements used for normal functioning of muscles, heart, nerves, and maintenance of body fluid composition as well as strong bone formation. The mineral composition of the gel and root of *A. percrassa* as shown in table 4 might be good source of minerals.

Table 4. Mineral analysis of the gel and root of *A. percrassa* on dry weight basis

Plant part	**Mineral composition (mg/100g; dry weight basis)**					
	Fe	**Na**	**Zn**	**Ca**	**P**	**K**
Root	9.39±1.02	378.03±4.38	1.23±0.16	871.03±5.44	45.02±4.7	387.86±5.6
Gel	3.02±0.06	92.46±6.21	3.55±0.19	782.76±5.55	280.15±3.17	4765.23±98.28

According to Sanoussi *et al.* (2016), the average mineral composition of tubers of different potato species on dry weight basis ranged from, 0.53 to 0.73 mg/100 g for Fe, 0.23 to 0.27 mg/100 g for Zn, 23.04 to 29.97 mg/100 g for Ca, 21.30 to 25.40mg/100 g for Mg, 42.00 to 46.33 mg/100 g for P, 308.67 to 328.67 mg/100 g for K and 29.00 to 34.00 mg/100 g for Na.

and root of Cassava plant also have 19-176 mg/100 g Ca, 6-152 mg/100 g P, 0.3-14 mg/100 g Fe, 250-720 mg/100g K (Salvador *et al.*, 2014), Concerning mineral content, *A. percrassa* plant is higher concentration than that of average result of ten sweet potatoes and six different varieties of cassava root plants.

In *A. percrassa*, K was the highest mineral content in the gel (4765.23 mg/100 g) on dry weight base, and even it was higher in concentration than that of banana (1116.89 mg/100 g) (Wall, 2006). Whereas, Ca was the highest in the root (871.03 mg/100g) but second next to K in the gel (782.76 mg/100 g) (Table 4). Generally, K, Ca, P, Zn, Fe and Na characterize the highest amount of minerals recorded in the gel and root of *A. percrassa*. *A. percrassa* could contribute different proportion of recommended dietary allowance of children and adults as shown in Table 5.

The Na/K ratio plays a very important role in a diet as it controls high blood pressure in the body. Studies showed that lower Na and higher K intake helps to reduce high blood pressure in hypertensive patients. The recommended Na/K ratio should be less than one (Jacob *et al.*, 2015). In the present study, the respective Na/K ratio values obtained from gel and root were 0.97 and 0.02, respectively (Table 5). Thus, consumption of the gel and root extract would help to prevent hypertension and might lower blood pressure in hypertensive patients.

The Ca/P ratio of *A. percrassa* root and gel were 19.35 and 2.79, respectively, which meet the standard ratio set greater than 0.5. High Ca/P ratio levels in food are required for favourable Ca absorption in the intestine for bone formation (Amoo *et al.*, 2008). The Ca/P ratio obtained in this study indicated that *A. percrassa* might help Ca absorption in the body and it could be a good source of mineral supplement for both humans and animals. Thus, the gel and the root of *A. percrassa* are remarkable sources of minerals in a best proportion for used to human. It could be an important diet for hypertensive patients and in bone formation.

Table 5. Mineral content of lyophilized gel and oven dried root powder of *A. percrassa*

	Mineral composition (mg/100g; dry weight basis)							
	Fe	Na	Zn	Ca	P	K	Na/K	Ca/P
Root	9.39	378.03	1.23	871.03	45.02	387.86	0.97	19.35
Gel	3.02	92.46	3.55	782.76	280.15	4765.23	0.02	2.79
RDA for children (mg/day)	10	400	10	800	800	1600	<1	>0.5
RDA for Adult (mg/day)	15	500	15	800	800	2000		
Contribution to RDA for children % (root)	93.90	94.51	12.30	108.88	5.63	24.24		
Contribution to RDA for Adult % (root)	62.60	75.61	8.20	108.88	5.63	19.39		
Contribution to RDA for Children % (gel)	30.20	23.12 %	35.50	97.85	35.02	297.83		
Contribution to RDA for Adult % (gel)	20.13	18.49 %	23.67	97.85	35.02	238.26		

RDA: Recommended Dietary Allowance

5.4 DPPH Radical Scavenging Activity

The antioxidant activity was increased in parallel with concentrations of the root and gel extracts, respectively (Table 6). Percent inhibition was plotted against concentration (Table 7), and the equation for the line was used to obtain the IC_{50} value (concentration of sample required to scavenge DPPH radical by 50%). A lower IC_{50} value indicates greater antioxidant activity. In this study, methanol extract of the gel and root of *A. percrassa* showed less antioxidant activity by DPPH radical scavenging method compared to the standard ascorbic acid and IC_{50} value found to be 0.12, 0.32 and 0.04 μg/mL for the gel, root and ascorbic acid, respectively (Table 6). This indicates that the root extract of *A. percrassa* has better antioxidant activity than that of the gel extract.

Table 6. Absorbance of methanol extract of root and gel of *A. percrassa* at different concentrations

Concentration(µg/mL)	Absorbance (Mean ± SEM)		
	Root	Gel	Ascorbic Acid
0.02	0.74±0.67	0.87±0.00	0.67±0.01
0.04	0.67±0.00	0.84±0.03	0.53±0.00
0.08	0.53±0.00	0.76±0.00	0.19±0.01
0.12	0.37±0.01	0.71±0.00	0.05±0.00
0.16	0.25±0.00	0.65±0.00	0.04±0.00
0.20	0.17±0.00	0.61±0.01	0.04±0.00
0.24	0.11±0.00	0.56±0.01	0.04±0.00
IC_{50}	**0.12**	**0.32**	**0.04**

SEM = Standard Error of the Mean

Table 7. DPPH percentage inhibition of methanol extract of root and gel of *A. percrassa*

Concentration (µg/mL)	Percent Inhibition (%)		
	Root	Gel	Ascorbic Acid
0.02	17.41	2.90	25.22
0.04	25.22	8.48	40.85
0.08	40.85	15.18	78.79
0.12	58.71	20.76	94.42
0.16	72.10	27.46	95.20
0.20	81.03	31.92	95.20
0.24	87.73	37.50	95.54
Regression equation	y=0.332x + 13.89	y=0.152x + 1.827	y=0.308x + 37.12
R-squared (R^2)	0.978	0.991	0.728

5.5 Anti-nutritional effects of *A. percrassa* gel and root

Phytate forms complexes with dietary minerals and causes mineral-related deficiency in humans. In addition, it adversely impacts protein and lipid utilisation. Phytate is one of a major concern for individuals who depend on plant based foods (Kumar *et al.*, 2010). Apart

from phytate, higher value of tannin in foods also interferes with protein absorption and digestive enzymes (Jacob *et al*, 2015). The result of anti-nutritional analysis of *A. percrassa* in this study was presented in (Table 8). The phytate content was 1497.20 mg/100 g in the root, which is slightly higher than common legumes like soybean (1430 mg/100 g) and lupine (1380 mg/100 g). In contrast, the gel contained 127.90 mg/100 g phytate which is lower than that of common cereals and legumes such as wheat (850 mg/100 g), maize (900 mg/100 g), barley (970 mg/100 g), oats (1010 mg/100 g), cowpea (420 mg/100 g), common bean (550 mg/100 g), and peas (1020 mg/100 g) (Hldvegi and Lasztity, 2002). Thus, the higher content phytate found in the root of *A. percrassa* might interfere on the absorption of the vital nutrients like Fe and Zn.

The concentration of tannin was 5.61 mg/100 g in root and 49.26 mg/100 g in the gel. The root constituted lower amount of tannin than the gel, and the gel also low concentration of tannin than *A. barbadensis* (155 mg/100 g), *A. percrassa* contains lower amount of tannin (Adesuyi *et al*., 2012). Therefore, *A. percrassa* could be considered as a good source of diet with low amount of tannin content.

Table 8. Phytate and tannin content of the root and gel

	Anti-nutrient	
Sample	**Phytate (mg /100 g)**	**Tannin (mg/100 g)**
Root	1497.20±7.69	5.61±0.23
Gel	127.90±2.17	49.26±3.2

Note: values are means of triplicate samples

5.6 Acute toxicity effects of methanol extract of *A. percrassa* root and gel

5.6.1 Observing physical signs of toxicity after oral administration of the extracts

Mice were observed for signs of abnormalities after the gel and root extract were given. There were no signs of toxicity and physical change during the study period following oral administration of 2, 4, and 6g/kg body weight of methanol extract of the gel and root of *A. percrassa.*

5.6.2 Determination of LD_{50} after oral administration of extracts of *A. percrassa*

The acute oral toxicity study of methanol extract of the gel and root of *A. percrassa* were evaluated on mice. The extract at the dose of 6g/kg body weight didn't produce treatment related signs of toxicity or mortality in any of the mice tested during 14 days observation period. Therefore, LD_{50} of the samples were estimated to be more than 6g/kg.

5.6.3 Effects of methanol extract of *A. percrassa* on body weight of mice

Effect of methanol root and gel extracts of *A. percrassa* on the body weight of the mice on the 14th day following administration was summarized in (Table 9 and 10). The extracts of both parts of the plant didn't produce significant change on the mean body weights of the mice at all doses offered in comparison to the control group ($P>0.05$).

Table 9. Effect of methanol extract of *A. percrassa* root on the body weight of mice

Dose	**Body weight (g) (Mean ± SEM)**			
(g/kg)	Initial	Final	Change	P-value
Control	22.20 ± 0.80	26.6 ±1.03	4.4 ± 0.51	
2	20.6 ± 0.51	26.2 ± 0.86	5.6 ± 0.98	0.346
4	22.4 ± 0.40	26.4 ± 1.17	4.0 ± 0.95	0.751
6	21.0 ± 0.32	24.8 ± 1.20	3.8 ± 0.97	0.634

*Significant (if p<0.05)

Table 10. Effects of methanol extract of *A. percrassa* gel on body weight of mice

Dose	Body weight (g) (Mean ± SEM)			
(g/kg)	Initial	Final	Change	P-value
Control	26.4 ± 0.40	29.6 ± 1.32	3.2 ± 0.97	
2	24.4 ± 0.25	29.0 ± 0.32	4.6 ±0.4	0.262
4	24.8 ± 0.37	29.6 ± 1.08	4.8 ±1.07	0.203
6	24.8 ± 0.49	28.4 ± 0.51	3.6 ± 0.81	0.744

*Significant (If p<0.05)

5.4.4 Effects of methanol extract of *A. percrassa* on packed cell volume (PCV) of mice

Effect of oral administration of *A. percrassa* root and gel on PCV of mice is indicated in Table 11 and 12. Analysis using unpaired *t* test showed no significant difference at dose rates of 2, 4 and 6 g/kg body weight of the root extract and 2 and 4 g/kg dose of the gel extract from that of the control group ($P > 0.05$). On the other hand, 6g/kg gel extracts showed significant difference from the control group ($P < 0.05$).

Table 11. Effect of methanol extract of *A. percrassa* root on PCV of mice treated at different doses

Dose (g/kg)	% PCV (Mean ± SEM)			
	Initial	Final	Change	P-value
Control	65.54 ±3.60	71.14 ±1.54	5.6±1.9	
2	67.38±0.65	66.98±2.92	0.4±2.79	0.493
4	59.82±3.61	69.53±1.50	9.04±4.04	0.169
6	61.73±2.31	71.51±1.30	9.78±2.70	0.394

*Significant (If p<0.05)

Table 12. Effect of methanol extract of *A. percrassa* gel on PCV of mice treated at different doses

Dose (g/kg)	% PCV (Mean ±SEM)			
	Initial	Final	Change	P-value
Control	73.50±0.57	56.68±1.47	16.82±1.34	
2	67.49±2.55	54.37±1.24	13.12±3.04	0.25
4	66.13±3.38	57.10±1.61	9.03±2.14	23
6	59.66±0.90	56.72±1.90	2.94±2.58	0.001*

*Significant (If p<0.05)

5.7 Sub-acute toxicity effect of methanol eextracts of *A. percrassa* root and gel

5.7.1 Observing physical signs of toxicity after oral administration

Long-term administration of different concentrations (2, 4, and 6 g/kg body weight) methanol extracts of both the gel and root of *A. percrassa*, elicit no changes in their appearances and signs of toxicity.

5.7.2 Effects of A. percrassa on haematological and biochemical parameters of mice

Measurements of serum biochemical parameters are useful for identifying toxic effects on vital organ (Ola-Davies *et al.*, 2014). The sub-acute toxic effect of methanol extract of the gel and root of *A. percrassa* on haematological and serum biochemical parameters is presented in (Table 13 and 14). Using one-way analysis of variance (ANOVA), there was no statistically significant difference in various serum biochemical parameters between mice treated with 2 g/kg, 4 g/kg and 6 g/kg of each of the sample extract and control mice. This suggests that the methanol extract of the gel and root of *A. percrassa* have no toxic effects on the serum biochemical parameters of the mice.

Likewise, statistical analysis (ANOVA) also revealed absence of significant difference in haematological composition of blood parameter among mice treated with different doses of the gel and root extracts ($P>0.05$).

Table 13. Biochemical parameters of methanol extract of *A. percrassa* treated mice at different dose rates

Part of plant	Dose g/kg	ALTL	ASTL	URE	CREA
Gel	2	21.9±6.1	141.4±23.5	40.0±7.4	0.19±0.02
	4	46.9±5.1	218.7±64.2	46.0±5.5	0.14±0.06
	6	42.4±3.2	109.5±53.6	51.5±0.7	0.20±0.01
Root	2	18.8±7.2	102.7±14.9	59.8±6.7	0.10±0.02
	4	26.4±9.0	189.8±21.3	39.5±2.4	0.10±0.09
	6	40.6±5.4	169.3±28.9	56.4±16.9	0.21±0.02
	Control	30.0±1.2	128.5±11.2	69.9±6.0	0.16±0.04
F-value		2.7	1.8	2.6	1.1
P-value		**0.062**	**0.166**	**0.057**	**0.435**

*Significant (If $p<0.05$)

	MCHC
5	34.6±0.6
3	35.3±0.4
3	34.1±0.7
3	29.4±5.2
4	33.4±1.0
2	33.9±0.4
6	34.0±0.7
	0.8
	0.571

5.7.3 Histopathological examination of mice treated with methanol extract of A. percrassa root and gel

5.7.3.1 Effects of methanol extract of *A. percrassa* on liver morphology of mice

Gross examination of the liver of sacrificed mice from both the control and nearly all of the treated group revealed absence of gross morphological abnormality. In addition, histological examination of sections of the livers of the control (Figure 3) and treated mice also showed similar morphological presentations i.e. normal architectural feature. There was no abnormality in the shape of the central vein, size of hepatic sinusoids and hepatocytes. However, there were mild focal periportal lymphocytic infiltrates or perivascular cuffing in the liver (hepatitis) of a mouse treated with 4 g/kg methanol extract of the root of *A. percrassa* (Figure 4). This change might not be attributed to toxicity of the extract as it is not seen at higher doses.

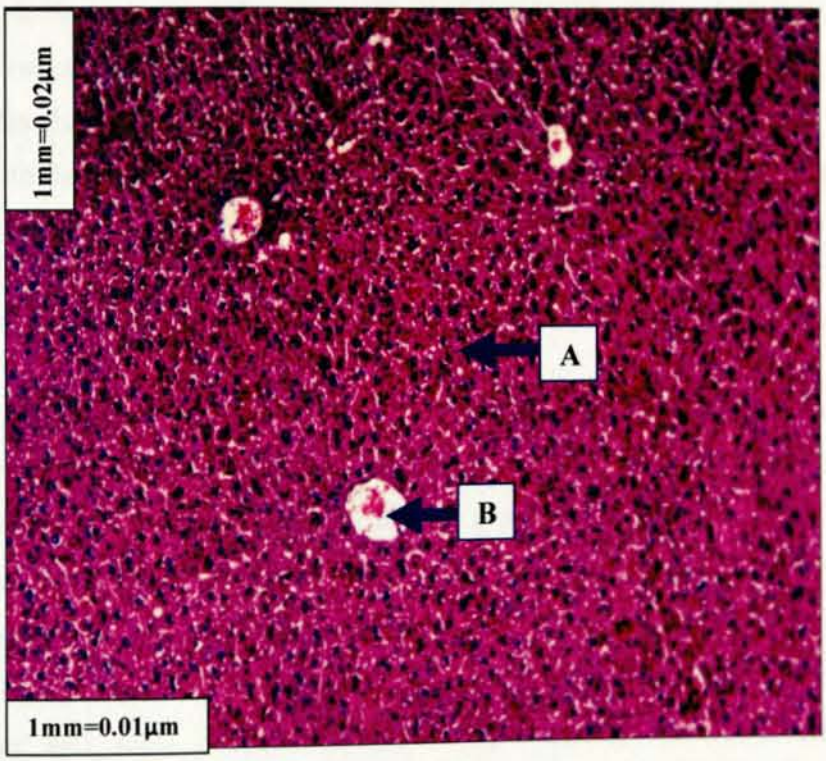

Figure 3. Photomicrograph of liver section of the control mouse. It shows normalperiportal vein and hepatocytes. (A) Hepatocytes (B) Periportal vein (Haematoxylin and Eosin stain, x 100) (1mm=0.01µm means 1mm in the paper is equals to 0.01µm in microscope)

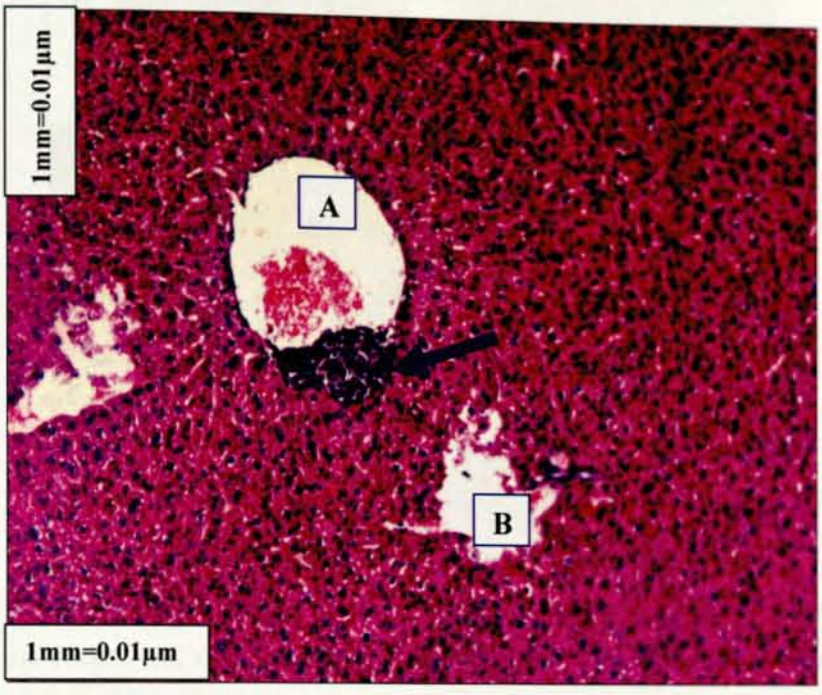

Figure 4. Photomicrograph of liver of mice treated with methanol extract of the root of *A. percrassa* at a dose of 4 g/kg b.wght. It shows mild focal periportal lymphocytic infiltrates (hepatitis) (Arrow). (A) Periportal vein (B) Hepatic sinusoids (Haematoxylin and Eosin stain, x 100), (1mm in the paper is equals to 0.01µm in microscope)

5.7.3.2 Effects of methanol extract of *A. percrassa* on kidney morphology of mice

The kidney of both the control and almost all of the treated mice showed no gross pathological changes. Histopathological examination also depicted that the kidney of the control (Figure 5) and nearly all of the treated mice consisted of normal cortex and medulla. The glomeruli and nephrons (renal tubules) i.e. the proximal and distal convoluted tubules exhibit a normal structure. However, microscopic observation of the kidney of a mouse treated with 2 g/kg b.wt of the root extract showed slight abnormalities comprising focal acute tubular injury and focal oedematous (Figure 6). Although those abnormalities were demonstrated, it is difficult to attribute it to the toxic effect extract as it was not dose dependent.

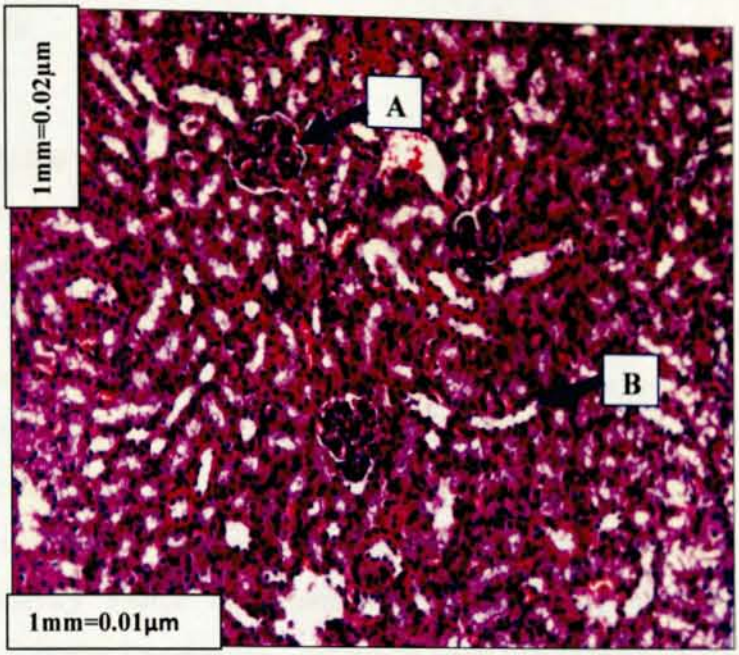

Figure 5. Photomicrographs of kidney sections of the control mouse. There are nromal glomeruli in the cortex and normal nephrons in the cortex and medulla. (A) Glomerulus (B) Nephron (Haematoxylin and Eosin stain, x 100), (1mm in the paper is equals to 0.01µm in microscope)

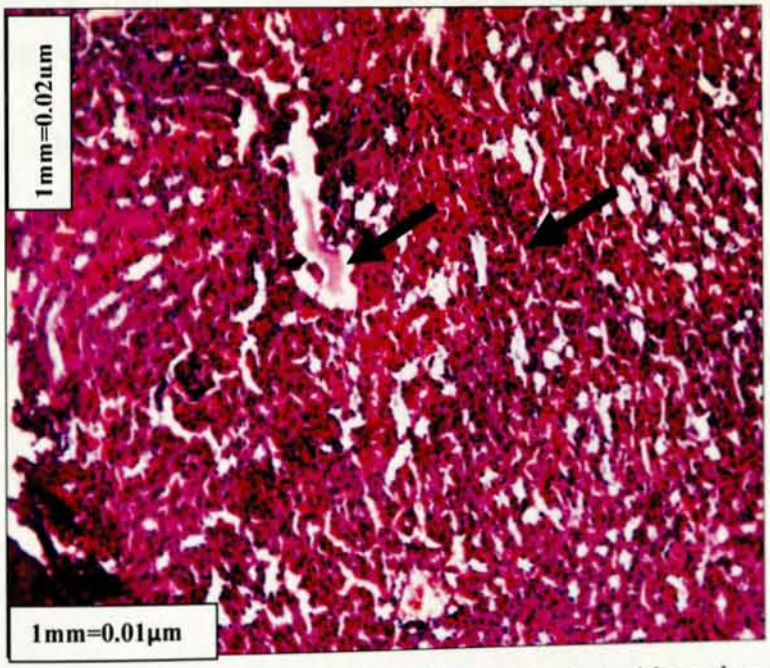

Figure 6. Photomicrographs of kidney sections of mouse treated with methanol extract of the root of *A. percrassa* at a dose of 2 g/kg. There is focal acute tubular injury (swollen tubular linings) and focal edematous changes (Arrow) (Haematoxylin and Eosin stain, x 100).

NCLUSIONS

ɔia is among the few countries gifted with unique geographical features essential for the ation of *Aloe* and other high potential food plants. *A. percrassa* is one of the indigenous peculiar to Ethiopia. Nonetheless, Ethiopia hasn't yet benefited from this plant. The t analysis carried out on *A. percrassa* revealed its nutritional potential, antioxidant ties with no toxic effects. The gel and root of *A. percrassa* is found to be a good source portant nutrients such as carbohydrate, fiber, minerals and protein. Both the gel and the an be considered as a valuable dietary supplement. *A. percrassa* contained major nts which are required for health. Therefore, the plant could be used as an important ementary dietary source of nutrients in a food based approach for combating nutrient deficiency. Despite, the presence of some anti-nutrients including phytate and s that could serve as mineral inhibitors, *A. percrassa* can be used as sources of valuable nutrients.

the study it was observed that the antioxidant potential of the gel is lower than that of andard (ascorbic acid). In contrast, the root extract exhibited comparable antioxidant ial to the standard. Thus, the root might be a source of Antioxidant.

h acute and sub-acute toxicity studies, no mortality was recorded on mice following oral istration of methanol extract of the gel and root of *A. percrassa* even at a higher dose f 6 g/kg b. wt. So, it can be concluded that the LD_{50} of this plant estimated to be greater 6 g/kg b. wt. Besides, the gel and root extract treated mice showed no statistically icant difference on the body weight, hematology and blood chemistry result in arison to the control group. However the observed results in histopathology findings on ver and kidney of few mice elicit doubt *A. percrassa* have slightly toxic for Swiss albino

COMMENDATIONS

e basis of the above conclusion, the following recommendations are forwarded.

Creation of awareness and provision of information to the stakeholders about the nutritive and antioxidant potential of *A. percrassa* is needed.

As the root and gel of *A. percrassa* are rich in carbohydrate, search for starch and subsequent extraction and commercialization must be instigates.

After reassuring its safety, *Aloe percrassa* should be considered as mineral source for mineral deficient individuals and diet for hypertensive patients as its root and gel are very rich in minerals and the Na/K ratio meets the standard.

As fiber is abundant in the root of *A. percrassa*, utilization of *A. percrassa* root as source of fiber for obese people, diabetic patients and people suffering from constipation must be taking into consideration.

A detailed investigation on parts of *A. percrassa* including whole leaf, gel, latex as well as the root in search for better antioxidant property using different solvents extraction methods and methods other than DPPH.

Further long-term studies involving hematological and serum biochemical parameters as well as histopathological observations of the liver, kidney and other vital organs in rodent and none-rodent animals are essential to confirm this innocuous nature of the gel and root of *A. percrassa.*

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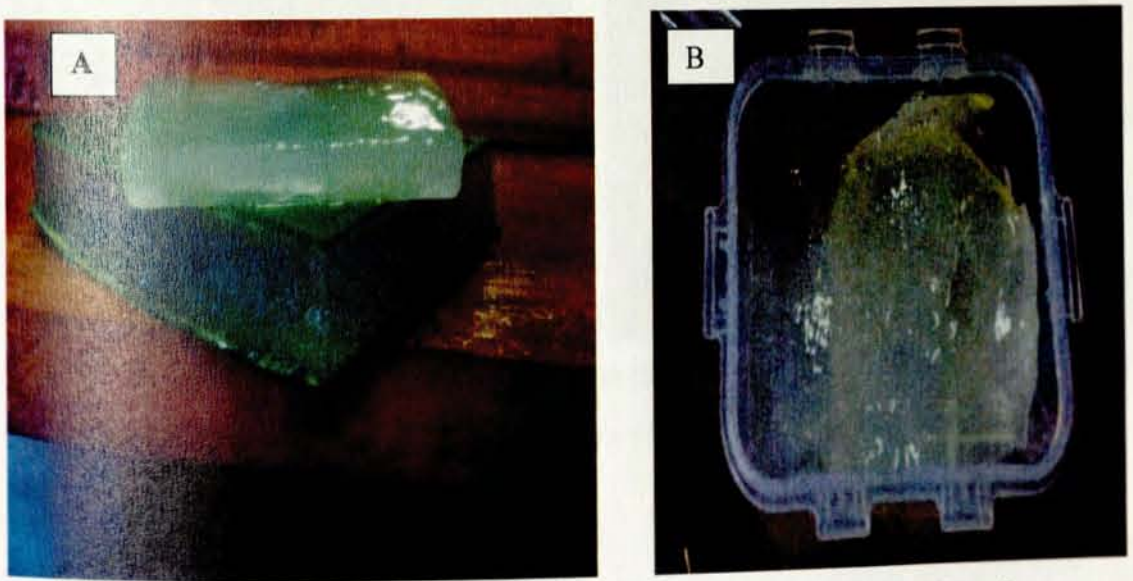
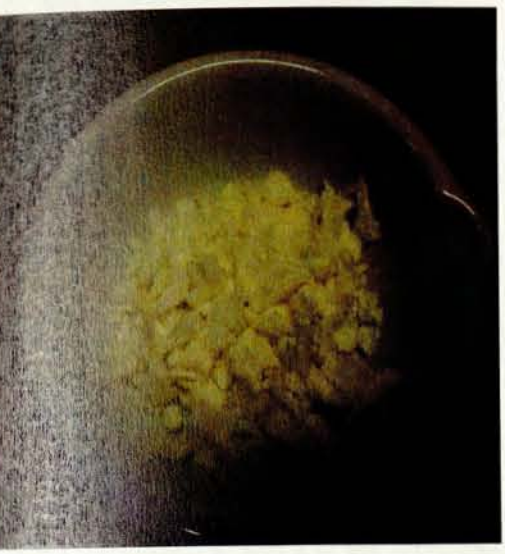
9. APPENDIX

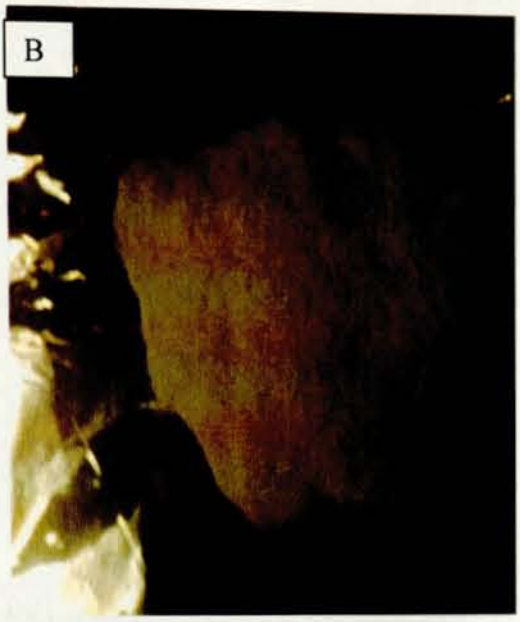
9.1 Parts of *Aloe percrassa*

Appendix 1. Leaf of *A. percrassa* (A) Intact Leaf (B) leaf-gel piled with plastic knife (Photo taken on March 23, 2016 at the laboratory of Center for Food Science and Nutrition)



Appendix 2. Leaf-gel of *A. percrassa* (A) Leaf cut and gel block (B) Gel collected (Photo taken on March 23, 2016 at the laboratory of Center for Food Science and Nutrition)





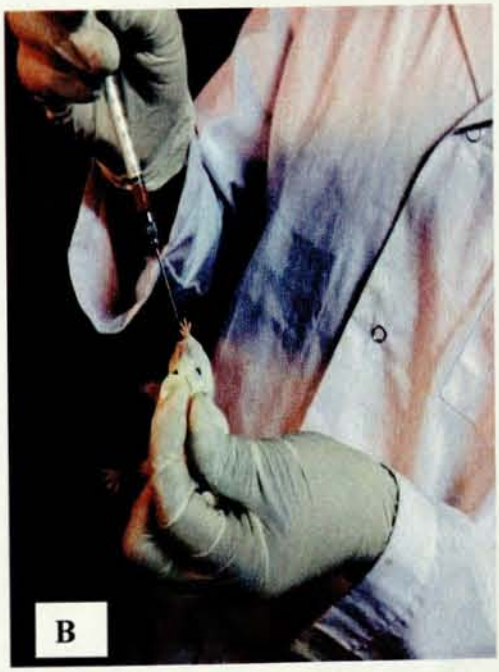
ndix 3. Dry gel of *A. percrassa* (A) After lyophilization (B) After grinding
o taken on April 8, 2016 at the laboratory of Center for Food Science and Nutrition)

ndix 4. Root of *A. percrassa* (A) Intact root (B) Chopped root.
o taken on March 23, 2016 at the laboratory of Center for Food Science and Nutrition)

lix 5. Powder of root of *A. percrassa*

aken on April 2, 2016 at the laboratory of Center for Food Science and Nutrition)

l Administration of Extracts of *Aloe percrassa*



dix 6. Oral administration of plant extracts (A) Mouse restraining (B) Oral stration using a gavage.

taken on October 10 at Biomedical laboratory)