Synthesis, Characterization and Application of Edible Coating from Taro

(Colocasia esculenta)

A Dissertation for

GBT-651 Dissertation

16 Credits

Submitted in partial fulfilment of Master's Degree in

Biotechnology

by

RAJAN DAMODAR AMONCAR

Seat Number 22P0470013

ABC ID: 716-181-620-566

PRN 201900293

Under the Supervision of

Dr. MEGHANATH PRABHU

School of Biological Science and Biotechnology (SBSB)

M.Sc. Biotechnology



GOA UNIVERSITY

Dates*April 2024

tong of



Seal of

Examined by:

And anitaha Sanitaha S. A

1

DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "Synthesis, characterization and application of edible coating from Taro (*Colocasia esculenta*)" is based on the results of investigations carried out by me in the MSc Biotechnology at the, Goa University under the Supervision of Dr. Meghanath Prabhu and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations / experimental or other findings given the dissertation.

I hereby authorize the University authorities to upload this dissertation on the dissertation repository or anywhere else as the UGC regulations demand and make it available to any one as needed.

RAJAN DAMODAR AMON CAR Signature and Name of Student

Seat no: 22 P04 70013

Date:8^hApril 2024

Place: Goa University

COMPLETION CERTIFICATE

This is to certify that the dissertation report "Synthesis, Characterization and application of edible coating From Taro (*Colocasia esculenta*)" is a Bonafide work carried out by Ms. Rajan Damodar Amoncar, under my supervision in partial fulfilment of the requirements for the award of the degree of master of science in the Discipline of Biotechnology at the school of biological sciences and biotechnology, Goa University.

911 Acr 6 m.

Signature and Name of Supervising Teacher

Date April 2024

Signature of Dean of the School

Date: gin April 2024

Place: Goa University

Dean of School of Biological Sciences & Biotechnology Goa University, Goa-403206

S

School Stamp

CONTENTS

Particular	Page
Preface	V
Acknowledgement	VI
Table and Figures	VII -VIII
Abbreviations	IX
Abstract	1
Introductions	2-4
1.1 Background	5
1.2 Aim and objectives	5
1.3 hypothesis	5
1.4 scope	5
Literature review	6-12
Methodology	13 -24
Analysis and Conclusions	25-38
Discussion	39
conclusion	40
Future prospect	41
References	42-50
Appendix	51-52

PREFACE

As the global concern over plastic pollution continues to escalate, the need for sustainable alternatives in various industries becomes increasingly urgent. One such industry is agriculture, where packaging and preservation methods often contribute significantly to environmental degradation.

During the rainy season, while observing the robust growth and unique properties of the Taro plant, a thought emerged: why not harness these qualities to develop an innovative solution for coating perishable fruits? Taro leaves possess remarkable attributes that make them ideal candidates for crafting edible coatings, offering a natural and eco-friendly alternative to conventional packaging materials.

This idea stems from a deep-seated desire to address the pressing issue of plastic waste while leveraging the untapped potential of indigenous resources. By exploring the feasibility of utilizing Taro leaves as an edible coating for fruits, we not only aim to extend the shelf life of produce but also to reduce the environmental footprint associated with packaging and preservation practices in the agricultural sector.

This preface sets the stage for an exploration into the concept of utilizing Taro leaves as an edible coating for perishable fruits, driven by a vision of sustainability and innovation.

ACKNOWLEDGMENT

I take this opportunity to express my sincere appreciation towards all those who have been supportive and helpful in the successful completion of my project.

I extend my deepest gratitude to my guide, Dr. Meghanath Prabhu whose unwavering support, guided me, helped me to fulfil my dream and complete my dissertation. Your mentorship has not only shaped my research but also my academic and personal growth. Sir, you mean a lot to me.

I would like to thank our Dean Prof. Bernard Rodrigues sir and senior prof. Sanjeev Ghadi, for providing facilities and resources.

I would also like to express my appreciation to all faculty members for their encouragement and insightful feedback, constructive criticism, which have contributed significantly to the refinement of my work.

Special thanks dedicated to laboratory staff Mr. Ashish T. Kuttikar, Mr. Sameer and Mr. Redulado Serrao.

I am thankful to all my classmates for camaraderie, collaborations and exchanges of ideas, discussions which have enriched my academic life.

I would like to thank Mr. Prajyot P. Chari for their assistance and expertise in conducting the FTIR analysis in the chemical science department. I would also like to thank fellow friends Varisha, Mark Rodrigues and Tanvi Tari for assistance in analysing the FTIR graph.

Lastly, I'm grateful to PhD Scholars for supporting and providing an environment for intellectual growth and learning.

I acknowledge with sincere appreciation the contribution of each individual mentioned above, without whom this endeavour would not be possible.

Table

Sr. No	Content	Page No
Table 1	Taro classification (Colocasia esculenta).	17
Table 2	Moisture content and total solid content of Taro leaves.	26
Table 3	The table shows the effect of coating with Taro extract on strawberry and keeping it at different temperatures with a control sample.	28
Table 4	Effect of Different Taro extract on shelf life of strawberries at 25 ± 2 °C	30
Table 5	Effect of Different Taro extract on shelf life of strawberries at 4°C temperature	32
Table 6	Phytochemical screening of Taro leaves	33
Table 7	The protein and carbohydrate estimation.	33
Table 8	Zone Inhibition shown by Taro leaves against Escherichia coli	56

Figure

Sr. No.	Content	Page No
Figure 1	Collection of strawberries from farm and sorting of strawberries	15
Figure 2	Picture of collected Taro leaves and locations of Taro plants.	25
Figure 3	Different extract obtained from Taro leaves	26
Figure 4	Showing the status of strawberries coated with different extract at 25 ± 2 °C	29
Figure 5	Effect of coating strawberries by different Taro leaves extract on shelf life of strawberries at 4°C	31
Figure 6	Graph of Antioxidant activity of Taro leaves extracts at different concentrations.	34
Figure 7	Taro leaves extract (aqueous) fourier transform infrared Spectra of Taro leaves extract.	35
Figure 8	Zone Inhibition shown by Taro against Escherichia coli	36
Figure 9	Zone of inhibition shown by <i>Rhizopus</i> on PDA upon addition of aqueous extract of taro leaves.	37
figure 10	Image of <i>Rhizopus</i> sp. observed under 40X objective of light microscope after staining with lactophenol cotton blue stain.	38

ABBREVIATIONS:

PDA: Potato Dextrose Agar

MHA: Muller Hilton agar

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

FTIR: Fourier Transform Infrared Spectroscopy

ABSTRACT

Fruits are highly nutritious and provide us with vitamins, minerals, etc, however, they are highly perishable and they get easily spoiled with change in temperature, pH and environmental conditions. This Research focuses on how the shelf life of fruits can be improvised and prevent loss to the food industry. At the same time reducing the burden of plastics by using edible coating from plants are found to be a novel and efficient way to preserve the perishable fruits as plastic bags only cause more harm to the environment. The coating solution was made from Taro (*Colocasia esculenta*) leaves and characterized for physico-chemical characteristics. The study showed that the coating of strawberries with Taro leaves extract increased the shelf life of the strawberries significantly by more than fifty percent. The antimicrobial study was also conducted using Taro leaves extract, and it observed that Taro leaves extract showed antimicrobial activity that preserve fruits from spoilage, thus increasing the shelf life of the perishable fruits.

KEYWORDS: Edible coating, Colocasia esculenta, strawberries, antimicrobial

CHAPTER ONE

INTRODUCTION

1.1 Background

Fruits are highly nutritious and provide us with vitamins, minerals, etc, however, they are highly perishable and they get easily spoiled with change in temperature, pH and environmental conditions. Every year farmers get a loss of 25%-50% of fruits due to physical or chemical or microbial contamination (Bancal and Ray, 2022). Food packaging is an essential field of food research because of its critical function in food protection and confinement and thus increasing the shelf life of these fruits and vegetables. Plastic packaging is most commonly used in the food industry (Corradini et al. 2013). Packaging made of plastic is used to protect food before consumption (Ozcalik and Tihminlioglu, 2013).

To prevent the degradation or spoilage of the fruits we use plastic bags or plastic airtight containers and then discard them after their purpose is fulfilled. For example, the apples produced in a particular area are packed fully with netted plastic wraps to prevent them from all types of possible damages during transportation to different parts. These plastic wraps are thrown out as soon as the fruits are purchased by the consumer. As of 2023, according to the Indian Institute of Science (IISc) and Praxis Global Alliance, Maharashtra, Gujarat and Tamil Nadu, is contributing thirty eight percent packaging material to the total plastic waste that is generated in India. Plastics derived polymers are difficult to decompose using abiotic as well as biotic elements (Ozcalik and Tihminlioglu, 2013; Akbar et al. 2013); thus, plastics are considered environmental polluters.

Currently, petroleum-derived materials control the food and beverage packaging field. These materials have increased the convenience and appeal of the agro, food, and packaging industries. Traditionally, petroleum-derived polymers have met the majority of packaging

material requirements. Petrochemical-based with excellent mechanical and barrier attributes such as tension and tear resilience, oxygen flexibility, carbon dioxide permeability, and aroma transmission include polystyrene, polypropylene, and polyvinyl chloride. These substances also brought with them challenges connected to the safe-disposal and sustainable use of these materials. Due to poor recyclability, derivability from non-renewable resources, and non-biodegradable nature of plastic packaging material, such polymers impose significant environmental constraints, resulting in serious ecological problems (Saurabh CK, Sharma et al. 2013).

Today's consumers are more worried about the environmental effect and health risks posed by these synthetic polymers. Because of growing concern about the impact on the environment of these materials, attention has shifted to the creation of better alternatives and promotion of ecofriendly packaging (Westlake et al. 2023; Skinner, 2015). In addition, the technology currently in use cannot preserve the fruits and vegetables for a longer period of time because fruits are living tissue which need oxygen for respiration Any changes in environment directly affect the fruits and vegetables. The use of alternative packaging materials with distinct biodegradable and renewable properties are more preferred. The edible film is seen to be a viable alternative for replacing plastic packaging and has received increased attention in this regard.

Edible coating or films are a thin layer of edible and environmentally friendly polymers that could be consumed and at the same time act as a barrier to gases, microbes and moisture to food products (Owusu-Akyaw Oduro, K., 2022). They are tasteless, can be colourless and also odourless they should have good mechanical properties. Edible coating is material used for packing of the item which will prevent or delay the deformation or spoilage of goods. Edible coatings are trending today due to their highly biodegradable characteristics, ease in process, and eco-friendly. Edible coatings/ films are fragile substances used for encasing or coating foods to boost their lifespans, which can be ingested either simultaneously or separately. The

edible means they can be consumable hence they must have the characteristic of safe-food components as defined by the Food and Drug Administration (FDA) as having Generally Recognized as Safe (GRAS) status. Edible coating helps in improving shelf life and looks. It also helps in maintaining the firmness of the fruits. Edible Packing (Film/coating) is easily biodegradable and the best sustainable solution for the future (Trajkovska Petkoska, A. T., 2021).

The edible coating manufacturing cost is readily reduced at certain levels due easy source of biomaterial and no requirement of sophisticated instruments. Edible coatings are used to reduce the loss to the farmers and keep the satisfaction in quality of fruits for consumers. Edible coating contains natural substances that increase the shelf life by preventing microbial spoilage. Various different kinds of environmentally friendly edible coatings, derived from renewable sources such as cellulose, starch, proteins, and fats are available to make biodegradable films which are safe to use in food applications (Benbettaeb et al. 2019).

Researchers are continuously studying novel materials and polymers for making edible films/coatings which may offer unique properties in terms of water barrier properties, sensory and organoleptic properties, adhesion, cohesion, stability, transparency, presence of phytochemical compounds, antimicrobial, antioxidants film, increasing shelf life of the product, providing mechanical strength, and reducing manufacturing cost (Erkmen O et al. 2018; Mir et al. 2018). This study was aimed at looking into the possibilities of using Taro leaves to make edible film to increase the shelf life of the highly perishable fruits.

1.2 Aim and Objective

Aim: The aim of research is to make edible coating from Taro leaves to increase the shelf life of highly perishable fruits.

Objective:

- Preparation of edible coating using Taro leaves.
- Characterization of prepared edible coating material from Taro leaves.
- To study the effect of prepared edible coating material from Taro leaves on shelf life of fruits.

1.3 Hypothesis

Hypotheses of study is that the coating made from Taro leaves extract increases the shelf life of perishable fruit (strawberry) due to its antimicrobial properties.

1.3 Scope

This study will provide an alternative method of edible coating on perishable fruits. It will increase the shelf life of fruits. The scope of this study will help to reduce the wastage of perishable fruits in markets which are at high demand.

CHAPTER TWO

LITERATURE REVIEW

Plant-based packaging is absolutely safe because it contains no dangerous chemicals or toxins; additionally, because it is biodegradable, it may simply be consumed alongside food without waste (Bajaj et al. 2023). Edible coating/film is the most advanced and trendy technology for coating fruits and vegetables (Raghav et al. 2016). This would not only reduce the plastic use but also improve the quality of the item coated by increasing the property of fruits such as look, texture colour, moisture content (Salgado P.R., 2015). Edible coatings have been used for a very long time since the 12th century Food (CPMA,2014). Industry applied wax coating on fruits and cellulose coating on meat casing (Jamie,2012). Edible coatings main advantage is that they are natural and cost effective free from toxic synthetic components and edibility (Prasad and Batra, 2015). Edible coatings like semi permeable barriers help in extension of shelf life by reducing the gas exchange, and damage by contamination as well as free radicals by reducing oxidation reaction rate (Baldwin et al.1996). Edible coating film performance depends on the barrier strength and oxidative barrier and mechanical barrier (Lin and Zhao, 2007).

There are various advantages of edible coating film: it maintains the natural quality of the fruits (Nunes C. et al.2023). Farmers and consumers are also benefited due to the increase in shelf life and appealing quality of fruits (Cabo, maria et al. 2015). There are several methods for coating or applying the coating substance, including the Dipping method, which involves simply dipping the fruits and then removing them. Then, using a brush, apply the solution to the fruits. Other processes include extrusion, spraying, Spray coating is a widely used method for putting edible coatings to food products (Owusu- Akyaw oduro et al. 2022). This method involves spraying a solution or dispersion of edible materials onto the surface of the food using specialized equipment such as spray guns or atomizers, spray bottles. Spray coating offers

several advantages, including uniform coverage, ease of application, and the ability to coat irregularly shaped or delicate food items (Owusu- Akyaw oduro et al. 2022). Dip coating is another widely used method for applying edible coatings to food products. In this method, the food item is immersed in a solution or dispersion of edible materials for a certain period, allowing the coating to adhere to the surface of the food (Owusu- Akyaw oduro et al. 2022) Brush coating involves manually applying an edible coating to the surface of food products using a brush or similar applicator. This method is often used for small-scale production or for coating food items with complex shapes or uneven surfaces (Owusu-Akyaw oduro et al. 2022). Extrusion coating is a more specialized method that involves extruding a molten or viscous edible material onto the surface of food products (Owusu- Akyaw Oduro et al. 2022). Electrostatic coating is a relatively new method of applying an edible coating to food products via electrostatic forces. According to this technique involves charging the coating material electrostatically before spraying it onto the food's surface, where electrostatic attraction causes it to stick (Oduro et al. 2022). Benefits of electrostatic coating include better adhesion, less overspray, and the capacity to coat porous or irregularly shaped food items. But compared to conventional coating techniques, this process might be more complicated and call for specialized equipment (Owusu-Akyaw Oduro et al. 2022). The choice of method depends on factors such as the type of food product, desired coating properties, production scale, and equipment availability. By understanding the different methods for preparing edible coatings, food manufacturers can select the most suitable approach to enhance the quality, safety, and shelf life of their products further research and innovation in this field are expected to drive the development of new and improved coating technologies for the food industry (Pinto et al., 2023; J.M. Valverde, at el. 2005). These were a few commonly used different methods for the preparation of edible coatings.

Edible coatings are classified based on Hydrocolloids: such as polysaccharides, proteins and alginate. Film-forming solutions are made from natural materials such as hydrocolloids (polysaccharides and proteins), lipids, or a combination of these. Lipids: e.g., fatty acids, triglycerides and waxes. Composites: e.g., protein/protein, polysaccharides/protein, lipid/polysaccharides (Pramod Kumar et al. 2016). Edible coatings serve as a protective layer on various food products, enhancing their shelf life and quality. They can be sourced from natural materials such as polysaccharides (like starches, cellulose, and gums), proteins (such as gelatine, wheat protein, and soy protein), lipids (including waxes and fatty acids), and composite materials combining these components. Polysaccharide-based coatings, derived from sources like fruits (pectin from citrus fruits), vegetables (pectin from apple pomace), and seeds (chia and flaxseed mucilage), offer biodegradability and sustainability. Proteins from milk, eggs, and soybeans contribute to film-forming properties, while lipids from sources like beeswax and shellac provide moisture barrier and glossiness. These diverse sources offer a range of functional properties and enable the development of edible coatings tailored to specific food applications, contributing to both food preservation and sustainability efforts (Priya and Thirunavukkarasu et al. 2023).

Taro (*C. esculenta*) is a member of the Araneae family. Taro has similarity to *Xanthosoma and Caladium*. Taro can be referred to as elephant ear. Taro has more than 1500 species and at least 100 different taxa (Mandal, 2013). Taro plant grows to a height of 1 to 2 m consisting of a central corm, lying just below the soil surface, from which leaves grow upwards, roots grow downwards, while cormel, daughter corms and runners grow laterally (Ubalua, 2016). Taro plant has different vitamins such as B complex vitamin C and various metals such as iron and zinc, copper and manganese (Quach, 2003). Taro shelf-life remains for months if kept properly (Lebot, 2009). This property aids values for using it for edible Packing. It also consists of wax (Kalita and Talukdar, 2018). Taro leaves contain bio wax which stables at even higher

temperatures. The bio-wax also possesses antibacterial properties (Kalita and Talukdar, 2018). When heated to 95-100 degrees Celsius, the extracted bio-wax maintains its hydrophobic characteristics (Kalita and Talukdar, 2018). Edible packing materials must have the following characteristics: They must be devoid of harmful compounds and suitable for human consumption; they must also have strong barriers against liquid, moisture, O2, CO2, and ethylene gas, as well as improve their visual and physical qualities in order to retain texture and structure (Otoni et al. 2017). Taro leaves are a promising raw material since they offer desirable qualities that can be used to create edible coatings. In this research paper, we will use Taro leaves to make edible packaging and investigate the physical properties of film. Taro (Colocasia), which thrives in tropical and subtropical climates, has heart-shaped leaves. Taro (Colocasia) is a plant species from the Araceae family that grows in Africa, the Pacific, South America, and India. Taro's other names include Kochai in Chhattisgarhi, Aravi in Hindi, Alupam in Sanskrit, Alavi in Gujarati, Alu in Marathi (Rashmi et al. 2018). Taro corms are widely cultivated as a food staple in Asian countries (Ahmed et al. 2013). The hydrophobic surfaces in biological form can be found in leaf surfaces such as the bio-wax extracted from Taro leaves, which contains 1-octacosanol, a common epicuticular component, and is responsible for the bio-wax's hydrophobicity properties (Ahmed et al. 2013). This property can be used to make better water-resistant film. High concentrations of antioxidants found in Taro leaves considerably lesser dangerous compounds for the body, particularly free radicals. If free radicals are not controlled, they can lead to a variety of harmful ailments like cancer, autoimmune disorders, and heart disease in addition to promoting inflammatory symptoms in the body (Shehata, Mehany et al. 2023). Taro leaves are a low-calorie green vegetable high in vitamins A and C, potassium, and folate. Taro leaves are essential since they are high in fibre and low in calories act as a crucial part in providing nutritional supplements for a diet that is balanced (Mitharwal, S., Kumar et al. 2022). Taro leaves also show antimicrobial properties against *Escherichia coli* due to the presence of flavonoids and saponins (Ahmad Shafwan S. Pulungan, 2018; Cazarolli, L. H. et al 2008).

The extract's antibacterial qualities will be strengthened by the flavonoid content (Badra et al, 2016). Vicenin-2, iso-vitexin, isovitexin 3'-O-glucoside, vitexin X''-O'Glucoside, iso-orientin, orientin7-O-glucoside, and luteolin 7-O'Glucoside are among the flavonoids present in the *C. esculenta* plant leaves extracts (Iwashina T, Konishi et al. 1999). FTIR can be used to detect the hydrophobicity of Taro leaves due to the presence of n-octacosanol (Noor s. Nasri et al. 2014).

Purpose of this research is to use such valuable plant sources to produce edible coating film.

The properties of *Colocasia* make it suitable for coating any edible item or to make them hydrophobic. These characteristics can be used to fill the gap in research of finding novel edible coating. The use of paper bags is recommended and to make paper bags more water resistant, hydrophobic components are used from the leaves of Taro (Castillo & Otoma, 2013). Similar principles can be our solution to growing food product packing manufacturing. We can make edible packaging out of it. Taro leaves also inspired the structural design of leaf imprinting (Kumar M. et. al 2020). The research on Taro making edible film will contribute to the environment as well as the food industry. It will help to reduce the loss after post-harvest of farmers. Edible coatings are significantly important to increase the appeal of fruits for customers.

Plasticizers are used in combination with edible coatings to improve film strength. Water can also be used as a blending solution. Coating film is used to improve the material's structural ability. Glycerol, fatty acids, sorbitol, propylene glycol, sucrose, polyethylene glycol, and monoglycerides are the most often used plasticizers (Pramod K. Raghav et al. 2016). Food packaging is important for food preservation across the food chain. Edible coatings have emerged as a promising technology in the food industry for enhancing food quality, extending shelf life, and reducing food waste (Popovic et al. 2018). The coatings are applied directly to the surface of food products to provide a protective barrier against moisture loss, oxygen, microbial contamination, and other environmental factors.

Edible coatings are made using a variety of techniques, each with specific benefits and drawbacks. A variety of edible coatings have been tested on highly perishable fruits, including peaches, blackberries, raspberries, cherries, and strawberries, in an effort to prolong their shelf life. Typical edible coatings for these fruits include the following: Chitosan Chitosan coatings, which are made from chitin, a naturally occurring polymer present in the shells of crustaceans like shrimp and crabs, have antimicrobial qualities that can stop bacteria and fungi from growing on fruits (Nunes et al. 2023).

Alginate: Obtained from brown algae, alginate coatings form a gel-like layer on the fruit surface, providing a barrier against moisture loss and microbial contamination (Senturk et al. 2018). Biowax natural waxes like carnauba wax, beeswax, and shellac are commonly used to coat fruits, creating a protective layer that reduces water loss and slows down decay. Starch-based coatings: Starches from sources such as corn, potato, and tapioca can be used to form edible films on fruit surfaces, offering protection against physical damage and microbial growth (Pashova et al. 2023). Protein-based coatings: Proteins like gelatine or soy protein isolate can be used to create edible coatings that provide a barrier against oxygen and moisture, thus extending the shelf life of fruits (Biswajit M. et al. 2020). Lipid-based coatings: Lipids such as edible oils or fatty acids can be applied as coatings to fruits, forming a protective layer that slows down deterioration processes (Milani and Nemati 2022). These coatings have been researched and tested for their effectiveness in prolonging the shelf life of fruits by reducing moisture loss, inhibiting microbial growth, and preventing physical damage. Many of these coatings are derived from natural sources, making them environmentally friendly and safe for consumption (Owusu-Akyaw Oduro, K. 2022). Research for novel edible coating from

biological sources is still going on hence study on Taro leaves is important as it has various properties which can be beneficial to prepare the edible coating solution at commercial scale.

CHAPTER THREE

METHODOLOGY

3.1 Collection of Taro leaves

Taro leaves were plucked from Majorda, (Latitude 15.297495°, Longitude 73.951188°) Goa 403601, India.

3.2 Preparation of Taro leaves extract

3.2.1 Preparation of Taro leaves Aqueous extract

The fifteen Taro leaves were brought to the laboratory and washed with tap water and then it was washed again with distilled water. The leaves were homogenised properly with the help of a blender. The homogenised leaves paste was then measured and accordingly mixed with hundred millilitre warm autoclaved distilled water in the flask. The flask was kept overnight. The extract was heated 60°C for one hour with the help of mantel. The extract was centrifuged (Sorvall ST 8 R, Thermofisher, Germany) at 9000 rpm for 5 min at 4°C and supernatant was collected (Shekhar. Kadam et al, 2016).

3.2.2 Preparation of Taro leaves extract using solvents.

a. The fifteen Taro leaves were washed with tap water and then it was washed again with distilled water. The leaves were homogenised properly using a blender. The homogenised leaves paste is measured. The twenty-five leaves paste was added to a hundred millilitre of ethanol and kept for incubation overnight. The plant extract was transferred in a beaker heated at 60°C for one hour with the help of a mantel. Then the extract was centrifuged at 9000 rpm for 5 min at 4°C the pellet was collected in that 0.4 N NaOH was added and again it was

centrifuged later the NaOH extract supernatant was collected and kept for later use. This extract was called alkaline extract.

b. Similarly sample preparation steps repeated and instead of NaOH the extract was suspended with 0.4N HCl then tubes centrifuged at 9000 RPM for 10 min at 4°C HCl Extract supernatant was collected and stored for later use. This extract was called an acid extract.

c. Another extract was prepared using equal volume of 0.4 NaOH and 0.4 HCl and extract was obtained in the same fashion explained above. The supernatant was called a mixed extract.

3.3 Collection of strawberry

The hundred strawberries were purchased from organic verelem strawberry farm which is located in Netravali. The Strawberry was selectively hand plucked. Only fresh and larger strawberries were chosen for the experiment. .

The strawberries were collected from Verlem farm. (Latitude: 15.045568218538495, Longitude:74.24800466931535). This farm (fig.1) is situated in Netravali south Goa. Netravali is 44.4 kilometres from Margao. The farm is taken care by Bhumika self-help group. The hundreds of strawberries were hand plucked from the farm and sorted with help of a local worker. The firm and healthy strawberries were selected for the experiment. The strawberries were packed properly and brought to the laboratory for further experiment. The strawberries were packed properly in boxes and brought to the laboratory. The strawberry which was brought from the farm was cleaned, washed with tap water firstly further with autoclaved distilled water and air dried and used for experiment.



Fig 1: Collection of strawberries from farm and sorting of strawberries

3.4 Coating Method

3.4.1 Effect of different Taro leaves extract on shelf life of highly perishable fruits (strawberry) at 25±2°C.

Dip method was followed in this experiment for coating the strawberries. Different extract concentration was used under each extract category: 1. Hundred percent extract concentration (aqueous extract, alkaline extract, acid extract, and mixed extract samples). 2. ninety eight percent extract with two percent glycerol. 3. 2% glycerol in distilled water. All coated strawberries were kept at 25 ± 2 °C and observed until visual spoilage of fruits was noticed. Controls without coating were also maintained. Shelf life of strawberries of sample coated and non-sample coated strawberries was analysed. This experiment set was performed in Triplicate.

3.4.2 Effect of different Taro leaves extract on shelf life of strawberries at 4 °C .

As similar to section 3.4.1, the sample was coated with different plant extracts and stored at 4 °C. Control samples without coating were also kept at 4 °C. The samples were kept at 4 °C temperature till noticeable spoilage was seen.

3.4.3 Shelf life of Taro leaves extract and estimation of quantity requirement of extract.

The shelf life of different extracts was analysed by keeping the extracts at 25 ± 2 °C and 4 °C. Visual growth of fungal contamination was monitored to check the spoilage. The quantity of extract to coat one strawberry was measured by calculating how much extracts per strawberries required to coat. This was done by taking 10 ml of coating solution and then coating the strawberries with it the amount which remained was calculated later.

3.5 characterization of Taro leaves

3.5.1 Classification of Taro

Kingdom	Plantae (Plants)
Sub- Kingdom	Tracheobionta (Vascular plants)
Division	Magnoliophyta (Flowering plants)
Class	Liliopsida (Monocotyledons)
Subclass	Arcidae
Family	Araceae (Arum family)
Genus	Colocasia Schot (Colocasia)
Species	Colocasia esculenta (L.) Schott (Cocoyam)
Synonyms	Alocasia dussil Dammer Alocasia illustris
	W. Bul

 Table 1. Taro classification (Colocasia esculenta).

Source:(Rashmi et al. 2018).

3.5.2 Morphology of Taro leaves

The Taro leaves are heart shaped green large leaves; the Taro leaves have one or more long petioles (Rashmi et al. 2018). It has corn at the base and this base is consumed by Indians mostly. Its height was measured. It has grown to a height of 1 metre. It consists of core corm that is just below the soil's surface that is also cooked and consumed by Indians. The leaves

and roots sprout from that bulb. Taro leaves have beautiful long laminate and they also have strong midribs. The height of the stalk is twenty-five to forty centimetres. The laminae are seven to fifteen cm in width and twelve to twenty-five centimetres in length. They have an entire, ovate to sagittate form with rounded basal lobes and an acuminate apex (Wilfred lee 1999). Drying can be the most effective method of preservation in which food is preserved by lowering the moisture content of the product (Divya Kashyap et al. 2023; Yashwant Kumar et al. 2015). These leaves have extremely hydrophobic characteristics. The Taro plant leaf shows self-cleaning property (Divya Kashyap et al. 2023; Kumar et al. 2020).

3.6 Total Solid (TS) and moisture content of Taro leaves

Taro leaves were collected and they were cut into small fragments. The five-gram leaves fragment was used to measure the Moisture content. The Moisture content of Taro leaves was measured by weighing the crucible (W1) weight and also measured weight of Taro leaves sample along with crucible (W2) then the sample was dried in the oven at 105 °C overnight (W3) then the weight was measured. Using this value, the moisture content and TS was measured using the below formula.

Moisture content:

Total moisture content = $\frac{(W2) \text{ sample weight - } (W3) \text{ dried sample weight } \times 100}{(W2) \text{ Sample weight}}$

Total solid (TS) = $\frac{(W3) dried weight - (W1) crucible weight \times 100}{(W2) Sample weight}$

3.7 Estimation of carbohydrates using the Anthrone method (E.E. Layne,1975; David T. Plummer ,1990)

- I. Prepared fresh anthrone reagent by dissolving two grams of anthrone in one litre of concentrated H₂SO₄.
- II. Glucose stock solution was prepared by dissolving one mg in one ml and from that working solution was prepared.
- III. Pipetted out a series of test tubes with a different volume of glucose solution from the stock solution and with help of distilled water volume was made one millilitre.
- IV. The first tube was considered as blank and (two to nine) other tubes are used for construction of standard curves. One tube which was marked as unknown is used for the sample.
- V. To each tube five millilitres of anthrone reagent was added and mixed well by vortex ting and tubes were kept cool for a while.
- VI. Then tubes were sealed with caps and kept in a water bath for ten min at 90°C in a boiling water bath.
- VII. The tubes were removed from the water bath and kept too cool to 25±2°C and then optical density was measured at 620 nm against blank using UV-Vis spectrophotometer (UV -1800 240V, Shimadzu Europa).
- VIII. The standard graph was plotted with concentration of glucose μ g vs optical density at 620 nm and with help of its unknown sample concentration was calculated.

3.8 Estimation of protein using Lowry's method

3.8.1 Reagent prepared

I. Reagent A; Reagent was prepared by dissolving two grams of sodium carbonated and four hundred milligram of sodium hydroxide in hundred millilitres of distilled water.

- II. Reagent B; Reagent was prepared by dissolving five hundred milligrams of copper sulphate (CuSO₄.5H₂O) and one gram of potassium sodium tartrate (KNaC₄O₆.4H₂O) this is dissolved in hundred ml of distilled water reagent b is prepared freshly.
- III. Alkaline Copper solution Reagent C; Reagent was prepared freshly by mixing one millilitre of reagent B and fifty millilitre of reagent A.
- IV. Diluted Folin's Reagent D; Dilute ciocalteu reagent with an equal volume of 0.1 N Sodium Hydroxide.
- V. Standard; Bovine serum albumin is used as standard; the fifty-milligram bovine serum albumin was dissolved in fifty millilitre distilled water in volumetric flask and from this solution ten millilitre is further added to fifty millilitres of distilled water.

3.8.2 Protocol

- I. Pipetted out a series of test tubes of different volumes of working solution of two hundred microlitre, four hundred microlitre, six hundred microlitre, eight hundred microlitre and one millilitre and test tubes were labelled accordingly. The distilled water is added to make up the volume one millilitre.
- II. The one test tube was labelled as unknown in which one millilitre of sample was added and another test tube with one millilitre distilled water was considered blank.
- III. In each test tube five millilitre of reagent C was added including unknown and blank, the tubes were mixed and allowed to stand for ten minutes.
- IV. Then five hundred microlitre of reagent D was added with immediate mixing and then kept for incubation at 25 ± 2 °C in dark for thirty minutes.
- V. The standard graph was plotted of bovine serum albumin concentration against optical density at 660 nm and unknown was calculated.

3.9 Phytochemical Screening of Taro leaves extract

3.9.1 Phenol test

To extract a few drops of two percent (FeCl₃) Ferric chloride solution was added, formation of blue-green yellow indicates presence of phenols (Ali Gamal AL-Kaf et al, 2019).

3.9.2 Saponin test

The two millilitre of extract was taken and added water to it then mixed vigorously for frothing, persists of foam indicates presence of Saponins (Arunachalam S and T.V Krishnapriya, 2017).

3.9.3 Flavonoids

To the one millilitre extract ten percent concentrated (H_2SO_4) sulfuric acid was added the presence of flavones is indicated by yellow – oranges red tint (R. Rajput et al. 2023).

3.10 Estimation of Antioxidant activity using DPPH (2,2- diphenyl-1- picrylhydrazyl) Assay

The DPPH is a molecule that acts as a radical Scavenging molecule. It is soluble in ethanol; it has violet colour and absorbance can be measured at 517 nm. Antioxidant molecules are able react with (DPPH) by donating hydrogen or electron and acceptance of electron or hydrogen by DPPH will reduce it to 2,2- diphenyl-1 hydrazine (DPPH-H) that is indicated by colour change to pale yellow or colourless that is detected by spectrophotometer (Athavale et al. 2012; Nilima S. Rajkumar et al. 2011).

Protocol

- I. Prepared fresh DPPH 0.3mM in ethanol solvent and stored in an amber bottle.
- II. Prepared fresh sample with different concentration twenty five percent, fifty percent seventy five percent and finally hundred percent concentrated concentration extract .
- III. The five test tubes were used in each test tube different concentrations of extracts were added and distilled water added to make up the volume three millilitre. In one of the test tubes only DPPH and Solvent was added and that is used as blank.
- IV. Then in each test tube one millilitre of DPPH was added. These test tubes were mixed well and also covered with aluminium foil. Then test tubes were kept in dark for 30 minutes of incubation and optical density was measured at 517 nm. Ascorbic acid one milligram per millilitre used as positive control.
- V. The graph was plotted with different concentration of extract vs scavenging activity of different extract concentrations.

The Antioxidant scavenging activity was calculated using the formula:

Antioxidant scavenging activity = $\frac{Ab-As}{Ab \times 100}$

Ab: Absorbance of blank - As; Absorbance of sample.

3.11 Fourier Transform Infrared Spectroscopy of Taro leaves extract.

FTIR identifies chemical bonds in a molecule by producing an infrared absorption spectrum. Liquid aqueous extract was added to the glass plate. This plate was kept in the oven for drying the sample. After drying, the sample was made into powder. This powder was later mixed with potassium bromide and the sample was inserted in a sample holder. Reading was measured using Alpha 2, Bruker FTIR and a graph was plotted using the software origin. Each peak indicates the functional group in the sample.

3.12 Antimicrobial Activities of Taro leaves extract

3.12.1 Antibacterial properties of Taro leaves extract against Escherichia coli

Diffusion method was performed to identify any antimicrobial properties of Taro leaves extract. *E. coli* is gram negative, facultative anaerobic, rod shape, coliform bacteria of the genus *E. coli* used as a test sample. The pathogenic *E. coli* was spread plated on (MH Agar) Muller Hinton agar in the Biosafety cabinet. The MH agar plates were prepared prior Aseptically. The freshly prepared plant extract was put into wells at the centre of plates in wells which were created with a sterile cork borer of nine millimetre. The control plate was kept without plant extract samples for reference. The inhibition zones were observed and measured. The *E. coli* was spread plated on the Muller Hinton Agar and control plate as well as the sample plates were measured and the test was performed in triplicates. The range of inhibition zones can be in 13mm to 20mm. *E. coli* was getting inhibited by Taro leaves extract and zone were observed and size was measured. The spread plate was carried out carefully in the biosafety cabinet for safety concern (Ahmad Shwans et. al. 2018).

3.12.2 Antifungal properties

The antifungal test was performed similarly like antibacterial with the help of a diffusion method. The fungus was isolated from spoiled strawberry and spot inoculated on (PDA) Potato Dextrose agar which was prepared prior aseptically. Agar plates and incubated for 24 hours at $25\pm2^{\circ}$ C after spread plating Then for antifungal test the plates were spread plated with a spreader in between flame and with help of sterile borer the well were created and extract was put at the centre of plates in wells and zones of inhibitions were observed.

3.12.3 Identification of fungal culture

Morphology under microscope

The fungus was isolated from spoiled strawberry and grown on PDA and kept at 25 ± 2 °C for incubation and one's spores was visible with help of teaser the spores and mycelium were plucked aseptically and kept on clean glass slide then the spores and mycelium were carefully separated and stained with lactophenol cotton blue, carefully coverslip was placed from the angle of 45° avoiding air bubbles and the slide was mounted under microscope and observed the slide under 40x magnification power. The pictures were used to identify the fungus. Lactophenol cotton blue consists of aniline which stains chitin and cellulose present in fungal cell walls.

CHAPTER FOUR

ANALYSIS AND CONCLUSIONS

4.1 Taro leaves collection

Taro leaves were plucked by hand from Majorda, Goa, (Latitude: 15.297495, Longitude: 73.951188). In (fig. 2)Taro leaves were identified based on their morphology; Taro leaves were brought to the laboratory for further processing.



Fig. 2: Picture of the collected Taro leaves and the location, Majorda Goa.

4.2 Different Taro extract

Different extracts (fig.3) were prepared from Taro leaves for preparation of coating such as aqueous extracts, alkaline extracts, acidic extracts and mixed extracts. Each extract was used to make control with 2% glycerol and 98% extracts for the coating and to study the shelf life of strawberries by comparing with 100% extracts.



Fig. 3: Different extract obtained from Taro leaves

4.3 Moisture content

Table 2: Moisture content	and total solid	content of Taro leaves
---------------------------	-----------------	------------------------

Crucible weight (W1) in g	Weight of sample in g	Sample with crucible (W2) in g	Dry weight (W3) in g	Moisture content (%)	Total solid (%)
43.74	2.02	45.76	44.13	80.50	19.49
43.58	2.013	45.59	43.96	80.97	19.02
46.10	2.04	48.14	46.49	80.81	19.18

Total moisture content = $\frac{(W2) \text{ sample weight } - (W3) \text{ dried sample weight } \times 100}{(W2) \text{ Sample weight}}$

The total moisture content is estimated to be 80.76 % in Taro leaves.

Total solid = $leaves \frac{(W3) dried weight - (W1) crucible weight \times 100}{(W2) Sample weight}$

The total solid is estimated to be 19.23% in Taro

According to literature the moisture content of Taro leaves extract is in the range of 60 - 83%

(Rashmi et al. 2018). In this study moisture content was estimated to be 80.76 %.

4.4 Morphology of Taro leaves.

Taro (Colocasia esculenta) is monocotyledonous and its family is Araceae. Its leaves are Green. It consists of tubers which are largely consumed by Asians. The Colocasia esculenta are herbaceous perennial plants. The plant's leaf is large and has a heart shaped structure. Taro plants can grow up to one to two in size. The height of the stalk is twenty-five to forty centimetres. The laminae are seven to fifteen cm in width and twelve to twenty-five centimetres in length. The petiole colour was light green. The leaf's main vein colour was greenish colour. Leaf blade margin was green and the margin of the leaf was entire. The Taro leaf shows best water repellent properties. It has super hydrophobic properties which makes a good substrate or extraction of bio wax as well an ideal substance for preparation of edible coating as edible coating should have hydrophobic characteristics so it can adhere to coated substances more firmly, and harness the water repellent property. This property of Taro leaves makes Taro leaves good for extraction of bio wax. The low surface vein colour was light green shade. The piko is the point of junction between the leaf blade and petiole. The piko colour is light green. The colour of primary blades is whitish green. The leaf blades are flare in shape; no cupped shape was observed. The blade orientation was downwards. The Taro leaves did not have any Variegation.

4.5 Coating of strawberries with different Taro leaves extract using dip method and checking shelf life at 25±2°C.

Different extracts (fig. 4) were used to coat the entire surface of strawberries by dip method. Upon quantifying, the amount of extract utilized for coating each strawberry was found to be 0.62 Millilitre. From (table 3), overall it can be clearly seen that the strawberries which were coated with extracts remained healthy for a longer period of time compared with the control sample that were treated with distilled water only. The strawberries which were coated with 100% aqueous extract and kept at 25 ± 2 °C remained healthy for seven days. Similarly, the strawberries coated with alkaline extract remained healthy up to the seventh day. However, it can be seen that, the strawberries coated with mixed extract got spoiled on the seventh day, strawberries coated with 98% extract and having 2% glycerol also got spoiled on the sixth day and strawberries coated with acidic extract as well as the control got spoiled on the fourth day (Table 4). This experiment shows that shelf life of strawberries is increased by 100% when coated with 100% aqueous extract compared to the control. From this it can be concluded 100% aqueous extract is the best coating material over alkaline extract as the latter involves the involvement of NaOH in the extraction process.

Table 3:The table shows the effect of coating strawberries with Taro leaves extract at 25 ± 2 °C along with a control sample on various days.

Days	Strawber Coated with Aqu extract	ries Ieous	Strawberries Coated with Acidic extract		Strawberries Coated with alkaline extract		Strawberries Coated with Mixed extract		Control
	100%	98% +2% G	100%	98% +2% G	100%	98% +2% G	100%	98% +2% G	No Coating
0	Н	Н	Н	Н	Н	Н	Н	Н	Н
1	Н	Н	Н	Н	Н	Н	Н	Н	Н
2	Н	Н	Н	Н	Н	Н	Н	Н	Н
3	Н	Н	Н	Н	Н	Н	Н	Н	Н
4	Н	Н	S	S	Н	Н	Н	Н	S
5	Н	Н	-	-	Н	Н	Н	Н	-
6	Н	Н	-	-	Н	Н	Н	Н	-
7	Н	S	-	-	Н	S	S	S	-
8	S	-	-	-	S	-	-	-	-

Key: S - SPOILED, H-HEALTHY, G-GLYCEROL, RT - 25±2°C,



Fig. 4: Showing the status of strawberries coated with different extracts at $25\pm^{\circ}C$ on various

days.

Sr. No.	Sample	Day at which strawberries got spoiled
1	Control sample (without coating) 25 ± 2 °C	Day 4
2	Strawberries coated with aqueous extract	Day 8
3	Strawberries coated with mixed extract	Day 7
4	Strawberries coated with acid extracts	Day 4
5	Strawberries coated with alkaline extracts	Day 8

Table 4 :- Effect of different Taro leaves extract on shelf life of strawberries at 25 ± 2 °C.

4.6 Effect of coating strawberries by different Taro leaves extract on shelf life of strawberries at 4°C.

The control strawberries were healthy for up to seventeen days (fig. 5) when stored at 4°C. The strawberries coated with alkaline extract and strawberries coated with mixed extracts were healthy up to day 20. The strawberries coated with acidic extract were healthy up to day 17. The best results were shown by aqueous extract, where coated strawberries remained healthy up to 21 days when stored at 4°C (table 5).



Fig. 5 Effect of coating from Taro leaves extract on shelf life of strawberries at 4°C.

It can be seen from (Table 5) that the aqueous extracts increased the shelf life of strawberries by 37.5 percent, compared to control when kept at 4°C.

Sr. No.	Sample	Days	Increase in shelf life (%)
1	Control sample (without coating) 4°C. temperature	16	-
2	Strawberries coated with aqueous extract	22	37.5%
3	Strawberries coated with mixed extract	21	31.25 %
4	Strawberries coated with acid extracts	17	6%
5	Strawberries coated with alkaline extracts	20	25%

Table 5:- Effect of Different Taro extract on shelf life of strawberries at 4°C.

From the results of these two above experiments, it was concluded that the aqueous extract of Taro leaves extract is the best in increasing the shelf life of the strawberries and hence only aqueous extract was characterized further for biochemical characteristics.

4.7 Shelf life and quantity of extract required for coating strawberry

It was observed that the aqueous extract stored at 25 ± 2 °C got spoiled in 2 months (observed with visual fungal growth) and extract stored in cold temperature remained uncontaminated from fungus for 3 months. It was estimated that, on an average, each strawberry required 0.62ml extract for coating.

4.8 The phytochemical analysis

The phytochemical constituents such as phenol, flavonoids and saponins were identified in Taro plant (*Colocasia esculenta*) leaves extracted upon qualitative estimation (Table 6). According to literature (Ashish Patel and Jay Singh 2023; R. Rajput 2023) it has also been proven the presence of flavonoids and other components in Taro leaf (*Colocasia esculenta*) extracts are vicenin-2, iso-vitexin etc.

Sr. No.	Test	Result
1	Phenol	Positive
2	Flavonoid	Positive
3	Saponin	Positive
4	Antioxidant	Positive

 Table 6: Phytochemical analysis of Taro leaves extracts

4.9 Protein and carbohydrates analysis

Table 7: The protein and carbohydrate estimation of Taro leaves extract

Sr. No.	Test	Method	concentration estimated
1	Carbohydrate estimation	Anthrone method	$5.85 \pm 0.816 \times 10^{-3}$
2	Protein estimation	Lowry Method	$2.40 \pm 0.478 \times 10^{-2}$

The carbohydrates are estimated to be $5.85 \pm 0.8164 \times 10^{-3}$

The proteins are estimated to be $2.40\pm0.478\times\!10^{-2}$

In this study the concentrations which were estimated are lesser than reported in the literature (Rashmi and Raghu ,2018) their values of carbohydrates they found 6.7 grams and protein 5 grams in Taro leaves.

4.10 Estimation of Antioxidant activity of Taro leaves using DPPH method

It can be clearly seen from (fig. 6) that as concentrations of Taro leaves extracts increase the scavenging activity increases in DPPH. At twenty five percent least, scavenging activity is measured. That was estimated to be 22.41 %. The highest scavenging activity is at hundred percent extracts that is 87.35% According to literature 65.80 % scavenging activity was seen (Christou et. al 2023).



Fig. 6: Graph of Antioxidant activity of Taro leaves extracts at different concentrations.

4.11 Fourier Transform Infrared Spectroscopy Analysis.

The Fourier Transform Infrared Spectroscopy (FTIR) identified functional groups in chemical bonds of molecules. The graph (fig.7) depicts that there is a hydroxyl group or alcohol group which shows stretching at 3425 cm⁻¹ wavenumbers can be due to the presence of phenolic compounds. Peak at 1583 cm⁻¹ wavenumber can be of OH or N-H group which is bending at 1032 cm⁻¹ wavenumber the presence of C-C interacting bond which is also attributed to C-O vibrations sugar functional groups. Peak observed at wavenumber 2921 cm⁻¹ confirms the presence of methyl functional groups present in protein. In this study, a similar peak was observed at wavenumber at 2921 cm⁻¹. Peak at 1311 cm⁻¹ is indicative of the presence of NO₂

bonds. Peaks obtained below 800 cm⁻¹ show presence of halogen groups in the compound. FTIR data confirm the existence of several groups such as hydroxyl groups. Peak at 1393 cm⁻¹ shows presence of CH₃ bending. However, a previous study observed the presence of sugar molecules such as galactose and arabinose at wavenumber 2932.12cm⁻¹. They also found N-H bending of protein molecules at wavenumber 1572.05 cm⁻¹ (Mansuari M. Tosfi et. al. 2023).



Fig. 7: Taro leaves extract (Aqueous) fourier transform infrared Spectra of Taro leaves

extract.

4.12 Antimicrobial activities of Taro leaves extract

Sr. No.	Inhibition Zone against Escherichia coli (in mm)
1	13
2	15
3	17

Table 8 Zone	Inhibition	shown b	ov Taro	leaves a	against	Escherichia	coli

The zone inhibition shown (fig. 8) by Taro leaves extract against *Escherichia coli* clearly shows the zone of inhibitions that indicates that plant extract is effective against. *E. coli*. The zone of inhibition on an average was 15 millimetres which was lower in accordance with literature which was 16.2 millilitres (Sarmistha Dutta and Biswajit Aich, 2017) and comparatively higher than value reported by (Mani M. et al. 2021) as they found 13 mm inhibition zone.



Fig. 8: Zone Inhibition shown by Taro against Escherichia coli.

4.13 Antifungal Activity

According to the literature it has been concluded that aqueous extract of taro leaves shows antifungal activity to various fungal species such as *Fusarium*, *alternaria solani*, *alternaria ricini*. (Shehata, M. G et al. 2023; Mengane, et al. 2015). The fungus isolated from spoiled strawberries was identified as *Rhizopus* based on its colony morphology such as white mycelium (fig. 9B) and microscopic observation (fig.10) using lactophenol cotton blue. When aqueous extract of taro leaves was tested for antifungal activity against isolated fungus showed an inhibition zone 15mm diameter (fig. 9A)



Fig. 9: Zone of inhibition shown by *Rhizopus* on PDA plate upon addition of aqueous extract of taro leaves. A)- test plate with *Rhizopus* and aqueous extract of taro leaves. B)- control plate with *Rhizopus* only.

The fungus involved in the strawberry spoilage was identified as belonging to *Rhizopus* sp. based on its sporulating bodies and structure (fig. 10). The handbook of soil fungi was referred to identify the fungi (Nagamani A. K3.12.3 unwar et.al 2006).



Fig. 10: Image of *Rhizopus* sp. observed under 40X objective of light microscope after

staining with lactophenol cotton blue stain.

Discussion

In this investigation, we explored the efficacy of Taro leaves extract edible coating applied at varying temperatures on the preservation of fresh strawberries. The findings indicate that the aqueous extract derived from Taro leaves effectively mitigates fungal decay and extends the shelf life of strawberries by up to 37.5% compared to uncoated counterparts. The shelf life of strawberries stored in cold conditions is approximately 2 weeks, while those stored at 25 ± 2 °C typically last 3-4 days (Romanazzi, 2009). In another study it was found that strawberries coated with encapsulated cannabidiol nanoparticles remain healthy for 15 days whereas in this study the Taro extract increases shelf life till the 22 days (Sukhavattanakul, P. et. al 2023). According to literature the strawberry which was coated with banana-starch-chitosan-aloe vera edible coating increased the shelf life up to 15 days. Whereas, the Taro leaves extract showed better results by increasing the shelf life up to 22 days (Pinzon, M. I., Sanchez, et. al 2019). Furthermore, this coating preserves the strawberries' properties, including their colour and firmness, thereby demonstrating its efficacy in maintaining fruit quality.

Conclusion

The edible coating from aqueous extract of Taro leaves showed best results compared to the alkaline extracts, acidic extracts and mixture extracts. The shelf life of the strawberries coated with aqueous extract increased by 100 % compared to the strawberries which were not coated. In terms of extending shelf life of strawberries increased at 4°Celsius Further, the shelf life of aqueous extract coated strawberries kept at 4° Celsius increased by 37.5%, compared to noncoated strawberries stored at 4° Celsius. The utilization of Taro leaves extract emerged as a promising avenue for the development of edible coatings, particularly for enhancing the shelf life of perishable fruits such as strawberries. The multifaceted properties exhibited by Taro leaves extract, including antioxidant activity, antimicrobial attributes, phytochemical compounds, adhesion capabilities, durability, etc. The antimicrobial shown by taro leaves extract against E. coli and Rhizopus fungal species, makes it suitable for prolonging shelf life of perishable fruits. Moreover, Taros' natural origin, cost-effectiveness, and low maintenance requirements position it as an environmentally friendly alternative to conventional plasticbased coatings. The findings of this study not only demonstrate the efficacy of Taro extract in extending fruit shelf life but also suggest its potential as a novel solution in the ongoing quest for sustainable edible coating solutions. By leveraging the abundant and renewable resources provided by Taro leaves, we can mitigate the environmental impact associated with plastic accumulation and contribute to a more sustainable approach to food preservation and packaging.

Future Prospects

- Identification of individual active molecular components can be studied in the Taro leaves extract.
- Further analysis such as NMR, XRD, SEM, Mass spectrometry, and other characterizations can be performed.
- The Future Prospects of this research is that the process of preparation of Taro leaves extracts can be upscaled at larger quantities and study can be tested on several different fruits and vegetable.

References

- Abdul Khalil, H. P. S., Banerjee, A., Saurabh, C. K., Tye, Y. Y., Suriani, A. B., Mohamed, A., Karim, A. A., Rizal, S., & Paridah, M. T. (2018, June 21). Biodegradable Films for Fruits and Vegetables Packaging Application: Preparation and Properties. Food Engineering Reviews, 10(3), 139–153. <u>https://doi.org/10.1007/s12393-018-9180-3</u>
- 2. Akash Kalita ,Nayan Talukdar Department of Biotechnology, Assam down town University, Guwahati,Assam,India(2018,April) *Colocasia esculenta* (1.) leaf bio-wax as a hydrophobic surface Coating Substance for Paper for preparing hydrophobic paper bags. International Journal of Pharmacy and Biological Sciences <u>www.ijpbs</u>online.com
- Agarwal, S., Gogoi, M., Talukdar, S., Bora, P., Basumatary, T. K., & Devi, N. N. (2020). Green synthesis of silver nanoplates using the special category of plant leaves showing the lotus effect. RSC Advances, 10(60), 36686–36694. https://doi.org/10.1039/d0ra06533a
- Andrade, R. M. S., Ferreira, M. S. L., & Gonçalves, D. C. B. A. (2016, January 14). Development and Characterization of Edible Films Based on Fruit and Vegetable Residues. Journal of Food Science, 81(2).<u>https://doi.org/10.1111/1750-3841.13192</u>
- Bizymis, A. P., & Tzia, C. (2021, June 8). Edible films and coatings: properties for the selection of the components, evolution through composites and nanomaterials, and safety issues. Critical Reviews in Food Science and Nutrition, 62(31), 8777–8792. https://doi.org/10.1080/10408398.2021.1934652
- Blancas-Benitez, F. J., Montaño-Leyva, B., Aguirre-Güitrón, L., Moreno-Hernández, C. L., Fonseca-Cantabrana, N., Romero-Islas, L. D. C., & González-Estrada, R. R. (2022, September). Impact of edible coatings on quality of fruits: A review. Food Control, 139, 109063. <u>https://doi.org/10.1016/j.foodcont.2022.109063</u>

 Biswajit Mohanty. (2020, January). Functionality of protein-based edible coatingreview.

https://www.researchgate.net/publication/348869001_Functionality_of_protein-Based_edible_coating-_review

- Baldwin, E., Nisperos, M., Chen, X., & Hagenmaier, R. (1996, November). Improving storage life of cut apple and potato with edible coating. Postharvest Biology and Technology, 9(2), 151–163. <u>https://doi.org/10.1016/s0925-5214(96)00044-0</u>
- Bancal, V., & Ray, R. C. (2022). Overview of food loss and waste in fruits and vegetables: From issue to resources. Fruits and Vegetable Wastes, 3–29. https://doi.org/10.1007/978-981-16-9527-8_1
- Bajaj, K., Adhikary, T., Gill, P. P. S., & Kumar, A. (2023). Edible coatings enriched with plant-based extracts preserve postharvest quality of fruits: A Review. Progress in Organic Coatings, 182, 107669. https://doi.org/10.1016/j.porgcoat.2023.107669
- Baghel, R. S., Suthar, P., Gajaria, T. K., Bhattacharya, S., Anil, A., & Reddy, C. R. K. (2020). Seaweed biorefinery: A sustainable process for valorising the biomass of brown seaweed. Journal of Cleaner Production, 263, 121359. https://doi.org/10.1016/j.jclepro.2020.121359
- 12. Buonaurio R., Iriti M., Romanazzi G., (2009). Induced resistance to plant diseases caused by Oomycetes and fungi. Petria Petria 19(3), 130-148
- Curt Joseph Jimenez Junio Technological Institute of the Philippines (June 2022) Taro leaves (*Colocasia esculenta*) as hydrophobic bio-wax coating for recycled grocery paper bags DOI: 10.13140/RG.2.2.36821.09449
- 14. Christou, A., Parisis, N. A., Venianakis, T., Barbouti, A., Tzakos, A. G., Gerothanassis,I. P., & Goulas, V. (2023). Ultrasound-assisted extraction of Taro leaf antioxidants

using natural deep eutectic solvents: An eco-friendly strategy for the valorization of crop residues. Antioxidants, 12(10), 1801. <u>https://doi.org/10.3390/antiox12101801</u>

- Corbo, Maria & Campaniello, Daniela & Speranza, Barbara & Bevilacqua, Antonio & Sinigaglia, Milena & Farris, Stefano. (2015). Non-Conventional Tools to Preserve and Prolong the Quality of Minimally-Processed Fruits and Vegetables. Coatings. 2015. 931-961. 10.3390/coatings5040931
- 16. Donhowe, i. g., & fennema, o. (1993, august). the effects of plasticizers on crystallinity, permeability, and mechanical properties of methylcellulose films. Journal of Food Processing and Preservation, 17(4), 247–257. <u>https://doi.org/10.1111/j.1745-4549.1993.tb00729.x</u>
- 17. Erkmen O, Barazi AO (2018) General Characteristics of Edible Films. Vol.2 No.1:
- Divya Kashyap, Aastha Vithalkar, Yashwant Kumar Patel (2023, February) Nutritional and Therapeutic Benefits of Taro. TIJER2302063 TIJER - International research journal Volume 10, Issue 2, www.tijer.org 448
- Gadhave, R.V.,Das, A., Mahanwar, P.A. and Gadekar, P.T. (2018) Starch Based Bioplastics: The Future of Sustainable Packaging. Open Journal of Polymer Chemistry, 8, 21-33. <u>https://doi.org/10.4236/ojpchem.2018.82003</u>
- 20. Gol, N. B., Patel, P. R., & Rao, T. V. R. (2013). Improvement of quality and shelf-life of strawberries with edible coatings enriched with chitosan. Postharvest Biology and Technology, 85, 185–195. https://doi.org/10.1016/j.postharvbio.2013.06.008
- 21. Hidayati, S., Zulferiyenni, Maulidia, U., Satyajaya, W., & Hadi, S. (2021, August).
 Effect of glycerol concentration and carboxy methyl cellulose on biodegradable film characteristics of seaweed waste. Heliyon, 7(8), e07799.
 https://doi.org/10.1016/j.heliyon.2021.e07799

- 22. V, I., Rawson, D. A., CK, S., & Jaganmohan, R. (2021, January 1). Food grade method of protein extraction of an under explored plant-based protein: Brief study on seaweed protein. International Journal of Chemical Studies, 9(1), 2931–2935. <u>https://doi.org/10.22271/chemi.2021.v9.i1ao.11675</u>
- 23. Lin, D., & Zhao, Y. (2007, June). Innovations in the Development and Application of Edible Coatings for Fresh and Minimally Processed Fruits and Vegetables. Comprehensive Reviews in Food Science and Food Safety, 6(3), 60–75. <u>https://doi.org/10.1111/j.1541-4337.2007.00018.x</u>
- 24. Le, K. H., Nguyen, M. D.-B., Tran, L. D., Nguyen Thi, H. P., Tran, C. V., Tran, K. V., Nguyen Thi, H. P., Dinh Thi, N., Yoon, Y. S., Nguyen, D. D., & La, D. D. (2021). A novel antimicrobial zno nanoparticles-added polysaccharide edible coating for the preservation of postharvest avocado under ambient conditions. *Progress in Organic Coatings*, 158, 106339. https://doi.org/10.1016/j.porgcoat.2021.106339
- 25. Kadam, S. U., Álvarez, C., Tiwari, B. K., & O'Donnell, C. P. (2017, September).
 Extraction and characterization of protein from Irish brown seaweed Ascophyllum nodosum. Food Research International, 99, 1021–1027.
 https://doi.org/10.1016/j.foodres.2016.07.018
- 26. Kumar, M., Bhardwaj, R. Wetting characteristics of *Colocasia esculenta* (Taro) leaf and a bioinspired surface thereof. Sci Rep 10, 935 (2020). https://doi.org/10.1038/s41598-020-57410-2
- 27. Khodaei, D., Hamidi-Esfahani, Z., & Rahmati, E. (2021, June). Effect of edible coatings on the shelf-life of fresh strawberries: A comparative study using TOPSIS-Shannon entropy method. NFS Journal, 23, 17–23. https://doi.org/10.1016/j.nfs.2021.02.003

- 28. Mohite, A. M., & chandel, D. (2020, October Formulation of edible films from fenugreek mucilage and Taro starch. SN Applied Sciences, 2(11). <u>https://doi.org/10.1007/s42452-020-03710-1</u>
- Milani, J. M., & Nemati, A. (2022). Lipid-based Edible Films and coatings: A review of recent advances and applications. Journal of Packaging Technology and Research, 6(1), 11–22. https://doi.org/10.1007/s41783-021-00130-3
- 30. Mitharwal, S., Kumar, A., Chauhan, K., & Taneja, N. K. (2022, July). Nutritional, phytochemical composition and potential health benefits of Taro (*Colocasia esculenta* L.) leaves: A review. Food Chemistry, 383, 132406.<u>https://doi.org/10.1016/j.foodchem.2022.132406</u>
- Mengane, S. K. (2015). Antifungal activity of the crude extracts of *Colocasia esculenta* leaves in vitro on plant pathogenic fungi. International Research Journal of Pharmacy, 6(10), 713–714. https://doi.org/10.7897/2230-8407.0610138
- 32. Nasri, N. S., Ahmed, M. M., Mohd Noor, N., Mohammed, J., Hamza, U. D., & Mohd Zain, H. (2014, October). Hydrophobicity Characterization of Bio-Wax Derived from Taro Leaf for Surface Coating Applications. Advanced Materials Research, 1043, 184– 188. <u>https://doi.org/10.4028/www.scientific.net/amr.1043.184</u>
- 33. Nagamani, A., Kunwar, I. K., & Manoharachary, C. (2006). Handbook of soil fungi.I.K International Pvt. Ltd..
- 34. Nunes, C., Silva, M., Farinha, D., Sales, H., Pontes, R., & Nunes, J. (2023). Edible coatings and future trends in active food packaging–fruits' and traditional sausages' shelf life increasing. Foods, 12(17), 3308. <u>https://doi.org/10.3390/foods12173308</u>
- 35. Nicolas, O., Pierre, A. E., Renan, E. T., Hemayoro, S., Pauline, B., Mahamadou, S., & Vincent, L. (2023a). Nutritional and phytochemical characterization of Taro [Colocasia

esculenta (L.) schott] germplasm from Burkina Faso. Journal of Plant Breeding and Crop Science, 15(1), 32–41. https://doi.org/10.5897/jpbcs2022.0999

- 36. O' Connor, J., Meaney, S., Williams, G. A., & Hayes, M. (2020). Extraction of Protein from Four Different Seaweeds Using Three Different Physical Pretreatment Strategies.
 Molecules (Basel, Switzerland), 25(8), 2005. https://doi.org/10.3390/molecules25082005
- 37. Owusu-Akyaw Oduro, K. (2022). Edible coating. Postharvest Technology Recent Advances, New Perspectives and Applications. <u>https://doi.org/10.5772/intechopen.101283</u>
- Popović, Senka & Lazić, Vera & Hromiš, Nevena & Suput, Danijela & Bulut, Sandra.
 (2018). Biopolymer Packaging Materials for Food Shelf-Life Prolongation.
 10.1016/B978-0-12-811449-0.00008-6.
- 39. Patel, A., & Singh, J. (2023, January 1). Taro (*Colocasia esculenta L*): Review on Its botany, morphology, ethno medical uses, Phytochemistry and pharmacological activities. The Pharma Innovation, 12(3), 05–14. https://doi.org/10.22271/tpi.2023.v12.i3a.18908
- 40. Pinzon, M. I., Sanchez, L. T., Garcia, O. R., Gutierrez, R., Luna, J. C., & Villa, C. C. (2019). Increasing shelf life of strawberries (fragaria SSP) by using a banana starch-chitosan-aloe vera gel composite edible coating. International Journal of Food Science & amp; Technology, 55(1), 92–98. <u>https://doi.org/10.1111/ijfs.14254</u>
- 41. Priya, K., Thirunavookarasu, N., & Chidanand, D. V. (2023). Recent advances in edible coating of food products and its legislations: A Review. Journal of Agriculture and Food Research, 12, 100623. <u>https://doi.org/10.1016/j.jafr.2023.100623</u>
- 42. Prabhu, M. S., Israel, A., Palatnik, R. R., Zilberman, D., & amp; Golberg, A. (2020).Integrated Biorefinery Process for Sustainable Fractionation of ulva ohnoi

(*Chlorophyta*): Process optimization and revenue analysis. Journal of Applied Phycology, 32(4), 2271–2282.<u>https://doi.org/10.1007/s10811-020-02044-0</u>

- 43. Pashova, S. (2023). Application of plant waxes in edible coatings. Coatings, 13(5), 911. https://doi.org/10.3390/coatings13050911
- 44. Pinto, T., Pinto, A., & Vilela, A. (2023). Edible coatings and films for preparation of grapevine by-product infusions and in freshly processed products. Coatings, 13(8), 1350. <u>https://doi.org/10.3390/coatings13081350</u>
- 45. Perez-Vazquez, A., Barciela, P., Carpena, M., & Prieto, M. A. (2023). Edible Coatings as a Natural Packaging System to Improve Fruit and Vegetable Shelf Life and Quality. Foods (Basel, Switzerland), 12(19), 3570. https://doi.org/10.3390/foods12193570
- 46. Quach & Melton, Laurence & Harris, Philip & Burdon, Jeremy & Smith, Bronwen.
 (2001). Cell wall compositions of raw and cooked corms of Taro (*Colocasia esculenta*).
 Journal of the Science of Food and Agriculture. 81. 311 318. 10.1002/1097-0010(200102)81:3<311::AID-JSFA816>3.0.CO;2-B.
- 47. Quero-García, J., Letourmy, P., Ivancic, A., Feldmann, P., Courtois, B., Noyer, J. L., & Lebot, V. (2009, April 12). Hybrid performance in Taro (*Colocasia esculenta*) in relation to genetic dissimilarity of parents. Theoretical and Applied Genetics, 119(2), 213–221. https://doi.org/10.1007/s00122-009-1030-5
- 48. Raghav, PramodK., Agarwal, N., & Saini, M. (2016). Herbal edible coatings of fruits
 & vegetables: A newer concept. International Journal of Advanced Research, 4(6), 1452–1458. https://doi.org/10.21474/ijar01/660
- Rashmi, D. R., Raghu, N., Gopenath, T. S., Palanisamy, P., Bakthavatchalam, P., Karthikeyan, M., & Basalingappa, K. M. (2018). Taro (*Colocasia esculenta*): an overview. Journal of Medicinal Plants Studies, 6(4), 156-161.

- 50. Suput, Danijela & Lazic, Vera & Popović, Senka & Hromis, Nevena. (2015). Edible films and coatings: Sources, properties and application. Food and Feed Research. 42. 11-22. 10.5937/FFR1501011S.
- 51. Sukhavattanakul, P., Thanyacharoen, T., Chuysinuan, P., Techasakul, S., & Ummartyotin, S. (2023). Influence of a transparent and edible coating of encapsulated cannabidiol nanoparticles on the quality and shelf life of strawberries. ACS Applied Materials & amp; Interfaces, 15(19), 23834–23843. https://doi.org/10.1021/acsami.3c04036
- Salgado P.R., Ortiz C.M., Musso Y.S., Di Giorgio L., Mauri A.N. (september, 2015)Edible Films and Coatings Containing Bioactives. *Curr. Opin. Food Sci.* 2015;5:86–92. doi: 10.1016/j.cofs.
- 53. Siska Wardhani, D. D., Warkoyo, Hidayat, R., & Sukardi. (2020, February 1). Physicmechanical properties of edible film based on Taro starch (*Colocasia esculenta* L. Schott) with glycerol addition. IOP Conference Series: Earth and Environmental Science, 458(1), 012039. <u>https://doi.org/10.1088/1755-1315/458/1/012039</u>
- 54. Senturk Parreidt, T., Müller, K., & Schmid, M. (2018). Alginate-based edible films and coatings for Food Packaging Applications. Foods, 7(10), 170. https://doi.org/10.3390/foods7100170
- 55. Shehata, M. G., Abd El-Aziz, N. M., Mehany, T., & Simal-Gandara, J. (2023). Taro leaves extract and probiotic lactic acid bacteria: A synergistic approach to improve antioxidant capacity and bioaccessibility in fermented milk beverages. LWT, 187, 115280. https://doi.org/10.1016/j.lwt.2023.115280
- 56. Temesgen, M., & Retta, N. (2015). Nutritional potential, health and food security benefits of Taro *Colocasia esculenta (L.)*: A review. Food Science and Quality Management, 36(0), 23-30

- 57. Tarique, J., Sapuan, S. M., & Khalina, A. (2021, July 6). Effect of glycerol plasticizer loading on the physical, mechanical, thermal, and barrier properties of arrowroot (*Maranta arundinacea*) starch biopolymers. Scientific Reports, 11(1). <u>https://doi.org/10.1038/s41598-021-93094-y</u>
- 58. Trajkovska Petkoska, A., Daniloski, D., D'Cunha, N. M., Naumovski, N., & Broach, A. T. (2021, February). Edible packaging: Sustainable solutions and novel trends in food packaging. Food Research International, 140, 109981. <u>https://doi.org/10.1016/j.foodres.2020.109981</u>
- 59. Tosif, M. M., Bains, A., Goksen, G., Ali, N., Rusu, A. V., Trif, M., & Chawla, P. (2023). Application of Taro (*Colocasia esculenta*) mucilage as a promising antimicrobial agent to extend the shelf life of fresh-cut brinjals (eggplants). Gels, 9(11), 904. https://doi.org/10.3390/gels9110904
- 60. Warkoyo, Y. W. N. H. (2019, January 13). Characterization of Edible Film from Starch of Taro (*Colocasia esculenta (L.) Schott*) with Addition of Chitosan on Dodol Substituted Seaweed (*Eucheuma cottonii L.*). Food Technology and Halal Science Journal, 1(1), 22. https://doi.org/10.22219/fths.v1i1.7544

Appendix I

PDA; Potato Dextrose Agar Compositions; 39 grams in 1000 ml distilled water.

Ingredients	Grams / litre
potatoes/ Infusion form	200.0g
Agar	15.0g
Dextrose	20.0g

MHA; Muller Hinton Agar;

Ingredients	Grams/Litre		
Beef extract	2.0g		
Acid hydrolysis of casein	17.50g		
Starch	1.50g		
Agar	17.0g		
Distilled water	1000 millilitres		

lactophenol cotton blue

Ingredients	grams/ litre		
Aniline blue	0.05g		
Phenol crystal	20.0g		
Glycerol	40.0 millilitres		
Lactic Acid	20.0 millilitres		
Distilled water	20.0millilitres		

Anthrone Reagent: 0.2 percent anthrone in concentrated sulfuric acid.

Standard Graph of Protein



Standard Graph of Carbohydrates

