# ANTI-BACTERIAL AND ANTI-INFLAMMATORY PROPERTIES OF DIFFERENT TYPES OF COOKING OILS

**Dissertation submitted to** 

# MAHATMA GANDHI UNIVERSITY, KOTTAYAM

# In partial fulfillment of the requirement for the degree of

**BACHELOR OF SCIENCE** 

Submitted by

# CATHERINE JOHNS (210021037717)

Under the supervision of

**Dr. Sherin Antony** 

Assistant Professor, Department of zoology



# BHARATA MATA COLLEGE, THRIKAKKARA

(Affiliated to Mahatma Gandhi University Accredited by NAAC with A+ Grade)

2021-2024

# DEPARTMENT OF ZOOLOGY BHARATA MATA COLLEGE THRIKKAKKARA



Date:

# **CERTIFICATE**

This is to certify that the project entitled	1 "	
		" is a bonafide work done
bywith	Register	No:
during 2023-24 in partial fulfilment o	f the require	ement for the award of the
Bachelor Degree of Science in Zoo	logy of Ma	hatma Gandhi University,
Kottayam.		

Head of the Department Dr. Simi Joseph P

# DECLARATION

I, **CATHERINE JOHNS** (210021037717), hereby declare that the dissertation work entitled "Anti-bacterial and anti-inflammatory properties of different types of cooking oils" submitted for the award of a Bachelor's degree in Bharata Mata College, Thrikkakara, is a partial fulfillment of the requirements. This work was done by me during the period from December 2023 to February 2024 under the supervision and guidance of Dr. Sherin Antony, Assistant Professor, Zoology Department. I affirm that this thesis is original and has not been submitted for any degree, fellowship, or similar qualification by any other candidate to any university.

Date:

Place: Thrikkakara

Signature:

CATHERINE JOHNS

# ANTI-BACTERIAL AND ANTI-INFLAMMATORY PROPERTIES OF DIFFERENT TYPES OF COOKING OILS

#### Abstract

Various cooking oils offer various health benefits attributed to their unique compositions. Cooking oils play a crucial role in culinary practices worldwide, offering various health benefits, culinary uses, and quality considerations. Health benefits include heart health, Antioxidant properties, Anti-inflammatory properties, Omega-3 fatty acids, and Vitamin E. Coconut oil (Cocos nucifera), rich in medium-chain fatty acids, exhibits antibacterial qualities, specifically associated with the presence of lauric acid. Its anti-inflammatory potential is bolstered by compounds that mitigate internal and external inflammation. Almond oil, (Prunus amygdales) with its antibacterial nature against E. coli, anti-inflammatory activity, and antioxidant effects. When chosen and utilized mindfully, these cooking oils can enhance culinary experiences and offer a range of health-promoting properties. We did a comparative analysis of the anti bacterial effect of coconut oil and almond oil.

# **INTRODUCTION**

Cooking oil is crucial in culinary traditions worldwide, its roots stretching back to ancient times. While the exact inception of cooking oils remains elusive, their history intertwines with the dawn of human civilization. Around 25,000 BC, the discovery of fire marked a pivotal moment as humans began harnessing animal fats for cooking purposes. As civilization progressed, the extraction of vegetable oils became a widespread practice. Early methods involved pressing various plants, seeds, and nuts to yield oils, albeit in crude and unrefined forms. These primitive oils lacked the sophistication of modern processing techniques, embodying a raw and natural essence. Throughout history, different cultures contributed to the rich tapestry of cooking oils. The Japanese and Chinese pioneered soy oil around 2000 BC, while olive oil production flourished in the Mediterranean regions as early as 4000 BC. In North America and Mexico, indigenous peoples innovated methods to extract oils from peanuts and sunflower seeds, employing techniques such as roasting and skimming. What sets these early cooking oils apart is their purity and simplicity. Untainted by additives and processed with minimal technology, they embody the essence of natural culinary practices. Through centuries of innovation and tradition, cooking oils have evolved into indispensable staples, enriching cuisines across the globe

In addition to the traditional cooking oils discussed earlier, the advancement of extraction technologies has led to the creation of various vegetable oil varieties. These innovations, coupled with refining machinery, have resulted in a plethora of single vegetable oils available in the market today. For instance, olive oil, a longstanding staple, now comes in different forms such as extra virgin, virgin, pomace, and extra

light. Furthermore, what was once considered waste products, such as watermelon seeds and grape seeds, are now utilized to produce oils, highlighting the ingenuity of modern extraction techniques. The transition from animal fats and basic plant oils to industrially processed oils marks a significant shift in culinary practices. While concerns exist regarding the health implications of oils derived from previously overlooked sources, certain associations advocate for their benefits. Moreover, the realm of vegetable oils has been influenced by advancements in genetic modification technology. A considerable portion of soybean, cotton, corn, and canola oils are derived from genetically modified organisms (GMO) seeds. Opinions on GMO products vary widely, with some expressing concerns about their potential health risks, while others argue that they are not fundamentally different from naturally produced oils. Regardless of the source, what remains paramount is the selection of oils that are low in unhealthy fatty acids, free from artificial additives, and rich in beneficial fats. Ultimately, the quest for healthier cooking oils underscores the importance of informed consumer choices in today's diverse culinary landscape.

Coconut oil, derived from pressing the meat inside coconuts (Cocos nucifera), is a versatile edible oil known for its unique properties. Solid at room temperature and liquid when heated, it comes in two main types: virgin and refined. Virgin coconut oil is extracted from fresh coconut meat, while refined oil is sourced from dried coconut meat, also known as copra. In culinary applications, coconut oil serves as a cooking fat, imparting a distinct flavor to dishes. Beyond the kitchen, it finds widespread use in skincare and hair care products due to its potent moisturizing properties. Despite claims touting various health benefits such as aiding in weight loss and preventing dementia, scientific evidence supporting these assertions remains inconclusive. Notably, coconut oil is rich in saturated fat, a factor linked to elevated cholesterol levels and increased risk of heart disease, according to the American Heart Association. Nevertheless, coconut oil has garnered attention for its array of potential health-promoting properties. Studies indicate that it exhibits antibacterial, antiinflammatory, and antioxidant effects. Virgin coconut oil (VCO) specifically boasts remarkable functional activities, including antioxidant, antibacterial, and antiviral properties. The antioxidant capacity of VCO is attributed to its phenolic compounds and medium-chain fatty acids (MCFAs). Research suggests that VCO can mitigate oxidative stress markers and suppress cytokine production, including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL6). Furthermore, VCO shows promise in protecting against oxidative stress induced by substances like gentamicin and improving renal function parameters. Dermatologically, it offers benefits such as antioxidant, anti-inflammatory, and skin-protective effects. In summary, coconut oil, particularly virgin coconut oil, exhibits notable antibacterial, anti-inflammatory, and antioxidant properties, hinting at its potential therapeutic applications across various domains. However, further research is warranted to fully elucidate its physiological effects and health implications.

Almonds(prunus dulcis) are renowned for their nutritional richness, boasting an array of healthy fats, fiber, phytochemicals, vitamins, and minerals. Extracting almond oil from these nutrient-dense nuts involves a meticulous process that begins with shelling to separate the edible kernel from the hard outer shell. Once shelled, almonds are typically ground or crushed into a coarse paste, which undergoes pressing or grinding to extract the oil. Traditional methods involve the use of hydraulic presses or expeller pressing techniques, applying mechanical pressure to release the oil from the almond paste. Almond oil, derived from Prunus dulcis almonds, offers versatility and numerous benefits. Depending on whether it's refined or unrefined, almond oil may appear as a pale liquid or a thicker, aromatic oil. Its mild flavor and nourishing properties make it a popular choice in cooking, skincare, and hair care. In culinary applications, almond oil adds a subtle nutty taste and is prized for its healthy fat content. In skincare, it's valued for its moisturizing and skin-friendly attributes, often used in massages and as a carrier oil for essential oils. The potential health benefits of almond oil are manifold, with studies indicating antibacterial, antioxidant, and antiinflammatory properties. Research suggests that almond oil may resist Escherichia coli (E. coli) bacteria, highlighting its antibacterial potential. Moreover, its antiinflammatory activity holds promise for reducing inflammation. Overall, almond oil stands as a versatile and beneficial

ingredient with applications spanning culinary, cosmetic, and therapeutic domains, offering a natural solution for various health and wellness needs.

Cooking oil provides many benefits, both nutritionally and health-wise. It is a good source of healthy fats, such as monounsaturated and polyunsaturated fats, which help to reduce cholesterol levels. It also contains essential vitamins and minerals that can help to boost the immune system and provide energy. Cooking oil is also beneficial for heart health. Studies have shown that consuming a moderate amount of cooking oil can help reduce the risk of heart disease. Additionally, cooking oil can help to reduce inflammation in the body, which can help to improve overall health. However, it's essential to be mindful of the potential health impacts of cooking oils, as excessive consumption, particularly of oils high in saturated fats, may contribute to health issues. Additionally, advancements in technology and genetic modification have influenced the production of cooking oils, introducing new varieties with varying nutritional profiles. Ultimately, making informed choices about cooking oils, and considering their properties, uses, and potential health benefits, can contribute to a well-rounded and nutritious culinary experience. The key is to strike a balance, using oils in moderation and opting for those that align with individual health goals and preferences. Cooking oils offer a range of benefits beyond their culinary uses, including notable properties that contribute to their potential as antimicrobial, antiinflammatory, and antioxidant agents:

Antimicrobial Properties, Some cooking oils, such as coconut oil and almond oil, contain compounds that exhibit antimicrobial activity. For instance, coconut oil contains lauric acid, which has been shown to have antimicrobial effects against bacteria, viruses, and fungi. Almond oil, on the other hand, has demonstrated resistance against bacteria like Escherichia coli (E. coli), indicating its potential as an antimicrobial agent.

Anti-Inflammatory Properties, Certain cooking oils possess anti-inflammatory properties that can help mitigate inflammation in the body. Olive oil, particularly extra virgin olive oil, contains oleocanthal, a compound with anti-inflammatory effects similar to ibuprofen. Additionally, linoleic acid in oils like sunflower oil has been associated with reducing inflammation, potentially offering relief from inflammatory conditions.

By incorporating cooking oils with these beneficial properties into your diet and skincare routine, you may reap the potential antimicrobial and anti-inflammatory benefits they offer, contributing to overall health and well-being. However, it's essential to use oils in moderation and choose high-quality, minimally processed options to maximize their potential benefits. Review of Literature

# Background

Cooking oil, derived from various edible vegetable sources such as olives, peanuts, and sunflowers, serves a multitude of culinary functions. It remains in a liquid state at room temperature and is commonly employed in the preparation of processed foods, frying, and the creation of salad dressings. Throughout history, people in diverse regions have engaged in the extraction of vegetable oils, using whatever resources were available to them. This practice dates back thousands of years, with early civilizations employing methods such as heating oily plant products using natural elements like the sun, fire, or ovens. e (Batugal et al., 1998)The Chinese and Japanese were early producers of soy oil around 2000 B.C., while olive oil production began in southern Europe as early as 3000 B.C. In other regions like Mexico and North America, peanuts and sunflower seeds were processed by roasting, grinding into a paste, boiling, and then skimming off the oil that rose to the surface. Similarly, Africans utilized techniques such as grating and beating palm kernels and coconut meat before boiling the resulting pulp and skimming off the oil. As extraction technology advanced, new oils became available, with corn oil emerging in the 1960s along with others like cotton oil, watermelon seed oil, and grapeseed oil. These oils, previously considered waste, have been utilized, highlighting the progress in extraction methods and resource utilization throughout history. (Mittaine and Mielke, 2012)

The initial endeavors to enhance production occurred independently in various regions including China, Egypt, Greece, and Rome. Employing primitive tools such as spherical or conical stone mortar and pestles, vertical or horizontal millstones, or even utilizing their feet, people commenced the process of crushing vegetable matter to augment its surface area. Subsequently, the pulverized material would be deposited into sieves, often shallow, flat wicker baskets, stacked in layers sometimes reaching up to 50 high. Following this, the material underwent pressing using a lever or wedge presses. The Greeks and Romans refined this technique by introducing edge runners for grinding and incorporating winches or screws to operate lever presses. This method persisted throughout the Middle Ages, representing a significant advancement in vegetable oil extraction.

The evolution of extracting vegetable oils has been marked by a series of advancements aimed at improving efficiency and maximizing yields. It began with early techniques like the stamper press from Holland in the 1600s, followed by innovations such as the rolling mill by John Smeaton in 1750 and the hydraulic press by Joseph Barmah in the late 18th century. These methods were gradually refined, leading to the development of the improved screw press in 1876 by V. D. Anderson in the United States, which utilized a continuous cage press operated by a screw to enhance oil extraction. Additionally, solvent extraction methods emerged, pioneered by Desis of England in 1856, building upon earlier experiments by Jesse Fisher in 1843. This involved the use of solvents like benzene, initially pumped through the material before evolving into continuous spraying systems developed independently by Bollman and Hildebrandt in Germany. Over time, both mechanical pressing and solvent extraction

techniques underwent continuous refinement, resulting in significantly improved oil extraction efficiency. Early methods may have only captured around 10% of the

available oil, while modern approaches such as solvent extraction can recover nearly all but 0.5 to 2 percent of the oil, showcasing the considerable progress in extraction methods within the vegetable oil industry.

# The coconut,

originating from the coconut palm (Cocos nucifera) is a versatile fruit widely utilized for its water, milk, oil, and delicious meat. Originally native to Southeast Asia and the islands between the Indian and Pacific oceans, coconut trees have become the most naturally widespread fruit tree globally, cultivated across various regions. Their popularity has surged due to their distinct flavor, culinary versatility, and perceived health benefits. (Abbott, I.A. 1992)

Despite claims of disease-fighting properties, many assertions about coconuts' health benefits lack solid scientific backing and may be exaggerated by food manufacturers. While coconut oil has received more attention in research, coconut meat—though potentially beneficial—has not been as extensively studied. It's worth noting that consuming coconut meat may offer certain benefits associated with coconut oil, although the meat yields significantly less oil per serving. Coconuts thrive year-round in tropical and subtropical climates, but it can take 12 to 13 years for a coconut tree to produce fruit after planting. At stores, you may encounter both brown and green coconuts, which are essentially the same variety but at different stages of maturity. Brown coconuts are fully mature with less juice, while green ones are younger with less meat. When selecting a coconut, opt for one that feels heavy for its size and lacks cracks, and listen for the presence of liquid when (shaken Bourdeix, R., J.L. Konan and Y.P. N'Cho. 2005)

# Known varieties

Two main types of coconut palms, Talls, and Dwarfs, are distinguished by their size and stature. Talls are the predominant variety cultivated worldwide. They are crosspollinated, resulting in considerable variability in characteristics such as fruit size, shape, color, husk thickness, endosperm weight, and yield. In contrast, Dwarfs are primarily self-pollinated, leading to a greater genetic uniformity among different dwarf varieties. This genetic consistency is evident in the more standardized appearance of various types of dwarf coconut palms. (Clarke, W.C., and R.R. Thaman (eds.). 1993)

# Leaves

During the first year of growth, coconut palm leaves remain intact. However, as the palm matures, the leaves, known as "fronds," gradually become more pinnate. The iconic coconut leaves typically exhibit paripinnate (even-pinnate) characteristics, featuring approximately 200–250 linear-lanceolate leaflets arranged in a single plane on each side of the rachis. These fronds measure between 4.5 to 5.5 meters (15 to 18 feet)

in length, with the petiole constituting a quarter of their total length. Leaflets are approximately 1.5 to 5 centimeters (0.6 to 2 inches) wide and 50 to 150 centimeters (20 to 60 inches) long. (Cox, P.A. 1991) The expanded base of the petiole ensures a sturdy attachment of the frond to the stem. The coloration of the petiole and rachis can

vary between green and bronze, serving as an indicator of the coconut fruit's color. In their prime, Talls typically produce around 12 to 18 leaves annually, while Dwarfs yield 20 to 22 leaves per year. As leaves naturally senesce approximately 2.5 years after unfolding, Talls typically maintain 30 to 35 leaves in their crown at any given time. (Enig, M. 2000)

#### Fruit

The coconut fruit is classified as a fibrous drupe, consisting of several layers arranged from the outside inwards. These layers include a thin, tough skin known as the exocarp, followed by a thicker fibrous layer called the mesocarp or husk. Beneath the husk lies the hard endocarp, commonly referred to as the shell. Inside the shell is the white endosperm, also known as the kernel, surrounding a large cavity filled with liquid, commonly referred to as "water." In its immature state, the exocarp typically appears green, occasionally taking on a bronze hue. There is considerable variability in fruit shape and size, both within different types of coconuts and among various populations. Coconut fruit shapes can range from elongated to nearly spherical, with mature fruits weighing between 850 and 3700 grams (approximately 1.9 to 8.1 pounds). (Fremond, Y., R. Ziller, and M. de Nuce de Lamothe. 1966.)

#### Seeds

The coconut seed consists of a dark brown shell and kernel, with the surrounding husk remaining intact and brown and dry upon maturity. When fruits are intended for planting purposes, they are commonly referred to as seednuts, distinguishing them from those used for non-propagation purposes such as drinking, consumption, and copra production. Seed nuts resemble the fruit in shape but are smaller and lighter due to the husk drying out and partial water loss from the cavity. Each coconut nut typically features three micropyles or "eyes," with one of them being soft, indicating the position of the viable embryo embedded in the kernel. While it is rare, a nut may contain more than one viable embryo. A bearing palm typically produces approximately 50 to 80 fruits per year. (Handy, E.S.C. 1985)

# Climate

Coconut palm thrives in a warm and humid climate, ideally with a mean annual temperature around  $27^{\circ}C$  (81°F) and evenly distributed rainfall ranging from 1500 to 2500 mm (60 to 100 inches) per year. Relative humidity above 60% further supports vigorous growth and high yields of the palm. Adequate moisture can be supplemented by a permanent water table accessible to coconut roots, compensating for insufficient

rainfall. However, excessive rainfall exceeding 2500 mm (100 inches) annually may lead to diseases affecting both the fruit and leaves.

These optimal conditions are typically found approximately 20 degrees north and south of the equator. Prolonged periods with mean daily temperatures below 21°C (70°F) can negatively impact the growth and yield of coconut palms. Frost poses a fatal threat to seedlings and young palms, particularly when the growing point remains close to the ground. (Thampan, P.K. 1982)

Coconuts can thrive and yield satisfactorily up to an altitude of 600 meters (1970 feet) near the equator. However, at latitudes of around  $23^{\circ}$ , optimal growth occurs only at sea level. In the extremities of its latitudinal range, coconut cultivation is most successful along the coastlines of large land masses, such as the east coast of Australia, Africa, and South America, as well as on islands where the maritime climate moderates temperature and humidity fluctuations.

#### GROWTH AND DEVELOPMENT

The growth trajectory of a coconut palm is characterized by its most rapid phase between the second and fifth years of life. Around the third to fourth year, a stem emerges beneath the crown, with initial elongation averaging between 30 to 50 centimeters (12 to 20 inches) annually. However, as the palm ages, this rate of stem elongation gradually slows down, especially in palms over 40 years old. (Thaman, R.R., and W.A. Whistler. 1996.)

After the sixth year, there is a noticeable shift in energy allocation towards fruit production, often at the expense of vegetative growth. From this point onward, growth stabilizes, and the palm sustains consistent yields for approximately 40 years. The age of the palm can be roughly estimated based on the length of its stem. Bearing palms are estimated to produce between 50 to 80 kilograms (110 to 176 pounds) of dry matter annually, contributing to their overall productivity and longevity in coconut cultivation. (Krauss, B.H. 1974)

#### Life span

Tall coconut varieties have the potential to live up to 100 years, although their productivity typically declines after reaching approximately 40 years of age. In contrast, dwarf coconut varieties generally have a shorter lifespan of around 70 years. (Handy, E.S.C. 1985)

# Coconut products

The Coconut Development Board categorizes coconut products into various groups based on their composition and processing. These categories include kernels, inflorescence, shells, water, and convenient food items. Examples of kernelbased coconut products include Virgin coconut oil, Desiccated coconut, Coconut milk, Coconut

skimmed milk, Spray-dried coconut milk powder, Coconut cream, Coconut chips, Coconut oil, Copra

These products are derived from the kernel of the coconut and are utilized in various culinary, industrial, and cosmetic applications. (Appaiah, Sunil, Kumar, & Krishna, 2015)

# Virgin coconut oil

(VCO) is produced through a meticulous process that begins with breaking down the white fleshy portion of the coconut. This flesh is then pressed in a screw press to extract coconut milk, which undergoes filtration to remove impurities. The cream is subsequently separated from the milk through centrifugation. The extraction of VCO from the cream involves a method called phase inversion. Unlike other extraction methods that involve heat treatment, wet processing is employed to extract VCO from fresh, mature coconuts. This method helps preserve the oil's natural

properties and ensures its nutrient density. The resulting VCO is crystal clear, possessing a distinct coconut aroma. It is rich in lauric fatty acid, known for its antiviral and antibacterial properties. One of the distinguishing features of high-quality VCO is its long shelf life, attributed to the presence of natural antioxidants. These antioxidants help maintain the oil's freshness and stability over time. VCO is highly sought after in both the cosmetics industry and global cuisine. Its tropical origins make it a prized ingredient in skincare and haircare products, while its unique flavor profile adds richness to various culinary creations worldwide. (Navin, 2019)

#### Desiccated coconut

Desiccated coconut meat, as described by (Jayasekara & Gunathilake 2007), is essentially grated and shredded dehydrated coconut. This product, commonly referred to as DC meat, finds widespread use in India, particularly in the confectionery and food sectors. It serves as a versatile ingredient, often employed as a substitute for shredded coconut in various dishes across culinary preparations.

#### Coconut water,

The clear liquid found inside a coconut is often hailed as the "perfect drink" due to its refreshing nature. In healthy coconuts, the water is sterile, making it a safe option for hydration. Its high sodium and potassium content makes it especially effective for rehydration purposes. Interestingly, during World War II, coconut water was administered intravenously to patients with blood loss when blood plasma was unavailable. In addition to its hydrating properties, coconut water serves as a readily available source of clean drinking water, particularly useful after natural disasters like cyclones or flooding. The taste and characteristics of coconut water vary with the age of the coconut. In very young coconuts (around 3-5 months old, before the endosperm develops), the water may be tasteless and slightly astringent. As the coconut matures, the water develops a slightly salty taste, though this saltiness may diminish in coconuts grown further inland. The optimal time to harvest a coconut for drinking purposes is

around 6-7 months of age, just as the jelly-like endosperm begins to form. At this stage, the water is at its sweetest and has low acidity. However, coconuts harvested at this age have a limited shelf life of 2-3 days before the water starts to sour. Banzon JA (1977)

# Coconut milk

It is a creamy emulsion made from the flesh of the coconut, consisting of coconut cream and coconut water. In industrial production, the sterilized coconut flesh is ground into a paste using a hammer mill. This paste then undergoes further processing, passing through a screw press and vibrating screens to extract coconut milk. To adjust the consistency and flavor, coconut milk is often diluted by blending it with coconut water and pure water. Additionally, sugar is typically added in the range of 10-12%, along with a 2% mixture of stabilizers, emulsifiers, and flavor enhancers. The resulting blend is sterilized to ensure preservation and then packaged in bottles for distribution and sale. This process, as described by (Sudheer 2018), enables the production of coconut milk on an industrial scale, providing consumers with a convenient and versatile ingredient for culinary applications.

# Coconut oil

Coconut oil, an edible oil consumed for millennia in tropical regions, is extracted from dried coconuts, also known as copra. This extraction process is facilitated by rotary and expeller machines, commonly utilized in places like the outskirts of Tanjore, Tamil Nadu, as depicted in Figure 1. Copra typically contains 60-65% oil content. There are two primary methods for extracting coconut oil: dry and moist extraction. In the dry extraction method, the coconut is dried to remove moisture while minimizing microbial contamination. Conversely, the wet extraction method does not involve drying the extraction process, various techniques such coconut. In the dry as heating, fermentation, cream centrifuge, pH adjustment, and chilling are employed to extract the oil. Large-scale enterprises typically prefer dry extraction, often combined with copra processing, although it necessitates more oil refinement. Mechanical extraction using a centrifuge is one method employed in this process. Additionally, enzyme extraction and solvent extraction are alternative methods for extracting coconut oil. These methods offer different advantages and may be utilized depending on specific production requirements. (Boateng, Ansong, Owusu, & Steiner-Asiedu, 2016; Marina, Man & Amin, 2009; Subroto, 2020)

# Copra

Copra production in India yields two main types: milling copra and edible copra, as highlighted by Ramakumar (2001). Edible coconut, typically consumed as a dry fruit, differs from milling copra, which is ground to extract oil. Copra boasts the highest oil content among various oilseeds. For over eight decades, the economy of Eastern Indonesia has relied on copra, often referred to as "green gold." The increased demand for cooking oil in European nations during the 1880s led to the recognition of copra oil

as a valuable cooking ingredient. Additionally, copra serves as a component in butter production and is utilized in soap manufacturing processes (Evita, 2020).

# Coconut vinegar

It is a natural product derived from the fermentation of coconuts, devoid of any additional preservatives or additives, as noted by Othaman, Sharifudin, Mansor, Kahar, & Long (2014). This vinegar is typically produced from either coconut water or fermented coconut sap. The fermentation process involves the use of Saccharomyces cerevisiae (yeast) and Acetobacter aceti (bacteria) to facilitate both ethanol and acetic acid fermentation, as described by Sudheer (2018). According to Sudheer (2018), coconut vinegar is created through the alcoholic fermentation of coconut water, typically with the addition of 10-12% sugar. This process involves fermenting the sugar into ethanol, followed by the oxidation of ethanol to produce vinegar. This traditional method results in a tangy and flavorful vinegar that retains the natural essence of coconuts. Ashburner GR, Thompson WK, Halloran GM (1997)

# Powder of coconut shells

Coconut shell powder finds extensive application in various industries, including plywood and laminated board manufacturing. It serves as a key ingredient in phenolic extruders and acts as a filler in synthetic resin glues, as well as in the production of mosquito coils and incense sticks.( Mueller-Dombois, D., and F.R. Fosberg. 1998) Due to its consistent quality and chemical composition, along with its superior characteristics, coconut shell powder is often preferred over alternatives such as bark

powder, furfural, and peanut shell powder. The main exporting countries of coconut shell powder include Sri Lanka, the Philippines, and Indonesia, as highlighted by Muralidharan (2011). These nations play a significant role in supplying coconut shell powder to meet the demands of various industries worldwide. (Little, E.L., and R.G. Skolmen. 1989)

The coconut tree boasts a diverse array of applications, exerting a significant influence on the lives of people globally. Beyond its utility as a source of food, the coconut tree offers various medical benefits and serves as a major income generator for communities. Indonesia, India, and the Philippines emerged as the top three countries benefiting economically from coconut tree cultivation. (Ohler, J.G., and S.S. Magat. 2002) Given the soaring demand for coconut-based products worldwide, it becomes imperative to raise awareness among farmers about the economic potential associated with coconut cultivation. By highlighting the economic value of coconut-based industries, farmers can be incentivized to increase production, thereby contributing to their livelihoods and the broader economy. Efforts to educate farmers about the profitability of coconut farming can lead to enhanced productivity and prosperity within coconut-producing regions. (Pukui, M.K. 1983)

#### The Almond

Almonds, members of the Rosaceae family, are categorized within the sub-family Prunoideae or Amygdaloideae. They are also grouped in their own family, known as Prunaceae or Amygdalaceae, leading to the hypothesis that the Prunus genus evolved from the Spiraeoidae subfamily. Almond trees are small deciduous plants, typically growing to heights of 4 10 m with a trunk diameter of around 30cm. Initially, young twigs are green but turn purple upon exposure to light. Their leaves measure between 3 to 5 inches in length. The almond flowers are characterized by pale pink petals, usually numbering five. These trees typically reach maturity in autumn, approximately 5 to 6 years after being planted.(Abdallah A, Ahumada MH, Gradziel TM. 1998)

#### Origin and history

The almond tree is native to the Mediterranean region of the Middle East, spanning from Syria and Turkey to Iran and extending eastward to Pakistan. In ancient times, it was found along the Mediterranean shores and spread into northern Africa and southern Europe. According to Zohary and Hopf, almonds were among the earliest fruit trees to be domesticated, thanks to the growers' ability to cultivate appealing varieties. Domesticated almonds can be traced back to the Early Bronze Age (3000-2000 BC). Initially, wild almonds were harvested as food, likely processed through leaching or roasting to remove toxicity. However, before cultivation and domestication, the sweet varieties of domesticated almonds were not toxic. (Navarro, Martínezsánchez, & Carbonellbarrachina, 2009)

Diamond proposes that a common genetic mutation resulted in the absence of glycoside amygdalin, making this mutant almond non-toxic. Early farmers unintentionally grew this mutant initially in waste heaps and later deliberately in their orchards.

Historically, a diverse range of plant oils have been utilized for numerous beneficial purposes, particularly in foods, cosmetics, and medicinal products. Today, there is increasing recognition of their potential in managing various skin disorders and other ailments, as well as in contributing to the protection and restoration of skin barrier homeostasis. The growing interest in natural ingredients has fueled a significant expansion of natural cosmetic products in recent years. However, this trend has also heightened the need for careful formulation and substantiated product claims when incorporating natural ingredients, including natural oils, into cosmetics. Consequently, there is a demand for comprehensive and thoughtful approaches to viewing natural ingredients, such as natural oils, as 'active' ingredients rather than merely serving as components for 'natural' claims. This approach is essential for ensuring the efficacy and integrity of natural cosmetic products in meeting consumer expectations and delivering desired outcomes. (Ouzir M, El Bernoussi S, Tabyaoui M, Taghzouti K 2020)

Sweet Almond Oil (SAO), Evening Primrose Oil (EPO), and Jojoba Oil (JJO) are three widely popular natural seed oils, extensively utilized in cosmetic, dermatological, and health markets. According to global business data, the collective value of these oils in 2020 was approximately 2080 million USD. Specifically, in 2020, the global market for Evening Primrose Oil was valued at 170 million USD [Moore E, Wagner C, Komarnytsky 2020], Sweet Almond Oil was worth 1756 million USD in 2019 [Bayles B, Usatine R 2009], and the market size for Jojoba Oil was valued at USD 133.2 million in 2019. Furthermore, all three oils are projected to experience growth ranging between 4 and 10% by the year 2027. This growth trajectory underscores the increasing demand and widespread application of these natural seed oils across various industries.

In conjunction with increased business development, the scientific literature has experienced a notable surge in research investigating the diverse composition of various natural oils, including Sweet Almond Oil (SAO), Evening Primrose Oil (EPO), and Jojoba Oil (JJO). These investigations focus on aspects such as (bio)chemistry, toxicity, and health benefits Additionally, the importance of extraction methods has garnered attention along with considerations regarding the effects of climate, genetics, and farming methods

# Types of almond

There are two main types of almonds: sweet almonds and bitter almonds. Sweet almonds are edible, while bitter almonds are considered non-edible or even poisonous due to their hydrogen cyanide content. Bitter almonds are slightly broader and shorter compared to sweet almonds. Sweet almonds are rich in fixed oil, making up about 50% of their composition.( L. Danielski *et al.*)

An almond consists of three parts: the kernel or meat, the mid shell, and the outer green shell. The kernel has a thin, leathery layer known as the brown skin or seed coat and is highly nutritious. Almonds are packed with essential vitamins, minerals, protein, and fiber. They can be consumed raw or roasted. It's important to note that almonds are drupes and are not classified as true nuts. (C.S.G. Kitzberger *et al.* 2007)

# Nutrition

Almonds are a rich source of various nutrients such as vitamin E, copper, magnesium, and high-quality protein. They also contain high levels of unsaturated fatty acids and bioactive molecules like fiber, phytosterols, vitamins, minerals, and antioxidants. These components contribute to the beneficial effects of almonds on cardiovascular health and help in managing cardiovascular disorders. (Sharma and Gupta (2006) )

Many natural oils contain species-specific compounds with a wide range of biochemical activities, including antioxidant, anti-inflammatory, and anti-pruritic properties This makes them attractive options for complementary treatment in conditions such as xerotic and inflammatory dermatoses, particularly those associated with epidermal barrier disruption and dysfunction It's important to note that Sweet Almond Oil (SAO),

also known as Prunus amygdalus dulcis oil, should not be confused with its bitter counterpart, Prunus amygdalus amara oil, as these oils possess distinct properties SAO, commonly used in cosmetic applications, is valued by the industry as an excellent carrier oil in various skin preparations. Evening Primrose Oil (EPO) derived from Oenothera biennis is highly acclaimed in cosmetic, dermatological, and health applications. Similarly, Jojoba Oil (JJO) extracted from Simmondsia chinesis is widely utilized in various skin applications due to its sebum-like characteristics. (V.J. Barwick 1997)

The biochemical composition of almond oil

The biochemical composition of almond oil is notable for its significant proportions of essential fatty acids, which are crucial for human health as they must be obtained from the diet since they are not synthesized by the body. Almond oil is particularly rich in beta-sitosterol, squalene, and alpha-tocopherol, all of which are important constituents for maintaining healthy-looking skin. These compounds contribute to the moisturizing, nourishing, and protective properties of almond oil, making it a popular choice in skincare products and cosmetic formulations. (L. Danielski *et al* 2007.)

Use of Almond

Almond oil

The kernel part of almonds produces a fixed oil called "oleum amygdalae." This oil is insoluble in water but soluble in chloroform and ether. It serves as a substitute for olive oil. Sweet almond oil, derived from the dried kernels of the plant, is utilized for various purposes such as massage therapy, anti-inflammatory properties, boosting immunity, and combating hepatotoxicity. (V.J. Barwick 2002)

# Almond milk

Almond milk has a creamy texture and a nutty flavor. It is free from cholesterol and lactose, making it suitable for individuals with dietary restrictions or preferences. Commercially, almond milk is often consumed sweetened and flavored to enhance its taste and appeal to different preferences. (Femenia et al. (2001)

# Almond syrup

Almond syrup typically consists of an emulsion of bitter and sweet almonds. Flavors are added to enhance its aroma. However, due to the cyanide content present in bitter almonds, modern almond syrups are primarily made from sweet almonds to ensure safety and avoid any potential health risks(. Kasai et al. (2003)

# Almond flour

Almond flour, made by grinding almonds, is often combined with sugar and honey. This combination is used as a gluten-free alternative to wheat flour in various cooking and baking recipes. (Femenia et al. (2001) LIPID PROFILES

In the cosmetic industry, researchers have extensively leveraged the knowledge of the chemical profiles of Sweet Almond Oil (SAO), Evening Primrose Oil (EPO), and Jojoba Oil (JJO), particularly their lipid profiles, to unlock several skin benefits and other pharmacological outcomes. The variations in the amounts of key fatty acids within these oils are influenced by seasonal changes, farming locations, extraction methods, and genetic variations. (King J, Cygnarowicz-Provost M, Favati F) Sweet Almond Oil (SAO) is notably rich in both mono- and polyunsaturated fatty acids, particularly oleic acid (up to 86%), with lower amounts of linoleic (up to 30%) and palmitic acids (up to 9%) respectively. Deng Y, Hua H, Li J, Lapinskas P.The predominant sterol in SAO is β sitosterol (up to 87%). Evening Primrose Oil (EPO), widely used in skincare and noncosmetic applications such as female health, primarily consists of the essential fatty acid C18:2 n-6 linoleic acid (74%), along with significant amounts of C18:3  $\gamma$ -linolenic acid (9%). EPO also contains high levels of  $\beta$ -sitosterol (64%). Jojoba Oil (JJO), often referred to as an 'oil' due to its liquid nature, is a liquid wax ester (98%). It resembles human sebum and is composed of straight-chain esters of monounsaturated long-chain fatty acids and long-chain primary fatty alcohols. Predominant fatty acids in JJO include oleic acid (30%-45%) and 11-eicosenoic acid (20%–40%). Jojoba is unique among plant species for synthesizing a 'liquid wax', which accounts for approximately 40% - 60% of the dry weight of mature jojoba seeds. Unlike other seed oils rich in triglycerides, JJO primarily contains liquid wax. ( El-Mallah M, El-Shami S. 2009)

# DERMATO-COSMETIC EFFECTS

Almond oil offers various benefits for different types of skin due to its nourishing, moisturizing, and soothing properties. Here's how almond oil benefits different skin types:

Dry Skin:

Almond oil is rich in fatty acids, including oleic and linoleic acids, which help moisturize and nourish dry skin. Its emollient properties help to soften and smooth rough, dry patches, leaving the skin feeling hydrated and supple. Almond oil can be used as a natural moisturizer to relieve dryness and prevent moisture loss, particularly in areas prone to dryness like elbows and knees. (Saeed A 2019)

#### Sensitive Skin:

Almond oil is gentle and hypoallergenic, making it suitable for sensitive skin types. Its anti-inflammatory properties help to soothe irritation and reduce redness, making it ideal for calming sensitive or reactive skin Almond oil can be used as a natural remedy for conditions like eczema and dermatitis, providing relief from itching and inflammation. (Sultana Y, Kohli K, Athar M, Khar RK, Aqil M 2007) Aging Skin:

Almond oil is rich in antioxidants, such as vitamin E, which help to protect the skin from damage caused by free radicals and environmental stressors. Its moisturizing properties help to hydrate the skin and improve elasticity, reducing the appearance of fine lines and wrinkles Almond oil can be used as an anti-aging treatment to promote a more youthful complexion and improve overall skin tone and texture. (Blaak J, Dähnhardt D, Dähnhardt-Pfeiffer S, Bielfeldt S, Wilhelm KP, Wohlfart R, Staib P 2017)

#### Oily or Acne-Prone Skin

Despite being an oil, almond oil has a lightweight texture that is easily absorbed by the skin without leaving a greasy residue. Its non-comedogenic properties make it suitable for oily or acne-prone skin types, as it won't clog pores or exacerbate breakouts. Almond oil contains anti-inflammatory compounds that help to reduce inflammation and redness associated with acne, while its antibacterial properties can help to prevent bacterial infections and breakouts. Almond oil is a versatile skincare ingredient that can benefit a wide range of skin types, providing hydration, nourishment, and protection for healthy, radiant skin. (Dähnhardt-Pfeiffer S, Surber C, Wilhelm KP, Dähnhardt D, Springmann G, Boettcher M, Foelster-Holst R 2012)

Almond oil, prized for its richness in macronutrients and micronutrients, serves as a sought-after ingredient in both the food flavoring and cosmetics industries. In recent times, there has been a surge in demand for high-quality almond oil, intended for human consumption. This review delves into various aspects of almond oil, including its chemical composition, storage conditions, and clinical evidence supporting its health benefits. Studies reviewed suggest that almond oil is predominantly composed of polyunsaturated and monounsaturated fatty acids, with oleic acid being the primary compound. Additionally, almond oil contains significant amounts of tocopherol and phytosterol content. Variations in the composition of almond oil may occur depending on the origin of the kernel and the extraction method employed. Emerging technologies such as ultrasonic-assisted extraction, supercritical fluid extraction, subcritical fluid extraction, and salt-assisted aqueous extraction are touted as promising techniques for the eco-friendly and efficient recovery of almond oil. Numerous clinical studies suggest that almond oil may play a role in managing cardiovascular risk, maintaining glucose homeostasis, reducing oxidative stress, offering neuroprotection, and exhibiting various dermatologic and cosmetic applications. However, further experimentation is required to validate almond oil's anticarcinogenic and fertility-related benefits. It emerges as a versatile and beneficial substance, offering many health and cosmetic advantages. Continued research and exploration of its properties and applications hold promise for further unlocking its potential benefits.( Choe C, Schleusener J, Lademann J, Darvin M 2017)

# Aim and Objectives

• To collect different types of cooking oils

• To analyze the antibacterial activity of coconut oil and almond oil • To analyze the anti-inflammatory activities of coconut oil and almond oil

# MATERIALS AND METHOD

Sample collection

Choose mature coconuts that are fully ripe and free from any visible damage or defects.

For almonds, gather a sufficient amount, considering both the number of nuts and weight needed for analysis Place the flesh in clean airtight containers to prevent contamination and maintain freshness store the sample in a cool and dry place to avoid direct sunlight

# 1. Extraction of sample

Coconut oil extracted using this method is derived from fresh coconut meat that has not been dried beforehand. The process begins with breaking open mature coconuts to access the white meat inside, ensuring its freshness and integrity. The fresh coconut meat is then grated or shredded, and coconut milk is extracted by adding water and pressing or squeezing the grated coconut.

Next, the coconut milk undergoes a separation process to extract the oil from the water content. This can be achieved through methods such as centrifugation, boiling, or natural settling. Centrifugation involves spinning the coconut milk in a machine to separate the heavier water component from the lighter oil, while boiling requires careful heating to evaporate the water and leave behind the oil. In traditional methods, natural settling allows the oil to rise to the surface over time, making it easier to collect.

Once the oil is separated, it is collected and stored in clean, airtight containers to preserve its freshness and quality. This method of extracting coconut oil from fresh coconut meat and milk is commonly used in traditional and small-scale production settings, resulting in high-quality, unrefined coconut oil with its natural flavor and aroma intact.( Jayasekara C, Gunathilake K (2007))

Almond oil extraction can involve both bitter and sweet almond varieties. Typically, the process entails cold pressing, although warm pressing may be used for sweet almonds. Initially, the almonds undergo thorough cleaning to remove impurities and are then separated from their shells. These cleaned seeds are gently warmed to the optimal pressing temperature and processed using a screw press to extract almond oil and

press cakes. These products are then either further processed or stored after undergoing cooling and crushing.

The extracted oil undergoes multiple purification stages. Alternatively, it can be left to settle for a few days after pressing to allow mucilaginous components to separate and be removed using filter presses. Once cold-pressed, the oil can be used in its natural form or undergo refining. It's important to note that essential almond oil, valued for its aroma and therapeutic properties, is specifically derived from bitter almonds, thus earning the name "bitter almond oil." (Abdallah A, Ahumada MH, Gradziel TM. 1998)

- 2. Materials used
- Coconut oil and almond oil Oils extracted from coconut and almond by cold pressing method
  - Preparation of nutrient broth and culture of bacteria

0.39 of nutrient broth powder is taken in a conical flask and 30 ml of water is mixed with it (1000ml,13g). Transfer this into 3 test tubes with cotton plugged and keep for sterilization in an autoclave under 121°C and 15 Lb pressure for 20 minutes. (open only after the pressure is completely released) then it is taken to a laminar airflow chamber (Laf) first sterilized the law, and switched on UV for 15 min After that transfer stored bacteria to nutrient broth using an inoculation loop and kept for bacterial growth (Marshall (ed.). 1993)

• Preparation of nutrient agar medium

13.3g of Meuller Hinton Agar (MHA) is mixed with 350ml of distilled water (38g in 1000ml) Heat this mixture while stirring to fully dissolve all components. Autoclave the dissolved mixture at 121 degrees Celsius for 15 minutes. Once the nutrient agar has been autoclaved, allow it to cool but not solidify. Pour nutrient agar into each plate and leave plates on the sterile surface until the agar has solidified. Replace the lid of each Petri dish and store the plates in a refrigerator. (Burnet, F. M. 1925)

- Petri dish It is a shallow transparent lidded dish that biologists use to hold a growth medium in which cells can be cultured
  - Pipette

A pipette is a type of laboratory tool commonly used in chemistry and biology to transport a measured volume of liquid,

Incubator

An incubator is a vital device utilized for cultivating and sustaining microbiological cultures or cell cultures. It plays a crucial role in maintaining optimal conditions such as temperature, humidity, and atmospheric composition, including CO2 and oxygen levels, within its enclosed environment. This controlled environment is essential for conducting various experiments in cell biology, microbiology, and molecular biology. Incubators are instrumental in culturing both bacterial and

eukaryotic cells, providing researchers with the ideal settings necessary for their growth and development. (Hackett and Dilts, 2004)

• Sterile swab.

Sterile swabs, sometimes referred to as sterile Q-Tips or sterile Q-tips, are similar in appearance to regular cotton swabs but are made from sterile materials to prevent contamination. These sterile swabs are typically used in medical settings and

laboratories for collecting samples, conducting tests, or applying medications to specific areas. Their absorbent material, often cotton, allows for efficient collection and transport of samples without compromising the integrity of the specimen.( P. J. Palin, L. S. Hanson, D. Barton, A. Frohwein 2017)

# • Spectrophotometer

A spectrophotometer is a device used to measure the intensity of light as it passes through a sample solution. Light is a versatile element that can be reflected, scattered, transmitted, and absorbed by different materials. Various materials have unique properties, such as absorption, reemission, or temperature-induced light emission (incandescence).

This optical instrument is designed to measure the intensity of light about its wavelength. It can be used to measure a wide range of materials, including plastic, paper, liquids, metals, and fabrics. As a color measurement device, a spectrophotometer ensures color consistency from the initial design phase to the final product delivery. Here spectrophotometer is used to measure the anti-inflammatory properties of oil by the intensity of light as it passes through the solution (Gabay C, Kushner I. 1999)

# 3. Analysis of Antimicrobial activities

# Coconut oil

Preparation of coconut oil - The cold pressing method for extracting coconut oil begins with the selection of mature coconuts, from which the fibrous outer husks are removed to access the white coconut meat inside. This meat is then cleaned grated, and optionally dried to reduce moisture content. The grated or dried coconut meat is fed into a hydraulic press or screw press machine, where mechanical pressure is applied to extract the oil without the use of heat. This gentle extraction process helps preserve the natural nutrients and flavor of the coconut oil. The extracted oil is collected and undergoes filtration to remove any remaining solids or impurities, ensuring the purity and clarity of the final product. The filtered coconut oil is then stored in airtight containers, labeled as "virgin" or "extra virgin" to denote its cold-pressed nature, and used for various culinary, cosmetic, and medicinal purposes due to its prized natural characteristics. (Jayasekara C, Gunathilake K (2007))

Preparation of Almond oil- Extraction of almond oil encompasses both bitter and sweet almond varieties, with the process primarily involving cold-pressing, although warm pressing may be suitable for sweet almonds. Initially, almonds undergo a thorough cleaning process to eliminate impurities before being separated from their shells. The cleaned almond seeds are then gently heated to the optimal pressing temperature and processed using a screw press to extract almond oil and press cakes. These resultant products may undergo further processing or be stored after undergoing cooling and crushing. The extracted almond oil proceeds through multiple purification stages. Alternatively, it may be left to settle for several days post-pressing, allowing mucilaginous components to separate and be removed using filter presses. Once cold pressed, the oil can be utilized in its natural state or undergo refining processes. It's essential to highlight that essential almond oil, renowned for its aroma and therapeutic benefits, is specifically derived from bitter almonds, hence the moniker "bitter almond oil." (Askin M, Baltab M, Tekintasc F, Kazankayab A, Balta F. 2007)

• Preparation of microbial culture

To prepare the nutrient broth for bacterial growth, start by measuring 0.39 grams of nutrient broth powder and adding it to a clean conical flask. Then, add 30 ml of distilled water to the flask and mix thoroughly until the powder is fully dissolved. Adjust the quantities proportionally if needed based on the concentration ratio, typically 13 grams of powder per 1000 ml of water. Transfer the nutrient broth solution into three test tubes and securely plug them with cotton to prevent contamination. Place these test tubes in an autoclave set to sterilize at 121°C and 15 pounds per square inch (psi) of pressure for 20 minutes. Ensure the pressure is completely released before opening the autoclave to avoid accidents.

Next, prepare the laminar airflow chamber (LAF) by sterilizing it and activating the UV light for at least 15 minutes to create a sterile environment. Using a sterilized inoculation loop, transfer a small amount of stored Escherichia coli (E. coli) and Staphylococcus saprophyticus cultures into separate test tubes containing the sterile nutrient broth. Perform these transfers within the sterilized LAF to prevent contamination. Seal the test tubes tightly with sterile cotton plugs or caps.

Finally, place the test tubes with the inoculated nutrient broth into an incubator set at the appropriate temperature and conditions for the growth of E. coli and S. saprophyticus. Regularly monitor the test tubes for signs of bacterial growth, such as turbidity or visible colonies, indicating successful bacterial propagation in the nutrient-rich broth. (S.W. Robinson *et al. 2014*)

• Mueller Hinton Agar (MHA) preparation

To prepare nutrient agar plates, begin by measuring out 13.3 grams of Mueller Hinton Agar (MHA) and adding it to 350 ml of distilled water. This concentration corresponds to

38 grams per 1000 ml of water. Heat the mixture while stirring continuously until all components are fully dissolved. Once dissolved, autoclave the nutrient agar solution at 121 degrees Celsius for 15 minutes to sterilize it. After autoclaving, allow the solution to cool down without allowing it to solidify. The agar should remain in a liquid state but be cool enough to handle safely. Carefully pour the nutrient agar into sterile Petri dishes, filling each to an appropriate depth. Place the plates on a sterile surface and allow them to sit until the agar solidifies, which typically takes around 30 minutes to an hour depending on ambient temperature. Once solidified, replace the lids on the Petri dishes to prevent contamination and store the plates in a refrigerator. Properly label each plate with the date of preparation and any relevant information. Following these steps will result in nutrient agar plates that are sterile and ready for use in microbiological experiments and culture growth. (Andrea J. Linscott, 2016.)

• Antimicrobial assay against E.coli and S.saprophyticus

The process is conducted within a laminar airflow chamber to maintain sterility and prevent contamination. Hands are wiped with alcohol before beginning the procedure. Two agar plates are required for the experiment. The first step involves swabbing the agar plate with a cotton swab dipped in Escherichia coli (E. coli) culture in one plate and Staphylococcus saprophyticus in the other, Ensuring even distribution. Using a cork borer heated in a flame to eliminate bacteria, wells are then created in the agar. A maximum of six wells are made on each plate.

The wells are labeled accordingly: "Co" for coconut oil, "Co+NP 1" and "Co+NP 2" for coconut oil with nanoparticle mixtures of different concentrations, "Ao" for almond oil, "Ao+NP 1" and "Ao+NP 2" for almond oil with nanoparticle mixtures of different concentration. The specified mixtures are poured into each well. Additionally, an antibacterial disc is placed at the center of the agar plates.

Once the agar plates are prepared, the lids are securely placed, and the plates are transferred to an incubator for the designated incubation period of 37 degrees Celsius for 24 hours before the inhibition zone around every sample disc is examined. This experimental setup allows for the evaluation of antimicrobial activity against bacterial strains, with specific attention to the effects of oil mixtures with nanoparticles. Later the inhibition zone was measured in diameter (mm)to indicate the presence of antibacterial activity in each sample and was compared.

• Data Collection and Analysis (writing measurements)

Table 1 Antibacterial activity of Coconut oil and Almond oil

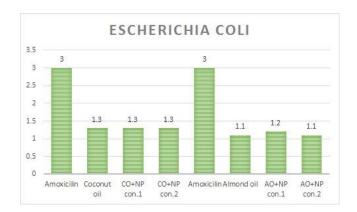
- 1. S.saprophyticus
- 2. Ecoli

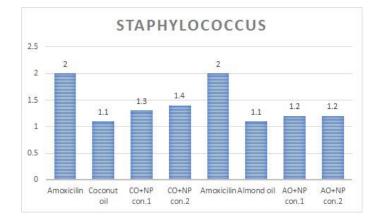
Coconut oil		
Antibacterial Amoxicillin	Yes	2cm
Coconut oil	Yes	1.1 cm
CO+NP con 1	Yes	1.3 cm
CO+NP con 2	Yes	1.4 cm

Almond oil		
Antibacterial Amoxicillin	Yes	2cm
Almond oil	Yes	1.1 cm
AL+NP con 1	Yes	1.2 cm
AL+ NP con 2	Yes	1.2 cm

Coconut oil		
Antibacterial Amoxicillin	Yes	3 cm
Coconut oil	Yes	1.3 cm
CO+NP con 1	Yes	1.3 cm
CO+NP con 2	Yes	1.3 \cm

Almond oil		
Antibacterial Amoxicillin	Yes	3 cm
Almond oil	Yes	1.1 cm
AO+NP con1	Yes	1.2 cm
AO+NP con2	Yes	1.1 cm







S.saprophyticus

E.coli

# • Result of antimicrobial properties

Coconut oil has greater Antimicrobial properties than almond oil

# Anti-inflammatory properties of coconut oil and almond oil using HRBC method

The Human Red Blood Cell (HRBC) membrane stabilization method is commonly used to evaluate the anti-inflammatory properties of natural compounds, including almond oil and coconut oil.

# Preparation of RBC suspension

The Human Red Blood Cell (HRBC) membrane stabilization method is commonly used to evaluate the anti-inflammatory properties of natural compounds, including almond oil and coconut oil. Here are the steps involved in conducting the HRBC membrane stabilization assay for assessing the anti-inflammatory properties of almond and coconut oil to prepare the Human Red Blood Cell (HRBC) suspension using Alsever's solution, the process begins by collecting whole blood from a healthy donor into an anticoagulant tube, typically containing EDTA to prevent clotting. This blood sample is then centrifuged at a low speed, around 1000 rpm, for approximately 10-15 minutes. The centrifugation process effectively separates the red blood cells (RBCs) from the plasma and other blood components.

After centrifugation, the packed RBCs are carefully collected from the bottom of the tube. To ensure purity and remove any remaining plasma proteins or contaminants, the RBCs undergo multiple washes with an isotonic saline solution, usually consisting of 0.9% NaCl. Each wash involves gently mixing the RBCs with the saline solution, followed by centrifugation to separate the RBCs from the supernatant.

Next, Alsever's solution is prepared by combining specific components, including dextrose 1g, sodium citrate 0.4g, citric acid 0.025g, sodium chloride (NaCl) 0.21g, and distilled water 50ml. The components are thoroughly mixed to create a homogeneous Alsever's solution. The washed and purified RBCs are then diluted with the prepared Alsever's solution to achieve the desired concentration for the HRBC suspension, typically ranging from 2% to 5%. This dilution process ensures that the HRBC suspension maintains isotonicity and stability, crucial for subsequent experimental assays. After centrifugation, the pellet obtained is mixed with an equal volume of isotonic saline solution (isosaline) and centrifuged again for a total of three times to ensure thorough washing and purification of the red blood cells (RBCs). The purpose of using isosaline, which typically contains 0.45 g NaCl in 50 ml of distilled water, is to maintain the isotonicity of the suspension and prevent damage to the RBCs during washing. Once the washing process is complete, the RBC pellet is transferred to a

clean the container along with the saline solution and kept refrigerated to maintain its stability. (Hartmann Joel A, Limbard Lee E, Goodmann Alfred 1998)

# • Preparation of Test Samples

Next, four test tubes are prepared for further experiments or analyses using the washed and suspended RBCs. These test tubes may be used for various assays or treatments to evaluate specific properties or responses of the RBCs under different conditions. Four test tubes are prepared as follows:

1. Test Tube 1: - 0.5 ml of HRBC suspension

- 1 ml of phosphate buffer (prepared by mixing 1.36 g of KH2PO4 and 6.15 ml of K2HPO4 in 10 ml of distilled water, then diluting with 90 ml of water to make 0.1 M phosphate buffer)

- 2 ml of hypo saline solution
- 1 ml of coconut oil
- 2. Test Tube 2:- 0.5 ml of HRBC suspension
- 1 ml of phosphate buffer
- 2 ml of hypo saline solution
- 1 ml of almond oil
- 3. Test Tube 3 (Control):
- 0.5 ml of HRBC suspension
- 1 ml of phosphate buffer
- 2 ml of hypo saline solution
- 4. Test Tube 4 (Standard Aspirin):
- 0.5 ml of hypo saline solution
- 1 ml of phosphate buffer
- 2 ml of hypo saline solution

- 1 ml of aspirin solution (standard concentration)

After adding the respective solutions to each test tube, they are mixed gently and then placed for incubation at 31 degrees Celsius for 30 minutes by closing it with a cotton plug. And placed in a spectrophotometer for the final reading. This experimental setup allows for the evaluation of the effects of coconut oil, almond oil, and aspirin on HRBC suspension under controlled conditions.

- Data Collection and Analysis (writing measurements)
- Result of antimicrobial properties

The result shows the difference in anti-inflammatory properties of coconut oil and almond oil using the herbs method it shows that coconut oil has more antiinflammatory properties than almond oil

# **RESULT AND DISCUSSION**

# Antibacterial Assay Results

The antibacterial activity of coconut oil and almond oil was evaluated using the agar diffusion method against two bacterial strains, Escherichia coli (E. coli) and Staphylococcus saprophyticus (S. saprophyticus). The zones of inhibition were measured in millimeters (cm) to determine the extent of bacterial growth inhibition.

Table 1: Antibacterial Activity of Coconut Oil and Almond Oil
---

Bacterial strain	Coconut oil (cm)	Almond oil (cm)
Staphylococcus saprophytic	1.1 cm	1.1 cm
Escherichia coli	1.3 cm	1.1 cm

Antibacterial Activity Comparison:

The results demonstrate that both coconut oil and almond oil exhibit antibacterial activity against the tested bacterial strains. Coconut oil showed a zone of inhibition of 1.3cm against E.coli and 1.1 cm against S.saprophyticus, while almond oil exhibited slightly lower activity with zones of inhibition of 1.1 cm and 1.1 cm against E.coli and S. saprophyticus, respectively. These findings suggest that coconut oil has stronger antibacterial properties compared to almond oil against these bacterial strains

# Discussion

The anti-inflammatory properties of almond oil and coconut oil have been investigated through various assays. assay. The results indicate that both oils possess significant anti-inflammatory effects, although coconut oil shows slightly stronger activity compared to almond oil.

In the present study, the anti-inflammatory activity of a substance was evaluated using the HRBC (Human Red Blood Cell) method. Blood samples were obtained from healthy volunteers and mixed with Alsevers solution, followed by centrifugation to separate the packed cells. These cells were then washed and used to create a 10% HRBC suspension. Different concentrations of the substance under study, along with a reference sample and a control, were mixed with appropriate buffers and the HRBC suspension. After incubating the mixtures, the supernatant liquid was removed, and the remaining cell suspension was analyzed to estimate hemoglobin content using a spectrophotometer.

Almond oil represents a notable reservoir of essential fatty acids crucial for human well-being. Within almond oil, two primary categories of essential fatty acids are noteworthy: omega-3 and omega-6 fatty acids. These particular fatty acids assume pivotal roles in an array of physiological processes, encompassing the maintenance of cellular architecture, facilitation of cognitive functions, inflammation regulation, and promotion of skin and hair health. Furthermore, almond oil emerges as a commendable source of alpha-tocopherol, a variant of vitamin E renowned for its potent antioxidant properties that shield cells against oxidative pressures and bolster overall immune responses. The integration of almond oil into one's dietary intake or its topical application can furnish these advantageous compounds, thereby conferring potential health advantages spanning from cardiovascular reinforcement to skincare enhancement. Nonetheless, skin to any ingestible or dermatological product, individual reactions may exhibit discrepancies, necessitating a thoughtful contemplation of personal health status and professional medical consultations when deemed necessary. (Covington AD. 2009)

The properties of almond oil can help fight infections caused by bacteria such as Staph and E. coli in several ways. Antibacterial effect, Almond oil contains compounds such as phytosterols and polyphenols, which have been shown to have antibacterial effects. These compounds can help inhibit the growth of bacteria such as staph and E. coli, reducing their ability to transmit disease. Anti-inflammatory properties As mentioned above, almond oil has anti-inflammatory properties due to its omega-3 fatty acids, vitamin E, and other components. Infections caused by bacteria cause an inflammatory response in the body. The antioxidant properties of almond oil can help reduce the inflammation associated with these diseases, relieve symptoms, and aid in the healing process. Moisturizing and healing, Almond oil is known for its moisturizing and healing properties. When applied topically, it can soothe irritated skin and promote healing of skin infections caused by bacteria such as S.saprophyticus Keeping your skin hydrated creates a barrier against bacterial infections. Natural Ingredients, Almond oil is considered a natural remedy and is often used in traditional medicine for its various health benefits. It is not a substitute for traditional herbal remedies for bacterial infections but can complement and support overall skin health. Almond oil may be effective against bacterial infections, but for severe infections, it is not recommended for treatment. Such as antibiotics (Mantzoros et.al 2006)

In studies comparing the effectiveness of coconut oil against E. coli, coconut oil has several advantages: Medium chain fatty acids (MCFA), Coconut oil is rich in medium chain fatty acids such as lauric acid, caprylic acid, and capric acid. These MCFAs have antibacterial properties. In other words, it can stop bacteria like E. coli, destroying their cell membranes and preventing their ability to multiply and spread. Lauric acid, Lauric acid is known for its powerful antibacterial properties. It is effective against many bacteria, viruses, and fungi. Lauric acid destroys the lipid membrane of bacterial cells and prevents cell death and bacterial growth. Antibacterial activity, Studies have

shown that coconut oil, especially coconut oil that contains MCFAs such as lauric acid, can reduce E. coli and other bacteria in the laboratory environment. This antibacterial effect makes coconut oil a natural remedy against bacterial infections. Vitamin Properties, Coconut oil contains antioxidants that help protect cells from oxidative stress and inflammation caused by bacterial infection. By reducing oxidative damage, coconut oil can support the body's immune response to fight bacterial attacks like E. coli. These properties are combined with coconut oil's ability to fight E. coli. coli and other bacteria because it is better than E. coli. nature. Other treatments for bacterial infections. However, it is important to note that more research is needed to fully understand the mechanisms and effects of coconut oil on bacterial strains in different clinical settings.(Mandal S., Kundu P., Roy B. and Mandal R. (2002)

Coconut oil has been studied against several pathogens, including Staphylococcus saprophyticus and E. coli. Coconut oil works against these bacteria as follows Antibacterial compounds Coconut oil contains medium chain fatty acids (MCFA) such as lauric acid, caprylic acid, and capric acid. These MCFAs have antibacterial properties. In other words, it can destroy the lipid membrane of bacteria such as Staphylococcus saprophyticus and E.coli. This disruption can cause cell death and prevent the growth and spread of these bacteria. The main component of coconut oil, lauric acid, is very effective against bacteria. It has been shown to have strong antibacterial activity against several pathogens, including Staphylococcus saprophyticus a closely related bacterium. Lauric acid can enter bacterial cells and inhibit their vital functions, which contributes to the antibacterial effect. Inhibition of biofilm formation, Coconut oil has also been shown to inhibit the formation of bacterial biofilms. Biofilms are bacterial communities trapped in a protective matrix that are resistant to antibodies and immune responses. Coconut oil can help prevent bacterial infections by preventing biofilm formation or making it more susceptible to treatment. Anti-inflammatory properties, Coconut oil contains antioxidants and antiinflammatory compounds that help reduce inflammation caused by bacterial infections. Inflammation is an important part of the immune response, but it can cause tissue damage and injury. By reducing inflammation, coconut oil can support the body's ability to fight bacterial infections. Coconut oil is an antibacterial agent for staphylococcus and E.coli bacteria, but more research is needed to understand its effectiveness in clinical settings and potential use. in the treatment of bacterial infections. Additionally, coconut oil should not replace standard treatment for serious illnesses, (Chou, C. T. (1997))

The antibacterial activity of coconut oil and almond oil can be attributed to the presence of bioactive compounds such as lauric acid, caprylic acid, and capris acid in coconut oil, and oleic acid, linoleic acid, and vitamin E in almond oil. These compounds have been reported (Muller et al. 2019) to possess antimicrobial properties by disrupting bacterial cell membranes, inhibiting bacterial enzymes, and interfering with bacterial metabolism.

Almond oil contains bioactive compounds such as polyphenols and phytosterols, which contribute to its anti-inflammatory properties.

Coconut oil, rich in lauric acid and other bioactive compounds, exhibited potent antiinflammatory activity in the assay.

**Iimplications and Further Research:** 

The observed antibacterial properties of coconut oil and almond oil have potential implications in natural medicine and food preservation. Further research is warranted to explore the specific mechanisms of action of these oils against different bacterial species and to investigate their potential synergistic effects with other antimicrobial agents.

In conclusion, both coconut oil and almond oil exhibit significant antibacterial activity against E. coli and S. saprophyticus. Coconut oil demonstrated stronger inhibitory effects, highlighting its potential as a natural antibacterial agent. These findings contribute to the growing body of evidence supporting the antimicrobial properties of plant-derived oils for various applications. This sample result and discussion section outlines the experimental findings, discusses the antibacterial activity of coconut oil and almond oil, and suggests areas for further research.

# Anti-inflammatory assay result

Both coconut oil and almond oil demonstrated significant anti-inflammatory activity in the HRBC membrane stabilization assay. The results showed dose-dependent inhibition of hemolysis, with higher concentrations of coconut oils leading to greater inhibition. The primary ingredient in coconut oil with anti-inflammatory qualities is lauric acid. The breakdown of lauric acid results in the production of monolaurin, an anti-inflammatory chemical. The antioxidants and other fatty acids in coconut oil also help to lessen inflammation in the body.

#### CONCLUSION

In conclusion, the antibacterial properties of almond oil and coconut oil were evaluated through agar well diffusion assays against bacterial strains such as E. coli and Staphylococcus saprophyticus. Based on the experimental findings and data analysis, the following conclusions can be drawn: Almond oil exhibited moderate antibacterial activity, as evidenced by zones of inhibition observed in agar diffusion assays. Coconut oil demonstrated strong antibacterial activity, showing larger zones of inhibition compared to almond oil. The presence of lauric acid and other antimicrobial compounds in coconut oil could have contributed to its potent antibacterial effects. Almond oil, while less potent, still exhibited antibacterial activity, indicating its potential as a natural antibacterial agent. Both almond oil and coconut oil hold promise as natural alternatives for combating bacterial infections. Incorporating these oils into topical formulations or using them as dietary supplements may offer antibacterial benefits and contribute to the management of bacterial-related conditions. The findings highlight the potential of almond oil and coconut oil as natural antibacterial agents, with coconut oil demonstrating stronger antibacterial properties. References

Abdallah A, Ahumada MH, Gradziel TM. 1998. Oil content And the fatty acid composition of almond kernels from different genotypes and California production regions. J. Am. Soc. Hortic. Sc. 123, 1029–1033.

Abaspour, M., Imani, A., & Hassanlo, T. (2012). Effects of almond genotype and growing location on oil percentage and fatty acid composition of its seeds. *Journal of Nuts*, 3(3), 5–12

Allan, F. J., et al. (2001). Serum lipoprotein cholesterol and triglyceride concentrations in pigs fed diets containing fish oil, milkfat, olive oil, and coconut oil. Nutrition Research, 21: 785–79

Alasalvar C, Pelvan E. 2011. Fat-soluble bioactives in nuts. Eur. J. Lipid Sci. Technol. 113, 943–949. <u>http://dx.doi</u>. Org/10.1002/ejlt.201100066

American Oil Chemists' Association (AOCS). (2016). Coconut oil boom. https://www.aocs.org/stay-informed/read-inform/featured-articles/coconut-oil-boommay-2016. Accessed 03 May 2018

Álvarez-Ortí M, Quintanilla C, Sena E, Alvarruiz A, Pardo J.E. 2012. The effects of a pressure extraction system on the Quality parameters of different virgin pistachio (Pistacia Vera L. var. Larnaka) oils. Grasas Aceites, 63, 260–266. http://dx.doi.org/10.3989/gya.117511

Askin M, Baltab M, Tekintasc F, Kazankayab A, Balta F. 2007. Fatty acid composition affected by kernel weight in almond (Prunus dulcis Mill.) genetic resources. J. Food Compos. Anal. 20, 7–12. <u>http://dx.doi.org/10.1016/j.jfca.2006.06.005</u>

Ayadi, M., Ghrab, M., Gargouri, K., Elloumi, O., Zribi, F., Ben Mimoun, M., Boulares, C., & Guedri, W. (2006). Kernel characteristics of almond cultivars under rainfed conditions. *Acta Horticulturae*, 726, 377–381. https://www.actahort.org/books/726/726\_61.htm.

Babayan, V. K. (1987). Medium chain triglycerides and structured lipids. Lipids, 22: 417–420.

Bail S, Stuebiger S, Unterweger H, Buchbauer G, Krist S. 2009. Characterization of volatile compounds and triacylglycerol profiles of nut oils using SPME-GC-MS and MALDI-TOF-MS. Eur. J. Lipid Sci. Technol. 111, 170–182. <u>http://dx.doi.org/10.1002/ejlt.200800007</u>

Barreira J, Casal S, Ferreira I, Peres AM, Pereira JBO. 2012. Supervised chemical pattern recognition in almond (Prunus Dulcis) Portuguese PDO cultivars: PCA- and LDA-based Triennial study. J. Agric. Food Chem. 60, 9697–9704. http://dx.doi.org/10.1021/jf301402t

BBC News. (2018). Is coconut oil a superfood? https://www.bbc.co.uk/news/health-42608071. Accessed 12 May 2018.Bezard J, Bugaut M, Clement G (1971) Triglyceride composition of coconut oil. J Am Oil Chem Soc 48:134–139

Bracco, U. (1994). Effect of triglyceride structure on fat absorption. The American Journal of Clinical Nutrition, 60: 1002S–1009S.

Bruüening, P., Haase, I., Matissek, R., & Fischer, M. (2011). Marzipan: Polymerase chain reaction-driven methods for authenticity control. *Journal of Agricultural and Food Chemistry*, *59*(22), 11910–11917. <u>https://doi.org/10.1021/jf202484a</u>

Culp, R. A., & Noakes, J. E. (1992). Determination of synthetic components in flavors by deuterium/hydrogen isotopic ratios. *Journal of Agricultural and Food Chemistry*, 40(10), 1892–1897. <u>https://doi.org/10.1021/jf00022a033</u>.

Chen CY, Lapsley K, Blumberg J. 2006. A nutrition and health Perspective on almond. J. Sci. Food Agric. 86, 2245–2250. <u>http://dx.doi.org/10.1002/jsfa.2659</u>

Cherif A, Sebei K, Boukhchina S, Kallel H, Belkacemi K, Arul J. 2004. Kernel fatty acid and triacylglycerol composition For three almond cultivars during maturation. J. Am. Oil Chem. Soc. 81, 901–905

Dodevska, M., Šobajić, S., & Djordjević, B. (2015). Fibre and polyphenols of selected fruits, nuts and green leafy vegetables used in Serbian diet. *Journal of the Serbian Chemical Society*, 80(1), 21–33. <u>http://www.doiserbia.nb.rs/img/doi/0352-5139/2015/0352-51391400062D.pdf</u>

Egea, G., González-Real, M. M., Baille, A., Nortes, P. A., Sánchez-Bel, P., & Domingo, R. (2009). The effects of contrasted deficit irrigation strategies on the fruit growth and kernel quality of mature almond trees. *Agricultural Water Management*, *96*(11), 1605–1614. https://doi.org/10.1016/j.agwat.2009.06.017.

Hasibuan DO, Silalahi J, Sitompul E.Antibacterial activity of the hydrolyzed virgin coconut oil against pathogenic and probiotic bacteria. Paper presented at the "Seminar NasionalFarmasi". FakultasFarmasi USU Medan, 29 September, 2012.

Kazantzis, I., Nanos, G. D., & Stavroulakis, G. G. (2003). Effect of harvest time and storage conditions on almond kernel oil and sugar composition. *Journal of Science and Food Agriculture*, 83(4), 354–359. <u>https://doi.org/10.1002/jsfa.1312</u>.

Kiani, S., Rajabpoor, S., Sorkheh, K., & Ercisli, S. (2015). Evaluation of seed quality and oil parameters in native Iranian almond (*Prunus* L. spp.) species. *Journal of Forest Research*, 26(1), 115–122. <u>https://doi.org/10.1007/s11676-014-0009-5</u>.

Kodad, O. (2017). Chemical composition of almond nuts. In R. Socias I Company & T. Gradziel (Eds.), *Almonds: Botany, production and uses* (pp. 428–448). Wallingford: CABI

Lee, J., Zhang, G., Wood, E., Castillo, C. R., & Mitchell, A. E. (2013). Quantification of amygdalin in nonbitter, semibitter, and bitter almonds (*Prunus dulcis*) by UHPLC-(ESI)QqQ MS/MS. *Journal of Agricultural and Food Chemistry*, *61*(32), 7754–7759. <u>https://doi.org/10.1021/jf402295u</u>

Lee, J., Zhang, G., Wood, E., Castillo, C. R., & Mitchell, A. E. (2013). Quantification of amygdalin in nonbitter, semibitter, and bitter almonds (*Prunus dulcis*) by UHPLC-(ESI)QqQ

MS/MS. Journal of Agricultural and Food Chemistry, 61(32), 7754–7759. https://doi.org/10.1021/jf402295u

Li, S. C., Liu, Y. H., Liu, J. F., Chang, W. H., Chen, C. M., & Chen, C. Y. (2011). Almond consumption improved glycemic control and lipid profiles in patients with type 2 diabetes mellitus. *Metabolism, Clinical and Experimental, 60*(4), 474–479. https://doi.org/10.1016/j.metabol.2010.04.009.

Lieberman S, Enig MG, Preuss HG. A Review of Monolaurin and Lauric Acid. Natural Virucidal and Bactericidal Agents. Alternative & Complementary Therapies. 2006; December:310- 314.

Marina AM, Che Man YB, Nasimah SAH, Amin I (2009) Chemical properties of virgin coconut oil. J Am Oil Chem Soc 86:301–307 Mittaine and mieklke, 2012 The globalisationn of international oilseeds <u>https://www.ocl-journal.org/articles/ocl/abs/2012/05/ocl2012195p249/ocl2012195p249.html</u>

# ACKNOWLEDGEMENT

I extend my gratitude to the individuals and organizations who collaborated with me on this college Project. I would like to thank Dr. Johnson K M, Principal of Bharata Mata College, Thrikkakara for Providing me with a platform to carry out my project work.

I extend my sincerest thanks to my project guide Dr. Sherin Antony, Department of Zoology, Bharata Mata College, Thrikkakara for their invaluable guidance, patience, and expertise throughout this project. Their insightful feedback and constructive criticism have been pivotal in Refining my work and fostering my academic and personal growth.

I am indebted to Dr. Simi Joseph, Head, Department of Zoology, Bharata Mata College, Thrikkakara For fostering an environment of learning and creativity within our college

I am also deeply grateful to the faculty members of Bharata Mata College, Thrikkakara especially to Dr. Sonia John & Jithin Johnson for providing an enriching academic environment. Their dedication To imparting knowledge and encouraging curiosity has been instrumental in my development as a Student and researcher.

I am also grateful to my fellow students who worked alongside me, sharing their knowledge and Skills. Our collaboration fostered a creative and productive environment, leading to the successful Completion of this project. Lastly, I would like to acknowledge the support received from Faculty member Mrs. Siji, lab Assistant, Department of Zoology for providing the necessary resources and encouragement that Enabled this collaborative effort.

Last but not least my soulful gratitude to the almighty, without his blessing this work has never been Seen in light. A word of apology to those I have not mentioned in person and a note of thanks to one and all who worked for the successful completion of this endeavor