NUTRITIONAL AND PHYTOCHEMICAL SCREENING OF HIGH ALTITUDE GROWN BEANS OF NEPAL

M. Sc. Thesis

(2019)

Submitted to

Central Department of Biotechnology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

A dissertation submitted as the partial fulfillment of the requirement for Master of Science in Biotechnology

By

Binod Shankar Neupane

Roll No. BT 302-072

TU Regd. No.: 5-2-0037-00336-2011

NUTRITIONAL AND PHYTOCHEMICAL SCREENING OF HIGH ALTITUDE GROWN BEANS OF NEPAL

A dissertation submitted as the partial fulfilment of the requirement for
Master of Science in Biotechnology
(2019)

Submitted to
Central Department of Biotechnology
Tribhuvan University
Kirtipur, Kathmandu, Nepal

Submitted by
Binod Shankar Neupane
Roll No. BT 302-072
TU Regd. No.: 5-2-0037-0336-2011

Supervisors

Ganga P. Kharel, Ph.D.
Professor
Central Department of Biotechnology
Tribhuvan University
Kirtipur, Kathmandu, Nepal

Deepa S. Shrestha
Senior Scientist
National Agriculture Research Council
(NARC-GeneBank)
Khumaltar, Lalitpur, Nepal

ACKNOWLEGMENTS

At first, I would like to acknowledge and offer sincere gratitude to my supervisors Prof. Dr. Ganga P. Kharel, Central Department of Biotechnology, Tribhuvan University and Mrs. Deepa S. Shrestha, Senior Scientist, Nepal Agriculture Genetic Resources Center (GeneBank) for their continuous inspirations, guidance, supervision, support and suggestions during this entire dissertation work.

I am extremely thankful to Prof. Dr. Krishna Das Manandhar, Head of the Department, Prof. Dr. Rajani Malla, former Head of the Department, Jarina Joshi, lecturers, along with all the faculty members and staffs of Central Department of Biotechnology. Thanks, are also due to lab assistants for their help during my lab work.

I am indebted to Sanjeev K. Karn (Director- General), Budha Kunwar (Food Research Officers) of Department of Food Technology and Quality Control (DFTQC), for their help in carrying out this work.

I would also like to show my appreciation to my friends Madhav Regmi, Dinesh Oli, Sarbesh Rizal, Tika Bahadur Budda, Ranjeeta Odari, Srijan Shrestha, Sabita Prajapati, Pranita Poudyal, Bidhya Kala Sunuwar, Manju Adhikari, Sunil Timalsina, Mahadev Bista, Inju Dhakal, Elisha Regmi, Sita Ghimire, Safalta Mallick, Manju Pun, Sabina Thapa and Kshitiz Subedi for their support.

Special thanks go to my seniors, Apsara Parajuli, Pratishya Mainali and Deepesh Thapa, my junior Archana Chauthat for their great support during the entire work.

I would also like to thank all the well-wishers who directly or indirectly helped me during this research.

Last but not the least, I would like to dedicate this work to my family members for their love, encouragement and constant support to come at this stage.

Binod Shankar Neupane

ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
ACE-1	Angiotensin converting enzyme-1
AOAC	Association of Official Analytical Chemists
CDBT	Central Department of Biotechnology
CGIAR	Consultative Group on International Agricultural Research
DFTQC	Department of Food Technology and Quality Control
DPPH	2,2'-Diphenyl-1-picrylhydrazyl
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
FAAS	Flame Atomic Absorption Spectrometry
FS	Flame Spectrometer
GAE	Gallic Acid Equivalent
GC-MS	Gas Chromatography Mass Spectrometry
GDP	Gross Domestic Product
HPLC	High performance Liquid Chromatography
HPLC-MS	HPLC- Mass spectroscopy
IC_{50}	Inhibitory Concentration 50
NARC	National Agricultural Research Centre
QE	Quercetin Equivalent
RARS	Regional Agricultural Research Station
NGLIP	National Grain Legume Improvement Program
NGLRP	National Grain Legume Research Program
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
USAID	United States Agency for International Development
WHO	World Health Organization

Table of Contents

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Phaseolus sp. are considered to be a good source of carbohydrate, protein, dietary fiber and other nutritional components. Besides, they are rich in a variety of bioactive compounds and thus possess health promoting effects in relation to prevention of chronic diseases including cancers, cardiovascular diseases, obesity and diabetes. This investigation provides analyses of nutrients and screening of bioactive compounds present in the common bean species which are cultivated in high altitude of Nepal. Eighteen varieties of bean commonly grown in high altitude of Nepal were analyzed. The proximate values range from 6.49-8.84% moisture, 61.94-67.35% carbohydrate, 19.40-24.55% crude protein, 1.98- 3.58% crude fat, 3.61-4.91% total ash and 4.02-4.96% crude fiber. The mineral content in the ash were determined and found to be ranged from 843.86-2763.62 mg/kg, 1540.63-2083.52 mg/kg, 38.93-73.69 mg/kg, 205.91-695.81 mg/kg, 13560.21- 16957.05 mg/kg, 2.72-8.77 mg/kg and 19.80-31.00 mg/kg dry weight for calcium, magnesium, iron, sodium, potassium, copper and zinc respectively. Besides nutrients, the presence of various bioactive compounds-alkaloids, flavonoids, phenolics, tannins, saponins, were screened and quantified in all eighteen samples. Higher concentration (more than 5 mg GAE/g) of phenolics were observed in four beans samples whereas eight samples ranged from 4-7 mg GAE/g and less than 4 mg GAE/g was observed in six samples. Similarly, higher concentration (more than 2 mg QE/g) of flavonoids were observed in five samples and less than 2 mg QE/g was observed in thirteen beans samples.

The composite flour of wheat (lacks lysine) and pulses (lacks s-amino acids) fulfills each other's deficiencies including amino acids and other nutritional and anti-nutritional components that are required for better health.

Key words: Common beans, high altitude, proximate composition, phytochemicals.

CHAPTER I
INTRODUCTION

1.1 Background

Legume seeds are an important part of the human diet in many countries throughout the world, particularly in tropical and subtropical areas, constituting an important source of protein, vitamins (especially B-group vitamins), and mineral elements such as potassium, phosphorus, zinc, and magnesium. They are also a good source of carbohydrate and food fibers. Of the legumes grown in the world, dry beans are the legume most widely consumed in the world as whole seed. Dry beans are especially cultivated in Latin America, India and Africa.

The family Leguminosae or Fabaceae comprises of 650 genera and 18000 species worldwide. About 100 genera and 379 species of legumes are widely distributed in varied agro-ecological zones ranging from Terai to the Alpine region of Nepal, with growth habit ranging from annual to perennial shrubs. Out of the 379 species of legumes, 262 are native and 20 species belonging to sub-group Papilionacae are used as pulses or grain legumes.

Food legumes are important crops of Nepal in terms of their contribution to the dietary proteins supply to human and livestock. They are rich source of micronutrients, play important role in crop diversification and intensification and maintenance of soil fertility through symbiotic nitrogen fixation. About 11% of cultivated area in the country is occupied by grain legumes that included lentil, chickpea, fababean, pigeonpea, soybean, blackgram, cowpea, mungbean, etc. Legumes are generally consumed after processing into various products like milling into dhal, puffing or roasting into snack foods, grinding into flour and also germinated grains for different food preparations. Traditional methods of processing and cooking legumes have been evolved to give acceptable, appetizing and nutritious products. Processing of legumes increases the digestibility and enhances the sensory qualities and nutritional attributes.

The dry common bean, or *Phaseolus vulgaris L*., is the most important edible legume for direct consumption in the world. There are many variations with regard to growth patterns, seed characteristics, maturation and adaptation, accounting for more than 40,000 varieties (Schneider, 2002). Twenty-three million tons of *Phaseolus* plants and over 12 million tons of dry beans are produced worldwide. This crop is produced under a diversity of systems and

environments in all regions: Asia (45.75%), the Americas (34.17%), Africa (17.56%), Europe (2.29%) and Oceania (0.24%). For the 2001– 2011 period, the largest production of cultivation was Brazil (16%), followed by India (15.9%), Myanmar (10.5%), China (8.9%), Mexico (5.8%) and USA (5.6%) (FAOSTAT, 2013; Secretaría de Economía, 2012). Among the highest consumers of beans are Brazil (19.7%), India (19.7%), Mexico (7.7%), the United States (6.6%), Tanzania (2.7%), and Uganda (2.7%) (FAOSTAT, 2013).

The dry beans, widely consumed throughout the world and it is recognized as the major source of dietary protein in many countries, has been associated with a decrease risk for such as cancer, obesity, diabetes and cardiovascular diseases. Beans are considered as a good source of high protein content, complex carbohydrates, dietary fiber and some vitamins and minerals. In addition, to these nutritional components, common beans are rich in a variety of several phytochemicals with potential health benefits such as polyphenolic compounds, fiber, lectins and trypsin-inhibitors.

Common beans are annual plants, cultivated in temperate and semitropical regions for their edible dry seeds that are variously called navy beans, kidney beans, red beans, black beans, pinto beans, and cranberry beans. They were first cultivated in Peru and Mexico around 8000 years ago and are now cultivated worldwide. They belong to the family Fabaceae. In the temperate regions, the green leaves and immature pods are edible as vegetables. Dry beans are mainly consumed in low- and middle-class families as the large portion of the protein. The main five domesticated species are the common bean (*Phaseolus vulgaris* L.), the lima bean (*Phaseolus lunatus*), the runner bean (*Phaseolus coccineus*), the tepary bean (*Phaseolus acutifolius*), and the year bean (*Phaseolus polyanthus*) (Gepts,2001).

1.2 Legume cultivation in Nepal

Nepal is a landlocked country rich in biodiversity due to its complex variation in geomorphology and phytogeography (topology, climate and altitudinal). Nepal occupies 0.09% of total surface of world's total area.

Nepal is situated on the southern slopes of the Central Himalayan and occupies total area of 1,471181 sq. km. extending from 26°22' N to 30°27' N latitudes and 80°4' E to 88°12' E longitudes. The altitude varies from some 60 m above sea level in the Terai to Mt. Everest (Sagarmatha) at 8848 m. Nepal is divided into four major agroclimatic zones, the Terai (in the south), the inner Terai, mid-hills and valleys, and high mountains (in the north). Out of total area of Nepal, about 42.5% is covered by forest, 26.6% is agricultural land, 11.8% pastures and the rest 19.1% is occupied by snow, lake, urban, roads, etc.

Agriculture is the mainstay of livelihood of 65.8% of population in Nepal (MoAD, 2014)

contributing to 32.62% of Gross Domestic Product (GDP). Nepalese agriculture is mainly characterized by subsistence farming with major concerns on household food security and poor nutrition. Grain legumes play an important role in Nepalese agriculture contributing towards food and nutritional security, nitrogen economy, crop intensification, diversification and sustainable farming systems. Grain Legumes (GL) rank 4th in terms of acreage (about 10.8% of total cultivated land) and 5th in production in Nepalese context.

Grain Legumes are grown in both summer and winter in Nepal. The main summer grain legumes are soybean (*Gycine max* L. Merr.), black gram (*Vigna mungo* L. Hepper), horse gram (*Macrotyloma uniflorum* L. Verde.), cowpea (*Vigna unguiculata* L. Walp.), mung bean (*Vigna radiata* L. Wilczek), and groundnut (*Arachis hypogaea* L.). Major winter grain legumes include lentil (*Lens culinaris* Medic), khesari *(Lathyrus sativus* L.; lathyrus; grass pea), chickpea (*Cicer arietinum* L.), and faba bean *(Vicia faba* L.). Pigeonpea (*Cajanus cajan* L. Millsp.) is sown early in the rainy season and harvested in the following spring/summer. Legumes are the second most important crops grown in rotation in rice-wheat cropping systems.

The Important legume grains cultivated in Nepal and used as various sources of food is shown in Table 1.1.

Table 1.1: Important legume grains of Nepal

S.N.	Common name	Botanical name	Region
1	Lentil	*Lens culinaris* subsp. Culinaris Medikus	Terai/Midhill
2	Chickpea	*Cicer arietinum* L.	Terai
3	Grasspea	*Lathyrus sativus* L.	Midhill/terai
4	Fababean	*Vicia faba* L.	Terai
5	Fieldpea	*Pisum sativum* L.	Terai
6	Pigeonpea	*Cajanus cajan* L.	Terai/High hill
7	Rajma/Simi	*Phaseolus vulgaris* L.	Terai/High hill
8	Soybean	*Glycine max* L.	Midhill
9	Blackgram	*Vigna mungo* L.	Midhill
10	Mungbean	(*Vigna radiata* L.)	Terai
11	Cowpea	(*Vigna unguiculata* L.)	Terai
12	Ricebean	(*Vigna umbellate* L)	Midhill
13	Horsegram	(*Macrotyloma uniflorum*)	Midhill

Source: Neupane and Shrestha, 2015

1.3 Research activities on legumes in Nepal

Grain legumes are the integral part of Nepalese farming system. With an objective of developing improved technology and disseminating to the farmers to increase the production and production and productivity of grain legumes in Nepal, research on grain legumes first time started in 1972 at Regional Agricultural Research Station (RARS), Parwanipur and Agronomy Division, Khumaltar. Grain legume program was upgraded to National Grain Legume Improvement Program (NGLIP) during 1985, after supports from IDRC and USAID were obtained for the creation of basic infrastructural facilities. After the establishment of Nepal Agricultural Research Council (NARC) as an autonomous institution, the program was named as National Grain Legume Research Program (NGLRP). Currently, thirteen legume grains research networks within NARC stations are established for research related to legumes.

National Grain Legumes Research program (NGLRP) of Nepal in collaboration with Consultative Group on International Agricultural Research (CGIAR) centers works for genetic improvement of lentil, chickpea, pigeonpea, soybean, mungbean, blackgram, grasspea, fababean and cowpea. Research activities at NGLRP are variety development, crop management, research outreach, source seed production and dissemination of technologies. GLRP has been working in collaboration with national (Department of Agriculture, NGO, community-based organizations, seed companies etc.) and international organizations/NARS such as ICARDA, ICRISAT, IVRDC, ACI AR /CLIMA, IITA, BARI, etc. for germplasm exchange, technology generation, funding, technical support (including experts visits) and capacity building. Grain legume improved technologies have been developed in collaboration with various research and extension partners including valuable support from farmers.

Grain legumes research for development in Nepal in collaboration with various international and national agricultural research organizations has led to the release of 35 grain legume varieties and popularization of several production technologies ultimately resulting into a substantial increase in grain legumes production, mainly because of area increase and yield increase during the last two and a half decades. Nepal has a great potential to produce different grain legume species because of her diverse agro- ecological environments. Nepal still have a great opportunity to incorporate grain legumes in the rice-based cropping system (RBCS) and hence there is further scope to increase area, production and productivity of grain legumes through development and popularization of suitable varieties and technologies, streamlining community-based seed production, addressing climate change issues and policy reforms for the promotion of grain legumes.

1.4 Production of legumes in Nepal

Grain legumes research for development has led to substantial increase in grain legumes production, mainly because of area increase and yield increase during the last two and a half decades. The current estimates for area, production and productivity of grain legumes in Nepal are 334323 ha, 319770 metric tons and 956 kg/ha, respectively (Gharti et al., 2014). Pulses rank fourth in terms of acreage and fifth in production after rice, maize, wheat and millet. Basically, all farmers in Nepal grow one or more species of grain legumes. Per capita consumption of pulses is rather low; its availability is influenced not only by production but also by economic status. In the mountains and hills, grain legumes are primarily for home consumption, while in the Terai and also in some warmer valleys, they are grown both for home consumption and market. The bulk of production in the Terai and inner Terai is from the winter grain legumes such as lentil, chickpea, field pea and grass pea and in the summer from pigeon pea. In hills, summer grain legumes such as soybean, black gram, horse gram and rice bean dominate while in the higher mountainous regions, peas and *Phaseolus* bean are the important grain legumes.

The cultivation of lentil has been increasing because of its increasing preference for its internal consumption and potential for export market. Nepalese lentils have greater demand in the international market of Bangladesh, Singapore, Sri Lanka, Germany, Korea, UK, Indonesia etc. Soybean is identified as the industrial crop and important ingredient for poultry industry. Winter grain legumes crops such as lentil, chickpea, kidney bean, grass pea, field pea and faba bean are grown entirely dependent on residual soil moisture after the harvest of rice (post rice) or seed broadcasted on standing rice about 7-15 days prior to rice harvest (relay cropping). While warm season grain legumes like soybean, mungbean, cowpea, blackgram and pigeonpea are grown during summer month (monsoon rain) in mono, mixed with maize/ finger millet or on paddy bunds.

1.5 Experimental Overview

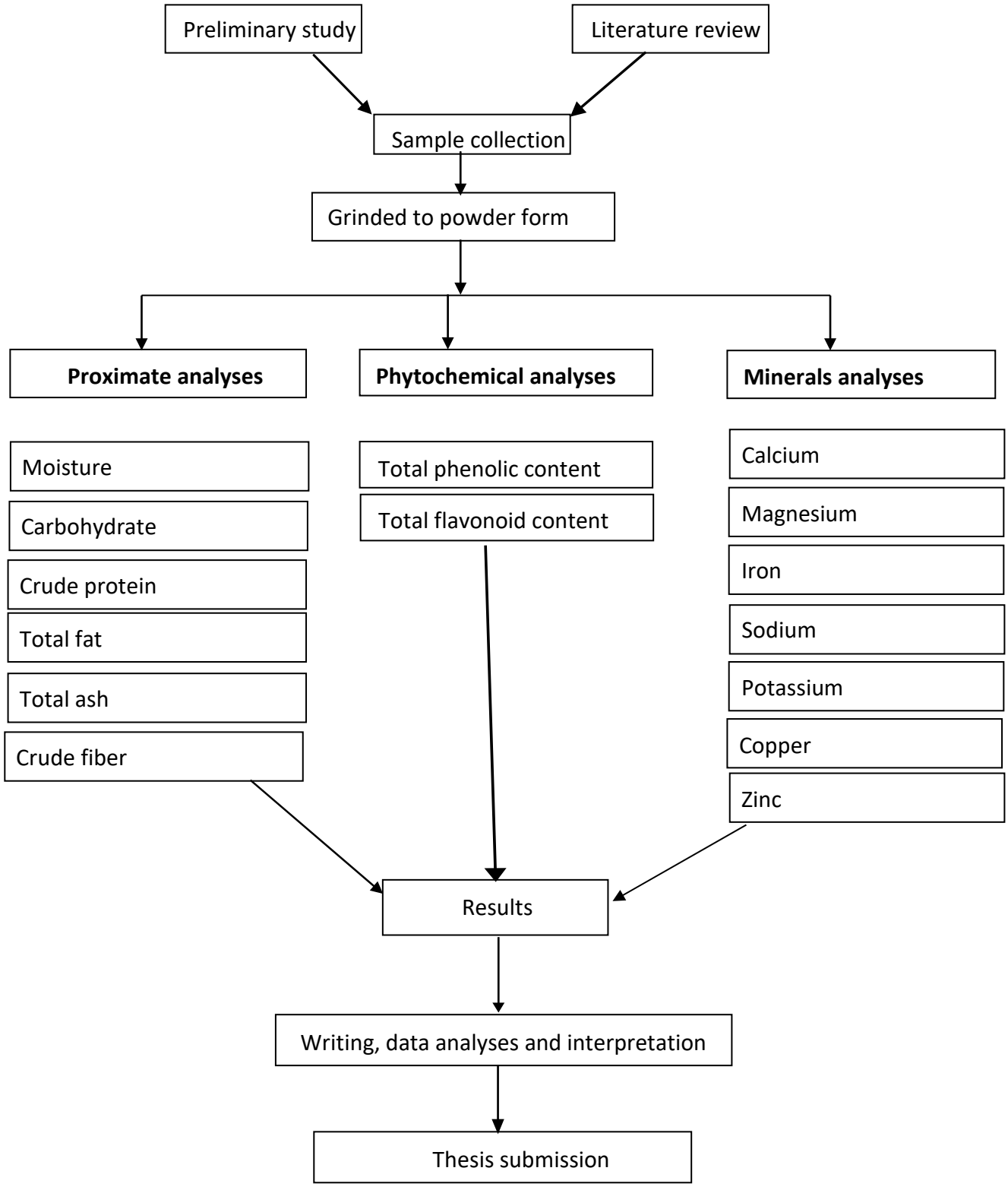


Fig. 1.1 Experimental overview of the present research work.

1.6 Research objectives

1. General objective:

The general objective of the research work was to analyze the nutritional and phytochemical attribute of the bean grown in high altitude of Nepal.

2. Specific objectives:

The specific objectives of the present research work were as follows:

1. To determine the proximate composition of high altitude grown common beans.
2. To determine the mineral contents of high altitude grown common beans.
3. To screen and quantification of phytochemical contents of high altitude grown common beans.

1.7 Significance of the study

Common bean is an important legume grown in virtually all parts of Nepal. It has generally been considered as low status food or the "meat of the poor" due to its low- cost relative to animal products. Bean provides a rich combination of carbohydrates (60-65%), proteins (21-25%), fats (less than 2%), vitamins and minerals (Ensminger et al., 1994). In fact, with increasing health concerns, most people especially the urban population are reducing consumption of animal proteins, and instead they are turning to pulses due to health benefits. The dry beans have been associated with a decrease risk for such as cancer, obesity, diabetes and cardiovascular diseases. Beans are rich in a variety of several phytochemicals with potential health benefits such as polyphenolic compounds, fiber, lectins and trypsin inhibitors, among others. Hence the rationale for emphasis in more bean research is self-evident. This study will help to investigate variation in profiles of alkaloids and flavonoids in high altitude bean extracts collected from different geographical regions in Nepal.

The research has huge impact on the applied aspects such that agricultural genetics. It is helpful for the establishment of the various nutrients present in different cultivated species of common beans. Truly speaking, it will make aware for the growers for production of better quality to use specialized or advanced technique.

Legumes research for development has led to substantial increase in grain legumes production, mainly because of area increase and yield increase during the last two and a half decades. The current estimates for area, production and productivity of grain legumes in Nepal are 334323 ha, 319770 metric tons and 956 kg/ha, respectively (Gharti et al., 2014). Pulses rank fourth in terms of acreage and fifth in production after rice, maize, wheat and millet. Basically, all farmers in Nepal grow one or more species of grain legumes. Per capita consumption of pulses is rather low; its availability is influenced not only by production but also by economic status. In the mountains and hills, grain legumes are primarily for home consumption, while in the Terai and also in some warmer valleys, they are grown both for home consumption and market. The bulk of production in the Terai and inner Terai is from the winter grain legumes such as lentil, chickpea, field peas and grass pea and in the summer from pigeon pea. In hills, summer grain legumes such as soybean, black gram, horse gram and rice bean dominate while in the higher mountainous regions, peas and *Phaseolus* bean are the important grain legumes.

CHAPTER II
LITERATURE REVIEW

2.1 Botanical information of beans

The Fabaceae or Leguminosae, commonly known as the legume, pea, or bean family comes from the defunct genus Faba, Latin term and appears to simply mean "bean". Leguminosae is an older name still considered valid, and refers to the fruit of these plants, which are called legumes. This family contains economically important flowering plants trees, shrubs, and perennial or annual herbaceous plants, which are easily recognized by their fruit (legume) and their compound, stipulate leaves. Many legumes have characteristic flowers and fruits. The family is widely distributed, and is the third- largest land plant family in terms of number of species, behind only the Orchidaceae and Asteraceae, with about 751 genera and about 19,000 known species (Christenhusz and Byng, 2016; Judd et al., 2002 and Stevens, 2006). The five largest of the genera are Astragalus (over 3,000 species), Acacia (over 1000 species), Indigofera (around 700 species), Crotalaria (around 700 species) and Mimosa (around 400 species), which constitute about a quarter of all legume species. The approximately 19,000 known legume species amount to about 7% of flowering plant species (Judd et al., 2002 and Magallón and Sanderson, 2001). The Fabaceae family includes a number of important agricultural and food plants, including *Glycine max* (soybean), *Phaseolus* (beans), *Pisum sativum* (pea), *Cicer arietinum* (chickpeas), *Medicago sativa* (alfalfa), *Arachis hypogaea* (peanut), *Ceratonia siliqua* (carob), and *Glycyrrhiza glabra*(liquorice).

The family Leguminosae or Fabaceae comprises of 650 genera and 18000 species worldwide. About 100 genera and 379 species of legumes are widely distributed in varied agro-ecological zones ranging from Terai to the Alpine region of Nepal, with growth habit ranging from annual to perennial shrubs. Out of the 379 species of legumes, 262 are native and 20 species belonging to sub-group Papilionacae are used as pulses or grainlegumes.

The generic name *Phaseolus* was introduced by Linnaeus in 1753, (Linnaeus, 1799) borrowed from the Latin word *Phaseolus*, a combination of phasēlus and the diminutive suffix -olus Fabaceae or Leguminosae, commonly known as the legume, pea, or bean family comes from the defunct genus Faba, Latin term and appears to simply mean "bean". This family contains economically important flowering plants trees, shrubs, and perennial or annual herbaceous plants, which are easily recognized by their fruit (legume) and their compound, stipulate leaves. Many legumes have characteristic flowers and fruits. The family is widely distributed,

and is the third-largest land plant family in terms of number of species, behind only the Orchidaceae and Asteraceae, with about 751 genera and about 19,000 known species. The five largest of the genera are *Astragalus* (over 3,000 species), *Acacia* (over 1000 species), *Indigofera* (around 700 species), *Crotalaria* (around 700 species) and *Mimosa* (around 400 species), which constitute about a quarter of all legume species. The plant genus *Phaseolus* is a member of the legume tribe Phaseoleae, subtribe Phaseolinae.

The ca. 19,000 known legume species amount to about 7% of flowering plant species. Fabaceae is the most common family found in tropical rainforests and in dry forests in the Americas and Africa. The Fabaceae family includes a number of important agricultural and food plants, including *Glycine max* (soybean), *Phaseolus* (beans), *Pisum sativum* (pea), *Cicer arietinum* (chickpeas), *Medicago sativa* (alfalfa), *Arachis hypogaea* (peanut), *Ceratonia siliqua* (carob), and *Glycyrrhiza glabra* (liquorice). A number of species are also weedy pests in different parts of the world, including: *Cytisus scoparius* (broom), *Robinia pseudoacacia* (black locust), *Ulex europaeus* (gorse), *Pueraria lobata* (kudzu), and a number of *Lupinus* species.

2.2 Commercial cultivars of beans in Nepal

The genus *Phaseolus* is a member of the legume tribe Phaseoleae, subtribe Phaseolinae. Numerous species of Phaseolus are cultivated all over the world primarily for their grains (beans), which are harvested when mature and marketed as dry products. They are generally known as pulses in the industry and commerce (Delgado- Salinas, 2012). Seeds from the cultivated species are consumed worldwide and constitute a vital source of protein and fiber for humans. Some of the cultivated species are often confused with those belonging to Vigna, another important genus of the subtribe and the seeds from both genera are together referred to as “beans”. In an attempt to discriminate between cultivars of the two genera, beans within Phaseolus are often referred to as “dry beans” and are distinguished by their shape, color and growth characteristics. They include the common bean or red kidney bean for P. vulgaris, butter bean or lima bean for *P. lunatus*, runner bean for *P. coccineus* and tepary bean for *P. acutifolius.*

Archeological studies reveal that beans from Phaseolus originated in the Americas, and in Mexico there have been discoveries of Phaseolus vulgaris dating from to 9000 years ago (Reyes-Rivas et al., 2008). The most consumed legumes worldwide are beans (*Phaseolus vulgaris*), chickpeas (*Cicer arietinum*), lentils (*Lentis esculenta*), peas (*Pisum sativum*), broad beans (*Vicia faba*), peanuts (*Arachys hipogea*), and soybeans (*Glycine max*), the latter being the most industrialized (Guillon and Champ, 2002) (Gepts, 2001).

Twenty three million tons of *Phaseolus* plants and over 12 million tons of dry beans are producedworldwide. This crop is produced under a diversity of systems in all regions: Asia (45.75%), the Americas (34.17%), Africa (17.56%), Europe (2.29%) and Oceania(0.24%). For the 2001– 2011 period, the largest production of cultivation was Brazil (16%),followed by India (15.9%), Myanmar (10.5%), China (8.9%), Mexico (5.8%) and USA (5.6%)(FAOSTAT, 2013; Secretaría de Economía, 2012).

The dry common bean is the most important edible legume for direct consumption in the world. There are many variations with regard to growth patterns, seed characteristics, maturation and adaptation, accounting for more than 40,000 varieties (Schneider, 2002). Among the highest consumers of beans are Brazil (19.7%), India (19.7%), Mexico (7.7%), the United States (6.6%), Tanzania (2.7%), and Uganda (2.7%) (FAOSTAT, 2013). In Latin America, beans are considered traditional nourishment, but in Africa beans are grown primarily for subsistence, and the Great Lakes region has the highest consumption per capita in the world (Jones, 2011).

2.3 Nutritional composition

The mature seeds of legumes are composed of seed coat, cotyledon, and embryonic axis. Within the cotyledon, there are protein bodies and starch granules, which constitute the anatomical structure of energy reserve of these seeds. The chemical composition of legumes varies between species (Muehlbauer and McPhee., 2002; El- Adawy and Alajaji., 2006; Brandon and Friedman., 2001); however, the high concentration of carbohydrates, proteins, and fiber, as well as the low lipid concentration are remarkable for each one.

Common beans do not differ mostly in their nutritional compositions; they differ slightly in taste, texture and cooking times (Mitchell et al*.,* 2009). Common beans play a vital role in the vegetarian diets and provide numerous health benefits connected with eating pattern (Tanzman and Haddad, 2003). They serve as a cost-effective source of nutrients. Health benefits of beans are generally acquired from direct attributes, including their high content of proteins, dietary fibers, low saturated fat content, vitamins, minerals, and phytochemicals, as well as replacement in the diet, when they substitute for animal products. These replacements of meat and other animal products with beans are highly linked with enhanced animal welfare and the decrease in inputs of environmental resources (Messina., 2014). The Food Habits in Later Life study conducted in Japanese, Greek and Australian populations have demonstrated that dried beans and other food legumes are the only foods linked with a reduced risk of mortality (Darmadi- Blackberry et al., 2004). Hence, health-promoting effects are directly proportional to increased bean intake.

2.3.1 Beans carbohydrates

Carbohydrates are the major component of beans found in variable amounts, accounting up to 50-60% of the dry matter (Vargas-Torres et al.,2004; Reynoso Camacho et al., 2006; Ovando-Martinez et al., 2011). Starch and non-starch polysaccharides constitute the major components of these carbohydrates along with considerable amounts of carbohydrate derivates such as oligosaccharides also found (Bravo et al*.,* 1998; Reynoso Camacho et al*.,* 2006). Among all carbohydrates, starch is the major carbohydrate found in beans in different shapes and sizes. Amylose and amylopectin are the two major forms of starch available in beans. Beans starch can be degraded into oligo-dextrin and glucose by different enzymes such as α- and β-amylases (Tharanathan, 2002; Zhou et al., 2004). On the basis of their susceptibility to amylose and the rate of glucose release and its absorption in the gastrointestinal tract, starches are classified into several types such as slowly digestible starch (SDS), rapidly digestible starch (RDS) and non-digestible starch (NDS) or resistant starch (RS) (Englyst et al., 1992; Tharanathan and Mahadevamma, 2003; Zhang et al., 2006). Beans contain slow digestion carbohydrates and a high proportion of non-digestible carbohydrates, which can be fermented in the large intestine. Non-digested carbohydrates that reach the colon include resistant starch, soluble and insoluble dietetic fibre and non-digestible oligosaccharides (Reynoso- Camacho, RamosGómez, & Loarca-Pina, 2006).

Beans also contain considerable quantities of dietary fiber which consist of edible parts of plant analogous carbohydrates such as cellulose, hemicellulose, pectins, oligosaccharides and lignins, that resist digestion and absorption in small intestine but are partially or completely fermented in the large intestine, thus imparting various physiological impacts with health implications (Hughes and Swanson, 1989; Costa et al., 2006). Both types of dietary fiber viz soluble and insoluble fiber fractions are well characterized for these beans (Kutos et al., 2003; Shiga and Lajolo, 2006). The insoluble fiber consists of cellulose, hemicellulose, and lignin which primarily improve the movement of material through the digestive system thereby improving laxation while soluble fractions consists of oligosaccharides, glucans, and glactomanan gums that help in lowering blood cholesterol and regulating blood glucose levels (Guillon and Champ, 2002; Tungland and Meyer**,** 2002; Rodriguez et al., 2006). Presence of these fibers in beans is helpful in controlling slow release of carbohydrates during digestion process and is considered valuable in the management of different diseases (Rizkalla et al., 2002; Jenkins, 2007).

2.3.2 Beans as a source of proteins

During growth phase, bean seeds accumulate proteins mainly in the cotyledon, which provides the free amino acids as well as ammonia and carbon skeletons during germination (Duranti, 2006). Protein concentration is influenced by environmental conditions and genetic factors (Segura and Jimenez, 1999; Hood-Niefer et al., 2012). The major fraction of proteins in legume seeds are storage proteins, which are classified into albumins, globulins, prolamins, and glutelins based on their solubility.

Beans are an excellent source of dietary proteins that play an important role in human nutrition by complementing other foods such as wheat and other cereals (Butt and Batool, 2010). Using protein rich beans along with cereals offer best strategy to combat problem of protein malnutrition (Batista et al., 2011). These types of complementary food strategies are in practice in Latin America and Eastern Africa, Brazil and most parts of the Asia (Broughton et al., 2003; Siddiq et al., 2010).

In contrast to the protein content of cereals, the protein content in beans is being equal to that of meat, ranging from 20-30% (Genovese and Lajolo, 2001; Costa et al., 2006). Globulins and albumins constitute the major protein fractions in pulse proteins while prolamin and glutelin exist as minor fractions (Adebowale et al., 2007). Contrary to other legume proteins, beans contain high amounts of glutelins (20-30%) versus (7-15%) in other legumes (Seena et al., 2005; Slupski, 2010). Globulins constitute the major fraction in beans proteins consisting of 50-70% of total proteins and are classified into 7S and 11S on the basis of their sedimentation coefficients (Tang and Sun, 2011a). Like other legume proteins, beans proteins also contain greater amounts of essential amino acids including lysine that is deficient in cereal grains. Therefore, beans and cereal proteins are nutritionally complementary with respect to essential amino acids and the combined consumption of beans and cereals alleviates these mutual deficiencies ensuring a balanced diet (Broughton et al., 2003; Iqbal et al., 2006; Slupski, 2010). The digestibility of beans proteins is about 79%; the amino acid score is 0.78 and protein digestibility between 0.57–0.68 (FAO/WHO, 1991).

2.3.3 Lipid constituents in beans

Within the micronutrients group, the lipid fraction is the smallest (1.5–6.2 g/100 g) and it is comprised of exogenic unsaturated fatty acids (Mabaleha and Yeboah, 2004). The major lipid components in beans are phospholipids and triacyclglycerols, while minor amounts of

diacylglycerols, hydrocarbons, steryl esters and hydrocarbons may also present. These lipids may also take the form of Phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol in beans (Yoshida et al., 2005). Common beans are also considered an essential source of unsaturated fatty acids, comprising of 61% of the total fatty acids with palmitic, oleic and linoleic acids being the dominant fatty acids. Linolenic acid is dominant among unsaturated fatty acid consisting of 43.1% of the fatty acids in beans (Grela and Gunter, 1995).

2.3.4 Minerals and vitamins in beans

Beans are an essential source of micronutrients such as minerals and vitamins and considered superior to cereals as a source of micronutrients (Welch et al., 2000). Beans have the highest level of mineral contents than other legumes, and are an important source of iron, zinc, copper, phosphorous and aluminum while other minerals are also found in appreciable amounts (Broughton et al., 2003; Shimelis and Rakshit, 2005). The level of iron is highest in beans with a range of 62-150 µg/g, which is mostly present in non-heme form (Elhardallon and Walker, 1992; Vadivel and Janardhanan, 2000). The levels of zinc, copper, phosphorus and aluminum in different beans varieties are found to be in the range of 10.1-10.9, 2.8-10.9, 15.8-64.6 and 6.7- 14.4 µg/g, respectively (Ojijo et al., 2000; Cabrera et al., 2003; Wu et al., 2005). Furthermore, bean seeds can provide 10- 20% of an adult's requirements in terms of minerals such as Fe, P, Mg, Mn, and, in a lower proportion, Zn, Cu, and Ca; nonetheless, the concentrations of Fe, Zn and Ca are lower when compared to food of animal origin. Correlations among mineral contents (Mn, Zn, Ca, Mg, K and P) in an intraspecific cross of two accessions of common beans were reported by Beebe et al.

However, although some studies have shown considerable variation between wild beans and modern cultivars, it seems that domestication does not affect the concentration of iron and zinc in the seed (Paredes et al., 2009). Both these minerals found in common beans are important for the populations of Latin America and Africa (Muhamba- Tryphone & Nchimbi-Msolla, 2010). Pressure-cooking and pre-soaking in water affect the retention of iron and zinc in cooked seeds, which should be complemented with the stock in which they are cooked (Carvalho et al.,2012).

Beans are also considered an important source of vitamins and variations in vitamin contents are observed in different classes of beans (Augustin et.al., 2000). Beans are good source of folate, tocopherols, thiamine, riboflavin, niacin, biotin and pyridoxamine (Broughton et.al., 2003; Campos-Vega et.al., 2010). Higher amount of folate (400-600

µg/g) in beans is sufficient to meet 95% of the daily requirement (Kadam and Salunkhe, 1989). Significant variations have been observed in the distribution of tocopherols in different bean cultivars (Bauerfeind, 1980). Thiamine, riboflavin, niacin, vitamin B6 and folic acid content of raw beans are in the range of 0.81-1.32, 0.112-0.411, 0.85-3.21, 0.299-0.659 and 0.148-0.676 mg/100 g, respectively (Augustin et al., 2000, Campos-Vega et al., 2010).

2.4 Anti-nutritional components

Antinutritional compounds (ANCs) are those biological compounds that reduce nutrient utilization or food intake, thereby contributing to impaired gastrointestinal and metabolic performance (Dunlop, 2004).Although legumes possess valuable nutritional composition, some people reject them because they contain substances, which are considered as non-nutritional, and their intake may have a positive or negative impact, depending on the dose consumed. The seeds accumulate these compounds as a defense mechanism against the attack of parasites, insects, fungi, and herbivorous animals mainly, and as a reserve to continue growing even in adverse conditions (Muzquiz and Wood, 2007).

Despite the relevant potential for human and animal nutrition, beans present several anti-nutritional factors — nowadays, explored also as bioactive compounds carrying on health benefits (Singh et al., 2017), classified into 2 major groups: the first of them corresponds to compounds of protein nature, including lectins, agglutinins, protease inhibitors (like trypsin and chymotrypsin inhibitors), and also bioactive peptides; and the second one, of nonprotein nature, which include alkaloids, phytic acid, phenolic compounds (tannins), and saponins. These factors affect the digestibility and bioavailability of nutrients and limit their consumption (Worku and Sahu, 2017).

Phytic acid, an antioxidant found in cereals, vegetables, nuts and natural oils, ranging from 0.4- 0.6% by weight on beans, can be considered an antinutritional component due to its ability to chelate with mineral cations like calcium, iron, magnesium and zinc, which hinders absorption in the gastrointestinal tract (Silva and Bracarense, 2016).

Trypsin inhibitors constitute an important class of protease inhibitors. As described by Adeyemo and Onilude, 2013, the presence of trypsin inhibitors in human foods interfere in protein digestion, causing pancreatic hyperplasia and metabolic disturbance. Protease inhibitors delay protein digestion, resulting in protein excretion and decreased bioavailability of sulfur-containing amino acids (Nikmaram et al., 2017).

The binding between tannins and minerals or proteins affect digestion process, resulting in reduced absorption and limited availability of nutrients. Because tannins bind to dietary proteins and also to digestive enzymes, formed complexes are not easily digestible

(Nikmaram et al., 2017; Raes et al., 2014). These substances can reduce protein digestibility, diminish nutrient absorption and mineral bioavailability, which may cause flatulence in human (Nikmaram et al., 2017). However, these anti-nutritional factors have antioxidant and prebiotic activities, and protect DNA damage against various cancers (Silva and Bracarense, 2016; Adeyemo and Onilude, 2013). Processing improves the nutritional quality of dry beans by reducing the content of anti- nutritional factors and, at the same time, diversifies their use as ingredients by altering their functional properties. The fact that dry beans, apart from being nutrient-rich, are gluten- free offers significant opportunities for exploiting bean flour use in different food systems (Fan and Beta, 2016).

2.5 Phytochemical compounds in beans

Plants are the source of medicines and produce amazing diversity of low molecular weight compounds. They contain hundreds of thousands of such compounds among which some are primary metabolites that are common to all organism and the rest are secondary metabolites whose biosynthesis is related to selected plant groups. These non- nutrient plant chemical compounds or bioactive compounds are referred to as phytochemicals for plant defense (Abo et al.,1991).

Several studies on beans have revealed that qualitative screening for common beans has shown the presence of alkaloids, anthraquinone, catechitannins, flavonoids, tannins, glucosides, polyphenols, saponins, steroids and terpenoids are present. Phytochemical screening showed the presence of some bioactive components such as alkaloids, anthocyanin, carbohydrate, catechin, fibers, flavonoids, phasine, phytic acid, quercetin, saponins, steroids, tannins and terpenoids and trypsin inhibitor. Analysis using HPLC-MS, identified compounds such as anthocyanins, flavanol monomers, and heterogeneous flavanol oligomers up to hexamers (Madhujith et al., 2004). The presence of catechin, delphinidin, cyaniding, and phenolic acids such as gallic, vanillic, caffeic, coumaric and ferrulic in the seed coat of common beans have been reported (Beninger and Hosfield, 2003). Phenolic compounds such as antocyanins, quercetin glycosides and protoanthocyanidins (condensed tannins), were isolated and identified in dark red kidney beans (Gabriela Espinosa-Alonso et al., 2006).

2.5.1 Phenolic compounds in beans

Plants synthesize secondary metabolites that often have widespread bioactivities, and are

known as phytochemicals. Polyphenol is one of the phytochemicals containing large bioactive structural phenolic units. The phenolic compounds are secondary metabolites essential for growth and reproduction of plants and act as protective agents against pathogens, being secreted as a defense mechanism during stress conditions such as infections and UV radiation, among others (Wink, 2013). The synthesis of phenolic compounds takes place from phenylalanine and tyrosine, and phenolic regulate metabolism and lignin synthesis (Dixon and Paiva, 1995). Polyphenols are classified into different groups based on the function of several phenyl rings, including flavonoids (flavones, flavonols, flavanones, isoflavones, anthocyanins, chalcones, dihydrochalcones, and catechins), phenolic acids (hydroxybenzoic hydroxyphenyl acetic, hydroxyphenyl pentanoic and hydroxyl cinnamic acids), stilbenes, and lignans.

Polyphenols are largely found in fruits, cereals, vegetables, food legumes, herbs, spices, nuts, wine, olive oil, tea, coffee, and chocolate. The major amounts of these phenolic compounds reside in the seed coat while cotyledons may also contain these nutraceutical ingredients but only in small amounts (De Mejia et al., 1999). The level of total phenolics is influenced by both genetic and environmental factors (Elizabeth et al., 2007) and is responsible for color of the seed coat due to diversification and variability in the composition of procyanidins, flavonol glycosides, and anthocyanidins (Feenstra, 1960).

The content of the phenolic compound is about 145 mg/g and represents about 11% of the total seed (Cardador-Martinez et al., 2002). These compounds are present in the edible beans in free form, extracted mainly by hydrophilic solvent water mixtures (e. g. 80% methanol). Moreover, these compounds can be insoluble conjugated form, bound with soluble oligosaccharides and peptides (can be released upon alkaline hydrolysis), and insoluble-bound form that are esterified to cell wall polysaccharides. Furthermore, Chen and co-workers showed that flavonoids are mainly found in free forms, while phenolic acids in conjugated and bound forms. The authors also reported a variation from 7 to 59%, from 28 to 76%, and from 8 to 18% for free, conjugated and bound phenolics, respectively, according to the bean varieties.

Giusti et al. determined the levels of phenolic compounds in different pulses. In fourteen varieties of common beans the levels reported were: gallic acid from 3.1 -7.1 mg/kg, chlorogenic acid from 24 -239.2 mg/kg, catechin from 10-614.3 mg/kg, epicatechin from 17.7- 279.2 mg/kg, syringic acid from 3.7-12.6 mg/kg, kaempferol-3-glucoside from 24.5-1486.3 mg/kg, ferulic acid from 1.7-21.5 mg/kg. Between all beans evaluated only in black beans had anthocyanins in their composition (delphinidin 3,5-diglucoside and cyanidin-3-glucoside) totaling 649.5 mg/kg.

The phenolic compounds give various beneficial health effects to consumers; the primary

biological activities reported for these molecules are antioxidant, anti-inflammatory (Oomah et al., 2010), and inhibition of α-amylase and amyl glucosidase enzymes involved in glucose regulation; it has also been reported that they have inhibitory effect on angiotensin converting enzyme-1 ACE-1 (Ranilla et al., 2010).

The primary functions of polyphenols are as anti-oxidants involved in the prevention of degenerative diseases such as cancer and metabolic syndromes. The health-promoting effects of polyphenols depend on the quantity consumed in the diet and their bioavailability. In addition, polyphenols are the active substances in many food legumes, which regulate the activity of a broad spectrum of cell receptors, enzymes and gene expression. Animal experimental studies showed that polyphenol in common beans possess anti-oxidant properties and have various biological activities including anti- diabetic, anti-obesity, anti-inflammatory, antimicrobial, anticancer, hepatoprotective, cardioprotective, nephroprotective, neuroprotective, and osteoprotective.

Furthermore, nowadays it is important to propose natural alternatives to supplement and preserving food products in substitution to synthetic additives or preservatives. Gan et.al. reported that flavonoids and pro-anthocyanidins from pigmented bean coat extracts showed high antioxidant and antibacterial activity, which can be potential candidates as food preservatives.

2.5.2 Antioxidant activity in beans

Antioxidants are very important compounds that protect the body against damages caused by free radical reactions offering protective function by getting oxidized themselves. Free radicals are atoms or molecules or ions with unpaired valence electrons. Free radicals are generated through normal body metabolism, environmental factors such as pollution, radiation, pesticides and cigarette smoke in which oxygen participates in the reaction. Extreme amounts of free radicals attack cellular components such as DNA, lipids and proteins which is thought to be an initiating factor for several chronic diseases (Yu et al., 2002).

The dry common beans have excellent anti-oxidant activities because of its phenolic acids, flavonoids, stilbenes, and tannins. These anti-oxidant activities are primarily due to the reducing capacity of polyphenols as they play vital functions in neutralizing free radicals and scavenging radicals or suppressing lipid peroxidation (Oomah et al., 2005). In addition, polyphenols involve chelation of metal ions, causing impairment/cessation of oxidative mechanisms. Generally, the anti-oxidant activity is elevated during digestion and absorption

of the common beans in the intestine. Common beans containing polyphenols have demonstrated the highest total anti-oxidant capacity measured by in- vitro methods of 2,2'-diphenyl-1-picrylhydrazyl (DPPH), β-carotene bleaching, ferric reducing anti-oxidant power, oxygen radical absorbing capacity, Trolox equivalent anti- oxidant capacity, and total radical-trapping anti-oxidant parameters (Frassinetti et al., 2015; Kumar and Baojun, 2017). Animal studies have also confirmed that common beans possess the highest anti-oxidant capacity, as measured in various biochemical parameters including thio-barbituric acid reactive substances, hydroperoxides, glutathione, superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, and glutathione S-transferase (Venkateswaran and Pari, 2002).

Sreeramulu et al. (2009) found a significant correlation between total phenolic compounds and antioxidant activity of different legumes including common beans. Recently, Akond et al. (2011) also observed variations in different beans genotypes and found that genotypes with high phenolic compounds exhibited high antioxidant activity.

nts are the source of medicines and produce amazing diversity of low molecular weight compounds. They contain hundreds of thousands of such compounds among which some are primary metabolites that are common to all organism and the rest are secondary metabolites.

2.6 Health promoting effects of beans

The consumption of beans has received increased attention because of their beneficial physiological effects in the prevention and control of broad range of chronic and degenerative diseases such as obesity, cardiovascular diseases, diabetes, and cancer. Similarly, to other health benefits, the chemoprotective activity of legumes is attributed to different anticancer agents, including phytoestrogens, protease inhibitors, saponins, phytates, phytosterols, fiber, proteins, and fatty acids (Daiz-Batalla et.al., 2006; Jenkins, 2007; Chung et.al., 2008).

CHAPTER III

MATERIALS AND METHODS

3.1 Study areas

The whole beans cultivated on high-altitude districts of Nepal were collected and brought at National Agriculture Genetic Resource Center (GeneBank), Lalitpur, Nepal for identification. After proper identification the samples were carried to Central Department of Biotechnology (CDBT), Tribhuvan University for laboratory analyses.

Table 3.1 Details of common beans collected from different locations of Nepal

S. No.	Accession No.	Location	Altitude(m.)	Part of plant used
01	EP 15-01-FB	Jumla	2968	Whole Seeds
02	EP-15-02-FB	Jumla	2968	Whole Seeds
03	EP 15-03-FB	Jumla	2968	Whole Seeds
04	EP 15-04-FB	Jumla	2968	Whole Seeds
05	EP 15-11-FB	Jumla	2968	Whole Seeds
06	EP 15-12-FB	Jumla	2968	Whole Seeds
07	EP 15-15-FB	Jumla	2968	Whole Seeds
08	EP 15-17-FB	Jumla	2968	Whole Seeds
09	KA-17-01-FB	Darchula	2146	Whole Seeds
10	KA-17-02-FB	Darchula	1536	Whole Seeds
11	KA-17-03-FB	Darchula	2146	Whole Seeds
12	KA-17-05-FB	Darchula	2146	Whole Seeds
13	KA-17-06-FB	Darchula	2146	Whole Seeds
14	KA-17-07-FB	Darchula	2146	Whole Seeds
15	KA-17-08-FB	Darchula	2146	Whole Seeds
16	KA-17-09-FB	Darchula	2146	Whole Seeds
17	Dolakha Beans 1 (DB-1)	Dolakha	2050	Whole Seeds
18	Dolakha Beans 2 (DB-2)	Dolakha	2050	Whole Seeds

3.2 Sample preparation

The collected samples of common beans were shed dried for about 10 days and grinded to fine powder. The fine powder was stored in air tight container for further use.

3.3 Proximate analyses

3.3.1 Determination of moisture

Moisture content was determined according to the AOAC (2016) as follows:
Two grams of powdered sample was weighed using a sensitive balance in clean dry and pre-weighed crucible and then placed in an oven at 105^{o}C and left overnight. The crucible was transferred to a desiccator and allowed to cool and then weighed. Further placements in the oven were carried out until approximately constant weight was obtained. The moisture content was calculated by reducing weight of fresh beans samples before drying and weighted.

3.3.2 Determination of carbohydrate

Determination of carbohydrate was based on differential method by AOAC (2016) method. The percentage of carbohydrate content in common beans were determined by taking sum of percentage of protein, crude fat, crude fiber and ash content subtracted from hundred percentage.

3.3.3 Determination of protein

The protein determination involves three steps which were based on Micro Kjeldahl method according to AOAC (2016).
A portion of 2 g of the powdered sample was weighed and placed in small digestion flask (50 mL). About 0.4 g catalyst mixture (96% anhydrous sodium sulphate and 3.5% copper sulphate) was added.3.5 mL of approximately 98% v/w of H_2SO_4 was added. The contents of the flask were then heated on an electrical heater for 2 h. or till the color changed to blue-green. The tubes were then removed from the digester and allowed to cool down until the distillation process.
The distillation apparatus used was an automatically operated one. The tubes were placed in the distillation chamber. Distilled water and 40% NaOH required for the distillation were stored in gallons and connected to the distillation unit. A program was set in the distillation

unit. The ammonia was received in 100 mL conical flask containing 10 mL of 2% boric acid plus 3-4 drops of methyl-red indicator. The distillation was continued until the volume reached 50 mL. The distillate (NH_3) obtained in the flask containing boric acid, was then titrated.

The boric acid having trapped ammonia was titrated with 0.02 N HCl. The color of boric acid having ammonia changed to pink.

3.3.4 Determination of fat

Fat determination was based on protocol of AOAC (2016) using Soxhlet apparatus as follows:

An empty clean and dry exhaustion flask was weighed. About 2 g of powdered sample was weighed and placed in a clean extraction thimble and covered with cotton wool. The thimble was placed in Soxhlet extractor (Cat 09- 551B, Fisher). 150 mL of petroleum ether was poured on the flask and refluxed for 8 hours with a heating mantle. Crude fat was extracted on the flask kept over for 2 hours. The residual ether was dried by evaporation. The flak was placed in an oven at 105^{o}C till it dried completely and then cooled in a desiccator and weighed. The percentage of crude fat content was determined by weight of flask with fat residue minus weight of empty flask divided by weight of original sample multiplied by hundred.

3.3.5 Determination of ash

Ash determination was based on Journal of AOAC (2016). Firstly, 5 g of powdered sample taken in a crucible. The sample charred over a low flame 80°C for 3 hours and kept in a muffle furnace set at 550°c for 5 hours until white ash was obtained. The crucible was taken out and kept in desiccator and weighted. The percentage of ash was calculated as weight of sample before heating in muffle furnace minus weight obtained after ash formed divided by weight of sample taken multiplied by 100.

3.3.6 Determination of crude fiber

Crude Fiber was determined according to AOAC. A portion (2.5 g) of the defatted common beans sample was taken in a beaker and boiled in 200 mL of 1.25% H_2SO_4 solution (0.26N) for 30 minutes. The content was then filtered and washed with distilled water to neutralize the content. The content was transferred again to the beaker and boiled in 250 mL of 1.25%

sodium hydroxide for 30 minutes. The crucible having crude fiber was weighted and crucible was set for heating at 550°C for 3 hours and weight was taken. The percentage of crude fiber was determined from the difference in the weight divided by weight of sample multiplied by 100.

3.4 Phytochemical analyses

3.4.1 Methanolic extract preparation

The extraction technique followed was percolation with intermittent sonication for which the powdered samples was taken in methanol such that the ratio of sample to solvent was maintained at 1:10(w/v). The solvent system was allowed to stand for three days at room temperature and was subjected to sonication. Sonication was done for 75 minutes per day for three days. On the fourth day, the supernatant was filtered through Whatman no.1 filter paper and collected into round bottom flask.

The filtrate so collected was concentrated using rotary vacuum evaporator under reduced pressure until the extract is concentrated enough. The concentrated extract was transferred to petri plates, allowed to dry at room temperature and the crude extract so obtained was scratched then sealed and stored at 4°C until use.

Crude extract (50 g) of each sample was weighed and dissolved in 1 mL methanol to prepare 50 mg/mL sock solution for quantification of total phenolics and total flavonoids.

3.4.2 Qualitative phytochemical analyses

The qualitative phytochemical analysis of crude methanolic extract for the presence of bioactive compounds was performed by using the standard following Harborn and Baxter, 1995, De et al., 2010 and Todkar et al., 2010. The analysis was done by colorimetric or by precipitate formation.

3.4.2.1 Test for alkaloids

a. Mayer's test

To 1 mL of test solution add few drops of Mayer's reagent (Potassium mercuric iodide solution). Cream precipitate indicates the presence of alkaloids.

b. Wagner's test

To 1 mL of test solution add equal volumes of Wagner's reagent (Iodine in Potassium Iodide). Reddish precipitate indicates the presence of alkaloids.

c. Hager's test

To 2 mL of test solution add few drops of Hager's reagent (Saturated Picric Acid Solution). Bright yellow precipitate indicates the presence of alkaloids.

3.4.2.2 Test for glycosides

a. Keller-Kiliani test

To 1 mL of test solution 1mL of glacial acetic acid was added, dissolved and then cooled. After cooling 2-3 drops of ferric chloride was added. Then carefully, 2 mL of conc. H_2SO_4 was added along the walls of test tube. Reddish brown ring at the junction of two layers indicates the presence of glycosides.

b. Molisch's test

To 1 mL of test solution 2-3 drops of Molisch reagent was added and mixed well. Then conc. H_2SO_4 was added along the walls of test tube. Reddish purple ring at the junction of two layers indicates the presence of glycosides.

c. Conc. H_2SO_4 test

To 1 mL of test solution 1mL of conc. H_2SO_4 was added and allowed to stand for 2 min. Red precipitate indicates the presence of glycosides.

3.4.2.3 Test for tannins

a. Ferric chloride test

Few drops of $FeCl_3$ solution was added to the test solution. Blackish precipitate indicates the presence of tannins.

b. Alkaline reagent test

The test solution was treated with sodium hydroxide solution. Yellow to red precipitate indicates the presence of tannins.

3.4.2.4 Test for flavonoids

a. Shinoda test (Magnesium Hydrochloride reduction test)

To 1 mL of the test solution, add few fragments of Magnesium ribbon. Then conc. HCl was added carefully along the walls of the test tube drop wise. Crimson red color indicates the presence of flavonoids.

b. Zinc Hydrochloride reduction test

To 1 mL of test solution a mixture of zinc dust and conc. HCl was added. Appearance of red color after few min. indicates the presence of flavonoids.

c. Conc. H_2SO_4 test

To 1 mL of test solution 1mL of conc. H_2SO_4 was added and allowed to stand for 2 min. Red precipitate indicates the presence of glycosides.

3.4.2.5 Test for phenols

a. Phenol test

To 2 mL of test solution 1 mL of $FeCl_3$ solution was added. Phenol is indicated by an intense color.

b. Ellagic acid test

To 2 mL of test solution add few drops of 5% (w/v) glacial acetic acid and 5% (w/v) sodium nitrate solution. Phenol is indicated by Niger brown precipitate.

3.4.2.6 Test for saponins

3 mL of test solution was taken in a test tube and vigorously shaken. Formation of foam indicates the presence of saponins.

3.4.2.7 Test for steroids

a. Salkowski test

To 1 mL of test solution 5 mL of chloroform was added and then few drops of Conc. H_2SO_4 was added to the above mixture and mixed well. Allowed the mixture to stand for some time, reddish precipitate in the lower layer indicates the presence of steroids.

3.4.3 Quantitative phytochemical analyses

3.4.3.1 Total phenolic content determination

Total phenolic content (TPC) of methanolic extracts were determined by Folin-Ciocalteu phenol reagent (Chang et.al*.,* 2002, Roy et al*.,* 2011 and Singleton & Rossi, 1965). 0.1 mL of each extract (2.5 mg/mL) was separately mixed with 1 mL Folin-Ciocalteu phenol reagent (Merck Ltd, India) (1:10 dilution with the distilled water) and 0.8 mL of aqueous 1M Na_2CO_3 solution. The reaction mixture was allowed to stand for about 15 minutes at room temperature. After completion of incubation, the absorbance of the reaction mixture was measured at 765 nm using the UV-Visible Spectrophotometer (Thermo fisher scientific, Genesystem- 10-5). A calibration curve was obtained using gallic acid (Moly chem, Mumbai, India) as standard in methanol and water mixture (50:50 v/v) with the concentration ranging from 25-250 µg/mL. Total phenolic content of plant extract was then quantified by using standard calibration curve generated. The result was expressed in terms of milligram of gallic acid equivalent per gm extract (mgGAE/g). For each extract, triplicates were performed to get more accurate result.

3.4.3.2 Total flavonoid content determination

Total flavonoid content (TFC) of methanolic extracts were determined using the aluminum chloride colorimetric method (Chang et al*.,* 2002; Roy et al., 2011). 0.25 mL of each extract

(10 mg/mL) was separately mixed with 0.75 mL of methanol, 0.05 mL of 10% aluminum chloride, 0.05 mL of 1M potassium acetate and 1.4 mL of distilled water. The reaction mixtures were shaken and incubated for 30 minutes at room temperature. The absorbance of the mixture was measured at 415 nm using the UV-Visible spectrophotometer (Thermo Fisher Scientific, Genesystem-10-5). Calibration curve was obtained with the help of quercetin (Sigma) as standard solution in methanol with the concentration ranging from 10-100 µg/mL. The total flavonoid content was then expressed in terms of milligram of quercetin equivalent per gram extract (mgQE/g). All the tests were carried in triplicates to get more accurate result.

3.5 Minerals analyses

Sample preparation for mineral were based on the Optimized protocol of DFTQC, which followed the methods of AOAC International. Total ash was taken for the analysis of mineral contents. Two mL of conc. HCl was added to the ash and heated for 2 minutes. One drop of hydrogen peroxide was added into the solution to remove turbidity. The solution was then transferred into a volumetric flask and total volume was made 100 mL by adding deionized water. This was then used to analyze the contents of minerals.

3.5.1 Solution preparation for mineral analyses

The mineral solution of all samples was prepared by dissolving the ash obtained after ashing the samples in a muffle furnace in dilute hydrochloric acid (1:1 v/v).
To the ash that was obtained was added 5 mL of a 1:1 solution of distilled water and fuming HCl. This mixture was then heated over a water bath to dryness before another 5 mL of the solution was added. It was heated further over the water bath until it started fuming and at this point, the crucible was retrieved and its contents filtered into a 100 mL volumetric flask using Whatman No.4 filter paper. After thorough rinsing of the crucible and the filter paper, the volume was made up to the mark with distilled water. Aliquots of this mineral solution were taken for the estimation of all the minerals in this study.

3.5.2 Estimation of calcium

The calcium content was estimated by precipitating it as calcium oxalate and titrating the solution of oxalate in dilute acid against standard potassium permanganate. To an aliquot

(25 mL) of the mineral solution was added a few drops of methyl red indicator and the solution was neutralized with ammonium until the pink color changed to yellow. The solution was heated to boiling and 10 mL of 6 % ammonium oxalate was added. The mixture was then boiled for a few more minutes and glacial acetic acid was added until the color turned distinctly pink. The mixture was kept overnight and when the precipitate settled down, the supernatant was tested with a drop of ammonium Oxalate solution to ensure the completion of the precipitate. The precipitate was then filtered through Whatman No.40 filter paper and washed with water until it was free of oxalate. The precipitate was then transferred along with the filter paper to free of oxalate. The precipitate was then transferred along with the filter paper to the same beaker and about 5 mL of 2N dilute H_2SO_4 was then titrated against N/$KMNO_4$ solution.

3.5.3 Estimation of iron

Iron was determined by colorimetric method. When potassium thiocyanate was added to the sample it turned red indicating the presence of iron in a sample (1 mL or less) of the micronutrient solution enough water is added (if necessary) to make up to volume of 6.5 mL followed by 1 mL of 30 % H_2SO_4, 1.0 mL of saturated potassium per sulphate and 1.5 mL 40 % KCNS solution. The red color that develops is measured within 20 min at 540 nm.

3.5.4 Estimation of copper

Copper content of beans samples were estimated by using atomic absorption spectrophotometer and the results were expressed as mg per 1000 g of beans sample. 100 ppm standard Cu^{2+} solution was prepared using 1000 ppm Cu^{2+} atomic absorption spectrophotometer solution and appropriate dilutions were made to get standard solution ranging from 0-0.6 ppm. These standards were fed to atomic absorption spectrophotometer as that of sample to get standard curve as a graph was fit. To this standard curve the sample readings were compared.

3.5.5 Estimation of magnesium

5 mL of the mineral solution was taken in porcelain dish and this was diluted with 25 mL of distilled water. A sufficient quantity of buffer solution (about 10 mL) was added, followed by 3- 5 drops of EBT indicator and this solution was titrated against standard EDTA solution

with constant stirring until a sky-blue end point reached. The titer volume was recorded.

3.5.6 Estimation of sodium

Sodium in solution was atomized into an oxyhydrogen or oxyacetylene flame. The flam excites atoms of sodium causing them to emit radiation at specific wave lengths. The amount of radiation emitted is measured on a spectrophotometer. Under standard conditions, it is proportional to the concentration of sodium in the solution. The sodium content of the sample was estimated from a standard curve prepared with standard sodium solution (range 0 - 150 ppm).

3.5.7 Estimation of potassium

Potassium in solution was atomized into an oxyhydrogen or oxyacetylene flame. The flam excites atoms of potassium causing them to emit radiation at specific wave lengths. The amount of radiation emitted is measured on a spectrophotometer. Under standard conditions, it is proportional to the concentration of potassium in the solution. The potassium content of the sample was estimated from a standard curve prepared with standard potassium solution (rang 0 - 150 ppm).

3.5.8 Estimation of zinc

Zinc content of the beans samples were estimated by using atomic absorption spectrophotometer and the results were expressed as mg per 1000 g of beans sample. 100 ppm standard Zn^{2+} solution was prepared using 1000 ppm Zn^{2+} atomic absorption spectrophotometer solution and appropriate dilutions were made to get standard solution ranging from 0-0.6 ppm. These standards were fed to atomic absorption spectrophotometer as that of sample to get standard curve as a graph was fit. To this standard curve the sample readings were compared.

3.6 Statistical data analyses

All the experiments were performed in triplicates for each sample. Statistical analysis was done using Microsoft Excel software (Microsoft Excel 2019 version 1910).

CHAPTER IV
RESULTS AND DISCUSSIONS

4.1 Proximate composition

The proximate analysis i.e. moisture, carbohydrate, crude protein, crude fat, total ash and crude fiber, has been tabulated in Table 4.1. All nutrient and minerals contents have been presented on dry basis except moisture content.

Moisture content is an index of storage ability of the flour and resistance to microbial growth. The moisture content of the 18 accessions ranged from 6.49±0.06% to 8.28±0.35%.

Carbohydrates are also a source of energy. The carbohydrate content was found to be ranged from 61.94±0.04% to 69.57±0.20%. Similar carbohydrate content has been reported by Chávez-Mendoza & Sánchez, 2017.

The crude protein of the 18 accessions of common beans ranged from 19.40±0.19% to 24.55±0.13%. This report is similar to that of who reported crude protein 16.50% (L, Kouakou, Brou, Kouadio, & Gnakri, 2010). Variations in protein content can be attributed to different environmental conditions, genotype, and analytical methods. In addition, protein content is sensitive to rainfall, light intensity, length of growing season, day duration, temperature and agronomic practices.

Result for ash content demonstrated the range from 3.61±0.05% to 4.95±0.10%. Similar result as of 3.61±0.00% was reported by Fan and Beta, 2016. Ash content is also an indicator of their mineral content.

Crude fiber refers to the indigestible plant material with significant health benefits such as lowering blood cholesterol, reducing hypertension, etc. The fiber content recorded in current study ranged between 3.39±0.09% to 4.96±0.13% supporting the findings as of 5.05% (Lee et al.,2010).

Table 4.1 Proximate composition of common beans.

Accession No.	Moisture (%)	Carbohydrate (% by difference)	Crude protein (%)	Crude fat (%)	Total ash (%)	Crude fiber (%)
EP 15-01-FB	6.59±0.20	67.16±0.16	22.50±0.08	2.07±0.05	3.91±0.05	4.92±0.06
EP-15-02-FB	6.81±0.08	66.92±0.39	21.04±0.23	3.47±0.06	4.52±0.16	4.96±0.13
EP 15-03-FB	7.86±0.05	63.22±0.17	24.55±0.13	2.50±0.04	4.95±0.10	4.94±0.12
EP 15-04-FB	6.97±0.07	65.69±0.40	19.40±0.19	2.87±0.09	4.91±0.30	4.38±0.25
EP 15-11-FB	6.75±0.18	66.94±1.52	22.97±0.24	2.58±0.09	3.98±0.16	3.39±0.09
EP 15-12-FB	8.84±0.19	64.91±0.10	21.05±0.23	2.98±0.09	4.82±0.09	4.02±0.05
EP 15-15-FB	7.78±0.06	66.90±0.08	21.82±0.29	2.74±0.12	4.69±0.06	4.59±0.10
EP 15-17-FB	7.71±0.16	66.95±0.69	21.93±0.13	2.18±0.10	3.94±0.23	4.95±0.07
KA-17-01-FB	6.94±0.12	66.10±0.54	22.32±0.11	1.98±0.06	4.41±0.20	4.16±0.20
KA-17-02-FB	7.21±0.21	66.92±0.15	20.96±0.09	2.78±0.10	4.81±0.06	4.94±0.40
KA-17-03-FB	7.00±0.08	66.82±0.66	21.95±0.08	2.88±0.12	4.88±0.10	4.84±0.12
KA-17-05-FB	8.33±0.16	66.32±0.57	21.95±0.22	3.01±0.03	4.79±0.06	4.71±0.10
KA-17-06-FB	7.71±0.16	66.96±0.38	21.98±0.35	2.28±0.11	4.88±0.06	4.37±0.19
KA-17-07-FB	8.25±0.15	67.35±0.51	20.55±0.39	3.58±0.06	4.89±0.09	4.80±0.06
KA-17-08-FB	8.60±0.06	61.94±0.04	22.93±0.11	2.83±0.18	3.93±0.13	4.59±0.09
KA-17-09-FB	7.90±0.09	63.14±0.41	21.69±0.21	2.43±0.13	3.82±0.17	4.73±0.18
*DB1	8.28±0.35	68.04±0.46	20.88±0.25	2.83±0.11	3.61±0.05	4.58±0.09
*DB2	6.49±0.06	69.57±0.20	19.95±0.08	2.20±0.18	3.97±0.05	4.08±0.18

Values are recorded as mean ±SD (standard deviation)

*DB1- Dolakha Beans-1

*DB2- Dolakha Beans-2

4.2 Yield of bean extract

The beans samples collected from various high-altitude of Nepal were subjected to ultrasonic extraction. The amount of methanolic extract extracted is shown in following Fig. 4.1.

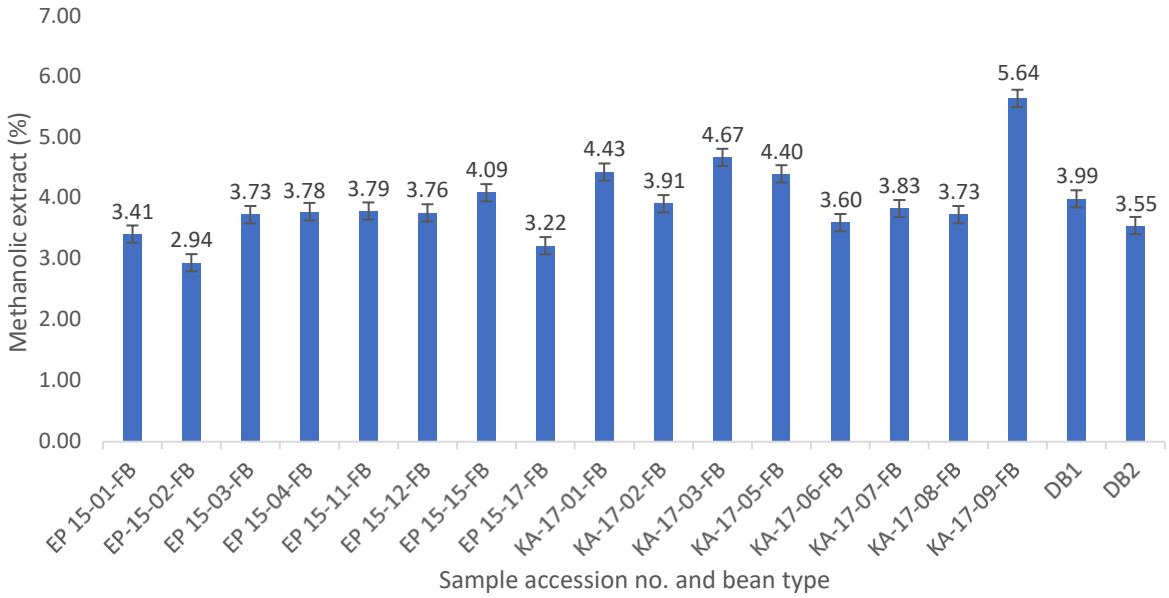


Fig. 4.1 Methanolic extract (%) of different bean samples after sonication

4.3 Preliminary phytochemical screening

The extract of selected samples was subjected to preliminary phytochemical analysis. Preliminary phytochemical screening indicated the presence of various pharmacologically important bioactive compounds such as alkaloids, flavonoids, phenols, etc. The medicinal properties of these bioactive compounds are due to the presence of chemical substances that produce physiological action on human body.

The phytochemicals act as antioxidant, anti-inflammatory and prevention of many diseases like obesity, aging, diabetes, heart related diseases and many health-related problems to cure and prevention of many physiological disorders as therapeutics.

Alkaloids are widely used as cancer chemotherapeutic agents as it interferes with the cell division in the plant.

Glycosides have been found to be effective in increasing the force of myocardial contraction and modify vascular resistance and capacitance.

Saponins are glycosides of both triterpenes and sterols having hypertensive and cardiac

Table 4.2 Preliminary phytochemical analysis of common beans

Tests	EP-15-01-FB	EP-15-02-FB	EP-15-03-FB	EP-15-04-FB	EP-15-11-FB	EP-15-12-FB	EP-15-15-FB	EP-15-17-FB	KA-17-01-FB	KA-17-02-FB	KA-17-03-FB	KA-17-05-FB	KA-17-06-FB	KA-17-07-FB	KA-17-08-FB	KA-17-09-FB	DB1	DB2
Alkaloid Test																		
Mayer's Test	++	++	++	++	++	++	++	+	+	+	+	+	+	+	+	+	++	++
$FeCl_3$ Test	++	++	++	++	++	++	+	+	+	+	++	+	+	++	+	+	++	++
Tannic Acid Test	++	++	++	+	++	++	++	+	+	+	++	+	+	+	+	+	++	++
Glycosides Test																		
Keller-Killani Test	+	+	+	+	+	+	+	+	+	+	++	+	+	++	+	+	++	++
Conc. H_2SO_4 Test	++	+	++	++	++	++	++	+	+	++	++	++	++	++	++	++	++	++
Tannin and Flavonoid Test																		
$FeCl_3$ Test	+	+	+	+	+	++	++	+	++	++	++	++	++	+	++	+	++	++
Alkaline Reagent Test	+	++	++	+	+	++	++	+	++	++	+	++	++	++	++	+	++	++
Saponins																		
Froth Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Note: +, presence; ++, highly presence

depressant properties, hence the presence of these metabolites in P. vulgaris seeds tend to support its medicinal uses (Trease & Evans, 2002).

The presence of tannins also, showed that the seed could be used as purgative, cough, asthma and hay fever (Ocho-Anini et al., 2010).

4.4 Total phenolic content

A calibration curve Y=0.0123x-0.0793, R2=0.9789 was obtained by using the standard solution of gallic acid ranging from concentration of 25-250µg/mL. Based on the calibration curve equation, the concentration of the total phenol content present in methanolic extract of eighteen common beans was determined and expressed as mgGAE/g.

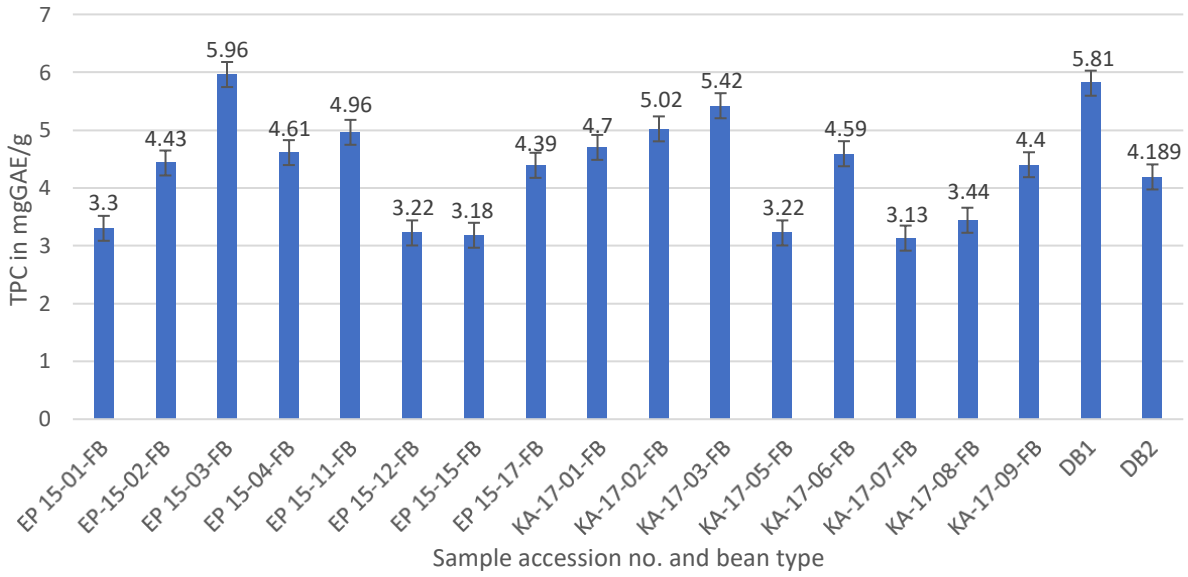


Fig. 4.2 Total phenolic content (TPC) of different bean samples

The total phenolic content of Phaseolus extract was found to be varied from 3.13 mgGAE/g to 5.96 mgGAE/g. Highest concentration (5.96 mgGAE/g) of phenol content was found in methanolic extract of EP-15-03-FB while lowest concentration (3.13 mgGAE/g) was found in methanolic extract of KA-17-07-FB.4 out of 18 bean samples contain phenol more than 5mgGAE/g, 8 bean samples in between 4-5 mgGAE/g and remaining 6 bean samples contain less than 4 mgGAE/g.

Phenolic compounds are secondary metabolites that are derivatives of pentose phosphate, shikimate, and phenylpropanoid pathways in plants. The phenolic compounds are among

the important class of compounds that acts as a good antioxidant and free radical terminators (Pourmorad et al., 2006).

4.5 Total flavonoid content

A calibration curve Y=0.0113x+0.011, R^2=0.9735 was obtained by using the standard solution of ranging from concentration of 10- 100 µg/mL. Based on the calibration curve equation, the concentration of the total flavonoid content present in methanolic extract of common bean was determined and expressed as mgQE/g.

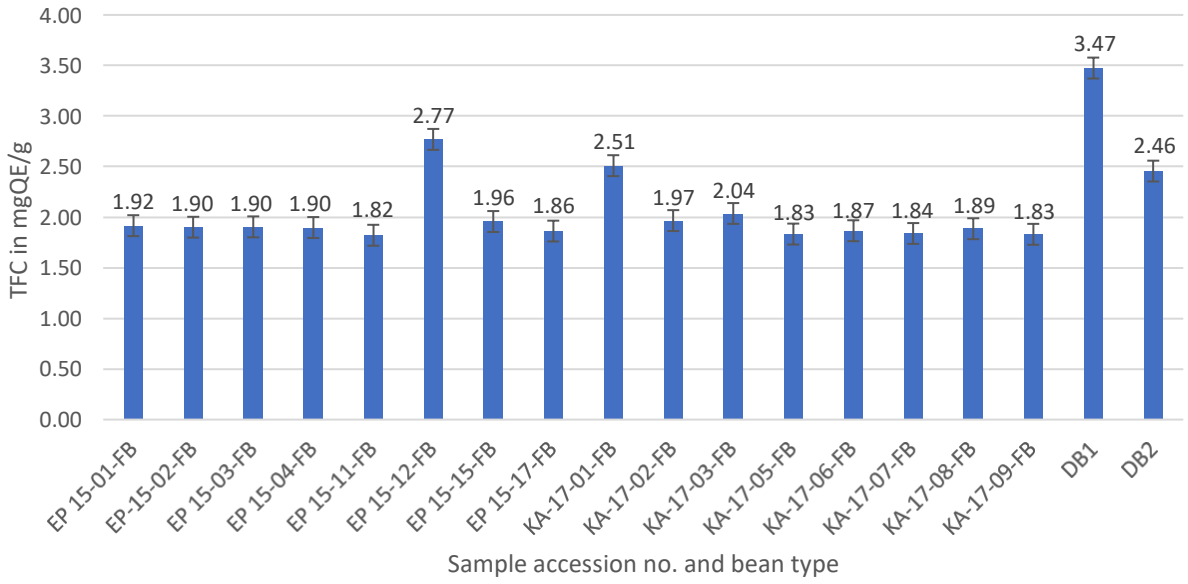


Fig. 4.3 Total flavonoid content (TPC) of different bean samples

Highest concentration (3.47 mgQE/g) of flavonoid content was found in methanolic extract of Dolakha Beans-1 (DB1) while lowest concentration (1.82 mgQE/g) was found in methanolic extract of EP-15-11-FB. Out of 18 bean samples, the flavonoid content of 5 samples were found greater than 2 mgQE/g and remaining 13 samples were found to contain flavonoid content less than 2 mgQE/g.

Flavonoids are a group of poly phenolic compounds with known properties which include free scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. It has been recognized that flavonoids show antioxidant activity and their effects in human health and nutrition is considerable (Lee et al., 2002).

4.5 Minerals content

Mineral are essential for human health. The amount or concentration of the mineral plays an important role in the different organs and cellular mechanisms to maintain the physical structure and strength of the body. Mineral status must be known before using them.

Table 4.3 Mineral analysis of common beans

Accession No	Mineral content(mg/kg)						
	Calcium	**Iron**	**Copper**	**Magnesium**	**Sodium**	**Potassium**	**Zinc**
EP 15-01-FB	1295.50	73.69	4.64	1895.10	695.81	16473.42	31.00
EP-15-02-FB	1234.05	69.10	2.72	1690.38	403.29	14504.81	26.31
EP 15-03-FB	1363.11	72.30	4.68	1890.78	468.43	15454.66	27.26
EP 15-04-FB	2241.37	59.83	4.92	1725.04	408.09	13849.17	24.95
EP 15-11-FB	1386.22	65.73	8.24	1863.27	414.99	13560.21	25.73
EP 15-12-FB	1035.21	38.93	2.95	1701.96	373.57	15339.06	21.15
EP 15-15-FB	1514.55	54.42	4.08	1771.35	205.91	15462.15	22.20
EP 15-17-FB	1999.51	68.74	5.70	1936.45	248.40	16957.05	26.67
KA-17-01-FB	1048.87	48.44	5.75	1976.33	232.64	14263.77	24.33
KA-17-02-FB	1671.68	54.90	2.82	2083.52	219.84	15544.14	19.80
KA-17-03-FB	1742.37	60.58	9.13	1870.15	330.94	14333.78	26.29
KA-17-05-FB	843.86	58.68	5.89	1708.54	254.70	16181.43	24.49
KA-17-06-FB	1061.91	56.43	5.84	1621.26	275.58	14891.17	25.57
KA-17-07-FB	1116.31	61.77	6.73	1540.63	305.51	16666.40	26.20
KA-17-08-FB	1295.61	69.86	7.28	1732.59	246.20	15146.08	25.10
KA-17-09-FB	1254.82	70.37	8.77	1606.76	382.54	13968.87	27.01
DB1	1567.92	59.19	7.98	1755.33	334.84	14283.75	24.86
DB2	2763.62	56.44	5.41	1982.69	387.94	13745.36	22.66

because it is necessary to know levels of toxicity. The presence of high and low amount of minerals showed typical disorders in physiological activities and construction of the body. The copper content in 18 accessions of common beans was found in the range from 2.71 mg/kg to 9.13 mg/kg with highest in KA-17-03-FB (9.13 mg/kg) and lowest in EP-15-03-FB (2.71 mg/kg). Copper acts as an antioxidant by protecting the brain and nervous system. Although, copper is an essential metal, it can also produce toxic effects when the metal intake is excessively high.

The magnesium content was found in the range from 1540.63 mg/kg to 2083.52 mg/kg with highest in KA-17-02-FB (2083.52mg/kg) and lowest in KA-17-07-FB (1540.63 mg/kg). Magnesium is an active component of several enzymes , regulates diverse biochemical reactions in the body, including protein synthesis, muscle and nerve functions, blood glucose control and blood pressure regulation of the body. It also keeps bones strong and heart rhythm steady.

The sodium content in the 18 accessions of beans were determined to be in range from 205.91 mg/kg to 695.81 mg/kg. The highest concentration of sodium was found in the sample EP-15- 01-FB (695.81 mg/kg) and lowest concentration was found in sample EP-15-15-FB (205.91 mg/kg). Sodium is the principal extracellular cation and is used for acid-base balance and osmoregulation. Sodium stimulates cell proliferation, protein synthesis and increase cell mass.

The iron content in the eighteen accessions of beans were found in the range from 38.93 mg/kg to 73.69 mg/kg. Iron aids in transport of oxygen in red blood cells and in muscles. It is an important constituent of succinate dehydrogenase and is also a part of the heme of hemoglobin, myoglobin and the cytochromes.

The calcium content in 18 accessions of common beans was found in the range from 843.855 mg/kg to 2763.620 mg/kg. Calcium functions in bone formation and blood coagulation (Seidu et al., 2015). Calcium is a co-factor for many enzymes; this means that without the presence of calcium, these important enzymes cannot work as efficiently. It also affects the smooth muscle that surrounds blood vessels, causing it to relax.

The potassium content in the 18 accessions of beans were determined to be in range from 13745.36 mg/kg to 16957.05 mg/kg. Potassium is the principal cation in intracellular fluid. It functions in acid-base balance, regulation of osmotic pressure, conduction of nerve impulse, muscle contraction particularly the cardiac muscle, and cell membrane function (Murray et al.,2000). Potassium is a building block of body tissue.

The zinc content in the eighteen accessions of beans were found in the range from 19.80 mg/kg to 31.00 mg/kg. Zinc is an important trace mineral that people need to stay healthy.

Of the trace minerals, this element is second only to iron in its concentration in the body. It is needed for the body's defensive (immune) system to properly work. It plays a role in cell division, cell growth, wound healing, and the breakdown of carbohydrates. During pregnancy, infancy, and childhood the body needs zinc to grow and develop properly. Zinc also enhances the action of insulin.

CHAPTER V
CONCLUSIONS

This is the first attempt to analyze proximate composition and bioactive compounds of the common beans grown in high altitude of Nepal. The proximate composition of moisture, carbohydrate, crude protein, crude fat, total ash and crude fiber ranged from 6.49 to 8.84, 61.94 to 67.35, 19.40 to 24.55, 1.98 to 3.58, 3.61 to 4.91 and 4.02 to 4.96 % respectively. The methanolic extract of the bean samples ranged from 2.94 to 5.64 %. The total phenolic content ranged from 3.22 to 5.96 mgGAE/g and total flavonoid content ranged from 1.82 to 3.47 mgQE/g. Similarly, the mineral content of calcium, iron, copper, magnesium, sodium, potassium and zinc ranged from 843.86 to 2763.62, 38.93 to 73.66, 2.72 to 9.13, 1540.63 to 2083.52, 205.91 to 695.81, 13560.21 to 16957.05 and 19.80 to 31.00 mg/kg respectively.

This study suggested that the common beans cultivated at high altitude are profuse of bioactive compounds and minerals as discussed above. Being rich in nutritional and phytochemical constituents, the bean flour could be used to complement conventional wheat flour which are low in protein, fiber, essential amino acids, and bioactive compounds. Variations in nutritional content and phytochemicals can be attributed to different environmental conditions and genotypes. Similarly, this study will reflect the condition of cultivation and the impact on human health created by beans consumed.

RECOMMENDATIONS

This study is focused only on the nutritional and phytochemical screening of common beans cultivated on high altitude regions of Nepal. Further recommendations on this research are listed below:

1. Since common beans are rich source of protein, minerals and bioactive compounds, their flour can be used to fortify foods with good nutritional value.
2. The study conducted on samples from few high-altitude regions of Nepal. Future works should focus on taking samples from as many as regions as possible.
3. This study is only focused on nutritional value present in samples. Further phyto-pharmacological study can be performed.

REFERENCES

Abo, K. A., Ogunleye, V. O., & Ashidi, J. S. (1999). Antimicrobial potential of Spondias mombin, Croton zambesicus and Zygotritonia crocea. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *13*(6), 494-497.

Adebowale, Y. A., Adeyemi, I. A., Oshodi, A. A., & Niranjan, K. (2007). Isolation, fractionation and characterization of proteins from Mucuna bean. *Food Chemistry*, *104*(1), 287-299.

Adeyemo, S. M., & Onilude, A. A. (2013). Enzymatic reduction of anti-nutritional factors in fermenting soybeans by Lactobacillus plantarum isolates from fermenting cereals. *Nigerian Food Journal*, *31*(2), 84-90.

Akond, A. S. M. G. M., Khandaker, L., Berthold, J., Gates, L., Peters, K., Delong, H., & Hossain,K. (2011). Anthocyanin, total polyphenols and antioxidant activity of common bean. *American Journal of Food Technology*, *6*(5), 385-394.

Alajaji, S. A., & El-Adawy, T. A. (2006). Nutritional composition of chickpea (Cicer arietinum L.) as affected by microwave cooking and other traditional cooking methods. *Journal of Food Composition and Analysis*, *19*(8), 806-812.

AL, O. A. A., Kouakou, T. H., Brou, K. D., Kouadio, Y. J., & Gnakri, D. (2010). Evaluation of bioactive components in seeds of *Phaseolus vulgaris* L.(fabaceae) cultivated in Côte d'Ivoire. *Journal of Applied Biosciences*, *31*, 1928-1934.

AOAC. (2016). Official Methods of Analysis.20th Edition. Association of Official Analytical Chemists, Washington DC Applied Science 7: 2216-2221.

Augustin, J., Beck, C. B., Kalbfleish, G., Kagel, L. C., & Matthews, R. H. (1981). Variation in the vitamin and mineral content of raw and cooked commercial Phaseolus vulgaris classes. *Journal of Food Science*, *46*(6), 1701-1706.

Batista, K. A., Prudêncio, S. H., & Fernandes, K. F. (2011). Wheat Bread Enrichment with Hard-to-Cook Bean Extruded Flours: Nutritional and Acceptance Evaluation. *Journal of Food Science*, *76*(1), S108-S113.

Beebe, S., Gonzalez, A. V., & Rengifo, J. (2000). Research on trace minerals in the common bean. *Food and Nutrition Bulletin*, *21*(4), 387-391.

Beninger, C. W., & Hosfield, G. L. (2003). Antioxidant activity of extracts, condensed tannin fractions, and pure flavonoids from Phaseolus vulgaris L. seed coat color genotypes. *Journal of agricultural and Food chemistry*, *51*(27), 7879-7883.

Boualga, A., Prost, J., Taleb-Senouci, D., Krouf, D., Kharoubi, O., Lamri-Senhadji, M. & Bouchenak, M. (2009). Purified chickpea or lentil proteins impair VLDL metabolism and lipoprotein lipase activity in epididymal fat, but not in muscle, compared to casein, in growing rats. *European journal of nutrition*, *48*(3), 162-169.

Bradstreet, R. B. (1965). The Kjeldahl digestion. *The Kjeldahl method for organic nitrogen. Academic Press, New York, 9-88.*

Bravo, L., Siddhuraju, P., & Saura-Calixto, F. (1998). Effect of various processing methods on the in vitro starch digestibility and resistant starch content of Indian pulses. *Journal of Agricultural and Food Chemistry*, *46*(11), 4667-4674.

Broughton, W. J., Hernandez, G., Blair, M., Beebe, S., Gepts, P., & Vanderleyden, J. (2003). Beans (Phaseolus spp.)–model food legumes. *Plant and soil*, *252*(1), 55-128.

Butt, M. S., & Batool, R. (2010). Nutritional and functional properties of some promising legumes protein isolates. *Pakistan Journal of Nutrition*, *9*(4), 373-379.

Cabrera, C., Lloris, F., Gimenez, R., Olalla, M., & Lopez, M. C. (2003). Mineral content in legumes and nuts: contribution to the Spanish dietary intake. *Science of the Total Environment*, *308*(1-3), 1-14.

Campos-Vega, R., Loarca-Piña, G., & Oomah, B. D. (2010). Minor components of pulses and their potential impact on human health. *Food Research International*, *43*(2), 461- 482.

Cardador-Martinez, A., Castano-Tostado, E., & Loarca-Pina, G. (2002). Antimutagenic activity of natural phenolic compounds present in the common bean (Phaseolus vulgaris) against aflatoxin B 1. *Food Additives & Contaminants*, *19*(1), 62-69.

Carvalho, L. J., Corrêa, M., Pereira, E., Nutti, M., Carvalho, J. V., Ribeiro, E. G., & Freitas, S. (2012). Iron and zinc retention in common beans (Phaseolus vulgaris L.) after home cooking. *Food & Nutrition Research*, *56*(1), 15618.

Chávez-Mendoza, C., & Sánchez, E. (2017). Bioactive compounds from Mexican varieties of the common bean (Phaseolus vulgaris): Implications for health. *Molecules*, *22*(8), 1360.

Chen, P. X., Bozzo, G. G., Freixas-Coutin, J. A., Marcone, M. F., Pauls, P. K., Tang, Y. & Tsao, R. (2015). Free and conjugated phenolic compounds and their antioxidant activities in regular and non-darkening cranberry bean (Phaseolus vulgaris L.) seed coats. *Journal of functional foods*, *18*, 1047-1056.

Cho, K. M., Ha, T. J., Lee, Y. B., Seo, W. D., Kim, J. Y., Ryu, H. W., ... & Lee, J. H. (2013). Soluble phenolics and antioxidant properties of soybean (Glycine max L.) cultivars with varying seed coat colours. *Journal of Functional Foods*, *5*(3), 1065-1076.

Christenhusz, M. J., & Byng, J. W. (2016). The number of known plants species in the world and its annual increase. *Phytotaxa*, *261*(3), 201-217.

Chung, H. J., Liu, Q., Pauls, K. P., Fan, M. Z., & Yada, R. (2008). In vitro starch digestibility, expected glycemic index and some physicochemical properties of starch and flour from common bean (Phaseolus vulgaris L.) varieties grown in Canada. *Food Research International*, *41*(9), 869-875.

Crujeiras, A. B., Goyenechea, E., & Martínez, J. A. (2010). Fruit, vegetables, and legumes consumption: Role in preventing and treating obesity. In *Bioactive foods in promoting health (pp. 359-380).* Academic Press.

Darmadi-Blackberry, I., Wahlqvist, M. L., Kouris-Blazos, A., Steen, B., Lukito, W., Horie, Y., & Horie, K. (2004). Legumes: the most important dietary predictor of survival in older people of different ethnicities. *Asia Pacific Journal of Clinical Nutrition*, *13*(2), 217-220.

de Almeida Costa, G. E., da Silva Queiroz-Monici, K., Reis, S. M. P. M., & de Oliveira, A. C. (2006). Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. *Food chemistry*, *94*(3), 327-330.

de Mejıa, E. G., Castano-Tostado, E., & Loarca-Pina, G. (1999). Antimutagenic effects of natural phenolic compounds in beans. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *441*(1), 1-9.

Delgado-Salinas, A. O. (2012). La historia natural del frijol (Phaseolus). *Fundación Herdez. El*

frijol. Un regalo de México al mundo [online]. Available from http://www. fundacionherdez. com. mx [accessed 10 Jul. 2013].

Díaz-Batalla, L., Widholm, J. M., Fahey, G. C., Castaño-Tostado, E., & Paredes-López, O. (2006). Chemical components with health implications in wild and cultivated Mexican common bean seeds (Phaseolus vulgaris L.). *Journal of Agricultural and Food Chemistry*, *54*(6), 2045-2052.

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

Duranti, M. (2006). Grain legume proteins and nutraceutical properties. *Fitoterapia*, *77*(2), 67-82.

Elhardallou, S. B., & Walker, A. F. (1992). Binding of iron by three starchy legumes in the presence of iron alone, with calcium or with calcium, zinc, magnesium and copper. *International Journal of Food Sciences and Nutrition*, *43*(2), 61-68.

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, *46*, S33-50.

Espinosa-Alonso, L. G., Lygin, A., Widholm, J. M., Valverde, M. E., & Paredes-Lopez, O. (2006). Polyphenols in wild and weedy Mexican common beans (Phaseolus vulgaris L.). *Journal of Agricultural and Food Chemistry*, *54*(12), 4436-4444.

Evans, W. C. (2002). Trease and Evans. Pharmacognosy, WB Saunders. *Edinburgh, London*, *72*. Harborne J, Phytochemical methods. Chapman and Hall, Ltd. London; 1973, p.49-88.

Fan G, Beta T (2016): Proximate composition, phenolic profiles and antioxidant capacity of three common bean varieties (Phaseolus vulgaris L.). Journal of Food Chem Nanotechnology, 23: 147-152.

FAOSTAT. (2013). Food and agriculture organization of the United Nations. Bean production in Mexico.

Feenstra, W. J. (1960). *Biochemical aspects of seedcoat colour inheritance in Phaseolus vulgaris L* (Doctoral dissertation, Veenman).

"Food Analytical Methods." (2009):110-115.

Friedman, M., & Brandon, D. L. (2001). Nutritional and health benefits of soy proteins. *Journal of Agricultural and Food Chemistry*, *49*(3), 1069-1086.

Ganesan, K., & Xu, B. (2017). Polyphenol-rich dry common beans (*Phaseolus vulgaris* L.) and their health benefits. *International journal of molecular sciences*, *18*(11), 2331.

Garrido, A., de la Maza, M. P., & Valladares, L. (2003). Potential health benefits of phytoestrogens. *REVISTA MEDICA DE CHILE*, *131*(11), 1321-1328.

Genovese, MI, & Lajolo, FM (2001). Trypsin inhibitory activity of beans (Phaseolus vulgaris L.): critical evaluation of the determination methods. *Latin American* files *of Nutricion* , *51*(4), 386-394.

Gepts, P. (2001). Phaseolus vulgaris (Beans). Retrieved from http:// www.plantsciences.ucdavis.edu/gepts/a1749.pdf.

Grela, E. R., & Günter, K. D. (1995). Fatty acid composition and tocopherol content of some legume seeds. *Animal Feed Science and Technology*, *52*(3-4), 325-331

Guillon, F., & Champ, M. J. (2002). Carbohydrate fractions of legumes: uses in human nutrition and potential for health. *British Journal of Nutrition*, *88*(S3), 293-306.

Haddad, E. H., & Tanzman, J. S. (2003). What do vegetarians in the United States eat? *The American Journal of Clinical Nutrition*, *78*(3), 626S-632S.

Hayat, I., Ahmad, A., Masud, T., Ahmed, A., & Bashir, S. (2014). Nutritional and health perspectives of beans (Phaseolus vulgaris L.): an overview. *Critical Reviews in Food Science and Nutrition*, *54*(5), 580-592.

Hermsdorff, H. H. M., Zulet, M. A., Abete, I., & Martínez, J. A. (2011). A legume-based hypocaloric diet reduces proinflammatory status and improves metabolic features in overweight/obese subjects. *European Journal of Nutrition*, *50*(1), 61-69.

Hood-Niefer, S. D., Warkentin, T. D., Chibbar, R. N., Vandenberg, A., & Tyler, R. T. (2012). Effect of genotype and environment on the concentrations of starch and protein in, and the physicochemical properties of starch from, field pea and fababean. *Journal of the Science of Food and Agriculture*, *92*(1), 141-150.

Hughes, J. S., & Swanson, B. G. (1989). Soluble and insoluble dietary fiber in cooked common bean (Phaseolus vulgaris) seeds. *Food Structure*, *8*(1), 4.

Hutchins, A. M., Winham, D. M., & Thompson, S. V. (2012). Phaseolus beans: impact on glycaemic response and chronic disease risk in human subjects. *British Journal of Nutrition*, *108*(S1), S52-S65.

Iqbal, A., Khalil, I. A., Ateeq, N., & Khan, M. S. (2006). Nutritional quality of important food legumes. Food chemistry, 97(2), 331-335.

Janardhanan, V. V. K. (2000). Nutritional and anti-nutritional composition of velvet bean: an under-utilized food legume in South India. *International Journal of Food Sciences and Nutrition*, *51*(4), 279-287.

Jenkins, A. L. (2007). The glycemic index: Looking back 25 years. Cereal Foods World, 52(2), 50-53.

Jones, A. L. (1999). "Phaseolus bean: Post-harvest operations." *Post-harvest compendium. (Food and Agriculture Organization of the United Nations: Rome)* (1999).

Jones, A. L. (2011). Phaseolus bean. Post-harvest operation. Food and Agriculture Organization of the United Nations.

Judd, W. S., Campbell, C. S. Kellogg, E. A. Stevens, P.F. Donoghue, M. J. (2002), Plant systematics: a phylogenetic approach, Sinauer Axxoc, 287-292. ISBN 0-87893-403.

Kadam, S. S. and Salunkhe, D. K. (1989). Minerals and vitamins. In: Handbook of World Food Legumes, pp. 117–121. Salunkhe, D. K., Ed., CRC Press, Boca Raton, FL.

Kuwar R.M, Nepal B.K, Kshhetri H.B, Rai S.K, Bussmann R.W, (2006). Ethnomedicine in Himalayan: a case study from Dolpha, Humla, Jumla and Mustang districts of Nepal. Journal of Ethinobilogy and Ethnomedicine.2, 27.

KIMURA, T., & KOAZE, H. (2000). Composition, soaking and softening characteristics of some Kenyan beans (Phaseolus vulgaris L.). *Food science and technology research*, *6*(1), 12-18.

Kutoš, T., Golob, T., Kač, M., & Plestenjak, A. (2003). Dietary fibre content of dry and processed beans. *Food chemistry*, *80*(2), 231-235.

Lewis G., Schrire B., Mackinder B. and Lock M. 2005. (eds.) Legumes of the world. The Royal Botanic Gardens, Kew, Reino Unido. 577 pages. 2005. ISBN 1-900347-80-6. Linnaeus, Charles. *Species plantarum*. Vol. 3. Impensis GC Nauk, 1799.

Mabaleha, M. B., & Yeboah, S. O. (2004). Characterization and compositional studies of the oils from some legume cultivars, Phaseolus vulgaris, grown in Southern Africa. *Journal of the American Oil Chemists' Society*, *81*(4), 361-364.

Macarulla, M. T., Medina, C., De Diego, M. A., Chavarri, M., Zulet, M. Á., Martínez, J. A., ... & Portillo, M. P. (2001). Effects of the whole seed and a protein isolate of faba bean (Vicia faba) on the cholesterol metabolism of hyper-cholesterolaemic rats. *British Journal of Nutrition*, *85*(5),607-614.

Madhujith, T., Amarowicz, R., & Shahidi, F. (2004). Phenolic antioxidants in beans and their effects on inhibition of radical-induced DNA damage. *Journal of the American Oil Chemists' Society*, *81*(7), 691-696.

Magallon, S., & Sanderson, M. J. (2001). Absolute diversification rates in angiosperm clades. *Evolution*, *55*(9), 1762-1780.

Mathers, J. C. (2002). Pulses and carcinogenesis: potential for the prevention of colon, breast and other cancers. *British Journal of Nutrition*, *88*(S3), 273-279.

McPhee, K. E., & Muehlbauer, F. J. (2002). Improving the nutritional value of cool season food legumes. *Journal of Crop Production*, *5*(1-2),191-211.

Messina, V. (2014). Nutritional and health benefits of dried beans. *The American Journal of Clinical Nutrition*, *100*(suppl_1), 437S-442S.

Messina, M. J. (1999). Legumes and soybeans: overview of their nutritional profiles and health effects. *The American journal of clinical nutrition*, *70*(3), 439s-450s.

Mitchell, D. C., Lawrence, F. R., Hartman, T. J., & Curran, J. M. (2009). Consumption of dry beans, peas, and lentils could improve diet quality in the US population. *Journal of the American Dietetic Association*, *109*(5), 909-913.

Murray RK, Granner DK, Mayes PA, Rodwell VW (2000). Harper's Biochemistry, 25th

Edition, McGraw-Hill, Health Profession Division, USA.

Muzquiz, M., & Wood, J. A. (2007). Antinutritional factors. *Chickpea breeding and management*, 143-166.

Nikmaram, N., Leong, S. Y., Koubaa, M., Zhu, Z., Barba, F. J., Greiner, R., ... & Roohinejad, S. (2017). Effect of extrusion on the anti-nutritional factors of food products: An overview. *Food Control*, *79*, 62-73.

Ocho-Anin, A. A. L., Kouakou, T. H., Brou, K. D., Kouadio, Y. J., and Gnakri D. (2010). Evaluation of bioactive components in seeds of *Phaseolus vulgaris* L. (fabaceae) cultivated in Cote d'Ivoire O*.J. Appl. Biosci*. **31**: 1928 – 1934.

Ojijo, N. K. O., Kimura, T., and Koazei, H. (2000). Composition, soaking and softening characteristics of some Kenyan beans (*Phaseolus vulgaris* L.). *Food Sci. Tech, Res.* **6**(I): 12-18.

Oomah, B. D., Cardador-Martınez, A., and Loarca-Pina, G. (2005). Phenolics and antioxidative activities in common beans (*Phaseolus vulgaris* L). *J. Sci. Food Agric.* **85**: 935–942.

Oomah, B. D., Corbé, A., & Balasubramanian, P. (2010). Antioxidant and anti-inflammatory activities of bean (Phaseolus vulgaris L.) hulls. *Journal of agricultural and food chemistry*, *58*(14), 8225-8230.

Oomah, B. D., Cardador-Martínez, A., & Loarca-Piña, G. (2005). Phenolics and antioxidative activities in common beans (Phaseolus vulgaris L). *Journal of the Science of Food and Agriculture*, *85*(6), 935-942.

Ovando-Martínez, M., Bello-Pérez, L. A., Whitney, K., Osorio-Díaz, P., & Simsek, S. (2011). Starch characteristics of bean (Phaseolus vulgaris L.) grown in different localities. *Carbohydrate polymers*, *85*(1), 54-64.

Paredes, M., Becerra, V., & Tay, J. (2009). Inorganic nutritional composition of common bean (Phaseolus vulgaris L.) genotypes race Chile. *Chile. Journal of. Agriculture. Research*, *69*, 486-495.

Pourmorad, F., Hosseinimehr, S. J., & Shahabimajd, N. (2006). Antioxidant activity, phenol and

flavonoid contents of some selected Iranian medicinal plants. *African journal of biotechnology*, *5*(11).

Raes, K., Knockaert, D., Struijs, K., & Van Camp, J. (2014). Role of processing on bioaccessibility of minerals: Influence of localization of minerals and anti-nutritional factors in the plant. *Trends in Food Science & Technology*, *37*(1), 32-41.

Ranilla, L. G., Kwon, Y. I., Apostolidis, E., & Shetty, K. (2010). Phenolic compounds, antioxidant activity and in vitro inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. *Bioresource technology*, *101*(12), 4676-4689.

Reyes Rivas, E., Padilla-Bernal, L. E., Pérez Veyna, O., & López-Jáquez, P. (2008). Historia, naturaleza y cualidades alimentarias del frijol. *Investigación Científica*, *4*(3), 1-21.

Reynoso-Camacho, R., Ramos-Gomez, M., & Loarca-Pina, G. (2006). Bioactive components in common beans (Phaseolus vulgaris L.). *Advances in Agricultural and Food Biotechnology*, *217.*

Rizkalla, S. W., Bellisle, F., & Slama, G. (2002). Health benefits of low glycaemic index foods, such as pulses, in diabetic patients and healthy individuals. *British Journal of Nutrition*, *88*(S3), 255-262.

Rodríguez, R., Jimenez, A., Fernández-Bolaños, J., Guillén, R., & Heredia, A. (2006). Dietary fibre from vegetable products as source of functional ingredients. *Trends in Food Science & Technology*, *17*(1), 3-15.

Schrire, B. D.; Lewis, G. P.; Lavin, M. (2005). "Biogeography of the Leguminosae". In Lewis, G; Schrire, G.; Mackinder, B.; Lock, M. *Legumes of the world*. Kew, England: Royal Botanic Gardens. pp. 21–54. ISBN 978-1-900347-80-8.

Seena, S., Sridhar, K. R., & Bhagya, B. (2005). Biochemical and biological evaluation of an unconventional legume, Canavalia maritima of coastal sand dunes of India. *Tropical and subtropical Agroecosystems*, *5*(1), 1-14.

Schneider, A. V. (2002). Overview of the market and consumption ofpulses in Europe. British Journal of Nutrition, 88(Suppl 3), S243–50.

Secretaría de Economía. (2012). Dirección General de Industrias Básicas. Análisis de la cadena de valor del frijol. Mexico: Proyecto Gobernanza de la Bodiversidad. Biblioteca Digital.

Segura-Nieto, M., & Jiménez-Flores, R. (1999). Genetic modifications of plant seed storage proteins for food production. *Molecular Biotechnology for Plant Food Production*, 411-474.

Seidu, K. T., Osundahunsi, O. F., Olaleye, M. T., & Oluwalana, I. B. (2015). Amino acid composition, mineral contents and protein solubility of some lima bean (Phaseolus lunatus L. Walp) seeds coat. *Food Research International*, *73*, 130-134.

Shiga, T. M., & Lajolo, F. M. (2006). Cell wall polysaccharides of common beans (Phaseolus vulgaris L.)—Composition and structure. *Carbohydrate Polymers*, *63*(1), 1-12.

Shimelis, E. A. and Rakshit, S. K. (2005). Proximate composition and physicochemical properties of improved dry bean (Phaseolus vulgaris L.) varieties grown in Ethiopia. LWT. 38:331–338.

Siddiq, M., Ravi, R., Harte, J. B., & Dolan, K. D. (2010). Physical and functional characteristics of selected dry bean (Phaseolus vulgaris L.) flours. *LWT-Food Science and Technology*, *43*(2), 232-237.

Silva, E. O., & Bracarense, A. P. F. (2016). Phytic acid: from antinutritional to multiple protection factor of organic systems. *Journal of Food Science*, *81*(6), R1357-R1362.

Singh, B., Singh, J. P., Shevkani, K., Singh, N., & Kaur, A. (2017). Bioactive constituents in pulses and their health benefits. *Journal of food science and technology*, *54*(4), 858-870.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, *16*(3), 144-158.

Slinkard, K., & Singleton, V. L. (1977). Total phenol analysis: automation and comparison with manual methods. *American journal of enology and viticulture*, *28*(1), 49-55.

Słupski, J. (2010). Effect of cooking and sterilisation on the composition of amino acids in immature seeds of flageolet bean (Phaseolus vulgaris L.) cultivars. *Food Chemistry*, *121*(4), 1171-1176.

Sreeramulu, D., Reddy, C., & Raghunath, M. (2009). Antioxidant activity of commonly consumed cereals, millets, pulses and legumes in India.

Stevens, P. F. "Fabaceae". Angiosperm Phylogeny Website. Version 7 May 2006.

Tang, C. H., & Sun, X. (2011). Structure–physicochemical function relationships of 7S globulins (vicilins) from red bean (Phaseolus angularis) with different polypeptide constituents. *Food hydrocolloids*, *25*(3), 536-544.

Tharanathan, R. N., & Mahadevamma, S. (2003). Grain legumes—a boon to human nutrition. *Trends in Food Science & Technology*, *14*(12), 507-518.

Tharanathan, R. N. (2002). Food-derived carbohydrates—structural complexity and functional diversity. *Critical Reviews in Biotechnology*, *22*(1), 65-84.

Tryphone, G. M., & Nchimbi-Msolla, S. (2010). Diversity of common bean (Phaseolus vulgaris L.) genotypes in iron and zinc contents under screenhouse conditions. *African Journal of Agricultural Research*, *5*(8), 738-747.

Tungland, B. C., & Meyer, D. (2002). Nondigestible oligo-and polysaccharides (Dietary Fiber): their physiology and role in human health and food. *Comprehensive Reviews in Food Science and Food Safety, 1(3),* 90-109.

Venkateswaran, S., & Pari, L. (2002). Antioxidant effect of *Phaseolus vulgaris* in streptozotocin-induced diabetic rats. *Asia Pacific Journal of Clinical Nutrition*, *11*(3), 206-209.

Vadivel, V., and Janardhanan, K. (2000). Nutritional and anti-nutritional composition of velvet bean: An under-utilized food legume in South India. *Int. J. Food Sci. Nutr*. **51**: 279–287.

Vargas-Torres, A., Osorio-Dıaz, P., Islas-Hernandez, J. J, Tovar, J., Paredes-Lopez, O., and Bello-Perez, L. A. (2004). Starch digestibility of five cooked black bean (*Phaseolus vulgaris* L.) varieties. *J. Food Comp. Anal.* **17**: 605–612.

Welch, R. M., House, W. A., Beebe, S., & Cheng, Z. (2000). Genetic selection for enhanced bioavailable levels of iron in bean (Phaseolus v ulgaris L.) seeds. *Journal of Agricultural and Food Chemistry*, *48*(8), 3576-3580.

WHO (1996) World Health Organization Technical series Trace elements in human nutrition and health World Health Organization, Geneva 199- 205.

Wink, M. (2013). Evolution of secondary metabolites in legumes (Fabaceae). *South African Journal of Botany*, *89*, 164-175.

Worku, A., & Sahu, O. (2017). Significance of fermentation process on biochemical properties of Phaseolus vulgaris (red beans). *Biotechnology reports*, *16*, 5-11.

Wu, X. J., James, R., & Anderson, A. K. (2005). Mineral contents in seed coat and canning quality of selected cultivars of dark red kidney beans (Phaseolus vulgaris L.). *Journal of Food Processing and Preservation*, *29*(1), 63-74.

Xu, B., & Chang, S. K. (2009). Total phenolic, phenolic acid, anthocyanin, flavan-3-ol, and flavonol profiles and antioxidant properties of pinto and black beans (Phaseolus vulgaris L.) as affected by thermal processing. *Journal of agricultural and food chemistry*, *57*(11), 4754-4764.

Yao Y, Cheng X, Wang S, Wang L, Ren GX. 2012. Influence of altitudinal variation on the antioxidant and antidiabetic potential of azuki bean (Vigna angularis). J Food Sci 75:C9–C13.

Yoshida, H., Tomiyama, Y., & Mizushina, Y. (2005). Characterization in the fatty acid distributions of triacylglycerols and phospholipids in kidney beans (*Phaseolus vulgaris L.*). *Journal of Food Lipids*, *12*(2), 169-180.

Yu, L., Perret, J., Davy, B., Wilson, J., & Melby, C. L. (2002). Antioxidant properties of cereal products. *Journal of Food Science*, *67*(7), 2600-2603.

Zhang, G., Ao, Z., & Hamaker, B. R. (2006). Slow digestion property of native cereal starches. *Biomacromolecules*, *7*(11), 3252-3258.

Zhou, Y., Hoover, R., & Liu, Q. (2004). Relationship between α-amylase degradation and the structure and physicochemical properties of legume starches. *Carbohydrate polymers*, *57*(3), 299-317.

Appendix I

Chemicals, glasswares and solutions

1) Required materials for protein estimation

Conc. H_2SO4, NaOH, kjeldahl digestion and distillation apparatus, Kjeldahl tubes,250 mL Erlenmeyer flasks, glass beads, digestion mixture (potassium sulphate and cupric sulphate) and indicator (methylene red and bromocresol green).

2) Required materials for fat estimation

Petroleum ether, Soxhlet extraction apparatus, oven set at 105°c, extraction thimbles and desiccator (60°- 80°C).

3) Required materials for fiber estimation

Soxhlet, condenser, buchner funnel and flask, whatman filter no.41, desiccators, and gooch crucible with asbestos fiber, oven, muffle, petroleum ether, sulfuric acid, and sodium hydroxide.

4) Required materials for ash estimation

Porcelain crucible, crucible furnace, dryer, desiccators.

5) Preparation of 4% boric acid and indicators

The 4% boric acid solution was prepared by dissolving 40 g of boric acid powder as added in volumetric flask. Hot water was added to dissolve the boric acid powder. The indicators were added to solution in the ratio of 12:8 (bromocresol green: methyl red) and volume was made up to 1000 mL.

0.1% indicators were prepared by mixing 0.1 g of methyl red in 100 mL of 95% alcohol and 0.1 g bromocresol green in 100 mL of 95% alcohol.

6) Preparation of 1 M Na_2CO_3-100 mL

10.599 g of the Na_2CO3 (Merck Specialties Pvt. Ltd, Mumbai, India) was carefully weighed and then dissolved in distilled water, and the volume was adjusted to 100 mL at the end.

7) Preparation of glacial acetic acid (20%)-200 mL

40 mL of the commercially supplied glacial acetic acid (Thermo Fisher Scientific, India) was taken and mixed with ethanol. Finally, the volume was adjusted to 200 mL by the addition of ethanol.

8) Preparation of aluminum chloride (10%)-100mL

10 g of the commercially supplied aluminum chloride (Merck Specialties Pvt. Ltd, Mumbai, India) was weighed and dissolved in water. Finally, the volume was maintained to 100 mL.

9) Preparation of 1 M potassium acetate (CH3COOK)-100mL

9.814 g of the commercially supplied potassium acetate (Merck Specialties Pvt. Ltd, Mumbai, India) was weighed and dissolved in water. Finally, the volume was maintained to 100 mL by the addition of water.

10) Preparation of the Folin-Ciocalteu phenol reagent (1:10dilution)

6 mL of the commercially supplied Folin-Ciocalteu phenol reagent (Merk Specialities Pvt. Ltd, Mumbai, India) was taken and mixed it with 54 mL of the distilled water to prepare the 60 mL of 1;10 dilution of Folin-Ciocalteu phenolreagent.

11) Preparation of 0.2 mM DPPH solution-100 mL

100 mL of 0.2mM solution of 1,1-diphenyl-2-picryhydrazyl (DPPH) was prepared by weighing 7.886 mg of the DPPH and dissolving it in an ethanol and finally maintaining the volume of 100 mL by addition of ethanol.

12) Required materials for phosphorus estimation

Citric-Molybdic acid reagent, quinoline solution, quinovic reagent, citric acid, conc. HNO_3, conc. HCl, sodium hydroxide, sodium molybdate, acetone, 500 mL volumetric flasks, 250 mL volumetric flasks, burette, Whatman's filter paper no.41, gooch filter, desiccators and oven.

13) Required materials for iron estimation

Conc. HCl, AAS, ferric nitrate solution as standard compound, 200 mL volumetric flasks, micro pipette, deionized water.

14) Required materials for calcium estimation

Conc. HCl, AAS, lanthanum (0.4%, w/w), 200 mL volumetric flasks, falcon tubes (big and small), calcium carbonate, pipettes and deionized water.

15) Reagent preparation for phosphorus estimation

For preparation of Citric molybdic acid reagent,54 g of 100% molybdic anhydric (MoO_3) and 12 g NaOH with stirring in 400 mL hot water and cooled. 60 g of citric acid in a mixture of 140 mL HCl and 200 mL water was added and cooled. It was then filtered and diluted to one liter.

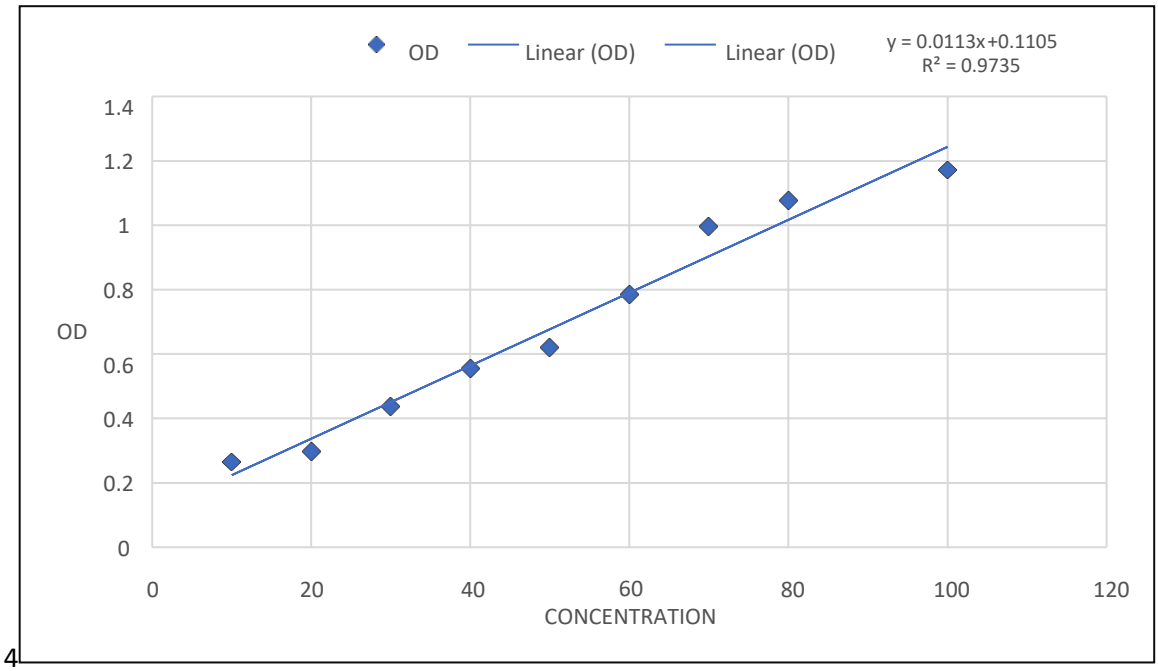
Quinolone solution was prepared by mixing 50 mL of synthetic quinolone, dissolving in a mixture of 60 mL HCL and 300 mL water by stirring. It was then cooled and diluted to one liter and then filtered.

Quinovic reagent was prepared by dissolving 70 g of sodium molybdetate in 150 mL H_2O, 60 g of citric acid dissolved in the mixture of 85 mL HNO_3 and 150 mL H_2SO_4 and cooled. Molybdate solution was gradually added to citric acid and HNO3 with stirring. Percentage of synthetic quinolone was dissolved in mixture of 35 mL HNO_3 and 100 mL H_2O, this solution was gradually added to molybdate citric acid. HNO_3 solution mixed and let it stood for 24 hours. It was then filtered, 280 mL acetone added, it was diluted to one-liter mixing with H_2SO_4.

16) Sample preparation for calcium estimation

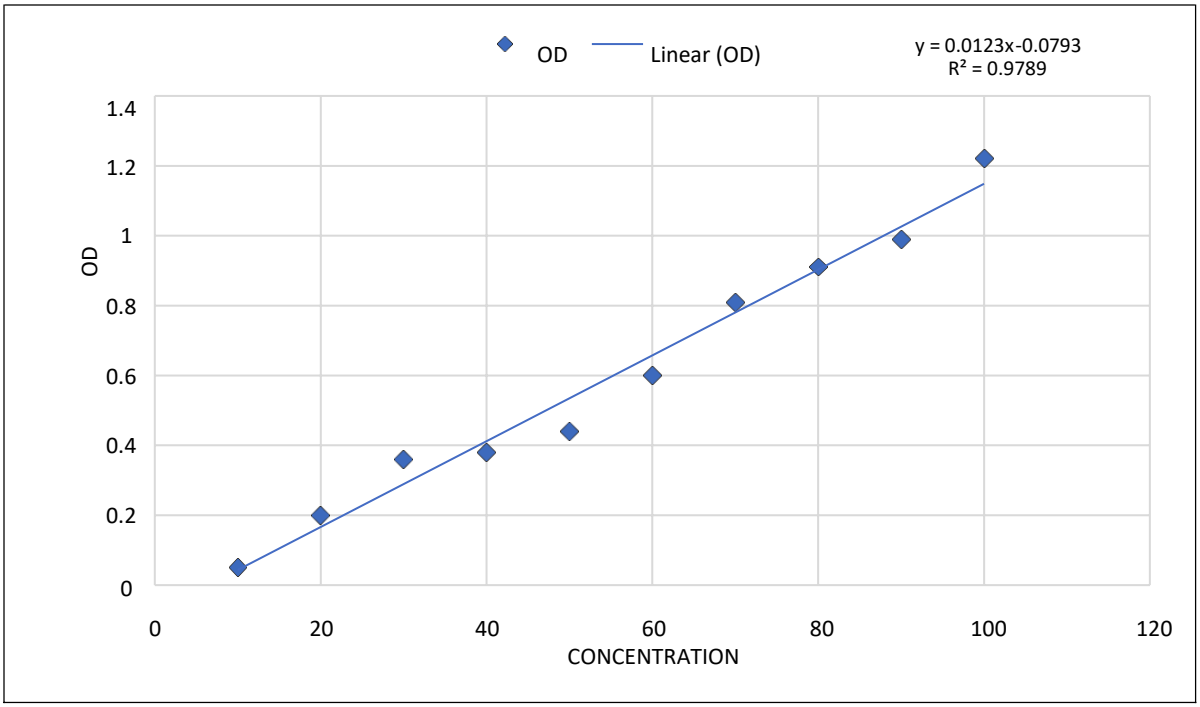
Sample preparation for calcium was based on the optimized protocol (DFTQC, 2014). For sample preparation 200 mL volumetric flasks were used in which 200 µL of standard solution of calcium carbonate was kept.1 mL of lanthanum carbonate solution was poured to each respective conical flask. Final volume was made to 100 mL of deionized water having concentration of 20 ppm. 9 mL conc. HCl was added to new volumetric flasks. From standard solution of 20 ppm, 5 mL, 10 mL, 15 mL and 20 mL were drawn into respective volumetric flasks which contained 1 ppm, 2 ppm, 3 ppm and 4 ppm respectively. The final volume was made 100 mL by adding distilled water to those working solutions. The blank was made which by adding 9.5 mL 1:1 HCl and 89.5 mL deionized water. Two controls were made 1 mL lanthanum, 9.5 mL 1:1 HCl and 2 ppm and 3 ppm spiked calcium itself.

Appendix II
Standard curves for analyses



4

Curve 1 Standard curve of quercetin



Curve 2 Standard curve of gallic acid

Appendix III
Bean sample and photographs

Photo 1 Bean samples collected from different locations of Nepal

Photo 2 Solvent extraction for phytochemical analysis

Photo 3 Fat estimation using Soxhlet apparatus

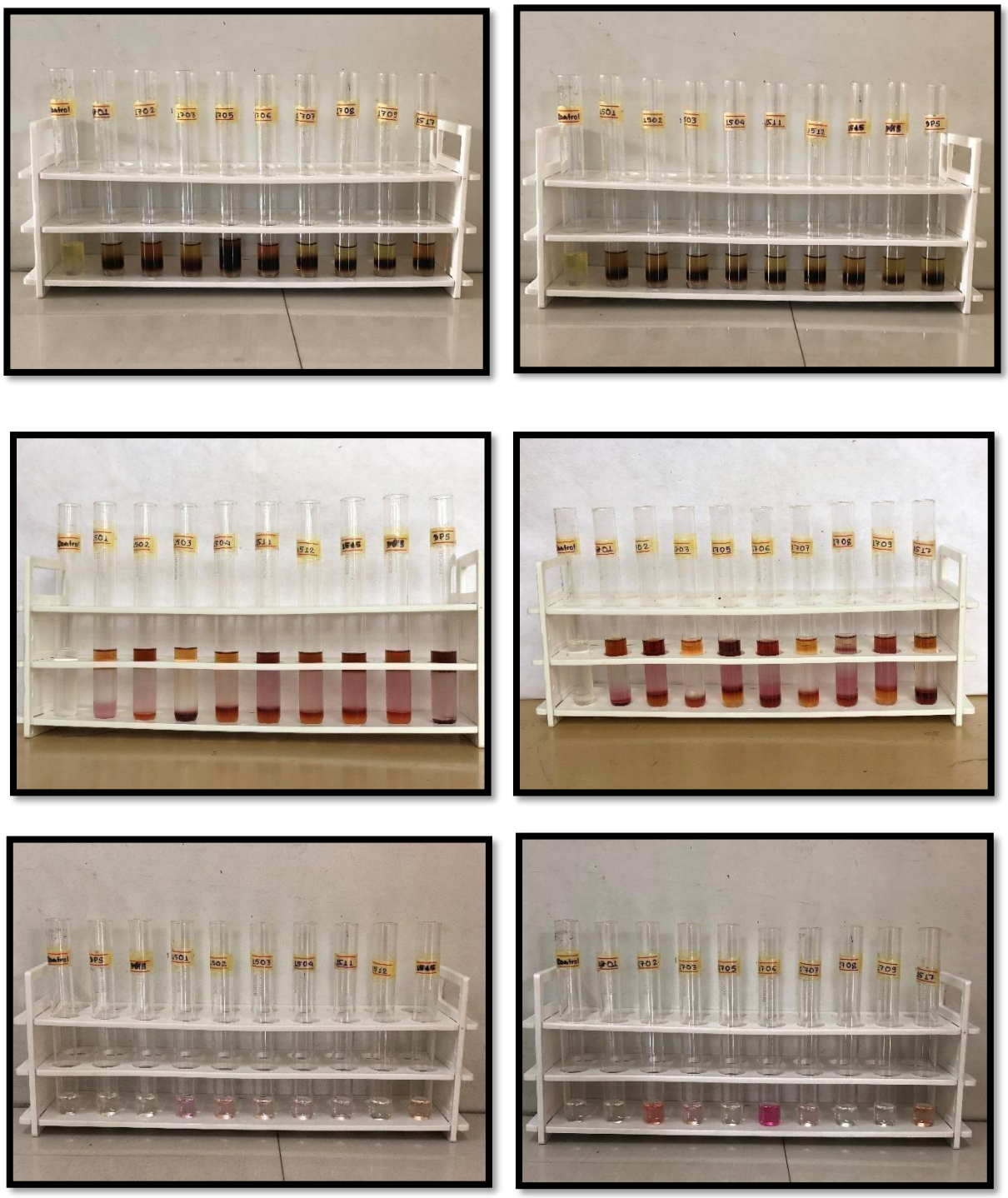


Photo 4 Qualitative phytochemical screening.

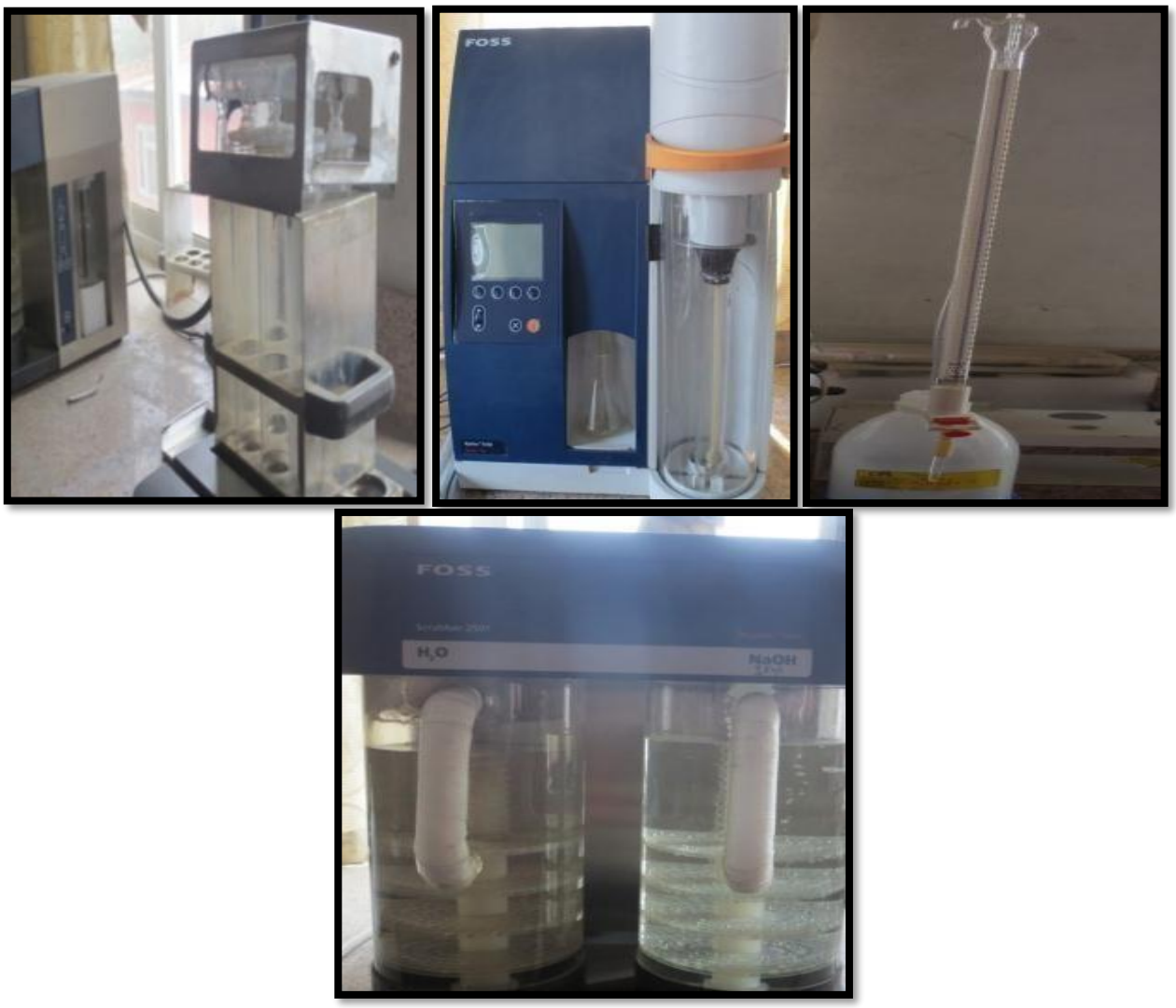


Photo 5 Protein determination (digestion, distillation, titration).