In-Vitro Antacid Screening of Methanolic Leaf Extracts Of *Elaeocarpus floribundus*

By

Ishrat Jahan
15146035

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

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Declaration

It is hereby declared that

1. The thesis submitted is my/our 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.

Ishrat Jahan
15146035

Approval

The thesis/project titled "In-vitro antacid screening of methanolic leaf extracts of *Elaeocarpus floribundus"*submitted by Ishrat Jahan (15146035) of spring 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on February 27th, 2020

Examining Committee:

Supervisor:
(Member)

Namara Mariam Chowdhury
Lecturer, 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 and Chairperson, Department of Pharmacy
Brac University

Ethics Statement

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

Abstracts

Elaeocarpus floribundus is a sort of tropical and subtropical evergreen trees and shrubs. Elaeocarpaceae is a family which has very nearly 615 types of plants in 12 genera. Among them with roughly 350 species Elaeocarpus is the biggest genera which has anti-ulcer, anti-inflammatory, anti-hypertensive, anti-diabetic, anti-bacterial property. This present study focuses on the in-vitro antacid screening of methanolic leaf extracts by doing preliminary antacid test, acid neutralizing capacity, acid neutralizing effect of extracts on GSF and lastly acid buffering capacity. Concentration of 1 mg/mL of leaf extract and 1 mg/mL of standard sodium bicarbonate was used for conducting all the tests. Methanolic extract of leaves shows very poor antacid properties. Using methanol instead of ethanol or aqueous extraction can be a reason for not showing proper result. However, a comparative study between methanolic and ethanolic extracts can be done in future to evaluate better results. Moreover, an anti-ulcer test can also be done from the leaf extracts.

Keywords: *Elaeocarpus floribundus,* methanolic extract, antacid, anti-ulcer

Dedication

Dedicated to my parents and family for their immense support and for having faith in me.

Acknowledgement

First of all, I would like to thank almighty God for giving me an opportunity to complete my project and overcome all the obstacles faced doing this project. I would never finish this project without His mercy. I would also like to thank and express deepest gratitude to my most esteemed supervisor Namara Mariam Chowdhury, Lecturer, Department of Pharmacy, Brac University for allowing me to work under her supervision and without her continuous encouragement, motivation, support, help and guidance; I would never be successful to finish my project. I also want to express my gratitude to Professor Dr. Eva Rahman Kabir, Chairperson, Department of Pharmacy. I also want to thank all the Lab Officers and Lab Assistants for their utmost help

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List of Acronyms

mmol	Millimole
mg	Milligram
mL	Milliliter
GSF	Artificial Gastric Juice
HCl	Hydrochloric acid
$NaHCO_3$	Sodium bicarbonate
SEM	Standard Mean Error
ACN	Acid Neutralizing Capacity

Chapter 1

Introduction

For centuries, people have been using plant sources for treating diseases. Different types of human diseases are treated with drugs and among them 87% are from plant sources(Zaman et al., 2016)Specifically, medicinal plants are rich in various chemical constituents or active compounds such as thymol, carvacol, saponin, terpenoids, flavonoid, phenol, etc. which are used in drug development and synthesis. All these active components have different therapeutic agents including antimicrobial, antihypertensive, laxative, ant diabetic, antiulcer, anticancer etc. from plants. For example, Papaver somniferum (poppy) is a plant from which Morphine was obtained. Morphine is the main alkaloid of opium and also precursor to other opioids such as codeine, fentanyl, methadone, hydrocodone, hydromorphone, meperidine, and oxycodone. Morphine is one of the most potent analgesics for reducing chronic pain. Despite of being potent its use is limited due to severe withdrawal syndrome, induction of tolerance and high risk of relapse and abuse. Plants with different medicinal properties can be the best source for obtaining variety of drugs according to WHO (World Health Organization). Medicinal plants are evidenced to be useful against microbial agents, open sore or ulcer. Huge numbers of medicinal plants are naturally available which can be used as antiulcer agents. Peptic ulcer is a chronic disease affecting up to 10% of the world's population. Development of open sores on the lining of stomach, lower esophagus or in the small intestine defines peptic ulcer. The formation of peptic ulcers depends on the presence of H. Pylori bacteria, gastric juice pH, erosion of stomach acid, and the decrease in mucosal defenses. When the amount of hydrochloric (HCl) acid secretion increases or mucus secretion decreases the protective layer of stomach lining breaks down and develops an open sore which is commonly known as ulcer. One of the major complications of peptic ulcer includes bleeding which is dangerous as it can cause hemorrhage. Few other complications are

perforation, penetration and gastric outlet obstruction. The management of peptic ulcer disease and its complications remain a challenge and for that the demand of new anti-ulcer drug is increasing. Chemical constituents like secondary metabolites, alkaloids, flavonoids, terpenoids shows great anti-ulcer activity. Antacids can also be included in drug regimen to treat ulcer as it neutralizes the stomach acid and provides rapid relief from pain. Moreover, it reduces the pepsin activity and helps to bind bile acids. Calcium carbonate, sodium bicarbonate, aluminum hydroxide and magnesium hydroxide are the common active ingredients which are used in antacid preparation. Generally, antacids are used in combination such as Aluminum Hydroxide, Magnesium Hydroxide are available. These types of antacid preparations are effective in stomach acid neutralization, binding bile acids, reduction of pepsin activity and it might stimulate local prostaglandin production. Adverse effects like constipation, diarrhea and chronic renal failure may occur if these preparations are used frequently. As a result an alternative antacid should be introduced this will give more beneficial effects with less side effects. (Cua et al., 2018)

Elaeocarpaceae is a family which has almost 615 species of plants in 12 genera. Among them with approximately 350 species Elaeocarpus is the largest genera. Elaeocarpus Floribundus is a species of this genus which has medicinal properties. This plant is readily available in India, Myanmar and in few regions of Bangladesh. Few researches were conducted to find out the phytochemical, antibacterial and antioxidative properties of this plant. However, very little or less amount of studies has been conducted on Elaeocarpus Floribundus. One of the major one was extraction from leaves which shows activity against CEM-SS cancer cell. Leaves and barks of Elaeocarpus Floribundus has anti-ulcer properties so this might give antacid effect. Other than that, an infusion of the bark and leaves is used as a mouthwash for inflamed gums. For treating hypertension and diabetes leaf decoration was

used in Mauritius.(*Indian Olive*, 2017) Moreover, enough research scope is there particularly for this species.

1.1 Other Species of Elaeocarpus

Elaeocarpus Sphaericus

Elaeocarpus Sphaericus which is mainly known as Rudraksha (Hindi) or Bead tree (English) is very famous in India. From Myanmar to the whole North-East India, Bangladesh, Nepal, Bhutan this species is available in different names. Rudraksha is an evergreen, perennial broad-leaved tree with a large spreading crown. The dried fruit is called bead which has electromagnetic property and amazing healing power. It's a belief that the beads have positive effect on those who suffer from anxiety and reduce body temperature and also have calming effect. On the other hand, E. Sphaericus has vast use in the treatment of depression,

Figure 1: E. Sphaericus Tree

Figure 2: E. Sphaericus fruit

palpitation, stress, hypertension, migraine, epilepsy, indigestion and so on. This species contains a lot of chemical constituents like alkaloids, glycosides, steroids, flavonoid (quercetin), tannins (gallic and ellagic acids), fatty acids (palmitic and linoleic acids), carbohydrates, proteins and ash(Pant et al., 2013)

Elaeocarpus Serratus

Elaeocarpus Serratus is commonly known as Ceylon olive. It's an 18-meter-tall evergreen tree which has a few medicinal applications. This tree is available in the region of E. Asia - India, Nepal, Sri Lanka, Myanmar, Malaysia, Indonesia. Only the fruit part of this tree is edible which is rich in starch and sugar and have small amount of iron and protein. Moreover, this can be used in the treatment of dysentery and diarrhea. The leaves are used as antirheumatic agent and an antidote to poisoning. Wood is suitable for general planking, boxes, crates, wooden pallets and plywood.

Figure3: E. Serratus leaf

Figure 4: E. Serratus flower

Figure 5: E. serratus fruit

Elaeocarpus Angustifolius

Elaeocarpus Angustifolius is commonly known as blue marble tree and open canopy can grow up to 35 meters tall. It is found in the region of E. Asia - China, Indian subcontinent, Myanmar through Malaysia, Cambodia to Australia and the Pacific. Though it has very minor medicinal applications but grown as ornamental. This species is also edible. The fruit is fleshy and used in the treatment of epileptic fits. The leaf sap can be used to treat stomach

ache or chest pain. Seeds of *E. Angustifolius* is very useful and used as remedy of blood pressure and heart ailments. Once in Philippines bark of this tree was used to treat enlarged spleen. This species is moderately used in other commercial purpose like general planking, shuttering, boxes, crates, wooden pallets, boat planking, racing oars, match splints, veneer and plywood and also useful for lightweight carving timber, also suitable for barrels.

Figure 6: E. Angustifolius fruit

Figure 7: E. Angustifolius tree

1.2 Description of the plant *Elaeocarpus Floribundus*

Elaeocarpus Floribundus which is an evergreen tree with a spreading crown. This species often grows smaller though the height could be around 30-40 meters. It is generally found in the wild as well as in the house garden for edible parts. General habitat of this species is lowland rainforest and lower mountain forest, up to 1500 m altitude. Elaeocarpus Floribundus can be propagated by stones, which should be sown in the shade and have about 15% germination in 4–8 months. (Sircar & Mandal, 2017)E. Floribundus is widely distributed in India, Bangladesh, Myanmar, Bhutan, Malaysia and Indonesia. The wood is soft to moderately hard, light in weight to moderately heavy, not very durable.

Figure 8: E. Floribundus Tree

Taxonomical Classification

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Oxalidales

Family: Elaeocarpaceae

Genus: Elaeocarpus

Species: Elaeocarpus Floribundus

Common Names

Assamese-Jolphai, Jalphai, Jalpai

Bengali-Belphoi

English-Indian Olive

Hindi-Jalpai

Karbi-Theng koreng

Nepali-Koving

Others-Indian Olive

Synonyms of E. Floribundus

Elaeocarpus floribundas var. tahanensis (Hend.) Ng
Elaeocarpus grossus Wall. [Invalid]
Elaeocarpus lobbianus Turcz.
Elaeocarpus pseudosepicanus O.C. Schmidt
Elaeocarpus tahanensis M.R. Hend

1.3 Different Parts of the Plant

Leaf-

Leaves of *E. Floribundus* are simple, alternate and spiral. They are 3-5cm long and crowded at the end of twigs, petiole 1-5.5cm long with joint at apex. The leaves are ovate, elliptic, often rounded at the base. The margin of leaf usually toothed and pinnately veined with 5–7 pairs of lateral veins. They are normally dark green in color but become red before falling. Leaves of Elaeocarpus Floribundus is very useful in the treatment of diabetes and hypertension. Once it was used to treat inflamed gums in the form of mouthwash by the people of Philippines.

Figure 9: E.floribundus Dry leaf (Red)

Figure 10: E.floribundus Green leaf

Flower-

Elaeocarpus Floribundus flowers are generally white in color and small in size. It has triangular sepals which is narrow and petals are obovate-oblong, ellipsoid flower-buds, 25-40 stamens. This flower is bisexual and has tiny- hairy part, filaments 1 mm long, slender, minutely puberulous, anthers 2 mm long, oblong, bearded disc silky villous, ovary 3-loculed, silky villous. Suitable flowering time is from May- August.

Figure 11: Flower

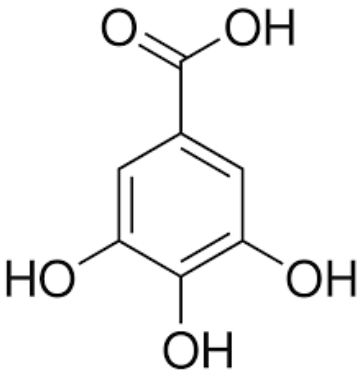
Fruits-

Elaeocarpus floribundus fruit is sour in taste. It is commonly known as Jalpai or belphoi. Fruits are harvested during August to October. This fruit is pale green in color and oval in shape. Edible portion of this fruit is the mesocarp around the seed and single seeded.(Mani & Bhowmick, 2017) The green fleshy drupes are about 22m long and slightly acidic. This fruit can be eaten as raw or in the form of pickle.

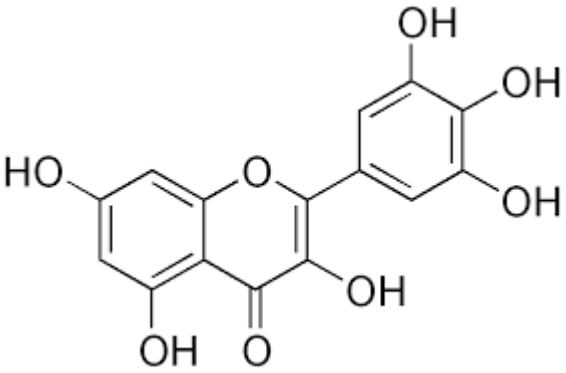
Figure 12: Fruits

1.4 Chemical constituents of *Elaeocarpus Floribundus*

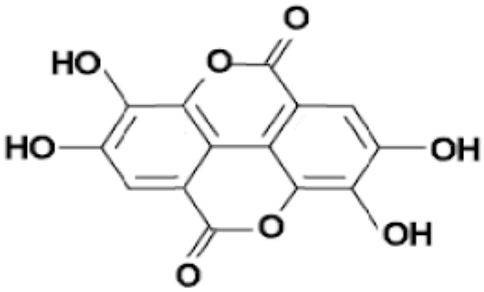
Elaeocarpus belongs to the family Elaeocarpaceae. This genus shows different biological activities like affinity for opioid receptor, cytotoxicity and antioxidative property and for those they contain various compounds. Elaeocarpus Floribundus is a plant of tropical and subtropical region and widely distributed throughout Asia. In a report it was shown that 15 components and 3 additional components as mixture were isolated from the leaves. This includes four fatty acids, three diterpenoids, one triterpene alcohol, two fatty alcohols, three phaeophytins, one phytosterol, one sesquiterpene, and three hydrocarbons from the hexane extract of the leaves. (Ayorinde Victor Ogundele & Archana Moni Das (2019) Epifriedelanol and Friedelanol are the two components which were found active against cancer cells. The fruit which is mainly known as Indian olive is slightly acidic in taste as it has excessive amount of citric acid in it. Other than citric acid it contains tannin, rhamnose (11%), arabinose (26%), galactose (35%) and uronic acid. Vitamin-C, myricitrin, myricetin, mearnestin and ellagic acid are compounds which was found in leaves (Sonia Zama,2016).



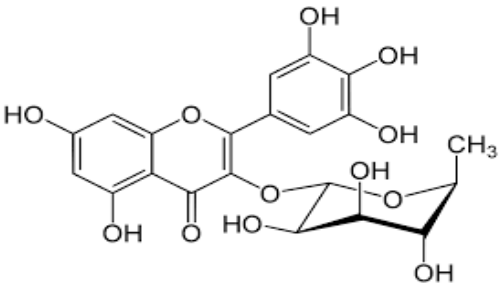
Gallic acid



Myricetin



Ellagic Acid



Myricitrin

1.5 Aims and Objectives of this study

Aim-

The aim of this study is to evaluate the in vitro antacid activity of E. Floribundus as it shows anti-ulcer properties using preliminary antacid test, acid neutralizing capacity, neutralizing effect of extracts on GSF and acid buffering capacity.

Objectives-

The objectives of this study are-

- Evaluating the antacid effect of the plant extract compared to the standard.
- Performing preliminary antacid test of the plant extract compared to the standard.
- Determination of acid neutralization capacity of the plant extract compared to the standard.
- Calculate acid buffering capacity of the plant extract compared to the standard.
- Determination of neutralizing effect of extracts on GSF of the plant extract compared to the standard.

Chapter 2

Methodology

2.1 Collection and identification of plant material

In the month of November-December Elaeocarpus Floribundus plant was collected from Moulovibazar, Lowachora, Bangladesh. This tree is locally known as “Bon Jolphai”. The leaves were collected carefully and shade dried. The dried leaves were powdered and stored in an air tight container. The taxonomic authentication of Elaeocarpus Floribundus was conducted by Bangladesh National Herbarium, Mirpur, Dhaka under the accession number 51029. This plant was selected as no studies were conducted before on the antacid properties of Elaeocarpus Floribundus.

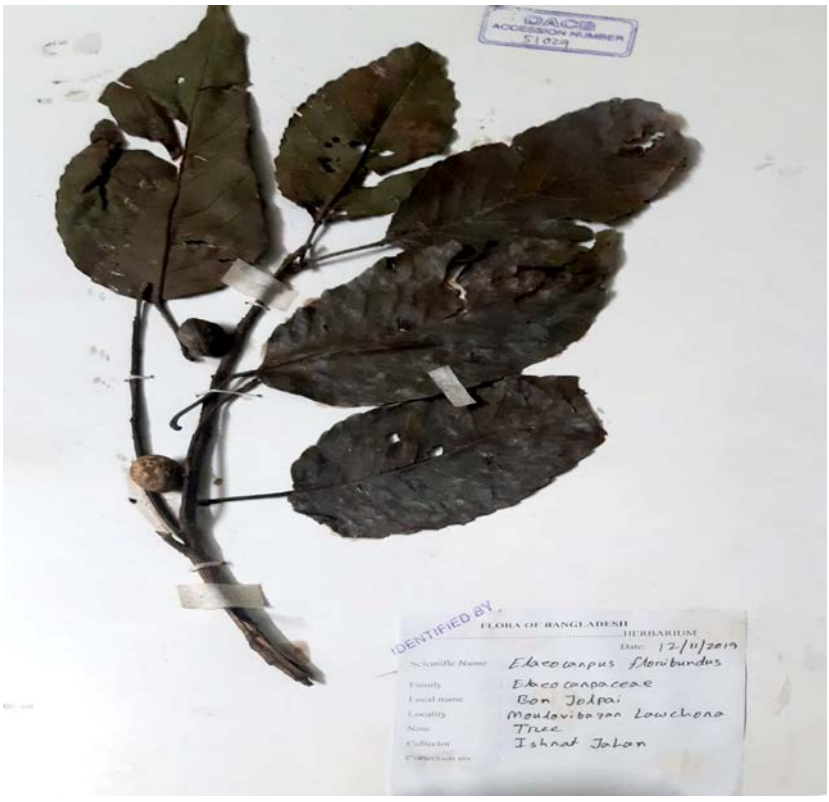
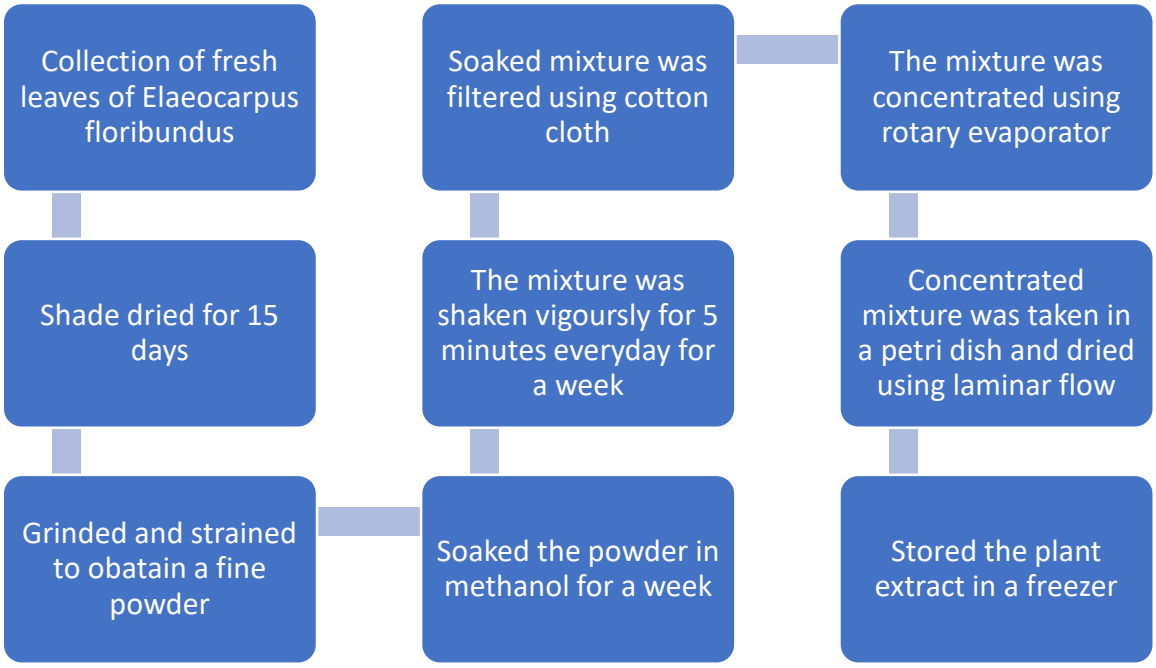


Figure 22: Accession number copy

2.2 Preparation of plant material

After collection fresh leaves were separated from the undesirable parts. The leaves were shade dried for 15 days in an open space and away from direct sunlight. The leaves were then ground and strained after which a smooth powder was obtained. The powder was stored in a clean and air tight glass jar at a room temperature.

2.3 Extraction process of plant material



200gm of dried and fine powdered plant material of Elaeocarpus floribundus were soaked in 1.2L of methanol. The mixture was kept in air tight glass jar for 7 days in a cool dark place at room temperature. During this time, it was shaken vigoursly every day for 5 minutes. The extract was filtered using a piece of cotton cloth and dried using rotary evaporator. Further it was dried in laminar flow by keeping the concentrated mixture in a petri dish. The final product was a heavy sticky gel like dark greenish mass (162.148 gm) (Bulbul et al., 2013).

Figure 14: Rotary Evaporator

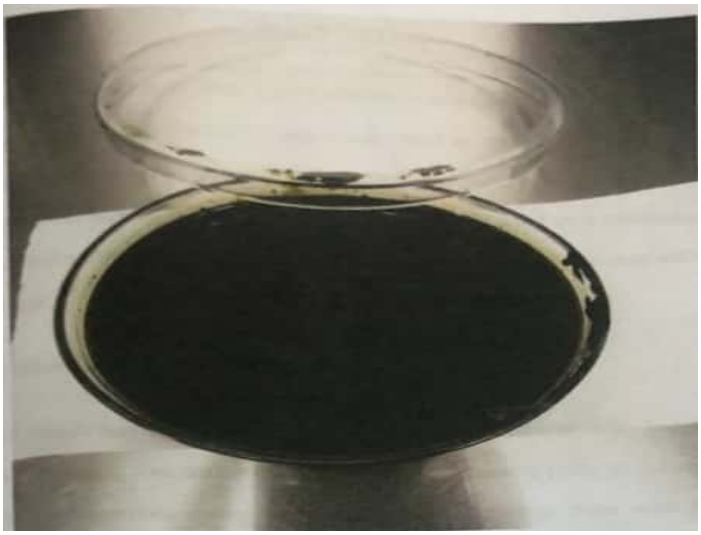


Figure 14: Extract in petri dish

2.4 Chemicals and apparatus

Chemicals	Apparatus
• Methanol • HCl • NaOH • Sodium Bicarbonate • NaCl • Pepsin	• Petri Dish • Beaker • Test tube • Pipette • Micro pipette • Burette • Volumetric flask • Round bottom flask • Oahu's Starter 300 pH meter

2.5 Preparation of reaction mixture

Stock Solution

Stock arrangement of the methanolic concentrates of *E. floribundus* (100 mg/mL) were at first arranged in absolute methanol and deionized water individually.

Test solution

For the test solution a final concentration of 1mg/mL from stock solution was prepared in triplicates. The positive controls were used sodium bicarbonate and combination of aluminum hydroxide and magnesium hydroxide both in 1mg/mL in methanol. For negative control methanol were used. All the experiments were maintained at 37 ℃. The pH was measured using the Oahu's Starter 300 pH meter. (Lirazan et al., 2018)

Preparation of GSF

The counterfeit gastric juice was set up as per the simulated gastric liquid test solution by the United States Pharmacopeia (USP). 2g of sodium chloride and 3.2 mg of pepsin were disintegrated in 500 mL deionized water. After that 7 mL of concentrated hydrochloric acid was included. In a volumetric cup the volume was balanced up to 1000mL and pH was changed in accordance with 1.2.(Lirazan et al., 2018)

2.6 Preliminary Antacid test

40 mL of each test solution (1mg/mL sample solution, positive control, negative control) was stirred continuously for 1 minute at 37℃ in order to conduct preliminary antacid test.

10mL of standardized 0.5M HCl was added in this and it was stirred for about 10 minutes constantly and then PH was measured. (Cua et al., 2018)

2.7 Determination of the Acid Neutralization Capacity (ANC) Using the Titration Method of Fordtran's Model

Acid neutralization capacity was resolved utilizing the titration method for Fordtran's model. Fifty milliliters (50 mL) of each test solution that was warmed to 37℃, was persistently stirred at 30 rpm to impersonate stomach movements and then titrated with artificial gastric juice (GSF) or HCl to the endpoint of pH 3. Calculation of the total devoured hydrogen ions (mmol H^+) was finished by multiplying the concentration of counterfeit gastric juice utilized by its volume that was added to the sample.(Lirazan et al., 2018)

2.8 Determination of the Neutralizing Effect of Extracts on Artificial Gastric Juice

Fifty milliliters (50 mL) of each test solution (1mg/mL) was added to 55 mL artificial gastric juice at pH 1.2. The reaction was equilibrated for 1 minutes. After that pH was measured.

2.9 Acid-Buffering Capacity Assay

The Buffering limit was assessed dependent on the Official Methods of Analysis of the Association of Official Analytical Chemists. Forty milliliters (40 mL) of each test arrangement was ceaselessly stirred at 60 rpm at 37℃. At that point the pH was estimated and recorded. Forward titration was performed by bit by bit incorporation of 0.5 mL of standard 1M HCl until the pH decreased to 1.5 and volume was recorded at that point. The examples were then back titrated by gradual inclusion of 0.5 mL of standard 1M NaOH until the pH expanded to 10. At this point the volume was likewise recorded. All the pH values were measured following a 1 minute equilibrium period. The acid buffering capacity (BC) was assessed by utilizing the equation by Van Slyke: total volume of acid added to each sample multiplied by the acid molarity, divided by the total change in pH.(Cua et al., 2018)

Chapter 3

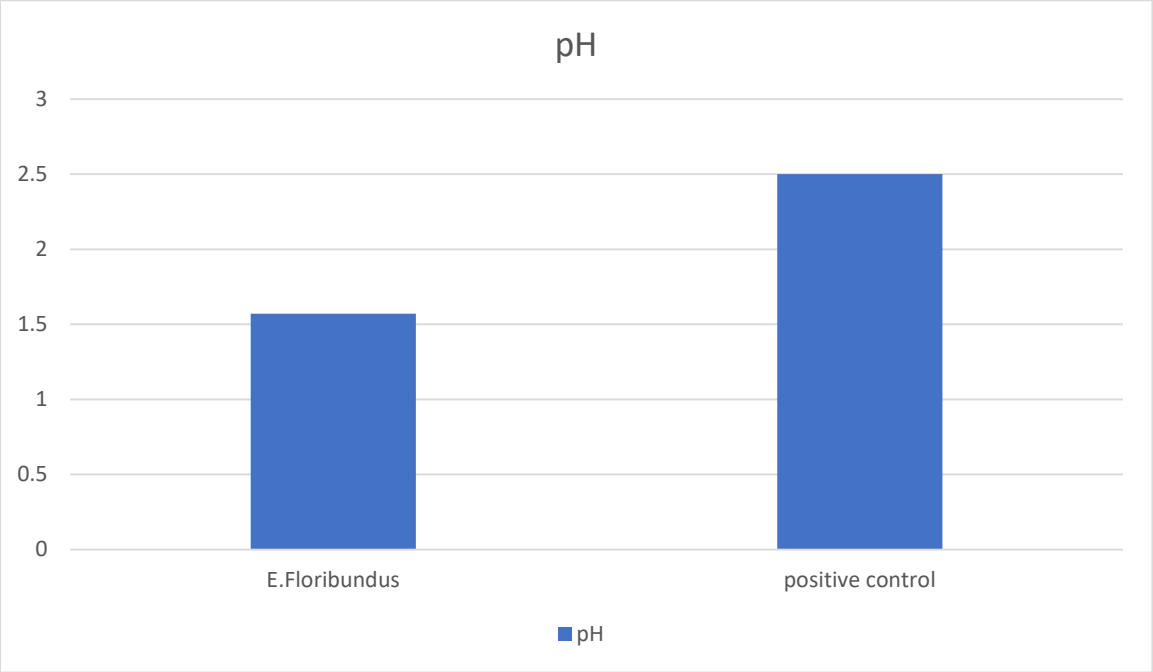
Results

3.1 Neutralizing effect of extracts on GSF

Table 1: *Acid neutralizing effect on gastric juice of the negative and positive controls and plant extracts.*

Sample	**pH**
E.floribundus (1 mg/mL)	1.57
$NaHCO_3$ (1 mg/mL)	1.69
Negative control	-

Here, pH of sample is 1.57 and pH of standard sodium bicarbonate is 1.69. pH of standard is higher than the sample.

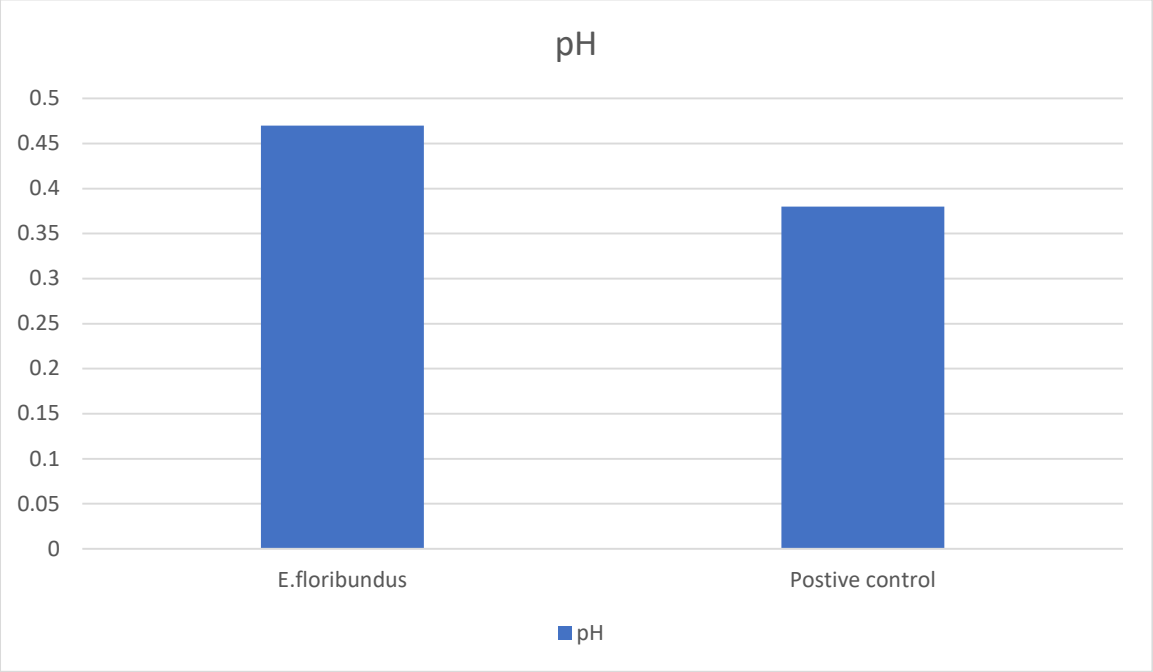


3.2 Preliminary Antacid Test

Table 2: *Preliminary antacid test results of negative and positive controls, and plant extracts. Result shown is average pH ± SEM*

Sample	**pH**
E.floribundus (1 mg/mL)	0.47 ± 0.017
$NaHCO_3$ (1 mg/mL)	0.38
Negative Control	-

In this table pH of sample is 0.47 and standard mean error is 0.017 and pH of standard is 0.38 which is lower than sample.



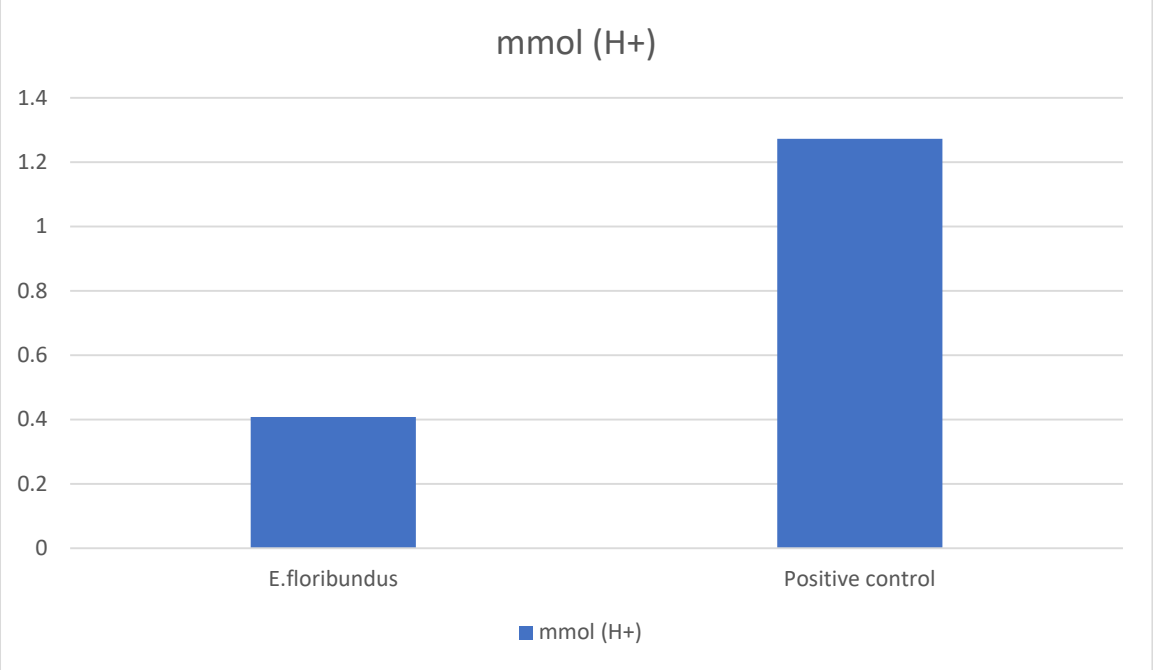
3.3 Acid neutralizing capacity

Table 3:

Acid neutralization capacity (ANC) effect of the negative and positive controls and plant extracts. Results shown as average ANC ± SEM

Sample	**Acid neutralization capacity (mmol H^+)**
E.floribundus (1 mg/mL)	0.408±0.0058
$NaHCO_3$ (1 mg/mL)	1.2724±0.0379
Negative control	-

Here, according to this table ACN capacity of sample is 0.408 and SEM is 0.0058 and standard is 1.2724 and amount of error is 0.0379.

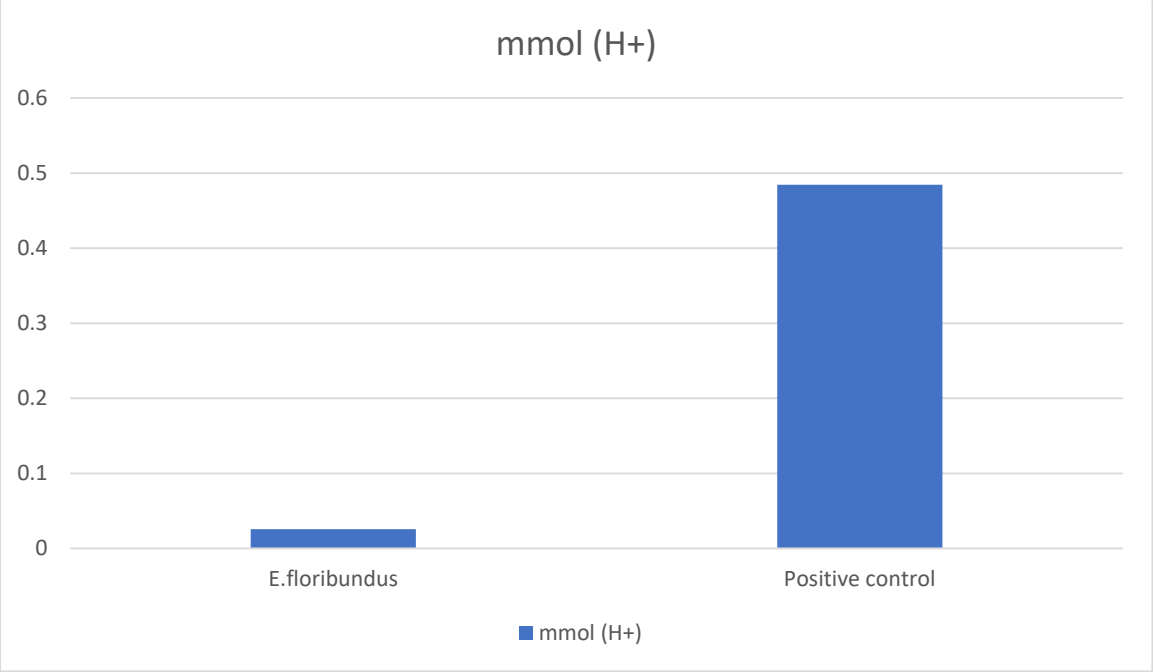


3.4 Acid-Buffering Capacity

Table 4: *Acid buffering capacity of positive control and aqueous plant extracts (1 mg/mL). Acid buffering capacity was determined by dividing the titratable alkalinity (mmol H+) by the total change in pH units.*

Sample	**Acid-Buffering Capacity (mmol H^+)**
E.floribundus (*1* mg/mL)	0.0257 ± 0.0053
$NaHCO_3$ (1 mg/mL)	0.4843 ± 0.0052
Negative control	-

In this table Acid-Buffering Capacity of sample is 0.0257 and SEM is 0.0053 which is lower than standard 0.04843 and SEM is 0.0052.


mmol (H+)
0.6
0.5
0.4
0.3
0.2
0.1
0
E.floribundus
Positive control
mmol (H+)

Chapter 4

Discussion

4.1 Neutralizing effect of extracts on GSF

The neutralizing effect on artificial gastric juice can be utilized as a proportion of the beginning of activity of stomach settling agents. In this case the determination of pH depends on the addition of fixed volume of artificial gastric juice to the sample solution. It is a significant factor and should be considered while assessing the potency of antacid since one model of an ideal antacid is that it must respond quickly with acids. From the table 1 it is observed that the methanolic extracts shown a decreased pH than standard sodium bicarbonate.

4.2 Preliminary antacid test

The preliminary antacid test was utilized to decide whether a solitary portion of a substance is fit for expanding the pH of a 10 mL solution of 0.5 M HCl to more prominent than or equivalent to 3.5 following 10 minutes of response, as expressed in the United States Pharmacopeia national model; substances that fulfill this rule are considered as “antacid”. According to the table 2 it is evident that plant extract has more antacid potential. Though, positive control has lower pH than methanolic extraction.

4.3 Acid neutralizing capacity

Acid neutralizing capacity (ANC) is regularly used to assess the adequacy of various antacid. Acid neutralizing capacity is the measure of hydrochloric corrosive an acid neutralizer can kill, communicated as mmol of H+. Furthermore, according to the table 3 neutralizing capacity of methanolic extract was higher but lower than the standard sodium bicarbonate.

4.4 Acid-buffering capacity assay

Table 4 shows the buffering capacity of methanolic extract and standard sodium bicarbonate and a negative control. According to the table it is evident that methanolic extract has higher buffering capacity but significantly lower than the standard sodium bicarbonate.

All the result shows that methanolic plant extracts has poor antacid properties. Previously all the tests were conducted with ethanolic and aqueous extracts but here in this project methanol was used for the extraction which could be a potential reason for not getting proper result. Moreover, 37°C needed to be maintained throughout all the tests which was not strictly maintained. Another reason could be measurement accuracy of all the reagents and solvents which is also an important factor in this work. As *Elaeocarpus floribundus* leaves has anti-ulcer properties anti-ulcer test could be done in future.

Chapter 5

Conclusion

In conclusion, it can be said that this study shows that *Elaeocarpus floribundus* shows poor antacid properties though the leaves has anti-ulcer properties. In future anti-ulcer test can be done to see whether it is a potent anti-ulcer agent or not. Here methanolic extraction was used instead of ethanolic or aqueous extract so a comparison can also be done between the methanolic and ethanolic and aqueous extract.

Chapter 6

Future work

- Bark infusion of *Elaeocarpus floribundus* can be used as anti-diabetic agent so anti-diabetic test can be done.
- For the treatment of inflamed gums *Elaeocarpus floribundus* can be used so an anti-inflammatory test can be conducted.
- Anti-hypertensive test can also be conducted from this plant extraction.
- Comparative study between the methanolic and ethanolic extracts of antacid test
- As it has anti-ulcer properties so anti-ulcer test can be done.

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