An *In-vitro* Study on Antioxidant Properties of *Alcea rosea* Leaves

By

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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.

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Approval

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Ethics Statement

This study does not involve any kind of any animal or human trial.

Abstract

The aim of the study is to determine free radical scavenging capacity, total phenolic content and total flavonoid content and to investigate the *in-vitro* antioxidant potential of methanolic leaves extract of *Alcea rosea*. The free radical scavenging capacity, TPC and TFC were performed using DPPH scavenging assay, Folin-Ciocalteu reagent assay and aluminium chloride colorimetric assay respectively. The result of the study indicates that *Alcea rosea* posses significant scavenging activity against DPPH and result is very much similar to standard. The IC_{50} value of the sample was 255.13 ± 8.35 µg/mL and comparable with the standard 167.84 ± 5.11 µg/mL. The study of total phenolic content and total flavonoid content revealed that the leaves of *Alcea rosea* has significant amount of phenolic content 52.2 ± 1.1 mg GAE/g and flavonoid content 29.4 ± 0.707 mg QE/g. The obtained results suggest that *Alcea rosea* exhibits antioxidant activity. But further studies are needed to validate its medicinal uses.

Keywords: *Alcea rosea*, Antioxidant, DPPH, Phenolic content, Flavonoid content.

Dedication

I have dedicated my project work to my beloved parents.

Acknowledgement

I would like to acknowledge and express my sincere thanks to those who have supported in my project accomplishments. First and foremost, I am grateful to Allah Almighty for giving me the strength and ability to complete my project work and the whole academic session. Then I would like to express my deepest thanks to my supervisor Faria Tahsin, Lecturer, Department of Pharmacy, Brac University for her constant guidance and suggestions throughout the thesis work. My gratitude also goes to Dr. Eva Rahman Kabir, Chairperson, Department of Pharmacy, Brac University for providing an opportunity and the support to carry out the project work. Finally, I take this opportunity to thank all my faculty members of Department of Pharmacy, Brac University for their inspiration and the lab officers for their assistance.

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List of Acronyms

mg	Milligram
g	Gram
mL	Millilitre
nm	Nanometre
pm	Pico meter
cm	Centimetre
DMSO	Dimethylsulfoxide
SEM	Standard Error of Mean

Chapter 1

Introduction

Free radicals are biological molecules which are highly reactive as they contains unpaired electron on their outer orbit. Continual production of free radicals take place in the cells through enzymatic and non-enzymatic reaction as it is one of the vital sections of normal cellular function in living organism. Along with that, excessive amount of radicals are produced due to exposure to ozone, x-ray, air pollution, cigarette smoking, fertilizer and industrial chemicals (Sultana et al., 2014).

Antioxidant therapy has been caught the great attention in the treatment of the diseases which are linked to stress caused by oxidation. Over production exceeds the level of defending mechanism of our body then it leads to the damage of tissue and different degenerative diseases like cardiovascular diseases, cancer, neuro-degeneration, central nervous system disease, diabetics, dementias, autoimmune disorder, cataracts, rheumatoid arthritis, Parkinson's disease, aging, atherosclerosis, tumour formation and so many (Huang, 2018).

Antioxidants are foremost substances that participate in prevention of oxidative chain reactions and provide providence to human health. On account of this capacity, it is also used as oxygen scavengers. Natural antioxidants are founds in majority of the plants. Scientific research accounts that phenolic compound, polyphenols and flavonoids are widely distributed in most of the plants. They show a great amount of therapeutic action such as antiviral, antioxidant, hepatoprotective, anti-inflammatory and anti-carcinogenic (Gülçin, Huyut, Elmastaş, & Aboul-Enein, 2010).

.

At present market several synthetic antioxidants are available which includes propyl gallate, tertiary butylhydroquinone, butylated hydroxyltoluene (BHT) and butylated hydroxyanisole (BHA) etc. In the food industry they are utilizing widely to prevent lipid oxidation. Moreover, chemical supplements are also used along with diet to overcome the lack of antioxidant. However, they are not safe enough for the human health. Their toxic effects due to their chemical composition has been arisen question of safety (Pham-Huy, He, & Pham-Huy, 2008).

Medical plants can play a vital role to resolve this problem by providing natural antioxidants. Extracted antioxidants from plant derivatives indicates original source. The natural antioxidant has gain overriding in order to replace synthetic antioxidant. Medical plants are available in huge diversity among the whole world and their demands are increasing day by day. Medical herbal drugs are widely use as they are readily available, cost efficient, effective, less side effects etc. For better potency scientific research are being conducted with a great concern. However, sufficient data on antioxidant capacity of most of the plants are not available in Bangladesh (Sultana et al., 2014).

Notably, *Alcea rosea* has great medicinal benefits and it's available in Bangladesh and tropical areas. Several scientific studies accounts that the constituents of the plants associated with large number of therapeutic activity such as inflammation, constipation, diarrhoea, urinary tract infection, intestinal tract infection, malaria, jaundice, angina etc. The plant extracts of different parts have also showed antimicrobial activity, antioxidant activity, hepatoprotective activity and hypoglycaemic activity (Hussain, Akash, Tahir, Rehman, & Ahmed, 2014). But, there is no scientific approved study or articles have been reported about the antioxidant properties of *Alcea rosea.* So, *in-vitro* methods were performed to investigate the antioxidant capacity of this plant.

1.1 Other species of *Alcea rosea*

1.1.1 *Alcea setose*

Alcea setose also known as bristly hollyhock is an extremely beautiful due to its large pink coloured bisexual flower and most favourite an ornamental plant. The plant is almost 6 feet tall which is a prominent of height. The species is mostly found in Jordan, Turkey, and Israel etc. The plant is also very common in some places such as mountain, abandoned fields and alongside landscape road. Before spring, vertical flower and flowering stem starts to grow along with inflorescence stalk. The growth of the plant is faster. The margin of the leaves is smooth. The leaves are carried in petiole. The fruits have several units containing seeds. The bud of these pink flowers is used as a main ingredient for herbal tea. Moreover, the plant is used to treat injuries, burns, cough, inflammation, respiratory tract inflammation etc in folk medicine. Recently, several scientific studies reported that the plant have effective therapeutic effect such as diuretic, emollient, reduce stomach and intestinal pain, inflammation, asthma, skin laxative, urinary tract infection, cough, wound relief and many others (Erga Aloni, 2016).

1.1.2 *Alcea rugosa*

Alcea rugosa is old garden choice and used as an ornamental plant. This species is mostly available in Ukraine, Russia, cremia and others. The plant is short lived, self seeding perennial plant. The removal of flower spike and dead flower makes it act like perennials. This plant is 4 to 9 feet high and the leaves are deeply lobed. In midsummer pale yellow single outward-facing flowers blooms. The leaves get smaller as time passes and the flowers show stamina column at centre. *Alcea rugos* is popular for its extreme resistance to rust. *Alcea rugosa* reported that it has therapeutic activity for respiratory illness (Azab, 2016).

1.1.3 *Alcea biennis*

Alcea biennis is perennial plant about 30-120 cm tall. The plant is branched at the base. The stem is cylindrical, hairy and sometimes forwarded. The leaves are orbicular, hairy and lobed. 1-3 flowers are at leave axils. 5 petals consist of pink, purple, yellow and white coloured. Stamens are yellow in colour and cylindrical in size. The flowers is used in treatment of bronchitis (Erkan & Vural, 2012).

1.1.4 *Alcea nudiflora*

The most lovely, rare hollyhock is native to China and Kazakhstan. The wild special flower is large contains graceful dedicated green yellow centre. The lifecycle of this plant are both biennial and perennial. *Alcea nudiflora* are almost similar in many aspects to *Alcea rosea* though differs in some parts. The central lobe of the leaves is longer in *Alcea nudiflora*. Furthermore is has longer pedicels, white or greenish yellow corolla and no leafy bracts. It is about 80 cm tall in height. It is used to treat diarrhoea (Azab, 2016).

1.1.5 *Alcea ficifolia*

Alcea ficifolia is very less common but is famous for its beautiful stains. The plant is 6-8 feet tall. It is a self seeding biennial or short perennial herb. This is mostly known as Antwerp and Fig leaved hollyhock because of its attractive foliage which is both appealing to butterfly and hummingbirds. The stakes of the flowers are tall and the flowers are 8-12 cm wide in size. The colour variations are range from cream to gold, pink, rosy etc. This plant is most resistant to rust than other species. They are most impressive as ornamental plant and for background presentations. The plant has no modern use as medication although it was used as emollient, pain reliever and diuretics in past years (Pomona Belvedere, 2008).

1.2 *Alcea rosea* plant description

Alcea rosea or hollyhock from mallow family is biennial to short lived perennial herb. The plant is 4-8 feet tall with a wide spread root system. The leaves are simple, alternate, palmately lobed with stipuli. The mild green surface of leaf blade is oval or orbicular in size. The green stem is un-branched and cylindrical. This is an annual flowering plant in particular the flowering time is from June to September. The flowers are cyclic, complete with a wide variety of colours. Besides, the seeds are brown, oval and flattered (Bruneni, 2014).

Figure 1: Alcea rosea (Hussain et al., 2014)

Taxonomic classification:

Domain - Eukaryota

Kingdom – Plantae

Phylum – Spermatopyta

Subphylum – Angispermae

Class - Dicotyledonae

Order – Malvales

Family – Malvaceae

Genus – Alcea

Species – *Alcea rosea*

(Kazemi, M. ; Aran, M. ; Zamani, 2011).

Vernacular names:

English – Hollyhock

Nepali – Citra Seavati

Urdu – Gulkhaira

Spanish – Malva real

French – Rose papale

(Rita Singh, 2019).

1.3 Different parts of *Alcea rosea*

Leaves: Leaves are alternate, stipulate, stalked, coarse and orbicular in size. Additionally, both surfaces are hairy. The blades are coordinated usually with 3-5 lobed, toothed, wrinkled and oval in size roundly coordinated.

Flowers: The flower has variety of delightful colours along with magnificent fragrance. The flower colour are ranging from white to bright, yellow, pale orange, various pink, rosy, purple, deep purple and almost black. The flowers are regular, complete, cyclic and 5-10 cm wide. It contains 5 petals, 5-lobed calyx, 6-7 lobed green epicalyxs. The spikes of the flowers are usually grown to 5-7 feet high.

Fruits: The fruits are schizocarp; shape is spherical like capsule, 1.5-2.5 cm in size, brown colour, and wrinkled surface. The fruits are many parted and contain many seeds.

Seeds: The seeds are ribbed, brown, hairy, reniform. Furthermore, they are many in quantity and 0.4-0.5 cm in size (Bruneni, 2014).

***Alcea rosea* leaves**

***Alcea rosea* flower**

***Alcea rosea* fruit**

***Alcea rosea* seeds**

Figure 2: Different parts of Alcea rosea

1.4 Chemical components of *Alcea rosea*

Alcea rosea contains amino acid, glucorunic acids, acidic polysaccharide, monosaccharides, pictinic polymers of rhamnose, galactorunic and phenolic acids such as vanillic, ferulic, syringic, p-coumaric, caffeine. In addition, it contains phenolic compounds, six types of

flavonoids such as dihyrokaempferol-4-O-ß-D-Glucopyranoside (Antioxidant activity), dihyrokaempferol (Antioxidant activity), kaempferol-3-O-6-ß-D-Glucopyranoside (Cytotoxic activity), kaempferol-3-O-ß-D-Glucopyranoside (Immune stimulant activity), Apigenin and kaempferol-3-O-α-L-rhamnopyranosyl-ß-D-Glucopyranoside etc (Azizov et al., 2007).

1.5 Medicinal uses of *Alcea rosea*

Alcea rosea has significant beneficial therapeutic effects. The flowers are used as laxative, depurative, bone fracture, gum bleeding and mouth wounds etc. The leaves have beneficial effects such as cough, cold, bronchitis, oxidative stress and anti-inflammatory. Beyond these, the plant as a whole has been reported a wide range of medicinal uses such as urinary tract infection, kidney problem, asthma, throat infection, dermatitis, dandruff, cancer, goitre, analgesic, kidney stone, diabetes, joint pain, muscles pain, dysmenorrhoea and so on (Marzieh Ahmadi, Mousa-Al-Reza Hadjzadeh, 2012).

1.6 Background study

1.6.1 Antimicrobial activity of *Alcea rosea*

To determine antimicrobial activity, 24 hours cultures of microorganisms are used. They are diluted by sterile water to achieve equivalent to 0.5 Mc Farland standards of turbidity. 24 hours culture of yeast was done to achieve 107 cfu/ml in dextrose broth. On sterile 6mm disk under aseptic condition, the absorbance of crude extract was obtained at 50°C to obtain 30 mg extract/disk. After that, the dried disks were place on the plate containing sterile microorganism. 40 mL of sterile 10% aqueous DMSO was used as control. At 37°C ager plate which contained bacteria was kept for incubation for 24 hours. The plate which contained yeast was stand in incubation for 48 hours at 27°C. Ceftazidim (30 mg/disk), an antimicrobial

agent as used positive control for bacteria. For yeast, Nystatin (25 mg/disk) which is a potential antifungal agent was using as positive control. The inhibition of the microorganism were measured to determine the antimicrobial activity (Tuba Mert, Tuğçe Fafal, 2010).

1.6.2 Hepatoprotective effects of *Alcea rosea*

Hepatoprotective analysis was done by using mice. Generously all the mice have been killed for the study. After that, liver tissue of these mice were vivisected and disinfected with saline. After that, dehydration of the tissue by ethanol was performed. Then they were washed by using xylene. Further paraffin infiltration was conducted. After all the process, tissues were cut in size of 4-5 µg. Rehydrated by using isopropyl alcohol were performed. In this process, a little amount of water was used. Further that, by xylene these were de paraffinised. The slides were kept in oven for drying to remove water. When the tissue fixation was performed, by hematoxylin and eosin these were stained. Stained section of the slides was observed by high resolution microscope. Enough amounts of photographs were taken for the further investigation. Further to measure the effect Statistical analysis needed to performed (Hussain et al., 2014).

1.6.3 Cytotoxic activity of *Alcea rosea*

The flowers are reported to have cytotoxic activity. 3.8 g sea salt was dissolved in 100 mL water and filtered. Brine shrimp eggs were used in this method. The shrimp were kept into the saline water which should be from sea. The incubated for 48 hours at 28° C. After this process, at 2000, 200 and 20 ppm each one of extract were tested. In 2 mL chloroform 20 mg extract solution was solvated and concentration of this solution is 10 mg/mL. To evaporate 500 mL, 50 mL and 5 mL were kept in vials. When evaporation was done properly, 5 mL of sea salt solution added to each vials and prepare concentration of 2000, 200 and 20 ppm. As

control by occupying chloroform (500 mL) a vial was prepared. After incubation, 10 larvae were introduced into vials of extract of different concentration of 100 ppm, 100 ppm and 10 ppm. After 24 hours the numbers of survived larvae were counted. The data was analysed for further details (Tuba Mert, Tuğçe Fafal, 2010).

1.7 Aim and objectives of the study

Aim of the study

The aim of the study is to find out the antioxidant activity of *Alcea rosea* by determining free radical scavenging, total phenolic content and total flavonoid content.

Objectives of the study

The objectives of the study are to

- Estimate the percentage of inhibition through radical scavenging in case of both the test drug and the standard drug.
- Determine the concentration at which both the test drugs and the standard drug provide 50% inhibitory effects.
- Estimate the total phenolic content compared to the reference galic acid.
- Estimate the total flavonoid content compared to the reference quercetin.

Chapter 2

Methodology

In- vitro methods such as DPPH scavenging assay, total phenolic content (TPC) assay and total flavonoid content (TFC) assay were selected to investigate the antioxidant properties of *Alcea rosea.*

2.1 DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging assay

Conduction of DPPH scavenging assay by using L-ascorbic acid as standard to analyze free radical scavenging of *Alcea rosea* leaves (R, Sakthidevi, & Mohan, 2013).

2.1.1 Chemicals and reagents

In the DPPH scavenging method, the requisite chemicals and reagents are tabulated.

Table 1: The chemicals and reagents requisite to estimate the DPPH scavenging capacity

Name of reagents/chemicals	Manufacturer
DPPH	Sigma-Aldrich Corporation, U.S.A
Methanol	Merck KGaA, Germany
L-ascorbic acid	Merck KGaA, Germany

2.1.2 Sample solution preparation

To prepare stock solution of 1 mg/mL concentration, in 100 mL methanol 100 mg of leaves extract was solvated. 0.5 mL, 1 mL, 2 mL, 4 mL and 8 mL were taken from the stock solution and methanol was added up to 10 mL to prepare different sample concentrations.

2.1.3 Flow diagram of sample solution preparation

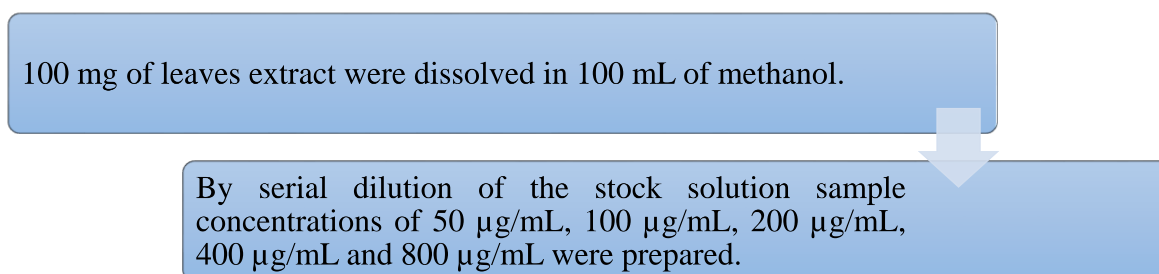

Figure 3: Preparation of sample solution

2.1.4 Standard solution preparation

To prepare stock solution of 1 mg/mL concentration, in 100 mL methanol 100 mg L-ascorbic acid was solvated. 0.5mL, 1 mL, 2 mL, 4mL and 8 mL were taken from the stock solution and methanol was added up to 10 mL to prepare different standard concentrations.

2.1.5 Flow diagram of standard solution preparation

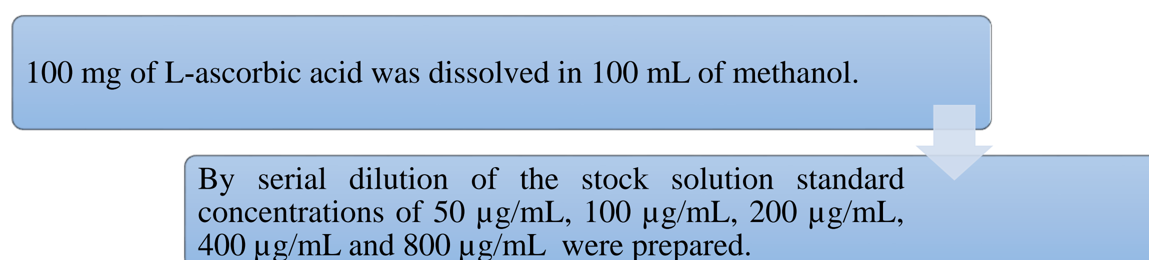

Figure 4: Preparation of standard solution of ascorbic acid

2.1.6 Reagent preparation

DPPH reagent is applied in this method. The molecular weight of DPPH is 394 g/mol. To prepare 1 M in 1000 mL of methanol 394 g DPPH need to be dissolved. For 1 mM solution of 1000 mL 394 mg DPPH requisite. So, for 0.1 mM in 1000 mL DPPH required 39.4 mg. To prepare the required solution for the experiment, in 100 ml methanol 3.94 mg DPPH was solvated for 0.1 mM solution.

2.1.7 Experimental procedure

- 3 mL of sample and standard solution of every concentration were placed in test tubes.
- In each and every tube 1 mL of DPPH was added.
- Control solution was composed of 1 mL DPPH and 3 mL of methanol.
- Blank was composed of 4 mL of methanol.
- All the solution including sample, standard and control solution were incubated at room temperature in dark for 30 minutes.
- Absorbance was taken against blank at 517 nm by using a spectrophotometer (UV 1280 Spectrophotometer).
- The following equation was applied to calculate the percentage of free radical scavenging.

$$\text{DPPH scavenged (\%)} = \frac{(\text{Acon} - \text{Atest})}{\text{Acon}} \times 100$$

Acon = the absorbance of the control solution

Atest = the absorbance of the sample/standard solution

- At last, the % of inhibitions against concentration was plotted. The equation which was acquired from the calibration curve was occupied to calculate IC_{50}. IC_{50} value refers the concentration at which inhibition of free radical is 50%.

2.1.8 Flow diagram of experimental procedure

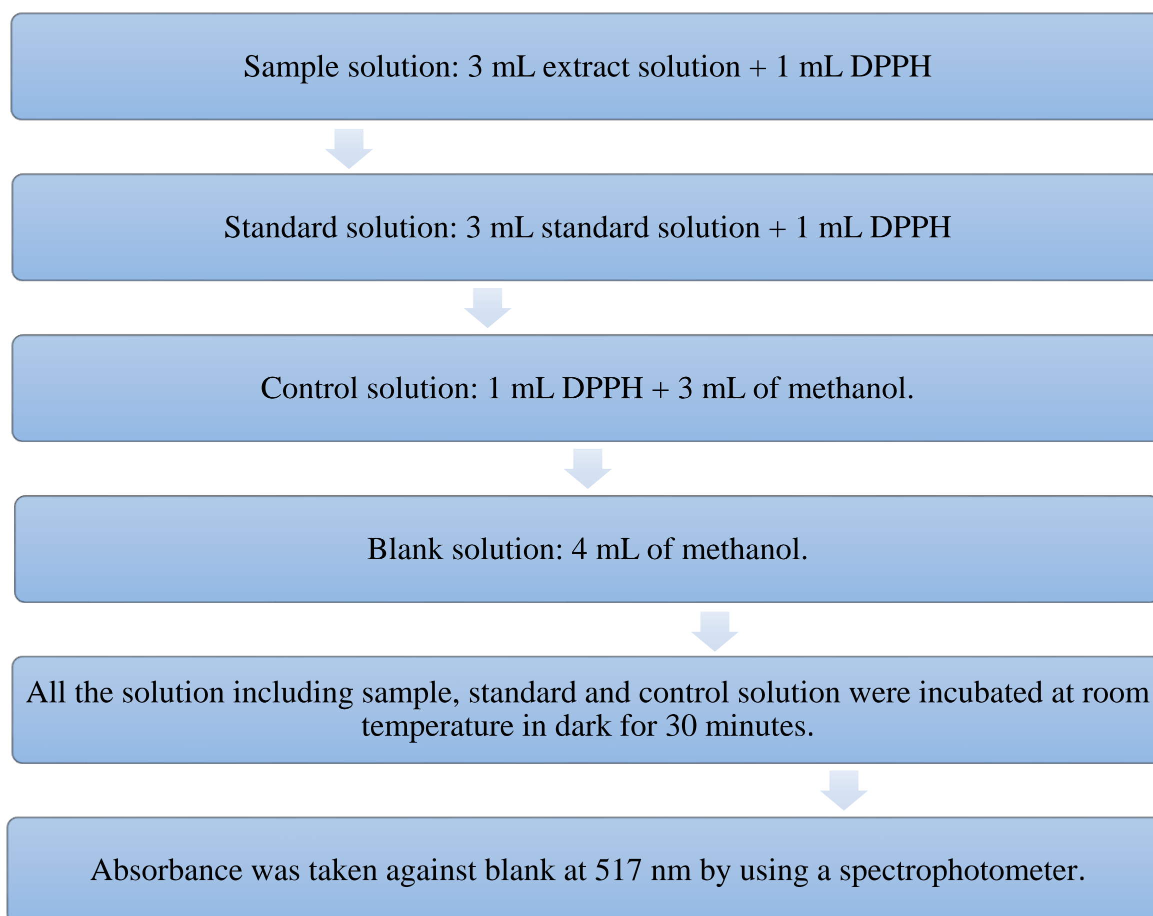

Figure 5: The method of DPPH scavegning assay

2.2 Determination of total phenolic content

In Folin-Ciocalteu reagent (FCR) assay to inspect total phenolic content was carried out by applying Folin-Ciocalteu reagent (Alhakmani, Kumar, & Khan, 2013a).

2.2.1 Chemicals and reagents

Folin-Ciocalteu reagent (FCR) assay, the requisite chemicals and reagents are tabulated.

Table 2: The chemicals and reagents requisite to the estimate total phenolic content

Name of chemicals/reagents	Manufacturer
Folin and Ciocalteu phenol reagent	Loba Chemie Pvt. Ltd., Mumbai, India
Galic acid monohydrate	Sigma-Aldrich Corporation, USA
Sodium carbonate anhydrous	Merck Specialties Pvt. Ltd.,Mumbai, India
Methanol	Merck KGaA, Germany

2.2.2 Sample solution preparation

In 100 mL methanol 10 mg extract were measured and dissolved to prepare stock sample solution of the concentration of 100 µg/mL.

2.2.3 Standard solution preparation

To prepare stock solution of 100 µg/mL concentration 10 mg of galic acid were solvated in 100 mL of methanol. 1 mL, 2 mL, 4 mL, 6 mL and 8 mL were taken from the stock solution and methanol was added up to 10 mL to prepare different standard concentrations.

2.2.4 Flow diagram of standard solution preparation

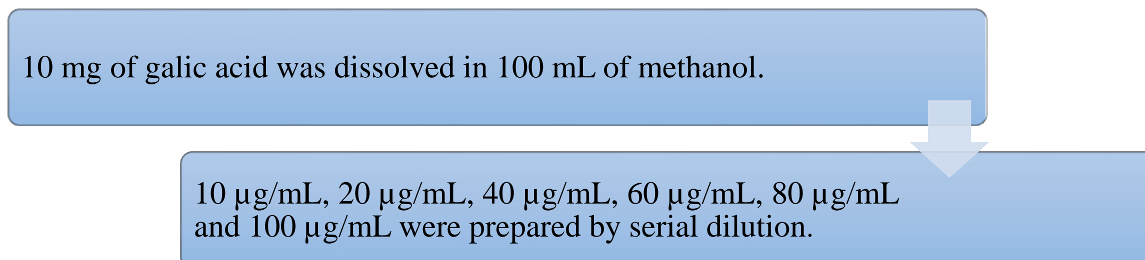

Figure 6: Preparation of standard solution of galic acid

2.2.5 Reagent and chemical preparation

To prepare reagent for the experiment 5 mL of Folin-Ciocalteu reagent (FCR) was diluted with 45 mL of distilled water which was de-ionized to produce total 50 mL volume (1:10). 7.5 g sodium carbonate anhydrous was measured and placed in volumetric flask and then added distilled water up to 100 mL (7.5% w/v).

2.2.6 Preparation of blank

The blank solution composed by 2 mL Folin-Ciocalteu reagent, 4 mL of sodium carbonate anhydrous and 0.5 mL of methanol instead of leave extract or galic acid. The total volume of the blank solution was 6.5 mL as same as sample or standard solution.

2.2.7 Experimental procedure

- 0.5 mL of sample and standard solution from different test tubes of different concentrations were placed.
- 2 mL of Folin-Ciocalteu reagent were added to each tube.
- After 3 minutes, 4 mL of sodium carbonate anhydrous was added to neutralize the solutions.

- At room temperature, all the reaction mixtures were incubated in dark for 30 minutes for colour formation.
- Absorbance was taken against blank at 765 nm by using a spectrophotometer (UV 1280 Spectrophotometer).
- Galic acid standard curve generated linear equation from which total phenolic content was calculated. The total phenolic content ware illustrated as mg/g galic acid equivalent (GAE) of dry extract.
- The formula for calculation is C = cV/m

  Where, C = total phenolic content mg GAE/g dry extract

  c = concentration of galic acid acquired from the calibration curve in mg/mL

  V = extract volume in mL

  m = leaves extracts mass in gram.

2.2.8 Flow diagram of experimental procedure

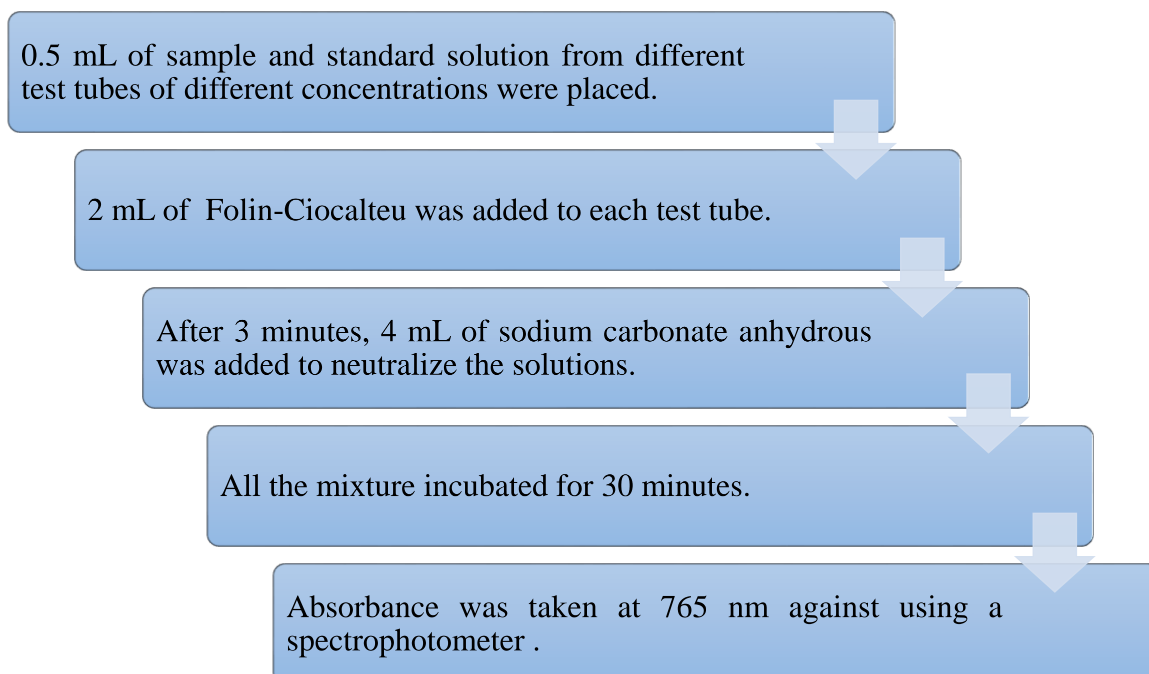

Figure 7: The method of total phenolic content assay

2.3 Determination of total flavonoid content

Aluminium chloride colorimetric (ACC) assay was carried out to evaluate total flavonoid content of *Alcea rosea* leaves (Chintalapani, Swathi, & Narasu, 2018).

2.3.1 Chemicals and Reagents

In aluminium chloride colorimetric (ACC) assay, the requisite chemicals and reagents are tabulated.

Table 3: The chemicals and reagents requisite to estimate the total flavonoid content

Name of chemicals/reagents	Manufacturer
Quercetin	Sigma-Aldrich Chemie Gmnh, Germany
Potassium acetate	Merck KGaA, Germany
Aluminium chloride	Active Fine Chemicals LTD, Bangladesh
Methanol	Merck KGaA, Germany

2.3.2 Chemical preparation

Potassium acetate was whose molecular weight is 98.15 g/mol. For 1000 mL solution of 10% of 1 M potassium acetate 98.14 g needed to dissolve. So, to formulate 100 mL of 10% of 1 M potassium acetate, 9.81 g was measured and placed in a volumetric flask and after that water was added up to 100 mL mark. 10 g aluminium chloride was measured and to formulate 100 mL of 10% aluminium chloride, 100 mL water was added up to 100 mL mark.

2.3.3 Sample solution preparation

In 100 mL methanol 10 mg extract were measured and dissolved to prepare stock sample solution of the concentration of 100 µg/mL.

2.3.4 Blank preparation

The blank solution contained 0.1 mL potassium acetate and 0.1 mL aluminium chloride as occupied in standard solution and 0.1 mL of methanol instead of extract or quercetin. The total volume of the blank solution was 4 mL.

2.3.5 Standard solution preparation

To prepare stock solution of 100 µg/mL concentration, in 100 mL methanol 10 mg of quercetin were dissolved. 1 mL, 2 mL, 4 mL, 6 mL and 8 mL of solution were taken from the stock solution and methanol was added up to 10 mL to prepare different standard concentrations.

2.3.6 Flow diagram of standard solution preparation

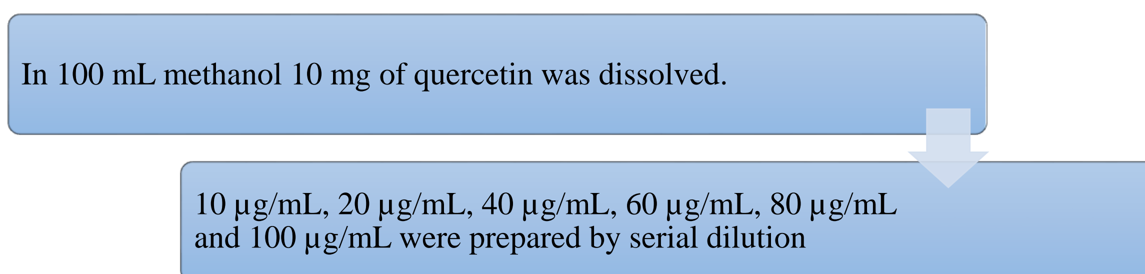

Figure 8: Preparation of standard solution of quercetin

2.3.7 Experimental procedure

- 1 mL of sample and standard solution from each concentration were placed in test tubes.
- 0.1 mL of 10% of aluminium chloride was added, 0.1 mL of 10% of 1 M potassium acetate and 2.8 mL of distilled water added to each test tube.
- The reaction mixtures were incubated at room temperature.
- Absorbance was taken against blank at 415 nm using a spectrophotometer (UV 1280 Spectrophotometer).
- Quercetin standard curve generates linear equation from which total flavonoid content was calculated. The total flavonoid content ware illustrated as mg/g quercetin equivalent (QE) of dry extract.
- The formula for calculation is A = cV/m

  Where, A = total flavonoid content mg QE/g dry extract

  c = concentration of quercetin acquired from the calibration curve in mg/mL

  V = extract volume in mL

  m = leaves extract mass in gram.

2.3.8 Flow diagram of experimental procedure

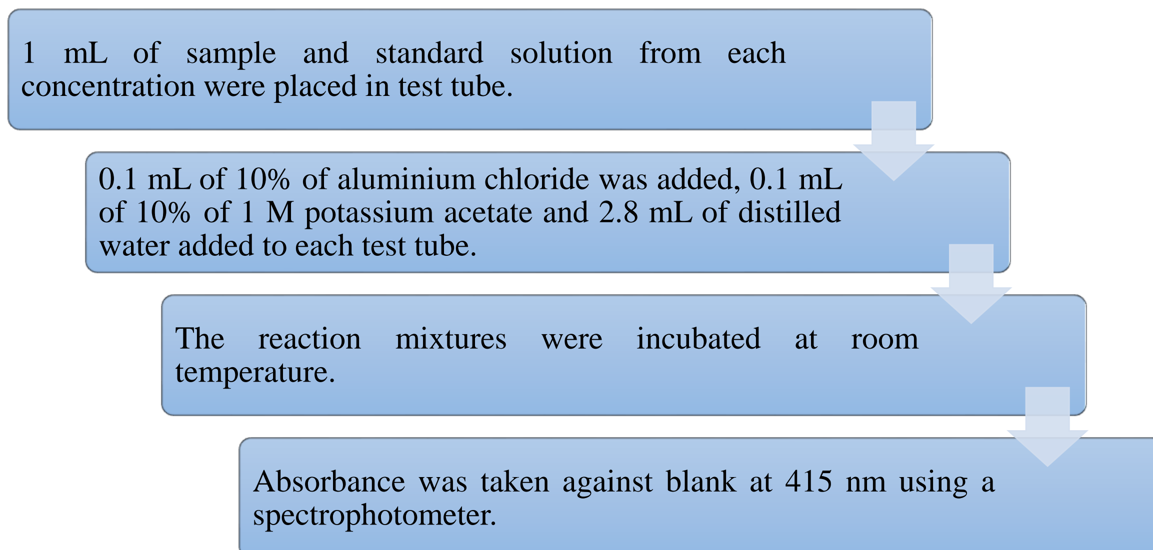

Figure 9: The method of total flavonoid content assay

Chapter 3

Results

3.1 DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging assay

3.1.1 % of inhibition in DPPH free radical scavenging assay

The concentration along with % of scavenging of *Alcea rosea* and L-ascorbic acid are given below in the table.

Table 4: % of inhibition of DPPH free radical of Alcea rosea and standard L-ascorbic acid

Concentrations (µg/mL)	**% of scavenging of L-ascorbic acid (Mean ± SEM)**	**% of scavenging *Alcea rosea* (Mean ± SEM)**
50 µg/mL	25.19 ± 0.927	18.83 ± 0.415
100 µg/mL	46.81 ± 0.308	42.64 ± 0.416
200 µg/mL	64.05 ± 0.929	55.11 ± 1.20
400 µg/mL	76.58 ± 0.329	69.40 ± 1.328
800 µg/mL	89.49 ± 0.379	84.33 ± 0.696

Interception: DPPH free radical inhibition increases with the increased of concentration of both leaves extract and standard. The highest concentration showed the highest percentage of inhibition. However, the % of inhibition was slightly higher in standard than the sample. At 800 µg/mL of plant extract (*Alcea rosea*) the highest % of inhibition was found which was 84.33 ± 0.696 and in L-ascorbic acid 89.49 ± 0.379.

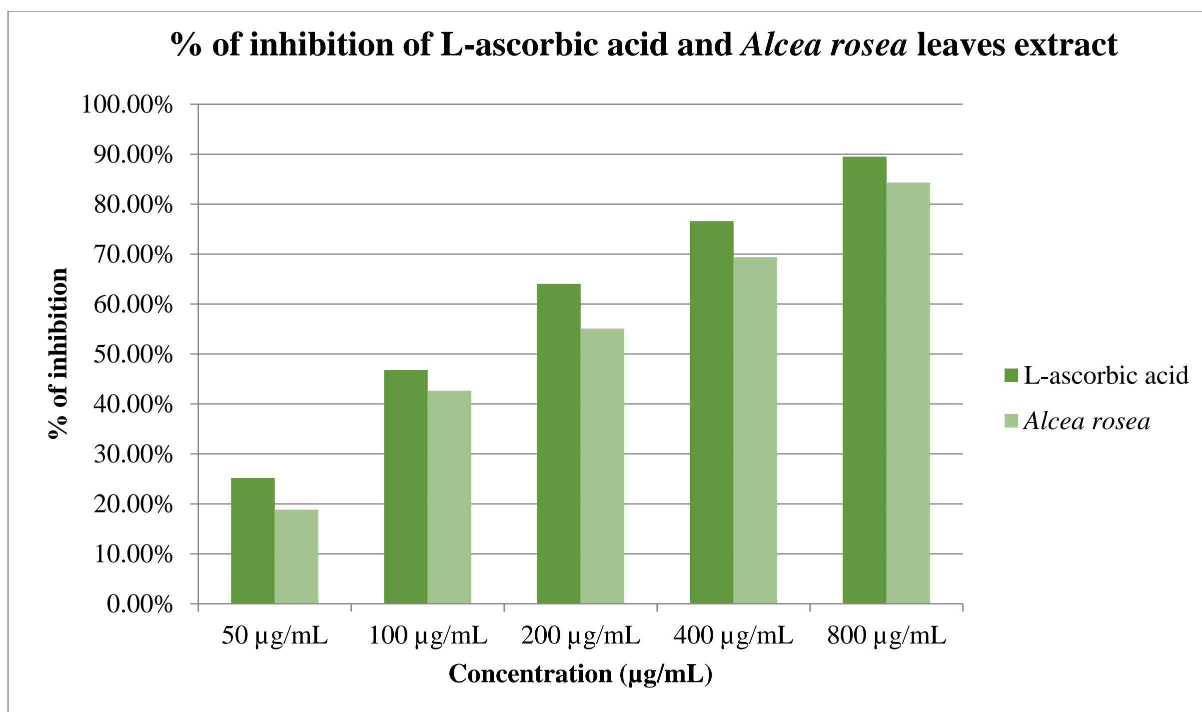

Figure 10: % of inhibition of L-ascorbic acid and Alcea rosea leaves extract

3.1.2 IC_{50} estimation of L-ascorbic acid and *Alcea rosea* leaves extract

Table 5: IC_{50} *of Alcea rosea and L-ascorbic acid*

Name of sample	IC_{50} (µg/mL)
Alcea rosea	255.13 ± 8.35 µg/mL

Name of standard	IC_{50} (µg/mL)
L-ascorbic acid	167.84 ± 5.11 µg/mL

Interpretation: IC50 value indicates the concentration to inhibit 50% of DPPH free radical scavengers. In plant extract, the IC_{50} value was 255.13 ± 8.35 µg/mL and in L-ascorbic acid was 167.84 ± 5.11 µg/mL. The result concludes that slightly higher concentrations of plant

extract was required to inhibit 50% DPPH free radical scavengers compared to L-ascorbic acid.

3.2 Total phenolic content of *Alcea rosea*

The acquired equation from the calibration curve is given below:

$y = 0.009x+0.102$

$R^2 = 0.982$

Here,

y= absorbance of the extract

m= Slope = 0.0091

c= Intercept = 0.102

Table 6: Total phenolic content of Alcea rosea

Sample solution (µg/mL)	Weight extract per mL (g/mL)	Absorbance of the extract	GAE conc. (µg/mL)	GAE conc. (mg/mL)	TPC mg GAE/g	TPC (Mean ± SEM)
100 (µg/mL)	0.0001	0.213	12.33	0.012	53.3	52.2 ± 1.1
		0.210	12.00	0.012	53.3	
		0.198	10.66	0.010	50	

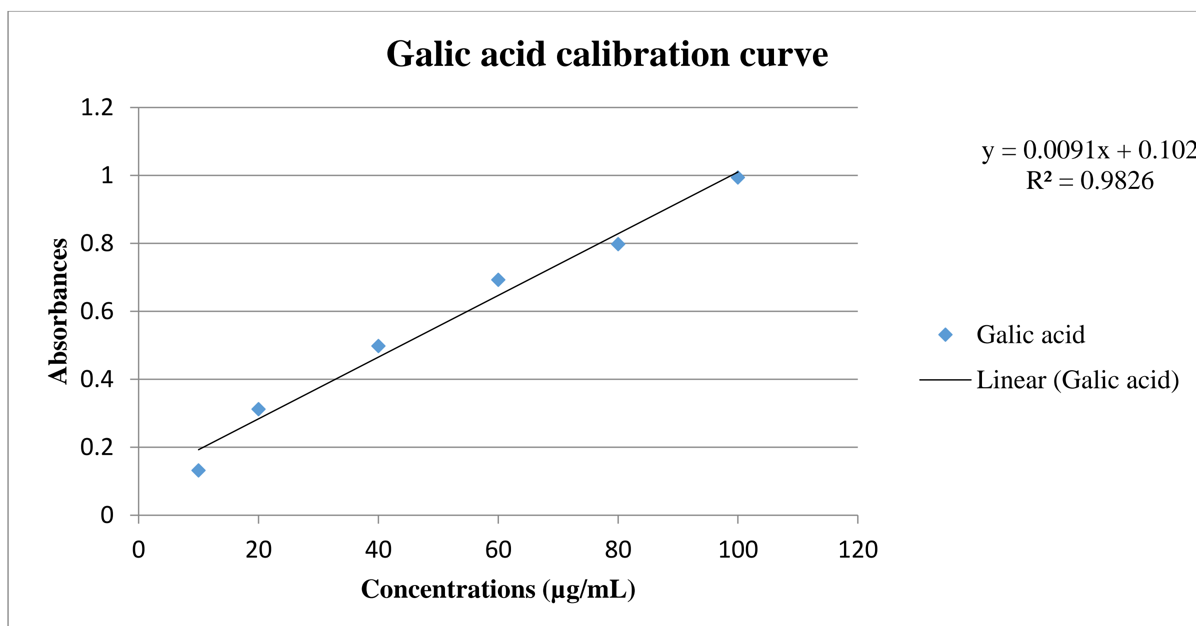

Figure 11: Standard curve of galic acid

Interception: 100 µg/mL of plant extract contains 52.2 ± 1.1 mg GAE/g. The obtained result exhibits that the plant has antioxidant activity.

3.3 Total flavonoid content of *Alcea rosea*

The acquired equation from the calibration curves is given below:

$y = 0.019x+0.167$

$R^2= 0.960$

Here,

y= absorbance of the extract

m= Slope = 0.019

c= Intercept = 0.167

Table 7: Total flavonoid content of Alcea rosea

Sample solution (µg/mL)	**Weight extract per mL (g/mL)**	**Absorbance of the extract**	**QE conc. (µg/mL)**	**QE conc. (mg/mL)**	**TFC mg QE/g**	**TFC (Mean ± SEM)**
100 (µg/mL)	0.0001	0.224	2.92	0.00292	29.2	29.4 ± 0.707
		0.222	2.82	0.00282	28.2	
		0.227	3.08	0.00308	30.8	

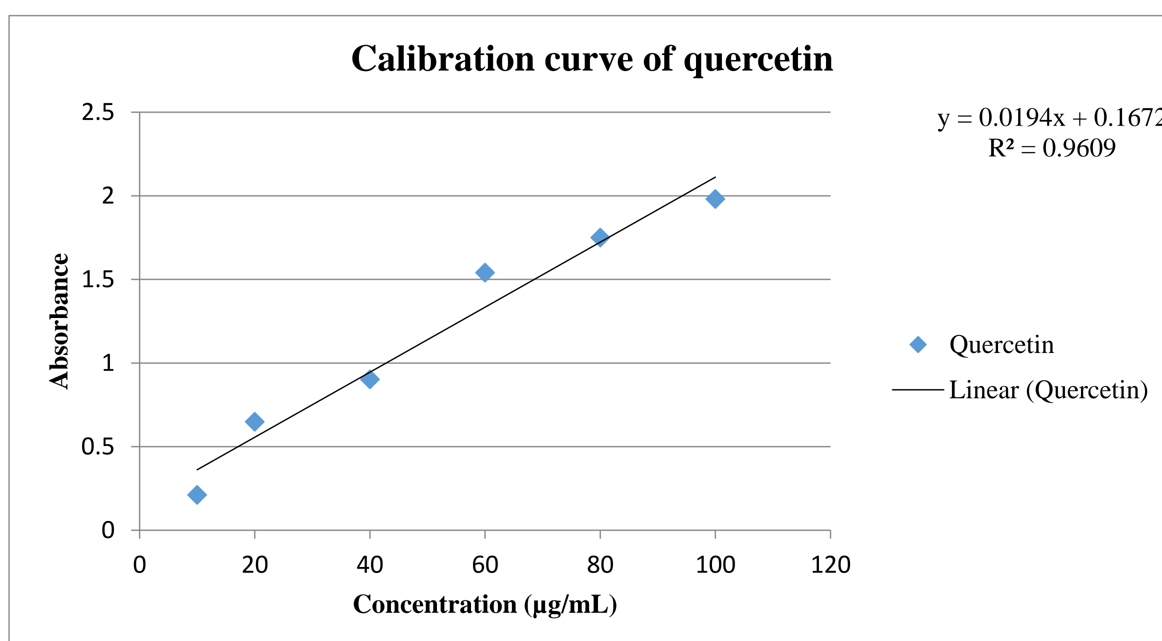

Figure 12: Standard curve of quercetin

Interception: 100 µg/mL of plant extract contains 29.40 ± 0.707 mg QE/g. Total flavonoid content exhibits the antioxidant activity of the plant.

Chapter 4

Discussion

The obtained results of the conducted experiment on *Alcea rosea* show antioxidant property. In higher concentration the % of inhibition for L-ascorbic acid and plant extract were 89.49 ± 0.379 and 84.33 ± 0.696. The IC_{50} value of both standard and sample were 167.84 ± 5.11 µg/mL and 255.13 ± 8.35 µg/mL. The estimated phenolic content and flavonoid content of leaves extract were 52.2 ± 1.1 mg GAE/g and 29.4 ± 0.707 mg QE/g accordingly in 100 µg/mL.

DPPH scavenging capacity of *Alcea rosea* was measured by comparing with L-ascorbic acid. L-ascorbic acid is a potential natural antioxidant. DPPH scavenging method is the extensively applied method for inspection of antioxidant property. DPPH is a free radical which willingly to be more stable. Consequently when DPPH free radicals react with antioxidant it accepts hydrogen radical from antioxidant. Thus there is a decrease of free radical which was observed. In this experiment at different concentration of standard exhibits higher scavenging capacity compared to the *Alcea rosea* leaves extract. The increased % of inhibition was detected along with the increased concentration of both standard and leaves extracts. However, *Alcea rosea* leaves extract seemed to give comparative results with L-ascorbic acid to inhibit 50% DPPH free radical scavengers. Thus the scavenging capacity of free radical by *Alcea rosea* leaves extracts indicates that the plant is a great source of antioxidant.

Medicinal plants demonstrate great pharmacological activity due to their secondary metabolites and phenolic compounds are most obtainable secondary metabolites in the majority of the plants. It is a predominant constitutes because it has great antioxidant activity due to its redox property and scavenging activities (Alhakmani, Kumar, & Khan, 2013b). Total phenolic content assay of *Alcea rosea* showes that the plant contains notable amount of

phenolic content. It is assumed that greater radical scavenging is shown due to higher concentration of phenolic compound present in the plant. In this assay, galic acid was applied as standard as it's a predominant phenolic compound and it is equivalent to other phenolic compound on a mass basis.

Secondary metabolites of plants also include flavonoid compounds which have the antioxidant capacity of the plants due to its antiradical property. The procured results designate that the plant has flavonoids which is used to treat many diseases for its antiradical activities.

Overall, DPPH scavenging assay, total phenolic content and total flavonoid content assay confirms that *Alcea rosea* exhibits antioxidant property. But further research should be conducted to validate possible clinical use of the extract.

Chapter 5

Conclusion

The results acquired from *in-vitro* assay on *Alcea rosea* leaves extract disclose potential antioxidant properties. In DPPH scavenging method the higher concentration displayed the higher percentage of free radical scavenging. The highest percentage of inhibition found from both plant extract and L-ascorbic acid were 84.33 ± 0.696 and 89.49 ± 0.379. L-ascorbic acid has higher antioxidant property compared plant extract. IC_{50} value of both plant and standard are 255.13 ± 8.35 μg/mL and 167.84 ± 5.11 μg/mL. Elevated concentration of extract was requisite for inhibition of 50% of free radicals compared to standard as L-ascorbic acid. The phenolic compounds and flavonoid content are the predominant constitutes of the plants which have antiradical property. The results derived from total phenolic content assay and total flavonoid content assay were 52.2 ± 1.1 mg GAE/g and 29.4 ± 0.707 mg QE/g accordingly in 100 μg/mL. These results imply that plant extract has dose dependent antioxidant properties which was comparable to the standard drug.

Chapter 6

Future work

This study established that *Alcea rosea* leaves extract is a strong antioxidant. Further study is necessary to gather a wide range of information.

- Comprehensive antioxidant study can help to understand the structural characteristics of the molecules involved further investigation.
- Other part of the plant such as bark, root, stem, flower, fruits need to be investigated for antioxidant property.
- Different solvent system can be used to investigate the antioxidant property to get more accurate and clear result.
- More *in-vitro* method should be conducted to highlight the outcomes of the plant and to analyse the mechanism of action more accurately.

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