ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

CENTER FOR FOOD SCIENCE AND NUTRITION

Chemical composition and antihypertensive properties of salt extracted from ash of Marsh barbel (*Hygrophilia schulli)* grown on Gambella, Ethiopia

By:

Degsew Mengistu Amare

Advisors: Paulos Getachew (PhD)

Kaleab Baye (PhD)

A Thesis Submitted to College of Natural and Computational Sciences of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Science and Nutrition

January, 2019

Addis Ababa

ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

CENTER FOR FOOD SCIENCE AND NUTRITION

Chemical composition and antihypertensive properties of salt extracted from ash of Marsh barbel (*Hygrophilia schulli*) grown Gambella, Ethiopia

A Thesis Submitted to College of Natural and Computational Sciences of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Science and Nutrition

By

Degsew Mengistu Amare

Approval by:	Signature	Date
_____________________ (External examiner):	_________________	______________
______________________ (Internal examiner):	_________________	______________
______________________ (Chairperson)	_________________	______________

Advisors:

Dr. Paulos Getachew	_________________	______________
Dr. Kaleab Baye	_________________	______________

DECLARATION

I, the undersigned, declare that, this is an original work and has never been presented in any other university as well as research institutes and all the source material used for this thesis have been fully acknowledged.

Degsew Mengistu Amare

Signature _________________

Date _________________

The thesis has been submitted for examination with the approval of my University advisors.

Name: Paulos Getachew (PhD)	Name: Kaleab Baye (PhD)
Signature __________________	Signature ______________

Date and place of Submission: (office of Research and Graduate Programs)

Addis Ababa University

January, 2019

ACKNOWLEDGMENTS

First of all, I would like to thank and praise the almighty God for his support and always blessing my works.

I would like to extend my heartfelt gratitude to my advisors Dr.Paulos Getachew and Dr.Kaleab Baye for their supervision, guidance from the starting day of this research and sharing me great experiences and encouragements with constructive comments based on their inconceivable experiences throughout my research work. I would like to express my appreciation to Kotebe Metropolitan University for the sponsorship. I would like to extend my sincere thanks to Kotebe Metropolitan University staff, especially Mr.Zerihun Tadesse in Chemistry Department and all Laboratory assistants in Biology Department for the technical support.

I would also like acknowledge laboratory staff members of Bless Agri Food Laboratory Services; especial thanks goes to Mr. Henok Ashagrie for his technical support. I would like to extend Ethiopia Leather Industry Development Institute for the support in mineral analysis, Ethiopian Public Health Institute (EPHI), especially to Mr.Meseret, Mr. Abiy Abebe and Mr Yirgalem Tadesse for their consistent support for the success of the animal based study. I would also like to acknowledge Center for Food Science and Nutrition of Addis Ababa University and staff members of the Center for being core assistance for the completion of this study.

Finally, I would like to thank all my friends and colleagues who encouraged me in my work and contributed a lot in one way or another. I would like to express my thankfulness to Mr. Fikru Gashaw and Ms.Tsehay Mitku for economic support. My special thanks also goes to Chef Yohannis for flight fare sponsorship during plant collection and for providing core information on ash salt processing.

TABLE CONTENTS

Title Page No

ACKNOWLEDGMENTS i

LIST OF TABLES v

LIST OF FIGURES........ vi

LIST OF APPENDIXES vii

ABBREVIATIONS........ viii

ABSTRACT ix

1. INTRODUCTION 1

1.1. Background of the study 1

1.2. Statement of the problem 4

1.3. Objectives 7

1.3.1. General objective 7

1.3.2. Specific objectives 7

2. LITERATURE REVIEW 8

2.1. Overview of Commercial Salt 8

2.1.1. Production of Sea Salt 8

2.1.2. Unrefined Salt 8

2.1.3. Refined Salt 9

2.1.4. Iodized Salt 9

2.1.5. Risks of excess salt consumption 10

2.2. Vegetable (ash) Salt 12

2.2.1. Distribution of *H. schulli* 14

2.2.2. Biology of *H. schulli* 14

2.2.3. Uses of *H. schulli* 15

3. MATERIAL AND METHODS 20

3.1. Experimental area 20

3.3. Sample collection and sampling site 20

3.4. Salt extraction method 21

3.5. Analytical methods 23

3.5.1. Macro and micro metals analysis 23
3.5.2. Toxic metal analysis 23
3.5.3. Method of iodine (I_2) determination 25
3.5.4. Alkalinity determination 26
3.5.5. Sulphate determination 26
3.5.6. Chloride determination 27
3.5.7. pH measurement 27
3.5.8. Determination of water insoluble matter 27
3.5.9. Moisture content determination 28
3.6. Anti-hypertensive test 28
3.6.1. Experimental animals and housing 28
3.6.2. Heating (warming) 29
3.6.3. Body weight and feed intake measurement 29
3.6.4. Blood Pressure Measurement 29
3.7. Sensory acceptability test of *H. schulli* salt 32
3.7.1. Determination of taste detection and recognition thresholds 32
3.7.2. Sensory acceptability test 32
3.8. Data analysis 34
4. RESULTS AND DISCUSSIONS 35
4.1. Percent of salt obtained from *H. schulli*, calculated from ash and raw material 35
4.2. Quality factors of the salt 36
4.2.1. Moisture content 36
4.2.2. Matter insoluble in water 37
4.2.3. pH 37
4.3. Anion composition of vegetable salt *H. schulli* and common salt 38
4.3.1. Carbonate (alkalinity) content 39
4.3.2. Sulphate content 39
4.3.3. Chloride content 40
4.4. Macro mineral composition 41
4.4.1. Sodium 41

4.4.2. Potassium 43
4.4.3. Calcium 44
4.4.4. Magnesium 44
4.5. Micro mineral composition 45
4.5.1. Iron 45
4.5.2. Zinc 46
4.5.3. Iodine 46
4.6. Toxic metal composition of salt 48
4.6.1. Cadmium (Cd) 49
4.6.2. Chromium 49
4.6.3. Lead 49
4.6.4. Mercury and arsenic (Hg and As) 50
4.6.5. Nickel 50
4.7. The sensory quality of salt extracted from *H. schulli* plant 51
4.7.1. Taste detection and recognition threshold 51
4.7.2. Paired comparison difference and preference tests 53
4.7.3. Rating sensory evaluation of vegetable salt extracted from *H. schulli* and common salt…………………… 53
4.8. Anti-hypertensive test 55
4.8.1. Effects of 4% salt extracted from *H. schulli* and 4% common salt on systolic, mean and diastolic blood pressure of Wistar rats 55
5. CONCLUSION AND RECOMMENDATIONS 60
5.1. Conclusion 60
5.2. Recommendations 61
REFERENCES 62

List of Tables

Table 1: Percent of salt obtained from stem and leaf of *H. schulli*, calculated from ash and raw material used (%)....... 36

Table 2: Matter insoluble in water, moisture content and pH of the salt 36

Table 3: Anion composition of salt extracted from *H. schulli* and common iodized salt (%) . 39

Table 4: Macro mineral composition of salt extracted from *H. schulli* and common iodized salt (%)....... 45

Table 5: Micro mineral composition of salt extracted from *H. schulli* and common iodized salt (ppm) 48

Table 6: Toxic metal composition of salt extracted from *H. schulli* and common iodized salt (ppm) 51

Table 7: Detection and recognition threshold of salt extracted from *H. schulli* (%)....... 52

Table 8: Rating sensory test results on the *H. schulli* and common salt 54

Table 9: Sensory preference test between *H. schulli* and common salt (%) 54

Table 10: showed the Body weight gain, fed intake and Fed Efficiency Ratio obtained from normal control, 4% common salt and 5% vegetable salt fed rats for 5 weeks....... 55

Table 11: Average 5 weeks Systolic, Mean and Diastolic Blood Pressure of Wistar rats fed with a diet containing 4% salt extracted from H. schulli and 4% common salt....... 57

List of figures

Figure 1: Edible salt processing through evaporation of saline water....................................10

Figure 2: Steps of vegetable salt production. 13

Figure 3: Pictures of *H. schulli* grown in Gambella region, Ethiopia. 14

Figure 4: A and B Chemical structure of gallic acid and quercetin 16

Figure 5: Map of Gambella Region, Ethiopia. 21

Figure 6: Traditional and scientific vegetable salt production methods..................................22

Figure 7: Procedures of anti-hypertensive test on Wistar rats..31

Figure 8: pH values of ICs, NICs and Vs. 38

Figure 9: Weekly Systolic Blood Pressure records of rats fed with 4% Vs, 4% Cs and normal diets...57

Figure 10: Weekly Mean Blood Pressure records of rats feed with 4% Vs, 4% Cs and normal diets...58

Figure 11: Weekly Diastolic Blood Pressure records of rats feed with 4% Vs, 4% Cs and normal diets. 58

LIST OF APPENDIXES

Appendix 1: Ethical clearance ……..……………………….…………………………….………77

Appendix 2: Vegetable salt production procedures from *H. schulli*……………….……….......78

Appendix: Photographic picture of vegetable salt extracted from *H. schulli*.........................79

Appendix 4: Formant for Paired comparison difference sensory test…...……......…………80

Appendix 5: Format for paired comparison preference test…...……………...……….……..81

Appendix 6: Format for multiple comparison difference test.…………..…...……….……..82

Appendix 7: Rating sensory panelist evaluation of both common and vegetable salt………..83

Appendix 8: The graph of weekly systolic and diastolic blood pressure (mmHg) of 4% Vegetable Salt (Vs) group of Wistar rats using tail cuff……………………………..…….84

Appendix 9: Graph obtained from weekly Systolic and Diastolic Blood pressure (mmHg) of normal control fed group of Wistar rats using tail cuff…….……………….……………......…85

Appendix 10: Graph of weekly Systolic and Mean Blood pressure for 4% common salt containing rat fed……….…………………….……….…..…………………….………………86

Appendix 11: Tail cuff Blood pressure analyzer and warming chamber................................87

ABBREVIATIONS

ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemists
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DPI	Dietary Potassium Intake
DSI	Dietary Sodium Intake
DSPR	Dietary Sodium Potassium Ratio
FAAS	Flame Atomic Absorption Spectroscopy
FER	Feed Efficiency Ratio
IARC	International Agency for Research on Cancer
IDD	Iodine Deficiency Disorder
MBP	Mean Blood Pressure
NCD_s	Non communicable Diseases
RDA	Recommended Dietary Allowance
SBP	Systolic Blood Pressure
SPSS	Statistical Package for Service Solution
TIAs	Taste-Improving Agents
WHO	World Health Organization

ABSTRACT

In some parts of Ethiopia particularly, in Gambella (Anuaq), vegetable salt extracted from ash of plant parts is consumed as dietary salt and for therapeutic purposes of heart disease, liver disease, kidney failure, blood pressure and diabetics traditionally. The aim of this study was to investigate selected minerals composition, sensory acceptability and anti-hypertensive properties of vegetable salt extracted from Hygrophilia schulli. The salt was prepared from the ash obtained by burning stems of the Hygrophilia schulli. The Macro and micro minerals were determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) and toxic metals were analyzed by Flame Atomic Absorption Spectroscopy (FAAS) accordingly. The mineral analysis showed that the salt was potassium rich and sodium poor with sodium to potassium ratio of 0.05. The vegetable ash was free from harmful metals like mercury, cadmium, chromium, arsenic and other analyzed toxic metals (Lead and Nickel) were below the Provisional Tolerable Weekly Intake (PTWI) specified European Union. The values were 0.0026mg/Kg for lead and 0.0052mg/Kg for Nickel. The major anions in this salt were chloride, carbonate and sulfate but chloride was significantly ($p<0.05$) greater than carbonate and sulfate. The sensory acceptability (taste attribute) of vegetable salt was relatively low value (20%) when compared to common salt (80%). However, the sensory acceptability of the combination of vegetable and common (commercial) salt in different proportion was highly accepted than common salt. Finally, the antihypertensive property of the salt was determined invivo Wistar rat experiment. Based on this, rats fed on salt from Hygrophilia schulli showed a significant ($p<0.005$) lower systolic, diastolic and mean blood pressure compared to normal fed and common salt fed rat groups. Results from this study indicated that vegetable salt extracted from Hygrophilia schulli can be a potential substitute of common salt with comparable sensory attributes, especially for people with hypertension and related chronic diseases.

Keywords: *Vegetable salt, Hygrophilia schulli, Hypertension, Blood pressure, Sodium-potassium ratio and Common salt*

CHAPTER ONE

1. Introduction

1.1. Background of the study

Edible salt is a mineral compound which is known as common salt, primarily used as an essential dietary item for both human and domestic animals. Salt has been used from pre-historic times for flavoring, pickling, preserving, and curing meat and fish, and for tanning. These features have made salt an important part of human culture and civilization (Morris *et al*., 2008).

The major components of edible salt produced from sea, lake and rock are sodium and chloride ions. Magnesium, calcium, sulfate, phosphate, carbonate, iron, zinc, nickel, chromium, cadmium, manganese, copper, lead, mercury, cobalt and clay are present in minute quantities (Gong *et al*., 1997). Edible salt can be produced from sea, lake and rock sources commercially. In 2007 annual world salt production was 244 million tons and now reached 327 million tones. The top six world salt producing countries are China, USA, India, Germany, Canada and Australia (Qadir *et al*., 2005).

From global salt production, around 5 million tons is produced in Africa. Angola, Botswana, Ethiopia, Ghana, Mozambique, Namibia, Senegal, South Africa, Sudan, and Tanzania are the ten key salt producing countries in Sub Saharan Africa (Mannar and Yusufali, 2013). Salt production also has a long history in Ethiopian, Afar region in particular. In fact, a small amount of salt is available in underground water in Tigray and from rocks in Somali region. However, abundant salt is found in the Afar National Regional State, particularly from Lake Afdera. At present, Lake Afdera, located close to the Eritrean border, produces 95% of the total salt requirement of Ethiopia. Ethiopia's annual domestic salt demand is estimated at 350,000 tons, whereas Afdera, alone has an annual production capacity of 1.2 million tons of salt (Feyissa, 2011).

In fact, worldwide and Ethiopian edible salt production is enough for daily consumption of the population. However, consumption of High common salt leads to rising of sodium ion in human blood, resulting in high water flow into the blood. The increase in fluid amount in the blood vessels thus causes increase in blood pressure (i.e hypertension) (MacGregor and De Wardener, 2002).

Hypertension may result in cardiovascular disease, stroke and diabetics (Ortells-Abuye *et al.*, 2010). Cardiovascular disease is the single largest risk for mortality in both developed and developing countries, killing an estimated 17 million people each year. Thus, WHO has a strong recommendation of less than 5g/day salt. Furthermore, since edible salt is consumed daily, any contamination (i.e. toxic metals), even at low level can be hazardous to the consumer's health. As a mitigation, avoiding common salt consumption is major risk factor that can cause iodine deficiency disorder because salt is the best vehicle used for Universal Salt Iodization (Mohan and Campbell, 2009).

Instead lower sodium salts (i.e. potassium rich salts) are important to use alternative salt sources to prevent chronic diseases. Accordingly, potassium rich edible salt has been produced from wild and domestic vegetables traditionally by lixiviation method. This is a process of salt making from plant parts after burning, dissolution and evaporation. As such salt types are called vegetable or ash salt. Vegetable salts have been produced and consumed by many people from Oceania, Central Africa and America. Portères (1950) and (1957) reported that, 158 different plant species have been used in Africa for vegetable salt preparation. For instance, in Central Africa, 10 % and 35% ash salt was produced from the fresh plant and ash of *Hygrophilia auriculata* (Mianpeurem, 2012). Ash salt is highly composed of potassium salts (i.e. potassium sulfate, potassium carbonate, potassium chloride etc) and lower amount of sodium. It has been used for therapeutic purposes to treat/prevent cardiovascular diseases, diabetes and hypertension traditionally (Okayama *et al*., 2016).

Vegetable salt is also produced and consumed in Ethiopia particularly in Gambella region from *H. schulli* species. As to our knowledge, there is no study or related document on ash salt production, composition and consumption in Ethiopia. A year ago, a popular TV show host named “Chef Yohannes” in EBS broadcasted a short documentary on ash salt production and consumption in Ethiopia (i.e. Gambella region). According to the show, the widely used plant to produce ash salt is *H. schulli*. *H. schulli* is widely distributed throughout India, Srilanka, Burma, Malaysia, Nepal and Tropical Africa. The whole parts of the plant including ashes used in traditional medicine for various types of diseases (Lobo *et al*., 2010).

Therefore, the main objective of this study was to investigate the composition of minerals and toxic metals in the ash salt from *H. schulli*. Also, the study aimed to evaluate the sensory quality of the salt as compared to commercial salt. Ultimately, this study investigated the anti-hypertensive property of the ash salt using Wistar rat *in vivo* study.

1.2. Statement of the problem

Average salt consumption worldwide is exceeding the allowed limit (5g/day) for physiological function of human beings that is recommended by Joint World Health Organization/Food and Agriculture Organization of the United Nations expert consultation (2002) and WHO (2007) guideline. This excessive salt intake will lead to excess sodium intake, which is a risk factor causing hypertension and other non-communicable diseases (Aburto, 2013).

Briefly, 95% of sea salt is sodium chloride; sodium raises blood pressure through which moves into the bloodstream, water follows. The reabsorbed water increases the blood volume. Therefore, the blood pressure will be created. Sodium is retained predominately extracellular in contrast to potassium which is almost entirely intracellular. Excessive salt ingestion is associated with excessive thirst because of the body's need to maintain the istonicity of extracellular fluid and increases the amount of fluids in the blood vessels, thus creating the pressure (Frisoli *et al*., 2012). This will lead to cardiovascular disease, diabetes, kidney failure and stroke (non-communicable diseases (NCD_S)), which are the leading causes of death globally. In 2005, cardiovascular diseases accounted for 30% of all other deaths (WHO, 2010). Increased blood pressure or hypertension is a big factor that leads to cardiovascular diseases contributing to 49% of all coronary heart disease and 62% of all stroke related incidences. At present, hypertension affects around half of adults globally (WHO, 2012).

Commonly salt produced from sea also has a chance to be cross-contaminated with harmful trace/toxic metals and other impurities from many sources. The common toxic metals include lead, mercury, arsenic, chromium and cadmium. Toxicity of harmful metals can lead to inflammation in arteries and tissues, organ specific toxicities (i.e. .neurotoxic, nephrotoxic, fetotoxic and teratogenic effects) (Wuana and Okieimen, 2011). With three meals per day, each meal containing salt, it is intuitive to assume the excessive salt/sodium and toxic metal intake.

Due to these concerns, consumers are developing a trend to avoid salt from their menu. However, edible salt is the most commonly used vehicle for iodization process because of its safety, sustainability and cost effectiveness in order to mitigate iodine deficiency disorder (IDD). Iodine deficiency in children results in learning disabilities and reduced achievement (Baguune, 2012). Even mild to moderate iodine deficiency has been shown to cause abnormalities in psychomotor and intellectual development in children (WHO, 2007).

Globally, nearly two billion people are at risk of iodine deficiency (ID), while one-third lives in areas where natural source of iodine is low. Among the highest prevalence of ID is documented in Africa (42%), the largest burden being in Ethiopia (Abebe, Tariku and Gebeye, 2017). Apparently, the consequence of avoiding edible salt from diet will lead to other malnutrition burdens. Hence, it is necessary to investigate other salt alternatives with lower sodium to potassium ratio and acceptable sensory attributes.

Alternative salts with lower sodium to potassium ratio were produced from plant parts worldwide. Specifically from plants with *Hygrophilia genera*, vegetable ash has been produced and consumed in different parts of the world (Africa, Oceania etc). For instance; Portères (1957) reported that, 158 different plant species have been used in Africa for ash salt preparation. Ash salt is highly composed of potassium salts (i.e. potassium sulphate, potassium carbonate, potassium chloride etc) and lower amount of sodium. It is has been used for therapeutic purposes to teat/ prevent cardiovascular diseases diabetes and hypertension traditionally (Okayana *et al*., 2016).

With this regard, different studies were conducted worldwide. For instance, Witoto ash salts from the Amazon (Echeverri, 2011), ash salt from Chad (Mianpeurem *et al*., 2012) and the ash salt extracted from Chirchita (*Achyranthes aspera*) belongs to the family of Amaranthaceae is used for therapeutic purpose. The plant contains variety of organic salts like, chloride, phosphorus, potassium and magnesium in variable amount. This salt used as therapeutic medicine for urinary and respiratory disorders (Tarannum, 2015).

All these studies have a significant role by showing the chemical composition and the purpose of vegetable salt (potassium rich salt) for medicinal purpose but the studies also have its own limitations. Because, most studies were focused only theoretical aspect of vegetable salt, they did not focus on animal model experiment using ash salt of the plant.

Similarly, vegetable salt is produced and consumed in Ethiopia particularly in Gambella region from *H. schulli*. However, as to our knowledge, there is no study or related document on ash salt production, composition and consumption in Ethiopia. Therefore, the main objective of this study was to investigate the composition of minerals and toxic metals in the ash salt from *H. schulli*. Also, the study aimed to evaluate the sensory quality of the salt as compared to commercial salt. Ultimately, this study investigated the anti-hypertensive property of the ash salt using Wistar rat *in vivo* study.

1.3. Objectives

1.3.1. General objective

To evaluate chemical composition and anti-hypertensive properties of vegetable salt produced from ash of *Hygrophilia schulli*.

1.3.2. Specific objectives

The specific objectives of the study were to:

- Evaluate mineral compositions and toxic metal levels of vegetable salt extracted from *Hygrophilia schulli*
- Evaluate anti-hypertensive properties of vegetable salt of *Hygrophilia schulli*
- Compare some physico-chemical characteristics of vegetable salt with common edible salt
- Evaluate sensory acceptability of the salt extracted from *Hygrophilia schulli*

CHAPTER TWO

2. Literature Review

2.1. Overview of Commercial Salt

2.1.1. Production of Sea Salt

Salt for human consumption is produced in different forms: unrefined salt (sea salt), refined salt (table salt) and iodized salt. It is a crystalline solid, white, pale pink or light gray in color, normally obtained from seawater or rock deposits. Commonly salt is manufactured from underground deposits and from solar evaporation of saline seawater (Figure 1). Upon solar evaporation of seawater, certain trace minerals will remain in the salt. These minerals add flavor and color to the salt (i.e. slightly grayish). Sea salt consists 95-98% of sodium chloride. The remaining percent is constituted by essential minerals, toxic metals and other impurities (Cole *et al*., 2003).

2.1.2. Unrefined Salt

Unrefined salt does not under go through the purification process. In developing countries, salt after extraction from the sea consist of crude lumps or granules which contain in addition to sodium chloride, sand, mud, other salts and organic matter. The salt is off-white or yellowish-brown in colour. This crude salt is often used for some industrial purposes, for livestock use, in some developing countries for human use, bathing additives and cosmetics product (Heshmati *et al*., 2014).

Unrefined salt is +also referred as the Industrial salt. A few mining operations used in mining are packing and its transport (Celik and Oehlenchlar, 2007). The salts are 96 % pure and have some essential trace minerals such as Mg, Ca, S, N and I_2 etc. Unrefined salt is still the preferable choice of consumers in developing countries despite of the fact that several health agencies have discouraged its usage (Hassana *et al*., 2017).

2.1.3. Refined Salt

Refined salt is very pure (NaCl content 99.5%), dry, and white with uniform grain size (0.3 - 1.0 mm). it is sold with or without additives. additives are anti-caking agents to prevent adjacent grains to form a hard solid mass. The common additives are potassium or sodium ferrocyanide in the range 5-15 parts per million. Refined salts may be free flowing agents, to provide the crystals with a mechanical coating that absorbs any water vapour within the package after sealing. The common flowing agents are magnesium carbonate, calcium silicate and etc (Gidey *et al*., 2012). Refined salt is most widely used presently. Food grade salt accounts for only a small part of salt production in industrialized countries (3 % in Europe) although worldwide, food uses account for 17.5% of salt production. The majority is sold for industrial use. Salt has great commercial value because it is a necessary ingredient of wide applications (Mannar and Dunn, 1995).

2.1.4. Iodized Salt

Iodized salt is table salt mixed with a minute amount of potassium iodide or potassium iodate. Iodized salt is used to mitigate the incidence of iodine deficiency in human beings (Zimmermann, 2007). Iodine deficiency in children results in learning disabilities and reduced achievement (Baguune, 2012). Even mild to moderate iodine deficiency has been proved to cause abnormalities in psychomotor and intellectual development in children (WHO, 2007). About 38 million newborns in developing countries every year remain unprotected from the lifelong consequences of brain damage associated with iodine deficiency disorder (UNICEF, 2008).

Other consequences of iodine deficiency include hypothyroidism and cretinism. Hypothyroidism is detected by low levels of thyroid hormones in the blood resulting in sluggishness, sleepiness, dry skin, cold intolerance and constipation. Cretinism refers to the very consequences of hypothyroidism occurring at the fetal or neonatal life. In addition to having severe irreversible mental retardation, they may also present several other conditions including deaf mutism, short stature and retarded development of the musculo-skeleton system (Geertman, 2000).

Figure 1*:* Edible salt processing through evaporation of saline water (Mannar and Dunn, 1995).

2.1.5. Risks of excess salt consumption

The strong correlation between excess dietary salt intake and increased blood pressure is well established through epidemiological and intervention studies. Most adult populations around the world have average daily salt intake higher than 6g. In Eastern Europe and Asia the intake even exceeds 12g per day. However, international dietary guidelines recommend a daily salt intake of less than 5-6 g (WHO, 2012).

Population based intervention studies and randomized controlled clinical trials have shown the significant reductions in blood pressure with reduced salt intake in people with and without hypertension. Based on the effects of high salt intake on blood pressure and consequently resulting in cardiovascular diseases, it has been recommended that a population-wide reduction in salt intake could substantially reduce the incidence of cardiovascular disease (WHO, 2007).

Approximately two-thirds of adults in the U.S. have either hypertension, defined as untreated systolic blood pressure (SBP) above139 mmHg or diastolic blood pressure (DBP) above 89 mmHg, or pre-hypertension with SBP 120 to 139 mmHg or DBP 80 to 89 mmHg. Untreated hypertension is associated with increased incidences of diabetes, heart disease, stroke, and kidney disease. Therefore, there is universal agreement for intervention that reduces or prevents development of high blood pressure (Havas *et al*., 2007).

Age, Body Mass Index (BMI), physical activity levels, and dietary sodium and potassium intake are all known to affect blood pressure. However, among these factors some analysts underline dietary sodium intake as a major risk factor of hypertension (Hollenberg, 2006). Approximately 98% of dietary sodium is absorbed in intestine. Excess sodium is excreted mainly by the kidneys and some is lost with perspiration. In healthy adult humans at steady state conditions, urinary sodium excretion roughly equals intake. Sodium is an essential nutrient, mainly responsible for regulating extracellular fluid and plasma volume. It also determines membrane potential of cells and participates in the active transport of some molecules across cell membranes (Adrogué and Madias, 2007).

Several hormones and the sympathetic nervous system enable healthy humans to adapt different dietary salt levels. This will help to maintain plasma levels of sodium within an optimal range by altering the excretion of sodium in response to changes in dietary sodium intake. However, people age develop certain chronic diseases, kidney function may decline by affecting homeostatic regulation of electrolytes. As the efficiency of excretion of excess sodium diminishes, plasma volume may increase and stress the cardiovascular system by inducing hypertension. Hypertension, in turn, is correlated with higher risk for coronary heart disease, stroke and end-stage renal disease (MacGregor *et al.*, 2007).

This edible sea salt can be contaminated with toxic trace metals because it is one of the most ubiquitous food ingredients. Some studies of heavy metals in salt had been previously conducted in Iran (Hamshahri, 2006). Because, toxic metals set up conditions that lead to inflammation in arteries and tissues, causing more calcium to be drawn to the area as a buffer, this contributes to the hardening of the artery walls with progressive blockage of the arteries and osteoporosis. Generally speaking harmful trace metals have no physiological function in human body and can be highly toxic. They are systemic toxins with specific neurotoxic, nephrotoxic, fetotoxic and teratogenic effects. Several reports have focused on residues of numerous heavy metals in food stuffs (Jahed *et al.,* 2005).

Copper and zinc are known to be essential and may enter the food chain from the soil through mineralization by crops; food processing or environmental contamination. The toxicity of them is due to excessive intake but toxic trace metals like lead, mercury and cadmium cause toxicity in small concentration (Kuntz, 1994).

2.2. Vegetable (ash) Salt

Salts that are produced from ashing plant (plant bodies) called vegetable salt or ash salt. Vegetable salt has been used in China, Africa and New Guinea for religious rites. Historically, salt used to be a currency to pay taxes and salary in Europe. Excess intake of edible salt has been linked with hypertension worldwide. For instance in France 25,000 related deaths per year causing hypertension. As a result, many consumers and especially in Africa prefer vegetable salt which is obtained by burning plant parts. In Africa, this salt was widely used before colonilization (Thomson, 2012). With the colonization, the white commercially produced salt appeared. It is collected from salt mines or produced industrially. This newly introduced salt is cheaper; Because of this reason, salt of Vegetable ash has been abandoned in many places (Porteres, 1950 and 1957).

Vegetable salt production method proceeds largely by lixiviation. This is a process in which plant parts will be burned, ashed, dissolved in water, filtered and evaporated leaving crystal salt (Figure 2). The plants used for the preparation are mostly waste parts of cultivated plants like stems of maize, millet, sorghum and some widespread plants like from *Hygrophilia species*. Also false trunk of pawpaw and banana are used to produce vegetable salt traditionally (Allaramadji, 2011).

Studies on chemical composition of vegetable salt are limited. For instance, Zerries (1964) reported composition of vegetable salt conducted by Martius in the Xingu River and by Sick in the British Guyana. Similarly Schmeda-Hirschmann (1994) Roman-Jitdutjaano (2011) analyzed vegetable salts from the Papaguayan Chaco and reported the mineral contents. Roman-Jitdutjaano (2011) has reported the chemical analyses results of ash salts from 57species used by the Witoto Indians of Amazon. Also there are very few studies on Africa vegetable ash salt has investigated the elemental composition of four vegetable salts produced from ash of burning stems of maize (Zea *mays*), stems of sorghum *(Sorghum bicolori*), false trunks of pawpaw *(Carica papaya)* and whole parts of *Hygrophilia auriculata*. The elemental analysis showed that all the four salts are potassium rich and sodium poor in their study. Maize and sorghum salts mainly contain potassium chloride and potassium sulphate, whereas salts from pawpaw and *H. auriculata* are composed of three potassium salts: potassium chloride, potassium carbonate and potassium sulfate. All the salts did not contain heavy metals. It was recommended that, these salts can be used as dietetic salts and substituting commercial salt which contains mostly sodium chloride (Allaramadji, 2011).

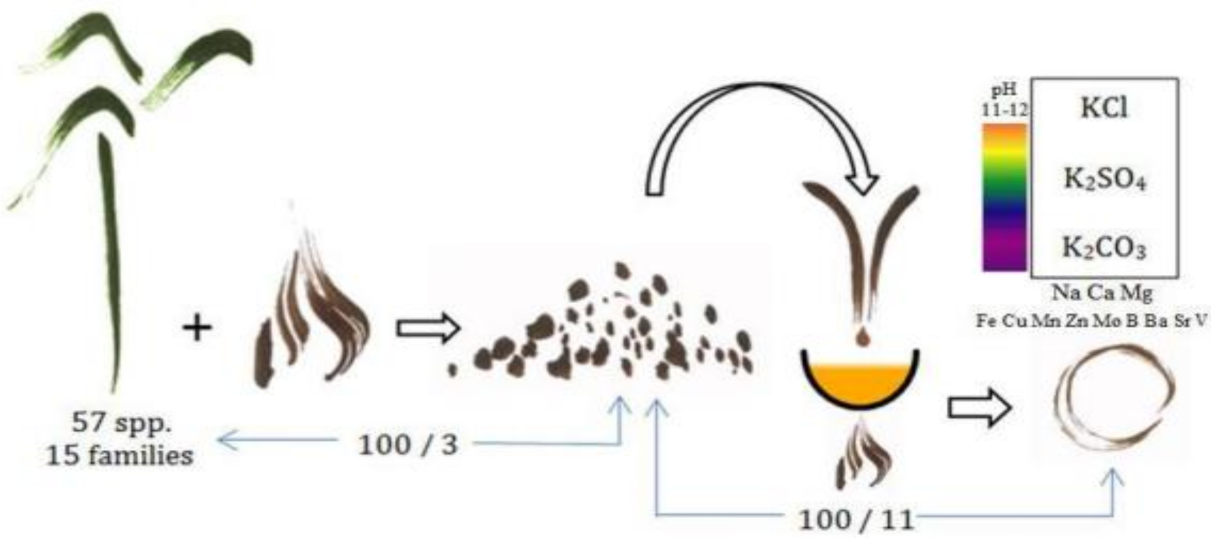


Figure 2: Steps of vegetable salt production (Echeverri, 2011).

Vegetable salt is also produced and consumed in Ethiopia particularly in Gambella region from *H. schulli*. As to our knowledge, there is no study or related document on ash salt production, composition and consumption in Ethiopia. A year ago, the popular TV show "Chef Yohannes" in Ethiopian Broadcast Satellite broadcasted a short documentary on ash salt production and consumption in Ethiopia; this is the basis and initiative for this study. According to the show, the widely used plant to produce ash salt is *H. schulli.*

2.2.1. Distribution of *H. schulli*

H.schulli (Buch.-Ham.) M.R.Almeida and S.M.Almeida (family Acanthaceae), Marsh barbel (Eng), Kurumbeye (Amha), Darbate (Orom) is unbranched herb with straight curved thorns of the family Acanthaceae that commonly grows in moist places, on the banks of rivers, ditches and paddy fields (Figure 3). The plant is widely distributed throughout India, Sri-Lanka, Myanmar, Indo-China, Malaya and tropical African countries including Ethiopia (Sufian and Haque, 2015).

Figure 3: Pictures of H. schulli grown in Gambella region, Ethiopia.

2.2.2. Biology of *H. schulli*

H. schulli is an erect herb, perennial plant growing 1-1.5 m in height. The stems are sub-quadrangular and numerous. The flowers are bluish purple in the axils of leaves, amidst the spines. The seeds are slimy to taste. The plant flowers during October to December. It is common in water logged places (Hussain *et al*., 2010).

Leaves are sub-sessile or linear lanceolate, and spines are yellowish brown, 2-3 cm long, flowers 2-3 cm long, purple–blue, compressed about 8cm long, pointed, four to eight seeded.

Seeds are ovate, flat or compressed 0.20-0.25 cm long and 0.10-0.15 cm wide, hairy but appearing smooth; when soaked in water, they immediately get coated with mucilage, light brown: Taste slightly bitter and odor is not distinct (Kannur *et al*., 2012).

2.2.3. Uses of *H. schulli*

H. schulli has an important role in traditional medicine and ash salt source in different nations worldwide like India, Central Africa specifically in Chad, Ethiopia (Gambella). The detail uses of the plant are discussed below.

2.2.3.1. Medicinal value of *H. schulli*

H. schulli (K. Schum) has been used in traditional practice for many years. The plant contains various groups of phyto-chemicals (Dash *et al.*, 2012). These phyto-constituents are phytosterols, fatty acids, minerals, polyphenols, proanthocyanins, mucilage, alkaloids, enzymes, amino acids, carbohydrates, hydrocarbons, flavonoids, terpenoids, vitamins, glycosides, etc. These non-nutrient components of plants are useful in the treatment of diseases of urino-genital tract, dropsy of chronic disease, hyperdipsia, vesical calculi, diarrhea, dysentery, leucorrhoea, gonorrhoea, asthma, blood and gastric diseases (Thorat, 2018).

In the rural areas of West Bengal, feeding the hot water infusion of succulent aerial parts of pre-flowering and flowering plant to pregnant women is a common practice to prevent anaemia. The whole parts, leaves, seeds, roots and ash of the *H. schulli* are broadly used in traditional system of medicine for the treatment of diabetic, dysentery and other disorders. *H.schulli* is known by possessing antibacterial, anti fungal, hypoglycemic, hepatoprotective and antitumor characteristics (Jain *et al*., 2008). Some of isolated and elucidated phyto-chemicals from Hygrophilia plant include gallic acid 3, 4, 5-trihydroxybenzoic acid and quercetin (2-(3, 4- dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one) (Figure 4).

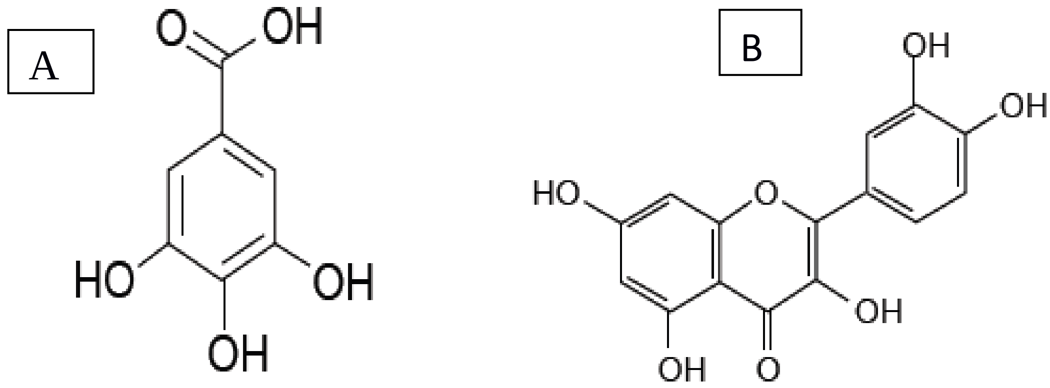


Figure 4: A and B Chemical structure of gallic acid and quercetin (Hussain, 2012)

Studies have shown that different flavonoid glycosides (gallic acid and quercetin) are preferentially absorbed in small intestine through various uptake mechanisms, suggesting its bio-effectiveness (Amiot-carlin *el al.*, 2011). Studies have reported that quercetin, which is the major flavonol present in *H. schulli,* exhibits anti-cancer, anti-inflammatory, antiviral activities and may also prevent cardiovascular disease in humans (Hussain, 2012).

2.2.3.2. Anti-hypertensive and anti-diabetic activities of *H. schulli*

Hygrophilia auriculata and *H. schulli* (K. Schum) Heine, a generally occurring wild herb belonging to Acanthaceae family has been used for the treatment of diabetes and dysentery. Roots, seeds, and aerial part of the plant have been used in the treatment of hypertension, hepatic obstruction, inflammation, urinary infection, malaria and impotence (Manikandan and Lakshminarasimhan, 2012). The plant has been reported to contain flavonoids (apigenin 7-O-glucuronide, apigenin 7-Oglucoside), alkaloids, aliphatic esters (25-/ oxo/ -/ hentricontyl/ acetate, methyl/ -8- hexyltetracosanoate), and minerals like iron, sterols (stimagsterol), triterpenes (lupeol, hentricotane, betulin, luteolin, luteolin -7- /O-/ rutinosides) and essential oils. Phenolic bioactives compounds. These phenolic bioactive compounds have potential to manage early stages of type 2 diabetes and hypertension (Phoboo, 2015).

Hydrolysis of dietary carbohydrates such as starches is the major source of glucose in the blood glucose. The enterocytes of the small intestine can only absorb monosaccharide such as glucose and fructose from our diet. Therefore, the dietary polysaccharides need to be broken down to monosaccharide before they can be absorbed. This hydrolysis is carried out by a group of hydrolytic enzymes called α-glucosidases which includes sucrase, maltase, gluco-amylase, dextrinase and the pancreatic α-amylase. It is now believed that inhibition of these enzymes involved in the digestion of carbohydrates can significantly decrease the increase of blood glucose level after a mixed carbohydrate diet and therefore can be an important strategy in the management of type-II diabetes and hypertension (ElObeid *et al*., 2015).

2.2.3.3. Antimicrobial activities of *H. schulli*

H. schulli mainly contains lupeol, stigmasterol, isoflavone glycoside, an alkaloid and small quantities of uncharacterized bases. *H. schulli* is one of the most important medicinal plants of the family Acanthaceae and leaf extract possesses potent antibacterial property. The Plant extracts are more pronounced in their antibacterial response against bacteria species including *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli* and *Bacillus subtilis* (Chandran *et al*., 2013). The plant also has been reported to be used in dropsy, diarrhea, cough, jaundice, and urinogenital diseases. Seed juice of the plant is used during child birth and leaf paste applied externally to lumbago and rheumatism (Gulshan *et al*., 2012).

2.2.3.4. Nutritional benefits of the *H. schulli*

There are very few scientific investigations on the nutritional composition of *Hygrophilia schulli*. The existing reports indicated that the plant is a good source of oils with linoleic, palmitic, and stearic acids (Mazumdar, 1978). Polysaccharides like xylose, glucose, rabinose and mannose also found in the plant. It is also a good source of nutrients like uronic acid, histidine, lysine, phenylalanine and sitosterol. Vitamins like ascorbic acid, nicotinic acid, are also present in significant amount in the plant (Ali, 1967).

Proximate content of leaf on dry matter basis of *Hygrophilia auriculata* plant were found to be crude protein (18.41±0.26%), ash (14.21±0.38%), which differ from the values of crude protein (20.6%), ash (18.3%), crude fat (3.4%), crude fiber (21.3%). Zn, Fe, Co, Ca, Mg and P were analyzed in high concentration, but K concentrations were found significantly higher ($P \leq 0.05$) in leaf extract of the plant, compared to others. Fe, Ni, Cr, Na, K, Al, and Sr were also isolated from the plant using flame photometer, atomic absorption spectrometer and inductively coupled plasma (Dash, 2012).

2.2.3.5. Antioxidant properties of *H. schulli*

Most of the diseases that affect our body are mainly related to oxidative stress due to free radicals. Free radicals are basically any biochemical process and represent an essential part of aerobic life and metabolism. The most common reactive oxygen species (ROS) include superoxide (0_2.), Anion, hydrogen peroxide (H_2O_2), peroxyl (ROO-) radicals, and reactive hydroxyl (OH.) radicals (Tiwari, 2001). Antioxidants are chemical substance basically found in plants and have an important role in inhibiting and scavenging free radicals. Plant originated polyphenols and flavnoids have powerful antioxidant activities. These photochemicals are able to scavenge a wide range of reactive oxygen species, inhibiting lipid peroxidation in human tissues (Lobo *et al*., 2010). Polyphenols in plants include simple phenols, phenolic acids (both benzoic and cinnamic acid derivatives), coumarins, flavnoids, hydrolyzable and condensed tannins (Dixon and Paiva, 1995). Thus, current food related studies are focusing on natural antioxidants originated from plants due to safe therapeutics. Similarly, *H. schulli* has been reported as a potential antioxidant compound source. For instance, the extracts from the plant has been used in nations like India as traditional medicine for a wide range of ailments (Lobo, 2010).

2.2.3.6. *H. schulli* used as edible salt

H. schulli is reported in different studies as a potential source of ash salt. In fact in Africa, vegetable salts were used formerly in economic activities before colonization. Different plant species were used for the preparation of ash salt. Commonly *Hygrophilia* species was well documented as a source of ash salt. In Central Africa, Chad, 10% ash salt was obtained through lixiviation from the whole *H. auriculata* (Allaramadji, 2011). Similarly, in Ethiopia (Gambella region) people consume ash salt from *H. schulli* traditionally. People in the region believe that the salt produced from *H. schulli* has therapeutic effect against heart and liver diseases, hypertension, diabetics and stomach disorder. The consumers also witnessed cure of fresh wounds using ash salt. With this regard, the data from Ethiopian Ministry of Health (2007, 2008 and 2009) demonstrated that the mortality and morbidity by hypertension, stroke, diabetics and heart diseases are the least in Gambella region among the other regions.

Apparently, these evidences indicate the potential of vegetable salt to mitigate chronic diseases. However, as to our knowledge, there is no study on ash salt production, composition and consumption in Ethiopia. Therefore, the main objective of this study was to investigate the composition of minerals and toxic metals in the ash salt from *H. schulli*. Also, the study aimed to evaluate the sensory quality of the salt as compared to commercial salt. Ultimately, this study investigated the anti-hypertensive property of the ash salt using wistar rat *in vivo* study.

CHAPTER THREE

3. Material and Methods

3.1. Experimental area

The chemical analyses in the study were conducted in different food laboratories at Center for Food Science and Nutrition of Addis Ababa University, Kotebe Metropolitan University, Bless Agri Laboratory Service PLC, Ethiopian Leather Industry Institute and Ethiopian Public Health Institute.

3.2. Chemicals

All chemicals for this study were Analytical Grade (AR) bought from the market, Addis Ababa, Ethiopia. De-ionized water was used for all dilutions and rinsing purposes throughout the study.

3.3. Sample collection and sampling site

The sample of *H. schulli* parts were collected to prepare ash salt for this study. The plant was collected from Gambella and Abobo woreda of Gambella region, around the Shore of Baro River, Ethiopia in December, 2017. In addition to the plant, already prepared ash salt from *H. schulli* was collected from open markets in Anuaq (Wolenga) /Addis sefer/, Gambella region. Gambella People's National Regional State is found in Ethiopia between latitudes 6^0 30' and 8^0 30' N and longitudes 33^0 00' 35^0 45' E, with total area of 26,000 Km^2. Gambella region is bound by Oromia Regional State to the North, Southern Peoples′ Nations and Nationalities Regional State to the East and the Republic of Sudan to the South and West (Awas, 2001) (Figure 5). Plant and salt samples were packed in polyethylene (PE) plastic bags respectively and transported to the laboratory of Center for Food Science and Nutrition of Addis Ababa University. Then, the plant sample was oven dried (100^0C) and burned to obtain ash according to the method described in section 3.4 below. The common edible salt used in this study was bought randomly from open markets in Addis Ababa.

Figure 5: Map of Gambella Region, Ethiopia (Seide, 2017).

3.4. Salt extraction method

The salt was extracted using the method of Mianpeurem, (2012). This salt preparation method, was adapted from the indigenous ash salt processing method with some modifications. Collected plant material (2935.47g) was burnt on broken pot (open air) from the stem and 1320g from the leaf. Then 100gm ash was dissolved in 1.5L distilled water. The solution was mixed well and kept at room temperature for 1hr. Then it was mixed again for 30 minutes to dissolve all salts in the ash. Afterwards, the solution was passed through a filter paper (Watman international Ltd, 11.0cm, Cat N0 10936211) to separate the salt and impurities. Finally, the filtrate was placed in drying oven (Model 105, Industrial Estate, Ambala Cantt-India) at 105°C until it crystallizes. The crystallized salt was collected from the oven, sundried and kept at room temperature until analysis.

Figure 6: Traditional and scientific vegetable salt production methods (from the society)

3.5. Analytical methods

3.5.1. Macro and micro metals analysis

One g of each test sample (both vegetable and common salt samples) was weighed and transferred in to conical flask containing 400mL 10% nitric acid solution and stirred. Then the solution was placed in digester (Milestone start D microwave digester, Switzerland) to digest for 15 minutes. Finally 1mL of the sample solution was dissolved in 50mL 10% nitric acid and run in Inductively Coupled Plasma-Optical Emission Spectroscopy (Agilent 700 series, USA) to analyze metal ions by using ISO 11885:2012 test method. Then concentration of metal was calculated using an equation 1:

Conc. of metals (ppm) = $(s_C - b_C) * E_V * Df$……………………….……………… *eq.*1

Where;

S_{C} = concentration of sample

B_{C} = concentration of blank

E_V = Extracted volume

Df = Dilution factor

3.5.2. Toxic metal analysis

Concentrations of toxic metals (Pb, Cd, Ni and Cr) in both samples were determined by ICP-OES using the same extraction method. As by Graphite Atomic Absorption Spectrophotometer (Model G8432A, Agilent Technology, Malaysia) and Hg was determined by cold vapor Atomic Absorption Spectrophotometer (model G8436A, Agilent Technology, Malaysia) using ISO 17025: 2005 test method.

3.5.2.1. Method of mercury (Hg) quantification

Ten g of salt was weighed and transferred into100 ml conical flask. Then 30 ml of de-ionized water, few glass beads, 4 ml of 6 mol/L hydrochloric acid and 3 ml of 10% sodium chlorate solution was added. The solution was heated and kept boiling for 5 minutes, then was allowed to cool. The whole content was transferred into 100 ml volumetric flask, diluted to the mark and mixed.

Then, 10ml of the solution and 3 ml of 100g/L hydroxyl ammonium chloride were transferred into reaction bottle and filled to the mark (60 ml) with de-ionized water. Two ml of 100g/L tin (II) chloride was added and whole content was placed into the AAS device. Swirl to mix well and allowed to stand for few minutes. Nitrogen was passed through the reaction bottle and the maximum of absorbance was determined using EuSalt/AS 012:2015 test method. The mercury content of the sample is calculated using an equation 2:

Total Hg Content (µg/kg) is = $\frac{1000}{M} \times (m_1 - m_0)$ ………………………………… *eq*. 2

Where;

M = Sample weight

m_1 = Mass of mercury Hg (µg) analyzed in the test solution

m_o = mass of Hg (µg) analyzed in the blank solution

3.5.2.2. Method of arsenic (As) quantification

Ten g of salt sample was weighed and transferred into 100 ml conical flask containing 50 ml of water and 10 ml 37% of hydrochloric acid. Then 5m silver diethyldithiocarbamate solution was transferred into the absorption vessel which is attached with the connecting tube with a safety clip. Upon which, 3 ml of 100 g/l potassium iodide, 2 ml of 400 g/l tin (II) chloride solution were added, swirled and allowed stand for 15 minutes. Further, allowed to stand for 45 minutes to complete the reaction. Then, the absorption vessel was swirled to disperse the red deposits. The color of the solution was stable for about 2 hours in the absence of light. For the photometric measurements the apparatus was adjusted to zero absorbance with pyridine. The photometric measurements were conducted using FAAS with maximum of absorption (wavelength around 540 nm) using EuSalt/AS 011:2015 test method.

The arsenic content of the sample, (As), of salt is given by the equation 3:

$$\text{As (mg/Kg)} = \frac{(m1-m0)}{M} \;\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; eq.\ 3$$

Where;

M = Mass (g) of the test portion

m_1 = Mass of arsenic (µg) analyzed for the test solution

m_0 = Mass of arsenic (µg) analyzed for the blank solution

3.5.3. Method of iodine (I_2) determination

Iodine content of both common and vegetable salts was determined using Association of Official Analytical Chemists (AOAC, 1998). In this method iodate was reduced to iodine (I_2) and then titrated with sodium thiosulfate using a starch indicator. Briefly, 50 grams of the dried salt samples were dissolved in de-ionized water and make up the volume to the mark in 250ml volumetric flask. Then 100ml of the solution was pipetted in to 500ml conical flask and diluted to 300ml de-ionized water. Then 0.01% methyl orange indicator was added and neutralized with 85% (m/v) phosphoric acid. Afterwards, 22 g of bromine was added and the solution was boiled gently until it becomes colorless and then for 5 minutes more.

Then, few crystals of salicylic acid were added and the solution was cooled at room temperature. On the cooled solution, 1 ml of 85% phosphoric acid and 0.5 g of potassium iodide were added followed by titration with 0.005mol/L sodium thiosulphate. Then 1percent starch solution was added near the end of the titration. Blank determination was carried out on with the same procedure without salt samples.

Iodine concentration was determined using an equation 4:

$$\text{Iodine, mg/kg} = \frac{52875(V1-V2)}{\mathrm{m}} \;\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; eq.\ 4$$

Where;

V_1 = Volume (ml), of standard sodium thiosulphate solution titrating the sample

V_2 = volume (ml), of standard sodium thiosulphate solution titrating the blank solution

m = Mass (g) salt sample

52875 = Constant for titration of iodine

3.5.4. Alkalinity determination

Alkalinity as sodium carbonate was determined by AOAC (1998). Briefly, 6 g of salt in a flask was dissolved in 300 ml de-ionized water followed by filtration. Then, the residue was discarded and 50mL of the filtrate was titrated against 0.1N hydrochloric acid using methyl orange as an indicator.

The percentage by mass of alkalinity (as Na_2CO_3): $\frac{5.3}{M} \times V$………….………… *eq*.5

Where;

V = Volume (ML) hydrochloric acid used in the titration

M = Mass (g) of salt tested

3.5.5. Sulphate determination

Sulphate content in the salt is determined as barium sulfate by gravimetric method. Briefly, 4g of both vegetable and common salt samples were dissolved in 200ml of distilled water, followed by the addition of 1.0 ml of 4N hydrochloric acid and heated to boiling with continued stirring. Then, 6ml of 10% barium chloride was added drop by drop and placed in boiling thermostatic water bath (YCW-010, Gemmyco-Industrial Corp, Taiwan) for 1h. The precipitate was filtered through ashless filter paper, and washed with boiling water. Chloride ion was detected when silver solution was added to the filtrate. Hence, the residue was washed over and over again until no more white precipitate formed on testing of the drop of the filtrate with silver nitrate solution. The filter paper and its content were completely dried in oven at 110^0C. Then, the filter paper was ignited in an electric furnace (J.P.selecta, s.a, code 20000366, Spain) at 825^oC, cooled in a desiccator and weighted.

The percent by mass of sulphate (SO_4): $41.13\,\frac{M1}{M2}$…………………………….. *eq*.6

Where;

M_1 = mass (g) of barium sulphate

M_2 = mass (g) of dried sample

3.5.6. Chloride determination

Chloride content of the salt was determined using the protocol by Potentiometric titration method (EuSalt/AS 016-2005 test method). Briefly, 10 g salt was transferred into 1000 ml volumetric flask and dissolved in water to the mark. Then, 10ml of 10g/L sodium chloride, 2 ml of 1.4 mol/L nitric acid, 5ml of 2g/L PVA solution was transferred into the titration vessel, followed by dilution with 50 ml water. Finally, the chloride was titrated with the 0.100 mol/L silver nitrate solution.

The chloride content was calculated using an equation 7:

$$\text{Chloride amount (g/kg)} = V \times C\,(AgNO_3) \times \frac{1000}{10 \times m} \times 35.453 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; eq.7$$

Where;

M = Mass (g) of the test portion

C ($AgNO_{3)}$ = concentration of $AgNO_3$

V = Volume of $AgNO_3$ used for the titration

3.5.7. pH measurement

Ten g of vegetable and common salt samples were dissolved in 50 ml distilled water and the pH was measured using 009 (1) pen type digital pH meter. The Calibration was set between pH 4 and 14.

3.5.8. Determination of water insoluble matter

Water insoluble matter was determined using (ES ISO 2479). Briefly, about 25gm of salt sample was dissolved in 100 ml of distilled water and heated below 100^0C for 10 minutes with stirring. Then, the whole content was kept in a boiling water bath for 30 minutes. This was followed by vacuum filtration on filter crucible which was previously dried at 110^0C, cooled in a desiccator and weighed. Then, the insoluble matter was washed successively using distilled water in order to bring the insoluble matter into suspension for approximately one minute. After repeated washing, silver nitrate was added to the filtrate to check the presence for chloride ion. Finally, the crucible and its contents were dried in drying oven at 112^0C and weighed.

The water insoluble matter was calculated using an equation 8:

$$\% \text{ of water insoluble matter} = \frac{M2 \times 100}{\text{M1}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; eq.8$$

Where;

M_1 = Mass (g) of sample

M_2 = mass (g) dried water insoluble matter

3.5.9. Moisture content determination

Moisture content was determined by oven drying method (AOAC, 1998). Briefly, empty dishes were dried using air drying oven for an hour at 105^{o}C, transferred to desiccator, cooled for 30 minutes and weighed. Homogenized salt sample was transferred to the dried dishes and weighed. The whole content was kept in drying oven for 3 hours at 110^{0}C. Then, the dishes were cooled in desiccator at room temperature and weighed.

The moisture content (%) was determined using an equation 9:

$$\text{Percent of moisture} = \frac{(Wt.Bd - \text{Wt.}Ad) \times 100}{\text{Wt.Bd}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; eq.9$$

Where;

Wt.Bd = weight of sample (g) before dried

Wt.Ad = weight of sample (g) after dried

3.6. Anti-hypertensive test

3.6.1. Experimental animals and housing

The experiment was done using the method of Boegehold and kitchen, (1990). Four-weeks-old male Wistar rats (weight between 200g to 270g) were purchased from Ethiopian Public Health Institute (EPHI). The rats were housed at 22 ± 3°C room temperature with normal humidity (40% - 70%). The fed were supplied to rats for five weeks after two weeks adaptation period. The rats were randomly assigned to 3 groups: normal diet fed group (control) (group 1, n = 6), 4% *H. schulli* salt containing diet feed group (group 2, n = 6) and 4% common salt containing diet feed group (group 3, n = 6). Then fed and water (tape) were provided *ad libitum* throughout 5 weeks feeding time. 4% salt containing rat fed was prepared by 252g of salt mixing with 6300g of normal fed. Briefly: 252g salt and 252g of salt free fed was mixed manually (504g salt mixture). 504g salt mixture and 504d of salt free feed was mixed (1008g). Next 1008g of mixture and 1008g salt free fed was mixed (2016g). Then 2016g mixture and 2016g salt free fed was mixed (4032g). Finally 4032g mixture and 2520g of salt fed was mixed (6552g). Then the pellet was prepared.

3.6.2. Heating (warming)

The rats in the cage were moved close to the platform next to the tail cuff machine (Blood pressure analyzer) (Model 179, 23924 Victory Blvd Wood land Hills CA91367, USA, blood pressure analyzer) and left for 15 minutes without handling. Then, each rat was picked up from the cage and held on hand for 30 seconds and then was returned to the cage for further recording over 15 minutes. Afterwards, each rat was removed from the cage and placed into the restraint tube and transferred into heater (the part of tail cuff machine with the temperature maintained at 30 to 33°C). The restraint period was lasted for 15 minutes. Once the rats within the restraint tube were heated, finally blood pressure was measured by the tail-cuff method (Wilde *et al.*, 2017).

3.6.3. Body weight and feed intake measurement

Body weight of the rat was measured once a week individually during the same time and group feed intake was estimated twice a week by measuring the fed remaining in the cages after 24 hours (Cho *et al*., 2006). Then, Fed Efficiency Ratio (FER) was calculated using an equation 10:

$$\text{FER} = \frac{\text{Fed intake}}{\text{Weight gain} \times 100} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; eq.10$$

3.6.4. Blood Pressure Measurement

Arterial blood pressure was measured by indirect method using the tail cuff method. Ahead of the experiment each rat was trained for one week until the blood pressure was steadily recorded with minimal stress in the restraint holder. Blood Pressure Reading (BPR) Cuff Tubing was secured in the notch on the top rear of the holder and finally the cuff was attached to the controller (restrainer). The first measured base line blood pressure was discarded and the mean of 5 subsequent measurements were recorded. Accordingly, systolic, diastolic and mean blood pressures was measured weekly for 5 weeks by indirect tail-cuff method (Balaraman *et al.*, 2006). Systolic and mean BP was taken from the reading (graph) of the machine and diastolic BP was calculated using an equation 11:

$$DBP = \frac{1}{2}(3MBP\text{-}SBP) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textit{Eq.}11$$

Where;

MBP = Mean Blood Pressure

SBP = Systolic Blood Pressure

DBP = Diastolic blood pressure

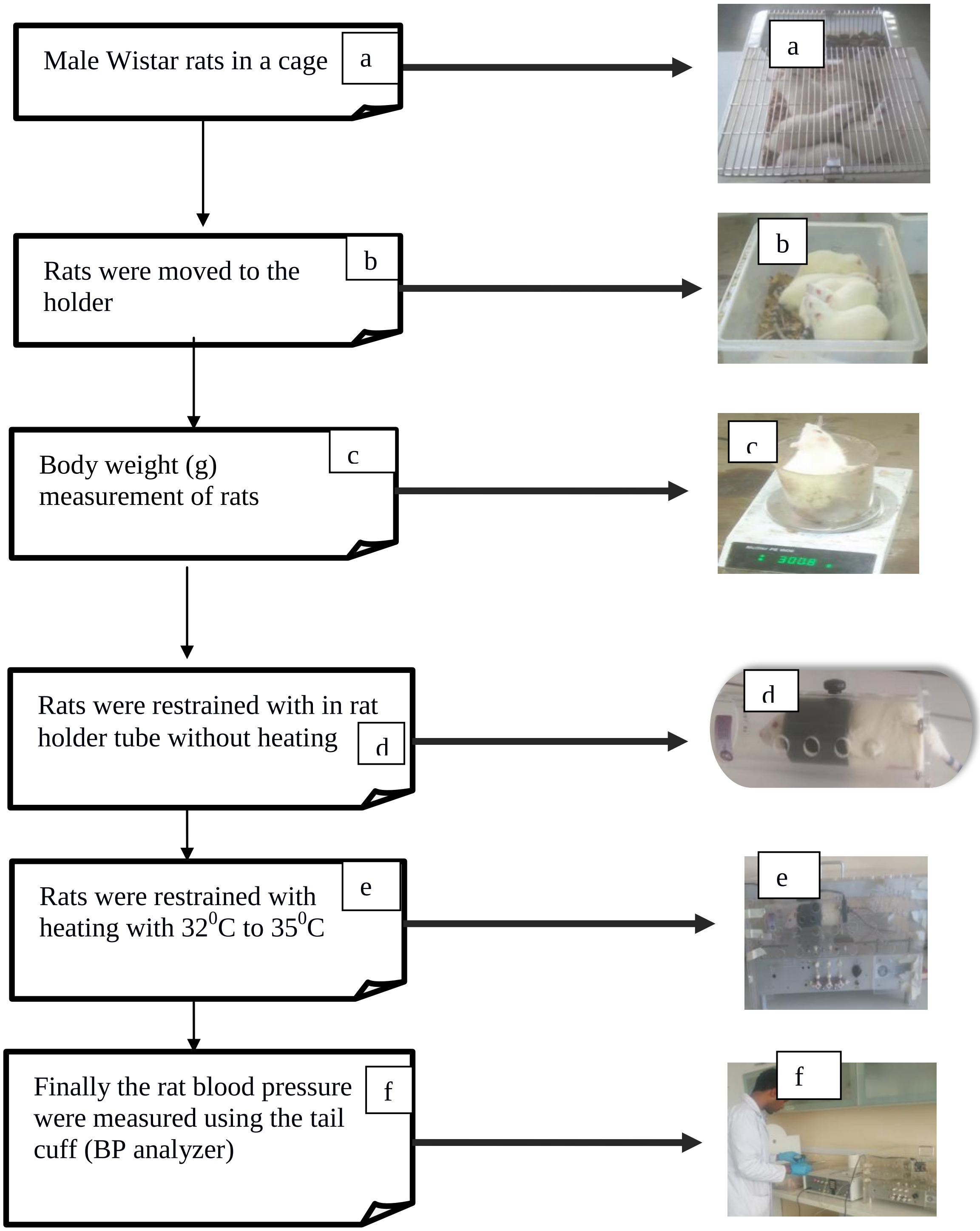


Figure 7: Procedures of antihypertensive test on Wistar rats

3.7. Sensory acceptability test of *H. schulli* salt

3.7.1. Determination of taste detection and recognition thresholds

Group taste thresholds for salt detection and recognition were determined using a modified procedure previously described by the American Society for Testing and Materials (ASTM E, 679) and by the International Organization for Standardization (ISO, 13301). Briefly, subjects received a series of 14 presentations. The first presentation was blank sample distilled water to introduce the participant to the procedure. The next 13 presentations were ascending concentrations of salt solution (0.01, 0.03, 0.05, 0.075, 0.10, 0.125, 0.150, 0.200, 0.300, 0.400, 0.500, 0.600 and 0.700 %). For each presentation, subjects were asked to take the samples sequentially from left to right and to put each sample solution in their mouth all at once and to carefully gargle the solution to ensure the whole mouth was exposed to the tested solution. Subjects rinsed their mouth with distilled water between each series of presentations. Then participants were asked to indicate which of the samples contained the taste (detection threshold) and to describe the taste (recognition threshold). If a subject perceived no difference between the three samples, they were asked to choose one (forced choice). Each cup containing the salt solution was labeled with a 3-digit numbers. Thresholds were estimated using group thresholds approach. The group best-estimated detection threshold (BET), as proposed in ASTM E679, was obtained by calculating the mean of the population.

3.7.2. Sensory acceptability test

Sensory acceptability of the taste, odor and color attributes of the *H. schulli* salt were evaluated using paired of comparison, multiple comparison and rating sensory test using seven-point hedonic scale (like extremely (7), like moderately (6), like slightly (5), neither like nor dislike (4), dislike slightly (3), dislike moderately (2) and dislike extremely (1)) tests (*Linda et al.*, 1991). The sensory acceptability was done by involving 12 for paired comparison test and 17 for descriptive and multiple comparison untrained panelists of (age between 22 and 47) who were randomly selected among staff and graduating class students of Center for Food Science and Nutrition, Addis Ababa University, Ethiopia.

3.7.2.1. Rice preparation for the sensory test

Rice preparation was done by using the method of Saavedra-Garcia *et al*., (2015). For the paired comparison test, 100% common iodized salt and 100% vegetable salt, and for multiple comparison tests, 20%, 40%, 60%, 80% and 100% potassium-enriched marsh barbell (*H. schulli*) salt and 100% sodium chloride (common table salt) as reference were used in the preparation of rice. Rice was chosen because it is the most common staple food all over the world and will not mask the taste of the salt. Briefly, rice (150 g), combined salt (5 g), and water (400 g) were combined in a metal pot and then boiled for 30 minutes. Then, the rice was cooled for 5 minutes. Finally, the rice was served in plastic plates for the panelists.

3.7.2.2. Sensory test (evaluation)

The panelists conduct the sensory tests to identify difference/similarity and the preference of sodium or potassium rich salts after eating cooked rice with the salts. Hence, rating sensory evaluation test was applied in this study. All samples were coded with a three-digit random number and participants were presented with two food samples. Each different sample for paired comparison test was presented simultaneously in a predetermined counterbalanced order. The samples were randomly presented to avoid positional bias.

Experimental samples were coded three letters: the rice prepared by 100% vegetable salt extracted from *H. schulli* coded as BAB and the rice prepared by 100% sodium chloride coded ABB for paired comparison. For rating sensory evaluation tests coded BAA (20% vegetable salt), AAB (40% vegetable salt), ABB (60% vegetable salt) and ABA (80% vegetable salt) and R (reference = 100% sodium chloride) for multiple comparisons sensory test. After orientation, two-coded samples for paired comparison and 5 coded samples including reference for multiple comparison tests were given in random order to the tasters along with the cup of water to clean their mouth between sample tasting, to avoid carry over bias. The experiment then was done with rice using common salt and vegetable salt from *H. schulli* and the sensory evaluation test were done by selected panelists.

3.8. Data analysis

The data were performed in at least triplicate, and presented as means $\pm$ SE. Statistical comparisons of the mean values were performed by Chi-square test, paired samples t - test and analysis of variance (ANOVA), followed by Duncan's multiple range test using SPSS software (version 23). Means were considered significantly different at $p < 0.05$.

Ethical clearances for animal based experiment and sensory evaluation tests were approved by the College of Natural and Computational Science Institutional Review Board (CNS-IRB) Committee.

CHAPTER FOUR

4. Results and Discussions

4.1. Percent of salt obtained from *H. schulli*, calculated from ash and raw material

Potassium rich salts accumulate in different parts of Marsh barbell (*H. schulli*). Hence, in this study, salt was extracted from the stem and leaf of the plant. The salt amount (%) was calculated from the total ash and raw material used for the preparation (Table 1). A total of 2935.47g (2.94 Kg) of fresh *H. schulli* stem was collected from Gambella region of Ethiopia. From this amount 298.589g (0.299 Kg) of ash was obtained after kindling the pyre in open air. In the same way, from the total of 1.32 Kg (1320 g) of fresh *H. schulli* leaves were yielded 363.8 g (0.364 Kg) ash. So, the average percent of ash-to-raw material weight was 0.299 Kg and 0.364 Kg from the stem and leaf of Marsh barbell (*H. schulli*), respectively. Similarly, the average ratio of salt to raw material from the stem and leaf of Marsh (*H. schulli*) was 5.00% and 0.50% respectively. Apparently, the salt yield was higher when considering the ratio from the total ash (i.e. 48.00 % and 2.23 % from the stem and leaf of the plant, respectively) (Table 1).

There are very few studies on vegetable salt extraction, composition etc, to correlate with the present study. One of these few studies from Chad reported elemental composition of vegetable salt from four common plant species maize (*Zea mays*), stems of sorghum (*Sorghum bicolori*), false trunks of pawpaw (*Carica papaya*) and stems of *Hygrophila auriculata*)). Among the four species, the highest amount of salt was extracted from *H. auriculata* (34.0% from ash and 9.5% for raw stem). Because of the high yield *H. auriculata* is the most traditionally used plant in the south of Chad to produce vegetable salt (Mianpeuren, 2012).

Also, Echeverri and Roman-Jitudutjaano, (2011) have mentioned another species of the genius, *Hygrophila spinosa* as dominant plant in Africa used to produce vegetable salt. The salt yield in the present study is lower than the value of 9.5% reported by Mianpeuren, (2012). This difference might arise from nature of the soil, plant species variation and the environment conditions etc. However, the salt yield from the stem expressed as percentage of ash (48%) is higher than the value in their study (34%). Besides, the salt yield in the present study is higher than the reports from other plants including raw fern (0.42%), raw vine (0.55%), raw willow (0.55%) and raw oak (0.15%) (Mianpeuren, 2012).

Table 1: Percent of salt obtained from stem and leaf of *H. schulli*, calculated from ash and raw material used (%).

Marsh barbel parts	Weight of raw material (g)	Weight of salt to ash	Weight of salt to raw material (%)
Stem	2935.47	48.90	5.00
Leaf	1320	2.31	0.64

4.2. Quality factors of the salt

Quality factors including matter insoluble in water, moisture content and pH of vegetable salt from *H. schulli* was determined and results are presented in Table 2 and are compared with the values in common salt and set standards (Ethiopian/Codex).

Table 2: Matter insoluble in water, moisture content and pH of the salt

Salt	Matter Insoluble in water (%)	Moisture content (%)	PH
Cs	0.29 ± 0.004*	0.28 ± 0.01*	7.13 ± 0.12*
Vs	0.13 ± 0.01	1.77 ± 0.05	9.97 ± 0.03
ES/codex	1.00	0.50-4.00	7.00-8.00

Data are expressed as mean ± SEM (n=3) and *Means in the same column are significantly different at $p<0.05$.
Cs: Common salt; Vs: Vegetable salt; ES: Ethiopian Standard

4.2.1. Moisture content

Moisture is an important factor in edible salt, which determines the stability of iodine (Usman and Filli, 2011). In the present study, the moisture contents of the vegetable and common edible salts were significantly different ($p< 0.05$), Common salt being more dried. The moisture content of the common salt is lower than the value specified in ES (Table 2). Meanwhile, for the vegetable salt the moisture content is within the standard. In fact, moisture contents of salt is highly influenced by factors including absence of anti-caking agents during re-processing steps, incomplete drying after washing, season of processing and so on. For instance, one study in Morocco showed that the moisture content of salts was 1% during the dry season, 3–4% during the moist season and 0.55 % of moisture in common salt was reported by (Zimmermann *et al*., 2004).

4.2.2. Matter insoluble in water

Similar with moisture content, water insoluble matter in common edible salt can affect the stability of iodine. Contents of insoluble matter in water between the two salt samples were significantly different ($p<0.05$). These amounts are within the allowed Ethiopian Standard. The amount is higher in common salt than in the vegetable salt (Table 2). This might be due to the exposure of common salt during production to foreign matters like sand, dust, ash etc (Usman *et al.*, 2014). For instance, salts obtained by solar evaporation in Uganda contained 2.50 % water insoluble matter. In contrast, chemically processed salt contained only 0.001% of water insoluble matter due to less exposure to foreign materials (Kirabira *et al*., 2013). Since, the production of vegetable ash is commonly in confined areas, there is a lesser chance of being contaminated with water insoluble matter.

4.2.3. pH

In this study, the pH value between vegetable and common salt was significantly different ($p<0.05$). The pH of the Vs was higher than the value both in common salt and setted standard of Ethiopia (Table 2). The pH of salt extracted from *H. schulli* is alkaline. Also, previous reports mentioned that salts produced from ash of plants are basic to strongly basic (pH ranging from 11-14). As a result, vegetable salts are mostly employed by societies like the Witoto as an alkaline reagent to liberate alkaloids from alkaloid-rich plant foods (Echeverri, 2011). In contrast, the pH of common edible salt is acidic. For example, the pH of common edible salt produced from Assale, Ethiopia was found to be 6.03 (Binega, 2006). Similarly, the pH of common non iodized and iodized salt from Afdera, Ethiopia was reported to be 6.10 and 7.20, respectively. The pH values are lower than the value in vegetable salt in the present study. Among these, only iodized common salts meet African standard specification (7-8) (AOS, 2012). Alkaline pH of salt obtained from ashing plants may due to potassium and/or sodium carbonate, hydroxides of potassium and sodium. However, the levels of hydroxides are lower than carbonates and both hydroxide and carbonate contents of K are lower than Na. this implies that alkaline salt are carbonate of K (Israel and Akpan, 2016).

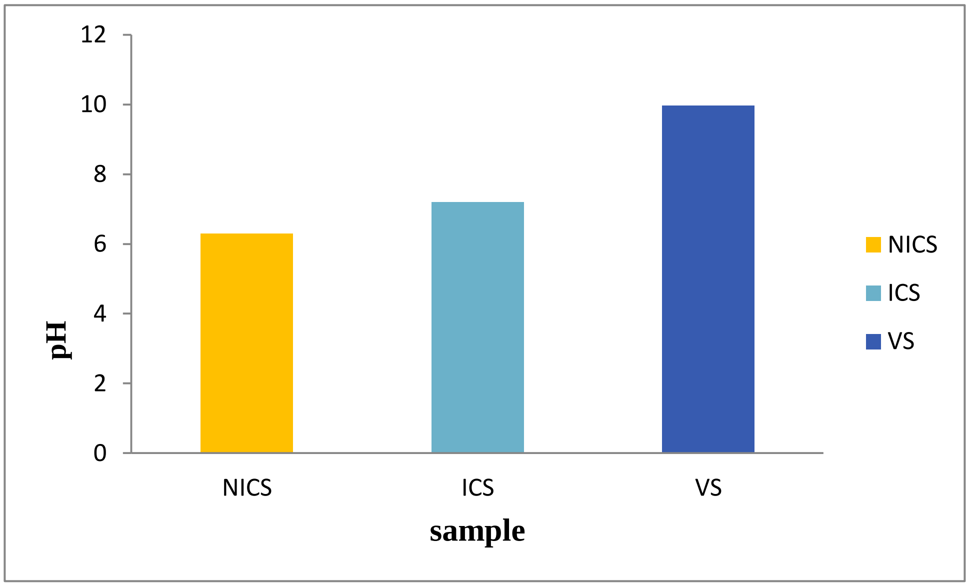


Figure 8: pH values of ICs, NICs and Vs, ICs = iodized common salt, NICs = non iodized common salt and Vs = Vegetable salt of *H. schulli.*

4.3. Anion composition of vegetable salt *H. schulli* and common salt

The predominant anions commonly analyzed from ash of plants are chloride, carbonate and sulfate. These anions are basically found in a form of potassium compound. Potassium sulfate, carbonate and chloride salts from vegetables are soluble in water, thus cause cannot health problems even at higher concentrations (Echeverri and Román, 2001). These anions are highly correlated with perceived taste of salts. Salts with a high concentration of chloride are perceived as “sweet and cool”, whereas salts with a high concentration of carbonate are perceived as “strong and hot.” The presence of sulfate combines with the sensation produced by the other two anions: it may moderate the strong flavor of carbonates into biting blunt the sweet flavor of chloride into “insipid” (produce mixed perceptions).

Table 3: Anion composition of salt extracted from *H. schulli* and common iodized salt (%)

Salts	Carbonate	Sulfate	Chloride ion
Cs	0.28 ± 0.004*	0.10±.00*	59.80±06*
Vs	3.82 ± 0.01	0.67±.012	12.43 ± 09
ES/Codex	0.20	0.50	59.00-60.00

Data are expressed as mean ± SEM (n=3) and *Means in the same column are significantly different at $p<0.05$. Cs: Common salt; VS: Vegetable salt; ES: Ethiopian Standard

In the present study, the anion composition of both common and vegetable salts was dominated by chloride ions. In fact, this is as per the set by Ethiopian standard also. Between the two salts, the vegetable salt has lower chloride content (Table 3).

4.3.1. Carbonate (alkalinity) content

Carbonates have a long history of use in food stuff as edible salt, feed and industrial processes. Carbonate ion is a principal buffer in blood and interstitial fluids in vertebrates. In this study, there was a significant difference in carbonate content between the *H. schulli* salt and common edible salt ($p<0.05$) (Table 3). The carbonate content in the common salt is within the standard. Similar amounts of carbonate (0.3%) in common salt were reported in Uganda (Kirabira *et at*., 2013). Meanwhile the vegetable ash contained higher amount of carbonate compared with both common salt and set standard. In commonly consumed *H. auriculata* salt in Chad, the content of carbonate ions was 213.5ppm.Similarly Echeverri. (2011) reported carbonate amount in vegetable ash collected in Witoto Amazon ranged between 0.38% to 34.07% from different plant species. The value in the present study lies in this range. The higher amounts of carbonate in the vegetable ash make the pH alkaline. As a result, in this study, the pH of the *H. schulli* salt was higher than in the common salt (Table 2).

4.3.2. Sulphate content

Normally, sulphate present in the salt as a contaminant and but does not cause any health problem (Kumar, 2001). The sulfate content of salt produced from Assale Lake is reported to be 0.09% which is below the limit set by the ES (0.5). 2.5% of sulphate in the salt was found in Uganda by (Kirabira *et al*., 2013). Excess consumption of sulphate will results in cathartic effects due to purgation of the alimentary. However, with time human appear to be able to adapt to higher sulfate concentrations. Dehydration has also been reported as a common side effect following the ingestion of large amounts of sulfate (Müller, 2005). Vegetable salt and common salt samples are significantly different from each other ($p<0.05$). The concentration of sulphate ions of common salt and ash salt of *H. schulli* are 0.1% and 0. 68%, respectively (Table 3). Sulphate content analyzed from common edible salt can fulfill specification of ES.

The sample of common salt used in this research was iodized; it is true that iodized common salts will have less concentration of sulphate because of reprocessed during salt production. Sulphate concentration of ash salt from *H. schulli* is higher than Ethiopian standard specification limit (0.5%). Reports of different research papers show that sulphate concentration of ash salt obtained from vegetables are high. For example, basic composition of Pawpaw and *Hygrophilia auriculata* salts are potassium chloride, potassium carbonate and potassium sulphate. Porters (1950) reported that salts used in central Africa from ash of some vegetables have high concentration of sulphate.

4.3.3. Chloride content

The major component of Common edible salt is sodium chloride. From this major component about 59% of the salt is chloride ion (Nafees, 2013). In this study, Chloride content of vegetable and common salt is significantly different at $p<0.05$ (Table 3). The chloride content of the common salt is within Ethiopian set standard (59-60%). However, the major components of salt from ashing of vegetables are in a form of potassium chloride, potassium sulfate and potassium carbonate. Hence, chloride content of vegetable salt is much lower than that of common edible salts. But, still within the vegetable ash chloride ion content is the highest composition (12.43%) (Table 3).

This is the fact that determines the taste of the salt in food. There is always a relationship between the concentration of sulfate, carbonate, chloride ion and the perceived taste of the salts; the “sweet” flavor of chloride being the preferred taste (Echeverri *et al*., 2013). Therefore, vegetable salt can be an alternative salt keeping the expected taste qualities of salt.

However, the Health authorities recommended limitations for intake of chloride ion from salt are 3400 mg/day (Nafees *et al*., 2013).

4.4. **Macro mineral composition**

The macro minerals (Na, K, Ca and Mg) results are presented (Table 4).

4.4.1. Sodium

There was a significant difference in sodium content between the common and vegetable salt of *H. schulli* ($p<0.05$). Significantly, higher amount of sodium was found in the common salt. Similarly, from other related research 37- 40 % of sodium ion was analyzed in common salt and 0.005% to 0.63% sodium ion was analyzed from salt of different plant species in USA.

Common salt is a crucial food ingredient with several important functions: source of minerals, taste-enhancing and modifying ingredient; osmotic preservative for meat and some other food products and so on (Israel, 2016). Despite these important functions, health authorities including WHO have recommended limitations for salt intake, which is about 5-6g/day. Along with this total amount the recommended sodium and chloride in take per day is 2400 mg and 3400 mg, respectively. For people with high blood pressure even a lower sodium intake is recommended daily (i.e. 1500mg).

However, in the present world, modern people all over the world consume too much salt with their regular diet exceeding the recommended level by WHO. For instance, in selected European countries, the average salt intake of women is 7.3–10.0 g/day and that of men is 9.4–13.3g/day. Similarly, epidemic studies showed that dietary sodium in a form of sodium chloride intake in most countries in the world is about 9 to 12 g/day, which is equivalent to 3.6 to 4.8 g/d of sodium (Rust, 2016).

High Dietary Sodium Intake (DSI) via common salt consumption is generally associated with hypertension and increased risk of cardiovascular diseases (CVD). Hence, it is a common health challenge worldwide. Thus, a dietary pattern which is low in sodium and high in potassium has been recommended for blood pressure lowering, and is well supported by numerous randomized controlled trials. The WHO 2013–2020 Global Action Plan for the Prevention and Control on Non-communicable Diseases (NCDs) identifies nine voluntary global targets including a 25% reduction in risk of cardiovascular disease and raised blood pressure, and a 30% reduction in mean population salt intake by 2025 (WHO, 2014). To attain these objectives there are different strategies to reduce salt consumption within the allowed standard. Example, government policies, consumer awareness and monitoring of population salt intake.

Usage of edible salt is inevitable because of the mentioned reasons, specifically due to the required taste by the consumers. Therefore, rather than avoiding salt to prevent non-communicable diseases, it is better to find alternative healthier salts. Salts extracted from plants are as such an item, in which this study focused on. As reported in Table 4, the vegetable salt has lower sodium content than the common salt in this study. Mainpeurem *et al*., (2012) also reported a lesser amount of sodium ion in a salt sample extracted from different plant species. Sodium amount reported in *Hygrophilia auriculata* was 6mg/L, the value in the present study was 7000mg/L. In both studies the sodium amount is lower than the potassium as compared to the value in common salt. But, the sodium value in the present study is much higher than in the previous report. May be due to the nature of soil type, sodium is found in many minerals when they break down over time, from concentrated ruff of pesticides, fertilizers and fossils of the soil (Blumwald, 2000).

For instance, there is variation in sodium and other mineral contents in vegetable salts based on the species type, ecology etc. With this regard Idris (2015) reported a higher amount of sodium (20.12%) in a salt sample produced from *Achyranthes aspera* through lixiviation. In a Dietary Approach to Reduce Hypertension (DARH), sodium to potassium ratio less than one is very important.

Park *et al*., (2016) reported that Na:K ratio had a significant effect on hypertension prevalence and blood pressure. The mechanisms by which sodium and potassium affect blood pressure are multiple. In salt-sensitive individuals, salt ingestion causes sodium and water retention and extracellular volume expansion, which results in the release of substances increasing heart and blood vessel contraction and affecting renin-angiotensin-aldosterone system. Potassium increases urinary sodium excretion which diminishes body sodium.

In addition, potassium is thought to induce vascular smooth muscle relaxation and thus decrease peripheral resistance. Na:K ratio had a stronger effect on the risk of cardiovascular disease than sodium or potassium alone. Therefore, people should be more concerned about the Na:K ratio in their diet than the level of sodium. Accordingly in the present study, the amount of sodium is significantly ($P<0.05$) lower in the vegetable salt than in the common salt. Also, the Na:K ratio for the vegetable and common salt was 0.05 and 164 respectively (Table 4). A Na: K intake ratio of less than one has been recommended for optimal health, with higher ratios associated with elevated blood pressure and increased risk of cardiovascular disease (Rachael *et al*., 2015). Therefore, the vegetable ash from *H. schulli* was demonstrated to be a healthier alternative to the common salt.

4.4.2. Potassium

Potassium is an essential element for all living organisms. Animals need large quantities of K^+ (i.e. 3 to 4 gm of KCl is necessary for a daily diet of 2000 calories). Potassium is needed for maintenance of total body fluid volume, acid and electrolyte balance, and normal cell function. Average potassium consumption in many countries is below 70-80 mmol/day (Elliott *et al.*, 1996). In general, lower potassium consumption has been associated with elevated blood pressure, hypertension, and stroke and higher levels of consumption could be protective against these conditions. Public health interventions aimed at increasing potassium intake from food are potential cost effective measures for reducing the burden of morbidity and mortality from non-communicable diseases (Dyer *et al.*, 1994). Increased potassium intake lowers blood pressure in adults with and without hypertension (Cappuccio *et al.*, 1991). A recent meta-analysis of cohort studies reported an inverse association between potassium intakes (Whelton *et al.*, 1998).

The value recommended by the 2002 Joint World Health Organization (WHO)/Food and Agriculture Organization Expert Consultation is 90 mmol/day (Aburto *et al.*, 2013). In the current study, potassium concentration in the vegetable salt was significantly higher than in the common salt ($p<0.05$) (Table 4). But the K concentration in this study was much higher than previous reports. For instance, Mianpeurem *et al*., (2012) reported 47.5 mg/L K concentration in *H. auriculata* salt. According to Weaver (2013), a key parameter is actually the relative ratio of Na:K, than the individual elements amount. Thus, a Na: K intake ratio of less than one has been recommended for optimal health (Rachael *et al*., 2015). Therefore, a prolonged dietary imbalance with high DSI, where in the Na:K is much higher than 1.2, would lead to increased risk of hypertension and related CVD. In this study, the Na:K was 0.05 indicating that the salt produced from *H. schulli* has a significant role to reduce hypertension and other related diseases.

4.4.3. Calcium

Calcium concentration in the salt samples were significantly different at $p<0.05$ (Table 4), but the difference was not large as in the case between Na and K. According to WHO standard the level of calcium should not exceed the value 5g/kg in table salt. Both the common and vegetable salt in this study are within this limit. Also, similar calcium concentration was reported from vegetable salt value from Witoto, Amazon (0.39%) But a much lower calcium concentration (20mg/L) was reported by from another finding (Mianpeurem *et al.,* (2012).

4.4.4. Magnesium

In this study, both common and vegetable salts had a magnesium concentration which is within the set by Ethiopian Standard for edible salt (0.5%). Compared with the report by Mianpeurem *et al*., (2012), the magnesium content in the present study was higher, which is 0.15% for both common salt and salt *H. schulli* (Table 4). Similarly, magnesium concentration in the other research had valued 0.3% from common salt and 0.62% from salt of plant ash (Filli, 2013).

Table 4: Macro mineral composition of salt extracted from *H. schulli* and common iodized salt (%)

Salt type	Na	K	Ca	Mg
Cs	39.37 ± 0.09*	0.24 ± 0.00*	0.34 ± 0.00*	0.15 ± 0.00
Vs	0.71 ± 0.01	13.40 ± 0.00	0.30 ± 0.00	0.15 ± 0.00
ES/codex	NS	NS	0.5	0.5

Data are expressed as mean ± SEM (n=3), *Means in the same column are significantly different at p<0.05. Cs: Common salt; VS: Vegetable salt; ES: Ethiopian Standard. "NS"No Standard

4.5. Micro mineral composition

Micro minerals can involve in several biological processes, such as component of metallo-enzymes and enzyme co-factors. They function as activator of enzymes involved in intracellular detoxification mechanism of free radicals and in stabilization of secondary molecules (Kumar *et al.*, 2011).

4.5.1. Iron

As reported in Table 5, there is no significant difference in iron content between the common and vegetable salt (p>0.05). In common salt iron concentration is found between the ranges of 12-273 mg/kg across different studies ((Nafees *et al.*, 2013). In vegetable salt produced from *H. auriculata*, 31ppm iron concentration was reported (Mianpeurem *et al*., 2012). This is a comparable amount of iron with the current study. Iron deficiency due to inadequate diet is a global problem in which the effects of iron-lack anemia on work efficiency have a large economic impact. Perhaps even more profound is the diminution of the rate of normal cognitive development in iron-deficient children (Hemalatha, 2007). Iron has a vital role in lipid metabolism (Cunnane, 1987), part of hemoglobin that transports oxygen through human body etc (Chung *et al*., 2011). Therefore, ash salt extracted from *H. schulli* can be used as an alternative edible salt with reduced health risks being a good source of micro elements like iron.

4.5.2. Zinc

The zinc concentration in the *H. schulli* salt is significantly higher than in common salt ($p<0.05$) (Table 5). From previous reports, zinc concentration in edible salt is between the ranges of 4ppm to 9ppm (Nafees, 2013). This is a lower concentration compared with the value in the common salt in this study. Mianpeurem *et al*., (2012) reported a value of 0.007ppm maximum concentration of zinc in salt sample extracted from *Zea mays*, which is a very low concentration as compared to the value (52.6ppm) in this study. In contrast to the present study, the zinc concentration in *H. auriculata* salt was below detection in the same report. A high concentration of zinc (500ppm) was reported from vegetable salt of *Bactris humilis*, also known as women plant in witoto. This salt is required for pregnant and lactating women (Merialdi *et al*., 1999) and is important in the production of insulin, in which the fetus requires from the 4th month of gestation onwards (Echeverri, 2011). Similarly, in Gambella, Ethiopia, salt extracted from *H. schulli* is used as traditional medicine for women traditionally based on the society.

Zinc deficiency may lead to loss of appetite, decreases sense of taste and smell, slow wound healing and skin sores and in extreme shortage, it causes birth defects. The recommended daily intake for zinc is 15mg/day (Oti-Boakye, 2011). Deficiency of zinc is a major public problem in developing countries (Prasad, 2003). Cereals and legumes are the primary sources of zinc in developing countries legumes (Hemalatha *et al*., 2007). These grains are full of plant anti- nutrient materials, which render the bioavailability of micronutrients. Therefore, this vegetable salt produced from *H. schulli* can be a potential alternative to common salt being a good source of zinc besides the mentioned health benefits.

4.5.3. Iodine

Iodine was determined from three samples (i.e., from non-iodized common salt, iodized common salt and vegetables salt from *H. schulli*). Concentration of iodine from none iodized common salt was not detected, from iodized common salt was 69 ± 0.367 and from vegetable salt of *H. schulli* salt was 12±0.5552ppm (Table 5). Similarly, 40 to 72ppm iodine was detected from iodized common salt (Vithanage, 2016). The source of iodine may come from the soil and the river, Iodine in rivers has two significant sources: oceanic cyclic and iodine weathered from soils and rocks (Moran *et al*., 2002).

Iodine is naturally found in seaweeds, sponges and other related materials. It is assimilated by seaweeds and sponges and is found in Chilean saltpeter, caliches, and brine associated with salt deposits. Iodine is essential element for the production of thyroid hormone but the body does not make iodine. Unless supplemented from food iodine deficiency can lead to enlargement of the thyroid hypothyroidism and to mental retardation in infants and children whose mothers were iodine deficient during pregnancy (Becker, 2006).

Salt is the best vehicle chosen to mitigate this problem. Thus, Universal Salt Iodization (USI) is the recommended intervention for preventing and correcting iodine deficiency. USI is based on the assumption of average salt consumption of 5-10g/day. But consumers being concerned with chronic diseases are reducing salt from their meals. This will ultimately affect the expected daily iodine intake, especially for the case of pregnant women. Therefore, to keep up with USI to increase iodine coverage, searching for alternative healthier edible salt is important. In this study, 12ppm iodine was found in the salt extracted from *H. schulli*. Hence, besides being an alternative vehicle to USI, this salt can reduce the cost of iodization due to the inherent iodine content. The other microelements quantified from the salt samples were cobalt, copper and manganese. There is no significant difference in the cobalt concentration between the two salt samples ($p>0.05$). Copper concentration was below the detection limit (BDL) of the ICP-OES value of 0.01 mg/Kg (Table 5). Similarly, copper concentration in the salt from *H. auriculata* was BDL of the instrument used in the report by Mianpeurem *et al*. (2012). In fact, copper concentration in other vegetable salts was also reported BDL (Israel, 2016). Manganese is an essential metal for human health having a big role to maintain function of central nervous system, bone growth, reproduction and metabolizes cholesterol, carbohydrates (Karanja *et al*., 2010). In this study, the manganese concentration of the *H. schulli* and common salt were 3.03 and 1.70ppm, respectively ($p<0.05$) (Table 5).

Table 5: Micro mineral composition of salt extracted from *H. schulli* and common iodized salt (ppm)

Essential minerals	Salt code		
	Cs	Vs	ES/codex
Co	0.08 ± 0.00	0.08 ± 0.00	___
Cu	< 0.01	<0.01	2
Fe	34.81 ± 0.01	34.61 ± 0.00*	50
Mn	3.03 ± 0.03	1.70 ± 0.00*	___
Zn	33.30 ± 0.00	52.60 ± 0.00*	___
I_2	ND	12.00 ± 0.56*	___

Data are expressed as mean ± SEM (n=3) and *Means in the same row are significantly different at p<0.05. Cs: Common salt; VS: Vegetable salt; ES: Ethiopian Standard.

4.6. Toxic metal composition of salt

Heavy metals are inorganic elements that are non-biodegradable and cannot be metabolized by the body: thus accumulated in the body through time. These metals include Pb, Hg, Cr, As, Cd (Annadurai, 2013) and the adverse effects of exposure to heavy metals include carcinogenic, neurotoxic, nephrotoxic and fetotoxic (Jahed *et al*., 2005). Unfortunately foods and food containers is one of the major routes of heavy metal contamination in general population. Thus, it is important to determine the heavy metal concentrations in daily consumed food ingredients like salt. In Ethiopia, the major edible salt production is in Afar region, Afdera. Some reports indicated that this area is frequently under volcanic eruption, so as the assumption of harmful metals contamination in edible salt in Ethiopia. The results of Table 6 showed the toxic metals found in *H. schulli* extracted salt and common iodized salt.

4.6.1. Cadmium (Cd)

The concentration of cadmium in the common edible salt and *H. schulli* salt was not detected by ICP-OES, which was less the value 0.04mg/Kg). International Agency for Research on Cancer (IARC) has reported cadmium as a carcinogenic agent which is a major cause of kidney dysfunction (WHO, 1989). Cadmium also is tremendously toxic metal which can cause lung cancer; produce bone defects (Gopalani *et al*., 2007) and adverse pulmonary effects like emphysema, bronchiolitis and alveolitis in humans (Yang *et al*., 2012). Thus, the food sanitary standard of Cd in edible salt concentration value 2 mg/kg by Ethiopian standard, in which the concentration in the present study is below the detection limit in his research (Table 6).

4.6.2. Chromium

Similarly chromium concentration both in the *H. schulli* and common salt was below detection limit, which was less than the value of 0.0075 mg/kg (Table 6). Mainpeurem *et al*., (2012) reported a 0.007ppm chromium concentration in salt extracted from *H. auriculata*. Chromium in hexavalent form is toxic metal (Seema *et al.,* 1997), which induces mutagenic and carcinogenic effects in humans (Goyer *et al*., 2001). The permissible limit of chromium in common edible salt according to Ethiopian Standard is 2ppm, which is a higher value than in this study value (below the detection limit).

4.6.3. Lead

Lead is a very toxic metal for human which enter to the body via ingestion ((Yousefi *et al*., 2016). Lead is highly toxic for plants, animals and microorganisms and the main sources of lead pollution in agriculture and plants are lead mines, fuel combustion, sewage sludge and volcanic eruption etc. Long-term exposure to lead can result anemia, pale skin, a decrease handgrip strength, abdominal pain, nausea, vomiting, paralysis of the wrist joint, kidney damage, decreased fertility and/or increased chance of miscarriage or birth defects (Ciobanu *et al*., 2012). In this study, the lead concentration in the common and *H. schulli* salt was 0.61 and 2.27ppm, respectively (Table 6) ($p<0.05$). A lower lead concentration in *H. auriculata* salt value of 0.036ppm was reported by Mianpeurem *et al*., (2012).The maximum permitted level of lead in food grade salt is 2.0 ppm according to the Codex legislation (Codex, 2010). The concentration in the *H. schulli* salt was higher than this value by 0.27ppm. Due to this concern, Provisional Tolerable Weekly Intake (PTWI) was calculated to check the safety of *H. schulli* salt from lead contamination.

The PTWI of lead is 25 mg/kg body weight (WHO, 2004). With the assumption that a 60 kg adult will at average consumes 10g salt per day, the weekly lead intake for one person through common edible and *H. schulli* salts is 0.0007 and 0.0026 mg/kg body weight, respectively. These values are 0.0028 and 0.0104% of the set PTWI value by WHO/FAO, respectively. Thus, the lead concentration in the *H. schulli* salt is safe in accordance with the assumption of PTWI.

4.6.4. Mercury and arsenic (Hg and As)

The concentration of mercury in common edible salt and ash salt from *H. schulli* were below the detection limit (< 0.03 mg/kg) (Table 6). Similarly, Mianpeurem *et al.* (2012) mercury concentration was below detection limit in the salt extracted from *H. auriculata*. The allowed mercury concentration in edible salt according to codex and Ethiopian Standard is 0.1 mg/kg. Both salt samples are below this limit. The concentration of arsenic in common edible salt and ash salt from *H. schulli* was below detection limit (< 0.20 mg/kg). The edible salt allowed arsenic amount according to ES is 0.5 mg/kg, in which both salts in this study was not detected.

4.6.5. Nickel

As reported in Table 6, the concentration of nickel in vegetable salt of *H. schulli* was below detection limit (< 0.015 mg/kg) but the concentration of nickel in common salt was 4.46mg/Kg. The permissible limit for nickel in edible salt is 0.16 mg/day (Nafees *et al*., 2013). Nickel is toxic element and is present in air, water and soil in traces (Brown *et al*., 1987). The sources which introduce nickel into the environment are metallurgy and refining industries, coal combustion, diesel and fuel oil and sewage (Cempel, 2006). Humans are exposed to nickel from food, jewellery, coins, and dental restorations (Vahter *et al*., 2007). Nickel is carcinogenic with many adverse health effects on respiratory tract causing lung cancer, lung inflammation (NTP, 1996), nose cancer, larynx cancer, respiratory-failure, birth defects, asthma, allergic reactions such as skin rashes.

The PTWI of nickel is 35 mg/kg body weight (Gulson *et al*., 1995).With Assumption 60 kg adult and consumption of 10g salt per day, the weekly nickel intake for one person through common salt in this study is 0.0052mg/Kg, Which is less than PTWI set by WHO/FAO. Therefore the salt is safe to consume. The concentration of nickel for common salt is higher than the concentration of nickel in common edible salt.

Table 6: Toxic metal composition of salt extracted from *H. schulli* and common iodized salt (ppm)

Salt code	Cd	Cr	Pb	Hg	As	Ni
Cs	< 0.004	< 0.0075	0.61 ± 0.00*	< 0.03	< 0.20	4.46 ± 0.00*
Vs	< 0.004	< 0.0075	2.27± 0.00	< 0.03	< 0.20	<0.015
ES/codex	0.50	2.00	2.00	0.10	0.50	____

Data are expressed as mean ± SEM (n=3) and *Means in the same column are significantly different at p<0.05. Cs: Common salt; VS: Vegetable salt; ES: Ethiopian Standard.

4.7. The sensory quality of salt extracted from *H. schulli* plant

4.7.1. Taste detection and recognition threshold

Prior to the sensory test, *H. schulli* salt detection and recognition threshold in distilled water were determined by 10 participants. Briefly, subjects received a series of 14 presentations. The first presentation was blank sample (water without salt) to introduce the participant to the procedure. The next 13 presentations were ascending concentrations of salt solution (0.01, 0.03, 0.05, 0.075, 0.10, 0.125, 0.150, 0.200, 0.300, 0.400, 0.500, 0.600 and 0.700 percent). For each presentation, subjects were asked to take the samples sequentially from left to right and to put each sample solution in their mouth all at once and to carefully gargle the solution to ensure the whole mouth was exposed to the tested solution. Then participants were asked to indicate which of the samples contained the taste (detection threshold) and to describe the taste (recognition threshold). If a subject perceived no difference between the three samples, they were asked to choose one (forced choice). Each cup containing the salt solution was labeled with a 3-digit numbers. Thresholds were estimated using group best-estimated approach. The group best-estimated detection threshold (BET), as proposed in ASTM E679, was obtained by calculating the geometric mean of the participants last missed concentration and the next higher concentration detected, assuming that the first all subsequent concentration correctly answered.

Table 7: Detection and recognition threshold of salt extracted *from H. schulli* (%)

Panelist	Detection threshold	Recognition threshold
1	0.100	0.150
2	0.075	0.125
3	0.050	0.125
4	0.050	0.150
5	0.075	0.100
6	0.050	0.150
7	0.030	0.10
8	0.050	0.125
9	0.075	0.100
10	0.050	0.125

The mean detection and recognition thresholds of *H. schulli* salt in distilled water for 10 participants of (age range from 22 to 44 females) were 0.06 ± 0.007 and 0.10 ± 0.009 %, respectively. The mean value of detection and recognition thresholds of sodium chloride in distilled water for the population of 221 (age range 19 to 56) were 0.031% and 0.09%, respectively (de Loryn *et al*., 2014). The recognition threshold, were the salty taste quality become apparent, was higher and similar to the value of 0.05% (Hatae *et al.*, 2009).

Detection and recognition threshold values of sodium chloride reported in previous studies and for the *H. schulli* salt in the present study are almost the same. Thus, the same concentration was used to prepare the food for sensory analysis in this study.

4.7.2. Paired comparison difference and preference tests

The result showed, rice cooked with 100% *H. schulli* salt had distinguishable taste from 100% common salt cooked rice. All the 12 panelists indicated taste difference between the two samples. Among the panelists, 83% preferred the rice prepared with 100% common salt and only 16.7% of the participants (only 2) preferred the rice prepared with 100% *H. schulli* salt. This is might be due to the characteristic metallic and bitter taste of *H. schulli* salt as a result of its major composition KCl (Sinopoli, and Lawless, 2012). Besides, the participants were not vegetable salt consumers, thus were not familiar with the taste of the vegetable salt. Therefore, for future successful use of vegetable salt (potassium rich salt), addition of some effective taste-improving agents (TIAs) have to be introduced to overcome its sensory drawbacks (Saavedra-Garcia *et al.*, 2015).

4.7.3. Rating sensory evaluation of vegetable salt extracted from *H. schulli* and common salt

Descriptive sensory attributes of the two salts were evaluated by 17 participants. These sensory attributes were appearance, taste, color, odor and overall acceptability (Table 8). Taste is the important sensory attribute in edible salt and specifically is the major concern in vegetable salt due to its composition. In this study, there was no significant difference ($p>0.05$) in overall appearance, color and odor between the two salt types (Table 8). In contrast, there was a significant perceived taste difference between the two samples, in which the rice cooked with common salt gave better taste.

There is a correlation between the concentration of chloride, carbonate and sulfate ions and perceived taste of the salts. For instance, Salts with a high concentration of chloride, and low concentration of carbonate and sulphate are perceived as sweet and cool. Salts with a high concentration of carbonate and, low concentration of chloride and carbonate are perceived as strong and hot or biting. High concentration of sulphate perceived as sweet or insipid (Echeverri, 20011). Carbonate concentration of *H. schulli* salt in this study is high next to chloride ion. May due to this reason, the taste perception was less accepted by the participants. However, it is possible to enhance the taste quality of the *H. schulli* salt through the addition of improving agents. Some of these agents that can be used to improve or modify the salt taste include umami ingredients, spices, vegetables and other flavoring agents taste Cepanec *et al*., 2017).

Table 8: Rating sensory test results on the *H. schulli* and common salt

Sample code	Overall Appearance	Color	Taste	Odor	overall acceptance
Cs	6.75 ± 0.17	6.75 ± 0.179	5.92 ± 0.23*	5.66± 0.497	5.58 ± 0.416*
Vs	6.74 ± 0.41	6.73 ± 414	4.08 ± 0.58	5.50 ± 0.261	5.31± 0.312

Data are expressed as mean ± SEM (n=3) and *Means in the same column are significantly different at $p<0.05$. Cs: Common salt; VS: Vegetable salt.

4.7.4. Multiple comparison sensory test of different proportion of *H. schulli* salt with common salt

For the multiple comparison test 20%, 40%, 60%, 80% and 100% potassium-enriched *H. schulli* salt and 100% sodium chloride (common salt) as reference were used in the preparation of rice. Rice was choosen because it is the most common staple food all over the world and will not mask the salt taste (Saavedra-Garcia *et al*., 2015).

Table 9: Sensory preference test between *H. schulli* and common salt (%)

Replacement Level	Total Participants	Comparable to R	Different to R	Better than R	Inferior to R
20% Vs	17	82%	17%	17%	0%
40% Vs	17	35%	65%	53%	12%
60% Vs	17	17%	82%	59%	23%
80% Vs	17	17%	82%	47%	35%

Vs = Vegetable salt from *H. schulli,* R = Reference (common salt) which was partially substituted with vegetable salt (20, 40, 60, 80%)

The result in Table 9 showed that the substitution rate of common salt with Vs increased, the acceptability compared with R decreased (i.e. from 82% to 17%). Interestingly, the 40% Vs cooked rice had a 35% comparable taste acceptance with R cooked rice. Furthermore, the % of panelists detecting a difference as the Vs incorporation increased (i.e. from 17% to 82%).

With 40 and 60% Vs replacement of common salt, 53 and 59% of the panelists perceived a better taste than 100% R cooked rice. These data indicated that even without additional taste improvers, with blending the Vs with Cs an acceptable taste can be induced for the consumer. This blending will also provide a reduced/recommended Na/K for the consumers. For instance, with the 40% Vs, the Na/K ratio would be 4.34 as compared with the value in Cs 164. Therefore, with the right combination of Vs with Cs, an alternative healthier salt product maintaining the expected sensory taste can be produced. The correct replacement level of sodium chloride with potassium rich salt can improve the taste (Cepanec *et al*., 2017).

4.8. Anti-hypertensive test

4.8.1. Effects of 4% salt extracted from *H. schulli* and 4% common salt on systolic, mean and diastolic blood pressure of Wistar rats

Four-weeks-old male Wistar rats (weight between 200g to 270g) were purchased from Ethiopian Public Health Institute (EPHI). The rats were housed at 22 ± 3°C room temperature. The feed were supplied to rats for 5 weeks after two weeks of adaptation period. The rats were randomly assigned to 3 groups: normal diet fed group (control) (group 1, n = 6), 4% *H. schulli* salt containing diet fed group (group 2, n = 6) and 4% common salt containing diet fed group (group 3, n = 6). The basic composition of the rat's diet is protein 14%, carbohydrate 48%, fat 4%, crude energy 16.5 KJ/g and digestible energy 12.1 KJ/g (Oliva *et al*., 2017).

a) Body weight and Feed Efficiency Ratio (FER)

Table 10: showed the Body weight gain, fed intake and Fed Efficiency Ratio obtained from normal control, 4% common salt and 5% vegetable salt fed rats for 5 weeks

Fed group	N	Body weight gain (g)	Fed intake (g)	FER
Normal fed	6	80.33 ± 7.80	950.85 ± 2.50^{b}	11.83 ± 1.21^{b}
4% Vs	6	83.83 ± 2.18	922.07 ± 2.45^{c}	11.07 ± 0.90^{a}
4% Cs	6	67.83 ± 9.21	964.27 ± 1.05^{a}	14.22 ± 0.60^{c}

Data are expressed as mean ± SE. Mean values in the same column with different superscripts are significantly different at $p<0.05$. Vs: Vegetable salt; Cs: Common FER = Fed Efficiency Ratio

Rats fed with 4% Vs containing diet relatively gained more weight than the other fed groups. But the fed intake in this group was significantly the lowest compared with the other two feed groups (Table 10). This might be the less palatable nature of the Vs (metallic taste), which was observed in the sensory tests in this study. Similarly, the FER of 4% Vs diet fed mice was the lowest. Low FER means that the diet is more performed on the growth of rat (Adeyemi *et al*., 2015).

b) Blood pressure measurement

SBP and MBP were measured on the 1^{st} week of the experiment before fed with 4% common salt and vegetable salt containing diets and this blood pressure was recorded as BP0. Accordingly, SBP0 ≤ 192mmHg, MBP0 ≤ 150mmHg and DBP0 ≤ 132mmHg were reported in the 1^{st} week. Then, BP was measured every week for 5 weeks using BP analyzer. The SBP and MBP were read directly from the pulse tracing, DBP was calculated as follows:

$$DBP = \frac{1}{2}(3MBP - SBP)$$

As reported in Table 11, SBP, MBP and DBP of the rats fed with 4% *H. schulli* salt decreased significantly ($p<0.05$) as compared with the normal diet fed rats for 4 weeks. In contrast, in the 4% common salt incorporated diet fed rats the SBP and DBP increased compared with the other groups ($p<0.05$). The potassium to sodium ratio was higher in the *H. schulli* salt compared with common salt in this study. Hence, the reduced sodium and increased potassium intake might contribute for the significant systolic and diastolic blood pressure reduction in the rats fed with Vs incorporated diet (Ravi *et al*, 2016; Geleijnse *et al*., 2003).

Table 11: Average 5 weeks Systolic, Mean and Diastolic Blood Pressure of Wistar rats fed with a diet containing 4% salt extracted from *H. schulli* and 4% common salt

Group	N	SBP (mmHg)	MBP (mmHg)	DBP (mmHg)
Normal control	6	195.6 ± 0.88^{b}	146.4 ± 0.50^{b}	121.5 ± 0.66^{b}
4% Vs	6	189.5 ± 1.12^{c}	137.7 ± 1.87^{c}	111.3 ± 1.40^{c}
4% Cs	6	205.8 ± 0.99^{a}	153.5 ± 1.07^{a}	127.0 ± 1.32^{a}

Data are expressed as mean ± SEM (n = 6), Mean values in the same column with different superscripts are significantly different at $p < 0.05$.Vs = Vegetable salt, Cs = Common salt, SBP = Systolic Blood Pressure, MBP = Mean Blood Pressure and DBP = Diastolic Blood Pressure.

On the weekly basis BP measurement, the SBP was always in the order of 4% Cs > normal diet > 4% Vs fed rats between week 1 and 5. This is again related with the high potassium to sodium ratio in the *H. schulli* salt compared with common salt. In all the diet groups the SBP of the rats decreased until week 4, then again the BP raised up in the 5th week.

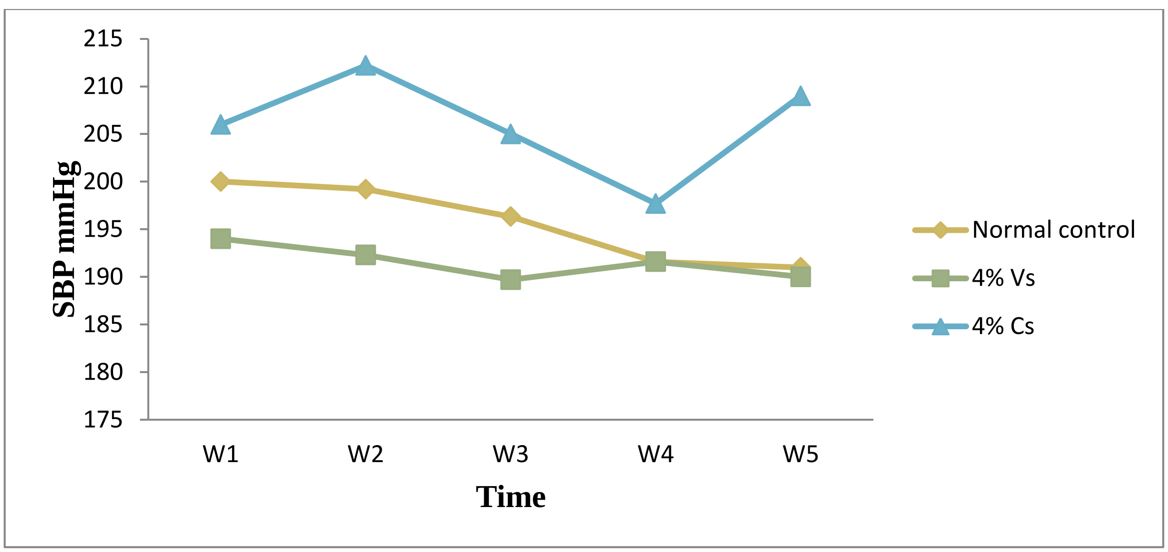


Figure 9: Weekly Systolic Blood Pressure records of rats fed with 4% Vs, 4% Cs, and normal diets. Vs: Vegetable salt from *H. schulli*, Cs: Common salt, SBP: Systolic blood pressure, Hg: mercury

As shown in figure10, the MBP was always in the order of 4% Cs > normal diet > 4% Vs fed rats between week 1 and 5. This is again related with the high potassium to sodium ratio in the *H. schulli* salt compared with common salt.

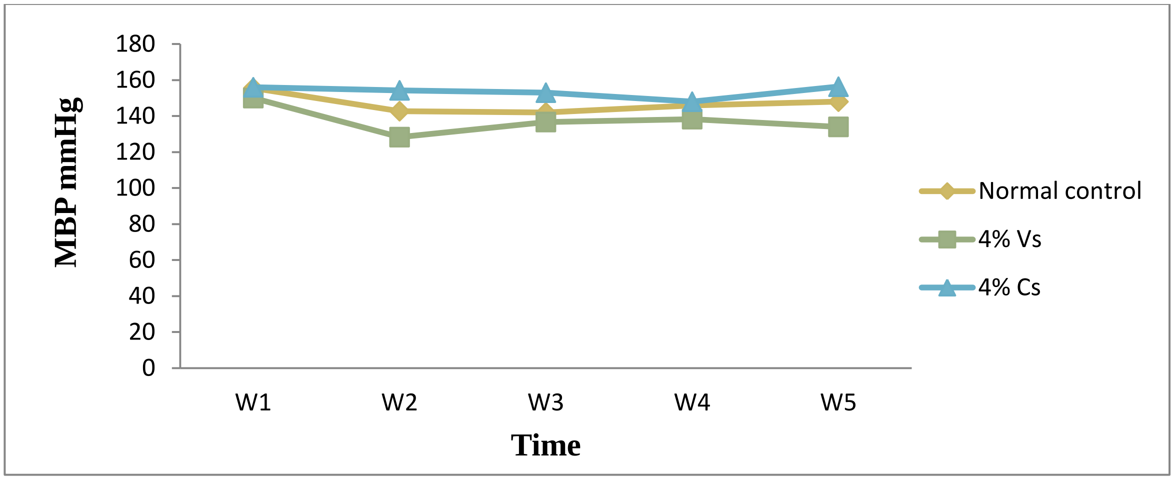


Figure 10: Weekly Mean Blood Pressure records of rats fed with 4% Vs, 4% Cs, and normal diets. Vs: Vegetable salt from *H. schulli*, Cs: Common salt, MBP: Mean blood pressure

As shown in figure11, the DBP was always in the order of 4% Cs > normal diet > 4% Vs fed rats between week 1 and 5. This is again related with the high potassium to sodium ratio in the *H. schulli* salt compared with common salt.

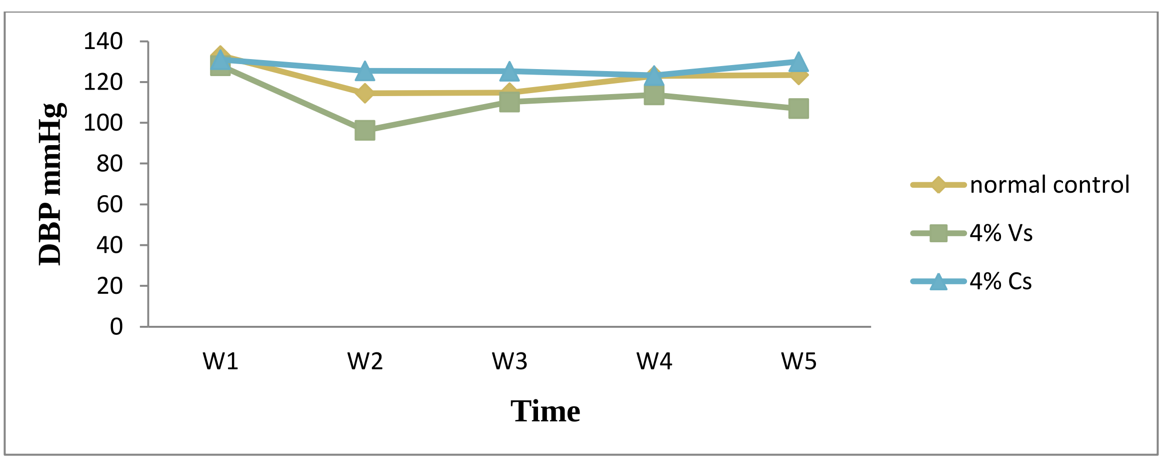


Figure 11: Weekly Diastolic Blood Pressure records of rats feed with 4% Vs, 4% Cs, and normal diets. Vs: Vegetable salt from H. schulli, Cs: Common salt, DBP: Diastolic BP

SBP and DBP in this finding agrees with the result recorded by tail cuff from normal control fed group by Bugag (1973) in which average normal tail cuff SBP and DBP are 190 mmHg and 119 mmHg. This is an indication of the salt extracted from *H. schulli* has a potential to reduce SBP and DBP and can be used as alternative edible salt source.

5. Conclusion and Recommendations

5.1. Conclusion

- This study focused on chemical composition, anti-hypertensive properties and sensory quality of vegetable salt extracted from *H. schulli.* Accordingly, the major composition of the salt was potassium from macro minerals and chloride from anions.
- Sodium-potassium ratio of the *H. schulli* salt was less than one. Also this ratio is lower than the value in common salt, which indicated that *H. schulli* salt has anti-hypertensive property.
- The anti-hypertensive property of the salt was further proved through *in vivo* Wistar rat feeding experiment. The rats fed with the 4% *H. schulli* diet had significantly a lower both systolic and diastolic blood pressure compared to normal and common salt containing diet fed rats blood pressure, this is because of high content of potassium. *H. schulli* salt was also found with non-detectable concentrations of harmful metals like Cd, Cr, As, Ni and Hg. Also the detected concentrations of Pb were very low as compared with the Provisional Tolerable Weekly Intake of the metals.
- The sensory acceptability (particularly the taste attribute) of the *H. schulli* salt was relatively low compared to the common edible salt. However, the sensory acceptability of *H. schulli* salt increased when it was blended with different amount of common salt.

5.2. Recommendations

- Anti-hypertensive properties should be checked again and other health benefits of the salt should be studied in the future. For instance, anti diabetic activities, antimicrobial activities and etc.
- Anti-hypertensive properties
- Studies to improve the taste of the salt more than the acceptability rate in the present value should be carried.
- Shelf stability of the salt should also be studied.
- The potential of this salt as a vehicle of Universal Salt Iodization should be carried. This will ultimately address both the concerns of non-communicable diseases and iodine coverage.

REFERENCES

Abebe, Zegeye., Tariku, Amare., and Gebeye, Ejigu. (2017). Availability of adequately iodized in Northwest Ethiopia: a cross-sectional study. *Arch of Pub Health, 75* (1): 33.

Adeyemi, O. T., Osilesi, O., Adebawo, O. O., Onajobi, F. D., and Oyedemi, S. O. (2015). Variations in the levels of total protein, urea and ureate in weaned male albino rats feed on processed Atlantic horse mackerel. *Variations*, *5*(6).

Adrogue, HJ Ali, M. A. (1967). Chemical investigation on the seeds of *Hygrophila spinosa*. *Pak. J. Sci. Ind. Res*, *10*: 82-83.

Ali, M. A. (1967). Chemical investigation on the seeds of *Hygrophila spinosa*. *Pak. J. Sci. Ind. Res*, *10*: 82-83.

Andrews, N. C. (1999). Disorders of iron metabolism. *N Engla J of Med*, *341*(26); 1986-1995.

Annadurai, G., Juang, R. S., and Lee, D. J. (2003). Adsorption of heavy metals from water using banana and orange peels. *Water sci and tech*, *47*(1): 185.

Araya, Henok. (2016). *Adequacy of Iodine Content and Level of Contaminants in Edible Salts Produced In Ethiopia* (Doctoral dissertation, Addis Ababa University).

Awas, Tesfaye., Bekele, Tamrat., and Demissew, Sebsebe. (2001). An ecological study of the vegetation of Gambella region, southwestern Ethiopia. *SINET: Ethio J of Sci*, *24*(2): 213-228.

Balaraman, R., Dangwal, S., and Mohan, M. (2006). Antihypertensive effect of *Trigonella foenum-greacum*. Seeds in experimentally induced hypertension in rats. *Pharmacol biol*, *44*(8): 568-575.

Bauer, A. W., Kirby, W. M., Sherris, J. C., and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *Amer J Clin Patho, 45* (4): 493-496.

Beard, J. L. (2001). Iron biology in immune function, muscle metabolism and neuronal functioning. *J of nutri*, *131*(2): 568S-580S.

Becker, D. V., Braverman, L. E., Delange, F., Dunn, J. T., Franklyn, J. A., Hollowell, J. G., and Rovet, J. F. (2006). Iodine supplementation for pregnancy and lactation—United States and Canada: recommendations of the American Thyroid Association. *Thyroid*, *16*(10): 949-951.

Binega, Y. (2006). Chemical analysis of the Assale (Ethiopia) rock salt deposit. *Bulletin of the Chemical Society of Ethio*, *20*(2): 319-324.

Boegehold, M. A., and Kotchen, T. A. (1990). Effect of dietary salt on the skeletal muscle microvasculature in Dahl rats. *Hypert*, *15*(4): 420-426.

Broding, H. C., Michalke, B., Gӧen, T., and Drexler, H. (2009). Comparison between exhaled breath condensate analysis as a marker for cobalt and tungsten exposure and biomonitoring in workers of a hard metal alloy processing plant. *Int arch of occupa and env health*, *82*(5): 565-573.

Brown, P. H., Welch, R. M., and Cary, E. E. (1987). Nickel: A micronutrient essential for higher plants. *Plant physiol*, *85*(3): 801-803.

Bunag, R. D. (1973). Validation in awake rats of a tail-cuff method for measuring systolic pressure. *J of Appl Physiol 34*(2), 279-282.

Buxton, C., and Baguune, B. (2012). Knowledge and practices of people in Bia District, Ghana, with regard to iodine deficiency disorders and intake of iodized salt. *Arch of Pub Health*, *70*(1), 5.

Cappuccio, F. P., and MacGregor, G. A. (1991). Does potassium supplementation lower blood pressure? A meta-analysis of published trials.

Celik, U.O., Oehlenchlar, J. (2007). High contents of cadmium, lead, zinc and copper in popular fishery products sold in Turkish supermarkets. *Food Control,* 18: 258-261.

Cempel, M., and Nikel, G. (2006). Nickel: A review of its sources and environmental toxicology. *Polish J of Env Studies*, *15*(3).

Cepanec, K., Vugrinec, S., Cvetković, T., and Ranilović, J. (2017). Potassium Chloride-based salt substitutes: A critical review with a focus on the patent literature. *Compr Rev in Food Sci and Food Saf*, *16*(5): 881-894

Chandran, R. P., Manju, S., Vysakhi, M. V., Shaji, P. K., and Nair, G. A. (2013). *In vitro* antimicrobial activities of *Hygrophila schulli* (Buch.-Ham) leaf and root extracts against clinically important human pathogens. *Biomed Pharmacol J*, *6*: 421-28.

Cheraghali, A. M., Kobarfard, F., and Faeizy, N. (2010). Heavy metals contamination of table salt consumed in Iran. *Iranian j of pharmace res: IJPR*, *9*(2): 129.

Cho, S. H., Lee, S. M., Park, B. H., and Lee, S. M. (2006). Effect of feeding ratio on growth and body composition of juvenile olive flounder *Paralichthys olivaceus* fed extruded pellets during the summer season. *Aqua*, *251(1)*: 78-84.

Chong, A., Cohen, I., Field, E., Nakasone, E., and Torero, M. (2016). Iron deficiency and schooling attainment in peru. *Amer Eco J: Appl Eco*, *8*(4): 222-55.

Ciobanu C., Slencu B. G., and Cuciureanu R. (2012). Estimation of dietary intake of cadmium and lead through food consumption. *Rev Med Chir Soc Med Nat Iasi*, *116(2)*, 617-623

Codex Alimentarius Commission. (2006). The Codex Standard for Food Grade Salt. *Codex Stan 150*.

Cole, I. S., Paterson, W. Ganther. (2003). Holistic model for atmospheric corrosion Part1. Theoretical framework for production, transportation and deposition of marine salts. *Corr Eng, Sci and Technol: 38(2)*:129-134(6).

Dash, A. K., Dutta, G. K., Sardar, K. K., and Sahoo, G. (2012). Ethnomedicinal importance of *Hygrophila spinosa* T. Anders: A review. *Plant Arch*, *12(1)*: 5-9.

de Loryn, L. C., Petrie, P. R., Hasted, A. M., Johnson, T. E., Collins, C., and Bastian, S. E. (2014). Evaluation of sensory thresholds and perception of sodium chloride in grape juice and wine. *Amer J of Eno and Viticul*, *65*(1): 124-133.

Dickinson B. D, and Havas S. (2007). Reducing the population burden of cardiovascular disease by reducing sodium intake: a report of the Council on Science and Public Health. *Arch Intern Med*, *167*: 1460–1468.

Dixon, R. A., and Paiva, N. L. (1995). Stress-induced phenylpropanoid metabolism. *The plant cell*, *7*(7): 1085.

Dizman, S., Görür, F. K., and Keser, R. (2017). Assessment of human health risk from heavy metals levels in water and tissues of two trout species (Oncorhynchus mykiss and Salmo coruhensis) from the Fırtına and Güneysu Rivers in Turkey. *Toxin Reviews*, *36*(4): 306-312.

Dyer, A. R., Shipley, M., Elliott, P., and INTERSALT Cooperative Research Group. (1994). Urinary electrolyte excretion in 24 hours and blood pressure in the INTERSALT study: I. Estimates of Reliability. *Amer J of Epid*, *139(9)*: 927-939.

Echeverri, J. A. (2011). Witoto ash salts from the Amazon. *J of ethnopharmacol*, *138*(2): 492-502.

Echeverri, J. A., and Román-Jitdutjaaño, Ó. E. (2013). Ash salts and bodily affects: Witoto environmental knowledge as sexual education. *Env res letters*, *8*(1: 015034.

Elliott, P., Stamler, J., Nichols, R., Dyer, A. R., Stamler, R., Kesteloot, H., and Marmot, M. (1996). Inter salt revisited: further analyses of 24 hour sodium excretion and blood pressure within and across populations. *Bmj*, *312(7041)*: 1249-1253.

Emmanuel, S., Rani, M. S., and Sreekanth, M. R. (2010). Antidiabetic activity of *Cassia occidentalis* Linn. In streptozotocin-induced diabetic rats: a dose dependent study. *Int J Pharmacol Biol Sci*, *1(4)*: 15-25.

Fardousi, M. (2012). *Determination of iodine content in different brands table salt of Bangladesh* (Doctoral dissertation, East West University).

Feyissa, D. (2011). The political economy of salt in the Afar Regional State in northeast Ethiopia. *Review of Afric Polit Eco*, *38*(127): 7-21.

Frisoli, T. M., Schmieder, R. E., Grodzicki, T., and Messerli, F. H. (2012). Salt and hypertension: is salt dietary reduction worth the effort?. *The Amer j of med*, *125*(5): 433-439.

Geertman, R. M. (2000). Sodium chloride: Crystallization. *Reference Module in Chem, Molecu Sci and Chemi Engi*, 4127-4134.

Geleijnse, J. M., Kok, F. J., and Grobbee, D. E. (2003). Blood pressure response to changes in sodium and potassium intake: a metaregression analysis of randomised trials. *J Human Hypert*, *17(7)*: 471

Gidey, B., Alemu, K., Atnafu, A., Kifle, M., Tefera, Y., and Sharma, H. R. (2015). Availability of adequate iodized salt at household level and associated factors in rural communities in Laelay Maychew District, Northern Ethiopia: a cross sectional study. *J of Nutri and Healh Sci*, *2*(1): 1.

Gong, S. L., Barrie, L. A., and Blanchet, J. P. (1997). Modeling sea salt aerosols in the atmosphere: 1.Model development. *J Geophy Res: Atmosp* (1984–2012), 102(D3):3805-3818.

Gopalani, M., Shahare, M., Ramteke, D. S., and Wate, S. R. (2007). Heavy metal content of potato chips and biscuits from Nagpur city, India. *Bulletin of env conta and toxico*, *79*(4): 384-387.

Goyer, R. A., and Clarkson, T. W. (1996). Toxic effects of metals. *Casarett and Doull's Toxicology. The Basic Science of Poisons, Fifth Edition, Klaassen, CD [Ed]. McGraw-Hill Health Prof Div, ISBN*: *71054766*.

Gulshan, A. B., Dasti, A. A., Hussain, S., Atta, M. I., and Amin-ud-Din, M. (2012). Indigenous uses of medicinal plants in rural areas of Dera Ghazi Khan, Punjab, Pakistan. *J of Agri and Biol Sci*, *7*(9): 750-762.

Gulson, B. L., Davis, J. J., and Bawden-Smith, J. (1995). Paint as a source of recontamination of houses in urban environments and its role in maintaining elevated blood leads in children. *Sci of the Total Env*, *164*(3): 221-235.

Hamshahri Organization (2006). Health aspects of edible salts.Food and Drug Department.Ministry of Health and Medical Education (MOHME). Hamshahri Organization Iran. http://www.hamshahri.org/ Categories.

Hatae, K., Takeutchi, F., Sakamoto, M., Ogasawara, Y., and Akano, H. (2009). Saltiness and acidity: detection and recognition thresholds and their interaction near the threshold. *J of food sci*, *74*(4): S147-S153.

Havas, S., Dickinson, B. D., and Wilson, M. (2007). The urgent need to reduce sodium consumption. *Jama*, *298*(12): 1439-1441.

Hemalatha, S., Platel, K., and Srinivasan, K. (2007). Influence of germination and fermentation on bioaccessibility of zinc and iron from food grains. *Euro J of clin nutri*, *61*(3): 342.

Heshmati, A., Vahidinia, A., Salehi, I. (2014). Evaluation of heavy metal contamination of unrefined and refined table salt samples. *Int J of Res Studies in Biosci*, 2: 21-24.

Hollenberg, N. K. (2006). The influence of dietary sodium on blood pressure. *J of the Amer College of Nutri*, *25*(sup3): 240S-246S.

Hoppu, U., Hopia, A., Pohjanheimo, T., Rotola-Pukkila, M., Mäkinen, S., Pihlanto, A., and Sandell, M. (2017). Effect of salt reduction on consumer acceptance and sensory quality of food. *Foods*, *6(12)*, 103.

Hussain, M. S., Ahamed, K. F., Ravichandiran, V., and Ansari, M. Z. (2009). Evaluation of in vitro free radical scavenging potential of different fractions of Hygrophila auriculata (K. Schum) Heine. *Asian J Trad Med*, *4*: 179-187.

Hussain, S., Fareed, S., and Ali, M. H. (2010). Ethno botany, photochemistry and pharmacology. *Asian J Tradi Med*, *5*:122-31

Idris, T. M. (2015). Salt of *Achyranthes aspera* (Chirchita): By lixiviation. Indo Amer J of Pharmacol Res *5(05)*: 2016-2020.

Israel, A. U., and Akpan, I. A. (2016). Mineral composition of flashed and charred Palm (*Elaeisguineensis*) bunch and plantain (*Musa paradisiaca*) Peel. *Brit J Appl Sci and Techno*, 16(5): 1-9.

Jahed, K., Gh, R., and, Zazoli M., A. (2005).Cadmium and lead contents in rice *(Oryza sativa)* in the north of Iran. *Int .J. Agric. BioI.Sci*., *7:1026*-1029.

Jain, A., Soni, M., Deb, L., Jain, A., Rout, S. P., Gupta, V. B., and Krishna, K. L. (2008). Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Momordica dioica* Roxb. Leaves. *J Ethnopharmacol, 115(1)*: 61-66.

Joutey, N. T., Sayel, H., Bahafid, W., and El Ghachtouli, N. (2015). Mechanisms of hexavalent chromium resistance and removal by microorganisms. In *Reviews of Env Conta and Toxico Volume 233* (pp. 45-69).

Kannur, D. M., Dongre, P. P., Kumbhar, S. T., and Khandelwal, K. R. (2012). Anti-inflammatory and antinociceptive activities of *H. schulli* seed extracts. *Inter J Green Pharmacol (IJGP)*, *6*(3).

Karanja, N. N., Mutua, G. K., Ayuke, F., Njenga, M., Prain, G., and Kimenju, J. (2010). Dynamics of soil nematodes and earthworms in urban vegetable irrigated with wastewater in the Nairobi river basin, Kenya. *Tro and Subtro Agroeco*, *12*(3): 521-530.

Khaniki, G. R. J., Dehghani, M. H., Mahvi, A. H., and Nazmara, S. (2007). Determination of trace metal contaminants in edible salts in Tehran (Iran) by atomic absorption spectrophotometry. *J Biol Sci*, *7*(5): 811-814.

Kirabira, J. B., Kasedde, H., and Semukuuttu, D. (2013). Towards the improvement of salt extraction at Lake Katwe. *Inter J Sci and Technol Res*, *2(1)*: 76-81.

Kumar, S., Pandey, A. K., Razzaque, W. A. A., and Dwivedi, D. K. (2011). Importance of micro minerals in reproductive performance of livestock. *Vet Word*, *4(5)*: 230-233.

Kuntz, L. A. (1994). The many benefits of salt. Weeks Publishing Company. www.foodproductdesign.com.

Kwon, Y. I. I., Vattem, D. A., and Shetty, K. (2006). Evaluation of clonal herbs of *Lamiaceae* species for management of diabetes and hypertension. *Asia Paci J of Clin Nutri*, *15(1)*: 107-118.

Lendinez, E., Lorenzo, M. L., Cabrera, C., and López, M. C. (2001). Chromium in basic foods of the Spanish diet: seafood, cereals, vegetables, olive oils and dairy products. *Sci of the total env*, *278*(1-3): 183-189.

Leone, S. (2012). "Would have to be lined as the Aldabra clay is." Handbook of World Salt Resources: 297.

Lobo, V. C., Phatak, A., and Chandra, N. (2010). Antioxidant and free radical scavenging activity of *Hygrophila schulli* (Buch.-Ham.) Almeida and Almeida. Seeds. *Adv Biores*, *1(2)*: 72-78.

Lobo, V. C., Phatak, A., and Chandra, N. (2010). Antioxidant and free radical scavenging activity of *Hygrophila schulli* (Buch.-Ham.) Almeida and Almeida. Seeds. *Adv Biores*, *1*(2): 72-78.

MacGregor, G., and De Wardener, H. E. (2002). Commentary: salt, blood pressure and health. *Intl J of Epidem*, *31*(2): 320-327.

MacGregor, GA. (2007). Dietary salt, high blood pressure and other harmful effects on health. In: Kilcast D, Angus F, editors. Reducing salt in foods. *Boca Raton, Fla.: CRC Press*. P 18–54.

Madias, NE. (2008). Sodium and potassium in the pathogenesis of hypertension. *N Engl J Med, 356*:1966–78.

Mannar, M. V., Dunn, J. T., Micronutrient Initiative, International Council for Control of Iodine Deficiency Disorders, and UNICEF. (1995). *Salt iodization for the elimination of iodine deficiency*. ICCIDD, NL.

Mannar, V., andYusufali, R. (2013). Salt production and trade in Africa. *IDD Newsletter: Zurich, Switzerland*. Chuko, T., Bagriansky, J., and Brown, A. T. (2015). Ethiopia's long road to USI. *IDD Newsletter, May*.

Mazumdar, UK, Sengupta, A. (1978). Triglyceride composition of *H. spinosa* seed oil. *Ind. J Pharmacol Sci, 40*:119-20.

Merialdi, M., Caulfield, L.E., Zavaleta, N., Figueroa, A., and DiPietro, J.A (1999). Adding zinc to prenatal iron and folate tablets improves fetal neurobehavioral development. *Amer J Obst and Gynecol, 180 (2)*: 483-490.

Miah, M. Y., Volchek, K., Kuang, W., and Tezel, F. H. (2010). Kinetic and equilibrium studies of cesium adsorption on ceiling tiles from aqueous solutions. *J Haz Mat*, 183(1-3): 712-717.

Mianpeurem, T., Mbailao, M., Nambatingar, N., Yaya, M., and Ngarmadji, A. (2012). Four common plants species from Chad. *Inter J Pharmacol*, *8(6)*: 582-585.

Mianpeurem, T., Mbailao, M., Nambatingar, N., Yaya, M., Ngarmadji, A., Alderman, M. H., and Stan, C. (1998). The Cultural and Historical Role of Salt in the Waika Indians of the Upper Orinoco in Connection of Southern America. *Int J of Pharmacol*, *8*(6): 781-785.

Miller, A. L. (1996). Antioxidant flavonoids: structure, function and clinical usage. *Alt. Med. Rev. 1*: 103-111.

Mohan, S., and Campbell, N. R. (2009). Salt and high blood pressure. *Clinic Sci*, *117(1)*: 1-11.

Moran, J. E., Oktay, S. D., and Santschi, P. H. (2002). Sources of iodine and iodine 129 in rivers. *Water resou res, 38*(8).

Morris, M. J., Na, E. S., and Johnson, A. K. (2008). Salt craving: the psychobiology of pathogenic sodium intake. *Physiol and Behav*, *94* (5): 709-721.

Müller-Lissner, S. A., Kamm, M. A., Scarpignato, C., and Wald, A. (2005). Myths and misconceptions about chronic constipation. *The Amer J of Gastroentro*, *100(1)*: 232.

Nafees, M., Khan, N., Rukh, S., and Bashir, A. (2013). Analysis of rock and sea salts for various essential S and inorganic elements. *J Sci and Technol*, *37(1)*: 09-20.

Neharkar, V. S., Kshirsagar, S., and Pandhare, R. (2015). Acute toxicity study of *Hygrophila auriculata* L. Leaves methanolic extract in Albino rats. *J. Pharmacol. Chem. Biol.*, *3(3)*: 388-395.

Ojeda, C. B., Rojas, F. S., and Pavón, J. M. C. (2012). Determination of cobalt in food, environmental and water samples with preconcentration by dispersive liquid-liquid microextraction. *Amer J of Analy Chem*, *3*(02): 125.

Okayama, A., Okuda, N., Miura, K., Okamura, T., Hayakawa, T., Akasaka, H., and Takashima, N. (2016). Dietary sodium-to-potassium ratio as a risk factor for stroke, cardiovascular disease and all-cause mortality in Japan: the NIPPON DATA80 cohort study. *BMJ open*, *6(7)*: 011632.

Okayama, A., Okuda, N., Miura, K., Okamura, T., Hayakawa, T., Akasaka, H., and Takashima, N. (2016). Dietary sodium-to-potassium ratio as a risk factor for stroke, cardiovascular disease and all-cause mortality in Japan: the NIPPON DATA80 cohort study. *BMJ open*, *6*(7): e011632.

Oliva, L., Aranda, T., Caviola, G., Fernández-Bernal, A., Alemany, M., Fernández-López, J. A., and Remesar, X. (2017). In rats fed high-energy diets, taste, rather than fat content, is the key factor increasing food intake: a comparison of a cafeteria and a lipid-supplemented standard diet. *PeerJ*, *5*: e3697.

Oliver, W. J., Cohen, E. L., and Neel, J. V. (1975). Blood pressure, sodium intake, and sodium related hormones in the Yanomamo Indians, a" no-salt" culture. *Circu*, *52*(1): 146-151.

Ortells-Abuye, N., Busquets-Puigdevall, T., Díaz-Bergara, M., Paguina-Marcos, M., and Sánchez-Pérez, I. (2014). A cross-sectional study to compare two blood collection methods: direct venous puncture and peripheral venous catheter. *BMJ open*, *4*(2): e004250.

Oti-Boakye, A. (2011). *Levels of some heavy metals in omega-3 food supplements* (Doctoral dissertation).

Park J., Kwock C. K. and Yang Y. J. (2016) The effect of the sodium to potassium ratio on hypertension prevalence: A propensity score matching approach. Nutrients *8 (482)*: 1.

Phoboo, S., Shetty, K., & ElObeid, T. (2015). In Vitro Assays of Anti-diabetic and Anti-hypertensive Potential of Some Traditional Edible Plants of Qatar. *Journal of Medicinally Active Plants*, *4*(3): 22-29.16.

Pongpaew, P., Saowakontha, S., Tungtrongchitr, R., Mahaweerawat, U., and Schelp, F. P. (2002). Iodine deficiency disorder—an old problem tackled again: a review of a comprehensive operational study in the northeast of Thailand. *Nutri res*, *22*(1-2): 137-144.

Portères, R. (1950). sels alimentaires, cendres d'origine végétale, sels de cendres comme succédanés du chlorure de sodium alimentaire et catalogue des plantes salifères en Afrique intertropicale et à Madagascar.

Portères, R. (1957). Le Sel culinaire et les Cendres de plantes en dehors de l'Afrique. *J d'agri tradi et de bota appliq*, *4*(3): 157-158.

Pourgheysari, H., Moazeni, M., and Ebrahimi, A. (2012). Heavy metal content in edible salts in Isfahan and estimation of their daily intake via salt consumption. *Int J of Env Health Engi*, *1*(1): 8.

Qadir, H., Farrukh, M., and Aurangzaib, M. (2005). Production of table salt from Kohat rock salt. *J of Appl Sci*, *5*(1): 12-14.

R. F. (2004). Dual fortification of salt with iodine and micronized ferric pyrophosphate: a randomized, double-blind, controlled trial. *The Amer jo of clin nutri*, *80*(4): 952-959.

Rankin, C. W., Nriagu, J. O., Aggarwal, J. K., Arowolo, T. A., Adebayo, K., and Flegal, A. R. (2005). Lead contamination in cocoa and cocoa products: isotopic evidence of global contamination. *Env Health Pers*, *113(10)*: 1344.

Reilly, C. (2008). *Metal contamination of food: its significance for food quality and human health*. John Wiley and Sons.

Saavedra-Garcia, L., Bernabe-Ortiz, A., Gilman, R. H., Diez-Canseco, F., Cárdenas, M. K., Sacksteder, K. A., and Miranda, J. J. (2015). Applying the triangle taste test to assess differences between low sodium salts and common salt: evidence from Peru. *PloS one*, *10(7)*: e0134700.

Sakat, S. S., Wankhede, S. S., Juvekar, A. R., Mali, V. R, Bodhankar, S. L. (2009). Antihypertensive effect of aqueous extract of *Elaeocarpus ganitrus*. Seeds in renal artery occluded hypertensive rats. *Int. J. Pharmacol Tech Res*, *1(3)*: 779-82.

Schmeda-Hirschmann, G. (1994). Tree ash as an Ayoreo salt source in the Paraguayan Chaco. *Econo bota*, *48*(2): 159-162.

Seide, W. M. (2017). *The Nuer pastoralists: between large scale agriculture and villagization: a case study of the Lare District in the Gambella Region of Ethiopia*. Nordiska Afrikainstitutet.

Shentema, A. (2012). *Ecological Study of Shrubland Vegetation along the Escarpments between Addis Alem and Wolenkomi, West Shewa, Oromoia National Regional State*(Doctoral dissertation, Addis Ababa University Addis Ababa, Ethiopia).

Sinopoli, D. A., and Lawless, H. T. (2012). Taste properties of potassium chloride alone and in mixtures with sodium chloride using a check-all-that-apply method. *J of food sci*, *77*(9): S319-S322.

Spears, J. W. (2000). Micronutrients and immune function in cattle. *Proceedi of the nutri soci*, *59*(4): 587-594.

Sufian, M. A., and Haque, M. R. (2015). Cytotoxic, thrombolytic, membrane stabilizing and anti-oxidant activities of *Hygrophila schulli*. *Bangla J of Pharmacol*, *10*(3): 692-696.

Thomson, I. S. I. (2012). Tarkodjiel Mianpeurem, Mbaiguinam Mbailao, Ngaram Nambatingar, Mahmout Yaya and Allaramadji Ngarmadji. *Interof Pharmacol*, *8*(6): 582-585.

Thomson, I. S. I. (2016). Nader Yousefi, Maryam Meserghani, Hamideh Bahrami and Amir Hossein Mahvi. *Research J of Env Toxicol*, *10*(1): 75-81.

Thorat, B. (2018). Chemical extraction and biomedical importance of secondary organic metabolites from plants–A review. *J Biomed and Therap Scie, 5(1)*: 9-42.

Tiwari, A. (2001). Imbalance in antioxidant defense and human diseases: Multiple approach of natural antioxidants therapy. *Curr. Sci*, *81*: 1179-1187

ul Hassan, A., Ali, S., Din, A. M. U., and Muhsen, S. (2016). Chemical Characterisation of Unrefined Rock Salt Deposits of Pakistan. *Pak J of Scienti and Indu Res Series A: Physi Sci*, *59*(3): 126-129.

ul Hassana, A., Dina, A. M. U., and Ali b, S. (2017). Chemical characterization of Himalayan rock salt. *AIMS and SCOPE*, 67.

UNICEF. (2008). *The state of the world's children 2009: maternal and newborn health* (Vol. 9). Unicef.

Usman, M. A., and Filli, K. B. (2011). Determination of essential elements and heavy metals contained in table salt. *J Res in Nat Dev, 9(2)*: 73-78

Vahter, M., Åkesson, A., Lidén, C., Ceccatelli, S., and Berglund, M. (2007). Gender differences in the disposition and toxicity of metals. *Env res*, *104*(1): 85-95.

Van Dam, R. M., Hu, F. B., Rosenberg, L., Krishnan, S., and Palmer, J. R. (2006). Dietary calcium and magnesium, major food sources, and risk of type 2 diabetes in US black women. *Diab care*, *29*(10): 2238-2243.

Van Oss, H. G. (2013). US Geological Survey, Mineral Commodity Summaries, January 2013.

Vithanage, M., Herath, I., Achinthya, S. S., Bandara, T., Weerasundara, L., Mayakaduwa, S. S., . and Kumarathilaka, P. (2016). Iodine in commercial edible iodized salts and assessment of iodine exposure in Sri Lanka. *Arch of Pub Health*, *74*(1): 21.

Wang, C., Zhang, S. H., Wang, P. F., Hou, J., Zhang, W. J., Li, W., and Lin, Z. P. (2009). The effect of excess Zn on mineral nutrition and antioxidative response in rapeseed seedlings. *Chemos*, *75*(11): 1468-1476.

Weaver, C. M. (2013). Potassium and Health–. *Advances in Nutrition*, *4*(3): 368S-377S.

Wegmüller, M., Zimmermann, V., Bühr, E. and Windhab, R. (2006). Development, stability, and sensory testing of microcapsules containing iron, iodine, and vitamin A for use in food fortification. *J Food Sci*, *71*(2): S181-S187.

Whelton, P. K., He, J., Cutler, J. A., Brancati, F. L., Appel, L. J., Follmann, D., and Pope, W. D. B. (1998). Effects of oral potassium on blood pressure: Meta-analysis of randomized controlled clinical trials. *Survey of Anesthesiol*, *42(2)*: 100.

Wilde, E., Aubdool, A. A., Thakore, P., Baldissera Jr, L., Alawi, K. M., Keeble, J., and Brain, S. D. (2017). Tail-cuff technique and its influence on central blood pressure in the mouse. *J of the Amer Heart Associ*, *6*(6): e005204.

World Health Organization (2007). Reducing salt intake in populations: report of a WHO forum and technical meeting. WHO: 1-60.

World Health Organization (WHO, 2012). Guideline: Sodium intake for adults and children; World Health Organization: Geneva, Switzerland.

World Health Organization and Food and Agricultural Organization of the United Nations (2006). "Guidelines on food fortification with micronutrients," 2006. (Online). Available: http://whqlibdoc.who.int/publications/2006/9241594012. (Accessed 7 March 2012).

World Health Organization. (2012). Effect of increased potassium intake on blood pressure, blood lipids and other potential adverse effects in children.

World Health Organization. (2012). *Guideline: Sodium intake for adults and children*. World Health Organization.

World Health Organization. Global status report on non communicable diseases. WHO, 2010:164.

Wuana, R. A., and Okieimen, F. E. (2011). Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. *Isrn Ecol*, *2011*.

Yang, L. S., Zhang, X. W., Li, Y. H., Li, H. R., Wang, Y., and Wang, W. Y. (2012). Bioaccessibility and risk assessment of cadmium from uncooked rice using an in vitro digestion model. *Biologi trace element res*, *145*(1): 81-86.

Yousefi, N., Meserghani, M., Bahrami, H., and Mahvi, A. H. (2016). Assessment of human health risk for heavy metals in imported rice and its daily intake in Iran. *Res J of Env Toxico*, *10*(1): 75-81.

Zarei, M., Eskandari, M. H., and Pakfetrat, S. (2011). Determination of heavy metals content of refined table salts. *Amer-Eura J of Toxico Sci*, *3*(2): 59-62.

Zerries, O. (1964). *Waika: Die kulturgeschichtliche Stellung der Waika-Indianer des oberen Orinoco im Rahmen der Völkerkunde Südamerikas* (Vol. 1). K. Renner.

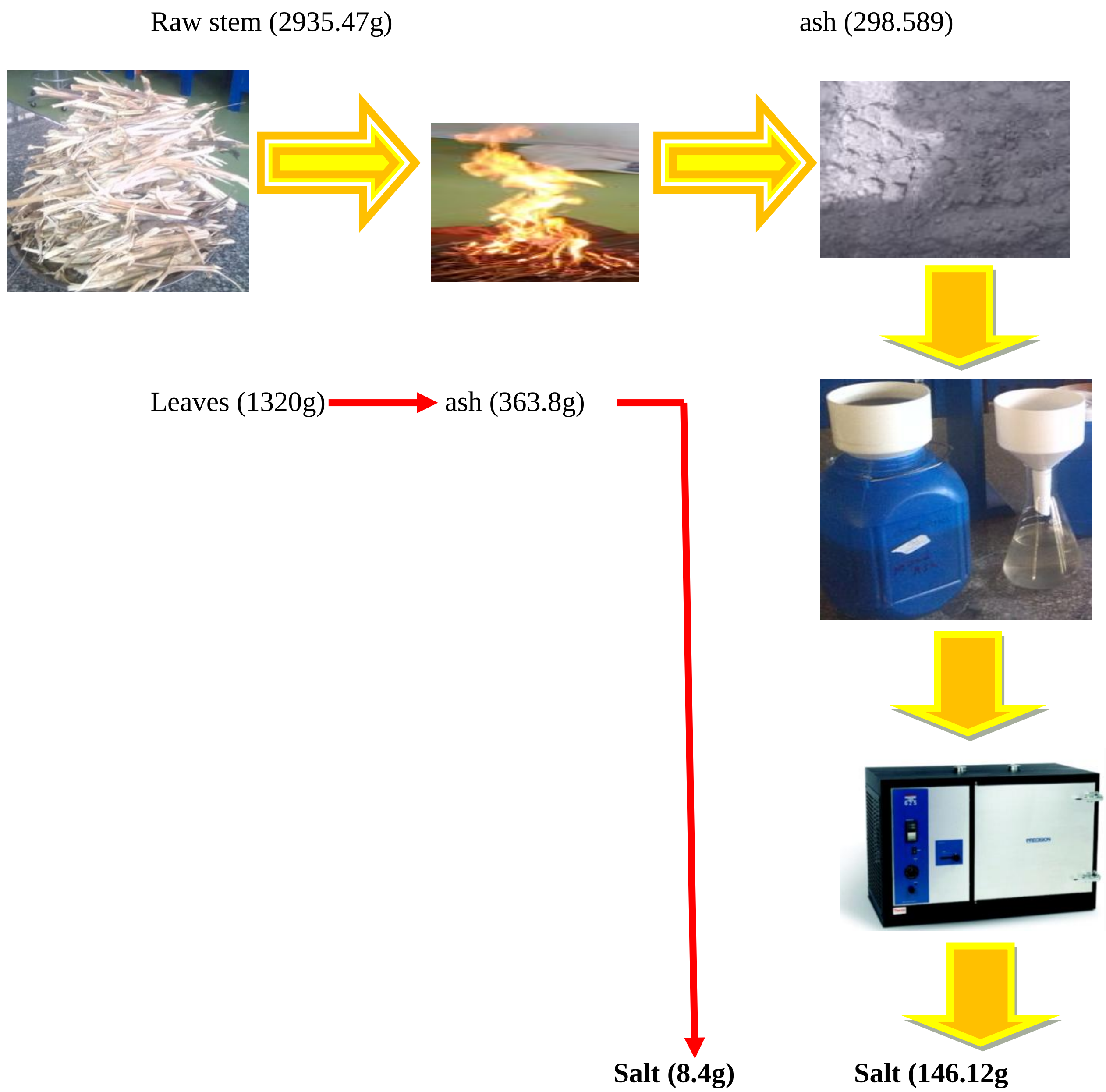
Zimmermann, M. B. (2007). The adverse effects of mild-to-moderate iodine deficiency during pregnancy and childhood: a review. *Thyroid*, *7*(9): 829-835.

Zimmermann, M. B., Jooste, P. L., and Pandav, C. S. (2008). Iodine-deficiency disorders. *The Lancet*, *372*(9645): 1251-1262.

Zimmermann, M. B., Wegmueller, R., Zeder, C., Chaouki, N., Rohner, F., Saïssi, M., and Hurrell, R. F. (2004). Dual fortification of salt with iodine and micronized ferric pyrophosphate: a randomized, double-blind, controlled trial. *The Amer j of clin nutri*, *80*(4): 952-959.

Appendixes

Appendix 1. Vegetable salt production procedures from *H. schulli* flow diagram



Appendix 2. The picture of vegetable salt produced from *H. schull*

Appendix 3. Formant for Paired comparison difference sensory test

DATE _____________ TIME _________ ASSESSOR ________________ PRODUCT _________

Dear participant, here are two samples for evaluation. Is there a difference in overall appearance, color, taste, odor (flavor) between the two samples?

Yes _____ No _____

Comments (if any)

……….

…………………………………………………………………………………..

Thank you!

Appendix 4. Format for paired comparison preference test

PAIRED COMPARISON PREFER

Product …………………… **Date** …………. **Time** ………. **Assessor** …………………….

Dear participant, here are two samples for evaluation. Please examine left to right and

Put **‘**√**’** sign on the sample you prefer.

Which sample do you prefer?

Please tick the appropriate box.

Code	**ABB**	**BAB**
Place tick		

You must make a choice

Thank you!

Indicate the degree of preference between the two samples.

Slightly _____

Moderately ___

Very much ____

Extremely ____

Appendix 5. Format for multiple comparison difference tests

Product _________________ Date_________________ time________________

Dear participants, there are five samples presented for this sensory analysis that will be tasted by you, the first sample is reference (**R**) and the other four samples are coded as **BAA**, **AAB**, **ABB** and **ABA**. Please taste each of the coded samples that presented from left to right and show whether it is different, better than, comparable and inferior to the reference. Then mark the amount of difference that exists.

Samples code	**BAA**	**ABB**	**ABA**	**AAB**
Comparable to **R**	__________	__________	__________	__________
Different to **R**	__________	__________	__________	__________
Better than to **R**	__________	__________	__________	__________
Inferior to **R**	__________	__________	__________	__________

Please indicate the amount difference between the samples

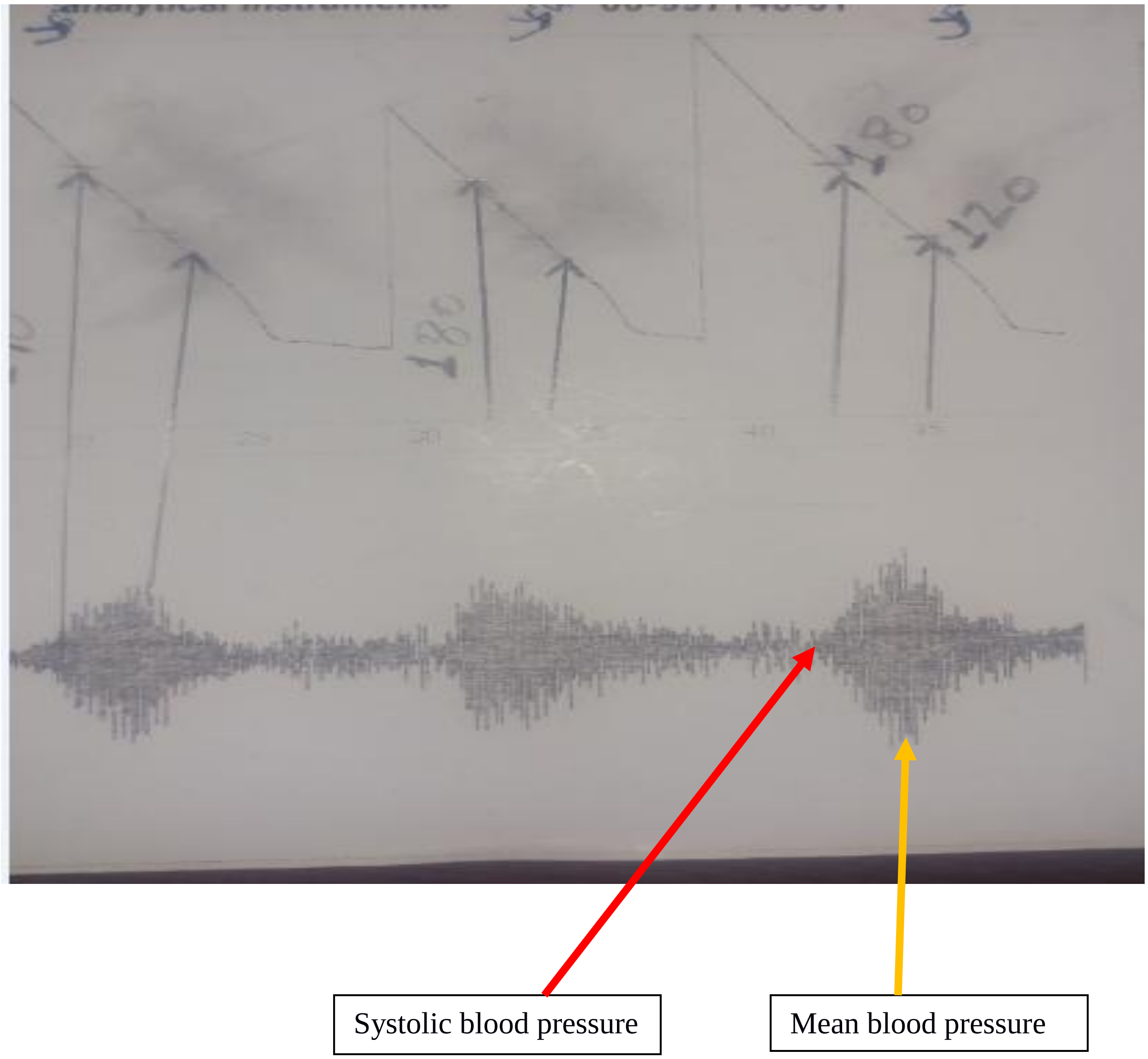
None	__________	__________	__________	__________
Slight	__________	__________	__________	__________
Moderate	__________	__________	__________	__________
Much	__________	__________	__________	__________
Extreme	__________	__________	__________	__________

Please you can put any comments you feel about the taste of the sample

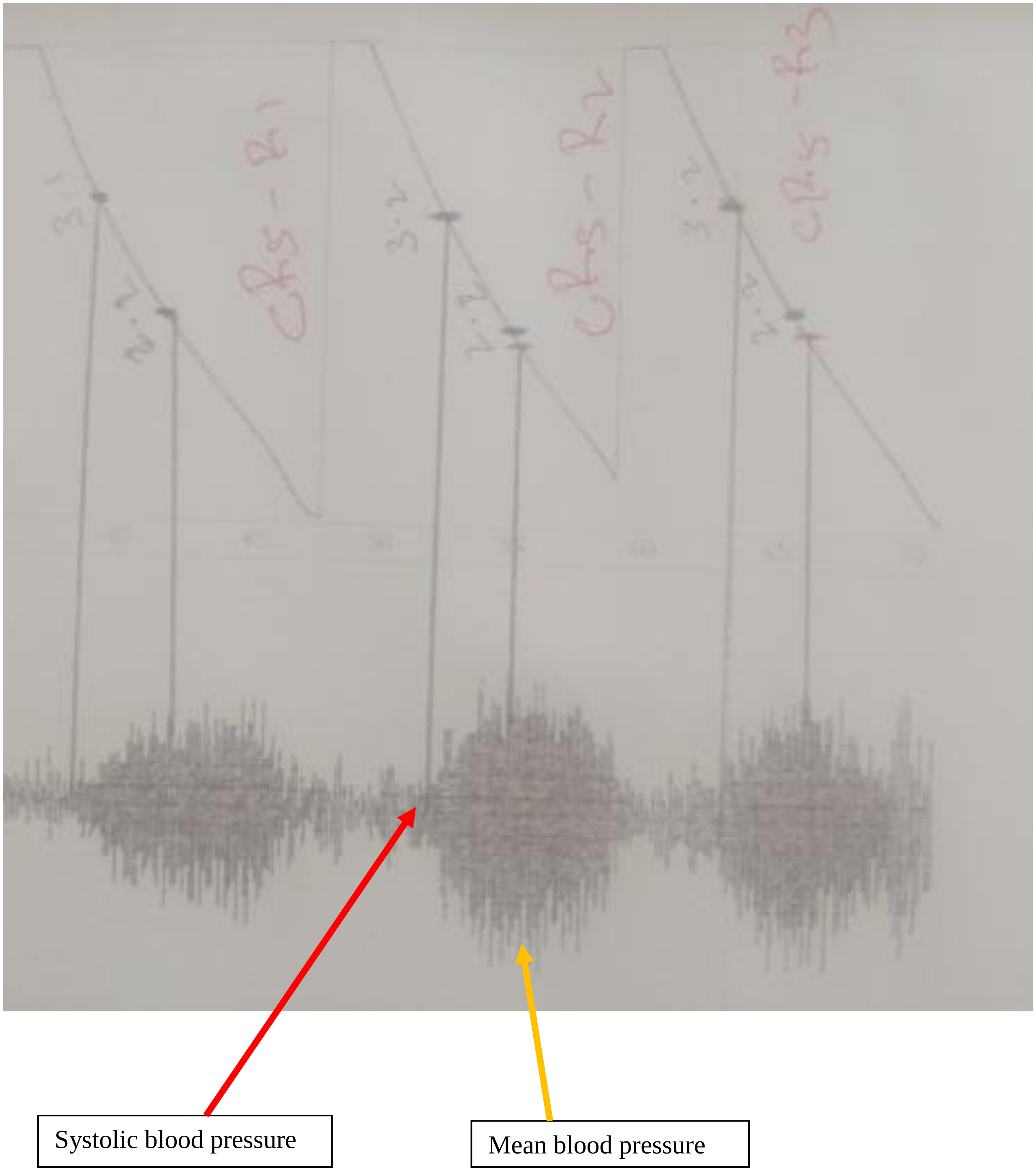
Appendix 6. Descriptive sensory panelist evaluation of both common and vegetable salt

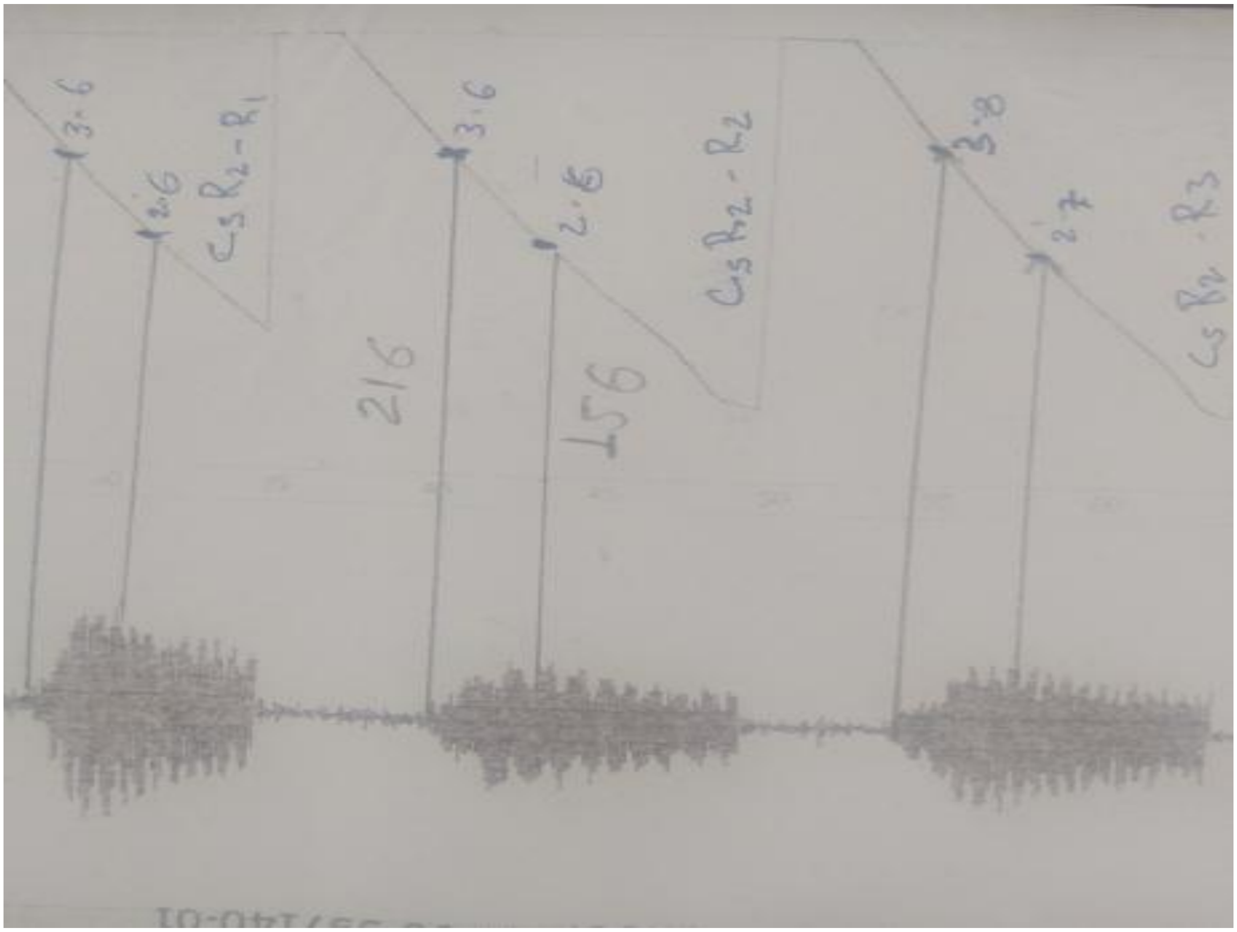
Sample Code	Panelist code	Overall appearance	Color	Taste	Odor	overall acceptance
1	1	7	6	6	6	7
1	2	7	7	7	7	7
1	3	5	6	5	7	6
1	4	6	5	7	5	5
1	5	7	6	5	5	6
1	6	7	6	6	5	7
1	7	7	6	7	6	7
1	8	7	7	7	7	7
1	9	7	4	1	2	5
1	10	7	6	5	5	7
1	11	7	6	5	7	6
1	12	7	6	7	5	6
2	1	4	3	3	3	3
2	2	5	5	5	5	5
2	3	2	1	6	6	5
2	4	5	6	6	6	5
2	5	6	1	6	7	2
2	6	7	5	6	5	3
2	7	5	5	5	5	5
2	8	7	5	6	5	6
2	9	7	5	6	5	5
2	10	6	6	6	7	7
2	11	5	6	5	5	5
2	12	5	1	6	6	3

Appendix 7. The graph obtained from weekly Systolic and Diastolic Blood pressure (mmHg) of 4% Vegetable Salt (Vs) group of Wistar rats using tail cuff.



Appendix 8. Graph obtained from weekly Systolic and Diastolic Blood pressure (mmHg) of normal control feed group of Wistar rats using tail cuff.





Appendix 9. Graph of weekly Systolic and Mean Blood pressure for 4% common salt containing rat feed. 216 = Systolic Blood pressure, 156 = Diastolic Blood pressure, CS R3 R2 = common salt rat 3 reading 2, CsR3R3 = Common salt rat 3 reading 3.

Appendix 10. Tail cuff Blood pressure analyzer and warming camper