ANTIOXIDANT AND ENZYME-INHIBITORY ACTIVITIES OF WATER-SOLUBLE EXTRACTS FROM BANGLADESH VEGETABLES

By

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ABSTRACT

The interest in the possible health benefits of fruit and vegetable-rich diets is on the increase, partly because it has been recognized that increased consumption of vegetables may decrease the risk of developing non communicable diseases (NCDs) . The main attributes that can contribute to the antioxidants and enzyme inhibition properties of vegetables are polyphenolic compounds, minerals and vitamins that are present in abundance. Therefore, the aim of this thesis was to determine the NCD-related antioxidant and enzyme inhibition properties of nine water-soluble extracts of Bangladesh vegetables including ash gourd, bitter gourd, brinjal, Indian spinach, kangkong, okra, ridge gourd, snake gourd and stem amaranth. Aqueous polyphenol extracts were aquired to carry out all the assays because of the hydrophilic nature of the human gastrointestinal tract in addition to the high extraction yield and cheaper cost. The *in vitro* antioxidant and enzyme inhibition properties showed that almost all the vegtables were able to scavenge free radicals, chelate metal ions and reduce ferric ions, in addition to the inhibition of α-amylase, α-glucosidase, pancreatic lipase, angiotensin converting enzyme and renin. Indian Spinach, kangkong and okra showed the best activity to scavenge free radicals whereas Indian spinach and brinjal extracts performed better for inhibiting digestive enzymes responsible for the progression of NCDs. The quantities of amino acids, minerals and β-carotene available in these vegetables were also determined.

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FOREWORD

The manuscript format was followed in producing this Masters thesis.The work consists of two manuscripts bookended by a ***general introduction*** (Chapter1) and a ***literature review*** (Chapter 2) portion, and a ***conclusion*** part (Chapter 5). Each manuscript was prepared according to the style of the journal it is intended to be/has been published. Manuscript 1 (Journal of Food Biochemistry) deals with the Chemical composition and *in vitro* antioxidant properties of water-soluble extracts obtained from Bangladesh vegetables while manuscript 2 ("Foods" journal) examines the inhibitory activities of aqueous vegetable extracts against α-amylase, α-glucosidase, pancreatic lipase, renin, and angiotensin converting enzyme. A transition statement is added at the end of each manuscript and links it to the next chapter to provide coherence. Finally, the last chapter (Chapter 5) contains conclusions along with directions for future research.

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LIST OF ABBREVIATIONS

4MU	4-methyl umbelliferone
4MUO	4-methyl umbelliferyl oleate
ACE	Angiotensin I converting enzyme
ALS	Amyotrophic lateral sclerosis
ASP	Aspartic + asparagine
BDHS	Bangladesh health and demographic survey
BHT	Butylated hydroxytoluene
COPD	Chronic obstructive pulmonary diseases
CVD	Cardiovascular diseases
DNA	De-oxy ribo nucleic acid
DPPH	2,2- diphenyl-1 picrylhydrazil radical
DRSA	DPPH radical scavenging activity
FAO	Food and agriculture organization
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent
GLU	Glutamic +glutamine
LDL	Low density lipoprotein
MCA	Metal chelation activity
PL	Pancreatic lipase
PPHG	Postprandial hyperglycemia
RAS	Renin-angiotensin system
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
T2DM	Type 2 diabetes mellitus
TFC	Total flavonoid content

TPC	Total phenolic content
TPTZ	2,4,6- tripyridyl-s-triazine
WHO	World health organization
XO	Xanthine oxidase

CHAPTER ONE

1.0 INTRODUCTION

Bangladesh is known as a developing and densely populated country, however majority of the population live below the poverty line. Owing to quality, affordability, and popularity, local and indigenous foods are always on the daily meal.There are approximately more than sixty indigenous exotic vegetables available in Bangladesh. These plants are considered as sources of nutritional and antioxidant compounds for the local communities (Hamid et al., 2011).

Cardiovascular diseases, cancer, chronic diseases, and diabetes are considered major non-communicable diseases (NCDs), which have become a significant public health problem in Bangladesh. In 2014, a global status report on NCDs from the World Health Organization (WHO) recorded that about 17.5% of premature deaths in people aged 30 to 70 years occur due to NCDs (Zaman et al., 2015). In 2013, about 38 million people died due to NCDs around the world. At least 61% of deaths in Bangladesh can be attributed to NCDs, and this number is on the increase (Panja, 2018).

According to the World Health Organization (WHO), around 41 million people die each year all over the world from NCDs, which is about 71% of global death. Worldwide 15 million people between the ages of 30 and 69 die from NCD every year, and 85 % are from low and middle income countries.("Noncommunicable Diseases", 2020). WHO also declared that, among all NCDs, the annual rates for cardiovascular disease, cancers, respiratory diseases, and diabetes, are 17.9 million, 9 million, 3.9 million, and 1.6 million, respectively. In Bangladesh, approx. 580000 people die each year due to NCDs, which represent 67% of total deaths ("Noncommunicable Diseases", 2020)

The causative factors related to the onset of NCDs are low intake of fruits and vegetables, low level of physical activity, tobacco use, harmful use of alcohol, obesity, raised blood pressure, increased blood cholesterol and glucose. In Bangladesh, these high levels of NCDs are on the growing trend due to increase in the number of people with high blood pressure and diabetes (Zaman et al., 2015). The prevelance of NCDs is higher in developing countries compared to developed nations (Hosseinpoor et al., 2012). WHO confirmed that 38 million people die from NCDs annually, which is 82% of all annual deaths (Mwenda et al., 2018). According to WHO, the recommended amount of fruits and vegetables is 400 g/day though much lower intakes are recorded for both high income and low-income countries (Kjøllesdal et al., 2016).

In the research community, it has been widely studied that by consuming a large amount of fruits and vegetables, several diseases can be controlled, such as obesity, diabetes, hypertension, and all other diseases linked with CVDs. It was also recorded that fruits and vegetable consumption is the best method to decrease sugar and fat content in the body and consequently to prevent obesity. Fruits and vegetables contain a wide range of enzyme inhibitors, which can modulate metabolic efficiency, as well as insulin secretion and action to consequently control body weight (Bazzano, Serdula, & Liu, 2003)

Selection of the most appropriate solvent is an important extraction factor property, because different solvents produce extracts that differ in structure and matrix composition, which influences bioactive properties. For example, a better extraction yield was observed from water extracts when compared to methanol and ethanol (Fernández-Agulló et al., 2013), whereas in some cases, acetone mixtures produced more active extracts (chiou-Dziki et al., 2013). Therefore, the type or nature of the starting material (sample) may also have influence on the yield and bioactive

properties due to variations in chemical composition. Plant-synthesized secondary metabolites are called phytochemicals, and their bioactive effectiveness are considered beneficial to human health. For example, antioxidant properties of phytochemicals can prevent oxidative stress caused by free radicals; this property can be used to control a broad range of diseases such as cardiovascular, neurodegenerative, and cancers. The necessity of food has now changed for consumers because of not only its need in the diet as a nutritive agent but also the disease prevention activities (Mazzucotelli et al., 2018). Vegetables contain different phenolic and antioxidant activities that emphasize the necessity to determine their polyphenol and antioxidant capacity so that health-promoting diets can be formulated adequately (Liu, 2013). Antioxidant and polyphenol activities of some vegetables are already recorded in the literature. However, various factors like geographical regions, variety, lights, and even storage conditions can cause variations (Santos, Oliveira, Ibanez, &Herrero, 2014). On the other hand, the assessment of nonconventional vegetables is intended to sensitize consumers towards new sources of bioactive compounds, which can help increases their consumption and add value to certain parts of the plant that are sometimes considered as waste products (Mazzucotelli et al., 2018).

In addition to polyphenolic compounds, the amino acid contents of vegetables can be used as a building block of proteins and as antioxidants that protect the human body from toxic free radicals (Elias, McClements, & Decker, 2005; Udenigwe & Aluko, 2010).

The prevalence of obesity continues to intensify with nearly 300 million people afflicted worldwide. Obesity condition is attained when the body mass index becomes more than 30 kg/m^2 or higher. (Schröder, 2007).

An increasing incidence of diabetes follows the rising obesity epidemic. Type 2 diabetes known as non-insulin-dependent diabetes is mainly an adult-onset hyperglycemic condition that now

occurs worldwide.(Schröder, 2007). The number of cases of diabetes has risen from almost 150 million in 1980 to nearly 350 million in 2008 and 382 million in 2013 in the global population, with a projected growth to 592 million by 2035 (Wu, Zhang, Jiang & Jiang, 2015).

Obesity, along with diabetes, can lead to different diseases, especially cardiovascular diseases and strokes; about 42% of health care costs are associated with obesity and associated symptoms when compared to all other diseases (Schröder, 2007). Moreover, diabetes treatments cost two times more than non-diabetes. Together, obesity and diabetes have contributed to elevate the increased complexity of metabolic disorders such as cardiovascular diseases and strokes. An estimated 422 million people had diabetes worldwide in 2014, with 60% of the cases occurring in Asia. Most diabetes patients are aged between 40-59 years old while 179 million people are believed to suffer from this condition but remain undiagnosed. Therefore, it is expected that the number of diagnosed diabetics will increased to 642 million by 2040 worldwide (Dias & Imai, 2017)

While there are several synthetic drugs available for the management of NCD symptoms, their long-term use harms the human body. Therefore, natural alternatives such as vegetable phytochemicals are considered effective and safe compounds for the prevention and treatment of NCDs when compared to synthetic compounds (drugs). It has been suggested that diabetes can be prevented by consuming vegetables containing fiber, phytochemicals, vitamins, and minerals (Dias et al., 2017).

The available forms of dietary polyphenols are in the glycosylated form with the residue of sugar paired with either hydroxyl group or aromatic ring, which contributes to their lower absorption from the gastrointestinal tract. Polyphenols, because of the substituted phenolic rings are less lipophilic than hydrophilic. Hence, polyphenolic compounds containing aglycones, glycosides,

and oligomers can be extracted easily by using different solvents (Brglez Mojzer, Knez Hrnčič, Škerget, Knez, & Bren, 2016). The most commonly used extraction solvents are water, methanol, ethanol, and their different mixtures .

In the past two decades, the diseases associated with overweight and obesity have increased significantly. In the USA, more than 30% of the population are affected with diabetes, and it is assumed that one in three children will be affected in the early parts of this century. However, different studies have shown that consumption of polyphenol-containing foods can be used to change lipid and energy metabolism along with weight loss. The mechanism involved in the effectiveness of polyphenols are: fat absorption suppression from the gut, glucose uptake from skeletal muscles, anabolic pathway suppression, catabolic pathways stimulation in adipose tissues, angiogenesis inhibition in adipose tissues, preadipocytes to adipocytes formation inhibition and consequently reduced chronic inflammation associated with adiposity (Meydani & Hasan, 2010).

Diabetic conditions arise mainly from excessive consumption of carbohydrates and fat. Carbohydrate digestion is mainly facilitated by two enzymes α-amylase and α-glucosidase. Carbohydrate breakdown by α-glucosidase occurs at the brush border of jejunum, which leads to increased blood glucose level (Patil, Mandal, Tomar, & Anand, 2015). In contrast, lipase enzyme breaks down fats to produce absorbable free fatty acids and monoglycerides (Tan & Chang, 2017). Polyphenols in fruits and vegetables can bind these enzymes and prevent diseases (Tan & Chang, 2017). For example, it was has been reported that polyphenol extracts could reduce the blood level of glucose, triglycerides, and low-density lipoprotein (LDL) cholesterol. Also, they are beneficial for increasing fat oxidation, energy expenditure as well as reduce body weight and adiposity (De La Garza, Milagro, Boque, Campión, & Martínez, 2011).

Hypertension is another risk factor for developing, diabetes, arteriosclerosis, congestive heart failure, coronary heart disease, end-stage renal diseases, myocardial infarction, and stroke, all of which contribute to increased mortality (Abegaz, Shehab, Gebreyohannes, Bhagavathula, & Elnour, 2017). By blocking the principal agents that control the renin-angiotensin system (RAS), hypertension can be controlled (Pechanova et al., 2019). Though synthetic inhibitors like captopril can also the inhibit activities of RAS, they might have some negative impact on the human body, such as dyspnea, cough, hair loss, headache, edema, and flushes. Due to this, the importance of natural drugs or natural sources arises (Kumbhare et al., 2014). For example, phenolic extracts have been found to inhibit RAS enzymes and could become important agents to control hypertension (Henriksen, Diamond-Stanic, & Marchionne, 2011). Moreover, modulation of RAS activities under pathological conditions can lower blood pressure, increase glomerular filtration rate and prevent chronic kidney diseases (Oboh, Akinyemi, Ademiluyi, & Bello, 2014; Chiou et al., 2018).

1.1 HYPOTHESES

a) Water-soluble polyphenolic vegetable extracts will possess *in vitro* antioxidant properties.

b) Water-soluble polyphenolic vegetable extracts will inhibit *in vitro* activities of digestive enzymes (α-amylase, α-glucosidase, pancreatic lipase) associated with excessive glucose and fatty acid absorption.

Water-soluble polyphenolic vegetable extracts will inhibit *in vitro* activities of ACE and renin, the two main factors responsible for mammalian hypertension.

1.2 AIM AND OBJECTIVES

The overall aim of this research was to determine the antioxidant and enzyme inhibition properties of water-soluble vegetable extracts.

Therefore, the specific objectives of this research are:

1. To determine the *in vitro* antioxidant properties of aqueous extracts of common Bangladesh vegetables.
2. To determine the *in vitro* inhibitory activities of the extracts against ACE and renin activities, the two main factors responsible for systemic hypertension in human beings.
3. To determine the ability of the extracts to inhibit *in vitro* activities of pancreatic lipase, α-amylase, and α-glucosidase, which have been implicated in the pathogenesis of obesity and diabetes.

Therefore, this study used aqueous (water) extraction to produce polyphenol-rich extracts from these readily available Bangladesh fruits and vegetables. The use of water as an extracting solvent is a more socially and economically acceptable process to produce polyphenols for human consumption as consumers are more aware of what they consume and therefore, tend to purchase foods with clean and environmentally friendly ingredients.

Also, the ability to successfully obtain polyphenolic rich extracts that mimics how these fruits and vegetables are traditionally prepared for consumption (water cooking of steaming) will answer the proposed research questions of this study.

1.3 ROLE AND CONTRIBUTION OF THE RESEARCH TO IDRC-SPONSORED PROJECT

The present thesis research on the antioxidant and enzyme-inhibitory activities of water-soluble extracts from vegetables in Bangladesh has been one of the major components of the International Development research Centre (IDRC), Ottawa, Canada funded and sponsored research project, entitled "Promoting balanced diet and vegetable consumption for reducing dietary related risks associated with NCDs in Bangladesh". The broad goals of the IDRC-sponsored research project were to i) enhance capacity in understanding and contributing to the promotion of coordinated actions of NCD related policies and practices across sectors, and ii) thus effectively promote balanced diet and vegetable consumption and associated benefits across multiple stakeholders and reduce dietary risks associated with non-communicable diseases in Bangladesh. The Project was implemented in Bangladesh and Canada during 2016-2020. In perspective of these goals and considering the knowledge and data gaps regarding nutritional values of indigenous vegetable species of Bangladesh, an objective the Project was to analyse the macro and micro-nutrient contents of 9 indigenous vegetable species of Bangladesh which specifically created the need for the present thesis research. It is in this context, the present thesis research aimed to contribute to the understanding of the antioxidants and enzyme inhibition properties of 9 selected vegetables, and in turn, to help disseminating the results among the current and potential consumers in Bangladesh. It is anticipated with improved scientific information on these properties, consumers' capacity to assess and market decision would be enhanced. These would likely to help reducing NCDs in Bangladesh in future.

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CHAPTER TWO

2. LITERATURE REVIEW

2.1 VEGETABLES

The nutrient composition of leafy vegetables makes them a popular nutritional staple worldwide. The popularity of vegetables in Bangladesh cannot be overstated; however, scientific data on the nutritional composition and how they affect health is lacking. (Hossain, Sultana, Iftekharuzzaman, Hossain, & Taleb, 2015). The vegetables that are being taken into consideration in this research include ash gourd, bitter gourd, brinjal, Indian spinach, kangkong, okra, ridge gourd, snake gourd, and stem amaranth.

2.1.1 Ash gourd

Ash Ggourd (*benincasa hispida),* a member of the Cucurbitaceae family, is also commonly referred to as tallow gourd, Chinese watermelon, white gourd, or wax gourd. It is popularly called Chal kumra in Bangladesh. While it originated from Java seashore, China has cultivated it from ancient times (Childhood, Project, & Aged, 2013). Other Asian countries such as India, China, Indonesia, Malaysia, Philippines, Taiwan, Indonesia, and Bangladesh cultivate it as well. In Bangladesh, the edible part of the ash gourd is the green immature fruit and its young twigs. Ash gourd is one of the most commonly grown summer vegetables and the only vegetable that is a foreign exchange earner (Ramesh et al., 1989). For marginal farmers, ash gourd gives a higher economic return. It grows throughout the year so that when vegetables are scarce, ash gourd can help meet up the demand. (Rahman, 1994). According to Bangladesh Bureau of Statistics 2010, the total ash gourd production was 134,000 metric tons (Childhood et al., 2013). Ash gourd consumption has been discovered to manage diseases such as peptic ulcer, urinary infection,

internal organs hemorrhages, epilepsy, and other nervous disorders. The fruit extracts are also recommended for their anti-ulcer, anti-angiogenic, and antihistaminic. Seeds are removed from the fruits to make sweets and the seeds are also traditionally used for the therapeutic control peptic ulcer and as vermifuges (Gill, Dhiman, Bajwa, Sharma, & Sood, 2010).

2.1.2 Bitter gourd

Bitter gourd (*Momordica charantia*) is also a member of the Cucurbitaceae family and another popular vegetable in South Asia. It grows throughout the tropical and subtropical areas as well as East Africa, Asia, the Caribbean, and South America. It is an economically important vegetable also referred to as balsam pear, karela, and bitter melon (Paul, Mitter, & Raychaudhuri, 2009). All parts of the bitter gourd are edible, including the fruits, seeds, roots, and leaves. It is used as a remedy for diabetes, wounds, stomach pain, tumors, inflammation, malaria, fever, and measles. Bitter gourd contains insulin-like molecules and that is why it is used for diabetic and pre-diabetic treatment (Mahwish et al., 2017). It was also recorded as being effective for lowering blood glucose levels, cholesterol, and visceral fat. In animal models, bitter gourd extracts were found to improve high-fat diet-induced obesity and hyperlipidemia (Chen et al., 2012). Two types of bitter gourd are generally found in Bangladesh. The small one is called “ucche” and the large korolla. Some other wild species are also known. While the worldwide use of bitter gourd is for diabetes treatment, it is also used for the treatment of dysmenorrhea, eczema, emmenagogue, gout, jaundice, leprosy, piles, pneumonia and scabies as well. Besides this, bitter gourd extracts are found to be beneficial for cancer, ulcers, malaria, pain, dyslipidemia, hypertension, and inflammation (Alam et al., 2015).

2.1.3 Brinjal

Brinjal (*Solanum melongena*) belongs to the family Solanaceae and is also known as the eggplant. It is an important, cheap, and well-known vegetable that is considered the second most important vegetable produced in Bangladesh, and is cultivated on over 50,000 hectares nationwide, providing a source of income to poor Bangladeshi farmers (Rahman, Kabir, & Khan, 2016). It is ranked third amongst all vegetable productions worldwide (Meherunnahar, 2009). It is also called aubergine, berenjena or guinea. Good antioxidant activity (anthocyanin and phenolic acids) has been recorded from brinjal fruit. It was also recorded for skin problem treatment, used as a purgative, and to ease urination (Gürbüz, Uluişik, Frary, Frary, & Doğanlar, 2018)

2.1.4 Indian spinach

Indian spinach (*Basella alba* L.), is an indigenous, rapidly growing, tropical leafy vegetables belonging to the family Basellaceae. It is popular in tropical and subtropical regions, and that includes Asia, America, Africa, and Madagascar. It has different common names; it is known as Ceylon spinach in China, spinach in Malabar, mongtoi in Vietnamese, alugbati in the Philippines and puishag in Bangladesh. It has low carbohydrate content but has been reported to contain a good amounts of vitamins, minerals, irons, calcium, and antioxidants (Hasan, Binta Islam, Naznin, Islam, & E-Mustarin, 2016).

2.1.5 Kangkong

Kangkong, (*Ipomoea aquatica*), from the botanical family Convolvulaceae is a perennial herb commonly known in Bangladesh as kalmi shak or water spinach. It is a herbaceous perennial plant with hollow and tiny stems; the roots come from the nodes and can penetrate the soil easily (Göthberg, Greger, & Bengtsson, 2002). A high number of alkaloids, reducing sugars, flavonoids,

soluble carbohydrates, β-carotene, tannins, and phenols have been discovered from the phytochemical screening of kangkong. The nutritive and antioxidant activities of different kangkong extracts have also been researched. The vegetable is cheap, readily available, and environment friendly because of the agronomic practices involved in its cultivation (Sharmin MBBS et al., 2016).

2.1.6 Okra

Okra (*Abelmoschus esculentus*) belongs to the family malvaceae, and it is another essential vegetable grown all year-round in Bangladesh, an average minimum yield of 1.33 tons/ha (Seiwert, Baldrian-Hussein, Mittag, Findeisen, & Sprenger, 1994; Durazzo et al., 2019). Okra originated from the tropical and subtropical regions of Africa. Okra is also grown in India, Japan, Turkey, Iran, Yugoslavia, Pakistan, Myanmar, Malaysia, Thailand, Brazil, and in the southern parts of the United States commercially. Okra is a multipurpose crop because all the plant parts can be consumed (leaves, buds, pods, flowers, seeds, and stems). Apart from its vegetables, the fruits can also be used in salads and in soups to increase consistency. Okra's mucilage has been found to have the ability to bind cholesterol and bile acids that are responsible for binding toxins (Gemede, 2015).

2.1.7 Ridge gourd

Ridge gourd *(Luffa acutangula),* belongs to the family Cucurbitaceae and grows throughout the summer and spring in tropical and subtropical countries (Karmakar et al., 2013). This fruit is known as a nutrition powerhouse because of the presence of vitamin A, thiamin, riboflavin, dietary fiber, and vitamin C besides low fat and zero cholesterol contents. It is also a rich source of carbohydrates, protein, and minerals such as Mg, Ca, Na, Zn, Fe, and Mn. The whole plant is

considered important for the treatment of ulcers and sores (Swetha & Muthukumar, 2016). The fruit juice has also been used in the treatment of jaundice, improvement of liver function, and regulation of blood glucose levels. The antidiabetic, anti-cancerous, and antioxidant activities have also been reported (Bor, Chen, & Yen, 2006).

2.1.8 Snake gourd

Snake gourd (*Trichosantes cucumerina* L.) is generally believed to have originated from India. Though it originated in a wild state, it later became domesticated. The wild species can still be found in India and other parts of South East Asia and some parts of Australia, while the domesticated crop is cultivated in India, parts of South East Asia, Australia, West Africa, Latin America, and the Caribbean (Idowu, Fashina, Kolapo, & Awolusi, 2019). In Bangladesh, snake gourds usually grow in fields and homesteads from March to October when there is a shortage of other vegetables. It is one of the most economically important vegetables in Bangladesh because of its nutritional quality. It contains a high amount of protein, fat, minerals, fiber, and carbohydrates (Amin, Ahmed, Zahid, & Swaraz, 2015).

2.1.9 Stem amaranth

Amaranth (*Amaranthus tricolor*) is a widely grown vegetable from the family amaranthaceae, and is considered a poor man's vegetable in Bangladesh because it is cheap and readily available. In Bangladesh, amaranth is mainly cultivated in the summer, and to a lesser extent in the winter. The leaves and stems of amaranth are consumed regularly because of the abundant presence of protein, fat, phosphorus, riboflavin, β carotene, ascorbic acid, and iron (Ahammed, Rahman, & Hossain, 2015).

2.2 DIET AND HEALTH

The FAO and World Health Organization (WHO) recommend that adults consume 400 g of fruits and vegetables per day. The 2003 dietary guidelines for Bangladesh recommend 300 g of vegetables per day for adults. In Bangladesh, people consume an average of 23g of leafy vegetables, 89g of non-leafy vegetables, and 14 g of fruit, which amounts to 126 g of vegetables and way below the recommended daily recommendation. Vegetables play a significant role in the human diet throughout the world because they generally contain phytonutrients in addition to different kinds of vitamins such as A, B1, B6, B9, C and E, minerals and, dietary fiber (Silva Dias & Ryder, 2011).

Unhealthy diets are the root causes of ill health as this leads to a cascade of diseases such as obesity, diabetes, oxidative stress, anemia, gastric ulcer, rheumatoid arthritis, several forms of cancer and cardiovascular diseases. Type 2 diabetes risk is linked with higher body circumference and body mass index (BMI) and this risk varies in different populations (Dias, 2012). General physical health, gastrointestinal health, and vision can be improved by incorporating vegetables into the regular diet. WHO reported in 2007 that 2.7 million deaths occur each year due to the low consumption of vegetables and consequently low carbohydrate and dietary fiber levels, resulting in preventable death (Dias, 2012).

Diabetes is linked to several other diseases like blindness, vision loss, kidney failure, heart attack, stroke, leg amputation, nerve damage, Alzheimer's disease and dementia. Therefore, by systematically controlling the diet, a wide range of nutrition-related illnesses can be controlled. WHO has projected that by 2030, diabetes will be 7^{th} major disease-causing death in the world (Silva Dias & Ryder, 2011). Different vegetables in combination with dietary fiber help maintain overall health in various ways such as improving bowel movement, lowering cholesterol,

maintaining the glucose level in the blood, and helping to transport a substantial quantity of minerals and phytochemicals through the body system. To maintain a healthier diet, it is important to reduce consumption of saturated fats, trans fats, and foods containing high calories. And that can be done by increasing vegetable consumption. The phytonutrient, vitamin, mineral, and dietary fiber contents of vegetables are dependent on the specie. Therefore to harness the maximum health benefits from vegetables, it is better to consume different types (Hossain et al., 2015).

The important role of diet is because of the assumed role it can play in prevent one-third of all cancers in the United States. The 2010 dietary guidelines for Americans recommend that it is good to consume 9 servings of fruits and vegetables in a day, and 5 servings must be from vegetables. And it was also recommended that the selection of a wide variety is also important because the amount and type of bioactive compounds vary with vegetables; therefore, different mechanisms of action may be associated with each (Liu, 2013; Gao et al., 2014).

2.3 ROLE OF PHYTONUTRIENTS IN PREVENTING DISEASES ASSOCIATED WITH FREE RADICALS AND OXIDATIVE STRESS

2. 3.1 Free radical and oxidative stress

During general metabolic processing, free radicals are produced that are not only reactive and unstable under physiological conditions, but they also become potentially harmful. Enzymatic antioxidant systems control several cellular redox pathways while chemical scavengers such as endogenous enzymes, dietary antioxidants and certain hormones play different roles in preventing or suppressing oxidative stress (Terao, Piskula, & Yao, 1994). Free radicals are atoms or molecules, having one or more unpaired electrons in the outer shell. When the electron number of free radicals is odd and unpaired, they become unstable, short-lived and highly reactive, which

makes attracting electrons from other compounds to make a pair. However, the compound that donate electrons also become a free radical that can attack other compounds. Consequently, this process leads to the production of several free radicals that can attack vital cellular molecules like proteins, lipids and DNA; eventually, these never-ending cycles destroy healthy living cells and can lead to diseases (Phaniendra, Jestadi, & Periyasamy, 2015). Therefore, overproduction of free radicals and the insufficient levels of cellular enzymatic and non-enzymatic antioxidants can lead to a significant increase in the production of free radicals, which overwhelm the antioxidant defense and placing oxidative stress on the physiological system (Kähkönen et al., 1999).

Oxidative stress is responsible for the damage of lipids, proteins, or DNA in cells, which leads to the inhibition of normal body functions and has been associated with several degenerative diseases, such as atherosclerosis, coronary heart disease, aging, and cancer. A healthy physical condition can be promoted by minimizing oxidative stress and preventing certain free radical-mediated degenerative diseases. To maintain a healthy biological system; therefore, the balance between oxidation and antioxidation is very critical. The excessive generation of reactive oxygen species (ROS) causes oxidative stress spreading across all cell targets (DNA, lipids, and proteins). Consumption of polyphenols, flavonoids, anthocyanins, and vitamins has been reported to demonstrate antioxidant properties that can assist in providing a healthy oxidative balance (Terao et al., 1994). Synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole are used in the food industry today to maintain freshness and prevent oxidative spoilage. However, there is several agreement that synthetic antioxidants should be replaced with natural antioxidants as some synthetic antioxidants have demonstrated potential health risk and toxicity, such as skin allergies, gastrointestinal tract and in most notably cancerous effects. (Lourenço, Moldão-Martins & Alves, 2019)

In this regard, vitamin C and E, carotenoids, and other polyphenols are significant free radical chain breaking natural antioxidants used in the food industry as preservatives (Číž et al., 2010)

2.3.2 Sources of free radicals

ROS and reactive nitrogen species (RNS) are two classes of free radicals derived from endogenous and exogenous sources. Endogenous sources are mitochondria, peroxisomes, endoplasmic reticulum, and phagocytic cells while exogenous sources include pollution, alcohol, tobacco smoke, heavy metals, transition metals, industrial solvents, pesticides, drugs like halothane, paracetamol, and radiation. Both ROS and RNS have two other subgroups; radical and non-radical. Radicals are independent and have at least one free electron whereas non-radicals are not independent like free radicals but they assist free radicals to initiate reactions in living cells (Phaniendra et al., 2015)**.**

2.3.3 Deleterious effects of free radicals and oxidative stress

Oxidative stress causes damage to DNA and consequently DNA mutations, that can initiate cancer, chromosomal defects, and oncogene activation. Below is a list of a few illnesses that can be triggered when the body is compromised in combination with oxidative stress.

a) Cardiovascular diseases with oxidative stress: Neurological diseases like Alzheimer's, Parkinson's, multiple sclerosis, amyotrophic lateral sclerosis (ALS), memory loss, depression have been recorded from oxidative stress. Losses of neurons and dementia have occurred from oxidative damages based ona clinical survey. Alzheimer's disease that is caused by the accumulation of toxic beta-amyloid has been found in the brain of a patients

Table 2.1: Sources and symbols of free radicals.

Free Radicals						
Reactive Oxygen Species				Reactive Nitrogen Species		
Radicals	Symbol	Half-life		Radicals	Symbol	Half-life
Superoxide	O_2	10^{-6} s		Nitric Oxide	NO	s[a]
Hydroxyl	OH	10^{-10} s		Nitrogen dioxide	NO_2	s
Alkoxyl Radical	RO	10^{-6} s				
Peroxyl Radical	ROO	17 s				
Non-radicals	Symbol	Half-life		Non-radicals	Symbol	Half-life
Hydrogen peroxide	H_2O_2	Stable		Peroxynitrite	$ONOO^-$	10^{-3} s
Singlet oxygen	$^1O_2$	10^{-6} s		Nitrosyl cation	NO^+	s
Ozone	O_3	S		Nitroxyl anion	NO^-	s
Organic peroxide	ROOH	Stable		Dinitrogen trioxide	N_2O_3	s
Hypochlorus acid	HOCl	Stable (min)		Dinitrogen tetraoxide	N_2O_4	s
Non-radicals	Symbol	Half-life		Non-radicals	Symbol	Half-life
Hypobromous acid	HOBr	Stable (min)		Nitrous acid	HNO_2	s
				Peroxynitrous acid	ONOOH	Fairly stable
				Nitryl chloride	NO_2Cl	s

* S- Seconds, a- The half life of some radicals depends on the environmental medium

b) Pulmonary disease and oxidative stress: Asthma and chronic obstructive pulmonary diseases (COPD), known as inflammatory lung diseases have been attributed to oxidative stress. Kinases and redox transcription activation play roles for inflammation.

An appropriate measure to prevent these diseases is the consumption of natural antioxidant containing diets. While polyphenols were originally not considered important in the human diet, their importance in health promotion is currently being taken into consideration. There is also evidence on the antioxidant effects of plant polyphenolic compounds beyond the antioxidant activities of vitamins present in plants (Cieślik, Gręda, & Adamus, 2006).

2.4 NUTRITIONAL IMPORTANCE OF VEGETABLES

Fresh vegetables are edible parts of plants that play a significant role in maintaining health and preventing human diseases. The nutrients available in vegetables are important for building immunity, repairing body tissues, and maintaining alkaline reserves in the body. Vegetables contribute to diets differently concerning nutrition; for example, carrot is a good source of vitamin A, which helps to maintain regular vision. Spinach and tomato are good sources of vitamin C, which is important in the prevention of scurvy, while the gourds are sources of dietary fibers that are important in preventing constipation (Hanif, Iqbal, & Iqbal, 2006).
In Bangladesh, the extent of micronutrient deficiency is much larger than energy malnutrition. Around 60% of the population suffer from micronutrient deficiency and that consequently causes serious health problems. Micronutrients are found in high quantities in vegetables, therefore, the consumption of green leafy vegetables might be a necessary nutritional requirement that can help prevent micronutrient deficiency and minimize deficiency (Ebert, 2014).

It was also recorded from different epidemiological studies that aging, inflammation, cancer, cardiovascular diseases, Alzheimer's and Parkinson’s diseases can be reduced by

consuming fruits and vegetables (Hossain et al., 2015). It is presumed that the health benefits of vegetables are attributed to the actions of bioactive compounds. Bioactive phytochemicals presents in fruits, vegetables, and other plant-based foods can reduce the incidence of chronic diseases (Liu, 2013). Micronutrients comprise vitamins and minerals, which are important for maintaining a healthy human body. Calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), chloride (Cl), phosphorus(P), and sulfur (S) are considered major minerals (Nabavi, Nabavi, Ebrahimzadeh, Eslami, & Jafari, 2013). Minerals are essential not only to build strong bones but also to transmit nerve impulses. Some minerals are good for teeth structure such as Ca, P and F while Ca, Mg, Mn, P, and Fe are for healthy bones. Minerals are also important for immune response (Ca, Mg, Cu, Se, and Zn) and for normal functioning of the brain (Cr and Mn). Mineral deficiency is prevalent worldwide with 60% of the population having Fe deficiency, 30% for both Zn and I, and 15% deficient in Se. Around 20% of mortality for children less than 5 years old was due to deficiency of Zn, Fe, and I in association with vitamin A (White, White, & Broadley, 2009).

Minerals, vitamins, and fiber can be added to the regular diet mostly by consuming vegetables. In maintaining the regular metabolic activities in body tissues, minerals are vital. Twenty-five minerals are found in a living organism, where 92 occurs normally. They are essentially important for bones, teeth, muscles, blood, hair, and nerve cells. It has been observed that despite the presence of mineral balance in the body, it is usually difficult for vitamins to be adequately assimilated. Therefore, vitamins are needed in a fairly higher amount in the diet. This is because vitamins are essential for maintaining good skin, mucous membranes, bones, teeth, hair, vision, and the reproductive system. Additionally, they are also important for bone growth and maintenance, preventing blood clots, and promoting normal activities of the nervous system and endocrine glands (Hanif, Iqbal, & Iqbal, 2006). Among minerals, Na and K

play a significant role in the human body to transport metabolites. The ratio of Na to K is also significant in regulating blood pressure and preventing cardiovascular disorder. Consumption of high Na and low K can increase the risk of hypertension. To maintain healthy cardiovascular health, a NA: K ratio of less than one is recommended (Akubugwo, Obasi, Chinyere, & Ugbogu, 2007).

Growth and maintenance of teeth, bones, muscle, and heart functions are regulated by Ca, while the human RNA and DNA synthesis occurred using Mg. It is also important to metabolize Ca and regulate potassium fluxes (FAO/WHO Expert Consultation, 2005). The main ingredient important for the formation of hemoglobin is Fe, and it is also responsible for the proper functioning of the central nervous system as well as carbohydrate, protein, and fat metabolism (Gupta, Jyothi Lakshmi, Manjunath, & Prakash, 2005). Consuming 40 g of fresh or dry vegetables daily can help meet the average daily recommendation of Fe, which is 400 g/day. Besides this, Cu, and Fe are also important for hemoglobin formation to prevent anemia while Fe along with Zn, also assist in enzyme activities (Osredkar, 2011). In the absence of Zn, different complications arise during pregnancy and after birth, such as low birth weight, premature birth of the child, loss of appetite, and weakness. Zn is also important for maintaining normal growth, mental ability, immune system, reproduction, and heart health (Deshpande, Joshi, & Giri, 2013). Thyroid gland enlargement called goiter occurs due to iodine deficiency (Nabavi et al., 2013). It is always important to know the recommended limits of iodine consumption because it may be toxic if safe limits are exceeded (Ogwok, Bamuwamye, Apili, & Musalima, 2014).

While proteins arguably the most important nutrient for good health, it is still mostly absent or limited in the diets of developing countries. One of the cheapest and most abundant sources of protein is green vegetables. Vegetables can synthesize amino acids from water, CO_2, and also from

atmospheric nitrogen. Therefore, incorporating them in the meals with a plan to improve the protein base can help increase the quantity of protein consumed (Aletor, Oshodi, & Ipinmoroti, 2002). In-plant and animals, amino acids are building blocks of proteins and play roles in cellular metabolism. Proteins provide the structural materials in humans and also function as enzymes, hormones, and antibodies. The digestion of proteins forms amino acids with the help of enzymes before absorption from the small intestine. A range of amino acids is synthesized by the human body, including alanine, asparagine, aspartic acids, cysteine, cystine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine. Nine others are referred to as essential amino acids because the body is not capable of synthesizing them. Therefore, they have to be consumed as part of the diet: arginine, histidine, isoleucine, leucine, lysine, methionine, tryptophan, valine, and phenylalanine (Hounsome, Hounsome, Tomos, & Edwards-Jones, 2008). Almost all the essential amino acids can be found in vegetables. However, some have more of the limiting amino acids than others. Therefore, it is important to combine a variety of vegetables to obtain all the essential amino acids. The consumption of whole food is better than refined foods or dietary supplements. It was also observed in surveys from France, Italy, Netherlands, and Spain that carotenoid-rich fruits and vegetable consumption is better than dietary supplements in reducing DNA damage and improving resistance to LDL oxidation (Kader, Perkins-veazie, & Lester, 2000).

2.5 THE IMPORTANCE OF POLYPHENOLS AS ANTIOXIDANTS

Polyphenols are widely distributed in nature as secondary plant metabolites, abundant in fruits and vegetables. Polyphenols contain different compounds and different classes based on their chemical structures such as phenolic acids, flavonoids, stilbenes, and lignans. About 8000 phenolic structures have been discovered to be present in over 100 edible vegetables. A strong relationship has been observed between phenolic compounds and NCD prevention (Hurtado-Barroso et al.,

2018). There is also scientific evidence that the antioxidant activity of polyphenols is helpful in reducing cardiovascular diseases. Other epidemiological studies have shown that long term consumption of plant polyphenols can protect the human body from cancers, cardiovascular diseases, diabetes, osteoporosis, and neurodegenerative diseases (Pandey & Rizvi, 2009). Apart from disease preventions, polyphenols contribute to control disease propagation, suppress progression, and meanwhile take part in the healing process (González-Vallinas, González-Castejón, Rodríguez-Casado, & Ramírez de Molina, 2013). Polyphenols also include hydrolyzable tannins and phenylpropanoids like lignins, flavonoids and condensed tannins (Pandey & Rizvi, 2009). Hydroxyl group-containing polyphenols occur in the ortho or para positions and are readily involved in redox reactions. Phenolic compounds can easily participate in reactions that reduce oxidation because they carry protons and electrons (Shahidi, Janitha, & Wanasundara, 1992).

Antioxidants present in plants helps thrombocytes sealing and stop oxidation of LDL cholesterol and blood lipids and thus enhance sclerotic processes Consequently, this helps to maintain the normal cardiovascular system. Antioxidants also help prevent resistance to damage to the elasticity and integrity of the blood vessel wall. The daily recommended polyphenol consumption is about 1-2 g (Cieślik et al., 2006). Phenolic contents available in plant cells are not uniform. Some are available in the tissues, while others are present at the cellular and subcellular levels. Soluble and insoluble phenolics are present in plant cell vacuoles and cell walls, respectively. For specificity, flavones and isoflavones are only found in individual plants while polyphenols such as quercetin are present in fruits, vegetables, and cereals. Phenolic contents are usually higher in the outer layer of plants compared to the core parts (de Simón, Pérez-Ilzarbe, Hernández, Gómez-Cordovés, & Estrella, 1992). There are several factors associated with the significant differences in the polyphenolic contents present in plants. Factors include the degree of

ripeness, harvesting time, processing, storage, as well as environmental and climatic factors (rainfall, geographic location of the plant). The amount of digested polyphenols also varies depending on the absorption site. Absorption occurs mainly within the gastrointestinal tract (Pandey & Rizvi, 2009).

2.5.1 Phenolic compounds

Phenolic compounds are widely available in edible and nonedible plants; multiple biological effects have been reported, including antioxidant activity (Kähkönen et al., 1999). Phenolic compounds have different structures and can conjugate various hydroxyl groups, and therefore, act as a defensive mechanism both for oxidative stress and UV radiation. Insoluble polyphenolic complexes were associated with the nutritional value decrease observed as a result of their inclusion in animal diets (Cirkovic Velickovic & Stanic-Vucinic, 2018).

2.5.2 Carotenoids

Carotenoids are natural pigments, comprising more than 700 structures. They are synthesized not only in plants but also in other photosynthetic organisms, like bacteria, fungi, algae, yeasts, and molds (Nahak, Suar, & Sahu, 2014). The chemical, biochemical, and physical properties of carotenoids are influenced due to the presence of conjugated double bonds in their system (El-Qudah, 2009). In foods, the most occurring carotenoids are β-carotene, α-carotene, and β-cryptoxanthin. All these carotenoids are converted into vitamin A or retinol, an active form of vitamin A. Among them, β-carotene is the most important because of it is safe, available in foods and essential for proper immune function, growth, and development (Sass-Kiss, Kiss, Milotay, Kerek, & Toth-Markus, 2005). Apart from their antioxidant properties, carotenoids also take part

in cell growth regulation, inhibition of cancer cell growth, and stimulation of the gap junctional communication (El-Qudah, 2009).

2.5.3 Flavonoids

Flavonoids are the most abundant plant antioxidants and non-nutritive phytochemicals. The daily total flavonoid intake can be up to 1 g and that is more than other phytochemicals (Toh, Tan, Lim, Lim, & Chong, 2013; Scalbert et al., 2005). Flavonoids are the largest group of polyphenols. Flavonoids, which are secondary plant metabolites, have strong beneficial health effects because of their antioxidants and chelating properties; therefore, they make significant contributions to the antioxidant capacity of vegetables (Vijayalaxmi, Jayalakshmi, & Sreeramulu, 2015a). Also, the anti-inflammatory, anti-mutagenic and anti-carcinogen properties of flavonoids have been reported along with their ability to exert inhibitory activities against different enzymes such as xanthine oxidase(XO), cyclo-oxygenase, lipoxygenase, and phosphoinositide 3 kinase (Panche, Diwan, & Chandra, 2016). An inverse relationship has been observed between flavonoid intake and cardiovascular diseases (CVD) mortality (Toh et al., 2013).

2.5.4 Bioavailability of polyphenols

There is no association between the amount of polyphenols in food and the bioavailability of each polyphenol in the human body. The small intestine can absorb aglycones; though most of the time esters, glycosides, and polymers are the available forms of polyphenols in foods, which must be hydrolyzed by intestinal enzymes before absorption can occur. Polyphenols undergo substantial alteration during absorption; in fact, they are conjugated by methylation, sulfation, and/or glucuronidation in the intestinal cells and later in the liver. The activities of polyphenols also differ depending on their concentrations and absorption sites. Some polyphenols are absorbed in the

upper gastro-intestinal tract, while others are absorbed in the large intestine (Pandey & Rizvi, 2009).

2.5.5 Functional properties of polyphenols

Functional foods and nutraceutical products can be developed using phenolic compounds and other nutrients. Some parameters are used to indicate the functional properties of phenolic compounds. Such include antioxidant capacities, phenolic compound contents, anti glycemic, and antihypertensive activities. It was also recorded that anti glycemic and antihypertensive properties of vegetables are attainable due to the abundance of phenolic compound (Da Rocha Rodrigues, Alves, Machado, Barbosa, & Barbosa Junior, 2018). In order to isolate polyphenols from vegetables, the first step is to carry out an extraction process, with maximum recovery dependent on technique and type of solvents used. Polyphenolic compounds have aromatic structures containing one or more hydroxyl groups. Due to enzymatic actions and chemical reactions in plants, several physical and chemical changes occurr at these hydroxyl groups. Thus, physical alternations occurr in the vegetable matrix. Therefore, to release bioactive compounds rupturing plant tissues through diffusion process extraction is important (Álvarez, Araya, Navarro-Lisboa, & de Dicastillo, 2016). The type of extraction solvent and its polarity may, therefore, have a significant impact on the amount of the extracted polyphenols. The polyphenol polarities vary from polar to nonpolar. Polar solvents have better solvent efficiency due to the interactions between polar sites of antioxidants and the solvent of the nonpolar one. Thus, water and sometimes water with some other aqueous mixture is used frequently to recover polyphenols (Thouri et al., 2017).

The types of solvents usually used for vegetable polyphenol extraction include ethyl acetate, ethanol, methanol, and water (Vijayalaxmi, Jayalakshmi, & Sreeramulu, 2015b). A better solvent is distinguished by its optimum extraction and its ability to keep the chemical str

-ucture of desirable compounds stable. It was also observed from a study that phytochemical content (total polyphenols, flavonoids), antioxidant activities (DPPH, ABTS, FRAP)) are affected significantly depending on extracting solvents. Solvents also have an impact on the inhibition capacity of biological activities such as inflammation and key enzymes involved in hyperglycemia (Thouri et al., 2017). Aqueous ethanol extracts were reported to be superior to methanol and ethanol while extracting flavonoids from tea, whereas for another experiment that involved tea catechin extraction, water was a better solvent than methanol and ethanol. Above all, water alone or mixed with other solvents is used mostly because of its low toxicity and high yield of extraction along with the benefit of modulating the polarity of organic solvents (Vijayalaxmi et al., 2015b).

2.6 NON-COMMUNICABLE DISEASES (NCDs)

Non-communicable diseases, commonly known as NCDs are the major leading cause of death worldwide, and they are associated with low consumption of fruits and vegetables. In low-income countries, NCDs such as CVDs, diabetes mellitus (DM), cancers, and chronic pulmonary disorders (CPD) are considered a burden and they are responsible for 50% of the global mortality rate. This is set to increase according to the WHO (1997), if not controlled. The two leading causes of diseases in Southeast Asia are ischaemic heart disease and stroke, whereas diabetes is the eighth leading cause of death in that region (Kjøllesdal et al., 2016). About 80% of heart diseases, 40% of cancer cases, strokes and type 2 diabetes can be prevented if they are identified early as this enables development of plans and taking precautions that eliminate the key risk factors such as poor diet, physical inactivity, and smoking (Phaswana-Mafuya, Tassiopoulos, Mkhonto, & Davids, 2011).

According to the WHO, Regions of Southeast Asia, (including Bangladesh) accounted for 57% annual mortality and 54% are sufferings from NCDs. About 61% of NCDs are identified as risk factors for all other types of diseases. According to the 2011 Bangladesh Health and Demographic Survey (BDHS), the age-adjusted prevalence of diabetes, pre-diabetes, prehypertension, and hypertension were 9.7%, 22.4%, 27.1%, and 24.4%, respectively. Besides this, the overall obesity prevalence is 11.6%. The survey revealed that among Bangladeshi people, 98.7% have a minimum of one potential risk, 77.4% have two or more risk factors, and 28.3% have three or more risk factors of developing NCDs; these levels are on the increase (Biswas, Islam, Linton, & Rawal, 2016). In 2005, about 35 million people died due to NCDs with 80% of these from low and middle-income countries, and 16 million people being under the age of 70 years. It has been projected that in 20 years, the worldwide disease burden will be caused mostly by NCDs. Therefore, a healthy diet such as the consumption is 400 g for fruits and vegetables per person is an important measure that has been recommended to reduce NCD risks (Habib & Saha, 2010). It is estimated that about 2.7 million lives can be saved each year by consuming the recommended levels of fruits and vegetables. About 85% of CVDs, 31% of ischemic heart diseases, 11% of strokes, and 19% of gastrointestinal cancers are believed to be caused mainly because of the low consumption of fruits and vegetables (Kanungsukkasem et al., 2009).

2.7 NUTRITIONAL FUNCTIONALITIES OF POLYPHENOLS.

a) Antioxidant activity
b) Anti obesity activity
c) Antidiabetes activities
d) Anticancer activity
e) Anti cardiovascular activity

2.7.1 Antioxidants activities

To extend the shelf life of foods, antioxidants are the substances that delay or inhibit a target molecule's oxidative damage, as they act as scavengers, and prevent damage to cells and tissues. By scavenging free radicals, antioxidants can stop or delay the oxidation process, especially lipid peroxidation that leads to the production of rancidity in foods containing oil. Agricultural residues and vegetables, many of which are polyphenols have great potential as cost-effective sources of antioxidants. Natural antioxidants instead of synthetic antioxidants that adversely affect human health are gaining popularity and consequently, high demand due to consumers' health awareness (Duistermaat & Kolk, 2011). Therefore, the production of safer food can be promoted by replacing synthetic antioxidants with natural plant antioxidants. Antioxidants have proved beneficial for human health. From a recent meta-analysis, it was observed that high antioxidant containing foods can reduce the risk of CVD-related mortality, CVD, cancers, and mood disorders (Cicero & Colletti, 2015). Polyphenols containing bioactive compounds can increase gene transcription factors and antioxidant activities can be regulated (Na & Surh, 2008). Polyphenols can activate peroxisome proliferation, which can trigger the tissues responsible for insulin sensitivity, inflammation, and lipid metabolism (Cho et al., 2010). Free radicals such as peroxide, hydroperoxide, or lipid peroxyl can be scavenged by phenolic acids, polyphenols, and specifically the flavonoids. These activities can inhibit oxidation production as well as degenerative diseases. A proven number of clinical studies showed that fruits, vegetables, and tea containing antioxidants are effective in reducing chronic disease incidences such as heart disease and cancers (Nahak et al., 2014). Antioxidants can alsocontrol the expression of gene encoding antioxidant enzymes, restore the oxidative damage done by radicals, and accelerate the removal of damaged molecules. In the industry, the use of antioxidants is important because it not only increases shelf life but also

helps to prevent oxidation (Coelho, Bellato, Santos, Ortega, & Tsai, 2007).

2.7.2. Anti-obesity activity

Obesity is considered a global problem because obesity is the root cause of several chronic diseases such as dyslipidemia, fatty liver, osteoarthritis, hypertension, gastrointestinal cancer, coronary artery diseases, stroke, and heart failure as well. Around 1.4 billion people above 20 years of age are obese with one-third of the global population suffering from this condition (Seyedan, Alshawsh, Alshagga, Koosha, & Mohamed, 2015). When the body accumulates excess fat in the body, this results in an imbalance between energy intake and loss. Apart from genetic predispositions, environmental factors related to obesity are insufficient physical activity along with high calorie and consumption of high fat-containing food. Lifestyle modification can be the best therapeutic approach to get rid of obesity (Vasiljevic, Pechey, & Marteau, 2015).

The pancreatic lipase (PL), is the main enzyme responsible for the digestion of 50-70% of dietary triglycerides into monoglycerides and free fatty acids, which are then absorbed in the enterocytes. Fat absorption and energy uptake can be reduced by inhibiting PL activity, which can consequently facilitate reduction in obesity (Vasiljevic et al., 2015). Several studies have suggested that polyphenols can be used as anti-obesity drugs, especially to reduce fat storage in adipose tissues. Therefore, vegetables that are enriched in phytochemicals have the potential to be use as anti-obesity agents. As there is a relationship between obesity and inflammation, anti-inflammatory activity can also be helpful in reducing obesity (Vauzour, Rodriguez-Mateos, Corona, Oruna-Concha, & Spencer, 2010). The mechanism of action of vegetable phytochemicals include the ability to inhibit the precursor cell proliferation to reduce adipose tissue fat storage, increase apoptosis rate during adipocyte lifecycle, and inhibit dietary triglyceride absorption. Different scientific investigations from vegetable-derived polyphenols were carried out using cell culture

and animal models and it was observed that vegetables can induce lipolysis, decrease lipid accumulation, and induce apoptosis in adipose tissues (Vauzour et al., 2010).

2.7.3 Anti-diabetes activity

Diabetes is a major non-communicable disease, with 4.9 million people dying from it in 2014 alone (Poovitha & Parani, 2016). There are bascically two forms of diabetes, namely type I and type II. Long-term consequences of diabetes include gradual development of different complications such as retinopathy, which affects the eyes and lead to blindness. Diabetes also causes disturbed and altered renal functions, known as nephropathy with increased amputation risks known as neuropathy. Foot ulcers, sexual dysfunction can also develop due to diabetes (Pandey & Rizvi, 2009). Postprandial hyperglycemia (PPHG) is a condition where blood glucose becomes high after meal consumption. By controlling PPHG, diabetes, and complications related to diabetes like diabetic retinopathy, diabetic retinopathy, and cardiovascular diseases can also be controlled. Blood glucose increases due to the cleavage of α-D-(1,4) glycosidic linkage by α-amylase and this produces oligosaccharides, which are also cleaved to monosaccharides with the help of α-glycosidase. This glucose is absorbed from the gastrointestinal tract into blood circulation to increase blood glucose levels from where it is deposited in the body. Therefore, inhibiting α-amylase and α-glucosidase activities can reduce blood glucose level spikes and the PPHG level can be controlled (Poovitha & Parani, 2016).In developing countries where limited resources and modern treatment is not readily available, research has found that polyphenol-containing foods act the same way as insulin for utilizing glucose. These polyphenols also inhibit -amylase and α-glucosidase and consequently type 2 diabetes. Consistent with these findings, ithas been discovered that many plants have hypoglycemic properties (Nair, Kavrekar, & Mishra, 2013).

There are some enzyme inhibitory drugs such as acarbose, miglitol, and voglibose that are available to control PPHG. Acarbose can inhibit both α-amylase and α-glucosidase whereas miglitol and voglibose inhibit only α-glucosidase. While these drugs are inhibitors they are not desirable for long term use because of their negative gastrointestinal effects (Poovitha & Parani, 2016). Apart from the high price, other side effects recorded from these drugs are liver disorder, flatulence, and abdominal cramping, hence the need for safer natural inhibitors like polyphenols (Shobana, Sreerama, & Malleshi, 2009).

2.7.4 Anti-cancer activity

Cancer is the second death leading and major health problem worldwide. In the united states, 1762450 new cases and 606880 deaths occurred in the year of 2019 as projected by the American cancer society. Apart from massive death, cancer treatment costs are very high along with the physical and emotional difficulties. There are two sources of antioxidants that can reduce oxidative stress; either produced endogenously or by exogenous supplements from foods. Plant products are considered as major sources of highly available and effective antioxidants (Prasad & Srivastava, 2020). Cancer develops in stages, starting with initiation, promotion, and then progression. Oxidative damage and inflammation contribute to cancer development throughout all the stages. When cancer cells start to initiate, free radicals induce DNA oxidative damages. If it is not stopped, this damaged DNA stimulates mutation, cross-linking, single and double strands break, and chromosomal breaks as well (Liu, 2013). Fruits, vegetables, and whole grains containing antioxidants can prevent or stop cancer induction that arise from oxidative damages. Apart from the initiation stages, the promotion and progression stages can be modulated by regulating different signal transduction pathways. It was reported that more than 28 vegetable servings per week can reduce the risk of prostate cancer by 35% in comparison to less than 14 servings; reduced risk of

pancreatic cancer was also reported from fruits and vegetables consumption (Cohen, Kristal, & Stanford, 2000). An inverse relationship has been observed between colon and colorectal cancer and vegetable consumption (Liu, 2013).

Polyphenols work as anticancer agents because of their high accessibility, low toxicity, response specificity, and different biological effects. Polyphenols combine the cytoprotective effects against normal cells and cytotoxic effect against cancer cells and thus work as anticarcinogenic agents (Brglez Mojzer et al., 2016). Polyphenols regulate the growth factor receptor interactions and play a great role in carcinogenesis. It is also helpful that they encourage cell cycle arrest and affect cell survival and apoptosis of cancerous cells (Tabrez et al., 2013). Polyphenols take part in pro-oxidative actions and induce apoptosis and that depends on different factors like concentrations, environmental conditions, and target molecules (Scalbert et al., 2005). Due to these different factors, the interactions become different. Moreover, angiogenesis responsible for tumor growth is also inhibited by polyphenols, which promotes establishment of body immune systems and consequently act as inflammatory agents (Tabrez et al., 2013). Polyphenols have been found to reduce a number of tumors. Tests were carried out with different polyphenols such as quercetin, catechins, isoflavones, lignans, flavanones, ellagic acid, and it was observed that all of them showed protective actions against cancer (Pandey & Rizvi, 2009). In the case of cancers, polyphenols reduced the metastatic potential by lessening the adhesiveness and invasiveness of cells.

2.7.5 Anti-cardiovascular disease activity

In 2012, around 17.5 million people died from CVDs, which was 31% of global deaths recorded by WHO. Out of the 17.5 million, 7.4 million died due to heart diseases and 6.7 million due to strokes. Since vegetables and fruits are full of important nutrients it has been suggested that

consumption of fruits and vegetables can reduce CVD risks along with diabetes, cancer, and other chronic diseases (Collese et al., 2017). It is significant that those who suffer from a primary CVD event are 20% more predisposed to a second cardiovascular event without a mitigation treatment (Clemens et al., 2020). CVDs are responsible for kidney damages and heart attack and it arises from hypertension or excessive high blood pressure. It is also responsible for arteriosclerosis, stroke, and the last stages of renal disease. Blood pressure can be controlled by the renin-angiotensin system (RAS), influenced by angiotensin I converting enzyme (ACE) and renin. Angiotensin I is formed from angiotensinogen by the help of Renin whereas angiotensin II is converted from angiotensin I by ACE (Aluko, 2019). Once angiotensin II forms, the released aldosterone and blood sodium consequently leads to blood pressure increases. Also, the blood vessel relaxing ability decreases when ACE hydrolyzes a potent vasodilator, bradykinin. These RAS mechanisms can be controlled by two steps. Firstly, by inhibiting ACE activity, to prevent excessive formation of Ang II, and consequently, bradykinin destruction will be reduced. As a result, blood pressure will be reduced. Secondly, renin inhibition activity can inhibit the total RAS pathway and lower blood pressure (Sonklin, Alashi, Laohakunjit, Kerdchoechuen, & Aluko, 2019)

The available forms of dietary polyphenols are glycosylated forms containing sugar residues that are bonded to the hydroxyl group on an aromatic ring, which causes slow absorption in the stomach. Aglycones and some glycoside absorption occur in the small intestine whereas the colon is the absorption site for the rest. Also, the colon is not able to absorb polyphenols quickly, as it takes almost 9 hrs. Consequently, only 15-20% of intake is polyphenol absorbed. So, the more efficient absorption occurs in the glycosides form (Brglez Mojzer, Knez Hrnčič, Škerget, Knez & Bren, 2016).

LDL oxidation is considered as the main reason for atherosclerosis and polyphenols can inhibit this oxidation as well as atherosclerosis. A strong correlation has been observed between polyphenol-rich food consumption and CVD occurrence and it was recorded that people who consumed more polyphenol-rich foods have less myocardial infarction (Pandey & Rizvi, 2009). It was also reported that excess ROS production leads to promote chronic inflammation as well as atherosclerosis and CVD (Clemens et al., 2020).

For developing CVD, LDL oxidation works as an atherogenic factor; intimal LDL increased when LDL spread through the artery wall due to its oxidation. Intima-oxidized LDL is much more atherogenic than native LDL and acts as a chemotactic factor targeting the circulating monocytes and macrophages (Liu, 2013).

The oxidized LDL induces further production of inflammatory cytokines and promotes cell propagation in smooth muscle cells, cholesterol ester aggregation, and the development of foam cells by macrophages in the intima. The foam cell formation will produce a fatty streak in a blood vessel that could escalate to atherosclerotic disorder as well as further damage to the endothelial system. Consequently, phytochemicals containing antioxidants are able to scavenge free radicals to prevent or delay the progression of the atherosclerotic lesions (Sánchez-Moreno, Jiménez-Escrig, & Saura-Calixto, 2000). Apart from the above activities, dietary antioxidants can also reduce platelet aggregation, cholesterol synthesis, lipid absorption, blood pressure andas well as anti- inflammation (Song et al., 2010). In the western world, atherosclerosis is considered a major cause of mortality. It comprises of connections between different parts such as the arterial wall, blood cells, and plasma lipoproteins. Atherosclerosis, a chronic inflammatory disease is responsible for developing medium-sized arteries in lesion-prone regions. From this lesion, myocardial infarction, unstable angina and even sudden cardiac death can occur. Polyphenolic

compounds are potent inhibitors of LDL oxidation that can help control atherosclerosis development and stabilize the atheroma plaques (Pandey & Rizvi, 2009). The overall intake of polyphenol can be 1 g/day, and this can be more effective than all other antioxidants. For example, this level of polyphenol intake is 10 times more potent than vitamin C and 100 times more than vitamin E and carotenoids (Scalbert et al., 2005).

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CHAPTER THREE
MANUSCRIPT ONE

Chemical composition and *in vitro* antioxidant properties of water-soluble extracts obtained from Bangladesh vegetables

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3.0 Abstract

The aim of this study was to evaluate the nutritional value and antioxidant properties of aqueous extracts of some Bangladesh vegetables using, fruits of ash gourd, bitter gourd, brinjal, okra, ridge gourd, snake gourd, and leaves of Indian spinach, kangkong, and stem amaranth. Proximate composition showed that the dried extracts were composed mainly of crude protein (14.6-46.7%) and non-fibre carbohydrates (26.4-53.5%). With the exception of stem amaranth, all the extracts had >40% DPPH radical scavenging ability at 0.5 mg/mL. In contrast metal chelation was lower, except in Indian spinach with ~46%. The ferric reducing antioxidant power was highest for the kangkong (10.9 mM Fe^{3+} reduced), which is similar to the 9.9 mM for butylated hydroxytoluene (BHT). All the extracts suppressed linoleic acid oxidation better than BHT within the first five days of the incubation period. We conclude that the Indian spinach, kangkong, and okra could be considered as the most promising sources of antioxidant compounds.

3.1 Practical applications

Vegetables are commonly consumed as part of a regular diet but the high water and fibre contents usually mean that large quantities are required to provide long-term health benefits. Therefore, in this work, aqueous extracts of nine Bangladesh vegetables were prepared to provide a more concentrated form of nutrients and bioactive compounds. The extracts had strong nutritional value based on the high contents of crude protein, potassium, iron and non-fibre carbohydrates. The high content of polyphenolic compounds in the extracts can also provide health benefits, which was demonstrated through strong free radical scavenging, metal chelation, ferric iron reduction and inhibition of linoleic acid oxidation. These vegetable extracts have the potential to be used as

sources of bioactive compounds to prevent or treat non-communicable diseases that are associated with high oxidative stress.

KEYWORDS: Bangladesh vegetables, polyphenols, antioxidant, proximate composition, amino acid composition

3.2 INTRODUCTION

Non-communicable diseases (NCDs) such as cardiovascular diseases (CVDs), cancer and diabetes are associated with low levels of physical activity, tobacco use along with low intake of fruits and vegetables (Zaman et al., 2015). Fruits and vegetables are high in vitamins, minerals and fibre, which help to reduce blood pressure (BP) and oxidative stress with improved lipoprotein and insulin sensitivity (Kjøllesdal et al., 2016). Vegetables and fruits are rich in antioxidants, which provide a wide range of protective biological, pharmacological and chemical properties. The beneficial effects of antioxidants include the protection from diseases such as cancer, cardiovascular disease, diabetes, hypertension, stroke, paralysis and urinary disorder (Kumari, Verma, Nayik, & Solankey, 2017).

In developing countries, majority of the population depend on vegetables and fruits for their nutritional dietary needs because they are relatively cheaper than animal protein sources. The daily suggested consumption is about 400 g of vegetables along with nuts and pulses (Kumari et al., 2017; Oboh et al., 2016). Vegetables also contain several phytochemicals, which have strong antioxidant activity such as radical scavenging or metal ion chelation. Consequently, antioxidant activities are attributed to the presence of vitamins A, C, E and K, as well as carotenoids, terpenoids, flavonoids, polyphenols, saponins, minerals, and enzymes found in the vegetables (Kumari et al., 2017). It has also been reported that vitamin C and E, or carotenoids and

polyphenols are significant free radical chain breaking antioxidants used in the food industry as preservatives (Číž et al., 2010).

Through physiological metabolic processes in the human body and from some external sources like X-rays, cigarette smoke, industrial chemicals and air pollution, free radicals are produced, which are not only highly reactive but also unstable. As a consequence of enzymatic and non-enzymatic reactions, these free radicals are generated continuously (Lobo, Patil, Phatak, & Chandra, 2010). Antioxidants work in two ways, firstly, primary antioxidants provide an electron to the free radicals, which is known as chain breaking mechanism. Secondly, through the removal of reactive oxygen species and reactive nitrogen species by quenching chain initiating catalysts (Lobo et al., 2010). To maintain a healthy biological system therefore, the balance between oxidation and anti-oxidation is very critical. Polyphenols, flavonoids, anthocyanins, and vitamins consumptions have been reported to demonstrate antioxidant properties and could provide healthy oxidative balance (Terao, Piskula, & Yao, 1994).

Antioxidants also help delay the oxidation process, especially lipid peroxidation that leads to the production of rancidity in foods. In foods and feeds, lipid oxidation is ubiquitous, therefore, the amount may differ from food to food and the level of occurrence may be low, but lipid oxidation destroys food quality and reduces shelf life. In foods, oxidized lipids, even at low levels are responsible for losses such as colour, flavour and nutrients, which can also have a negative impact on human health (Wasowicz, Gramza, Hes, Malecka, & Jelen, 2004). Butylated hydroxytoluene (BHT) and hydroxyanisole (BHA) are frequently used synthetic antioxidants in the food industry to prevent the negative effects of free radicals. However, there is a universal agreement on the need for increased use of natural antioxidants such as polyphenols because the

synthetic antioxidants have potential health risks and toxicity, most notably cancerous effects (Číž et al., 2010).

In Bangladesh, cooked vegetables are consumed regularly as part of meals and are considered as good sources of health-promoting and essential nutrients such as amino acids, dietary fibre and minerals. Therefore, the vegetables could play important roles in the prevention and treatment of NCDs. Ash gourd, bitter gourd, brinjal, Indian spinach, kangkong, okra, ridge gourd, snake gourd and stem amaranth are some of the typical vegetables that are readily available in Bangladesh because they are widely grown across the country. However, information is scant on the nutritional and potential bioactive properties of the soluble compounds, especially polyphenols of these vegetables. Therefore, the main objective of this research work was to characterize the *in vitro* antioxidant properties of the polyphenol-rich water extracts from these vegetables in order to estimate potential uses as food preservatives and prevention of oxidative stress that have been associated with onset and progression of NCDs. In addition to the content of polyphenolic compounds, we also profiled the aqueous soluble vegetable extracts for their nutritional value (mineral and amino acid contents) as well as their contents of potentially bioactive phytochemicals such as chlorophylls and β-carotene.

3.3 MATERIALS AND METHODS

3.3.1 Materials

The 2,2- diphenyl-1 picrylhydrazil radical (DPPH), BHT, 3-(2- Pyridil)-5, 6-diphenyl-1,2,4-triazine-4',4"- disulfonic acid sodium salt (Ferrozine), 2,4,6- tripyridyl-s-triazine (TPTZ), Folin-Ciocalteu phenol reagent, gallic acid, catechin, myricetin, caffeic acid and rutin were purchased from Sigma Aldrich (Sigma Chemicals, St. Louis, MO). All other reagents were of analytical grade and purchased from Fisher Scientific (Oakville, ON, Canada). Ash gourd (*Benincasa hispida*)-

BARI CHALKUMRA 1, bitter gourd (*Momordica charantia*)- BARI KOROLA 1, brinjal (*Solanum melongena*)- BARI BEGUN 8, Indian spinach (*Ipomoea aquatica*) – BARI PUISHAK 1, kangkong (*Ipomoea aquatica*)- BARI GIMAKOLMI 1, okra (*Ebelmoschus esculentus*)- BARI DHEROSH 2, ridge gourd (*Luffa acutangula*) – BARI JHINGA 1, snake gourd (*Tricosanthes cucumerina*) – BARI CHICHINGA 1 and stem amaranth (*Amaranthus L.*)- BARI DATA 1 were supplied by the Bangladesh Agricultural Research Institute, Dhaka, Bangladesh. Each vegetable was harvested at matured stage, rinsed in water, destalked, oven-dried at 40 °C for 8-10 hrs, pulverized into a powder and stored at 4 °C.

3.3.2 Preparation of phenolic-rich water extract

A previously proposed method reported by Olarewaju et al., (2018), was used to extract the unbound water-soluble polyphenols with minor changes. Briefly, dried vegetable powders and distilled water (1:20) were mixed in a 500 mL beaker, adjusted to 60 °C and stirred for 2 h. Samples were allowed to cool to ambient temperature and centrifuged for 30 min at 5600 x *g*. The supernatant was filtered through a muslin cloth and the first filtrate collected. The residue was subjected to a second water extraction and the process repeated to collect a second filtrate, which was then combined with the first filtrate. The combined filtrates was evaporated in a rotary evaporator under vacuum at 60 °C, followed by freeze-drying of the residual aqueous solution; the dried material was then stored at -20 °C.

3.3.3 Total phenolic content (TPC) assay

TPC assay of the dried extracts was carried out using a previously reported method (Hoff & Singleton, 1977) with some modifications. A standard curve was constructed with 25-350 µg/mL gallic acid concentration prepared in 50% (v/v) methanol in water. Then the samples were diluted to 250 -500 µg/mL with the 50% methanol to obtain gross weights that are within the gallic acid

standard curve concentration range. This was followed by mixing 0.25 mL of gallic acid solution or diluted sample with 0.25 mL of Folin-Ciocalteu reagents and incubated for 5 min in a dark place at ambient temperature. A 0.5 mL aliquot of 20% (w/v) sodium carbonate solution and 4 mL of distilled water were added, vortexed to mix properly and further incubated for 1 h in a dark place. Absorbance of the samples at 725 nm was measured using an Ultraspec UV- visible spectrophotometer (GE Heathcare, Montreal, PQ, Canada). TPC was expressed as milligrams gallic acid equivalents (GAE) per gram of dry leaf powder (mg GAE/g).

3.3.4 Total flavonoid content (TFC) assay

TFC of extracts was determined as previously described (Nabavi, Nabavi, Ebrahimzadeh, Eslami, & Jafari, 2013) with slight modifications. Samples were dispersed in methanol and 30 µL mixed with 90 µL of methanol followed by 6 µL of 10% (w/v) aluminium chloride, 6 µL of 1 M potassium acetate and 168 µL of double distilled water. This mixture was incubated at ambient temperature in the dark for 30 min. The reaction mixture absorbance was then measured at 415 nm in a synergy H4 multi-mode microplate reader (Biotek Instrument, Winooski, VT, USA). TFC was calculated as rutin equivalent (RE) from a rutin calibration curve (0.05, 0.1, 0.125, 0.25, 0.5 and 1 µg/mL in methanol).

3.3.5 β-Carotene content assay

The method of Biswas, Sahoo, and Chatli (2011) was used to estimate the β-carotene content of the dried vegetable extracts. Samples (0.2 µg/mL) were dispersed in chilled acetone in test tubes. The mixture was shaken for 15 min at 4 ºC, vortexed and then centrifuged for 10 min. The supernatant was collected, and the precipitate re-extracted with acetone and the protocol repeated to collect a second supernatant. Both supernatants were combined, filtered through a Whatman No. 42 filter paper and the absorbance of the filtrate measured at a wavelength of 449 nm in the

Ultraspec UV- visible spectrophotometer (GE Heathcare, Montreal, PQ, Canada). Commercial β-carotene (95% purity) was used as the standard to calculate the content of β-carotene in the vegetable samples.

3.3.6 Determination of total chlorophyll, chlorophyll a and chlorophyll b

The amounts of total chlorophyll, chlorophyll a and chlorophyll b in the extracts were determined by following a previously described method (Rajalakshmi & Banu, 2015). A 0.1 g powder of the vegetable extracts was mixed with 40 mL of 80% acetone followed by centrifugation for 5 min at 4 ºC and 5600 x *g*. The collected supernatant was transferred into a glass container while the acetone extraction, was repeated until the residue became colourless. The supernatants were combined and absorbance's read at 645 nm and 663 nm, using acetone as a blank in the Ultraspec UV- visible spectrophotometer (GE Heathcare, Montreal, PQ, Canada). The amount of total chlorophyll, chlorophyll a and b were calculated by using the following equations:

Total chlorophyll: $20.2\ (A_{645}) + 8.02(A_{663})$

Chlorophyll a: $12.7(A_{663}) - 2.69(A_{645})$

Chlorophyll b: $22.9\ (A_{645}) - 4.68(A_{663})$

3.3.7 Proximate and mineral composition analyses

The proximate and mineral compositions of the dried vegetable extracts were determined using the standard methods of the Association of Official Analytical Chemists (AOAC, 1990).

3.3.8 UHPLC MS/MS analysis of polyphenolic compounds

UHPLC analysis of the vegetable extracts was performed using an Agilent 1290 UHPLC system (Santa Clara, CA, USA) coupled with an HSS T3 2.1x100 mm 1.7 µm column from Waters Corp (Milford, MA, USA). The samples (5 µL injection volume) were eluted at a flow rate of 0.5 mL/min using Buffer A and B (0.1% formic acid in water and 0.1% formic acid in acetonitrile

respectively) at 40 °C. The following gradients were used; initial holding time 0.5 min, buffer B ramped up to 50% after 5 min, 95% after 6 min, held for 1 min and re-equilibrated for 1.5 min. A diode array detector was used to identify samples at a wavelength range of 230- 640 nm in 2 nm increments and a frequency of 5 Hz. The mass spec was carried out using an Agilent 6550 QTOF at 200 °C, using a drying gas pressure of 18 psi, 40 psi nebulizer and 350 °C sheath gas, a pressure of 12 psi and 3500 V capillary with a 1000 V nozzle, ran in positive ion electrospray at a frequency of 3Hz and acquisition from 30-1,700 m/z. The MS/MS was performed at a narrow quadruple setting (1.3 atomic mass units), using 10, 20, and 40 eV collision energy and 30-1700 m/z. The compounds were identified using internal polyphenols standards.

3.3.9 Determination of amino acid composition

Samples were hydrolyzed with 6 M HCL and analyzed using an HPLC system as previously described (Bidlingmeyer, Cohen, & Tarvin, 1984). For cysteine and methionine contents, samples were hydrolyzed with performic acid (Gehrke, Wall, Absheer, Kaiser, & Zumwalt, 1985) while tryptophan was determined after alkaline hydrolysis (Landry & Delhaye, 1992).

3.3.10 DPPH radical scavenging assay

The method of described by Olarewaju et al., (2018).was used to determine the DPPH radical scavenging activity of samples in a 96-well clear (flat bottom) microplate. The leaf extracts were dissolved in 0.1 M sodium phosphate buffer, pH 7.0 containing 1% (w/v) Triton X-100 to obtain final concentrations of 0.125, 0.25 and 1 mg GAE/mL. DPPH was dissolved in methanol to obtain 100 µM final concentration and used as the blank. A 100 µL of sample solution was then mixed with 100 µL of DPPH solution in 96 well plate and incubated at ambient temperature for 30 min in the dark. The absorbance values of blank and sample solutions were measured at 517 nm using

the Synergy H4 multi-mode microplate reader with BHT as a standard. The following equation was used to determine the percentage of DPPH radical scavenging activity,

$$\text{DPPH (\%)} = \frac{Abs(blank) - Abs(sample)}{Abs\ (blank)} \times 100$$

3.3.11 Ferric reducing antioxidant power (FRAP) assay

The previously described method was used to measure FRAP activity as follows (Olarewaju et al., 2018). A 300 mM, pH 3.6 acetate buffer was mixed with 10 mM TPTZ prepared in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ at a ratio of 10:1:1 to make the FRAP reagent. Sample (40 µL) were then mixed with 200 µL of the FRAP reagent in a 96 clear well microplate to obtain final concentrations of 0.5-1.0 mg GAE/mL. The absorbance of each well was measured at 593 nm in the Synergy H4 multimode microplate reader. The results were expressed as mM of Fe^{2+} reduced/g extract using a 25-150 mM $FeSO_4.7H_2O$ calibration curve. BHT was used as positive control and assayed using the same protocol described for the samples.

3.3.12 Metal chelation activity (MCA) assay

MCA was estimated using a previously described method (Xie, Huang, Xu, & Jin, 2008) with slight modifications for a 96-well microplate as follows. A 1 mL aliquot of sample or BHT solution was added to 925 mL of water and 0.05 mL of 2 mM $FeCl_2$ in a reaction tube to give 0.25, 0.5 and 1 mg/mL final concentrations while water was used as the blank. The solution was vortexed and 25 µL of 5 mM Ferrozine was added and the solution vortexed again. The mixture was kept at room temperature for 10 min and 200 µL pipetted into the microplate wells. Absorbance values of the blank and samples were then measured at 562 nm in the Synergy H4 multi-mode microplate reader. By using the following equation, the percentage MCA values were calculated:

$$\text{Metal chelating activity (\%)} = \frac{Abs(blank) - Abs(sample)}{Abs\ (blank)} \times 100$$

3.3.13 Inhibition of linoleic acid oxidation assay

Linoleic acid oxidation was measured as previously described (Li, Jiang, Zhang, Mu, & Liu, 2008). Samples were dissolved in 1.5 mL of 0.1 M phosphate buffer, pH 7.0 to give a final concentration of 0.25 GAE/mL and 1 mL of 50 mM linoleic acid added in a glass test tube. The mixtures were kept in a dark place at a temperature of 60 ºC for 7 days. At 24 h intervals, 100 µL from each mixture was drawn and mixed with 4.7 mL of 75% aqueous ethanol, 0.1 mL of ammonium thiocyanante (30% w/v) and 0.1 mL of 0.02 M $FeCl_2$, prepared in 1 M HCl solution. After 3 min, absorbance of each mixture was then measured at 500 nm using the Synergy H4 multi-mode microplate reader with BHT as the standard.

3.3.14 Statistical analysis

A minimum of duplicate assays was used to find out the mean values and standard deviations. For statistical analysis, analysis of variance was been used while significant differences ($p < 0.05$) between mean values were determined by the Duncan's multiple range tests. The IBM SPSS statistical package (version 24) was used for all statistical analyses.

3.4 RESULTS AND DISCUSSION

3.4.1 Extract yield

For this work, the focus was on water-soluble polyphenols and other compounds because they are most likely to be absorbed during digestion in the gastrointestinal tract and have a stronger relevance to human health than the water-insoluble compounds. For example, a recent work showed that chlorophylls were assimilated and bioavailable in human epithelial colorectal adenocarcinoma cells (Caco -2 cells) through the process of aqueous micellization of dephytylated and oxidised chlorophyll derivatives obtained from seaweed (Chen & Roca, 2018). In addition, hydrophilic polyphenolic compounds such as malvidin-3-glucoside and phenolic acids are known

to be absorbed well from the stomach (Lewandowska, Szewczyk, Hrabec, Janecka, & Gorlach, 2013). Moreover, current trends in the food industry places less emphasis on the use of toxic solvents and more on the cheaper and environmentally friendly aqueous-based extractions. As shown in Table 1, Brinjal had the significantly ($p < 0.05$) highest yield, which indicates a high concentration of water-soluble compounds in this vegetable. The snake gourd, ridge gourd, and Okra extracts also had very high yields (>68%). In contrast, the content of water-soluble compounds were significantly ($p < 0.05$) lowest (<40%) in the Indian spinach and stem amaranth.

3.4.2 Total polyphenol content (TPC), total flavonoid content (TFC) and identification of major polyphenolic compounds

The TPC and TFC are the broad categories used in scientific literature to quantify and describe the presence of major plant phytochemicals that contain phenolic rings. Table 1 shows that the Indian spinach extract had a significantly ($p < 0.05$) higher TPC value than all the other vegetables. The values for the other extracts were similar except the stem amaranth and ash gourd extracts, which had the lowest TPC values. The results are in contrast to a previous work, which reported that kangkong had a higher TPC value of 27.65 ± 1.45 mg GAE/g when compared to 7.65 ± 0.47 mg/GAE for *Amaranth gangeticus*, 7.22 ± 0.31 mg GAE/g for *Amaranth viridis* and 2.71 ± 0.05 mg GAE/g for brinjal (Sharmin, Nazma, Mohiduzzaman, & Cadi, 2011). Similarly, Hossain, Sultana, Iftekharuzzaman, Hossain, & Taleb (2015) also reported that kangkong had a higher TPC value of 38.9 ± 1.7 mg GAE/g when compared to amaranth with 16.6 ± 0.7 mg GAE/g. The differences between our work and previous studies may be due to the type of extraction solvent used because some of the previous studies used solvents and not water. For example, the TPC of picralima seed from aqueous extraction was reported to be 435.87 ± 1.76 mg GAE/g whereas it was 356.53 ± 4.67 mg GAE/g for the methanol extract (Akinwunmi & Amadi, 2019). Similarly,

the TPC content of date plum water extract was 14.5 ± 0.20 mg GAE/g when compared to 1.6 ± 0.12 mg GAE/g for the acetone extract (Gao et al., 2014). Thouri et al. (2017) also reported higher TPC values for the water extract of date seed when compared to different concentration of methanol and acetone. The differences between the TPC of the vegetable varieties may be due to several factors such as variation in their response to water stress, temperature, and other environmental conditions (Rana, Alam, & Akhtaruzzaman, 2019).

Flavonoids are considered as one of the dominant polyphenolic compounds in plants and is the most important quality index for antioxidant activity (Zhao et al., 2018). Table 1 also shows that the highest ($p < 0.05$) TFC was found in kangkong (305.39 ± 2.93) while stem amaranth (62.22 ± 0.24) had the lowest value. The results are similar to those reported by Hossain et al. (2015), which showed that kangkong had the highest TFC when compared to 11 other vegetables. Noticeably, there was no direct correlation between the TPC and TFC values, which indicates varied levels of polyphenolic compounds in the samples. Results obtained from this study support the findings of Sarwar et al. (2019), who also reported that samples that contained higher TPC did not have high TFC. Overall, the results suggest that regular consumption of these vegetables could contribute to significant flavonoid intake. This is important because consumption of vegetables containing flavonoids had a direct relationship with reduced risks of chronic diseases such as stroke, cancers and other forms of CVDs (Bunney, Zink, Holm, Billington, & Kotz, 2017).

A total of ten phenolic compounds were identified by mass spectrometry to be present in all the vegetable samples, though some compounds were not found in all the samples while certain peaks could not be identified (Fig. 1). The identified compounds include vitexin ramnoside,

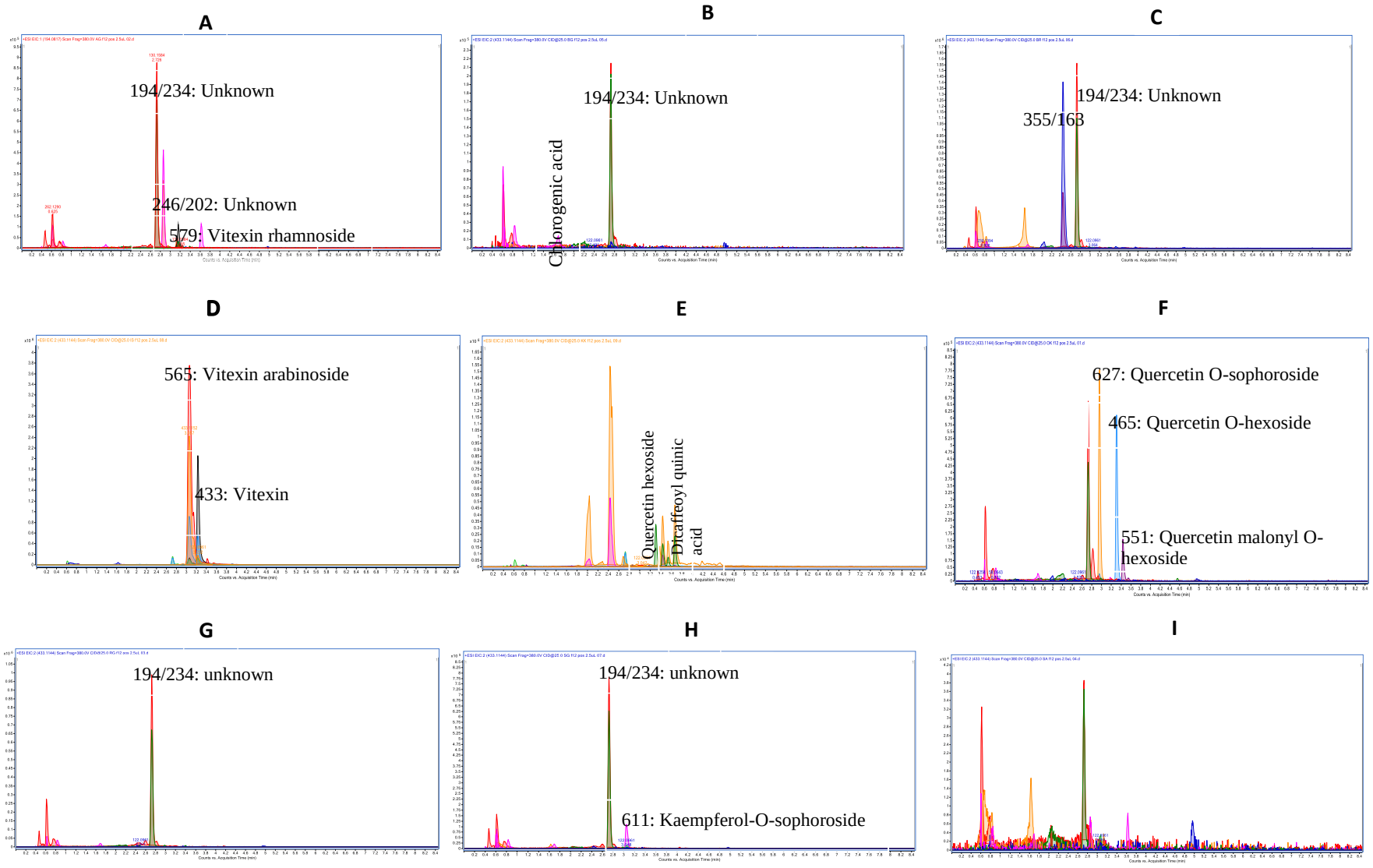


Fig. 3.1 MS chromatogram profile of aqueous extracts of dried vegetables: A, Ash gourd; B, Bitter gourd; C, Brinjal; D, Indian spinach; E, Kangkong; F, Okra; G, Ridge gourd; H, Snake gourd; I, Stem amaranth

chlorogenic acid, vitexin arabinoside, kaempferol O-rutinoside, dicaffeoyl quinic acid, quercetin hexoside, isochlorogenic acid, quercetin O-sophoroside, quercetin malonyl O-hexoside, kaempferol and O-sophoroside. Majority of the identified polyphenols are present in kangkong, okra and Indian spinach. Some unknown compounds were detected in ash gourd, bitter gourd, brinjal and ridge Gourd. For the stem amaranth, very low signals were detected, which suggest low levels of the phenolic compounds. The presence of glycosides may have enhanced water solubility of the polyphenolic compounds. Among all the compounds chlorogenic acid and quercetin are considered as potent antioxidative, anti-inflammatory and anti-carcinogenic agents (Silinsin & Bursal, 2018).

3.4.3 β-Carotene content

Carotenoids consist of a large group of phytochemicals that could have health benefits because of their antioxidant activities (Başkan, Tütem, Özer, & Apak, 2013; Biswas et al., 2011). Among the carotenoids, β-carotene is considered the main compound with pro-vitamin A activity. Moreover, it has been reported that leafy vegetables are good sources of β carotene when compared to other vegetables (Žnidarčič, Ban, & Šircelj, 2011). For example, it has been reported that vegetables have 4 times more β-carotene than fruits (Vargas-Murga, de Rosso, Mercadante, & Olmedilla-Alonso, 2016). From this work, the highest amounts of β-carotene were recorded for bitter gourd (121.27 ± 0.74) and kangkong (120.02 ± 0.88) Table 1. A previous work has also reported that bitter gourd solvent extracts had the highest β-carotene contents when compared with seven other vegetables (Yadav, Yadav, Yadav, & Garg, 2016). The lowest amount of β-carotene was found in snake gourd (51.55 ± 0.14). These results suggest that the vegetables are good sources for enhancing the β-carotene content of human diets.

Table 3.2: Extraction yield, and the total polyphenol, β-carotene, flavonoid, total chlorophyll, chlorophyll-a and chlorophyll-b contents of aqueous vegetable extracts (dry weight basis).

Samples	Extraction Yield (%)	Total polyphenol content (mg GAE/g)	Flavonoid Content (µg RE/g)	β-Carotene content (mg/g)	Total Chlorophyll (mg/g)	Chlorophyll-a (mg/g)	Chlorophyll-b (mg/g)
Ash gourd	62.8 ± 0.4^{c}	566.93 ± 0.06^{ab}	112.70 ± 0.01^{d}	97.84 ± 0.14^{d}	99.31 ± 0.01^{c}	29.18 ± 0.01^{a}	70.16 ± 0.01^{e}
Bitter gourd	64.1 ± 1.1^{c}	646.70 ± 0.39^{bc}	191.44 ± 0.01^{f}	121.27 ± 0.74^{e}	161.23 ± 0.01^{f}	87.02 ± 0.01^{g}	74.26 ± 0.01^{f}
Brinjal	72.3 ± 2.0^{e}	689.07 ± 0.09^{cd}	181.08 ± 2.93^{e}	59.27 ± 0.10^{ab}	147.09 ± 0.01^{e}	58.61 ± 0.01^{f}	88.53 ± 0.01^{h}
Indian spinach	37.9 ± 0.3^{a}	764.85 ± 0.37^{d}	226.66 ± 2.93^{h}	79.84 ± 0.46^{c}	165.85 ± 0.01^{g}	89.71 ± 0.00^{h}	76.19 ± 0.01^{g}
Kangkong	53.7 ± 0.5^{b}	667.86 ± 0.07^{cd}	305.39 ± 2.93^{i}	120.02 ± 0.88^{e}	299.57 ± 0.03^{h}	239.51 ± 0.01^{i}	60.14 ± 0.02^{c}
Okra	68.6 ± 0.4^{d}	683.08 ± 0.01^{cd}	212.15 ± 0.01^{g}	56.69 ± 0.77^{ab}	104.89 ± 0.03^{d}	44.04 ± 0.02^{e}	60.89 ± 0.01^{c}
Ridge gourd	69.8 ± 0.5^{d}	650.74 ± 0.36^{c}	91.99 ± 0.01^{c}	88.12 ± 0.55^{c}	105.60 ± 0.01^{d}	42.85 ± 0.02^{d}	62.79 ± 0.02^{d}
Snake gourd	70.3 ± 0.6^{d}	632.54 ± 0.36^{bc}	79.56 ± 0.01^{b}	51.55 ± 0.14^{a}	86.74 ± 0.00^{b1}	34.71 ± 0.02^{b}	52.06 ± 0.02^{b}
Stem amaranth	38.5 ± 0.9^{a}	501.08 ± 0.64^{a}	13.26 ± 0.01^{a}	62.22 ± 0.24^{b}	65.74 ± 0.01^{a}	36.59 ± 0.01^{c}	29.17 ± 0.01^{a}

Samples with different letters indicate significantly different values ($p < 0.05$) using the one-way analysis of variance. Means (n=2) ± standard deviation. GAE = gallic acid equivalent; RE = rutin equivalent

3.4.4 Chlorophyll content

Chlorophyll-a and chlorophyll-b are antioxidant compounds with potential bioactive effects that can help to reduce blood sugar, enhance digestion and remove food allergens (Başkan et al., 2013; Revatipadale et al., 2019). Their potential as dietary bioactives was recently demonstrated from experiments that showed they can be absorbed from the gastrointestinal tract (Chen et al., 2018). Chlorophyll-a is considered the primary photosynthetic pigment that is responsible for energy production in plants while chlorophyll-b consists of the accessory pigments (Srichaikul et. al., 2011; Sumanta, Haque, Nishika, & Suprakash, 2014). Significant differences in the total leaf chlorophyll content were found among the nine vegetables as shown in Table 1. Kangkong (299.57 ± 0.03) had the significantly ($p < 0.05$) highest total chlorophyll content while stem amaranth (65.74 ± 0.01) had the lowest. For chlorophyll-a, the highest ($p < 0.05$) was also found for kangkong (239.51 ± 0.01) and the lowest was present in ash gourd (29.18 ± 0.01). However,

chlorophyll-b content was highest ($p < 0.05$) in brinjal (88.53 ± 0.01 mg/g) and the lowest for stem amaranth (29.17 ± 0.01). Some of the vegetables in this work had higher chlorophyll-a content than chlorophyll-b, which agrees with previous reports (Bohm, Puspitasari-Nienaber, Ferruzzi, & Schwartz, 2002; Kopsell, Kopsell, Lefsrud, Curran-Celentano, & Dukach, 2004). However, others such as ash gourd, brinjal okra, ridge gourd and snake gourd had higher chlorophyll-b than chlorophyll-a, which is different from the data reported for *Brassica oleracea* leaves (Kopsell et al., 2004). It has been reported that chlorophyll-a is 3-times more abundant than chlorophyll b (Başkan et al., 2013), which is different with the results obtained in this work. Therefore, it is possible that some of the chlorophylls, especially chlorophyll-a were not solubilized by water during extraction, hence the expected natural ratio in plant tissues was not obtained. The highest chlorophyll contents obtained for kangkong in this work are lower than the values reported for a *B. oleracea* species, which had 278.03 ± 23.54 mg/100 g chlorophyll-a and 89.52 ± 1.65 mg/100 g chlorophyll-b (Kopsell et al., 2004). However, the chlorophyll-a contents obtained in this work are higher than those reported for other varieties like kale, Swiss chard and lettuce (Žnidarčič et al., 2011).

3.4.5 Proximate composition of vegetable extracts

Table 2 shows varied moisture contents for the dried vegetable aqueous extracts but generally, the snake gourd had the highest while stem amaranth had the lowest value. The high content of moisture can lead to increased activity of water-soluble enzymes and co-enzymes involved in the metabolism of these leafy vegetables (Arasaretnam, Kiruthika, & Mahendran, 2018). Therefore, the stem amaranth extract may possess better storage shelf life than the snake gourd. The reason for the varied moisture content is not clear but this may be due to difficulty in ensuring water evaporation during freeze-drying, especially if the samples contain different levels of compounds

with strong water-holding capacity. The highest and lowest amount of crude protein contents were found in the Indian spinach and ash gourd, respectively. The value obtained for Indian spinach in this work is higher than the 16.28% crude protein content reported

for another spinach variety (Arowora, Ezeonu, Imo, & Nkaa, 2017). It is noticeable than the stem amaranth contained the highest ($p < 0.05$) crude protein content, which suggest that the dried extract of this leaf could be a good source of high protein intake in the human diet. The reason behind the varietal differences in protein content may be due to soil nutrient variation, influence of environment and cultivation period as well. (Arowara et al., 2017). Crude fibres are considered an essential component of the human diet because of the positive effects on bowel movement and gut health. However, the vegetable extracts had very low contents of crude fibre, which reflects their poor solubility in water that was the extraction medium. The results show that the extracts cannot serve as good sources of dietary fibre, which is not surprising since polyphenols were the focus of this work. Similarly, the lipid contents were low, which also reflects the use of water for extraction and the naturally low levels of lipids in leaves. The low lipid level could enhance storage life since level of peroxidation will be low. With respect to ash content, the Indian spinach and ash gourd had highest and lowest values, respectively. A previous work (Karmakar, Muslim, & Rahman, 2013) reported a low value of 16.28% ash content for the Indian spinach, which may be due to difference is agronomic practices. The high (>20%) amounts of ash content, especially in Indian spinach, kangkong and bitter gourd indicate that these vegetables may be good sources of essential minerals. However, the Indian spinach contained the lowest amount of non-fibre carbohydrates while ash gourd had the highest level. Since the leaves are mainly photosynthesizing bodies, it is possible that the main components of the non-fibre carbohydrates are the soluble sugars.

Table 3.2: Proximate composition of freeze-dried aqueous vegetable extracts

	Moisture (%)	Dry Matter (%)	Crude Protein (%)	Crude Fibre (%)	Lipids (%)	Ash (%)	Non Fibre Carbohydrates (%)
Ash gourd	19.18 ± 0.11^{f}	80.82 ± 0.11^{bc}	14.58 ± 0.14^{a}	0.035 ± 0.03^{a}	1.83 ± 0.08^{a}	10.79 ± 0.06^{a}	53.5 ± 0.03^{g}
Bitter gourd	15.45 ± 0.01^{e}	84.54 ± 0.08^{c}	17.69 ± 0.09^{b}	0.07 ± 0.014^{a}	3.17 ± 0.00^{c}	20.08 ± 0.07^{c}	43.54 ± 0.77^{d}
Brinjal	18.85 ± 0.41^{f}	81.15 ± 0.41^{g}	16.38 ± 0.30^{f}	0.06 ± 0.01^{ab}	1.40 ± 0.08^{a}	12.50 ± 0.05^{h}	50.83 ± 0.74^{f}
Indian Spinach	9.77 ± 0.06^{b}	90.22 ± 0.06^{f}	25.04 ± 0.27^{d}	0.25 ± 0.18^{a}	0.55 ± 0.11^{d}	38.05 ± 0.09^{g}	26.35 ± 0.19^{a}
Kangkong	14.07 ± 0.01^{c}	85.94 ± 0.08^{e}	21.17 ± 0.09^{e}	0.16 ± 0.06^{ab}	1.72 ± 0.07^{b}	25.68 ± 0.03^{e}	37.22 ± 0.02^{c}
Okra	14.80 ± 0.11^{d}	85.2 ± 0.11^{b}	22.57 ± 0.09^{ab}	0.36 ± 0.04^{ab}	1.17 ± 0.08^{e}	15.89 ± 0.01^{ab}	45.22 ± 0.93^{e}
Ridge gourd	19.42 ± 0.01^{g}	80.56 ± 0.01^{a}	15.58 ± 0.74^{c}	0.78 ± 0.05^{ab}	1.93 ± 0.01^{e}	11.15 ± 0.05^{b}	51.15 ± 0.74^{f}
Snake gourd	21.65 ± 0.13^{h}	78.34 ± 0.13^{d}	18.76 ± 0.81^{c}	0.5 ± 0.16^{a}	1.92 ± 0.12^{e}	11.36 ± 0.36^{f}	45.85 ± 1.09^{e}
Stem amaranth	4.72 ± 0.09^{a}	95.28 ± 0.09^{h}	46.72 ± 0.09^{g}	1.15 ± 1.10^{b}	0.43 ± 0.01^{a}	13.04 ± 0.35^{d}	33.95 ± 1.27^{b}

*Mean ± standard deviation (n = 2); column values with different alphabets are significantly different ($p < 0.05$)

3.4.6 Mineral compositions of vegetable extracts

Mineral elements are considered as essential structural and functional materials for human body tissues, which meet the requirement for normal metabolism. Foods remain the major sources of mineral intake for humans because non-food sources are scanty (Yu, Guo, Jiang, Song, & Muminov, 2018). Since micronutrients are essential nutrients, any shortages can cause serious health problems, which emphasize the role of edible leafy vegetables in ensuring adequate mineral intake. The mineral composition recorded for these vegetables are shown in Table 3. The highest amount of Ca was found in stem amaranth while Brinjal and Indian spinach (0.03%) had the lowest. Calcium is considered as an essential mineral for bone formation, blood clotting and muscle contraction but the samples cannot be considered as major sources of this nutrient. Phosphorus was significantly ($p < 0.05$) highest in Kangkong and lowest in stem amaranth but all the samples had values higher than the 0.11% reported by Umar, Hassan, Dangogo and Ladan (2007) for similar species. Magnesium values were generally low <1%) and similar to the calcium contents. Of the major minerals, potassium was present at the highest levels with 18.24% in Indian spinach and 4.03% in snake gourd. With the exception of stem amaranth, sodium levels were also generally less than 1% and similar to the calcium contents. Potassium depresses while sodium raises blood pressure, which could make the high potassium levels coupled with low sodium levels as important attributes for use of the vegetable as dietary factors to avoid hypertension (Buendia, Bradlee, Daniels, Singer, & Moore, 2015).

Stem amaranth contains the highest copper content while bitter gourd had the lowest. The human body has been found to need 2-3 mg copper/day because this mineral is an integral part of metalloenzymes, specifically oxidases and is involved in the conversion of ferrous ion into ferric ion during iron metabolism (Singh et al., 2011). Therefore, the vegetables could partially satisfy

the daily copper requirement for maintaining a healthy human body. For most of the vegetables, iron was the most abundant minor mineral element, which makes these samples relevant for ensuring adequate iron intake. This is because iron is an important component of several proteins (enzymes, myoglobin and haemoglobin) that are critical for normal cellular metabolism. Moreover, anemia is a worldwide disease caused by iron deficiency, and mostly occur in children and women due to inadequate dietary intake (Singh et al., 2011). For this reason, iron-rich vegetables can be a good source of dietary intake for women and children (Knutson et al., 1999). The other minor minerals, manganese and zinc were also present at varying levels depending on the sample. However, the kangkong had a manganese level that was several fold higher ($p < 0.05$) than the other vegetables but okra had the highest zinc concentration. Manganese has been reported to have important roles in the biosynthesis of cholesterol and fatty acids because it is an integral part of several enzymes. Moreover, the mitochondrial super oxide dismutase (a powerful antioxidant enzyme) also contains manganese (Singh et al., 2011). Zinc is an important part of metalloenzymes and takes part in vitamin A metabolism (Singh et al., 2011) and is an essential component of angiotensin converting enzyme, one of the main enzymes that control human blood pressure (He, Aluko, & Ju, 2014). These findings show the mineral content diversity in the different vegetables and suggest regular consumption could play an important role in homeostasis maintenance.

Table 3.3: Mineral composition of freeze-dried aqueous vegetable extracts

	Calcium (%)	Phosphorus (%)	Magnesium (%)	Potassium (%)	Sodium (%)	Copper (mg/kg)	Iron (mg/kg)	Manganese (mg/kg)	Zinc (mg/kg)
Ash gourd	0.22 ± 0.00^{c}	0.56 ± 0.01^{a}	0.26 ± 0.01^{b}	4.69 ± 0.09^{a}	0.04 ±0.00^{a}	23.04 ± 0.76^{f}	82.93 ± 9.48^{d}	11.41±0.06^{c}	26.4±0.43^{b}
Bitter gourd	0.08 ± 0.01^{b}	1.19 ± 0.01^{f}	0.31 ± 0.01^{c}	9.64 ± 0.15^{c}	0.21 ± 0.01^{c}	1.72 ± 0.09^{a}	95.14 ± 1.59^{b}	24.85±0.88^{e}	49.89±4.48^{d}
Brinjal	0.03 ± 0.00^{a}	0.82 ± 0.01^{b}	0.17 ± 0.01^{a}	5.91 ± 0.042^{b}	0.07 ± 0.00^{a}	18.34 ± 0.41^{c}	18.04 ± 0.04^{a}	8.64±0.67^{b}	18.09±0.14^{a}
Indian spinach	0.03 ± 0.00^{a}	1.25 ± 0.02^{g}	0.97 ± 0.04^{f}	18.24 ± 0.90^{e}	0.48 ± 0.01^{e}	13.79 ± 0.38^{c}	129.81 ± 3.26^{f}	34.07±0.92^{f}	54.31±0.90^{e}
Kangkong	0.59 ± 0.04^{f}	1.54 ± 0.01^{h}	0.49 ± 0.01^{d}	10.48 ± 0.41^{d}	0.52 ± 0.03^{e}	6.16 ± 0.04^{b}	54.89 ± 0.79^{e}	130.84±1.17^{g}	48.63±0.31^{d}
Okra	0.59 ± 0.01^{f}	1.06 ± 0.01^{e}	0.93 ± 0.01^{e}	6.45 ± 0.07^{b}	0.13 ± 0.00^{b}	14.79 ± 0.03^{d}	22.13 ± 0.21^{a}	32.89±0.31^{f}	74.57±0.50^{f}
Ridge gourd	0.26 ± 0.00^{d}	0.89 ± 0.14^{c}	0.29 ± 0.01^{bc}	4.28 ± 0.08^{a}	0.36 ± 0.00^{d}	22.98 ± 0.30^{f}	43.4 ± 0.39^{b}	12.34±0.014^{c}	27.6±0.96^{b}
Snake gourd	0.51 ± 0.01^{e}	0.93 ± 0.01^{d}	0.45 ± 0.00^{d}	4.03 ± 0.23^{a}	0.67 ± 0.01^{f}	26.07 ± 0.14^{g}	57.33 ± 3.87^{c}	23.01±0.66^{d}	40.46±0.75^{c}
Stem amaranth	0.63 ± 0.00^{f}	0.55 ± 0.00^{a}	0.47 ± 0.00^{d}	17.87 ± 0.07^{e}	1.78 ± 0.04^{g}	29.11 ± 0.28^{h}	57.03 ± 1.19^{c}	6.72±0.28^{a}	27.03±1.07^{b}

*Mean ± standard deviation (n = 2); column values with different alphabets are significantly different ($p < 0.05$).

3.4.7 Amino acid contents

The amino acid compositions of the aqueous extracts of nine vegetables are shown in Table 4. Amino acids are important dietary components because they are essential building blocks for protein synthesis. Moreover, certain amino acids such as acidic residues (aspartic and glutamic) and sulfur-containing (cysteine and methionine) are excellent antioxidants that can contribute to reduced antioxidative stress (Udenigwe & Aluko, 2010). Since several of the amino acids cannot be synthesized by the human body, the amount of amino acid presents in the body fluid can be changed considerably by the diet (Arowora et al., 2017). The most abundant amino acids present in the vegetable extracts are GLU (glutamic + glutamine) and ASP (aspartic + asparagine). This is especially prominent in the okra extract where GLU+ASP constituted ~61% of the total amino acids. Other extracts like the ash gourd (~36%), brinjal (~40%), Indian spinach (~39%), kangkong (~38%), ridge gourd (~45%) and snake gourd (~34%) also had high contents of GLU+ASP. In contrast, the okra extract had lower amounts of methionine, branched-chain amino acids (BCAAs), and phenylalanine when compared to the other extracts. BCAAs are important for muscle tissue repair, growth hormone production, and blood sugar regulation (Arowora et al., 2017), hence the okra extract may lack these desirable effects. The brinjal extract was almost devoid of tryptophan, which was relatively high in Indian spinach extract. Tryptophan is considered important for sleep control in addition to amelioration of vascular migraine and rheumatoid arthritis; therefore the brinjal extract may not have these desirable effects when consumed but the Indian spinach extract could provide such benefits. Only the stem amaranth extract had amino acid values that can be considered similar to the normal distribution in plant tissues.

Table 3.4: Amino acid composition (%) of freeze-dried aqueous vegetable extracts

	Ash gourd	Bitter gourd	Brinjal	Indian spinach	Kangkong	Okra	Ridge gourd	Snake gourd	Stem amaranth
ASP	11.26	9.57	20.59	8.13	37.02	31.81	13.13	12.01	11.75
THR	4.16	3.18	2.21	3.42	3.12	2.43	1.73	2.63	2.31
SER	4.83	4.66	3.22	4.40	4.53	3.17	3.00	4.00	3.20
GLU	23.23	13.78	19.17	31.36	9.79	29.63	31.68	21.99	16.66
PRO	4.92	5.24	4.62	4.72	3.71	2.42	3.95	3.04	6.89
GLY	4.00	5.49	2.66	5.02	3.03	1.97	3.83	4.67	6.04
ALA	6.87	7.60	5.64	7.06	7.86	5.98	5.88	7.46	6.93
CYS	0.86	1.50	1.32	2.05	0.41	0.48	1.08	1.20	2.22
VAL	6.03	6.49	6.17	5.70	6.00	3.03	4.63	6.83	6.93
MET	1.13	1.44	0.76	1.07	0.74	0.41	1.27	1.22	1.86
ILE	4.59	3.56	3.49	3.18	3.43	1.63	2.60	4.46	4.78
LEU	6.32	5.66	3.69	4.99	3.83	1.84	3.83	6.60	6.45
TYR	2.16	3.01	2.33	0.53	1.91	1.23	1.77	2.23	4.34
PHE	3.71	3.87	2.80	4.32	4.62	1.63	2.19	3.94	6.28
HIS	6.34	5.36	9.15	3.84	3.42	5.01	9.81	6.42	3.85
LYS	3.79	5.01	4.27	4.07	2.63	1.88	3.16	4.85	4.95
ARG	4.89	13.90	7.49	4.52	3.03	4.99	6.14	6.00	3.56
TRP	0.90	0.01	0.41	1.60	0.91	0.45	0.32	0.45	0.97
AAA	6.77	6.88	5.54	6.45	7.44	3.31	4.29	6.62	11.59
BCAA	16.93	15.71	13.35	13.88	13.26	6.49	11.06	17.89	18.16
HAA	37.48	38.37	31.24	35.23	33.42	19.10	27.53	37.44	47.67
PCAA	15.02	24.27	20.90	12.43	9.08	11.89	19.11	17.27	12.36
SCAA	1.99	2.95	2.08	3.12	1.16	0.89	2.36	2.42	4.09

*Combined total of aromatic amino acids (AAA) = phenylalanine, tryptophan, and tyrosine; Branched-chain amino acids (BCAA) = leucine, isoleucine and valine; Hydrophobic amino acids (HAA) = alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, proline, methionine, and cysteine; Positively-charged amino acids (PCAA) = arginine, histidine, lysine; Sulfur-containing amino acids (SCAA) = cysteine and methionine.

3.4.8 DPPH radical scavenging activity (DRSA)

The highest DRSA was observed for BHT at 0.5 mg/mL (81.08 ± 0.65%) when compared to the vegetable extracts (Fig. 2). The weaker DRSA of the vegetable extracts may be because they are not pure extracts and contain a mixture of compounds unlike the BHT that is a pure synthetic

compound. Among vegetable extracts, the highest activity was observed for Indian spinach (65.20 ± 2.72 %) at 0.50 mg/mL. Ridge gourd (62.96 ± 0.73 %) and snake gourd (62.54 ±1.39%) also showed values that are comparable to the Indian spinach. The results differ from a previous work, which reported 100.9 ± 0.6 % DRSA at 200 µg/mL for kangkong and 71.6 ± 0.6% for Indian spinach (Hossain et al., 2015). Apart from brinjal and bitter gourd, all the other vegetable extracts had more than 50% DRSA. However, the DRSA was not totally concentration-dependent because a decrease in value was observed at the highest concentration (1 mg/mL) for most of the extracts. The decreased scavenging capacity at 1 mg/mL could be attributed to pro-oxidative effect or polyphenol aggregation. This is because at high concentrations, polyphenol aggregation could occur through hydrophobic interactions, which then reduce availability of binding sites and interactions with the DPPH free radical.

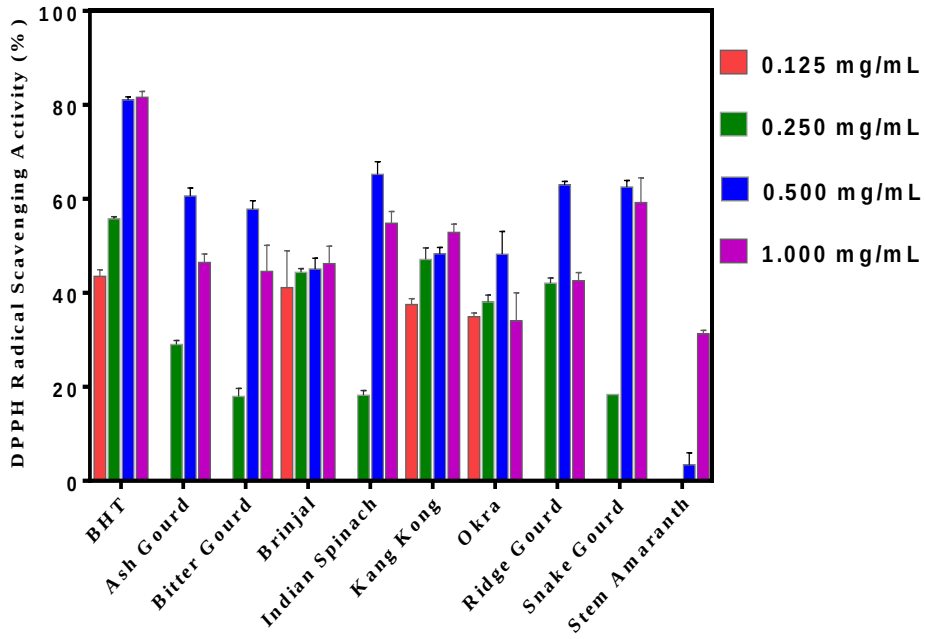


Fig 3.2 DPPH radical scavenging activity at different concentrations of aqueous extracts of dried vegetables. Bars are means (n=3) ± standard deviations. BHT, butylated hydroxytolueneThe results are

consistent with a previous report, which also showed decreased DRSA when polyphenol concentration exceeded 0.5 mg/ mL (Olarewaju et al., 2018).

3.4.9 Ferric reducing antioxidant power (FRAP)

Conversion of ferric (Fe^{3+}) to ferrous (Fe^{2+}) is the basis for the FRAP assay by measuring the electron-donating ability of antioxidants, which is reflected as a color change (Rana et al., 2019). As shown in Fig. 3, the FRAP activity increased when sample concentration was doubled from 0.5 mg/mL to 1.0 mg/mL. Interestingly, for most of the samples, the FRAP value at 1.0 mg/mL was more than twice that of the 0.5 mg/mL, which suggest a synergistic effect at the higher concentration. At 0.5 mg/mL, the BHT was more effective than the vegetable extracts. However, at 1.0 mg/mL, the kangkong was significantly ($p < 0.05$) more effective than BHT. The lowest values were obtained for stem amaranth and snake gourd extracts at both of 0.5 and 1 mg/mL concentrations. A previous work also reported that amaranth had a lower FRAP value when compared with some other leafy vegetables (Yadav et al., 2016).

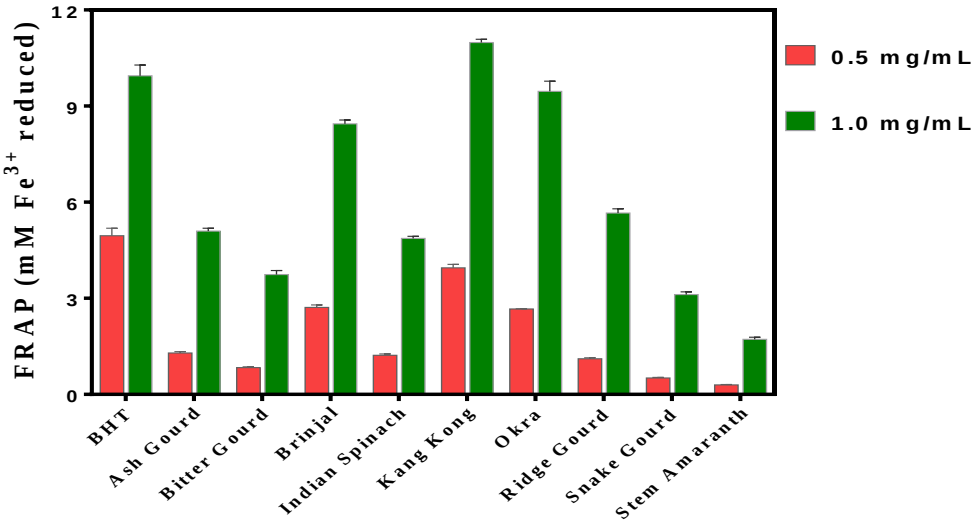


Fig. 3.3 Ferric reducing antioxidant power (FRAP) of aqueous extracts of dried vegetables. Bars are means (n=3) ± standard deviations. BHT, butylated hydroxytoluene

3.4.10 Metal ion chelating activity

Lipid peroxidation can be initiated by transition metal ions, especially Fe^{3+} and Fe^{2+}, which can also accelerate lipid oxidation by decomposing hydroperoxides into alkoxyl radicals that perpetuates the free radical chain reaction. Therefore, the ability of antioxidant compounds to chelate and inactivate these transition metal ions can prevent lipid oxidation, which reduces the negative impact of lipid oxidation on food shelf life and human health (Mohan, Balamurugan, Salini, & Rekha, 2012). Overall, the metal ion chelation activity of the vegetable extracts was enhanced at 1 mg/mL when compared to lower concentrations (Fig. 4). The poor metal ion binding capacity of BHT has been previously reported (Olarewaju et al., 2018) and was confirmed in this work. The results suggest that Indian spinach (46.27 ± 1.31%), kangkong (31.61 ±0.45%) and Okra (26.33 ± 1.88%) have the best potential for limiting metal ion-induced lipid peroxidation.

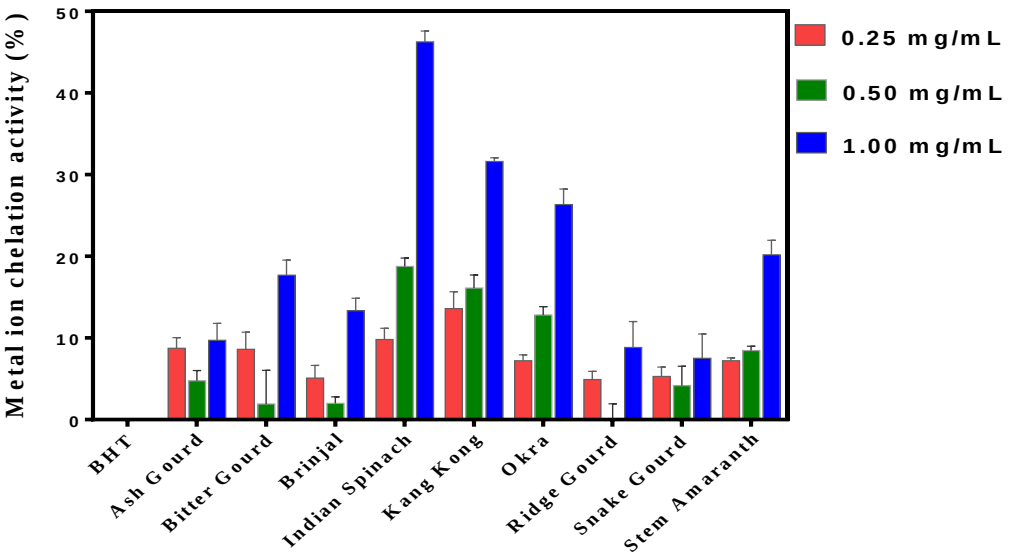


Fig 3.4 Metal ion chelation activity at different concentrations of aqueous extracts of dried vegetables. Bars are means (n=3) ± standard deviations. BHT, butylated hydroxytoluene

3.4.11 Inhibition of linoleic acid oxidation

Lipid peroxidation products will eventually undergo secondary breakdown to form toxic free radicals that raise their deleterious effects in foods and causes damage to mammalian cells. Generally, polyunsaturated fatty acids undergo lipid peroxidation through free radical-mediated removal of hydrogen atoms from the methylene carbons (Li et al., 2008). Therefore, estimation of total peroxides can be used to determine level of lipid peroxidation. Fig. 5 shows that all the vegetable extracts had significant ability ($p < 0.05$) to suppress linoleic acid peroxidation over the 7-day experimental period when compared to the blank that contained no extract. The vegetable extracts were particularly more effective than BHT in preventing linoleic acid peroxidation within the first 5 days of the experiment. The results differ from those reported for a herb root water extract, which had lower linoleic acid peroxidation inhibitory activity than BHT (Pervin et al. 2014). On days 6 and 7, the inhibitory power of the vegetable extracts was similar to that of BHT, which suggests slight weakening of the strength of the extracts. The results are similar to that of the root water extract, which was also shown to exhibit slight losses in inhibitory activity at days 6 and 7 (Pervin et al., 2014). In contrast, the absence of BHT or vegetable extracts led to continuous increase in peroxides as reflected in the data for the blank reaction. The stronger inhibitory effect of the vegetable extracts suggests synergistic interactions of the different polyphenolic compounds when compared to the single BHT molecule.

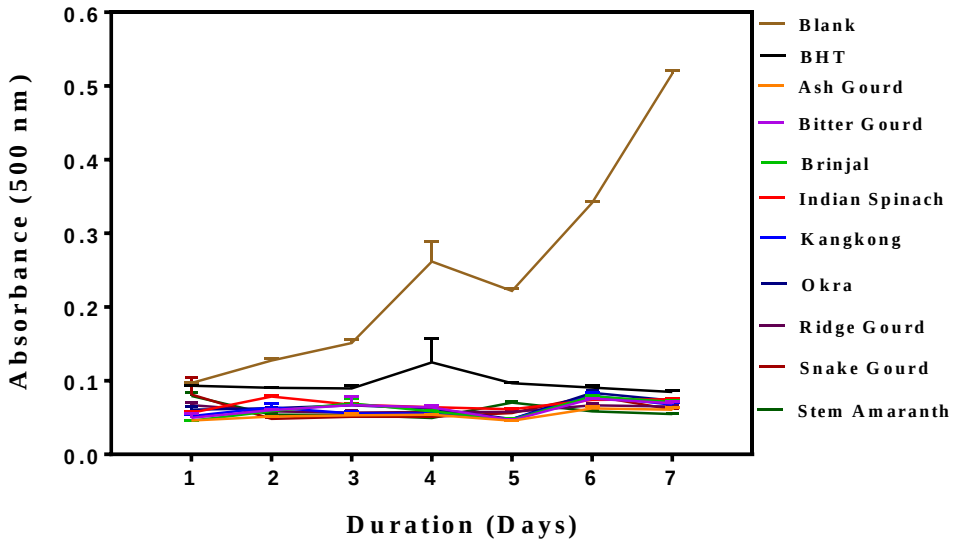


Fig 3. 5 Inhibition of linoleic acid oxidation of the aqueous extracts (0.25 mg/mL) of dried vegetables. Bars are means (n=3) ± standard deviations. BHT, butylated hydroxytoluene

3.5 CONCLUSION

The aqueous extracts of vegetables differed with respect to chemical composition and antioxidant properties, which reflect genetic differences as well as responses to agronomic and environmental factors. The high extract yields obtained for most of the vegetables reflect high contents of water-soluble compounds and indicate the potential for commercial extraction, which could accelerate their economic importance. Since the human digestive tract is an aqueous environment, the high extracts yields suggest a high potential for the bioactive constituents to be solubilized during digestion, which could increase bioavailability. Results from the antioxidant assays suggest that the Indian spinach, kangkong, and okra could be considered as the most promising sources of compounds that could reduce oxidative stress with relevance to prevention of NCDs. However, all the vegetable extracts were very effective in preventing linoleic acid peroxidation, which has relevance in preventing food quality deterioration and human health degeneration.

3.6 ACKNOWLEDGMENT

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3.8 TRANSITION STATEMENT

The purpose of this thesis is to characterize the antioxidant and enzyme inhibitory activities from water soluble extracts of nine vegetables grown in Bangladesh. The antioxdiant properties of the aqueous extracts these vegetables were determined in the preceding chapter. Among other findings, the study showed that almost all the vegetables possess good antioxidant activities. Among all the vegetables Indian Spinach, Kangkong and Okra showed the best antioxidant activity. However, NCD is a multi-factorial health impairment that requires other therapeutic agents in addition to antioxidants. Additionally the preceding chapter highlighted the chemical compositions such as amino acid contents, proximate composition, mineral compositions of the mentioned vegetables. Based on the different chemical composition available in these vegetables, these vegetables can be used as a safe food sources or as a replacements for synthetic drugs in the food industry. Due to the presence of different polyphenols and amino acid compositions, the work also confirms that these vegetables can be a potential source of proteins. However, in order to effectively control NCDs, it is mandatory to know the enzyme inhibition properties of these vegetables in additional to their antioxidant properties. These are enzymes that play critical roles in promoting NCDs through increased glucose and fatty acid uptake and excessive activities of the blood pressure-controlling renin-angiotensin system. Hence, the use of vegetables to attenuate the activities of these enzymes could enhance the use of diets to prevent or manage NCDs. Therefore, the next chapter (Chapter 4) addresses the enzyme inhibition activities of these specific vegetables.

CHAPTER FOUR

Inhibitory activities of aqueous vegetable extracts against α-amylase, α-glucosidase, pancreatic lipase, renin, and angiotensin converting enzyme

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Running title: Enzyme-inhibitory properties of vegetable extracts

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4.0 Abstract

The aim of the study was to determine the in vitro enzyme inhibition activities of aqueous polyphenolic extracts of nine popular Bangladeshi vegetables, namely ash gourd, bitter gourd, brinjal, Indian spinach, kangkong, okra, ridge gourd, snake gourd, and stem amaranth. Polyphenolic glycosides were the major compounds present in the extracts. Inhibition of α-amylase (up to 100% at 1 mg/mL) was stronger than α-glucosidase inhibition (up to 70.78% at 10 mg/mL). The Indian spinach extract was the strongest inhibitor of pancreatic lipase activity (IC_{50} = 276.77 µg/mL), which was significantly better than that of orlistat (381.16 µg/mL), a drug. Ash gourd (76.51%), brinjal (72.48%), and snake gourd (66.82%) extracts were the most effective inhibitors of angiotensin-converting enzyme (ACE), an enzyme whose excessive activities have been associated with hypertension. Brinjal also had a significantly higher renin-inhibitory activity than the other vegetable extracts. We conclude that the vegetable extracts may have the ability to reduce enzyme activities that have been associated with hyperglycemia, hyperlipidemia, and hypertension.

Keywords: Bangladesh; vegetables; polyphenols; amylase; glucosidase; renin; angiotensin-converting enzyme; lipase; mass spectrometry.

4.1 INTRODUCTION

Several physiological disturbances can be attributed to the effect of metabolic syndrome, which is a condition characterized by disease conditions such as obesity, dyslipidemia, hyperglycemia, and hypertension [1]. These metabolic syndrome-related diseased are considered major health concerns worldwide. Obesity is considered as one of the main factors in the pathogenesis of cardiovascular diseases such as hypertension, stroke, type 2 diabetes mellitus (T2DM), and cancer [2,3]. Postprandial hyperglycemia (PPHG), a major feature of T2DM, occurs when the blood glucose level increases after consuming a meal and this is an important factor taken into consideration for diabetes management. PPHG can be controlled by inhibiting the enzymes responsible for causing elevated blood glucose [4].

Several epidemiological and empirical studies have reported that consumption of fruits and vegetables containing polyphenol compounds play an important role in inhibiting carbohydrate-hydrolyzing enzymes such as α-amylase and α-glucosidase [5]. α-amylase (EC 3.2.1.1) is responsible for the hydrolysis of α-1,4 glucosidic bonds of starches, which are then converted to oligosaccharides. These oligosaccharides are further converted to maltose, glucose, and limit dextrins, causing blood glucose levels to increase [6]. The enzyme α-glucosidase (EC 3.2.1.20), located in the brush border of the small intestine enterocytes, is responsible for carbohydrate breakdown and synthesis. α-glucosidase is a carbohydrate-hydrolase that releases monosaccharides, and is the main enzyme responsible for increasing blood glucose levels after consumption of a meal. Therefore, subsequent to the initial α-amylase hydrolysis of dietary carbohydrates, α-glucosidase plays an important role in ensuring the release of absorbable monosaccharides in the intestinal tract [7]. To control PPHG, acarbose, miglitol, and voglibose are drugs that are used as α-glucosidase and α-amylase inhibitors. However, these drugs not only are

expensive but they also affect the gastrointestinal system negatively, with long-term use of these drugs causing flatulence and diarrhea [8].

Digestion of dietary triglycerides occurs in the small intestine by a key enzyme called pancreatic lipase (PL) to release 2-monoglyceride and fatty acids, which are then absorbed. Hence, inhibition of PL (EC 3.1.1.3) activity is regarded as a major approach for obesity prevention and treatment [9–11]. Although orlistat (a drug) has been used as a PL inhibitor, the negative side effects such as bloating, oily spotting, fecal urgency, steatorrhea, and fecal inconsistency have reduced acceptance [12,13].

Another reason for the development of cardiovascular diseases is hypertension, which causes arteriosclerosis, congestive heart failure, coronary heart disease, end-stage renal diseases, myocardial infarction, and stroke [14]. The renin–angiotensin system (RAS) has been found to be one of the major regulatory mechanisms in blood pressure regulation [15]. Within the RAS, renin (EC 3.4.23.15) acts on angiotensinogen to release angiotensin I, which is then cleaved by angiotensin-converting enzyme (ACE) to produce angiotensin II, a potent vasopressor. Therefore, ACE (EC 3.4.15.1) and/or renin inhibition help to manage hypertension and offer cardiovascular protection by limiting the physiological level of angiotensin II. Just like obesity and diabetes, current clinical management of hypertension involves mainly the use of drugs, which also have negative side effects [15].

Different in vitro and in vivo studies have observed that dietary phenolic compounds have many properties beneficial for maintaining human health [16]. In particular, studies have found that phenolic compounds are helpful for inhibiting lipase, alpha-amylase, alpha-glucosidase, ACE, and renin [17–20]. In fact, polyphenol-rich extracts have been shown to reduce blood pressure in spontaneously hypertensive rats and could serve as potential antihypertensive agents [21,22].

Therefore, this research investigated the enzyme inhibition ability of aqueous extracts of nine popular Bangladesh vegetables to determine their potential utility as agents against obesity (PL, α-glucosidase, α-amylase), diabetes (α-glucosidase, α-amylase), and hypertension (ACE, renin). Aqueous extracts were used in order to enhance the practical utilization of the research outcome, since the human gastrointestinal tract is highly hydrophilic and soluble compounds are more likely to be absorbed or interact with enzymes than hydrophobic compounds.

4.2 MATERIALS AND METHODS

4.2.1. Materials

Fresh vegetables, namely ash gourd (BINA CHALKUMRA 1), bitter gourd (BARI KOROLA 1), brinjal (BARI BEGUN 8), Indian spinach (BARI PUISHAK 1), kangkong (BARI GIMAKOLMI 1), okra (BARI DHEROSH 2), ridge gourd (BARI JHINGA 1), snake gourd (BARI CHICHINGA 1), and stem amaranth (BARI DATA 1), were collected from the Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. The vegetables were dried in the oven at 40 °C, ground into powders, and then stored at −20 °C. Porcine pancreatic α-amylase, porcine PL, rat intestinal acetone powder, acarbose, orlistat, 4-nitrophenyl α-D-glucopyranoside (PNP), N-(3-[2-furyl]acryloyl)-phenylalanyl glycyl glycine (FAPGG), and rabbit lung ACE were purchased from Sigma-Aldrich (St. Louis, MO, USA). A human recombinant renin inhibitor screening assay kit was purchased from Cayman Chemicals (Ann Arbor, MI, USA). Other analytical grade chemical reagents were obtained from Fisher Scientific Company (Oakville, ON, Canada).

4.2.2. Extraction of Polyphenolic Compounds

Water-soluble free (unbound) polyphenols were extracted using a previous method [23] with minor modifications as follows. Briefly, dried vegetable powders were weighed and one part mixed with 20 parts of distilled water (1:20) in a 500 mL beaker, which was then adjusted to 60 °C and stirred for 2 h [23]. After cooling to ambient temperature, the mixture was centrifuged for 30 min at 5600 × *g*, and the supernatant passed through a muslin cloth to collect filtrate #1. The residue was mixed with water (1:20) and the extraction, centrifugation, and filtration processes repeated to collect filtrate #2, which was then combined with filtrate #1. The combined filtrate was partially evaporated in a rotary evaporator (Heidolph Instruments GmbH & CO., Schwabach, Germany) under vacuum (~24 mm Hg) at 60 °C, and the aqueous residue freeze-dried and stored at −20 °C.

4.2.3. UHPLC MS/MS Analysis of Polyphenolic Compounds

An Agilent 1290 UHPLC system (Santa Clara, CA, USA) coupled with an HSS T3 2.1 × 100 mm 1.7 µm column from Waters Corp (Milford, MA, USA) was used to perform UHPLC analysis of the vegetable extracts. Samples were mixed with distilled water, vortexed, and passed through a 0.2 µm filter. Then, a 5 µL portion of the filtrate was injected onto the column followed by elution at a flow rate of 0.5 mL/min using mobile phases A and B (0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively) at 40 °C. The following gradients were used: initial holding time 0.5 min, mobile phase B ramped up to 50% after 5 min, 95% after 6 min, held for 1 min, and re-equilibrated for 1.5 min. Compounds were identified using a diode array detector at a wavelength range of 230–640 nm in 2 nm increments and a frequency of 5 Hz. The mass spec was carried out in an Agilent 6550 QTOF (Santa Clara, CA, USA) at 200 °C, using a drying gas

pressure of 18 psi, 40 psi nebulizer and 350 °C sheath gas, a pressure of 12 psi, and 3500 V capillary with a 1000 V nozzle, and ran in positive ion electrospray at a frequency of 3Hz and acquisition from 30–1700 *m/z*. The MS/MS was performed at a narrow quadruple setting (1.3 atomic mass units), using 10, 20, and 40 eV collision energy and 30–1700 *m/z*. The compounds were identified using MS/MS fragmentation patterns and quantified based on the MS peak area.

4.2.4. Inhibition of α-Amylase Activity

α-amylase inhibition assay was carried out using a previous method [14] with slight modifications. Plant extracts were dissolved in 1 mL of 0.2 mM sodium phosphate buffer, pH 6.9 containing 6 mM NaCl. Then, 100 µL of sample aliquot (0.03–10 mg/mL final concentration) and 100 µL of α-amylase solution were added together in a test tube and incubated for 10 min at 25 °C. A 100 µL amount of 1% starch (previously dissolved in the same buffer, heated, and cooled) was added to the mixture and incubated again at 25 °C for 10 min. Then, 200 µL of 96 mM dinitrosalicylic acid (DNSA), prepared in 2 M sodium potassium tartrate tetrahydrate, were added to terminate the reaction and heated in a water bath at 100 °C for 15 min. Subsequently, a 3 mL amount of double-distilled water was added after the reaction mixture was cooled down to room temperature. From this reaction mixture, a 200 µL aliquot was transferred to a 96-well microplate and absorbance read at 540 nm using a Synergy™ H4 microplate reader (Biotek™, Winooski, VT, USA) at 25 °C. The phosphate buffer was used as a blank and its absorbance subtracted from each well to calculate enzyme activity. Acarbose, a known α-amylase inhibitor, was used as standard and assayed concomitantly with the samples. The inhibitory activity of α-amylase was calculated using the following equation:

$$\text{Inhibition } (\%) = (\text{Ac} - \text{As}/\text{Ac}) \times 100$$

where Ac = Absorbance of the control (no inhibitor) and As = Absorbance of the sample.

4.2.5. Inhibition of α-Glucosidase Activity

α-glucosidase inhibitory activity of the samples was determined according to a previously described method [24] with the following modifications. First, a 300 mg portion of rat intestinal powder was mixed with 9 mL of 0.9% (*w*/*v*) NaCl solution and centrifuged at 5600 × *g* for 30 min, and the supernatant was used as the source of α-glucosidase activity. Plant extracts were dissolved (final concentration of 0.03–10 mg/mL) in 0.1 M sodium phosphate buffer pH 6.9 and 50 µL mixed with 50 µL of the α-glucosidase solution in a 96-well microplate followed by incubation for 10 min at 37 °C. Then, 100 µL 5 mM (PNP) also dissolved in the phosphate buffer were added to each well and the absorbance read at 405 nm in 30 s intervals for 30 min using the Synergy^{TM} H4 microplate reader with temperature maintained at 37 °C. A blank measurement was taken without the addition of the enzyme, and its absorbance was subtracted from each well. Acarbose, an α-glucosidase inhibitor, was used as standard and assayed using the same protocol. The following equation was used to determine the α-glucosidase inhibitory activity of the samples:

$$\text{Inhibition } (\%) = (\text{Ac} - \text{As})/(\text{Ac}) \times 100$$

where Ac = Absorbance of the control (no inhibitor) and As = Absorbance of the sample.

4.2.6. Inhibition of Lipase Activity

The method described by Tang et al. [25] with slight modifications was used to determine PL inhibition by measuring the release of 4-methyl umbelliferone (4MU) from 4-methyl umbelliferyl oleate (4MUO). PL solution (final concentration of 3.125 U/mL) was prepared in 13 mM Tris-HCl buffer, pH 8.0 containing 1.3 mM $CaCl_2$ and 25 µL added to the mixture containing 225 µL of a

0.5 mM 4-MUO solution and 25 µL of sample (different concentrations) to start the enzyme reaction, followed by incubation for 1 h at 37 °C. The Synergy^{TM} H4 microplate reader was set at 400 nm and used to measure the amount of 4MU released during the reaction. Orlistat, a commonly used pharmacological agent against PL, was used as the standard.

The following equation was used to calculate PL inhibition:

$$\text{Inhibition (\%)} = (\text{Ac} - \text{As})/(\text{Ac}) \times 100$$

where Ac = Absorbance of control (no inhibitor) and As = Absorbance of the sample.

4.2.7. ACE Inhibition Assay

The method described by Alashi et al. [21] was used to determine ACE-inhibitory activity of the polyphenolic extracts. ACE, FAPGG (ACE substrate), and samples were individually dissolved in 50 mM Tris-HCl buffer, pH 7.5 containing 0.3 M NaCl. A 10 µL aliquot of ACE (final reaction activity 25 mU) was added to each well containing 170 µL of 0.5 mM FAPPG and 20 µL of samples at 37 °C. The buffer was used as blank (uninhibited reaction), while captopril, an ACE-inhibitory drug, was used as standard and assayed using a similar protocol. Absorption was read at 345 nm at 1 min intervals for 30 min to determine the reaction rate. The slope of the blank or sample reactions was used to calculate the percentage ACE inhibition as follows:

$$\text{ACE inhibition (\%)} = \left(\frac{\text{Slope } \left(\frac{\Delta A}{\text{min}}\right)_{\text{blank}} - \text{Slope } \left(\frac{\Delta A}{\text{min}}\right)_{\text{sample}}}{\text{Slope } \left(\frac{\Delta A}{\text{min}}\right)_{\text{blank}}} \right) \times 100$$

4.2.8. Renin Inhibition Assay

The renin-inhibitory activity of samples was determined using a previously described method [26]. Briefly, 20 µL of the substrate, 160 µL of assay buffer, and 10 µL of distilled water were mixed and added to the background well. Then, 20 µL of the substrate, 150 µL of assay buffer, and 10 µL of water were mixed into the control (uninhibited) wells, whereas the sample (inhibited) wells contained same reagents except that the water was replaced with 10 µL of samples (0.5 mg/mL assay concentration). This was followed by the addition of 10 µL of renin solution (dissolved in the assay buffer) to the control and sample wells to initiate enzyme reaction; the microplate was shaken for 10 s to ensure adequate mixing of the reagents and then incubated at 37 °C for 10 min in the dark. Enzyme catalytic activity was measured as the fluorescence intensity (FI) measured at excitation and emission wavelengths of 340 and 490 nm, respectively. Enzyme inhibition was calculated as follows after subtracting the FI of the background well from the control and sample wells:

Renin inhibition (%) =(FI of control well-FI of sample well)/(FI of control well)×100

4.2.9. Statistical Analysis

A minimum of duplicate assays were used to find out the mean values and standard deviations. For statistical analysis, analysis of variance (Kruskal–Wallis ANOVA) was used, while significant differences ($p < 0.05$) between mean values were determined by the Duncan's multiple range tests. The IBM SPSS statistical package (version 24, Armonk, NY, USA) was used for all statistical analyses.

4.3. RESULTS

4.3.1. UHPLC MS/MS Analysis

The major polyphenolic compounds identified in the vegetable extracts are shown in Table 1. More polyphenolic compounds were detected from Indian spinach, kangkong, and okra as compared to other vegetable extracts. The identified compounds were mainly chlorogenic acid and the glycosides of quercetin, vitexin hexoside, and kaempferol. Kaempferol O-sophoroside was particularly present at a very high concentration in snake gourd and may be the main compound that determined the bioactive properties of this extract.

Table 4.1. Main polyphenolic compounds of aqueous extracts of vegetables *.

Samples	Polyphenolic Compounds	Retention Time (min)	*m/z* (Da)	MS/MS (Da)	Concentration (mg/g)	
					A [1]	B [2]
Ash Gourd	Vitexin ramnoside	3.2	579	433, 313, 283	1.23	0.77
Brinjal	Chlorogenic acid	2.4	355	163	5.63	4.07
Indian spinach	Vitexin arabinoside	3.0	465	433, 313, 283	8.96	3.40
	Vitexin	3.2	433	313, 283	2.82	1.07
	Kaempferol O-rutinoside	3.4	595	449, 287	0.12	0.05
Kangkong	Dicaffeoyl quinic acid	3.5	517	163	6.92	3.72
	Quercetin hexoside	3.4	465	163	4.46	2.40
	Chlorogenic, isochlorogenic acid	2.5	355	163	10.12	5.43
Okra	Quercetin O-sophoroside	3.0	627	301	3.33	2.28
	Quercetin O-hexoside	3.4	465	301	2.63	1.80
	Quercetin malonyl O-hexoside	3.5	551	301	0.66	0.45
Snake gourd	Kaempferol O-sophoroside	3.1	611	287	81.35	57.15

* Compounds in bitter gourd, ridge gourd, and stem amaranth could not be identified. [1] Dried polyphenolic extract. [2] Dried vegetable powder.

4.3.2. α-Amylase Inhibition

The inhibitory activity of α-amylase obtained in this study by the phenolic extracts was mostly dose-dependent, although at higher concentrations than acarbose, the standard compound (Figure 1). However, for brinjal and stem amaranth, decreases at sample concentrations >0.6 and 0.8 mg/mL, respectively, were observed. Only ash gourd had detectable activity at 0.2 mg/mL, whereas only brinjal, Indian spinach, and snake gourd had high inhibition levels at 0.4 mg/mL. Kangkong had no detectable activity at 0.2–0.6 mg/mL. Among the vegetable extracts, the ridge gourd achieved 100% inhibition at 1 mg/mL concentration, which is significantly ($p < 0.05$) better than the other extracts. The okra (40.82% ± 1.88%) and stem amaranth (36.84% ± 0.49%) showed the lowest levels of α-amylase inhibition, respectively, at 1 mg/mL.

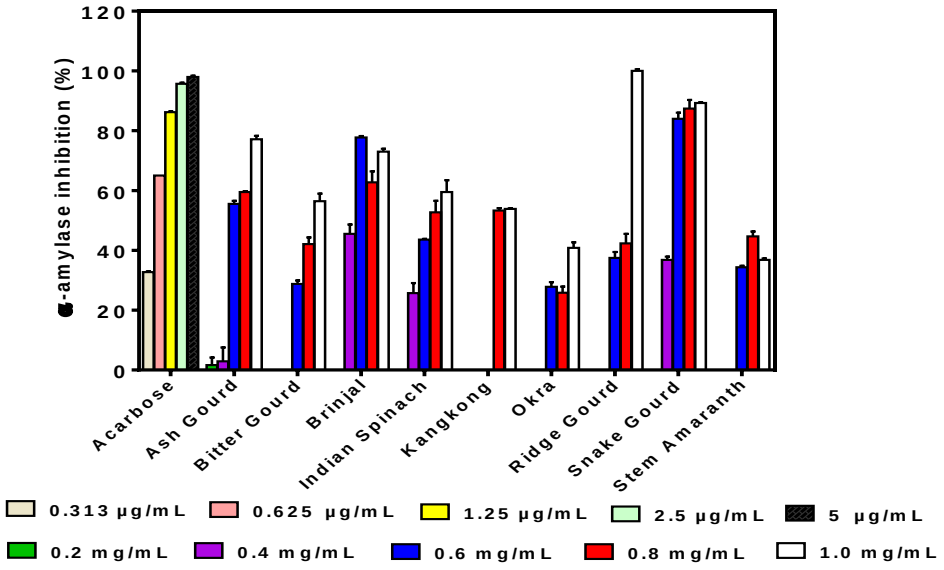


Fig 4.1. Inhibition of α-amylase activity at different concentrations of acarbose and aqueous polyphenolic extracts of vegetables. Bars are means ($n = 3$) ± standard deviation.

4.3.3. α-Glucosidase Inhibition

A mostly dose-dependent effect was also observed as the inhibitory activity of α-glucosidase increased with increasing concentration for almost all the samples (Figure 2). However, decreases in α-glucosidase inhibition were observed at sample concentrations >6 and 8 mg/mL for bitter gourd and stem amaranth, respectively. The highest inhibition was observed for brinjal (70.78% ± 3.45%) at 10 mg/mL, which is similar to that of acarbose (69.36% ± 0.80% at 0.5 mg/mL), a purified synthetic inhibitor of α-glucosidase. With the exception of brinjal (higher activity) and stem amaranth (lower activity), the inhibitory values obtained for the vegetable extracts were similar at 10 mg/mL, but snake gourd had the lowest inhibition at 0.2 mg/mL.

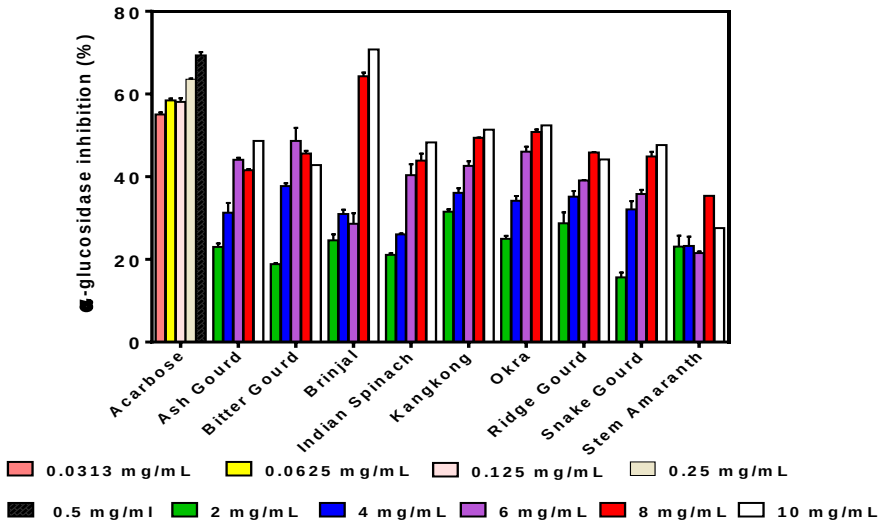


Fig 4.2. Inhibition of α-glucosidase activity at different concentrations of acarbose and aqueous polyphenolic extracts of vegetables. Bars are means (n = 3) ± standard deviation.

4.3.4. Pancreatic Lipase (PL)-Inhibitory Activity

PL inhibition was strong for all the vegetable extracts, which enabled IC_{50} calculation as shown in Figure 3. Because lower IC_{50} values indicate stronger inhibitory activity, the results

obtained show that Indian spinach (276.77 ± 4.95 µg/mL) was the most active with a significantly ($p < 0.05$) lower value than orlistat. Brinjal (397.22 ± 2.36 µg/mL), okra (427.94 ± 1.40 µg/mL), and kangkong (482.04 ± 0.67 µg/mL) also had strong activities, although lower than orlistat (381.16 µg/mL). It is also noticeable that the stem amaranth extract had the weakest PL inhibition (IC_{50} = 723.394 ± 2.36 µg/mL), just as it showed the lowest inhibitions of α-amylase and α-glucosidase inhibitory activities.

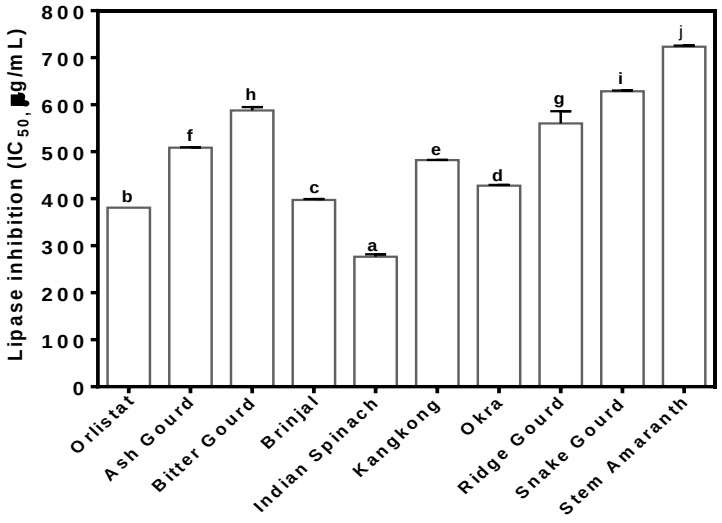


Fig 4. 3. IC_{50} values (n = 3 ± standard deviation) for the inhibition of pancreatic lipase (PL) activity by orlistat and aqueous polyphenolic extracts of vegetables. Bars with different letters have mean values that are significantly ($p < 0.05$) different.

4.3.5. Angiotensin-Converting Enzyme (ACE)-Inhibitory Activity

Different levels of ACE inhibition were obtained for the vegetable extracts but, in general, the ash gourd (76.51% ± 0.25%), brinjal (72.48% ± 0.02%), and snake gourd (66.82% ± 0.99%) were the most active (Figure 4). All the vegetable extracts had significantly ($p < 0.05$) weaker inhibition than captopril, the ACE-inhibitory drug. It is also observable that ash gourd also showed a good

record for inhibiting α-amylase and α-glucosidase. Stem amaranth showed the lowest activity for all the concentrations and the lowest ACE inhibition, which is consistent with the observed poor inhibitory activities against PL, amylase, and glucosidase.

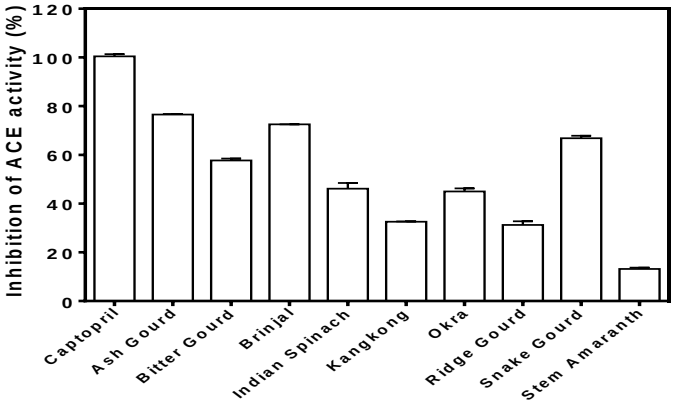


Fig 4.4 Inhibition of angiotensin-converting enzyme (ACE) activity (n = 3 ± standard deviation) by 1 mg/mL captopril and aqueous polyphenolic extracts of vegetables. Bars with different letters have mean values that are significantly ($p < 0.05$) different.

4.3.6. Renin-Inhibitory Activity

The highest renin inhibitory activity was exhibited by brinjal (79.64% ± 7.63%) at 0.5 mg/mL as compared to 99.11% ± 1.75% for aliskiren (drug) at 0.05 mg/mL (Figure 5). The snake gourd had the significantly lowest renin inhibition, which was not significantly ($p > 0.05$) different from the stem amaranth with no detectable renin inhibition.

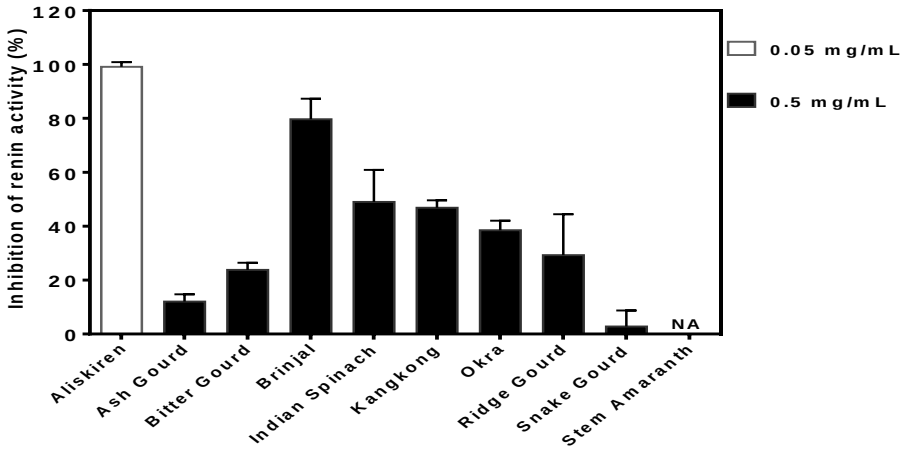


Fig 4.5. Inhibition of renin activity (n = 3 ± standard deviation) by aliskiren and aqueous polyphenolic extracts of vegetables. Bars with different letters have mean values that are significantly ($p < 0.05$) different.

4.4 DISCUSSION

UHPLC MS/MS analysis is considered an effective analysis method because it provides high selectivity, high sensitivity, and the potentiality for robust and accurate analysis identification of compounds [27]. As shown in Table 1, the dominant phenolic compounds were the glycosides, which reflect the aqueous extraction. The water solubility properties of the phenolic compounds could enhance their interactions with target enzymes within the hydrophilic environment of the gastrointestinal tract. For example, the aqueous extracts of some bean varieties were shown to have stronger lipase inhibition than the ethanolic extracts [28]. Inhibition of α-amylase was mainly dose-dependent except for brinjal and stem amaranth, where their decreases occurred at higher sample concentrations. However, the dose-dependent inhibitory activity pattern observed for most of the samples was also reported for α-amylase inhibition by bitter gourd extract [29]. The decreased α-amylase inhibition at high concentrations of brinjal and stem amaranth may be due to increased interactions between the polyphenol molecules, which reduced interactions with the enzyme protein. The weaker α-amylase inhibitory activity when compared to acarbose, which is an

approved drug, suggests lower enzyme binding intensity by the polyphenolic compounds. A previous study also reported that the ethanolic extract of bitter gourd exhibited lower α-amylase activity than acarbose [29]. The high inhibitory activity of snake gourd (89.26% ± 0.23%) may be attributed to the kaempferol O-sophoroside, although the contribution of other non-identified compounds cannot be discounted. The low inhibitory activity of okra suggests that the quercetin glycosides, which were the main identified compounds in the extract, are not very effective inhibitors of α-amylase. In contrast, other extracts that contained vitexin, chlorogenic acid, and dicaffeoyl quinic acid had higher inhibitions of α-amylase activity when compared to okra that contained mainly quercetin glycosides. α-amylase inhibitors are also referred to as starch blockers because they prevent starch absorption. Digestive amylase enzyme and other secondary enzymes play a critical role in breaking down complex carbohydrates such as starch, without which they cannot be absorbed because polysaccharides need to be broken down first into monosaccharides for absorption to take place [30]. The association between α-amylase inhibition and its potential contribution to the management of T2DM with phenolic extracts have been investigated for other vegetables [31,32].

α-glucosidase is another enzyme that makes glucose available in the body by breaking down oligo- and disaccharides, converting them into absorbable monosaccharides. Therefore, in order to reduce serum glucose levels and manage related diseases, α-glucosidase inhibition has been proposed as a suitable approach [33,34]. This work showed that the vegetable extracts had weaker α-glucosidase inhibition than acarbose, the drug. In fact, only brinjal extract at 8 and 10 mg/mL produced similar inhibitory values to acarbose at 0.25 mg/mL. In contrast, a nanoparticulated ethanolic extract of bitter gourd was reported to have stronger α-glucosidase inhibition than acarbose [29]. Nwanna, Ibukun, and Oboh [35] reported 50% inhibition of α-glucosidase activity

for a similar eggplant variety using 63.24 ± 0.30 µg/mL, which is stronger than the results obtained in this work. The difference may be due to their use of solvent extraction, which could have isolated more compounds than the aqueous extraction used in the present work. The observed variations in identifiable polyphenolic compounds of the vegetable extracts did not have strong effects in influencing α-glucosidase inhibition. However, the results obtained in this work agree with a previous work [30], which suggested that brinjal phenolics may have potential for use in controlling T2DM because of the strong glucosidase-inhibitory property. A previous work [36] reported 66.64% (at 2.5 mg/mL), which is higher than the 48.65% (at 6 mg/mL) obtained in this work. The α-glucosidase inhibitory properties of a sample have been shown to be dependent on the type of extracting solvent; for example, ethyl acetate extract had stronger activity than hexane, methanol, and chloroform extracts of bitter gourd [36]. Therefore, the weaker activity obtained for the bitter gourd extract in this work may be due to the aqueous extraction as compared to the ethyl acetate extract.

Lipase is secreted in the pancreas and is used as a catalyst to aid triglyceride hydrolysis in the stomach, and this hydrolysis is completed by intestinal lipase in the small intestine [28,37]. It has been estimated that about 50–70% of total dietary fats are hydrolyzed for absorption by PL [38], which emphasizes the importance of this enzyme in calorie release from diets. Surprisingly, orlistat is the only FDA-approved drug for inhibiting PL. It was reported that orlistat can prevent approximately 30% of dietary fat absorption; however, regular usage is associated with some undesirable side effects like flatulence, diarrhea, oily spotting, incontinence, abdominal cramping, and fecal urgency [39]. Therefore, the search for active PL inhibitors, especially natural compounds with potentially fewer side effects, has become important. Results from this work confirmed the PL-inhibitory activity of the vegetable extracts, especially the stronger effect of

Indian spinach as compared to orlistat. Therefore, the Indian spinach extract could serve as a potential agent for use in limiting PL-dependent digestion of dietary lipids. While there may be contributions from other polyphenolic compounds, the results suggest that the unique combination of vitexin with the kaempferol and vitexin glycosides contributed to the stronger PL inhibition by Indian spinach. This is because ash gourd and snake gourd, which contain vitexin and kaempferol glycosides, respectively, but not both types of polyphenols, were not as active as Indian spinach. The activities obtained in this work are weaker than the 92.0 ± 6.3 to 128.5 ± 7.4 μg/mL reported for the polyphenolic-rich extracts of common bean varieties [10]. The differences may be due to the type of sample and the extraction solvent since acetone was used as compared to aqueous extraction in the present work.

ACE is responsible for the formation of angiotensin II, a powerful vasoconstrictor; excessive physiological levels of angiotensin II lead to hypertension [15]. Therefore, ACE activity inhibition has been used to prevent and treat various diseases like heart failure, myocardial infarction, nephropathy, and even diabetes. However, apart from the different negative side effects associated with the use of pharmacological agents, ACE-inhibitory drugs are not permitted for use during pregnancy due to potential damage to the fetus [40]. Therefore, foods may serve as sources of ACE-inhibitory compounds with less harmful effects. For example, flavanols have been shown to inhibit in vitro activity of ACE as well as in isolated rat kidney membranes [41]. The vegetable extracts had lower ACE inhibition than captopril, which is consistent with the strong antihypertensive activity associated with standard drugs. However, the advantage of natural products is the reduced risk of negative side effects that could make them preferred antihypertensive agents when compared to synthetic drugs. Moreover, it is possible to incorporate these vegetable extracts into foods for regular consumption to combat the incidence of

hypertension [21]. The results suggest that the presence of multiple compounds as identified in this work produced no strong synergistic effect since the strongest ACE inhibition was associated with samples where one polyphenolic compound predominate. However, it should be emphasized that other non-identified compounds may have contributed to the observed activities. The results are consistent with previous reports, which have reported that ACE-inhibitory activity varies because of differences in polyphenolic compounds of different plant extracts [21,42–45].

Renin inhibition has also been shown to be an effective means of reducing blood pressure because it catalyzes the rate-determining step (conversion of angiotensinogen to angiotensin I) in the RAS pathway [15,46]. However, effective renin-inhibitory drugs are rare and only one (aliskiren) has so far been approved as an antihypertensive agent [47]. Therefore, natural compounds that inhibit renin activity could enhance the management of hypertension. This is possible because polyphenolic compounds such as saponins have been shown to bind renin protein [48] and produce blood pressure-lowering effects after oral administration to rats [49]. As shown in Figure 5, the vegetable extracts had weaker renin inhibition than aliskiren even though the drug was used at 10 times lower the concentration of the extracts. The weaker renin inhibition by the vegetable extracts is consistent with the high purity and chemical potency of the synthesized drug. However, brinjal extract had the strongest renin inhibition among the vegetables, which suggests that this sample could serve as a potential natural product to reduce renin activity. Unlike ACE inhibition, the presence of multiple polyphenolic compounds may have worked synergistically to contribute to the renin-inhibitory activity of Indian spinach, kangkong, and okra. Reports of polyphenol-dependent inhibition of renin activity are scant, but a previous work with aqueous vegetable leaf extracts reported <40% inhibitions, although at a lower concentration of 0.25 mg/mL [22]. Strong inhibition of in vitro renin activity was also reported for purified soybean

saponins with an IC_{50} value of 59.9 µg/mL [49]. The high purity of the saponin preparation could have contributed to the high renin inhibition when compared to the mixture of polyphenolic compounds used in this work.

4.5 CONCLUSIONS

Results from this work have shown that the aqueous extracts of vegetables could inhibit enzyme activities with respect to obesity, diabetes, and hypertension. The extracts were more effective inhibitors of α-amylase than α-glucosidase, which suggests a strong potential to limit excessive glucose release during digestion because starch hydrolysis is the rate-limiting step. Ability of the extracts to also inhibit PL suggests potential use as agents to limit the release of fatty acids during intestinal digestion, which could assist in reducing hyperlipidemia. Inhibition of ACE and renin activities are indications of potential blood pressure-reducing ability, which could also make the extracts function as antihypertensive agents. Overall, Indian spinach and brinjal extracts produced the most promising inhibitory effects on the five enzymes studied in this work, whereas stem amaranth extract was the poorest. However, in vivo determination of enzyme inhibitory activities using suitable animal disease models is required to confirm potential health benefits.

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CHAPTER FIVE

5.0 CONCLUSIONS

Finally, it can be concluded that Ash Gourd, Bitter Gourd, Brinjal, Indian Spinach, Kangkong, Okra, Ridge Gourd, Snake Gourd, and Stem Amaranth extracts have excellent antioxidant and enzyme inhibition activities. These vegetables have high mineral contents, proximate compositions, and almost all the essential amino acids. While a single vegetable did not exhibit all the activities, kangkong showed better activities for almost all the assays carried out. Kangkong also showed good activity for antioxidant activity even more than a synthetic antioxidant. Ash gourd, bitter gourd, brinjal, Indian spinach were also effective antioxidants. Indian spinach also showed an excellent lipase inhibition activity. Ridge gourd, snake gourd, and ash gourd showed excellent activity for α-amylase inhibition whereas brinjal, okra, kangkong, ash gourd, and snake gourd showed excellent activity against α-glucosidase. For inhibiting ACE, the best activities were observed from ash gourd, brinjal and snake gourd whereas brinjal showed the best activity for inhibiting renin. The inhibitory activity of vegetables against the enzymes was dose-dependent.

All the experiments carried out in this research have been done using a minimum of two replicates. Also, all the tests were carried out by following standard methods from published scientific literature. So, it is obvious that the data is reliable. In addition, the analysis of variance was done to determine the statistical significance of data and enable sample comparisons. Therefore, the results obtained from this research are deemed to be highly reliable.

From the above results, it was observed that the polyphenolic extracts of vegetables used in this research contained substantial amounts of antioxidants. So, these vegetables can reduce oxidative stress and NCDs that are related to oxidative stress. The results are consistent with published

scientific evidence that the antioxidant activity of polyphenols is helpful in reducing cardiovascular diseases. Similarly, other epidemiological studies have shown that long term consumption of plant polyphenols can protect the human body from cancers, cardiovascular diseases, diabetes, osteoporosis, and neurodegenerative diseases (Pandey & Rizvi, 2009).

Apart from enzyme inhibition, polyphenols inhibit glucose absorption. Glucose is transported by the Na+-dependent transporter, sodium-glucose cotransporter (SGLT1) through the apical membrane into the enterocytes. In response to a high load, the basolateral membrane (BLM), glucose transporter 2 (GLUT2) is thought to be inserted into the brush border membrane (BBM), contributing to the intake of this sugar. It has been reported that both of these transporters can be inhibited by polyphenols and polyphenol rich foods (Loureiro and Martel, 2019).

Therefore, these vegetables can serve as good sources of antioxidant and enzyme inhibitors, while also possessing high vitamin and mineral contents. Furthermore, these preliminary studies provide strong evidence that the vegetables can be beneficial for attenuating non-communicable diseases and can be used in the industries as ingredients for functional food formulations. There is no evidence that the polyphenolic compounds are destroyed at normal cooking temperatures, therefore cooked vegetable foods are recommended as good sources of these bioactive compounds.

5.1 DIRECTIONS FOR FUTURE RESEARCH

Further research is needed to find out the potential activities of these vegetables in animal or human models for possible replacement of synthetic drugs. Information is also required on the fate of polyphenolic compounds that are not absorbed in the upper intestinal tract but end up in the colon. The gut microbiota is important for the bioavailability of ingested polyphenols, as most parent

polyphenols are not well absorbed by the small intestine. Around 90 percent of ingested polyphenols enter the large intestine and are converted into bioavailable products within the range of the resident microbiota. Following ingestion of polyphenolic compounds, usually in their glycosylated form, bacteria in the GI tract convert dietary polyphenols to phenolic compounds of low molecular weight, such as phenolic acids which may be more extensively absorbed (Pasinetti et al., 2018). In addition to better-defined human intervention studies aimed at assessing physiological endpoints linked to disease, further research is also required regarding the bioavailability of polyphenols, particularly with regards to the effects of food matrices on absorption and the influence of age, gender and genotype on both absorption and metabolism. These studies are required to help determine the physiological, metabolic forms responsible for activity *in vivo*, as well as to help define adequate biomarkers of polyphenol intake. Therefore, at present, while the vast literature regarding the potential of polyphenols to improve human health is encouraging, more long-term, randomized, controlled, dietary intervention trials with appropriate controls are warranted to assess the full and unequivocal role that polyphenols play in preventing chronic human disease. The outcomes of these studies may ultimately be used to make specific dietary recommendations regarding the efficacy of polyphenols in preventing chronic disease risk and to fully validate polyphenols as the new agents against various chronic human diseases.

6.0 REFERENCES

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