An *In-vitro* Study on Anti-inflammatory Properties of *Alcea rosea*

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

Department of Pharmacy
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October 2019

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

The project titled "An *In-vitro* Study on Anti-inflammatory Properties of *Alcea rosea*" submitted by Prionti Saha (13146019) of Spring, 2013 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on October 10, 2019.

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Ethics Statement

This study does not involve animal trial or human trial. I have donated my own blood for the experiments done using blood in the current project. This work will only be published after ethical permission has been taken.

Abstract

Methanol extract of leaves of *Alcea rosea* (Family: Malvaceae) was assessed for its anti-inflammatory activity by *in-vitro* method. The present study focuses on the investigation of anti-inflammatory activity of methanolic extract of *Alcea rosea* by observing percentage (%) inhibition of haemolysis. *In-vitro* anti-inflammatory activity was evaluated by using membrane stabilization activity at different concentrations. Aspirin was used as a standard drug and for control normal saline was used. The result showed that *Alcea rosea* methanol extract at a concentration range of 100-500 µg/mL has different inhibitory action. At the concentration of 400 and 500 µg/mL the inhibition was more (13 and 14.5%). Heat induced haemolysis of erythrocyte was effective in inhibition at the concentration of 400 and 500 µg/mL. The results obtained in the present study indicate that methanol extract of the leaves of *Alcea rosea* have effective anti-inflammatory activity which needs further investigation.

Keywords: *Alcea rosea*; Aspirin; Anti-inflammation; Heat induced; Haemolysis

Dedication

I have dedicated my project work to the God almighty and my beloved parents.

Acknowledgement

I would like to acknowledge who have played the vital role in my academic accomplishments. First of all, God almighty who helped me to complete my project work and the whole academic journey. Then, I would like to mention my project supervisors Faria Tahsin, Lecturer and Namara Mariam Chowdhury, Lecturer, Department of Pharmacy, Brac University who were amazing by giving me the support which I needed to complete my project. I would also like to express my sincere gratitude to Dr. Eva Rahman Kabir, Chairperson, Department of Pharmacy, Brac University for providing me the opportunity and every academic support which I needed to carry out my project.

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List of Acronyms

RBC	Red blood cell
mg	Milligram
gm	Gram
ml	Millilitre
SEM	Standard Error of Mean

Chapter 1

Introduction

1.1 Inflammation

Inflammation is an adaptive response caused by injury to a living tissue, which involves an increase in the vascular permeability, increase in the protein denaturation and causes membrane alteration. Four primary indicators of inflammation can be pain, redness, heat or warmness and swelling. Again, inflammation can be caused from the response to stress. In response of tissue injury kinins, histamine, and prostaglandins are released. Moreover, an inflammatory cascade is formed consisting of physiological, behavioral, and immunologicalevents (J. Narayanan, 2018).

Inflammation is of two types. One is chronic inflammation and other is acute inflammation. Chronic inflammation is a long term dysregulated form of inflammation (Murakami & Hirano, 2012). Examples of diseases and conditions which can lead to chronic inflammation include asthma, tuberculosis, chronic peptic ulcer, rheumatoid arthritis, periodontitis, sinusitis, ulcerative colitis and Crohn's disease, active hepatitis (Ma et al., 2014).

Acute inflammation is a short term regulated form of inflammation (Murakami & Hirano, 2012). Examples of diseases, conditions and situations which can lead to acute inflammation include acute bronchitis, sore throat, infected ingrown toenail, scratch or cut on the skin, acute appendicitis, tonsillitis, dermatitis, infective meningitis, sinusitis, physical trauma (Ma et al., 2014).

The symptoms of inflammation include redness (*rubor*), heat (*calor*), swelling (*tumor*), pain (*dolor*) and immobility. During pain the inflammed area is likely to be painful, chemicals which stimulate the nerve endings are released, causes the area more sensitive. Due to the inflammation redness occurs which fills the capillaries in the area with more blood than

usual. Immobility causes loss of some function in the region of the inflammation. By an accumulation of fluid swelling is caused. Heat is caused due to the more blood flow in the affected area (Ma et al., 2014).

There are distinctive pharmacological changes which occurred during inflammation. Chemical mediators are accelerated the inflammatory process. The migration of leukocytes and the release of cytokines are played a significant role during the inflammation. Vasodilation is occurred by these chemicals which make the capillaries permeable. This is caused increased blood flow to the site of injury. Acute inflammation is occurred during the initial response of the body's harmful stimuli. During this phase plasma and leukocytes are moved progressively into the injured area. Chronic inflammation continues the progressive shift on the cells which are present at the site of inflammation. During this phase simultaneous breakdown of the tissues occur (Gunathilake, Ranaweera, & Rupasinghe, 2018).

For the treatment of inflammation, non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used. These drugs can lead to several adverse side effects including gastric irritation, gastric ulcers. Therefore, due to the presence of many biological activities, use of natural sources for the treatment of inflammation is advisable (Gunathilake, Ranaweera, & Rupasinghe, 2018).

Several experimental protocols of inflammation are used for evaluating the potency of drugs. The management of inflammation related diseases is a real issue in the rural community; the population in these areas uses many alternative drugs such as substances produced from medicinal plants (Leelaprakash & Mohan Dass, 2011).

The HRBCs (Human Red Blood Cells) membrane stabilization has been used as a method to study the *in vitro* anti-inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release. The lysosomal enzymes released during inflammation produce various disorders. The extra cellular activity of these enzymes are said to be related to acute or chronic inflammation. The non-steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane (Leelaprakash & Mohan Dass, 2011).

1.2 Other species of *Alcea rosea*

1.2.1 *Alcea angulata*

The flower of *Alcea angulata* have a significant medicinal use. It belongs to the family of Malvaceae. One of the chemical constituents of the plant is flavonoids. This species is used in the treatment of laxative, gum swelling, mouth wounds, bone fracture, depurative (Azab, 2016).

1.2.2 *Alcea apterocarpa*

The roots and shoots of *Alcea apterocarpa* have a significant medicinal use. It belongs to the Malvaceae family. This species is used in anti-inflammation, skin disorders, pulmonary disorders, urinary system disorders, intestinal disorders, stomach ailments, cough. Hexatriacontane is the main constituent of *Alcea apterocarpa* (Azab, 2016).

1.2.3 *Alcea kurdica*

Alcea kurdica belongs to the Malvaceae family. It is a widespread species found in east Iraq and west Iran. Mucilage is one of the main constituent of *Alcea kurdica*. This species is used in the treatment of gastric ulcers, duodenal ulcers, urinary tract infections, expectorant (Azab, 2016).

1.2.4 *Alcea setosa*

Alcea setosa is a medicinal plant belongs to the Malvaceae family. It is a striking perennial plant. It is generally growing with one long stem. This species is mainly used as diuretic, emollient, expectorant (Azab, 2016).

1.3 *Alcea rosea* plant description

Alcea rosea is a plant from Mallow family (Malvaceae). It is an ornamental plant. Hollyhock is a trival or common name of *Alcea rosea* widely distributed from the Mediterranean region to Central Asia. These plants are native to China and Greece, and also cultivated in Indian gardens. *Alcea rosea* is a perennial, herbaceous plant which height can go up to 100 to 200 cm, rarely up to 300 cm. Flowering period depends on location. Multi-colored, bowl-shaped flowers ranging in color from white to dark violet which have deciduous, rounded, dented, rough and big leaves (Fahamiya, Shiffa, & Mohd., 2016).

Figure 1: Alcea rosea

Taxonomic classification

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae

Order: Malvales

Family: Malvaceae

Genus: Alcea

Species: *Alcea rosea*

(Fahamiya et al., 2016)

Vernacular names

English - Hollyhock, Hock Herb, Round Dock

French – *Alcee*, *Alcee rose*, *Althee rose*

Greek – Altaia

Italian – Malvarose

(Fahamiya, Shiffa, & Mohd., 2016)

1.4 Chemical constituents of *Alcea rosea*

Acidic polysaccharides, phenolic acids (ferulic, syringic, vanillic, p-coumaric, p-hydroxyphenylacetic, p-hydroxybenzoic and caffeic), monosaccharides, amino acids, pictinic polymers consists rhamnose, galactorunic and glucorunic acids (Azab, 2016)**.**

1.5 Different parts of *Alcea rosea*

Flowers: Flowers contain nice fragrance and luminous colors. There are long spikes of flowers which grow from 5 feet to 8 feet high. The colors range from white to almost black and include shades of pink, rose-pink, salmon-rose, golden yellow, canary-yellow, dark red, purple-crimson, dark maroon and white.

Leaves: The leaves of *Alcea rosea* are coarse. The shape of the leaves are orbiculate. There is no fragrance in the leaves.

Fruits: The fruits of *Alcea rosea* are capsule in shape. The fruits are brown in color with a smooth surface (Talhouk et al., 2015).

1.6 Medicinal uses of *Alcea rosea*

The pharmacological studies have revealed that this plant have antibacterial, analgesic and anti-inflammatory activities. In Iranian traditional medicine the roots of *Alcea rosea* is used

for a wide range for treating diarrhea, constipation, bronchitis, inflammation, severe cough and angina (Wang DF, Shang JY & Yu QH, 1989). The flowers of *Alcea rosea* are having properties like, diuretic, cooling, demulcent, emollient, febrifuge and astringent (Fahamiya et al., 2016). Whole plant is beneficial in asthma, cough, jaundice, throat pain and its swelling, irritated stomach, kidney pain and urinary irritation (Lone, Bhardwaj, & Bahar, 2015).

1.7 Background study

1.7.1 Cytotoxic study of *Alcea rosea*

Cytotoxicity was evaluated by a method known as the brine shrimp lethality bioassay. The sea salt weighing 3.8 gm was dissolved in 100 mL of water and then filtered. Brine shrimp eggs were taken into the sea water and allowed to incubate for 48 hrs at 28℃ in a small tank. Each extract was tested at 1000, 100 and 10 ppm. 20 mg of plant extract was taken and dissolved in 2 mL of chloroform to prepare a stock solution of 10 mg/mL. 500, 50 and 5 mL was transferred to different vials from the stock solution and allowed to evaporate. After evaporation, 5 mL of sea salt solution was added to each vial to prepare different concentrations including 1000, 100 and 10 ppm. Each concentration was prepared in triplicate. For control a vial using chloroform (500 mL) was prepared. 10 brine shrimp larvae were introduced into vials after incubation. These vials were containing concentrations ranging from 10 to 1000 ppm of the test extracts. The number of surviving shrimps at each concentration of the extract was calculated after 24 hrs. The data was analyzed with Finney computer programme to determine the LC_{50} at 95% confidence interval (Kivçak et al., 2014).

1.7.2 Antimicrobial study of *Alcea rosea*

The disc diffusion method was used to determine the antimicrobial activities. 24 hrs cultures containing 108 cfu/mL of microorganisms were used and diluted with sterile distilled water to obtain equivalent to 0.5 Mc Farland's standards of turbidity. 24 hrs cultures of the yeast were prepared in Sabouraud Dextrose Broth to obtain 107 cfu/mL. 40 mL of reconstituted crude extracts were absorbed onto the sterile 6 mm discs under aseptic conditions to obtain 30 mg extract/disc and dried at 500℃. The dried discs were transferred on to the plates containing test organisms with sterile forceps. The control disc contained 40 mL of sterile 10 % aqueous DMSO. The agar plates containing bacteria were incubated at 37℃ for 24 hrs and those containing yeast at 27℃ for 48 hrs. The standard antibacterial agent Ceftazidime (30 mg/disc) was used as a positive control for bacteria and the standard antifungal agent Nystatin (25 mg/disc) was used as the positive control for yeast. All experiments were done in triplicate (Kivçak et al., 2014).

1.7.3 Antitussive effects of *Alcea rosea*

Marshmallow root extract and isolated mucilage polysaccharide were tested for antitussive activity in unanesthetized cats of both sexes at oral doses of 50 to 100 mg/kg body weight, in a cough induced by mechanical stimulation, in a comparison with the cough-suppressing effects of Althaea syrup (1000 mg/kg), prenoxdiazine (30 mg/kg), dropropizine (100 mg/kg) and codeine (10 mg/kg). Both the extract and isolated polysaccharide significantly reduced the intensity and the number of cough efforts from laryngopharyngeal and tracheobronchial areas. The root extract was less effective than the isolated polysaccharide. The antitussive activity was found to be lower than that of codeine, but higher than those of prenoxdiazine and dropropizine. Polysaccharides of marshmallow exhibited statistically significant cough

suppressing activity, which was noticeably higher than that of the non-narcotic drug used in clinical practice to treat coughing. By testing many plants, the most expressive antitussive activity was observed with the polysaccharide from marshmallow, containing the highest proportion of the uronic acid constituent (Al-Snafi, 2013).

1.8 Aim of the study

The aim of this research project was to evaluate the potential *in-vitro* anti-inflammatory activity of *Alcea rosea* by investigating its effectiveness in human red blood cells (HRBCs) membrane stabilization. The importance of this project lies in the fact that this species of *Alcea* has not yet been studied for this effect in the past.

1.9 Objectives of the study

The objectives of this study were:

- To calculate the percentage (%) inhibition of heat induced haemolysis of the plant extract compared to the standard drug.
- To determine whether the selected plant extracts can control membrane stabilization of human red blood cells.

Chapter 2

Methodology

2.1 Collection, identification and authentication of plant material

The plant was selected for the research because no previous studies were conducted on anti-inflammatory properties. Based on the natural availability of the plant as well as acquiring of the leaf samples *Alcea rosea* plant was selected. The leaves of *Alcea rosea* were collected from Bangladesh National Herbarium, Mirpur, Dhaka. The taxonomic authentication was conducted at the herbarium and a voucher specimen was issued mentioning the accession number (50109) which indicates that the plant was verified and authenticated by Bangladesh National Herbarium.

2.2 Preparation of the plant material

The fresh leaves were segregated after collection from the unwanted parts. The leaves were then shade dried for 10 days in an open space avoiding direct sunlight making them worthy for grinding. After grinding a uniform powder was obtained and stored in a properly cleaned air tight container in a dry place away from light and in room temperature (Bulbul et al., 2013).

2.3 Extraction process of the plant material

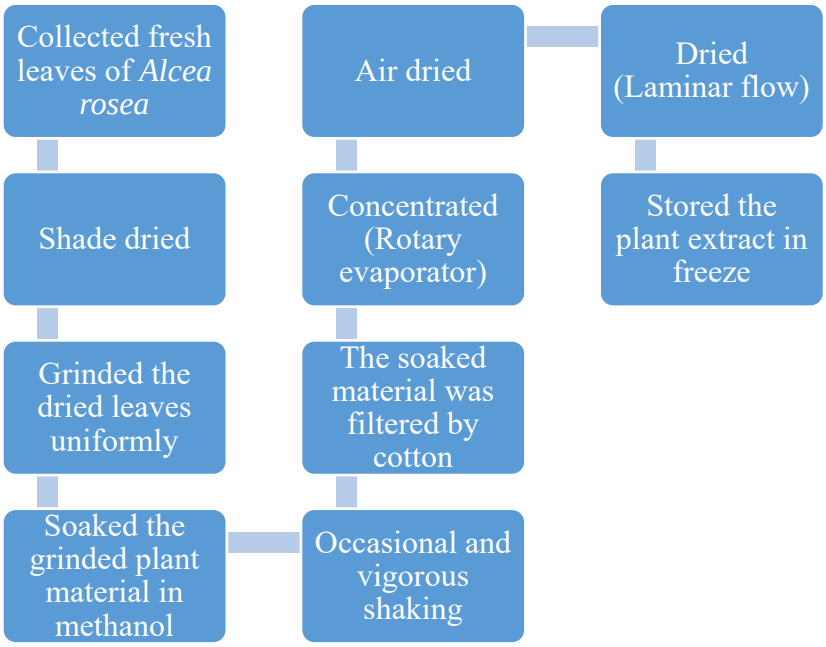

Figure 2: Extraction process of Alcea rosea

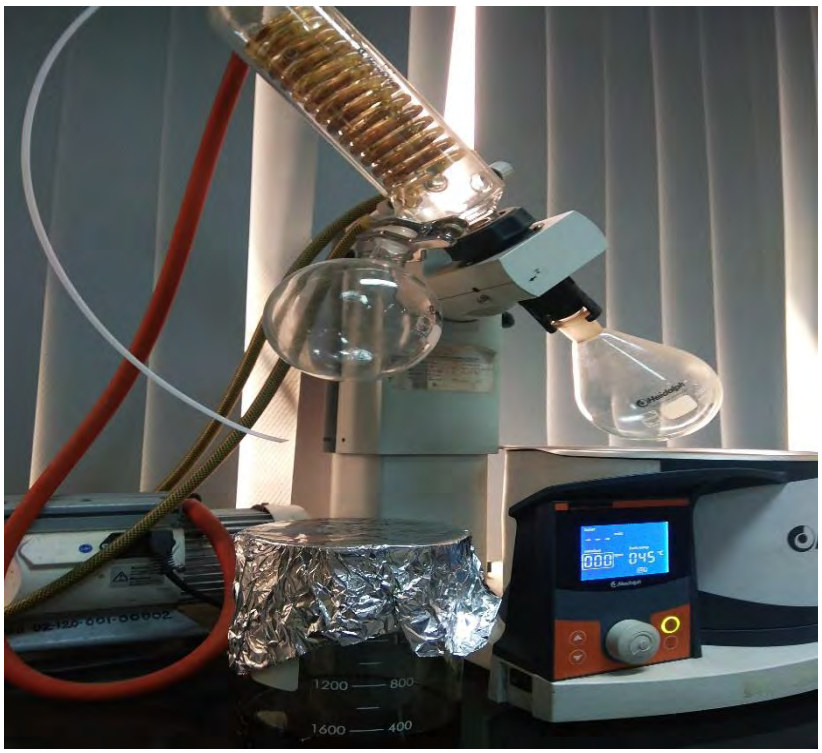

Figure 3: Alcea rosea leaves material in methanol

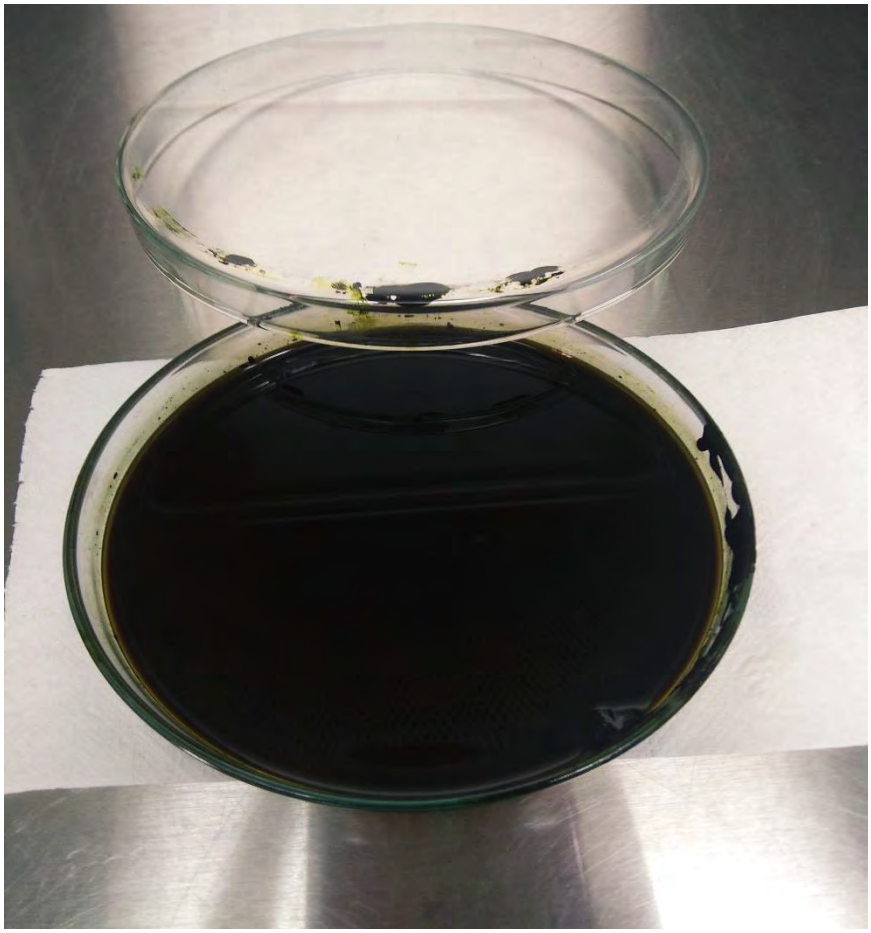

Figure 4: Methanolic extract of Alcea rosea

50 gm of dried and bristly powdered plant materials of *Alcea rosea* were soaked in 500 mL of methanol. For five days the methanol soaked plant materials were kept in an air tight container made of amber glass under cool, dark and dry conditions with occasional but vigorous shaking. The extract was then filtered through cotton filter and after then the filtrate was dried using rotary evaporator. After processing through rotary evaporator, a gel-like greenish mass was obtained which was kept in a petri dish and air dried first. Then it was kept in a laminar flow which resulted the final product which was a heavily sticky tar textured dark greenish mass (Bulbul et al., 2013).

2.4 Chemicals

Two different types of solvents were used in the formulation. Pharmaceutical grade methanol was purchased from Merck, Germany and it was used as an organic solvent. Normal saline was purchased from Beximco Pharmaceuticals Ltd. and used as an aqueous solvent. Aspirin was purchased from Square Pharmaceuticals Ltd. and was used as a reference drug for the anti-inflammatory activity test. Potassium oxalate was used as an anticoagulant, it was from Merck, Germany.

2.5 Preparation of red blood cells (RBCs) suspension

The 5 mL of blood was collected from healthy human volunteer who has not taken any NSAIDs (Non-steroidal Anti-inflammatory Drugs) for 2 weeks prior to the experiment and transferred to the centrifuge tubes. Prior to fill the centrifuge tube with human blood, anticoagulant was put into the tube to avoid the blood clot. After collecting the blood, the tubes were centrifuged at 3000 rpm for 10 minutes and were washed three times with equal volume of normal saline. The volume of blood was measured and reconstituted as 10% RBC suspension with normal saline (Leelaprakash & Mohan Dass, 2011).

2.6 Heat induced haemolysis

2.6.1 Preparation of standard drug of different concentrations:

Aspirin was used as a standard drug. The different concentrations of aspirin ranging from 100-500 µg/mL were prepared. 4 tablets of aspirin were taken and the average weight of the tablets were 150 mg. By calculation it was found that 100 mg in weight of crushed tablets consist of 50 mg of aspirin. Therefore, 100 mg crushed tablet was taken and dissolved in 100 mL of normal saline to make the concentration of the solution 500 µg/mL. After then, the

standard solution of 8 mL was taken containing standard drug and made the volume upto 10 mL by adding normal saline to dilute the concentration to 400 µg/mL. Moreover, 6 mL, 4 mL and 2 mL of solution were taken from 500 µg/mL concentrated stock solution and made it upto 10 mL by adding normal saline to make the final concentration respectively 300 µg/mL, 200 µg/mL and 100 µg/mL.

2.6.2 Flow diagram of preparation of standard drug with different concentrations:

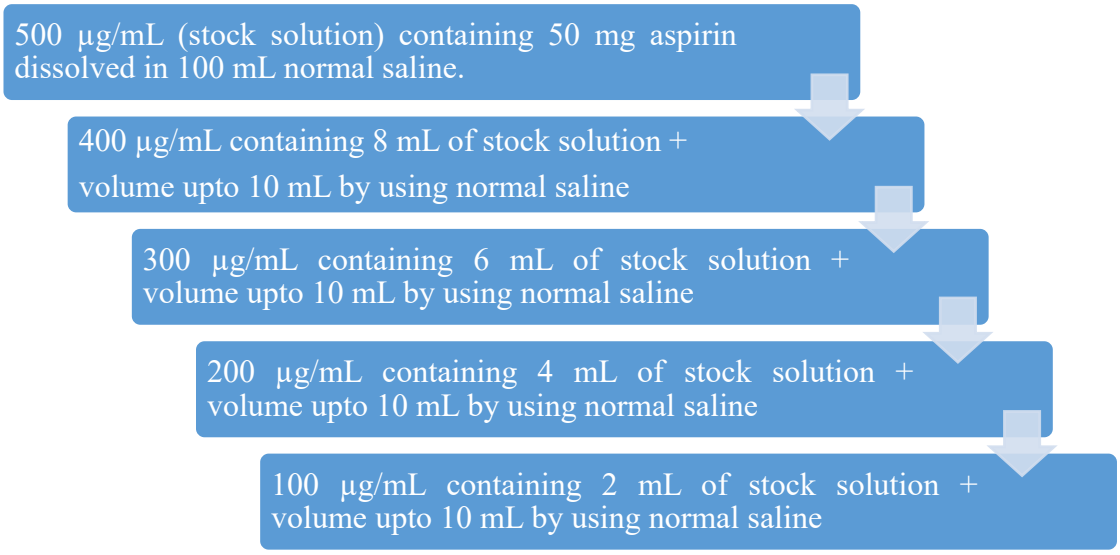

Figure 5: Flow diagram of preparation of standard drug (aspirin) with different concentrations

2.6.3 Preparation of extract of *Alcea rosea* with different concentrations:

50 mg of extract was taken. The extract was dissolved in 100 mL of normal saline to make the concentrations of 500 µg/mL. After this 8 mL was taken from the 500 µg/mL concentrated stock solution and made the volume upto 10 mL by using normal saline to dilute the concentration to 400 µg/mL. Moreover, 6 mL, 4 mL and 2 mL of solution was taken from

500 µg/mL concentrated stock solution (test drug) and made it upto 10 mL by using normal saline to make the final concentration respectively 300 µg/mL, 200 µg/mL and 100 µg/mL.

2.6.4 Flow diagram of preparation of test drug (*Alcea rosea*) with different concentrations:

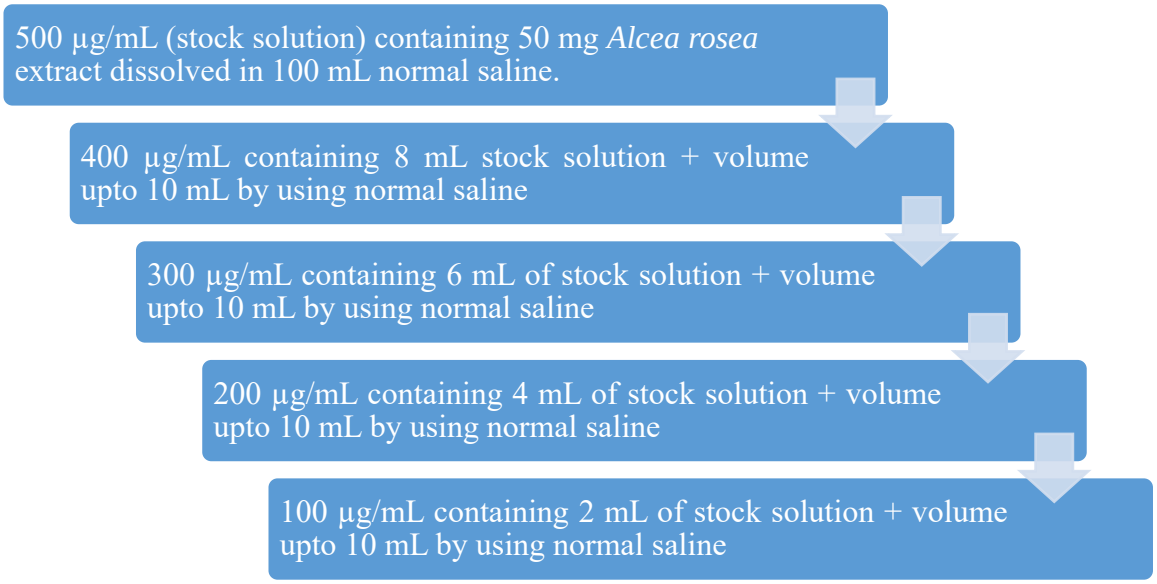

Figure 6: Flow diagram of preparation of Alcea rosea with different concentrations

2.6.5 Preparation of reaction mixtures

Control solution

In total 2 mL of volume, 1 mL of normal saline was added to 1 mL of 10% HRBCs suspension.

Test solution

In total 2 mL of volume, 1 mL of test solution of *Alcea rosea* extract with different concentration (100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, 500 µg/mL) was mixed with 1 mL of 10% HRBCs suspension.

Standard solution

In total 2 mL of volume, 1 mL of standard solution of aspirin with different concentrations (100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, 500 µg/mL) was mixed with 1 mL of 10% HRBCs suspension.

2.6.6 Procedure of heat induced haemolysis

- Different concentrations of extract of *Alcea rosea* were taken to prepare 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL and 500 µg/mL of test solution.
- Then different concentrations of standard drug (aspirin) were taken to prepare 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL and 500 µg/mL of standard solution.
- Reaction mixtures were prepared containing 2 mL of test solutions, 2 mL of standard solutions and 2 mL of control solution.
- The reaction mixtures were incubated in water bath for 30 minutes at 56℃.

- After completion of incubation, all the samples were kept under running tap water for cooling down into normal temperature.
- Then all the reaction mixture tubes were centrifuged for 5 minutes at 2500 rpm.
- Finally, the absorbance of the supernatant of each reaction mixture was measured using UV visible spectrophotometer at 560 nm.
- The experiment was done in triplicates manner for all the test samples.
- The percentage (%) of inhibition of protein denaturation was calculated by the following formula: Percentage (%) of Inhibition = (Abs control – Abs sample) × 100 / Abs control [Here, Abs = Absorbance]

(Leelaprakash & Mohan Dass, 2011)

2.6.7 Flow diagram of heat induced haemolysis method:

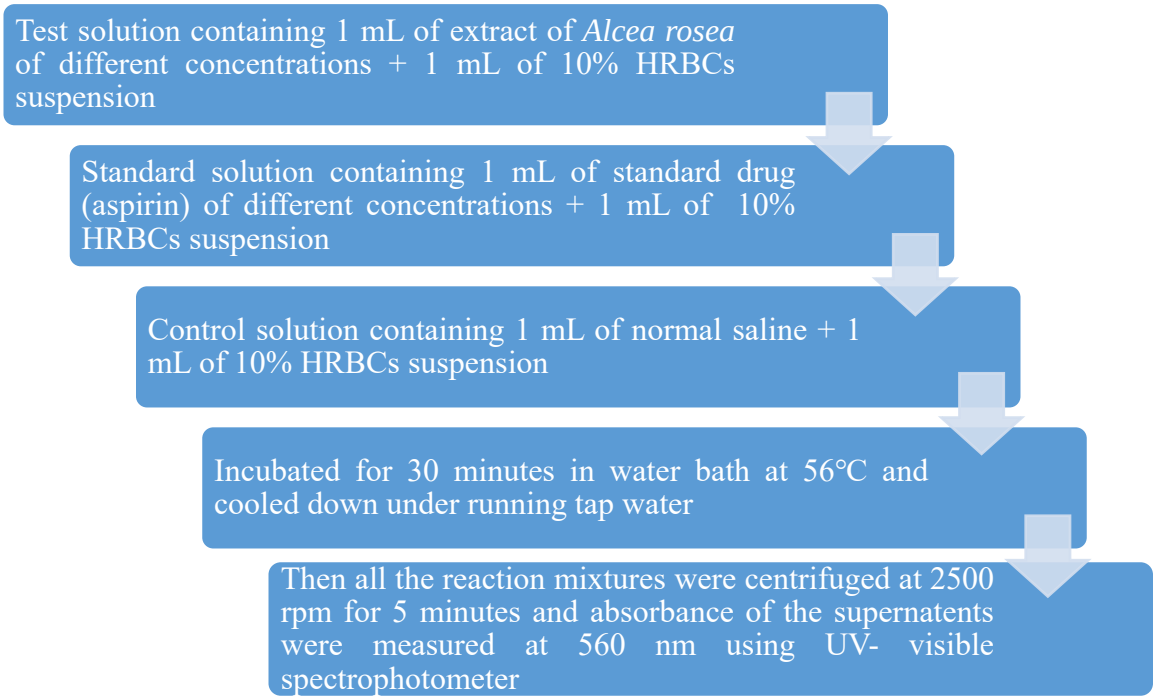

Figure 7: Flow diagram of anti-inflammatory activity testing procedure

Chapter 3

Results

3.1 Percentage (%) yield of plant extract

The total weight of the powdered plant:

50 gm of the powdered plant was measured by using automated balance which was enough for maceration process to be run.

The final weight of the plant extract:

After completion of the maceration process, it has been seen that the total weight of the powdered plant was decreased and the following table is showing the comparative weight variation:

Table 1: The total weight of the Alcea rosea extract after subsequent maceration process

Initial weight of Petri-dish	70.155 gm
Final weight of Petri-dish (Extract + Petri-dish), (W2)	74.602 gm
Final weight of the extract, (W1)	4.447 gm

Elucidation: 4.447 gm of plant extract in total was obtained from 50 gm of powdered plant as a result of maceration and subsequent drying process regarding methanolic extract of *Alcea rosea* that was performed to carry out the desired experiment.

Calculation of the percentage yield of *Alcea rosea* extract:

Formula: Extract yield (%) = (W1 × 100) / W2

Where, W1 = Net weight of the extract after maceration

W2 = Total weight of the powder taken for extraction

Therefore, percentage (%) yield of extraction = (4.447 × 100) / 50

= 8.894%

3.2 Table of haemolysis inhibitory activity of extract

The concentration, absorbance and percentage of inhibition of haemolysis done by extract (*Alcea rosea*) is shown below in the table:

Table 2: Effect of Alcea rosea on heat induced haemolysis of erythrocyte

Treatment(s)	Concentration (µg/mL)	Absorbance at 560 nm	Percentage (%) inhibition of haemolysis
Control	-	1.854	-
Alcea rosea	100	1.786 ± 0.002	3.67
Alcea rosea	200	1.776 ± 0.001	4.21
Alcea rosea	300	1.664 ± 0.002	10.24
Alcea rosea	400	1.599 ± 0.002	13
Alcea rosea	500	1.585 ± 0.005	14.5

Interpretation: From the table (2), it was seen that the percentage (%) of inhibition of haemolysis was increasing with the increasing number of concentration. The initial inhibition was 3.67% at concentration 100 µg/mL where the inhibition had increased to 14.5% at concentration 500 µg/mL. This indicates the dose dependent relationship of concentration and inhibition. The result showed that at 400 µg/mL and 500 µg/mL concentration protect the erythrocyte membrane against lysis induced by heat.

3.3 Table of haemolysis inhibitory activity of standard drug

The concentration, absorbance and percentage of inhibition of haemolysis done by standard drug (aspirin) is shown below in the table:

Table 3: Effect of aspirin on heat induced haemolysis of erythrocyte

Treatment(s)	Concentration (µg/mL)	Absorbance at 560 nm	Percentage (%) of inhibition of haemolysis
Control	-	1.854	-
Aspirin	100	1.647 ± 0.014	11.17
Aspirin	200	1.575 ± 0.004	15.05
Aspirin	300	1.472 ± 0.002	20.6
Aspirin	400	1.317 ± 0.002	28.96
Aspirin	500	1.254 ± 0.002	32.36

Interpretation:

From the table (3), it was seen that the percentage (%) of inhibition of haemolysis was increasing with the increasing number of concentration. The initial inhibition was 11.17% at concentration 100 µg/mL where the inhibition had increased to 32.36% at concentration 500 µg/mL. This indicates the dose dependent relationship of concentration and inhibition. The result showed that at 400 µg/mL and 500 µg/mL concentration significantly protect the erythrocyte membrane against lysis induced by heat.

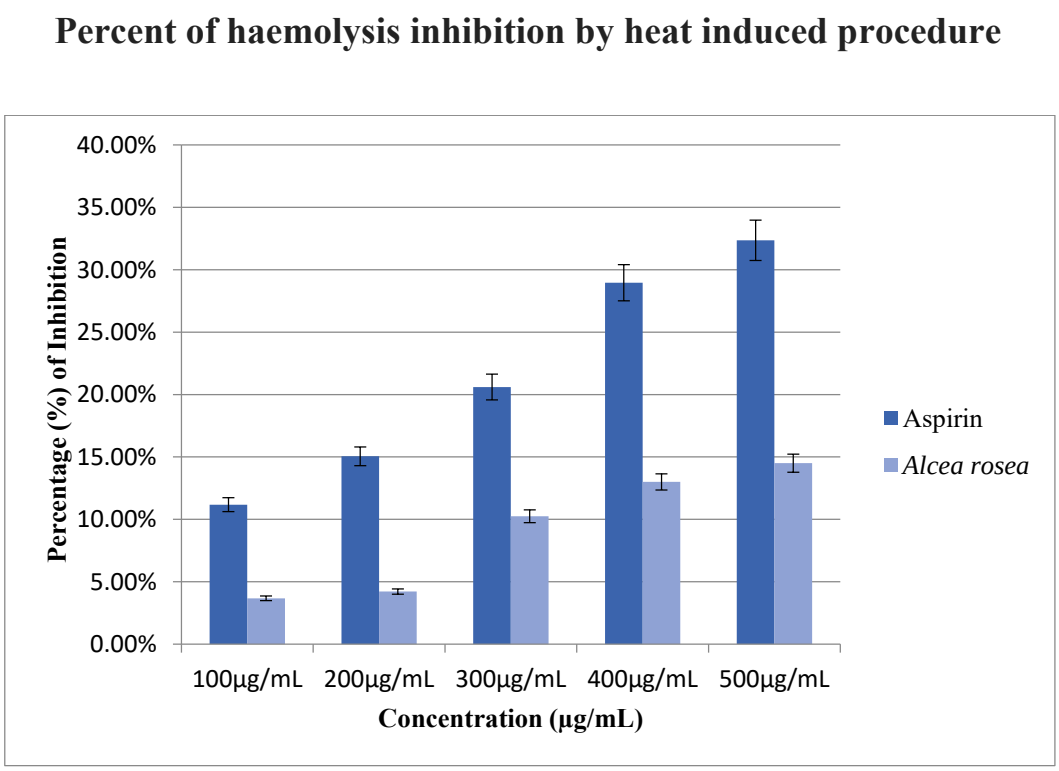

Figure 8: Percentage (%) haemolysis inhibition concentration (µg/mL)

Chapter 4

Discussion

Based on the heat induced haemolysis process, the *Alcea rosea* leaves extract was showed comparable anti-inflammatory activity with standard drug (aspirin).

The results showed that *Alcea rosea* at concentration 400 and 500 µg/mL have potential inhibitory activity of 13 and 14.5% (Table 2) whereas the standard drug (aspirin) offered a significant protection at 400 and 500 µg/mL concentration of 28.96 and 32.36% (Table 3).

So, from the above experiment regarding anti-inflammatory property investigation it has been found that the methanolic extract of *Alcea rosea* may be used for anti-inflammatory treatment for its effectiveness against inflammation.

Chapter 5

Conclusion

In the present study, results indicate that the methanol extracts of *Alcea rosea* leaves possess potential anti-inflammatory activities. Among the different concentrations, *Alcea rosea* leaves extract appeared to exhibit anti-inflammatory activity on heat induced haemolysis. The lower concentrations of the extract did not appear to show effective results. The aim of this study was to investigate the anti-inflammatory activity of *Alcea rosea*. It was done by observing percentage (%) inhibition of haemolysis by heat induced haemolysis where aspirin was used as standard drug. Moreover, further *in-vivo* and *in-vitro* studies and additional researches are needed to further prove whether the plant does possess this activity. In addition, further studies can be carried out on this plant like the isolation of the phytocomponents will be helpful. Finding out the actual mechanism by which *Alcea rosea* may show anti-inflammatory activity to provide inhibition against haemolysis is also a necessity to determine the molecular basis of how *Alcea rosea* exerts its effects.

Chapter 6

Future work

- There is specific mode of action done by the *Alcea rosea* leaves extract to exhibit the anti-inflammatory activity. To be more clear about the process of anti-inflammatory activity done by *Alcea rosea*, further phytochemical screening needs to be evaluated.
- Furthermore, more *in-vivo* and *in-vitro* studies should be conducted on anti-inflammatory and also other properties which have not proved yet.

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