ANALYSIS OF NUTRITIONAL COMPOSITION, IN VITRO ANTIOXIDANT AND ANTIDIABETIC EFFECT OF THREE *CHENOPODIUM* SPECIES (*C. album* L., *C. giganteum* L. & *C. murale* L.)

Salma Khatun

Roll No.: 0119/04

Registration No.: 662

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A thesis submitted in partial fulfillment of the requirements for the degree of Masters of Science in Applied Human Nutrition and Dietetics

Department of Applied Food Science and Nutrition

Faculty of Food Science and Technology

Chattogram Veterinary and Animal Sciences University

Zakir Hossain Road, Khulshi, Chattogram-4225, Bangladesh

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This is to certify that we have examined the above Master's Thesis and have found that it is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made.

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DEDICATATION

I DEDICATED MY SMALL PIECE OF WORK TO MY BELOVED FAMILY MEMBERS AND RESPECTED TEACHERS.

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Abbreviations

%	:	Percentage
&	:	And
AOA	:	Antioxidant activity
AOAC	:	Association of Official Analytical Chemists
℃	:	Degree Celcius
cm	:	Centimetre
CVASU	:	Chattogram Veterinary and Animal Sciences University
Dl	:	Deciliter
DPPH	:	2,2-diohenyl-1-picrylhydrazyl
et al	:	Et alii/et aliae/ et alia
etc.	:	Et cetera
g	:	Gram
GAE	:	Gallic acid equivalent
IC_{50}	:	Half inhibitory concentration
Kg	:	kilogram
ml	:	milliliter
mg	:	milligram
µg	:	microgram
ppm	:	parts per million
QE	:	Quercetin equivalent
RDA	:	Recommended Daily Allowance
SD	:	Standard Deviation
SEM	:	Standard Error of Mean
SPSS	:	Statistical Package for Social Science
T1DM	:	Type 1 diabetes mellitus
T2DM	:	Type 2 diabetes mellitus
WHO	:	World Health Organization
UV	:	Ultraviolet

Abstract

Considering the growing need to identify more plant-based nutritional sources, leaves of *Chenopodium album* (L.), *Chenopodium giganteum* (L.) and *Chenopodium mueale* (L.) from the Chenopodiaceae family were evaluated to find out the nutritional composition including major and trace minerals, in vitro antioxidants and antidiabetic effect in order to priorities their use as both food & medicine. These plants were found to have high protein (27.64 - 34.41 g/100gm) and low fat (2.85-3.51g/100gm). High ash content (17.49-26.70 g/100gm) revealed that they are good mineral source. The leaves of these plants are rich in essential minerals (Na, K, Ca, Mg, P) and sufficient trace elements (Fe, Zn, Cu). The presence of phytochemicals such as tannins, saponins and alkaloids was known through qualitative phytochemical screening of methanolic leaf extracts. The antioxidant activity of the extracts of *Chenopodium* species was determined with (DPPH) radical scavenging activity. Results showed that antioxidant activity of *C. album, C. giganteum* and *C. murale* was 76.99 %, 68.24 % and 79.45 % respectively with IC_{50} values of 14.25 µg/ml, 17.31 µg/ml and 13.86 µg/ml. Quantitative Analysis of phytochemical compounds showed that the phenolic content of *C. album, C. giganteum* and *C. murale* were 85.48, 88.19 and 94.10 mg GAE /100g respectively. The flavonoid content of *C. murale* 78.19 mg QE/100g was higher than *C. album* and *C. giganteum* which have flavonoid contents of 56.66 mg QE/100g and 73.57 mg QE/100g respectively. The in vitro antidiabetic effect of the extracts of *Chenopodium* species was evaluated by using α-amylase enzyme inhibition assay. Results showed that in vitro antidiabetic activity of *C. album, C. giganteum* and *C. murale* was 59.98 %, 67.32 % and 61.94% respectively with IC_{50} values of 80.93 µg/ml, 62.38 µg/ml and 76.84 µg/ml. So, important massages from this study are, these three leafy vegetables are important sources of essential nutrients and phytochemicals and provide potential antioxidant and antidiabetic effect.

Keywords: *Chenopodium* species, nutrient, phytochemicals, antioxidant, antidiabetic

Chapter I: Introduction

Green Leafy Vegetables (GLV) play an important role in human nutrition. They are made up of cellulose, hemi-cellulose and pectin substances that provide them their texture and firmness (Mohammed and Sharif, 2011). They supply an adequate amount of dietary fibers, minerals, vitamins and other nutrients to individuals in developing countries. Apart from the variety which they add to the menu (Asaolu et al., 2012), they are valuable sources of nutrients especially in rural areas where they contribute substantially to minerals, vitamins, fibers, proteins and other nutrients which are usually in short supply in daily diets. They are very important protective foods and useful for the maintenance of health and for the prevention of various diseases (Mohammed and Sharif, 2011). Leafy vegetables contain low energy densities hence suggested for weight management (Nwanekezie and Obiakor, 2014). The availability of indigenous vegetables has declined drastically because of the excessive cultivation of field crops. There is also growing ignorance among young people about the existence of these readily available nutritionally rich food plants (Odhav et al., 2007).

Elevation of the dependency on commercially cultivated major food crops has been responsible for the reduction of the food basket, for this reason humankind has been relying upon for Productions (Prescott-Allen 1990). Present crop production mostly involves entirely hundreds of the many thousands of well-known food plants globally. Ethnobotanical surveys indicate that thousands of traditional species are mostly ignored by scientific researchers and food processors. *Chenopodium album* (L.), *Chenopodium giganteum* (L.) and *Chenopodium mueale* (L.) of the family Chenopodiaceae are native plants of the Indian subcontinent, also falls under the explored category. These plants fall under the genus *Chenopodium* which has a worldwide distribution and contains about 250 species (Risi and Galwey, 1984). In the Indian subcontinent, it is represented by about 21 species, of which some are cultivated for an end use as vegetables and a few for the grains obtained from the plant (Yadav et al., 2007). These plants have additionally been reported to grow naturally as weeds within the fields of wheat, barley, mustard, gram and other crops (Khurana et al., 1986).

The tender shoots of these plants are eaten raw in salad or with curd; they are also cooked as a vegetable curry to eat with rice or used as an ingredient in paratha. The

dehydrated leaves of these plants can also be incorporated in various conventional food items as it can improve the nutritional quality of the product as well as add variety to the diet (Singh et al., 2007).

Along with commercially cultivated vegetables, wild vegetables are considered to be a potential source of essential nutrients such as vitamin C, minerals, vitamins, proteins, fibers (Afolayan and Jimoh, 2009; Gupta et al., 2005; Nguyen et al., 2016), and are also good dietary sources of phytochemical compounds such as flavonoids and other polyphenolic constituents which have strong antioxidant activity (Afolayan and Jimoh, 2009; Dasgupta and De, 2007).

Phytochemical compounds have extra nutritional constituents which are in small quantities. These compounds have a great impact on physiological, behavioural, and immunological. These compounds vary on the basis of chemical structure, function (Bernhoft et al., 2010). Phytochemical compounds prevent disease as a result of oxidative stress (Bernhoft, 2010; Kaur et al., 2001). The levels of the phytochemical compounds vary in foods in composition from various fruits, vegetables and genetic factors and environmental conditions such as light, maturity and postharvest treatments. These compounds have different beneficial effects like antioxidant activity, inhibition of enzymes, inhibition of receptor activities and inhibition of gene expression (Correia et al., 2012).

Green leafy vegetables are considered a popular source of life-saving natural antioxidants. Antioxidants are substances that can protect against cell damage due to free radicals. It acts as a radical scavenger, hydrogen donor, electron donor, peroxide decomposer, enzyme inhibitor, metal chelating agent. It performs as anti-cancer, anti-aging, anti-inflammation (Cai et al., 2004). Vegetables contain antioxidant compounds like phenol, Flavonoid, vitamins, carotenoids and minerals that have chemo preventive effects (Almeida et al., 2011). Evidence from epidemiological studies indicates that daily consumption of fruits and vegetables is correlated with a lower prevalence of many chronic diseases, including diabetes, infections, cardiovascular and neurological disorders and cancers (Johnsen et al., 2003; Vauzour et al., 2010; Murimi et al., 2018).

Diabetes mellitus, a chronic metabolic disorder, is characterized by elevation of glucose level in blood due to irregularity in insulin secretion, improper insulin action,

or both (American Diabetes Association, 2012). Nowadays, synthetic drugs and insulin are used as the main means for diabetes treatment (Babu et al., 2010). Oral hypoglycemic agents usually show side effects, such as hypoglycemia, drug-resistance, dropsy, and weight gain (Tahrani et al., 2010)). The board optimistic treatment in diabetes mellitus is to keep up optimal blood glucose level after the meal. Inhibitors of alpha-amylase are considered to be very effective in delaying glucose absorption. So from this point of view, many researchers have focused on the intensive search of novel enzyme inhibitors and natural products (Kawabata et al., 2008). Over the years, various medicinal plants have been reported to be effective in the treatment of diabetes (Paari et al., 2000). Plants are rich sources of phytochemicals such as flavonoids, alkaloids, glycosides, saponins and have been used as antidiabetic, antihyperlipedemic and antioxidant agents (Mata et al., 2015; Ramesh and Parasuraman, 2018).

Thus, the inhibitors of α-amylases, which break down long chain carbohydrates, show effectiveness in slowing down glucose absorption. The inhibition of α-amylase activity is considered to be an effective strategy for the control of diabetes. From this point of view, more researchers have focused on the search for more effective inhibitors of antidiabetic compounds from natural materials (Liu et al., 2012; Wang and Du, 2010).

In recent decades, a resurgence of interest has focused on wild edible plants for their nutritional and medicinal values to broaden the diversity of the human diet (Flyman and Afolayan, 2007; Afolayan and Jimoh, 2009). In many areas, people traditionally harvest a wide number of wild vegetables without any commercial cultivation due to cultural uses, taste habits or food shortage (Mahapatra et al., 2012). Nowadays wild vegetables have become a commercial crop with increasing market potential due to their nutritional importance, absence of residues from pesticides or fertilizers (Weng et al., 2001).

Several wild edible plants are traditionally consumed along with staple foods, especially in rural areas and a few urban communities, in Bangladesh. These plants play a vital role in fulfilling the demand for nutritional, minerals and antioxidant compounds in the diet of indigenous communities (Afolayan and Jimoh, 2009; Satter et al., 2016); besides these factors, they are also used in treating certain medical

conditions (Ocvirk et al., 2013). But there is a lack of descriptive scientific information about the nutritional value and functional properties of indigenous wild leafy vegetables like *C. album*, *C. giganteum* and *C murale*. The present study was carried out to investigate the nutritional composition, antioxidant potential and antidiabetic potential of the leaves of three species. The findings of the current study will serve the preliminary knowledge on the nutritional and nutraceutical potential of naturally grown edible plants in Bangladesh and thus could be incorporated into food composition databases and used for further utilization as dietary supplements or functional foods.

Objectives of the study

1. To determine the nutritional composition of *C. album*, *C. giganteum* and *C. murale* leaves.
2. To determine the concentration of phytochemical compounds and in vitro antioxidant activity of three species.
3. To analyze in vitro antidiabetic effect through α-amylase inhibition assay of these three *Chenopodium* species.

Chapter II: Review of Literature

Relevant literature of *Chenopodium album* (L.), *Chenopodium giganteum* (L.) and *Chenopodium murale* (L.) with their health benefits related to various nutrients, phytochemical compounds, antioxidant and antidiabetic properties have been reviewed. The review founding of the article is presented in this chapter.

2.1 Description of three *Chenopodium* species

Chenopodium is a genus of various species of perennial or annual herbaceous flowering plants which occur almost anywhere within the world. *Chenopodium album* (L.), *Chenopodium giganteum* (L.) and *Chenopodium murale* (L.) are members of the family Chenopodiaceae. The genus *Chenopodium* contains many plants of minor to moderate importance as food crops as leaf vegetables – used just like the closely related spinach (Bazan et al., 2012). It is a fast-growing, very common in temperate regions, growing almost everywhere in soils rich in nitrogen, especially on wasteland (Al-Snafi, 2015).

Figure 2.1: *C. album*

Figure 2.2: *C. giganteum*

Figure 2.3: *C. murale*

The leaves of these *Chenopodium* species are known as bathua sag in Bangladesh and some regions of India. In English, these plants are known as goosefoots, pigweed, lamb's quarters, melde, wild spinach and fat-hen (Kirtikar and Basu, 1999). The Greek name *Chenopodium* means (goose) and (foot), which refer to the shape of the leaves of these species. Though the names are generally applied for all of the three species of the genus *Chenopodium*, for this reason *C. album* is often distinguished as white goosefoot. The Latin species name album means white and alludes to the waxy covering on the plant (Agrawal et al., 2014). It tends to grow upright at first; reaching heights above 30 cm. Stems are rarely slender, angled, generally green in colour and rarely striped red or purple. Leaves are simple, rhomboid, deltoid to lanceolate, upper

entire, lower toothed or irregularly lobed, extremely variable in cultivated forms. The opposite leaves can be very varied in appearance. The first leaves, near the base of the plant, are toothed and roughly diamond-shaped. It has been found in green colour with smooth under surface. The leaves are waxy-coated, unwettable and mealy in appearance, with a whitish coat on the underside (Pande and Pathak, 2010).

Chenopodium giganteum, also known as tree spinach, is an annual, upright many-branched shrub with a stem diameter of up to 5 cm at the base, which can grow to a height of up to 3 m. Because of its height is also known as giant goosefoot. The younger leaves of *C. giganteum* are hairy with a magenta colour and the older become green as *C. album*. Other common names of *C. giganteum* are magenta spreen, purple goosefoot, giant lambquarters, tree spinach, mexican tree spinach. *C. murale* is another species of the family Chenopodiaceae known by the common names nettle-leaved goosefoot, Australian-spinach, salt-green, and sowbane, which grows up to 15-30 cm long. *C. murale* much resembles *C. album* but differs in its dark green coloured rhombic-ovate leaves with red leaf margin, vein and petiole. Leaves are lobed clearly cymose inflorescences and sharply keeled, closely pitted seeds. Stems are commonly reddish in colour. The large mature plant has a bushy appearance, tapering gradually toward the apex (Choudhary and Sharma, 2014).

2.2 Traditional use as food

The consumption by humans of edible wild members of the family Chenopodiaceae has been, in other times and cultures, much more extensive than now. The first European farmers that harvested or cultivated and consumed leaves and seeds of these species, as could be observed in archaeological deposits from Hascherkeller (Bavaria), dated from approximately 3000 years ago (Wells, 1984). Additionally, many current authors consider these species as edible for humans (Bois, 1927; Launert, 1982; Kunkel, 1983; Font-Quer, 1990; Grau et al., 1990).

Many wild species of *Chenopodium* are cultivated for human and consumption in several regions with a tropical climate, such as India, Bangladesh, South America, etc. These species are described as annual weeds, being collected in winter-spring, sometimes they're cultivated or sometimes they grow in orchards, fallow lands, gutters, corrals, etc. Their leaves are cooked like spinach, and seeds also are collected to make bread (Gupta and Wagle, 1988).

The young shoots and leaves of these plants are consumed as vegetable in the rural areas of the Eastern Cape Province and other parts of South Africa (Afolayan and Jimoh, 2009). The leaves and young shoots could also be consumed as a leaf vegetable; either steamed in its totality or cooked like spinach, but should be consumed in moderation because of high levels of oxalic acid (Johnson et al., 1995). The flower buds and flowers can also be eaten cooked (Benoliel and Doug, 2011). The leaves and young shoots of these plants are utilized in dishes like soups, curries, and paratha-stuffed breads, common in North India. The seeds or grains are used in phambra or laafi, gruel-type dishes in Himachal Pradesh, and in gently alcoholic fermented beverages. In Haryana state, the "bathue ki raita" i.e. the side dish (yogurt dip) made with bathua, is extremely fashionable in winters (Choudhary and Sharma, 2014). Bathua seeds conjointly double up for rice and pulses.

The young shoots and leaves of genus *C. giganteum* are often eaten up like spinach, another member of the Chenopodiaceae family. Most of the oxalic acid and saponins are removed during the cooking process, especially if boiled for 2 minutes at 100 °C (212 °F) (Ishii et al., 1991; Wang et al., 2018). Yet, the leaves are also edible raw in lower quantities, for instance as a salad. The seeds can be prepared similar to rice or quinoa or can alternatively be ground into flour, which is then mixed with cereal flour for bread making. Due to the partially pink coloured leaves, *C. giganteum* also has an ornamental value (Partap et al., 1998).

2.3 Nutritive value of *Chenopodium* species

Studies implemented in numerous places of the world indicate that *C. album* is a rich source of nutrients, antioxidants and important dietary elements (Afolayan and Jimoh, 2009; Hussain and Khan, 2009). Young shoots and mature plants of *C. album* contain vitamin-C and β-carotene, indicating that these vegetables could constitute an important source of these vitamins in the diet (Gqaza et al., 2013). However, many indigenous leafy vegetables including *C. album* are neglected and despised in the urban areas of Bangladesh, despite their nutritional richness and potential to contribute to healthier diets in the country.

This plant *C. gigantium*, a relative of quinoa, has edible seeds which may be hard-baked or ground into flour. The plant contains good amounts of vitamins A, C, and K,

and metallic elements like calcium, iron, phosphorus, and potassium, as well as saponins, which may have health advantages (Bhargava et al., 2006; Ward, 2000).

Many studies performed in different regions of the world for nutritional assessment of *C. album*. A study was carried out in the Eastern Cape Province of South Africa where widely grown *C. album* was nutritionally evaluated and found to be promising sources of protein, carbohydrate, fibre's and vitamins, with high energy values and essential micronutrients such as potassium, magnesium, phosphorus and iron. According to the result of this study, the values for carbohydrates, protein and fibre were 7.0(g/100g), 29.2(g/100g) and 36.5 (g/100g) respectively. The proximate analysis of *C. album* reported the moisture and ash content was 7.2% and 16.7% individually in the tested dried sample. As stated in the study the energy content was 800KJ/ 100gm (dry basis). The calcium, potassium and magnesium content were 18213.2(mg/1000g), 49028.6(mg/1000g) and 13821.5 (mg/1000g) respectively. The sodium content was ranges from 48.8 - 68.0 (mg/100g). The micronutrients iron, zinc and copper in the plant 120.4(mg/1000g), 23.0(mg/1000g) and 9.1 (mg/1000g) respectively. The amount of vitamin C was within a range of 5.2- 5.6(mg/100g). The amount of β-carotene was found 68 (μg/100g) in the leaves of *C. album*. The sucrose content was found within a range of 1.1-2.1(g/100gm) (Gqaza et al., 2013).

The nutritional composition analysis described by Guerrero and Isasa (1997) in three selected wild edible leafy vegetables indicated that wild edible leafy vegetables may be a good substitute to cultivated leafy vegetables for their high nutrient and mineral content. It reports the nutrient value of three leafy vegetables from *Chenopodium* species. These three are *C. album, C. murale* and *C. opulifolium* Shraeder. The proximate composition, mineral components (sodium, potassium, calcium, magnesium, phosphorus, iron, zinc, copper, and manganese.), fatty acids, vitamin C, carotenoids and oxalic acid contents were determined. The mean values for moisture percentage and ash percentage in *C. album* were 71.09% and 4.04% while for the *C. murale* were 82.02% and 4.23% respectively in fresh biomass. The carbohydrates, protein and fibre in the *C. album* were 5.89 (g/100g), 8.83 (g/100g) and 6.38 (g/100g) while those for the *C. murale* were 3.26(g/100g), 4.35(g/100g) and 4.13(g/100g) respectively. The lipid content is found to be higher in *C. album* (0.68g/100g) than *C. murale* (0.43g/100g). The energy value found in *C. album* was 266KJ/100gm and in

C. murale was 140KJ/100gm in fresh biomass. The measurements for vitamin C were 155(mg/100g) and 133(mg/100g) while carotenoids measured 12.5(mg/100g) and 10.2 (mg/100g) in leaves of *C. album* and *C. murale* respectively. Oxalic acid which is known as an anti-nutritional component was found little amount in both species *C. album* & *C. murale.* In case of fresh biomass the macro-elements Na, Mg in the *C. album* were 138 (mg/100gm) and 392.9 (mg/100g) while for the *C. murale* they were 453(mg/100g) and 435.3 (mg/100g) respectively. This data indicates that a lower amount of sodium and magnesium is present in the leaves of *C. album* than *C. murale*. Other macronutrients K, Ca, P in *C. album* were 1326.9 (mg/100g) , 312.9 (mg/100g) and 18.9 (mg/100gm) while for the *C. murale*, they were 837.1(mg/100g) , 21.1 (mg/100g) and 49.8 (mg/100g) respectively. These data showed in the result indicate the higher portion of K, Ca, and P content in *C. album* than *C. murale*. In humans, approximately 85% of phosphorus, the second most abundant mineral in the human body, is in bone, primarily compounded with calcium (Ca2+), the most abundant mineral and form calcium phosphate crystals which deposited on the collagen matrix (Broadus, 2003). The microelements of Fe, Zn, Cu in the *C. album* were 5.78(mg/100g), 1.85(mg/100g) and 0.33 (mg/100g) while for the *C. murale*, they were 8.62(mg/100g), 1.09(mg/100g) and 0.30 (mg/100g) respectively. Iron (Fe) was found in high percentages in *C. murale* according to the data reported in the study. The amount of copper (Cu) and zinc (Zn) were found higher in *C. album* than *C. murale.*

According to Pandy and Gupta (2014) the nutritional evaluation of dried bathua leaves show potential benefits as it has many beneficial nutrients. Considerably low (5.06%) moisture content was determined during the nutritional analysis of dry matter. There was a significant amount of carbohydrates (40.84% by weight) and protein (28.69 % by weight) detected. The fiber content was noticed (0.1%) which is a very tiny amount. These data indicate *C. album* as a good source of protein while a poor source of fiber. There is evidence that dietary fiber has a number of beneficial effects related to its indigestibility in the small intestine (Asp, 1996). The amount of fat was (4.41%) which is very low and helpful meal for overweight individuals. The energy value of *C. album* was determined in this study and the value attained 317.81 kcal. Nutritional analysis proved that it is a good source of many macro and micro minerals (potassium, sodium, calcium, phosphorous, magnesium, iron and zinc etc.). Amount of the macro-minerals sodium, potassium, calcium, phosphorous and

magnesium was found 5739 (µg/gm), 81252 (µg/gm), 14389 (µg/gm), 4197 (µg/gm) and 13101 (µg/gm) respectively. The micronutrients iron, zinc and Copper in the plant were 152 (µg/gm) (Pandy and Gupta, 2014).

Among all the macro-minerals found here, potassium content was highest. The calcium content is also higher than other macro-minerals. Among all the micronutrients, the richest amount of iron was found. High content of potassium can provide relief from stroke, blood pressure, heart and kidney disorders; also enhance muscle strength, water balance, electrolytic functions, and the nervous system. Calcium can play a crucial role in providing rigidity to the skeleton besides its involvement in neuromuscular functions, blood clotting, and many other metabolic processes (Campous et al., 2009). It also contains iron which is used against anemia, tuberculosis and disorders of growth (Claude and Paule, 1997).

2.4 Medicinal properties

Since the dawn of civilization, man used plants for their medicinal and edible value. By trial and error, and before the introduction of chemical medicines, man distinguished between the useful and toxic plants. Every population within the world developed its own traditional medical knowledge and experiences. World Health Organization estimates that about 80% of the world's population rely almost exclusively on traditional medicine for their primary healthcare needs (Al-Snafi, 2015).

The use of medicinal plant is growing worldwide because of the increased toxicity and allergic manifestations of synthetic drugs. Also, modern pharmacopoeia still contains at least 25% of drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants. Demand for plant-based medicine is increasing in both developing & developed countries because of the growing recognition of natural product, being non-narcotic, having no facet effects, simply medicinal plant sector has historically occupied a vital position within the socio cultural, spiritual and medicinal arena of rural & tribal group lives of India, Bangladesh, Nepal etc (Sharma et al., 2012).

In India, bathua is used as a laxative, diuretic, sedative and the infusion of the plant is used for the treatment of rheumatism (Arora et al., 2014). It was also used as an antidiarrhoeal, antiphlogistic, antirheumatic, contraceptive, odontalgic, cardiotonic,

antiscorbutic, blood purifier, digestive, carminative, aphrodisiac, for the treatment of dyspepsia, flatulence, strangury, seminal weakness, pharyngopathy, splenopathy, hemorrhoids, ophthalamopathy, cardiac disorder, hepatic disorder, spleen enlargement, biliousness, intestinal ulcers, and general debility (Agarwal et al., 2005; Panda, 2005; Pramila, 2006; Khare, 2007).

Alcoholic and aqueous extracts of the aerial parts of *C. album* at the doses of 200 and 400 mg/Kg were evaluated for hepatoprotective activity against paracetamol-induced hepatotoxicity using biochemical indicators and by histopathological technique. The aqueous extract at a dose of 400 mg/kg was found to be more potent when compared to Silymarin. Alcoholic and aqueous extracts [200 & 400 mg/Kg] showed significant hepatoprotective activity against paracetamol induced hepatotoxicity (Pal et al., 2011; Nigam and Paarakh, 2011).

Aqueous and alcohol extracts were prepared from bathua leaves and observed their antibacterial activity against human pathogenic bacteria Viz. *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Proteus vulgaris* and *Pseudomonas aueruginosa*. The significant results were obtained by aqueous as well as alcohol leaf extract on tested pathogens using the paper disc diffusion technique. The aqueous extract revealed the strongest antibacterial activity on *Staphylococcus aureus* and methanol leaf extract showed strongest antibacterial activity on *Pseudomonas aeruginosa* (Singh et al., 2011; Amjad and Alizad, 2012).

The crude extract prepared from bathua leaves and tested in vitro on intestinal smooth muscles of rabbit show spasmolytic and analgesic activity. The crude extract exhibited a dose-dependent increase in relaxation of smooth muscles, starting from 5 mg/ml and maximum effect was found at 20 mg/ml (92.86%) (Ahmad et al., 2012).

According to Dai et al. (2002) the ethanolic extract from the fruits of *C. album* showed a relatively weak antipruritic and antinociceptive effect. A study was aimed to investigate the effects of *Chenopodium* (leaves) on the growth of estrogen dependent and estrogen independent human breast cancer cell lines. The different solvent extracts (petroleum ether, ethyl acetate and methanol) were assessed for the bioassay. Among the various extracts studied for two cell lines, methanolic extract of *C. album* (leaves) exhibited the most antibreast cancer activity having (IC_{50}) (the concentration of an individual compound resulting in 50% inhibition) value 27.31

mg/ml against estrogen dependent cancer cell line. Remarkable percent inhibition (94.06%) within the methanolic extract of *C. album* (leaves) at 48 h of exposure and concentration 100 mg/ml ($p < 0.05$) against estrogen dependent cancer breast cancer cell line (Khoobchandani et al., 2009).

2.5 Phytochemical compounds

Phytochemicals are the bioactive non-nutrient plant compounds in the fruits, vegetables, grains and other plant foods that have been linked to minimizing the risk of major chronic diseases (Zhang et al., 2015). A type of chemical found in small amounts in plants and certain foods (such as fruits, vegetables, nuts, oils, and whole grains). Phytochemical compounds are extra-nutritional constituents that are found in small quantities in foods providing health benefits beyond the basic nutritional value of the product (Kitts, 1994). Phytochemical compounds have the capability of modulating metabolic processes and resulting in the promotion of better health. They exhibit as a receptor activator and inhibitor of gene expression (Correia et al., 2012). The inaccessibility of each phytochemical compound differs greatly and the most abundant compounds in ingested fruits and vegetables. Indeed, during studying the role and impact of phytochemical compounds in human health, bioavailability is not always well known (Carbonell-Capella et al., 2014). Carotenoids, flavonoids, carnitine, choline, coenzyme Q, dithiolthiones, phytosterols, phytoestrogens, glucosinolates, polyphenols and taurine etc. are some examples of phytochemical compounds. As vitamins and minerals express pharmacological effects, they can be categorized as bioactive phytochemical compounds as well (Hamzalioglu and Gokmen, 2016).

2.5.1 Common phytochemical compounds available in leafy vegetables

It is estimated that more than 5000 Phytochemicals have been identified but a large percentage still remains unknown (Shahidi and Naczk, 1995). Phytochemical compounds are found in vegetables and whole grains. The most common compounds found in leafy vegetables are polyphenol compounds, carotenoids, tocopherols, phytosterols, flavonoids, phenolic acids, isoflavones and anthocyanin compounds which have different chemical structures. Different species of *chenopodium* have different types and amounts of phytochemical compounds. Many scientific investigations identified the presence of tannin, saponin, alkaloids, terpinoids,

cumarins, flavonoids and phenols as the main phytochemical compounds in *chenopodium* species (Paliwal, 2015).

2.5.2 Phenolic compounds

In organic chemistry, phenols, sometimes called phenolics, are a class of chemical compounds consisting of one or more hydroxyl groups (—OH) bonded directly to an aromatic hydrocarbon group. Phenolic compounds are classified as simple phenols or polyphenols based on the number of phenol units in the molecule. Phenol – the simplest of the phenols. Phenols are both synthesized industrially and produced by plants and microorganisms (Hättenschwiler et al., 2000). Polyphenols have more than one hydroxyl group that are attached to benzene rings. The phenolic compounds have attention for their potential as antioxidants, their great abundance in our diet and their possible role in the prevention of various diseases associated with oxidative stress (Scalbert et al., 2005). The main dietary sources of phenols are vegetables, fruits and beverages (fruit juice, tea, coffee) legumes and cereals. Leafy vegetables are considered as the most enormous in phenolic content within all vegetables. Phenolic compound-rich food consumption has also been shown to be inversely associated with type-2 diabetes. By binding to the non-specific site of the enzyme, phenolic compounds can inactivate the starch-digesting enzyme α-amylase (Nwosu et al., 2011).

2.5.3 Flavonoids compounds

Flavonoids are polyphenol molecules containing 15 carbon atoms and they are water soluble. There are main three compounds such as flavones, flavanol and flavanone. Flavonoids have diverse biological properties and act as antioxidative, antimicrobial, antidiabetic, anticarcinogenic and cardio protective. Flavonol quercetin is a common flavonol-type flavonoid that is available in several foods such as onion, tea and apple and is consumed almost daily. In the Western diet, daily intake of quercetin is estimated and the range is 0-30 mg (D'Andrea, 2015). Flavanol catechins which are isolated into epicatechin, gallocatechin, epicatechin gallate, epigallocatechin, gallocatechin gallate and epigallocatechin gallate under flavanol group. Catechins are the major building blocks of tannins (Gadkari and Balaraman, 2015). Flavonoids show a wide range of biological activities such as inhibition of cell proliferation, induction of apoptosis, inhibition of enzymes and other antibacterial and antioxidant

effects (Cook and Samman, 1996; Middleton and Kandaswami, 1992).

2.5.4 Health benefits of phytochemical compounds

Bioactive phytochemical compounds have antioxidant capacity, scavenging free radicals and chelating action due to the presence of functional groups in their nuclear structure. The phytochemicals like phenols, flavonoids, isoflavonoid, etc., have garnered great interest for their potential role in the maintenance of human health particularly significant reduction in non-communicable diseases. They also assigned most of the health benefits from the consumption of phenolic and flavonoids. Both phenols & flavonoids act as antimutagenic and antitumoral activities. The flavonoids have the property of inhibiting many enzymes, such as oxygenases (prostaglandin synthase), required in the synthesis of eicosanoids. Thus, flavonoids have the capacity to inhibit hyaluronidase activity and help in maintaining the proteoglycans of connective tissues. The expansion of bacterial or tumour metastases would be prevented by this process (Havsteen, 2002).

Table 2.1: Health promoting property of various phytochemical compounds

phytochemical compounds	Health promoting property	References
Catechin	Resistance of LDL to oxidation, brachial artery dilation increased plasma antioxidant activity and fat oxidation	(Rasool et al., 2010)
Quercetin	Promotes overall cardiovascular health by encouraging blood flow	(Rasool et al., 2010)
Kaempferol	Reduce tumor initiation, platelet aggregation, increase HDL (high density lipoprotein)	(Rasool et al., 2010)
Gallic acid	Antioxidant and potential hepatoprotective effects	(Rasool et al., 2010)
Cinnamic acid	Is a precursor to the sweetener aspartame by the means of enzyme catalysed amination to phenylalanine	(Rasool et al., 2010)

p-Coumaric acid	Antioxidant properties and potentially reduce the risk of stomach cancer	(Rasool et al., 2010)
Gallocatechin gallate	Cholesterol reduction, reduce cell oxidation	(Rasool et al., 2010)
Epigallocatechin	Decreases triglycerides and high blood pressure and provide antioxidant action	(Rasool et al., 2010)
Trans-α carotene	Antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antiallergic, modulation of enzyme activity, antiviral and vasodilatory actions	(Rasool et al., 2010)
Trans-β carotene	Precursor to vitamin A and prevent cell oxidation	(Rasool et al., 2010)
Tannic acid	For the treatment of burns, it is used as medicinal agent	(Siang , 1983)
Violaxanthin	Reduce the risk of cardiovascular disease and cancer	(Li et al., 2011)
Cryptoxanthin	Used as a natural food colourant	(Li et al., 2011)
Stigmasterol	Decreases blood pressure, glucose levels, and heart beat rate	(Choudhary and Tran, 2011)
β - Sitosterol	Potential to reduce blood cholesterol levels and benign prostatic hyperplasia	(Choudhary and Tran, 2011)
Campesterol	Reduce the absorption of cholesterol in the human intestine	(Choudhary and Tran, 2011)

2.5.5 Quantitative assessment of phytochemical compounds of *Chenopodium* species

Pandey and Gupta (2014) investigated that total phenolic content was found in the amount (57.0 μg/GAE/mg) in ethyl acetate extract of *C. album* as compared to other extraction solvents. The plant contains 1.8 mg/100 g dry weight of alkaloid and lowers than the values reported for the leafy vegetables like *Aspilia africana, Bryophyllum pinnatum* and *Emilia coccinea.* The flavonoid contents are (42.74 μg/GAE/mg) in *C. album* (Adedapo et al ., 2011).

Another study reported that *C. album* contains simple phenols (72.50–101.007 mg GAE/100 g), total phenols (224.99–304.98 mg GAE/100 g), tannins (152.49–203.91 mg GAE/100 g), flavonoids (220.0–406.67 mg/100 g), phytic acid (238.3–268.33 mg/100 g), phytate phosphorus (67.16–75.62 mg/ 100 g), saponin (0.043–0.867 g/100 g), alkaloids (1.27–1.53 mg/100 g) and oxalates (394.19–477.08 mg/100 g) (Sood et al ., 2012). According to Ververidis et al. (2007) mainly two flavonoids are found in *C. album*, i.e., kaempferol and quercetin. They can be considered more into flavonols, isoflavones, flavanones, flavones, flavanonols, and flavans (catechins and proanthocyanidins) and anthocyanidins. Pachauri et al. (2017) reported that *C. album* contains 84 mg/100 g flavonoids on dry weight basis, which have health promoting properties. A study by Ibrahim et al. (2007) some flavonoid compounds were isolated from *C.album* namely quercetin, kaempferol, apiofuranosyl, rhamnopyranoside, glucopyranoside. Significant amount of phytochemical compounds such as phenols, flavonoids, tannins, saponins and cumarins were found in *C. murale* (Khan et al., 2019).

2.6 Antioxidants

Antioxidants are man-made or natural substances that are at low concentration in comparison with the main oxidizable substrate and prevent oxidation of that substrate (Halliwell, 2007). More than 170 antioxidants have been introduced in the current literature (Zou et al., 2015). There is a good impact in the body defense system against reactive oxygen species (ROS) (Boxin et al., 2002) and they have a good contribution to control blood pressure or blood sugar influencing substances, or can act as agents with anticarcinogenic, immunity-supporting, antibacterial, antifungal, antiviral, cholesterol-lowering, antithrombotic, or anti-inflammatory properties (Bub et al., 2003). The fruits and vegetables act as antioxidants due to the presence of fiber, polyphones, conjugated isomers of linoleic acid, trans-α carotene dlimolene, epigallocatechin gallate, isoflavonies, vitamins A, B, C, and E, Calcium, selenium, chlorophyllin, alipharin, sulphides, tetrahydrocurecumin, seasaminol, glutathione, indoles, thiocyanates and protease inhibitors (Karakaya et al., 2001). These phyto-constituents are strong reducing agents, singlet oxygen quenchers and hydrogen donors that contribute to minimizing oxidative stress by the scavenging action, due to the presence of hydroxyl groups (Morton et al., 2012).

2.6.1 Types of antioxidants

Natural antioxidant

Many fruits and vegetables have natural antioxidants and there is a great deal of public and scientific attention (Diwani et al., 2009). There happens constant oxidative stress from free radicals which are from all parts of plants reactive oxygen species, and prooxidants generated both exogenously (heat and light) and endogenously (H_2O_2 and transition metals). Many of these tissues are effective to control free radicals, lipid oxidation, oxidation intermediates, and secondary breakdown products by the use of the antioxidant system (Brown and Kelly, 2007). These antioxidant compounds act as reluctant and consist of flavonoids, phenolic acids, carotenoids and tocopherols that can inhibit Fe^{3+} induced oxidation, scavenge free radicals and natural antioxidants are found in natural sources such as fruits, vegetables and meats. There are various natural antioxidants that are available in everyday foods, the most common of which have vitamin C (ascorbic acid), vitamin E (tocopherols), vitamin A (carotenoids), various polyphenols including flavonoids, anthocyanins, and lycopene (Ozsy et al., 2009).

Phenols, flavonoids, ascorbates, tocopherols and carotenoids are well-known antioxidants and research shows the relation of the health benefits (Boskou et al., 2005). Leafy vegetables represent a valuable source of minerals, fiber and vitamin C, as well as vegetable pigments (carotenoids, chlorophylls) and polyphenolic compounds, with high biological activity. Most of these compounds are known to act as strong antioxidants that protect the human body against harmful effects of free radicals, such as heart diseases, tumors and aging processes (Panczenko-Kresowska , 1997; Rietjens et al., 2002; Wong et al., 2006; Bawa, 2004). Many investigations show the strong antioxidant activity of *Chenopodium* species. The antioxidant activity of aqueous and ethanolic extracts of C. album was 64.5% and 60.5% respectively (Kaur and Kapoor, 2002).

Synthetic antioxidant

Synthetic antioxidants are not found naturally, they are found by chemically synthesized (Shahidi et al., 1992). These antioxidants fall into two major categories on the basis of their mode of action primary antioxidants and secondary antioxidants. The primary antioxidants help to prevent the formation of free radicals during oxidation. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated

hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) are common antioxidants and widely used around the world for decades.

2.6.2 Functions of antioxidants

Antioxidants play a key role in the protective influence exerted by plant foods. The risk of chronic diseases is reduced by consuming fruits and vegetables regularly (Dembinska et al., 2008). Studies demonstrate that an antioxidant-rich diet has a beneficial health impact in the long run of life (Sin et al., 2013). Nowadays antioxidants make a relation between radicals and oxidative stress, cancer prophylaxis therapy and longevity (Kalcher et al., 2009). All antioxidants neutralize free radicals by giving up some of their own electrons and are responsible for the prevention of the damaging effects of free radicals and toxic products of their metabolism. However, there is a coordination system between these systems, the antioxidant (team) acts to control levels of free radical formation where deficiencies in one component impact the efficiency of others. These are hydrogen donation, electron donation by antioxidants and the addition of lipid to the antioxidants and then the formation of a complex between lipid and antioxidants. As food components work against chronic diseases, there is an attention to photo-chemicals, plant-derived molecules endowed with steady antioxidant power (Peter, 2007).

2.7 Diabetes Mellitus

Diabetes mellitus, a chronic metabolic disorder, is characterized by elevation of glucose level in blood due to irregularity in insulin secretion, improper insulin action, or both. It happens if the body is either not producing enough insulin or because the body cells do not properly respond to the insulin that is produced. Beta cells of pancreas maintain the adequate insulin secretion. However, a combination of genetic and environmental factors causes the beta-cell failure leads to hyperglycemia. This widely known metabolic disorder is affecting a large proportion of the population all over the world. Diabetes mellitus is a long term disease. This disease can directly or indirectly affect various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Rother, 2007; American Diabetes Association, 2012; Cho et al., 2018).

2.7.1 Epidemiology of diabetes

The number of diabetic patient is increasing worldwide. The increased number is alarmingly high in some parts of the world. Dramatic changes in sedentary lifestyle

and urbanization boosting the prevalence of diabetes worldwide. In 1980, the World Health Organization (WHO) estimated that there were 108 million people living with diabetes and this number increased fourfold in 2014 estimates (Zhou et al., 2016). It is estimated that 366 million people had diabetes mellitus in 2011; by 2030 this would have risen to 552 million (Anonymous, 2011). International Diabetes Federation (IDF) estimated the global prevalence to be 151 million in 2000, 194 million in 2003, 246 million in 2006, 285 million in 2009, 366 million in 2011, 382 million in 2013 and 415 million in 2015. In 2017 there were 451 million (age 18–99 years) people with diabetes worldwide. These figures were expected to increase to 693 million by 2045. Moreover, It was estimated that approximately 5.0 million deaths were attributable to diabetes among people aged 20– 99 years in 2017. Hence, diabetes accounted for 9.9% of the global all-cause mortality among people within this age range (Cho et al., 2018). Similarly, the risk of type 2 diabetes in South Asia is estimated to be more than 150% between 2000 and 2035 (Nanditha et al., 2016). A recent meta-analysis showed that the prevalence of diabetes among adults had increased substantially, from 4% in 1995 to 2000 and 5% in 2001 to 2005 to 9% in 2006 to 2010 (Saquib et al., 2012). Among urban residents, the prevalence of diabetes was 15.2% compared with 8.3% among rural residents. In total, 56.0% of diabetics were not aware they had the condition and only 39.5% were receiving treatment regularly. The likelihood of diabetes in individuals aged 55 to 59 years was almost double that in those aged 35 to 39 years (Akter et al., 2014). Almost one in ten adults in Bangladesh was found to have diabetes, which has recently become a major public health issue. Better detection, awareness, prevention and treatment are necessary to prevent the rise in diabetes.

2.7.2 Most Common types of diabetes mellitus

Diabetes mellitus is broadly divided into 2 groups, namely insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM). This classification is mainly based on the requirement of insulin for treatment.

Type 1 diabetes mellitus (T1DM)

T1DM is widely known as insulin dependent diabetes mellitus (IDDM), previously known as juvenile onset diabetes mellitus. This condition results from a cellular-mediated autoimmune destruction of the beta cells of the pancreas. Beta cell is responsible for proper insulin secretion. Multiple genetic predispositions and other

environmental factors are mainly the underlying causes of beta-cell destruction. Though the rate of beta-cell destruction is quite variable, the process is rapid in infants and children. However, the rate of destruction is slow in adults. That is the reason; type 1 diabetes mellitus only forms 5-10% of the total diabetes patients (American Diabetes Association, 2012).

Type 2 diabetes mellitus (T2DM)

The majority (95%) of diabetic patients have type II diabetes. T2DM is characterized by insulin insensitivity as a result of insulin resistance, declining insulin production, and eventual pancreatic beta-cell failure (Kahn, 1994). An impaired alpha-cell function is also associated with the pathophysiology of T2DM. These systematic problems in the body lead to the rising of glucagon and hepatic glucose levels during fasting. In addition to inadequate levels of insulin and increased insulin resistance, this condition ultimately results in hyperglycemia (Fujioka, 2007). Several studies show that insulin resistance precedes the defect in insulin secretion but individuals will develop diabetes only when insulin secretion is inadequate to maintain glucose concentration close to normal (Fujioka, 2007; Kaku, 2010). This disease is the most prevalent and chronic metabolic disorder expanding firmly all over the world. This condition was formerly known as non-insulin dependent diabetes or maturity onset diabetes. Other than genetic factors, physical inactivity, sedentary lifestyle, cigarette smoking and generous consumption of alcohol are the underlying causes of this condition (Olokoba et al., 2012).

Gestational Diabetes mellitus

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. Approximately 7% of all pregnancies are complicated by GDM, resulting in more than 200,000 cases annually. Gestational diabetes increases the risk of preeclampsia, stillbirth and delivery complications due to baby weight. Assessment of risks of developing gestational diabetes should take place during the first prenatal visit. However, these risks can be reduced by close monitoring of blood glucose through the whole pregnancy (American Diabetes Association, 2004).

Other specific types of diabetes

Some specific diseases, drugs, or genetic conditions/syndrome are associated with development of the chronic hyperglycemia. Pancreatic disease or removal of pancreatic tissue, endocrine diseases such as acromegaly, Cushing's syndrome,

pheochromocytoma, glucagonoma, somatostatinoma and primary aldosteronism, or the administration of certain hormones, drugs and chemicals can cause hyperglycemia. Genetic syndrome, abnormalities in insulin receptors may also cause a specific type of diabetes (Anonymous, 1979).

2.7.3 Treatment of diabetes mellitus

Treatment or management of diabetes mellitus is focusing on supporting people to live with minimum or no risk of complications. In terms of T1DM, patients are often required to use insulin analogues and mechanical technologies (insulin pumps and continuous glucose monitors) for improved treatment of type 1 disease (Hirsch, 2009). Setting of realistic goals in the management of T1DM is important for each child and family. Factors to be considered include a patient's age, developmental status, family involvement, and social situation, economic factors, and hypoglycemic history in persons with established disease. Moreover, nutritional education and psychological support need to be provided accordingly. Though, in T1DM, more focus is given on insulin management, optimal management requires proper recognition of the balance among insulin, exercise and food (Haller et al., 2005).

For T2DM, lifestyle modification along with nutritional and drug treatment can help in a great way. Maintaining body mass index an appropriate level together with a continuing diet rich in fiber, low in saturated fat, abstinence from smoking and alcohol can significantly reduce the complications of T2DM (Willi et al., 2007; Chen et al., 2011).

Insulin administration and oral glucose-lowering medications can help to manage diabetes mellitus. The glucose-lowering effectiveness of individual therapies and combinations of therapies depended not only on the intrinsic characteristics of the intervention but also on the duration of diabetes, baseline glycemia, previous therapy and other factors.

Insulin administration

As T1DM is insulin dependent, insulin should be administered to type 1 diabetic patient. Insulin is also suggested to T2DM patient as a supplement when oral drugs and nutritional therapy fails to control blood glucose concentrations. In the market, 4 types of insulin injections are available: rapid acting insulin, short acting insulin, intermediate acting insulin and long lasting insulin. The selected insulin regimen,

given alone or in combination with oral agents, should be tailored to the individual needs of the patient. Sometimes, an adverse effect such as hypoglycemia was also observed in some patients. It could be happened due to erratic meal timing, excessive insulin dosage or unplanned exercise (Chehade and Mooradian, 2000; Haller et al., 2005).

Oral anti-diabetic drugs

Sulphonylurea

Sulphonylurea works by binding to the sulfonylurea receptor on beta cell surface to stimulate the secretion of insulin from the pancreatic beta cells. Sulphonylureas are divided into two groups, 1st generation drugs (tolazamide, tolbutamide, acetohexamide and chlorpropamide) and 2nd generation drugs (glibenclamide glimepiride, glipizide and gliclazide). Second generation drugs are superior to first generation drugs. The occurrence of hypoglycemia was often associated under the usage of these drugs (Chehade and Mooradian, 2000; Olokoba et al., 2012).

Metformin

In most of the world, metformin is the only biguanide available. In a diabetic patient, biguanides suppress hepatic glucose production, increase insulin sensitivity, enhance glucose uptake, increase fatty acid oxidation and decreases the absorption of glucose from the gastrointestinal tract. It also enhances glucose uptake in the peripheral tissue, mainly the muscle (Chehade and Mooradian, 2000; Collier et al., 2006). It is noteworthy that individuals who are primary or secondary failures to sulphonylurea agents are unlikely to respond to metformin alone. However, when metformin is combined with sulphonylurea agents in those individuals who appear to be secondary failures, a substantial blood glucose lowering occurs (DeFronzo and Goodman, 1995).

Acarbose

Pancreatic α-amylase is an endo-hydrolase acting on 1,4-glucosidic linkages in linear regions of suitable length in starch, glycogen, and various oligosaccharides. The activity of the enzyme releases simpler sugars that are then converted into glucose for intestinal absorption by other enzymes. Therefore, the inhibition of α-amylase activity results in decreased bioavailability of oligosaccharides, absorbable sugars and consequently, in a decrease of the postprandial hyperglycemia. Alpha-amylase inhibitors (e.g., acarbose), competitively and reversibly inhibit the enzyme, but are reportedly associated with gastrointestinal side effects such as flatulence, abdominal pain, and diarrhea (Fujisawa et al., 2005; Singh et al., 2008).

2.7.4 Significance of plant source to improve diabetes mellitus

Nowadays, there is a growing interest in the use of phytochemical compounds in the treatment and management of diabetes, obesity and oxidative stress (Chukwuma et al., 2018). Over the last 2500 years, there are terribly well built ancient systems of drugs like Chinese, Ayurvedic, and therefore the Unani, born and practiced, additional within Asia. These traditions are still flourishing, since; approximately 80% of the people in developing countries rely on these systems of medicine for their primary health care needs (Tsay and Agrawal, 2005). In the last few years, there has been rapid growth in the field of herbal medicine. These medicines are gaining popularity both in developing and developed countries because of their natural and fewer side effects (Dubey and Mishra, 2017). Moreover, the awareness of the diabetic issue has led to a vast discovery of new medications as well as natural products extracted from herbal plants. Natural flavonoids of plants can promote hypoglycemia through increase glucose uptake and glycogen synthesis. Flavonoids also decrease the rate of glucose production by inhibiting the enzyme that initiates carbohydrate digestion (Chan et al., 2012). Around 800 plant species are recorded to hold antidiabetic properties. Several plant species have been used for the prevention or management of diabetes by the Native Americans, Chinese, South Americans and Asian Indians (Mentreddy et al., 2005). A review study revealed that 108 plant species were generally used for the treatment of diabetes (Dubey and Mishra, 2018). The fruits and leaves were most typically used plant components and different components (root, stem, bark, flower and whole plant) were conjointly helpful for natural action. In terms of diet, studies have also shown that individuals following a plant-based eating pattern typically consume fewer calories and less fat, saturated fat and cholesterol and have lower BMIs than non-vegetarians (Trapp and Levin, 2012). Both the American Academy of Nutrition and Dietetics and the American Diabetes Association (ADA) now include well-planned, plant-based consumption patterns (vegetarian and vegan) as a meal-planning choice in their nutrition guidance's for people with diabetes (Craig et al., 2009; Anonymous, 2012).

2.7.5 Alpha-amylase inhibition and diabetes mellitus

There are many digestive enzymes in humans and among them the most important one is alpha-amylase, that acts as catalyst in the reaction which involves the hydrolysis of the alpha-1,4 glycosidic linkages of the starch, amylopectin,

amylose, glycogen and numerous maltodextrins and is responsible for starch digestion. The enzyme alfa amylase initiates producing of glucose which is the final product of the digestive process of carbohydrates (Tundis et al., 2010).

The large molecules like starch are unable to cross the blood-brain barrier as glucose has to reach the brain thus; to beat this downside alpha-amylase cleaves the large starch molecules into tiny pieces of sugars so as to cross the blood-brain barrier. If there will be an extreme transformation of starch to sugars, it will raise the sugar quantity in blood, then the role of insulin will come into action by ordering cells to metabolise the surplus sugar moieties and reserve them as energy sources i. e. glycogen. This cycle is constantly happening in a healthy individual. However in several cases, because of excess activity of amylase enzyme and insulin deficiency or resistance to insulin, level of blood glucose arises which could ends up in hyperglycemia. To regulate hyperglycemia many studies on the inhibition of amylase enzyme activity are being studied. However, if there will be excessive inhibition of pancreatic alpha amylase, it might cause abnormal bacterial fermentation of undigested carbohydrates in the colon resulting in flatulence and diarrhea (Horii et al., 1986).

Acarbose, Miglitol and Voglibose are the enzyme inhibitors that are presently utilized for the management of PPHG (postprandial hyperglycemia). Though effective in controlling PPHG, these inhibitors are not desirable for long-term treatment due to their gastrointestinal side effects (Laar, 2008; Etxeberria et al., 2012). Given the fact that about 80 % of diabetic people are living in low and middle-income countries, these drugs are expensive also. Therefore, several groups have made their efforts to find α-amylase inhibitors from plants, bacteria, marine algae and fungi (Fatmawati et al., 2011; Konishi et al., 2012; Orhan et al., 2013; Panwar et al., 2014). The majority of them have studied the crude extracts (organic or aqueous), and some have studied pure compounds also (Ali et al., 2013; Kim et al., 2014). Most of the plant extracts and pure compounds were effective against α-amylase enzymes (Mohamed et al., 2012; Perez-Gutierrez and Damian-Guzman, 2012).

Synthetic medications are responsible for numerous side effects such as flatulence, abdominal distention and diarrhoea because of the excessive inhibition of pancreatic α-amylase. This ends up in abdominal bacterial fermentation of undigested

carbohydrates within the colon. Hence, at present, there is an increasing interest among food scientists to identify natural sources of α- amylase inhibitors for the dietary management of type II diabetes (Piette and Kerr, 2006). It is evaluated that over 800 plant species possess hypoglycemic activity, and over 450 plants have been analytically tested (Ciulei, 1982). A study by Kant et al. (2018) identified the presence of antidiabetic activity in methanolic extract of C. *album.* Methanolic extracts from *Chenopodium* species showed α-amylase inhibitory activity. Where acarbose was used as standard and the IC_{50} value for acarbose was 27 μg/ml (Rai et al., 2020). In vitro investigation of aqueous extracts from *C. album* show 54.52 ±0.38 % α- amylase inhibitory effect at the concentration of 5mg/ml, where the standard shows 99.21± 0.32 % inhibitory effects (Odhav et al., 2010).

Chapter III: Materials and Methods

3.1 Study period and Study area

The research work was conducted for a period of six months from August 2020 to December 2020. Experimental procedures were carried out in the laboratory of the Department of Applied Food Science and Nutrition, Department of Food Processing and Engineering, Department of Physiology, Biochemistry and Pharmacology, Department of Fishing & Post-Harvest Technology at Chattogram Veterinary and Animal Sciences University, Bangladesh.

Figure 3.1: Sample collection area

3.2 Experimental design

Firstly, one area was chosen from where selected plants of *Chenopodium* species were collected. After collection of samples, the leaves of plants were used for the preparation of powder from fresh leaves. Then this powder was used to determine proximate composition (ash, crude fat, protein, crude fiber and carbohydrate) and mineral (Sodium, Potassium, Calcium, Magnesium, phosphorus, Iron, Zinc and Copper) contents. The samples were also subjected to antioxidant activity, antidiabetic effect and phytochemical compounds assessment by UV-visible spectrophotometer. Antioxidant activity was measured by the DPPH scavenging method, antidiabetic effect was measured through α-amylase inhibition assay and two important phytochemical compounds like total phenolic contents (TPC) and total flavonoid content (TFC) were determined by suitable standards.

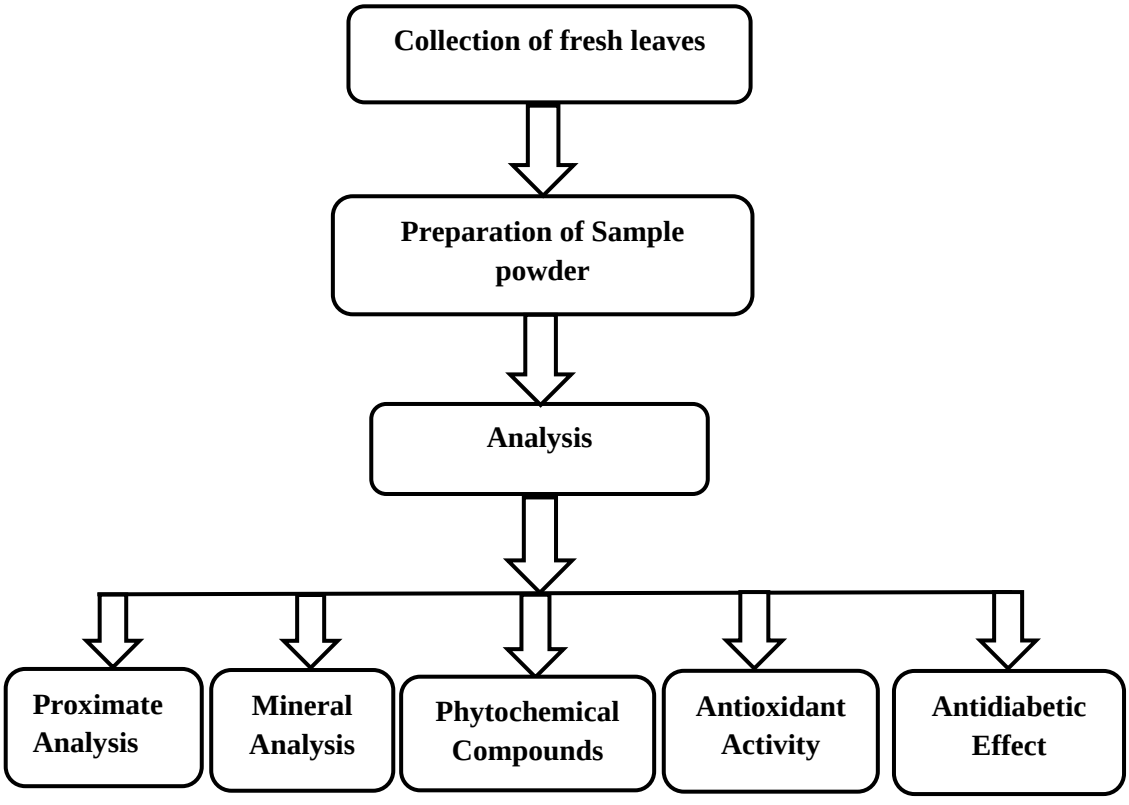

Figure 3.2: Study design

3.3 Collection of samples and preparation

Firstly, fresh plants of *C. album*, *C. giganteum* and *C. murale* were collected from local street vendors in Khulshi, Chattogram, Bangladesh. Then only the leaves were taken manually from the plants. The leaves were washed with water to remove adherences, dirt and other surface impurities. Then the leaves were dried in cabinet drier at 45℃ for one day. Dried samples were taken and ground into powder form with the help of a grinder. The powdered sample was then passed through a sieve. After that powder of samples was packed into zip-lock plastic bag and kept at 4℃ in the refrigerator for further examination.

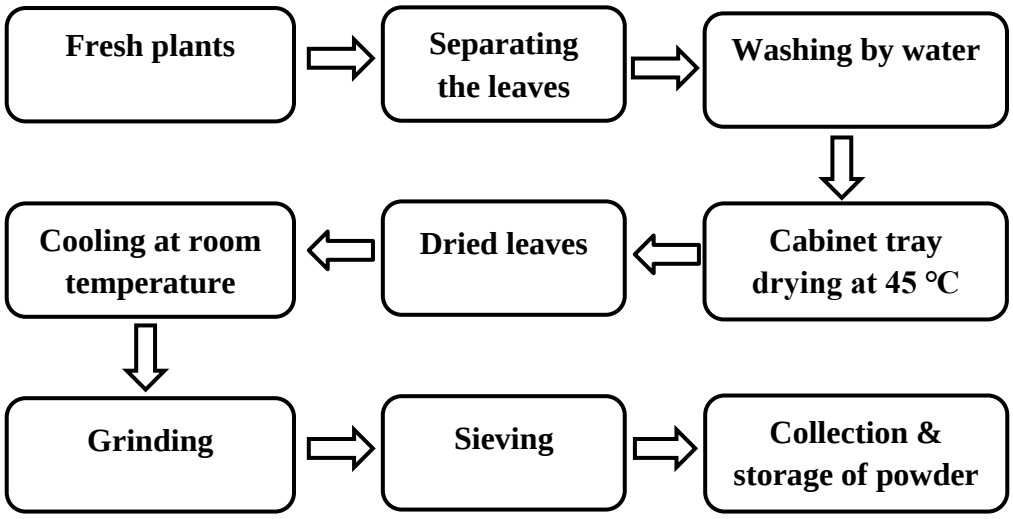

Figure 3.3: Flow Chart of sample powder processing

3.4 Proximate analysis

Moisture, protein, fat and ash contents of collected samples were measured in triplicate according to AOAC methods. The moisture was measured by oven drying at 105℃ to constant weight (AOAC, 2012). The crude protein content was measured by the Kjeldahl procedure (6.25×N). Lipid or fat was extracted by the AOAC (2012) method using the Soxhlet apparatus. Ash was measured gravimetrically in a muffle furnace by heating at 550℃ to constant weight (AOAC, 2012).

3.4.1 Moisture/Water

The moisture content of the samples was determined by the hot air oven drying method following the method described in AOAC. An empty crucible and 3 g of the sample were weighed in an analytical balance. The sample was taken in the crucible

and dried in a thermostatically controlled oven at 105℃ for 12 hours. After that, the crucible was removed and placed in a desiccator to cool at room temperature. The sample containing crucible was weighted and the value was recorded. This step was done repeatedly until a constant weight was found out. The moisture content of the sample was calculated from the loss of weight in the sample. From these weights, the percentage of moisture in food samples was calculated as follows:

$$\%\ \text{moisture content} = \frac{\text{Loss in weight}}{\text{weight of sample}} \times 100$$

3.4.2 Crude Protein Determination

Reagents used: Concentrated H_2SO_4 (98% pure), Digestion mixture ($CuSO_4$: K_2SO_4 =1:9), 4% Boric acid solution, NaOH (35%), mixed indicator solution (Bromocresol green + methyl red), Standard HCl (0.2N).

For estimation of protein, the steps were followed:

Digestion: 0.3g sample, 4g digestion mixture and 5 ml H_2SO_4 was taken in a kjeldahl digestion flask. It was heated at 320 ℃ for 30 minutes in a kjeldahl digestion and distillation apparatus. The digestion was completed when the color of the substance was pale yellow.

Distillation: After digestion 25ml water, 25 ml 35% NaOH and glass blitz were added to kjeldahl flask which contained about 10 ml 4% boric acid and 2-3 drops mixed indicator. Cooled tube and receiving solution were placed into the distillation unit. 25ml of 35% NaOH was automatically filled into the tube. The distillation process takes place for 3 minutes. The receiving solution turned green at the end of the process.

Titration: The solution collected was titrated with 0.2N HCl solution and titer value was recorded.

Calculation: The calculation of the percent of protein in the sample using protein factor 6.25.

$$\%\ \text{Nitrogen} = \frac{\text{ml of titrate} \times \text{Normality of acid} \times \text{meq. of Nitrogen}}{\text{Weight of sample(gm)}} \times 100$$

Where,

Normality of acid = 0.2N

meq. of N_2 = 0.014

% Protein = % Nitrogen × 6.25

3.4.3 Crude Fat Determination

Crude fat was determined by using a Soxhlet apparatus. The dried sample was weighted and transferred into a thimble and plugged with fat-free cotton. The thimble was placed into the fat extraction tube of the Soxhlet flask. 75 ml of anhydrous ether was taken into the flask and top of the fat extraction tube was attached to the condenser. The sample was extracted for 16hrs or longer period. At the end of the extraction, the thimble was removed and most of the ether was distilled off and collected through the Soxhlet tube. The ether from the tube was poured off as it was nearly full. When the volume of ether containing the fat particles of the sample was reached at a small volume, it was poured into a beaker using a funnel. The flask was rinsed filtered thoroughly using ether. Then the ether was evaporated on a steam bath at low heat. T was dried at 100℃ for 1 hour. After cooling, the weight was recorded carefully. The fat present in the sample is calculated by the formula given below:

$$\text{Fat } \% = \frac{\text{Loss of ether soluble material}}{\text{Weight of sample}} \times 100$$

3.4.4 Ash Content

First of all, an empty crucible was cleaned properly and dried in a hot air oven. It was placed in desiccators and cooled then the weight was recorded. 5 gm of the sample was weighed and placed in the crucible. It was allowed to burn until all the smoke was gone from the sample. The crucible was cooled and transferred to the muffle furnace at 550-600℃ for 8 hours. The process ends when formation of white ash accomplished. It was cooled at 150℃ and then placed to desiccator. When it cooled to mild warm the weight was recorded. The ash content was calculated using the following formula:

$$\text{Percentage of Ash} = \frac{W - W_1}{W_2} \times 100$$

Where, W= weight of the crucible with ash

W_1= weight of the empty crucible

W_2= weight of the sample

3.4.5 Crude Fiber Determination

Crude fiber was determined according to AOAC method. At first 2 gm of the sample was weighed and then taken into a beaker. Then 125ml of 1.25% sulfuric acid solution and 3-4 drops of n-octanol were added into the same beaker. N-octanol was used as an antifoaming agent. The beaker was boiled for 30 minutes at a constant volume. After that, the sample was washed three times to remove the acid. After washing 125ml of 1.25% sodium hydroxide and 3-5 drops of antifoam were added. It was again boiled for another 30 minutes at constant volume. The mixture was filtrated and again washed the residue like before. It was washed again with 1% HCL solution in order to remove the acid. Then the residue was dried in a hot air oven at 105℃ until a constant weight was found out. It was placed in a desiccator for cooling and the weight was recorded. Finally, the residue was burned up to smoke and ignited in the muffle furnace at 550-660℃ for about 3-4 hours until that turned into white ash. The ash particles were weighed and calculated to determine the crude fiber content of the sample.

$$\text{Percentage of crude fiber} = \frac{W - W_1}{W_2} \times 100$$

Where,

W= weight of crucible containing crude fiber and ash

W_1= weight of crucible containing ash

W_2 = weight of the sample

3.4.6 Carbohydrate Content

The available carbohydrate content was determined by subtracting the sum of the values of moisture, ash, protein and fat from 100 (per 100gm) (AOAC, 2012).

$$\%\ \text{of Carbohydrate} = 100 - (\text{Moisture}\ \% + \text{Ash}\% + \text{Protein}\% + \text{Fat}\ \% + \text{Fiber}\%)$$

3.4.7 Energy Content

The energy value of the samples was determined by multiplying the protein content by 4, carbohydrate content by 4 and fat content by 9 according to the standard James formula (James, 1995).

Energy value (Kcal/100g) = (Crud eprotein×4)+(crude fat×9)+(Total Carbohydrate×4)

3.5 Mineral analysis

Mineral contents (Sodium, potassium, magnesium, calcium, phosphorus, iron, zinc and copper) were determined by using biochemical analyzer (Humalyzer 3000). A commercially available biochemical kit (Randox®) was used for biochemical assay. The whole procedure was done in the Postgraduate Research lab under the Dept. of Physiology, Biochemistry and Pharmacology at Chattogram Veterinary and Animal Sciences University. All the analyses were done in triplicates and expressed in mg/100g.

3.5.1 Sample preparation

Apparatus: Beaker, Measuring pipets, Volumetric flask, Analytical balance, Heating mantle or hot plate, Filter paper, Whatman® No. 541

Required Reagent: Nitric acid and Perchloric acid

Procedure: One (01) gm of dry sample was weighted in a conical flask. For dried samples, 7.5 mL conc. HNO_3, and 2.5mL conc. $HClO_4$ in the ratio of 2:1 was prepared. For the wet sample, 5 mL HNO_3 and 1 mL $HClO_4$ ware added (HNO_3: $HClO_4$ = 5:1). Then the flask was placed in a hot plate at 200W for 1-2 hours until full digestion. After digestion, it was cooled at room temperature. Then transferred the digested samples into a 100 mL volumetric flask and diluted up to 100 marks with Deionized water and mixed well. Later, the solution was filtered through Whatman® filter paper No. 1 and transferred to Eppendorf Tube for mineral quantification.

3.5.2 Determination of Sodium (Na)

Sodium is precipitated as a triple salt with magnesium and uranyl acetate. The excess of uranyl ions is reacted with ferrocyanide in an acidic medium to develop a brownish color. The intensity of the color produced is inversely proportional to the concentration of sodium in the sample.

Procedure:

Table 3.1: Sodium (Na) determination

Step 1: Precipitation

	Pipette into cuvette	
	Blank	**Standard**
Precipitating Reagent(L1)	1.0 ml	1.0 ml
Sodium Standard	20 µl	-
Sample	-	20 µl

Mix well and let stand at room temperature for 5 minutes with shaking well intermittently. Centrifuge at 2500 to 3000 RPM to obtain a clear supernatant.

Step 2: Color Development

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Acid Reagent(L2)	1.0 ml	1.0 ml	1.0 ml
Supernatant from step 1.	-	20 µl	20 µl
Precipitating Reagent(L1)	20 µl	-	-
Colour Reagent(L3)	100 µl	100 µl	100 µl

Assay conditions:

Wavelength / filter:	530 nm (Hg 546) /Green
Temperature:	Room Temperature
Light path:	1 cm

Calculation:

$$\text{Sodium in } \frac{\text{mmol}}{\text{L}} = \frac{(\text{A})\text{ sample}}{(\text{A})\text{standard}} \times \text{Standard conc.} \left(\frac{\text{mg}}{\text{dl}}\right)$$

3.5.3 Determination of Potassium (K)

Sodium tetraphenyl boron reacts with potassium to produce a fine turbidity of potassium tetraphenyl boron. The intensity of turbidity is directly proportional to the concentration of potassium in the sample.

Procedure:

Table 3.2: Potassium (K) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Sample	-	-	0.02ml
Deionized water	0.02ml	-	-
Standard	-	0.02ml	-
K^+ Reagent	1.0ml	1.0ml	1.0ml

Assay conditions:

Wavelength / filter:	630 nm (Hg 623) /Green
Temperature:	Room Temperature
Light path:	1 cm

Calculation:

$$\text{potassium in } \frac{\text{mg}}{\text{dl}} = \frac{(\text{A})\text{ sample}}{(\text{A})\text{standard}} \times \text{Standard conc.} \left(\frac{\text{mg}}{\text{dl}}\right)$$

3.5.4 Determination of Calcium (Ca)

Calcium ions form a violet complex with O-Cresol phthalein complex one in an alkaline medium. Colorimetric method: O-Cresol phthalein complex one, without deproteinization.

Procedure:

Table 3.3: Calcium (Ca) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Sample	-	-	25µl
Distilled water	25 µl	-	
Standard	-	25 µl	-
Working Reagent	1.0ml	1.0ml	1.0ml

Assay conditions:

Wavelength / filter:	578nm Hg (550-590)
Spectrophotometer:	570nm
Temperature:	20-25^{O}C / 37^{O}C
Light path:	1 cm

Calculation:

$$\text{calcium in } \frac{\text{mg}}{\text{dl}} = \frac{\text{(A) sample}}{\text{(A)standard}} \times \text{Standard conc.} \left(\frac{\text{mg}}{\text{dl}}\right)$$

3.5.5 Determination of Magnesium (Mg)

The method is based on the specific binding of calmagite, a metallochromic indicator and magnesium at alkaline pH with the resulting shift in the absorption wavelength of the complex. The intensity of the chromophore formed is proportional to the concentration of magnesium in the sample.

Procedure:

Table 3. 2: Magnesium (Mg) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Sample	-	-	10µl
Standard	-	10µL	-
Reagent	1.0ml	1.0ml	1.0ml

Assay conditions:

Wavelength / filter:	520 nm, Hg 546 nm 500-550nm (Increase of absorbance)
	628 nm, Hg 623 nm, 570-650 nm (Decrease of absorbance)
Temperature:	20-25^{O}C / 37^{O}C
Light path:	1 cm
Measurement:	Against reagent blank

Calculation:

$$\text{Magnesium in } \frac{\text{mg}}{\text{dl}} = \frac{\text{(A) sample}}{\text{(A)standard}} \times \text{Standard conc.} \left(\frac{\text{mg}}{\text{dl}}\right)$$

3.5.6 Determination of Phosphorous (P)

Inorganic phosphate reacts with ammonium molybdate in the presence of sulfuric acid to form a phosphomolybdic complex which is measured at 340nm.

Procedure:

Table 3.3: Phosphorus (P) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Sample	-	-	10µl
Standard	-	10µL	-
Reagent	1.0ml	1.0ml	1.0ml

Assay conditions:

Wavelength / filter:	340 nm, Hg 334 nm, Hg 365 nm
Temperature:	20-25^{O}C / 37^{O}C
Light path:	1 cm
Measurement:	Against reagent blank

Calculation:

$$\text{phosphorus concentration } \frac{\text{mg}}{\text{dl}} = \frac{\text{(A) sample}}{\text{(A)standard}} \times \text{Standard conc.} \left(\frac{\text{mg}}{\text{dl}}\right)$$

3.5.7 Determination of Iron (Fe)

The iron is dissociated from transferring-iron complex in weakly acid medium. Liberated iron is reduced into the bivalent form by means of ascorbic acid. Ferrous ions give with Ferrozine a colored complex. The intensity of the color formed is proportional to the iron concentration in the sample.

Procedure:

Table 3.6: Iron (Fe) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Sample	-	-	200µl
Standard	-	200µl	-
Reagent	1.0ml	1.0ml	1.0ml

Assay conditions:

Wavelength / filter:	562nm
Temperature:	37^{O}C / 15-25^{O}C
Light path:	1 cm

Calculation:

$$\text{Iron in } \frac{\mu g}{dl} = \frac{(A)\text{ sample} - (A)\text{sample blank}}{(A)\text{standard}} \times \text{Standard conc.} \left(\frac{mg}{dl}\right)$$

3.5.8 Determination of Zinc (Zn)

Zinc in an aikaline medium reacts with Nitro - PAPS [2-(5-Nitro-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino) phenol disodium salt] to form a purple coloured complex. Intensity of the complex formed is directly proportional to the amount of Zinc present in the sample.

Procedure:

Table 3. 7: Zinc (Zn) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Working Reagent	1.0 ml	1.0 ml	1.0 ml
Distilled Water	50 µl	-	-
Zinc Standard	-	50 µl	-
Sample	-	-	50 µl

Assay conditions:

Wavelength/fiter	570nm (Hg 578 nm)/Yellow
Temperature	Room Temperature
Light path	1cm

Calculation:

$$\text{Zinc in } \frac{\mu g}{dl} = \frac{(A)\text{ sample}}{(A)\text{standard}} \times \text{Standard conc.} \left(\frac{mg}{dl}\right)$$

3.5.9 Determination of Copper (Cu)

Copper reacts with Di-Br-PAESA [4-(3,5-Dibromo-2-pyridylazo)-N-ethyl-N-(3-sulpho-propyl) aniline sodium salt monohydrate] to form a coloured complex. Intensity of the complex formed is directly proportional to the amount of Copper present in the sample.

Procedure:

Table 3.8: Copper (Cu) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Buffer Reagent (L1)	500 µl	500 µl	500 µl
Colour Reagent(L2)	500 µl	500 µl	500 µl
Distilled Water	50 µl	-	-
Copper Standard	-	50 µl	-
Sample	-	-	50 µ

Assay conditions:

Wavelength/fiter:	580 nm (Hg 578 m)/Yellow
Temperature:	Room Temperature
Light path:	1cm

Calculation:

$$\text{Zinc in } \frac{\mu g}{dl} = \frac{(A)\text{ sample}}{(A)\text{standard}} \times \text{Standard conc.} \left(\frac{mg}{dl}\right)$$

3.6 Phytochemical compounds analysis

3.6.1 Preparation of methanolic extract

Methanolic extract was Prepared according to a modified method described by (Ferreres et al., 2008). Cabinet-dried samples were transferred into respective beakers

added with absolute ethanol and left to shake on a shaker for 72 hr at room temperature. The solvent was then separated from residue by straining. The filtrate was collected and stored at room temperature while the residue was re-extracted twice, each time with fresh solvent.

Finally, all the filtrates were combined and evaporated under reduced pressure at 60℃ using a rotary evaporator to obtain the crude extracts. The crude extracts were stored at 4℃ until further analysis.

3.6.2 Qualitative screening of Phytochemical compounds

Phytochemical screening of the methanolic extracts of C. *album, C. giganteum* and *C. murale* were tested for the presence of tannins, saponins and alkaloids following the standard procedures described by (Bargah, 2015).

Test for Tannins

The presence of tannins was determined through Braymer's test. At first 2 ml of extract was mixed with 2 ml of distilled water and then 2-3 drops of 5% ferric chloride solution were added. The formation of green precipitation indicates the presence of tannins.

Test for Alkaloids

The presence of alkaloids was detected by Hager's test. About 3ml of extract was taken in the test tube and treated with a few drops of Hager's reagent (saturated picric acid solution). The presence of alkaloids was confirmed by the formation of a yellow color precipitate.

Test for Saponins

About 5 ml of extract was taken in a test tube. An equal volume of distilled water is also added and shaken vigorously then the test tube was warmed. The formation of emulsion or stable foam indicates the presence of saponins in the extract.

3.6.3 Total phenolic content (TPC)

TPC of the dried leaf powder extracts will be determined according to the method described with slight modifications (Azizi et al., 2010). The methanolic stock solution was used for the determination of total phenolic content. About 10 mg of gallic acid was dissolved into 10ml of distilled water to make the gallic acid (stock solution) and standard solutions of gallic acid (1.0, 2.0, 4.0, 6.0, 8.0 ppm) will be prepared.

Methanolic plant extracts or gallic acid standard solution (1 mL) will be pipetted into a test tube. Diluted Folin–Ciocalteu reagent (1.5 mL) will be then added and mixed. The mixture will be left for 3 min before adding 1.5 mL of sodium carbonate (75 g/L) solution and left for 60 min. The absorbance was read at wavelength 765 nm using a UV-vissible spectrophotometer and ethanol will be used as the blank. TPC will be calculated and expressed as milligrams of gallic acid equivalents (GAE) per gram of extracts (mg GAE/100g).

3.6.4 Total flavonoid content (TFC)

TFC of the (sample) extracts will be determined using the aluminum chloride colorimetric method described by (Chang et al., 2002). The methanolic stock solution was used for the determination of total flavonoid content. Quercetin will be dissolved in 80% ethanol to make standard solutions (1.0, 2.0, 4.0, 6.0, and 8.0 ppm) to plot a standard curve. Aliquots of 0.5 mL of diluted extract or standard solution will be mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 mol/L potassium acetate, and 2.8 mL of distilled water in the test tube. The mixture will be left at room temperature for 30 min. The absorbance will be read at wavelength 415 nm in UV-visible spectrophotometer. For the blank, 10% aluminum chloride will be substituted with distilled water of the same amount. TFC will be calculated and expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/100g).

3.7 Determination of antioxidant activity by DPPH scavenging method

Antioxidant capacity of the extracts was determined using DPPH assay as described by Nayira et al. (2013) with slight modifications. Different concentrations (2ppm, 4ppm, 8ppm, 16ppm, 32ppm) of sample extract solution and ascorbic acid were prepared with methanol. Methanolic DPPH solution was prepared by dissolving 6 mg of DPPH in 100mL methanol. The methanolic DPPH solution (2 mL) was added to 1 mL of each extract solution of different concentrations and the mixture was left for 30 min and the absorbance was read at wavelength 517 nm. Control was prepared by mixing 1 mL of methanol with 2 mL of DPPH solution while methanol was used as a blank. The scavenging activity was measured as the decrease in absorbance of the samples in comparison with the DPPH standard solution. Same concentrations of ascorbic acid solutions were also prepared and used as the standard in this method.

Antioxidant activity based on the DPPH free radical scavenging ability of extracts calculated using the following equation:

$$\text{Scavenging activity}(\%) = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100$$

Concentrations of extracts resulting in 50% DPPH free radical scavenging activity (IC_{50}) were determined graphically.

3.8 Determination of antidiabetic effect by α- amylase inhibition assay

Reagents used: α- amylase enzyme, phosphate buffer (pH 6.9), starch solution, 3,5dinitrosalicylic acid (DNS) color reagent, acarbose, Distilled water, methanolic plant extracts.

The α-amylase inhibitory activity for plant extracts was determined based on the spectrophotometric assay using acarbose as the reference compound (Dastjerdi et al., 2015). Different concentrations (20ppm, 40ppm, 60ppm, 80ppm, 100ppm) of sample extract solution and acarbose solution were prepared. The enzyme α -amylase solution was prepared of α-amylase in 40 mM phosphate buffer (pH 6.9). Add 1 mL of extracts from each concentration and 1mL of α-amylase enzyme. The solution was pre-incubated at 37°C for 10 min, after which 1ml of 1% starch solution was added and then further incubated at 37°C for 10min. The reaction was terminated by adding 1ml of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5min and cooled to room temperature. The absorbance was measured at 540 nm and a control reaction was carried out without the extract. Percentage inhibition was calculated by expression:

$$\%\ \text{inhibition} = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100$$

Concentrations of extracts resulting in 50% inhibition of enzyme inhibitory activity (IC_{50}) were determined graphically.

3.9 Statistical Analysis

Data were determined and stored in Microsoft Excel 2013 spread sheet to evaluate statistical analysis. All samples were in three replicates. Descriptive statistics (mean and standard deviation) were done for proximate composition and quantitative

phytochemical compounds. Data were sorted, coded and recorded in IBM SPSS Statistics 22. After that statistical analysis were conducted. Proximate composition and phytochemicals data were analysed by using One-way ANOVA procedures to assess significant level of variation at 95% confidence interval. Post hoc "Tukey" test was conducted to identify the variation within the sample groups. The statistical analysis was conducted at 5% level of significance ($p \leq 0.05$).

Chapter IV: Results

4.1 Proximate Analysis

The results of proximate analysis of *C. album*, *C. giganteum* and *C. murale* are presented in Table 4.1. The moisture content is presented as fresh weight basis and the other parameters (Protein, fat, fiber, ash, carbohydrate) are on a dry basis per 100g. The values for moisture, ash and carbohydrate content were found significantly different among the samples. Moisture content was highest (83.26±0.27%) in *C. album* and the lowest value (78.03±0.19%) was found in sample *C. murale.*

Protein content of *C. album* and *C. giganteum* showed no significant difference. *C. giganteum* had the highest protein content (34.41±0.46%) whereas *C. murale* had the lowest value (27.64±0.55%). Fat and fiber content of *C. giganteum* and *C. murale* were not significantly different. Fat and ash content found highest in *C. album*. Fat and ash content in *C. album* were found 3.51±0.33% and 26.70±0.03% respectively. Fat & Fiber contents of *C. giganteum* and *C. murale* were found in almost the same amounts. Fiber content was higher in *C. murale* (9.83±0.09%) whereas lower value (8.60±0.20%) was found in *C. album.* In case of Energy, *C. murale* carried the highest value (282.66±1.73) (Kcal/100g) and the lowest value (248.26±0.26) (Kcal/100g) was found in *C. album.*

Table 4.1 Proximate analysis

Species name	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	CHO (%)	Energy (Kcal/100g)
C. album	83.26±0.27[a]	34.19±0.35[a]	3.51±0.33[a]	8.60±0.20[b]	26.70±0.03[a]	19.96±1.00[c]	248.26±0.26[c]
C. giganteum	81.83±0.42[b]	34.41±0.46[a]	2.93±0.04[b]	9.82±0.05[a]	21.59±0.21[b]	25.09±0.78[b]	264.43±1.46[b]
C. murale	78.03±0.19[c]	27.64±0.55[b]	2.85±0.08[b]	9.83±0.09[a]	17.49±0.18[c]	36.56±0.83[a]	282.66±1.73[a]

Values are means ± standard deviations of triplicate determination. Values in the same column having the same super script letters are not significantly different ($p < 0.05$), Values in the same column having the different super script letters are differ significantly ($p < 0.05$).

4.2 Mineral contents of *C. album, C. giganteum* and *C. murale*

Table 4.2 showed the mineral contents of *C. album, C. giganteum* and *C. murale*. Potassium, Calcium, Phosphorus and Copper were higher in *C. album*. Magnesium, Iron and Zinc were higher in *C. murale*. On the other hand, the lowest values of Sodium, Potassium, Calcium and Phosphorus content were in *C. murale*. Magnesium, Iron, Zinc and Copper content were lowest in *C. giganteum* but the Sodium content was found highest. The highest Sodium, Potassium, Calcium, Magnesium, Phosphorous, Iron, Zinc and Copper were (378.25±0.09) mg/100gm, (6474.47±0.30) mg/100gm, (1284.91±0.96) mg/100gm, (850.84±0.31) mg/100gm, (1230.16±0.29) mg/100gm, (43.35±0.38) mg/100gm, (4.28±0.08) mg/100gm and (2.89±0.10) mg/100gm respectively.

Table 4.2 Mineral Content

Species name	Sodium mg/100gm	Potassium mg/100gm	Calcium mg/100gm	Magnesium mg/100gm	Phosphorus mg/100gm	Iron mg/100gm	Zinc mg/100gm	Copper mg/100gm
C. album	351.33±0.06^{b}	6474.47±0.30^{a}	1284.91±0.96^{a}	780.17±0.45^{b}	1230.16±0.29^{a}	40.37±0.22^{b}	3.48±0.32^{b}	2.89±0.10^{a}
C. giganteum	378.25±0.09^{a}	6318.60±0.18^{b}	1242.17±0.45^{b}	600.33±0.72^{c}	920.51±0.28^{b}	17.24±0.26^{c}	2.87±0.09^{c}	1.65±0.18^{c}
C. murale	307.87±0.12^{c}	4268.43±0.20^{c}	873.83±0.91^{c}	850.84±0.31^{a}	460.03±0.22^{c}	43.35±0.38^{a}	4.28±0.08^{a}	2.37±0.10^{b}

Values are means ± standard deviations of triplicate determination. Values in the same column having the same super script letters are not significantly different ($p < 0.05$), Values in the same column having the different super script letters are differ significantly ($p < 0.05$).

4.3 Qualitative screening of Phytochemical compounds

Phytochemical screening results of methanolic extracts of *C. album C. giganteum* and *C. murale* leaves are given in the Table 4.3.

Table 4.3: Qualitative phytochemicals screening test.

Phytochemical test	Extracts of *C. album*	Extracts of *C. giganteum*	Extracts of *C. murale*
Tannins	+	+	+
Alkaloids	+	+	+
Saponins	+	+	+

Here," +" indicates presence of the phytochemical

"- "indicates absences of the phytochemical.

4.4 Total Phenolic and total Flavonoid content

The methanolic extract of *C. album, C. giganteum* and *C. murale* leaves samples were subjected to measure total phenolic and flavonoid content. For the determination of total phenolic content, gallic acid was used as a reference. Quercetin was used as reference standard for the quantitative estimation of Flavonoid. Table 4.4 showed the total phenolic and flavonoid content of *C. album, C. giganteum* and *C. murale. C. murale* had the highest total phenolic content (TPC) 94.10±2.17 (mg GAE/100 g) and also had the highest flavonoid content (TFC) 78.19±2.17 (mg QE/100g).

Table 4.4 Total phenolic and total flavonoid content

Species name	**Total Phenolic Content (TPC) (mg GAE/100g)**	**Total Flavonoid Content (TFC) (mg QE/100g)**
C. album	85.48 ± 2.35^{b}	56.66 ± 0.58^{c}
C. giganteum	88.19 ± 1.33^{b}	73.57 ± 2.35^{b}
C. murale	94.10 ± 2.17^{a}	78.19 ± 2.17^{a}

Values are means ± standard deviations of triplicate determination. Values in the same column having the same super script letters are not significantly different ($p < 0.05$), Values in the same column having the different super script letters are differ significantly ($p < 0.05$)

4.5 In vitro antioxidant activity

(DPPH free radical scavenging assay)

DPPH free radical scavenging activity of methanolic extracts of *C. album, C. giganteum* and *C. murale* are given in the (table 4.5). The in vitro antioxidant activity (DPPH free radical scavenging) of *C. album, C. giganteum* and *C. murale* found 76.99 %, 68.24% and 79.45% respectively whereas standard ascorbic acid show 90.44% antioxidant activity. Figure 4.1 represents the % inhibition of the DPPH free radical in different concentrations of leaf extracts. The half inhibitory concentration (IC_{50}) was calculated from the (figure 4.1). The IC_{50} value for ascorbic acid was 9.56µg/ml. The IC_{50} value of *C. album, C. giganteum* and *C. murale* were 14.25 µg/ml, 17.31 µg/ml and 13.86 µg/ml respectively (Table 4.5). Ascorbic acid is regarded as a standard antioxidant. In comparison to ascorbic acid, all three samples possess antioxidant activity.

Table 4.5: Antioxidant activity (DPPH free radical scavenging activity)

Serial No	Concentration µg/ml	%inhibition of Ascorbic acid	% inhibition of *C. album*	% inhibition of *C. giganteum*	% inhibition of *C. murale*
01	2	27.80	16.75	13.91	18.13
02	4	36.78	27.32	30.67	31.33
03	8	54.05	49.17	41.19	47.24
04	16	69.17	62.44	56.47	60.17
05	32	90.44	76.99	68.24	79.45
IC_{50}(µg/ml)		9.56	14.25	17.31	13.86

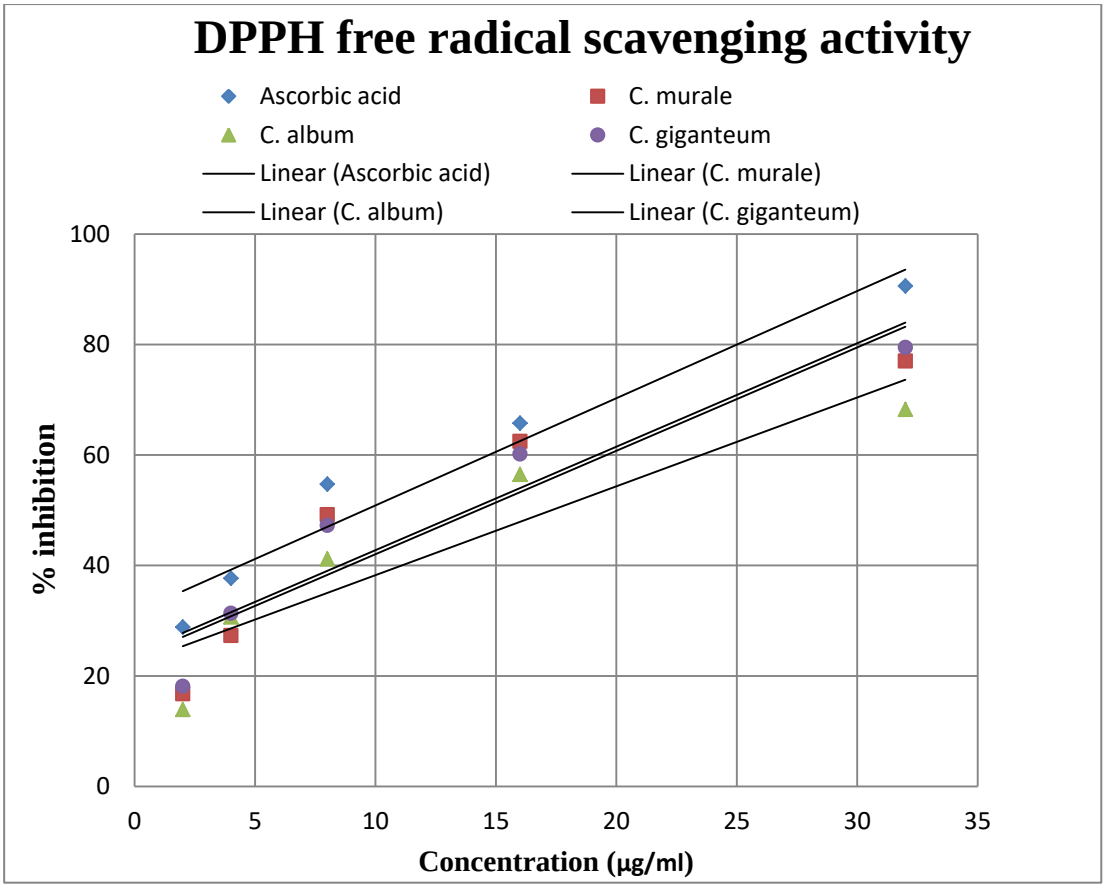

Figure 4.1: DPPH radical scavenging activity (% inhibition vs. concentration graph for standard and samples)

4.6 In vitro antidiabetic effect

(α-amylase enzyme inhibition assay)

α-amylase enzyme inhibition activity of methanolic extracts of *C. album*, *C. giganteum* and *C. murale* are given in the (Table 4.6). The in vitro antidiabetic effect (α-amylase enzyme inhibition) of *C. album, C. giganteum* and *C. murale* found 59.98 %, 67.32% and 61.94% respectively whereas standard acarbose show 90.06 % antidiabetic effect. Figure 4.2 represents the % inhibition of the α-amylase enzyme in different concentrations of the leaf extracts. The half inhibitory concentration (IC_{50}) was calculated from the (figure 4.2). The half inhibitory concentration (IC_{50}) value for enzyme inhibition by standard acarbose was 22.84 µg/ml. The IC_{50} value of *C. album*, *C. giganteum* and *C. murale* was 80.93 µg/ml, 62.38 µg/ml and 76.84 µg/ml respectively (Table 4.6). Acarbose is regarded as a standard enzyme inhibitor. In comparison to acarbose, all three samples possess an antidiabetic effect by inhibiting the enzyme α-amylase which breaks down long-chain carbohydrates.

Table 4.6: Antidiabetic effect (α- amylase enzyme inhibition assay)

Serial No	Concentration µg/ml	%inhibition of Acarbose	% inhibition of *C. album*	% inhibition of *C. giganteum*	% inhibition of *C. murale*
01	20	50.31	9.78	31.98	18.11
02	40	55.12	15.19	42.67	26.13
03	60	74.12	32.11	48.67	39.02
04	80	87.20	55.64	54.12	43.06
05	100	90.06	59.98	67.32	61.94
IC_{50}(µg/ml)		22.84	80.93	62.38	76.84

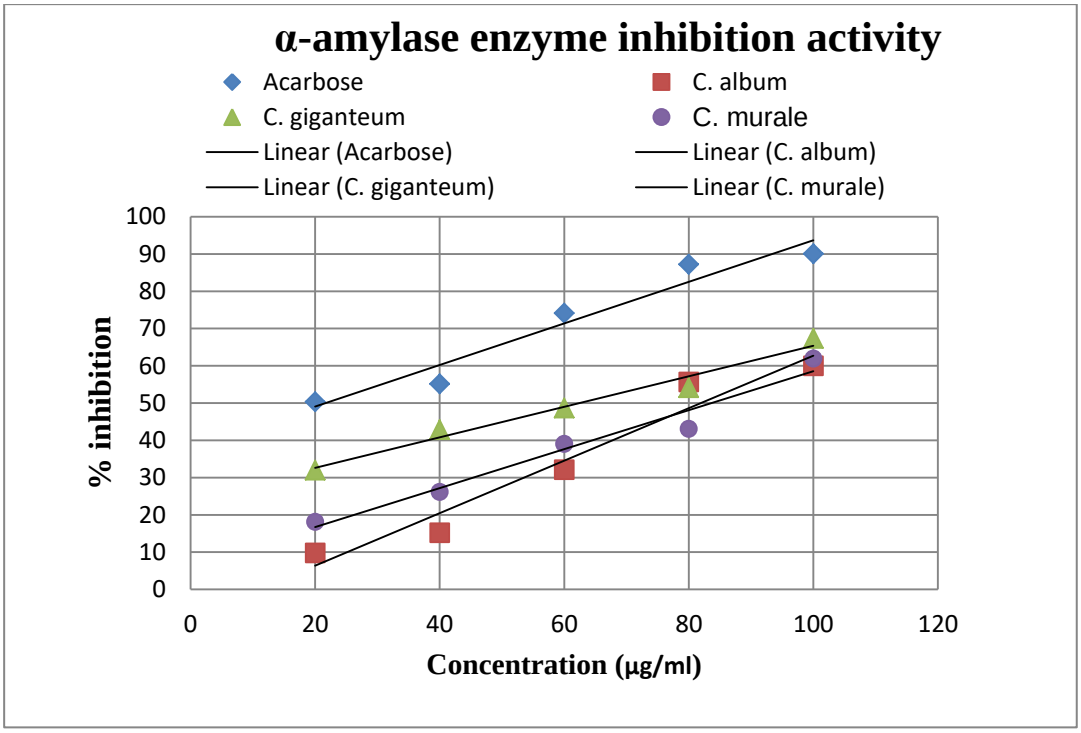

Figure 4.2: α- amylase enzyme inhibition activity (% inhibition vs. concentration graph for standard and samples)

Chapter V: Discussions

Nutritional composition analysis of three *Chenopodium* species was carried out and has been given in Table 4.1 and 4.2.

5.1 Nutritional Composition

5.1.1 Proximate Analysis

The moisture content of the fresh samples ranged from 78.03±0.19% to 83.26±0.27% which is similar to the results reported for some leafy vegetables (Agbaire, 2011; Adepoju and Oyewole, 2008). The result of this study is consistent with the study of Udo et al. (2013) who reported that leafy vegetables have a moisture content ranging from 72 to 93%. The high moisture content of vegetables indicates freshness and perishability, as well as indicating that they may play a key role in aiding the digestion of food (Adepoju and Oyewole, 2008). All the tested Green leafy vegetables have almost the same amount of fat content. The values observed in this study show similarity with the values obtained by Satter et al. (2016) for the fat content of some wild leafy vegetables consumed in Bangladesh. This is in agreement with the general observation that leafy vegetables contain low fat and plays a significant role in avoiding obesity (Nisha et al., 2012).

The ash content is usually recognized as a measure of quality for the assessment of the functional properties of foods (Hofman et al., 2002). The earlier findings showed that *C. album* has an ash content of 21% (Pandey and Gupta, 2014), which is lower than this study. However, the ash content of *C. giganteum* found out similar to the study of Karmakar et al. (2013) who reported that Goose foot leaves have an ash content of 22.60 ± 0.6 %. Commonly consumed leafy vegetables like Dhekishak (*Dryopteris filix-mas*), Helencha (*Enhydra fluctuans*), Kalmishak (*Ipomoea aquatica*) and Patshak (*Corchorus capsularis*) have an ash content of 13.26 ± 0.43%, 12.46 ± 0.55%, 9.11 ± 0.13% and 13.06 ± 0.13% respectively (Satter et al., 2016). This indicates that the studied leaves have high mineral content compared to the previous findings of other common leafy vegetables consumed in Bangladesh.

The earlier findings showed that *C. album* has protein content of 32.2±0.02% (Gqaza et al., 2013), which is slightly lower than this study. The results are much higher than some common leafy vegetables like Dhekishak , Helencha , Kalmishak and Patshak which have the protein content of 20.76 ± 0.59, 16.69 ± 0.36, 21.45 ± 0.32 and 21.98 ±

0.45% respectively (Satter et al., 2016). As compared to other leafy vegetables, these studied leaves have higher crude protein. So these investigated plants consider as a valuable source of protein and highly recommended for the mitigation of "Protein Energy Malnutrition".

Crude fiber provides many kinds of health benefits. It has a beneficial influence on the improvement of gastrointestinal function and helps in the prevention of metabolic diseases. By absorbing water it also works for the elimination of constipation (Chaturvedi et al., 2013). All studied leafy vegetables have moderate dietary fiber content and these values are slightly lower than the leafy vegetables *Enhydra fluctuans* (11.95 ± 0.18%) and *Corchorus capsularis* (10.06 ± 0.13%) (Satter et al., 2016). Total carbohydrate was most abundant (36.56±0.83 %) in *C. murale* compared to *C. album* which had the least total carbohydrate content (19.96±1.00%). Compared with other leafy vegetables reported by Satter et al. (2016) the carbohydrate content of the vegetables in this study is relatively low. The results from this study implied that the vegetables may be poor sources of dietary carbohydrates and so may not be recommended to vegetarians as the sole source of dietary carbohydrate and energy.

5.1.2 Mineral Content

The essential minerals Na, K, Ca, Mg , P and trace minerals Fe, Zn and Cu of the leafy vegetables on a dry weight basis are shown in Table 4.2.

Na and K are the most important minerals present in leafy vegetables. The earlier findings showed that leaves of *C. album* have a Na and K content of 68.0±3.6 and 4902.86 ±59 mg/100gm (Gqaza et al., 2013) which is lower than this present study. The ratio of Na/K in any food item is an important factor; too much Na and less K consumption contribute high prevalence of hypertension (Saupi et al., 2009; Tanase et al., 2011). The Na/K ratio in our body is very important to control high blood pressure and the ratio should be less than one (Akubugwo et al., 2007). In this study, all the leafy vegetables shown in Table 4.2 have the Na/K ration less than one, that indicates the consumption of these vegetables are helpful for human and might be able to control the high blood pressure of our body.

The Ca is an important macro-nutrient for the growth and maintenance of teeth, bone, muscle and heart function (Akubugwo et al., 2007). The results showed in (Table 4.2) from this present investigation are lower than the traditional leafy vegetables like

Amaranthus dubius and *Amaranthus spinosus* which contain 1686 mg/100gm and 3931 mg/100gm calcium respectively (Odhav et al., 2007) but higher for the wild green leafy vegetables traditionally consumed in Bangladesh (279.16 ± 1.33 - 909.13 ± 0.78) mg/100gm calcium (Satter et al., 2016). These data suggest that the studied leafy vegetables are used as a good source of dietary calcium. Phosphorus, an essential mineral, is a component of bones, teeth, DNA, RNA and the body's key energy source, ATP (Heaney, 2012). The Phosphorus content of the samples ranged from 460.03±0.22 mg/100gm to 1230.16±0.29 mg/100gm to which is higher than the result reported by Pandy and Gupta (2014) for *C. album.*

Mg is important for the synthesis of protein, RNA and DNA. This mineral acts as a cofactor of many enzymes and provides significant impact on the metabolism of Ca (Food and Agriculture Organization, 2002). All the three studied leafy vegetables have a great amount of Mg content and these values are comparatively much higher than the common leafy vegetables consumed in Bangladesh (57.38 ± 1.16 - 315.21 ± 1.24) mg/100gm (Satter et al., 2016). It may be concluded from the results that these leafy vegetables would be a rich source of dietary Mg.

Fe is important in the diet for the formation of hemoglobin, functioning of the central nervous system and in the metabolism of carbohydrates, proteins and fats (Kaya and Incekara, 2000; Gupta, 2014). The results obtained in this present investigation indicate similarity with the earlier findings showed that the iron content of 28 leafy vegetables consumed in Bangladesh varies from 11.78 to 78.24 mg/100gm of dry vegetable powder sample (Karmakar et al., 2013). This result provides an indication of the utility of leafy vegetables as a source of iron in our daily diet.

Zn is an essential mineral that plays catalytic, structural and regulatory roles as an integral part of many enzymes in the human body. It is essential for normal growth, mental ability, immune system, reproduction and healthy function of the heart (Afolayan and Jimoh, 2009; Deshpande et al., 2013). The WHO permissible limit of Zn is 6 mg/100gm. In this present research Zn content of three *Chenopodium* species shown in (Table 4.2) similar to earlier findings showed that *Chenopodium* species have Zn content of 2.62 and 4.86 mg/100gm respectively (Gqaza et al., 2013; Pandy and Gupta, 2014).

Cu is an important trace mineral for health, assisting in the formation of hemoglobin and takes part in many different enzyme activities (Osredkar and Sustar, 2011). In some cases, it may be toxic when its concentration exceeds the safe limit (Ogwok et al., 2014). The Cu levels in this study were observed between (1.65±0.18) mg/100gm to (2.89±0.10) mg/100gm which is below the WHO permissible limit (4 mg/100gm) in foods. The Cu levels in the leafy vegetables presented in this study (Table 4.2) exhibited with the levels reported in some wild leafy vegetables consumed in Bangladesh (Satter et al., 2016)

5.2 Phytochemical Compounds

The presence or absence of different phytochemicals, namely, tannins, saponins, and alkaloids was detected by the phytochemical screening methods with different chemical reagents. The presence of those chemical constituents in these plants is an indication that these plants if properly screened could provide medication of pharmaceutical significance. This is better supported by the fact that members of the family of these plants have been known to be involved in ethnomedicine in the management of various diseases (Sharma and Paliwal, 2013a; Sharma and Paliwal, 2013b). In the previous studies, it is reported that the presence of alkaloids, tannins and saponins in *C. album* leaves extracts (Paliwal, 2015). The presence of phytochemicals in this study correlates with the above reports for all three species.

To our knowledge, there are little or no available data in the literature about the TPC and TFC of the selected plants; thus, only a few papers could be found related to the TPC and TFC of *C. album* only. Compared to an earlier study by Thummakomma and Prashanthi (2019) using the most common leafy vegetables like mint, coriander, there are slightly lower TPC and TFC values in the studied leafy vegetables of the Chenopodiaceae family. On the other hand, phenol & flavonoid content observed from this present investigation was higher than a previous report for *C. album* (Poonia and Upadhayay, 2015). The results from the present study also found higher TPC in selected leafy vegetables than some plants, which are known as traditional medicinal plants in the Indian subcontinent (Sulaiman and Balachandran, 2012). However, it is well recognized that several factors, such as species, plant tissue, temperature, water stress and light conditions, as well as phonological development, can influence the TPC and TFC in the plants (Alam et al., 2016; Mendoza-Wilson et al., 2016). Thus, this

explains the large differences observed between the present study and previous findings.

5.3 Antioxidant Activity

The model method of scavenging the stable DPPH radical is usually used to analyze the radical scavenging ability of various samples in terms of antioxidant activity (Koleva et al., 2002). The present study suggests that the extracts of *C. album, C. giganteum* and *C. murale* leaves have potential antioxidant activity with the IC_{50} values of 14.25 µg/ml, 17.31 µg/ml and 13.86 µg/ml respectively, where the value of standard ascorbic acid 9.56µg/ml. The highest DPPH inhibition (79.45% inhibition) was observed in the *C. murale* leaves while the lowest inhibition (68.24% inhibition) was observed in *C. giganteum*. According to Kaur and Kapoor (2002) antioxidant activity of *C. album* found 60.5% which is lower than the antioxidant activity (76.99%) of *C. album* found in this present study. A previous study reported that *C. album* show 87.87% antioxidant activity which is higher than the findings of the present study (Kumar et al., 2015). The antioxidant activity of some leafy vegetables of Bangladesh ranged from (55.15%-86.65%) (Rana et al., 2019) . The results of this present study show consistency with the previous study for some leafy vegetables.

5.4 Antidiabetic effect

The potential of the plant extracts to provide antidiabetic properties was analysed through α-amylase enzyme inhibition assay. According to this present investigation, the extracts of *C. album, C. giganteum* and *C. murale* leaves have potential antidiabetic effect with the IC_{50} values of 80.93 µg/ml, 62.38 µg/ml and 76.84 µg/ml respectively, where the value of standard acarbose 22.84 µg/ml. The result revealed that the *C. giganteum* leaf extract showed the highest inhibitory activity against α-amylase (67.32% inhibition) whereas the leaf extracts of *C. murale* and *C. album* inhibited α-amylase by 61.94% and 59.98%. The previous findings by Odhav et al. (2010) finds out 54.14% α-amylase inhibitory effect of *C. album*, which is lower than this study. Another investigation by Kumar et al. (2015) showed 63.74% antidiabetic effect by α-amylase inhibition assay which is much higher than this study for *C. album* but lower than *C. giganteum* and *C. murale.* The result from the present study show resemblance with the study by Rana et al. (2019) where some Bangladeshi leafy vegetables were used for α-amylase enzyme inhibition % investigation and the enzyme inhibition was

found (56.16-70.95)%. The result of this present study is also comparable to previous study findings using other Bangladeshi and Indian plants ((Uddin et al., 2014: Rao and Mohan, 2017). Inhibiting or limiting the activity of α-amylase is one of the approaches in the prevention and/or management of type-2 diabetes. The inhibition of α-amylase delays carbohydrate absorption after food ingestion and thereby decreases the rate of glucose production and eventually lowers blood glucose levels (Hanhineva et al., 2010). Hence, the leaves of wild plants used in this study could be utilized as functional food ingredients for regulating and maintaining carbohydrate metabolism and postprandial hyperglycemia.

Chapter VI: Conclusion

Bangladesh is very fertile land for the growth of various leafy vegetables without commercial cultivation. 3 species of traditionally consumed leafy vegetables were analysed for proximate composition, mineral composition, qualitative and quantitative screening of phytochemicals, in vitro antioxidant and antidiabetic effect. This study indicates that the leaves can be considered as an alternative food source containing macro and micronutrients necessary for the human body. These results reveal that leaves from locally grown plants of leafy vegetables are valuable sources of health benefiting nutrients, hence they can be considered for use in food products formulation with the objectives of adding health benefits to foods such as increased nutraceutical potential. The present research also provides evidence that *C. album*, *C. giganteum* and *C. murale* contain phytochemicals like alkaloids, tannins, saponins, phenolic compounds and flavonoids. These compounds contribute to the potential antioxidant and antidiabetic effect and make them pharmacologically active.

Chapter VII: Recommendations & future perspectives

Now a day's people are more interested in alternative food source and plant-based medicine. These plants grow almost everywhere in Bangladesh without much effort. Considering the nutritional factors this can be a low-cost source of nutrients for people in our country. Naturally grown plants constitute an important and healthy part of the human diet, mainly owing to the presence of macro and micronutrients which play an essential role in human health, also to the presence of phytochemicals which have many medicinal properties like antioxidant and antidiabetic effect. This chapter provides the recommendations and future perspectives of the present study on the basis of the prevalence of nutritional composition, phytochemical compounds, AOA and antidiabetic potential in investigated plants of Bangladesh as follows:

The present study was carried out to determine nutrition content. A similar further research should be carried out to check the nutrition content of the plants from different areas and essential vitamins.

C. album, C. giganteum and *C. murale* leaves can be incorporated into different food products. High protein and mineral content suggest that it has potential food value and could be recommended as a functional food ingredient.

This study identifies some phytochemicals. A further qualitative and quantitative phytochemical analysis is recommended along with many other standard procedures.

Different methods of extraction, the use of different types of solvents for extraction and many distinct standard procedures are recommended to evaluate the antioxidant and antidiabetic activity. Further studies are needed to isolate and identify the active compounds and their mechanism in various disease conditions

However, studies are required in the animal model (in vivo investigation) and subsequently on the human subject to prove the efficiency in curing disease conditions. This will help for the development of new medicine from plant sources and will be safe for the treatment of various diseases.

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Appendix A: Standard Curves

Table: Concentration and absorbance for Standard solution for TPC

Sample ID	Type		Conc.	WL765.0	Wgt.Factor
1	STD1	Standard	1.000	0.763	1.000
2	STD2	Standard	2.000	0.780	1.000
3	STD3	Standard	3.000	0.920	1.000
4	STD4	Standard	4.000	1.007	1.000
5	STD5	Standard	5.000	1.074	1.000
6	STD6	Standard	6.000	1.115	1.000
7	STD7	Standard	7.000	1.230	1.000
8	STD8	Standard	8.000	1.314	1.000

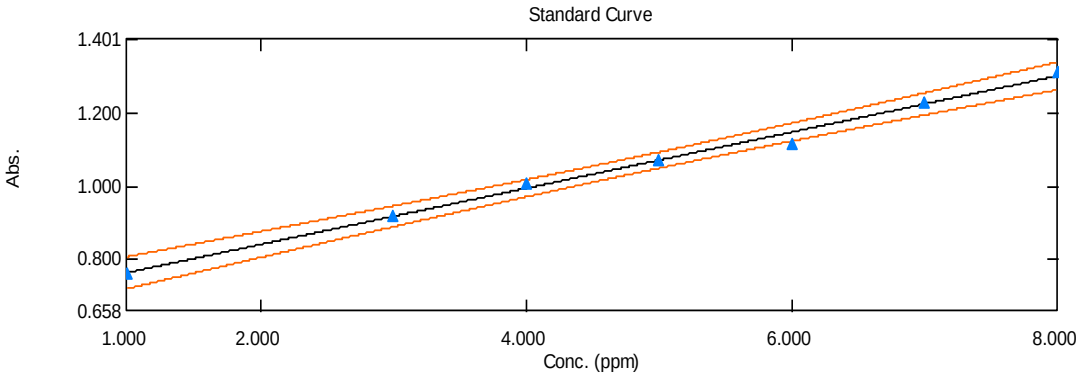


Figure: Standard curve for TPC determination test

Table: Concentration and absorbance for Standard solution for TFC

Sample ID	Type	Ex	Conc(ppm)	WL415.0	Wgt.Factor
1	Std_1	Standard	2.000	0.004	1.00
2	Std_2	Standard	3.000	0.010	1.00
3	Std_3	Standard	4.000	0.014	1.00
4	Std_4	Standard	6.000	0.020	1.00
5	Std_5	Standard	7.000	0.024	1.00
6	Std_6	Standard	8.000	0.029	1.00

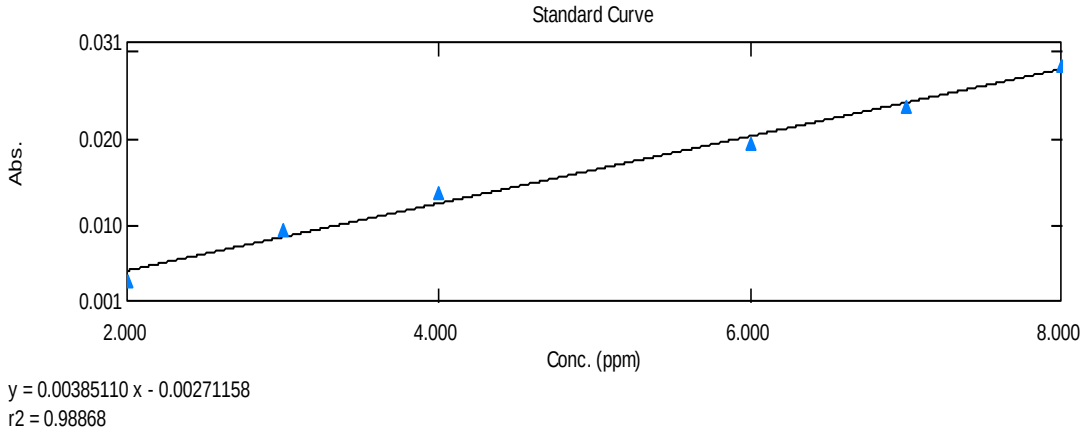


Figure: Standard curve for TFC determination test

Table: Concentration and Absorbance for Standard solution for AOA

Sample ID	Type	Ex	Conc.	WL517.00	Wgt. Factor
1	Std_1	Standard	2.000	0.740	1.000
2	Std_2	Standard	4.000	0.648	1.000
3	Std_3	Standard	8.000	0.471	1.000
4	Std_4	Standard	16.000	0.316	1.000
5	Std_5	Standard	32.000	0.098	1.000

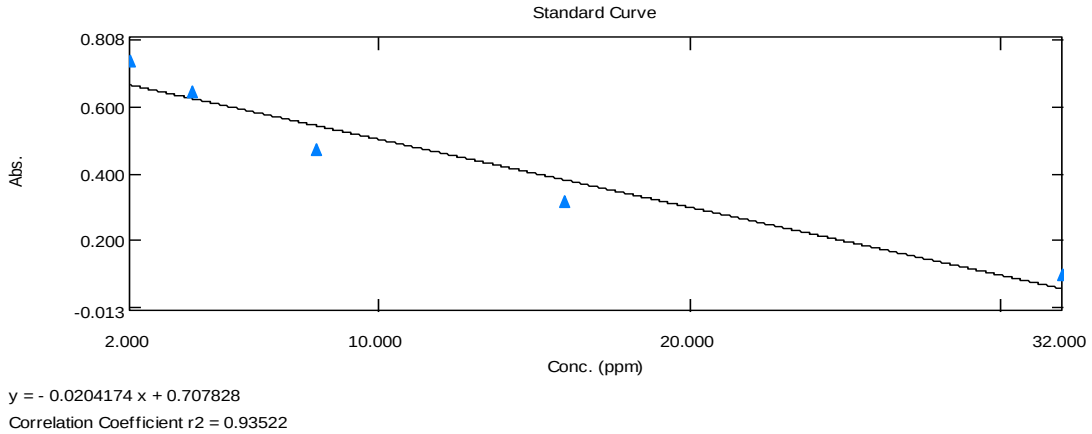


Figure: Standard curve for antioxidant activity determination test

Table: Concentration and Absorbance for Standard solution for Antidiabetic effect

Sample ID	Type	Ex	Conc.	WL540.00	Wgt. Factor
1	Std_1	Standard	20.00	0.468	1.000
2	Std_2	Standard	40.00	0.292	1.000
3	Std_3	Standard	60.00	0.172	1.000
4	Std_4	Standard	80.00	0.121	1.000
5	Std_5	Standard	100.00	0.094	1.000

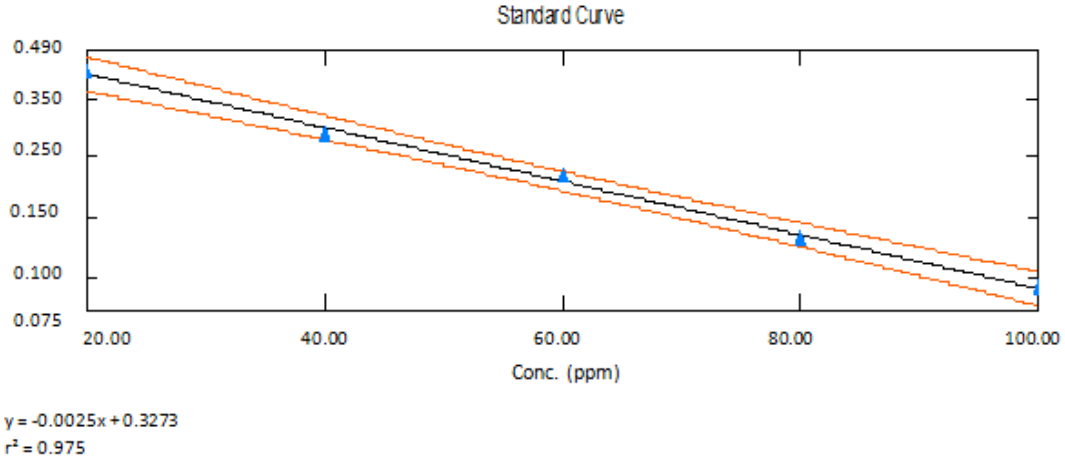


Figure: Standard curve for antidiabetic effect determination test

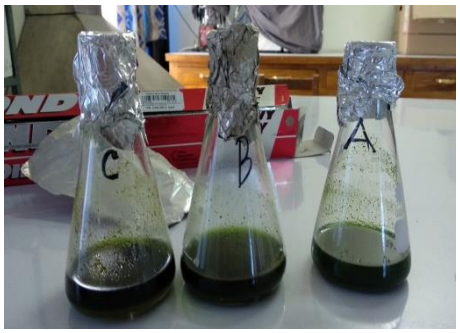
Appendix B: Picture Gallery

Drv leaves

Grinding

Sieving

Weighting



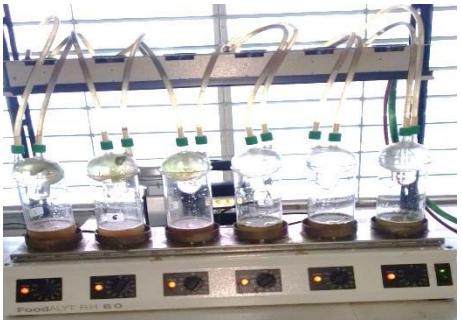
Sample with methanol

Filtration

Ash content determination

Protein analysis

Analysis of fat

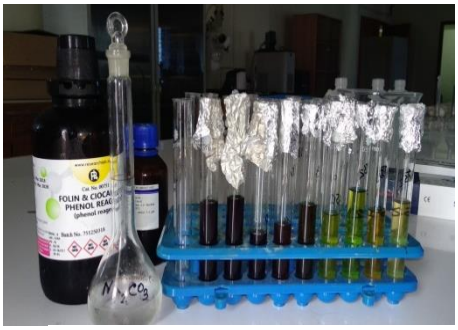


Crude fiber determination

Addition of acid

Digestion of sample in hot plate

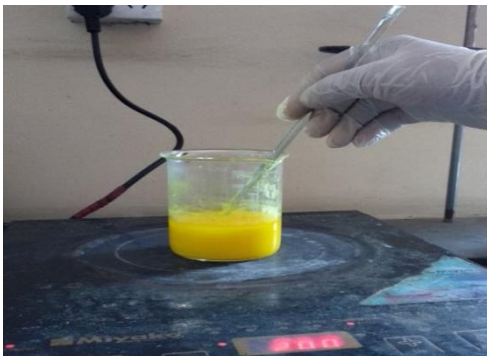
Mineral analysis in Humalyzer



Preparation for Phenolic content determination

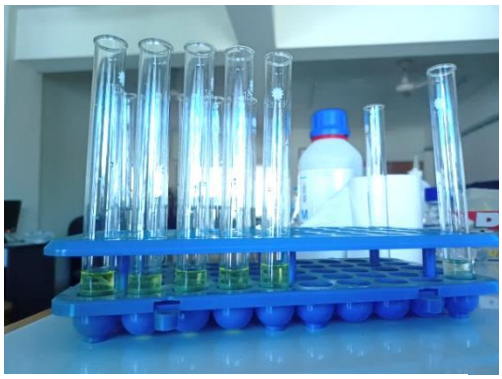
Preparation for Flavonoid Determination

Preparation for DPPH assay



Preparation of 3,5dinitrosalicylic acid (DNS)

Reagents for antidiabetic effect by (α-amylase inhibition assay)



Preparation for α-amylase inhibition assay

Preparing samples for Spectrophotometric analysis

Placing sample into UV-visible spectophotometer

Data analysis of UV-Visible spectrophotometer

Brief Biography

Salma Khatun passed the Secondary School Certificate Examination in 2010 and then Higher Secondary Certificate Examination in 2012. She obtained her B.Sc. (Hons.) in Food Science & Technology in 2018 from Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, she is a candidate for the degree of MS in Department of food Science and nutrition in Applied Human Nutrition and Dietetics under the faculty of Food Science & Technology, CVASU. She has immense interest to work in different food issues including nutrition, food chemistry, quality assurance, food quality control, environmental chemistry, product development and processing, malnutrition, reduction of nutritional changes in food etc.