Investigation of In Vitro Anti-inflammatory and Anti-oxidant Properties of Silver Nanoparticles of *Elaeocarpus serratus* Plant Extract

BY

Urmila Saha

21346066

A Thesis Submitted to the School of Pharmacy in Partial Fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

School of Pharmacy

BRAC University

2025

Declaration

It is hereby declared that-

1. The thesis submitted is my original work, completed during my Bachelor's degree at BRAC University.
2. The thesis neither contains any materials from previously published articles nor was written by any third parties, except where the data used has been fully cited and accurately referenced.
3. The thesis doesn’t contain material which has been previously accepted, submitted for any other degree or diploma at any other university or institution.
4. I have acknowledged all main sources of help.

Urmila Saha
21346066

Approval

The thesis/project titled **"Investigation of In Vitro Anti-inflammatory and Anti-oxidant Properties of Silver Nanoparticles of *Elaeocarpus serratus* Plant Extract**" submitted by Urmila Saha (21346066) of Summer 2021 has been accepted as satisfactory in partial fulfilment of the requirement for the degree of Bachelor of Pharmacy (Hons) in August 2025.

Examining Committee:

Supervisor:

(Member)

Namara Mariam Chowdhury
Senior Lecturer,
School of Pharmacy,
BRAC University.

Approved By:

Chairperson:

Dr. Raushanara Akter
Acting Chairperson and Professor,
School of Pharmacy,
BRAC University.

Dean:

A.F.M. Yusuf Haider, PhD
Acting Dean, School of Pharmacy
Professor, Department of Mathematics and Natural Sciences,
BRAC University.

Ethics Statement

This Study doesn’t involve any animal studies or human trials.

Abstract

Elaeocarpus serratus, also known as Ceylon olive, a plant commonly named as Jalpai in South Asian countries, is not just a mere fruit and vegetable but also a medicinal plant rich in bioactive phytochemicals like flavonoids and phenolic acids, demonstrating a great potential to be a lead molecule in treating drugs for various concerning diseases. This study focuses on synthesising silver nanoparticles (AgNPs) using a green method of the *E. serratus* leaf extract and compares and evaluates the in vitro antioxidant and anti-inflammatory activities of the crude extract and the synthesised nanoparticles. It was demonstrated that AgNPs demonstrated significantly greater membrane destabilising inhibition compared to the crude extract and the standard aspirin, especially at the concentration of 20µg/mL, as evaluated by the anti-inflammatory activity that was determined through heat-induced hemolysis. Moreover, the antioxidant activity evaluated through the DPPH scavenging assay was comparable between the extract and AgNPs. Both exert mostly similar high radical scavenging ability, though not as high as ascorbic acid, which is demonstrated by antioxidant potential estimated in the DPPH radical scavenging assay. It is also important to note that when used at a higher concentration, AgNPs were found to be less active, presumably because of cytotoxicity, so optimal dosing remains a crucial aspect. Therefore, it can be said that AgNPs synthesised by the green method using *Elaeocarpus serratus* could be utilised in the improvement of anti-inflammatory activity and maintenance of oxidative stress, making them excellent agents to develop a new therapeutic agent to treat oxidative stress and any inflammation-related disorder. However, for more accuracy, safety, efficacy and optimum dosage regimen, pharmacological in vivo tests should be conducted.

Key Words: *Elaeocarpus serratus*, Ceylon Olive, Medicinal Plants, Nanoparticles, Silver Nanoparticles, Lead molecules, Ethanolic extract, Anti-inflammatory, Anti-oxidant.

Dedication

I am dedicating this work to my beloved parents, my family and my partner for the immense support, encouragement, motivation, and faith in me throughout my life.

Acknowledgement

I would like to express my sincere gratitude to all those who have contributed to the success of my project. First and foremost, I offer my heartfelt gratitude to God for his continuous blessings, guidance, patience and strength throughout this research journey. Then, I would like to thank my supervisor, Namara Miss, for her invaluable guidance, constant support, motivation and encouragement that have really eased my working stages, and her insights and expertise in my work have greatly enriched the work. I couldn't be more blessed to have her as my project supervisor for this undergraduate life.

I am also thankful to the lab officers for their constant guidance throughout the experiments. For them, I could learn all the steps of my experiments more accurately and conduct the tests properly.

I would like to express my gratitude and thanks to my family, Ma, Baba, Kaku and my little sister, for whom today I am standing here to work on such a great project. Without them, my existence is nothing. It is their blessings, trust and support for me to be successful in life. Moreover, my friends were always there to motivate and cheer me whenever I felt down.

Finally, I would like to thank my partner for his love, trust and support in every step, who is the best critic in my life, and who always cheers me to be the best.

The study would not have been possible without the collective effort of all these individuals and organisations. Thank you all very much.

Table of Contents

List of Tables

List of Figures

List of Acronyms

AgNPs = Silver Nanoparticles

OLE = Olive Leaf Extract

DPPH = 2, 2-Diphenyl-1-picrylhydrazyl

RBC = Red Blood Cell

ROS = Reactive Oxygen Species

IC50 = Half maximal Inhibitory Concentration

NSAIDs = Non-steroidal Anti-Inflammatory Drugs

FRAP = Ferric Reducing Antioxidant Power

TAC = Total Antioxidant Capacity

nm = nanometer

Chapter 1: Introduction

1.1 Background

1.1.1 Medicinal Plants: The Groundwork of Traditional and Modern Pharmacology and the Source of Lead Molecules

Plants have always been central to traditional medicine, forming a backbone of all healthcare systems worldwide. A variety of drugs have been discovered based on natural products. Originally obtained as extracts of plants, fungi and microorganisms, and which may serve as lead molecules or structures in the development of other therapies. Bioactive compounds, namely alkaloids, flavonoids, terpenoids, glycosides, phenolic acids, and tannins, derived from these plants, possess broad-spectrum pharmacological properties, including anti-inflammatory, antioxidant, antimicrobial, anticancer, antidiabetic, etc. The significance of plant-derived molecules in the drug development process can also be explained by the fact that more than 50% of existing drugs are of natural product origins, directly or based on their chemical compounds (Chaachouay & Zidane, 2024).

Small-molecule drugs, constituting about 33.5% of the total number of drugs approved in the last four decades, are either natural products or related to natural product structures. Semi-synthetic derivatives and natural product mimics bring the figure to almost 65%, the sheer influence of natural compounds on contemporary pharmacotherapy being noted (Dzobo, 2021; Nasim *et al.*, 2022). In the case of anti-infective agents (antibiotics, antivirals), natural products and their derivatives form the largest part of most of the approved medicines, and this becomes an indispensable contribution in the fight against resistance. A great number of commonly used and classical drugs, including paclitaxel, morphine, artemisinin, tetracycline, and doxorubicin, are either derived from plants or are based on plant-derived chemical structures (Atanasov *et al.*, 2021).

1.1.2 The role of Inflammation and Oxidative Stress in Disease

Oxidative stress and inflammation are biomolecular processes that are closely related and are of central importance in human health and disease.

Inflammation is a type of physiological response that is provided by the immune system to a harmful stimulus, injury or infection. It is important for healing and defence. Although the elevated levels of acute inflammation result in a restoration of tissue homeostasis, the elevated levels of chronic inflammation are persistent and may cause tissue damage, which may lead to disease. The background of many diseases, such as cardiovascular disease, diabetes, rheumatoid arthritis, neurodegenerative diseases (Alzheimer's, Parkinson's) and cancer, is chronic inflammation. Chronic inflammatory mediators cause tissue damage and functional defects (Chen *et al.,* 2017).

On the other hand, oxidative stress occurs when there is an increase in levels of reactive oxygen species (ROS) production, with poor defence against these species by the body's physiology. The surplus ROS causes DNA damage, proteins, as well as lipids, leading to cell malfunction or death. It is intimately related to ageing, and oxidative stress has been described as the cause of pathogenesis of atherosclerosis, diabetes, cancer, neurodegeneration and inflammatory diseases (Sikder *et al.*, 2025).

Inflammation may be enhanced by oxidative stress. On the other hand, ROS production by inflammatory cells triggers a harmful feedback loop leading to an increase in disease development (Mittal *et al.*, 2024). A wide range of both acute and chronic illnesses is prevented and managed through addressing both inflammation and oxidative stress. Therapies that modulate inflammatory routes and boost antioxidant threshold are vigorously studied regarding their ability to mitigate disease progression and boost health.

1.1.3 Importance of *Elaeocarpus Serratus* in Pharmacological Research

Elaeocarpus serratus (popularly referred to as Ceylon olive) is one of the medicinal plants that have captured the attention of several researchers and herbalists because of its phytochemical abundance and long-term use in medicine. The species is a member of the Elaeocarpaceae family, comprising about 550 species in the world, characterised by their bioactive metabolites (Tropic, 2023; Mahomoodally & Sookhy, 2018).

In comparison with parts of E. serratus, some parts of them, like leaves, fruit, seeds, and the bark, contain very important constituents of nature like flavonoids (e.g., myricitrin, myricetin), phenolic acids (gallic acid), terpenoids, indolizidine alkaloids, cucurbitacin, glycosides and tannins (ellagic acid derivatives geraniin). The phytochemicals have been classified based on chemotaxonomic profiling, and various detailed methods of analysis, including UPLC, GC-MS, HPTLC and TLC that lead to their antioxidant along anti-inflammatory potentials (Geetha *et al.*, 2013).

The medicinal properties of the bioactive contents in *E. serratus* have proven to be quite favourable and show therapeutic effects in the chronic inflammatory diseases, oxidative stress-related diseases, diabetes and cancer. An example of such a flavonoid is myricitrin and myricetin, which are abundant in this plant. But there is a wide knowledge that they are potent inhibitors of oxidative damage and inflammation and can scavenge free radicals, hence, correcting the participants of the inflammatory process (Fayez *et al.*, 2023; Lins *et al.*, 2018). The presence of these compounds with gallic acid and gallic acid derivatives gives the plant powerful antioxidant properties, as illustrated by various tests such as DPPH free radical check and the total antioxidant capacity tests, where a plant with powerful efficacy indicates low IC50 values (Lim *et al.*, 2024; Salehi *et al.*, 2020). Pharmacologically, both in vitro and in vivo ethanolic *E. serratus* leaves and fruits have been proven to be incredibly anti-inflammatory.

For example, studies carried out using mice used the Freund's adjuvant arthritis model, which established that these extracts had the potential to effectively remedy the inflammation and could be as effective as ordinary anti-inflammatory medicines such as indomethacin (Silvestrini *et al., 2*023b). Studies also show that the *E. serratus* fruit extracts exhibit great antidiabetic effect in streptozotocin-induced diabetic rat model and therefore an expected development on the plant as a source of naturally occurring antidiabetic compounds (Villalva *et al.*, 2022).

Figure 1: Ceylon Olive Tree (Tropic, 2023)

1.1.4 *E. Serratus's* Contribution to Drug Development

Chemical diversity of the Elaeocarpus species, including the E. serratus, presents a source of lead molecules in the development of new drugs. Emine, the indolizidine alkaloids and cucurbitacins extracted from Elaeocarpus species are very commendable since they have cytotoxic, anticancer, and antiviral properties (Ezeoke, 2018). Widely studied representatives of triterpenoids are cucurbitacins used to develop many anticancer mechanisms, causing apoptosis and cancer cell proliferation.

In addition to that, other isolated triterpenoids, including friedelin and epifriedelanol in other species related to *E. serratus*, have exhibited considerable antioxidant and cytotoxicity activities, which have justified the possibility of use in chemoprevention and treatment (Sing *et al.*, 2023). The *E. serratus* leaves (ethyl acetate) extracts have an IC50 of about 54 µg/ml in this assay based (antioxidant) and had good free radical scavenging activity in FRAP (Ferric

Reducing Antioxidant Power) and TAC (Total Antioxidant Capacity) demonstrating significant improving effects on both these assays compared with the other solvent extracts that were tested (Lins *et al.,* 2018).

According to Najmi et al. (2022), natural products and their derivatives make up almost a 50-60% share of the approved anti-inflammatory and antioxidant drugs that have been licensed in the last few decades across the world. The integration of natural lead compounds of plant species such as *E. serratus* into the drug designing process has the potential to enhance the safety and efficacy of the designed drugs as compared to synthetic drugs. Its phytochemicals can be used directly as therapeutic agents and also as scaffolds or adjuvants in their pharmaceutical formulation, in the possibility of using them to address complex conditions with multi-target therapies that contribute to chronic inflammation and oxidative stress (Ebob *et al*., 2021).

Besides, as the number of patients in the developed world with chronic diseases of oxidative stress and inflammation increases, the natural, multi-functional drug substances are increasingly in demand. This unique phytochemical profile of E. serratus makes it a very promising candidate as a source of phytopharmaceutical medicine, nutraceutical or supplementary therapy.

1.2 Other Species of *E. Serratus*

Table 1: Different species of E. serratus

Species	**Distribution**	**Key Features**	**Uses**	**Conservative Status**
Elaeocarpus angustifolius	India, Bangladesh, China, Indochina, Indonesia, Australia, Papua New Guinea, and surrounding islands.	A very big, evergreen tree, which is in many cases not less than 40 metres in height. It is distinguished by its blue-marble-like drupe. The leaves have fine serrations and are glabrous, and the petals are profoundly divided at the top. It is occasionally with E.grandis, particularly in Australia, where the two are very close and even regarded as members of the same	They are religious beads (mala or prayer beads) made of sewn seeds (rudra ksitu) called rudraksha in Hinduism and Buddhism.	Widespread

		species.		
Elaeocarpus grandis	Australia	Quite allied to this, E. angustifolius, leaves large and shiny, fruit blue. The key differentiating factor is that it is regional and its morphological differences are minor, e.g. size of fruit and leaves.	Significant food supply to indigenous animals.	Widespread
Elaeocarpus stipularis	Southeast Asia, central Malaysia	Variable in the species, attaining a height of 40 meters, and frequently low-branched. It is remarkable in having persistent, palmate-shaped stipules and red senescent leaves. The racemose inflorescences are able to carry 90	Distribution in large habitats, including secondary and primary forests, riverine, peat, and freshwater swamps, as well as disturbed vegetation.	Common

		flowers. Fruits are not uniform in size and shape; they contain a tough stone.		
Elaeocarpus floribundus	Asia, Pacific	It is a characteristic example of the genus, except that it has simple leaves, racemose flowers, and blue drupes. Columns of fruit are reddish-brown, the endocarp being sculptured as usual in most Elaeocarpus species, where the leaves tend to turn red before falling.	Known by other names in popular language, Jalpai (Bengali), Belphoi (Sylhet) and Malangau (Philippines), it has local importance. Shows antimicrobial effects towards several bacteria, both Gram-positive and Gram-negative. Stunt the	widespread

			activities of some plants (phytotoxic).	
Elaeocarpus sphaericus	South Asia, Southeast Asia.	Blue fruit, Rudraksha beads. Morphologically similar to *Elaeocarpus angustifolius.*	Treatment of epilepsy, asthma, hypertension, and arthritis lowers inflammation, so it may be helpful in treating conditions such as arthritis.	widespread

(Banu *et al.,* 2024; SD, 2023; Aryal, 2021; Ogundele *et al.,* 2021; Prasannan *et al.,* 2020;Baruah *et al.,* 2019; Jawla & Rai, 2016).

1.3 Description of the Plant

Ellaeocarpus Serratus, commonly known as the ceylon olive, is a medium to large evergreen tree in the family Elaeocarpaceae, native to South and Southeast Asia, including Sri Lanka, India, Bangladesh, Nepal, Myanmar, Bhutan, Malaysia and Indonesia. The tree is valuable for its edible fruits, ornamental appeal, and traditional medicinal uses. Ceylon olive is a medium to large-sized evergreen tree which normally grows to a height of 15-20 m, but under favourable conditions it may reach a height of 60 m. Elaeocarpus serratus is a tropical and subtropical plant that grows in well-drained soils containing nutrients. It occurs in lowland rainforests, in evergreen forests, in hilly regions and on river banks, between sea level to approximately 2,000 meters (Raji & Siril, 2021).

1.4 Different Parts of the Plant

Leaves: Leaves are straightforward, alternate, elliptical to lanceolate, normally 615 cm in length and 2.45 cm broad. They are shiny, dark green with distinct veins, acuminate at the tip, and the edges are very frequently deeply notched or serrated. The leaves are red throughout the year, which makes it easy to identify (Ali, 2020; Raji & Siril, 2021).

Flowers: The tree bears small, aromatic, white or pale-green, bell-shaped flowers, which are normally clustered or racemes. The flowers contain five white to pale-olive-green calyxes, 4-6 mm long sepals, five white corollas with slightly fringed petals (4-5 mm long), and 18-30 stamens with slightly black anthers. Flowers fully open to their largest size in the late afternoon, probably to allow pollination by nocturnal insects like moths. It normally flowers between June and July.

Fruit: The fruit is a smooth, ovoid drupe, 1-2.5 cm long. When unripe, it is green in colour, and when ripe, it changes to purplish-black or dark purple. The fruit is very thin and contains one hard woody seed. The taste is said to be a little sweet, acidulous or styptic, or subacidulous. The fruit is eaten fresh, cooked, in curries or pickled (a common street food in Sri Lanka and

Bangladesh). It is rich in Vitamin C and has medicinal values, used in remedies for digestive problems or dysentery due to its astringent properties and other ailments (NV *et al.*, 2023).

Barks and Branches: It is large with a spreading crown and a straight trunk whose bark is brown to grayish-brown. Branches are hairy, and the tree is also distinct with its dense foliage and sometimes with red leaves, especially during the spring when the older leaves assume a blood red colour just before abscission. The barks are helpful for skill conditions and for inflammation (Raji & Siril, 2021).

1.5 Taxonomic Classification

Kingdom: Plantae

Phylum: Tracheophyta (Also known as Magnoliophyta)

Class: Magnolipsida (Also known as Angiospermae)

Order: Oxalidales

Family: Elaeocarpaceae

Genus: Elaeocarpus

Species: *Elaeocarpus serratus*

(Raji & Siril, 2021).

1.6 Vernacular Names

Jalpai- Bengali

Zolphai- Assamese

Veralu- Sinhala

Veralikkai- Tamil

Ceylon Olive- English

(Raji & Siril, 2021)

1.7 Chemical Constituents

Elaeocarpus serratus is found to have a wide range of bioactive chemical compounds, primarily isolated and identified in the leaves and the fruit by chromatographic and spectrometric techniques. According to a study, Major Chemical Constituents Identified by GC-MS in Leaf Extracts are- Methanol (20.57%), n-Dotriacontanol (a long-chain alcohol) (10.70%), n-Octadecanol (10.08%), Docosanoic acid, 1,2,3-propanetriyl ester (a fatty acid ester) (9.07%), n-Hexadecene (8.52%), Bis-(3,5,5-trimethylhexyl) ether (6.30%), Ethanone, 1-cyclopentyl- (4.81%), and Cyclohexane, ethyl- (4.05%). Other minor constituents are hexadecanoic acid methyl ester, ricinoleic acid, citronellyl isobutyrate and farnesol (Fernando *et al.*, 2024; Yeshma, 2015).

Bioactive Compounds in Fruits:

- Flavonoids: Contain myricitrin, myricetin, mearnsetin, kaempferol and quercetin
- Phenolic acids: Gallic acid, etc.
- Condensed tannins, Carotenoids, Vitamin C

The fruit is also a good source of antioxidants and has antimicrobial activity, which is in part due to these compounds (Sumanarathne *et al.*, 2020).

1.8 Description of Silver Nanoparticles

The 21st century is the beginning of a new century brought an amazing merger of biology, chemistry, and materials science, which has stimulated new trends in medicine and treatment. Some of the breakthroughs include nanotechnology, a cross-field where structures at the nanometer level, commonly 1-100 nanometers, are exploited to obtain materials and devices with specific physicochemical properties. The nanotechnology promises unparalleled opportunities in the field of biomedical research, pharmaceuticals, diagnostics and targeted therapy owing to its potential to handle matter on molecular and atomic levels, and thus the

resulting material is frequently more efficient and differently behaving than its bulk equivalent (S. Ullah *et al.*, 2023b).

1.8.1 Silver Nanoparticles: A Biogenic Innovation Spotlight

The nanoparticles of silver (AgNPs) are gaining popularity much quickly due to their peculiar property in the course of biological, electrical and optical applications, due to their high surface-to-volume ratio and due to quantum effects. Silver has a long history of antimicrobial characteristics; however, with the emergence of nanotechnology, this was increased over tenfold in AgNPs that are currently researched in terms of their antimicrobial, anti-inflammatory, antioxidant and anticancer capacity. The use of AgNPs in medical equipment and dressing, and their use in biosensing and drug delivery, significantly promises the biomedical relevance of AgNPs. Nevertheless, producing nanoparticles using the traditional method has several serious environmental and health issues in that the processes involve dangerous chemicals and rough physical settings (Barathi *et al.*, 2024; Ghosh *et al.*, 2022).

1.8.2 Green Synthesis: Plants for Sustainable Nanoparticles

With sustainability emerging as one of the core values in scientific advancement, what has changed is a paradigm shift towards the green production of nanoparticles, referred to as the green synthesis of nanoparticles. Plant extract in the synthesis of nanoparticles is an easy, cheap and non-toxic process when compared to chemical and physical means of producing nanoparticles. The reducing and capping agents found in plants (polyphenols, flavonoids, alkaloids, and other phyto-compounds) are the natural factories through which single nanoparticles can be formed under mild reaction conditions (Filho *et al.*, 2023). Green synthesis also lowers its toxicity, but in most cases, it gives nanoparticles extra bioactivity, provided by phytochemicals in plants and therefore, effective effects that are not achievable or are not as effective as solely synthetic ones.

1.8.3 The Interplay- Plants, Nanoparticles, and Bioactivity

Phytotherapy and nanotechnology stand to deliver fertile territory of discovery that is full of thousands of secret remedies that can be unlocked. Nanoparticles made out of plants often have higher bioactivity than the crude plant extracts and chemically made nanoparticles, due perhaps to the combination of the effects of the plant metabolites and the effects of the formed nanoparticles (Filho *et al.*, 2023). An example is that silver nanoparticles derived using plant extracts can have greater anti-inflammatory and antioxidant activity than that of the plant material. These features are of great pertinence towards fighting the dual burden of oxidative stress and inflammation in human health.

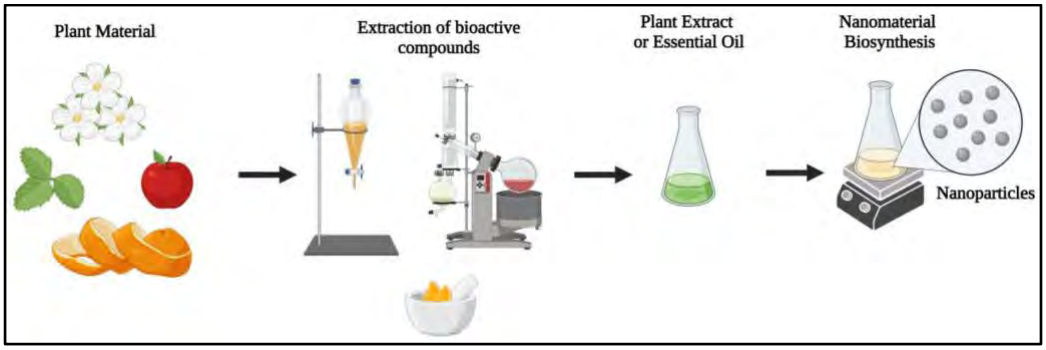

Figure 2: Biosynthesis of Nanoparticles (Filho et al., 2023).

The use of *Elaeocarpus serratus* as a natural reducing and stabilising agent in the green synthesis of silver nanoparticles aligns with the current and rising need for eco-friendly productions of nanomaterials. The high phytochemical diversity of the plant, with the presence of flavonoids, phenolic acids, alkaloids, and tannins, is used in both nanoparticle synthesis reactions. Ag+ ions are reduced to elemental silver (Ag0) and, at the same time, provide a substrate to prevent the aggregation of the formed nanoparticles (Pradeep *et al.*, 2021).

According to findings, Silver ions can be converted to AgNPs through *E. serratus* fruit or leaf extracts in less than 45 minutes and room temperature. Characteristic surface plasmon resonance (SPR) peaks were observed in UV-visible spectroscopy that confirmed the formation of nanoparticles.

Moreover, synthesised nanoparticles have uniform morphology, usually cuboid-like or spherical, with minimal size distribution of around 20 nm to 50 nm which as shown by the scanning electron microscopy (SEM) and X-ray diffraction (XRD). They are crystalline and face-centred cubic.

Besides, AgNPs that are produced using the *E. serratus* plant display much greater antibacterial effects on pathogens such as *Shigella dysenteriae* and *Staphylococcus aureus* when compared to the crude plant extract (Ghosh *et al.*, 2022). The AgNPs also turn out to be potent antioxidants (high DPPH radical scavenging), and are also capable of showing cytotoxicity against human colon adenocarcinoma cell lines (HT-29) with an IC50 of approximately 49.3 g/mL (Bedlovičová *et al.*, 2020).

Compared to the use of dangerous chemicals in a conventional synthesis, the green technique through E. serratus saves energy, is easy to expand on a large scale, and is environmentally sensitive. In studies involving related plant extracts, they have found that increased pH (alkaline conditions) and higher temperature increase reaction rates and modify the size and shape of nanoparticles. These parameters are translatable knowledge on the optimisation of *E. serratus*-mediated synthesis (S. Ullah *et al.*, 2023b; Islam *et al.*, 2021).

The inhibitory potential of AgNPs against inflammatory markers and mediators is blocked by disrupting their production or action. It is increased by the presence of the phytochemicals of the plant, so that the overall effect attained with AgNPs is higher than that with the plant extract alone in terms of preventing oxidative and inflammatory cascades from acting. The surface-to-volume proportion and unrivalled surface chemistry of the created nanoparticles interact more effectively with targeting biological substances and mediators of inflammation (Nguyen *et al.*, 2022).

1.9 Aims and Objectives

1.9.1 Aims

This study aims to evaluate the in vitro anti-inflammatory and antioxidant activity of E. serratus and synthesise silver nanoparticles from this plant, and compare their activity with that of the crude plant extract.

1.9.2 Objectives

The objectives of this study include-

- To synthesise silver nanoparticles (AgNPs) with the aqueous extract of *E. serratus* via a green, environment-friendly procedure.
- To calculate the percentage inhibition of heat-induced hemolysis of the nanoparticles compared to the crude extract and the standard drug.
- To determine and compare whether the *E. serratus* leaf extract and its bio-synthesised silver nanoparticles can control the membrane stabilisation of human RBC.
- To evaluate and calculate the antioxidant activities of the E. serratus plant extract and the synthesised silver nanoparticles in vitro, by using the DPPH radical scavenging test.
- To compare the biological activities of the plant extract and its silver nanoparticles, it is necessary to establish the increased activity of nanoparticles in the inhibition of inflammation and oxidative stress markers.
- To evaluate the novel activity of the silver nanoparticles extracted from Elaeocarpus serratus as a new therapeutic agent in the treatment of inflammatory diseases and diseases associated with oxidative stress.

Chapter 2: Methodology

2.1 Chemicals and Apparatus

Table 2: Chemicals and Apparatus used in the work

Chemicals	**Appartuses**
1. 95% Ethanol 2. Distilled Water 3. Aspirin 4. Sodium Citrate (Anti-coagulant) 5. Saline Water 6. DPPH Powder 7. $AgNO_3$	1. Beaker 2. Petri dish 3. Pippette 4. Micro Pippette 5. Water bath 6. Test tubes 7. Sonicator 8. Rotary Evaporator 9. Centrifuge Machine 10. UV Spectrophotometer

2.2 Collection and Identification of the Plant Material

For research purposes and to compare activities with nanoparticles, E. serratus was chosen and collected. In November, the plant leaves were collected from Matsha Vaba, Ramna, Bangladesh. The taxonomic authentication was conducted at the Bangladesh National Herbarium, Mirpur-1, Dhaka and a voucher specimen was issued mentioning the accession number **DACB 135353**, which indicates that the plant was verified and authenticated by the National Herbarium.

Figure 3:Plant Identification

2.3 Preparation of Plant Material for Extraction

Fresh leaves (1kg) were isolated after harvesting them from the unwanted portions. The leaves were dried in the shade for 15 days in an open area and out of direct sunlight. The leaves were ground and strained, and subsequently, a smooth powder was derived. The powder was kept in an air-tight and clean glass jar a room temperature.

Figure 4: Grounded powder of the E. serratus plant leaf

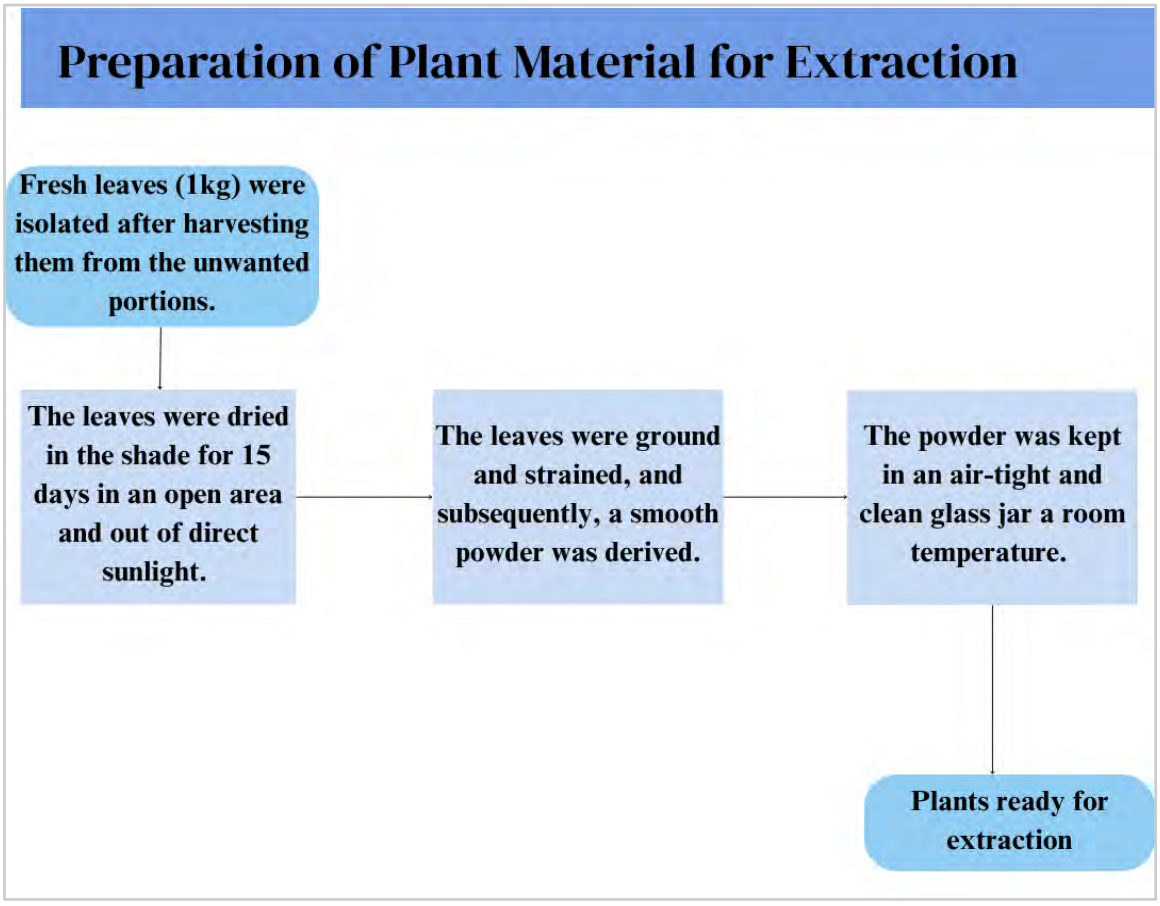

Figure 5: Flowchart of the Preparation of Plant Material for Extraction

2.4 Preparation of OLE

1.5 litres of ethanol were used to soak 500 g of dried and fine-powdered plant material of *Elaeocarpus serratus*. The mixture was stored in an air-tight glass jar, at room temperature, in a cool and dark place for 7 days. It was vigorously shaken daily during this period over a 5-minute period. The extract was filtered through a piece of cotton cloth and dried in a water bath (**60°C)**. The rotary evaporator was not used due to the high pressure and high volume. It was then dried in a laminar flow by leaving the concentrated mixture in a Petri dish. The end product was a thick, viscous, jelly-like dark greenish substance (Fernando *et al.*, 2024).

Figure 6: Preparation of OLE

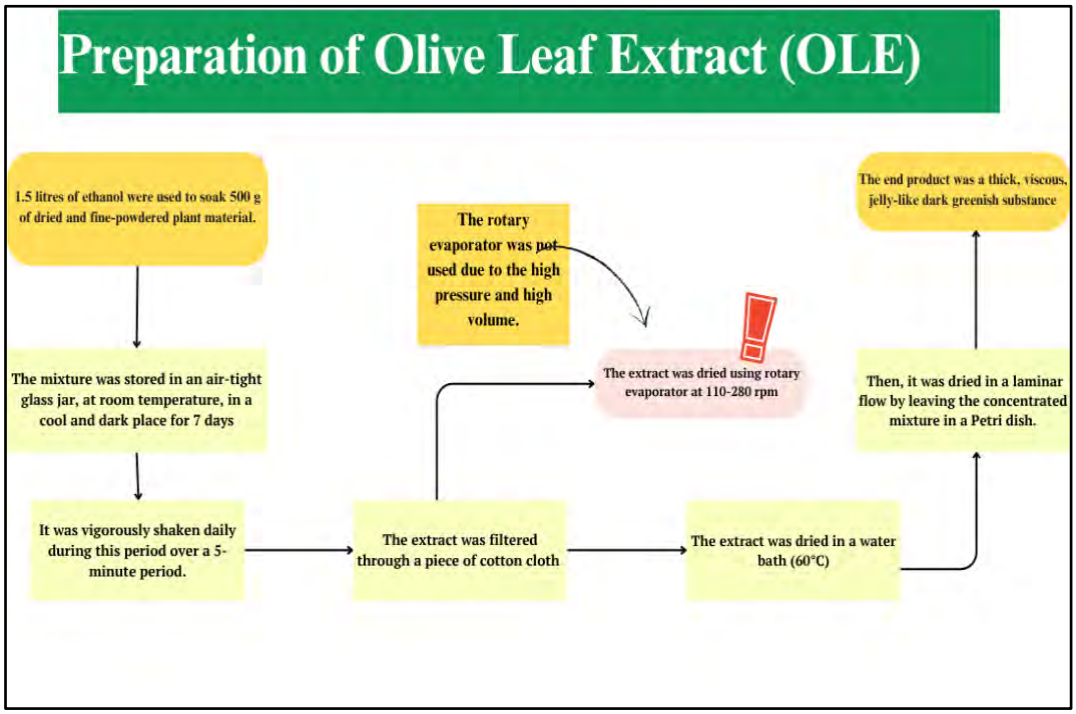

Figure 7: Flowchart of the Preparation of OLE

2.5 Biosynthesis of OLE-functionalized AgNPs

The synthesis of AgNPs@OLE was performed by combining 50 mL of a diluted OLE solution (100 µg/mL) with 50 mL of AgNO3 (100 mM) in a glass vial and irradiating it directly under diffused sunlight outdoors at various exposure times. We realise that the borosilicate glass vessel in which the synthesis was performed attenuates some of the UV light. So, IR and visible light predominated the light reaching the solution. The mixture turned colourless to light yellow and finally to brownish-yellow within a second, which denotes that AgNPs are successfully synthesised. It is to be noted that when the mixture solution was not exposed to sunlight, no change in colour was perceived, which means that it was necessary in the synthesis.

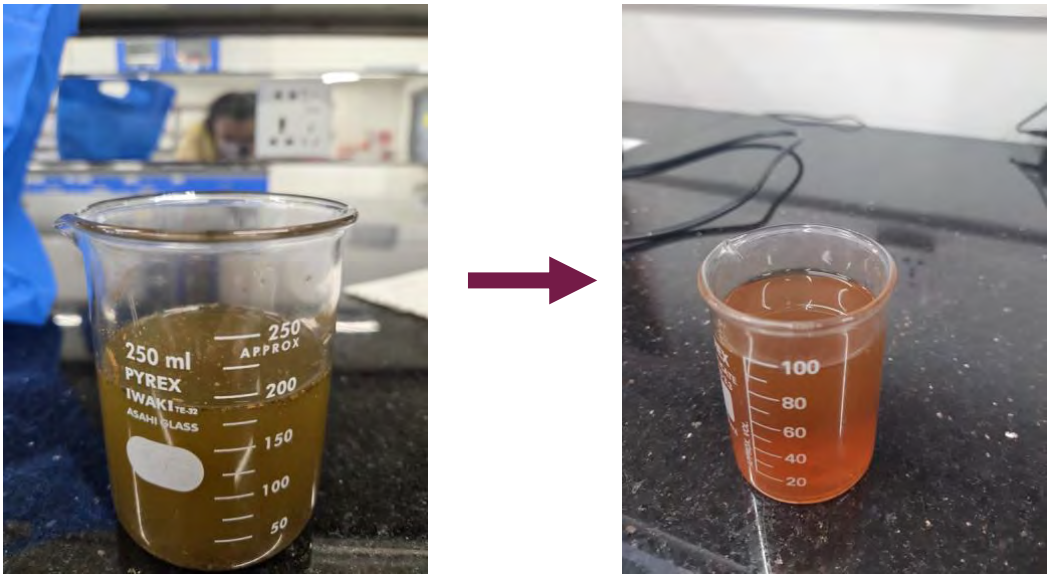

Figure 8: The colour changing process of OLE and AgNO3 solution

The AgNPs were next centrifuged at 4000 rpm for 120 min. The decantation technique was then used to separate the supernatant AgNPs suspension by discarding the upper liquid portion in the tube, leaving the solid portion at the bottom. The release of nanoparticles was then resuspended in distilled water (50 mL), after which it was again centrifuged for 30 mins. Again, through the process of decantation, the supernatant was isolated. This washing of AgNPs was done thrice to remove any unreacted extract or AgNO3. AgNPs were again redispersed in water (5 mL) by sonication in an ultrasonic bath (35 kHz) at 25°C for 15 min (Ali *et al.*, 2015).

The resultant AgNPs suspension was considered as the stock solution for subsequent spectroscopic characterisations and applications. A UV-vis spectrophotometer was used to analyse the progress of the reaction as a time-dependent variable of the formation of AgNPs. The synthesis of AgNPs was monitored under UV-Vis spectroscopy, and a typical result is viewed at around a 439 nm peak that indicates the presence of silver nanoparticles due to surface plasmon resonance (Fernando *et al.*, 2024; Ullah *et al.*, 2023).

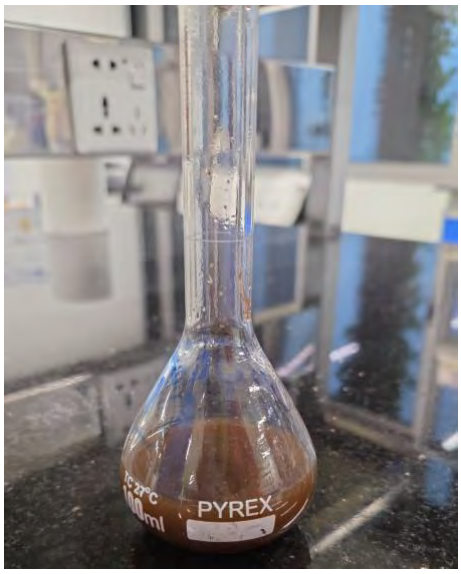

Figure 9: Synthesis of AgNPs-OLE

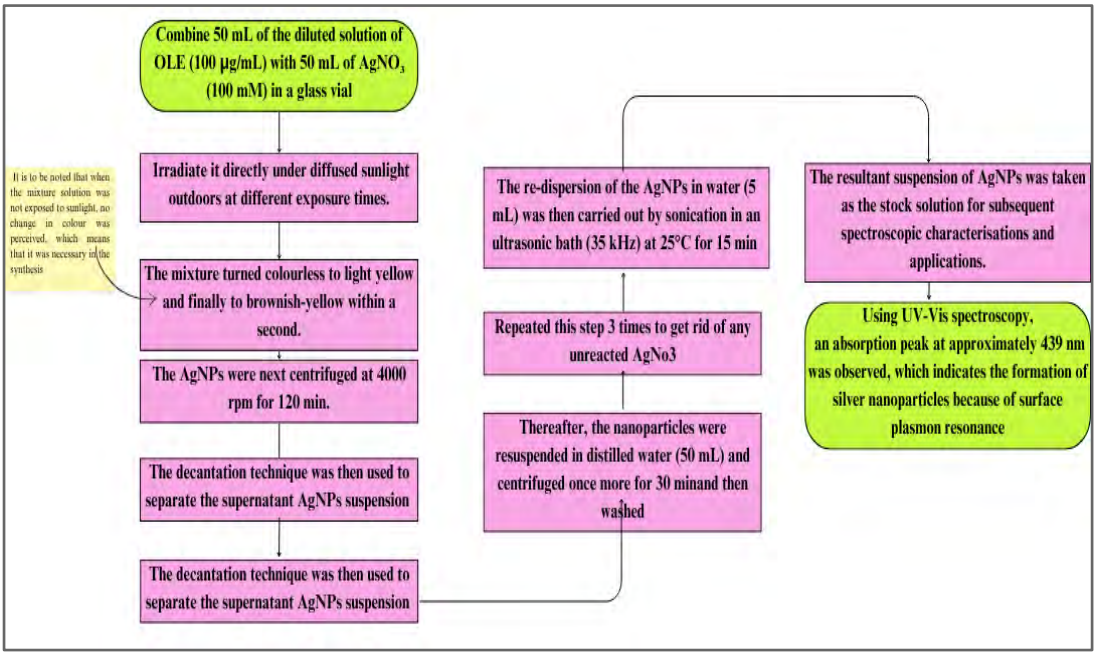

Figure 10: Flowchart of the Synthesis of AgNPs-OLE

2.6 Anti-inflammatory activity of AgNPs@OLE

2.6.1. Preparation of RBC Suspension

1. 6 mL of Blood samples were collected from a healthy volunteer (who had not taken any NSAIDs for 2 weeks before the experiment) and transferred to centrifuge tubes.
2. The anticoagulant was added to the tube to prevent clotting
3. The tubes were then centrifuged at 3000 rpm for 10 minutes.
4. Plasma (supernatant) was decanted or separated cautiously so as not to disturb the packed RBC layer.
5. To the packed RBCs, an equal volume (6 mL) of normal saline (0.9% NaCl) was added. The RBCs were then gently mixed and centrifuged at 1500–2000 rpm for 5 minutes.
6. Repeat washing 2-3 times is needed to remove any residual plasma or white cells.
7. To prepare a 20 mL 10% RBC suspension, we must add 2 mL of packed RBC and 18 mL of saline water. It is now constituted as a 10% RBC suspension (Itharat *et al.*, 2024; Silvestrini *et al.*, 2023).

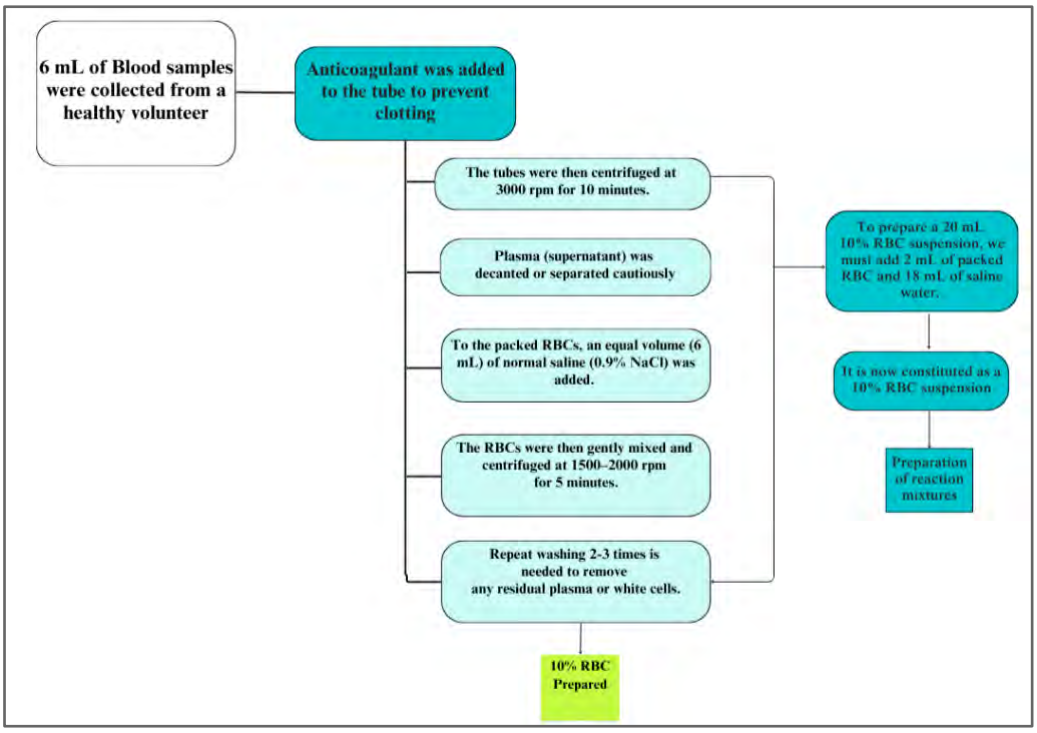

Figure 11: Flowchart of the preparation of the RBC Suspension

2.6.2. Preparation of Reaction Mixtures

Control Solution

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

Test Solution 1

a. **Stock 1:** To make 500 µg/mL, 50mg of OLE + 100 mL saline.
b. **Stock 2:** To make 100 µg/mL, 5mL from **stock 1** + 45mL saline (**Dilution factor** = 1:10)
c. Then, the serial dilution continues as follows-

Concentration	**Stock 2 volume**	**Saline Volume**
10 µg/mL	1 mL	9 mL
20 µg/mL	2 mL	8 mL
30 µg/mL	3 mL	7 mL
40 µg/mL	4 mL	6 mL
50 µg/mL	5 mL	5 mL

For the reaction Mixture, in total, 2 mL of volume, 1 mL of test solution of Ceylon olive extract with different concentrations (**10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, 50 µg/mL**) was mixed with 1 mL of 10% HRBCs suspension.

Test Solution 2

1. AgNPs-Extract (50µg/mL solution was already there; It is the stock)
2. Then, the serial dilution for the rest of the concentrations continues as follows-

Concentration	**Stock 2 volume**	**Saline Volume**
10 µg/mL	2 mL	8 mL
20 µg/mL	4 mL	6 mL
30 µg/mL	6 mL	4 mL
40 µg/mL	8 mL	2 mL

For the reaction Mixture, in total, 2 mL of volume, 1 mL of test solution of AgNPs-OLE with different concentrations (**10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, 50 µg/mL**) was mixed with 1 mL of 10% HRBCs suspension.

Standard Solution

a. **Stock 1:** To make 500 µg/mL, 50mg of aspirin Powder + 100 mL saline.
b. **Stock 2:** To make 100 µg/mL, 5mL from **stock 1** + 45mL saline (**Dilution factor** = 1:10)
c. Then, the serial dilution continues as follows-

Concentration	Stock 2 volume	Saline Volume
10 µg/mL	1 mL	9 mL
20 µg/mL	2 mL	8 mL
30 µg/mL	3 mL	7 mL
40 µg/mL	4 mL	6 mL
50 µg/mL	5 mL	5 mL

For the reaction Mixture, in total, 2 mL of volume, 1 mL of test solution of aspirin with different concentrations (**10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, 50 µg/mL**) was mixed with 1 mL of 10% HRBCs suspension.

2.6.3. Procedures of Heat-induced Hemolysis

1. Incubation of 30 minutes of reaction mixtures at 56 degrees Celsius was done in a water bath.
2. After the incubation period, all the samples were allowed to cool down to normal temperature by placing them under running tap water.
3. All the tubes of the reaction mixture were then centrifuged for five minutes at 2500 rpm.
4. Finally, the absorbance of the supernatant from each reaction mixture was plotted using a UV-Visible spectrophotometer at 560 nm (Itharat *et al.*, 2024).
5. Inhibition, % = **[(Abs control- Abs sample) x 100] /Abs control**

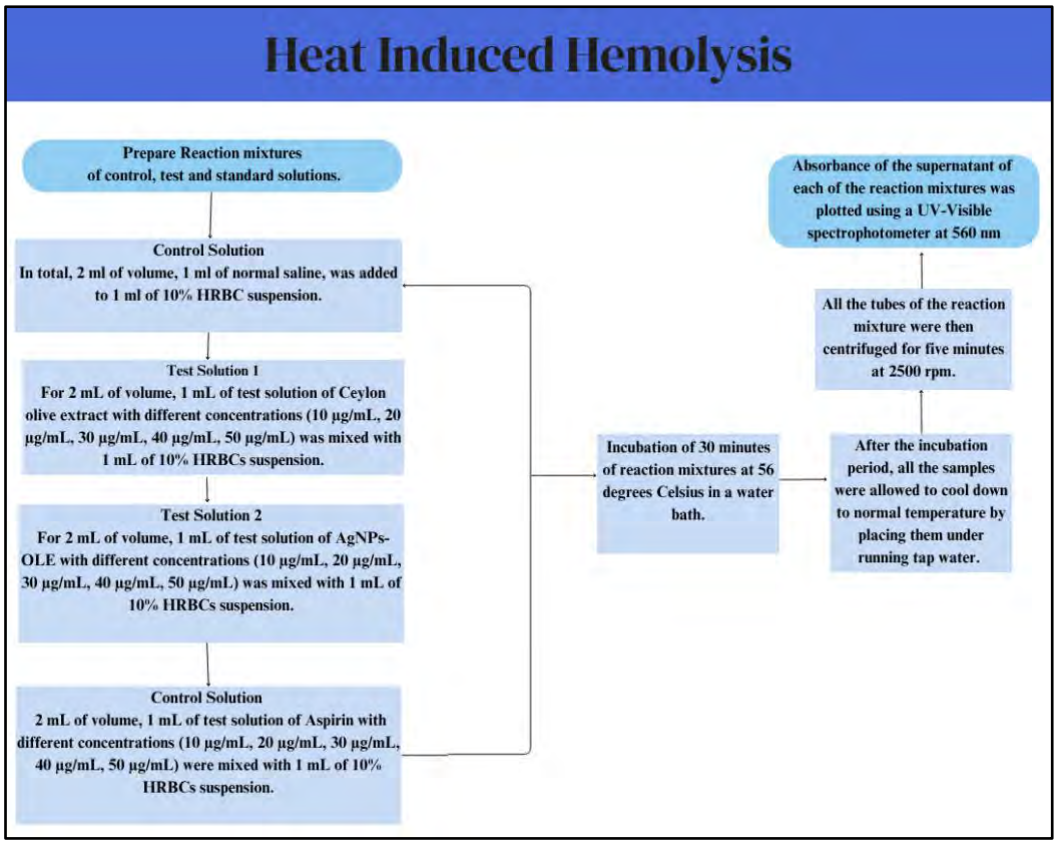

Figure 12: Flowchart of the process of Heat-Induced Hemolysis

2.7 Anti-oxidant activity of AgNPs@OLE

2.7.1. Preparation of DPPH solution

For 1 mL of a 1 mM solution, we will require the following:

Amount of DPPH (3.94 mg/100 mL)×1 mL= **0.0394 mg**

Therefore, for a 10mL solution, we will need 0.394mg DPPH

1. Weigh out accurately the DPPH of 0.394 mg.
2. Dissolve in ethanol
3. Put the weighed DPPH in a clean container.
4. Pour 10 mL of Ethanol into the container.
5. Stir or vortex the solution till the DPPH is dissolved.

2.7.2. Scavenging test

1. Now, from the prepared 10mL of DPPH solution, take 1ml and add it with 1ml of OLE (50µg mL^{-1}) in a test tube, and in another test tube, mix with 1ml of AgNPsOLE (50µg mL^{-1}).
2. The mixture was incubated at 30°C in the dark for 30 min.
3. The absorbance at 517nm was measured
4. The test was repeated 3 times.
5. Then, after getting all the absorbance values, calculate the %scavenging of the two solutions using the following formula:

   %Scavenging= **(Fc-Fs)/Fc x 100%**

Test Solution 1: 1ml DPPH + 1ml of OLE (50µg mL^{-1})

Test Solution 2: 1ml DPPH + 1ml of AgNPsOLE (50µg mL^{-1})

Control: 1ml DPPH + 1ml Ethanol

Blank: Pure Ethanol

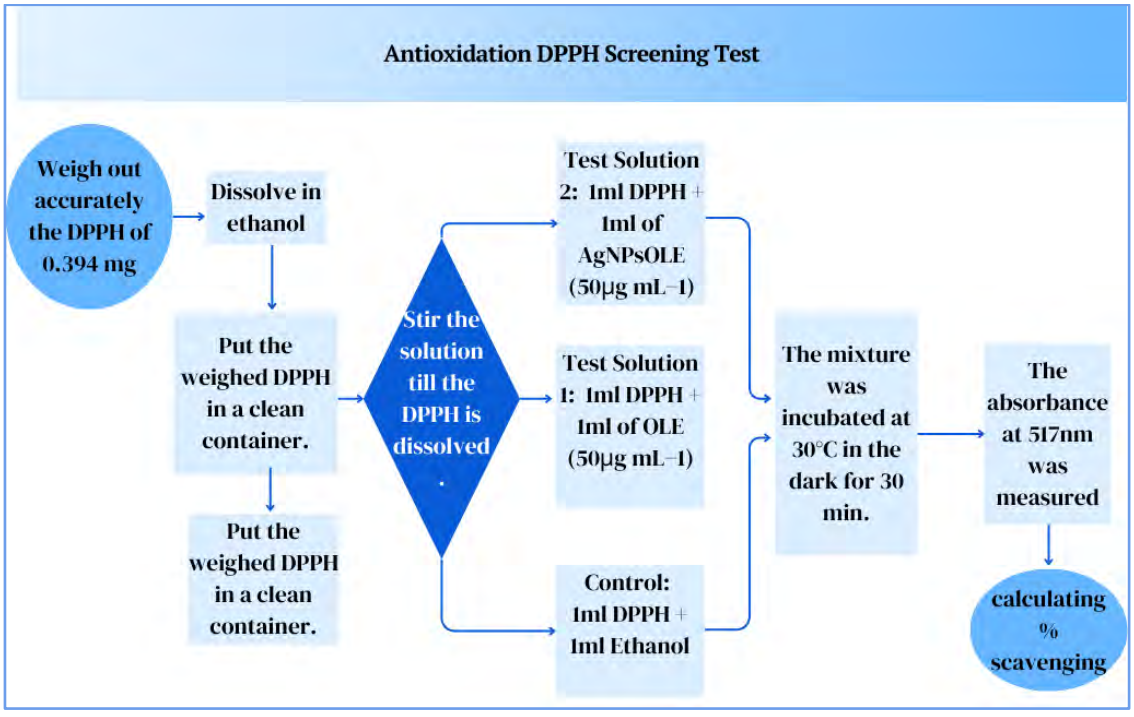

Figure 13: Flowchart of the DPPH Scavenging Test

Chapter 3: Results

3.1. AgNPs identification

After synthesising the prepared nanoparticles, they were checked in a UV-spectrophotometer to identify the absorption peak and confirm the presence of nanoparticles within the synthesised solution of Ceylon olive nanoparticles.

The peaks likely indicate the presence of nanoparticles of ceylon olive extract. The wavelength extends between 200 and 600 nm, and within this range, one can observe absorbance measures (in A) with measured peaks and troughs. Large absorption band within the visible region (400-600nm). The spectrum displays several absorbance peaks at **~400-500 nm**, often associated with the phenomenon of surface plasmon resonance (SPR), a characteristic of metallic nanoparticles, particularly silver nanoparticles.

3.2 Anti-Inflammatory Screening

After collecting the absorbance data from the UV-spectrometer and calculating the inhibition value of all the data of aspirin, OLE and AgNPS-OLE, the following values are measured and analysed further.

Table 3: The Absorbance data of Anti-inflammatory screening test

Treatment	**Aspirin**			**Extract**			**AgNPs**		
	% Inhibition of Haemolysis								
Conc. (µg/mL)	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
10	19.43	15.60	14.50	0.70	2.00	3.50	82.93	81.40	78.50
20	20.97	22.20	21.00	0.10	6.00	5.50	84.66	83.90	81.00
30	29.46	28.90	32.50	4.55	7.40	9.50	40.05	37.30	39.50
40	32.66	34.70	39.00	7.55	10.50	13.50	26.83	42.30	44.80
50	30.65	38.00	42.00	7.15	12.80	17.00	-40.22	-25.50	-0.40

The values from the table above indicate a clear analysis that the AgNPs values are much better than the extract alone and also the aspirin. This one is the most predicted outcome of this experiment. However, AgNPs also start to show toxic effects at 50µg/mL, but the aspirin had a good effect at that concentration, and the extract concentration was also maximum at that point. However, if we look at the table more closely, at 20µg/mL, the AgNPs give the most therapeutic inhibition value, and conversely, the OLE extracts give the lowest values at the same concentration.

The further analysis was conducted using Turkey's multiple comparison test using one-way ANOVA with the three groups, where we eventually analysed the most important and impactful concentration, which can give a better therapeutic effect and less toxicity compared to other concentrations.

3.2.1 Analysis for the 20µg/mL concentration

Table 4: Row Statistic analysis data of the 20µg/mL concentration

Concentration	**Control (Aspirin)**			**Extract Alone**			**AgNP Extract**		
X	Mean	SD	N	Mean	SD	N	Mean	SD	N
20	21.390	0.702	3	3.867	3.272	3	83.187	1.931	3

For the data above, the following graph can be illustrated-

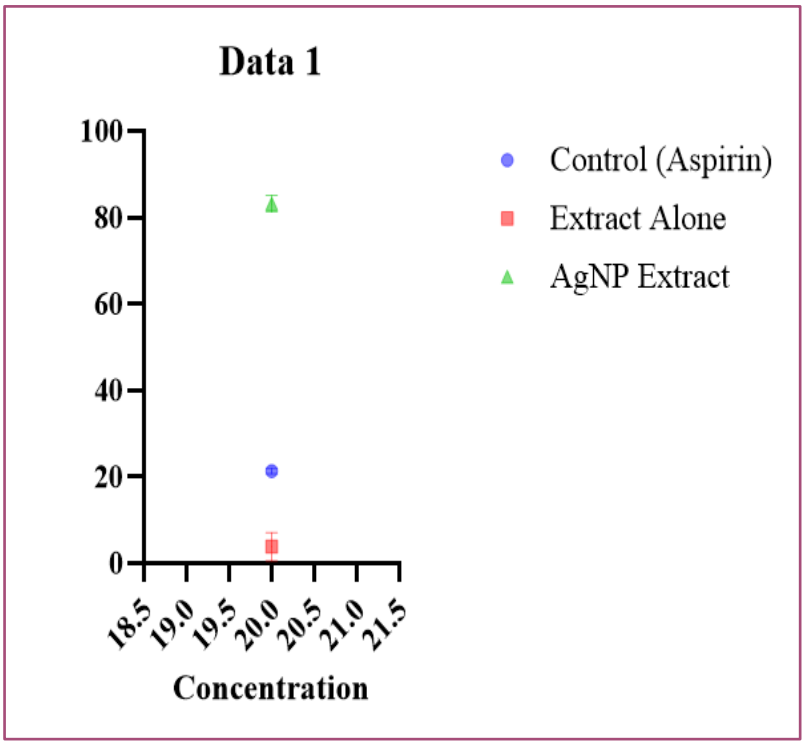

Figure 14: Comparative effect of the Anti-inflammatory effect at 20µg/mL

The graph illustrates the relative changes in the three treatment levels: Control (Aspirin), Extract Alone, and AgNP (Silver Nanoparticle). Values were recorded on a Y-axis (% inhibition), and biological parameters on the X-axis (concentration of 20.0). Concentration (x-axis) is kept constant throughout all the groups at 20.0, whereas the scale of the y-axis starts at 0 and goes up to 100, enabling one to see the differences in response level clearly.

All pairwise comparisons show statistically significant differences. AgNP Extract group shows the highest biological activity, indicating the mean value of nearly 83.2 and a low standard deviation (±1.93) with the shown narrow error bars. This will imply great efficacy and consistency in this circle. Meanwise, the Control (Aspirin) shows moderate activity with a mean of 21.4, and the standard deviation is slightly smaller in the form of ±0.70, which means that aspirin does affect the given process, but not quite as much as the AgNP formulation. The Extract Alone group has a low biological response of a mean of only 3.9 and a standard deviation of ±3.3, and this shows the low value and very low variability of the biological activity.

Overall, the evident data allow concluding that the bioactivity of the extract with the incorporation of silver nanoparticles is considerably higher than that of the extract where the silver nanoparticles are not added or the standard control, aspirin. The standard deviations of all the groups are relatively smaller, indicating consistency and replicability of the results. This observation shows that AgNP-based formulations have the potential to enhance the therapeutic or functional ability of natural extract, probably through enhanced bioavailability or synergistic effect between silver nanoparticles and natural components.

3.2.2 Statistical Analysis of the Whole Data Table (Table 03)

Tukey's multiple comparison test of two-way ANOVA shows that there are significant differences among treatments in different concentrations (table) :

Row 1 (10µg/mL): All the comparisons are statistically different. A significantly higher dose was obtained by AgNP Extract as compared with Aspirin and Extract Alone, **p < 0.0001**.

Row 2 (20µg/mL): Similar trends observed; all pairwise differences are extremely significant.

Row 3 (30µg/mL): There are significant differences between Aspirin vs Extract Alone **(*p= 0.0003)** and Extract Alone vs AgNP Extract **(**, p <0.0001)**. Nonetheless, the Aspirin vs AgNP Extract is a non-significant factor **(ns, p = 0.2396)**.

Row 4 (40µg/mL): The differences between the two comparisons of Aspirin vs Extract Alone and Extract Alone vs AgNP Extract are significant, but not between Aspirin vs AgNP Extract **(p = 0.8805).**

Row 5 (50µg/mL): Again, all the pairwise differences are significant, signifying the strong divergence, maybe due to the negative result in the AgNP group.

The following graph represents these interpretations.

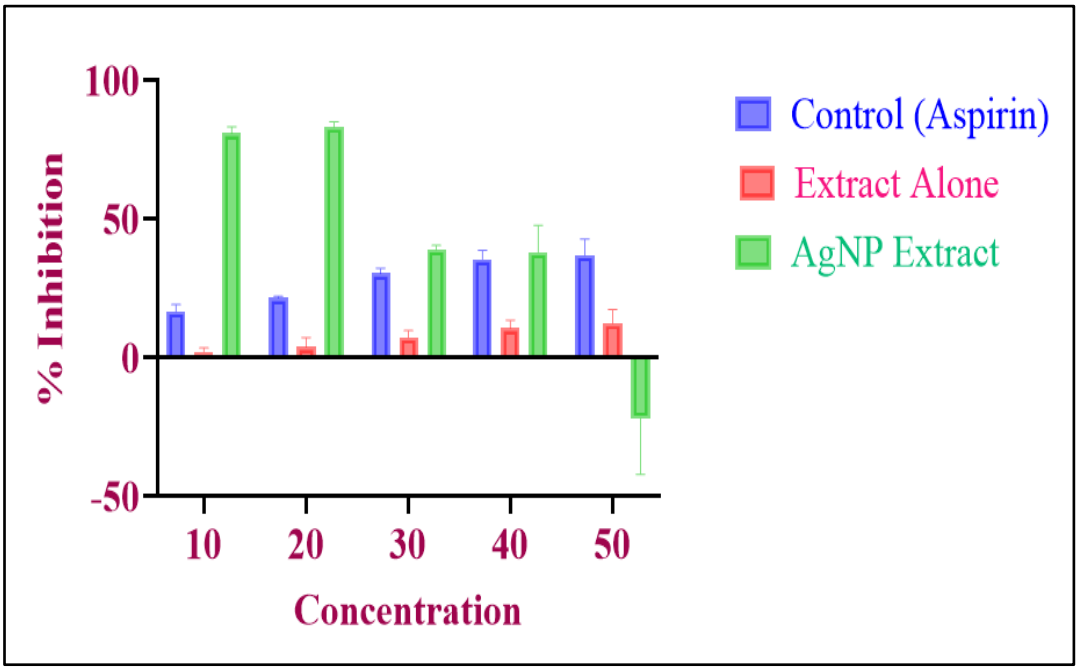

Figure 15: Comparison of the %inhibition of the five concentrations of the three groups

From the raw data and bar graph, there is a steady increase in inhibition of Control (Aspirin) that increases by 19.43% at 10µg/mL to 30.65% at 50µg/mL. The Extract Alone group shows low levels of inhibition at lower concentrations (0.70% to 7.15%), but this group shows slight

inhibition at higher doses. Conversely, inhibition is extremely high in the AgNP Extract group starting at low concentrations (as high as 84.66% at 20 µg/mL) but with an abrupt decrease, even turning into negative territory (-40.22%) at further increased concentrations, indicating that AgNP Extract may be cytotoxic or antagonistic at larger doses.

Also, the bar graph represents the statistical analytical data visually. At 10 and 20µg/mL, the % inhibition is maximum in AgNP Extract, unlike in both Aspirin and Extract Alone. The inhibition significantly reduces as concentration increases, and it even becomes negative at 50µg/mL. Comparatively, the inhibitory effect of Aspirin is consistent and proportionately increased in all concentrations, whereas the inhibition in Extract Alone is low and emerges slowly as the concentration increases. The trends in the graph suggest a dose-dependent reversal of the action of AgNP Extract, possibly due to toxicity at higher concentrations.

3.3 Antioxidant Screening

After collecting the absorbance data from the UV-spectrometer and calculating the %scavenging value of all the data of OLE and AgNPS-OLE, the following values are measured and analysed further.

Table 5: %scavenging values

	Extract			**AgNps**		
	Trial 1	**Trial 2**	**Trial 3**	**Trial 1**	**Trial 2**	**Trial 3**
50 µg mL^{-1}	78.93	74.97	88.95	72.72	83.18	68.92

After having all the data, it is clear that having both the OLE and AgNPs-ole shows a prominent antioxidant effect. However, for a better understanding, we conducted a statistical analysis using 2-way ANOVA by GraphPad Prism.

3.3.1 Two-way ANOVA Analysis of the Data Table

Here, at first, we undergo comparisons between all the trial values of the extracts and the nanoparticles. The two-way ANOVA analysis of the data on antioxidation activity shows that no factors (row or column) produced a statistically significant effect on the results. The p-values of both the row factor ($p = 0.9366$) and column factor ($p = 0.5378$) do not show a p-value less than 0.05, so there is no significant difference in trials and also between the two sample groups. The Extract had a slightly higher predicted mean antioxidation activity of 80.95 than that of AgNPs (74.94), but the difference (6.01) was not significant because it had a large standard error (8.153) and confidence interval (between -29.07 and 41.09). The power of the test is reduced by the small sample size (n=6) and the small degree of freedom. In general, the analysis indicates that the Extract and the AgNPs do not present a significant difference in the capacity of antioxidation, under the conditions tested.

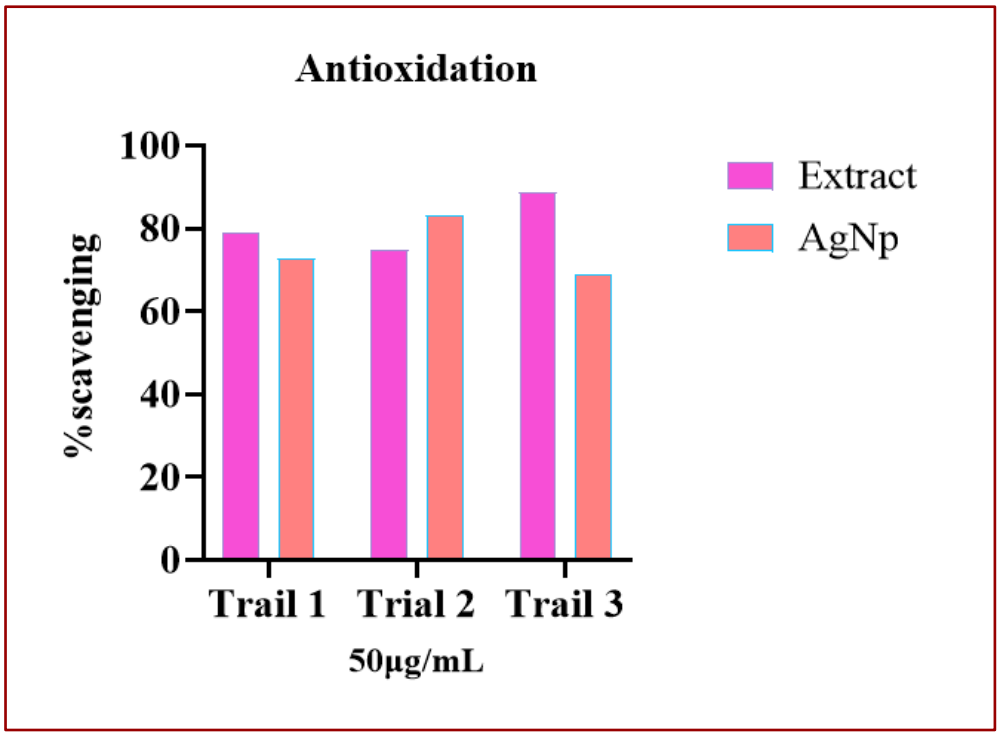

Figure 16: Comparison of the % scavenging of the Crude extract and AgNP-OLE

3.3.2 Row Statistics of the Data Table (Table 05)

We also conducted a row statistics test on the values of *Table 05* for further clarification, which also yielded the same results, highlighting that both treatments presented a strong antioxidant activity, but the Extract values were better. However, the difference between them is not necessarily statistically significant, as the standard deviations are also close, and so are the error bars.

Table 6: Row Statistics of Antioxidant Effects

Concentration	**Extract**			**AgNps**		
X	Mean	SD	N	Mean	SD	N
50µg/mL	80.050	7.206	3	74.940	7.385	3

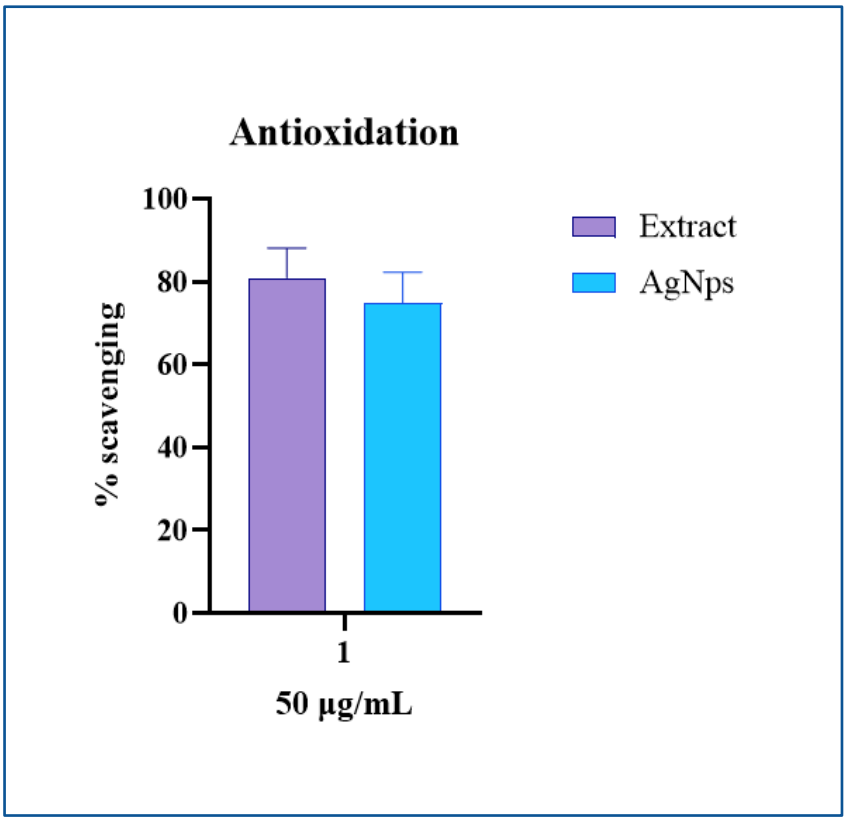

Figure 17: Comparative effect of the Anti-oxidation effect at 50µg/mL

3.3.3 Compare and Contrast with Ascorbic Acid

As we know, ascorbic acid exhibits strong and consistent antioxidant activity, typically at 50µg mL^{-1}, yielding antioxidant activity of 90-95%. When compared to the above two test values with ascorbic acid, the Extract and AgNPs demonstrate moderately strong antioxidative activity but are less likely to be effective. The slightly higher value of the mean antioxidant activity of the Extract (80.95%) compared to AgNPs (74.94%) was not significant ($p = 0.5378$). The test samples considered show promising inhibition rates are less potent than ascorbic acid. Because at nearly similar levels of concentration, ascorbic acid inhibits antioxidants by over 90%. Therefore, the Extract and AgNPs both possess high levels of antioxidant potential.

Chapter 4: Discussion

This project successfully assessed and compared the in vitro anti-inflammatory and antioxidant activity of the crude extract of *Ellaeocarpus serratus* and the silver nanoparticles(AgNPs) from *E. serratus* synthesised through green synthesis.

There was a striking difference in treatments during the anti-inflammatory screening.AgNPs were much more effective in preventing heat-induced hemolysis compared to the extract alone and aspirin, with noticeable inhibitory effects in 20 µg/mL, the effect of which is shown as follows: AgNPs (mean = 83.20), Crude extract (mean = 3.87) and Aspirin (mean = 21.40) respectively (*Table 04)*. Nonetheless, at the higher concentration (50 µg/mL), AgNPs exhibited significant reduction in activity with even negative inhibition, indicating that they may be cytotoxic at large doses. The significant difference between the treatment groups was further confirmed statistically by the Tukey's post hoc test, most significantly at 10 and 20 µg/ml concentrations, which shows the therapeutic effectiveness of AgNPs at low concentrations.

On the other hand, the DPPH scavenging test indicated that both the extract and AgNPs exhibited considerable activity. The mean inhibition values were 80.95% and 74.94%, respectively (Table 6). There was no statistically significant difference found ($p = 0.5378$). However, this shows similar antioxidant potential. Although they were very active, they were slightly less active than ascorbic acid (more than 90% which is usually inhibited at the same concentrations).

Overall, the integration of silver nanoparticles into *E. serratus* was found to be highly efficient in terms of increasing the anti-inflammatory potential of the given plant, especially when compared to its low concentrations. Though the antioxidant potential of AgNPs was lowered as compared to that of the crude extract, the difference was not found to be significant, which means that the nanoparticles had the essence of the same antioxidant power. These two types of action imply that AgNPs synthesised based on *E. serratus* may serve as a promising agent

in the treatment of inflammation-related illnesses and oxidative stress. Nevertheless, the presence of the cytotoxic effect observed at the higher dose explains the necessity of careful dose optimisation and additional in vivo research to assess the safety and effectiveness.

Chapter 5: Conclusion

To conclude, the study demonstrates that the silver nanoparticles (AgNPs) produced by green synthesis techniques using *Elaeocarpus serratus* leaf extract have a significant and potential anti-inflammatory effect as compared to that of the crude plant extract, as well as standard drugs, which is valuable in highlighting them as good source of lead molecules in the advancement of therapeutic agents. Even though the extract and AgNPs possess potent antioxidant capability, AgNPs uniquely enhance bioactivity owing to a synergy between the phytochemicals of the plant and the properties of nanoparticles. The green synthesis method is a sustainable process at a low cost to develop better effectiveness of these bioactive nanoparticles, especially in the inhibition of inflammation at low doses. Yet, cytotoxicity at increased doses of nanoparticles was observed, highlighting the necessity of dose optimisation in this particular area. The AgNPs produced by *E. serratus* are promising lead molecules to be used in future pharmaceutical development with respect to treating diseases of inflammation and oxidative stress.

Chapter 6: Future Work

1. **In Vivo Anti-Inflammatory, Antioxidant Studies and Toxicity Studies:** Extensive in vivo investigations, in animal models, can be carried out to determine the efficacy, pharmacokinetics and safety profile of *E. serratus*-derived silver nanoparticles.
2. **Mechanistic Investigations of Anti-Inflammatory Activity:** The anti-inflammatory molecular mechanisms of action of the silver nanoparticles (e.g., by modulation of certain signalling pathways, e.g., NF-κB, MAPK) can be elucidated (Ekambaram *et al*., 2021). It will help to understand their mode of action better, study the effect on pro-inflammatory cytokines, enzymes (COX-1, COX-2, LOX), and markers of oxidative stress.
3. **Advancements in antioxidant research in the area of functional nutrition and natural compounds:** Consumers are becoming more focused on functional nutrition – food and supplements rich in antioxidants that fight oxidative stress and inflammation. Future studies can investigate other dietary antioxidants, bioactive phytochemicals and the part these play in the avoidance or control of inflammation-related conditions, and check their biochemical mechanism.
4. **Drug Delivery and Formulation Development:** We can formulate nanoparticles into a delivery system, e.g., hydrogels, creams or injectable systems, to deliver the silver nanoparticles directly to where they are needed.
5. **Comparative Studies to other Medicinal Plants:** We can prepare nano-particles of silver with other anti-inflammatory and antioxidant medicinal plants extracts and compare their activity with that of *E. serratus* nanoparticles. It will help to determine synergistic mixtures of plant extracts with improved synthesis and bioactivity of nanoparticles.

6. **Potential for Novel Drug Leads:** The unique properties of nanoparticle extracts showing promising effects than the standard drugs may serve as a template or lead molecule for synthesising new drugs targeting cancer, diabetes, infectious diseases, other neurological disorders, etc.

References

Ali, M., Kim, B., Belfield, K. D., Norman, D., Brennan, M., & Ali, G. S. (2015). Green synthesis and characterisation of silver nanoparticles using Artemisia absinthium aqueous extract — A comprehensive study. Materials Science and Engineering C, 58, 359–365. https://doi.org/10.1016/j.msec.2015.08.045

Ali, M. (2020, June 27). Jolpai or Ceylon olive, Elaeocarpus serratus, Elaeocarpaceae. Flora of Bangladesh.https://www.floraofbangladesh.com/2018/11/jolpai-elaeocarpus serratus.html

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., Orhan, I. E., Banach, M., Rollinger, J. M., Barreca, D., Weckwerth, W., Bauer, R., Bayer, E. A., Majeed, M., Bishayee, A., Bochkov, V., Bonn, G. K., Braidy, N., Bucar, F., Cifuentes, A., D'Onofrio, G., Bodkin, M.,... Supuran, C. T. (2021). Natural products in drug discovery: advances and opportunities. Nature Reviews Drug Discovery, 20(3), 200–216. https://doi.org/10.1038/s41573-020-00114-z

Aryal, P. (2021). Medicinal value of Elaeocarpus sphaericus: A review. Asian Journal of Pharmacognosy, 6(3), 15-21.

Banu, Z., Poduri, R. R., & Bhattamisra, S. K. (2024). A comprehensive review on phytochemistry, health benefits, and therapeutic potential of Elaeocarpus angustifolius Blume. Ann. Phytomed, 13(1), 370-383. http://dx.doi.org/10.54085/ap.2024.13.1.37

Baruah, P. S., Deka, K., Lahkar, L., Sarma, B., Borthakur, S. K., & Tanti, B. (2019). Habitat distribution modelling and reinforcement of Elaeocarpus serratus L.: A threatened tree species of Assam, India for improvement of its conservation status. Acta Ecologica Sinica, 39(1), 42-49. http://dx.doi.org/10.1016/j.chnaes.2018.06.002

Barathi, S., Ramalingam, S., Krishnasamy, G., & Lee, J. (2024). Exploring the biomedical frontiers of Plant-Derived Nanoparticles: synthesis and biological reactions. *Pharmaceutics*, *16*(7), 923. https://doi.org/10.3390/pharmaceutics16070923

Bedlovičová, Z., Strapáč, I., Baláž, M., & Salayová, A. (2020). A brief overview of the antioxidant activity determination of silver nanoparticles. *Molecules*, *25*(14), 3191. https://doi.org/10.3390/molecules25143191

Chaachouay, N., & Zidane, L. (2024). Plant-Derived Natural Products: a source for drug discovery and development. Drugs and Drug Candidates, 3(1), 184–207. https://doi.org/10.3390/ddc3010011

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., & Zhao, L. (2017). Inflammatory responses and inflammation-associated diseases in organs. Oncotarget, 9(6), 7204–7218. https://doi.org/10.18632/oncotarget.23208

Dzobo, K. (2021). The role of natural products as sources of therapeutic agents for innovative drug discovery. In Elsevier eBooks (pp. 408–422). https://doi.org/10.1016/b978-0-12-820472-6.00041-4

Ebob, O. T., Babiaka, S. B., & Ntie-Kang, F. (2021). Natural products as potential lead compounds for drug discovery against SARS-COV-2. Natural Products and Bioprospecting, 11(6), 611–628. https://doi.org/10.1007/s13659-021-00317-w

Ekambaram, S. P., Aruldhas, J., Srinivasan, A., & Erusappan, T. (2021). Modulation of NF-κB and MAPK signalling pathways by hydrolysable tannin fraction from Terminalia chebula fruits contributes to its anti-inflammatory action in RAW 264.7 cells. Journal of Pharmacy and Pharmacology, 74(5), 718–729. https://doi.org/10.1093/jpp/rgab178

Ezeoke, M. C. (2018). Phytochemical Investigation of Alkaloids from the Leaves of Elaeocarpus tectorius (Lour.) Poir. And Elaeocarpus angustifolius Blume (Doctoral dissertation, University of Nottingham).

Fayez, N., Khalil, W., Abdel-Sattar, E., & Abdel-Fattah, A. M. (2023). In vitro and in vivo assessment of the anti-inflammatory activity of olive leaf extract in rats. Inflammopharmacology, 31(3), 1529–1538. https://doi.org/10.1007/s10787-023-01208-x

Filho, S. A., Santos, M. S. D., Santos, O. a. L. D., Backx, B. P., Soran, M., Opriş, O., Lung, I., Stegarescu, A., & Bououdina, M. (2023). Biosynthesis of nanoparticles using plant extracts and essential oils. *Molecules*, *28*(7), 3060. https://doi.org/10.3390/molecules28073060

Geetha, D. H., Rajeswari, M., & Jayashree, I. (2013). Chemical profiling of Elaeocarpus serratus L. by GC-MS. Asian Pacific journal of tropical biomedicine, 3(12), 985–987. https://doi.org/10.1016/S2221-1691(13)60190-2

Islam, R., Sun, L., & Zhang, L. (2021). Biomedical applications of Chinese Herb-Synthesised silver nanoparticles by Phytonanotechnology. *Nanomaterials*, *11*(10), 2757. https://doi.org/10.3390/nano11102757

Itharat, A., Thongmee, P., Piwngam, K., Inprasit, J., Makchuchit, S., Kuropakornpong, P., & Davies, N. M. (2024). In vitro and in vivo evaluation of anti-inflammatory activities of ethanol extract from Lom-Am-Ma-Pruek remedy for pain relief. Research in pharmaceutical sciences, 19(6), 683–697. https://doi.org/10.4103/RPS.RPS_26_24

Jawla, S., & Rai, D. V. (2016). Pharmacognostic studies on Rudraksh (Elaeocarpus angustifolius Blume) fruit. Advances in Biological Research, 10(6), 382-387.

Lim, H. S., Park, G., & Kim, Y. U. (2024). Anti-Oxidative Bioactivities of Medicinal Herbs in the Treatment of Ageing-Related Diseases. Antioxidants (Basel, Switzerland), 13(6), 631. https://doi.org/10.3390/antiox13060631

Lins, P. G., Pugine, S. M. P., Scatolini, A. M., & De Melo, M. P. (2018). In vitro antioxidant activity of olive leaf extract (Olea europaea L.) and its protective effect on oxidative

damage in human erythrocytes. Heliyon, 4(9), e00805. https://doi.org/10.1016/j.heliyon.2018.e00805

Mahomoodally, M. F., & Sookhy, V. (2018). Ethnobotany and Pharmacological Uses of Elaeocarpus floribundus Blume (Elaeocarpaceae). In Springer eBooks (pp. 125–137). https://doi.org/10.1007/978-3-319-93997-1_4

Mittal, M., Siddiqui, M. R., Tran, K., Reddy, S. P., & Malik, A. B. (2014). Reactive oxygen species in inflammation and tissue injury. Antioxidants & redox signalling, 20(7), 1126–1167. https://doi.org/10.1089/ars.2012.5149

Nasim, N., Sandeep, I. S., & Mohanty, S. (2022). Plant-derived natural products for drug discovery: current approaches and prospects. The Nucleus: an International Journal of cytology and allied topics, 65(3), 399–411. https://doi.org/10.1007/s13237-022-00405-3

Najmi, A., Javed, S. A., Bratty, M. A., & Alhazmi, H. A. (2022). Modern approaches in the discovery and development of Plant-Based natural products and their analogues as potential therapeutic agents. Molecules, 27(2), 349. https://doi.org/10.3390/molecules27020349

Nguyen, N. T. T., Nguyen, L. M., Nguyen, T. T. T., Nguyen, T. T., Nguyen, D. T. C., & Van Tran, T. (2022). Formation, antimicrobial activity, and biomedical performance of plant-based nanoparticles: a review. *Environmental Chemistry Letters*, *20*(4), 2531–2571. https://doi.org/10.1007/s10311-022-01425-w

NV, A., T, S., & M, S. (2023). A review on the pharmacological activities of Eleocarpus Serratus. Research Journal of Pharmacology and Pharmacodynamics, 66–68. https://doi.org/10.52711/2321-5836.2023.00013

Ogundele, A. V., Haldar, S., Yadav, A., & Das, A. M. (2021). Elaeocarpus floribundus Bl. Seeds as a new source of bioactive compounds with promising antioxidant and

antimicrobial properties. Zeitschrift für Naturforschung C, 76(3-4), 141-146. https://doi.org/10.1515/znc-2020-0114

Pradeep, M., Kruszka, D., Kachlicki, P., Mondal, D., & Franklin, G. (2021). Uncovering the Phytochemical Basis and the Mechanism of Plant Extract-Mediated Eco-Friendly Synthesis of Silver Nanoparticles Using Ultra-Performance Liquid Chromatography Coupled with a Photodiode Array and High-Resolution Mass Spectrometry. *ACS Sustainable Chemistry & Engineering*, *10*(1), 562–571. https://doi.org/10.1021/acssuschemeng.1c06960

Prasannan, P., Jeyaram, Y., Pandian, A., Raju, R., & Sekar, S. (2020). A review on taxonomy, phytochemistry, pharmacology, threats and conservation of Elaeocarpus L.(Elaeocarpaceae). The Botanical Review, 86(3), 298-328. http://dx.doi.org/10.1007/s12229-020-09229-9

Raji, R., & Siril, E. (2021). Genetic diversity analysis of promising Ceylon olive (Elaeocarpus serratus L.) genotypes using morphological traits and ISSR markers. Current Plant Biology, 26, 100201. https://doi.org/10.1016/j.cpb.2021.100201

Raji, R., & Siril, E. A. (2021). Alteration of media enables efficient in vitro cloning of mature *Elaeocarpus serratus* L. (Ceylon olive): a commercially important fruit tree. *Physiology and molecular biology of plants: an international journal of functional plant biology*, *27*(2), 429–443. https://doi.org/10.1007/s12298-021-00955-x

Salehi, B., Azzini, E., Zucca, P., Varoni, E. M., Kumar, N. V. A., Dini, L., Panzarini, E., Rajkovic, J., Fokou, P. V. T., Peluso, I., Mishra, A. P., Nigam, M., Rayess, Y. E., Beyrouthy, M. E., Setzer, W. N., Polito, L., Iriti, M., Sureda, A., Quetglas-Llabrés, M. M., . . . Sharifi-Rad, J. (2020). Plant-Derived Bioactives and Oxidative Stress-Related Disorders: A Key Trend towards Healthy Ageing and Longevity Promotion. *Applied Sciences*, *10*(3), 947. https://doi.org/10.3390/app10030947

SD, S. (2023). An overview of the ethnopharmacological, nutritional, and phytochemical perspectives of Elaeocarpus floribundus Blume. Current Pharmacology Reports, 9(5), 377-389. http://dx.doi.org/10.1007/s40495-023-00329-9

Sikder, M. M., Li, X., Akumwami, S., & Labony, S. A. (2025). Reactive Oxygen Species: Role in Pathophysiology, and Mechanism of Endogenous and Dietary Antioxidants during Oxidative Stress. *Chonnam Medical Journal*, *61*(1), 32–45. https://doi.org/10.4068/cmj.2025.61.1.32

Silvestrini, A., Giordani, C., Bonacci, S., Giuliani, A., Ramini, D., Matacchione, G., Sabbatinelli, J., Di Valerio, S., Pacetti, D., Procopio, A. D., Procopio, A., & Rippo, M. R. (2023). Anti-Inflammatory Effects of Olive Leaf Extract and Its Bioactive Compounds Oleacin and Oleuropein-Aglycone on Senescent Endothelial and Small Airway Epithelial Cells. *Antioxidants (Basel, Switzerland)*, *12*(8), 1509. https://doi.org/10.3390/antiox12081509

Singh, S. K., Shrivastava, S., Mishra, A. K., Kumar, D., Pandey, V. K., Srivastava, P., Pradhan, B., Behera, B. C., Bahuguna, A., & Baek, K. H. (2023). Friedelin: Structure, Biosynthesis, Extraction, and Its Potential Health Impact. *Molecules (Basel, Switzerland)*, *28*(23), 7760. https://doi.org/10.3390/molecules28237760

Sumanarathne, W., Ranaweera, L., Weebadde, C., & Sooriyapathirana, S. (2020). Assessment of the Morphometric Variation of Fruits and Phylogenetics of Elaeocarpus ganitrus (Woodenbegar) and Elaeocarpus serratus (Ceylon Olive). *Journal of Agricultural Sciences – Sri Lanka*, *15*(3), 362–377. https://doi.org/10.4038/jas.v15i3.9028

Tropic, T. (2023, March 16). *Elaeocarpus serratus (Common name: Ceylon Olive) | Top Tropicals Plant Encyclopedia*. TopTropicals.com - Rare Plants for Home and Garden. https://toptropicals.com/catalog/uid/elaeocarpus_serratus.htm

Ullah, S., Khalid, R., Rehman, M. F., Irfan, M. I., Abbas, A., Alhoshani, A., Anwar, F., & Amin, H. M. A. (2023). Biosynthesis of phyto-functionalized silver nanoparticles using olive fruit extract and evaluation of their antibacterial and antioxidant properties. *Frontiers in Chemistry*, *11*. https://doi.org/10.3389/fchem.2023.1202252

Villalva, M., Silvan, J. M., Guerrero-Hurtado, E., Gutierrez-Docio, A., Del Hierro, J. N., Alarcón-Cavero, T., Prodanov, M., Martin, D., & Martinez-Rodriguez, A. J. (2022). Influence of In Vitro Gastric Digestion of Olive Leaf Extracts on Their Bioactive Properties against H. pylori. *Foods*, *11*(13), 1832. https://doi.org/10.3390/foods11131832

Yeshma, M. (2015). *Quality evaluation and value addition of fruits of Elaeocarpus serratus L.(Ceylon olive tree)* (Doctoral dissertation, Department of Forest Management and Utilisation, College of Forestry, Vellanikkara).

Project

ORIGINALITY REPORT

7%	6%	5%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	dspace.bracu.ac.bd Internet Source	3%
2	www.ncbi.nlm.nih.gov Internet Source	1%
3	www.dixdecoeur.com Internet Source	1%
4	Submitted to Vision West Nottinghamshire College Student Paper	1%
5	www.apjtb.com Internet Source	1%
6	"Green Metal Nanoparticles", Wiley, 2018 Publication	1%

Exclude quotes Off
Exclude bibliography On
Exclude matches