Comparison of HPLC Metabolite Profile and Antioxidant Activity of Aqueous Extract of *Ginkgo Biloba* from Two Commercial Products Available in Bangladesh Market

By

Sarin Ahmed
15346038

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Bachelor of Pharmacy (Hons.)

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

Sarin Ahmed

Sarin Ahmed

15346038

Approval

The thesis titled "Comparison of HPLC Metabolite Profile and Antioxidant Activity of Aqueous Extract of *Ginkgo Biloba* from Two Commercial Products Available in Bangladesh Market" submitted by Sarin Ahmed (15346038) of Spring 2020 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on February, 2020.

Examining Committee:

Supervisor:
(Member)

_____

Dr. Raushanara Akter
Associate Professor, Department of Pharmacy
Brac University

Program Coordinator:
(Member)

_____

Dr. Hasina Yasmin
Professor, Department of Pharmacy
Brac University

Departmental Head:
(Chair)

_____

Dr. Eva Rahman Kabir
Professor, Department of Pharmacy
Brac University

Abstract

The availability of several brands of *Ginkgo biloba* in Bangladesh market places the health professionals in a confused situation when selecting the desired quality product for patients. This study was aimed to evaluate the quality by comparing antioxidant activities and HPLC metabolite profile of two brands formulated from *Ginkgo biloba*, purchased from retail pharmacies. These drugs are widely used in blood disorders, COPD symptoms, Alzheimer's disease, Raynaud's disease, peripheral vascular disease. In DPPH free radical scavenging assay, the highest % of inhibition for sample 1 and 2 was 88.022% and 86.892%. At the highest concentration of 1200 µg/mL, sample 1 extract showed higher value in TPC, TFC and TAC test. In HPLC metabolite profile, quercetin and kaempferol amount for sample 1 was also better than sample 2. Based on the study findings, sample 1 is expected to produce more therapeutic effect than sample 2.

Keywords: *Ginkgo biloba*; Antioxidant activity; Metabolite profile; DPPH; TPC; TFC

Dedication

Dedicated to my family to whom I owe my achievements and success

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List of Acronyms

ROS - Reactive Oxygen Species

WHO - World Health Organization

DPPH - 1, 1-diphenyl-2-picryl hydroxyl

ASA - Ascorbic Acid

FCR - Folin-Ciocalteu reagent

GAE - Gallic Acid Extract

TPC -Total Phenol Content

TFC -Total Flavonoid Content

TAC - Total Antioxidant Capacity

HPLC - High Performance Liquid Chromatography

Chapter 1

Introduction

1.1 Perspectives of the Commercially Available Drugs in Bangladesh Market

With a large number of drug industries in Bangladesh, the amounts of generic drug products have increased in number. However, this also raised argument on drug product quality because of the preference on favoring a more established manufacturer brand. This unconscious preference of people for the drugs of renowned manufacturing companies over the less renowned generic drugs may be associated with undesirable high cost. Generic substitution refers to the prescribing of different brand or an unbranded drug containing the same API at similar strength and dosage form (Posner & Griffin, 2011). Drug products of top pharmaceutical companies are known to be better as well as costly. It is a common psychology that drug products manufactured by top pharmaceutical companies are better in comparison with the products manufactured by small scale companies. However, poor people of under-developed and developing countries can hardly afford them in comparison with the products manufactured by the low scale companies (Oishi et al., 2017). Therefore, it has become necessity to determine the parameters of tablets during manufacturing in order to ensure that the product is of quality (Hailu et al., 2013) and to identify counterfeits. As nowadays, drugs can be obtained from more than one sources, there might be a chance of the presence of some superiors along with sub-standard drugs (Oishi et al., 2017). This makes the patients and prescribers conscious about the selection of medicines considering safety, effectiveness as well as economy (Tamader et al., 2016). The drug regulatory authority of the country has the responsibility to ensure the quality of drug, as drugs not meeting the quality criteria may cause serious health complications, drug adverse reactions, may produce drug

resistance in the individual as well as can increase the risk of morbidity and mortality (Binega et al., 2013). To assess the quality, therapeutic efficacy and safety of the drugs available commercially, post market monitoring serves as a confidential tool (Hailu et al., 2013). The existing regulations and product development can be moved rapidly by obtaining information from such monitoring (Chandrasekaran et al., 2011).

In this research, several parameters like HPLC metabolite profile and antioxidant study were evaluated on the aqueous extract of *Ginkgo biloba* from two commercially available products present in Bangladesh market to make quality comparison between them. Till now no such evaluation on the aqueous extract of *Ginkgo biloba* from commercially products available in the local market was carried out before. These facts directed interest to assess the quality of commercially available capsules in the Bangladeshi market where one of them is from a mainstream pharmaceutical company and the other one is from a herbal company.

1.2 Phytochemical Screening of Medicinal Plants

Phytochemical screening carries an important part of the procedure to get bioactive constituents, more specifically secondary metabolites from plant materials. Phytochemical screening is usually carried out on whole plant or sometimes on a particular part of plant to get the desired bioactive compounds (Sulaiman & Balachandran, 2012).The extract of medicinal plants contains several phytochemicals providing powerful solution to many diseases and pathological conditions. These compounds incorporate substances like flavonoids, alkaloids, saponins, glycosides, tannins and phenolic compounds (Gul et al., 2017). Various studies show that the compounds that are isolated from the natural sources are superior to those of the synthetic analogues due to their environmental safety and biodegradability. Hence, isolating phytochemicals and conducting study on them has created opportunities to link between conventional and modern drug compounds by identifying

natural compounds and formulating drugs from them. About 50% of all marketed drugs were derived from natural sources and their derivatives (LIU et al., 2006). In present world, many global researches are being conducted to discover different natural compounds with strong biological activity from distinct parts of medicinal plants to achieve a vast number of therapeutic effects (Archit et al., 2014).

1.3 Free Radicals and Reactive Oxygen Species Contributing in Damage of Organs and Tissues

Free radicals can be defined as any unpaired electron in an atomic orbital where molecular species can exist independently (Liu & Nair, 2010). Reactive oxygen species (ROS) produces due to the molecular oxygen reduction through exposure of high energy or reaction regarding electron transfer (Sailaja Rao et al., 2011). Free endogenous radicals act within the cell and later released into the surrounding area. If free radicals are exposed for a long period of time, it can have thoughtful effect on tissue damage, ultimately diseases generation.

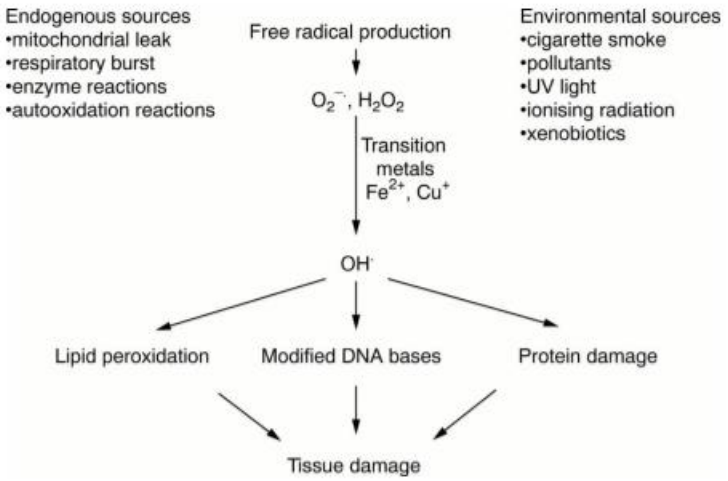

Figure 1: Tissue damage by free radicals

Reactive oxygen species (ROS) which is produced from mitochondria, endoplasmic reticulum, peroxisomes, membrane with the help of several enzymes is responsible for the

production of oxidative stress (Ogura & Shimosawa, 2014). It is highlighted in the above figure that reactive oxygen species (ROS) accumulate oxidative stress in various organs and eventually destroys the cells which can lead to being affected with various diseases. In the brain, ROS damage the nerve cells causing neurodegenerative diseases associated with Alzheimer's disease and Parkinson's disease (Sailaja Rao et al., 2011). ROS also damage the gut barrier and ultimately produce inflammatory bowel disease. In addition, beta cells may get damaged by the inhibition of insulin and insulin growth factors leading diabetes. In terms of cancer, normal cell cycles may become damaged due to the imbalance of transforming growth factors in DNA (He et al., 2017).

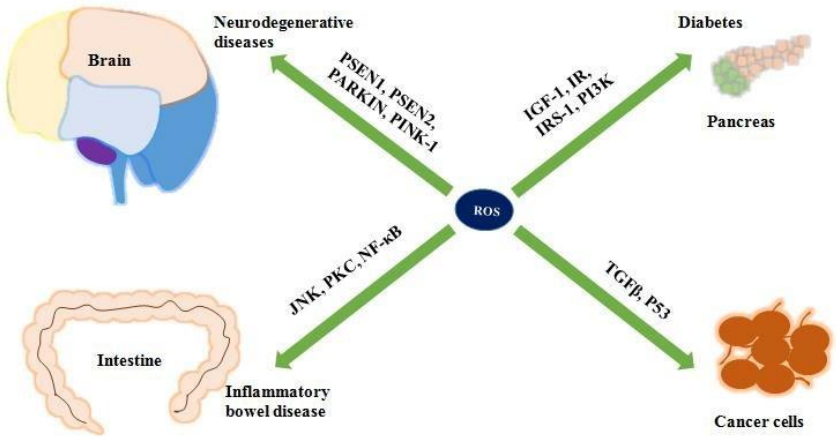

Figure 2: Organs affected by oxidative damage

1.4 Antioxidants Defense System

Antioxidants are defined as the substances that can delay or inhibit the oxidation of the substrate significantly when present at low concentrations compared to that of an oxidizable substrate (Ramful et al., 2011). They guard the cells from the harmful effects of reactive oxygen species that causes oxidative stress which ultimately lead to cell damage (Hamid et al., 2010). They shield the body from destructive molecules that are known as free radicals. Free radicals are produced by oxidation in the body. Oxidation is one type of biochemical

reaction leading to the initiation of extemporaneous chain reaction that causes damage to the cells of human body. Free radicals play a common and probably the most important role in the damage of our body cell which ultimately causes aging problem (Sailaja Rao et al., 2011). They have the potential to damage healthy cells of body by destroying their structure and functions. Antioxidants act as defense system by two types of processes. One process halts the chain reaction by eliminating the free radicals and oxidation reaction while the other process involves donation of electrons to the free radicals in the system (Flora, 2009). That is how antioxidant neutralizes free radicals excess, cytotoxicity, oxidative stress, the availability of oxidants. This lead to the reduction of cell death, organ damage and prevent diseases like early ageing, degenerative diseases, diabetes, cancer etc (Elkhamlichi et al., 2017). Thus, scientists are putting their interest on different natural sources such as plant extracts to obtain natural potential antioxidants.

1.5 *In-vitro* Antioxidant Assay

Radical scavenging activity can be determined by DPPH, TPC, TFC and TAC assay. These methods are most reliable, cheap and accurate method compared to other methods that determine the radical scavenging activity (Alam et al., 2013). On the other hand, FRAP assay is established for assessing "antioxidant power" which is an automated test measuring the ferric reduction capacity (Benzie & Strain, 1996).

1.5.1. Principle of DPPH Free Radical Scavenging Assay

DPPH is quick, simple and cheap in comparison with other test models among free radical scavenging methods (Alam et al., 2013). The reaction between DPPH and antioxidants (H-A) is given below:

DPPH + HA : DPPH-H + HA

In the above reaction, DPPH(1, 1-diphenyl-2-picrylhydrazyl) turns into yellow color from red color by the reaction of substrate. Antioxidants react with an antioxidant and form DPPH-H with the loss of red color. This degree of discolorations designates the scavenging activity of antioxidant compounds or extracts (Brewer, 2011).

1.5.2 Principle of Total Phenolic Content (TPC) Determination

Folin-ciocalteu reagent (FCR) is used in TPC determination to measure the reducing ability of the sample. This compound undergoes reduction reactions that involves transfer of electrons and forms blue colored complex (Wolfe et al., 2003). In the complex, molybdenum is reduced due to electron transfer between the reducing agent and Mo (VI).

$$\mathbf{Mo\ (VI) + e^- : Mo\ (V)}$$

1.5.3 Principle of Total Flavonoid Content (TFC) Determination

An acidic complex is produced between 3 substrates of C-4 keto group with either C-3 or C-5 hydroxyl group of the flavanols and flavones present in the extract sample with aluminium chloride. Moreover, acid labile complexes is formed by $AlCl_3$ with the ortho-dihydroxyl groups in the A or B ring of the flavonoids (Kumaran & Joel Karunakaran, 2007).

1.5.4 Principle of Total Antioxidant Capacity (TAC) Determination

The basis of this test relies on the reduction reaction between Mo(VI) to Mo(V) by the sample extract. A green colored phosphate-molybdenum(V) complex at an acidic pH is formed by the interaction (Prieto et al., 1999).

1.6 High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) formerly referred as high pressure liquid chromatography is a preparative or an analytical technique. The technique is used for

separation, purification and qualitative and quantitative identification of a unique chemical compound from a mixture of compounds. The advantages of this technique includes sensitivity and fast process that allow using smaller particle size (Malviya & Sharma, 2014).

1.6.1 Principle of HPLC Profiling Analysis

HPLC mainly utilizes a column that holds packing material (stationary phase). The instrumentation also includes a pump that can move the mobile phase through the column and a detector that can show the retention times of the molecules. Retention time at which a specific analyte elutes or comes out depends on the interactions between the stationary phase, the molecules being analyzed, and the used solvent types. The sample to be analyzed is placed in a small amount to the stream of mobile phase. Then chemical or physical interactions occur with the stationary phase. The nature of the analyte and composition of both stationary and mobile phase is dependent on the amount of interaction. The gradient separates the mixtures of analyte according to the affinity for mobile phase during analysis. This depends on the variation of mobile phase composition. This is known as gradient elution. A computer connected with the instrumentation helps to analyze the data and show the output in display. When the mobile phase leaves the detector, it can be sent to waste or collected as desired (Malviya & Sharma, 2014).

1.7 Selection of *Ginkgo biloba* for the Study

After going through overview on *Ginkgo biloba,* it was found that *in-vitro* antioxidant potential of the aqueous extract of the plant from marketed samples was never performed before. Thus, it was taken as initiative to determine its antioxidant, phytochemical activity and HPLC profile. The plant *Ginkgo biloba* was found to contain flavonoids like quercetin, kaempferol and isorhamnetin (Belwal et al., 2018). Quercetin which contains flavonol in abundant has potent antioxidant property because of the right molecular structure for showing

potent free radical scavenging activity (Pal et al., 2009). That is why, aqueous extract of the marketed samples containing the plant constituents is selected for the present study.

1.7.1 General Description of *Ginkgo biloba*

Figure 3: Ginkgo biloba plant

- **Scientific name:** *Ginkgo biloba*
- **Common names:** Ginkgo, Maidenhair tree, Baogou, Yin-hsing
- **Family:** Ginkgoaceae
- **Part used:** Leaves and seeds
- **Mean molecular mass:** 756.7 (United & Pharmacopeia, 2018)
- **Chemical constituents:**

1) Terpenetrilactones (TTL) or Terpenoids [5.4-6.6%, approx. 6%]
    a. Ginkgolide A (GKA), B (GKB), C (GKC) and J (GKJ) [2.8-3.4%]
    b. Bilobalide (Bb) [2.6-3.2%]
2) Flavonoids or Flavone glycoside [22-27%, approx. 25%]
    a. Quercetin [dominant, 64.8-81.5%]
    b. Kaempferol [13.3-28.9%]
    c. Isorhamnetin [2-8.4%]
3) Others

a. 6-hydroxy kynurenic acid

b. Ascorbic acid

c. Carotenoids

d. Ginkgolic acid

e. Dimeric flavones (Fermino et al., 2015)

- **Taxonomic hierarchy:**

Domain: Eukarya

Kingdom: Plantae

Phylum: Ginkgophyta

Class: Ginkgoopsida

Order: Ginkgoales

Family: Ginkgoaceae

Genus: *Ginkgo*

Species: *Ginkgo biloba*

1.7.2 Distribution and Habitat of *Ginkgo biloba*

There is only one species of Ginkgo remaining on earth which is named *Ginkgo biloba* around 250million years ago. *Ginkgo biloba* is native to China, also widely cultivated in Japan, Korea, Europe and the southern United States. The ginkgo is a tree that grows to around 30m and has fan-shaped leaves with a unique vein pattern. The leaves turn yellow each autumn before falling off (Miranda, 2011).

Figure 4: Leaves of Ginkgo biloba

1.7.3 Therapeutic Uses of *Ginkgo Biloba*

Ginkgo has a long history in traditional Chinese medicines. Some of the therapeutic indications of the plant are mentioned below (Belwal et al., 2018; Fermino et al., 2015)(Mullaicharam, 2016):

- Improves cognitive function and mild to moderate cerebrovascular insufficiency
- Enhances blood circulation in brain and to the entire body
- As anti-depressant, reduce thrombosis and inhibit platelet aggregation
- Improve concentration and combat short-term memory loss
- Treat blood disorders, COPD symptoms, Alzheimer's disease, Raynaud's disease, peripheral vascular disease and arterial occlusive disease
- Treat dementia, thinking problems, vision problems, vertigo, tinnitus symptoms
- Other potential uses include treating acute cochlear deafness, sexual dysfunction associated with SSRI use and protective action in hypoxia.

1.7.4 Side Effects of *Ginkgo biloba* and Precautions

No significant side effect has been observed following proper administration of designated therapeutic dosage. Large amounts or concentrations may cause gastrointestinal disturbance such as diarrhea, nausea, headache, vomiting, restlessness, weakness and skin irritation.

Moreover, it is better to avoid in case of hypersensitivity and bleeding disorders. Therefore, it is safe to use with caution in taking anticoagulant or antiplatelet agents (Belwal et al., 2018).

1.7.5 Available Commercial Products of *Ginkgo biloba* in Bangladesh Market

The table 1showing the available commercial products of *Ginkgo biloba* in Bangladesh market are given below from where two of them have been selected for this study:

Table 1: Available commercial products of Ginkgo biloba in Bangladesh market

Manufacturer name	Brand name	Dosage form	Dose (mg)	Dosage & administration
Incepta Pharmaceuticals	Premelos	Capsule	60/120 mg	For adults: 120-240 mg/day 1 or 2 capsule 2 to 3 times daily
Square Pharmaceuticals Ltd	Giloba		60/120 mg	
Radiant Nutraceuticals Limited	Ginoba		60 mg	
Drug International Ltd	Biloba		60 mg	
Hamdrad Laboratories (WAQF) Bd	Kobi		60/40 mg	
Purnava Ltd (Renata ltd)	JeeBee		60 mg	

1.8 Rationale of the Study

The rationale of this project is to compare the quality and antioxidant activities of two marketed products formulated from *Ginkgo biloba* extract which are indicated to treat blood disorders, thrombosis, COPD symptoms, Alzheimer's disease, Raynaud's disease, short-term memory loss, dementia tinnitus etc. Among the available brands in Bangladesh market, two

were selected for evaluating the HPLC metabolite profile and antioxidant activity from the aqueous extract of *Ginkgo biloba* where one of them is from a mainstream pharmaceutical manufacturer company and another is from a herbal company.

1.9 Aim of the Study

The aim of the study is to investigate *in-vitro* antioxidant assays and HPLC metabolite profile of *Ginkgo biloba* from aqueous extract of two commercially available drug formulations and make comparison of quality between the both samples.

1.10 Objectives of the Study

The objectives of the project are as follows:

- To carry out phytochemical screening test with the purpose of qualitative determination of the existence of phyto compounds.
- To evaluate and compare the antioxidant properties of the aqueous extract of two commercially available products formulated from the plant *Ginkgo biloba* by determining DPPH free radical scavenging activity, total phenolic content, total flavonoid content and total antioxidant capacity.
- To perform qualitative and quantitative analysis to identify the present constituents by HPLC metabolite profiling.

Chapter 2

Literature Review

2.1 Previous Studies on *Ginkgo biloba*

Literature review of Ginkgo biloba has been carried out to find out the previous studies.

2.1.1 Previously Studied Pharmacological and Antioxidant Studies on *Ginkgo biloba*

Previous studies showed toxicological and positive pharmacological effects of *Ginkgo biloba*. One review study by (Yotova et al., 2018) discusses about the potential health hazards of the plant as carcinogen. Study by (Box, 2013) reported on toxicology and carcinogenesis studies of the plant. Another review focuses on the evidence-based practice of *Ginkgo biloba* on brain health (Mullaicharam, 2016). Moreover, an established monograph talked about the detailed description of this plant (Pae, 1998). Furthermore, biological analysis study had been conducted by (Dumitru & Câmpean, 2016). The properties are extended in the journal by (Belwal et al., 2018) and multifaceted therapeutic benefits are explained by (Reviews & Science, 2008).

The phenolic content and antioxidant capacity of Ginkgo teas had been conducted by (Science, 2016). Another study by (Raafat et al., 2017) discussed about lead ions chelation process optimization after chelation with *Ginkgo Biloba* combined with usage of magnetic treated water as drinking water.

2.1.2 Previous Studies on HPLC Profiling of *Ginkgo biloba*

The previously studied HPLC metabolite profiling studies of *Ginkgo biloba* are given in table 2.

Table 2: Previous studies on HPLC profiling of Ginkgo biloba

Specificity of the studies	**References**
HPLC-UV analysis of flavonoids from *Ginkgo biloba* extracts	(Aqeel et al., 2012)
Simultaneous identification of characteristic components in HPLC- PDA-ELSD fingerprint profile of *Ginkgo biloba* leaves extract	(Wan et al., 2019)
Isolation of flavonoids from *Ginkgo biloba* leaf using the waters prep 150 LC system	(Aubin, 2013)
Analysis of terpenelactones in *Ginkgo biloba* by High performance liquid chromatography and evaporative light scattering detection	(Anzera et al., 2001)
Quantitative analysis of the flavonoid glycosides and terpenetrilactones in the extract of *Ginkgo biloba* and evaluation of their inhibitory activity towards fibril formation of β-Amyloid Peptide	(Xie et al., 2014)
Phytomedicine: An effective identification and quantification method for *Ginkgo biloba*flavonol glycosides with targeted evaluation of adulterated products	(Ma et al., 2016)
Comparative Study on the Pharmacokinetics of *Ginkgo biloba* Extract between Normal and Diabetic Rats by HPLC-DAD	(Tang et al., 2009)
Screening and Identifying Antioxidative Components in *Ginkgo biloba* Pollen by DPPH- HPLC-PAD Coupled with HPLC-ESI-MS 2	(Qiu et al., 2017)

Chapter 3

Methodology

3.1 Collection and Authentication

After numerous plant studies and having reviewed many literatures, it was strongly observed that many individual and combined studies have been conducted on the plant. Many studies have been conducted to investigate the antioxidant activity of methanolic extract of *Ginkgo biloba*. Fortunately, antioxidant activity of aqueous extract has not yet been investigated. It was decided to be selected for *in-vitro* antioxidant activity study of *Ginkgo biloba* with the help of comprehensive literature study of this plant from two selected commercially marketed drug samples in Bangladesh. The two commercially available drug samples were bought from a local authorized pharmacy shop in Mohakhali, Dhaka. General description of these two marketed samples is listed in below table 3.

Table 3: Description of the two commercially available drugs in Bangladesh market

Sample no	Dosage form	Dose (mg)	Dosage and administration	Batch no
Sample 1	Capsule	60	For adults: 120-240 mg/day 1 or 2 capsules (2 to 3 times daily)	8MO2112
Sample 2	Capsule	60		003

3.2 Phytochemical Tests Performed

Preliminary phytochemicals screening was performed following by the methodology (Hossain et al., 2013) and (Gul et al., 2017).

3.2.1 Test for Alkaloids:

a) **Mayer's test:** 1 mL from both extracts was taken in separate test tubes with the addition of Mayer's reagent (Potassium Mercuric Iodide Solution) drop by drop. The deposition of a white color precipitation designates the existence of alkaloids.

b) **Wagner's test:** 1 mL from both extracts was taken in separate test tubes and 1 mL of Wagner's reagent were introduced. Development of a brown or reddish-brown color precipitation confirms existence of alkaloids.

c) **Hager's test:** 1 mL from both extracts were taken in separate test tubes and few drops of Hager's (Saturated picric acid solution) reagent was added which gave bright yellow shaded precipitate and designates the existence of alkaloids.

3.2.2 Test for Glycosides:

a) **Molish's test:** To both extracts, about 2-3 droplets of molish reagent were added further and the mixtures were mixed properly. Now to these mixtures few drops of conc. H_2SO_4 were introduced cautiously. Reddish-purple shaded ring development at the intersection of two layers displays existence of glycosides.

b) **Conc. H_2SO_4 test:** 1 mL of conc. H_2SO_4 was added with 1 mL from both extracts and permitted to standstill for 2 minute which gives a reddish color precipitate specifies the existence of glycosides.

c) **Keller Killiani test:** 1 mL of both extracts were dissolved in 1 mL of glacial acetic acid and further cooled for few minutes. After cooling 2-3 droplets of ferric chloride accompanying with 2 mL of concentrated H_2SO_4 was added. A reddish-brown shaded disc development intersecting the two covers confirms existence of glycosides.

3.2.3 Test for Tannins:

a) **Ferric chloride test:** Both extracts were used to treat separately with couple of drops of $FeCl_3$ solution. Construction of black color precipitation designates existence of tannins.

b) **Lead acetate test:** 1-2 mL of both extracts was treated with basic lead acetate. Construction of bulky red color precipitation designates the existence of tannins.

c) **Alkaline Reagent test:** A mixture of Sodium hydroxide used for treating 1-2 mL of both extracts. Construction of yellow to red color designates existence of tannins.

3.2.4 Test for Flavonoids:

a) **Zinc-HCl reduction test:** In both extracts, a nip of zinc powder accompanying with few droplets of conc. HCl was added. Construction of deep red color designates existence of flavonoids.

b) **Lead-acetate test:** 1-2 mL of both extracts was treated with basic lead acetate. Construction of reddish-brown color precipitation designates existence of flavonoids.

c) **$FeCl_3$ test:** 1-2 mL of both extracts was treated with neutral ferric chloride solution. Construction of blackish red color precipitation designates existence of flavonoids.

3.2.5 Test for Sterols:

a) **Liebermann-Burchard test:** In 1-2 mL of both extracts, a few droplets of solution of acetic anhydride were introduced. Further addition of few droplets of conc. H_2SO_4 along the walls of the test tube was done cautiously. Construction of reddish-brown color ring at the intersection of two layers designates existence of steroids.

b) **Salkowski test:** In 1-2 mL of both extracts, 5 mL of chloroform accompanying with 1 mL of conc. H_2SO_4 was introduced cautiously sideways of the tube with mixing. Construction of reddish color in the bottom layer indicates the presence of steroids.

3.2.6 Test for Coumarin:

a) **Fluorescence test:** 1-2 mL of both extracts was taken in separate tubes to be enclosed with a piece of paper soaked in NaOH and further heated. After heating, these tubes yielding a yellow fluorescence under UV light designates existence of coumarins.
b) **NaOH test:** About 1.5 mL of 10% NaOH was added to 1 mL of both extract solutions. The formation of yellow color indicates the presence of coumarin.

3.2.7 Test for Resins:

In both extracts, 3-4 mL of solution of $CuSO_4$ was introduced with vigorous shaking for 1-2 minutes. The mixture was then kept until separation. Construction of green color precipitation designates existence of resins.

3.2.8 Test for Phenols:

a) **Ellagic acid test:** Couple of droplets of 5% (w/v) glacial acetic acid accompanied with 5% (w/v) mixture of $NaNO_2$ was added in1 mL of both extracts. Construction of muddy brown color designates existence of phenols.
b) **Phenol test:** 2 mL of both extracts were individually treated with 1 mL solution of $FeCl_3$. Construction of an intense color designates existence of phenols.

3.2.9 Test for Saponins:

5 mL of both samples were taken in separate test tubes and shaken vigorously for obtaining stable froth. 5-6 droplets of olive oil were introduced to these frothy solutions. Construction of an emulsion designates existence of saponins.

3.2.10 Test for Terpenoids:

2 mL of chloroform was added with 5 mL of both aqueous extract and evaporated on the water bath and then boiled with 3 mL of conc. H_2SO_4. A grey/brownish color formed which showed the entity of terpenoids.

3.3 *In-vitro* Antioxidant Assay Performed

There are various *in-vitro* methods for estimation of antioxidant activities of plant extracts (Alam et al., 2013). Among all of the various methods, these four methods were selected to estimate the antioxidant activity of the aqueous extract of the selected samples.

- DPPH free radical scavenging assay
- total phenolic content (TPC)
- total flavonoid content (TFC) and
- total antioxidant capacity (TAC)

3.3.1 DPPH (1, 1-diphenyl-2-picryl hydrazyl) Free Radical Scavenging Assay

The DPPH free radical scavenging assay of aqueous extract of *Ginkgo biloba* from two marketed samples was determined using the method described by (Lalhminghlui & Jagetia, 2018).

3.3.1.1 Reagents and chemicals:

The reagents and chemicals for the determination of DPPH scavenging activity are listed below in table 4 with their chemical source.

Table 4: Reagents and chemicals required to determine DPPH scavenging activity

Name of reagent/chemical	Source
DPPH	Sigma Aldrich U.S.A
Methanol	Active Fine Chemicals Ltd, Bangladesh
L Ascorbic acid	Merck, Germany

3.3.1.2 Reagent Preparation:

0.004% (w/v) DPPH solution was prepared by dissolving 2mg of DPPH in 50mL methanol to give purple colored solution and then stored in the refrigerator at -4°C till before use.

3.3.1.3 Sample and Standard Solution Preparation:

Stock solutions: 120mg of extract of sample 1 and sample 2 was individually measured and dissolved in 10mL of distilled water to produce a concentration of 12 mg/mL. This became the sample stock solution 1 and stock solution 2.

Sample solutions: The Concentrations of sample 1 and 2 were prepared by serial dilution of the sample stock solutions to derive 6 serially diluted concentrations: 1200, 800, 400, 200, 100 and 50.

Standard solutions: The standard used was L-ascorbic acid and it was prepared in the same manner as the extract resulting in 6 serially diluted concentrations, ranging from 1200-50 µg/mL.

Blank solution: Blank was also prepared for the investigation by mixing 2 mL of methanol and 1 mL of distilled water.

3.3.1.4 Experimental Procedure:

(a) 1mL of each of the fractions of two samples and standard (L-ascorbic acid) were taken in 3 separate test tubes.

(b) To each of the test tubes, 2mL of 0.004% (w/v) DPPH solution was added.

(c) Then, the test tubes were incubated for 30 minutes at room temperature.

(d) After that, the absorbance of the resulting solutions and control (DPPH and methanol) were measured at 517nm against blank using a spectrophotometer (U-1800 SHIMADZU UV-Vis Spectrophotometer).

(e) The percentage of free radical scavenging activity (% FRS) was then calculated from the equation as follows:

$$\textbf{\% Free radical Scavenging (\%FRS) activity} = (A_0 - A_1)\, A_0 \times 100$$

Where,

A_0 = absorbance of the control

A_1 = absorbance of the sample/standard

(f) Finally, the % Scavenging activity was plotted against concentration from which IC_{50} value was calculated (IC_{50} value is defined as the concentration at which 50% of total DPPH free radical is reduced by the antioxidants which can be determined by plotting % inhibition against the corresponding concentrations).

3.3.2 Determination of Total Phenolic Content (TPC)

The TPC of the plant extract of mixture of sample 1 and 2 was determined by the modified Folin-Ciocalteu method (Chandra et al., 2014).

3.3.2.1 Reagents and Chemicals:

The reagents and chemicals for the determination of total phenolic content is listed below in Table 5.

Table 5: Reagents and chemicals required to determine total phenolic content

Name of reagent/chemical	Source
Folin-Ciocalteu reagent (FCR)	LOBA Chemicals Pvt. Ltd., India
Gallic Acid Monohydrate	Sigma Aldrich, USA
Sodium Carbonate	Merck Specialties Pvt. Ltd., Mumbai
Methanol	Active Fine Chemicals Ltd., Bangladesh

3.3.2.2 Reagent Preparation:

FCR solution: 20mL of 10% Folin-Ciocalteu reagent (FCR) solution was prepared by taking 2mL of FCR in a volumetric flask and diluting it with distilled water.

Na_2CO_3 solution: 100mL of 7.5% (w/v) Sodium carbonate was prepared by measuring out 7.5g of Na_2CO_3 in a 100mL volumetric flask and then diluting it with distilled water to 100mL mark.

3.3.2.3 Sample and Standard Solution Preparation:

Stock solutions: 120mg of two sample extracts were measured and dissolved in 10mL of methanol to produce a concentration of 12mg/mL. This became the sample stock solution.

Sample solutions: The sample concentrations were prepared by serial dilution of the sample stock solution to derive four serially diluted concentrations: 1200, 800, 400 and 200µg/mL.

Standard solutions: Gallic acid was used as the standard and the stock solution was prepared in the same manner as the extract resulting in four serially diluted concentrations, ranging from 1200, 800, 400 and 200µg/mL.

3.3.2.4 Preparation of the Blank:

The blank solution contained the same volume of FCR solution and sodium carbonate was used in the experiment i.e. 5mL and 4mL, respectively, and 1mL of distilled water was used

to make the volume up to 10mL.

3.3.2.5 Experimental Procedure:

(a) 1mL of each of the fractions of two samples and standard (Gallic acid) concentrations were taken in 3 separate test tubes.

(b) To which 2.5mL of FCR solution was added.

(c) 2.5mL of sodium carbonate solution was added.

(d) Then each of the mixtures was vortexed for 15s and then allowed to stand for 30min at 40°C in a water bath.

(e) Finally, the absorbance of standard and sample solutions was measured against blank at 765nm using spectrophotometer (U-1800 SHIMADZU UV-Vis Spectrophotometer).

(f) The total phenolic content, C, for each of the fractions was expressed as Gallic Acid Equivalents (GAE) using the following equation:

$$\mathbf{C= (c \times V)/m}$$

Where,

C = Total content of phenolic compounds, milligram of Gallic acid per gram of dried plant extract, expressed as Gallic Acid Equivalent (GAE)

c = Concentration of Gallic acid obtained from calibration curve (mg/mL)

V = Volume of sample solution (mL)

m = Weight of the sample (g)

The experiment was repeated to confirm reproducibility.

3.3.3 Determination of Total Flavonoid Content (TFC)

The total flavonoid content of the extract of *Ginkgo biloba* from two marketed products was determined according to the following method (Rebaya et al., 2015).

3.3.3.1 Reagents and Chemicals:

The reagents and chemicals for the determination of total flavonoid content are listed below in Table 6.

Table 6: Reagents and chemicals required to determine total flavonoid content

Name of reagent/chemical	Source
Potassium Acetate	Merck KGAA, Germany
Quercetin (standard)	Sigma Lldrich, German
Methanol	Active Fine Chemicals LTD., Bangladesh
Aluminum Chloride	Merck specialties Pvt. Ltd., Mumbai

3.3.3.2 Reagent Preparation:

$AlCl_3$ solution: 100mL of 10% aluminum chloride solution was prepared by measuring 10g of aluminum chloride in a 100mL volumetric flask and diluting it with distilled water to 100mL mark.

$C_2H_3KO_2$ solution: 100mL of 1M potassium acetate solution was prepared by measuring 9.815g of potassium acetate in a 100mL volumetric flask and diluting it with distilled water up to 100mL mark.

3.3.3.3 Sample and Standard Solution Preparation:

Stock solutions: 120mg of both sample extract was measured and dissolved in 10mL of

distilled water to produce a concentration of 12mg/mL. This became the sample stock solution.

Sample solutions: The two sample concentrations were prepared by serial dilution of the sample stock solution to derive 4 serially diluted concentrations: 1200, 800, 400 and 200µg/mL.

Standard solutions: Quercetin was used as the standard and the stock solution was prepared in the same manner as the extract resulting in four serially diluted concentrations, ranging from 1200, 800, 400 and 200µg/mL.

3.4.3.4. Preparation of the Blank:

The blank solution contained 200µL of 10% aluminium chloride solution, 200µL of 1M potassium acetate solution and 9.6mL of distilled water such that the final volume of the solution was 10mL.

3.3.3.5 Experimental Procedure:

(a) 1mL of each of the fractions of two samples and standard (Quercetin) concentrations were taken in test tubes.

(b) To which 3mL of distilled water was added.

(c) And 200µL of 10% aluminium chloride solution and 200µL of 1M potassium acetate solution was added to each of the test tubes using 1000µL micropipette.

(d) Finally, 8.6mL of distilled water was added to the test to make the final volume of the solution 10mL.

(e) The test tubes were then incubated at room temperature for 30min.

(f) Afterwards, the absorbance of each of the sample and standard solutions were measured at

415nm against blank using spectrophotometer (U-1800 SHIMADZU) UV-Vis Spectrophotometer).

(g) The total flavonoid content, C, for each of the fractions were expressed as Quercetin equivalents (QE) using the following equation:

$$C = (c \times V)/m$$

Where,

C = Total content of flavonoid compounds, milligrams of quercetin per gram of dried plant extract, expressed as quercetin equivalents (QE)

c = Concentration of Gallic acid obtained from calibration curve (mg/mL)

V = Volume of sample solution (mL)

m = Weight of the sample (g)

The experiment was repeated to confirm reproducibility.

3.3.4 Determination of Total Antioxidant Capacity (TAC)

The total Antioxidant capacity of the extract of *Ginkgo biloba* from two marketed productswas determined by using the following method (Prieto et al., 1999).

3.3.4.1 Reagents and Chemicals:

The reagents and chemicals for the determination of total antioxidant capacity are listed below in table 7.

Table 7: Reagents and chemicals required to determine total antioxidant capacity

Name of reagent/chemical	Source
Ammonium Molybdate	Active Fine Chemicals Ltd., Bangladesh
L-ascorbic acid	Merck, Germany
Trisodium Phosphate (Na3PO4.12H2O)	Merck, Germany
Concentrated Sulfuric Acid	Merck, Germany
Methanol	Active Fine Chemicals Ltd., Bangladesh

3.3.4.2 Reagent Preparation:

H_2SO_4 solution: 100mL of 0.6M Sulfuric acid was prepared by measuring 3.28mL of 98% concentrated sulfuric acid in a 100mL volumetric flask and diluting it with distilled water to 100mL mark.

$(NH_4)_2MoO_4$ solution: 100mL of 0.004M ammonium molybdate solution was prepared by measuring 4.494g of ammonium molybdate in a 100mL volumetric flask and diluting it with distilled water up to 100mL mark.

$Na_3PO_4.12H_2O$ solution: 100mL of 0.028M trisodium phosphate solution was prepared by measuring 1.0645g of trisodium phosphate in a 100mL volumetric flask and diluting it with distilled water to 100mL mark.

3.3.4.3 Sample and Standard Solution Preparation:

Stock solutions: 120mg of both extracts were measured and dissolved in 10mL of distilled water to produce a concentration of 12mg/mL. This became the sample stock solution.

Sample solutions: The sample concentrations were prepared by serial dilution of the sample stock solution to derive 4 serially diluted concentrations: 1200, 800, 400 and 200µg/mL.

Standard solutions: Ascorbic acid was used as the standard and the stock solution was prepared in the same manner as the extract resulting in four serially diluted concentrations,

ranging from 1200, 800, 400 and 200µg/mL.

3.3.4.4 Preparation of the Blank:

3mL of reagent solution (0.6M sulfuric acid, 0.028M sodium phosphate and 0.004M ammonium molybdate) and 300µL of distilled water was used for preparing the blank solution.

3.3.4.5 Experimental Procedure:

(a) 300µL of each of the fractions of both samples and standard (L-ascorbic acid) concentrations were taken in 3 separate test tubes.

(b) 3mL of prepared reagent solution was added into the test tubes.

(c) The test tubes (sample, standard and blank) were then incubated at 95°C in a water bath for 90min.

(d) Finally, the absorbance of the sample and standard solutions was measured against blank at 695nm using spectrophotometer (U-1800 SHIMADZU UV-Vis Spectrophotometer).

(e) The total antioxidant capacity, A, for each of the fractions was expressed as Ascorbic Acid Equivalents (AAE) using the equation as follows:

$$\mathbf{C = (c \times V)/m}$$

Where,

C = Total antioxidant capacity, milligram of ascorbic acid per gram of dried plant extract, expressed as ascorbic acid equivalent (AAE)

c = Concentration of ascorbic acid obtained from calibration curve (mg/ mL)

V = Volume of sample solution (mL)

m = Weight of the sample (g)

The experiment was repeated to confirm reproducibility.

3.4 High Performance Liquid Chromatography (HPLC) Profiling

High Performance Liquid Chromatography **(**HPLC**)** was performed to quantitatively identify the constituents quercetin and kaempferol following by the methodology (United & Pharmacopeia, 2018).

3.4.1 Materials, reagents and solvents:

The materials, reagents and solvents for the determination of high performance liquid chromatography are listed below in table 8.

Table 8: Materials, reagents and solvents required to determine HPLC profiling

Materials	Reagents	Solvents
Reflux condenser (250 mL)	Phosphoric acid	Methanol
Water bath	Quercetin	Hydrochloric acid (HCl)
Volumetric flask (100 mL)	Kaempferol	Distilled water

3.4.2 Extraction Solvent Preparation:

Extraction solvent was prepared by mixing methanol, hydrochloric acid (HCl) and distilled water in a 100 mL volumetric flask in a ratio of 25:4:10.

3.4.3 Mobile Phase Preparation:

Mobile phase was prepared by mixing methanol, water and phosphoric acid in a 100 mL volumetric flask in a ratio of 100:100:1.

3.4.4 Standard Solution Preparation:

- **Standard solution A:** 0.02 mg of USP Quercetin RS was measured and dissolved in

1 mL methanol.

- **Standard solution B:** 0.02 mg of USP Kaempferol RS was measured and dissolved in 1 mL methanol.

3.4.5 Sample Solution Preparation:

(a) 1.0g of the both finely powdered marketed samples of *Ginkgo biloba* were transferred to a 250 mL reflux condenser and flask fitted.

(b) 78 mL of the extraction solvent (methanol, HCl and water in 25:4:10) was added in the reflux condenser.

(c) The reflux was put on a hot water bath for 35 min until the solution will turn into deep red and allowed to cool at room temperature which was decanted to a 100mL volumetric flask.

(d) Again, 20mL of methanol was added to the 250mL flask and sonicated for 30 minutes.

(e) It was then filtered and the filtrate was collected in the 100 ml volumetric flask.

(f) The residue was washed on the filter with methanol and the washing was collected in the same 100 mL volumetric flask which was later diluted with methanol to volume and mix.

(g) Then, 20µL solution was taken as injection volume to detect chromatography at UV 370 nm at 1.5mL/min flow rate on LC mode through 4.6mm×25cm (packing L1) column. It is noted here that the relative retention times for standard solution A, Quercetin and standard solution B, Kaempferol are about 1.0 and 1.8 respectively.

(h) Finally, the total percentage of each flavone glycoside in the portion of Ginkgo taken from the two samples was then calculated from the equation as follows:

$$\textbf{Result} = (r_u/r_s) \times (Cs/W) \times F \times 10$$

Where,

r_u = Peak area of the relevant analyte from the Sample solution

r_s = Peak area of the relevant analyte from the Standard solution A/ Standard solution B

Cs = Concentration of the relevant analyte in Standard solution A/ Standard solution B

W = Weight of sample taken to prepare the Sample solution.

F = Mean molecular mass factor to convert each analyte into flavone glycoside with a mean molecular mass of 756.7: 2.504 for Quercetin and 2.588 for Kaempferol.

Chapter 4

Result

4.1 Preliminary Phytochemical Screening Result

The result of preliminary phytochemical screening tests of aqueous extracts of *Ginkgo biloba* from two marketed products is listed below in table 9 where (+) sign means presence and (-) sign means absence of compounds in screening tests.

Table 9: Result of phytochemical screening tests

SL No	Phytochemical Tests Name		Sample 1	Sample 2
01.	Test for Alkaloids	Mayer's test	_	_
		Wagner's test	_	_
		Hager's test	_	_
02.	Test for Glycosides	Molisch's test	_	_
		Keller Killiani test	_	_
		Conc. H_2SO_4 test	_	_
03.	Test for Tannins	Ferric chloride test	+	+
		Lead-acetate test	+	+
		Alkaline reagent test	+	+
04.	Test for Flavonoids	Zinc-HCl reduction test	+	+
		Lead-acetate test	+	+
		$FeCl_3$ test	+	+
		Alkaline reagent test	+	+
05.	Test for Sterols	Liebermann-Burchard test	_	_
		Salkowski test	_	_

06.	Test for Coumarin	Fluorescence test	+	+
		NaOH test	+	+
07.	Test for Resins	$CuSO_4$ test	_	_
08.	Test for Phenols	Ellagic acid test	+	+
		Phenol test	+	+
09.	Test for Saponins	Froth test	_	_
10.	Test for Terpenoids	Terpenoid test	+	+

4.2 Evaluation of Antioxidant Activity

4.2.1 DPPH ((1, 1-diphenyl-2-picryl hydroxyl) Free Radical Scavenging Assay

Figure 5: DPPH free radical scavenging assay

- **Standard Ascorbic acid (ASA):**

The concentration to produce 50% reduction of the DPPH (IC_{50}) value by Standard (ASA) depending on the concentration and absorbance values are given below in the table 10.

Table 10: IC_{50} value of DPPH free radical scavenging assay by standard (L-ascorbic acid)

Absorbance of the control	Concentration (µg/mL)	Absorbance of the standard	Average of the absorbance	% of inhibition (%SCV) = (A_0-A_1) A0×100	IC_{50} (µg/mL) x= (y-c)/m
0.885	50	0.620	0.639	27.797	300.504
		0.657			
		0.641			
	100	0.533	0.534	39.661	
		0.494			
		0.575			
	200	0.415	0.420	52.542	
		0.434			
		0.412			
	400	0.347	0.347	60.790	
		0.343			
		0.351			
	800	0.205	0.206	76.723	
		0.211			
		0.202			
	1200	0.135	0.127	85.650	
		0.121			
		0.126			

The percentage of inhibitions that was found from the above table 10 was plotted against concentrations in a calibration graph. Graphical presentation of % of inhibition of DPPH by

Standard (L-Ascorbic acid) is shown below in figure 6.

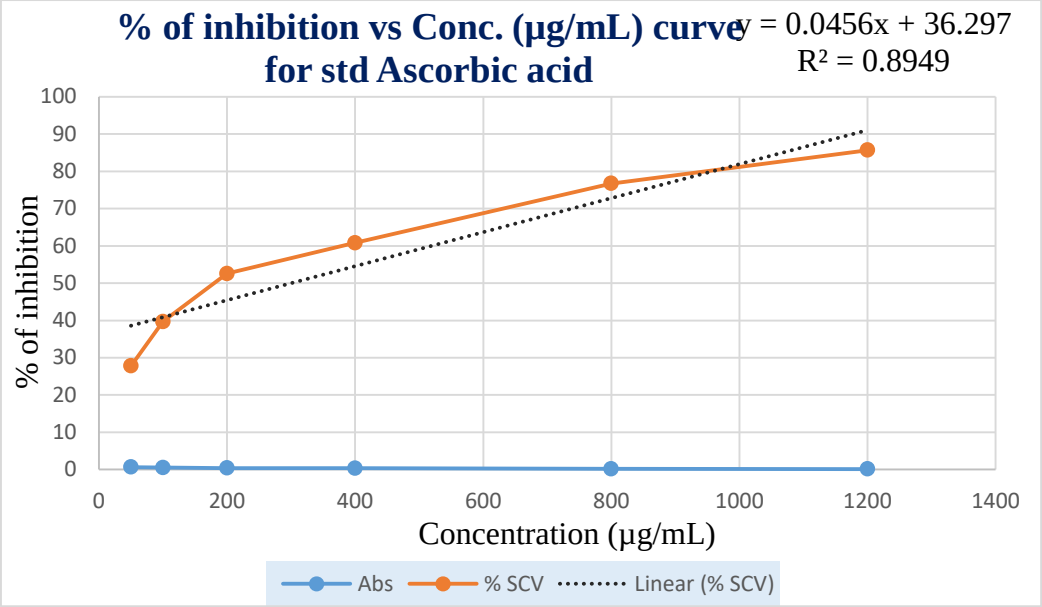

Figure 6: Standard curve of std (ASA) for DPPH free radical scavenging assay

- **Sample 1:**

The concentration to produce 50% reduction of the DPPH (IC_{50}) value by Sample 1 depending on the concentration and absorbance values are given below in the table 11.

Table 11: IC_{50} value of DPPH free radical scavenging assay by sample 1

Absorbance of the control	Concentration (µg/mL)	Absorbance of sample 1	Average of the absorbance	% of inhibition (%SCV) = (Ao-A1)A_o*100	IC_{50} (µg/mL) x= (y-c)/m
	50	0.790	0.760	14.124	
		0.769			
		0.721			
	100	0.707	0.708	20	
		0.712			

0.885		0.705			561.655
	200	0.587	0.569	35.706	
		0.542			
		0.578			
	400	0.467	0.469	47.005	
		0.464			
		0.475			
	800	0.366	0.365	58.757	
		0.349			
		0.381			
	1200	0.079	0.106	88.022	
		0.112			
		0.127			

The percentage of inhibitions that was found from the above table 11 was plotted against concentrations in a calibration graph. Graphical presentation of % of inhibition of DPPH by Sample 1 is shown below in figure 7.

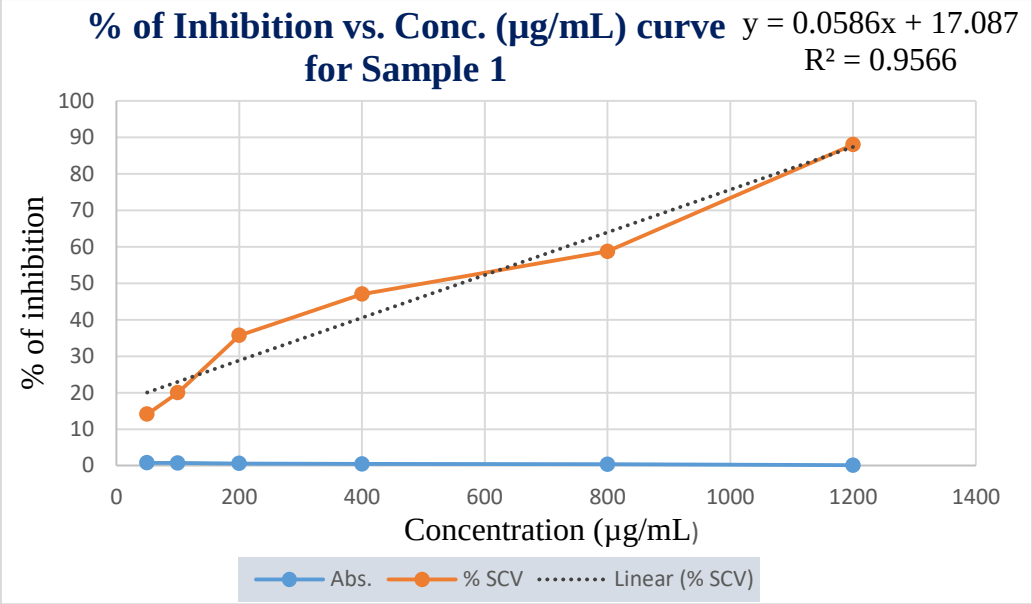

Figure 7: Standard curve of sample 1 for DPPH free radical scavenging assay

- **Sample 2:**

The concentration to produce 50% reduction of the DPPH (IC_{50}) value by Sample 2 depending on the concentration and absorbance values are given below in the table 12.

Table 12: IC_{50} value of DPPH free radical scavenging assay by sample 2

Absorbance of the control	Concentration (µg/mL)	Absorbance of sample 2	Average of the absorbance	% of inhibition (%SCV) = (Ao-A1)A_o*100	IC_{50} (µg/mL) x= (y-c)/m
	50	0.847	0.850	3.954	
		0.851			
		0.852			
	100	0.720	0.749	15.367	
		0.769			
		0.758			
		0.403			

0.885	200	0.390 0.393	0.395	55.367	416.521
	400	0.243 0.249 0.195	0.229	74.124	
	800	0.167 0.171 0.182	0.173	80.451	
	1200	0.122 0.111 0.115	0.116	86.892	

The percentage of inhibitions that was found from the above table 12 was plotted against concentrations in a calibration graph. Graphical presentation of % of inhibition of DPPH by Sample 2 is shown below in figure 8.

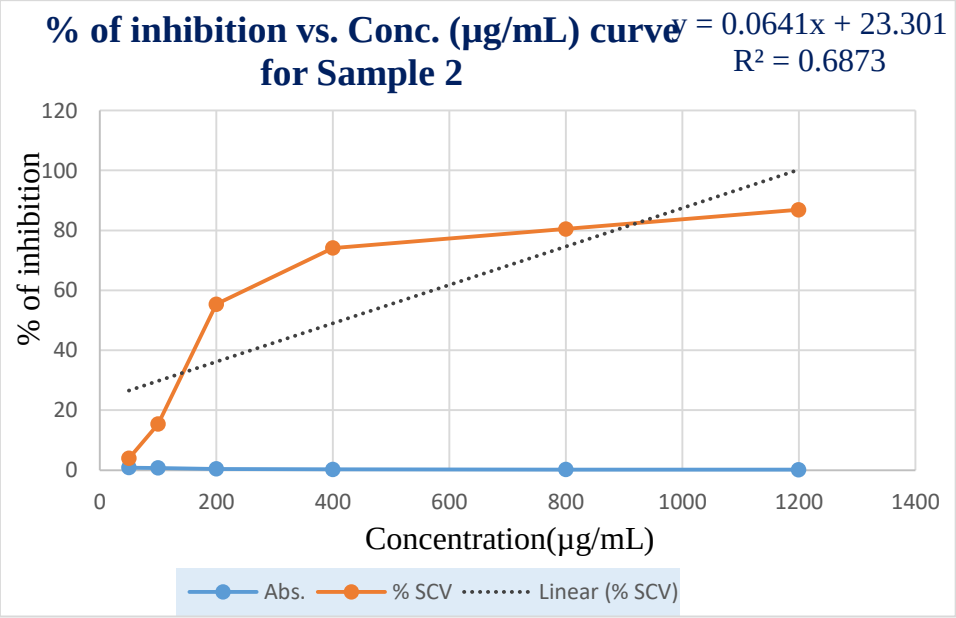

Figure 8: Standard curve of sample 1 for DPPH free radical scavenging assay

Lastly, a comparison data of Absorbance(nm) vs Concentration(µg/mL) value for Standard Ascorbic acid, Sample 1 and Sample 2 in DPPH assay is given below in table 13.

Table 13: Comparison of absorbances for different concentrations in DPPH assay

Concentration(µg/mL)	Abs. of Standard	Abs. of Sample 1	Abs. of Sample 2
50	0.639	0.760	0.850
100	0.534	0.708	0.749
200	0.420	0.569	0.395
400	0.347	0.469	0.229
800	0.206	0.365	0.173
1200	0.127	0.106	0.116

A comparison calibration graph of DPPH: Absorbance vs. Concentration of sample 1, sample 2 and std (ASA) is shown below in figure 9.

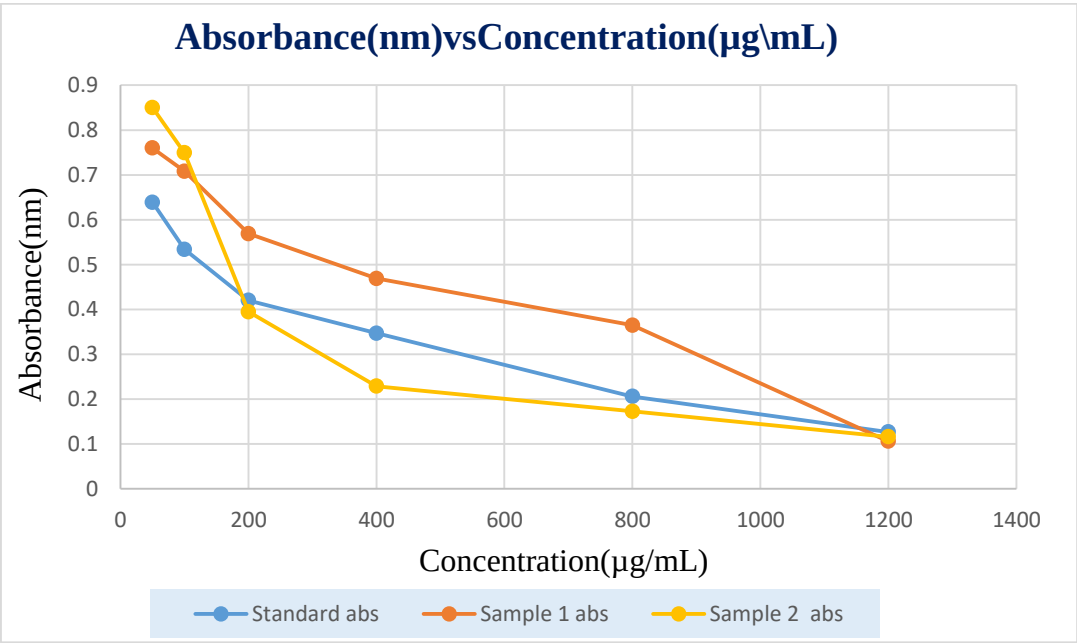

Figure 9: Graph of DPPH: absorbance vs. concentration for sample 1, sample 2 and std (ASA)

4.2.2 Total Phenolic Content (TPC) Determination

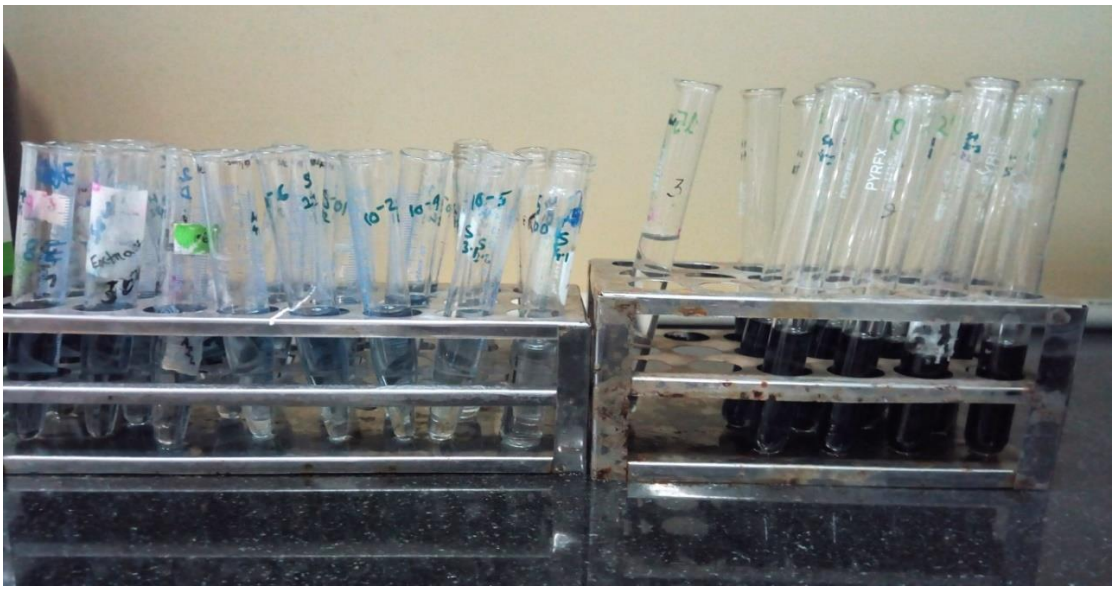

Figure 10: Total Phenolic Content (TPC) test

- **Standard Gallic acid:**

The total content of phenolic compounds, C expressed as Gallic Acid Equivalent (GAE) produced by standard Gallic acid depending on the concentration and absorbance values are given below in the table 14.

Table 14: GAE values of Total Phenolic Content (TPC) test by standard Gallic acid

Conc	Abs	Avg Abs.	Vol. Of sol^{n} in mL (V)	Wt of sample in g/ml (m)	GAE in µg/mL from curve	GAE in mg/ml (c)	TPC as GAE in mg/g (C=((c×V)/m)
200	0.063 0.077 0.066	0.068	1	0.0002	143.5	0.1435	717.5
	0.086						

400	0.100 0.109	0.098	1	0.0004	293.5	0.2935	733.75
800	0.162 0.158 0.151	0.157	1	0.0008	588.5	0.5885	735.625
1200	0.216 0.220 0.219	0.218	1	0.0012	893.5	0.8935	744.583

The concentrations of Gallic acid that was found from the above table 14 was plotted against concentrations in a calibration graph. Graphical presentation of absorbance vs concentration of TPC test by Gallic acid is shown below in figure 11.

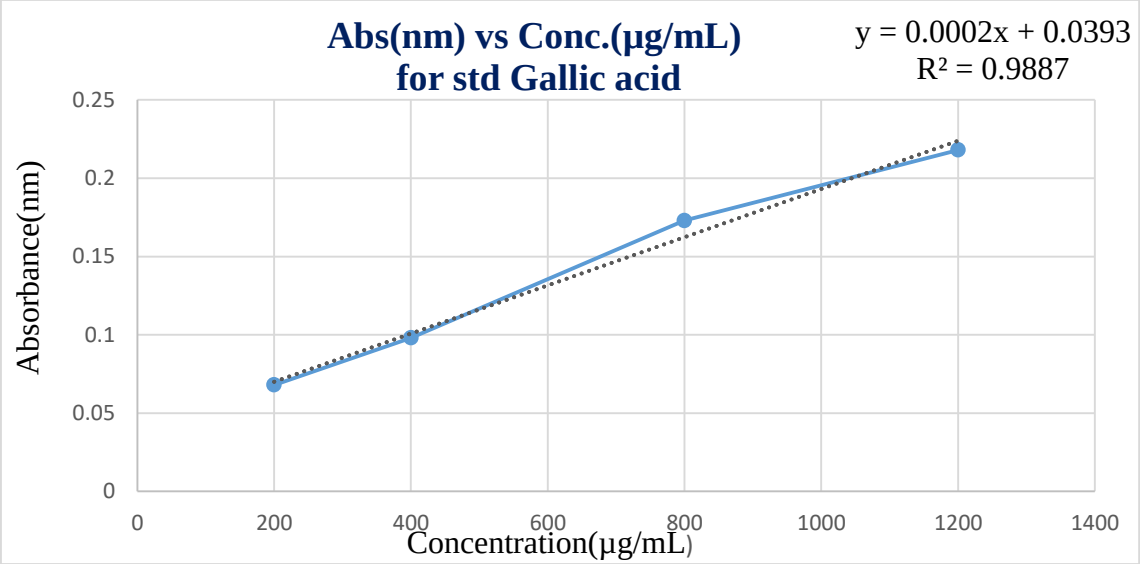

Figure 11: Standard curve of std Gallic acid for Total Phenolic Content (TPC) test

- **Sample 1:**

The total content of phenolic compounds, C expressed as Gallic Acid Equivalent (GAE) produced by Sample 1 depending on the concentration and absorbance values are given in the

table 15.

Table 15: GAE values of Total Phenolic Content (TPC) test by Sample 1

Conc	Abs	Avg Abs.	Vol. Of sol^{n} in mL (V)	Wt of sample in g/ml (m)	GAE in µg/mL from curve	GAE in mg/ml (c)	TPC as GAE in mg/g (C=((c×V)/m)
200	0.307	0.346	1	0.0002	170.8	0.1708	854
	0.369						
	0.362						
400	0.420	0.445	1	0.0004	368.8	0.3688	922
	0.461						
	0.454						
800	0.693	0.664	1	0.0008	806.8	0.8068	1008.5
	0.675						
	0.624						
1200	0.960	0.926	1	0.0012	1330.8	1.3308	1109
	0.898						
	0.921						

The concentrations of Sample 1 that was found from the above table 15 was plotted against concentrations in a calibration graph. Graphical presentation of absorbance vs concentration of TPC test by Sample 1 is shown below in figure 12.

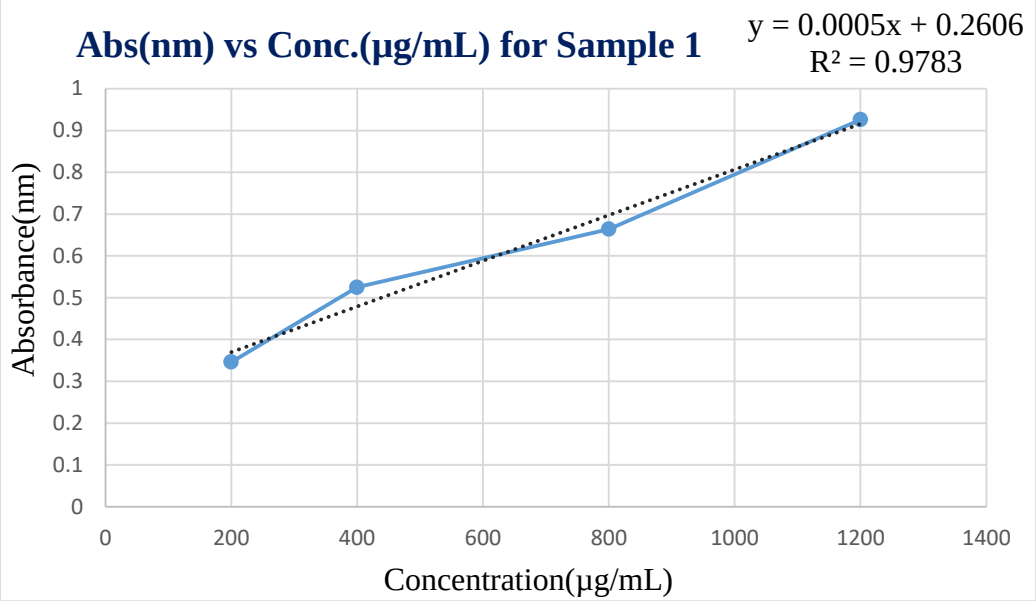

Figure 12: Standard curve of sample 1 for Total Phenolic Content (TPC) test

- **Sample 2:**

The total content of phenolic compounds, C expressed as Gallic Acid Equivalent (GAE) produced by Sample 2 depending on the concentration and absorbance values are given below in the table 16.

Table 16: GAE values of Total Phenolic Content (TPC) test by Sample 2

Conc	Abs	Avg Abs.	Vol. Of sol^{n} in mL (V)	Wt of sample in g/ml (m)	GAE in µg/mL from curve	GAE in mg/ml (c)	TPC as GAE in mg/g (C=((c×V)/m)
200	0.116 0.107 0.101	0.108	1	0.0002	151	0.151	755
400	0.182 0.168	0.176	1	0.0004	321	0.321	802.5

	0.178						
800	0.328 0.329 0.346	0.334	1	0.0008	716	0.716	895
1200	0.488 0.471 0.478	0.479	1	0.0012	1078.5	1.0785	898.75

The concentrations of Sample 2 that was found from the above table 16 was plotted against concentrations in a calibration graph. Graphical presentation of absorbance vs concentration of TPC test by Sample 2 is shown below in figure 13.

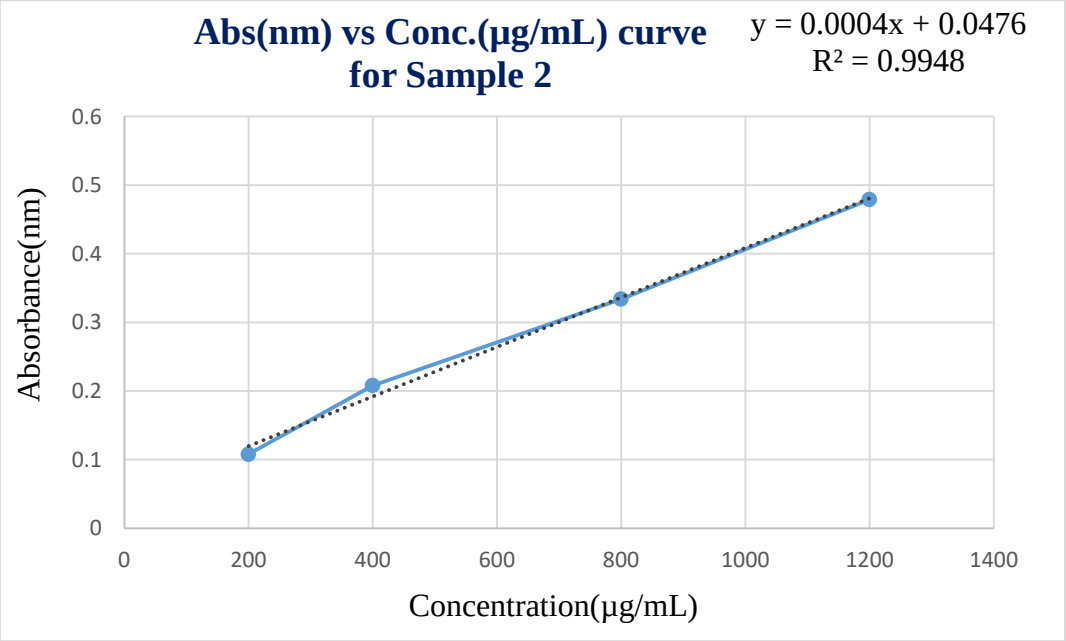

Figure 13: Standard curve of sample 2 for Total Phenolic Content (TPC) test

Lastly, a comparison data of Absorbance(nm) vs Concentration(µg/mL) value for Standard Gallic acid, Sample 1 and Sample 2 in TPC test is given below in table 17.

Table 17: *Comparison of absorbances for different concentrations in TPC test*

Concentration(µg/mL)	Abs. of Standard	Abs. of Sample 1	Abs. of Sample 2
200	0.068	0.346	0.108
400	0.098	0.445	0.176
800	0.157	0.664	0.334
1200	0.218	0.926	0.479

A comparison calibration graph of TPC: Absorbance vs. Concentration of sample 1, sample 2 and std (Gallic acid) is shown below in figure 14.

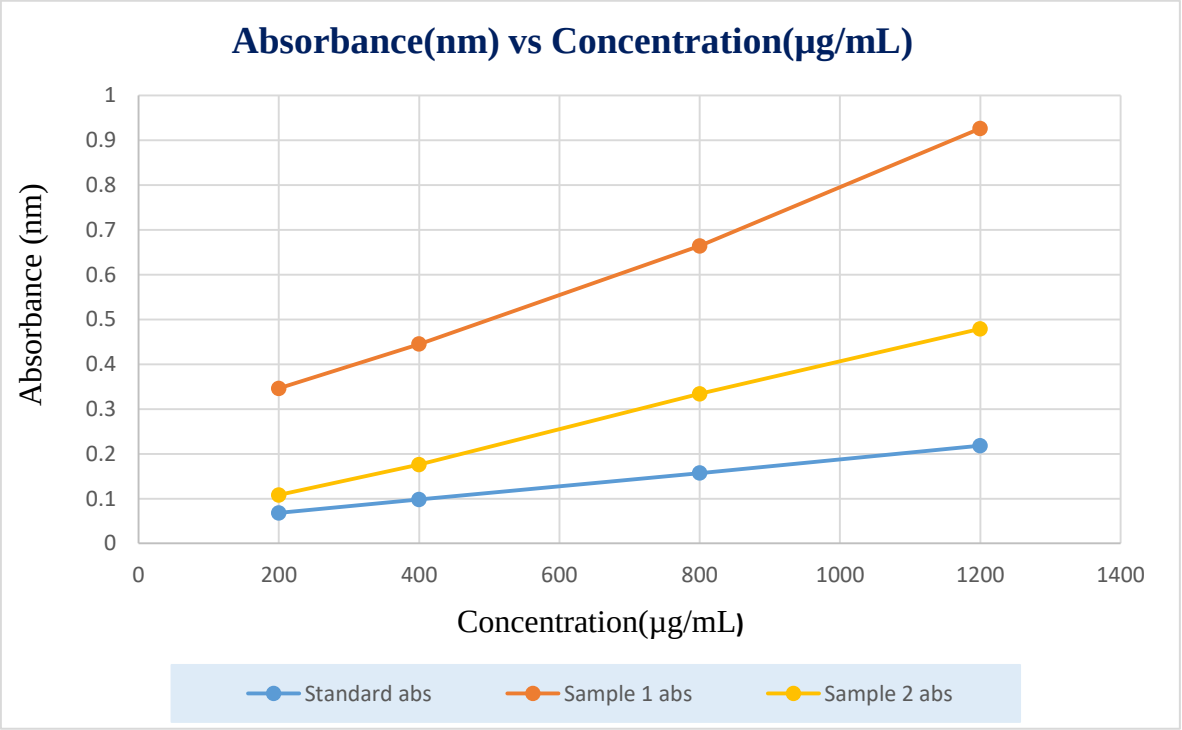

Figure 14: Graph of TPC: absorbance vs. concentration for sample 1, sample 2 and std (Gallic acid)

4.2.3 Total Flavonoid Content (TFC) Determination

Figure 15: Total Flavonoid Content (TFC) test

- **Standard Quercetin:**

The total content of flavonoid compounds, C expressed as Quercetin Equivalent (QE) produced by standard Quercetin depending on the concentration and absorbance values are given below in the table 18.

Table 18: QE values of Total Flavonoid Content (TFC) test by standard Quercetin

Conc	Abs	Avg Abs.	Vol. Of sol^{n} in mL (V)	Wt. of sample in g/ml (m)	QE in µg/mL from curve	QE in mg/ml (c)	TFC as QE in mg/g (C=((c×V)/m)
200	0.106 0.104 0.112	0.107	1	0.0002	108.5	0.1085	524.5
	0.139						

400	0.145 0.140	0.141	1	0.0004	278.5	0.2785	696.25
800	0.210 0.213 0.216	0.213	1	0.0008	638.5	0.6385	798.125
1200	0.286 0.298 0.302	0.295	1	0.0012	1048.5	1.0485	873.75

The concentrations of Quercetin that was found from the above table 18 was plotted against concentrations in a calibration graph. Graphical presentation of absorbance vs concentration of TFC test by Quercetin is shown below in figure 16.

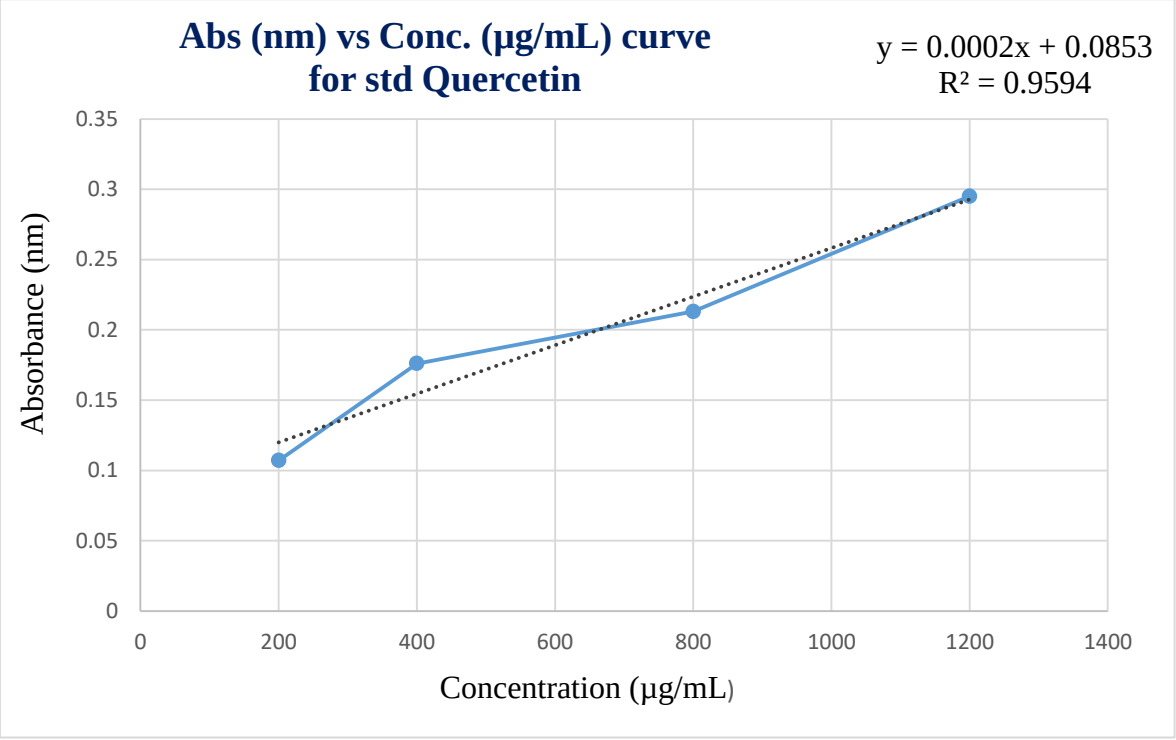

Figure 16: Standard curve of std Quercetin for Total Flavonoid Content (TFC) test

- **Sample 1:**

The total content of flavonoid compounds, C expressed as Quercetin Equivalent (QE) produced by Sample 1 depending on the concentration and absorbance values are given below in the table 19.

Table 19: QE values of Total Flavonoid Content (TFC) test by Sample 1

Conc	Abs	Avg Abs.	Vol. Of sol^{n} in mL (V)	Wt. of sample in g/ml (m)	QE in µg/mL from curve	QE in mg/ml (c)	TFC as QE in mg/g (C=((c×V)/m)
200	0.109 0.103 0.094	0.102	1	0.0002	102.5	0.1025	512.5
400	0.137 0.141 0.152	0.143	1	0.0004	307.5	0.3075	768.75
800	0.228 0.219 0.216	0.221	1	0.0008	697.5	0.6975	871.875
1200	0.317 0.311 0.308	0.312	1	0.0012	1152.5	1.1525	960.417

The concentrations of Sample 1 that was found from the above table 19 was plotted against concentrations in a calibration graph. Graphical presentation of absorbance vs concentration

of TFC test by Sample 1 is shown below in figure 17.

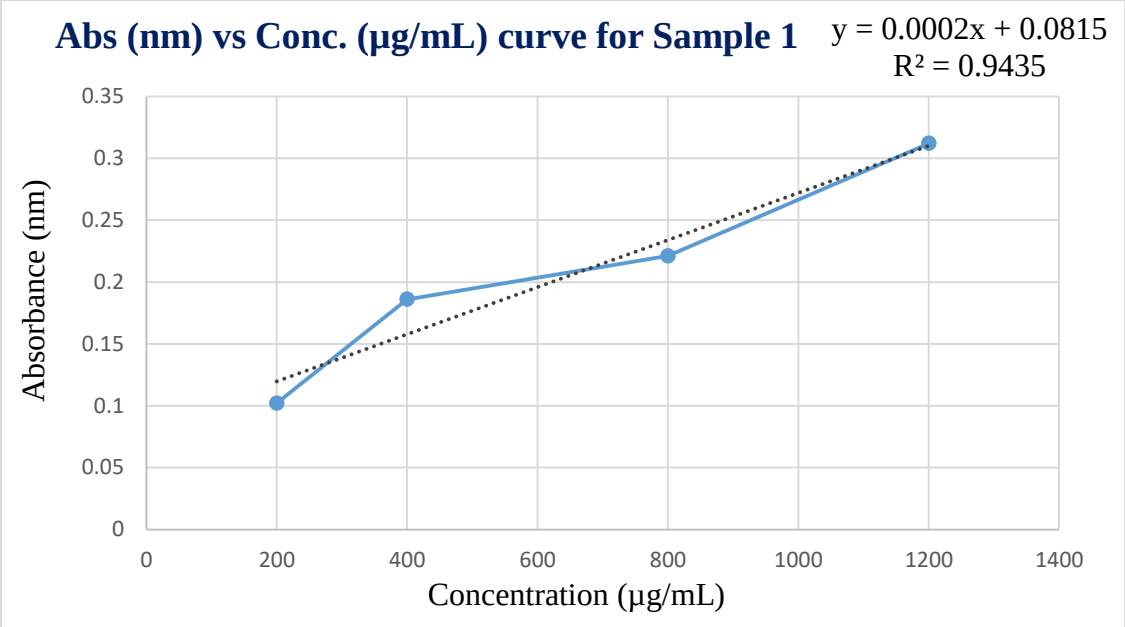

Figure 17: Standard curve of sample 1 for Total Flavonoid Content (TFC) test

- **Sample 2:**

The total content of flavonoid compounds, C expressed as Quercetin Equivalent (QE) produced by Sample 2 depending on the concentration and absorbance values are given below in the table 20.

Table 20: QE values of Total Flavonoid Content (TFC) test by Sample 2

Conc	Abs	Avg Abs.	Vol. Of sol^{n} in mL (V)	Wt. of sample in g/ml (m)	QE in µg/mL from curve	QE in mg/ml (c)	TFC as QE in mg/g (C=((c×V)/m)
200	0.097 0.103 0.101	0.1	1	0.0002	171	0.171	855

400	0.181 0.172 0.169	0.174	1	0.0004	356	0.356	890
800	0.318 0.338 0.317	0.324	1	0.0008	731	0.731	913.75
1200	0.481 0.474 0.476	0.477	1	0.0012	1113.5	1.1135	927.917

The concentrations of Sample 2 that was found from the above table 20 was plotted against concentrations in a calibration graph. Graphical presentation of absorbance vs concentration of TFC test by Sample 2 is shown below in figure 18.

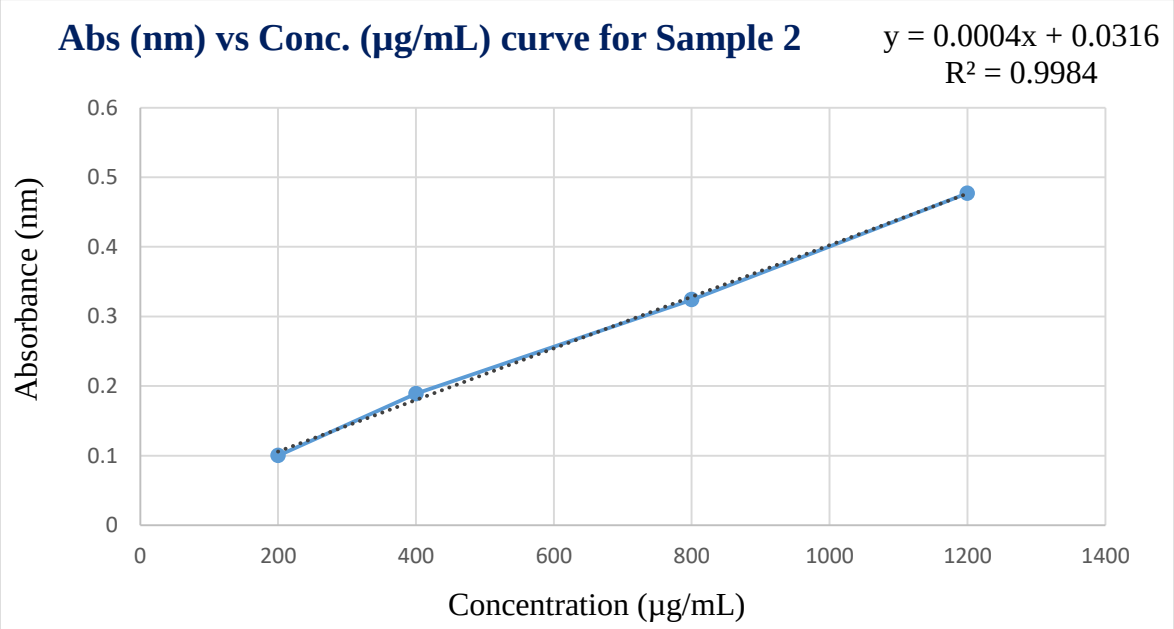

Figure 18: Standard curve of sample 2 for Total Flavonoid Content (TFC) test

Lastly, a comparison data of Absorbance(nm) vs Concentration(µg/mL) value for Standard Quercetin, Sample 1 and Sample 2 in TFC test is given below in table 21.

Table 21: Comparison of absorbances for different concentrations in TFC test

Concentration(µg/mL)	Abs. of Standard	Abs. of Sample 1	Abs. of Sample 2
200	0.107	0.102	0.1
400	0.141	0.143	0.174
800	0.213	0.221	0.324
1200	0.295	0.312	0.477

A comparison calibration graph of TFC: Absorbance vs. Concentration of sample 1, sample 2 and std (Quercetin) is shown below in figure 19.

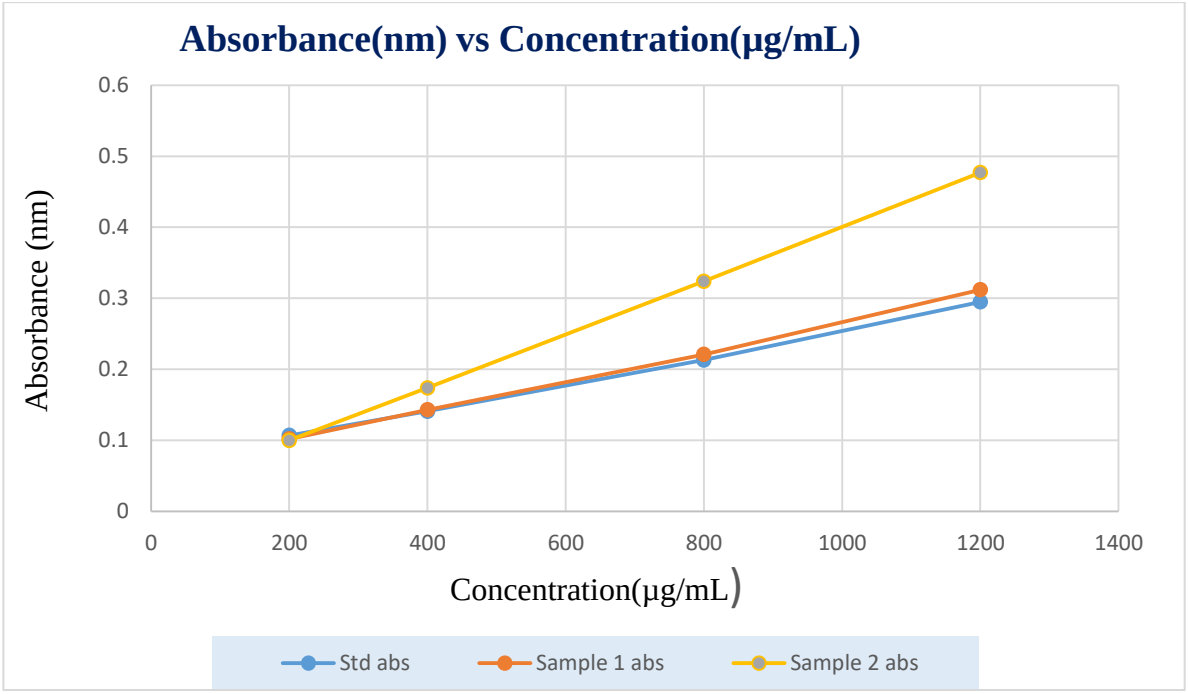

Figure 19: Graph of TFC: absorbance vs. concentration for sample 1, sample 2 and std (Quercetin)

4.2.4 Total Antioxidant Capacity (TAC) Determination

Figure 20: *Total Antioxidant Capacity(TAC) test*

- **Standard Ascorbic acid (ASA):**

The total antioxidant capacity, C expressed as Ascorbic Acid Equivalent (AAE) produced by standard Ascorbic acid depending on the concentration and absorbance values are given below in the table 22.

Table 22: AAE values of Total Antioxidant Capacity (TAC) test by standard Ascorbic acid

Conc	Abs	Avg Abs.	Vol. Of sol^{n} in mL (V)	Wt. of sample in g/ml (m)	AAE in µg/mL from curve	AAE in mg/ml (c)	TAC as AAE in mg/g (C=((c×V)/m)
200	0.115 0.111 0.110	0.112	1	0.0002	137	0.137	685
400	0.116 0.169	0.147	1	0.0004	312	0.312	780

	0.156						
800	0.223 0.211 0.214	0.216	1	0.0008	657	0.657	821.25
1200	0.268 0.291 0.308	0.289	1	0.0012	1022	1.022	851.667

The concentrations of L-Ascorbic acid that was found from the above table 22 was plotted against concentrations in a calibration graph. Graphical presentation of absorbance vs concentration of TAC test by Ascorbic acid is shown below in figure 21.

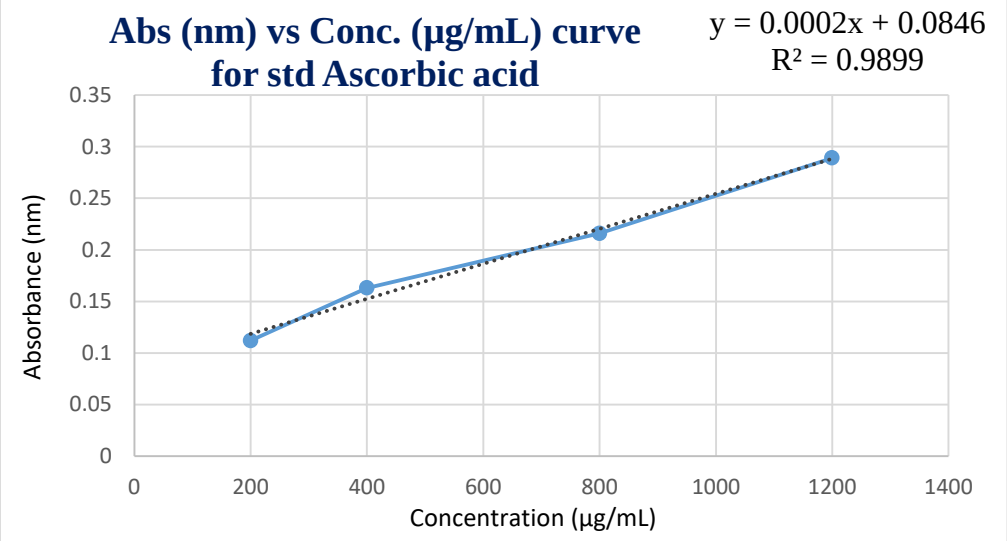

Figure 21: Standard curve of std Ascorbic acid for Total Antioxidant Capacity (TAC) test

- **Sample 1:**

The total antioxidant capacity, C expressed asAscorbic Acid Equivalent (AAE) produced by Sample 1 depending on the concentration and absorbance values are given below in the table 23.

Table 23: AAE values of Total Antioxidant Capacity (TAC) test by Sample 1

Conc	Abs	Avg Abs.	Vol. Of sol^{n} in mL (V)	Wt. of sample in g/ml (m)	AAE in µg/mL from curve	AAE in mg/ml (c)	TAC as AAE in mg/g (C=((c×V)/m)
200	0.083 0.091 0.108	0.094	1	0.0002	175	0.175	875
400	0.130 0.141 0.125	0.132	1	0.0004	365	0.365	912.5
800	0.210 0.216 0.213	0.213	1	0.0008	770	0.77	962.5
1200	0.325 0.318 0.320	0.321	1	0.0012	1310	1.31	1091.667

The concentrations of Sample 1 that was found from the above table 23 was plotted against concentrations in a calibration graph. Graphical presentation of absorbance vs concentration of TAC test by Sample 1 is shown below in figure 22.

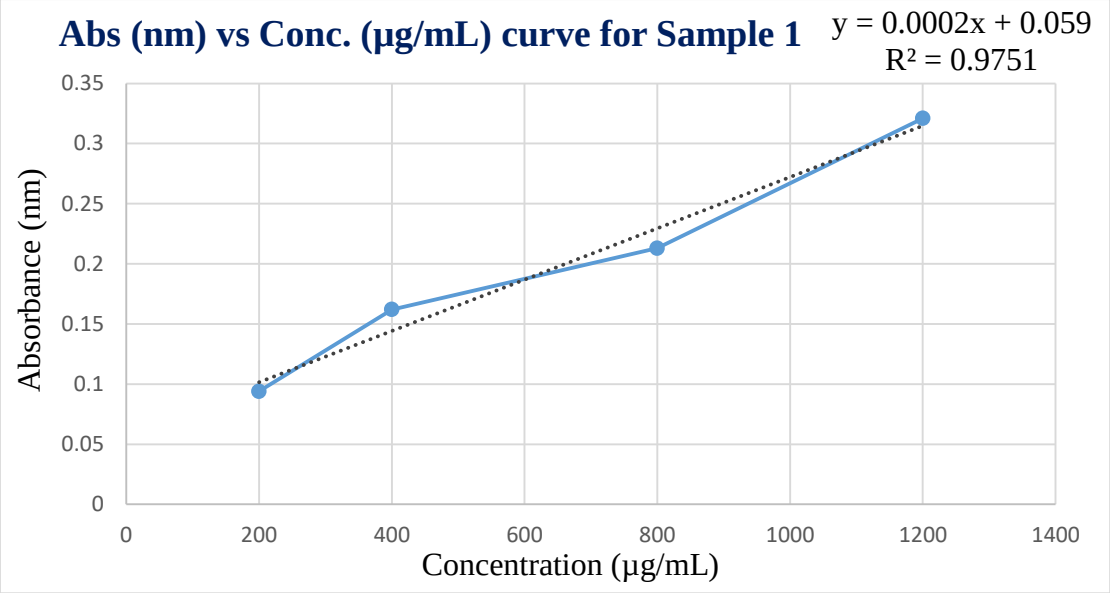

Figure 22: Standard curve of sample 1 for Total Antioxidant Capacity (TAC) test

- **Sample 2:**

The total antioxidant capacity, C expressed as Ascorbic Acid Equivalent (AAE) produced by Sample 2 depending on the concentration and absorbance values are given below in the table 24.

Table 24: AAE values of Total Antioxidant Capacity (TAC) test by Sample 2

Conc	Abs	Avg Abs.	Vol. Of sol^{n} in mL (V)	Wt. of sample in g/ml (m)	AAE in µg/mL from curve	AAE in mg/ml (c)	TAC as AAE in mg/g (C=((c×V)/m)
200	0.132 0.136 0.146	0.138	1	0.0002	124.5	0.1245	622.5
400	0.171 0.182	0.176	1	0.0004	314.5	0.3145	786.25

	0.175						
800	0.207 0.269 0.262	0.246	1	0.0008	664.5	0.6645	830.625
1200	0.394 0.375 0.326	0.365	1	0.0012	1259.5	1.2595	1049.583

The concentrations of Sample 2 that was found from the above table 24 was plotted against concentrations in a calibration graph. Graphical presentation of absorbance vs concentration of TAC test by Sample 2 is shown below in figure 23.

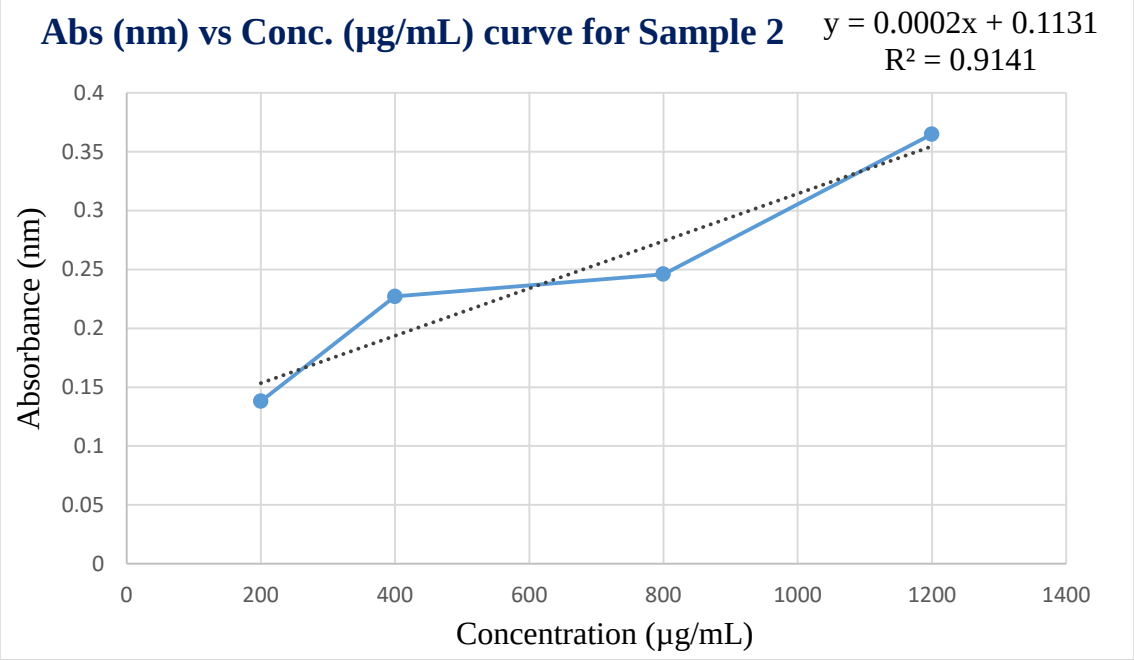

Figure 23: Standard curve of sample 2 for Total Antioxidant Capacity (TAC) test

Lastly, a comparison data of Absorbance(nm) vs Concentration(µg/mL) value for Standard L-Ascorbic acid, Sample 1 and Sample 2 in TAC test is given below in table 25.

Table 25: Comparison of absorbances for different concentrations in TAC test

Concentration(µg/mL)	Abs. of Standard	Abs. of Sample 1	Abs. of Sample 2
200	0.112	0.094	0.138
400	0.147	0.132	0.176
800	0.216	0.213	0.246
1200	0.289	0.321	0.365

A comparison calibration graph of TAC: Absorbance vs. Concentration of sample 1, sample 2 and std (Ascorbic acid) is shown below in figure 24.

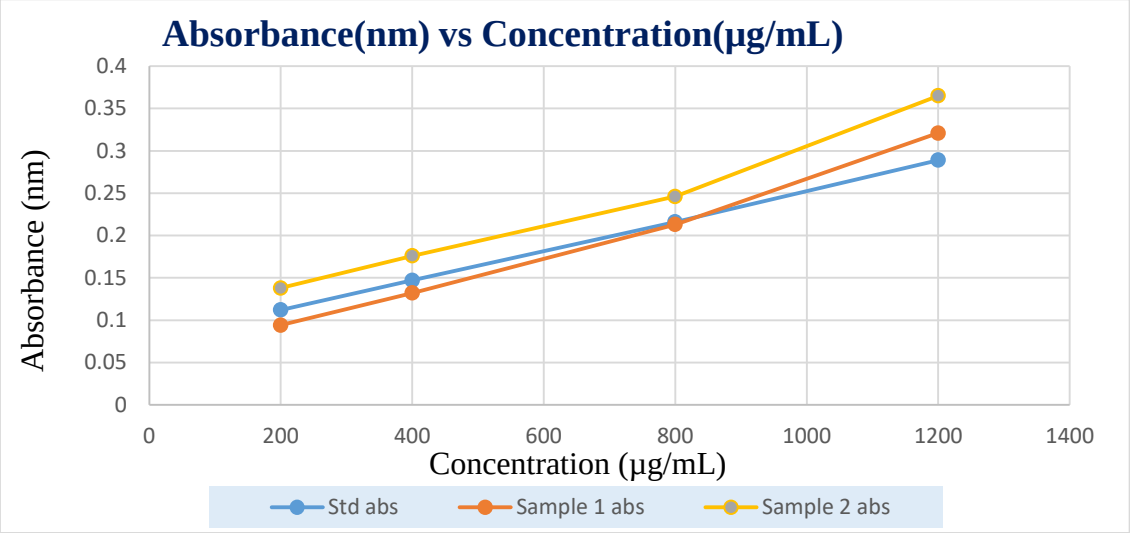

Figure 24: Graph of TAC: absorbance vs. concentration for sample 1, sample 2 and std (Ascorbic acid)

4.3 High Performance Liquid Chromatography (HPLC) Determination

4.3.1 Identification of the Standard A and B

- **Standard solution A, Quercetin:**

The standard Quercetin was identified quantitatively by high performance liquid

chromatography (HPLC) profile and graphical presentation is shown below in the figure 25.

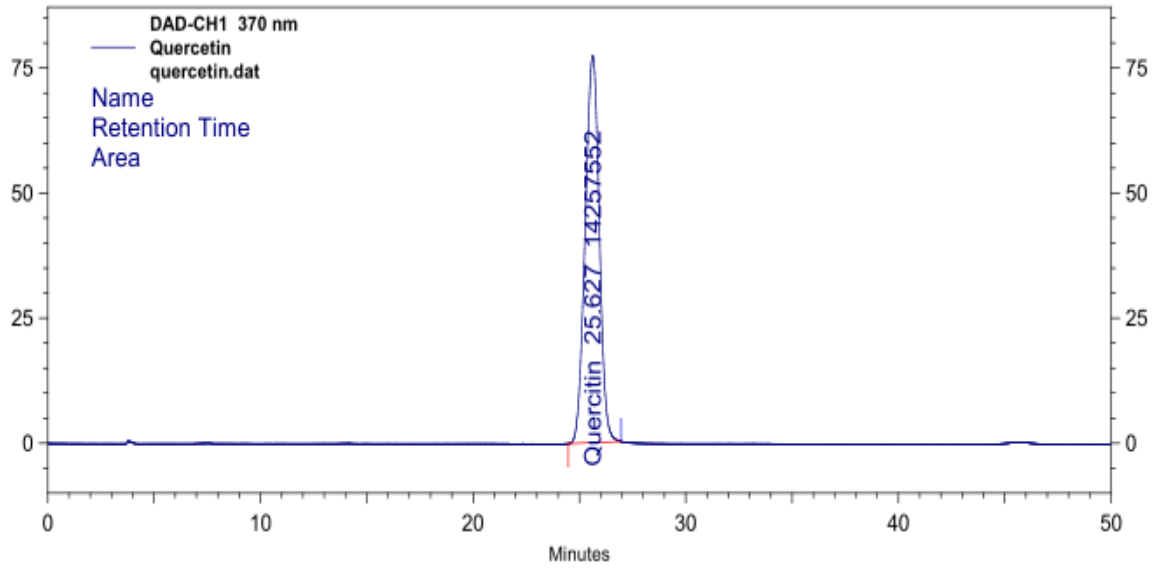

Figure 25: Identification of standard Quercetin by HPLC metabolite profile

- **Standard solution B, Kaempferol:**

The standard Kaempferol was identified quantitatively by high performance liquid chromatography (HPLC) profile and graphical presentation is shown below in the figure 26.

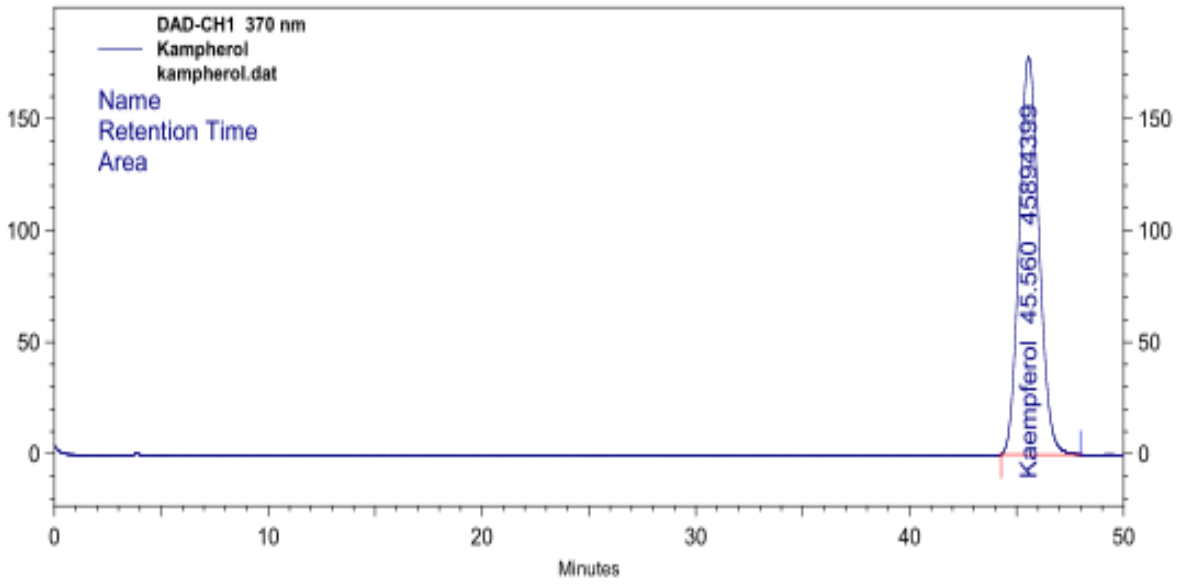

Figure 26: *Identification of standard Kaempferol by HPLC metabolite profile*

4.3.2 Identification of the Sample 1 and 2

- **Sample solution 1:**

The constituents Quercetin and Kaempferol present in Sample 1 were identified quantitatively by high performance liquid chromatography (HPLC) profiling and graphical presentation is shown below in the figure 27.

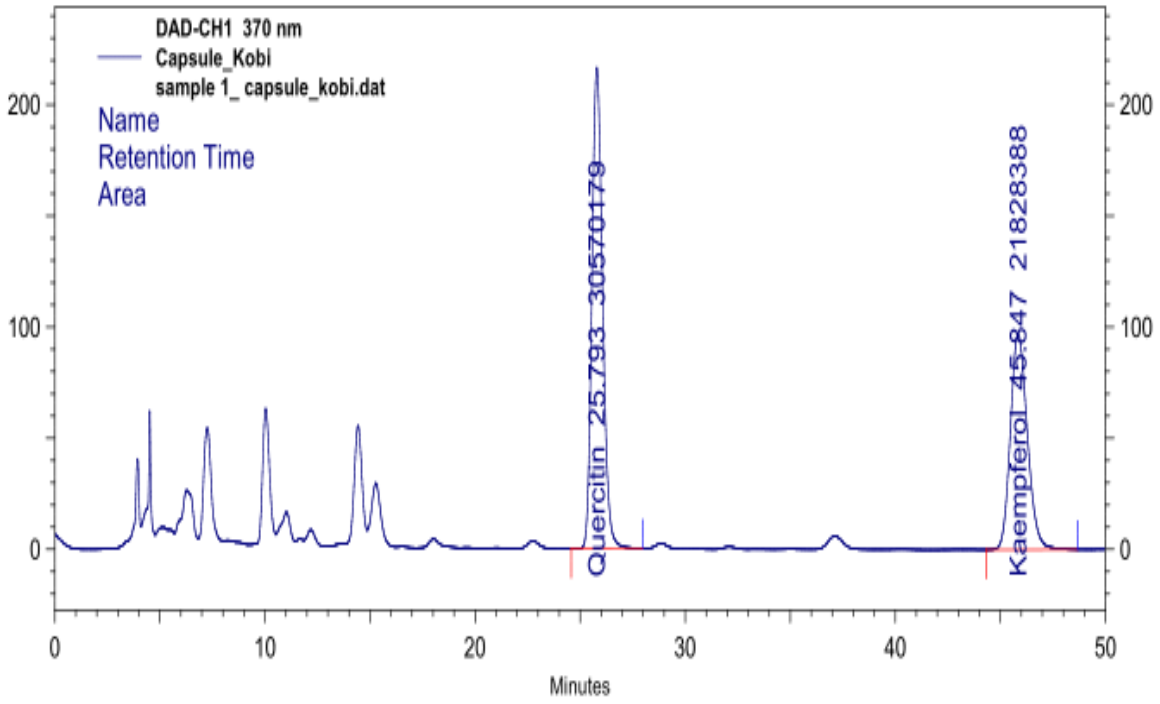

Figure 27: Identification of Quercetin and Kaempferol present in sample 1 by HPLC profile

- **Sample solution 2:**

The constituents Quercetin and Kaempferol present in Sample 2 were identified quantitatively by high performance liquid chromatography (HPLC) profile and graphical presentation is shown below in the figure 28.

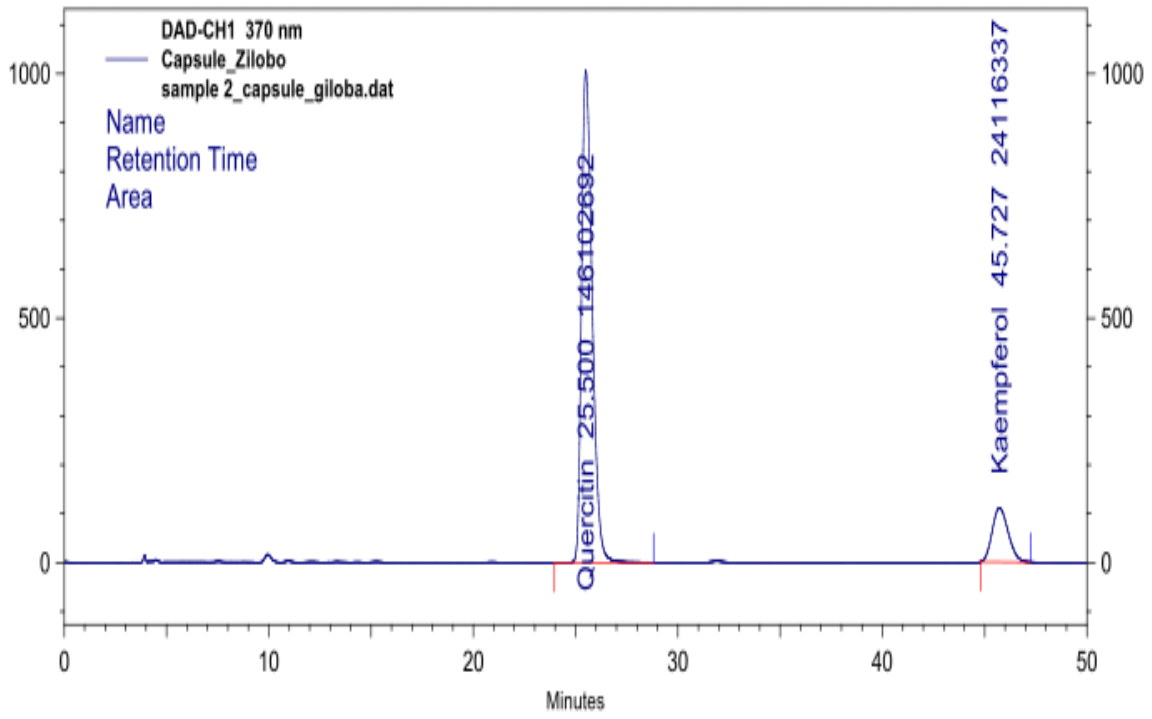

Figure 28: Identification of Quercetin and Kaempferol present in Sample 2 by HPLC profile

4.3.3 Comparison data of Sample 1 and 2

Lastly, a comparison data of the contents (Quercetin and Kaempferol) present in Sample 1 and 2 is shown below in table 26.

Table 26: Comparison data of the contents present in Sample 1 and 2

Sample name	Quercetin	Kaempferol
Sample 1	6.01	0.29
Sample 2	1.34	0.28

A bar graph of the constituents (Quercetin and Kaempferol) present in sample 1 and sample 2 for visual comparison is shown below in figure 29.

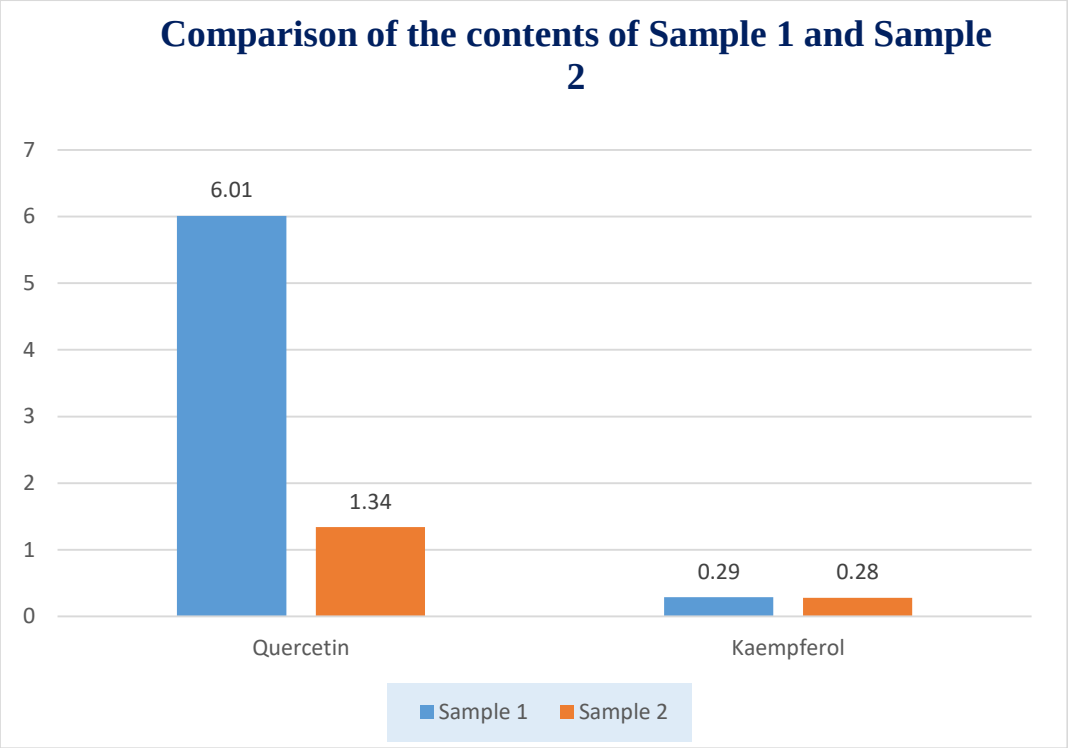

Figure 29: Bar graph showing visual comparison of the contents present in sample 1 and sample 2

Chapter 5

Discussion

The study was performed to evaluate the phytochemical screening, antioxidant activity and HPLC profiling of the aqueous extract of *Ginkgo biloba* from two commercially available marketed products.

5.1 Phytochemical Screening Tests

The phytochemical screening of aqueous extract of *Ginkgo biloba* from two marketed products was successfully carried out following by the methodology (Hossain et al., 2013) and (Gul et al., 2017). It has been noted before that the (+) sign means presence and (-) sign means the absence of compounds in the tests. The screening showed the presence of flavonoids, phenols, tannins, coumarin and terpenoids, while there was absence of alkaloids, glycosides, sterols, saponins and resin (Table 9). The existence of these constituents provides us chance to speculate the further activities and may justify its use in traditional remedies to treat different diseases. For example, flavonoid and phenol confirms the presence of antioxidant capacity. Phenol possesses effective free radicals scavenging property, anti-microbial, anti-fungal and anti-viral activity. Flavonoids also perform some other roles as chemical messenger, physiological regulators, cell cycle inhibitors (Galeotti et al., 2008). It is predicted in case of the presence of tannins that they have a significant impact on animal nutrition, on inhibition of growth rate digestive enzymes as well as properties like antimicrobial activity, anti-carcinogenic and antiseptic (Bennick, 2002). That is why phytochemical screening tests were carried out to examine the potential of the aqueous extract of the plant from two commercially available drugs.

5.2 *In- vitro* Antioxidant Activity

As aqueous extract of this plant from marketed products was never used before to estimate the antioxidant activity, which is why *in-vitro* antioxidant tests were carried out to examine the antioxidant potential of aqueous extract of plant *Ginkgo biloba* from two commercially available products. DPPH free radical scavenging assay, Total phenolic contents (TPC), Total flavonoid contents (TFC) and Total antioxidant capacity (TAC) tests were used to evaluate the antioxidant capacity of aqueous extract of the plant from two marketed products where data provided significant evidence that they possess strong antioxidant capacity.

5.2.1 DPPH Free Radical Scavenging Assay

Natural antioxidants are effective free radical scavengers (Brewer, 2011)and thus received great attention. The present study was designed to determine the antioxidant potential of the aqueous extract of *Ginkgo biloba* from two marketed products that were tested at 517 nm for free radical scavenging activity by using the method of Lalhminghlui & Jagetia, 2018. Reference standard used was Ascorbic acid (ASA).

When the concentration is increasing gradually from 50 μg/mL to 1200μg/mL, the absorbance values of standard Ascorbic acid are decreased slowly from 0.639 to 0.127 (Table 10). Similarly, the absorbance values are also decreased slowly from 0.760 to 0.106 for sample 1 (Table 11) and 0.850 to 0.116 for sample 2 (Table 12) when the concentration is increasing gradually from 50 μg/mL to 1200μg/mL. From the absorbance values of standard, sample 1 and sample 2, we can imply that concentration (from50μg/mL to 1200μg/mL) is inversely proportional to the absorbance of it.

Moreover, we can observe that % inhibition of DPPH free radical scavenging activity gradually increased corresponding to their concentration (50μg/mL to 1200μg/mL) in both of the samples and standard. The highest DPPH free radical scavenging activity for standard

Ascorbic acid was found to be 85.650% (Table 10, Figure 6) whereas 88.022% for sample 1 (Table 11, Figure 7) and 86.892% for sample 2 (Table 12, Figure 8) at 1200μg/mL concentration. It implies that % inhibition or % SCV of DPPH free radical scavenging activity by standard ascorbic acid was slightly lower than the value of % of inhibition by both of the samples and sample 1 showed the highest % of inhibition.

Furthermore, in this research, aqueous extract solution of standard Ascorbic acid presented notable free radical scavenging activity with IC_{50} value of 300.504 μg/mL (Table 10) whereas IC_{50} values for sample 1 was 561.655 μg/mL (Table 11) and sample 2 was 416.521 μg/mL (Table 12). This means that the free radical scavenging activity of the both samples was found to be much better than the standard Ascorbic acid. Additionally, sample 2 required less value of IC_{50} to produce 50% inhibition of the DPPH free radical than sample 1 indicating better result than sample 1.

Thus, finally we can conclude that IC_{50} value and % inhibition value both are comparable to the sample 2 which exhibited better antioxidant potential of aqueous extract of plant *Ginkgo biloba* from marketed products.

5.2.2 Total Phenolic Contents (TPC) Test

Phenolic compounds act as antioxidants since they have the redox properties (Soobrattee et al., 2005). The present study was designed to determine the antioxidant potential of the aqueous extract of *Ginkgo biloba* from two commercially available products that were tested at 765 nm for Total phenolic contents (TPC) by using the method of Chandra et al., 2014. Reference standard used was Gallic acid. The total phenolic content of the extract was expressed in terms of Gallic Acid Equivalent (GAE).

When the concentration is increasing gradually from 200 μg/mL to 1200μg/mL, the absorbance values of standard Gallic acid are increased slowly from 0.068 to 0.218 (Table 14). Similarly, the absorbance values are also increased slowly from 0.346 to 0.926 for sample 1 (Table 15) and 0.108 to 0.479 for sample 2 (Table 16) when the concentration is increasing gradually from 200 μg/mL to 1200μg/mL. From the absorbance values of standard, sample 1 and sample 2, we can imply that concentration is directly proportional to the absorbance of it. Moreover, the equation of the calibration curve of standard Gallic acid (Figure 11), sample 1 (Figure 12) and sample 2 (Figure 13) were used to determine the total phenolic content. A regression coefficient (R^2) of 0.9887 for standard, 0.9783 for sample 1 and 0.9948 for sample 2 were also obtained indicating a positive correlation between the concentrations and the absorbances.

In addition to that, it is observed that concentration of aqueous extract of *Ginkgo biloba* from marketed samples have proportional relationship with the TPC. When the concentration (200μg/mL to 1200μg/mL) increased, total phenolic content, C as GAE also increased from 717.5mg to 744.583mg of gallic acid per gram of dried extract for standard Gallic acid (Table 14), 854 to 1109 for sample 1 (Table 15) and 755 to 898.75 for sample 2 (Table 16). However, it additionally indicates that with the increase of total phenolic content, its antioxidant activity also increases.

Finally, we can conclude that the total phenolic content of the both samples were found to be much better than the standard Gallic acid. Additionally, sample 1 showed higher value of GAE than sample 2. Therefore, it indicates a positive correlation between total phenolic content of aqueous extract of *Ginkgo biloba* from two marketed samples and its antioxidant activity.

5.2.3 Total Flavonoid Contents (TFC) Test

The present study was designed to determine the antioxidant potential of the aqueous extract of *Ginkgo biloba* from two marketed products that were tested at 415 nm for Total flavonoid contents (TFC) by using the method of Rebaya et al., 2015. Reference standard used was Quercetin. The total flavonoid content of the extract was expressed in terms of Quercetin Equivalent (QE).

When the concentration is increasing gradually from 200 μg/mL to 1200μg/mL, the absorbance values of standard Quercetin are increased slowly from 0.107 to 0.295 (Table 18). Similarly, the absorbance values are also increased slowly from 0.102 to 0.312 for sample 1 (Table 19) and 0.1 to 0.477 for sample 2 (Table 20) when the concentration is increasing gradually from 200 μg/mL to 1200μg/mL. From the absorbance values of standard, sample 1 and sample 2, we can imply that concentration is directly proportional to the absorbance of it. Moreover, the equation of the calibration curve of standard Quercetin (Figure 16), sample 1 (Figure 17) and sample 2 (Figure 18) were used to determine the total flavonoid content. A regression coefficient (R^2) of 0.9594 for standard, 0.9435 for sample 1 and 0.9984 for sample 2 were also obtained indicating a positive correlation between the concentrations and the absorbances.

In addition to that, it is observed that concentration of aqueous extract of *Ginkgo biloba* from marketed samples have proportional relationship with the TFC. When the concentration (200μg/mL to 1200μg/mL) increased, total flavonoid content, C as QE also increased from 524.5mg to 873.75mg of quercetin per gram of dried extract for standard Quercetin (Table 18), 512.5 to 960.417 for sample 1 (Table 19) and 855 to 927.917 for sample 2 (Table 20). However, it additionally indicates that with the increase of total flavonoid content, its antioxidant activity also increases.

Finally, we can conclude that the total flavonoid content of the both samples were found to be much better than the standard Quercetin. Additionally, sample 1 showed higher value of QE than sample 2. Therefore, it indicates a positive correlation between total flavonoid content of aqueous extract of *Ginkgo biloba* from two marketed samples and its antioxidant activity.

5.2.4 Total Antioxidant Capacity (TAC) Test

The present study was designed to determine the antioxidant potential of the aqueous extract of *Ginkgo biloba* from two marketed products that were tested at 695 nm for Total antioxidant capacity (TAC) by using the method of Prieto, Pineda, & Aguilar, 1999. Reference standard used was Ascorbic acid. The total antioxidant capacity of the extract was expressed in terms of Ascorbic acid Equivalent (AAE).

When the concentration is increasing gradually from 200 μg/mL to 1200μg/mL, the absorbance values of standard Ascorbic acid are increased slowly from 0.112 to 0.289 (Table 22). Similarly, the absorbance values are also increased slowly from 0.094 to 0.321 for sample 1 (Table 23) and 0.138 to 0.365 for sample 2 (Table 24) when the concentration is increasing gradually from 200 μg/mL to 1200μg/mL. From the absorbance values of standard, sample 1 and sample 2, we can imply that concentration is directly proportional to the absorbance of it. Moreover, the equation of the calibration curve of standard Ascorbic acid (Figure 21), sample 1 (Figure 22) and sample 2 (Figure 23) were used to determine the total antioxidant capacity. A regression coefficient (R^2) of 0.9899 for standard, 0.9751 for sample 1 and 0.9141 for sample 2 were also obtained indicating a positive correlation between the concentrations and the absorbances.

In addition to that, it is observed that concentration of aqueous extract of *Ginkgo biloba* from marketed samples have proportional relationship with the TAC. When the concentration

(200µg/mL to 1200µg/mL) increased, total antioxidant capacity, C as AAE also increased from 685mg to 851.667mg of ascorbic acid per gram of dried extract for standard Ascorbic acid (Table 22), 875 to 1091.667 for sample 1 (Table 23) and 622.5 to 1049.583 for sample 2 (Table 24). However, it additionally indicates that with the increase of total antioxidant capacity, its antioxidant activity also increases.

Finally, we can conclude that the total antioxidant capacity of the both samples was found to be much better than the standard Ascorbic acid. Additionally, sample 1 showed higher value of AAE than sample 2. Therefore, it indicates a positive correlation between total antioxidant capacity of aqueous extract of *Ginkgo biloba* from two marketed samples and its antioxidant activity.

5.3 HPLC Metabolite Profile Analysis

HPLC profile of selected samples were performed at 370 nm following standard protocol (United & Pharmacopeia, 2018) to identify the compounds present in the samples. From the chromatogram, the presence of two flavonoids (Quercetin and Kaempferol) were identified and quantified with comparison to those of standard samples respectively. The result showed that the retention time of standard quercetin and kaempferol was found to be 25.627 minutes (Figure 25) and 45.560 minutes (Figure 26). In case of the samples, sample 1 showed retention time of 25.500 minutes for quercetin and 45.727 minutes for kaempferol (Figure 27). On the other hand, sample 2 showed retention time of 25.793 minutes for quercetin and 45.847 minutes for kaempferol (Figure 28). The retention times of the flavonoids present in the both samples are very close to the retention times of standard quercetin and kaempferol.

It is noted that in the total 25% of ginkgo flavonoids present in plant from natural source, percentage of quercetin is 64.8-81.5% of the total content (dominant), the percentage of

kaempferol is 13.3-28.9% of the total content and 2-8.4% for isorhamnetin which is very low compared to the other two constituents (Van Beek, 2002). The acceptance criteria is not less than 0.5% of flavonoids, as flavone glycosides with a mean molecular mass of 756.7 on the dried basis (United & Pharmacopeia, 2018). From the quantitative analysis, the amount of quercetin for sample 1 is 6.01mg and 1.34mg for sample 2 in 60mg capsule. In natural plant extract, the amount of quercetin is approximately 11mg. The amount of kaempferol is 0.29mg and 0.28mg for sample 1 and sample 2 in 60mg capsule. However, in the plant extract, the amount of kaempferol is 3.15mg. This means the content of the constituents present in these two commercially manufactured samples are much lower compared to the natural plant extract. This is may be due to the presence of adulteration in sample products. Moreover, the result interprets that the contents of the constituents present in sample 1 is higher than sample 2 (Table 26). This statement is supported by analyzing the comparison of the contents present in sample 1 and 2 which is presented visually in the Bar graph (Figure 29). This means the constituents (quercetin and kaempferol) present in sample 1 gave relatively better result than sample 2.

Chapter 6

Conclusion

In this present investigation, the aqueous extract of *Ginkgo biloba* from marketed samples was subjected to phytochemical screening as well as to anti-oxidant activity and HPLC metabolite profile. These drugs are widely used to cure various disorders like blood disorders, COPD symptoms, Alzheimer's disease, Raynaud's disease, peripheral vascular disease and to enhance blood circulation as well as possess therapeutic effects such as anti-depressant, antioxidant and inhibit platelet aggregation in order to control blood coagulation in human body. Among the available brands in Bangladesh market, two of them were selected for the study to compare their antioxidant activity and HPLC metabolite profile where one of them is from a mainstream pharmaceutical company while the other one is from herbal company. The phytochemical screening indicated the presence of phytochemical constituents namely, tannins, flavonoids, coumarin, phenolic compounds and terpenoids in both of the samples. This study also exhibited strong antioxidant properties indicating the potential of the both samples as antioxidant drugs. In the antioxidant activity studies, sample 2 showed better result in DPPH free radical scavenging assay whereas sample 1 showed better result in total phenol contents, total flavonoid contents and total antioxidant capacity tests. Moreover, in HPLC metabolite profile analysis, the presence of two flavonoids was detected quantitatively. Therefore, the outcome from the current study resulted in sample 1 extract producing more therapeutic effect than sample 2 extract.

Future Direction

- Different antioxidant test methods such as: nitric oxide, hydrogen per-oxide, lipid per oxidation method can be conducted to find more significant antioxidant activity.
- This study involves the investigation of two marketed products of *Ginkgo biloba*. Studies can be conducted involving the other brand products for further comparison.
- Studies can be conducted to observe the comparative difference in quality, dissolution time, potency and purity of the same class of drugs among different marketed products.
- Further investigation can be performed on different solvent system and the presence of terpenetrilactones (TTL) can be detected by using HPLC metabolite profile.

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